

Janssen Research & Development ***Clinical Protocol**

Protocol Title

Randomized, Double-blind, Placebo-controlled, Multicenter Phase 3 Study to Assess the Efficacy, Safety And Immunogenicity of Vaccination With ExPEC9V in the Prevention of Invasive Extraintestinal Pathogenic Escherichia coli Disease in Adults Aged 60 Years And Older with a History of Urinary Tract Infection in the Past 2 Years

**Protocol VAC52416BAC3001; Phase 3; E.mbrace
Version: Amendment 8**

VAC52416 (JNJ-78901563 [ExPEC9V])

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GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

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PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	Date
Amendment 8	09 August 2024
Amendment 7	17 November 2023
Amendment 6	20 July 2023
Amendment 5	2 December 2022
Amendment 4	02 June 2022
Amendment 3	20 September 2021
Amendment 2	19 May 2021
Amendment 1	10 May 2021
Original Protocol	26 January 2021

Amendment 8 (09 August 2024)**Overall Rationale for the Amendment:**

The main purpose of the amendment is to re-order study objectives such that the second primary objective becomes the sole primary objective. The VAC52416BAC3001 study design per Protocol Amendment 7 included two primary objectives for the prevention of invasive *E. coli* disease (IED). The first primary objective was prevention of first IED with microbiological confirmation from blood, other sterile sites, or urine. The second primary objective was prevention of first IED with microbiological confirmation from blood or other sterile sites, excluding urine only. With the original design, a hierarchical testing strategy would be applied with the first primary objective being tested before the second primary objective could be tested. Based on initial projections, the requisite number of IED cases from blood/sterile sites/urine was anticipated to be reached earlier in time, thus allowing for an earlier assessment of the efficacy of ExPEC9V in the prevention of IED. However, to date, the observed incidence of IED cases has been lower than expected and the majority of these cases have had microbiological confirmation from blood. Consequently, the projections to reach the requisite number of events for either primary IED objective are very similar. Given differences in clinical severity, ExPEC9V vaccine efficacy for the prevention of IED cases from blood/sterile sites is expected to be higher relative to efficacy for the prevention of IED cases from blood/sterile sites/urine. Therefore, to remove the risk of not being able to analyze the second primary objective if the first primary objective fails, the objectives have been re-ordered such that prevention of IED cases from blood/sterile sites is the sole primary objective. With the re-ordering of the objectives, corresponding changes have been made in the statistical plan with regard to the number of events necessary for analyses of IED cases from blood/sterile sites. The secondary endpoint definitions and the hierarchical testing strategy for these endpoints is unchanged.

An update has been made to the O-serotyping testing scheme. Based on the specificity and sensitivity observed during additional validation work on the agglutination assay, only agglutination will be performed to serotype *E. coli* isolates for the primary and secondary objectives.

To enhance the operational feasibility of the study and achieve the number of IED cases from blood/sterile sites required for final analysis of the primary objective, the sample size has been re-estimated at approximately 19,800 participants, and the duration of follow up has been extended from 3 to 4 years.

An update has also been made to discontinue site reporting of suspected urinary tract infection (UTI) and acute bacterial prostatitis (ABP) events unless the events are suspected to be pyelonephritis. The primary rationale for this change is to reduce participant and site burden during follow up and refocus study procedures on the collection of IED and pyelonephritis events. At the time of implementation of this protocol amendment, a sufficient number of UTI cases are projected to have been accrued to assess vaccine efficacy for the prevention of UTI.

The changes made to the clinical protocol VAC52416BAC3001 as part of Protocol Amendment 8 are listed below, including the rationale of each change and a list of all applicable sections. Changes made in previous protocol amendments are listed in Section 10.17 Appendix 17: Protocol Amendment History.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 2.1 Study Rationale 3. Objectives and Endpoints 4.1 Overall Design 4.2 Scientific Rationale for Study Design 6.3 Measures to Minimize Bias: Randomization and Blinding 8.1 Efficacy Assessments 8.1.2 IED Clinical Case Definition 9.1 Statistical Hypotheses 9.2.1 Efficacy 9.4.1.1 Primary Endpoint 9.4.1.2 Secondary Endpoints 9.5.1 Interim Analyses 9.5.1.1 Interim Analyses for the Primary Endpoint 9.5.2 Final Analysis 10.12 Appendix 12: Statistical Methodology for Sample Size Calculations	The study objectives were re-ordered such that prevention of first IED with microbiological confirmation from the blood or other sterile sites becomes the sole primary objective and prevention of first IED with microbiological confirmation from the blood, other sterile sites, or urine becomes the first secondary objective. Corresponding changes were made in the statistical plan.	To remove the risk of not being able to analyze the efficacy of ExPEC9V in the prevention of IED with microbiological confirmation from the blood or other sterile sites if the objective for prevention of IED with microbiological confirmation from the blood, other sterile sites, or urine fails.
8.5 Microbiological Assessments 9.4.1.2 Secondary Endpoints 10.15 Appendix 15: Decision Tree for Isolate Characterization	To O-serotype <i>E. coli</i> isolates for the primary and secondary objectives, only agglutination will be performed. The decision tree was removed as the O-serotype testing scheme is described in the text.	Based on the specificity and sensitivity observed during additional validation work on the agglutination assay, using this assay alone will produce reliable O-serotyping results while reducing operational complexity.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 4.1 Overall Design 6.3 Measures to Minimize Bias: Randomization and Blinding 9.2.1 Efficacy 9.2.3 Safety Subset 9.5.1 Interim Analyses 10.12 Appendix 12: Statistical Methodology for Sample Size Calculations	The target number of participants to be enrolled was increased from 18,556 to approximately 19,800.	To enhance the feasibility of the study to assess the efficacy of ExPEC9V in the prevention of IED with confirmation from blood or other sterile sites.
1.1 Synopsis 1.2 Schema 1.3.1 Schedule of Activities – Assessments for All Participants 3 Objectives and Endpoints 4.1 Overall Design 4.4 End of Study Definition 8 Study Assessments and Procedures 8.1 Efficacy Assessments 8.1.7 Frailty Assessments 8.4 Immunogenicity Assessments 9.2.1 Efficacy 9.4.3 Immunogenicity Analyses 9.5.1 Interim Analyses 9.5.2 Final Analysis 9.5.3 End-of-study Analysis 10.12 Appendix 12: Statistical Methodology for Sample Size Calculations	The duration of follow up was increased from 36 to 48 months with a site visit (immunogenicity subset) or telephone contact (all other participants) added at Day 1,461. For participants enrolled prior to Protocol Amendment 8 who are not reconsented on an updated ICF, the end of study visit will be conducted at Year 3 (Day 1,096).	To enhance the feasibility of the study to assess the efficacy of ExPEC9V in the prevention of IED with confirmation from blood or other sterile sites.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.2 Schema 1.3.1 Schedule of Activities – Assessments for All Participants 1.3.3 Schedule of Activities – Assessments for Participants with Suspected Pyelonephritis 3 Objectives and Endpoints 4.1 Overall Design 6.8 Concomitant Therapy 8 Study Assessments and Procedures 8.1 Efficacy Assessments 8.1.3 Pyelonephritis Clinical Case Definition and Procedures 8.1.4 UTI Clinical Case Definition 8.1.5 ABP Clinical Case Definition 8.1.6 Patient-reported Outcomes 8.3.6 Disease-related Events and Disease-related Outcomes not Qualifying as Adverse Events or Serious Adverse Events 8.4 Immunogenicity Assessments 8.5 Microbiological Assessments 8.6 Medical Resource Utilization and Health Economics 10.18 Appendix 18: UTI and ABP Study-specific Procedures Prior to Protocol Amendment 8	Changes were made to indicate that site-related study procedures will no longer be performed for suspected uncomplicated or complicated UTI or ABP events unless these events are suspected to be pyelonephritis. For all participants, such events will no longer require study visits, data entry in the CRF, eCOA surveys, and retrieval of bacterial isolates (<i>E. coli</i> or <i>P. aeruginosa</i>) to send to the central lab. Participants will still be required to inform sites about events that could be potential IEDs, pyelonephritis, UTI, or ABP cases. For participants in the immunogenicity subset, such events will no longer require immunogenicity sample collection. MRU data will no longer be collected for UTI and ABP events, including pyelonephritis.	To reduce participant and site burden during follow up and refocus study procedures on collection of IED and pyelonephritis events.
1.1 Synopsis 3 Objectives and Endpoints	The wording of a secondary objective was updated to clarify that MRU will be determined for IED, UTI, and ABP events caused by ExPEC9V O-serotypes rather than <i>E. coli</i> .	To clarify a secondary objective assessing medical resource utilization.
1.1 Synopsis 3 Objectives and Endpoints	The wording of an exploratory objective was updated to state that the efficacy of ExPEC9V will be evaluated in the prevention of the first IED caused by ExPEC9V O-serotypes resistant to ≥ 1 , ≥ 2 , and ≥ 3 antibiotic classes rather than antibiotics.	To clarify an exploratory objective assessing efficacy of ExPEC9V in the prevention of IED caused by antibiotic-resistant ExPEC9V O-serotypes.

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Schedule of Activities – Assessments for All Participants 5.1 Inclusion Criteria 10.16 Appendix 16: Medical Conditions of Interest for the Charlson Comorbidity Index, the Frailty Index, Additional IED Risk Factors, and the Risk Factors for Complicated UTI	The requisite timing of selected medical conditions relative to randomization was added to Inclusion Criterion 11, with further clarification provided in Appendix 16.	To clarify and resolve inconsistencies between eligibility criteria.
1.3.1 Schedule of Activities – Assessments for All Participants 10.16 Appendix 16: Medical Conditions of Interest for the Charlson Comorbidity Index, the Frailty Index, Additional IED Risk Factors, and the Risk Factors for Complicated UTI	The schedule of activities was updated to highlight the need for assessment of complicated UTI risk factors to allow for IWRS stratification. The tabular presentation of medical conditions considered risk factors for complicated UTI and requisite toxicity grade for stratification purposes was updated.	To clarify assessment of complicated UTI risk factors required for stratification and improve the tabular presentation of requisite toxicity grading.
1.1 Synopsis 4.1 Overall Design 4.2 Scientific Rationale for Study Design 9.5.1 Interim Analyses	Text was updated to clarify that participants will be followed up for at least 6 months post-vaccination in the event of the study being stopped for futility.	To clarify participant follow up in the event of the study being stopped for futility.
10.3.3 Informed Consent Process	Text was updated to clarify that remote consent may be obtained at the participant's home.	To clarify the acceptable procedures for obtaining remote consent.
1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 1.3.3 Schedule of Activities – Assessments for Participants with Suspected Pyelonephritis	The schedules of activities were updated to include the volume of the immunogenicity blood sample to be taken, if feasible, in participants with suspected IED or suspected pyelonephritis.	To clarify the immunogenicity blood sample volume in case of suspected IED or suspected pyelonephritis.
1.1 Synopsis 1.3.1 Schedule of Activities – Assessments for All Participants 3 Objectives and Endpoints 4.2 Scientific Rationale for Study Design 4.2.1 Study-specific Ethical Design Considerations 8.7 Participant Medical Information Prior To, During, And After the Study (Real-world Data) 11 References	The exploratory endpoint on the use of tokenization and generation of real-world data in US participants was removed.	Since tokenization was introduced to the protocol, data on the US incidence of IED have become available outside the study. Removing tokenization from the protocol will reduce US site burden.

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Schedule of Activities – Assessments for All Participants 4.1 Overall Design 8.1 Efficacy Assessments	The frequency of direct site communication with participants was updated from every 3 months at a minimum to every 3 months $\pm$ 2 weeks.	To update the frequency of surveillance contact.
8.1.1 Identification of Suspected IED Clinical Cases	The requirement for negative blood, sterile site, or urine cultures was removed from the definition of IED recovery.	To align the definition of IED recovery with assessments performed in clinical practice.
8.1.3 Pyelonephritis Clinical Case Definition and Procedures	Text was updated to clarify that the outcome of a suspected pyelonephritis case will only be assessed in case of microbiological confirmation of <i>E. coli</i> or <i>P. aeruginosa</i> .	To clarify the data entry requirements for pyelonephritis events that are not microbiologically confirmed as <i>E. coli</i> or <i>P. aeruginosa</i> .
1.1 Synopsis 1.3.1 Schedule of Activities – Assessments for All Participants 3 Objectives and Endpoints 8.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information	Text was added to clarify that additional SAE reporting may be performed in some countries per local regulatory requirements.	To facilitate additional SAE reporting if requested by health authorities in certain countries.
1.3.1 Schedule of Activities – Assessments for All Participants 6.8 Concomitant Therapy	Text was updated to clarify that the use of licensed <i>E. coli</i> vaccines is prohibited.	To clarify wording regarding the use of licensed <i>E. coli</i> vaccines in order to ensure accurate protocol deviation reporting and exclusion from the per protocol efficacy/per protocol immunogenicity populations.
6.3 Measures to Minimize Bias: Randomization and Blinding	Text was updated to clarify that the subset in which pre-selected sites enrol participants can be changed during the study, although participants at that site will only be enrolled into a single subset at any given time.	To provide flexibility in subset enrollment in order to meet regulatory commitments.
10.4.2 Attribution Definitions	Guidance on the causality assessment of AEs was added.	To align with the sponsor's most recent template update.

Section Number and Name	Description of Change	Brief Rationale
1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 1.3.3 Schedule of Activities – Assessments for Participants with Suspected Pyelonephritis 8.1.1 Identification of Suspected IED Clinical Cases 8.1.3 Pyelonephritis Clinical Case Definition and Procedures	Measurement of red blood cell count was removed from the laboratory procedures to be performed for evaluation of IED and pyelonephritis cases.	Red blood cell count was considered unnecessary for the evaluation of IED and pyelonephritis cases as hemoglobin and hematocrit are measured.
1.1 Synopsis 4.1 Overall Design	Text was added to state that over enrollment may occur for the Immunogenicity Subset due to local requirements.	To allow for over enrollment in the Immunogenicity Subset due to local requirements.
7.2.1 Withdrawal From the Use of Study Samples 10.3.5 Storage, Use, Transfer, and Retention of Data and Samples	Changes were made to the protocol wording on the use of study data and samples.	To align with the sponsor's most recent template update.
10.3.7 Use of Information and Publication	Text on the policies for publications and dissemination of clinical data was revised.	To align with the sponsor's most recent template update.
2.1.1 ExPEC9V	Text was added to provide background information on the immunogenicity of conjugate vaccines.	To provide additional context to the structure of ExPEC9V.
Throughout the protocol	Minor corrections and changes to make the protocol consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.

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1. PROTOCOL SUMMARY

1.1. Synopsis

Randomized, Double-blind, Placebo-controlled, Multicenter Phase 3 Study to Assess the Efficacy, Safety And Immunogenicity of Vaccination With ExPEC9V in the Prevention of Invasive Extraintestinal Pathogenic *Escherichia coli* Disease in Adults Aged 60 Years And Older with a History of Urinary Tract Infection in the Past 2 Years

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ExPEC9V (JNJ-78901563, primary compound number: VAC52416) is a 9-valent vaccine candidate in development for the prevention of invasive *Escherichia coli* disease (IED; previously referred to as invasive extraintestinal pathogenic *Escherichia coli* [ExPEC] disease) in adults 60 years of age and older. ExPEC9V consists of the O-antigen polysaccharides (PSs) of the ExPEC serotypes O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75 separately bioconjugated to the carrier protein, a genetically detoxified form of exotoxin A derived from *Pseudomonas aeruginosa* (EPA).

OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood or other sterile sites, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75	First IED event^a, with microbiological confirmation from blood or other sterile sites^b, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75
Secondary	
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes^c	First IED event^{a,d}, with microbiological confirmation from blood, other sterile sites^b, or urine, caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of all IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes^c	All IEDs^d (including multiple IEDs per participant) caused by ExPEC9V O-serotypes

^a Refer to Section 8.1.2 for the IED clinical case definition.

^b Refer to Section 10.15, Appendix 15 for the sterile sites' definition.

^c Key secondary efficacy objectives, part of the confirmatory testing strategy.

^d Indicated secondary endpoints related to IEDs will include events with *E. coli* microbiological confirmation from blood, other sterile sites, or urine.

Objectives	Endpoints
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first hospitalized IED event caused by ExPEC9V O-serotypes^c	First hospitalized IED event^d caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes^c	First IED event^d meeting criteria for sepsis caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first bacteremic (ie, microbiological confirmation from blood) IED event caused by ExPEC9V O-serotypes^c	First bacteremic IED event caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first pyelonephritis event caused by ExPEC9V O-serotypes^c	First pyelonephritis event caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first urinary tract infection (UTI) event caused by ExPEC9V O-serotypes^c	First UTI event^e caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of all UTIs (including multiple UTIs per participant) caused by ExPEC9V O-serotypes^c	All UTIs^e (including multiple UTIs per participant) caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event caused by <i>E. coli</i>^c	First IED event^d caused by <i>E. coli</i>
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first pyelonephritis event caused by <i>E. coli</i>^c	First pyelonephritis event caused by <i>E. coli</i>
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first UTI event caused by <i>E. coli</i>^c	First UTI event^e caused by <i>E. coli</i>

^c UTI and ABP events that occur after implementation of Protocol Amendment 8 will no longer be included in analyses.

Objectives	Endpoints
To evaluate the immunogenicity of ExPEC9V in the Immunogenicity Subset	Antibody titers to vaccine O-serotype antigens and EPA in the Immunogenicity Subset, as determined by multiplex electrochemiluminescent (ECL)-based immunoassay and antibody titers to vaccine O-serotype antigens, as determined by multiplex opsonophagocytic killing assay (MOPA) on Day 1 (pre-vaccination), Day 30, Day 181, Year 1, Year 2, Year 3, and Year 4
To evaluate the safety and reactogenicity of ExPEC9V	- Solicited local and systemic adverse events (AEs) (collected until 14 days post-vaccination [from Day 1 to Day 15] in the Safety Subset) - Unsolicited AEs (collected until 29 days post-vaccination [from Day 1 to Day 30] in all participants) - Serious adverse events (SAEs)^f in all participants
- To evaluate the preservation of health status and health-related quality of life (HRQoL) of ExPEC9V compared to placebo as measured by the Short Form-36 (SF-36) survey and the 5-level EuroQol 5-dimension questionnaire (EQ-5D-5L) descriptive system - To evaluate the impact of IED, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL as measured by the SF-36 and the EQ-5D-5L - To evaluate the impact of pyelonephritis, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL as measured by the SF-36 and the EQ-5D-5L - To evaluate the impact of UTI^e, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL, as measured by the SF-36 and the EQ-5D-5L	SF-36 and EQ-5D-5L responses at scheduled time points
To assess the degree of frailty in participants who received ExPEC9V versus placebo at baseline, Year 1, Year 2, Year 3, Year 4, and at the time of an IED	Frailty Index as a measure of frailty at baseline, Year 1, Year 2, Year 3, Year 4, and at the time of an IED^d

^f All SAEs until the end of Day 181 and only SAEs related to study vaccine, study procedures, or non-investigational sponsor products from Day 182 until the end of the study. Additional SAEs may be reported per local regulatory requirements.

Objectives	Endpoints
To determine the medical resource utilization (MRU) for IED, UTI, and acute bacterial prostatitis (ABP) events caused by ExPEC9V O-serotypes	- MRU for IED events^d caused by ExPEC9V O-serotypes - MRU for UTI events^e (Immunogenicity Subset only) and ABP events^e (Immunogenicity Subset only) caused by ExPEC9V O-serotypes - Hospitalization and length of stay in the hospital, including intensive care unit (ICU) hospitalization and ICU length of stay, for IED^d, UTI^e, or ABP events^e caused by ExPEC9V O-serotypes
To determine the mortality associated with IED	IED-related^d and all-cause mortality
Exploratory	
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event caused by ExPEC9V O-serotypes resistant to ≥ 1, ≥ 2, and ≥ 3 antibiotic classes	First IED event caused by ExPEC9V O-serotypes showing resistance to ≥ 1, ≥ 2, and ≥ 3 antibiotic classes in the microbiological profile
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of first uncomplicated, complicated, and recurrent UTI events caused by ExPEC9V O-serotypes	- First uncomplicated UTI event^e caused by ExPEC9V O-serotypes - First complicated UTI event^e caused by ExPEC9V O-serotypes - First recurrent UTI event^e caused by ExPEC9V O-serotypes - First recurrent uncomplicated UTI event^e caused by ExPEC9V O-serotypes
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first ABP event caused by ExPEC9V O-serotypes	First ABP event^e caused by ExPEC9V O-serotypes
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first ABP event caused by <i>E. coli</i>	First ABP event^e caused by <i>E. coli</i>
To evaluate antibody titers to vaccine O-serotype antigens and EPA in participants with IED	Antibody titers to vaccine O-serotype antigens and EPA, as determined by multiplex ECL-based immunoassay and antibody titers to vaccine O-serotype antigens, as determined by MOPA assays in participants with IED, with sera obtained at the time of diagnosis of the suspected IED
To evaluate the severity of IED	Severity of IED (presence of sepsis or septic shock) in participants with IED

Objectives	Endpoints
To identify a correlate of protection for the vaccine-mediated immune response associated with vaccine efficacy (VE)	Antibody titers for ExPEC9V determined by multiplex ECL-based immunoassay and MOPA assays at Day 1 pre-vaccination, Day 30 and at the time of diagnosis of the suspected IED (all participants) or at the time of a UTI or ABP event^c (Immunogenicity Subset only), in association with VE
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first invasive disease event caused by <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) and the first <i>P. aeruginosa</i> UTI/ABP event	- First invasive disease event caused by <i>P. aeruginosa</i> - First <i>P. aeruginosa</i> UTI event^c - First <i>P. aeruginosa</i> ABP event^c

Hypotheses

For the primary objective, the following null and alternative hypotheses will be used:

H_0 : VE $\leq 20\%$

versus

H_a : VE $> 20\%$

For the key secondary objectives related to IED, the following null and alternative hypotheses will be used:

H_0 : VE $\leq 0\%$

versus

H_a : VE $> 0\%$.

For the key secondary objectives related to pyelonephritis and UTI, the following null and alternative hypotheses will be used:

H_0 : VE $\leq 10\%$

versus

H_a : VE $> 10\%$.

OVERALL DESIGN

This is a randomized, double-blind, placebo-controlled, parallel group, multicenter, interventional Phase 3 study to be conducted in approximately 19,800 medically stable adults aged ≥ 60 years and with a history of UTI in the past 2 years.

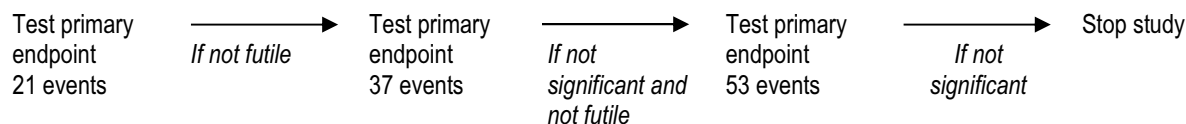
This study incorporates an inferentially seamless group-sequential design.

The target number of participants to be enrolled is approximately 19,800. Note that over enrollment may be allowed if required by local health authorities for the purpose of local health authority consideration. When the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, an immunogenicity interim analysis will be conducted and the sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation). Enrollment will not be paused during this immunogenicity interim analysis. The purpose of this immunogenicity interim analysis is to obtain an initial result of the immune response, no

decision will be taken on the remainder of the study and therefore no type I error adjustment is required. In addition, once the first 500 randomized participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, the independent data monitoring committee (IDMC) will review unblinded safety data. In case of serious safety concerns during this IDMC safety review, the IDMC may recommend the sponsor to stop the study. The safety data will include SAEs and unsolicited AEs from all participants and solicited AEs from participants in the Safety Subset. According to the ratio of the Safety Subset versus the initial total sample size of the study ($4,000/18,556=21.6\%$), approximately 108 participants are targeted in the Safety Subset among the first 500 randomized participants. Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety review will be conducted at least every 6 months. When 21 of 53 (40%) vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites^g) have been observed, an interim analysis for the primary endpoint (futility) will be performed. Based on the outcome of this interim analysis, the IDMC may recommend stopping enrollment of participants and stopping the study (in case of futility) or to continue the study. In the event of the study being stopped for futility, all participants will be followed up for at least 6 months post-vaccination. The futility analysis will be based on the Go/No Go approach. In addition, if the event rate is lower or higher than expected, a blinded sample size re-estimation (ie, based on the event rate combining all participants from both the ExPEC9V and placebo groups) may be performed by the sponsor at the time of this interim analysis; however, the sample size will not be increased by more than 50%.

A second interim analysis for the primary endpoint (efficacy/futility) will be conducted when 37 of 53 (70%) vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed. As with the first interim analysis, an independent statistician will perform the second interim analysis and will present the results to the IDMC. The final analysis for the primary endpoint will occur when 53 vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed in the study or at the latest when the last participant has been followed up for 48 months post-vaccination.

Figure: Interim and final analyses for the primary endpoint



The study consists of screening, randomization, and vaccination for each participant on Day 1 (selected screening procedures may be performed up to 8 days prior to vaccination on Day 1). Each participant will be followed for a maximum of approximately 48 months (1,461 days; 4 years) post-vaccination. At screening/Day 1, demographic data, Short Physical Performance Battery (SPPB) score, and medical conditions of interest will be collected. All participants will be enrolled and randomized in parallel in a 1:1 ratio to either ExPEC9V or placebo and receive the study vaccine on Day 1. Participants will be stratified at randomization according to their age (≥ 60 to < 65 years of age versus ≥ 65 to < 75 years of age versus ≥ 75 years of age) and according to the presence of at least one risk factor for complicated UTIs (cUTIs) at baseline.

An IDMC and Independent Endpoint Adjudication Committee (IEAC) will be commissioned for this study.

^g Refer to Section 10.15, Appendix 15 for the sterile sites' definition.

BENEFIT-RISK ASSESSMENT

Risks related to study participation include general risks related to intramuscular (IM) injection such as, local itching, warmth, pain, tenderness, erythema/redness, induration, swelling, arm discomfort, or bruising of the skin. Additionally, participants may exhibit general signs and symptoms associated with injection of a vaccine and/or placebo, including fever, chills, rash, myalgia, nausea/vomiting, headache, dizziness, arthralgia, general itching, and fatigue. These side effects will be monitored but are generally short-term and do not require treatment.

Syncope can occur in association with administration of injectable vaccines. Syncope can be accompanied by falls. Procedures should be in place to avoid falling injury. If syncope develops, participants should be observed until the symptoms resolve. Fear of injection might lead to fainting and fast breathing.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, urticaria, or even anaphylaxis. Severe reactions are rare.

Late onset solicited AEs were observed in ExPEC10V development program. The most frequent solicited events, pain/tenderness, fatigue, myalgia, and headache occurred mostly as early onset events. Injection site erythema and swelling occurred as late onset events (time to first onset >5 days after vaccination) in the majority of participants from the combined ExPEC10V groups. A trend towards increased local reactogenicity with higher doses of ExPEC10V was observed.

Potential benefits include the benefit from clinical testing and physical examination and the benefit from the knowledge that they may aid in the development of a vaccine for the prevention of IED.

Based on the available data and proposed safety measures, the overall benefit-risk assessment for this clinical study is considered acceptable.

NUMBER OF PARTICIPANTS

Overall, approximately 19,800 participants will be enrolled in the study. If the event rate is lower or higher than expected, a blinded sample size re-estimation may be performed by the sponsor at the time of the first interim analysis of the primary endpoint; however, the sample size will not be increased by more than 50%. Note that over enrollment may be allowed if required by local health authorities for the purpose of local health authority consideration. Efforts will be made to ensure good representation in terms of race, ethnicity, age, and gender. The sponsor expects to see the same proportional distribution of diversity during recruitment as in each site's patient database.

VACCINATION GROUPS AND DURATION

Description of Vaccinations

ExPEC9V (VAC52416): *Escherichia coli* (*E. coli*) bioconjugate vaccine in phosphate buffered solution containing O-antigen PSs of ExPEC serotypes O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75 separately bioconjugated to the EPA carrier protein. A single 0.5 mL IM (deltoid) injection of ExPEC9V will be administered on Day 1. ExPEC9V will be supplied at a concentration of 176 µg PS/mL. The PS content of the dose to be tested in the present study is 88 µg/dose. The EPA concentration in the currently manufactured ExPEC9V clinical batches is 290 µg/dose. The maximum allowed EPA concentration in ExPEC9V is 382.5 µg/dose.

Placebo: Sodium chloride 0.9% w/v. A single 0.5 mL IM (deltoid) injection of placebo will be administered on Day 1. The placebo for this study will be supplied by the sponsor as a commercially available injectable solution of 9 mg/mL sodium chloride (0.9% w/v).

The participants, study-site personnel, and investigators will be blinded to study vaccine group allocation throughout the study, except for the designated pharmacist(s) or qualified staff member(s) with primary responsibility for study vaccine preparation. These unblinded members will not be part of the team performing the evaluations. The vaccine administrator will be blinded and can perform other study evaluations. At the time of the interim analyses, the participants, investigator(s), and site staff will remain blinded to individual participants' study vaccination assignment. The sponsor will be blinded to study vaccine allocation until the database release of the final analysis of the primary endpoint. However, in case of positive efficacy results at an earlier interim analysis (ie, when the IDMC informs the sponsor committee that the superiority criteria have been met for the primary endpoint), the sponsor might be unblinded to start preparation of regulatory submissions. In this case, the study will not be stopped, but follow-up of participants will be continued for efficacy, safety, and immunogenicity.

EFFICACY EVALUATIONS

The primary objective of the study will be to demonstrate:

- the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood or other sterile sites, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75.

Events defined as IED or pyelonephritis will be collected according to study-specific procedures for all participants for the entire study duration (1,461 days; 4 years). As of Protocol Amendment 8, site study-specific procedures will not be performed for uncomplicated or complicated UTI or ABP events unless these events are suspected to be pyelonephritis. As part of the IED and pyelonephritis catchment strategy, sites are required to perform active surveillance through medical health records or by any other means. During study follow-up, participants are expected to inform the study site if they experience any signs or symptoms of UTI or ABP, or if they experience any new onset or worsening of symptoms that could be caused by a pyelonephritis or a systemic infection. Participants will be questioned about past hospitalizations or medical events that could have been potential IEDs or pyelonephritis cases that were not captured by the site in real time. Participant surveillance via eCOA will begin following the Day 30 visit and continue until the study end or participant early withdrawal. MRU data will be collected for IED (all participants). As of Protocol Amendment 8, MRU data will no longer be collected for UTI and ABP events, including pyelonephritis. Two electronic patient-reported outcome (ePRO) instruments will be used to measure HRQoL: the SF-36 version 2 and the EQ-5D-5L. In addition, the Charlson Comorbidity Index will be calculated based on the medical conditions of interest and frailty will be assessed using the SPPB and the Frailty Index.

IMMUNOGENICITY EVALUATIONS

For assessment of immunogenicity, serum immunoglobulin G (IgG) antibody levels elicited by the vaccine against each of the 9 vaccine O-serotypes and the carrier protein EPA will be measured by a multiplex ECL-based immunoassay and vaccine O-serotype-specific functional antibodies will be measured by a MOPA. Immunogenicity analyses will be performed for participants in the "Immunogenicity Subset", ie, for approximately 1,200 participants from selected study sites who have given informed consent prior to randomization for additional immunogenicity assessments and for all participants with a confirmed IED if feasible. Due to local requirements, over enrollment may occur for the Immunogenicity Subset. As of Protocol Amendment 8, immunogenicity samples will be collected from participants in the Immunogenicity Subset in the event of pyelonephritis, but not during other UTI or ABP events. Day 1 and Day 30 immunogenicity blood samples will be collected from all participants.

SAFETY EVALUATIONS

Key safety assessments include SAEs, physical examination, and vital signs. In addition, solicited local and systemic AEs will be recorded through 14 days post-vaccination for participants in the "Safety Subset", ie,

approximately 4,000 participants from selected study sites who have given informed consent prior to randomization for the additional assessments. Due to local requirements, over enrollment may occur for the Safety Subset. Unsolicited AEs will be recorded for all participants through 29 days post-vaccination.

STATISTICAL METHODS

Sample Size Calculation

To meet the primary objective of the study, it is estimated that with 53 participants developing at least 1 IED event with microbiological confirmation in blood or other sterile sites caused by ExPEC9V serotypes, this objective has statistical power of approximately 90% to demonstrate pivotal efficacy for a vaccine with 70% VE at a 0.025 (1-sided) level of significance, considering a futility interim analysis after 21 events, an efficacy/futility interim analysis after 37 events, and a final analysis after 53 events will be performed.

The study is designed to reject the null hypothesis that the true VE is $\leq 20\%$ for the primary objective. Assuming a 10% yearly dropout rate and assuming a yearly vaccine serotype-specific incidence rate of 0.17% among participants who received placebo (with an IED incidence rate for the primary endpoint of 0.26% in the placebo group and a vaccine serotype coverage rate of 65%), and assuming that on average a participant will be followed up for 3.5 years, a total of approximately 19,800 participants (1:1 ExPEC9V:Placebo) will be needed for this study.

Efficacy Analyses

The primary analysis of the primary endpoint will evaluate

- the number of participants with at least 1 IED event with microbiological confirmation in blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75 with onset at least 29 days after vaccination (from Day 30) in the active vaccine (ExPEC9V) group compared to the placebo group in the PPE population.

The follow-up time will also be taken into account. For participants with an IED event, the follow-up time is defined as the time between vaccination and the occurrence of the first event. For participants without an IED event, it is the time between vaccination and last visit (or last contact for participants that discontinued the study). The null hypothesis of $VE \leq 20\%$ will be tested versus the alternative hypothesis $VE > 20\%$ for the primary endpoint.

A hierarchical testing strategy will be applied to the following endpoints:

Primary:

- First IED with microbiological confirmation from blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes

Secondary:

- First IED, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes
- All IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes
- First hospitalized IED event caused by ExPEC9V O-serotypes
- First IED event meeting criteria for sepsis caused by ExPEC O-serotypes
- First bacteremic IED event caused by ExPEC9V O-serotypes
- First pyelonephritis event caused by ExPEC9V O-serotypes

7. First UTI caused by ExPEC9V O-serotypes
8. All UTIs (including multiple UTIs per participant) caused by ExPEC9V O-serotypes
9. First IED event caused by *E. coli*
10. First pyelonephritis event caused by *E. coli*
11. First UTI caused by *E. coli*

In this testing strategy, the first key secondary endpoint (first IED event, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes) will only be tested when the primary endpoint shows statistical significance (at the interim or final analyses). If the first key secondary endpoint shows statistical significance, the other secondary endpoints will be tested according to the above hierarchical testing strategy.

Safety Analyses

No formal statistical testing of safety data is planned. The safety analysis will include the descriptive summary of solicited local AEs, solicited systemic AEs, unsolicited AEs, and SAEs. The overall frequencies per group as well as frequencies according to severity and duration will be calculated for solicited and unsolicited AEs. Safety analyses will be done on the full analysis set (FAS), which is the primary safety population. The analysis of solicited AEs will be restricted to a subset of the FAS (Safety Subset).

Immunogenicity Analyses

The primary immunogenicity analysis will be done on the per protocol immunogenicity population.

For ExPEC9V serotype antibodies as measured by multiplex ECL-based immunoassay and MOPA, and EPA as measured by multiplex ECL-based immunoassay only, the following measures of immunogenicity will be evaluated and tabulated by the study vaccination groups, for all immunogenicity timepoints:

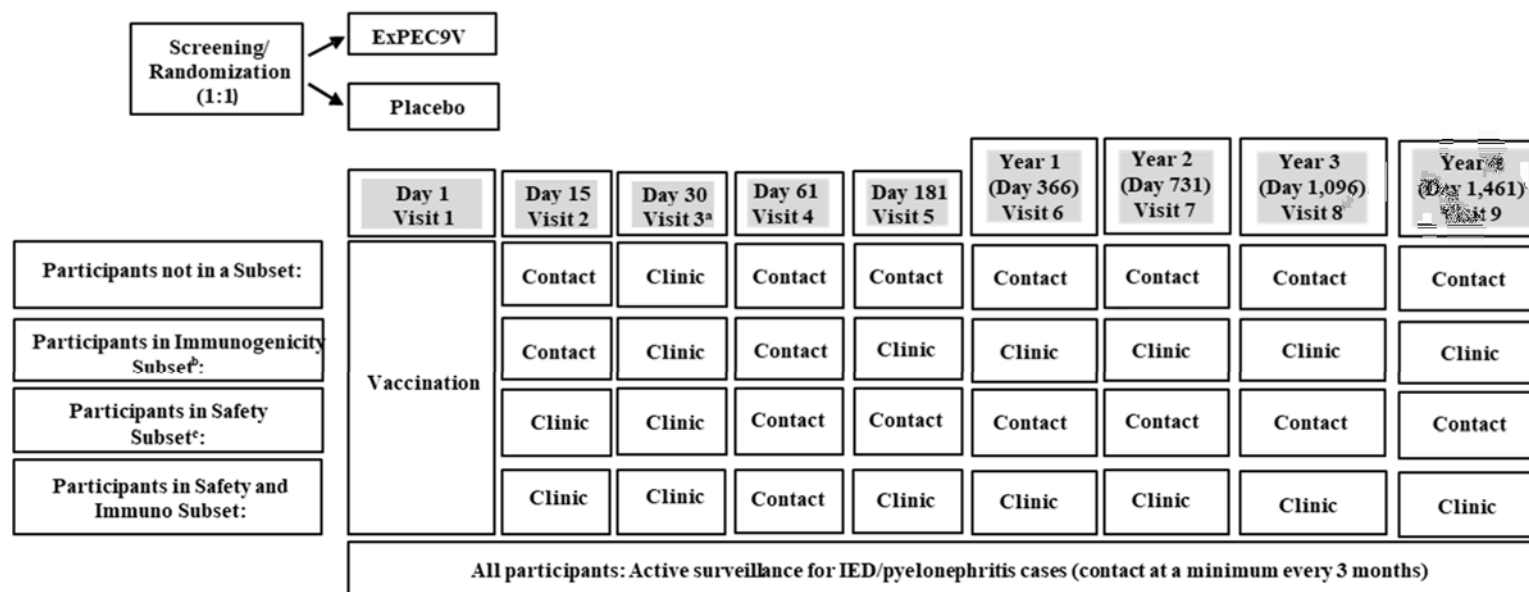
- proportion of participants with a ≥ 2 -fold and ≥ 4 -fold increase in serum antibody titers from Day 1 (pre-vaccination)
- geometric mean titer
- geometric mean of fold change from baseline, calculated from the post-baseline/baseline value.

Graphical representations of immunological parameters will be made as applicable.

For the 4-year follow-up period, descriptive summaries of immunogenicity will be presented for each serotype.

1.2. Schema

Figure 1: Schematic Overview of the Study



Contact=telephone contact or other means of communication; ExPEC9V=9-valent extraintestinal pathogenic *Escherichia coli* vaccine; IDMC=Independent Data Monitoring Committee; IED=invasive *Escherichia coli* disease.

- An immunogenicity interim analysis will be conducted when the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3) or discontinued earlier. The sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation). Enrollment will not be paused during this immunogenicity interim analysis. Once the first 500 randomized participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, the IDMC will review unblinded safety data. The safety data will include serious adverse events and unsolicited adverse events from all participants and solicited adverse events from participants in the Safety Subset. Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety review will be conducted at least every 6 months.
- The Immunogenicity Subset refers to approximately 1,200 participants (approximately 600 ExPEC9V participants and 600 placebo participants) from selected study sites who have given informed consent (prior to randomization) for additional immunogenicity assessments at Days 181, 366, 731, 1,096, and 1,461. Immunogenicity assessments are described in Section 8.4.
- The Safety Subset refers to approximately 4,000 participants from selected study sites who have given informed consent (prior to randomization) for additional safety assessments (approximately 2,000 ExPEC9V participants and 2,000 placebo participants). Safety assessments are described in Section 8.2 and Section 8.3. Safety Subset and Immunogenicity Subset participants will be enrolled only at a selection of sites. Participants can be included in both the Immunogenicity Subset and the Safety Subset. For further details of study assessments, refer to Section 1.3.

1.3. Schedule of Activities (SoA)

1.3.1. Schedule of Activities – Assessments for All Participants

Study Phase	Screening ^a / Vaccination	Follow-up								Early Exit ^b
Clinic Visit #	1 ^c	2 ^d ☎	3 ^e	4 ☎	5 ^f ☎	6 ^f ☎	7 ^f ☎	8 ^{f,h} ☎	9 ^f ☎	
Visit Timing	VACC	VACC + 14 days	VACC + 29 days	VACC + 60 days	VACC + 180 days	VACC + 1 Year	VACC + 2 Years	VACC + 3 Years	VACC + 4 Years	
Visit Day	1	15±2d	30±7d	61±14d	181±14d	366±14d	731±28d	1,096±28d	1,461±28d	
Visit Type	Vaccination	Safety ^d	Safety and Immuno	Safety	Safety and Immuno	Safety and Immuno	Safety and Immuno	Safety and Immuno	Safety and Immuno	
All participants										
Informed consent ^g	●									
Eligibility criteria ⁱ	●									
Demographics ^k	●									
Medical conditions of interest ^l	●					●	●	●	●	●
ePRO instruments (SF-36 version 2 and EQ-5D-5L) ^m	●	● ⁿ	● ⁿ		● ⁿ	● ⁿ	● ⁿ	● ⁿ	● ⁿ	●
Body weight and height	●									● ^{o,p}
Physical examination	● ^q									● ^{p,r}
Vital signs ^s including body temperature	● ^j									● ^p
Complicated UTI risk factor assessment ^t	●									
Randomization ^u	●									
SPPB ^v	●									
Vaccination	●									
30-min post-vaccination observation ^w	●									
Provision of the Participant Wallet Card	●									
Unsolicited AE recording	----- Continuous through Day 30-----									● ^x
SAEs recording ^y	----- Continuous through Day 181-----									●
SAEs related to study vaccine ^y	-----Continuous-----									●
AEs related to study procedures and non-investigational (concomitant) sponsor products ^z	-----Continuous-----									●
Concomitant medication	● ^{aa,bb}	● ^{aa,bb}	● ^{aa,bb}	● ^{aa,bb}	● ^{aa,bb}	● ^{bb,cc}	● ^{bb,cc}	● ^{bb,cc}	● ^{bb,cc}	●

Study Phase	Screening ^a / Vaccination	Follow-up								Early Exit ^b
Clinic Visit #	1 ^c	2 ^d ☎	3 ^e	4 ☎	5 ^f ☎	6 ^f ☎	7 ^f ☎	8 ^{f,h} ☎	9 ^f ☎	
Visit Timing	VACC	VACC + 14 days	VACC + 29 days	VACC + 60 days	VACC + 180 days	VACC + 1 Year	VACC + 2 Years	VACC + 3 Years	VACC + 4 Years	
Visit Day	1	15±2d	30±7d	61±14d	181±14d	366±14d	731±28d	1,096±28d	1,461±28d	
Visit Type	Vaccination	Safety ^d	Safety and Immuno	Safety	Safety and Immuno	Safety and Immuno	Safety and Immuno	Safety and Immuno	Safety and Immuno	
Blood sampling for immunogenicity (non-Immuno Subset participants) ^{dd, ee}	① ^j 10mL		● 10mL							
Blood sampling for assay management (at selected sites only)			● 50mL ^{ff}							
IED/pyelonephritis active surveillance via eCOA ^{gg}				----- Continuous until end of study -----						
Direct participant contact ^{hh}	-----Contacts/visits every 3 months ± 2 weeks-----									
Study procedures in case of signs and symptoms of IED or pyelonephritis ⁱⁱ		----- Continuous until end of study -----								
Immunogenicity subset only										
Blood sampling for immunogenicity ^{dd, ee}	① ^j 10mL		● 10mL		● 10mL	● 10mL	● 10mL	● 10mL	● 10mL	● 10mL ^p
Safety subset only										
Issue participant eDiary ^{jj}	●									
Participant eDiary review by site staff ^{kk}		●	● ^{kk}							● ^{ll}
Solicited AE recording	Continuous through Day 15									● ^{ll}
Physical examination		● ^r	● ^r							

ABP=acute bacterial prostatitis; AE=adverse event; d=day; COVID-19=coronavirus disease 2019; eCRF=electronic case report form; eCOA=electronic clinical outcome assessment; eDiary=electronic diary; ePRO=electronic patient-reported outcome(s); EQ-5D-5L=5-level EuroQol 5-dimension questionnaire; ExPEC9V=9-valent extraintestinal pathogenic *Escherichia coli* vaccine; ICF=informed consent form; IED=invasive *Escherichia coli* disease; IWRS=interactive web response system; SAE=serious adverse event; SF-36=Short Form-36; SPPB=Short Physical Performance Battery; UTI=urinary tract infection; VACC=vaccination.

☎ telephone contact or other means of communication; ① pre-vaccination procedures may be performed up to 8 days prior to vaccination ② should preferably be completed on the day of vaccination, prior to vaccination.

Footnotes:

- Participant screening must be completed, and all eligibility criteria must be fulfilled prior to randomization.
- Participants who are unable to continue participation in the study, but who do not withdraw consent, should be encouraged to complete an Early Exit visit, at the investigator's discretion. Participants who wish to withdraw consent from participation in the study will be offered an Early Exit visit for safety follow-up prior to

- formal withdrawal of consent. If the Early Exit visit would occur shortly after the Day 181 visit or an annual visit, the investigator may decide to have the Early Exit visit replaced by a telephone contact.
- c. Decentralized sites may conduct the Day 1 visit as home health visit.
 - d. Clinic visit 2 for participants in the Safety Subset and telephone contact or other means of communication for participants other than the Safety Subset.
 - e. Scheduled visits which cannot be conducted in person at the study site may be conducted as home health visit after sponsor approval.
 - f. Clinic visit for participants in the Immunogenicity Subset and telephone contact or other means of communication for participants other than the Immunogenicity Subset.
 - g. Signing of the ICF needs to be done before the first study-related activity.
 - h. For participants enrolled prior to Protocol Amendment 8 who are not reconsented on an updated ICF, the end of study visit will be conducted at Year 3 (Day 1,096). These participants will be considered to have completed the study if they have completed the Year 3 (Day 1,096) visit.
 - i. For information on the requisite timing of medical conditions considered additional risk factors for IED for eligibility, please see Appendix 10.16, Table 10.
 - j. If screening procedures are split over different days (ie, maximum 8 days prior to vaccination), eligibility criteria must be rechecked, pre-vaccination vital signs and pre-vaccination blood samples for immunogenicity should be collected on the day of vaccination (Day 1).
 - k. Participant's current living situation will be collected in the eCRF.
 - l. Relevant medical conditions of interest will be collected. Additional medical history related to the last UTI event in the past 2 years, including information regarding pathogens linked to UTIs, will be collected for all participants, as available. Medical conditions of interest, required for the Frailty Index calculation and the Charlson Comorbidity Index Score, will be collected (refer to Section 8.1.7 and Section 10.16 for more details).
 - m. The ePROs (SF-36 version 2 and the EQ-5D-5L) should be completed on the visit date, preferably before any tests, procedures, or other consultations to prevent influencing of the participant. Alternative options are available for the collection of ePRO data if participants are unable or unwilling to work with the eCOA system or if the eCOA capture system is unavailable (please refer to Section 8.1.6 for further details). Best practices on the collection of ePRO data are provided in the ePRO guidelines.
 - n. A time window of +4 days is acceptable.
 - o. Body weight only.
 - p. Not applicable if the Early Exit visit is replaced by a telephone contact.
 - q. General physical examination; it is at the investigator's discretion how detailed the examination (which body systems are to be covered) is deemed needed.
 - r. Targeted physical examination only, at the discretion of the investigator (based on complaints/symptomatology and health status of participant).
 - s. Vital signs (respiratory rate, systolic and diastolic sitting blood pressure and pulse/heart rate) including body temperature will be measured both before vaccination (and preferably before blood draws) and after the end of the 30-minute observation period (no later than 45 minutes after vaccination). Measurements should be done after 5 minutes of seated rest. Investigator must check for body temperature $\geq 38.0^{\circ}\text{C}$ (100.4°F) at the time of vaccination, preferably by oral temperature measurement.
 - t. Assessment of complicated UTI risk factors for IWRS stratification. Refer to Section 10.16 Table 10 for details of the medical conditions considered risk factors for complicated UTI and the requisite toxicity grading for stratification purposes.
 - u. Randomization to ExPEC9V or placebo (1:1) on Day 1.
 - v. Frailty will be assessed at baseline based on the SPPB score²¹ and additionally, the Frailty Index which will be calculated using participants self-reported information regarding health status (SF-36 version 2 and EQ-5D-5L) and baseline health conditions collected through the participant's medical conditions of interest, see above.
 - w. For participants in the Safety Subset, any solicited AEs will be documented in the eCRF by study-site personnel following the 30-minute observation period (no later than 45 minutes after vaccination).
 - x. If the Early Exit visit occurs before Day 30 visit.
 - y. All SAEs will be collected for all participants from the administration of the study vaccine until the end of Day 181. SAEs that are related to the study vaccine will be collected from Day 182 until the end of the study. Additional SAE reporting may be performed per local regulatory requirements. Death will be recorded for the duration of the study.

- z. AEs, whether serious or non-serious, that are related to study procedures or that are related to non-investigational sponsor products will be reported from the time a signed and dated ICF is obtained until the end of the study/early withdrawal.
- aa. Relevant prestudy specific therapies (ie, non-steroidal anti-inflammatory drugs, corticosteroids, antihistamines, vaccinations, antivirals, and all antibacterials [including antibiotics and urinary tract antiseptics]) administered up to 30 days before dose of study vaccination must be recorded at screening. Concomitant therapies associated with an SAE meeting the criteria outlined in Section 8.3.1 will be collected and recorded in the eCRF from ICF signature through the end of the study. For all participants, concomitant therapies (such as non-steroidal anti-inflammatory drugs, corticosteroids, antihistaminic, vaccinations, antivirals, and all antibacterials [including antibiotics and urinary tract antiseptics]) associated with unsolicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 29 days after vaccination (Day 30) or until clinical resolution or stabilization. Concomitant therapies associated with solicited AEs will be collected for the participants (in the Safety Subset) in the eCRF from the time of vaccine administration through 14 days after vaccination (Day 15) or until clinical resolution or stabilization.
- bb. Use of licensed *E. coli* vaccines must always be recorded as concomitant therapy for the entire duration of the study. See Section 6.8 for further details.
- cc. Only medications in conjunction with serious adverse events related to the study vaccine or study procedures should be recorded.
- dd. Blood samples will be collected from all participants for immunogenicity at Day 1 (pre-vaccination) and Day 30. For participants in the Immunogenicity Subset, blood samples will also be collected on Day 181, Year 1, Year 2, Year 3, and Year 4.
- ee. Antibacterials (including antibiotics and urinary tract antiseptics) administered up to 30 days before immunogenicity sampling must be recorded.
- ff. This additional blood sample will be taken at the discretion of the PI and will only be collected at selected sites.
- gg. For participants using study devices, (either provisioned or bring your own device), participant surveillance via eCOA will begin after the Day 30 visit and continue until the study end or participant early withdrawal. This eCOA surveillance method does not apply to participants exclusively using interview mode.
- hh. Sites should have direct contact with participants every 3 months $\pm$ 2 weeks. Every direct surveillance contact must be documented and recorded in the eCRF in the folder 'Surveillance Contact'. During these direct contacts, sites should monitor participant eCOA surveillance adherence and review medical events that have occurred during the past interval. Additional forms and folders relevant for IED/UTI/ABP data need to be completed in case a suspected study endpoint occurred since the previous study visit or contact.
- ii. For more details refer to Section 1.3.2 for participants with suspected IED and Section 1.3.3 for participants with a pyelonephritis. As of Protocol Amendment 8, site study-specific procedures will not be performed for uncomplicated or complicated UTI or ABP events unless these events are suspected to be pyelonephritis.
- jj. At Visit 1, participants in the Safety Subset will be provided with an eDiary (to record solicited local and systemic AEs), a thermometer (to measure body temperature), and a ruler (to measure diameter of any erythema and swelling) and will be instructed to measure and record solicited local and systemic AEs and body temperature daily for 14 days post-vaccination (day of vaccination and the subsequent 14 days). Alternative options are available for collection of safety data if the eDiary system is unavailable (please refer to Section 8 for further details).
- kk. If Visit 2 occurs before the end of the eDiary period after the vaccination, review of the eDiary will still take place, but the eDiary will be returned by the participant at the next study visit (Day 30). If the eDiary review is missed, the eDiary will be reviewed during the following visit.
- ll. If the Early Exit visit occurs before Day 15 visit.

1.3.2. Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification

IED ASSESSMENT ^a	IED-Day 1: IED Case Evaluation ^{a, b, c}	Day of Resolution OR IED-Day 29±3d ^{a, b, d, f}	Post-IED Follow-up (IED-Day 91±7d) ^{e, f, g}	Post-IED Follow-up (IED-Day 366±14d) ^{a, b, f, h}
Relevant existing conditions, pre-IED therapy, and pre-IED baseline SOFA score (if available) ⁱ	X		X	X
ePRO instruments (SF-36 version 2 and EQ-5D-5L) ^j	X ^k	X	X	X
Collection of IED signs and symptoms ^l	X ^m	X ^m		
Laboratory data ⁿ	X ^m	X ^m		
Sample collection from blood or any other normally sterile site (if applicable), and urine samples for culture (per standard of care) ^o	X ^m	X ^m		
Blood sample for immunogenicity assessments ^p	X 10mL			X 10mL
Confirmation of IED based on clinical case definition	X			
Identification of potential source of infection and type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) ^q	X			
Collection of information on treatment for IED ^r	X	X		
MRU ^s	X	X	X	X
Recovery status (outcome and complications of IED)		X	X	X

IED=invasive *Escherichia coli* disease; eCOA=electronic clinical outcome assessment; eDiary=electronic diary; ePRO=electronic patient-reported outcome(s); EQ-5D-5L=5-level EuroQol 5-dimension questionnaire; MRU=medical resource utilization; SF-36=Short Form-36.

Footnotes:

- Assessments will be done at the reference hospital/urology clinic/study site where the participant presents with signs or symptoms of suspected IED.
- Scheduled visits which cannot be conducted in person at the study site may be conducted as home health visit. Prior sponsor approval is not required for these home health visits.
- IED-Day 1 is the day on which the investigator suspects a systemic infection (Section 8.1.1). As of this day, study procedures should be performed, and data should be collected until the IED is excluded.
- Data collected on the day of resolution of IED signs and symptoms or on Day 29 post-IED identification (IED-Day 29) (whichever comes first). Follow-up of the suspected IED case should also be performed for cases

- for which confirmation of the IED diagnosis is still pending but for which the collected cultures are positive for *E. coli* or *P. aeruginosa*. The Medical Monitor should be contacted for further guidance. A study visit may be required to collect follow-up information.
- e. This visit may be conducted remotely (eg, via telephone call).
 - f. These procedures will only be performed for IED cases that were confirmed based on the clinical case definition.
 - g. Day 91 post-IED identification (IED-Day 91) or at the end of the study (whichever comes first).
 - h. Day 366 post-IED identification (IED-Day 366) or at the end of the study (whichever comes first).
 - i. Relevant medical conditions of interest, ie, conditions with increased risk for IED and other relevant co-existing medical conditions, history of IED. Any relevant treatment received in the last 90 days (ie, 3 months) prior to the IED, any antibiotic use (prophylactic or therapeutic) within the prior 90 days (ie, 3 months). Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures. Medical conditions of interest, required for the Frailty Index calculation and the Charlson Comorbidity Index Score, will be collected (refer to Section 8.1.7 and Section 10.16 for more details). The pre-IED baseline SOFA score should be captured if available.
 - j. The ePROs (SF-36 version 2 and the EQ-5D-5L) should be completed on the visit date, preferably before any tests, procedures, or other consultations to prevent influencing of the participant. Alternative options are available for the collection of ePRO data if participants are unable or unwilling to work with the eCOA system or if the eCOA capture system is unavailable (please refer to Section 8.1.6 for further details). Best practices on the collection of ePRO data are provided in the ePRO guidelines.
 - k. The ePROs (SF-36 version 2 and the EQ-5D-5L) can be completed up to 7 days post IED confirmation. ePROs should be collected per Section 8.1.1 for participants meeting the IED clinical case definition criteria (see Section 8.1.2) including participants who test positive for *Pseudomonas aeruginosa*, and for participants with suspected IEDs.
 - l. Evaluation of IED (signs/symptoms, and laboratory assessments), when the participant presents with the signs or symptoms of systemic infection, based on clinical case identification/definition criteria (presence of positive systemic inflammatory response syndrome or quick sequential organ failure assessment score or change in total sequential organ failure assessment score ≥ 2 , see Section 8.1.1 and Section 8.1.2) by the investigator/trained healthcare provider. In case the IED is confirmed, the participant will be followed up until the day of resolution of IED signs and symptoms or IED-Day 29 (whichever comes first) to check the evolution of the disease condition and disease management.
 - m. Collection of relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care (see Section 8.1.2). These will be recorded as unscheduled evaluations after IED-Day 1.
 - n. If a systemic infection cannot be excluded, the following laboratory procedures are expected to be performed per standard of care and upon medical judgement of the treating physician: hematology (white blood cell count with differential [absolute and %], hemoglobin, hematocrit, and platelets), serum chemistry (glucose, creatinine, bilirubin, alanine aminotransferase, aspartate aminotransferase, C-reactive protein, and lactate), urinalysis (at minimum white blood cell count), and arterial blood gas. If above mentioned procedures are not collected per standard of care, these procedures are not mandatory. On the day of resolution of IED signs and symptoms or on IED-Day 29 (whichever comes first), hematology, serum chemistry and urinalysis are optional (it is recommended to repeat relevant procedures performed for IED diagnosis – this includes data already collected to assess recovery/discharge before actual Day 29).
 - o. Blood cultures (or cultures from any other sterile site or urine, as applicable) will be taken, before initiating treatment including antibiotic(s) if possible. Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures. Standard clinical practice should be followed for collection of cultures. The relevant cultures should be collected as study procedures if not part of the standard of care. Microbiological assessments will be performed as detailed in Section 8.5.
 - p. If feasible, an immunogenicity blood sample should be taken for all suspected systemic infection cases, if possible before any treatment or antibiotic(s) is given. If sample collection prior to treatment initiation is not possible, the samples should be collected as soon as possible and within approximately 48 hours after a systemic infection is suspected by the investigator. Immunogenicity assessments will be performed as detailed in Section 8.4.
 - q. Please see Section 10.1 for the definitions of community-acquired, hospital-acquired, and healthcare-associated infections.
 - r. Concomitant medications associated with IED will be recorded for all participants experiencing such an episode.
 - s. MRU data over the last 3 months will be collected by interview with the participant or caregiver as soon as feasible, but no later than 7 days after IED confirmation, and recorded in the eCRF by the investigator and study-site personnel at IED-Day 1. In addition, MRU data will be collected on IED-Day 29, IED-Day 91, and

IED-Day 366. At these timepoints, the data collection window will be from the previous visit to the current visit (eg, at the IED-Day 29 visit/contact, MRU data between IED-Day 1 and IED-Day 29 will be collected). At IED-Day 366, only medical encounters related to IED complications will be collected.

1.3.3. Schedule of Activities – Assessments for Participants with Suspected Pyelonephritis

As of Protocol Amendment 8, site study-specific procedures will not be performed for uncomplicated or complicated UTI or ABP events unless these events are suspected to be pyelonephritis. Participants will still be required to inform sites about events that could be potential IEDs, pyelonephritis, UTI, or ABP cases. Refer to Section 10.18, Appendix 18 for site study-specific procedures for suspected UTI and ABP events prior to Protocol Amendment 8.

ASSESSMENT ^a	UTI/ABP-Day 1: Pyelonephritis Diagnosis ^{b, d}	Day of Resolution or Day 29 Post-Pyelonephritis Diagnosis (UTI/ABP-Day 29±3d) ^{c, d, e}
Medical conditions of interest and pre-UTI therapy ^f	X	
ePRO instruments (SF-36 version 2 and EQ-5D-5L) ^g	X	X
Collection of UTI or ABP signs and symptoms ^h	X	X ⁿ
Type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) ⁱ	X	
Laboratory data ^j	X ^k	X ⁿ
Blood sample for immunogenicity assessments ^l	X 10mL	
Collection of urine samples for urinalysis and urine culture ^m	X	X ⁿ
Collection of information on treatment for pyelonephritis ^o	X	X
Recovery status (outcome of pyelonephritis)		X

ABP=acute bacterial prostatitis; eCOA=electronic clinical outcome assessment; ePRO=patient-reported outcome(s); EQ-5D-5L=5-level EuroQol 5-dimension questionnaire; IED=invasive *Escherichia coli* disease; MRU=medical resource utilization; SF-36=Short Form-36; UTI=urinary tract infection.

Footnotes:

- If case identification criteria for IED are met (see Section 8.1.1), follow procedures per Section 1.3.2 for participants with suspected IED.
- Day 1 of pyelonephritis identification (UTI/ABP-Day 1) is the day on which a pyelonephritis is clinically diagnosed. As of this day, study procedures should be performed if the event is a suspected pyelonephritis, and data should be collected for further evaluation of the pyelonephritis protocol case definition.
- Data collected on the day of resolution of pyelonephritis (resolution of UTI/ABP signs and symptoms) or on UTI/ABP-Day 29 (whichever comes first).
- Scheduled visits which cannot be conducted in person at the study site may be conducted as home health visit. Prior sponsor approval is not required for these home health visits.
- If the participant has recovered and laboratory testing is not required, the Day 29 visit may be conducted remotely (eg, via telephone call).

- f. Medical conditions of interest, ie, conditions with increased risk for UTI and other relevant co-existing medical conditions (please refer to Section 10.16 for additional details). Any relevant treatment received in the last 90 days (ie, 3 months) prior to the pyelonephritis, any antibiotic use (prophylactic or therapeutic) within the prior 90 days (ie, 3 months). Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures.
- g. The ePROs (SF-36 version 2 and the EQ-5D-5L) should be completed on the visit date, preferably before any tests, procedures, or other consultations to prevent influencing of the participant. Alternative options are available for the collection of ePRO data if participants are unable or unwilling to work with the eCOA system or if the eCOA capture system is unavailable (please refer to Section 8.1.6 for further details). Best practices on the collection of ePRO data are provided in the ePRO guidelines.
- h. Identification of UTI/ABP (signs/symptoms and laboratory assessments), when the participant presents with UTI or ABP symptoms that meet the case definition for pyelonephritis (for the clinical case definition of pyelonephritis see Section 8.1.3), as assessed by the investigator/trained healthcare provider. For all clinically diagnosed pyelonephritis cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa*, the participant will be followed up until 29 days post-pyelonephritis diagnosis or until resolution of UTI/ABP signs and symptoms (whichever comes first) to check the evolution of the infection condition and infection management.
- i. Please see Section 10.1 for the definitions of community-acquired, hospital-acquired, and healthcare-associated infections.
- j. Laboratory procedures for pyelonephritis cases include dipstick urinalysis, urine microscopy, or urine flow cytometry in a mid-stream void specimen urine. **In case dipstick urinalysis yields abnormal/unclear results, it must be followed by urine microscopy or flow cytometry.** Additional procedures may be applicable in case of pyelonephritis: hematology test (white blood cell count and differential), computed tomography scan, or magnetic resonance imaging, and/or ultrasonography. **If not part of the standard of care, urine microscopy or urine flow cytometry are to be performed at a local lab as study procedures (at a minimum quantitative urine white blood cell/leukocyte count should be available.** Abnormal leukocyte count on dipstick results alone will not be sufficient evidence of pyuria to meet the pyelonephritis case definition).
- k. A full urinalysis (microscopy or flow cytometry) is a mandatory study procedure on Day 1 in order to verify the protocol case definition of pyelonephritis.
- l. Blood sample collection for immunogenicity assessments is only applicable for the Immunogenicity Subset. If feasible, an immunogenicity blood sample should be taken at the time of pyelonephritis diagnosis, if possible before any treatment or antibiotic(s) is given. If sample collection prior to treatment initiation is not possible, the samples should be collected as soon as possible and within approximately 48 hours after pyelonephritis diagnosis. Immunogenicity assessments will be performed as detailed in Section 8.4.
- m. Urine cultures (from mid-stream urine) will be taken, if possible before initiating treatment including antibiotic(s). Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures. If not part of the standard of care, the urine culture is a mandatory study procedure on UTI/ABP Day 1. Standard clinical practice should be followed for collection of cultures. If on Day 1 the urinalysis (or dipstick) does not confirm a pyelonephritis, the urine culture does not need to be performed (see Section 8.1.3).
- n. Collection of relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care (see Section 8.1.3). These will be recorded as unscheduled evaluations after UTI/ABP-Day 1.
- o. Concomitant medications associated with suspected pyelonephritis will be recorded for all participants experiencing such an episode.

2. INTRODUCTION

ExPEC9V (primary compound number: VAC52416) is a 9-valent vaccine candidate in development for active immunization for the prevention of invasive *Escherichia coli* disease (IED; previously referred to as invasive extraintestinal pathogenic *Escherichia coli* [ExPEC] disease) in adults 60 years of age and older. ExPEC9V consists of the O-antigen polysaccharides (PSs) of the ExPEC serotypes O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75 separately bioconjugated to the carrier protein, a genetically detoxified form of exotoxin A derived from *Pseudomonas aeruginosa* (EPA).

For the most comprehensive nonclinical and clinical information regarding ExPEC9V, refer to the latest version of the ExPEC10V/ExPEC9V Investigator's Brochure (IB).²⁹

The term "study intervention" throughout the protocol refers to the study vaccine. The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document. The term "participant" throughout the protocol refers to the common term "subject".

2.1. Study Rationale

ExPEC is a leading and increasing cause of bacteremia/bloodstream infections worldwide and comprises 17% to 37% of blood isolates.^{16,32,39} In the United States, the overall annual incidence of ExPEC bloodstream infections in adults ranges between 30 and 50/100,000 person-years.^{2,71} In Europe, the number of cases of ExPEC bacteremia annually increased by 8.1% between 2002 and 2008.¹⁶ A retrospective 15-year evaluation of bloodstream infections in Denmark (1992-2006) also showed an increasing incidence of gram-negative bloodstream infections, with the majority of community-acquired (40-50%), hospital-acquired (10-25%), and healthcare-associated (30-40%) bacteremia caused by ExPEC, and mostly in the older adults of >60 years of age.⁶¹ A recent study found an annual incidence of 63.5/100,000 person-years in England (2013-2014).⁸ The worldwide emergence of multidrug resistance (ie, resistance to 3 or more antibiotic categories) among ExPEC strains, such as *Escherichia coli* (*E. coli*) sequence type (ST) 131:O25B, represents a major challenge for prevention and management of ExPEC infections.^{44,47}

IED is defined as an acute illness consistent with systemic bacterial infection, which is microbiologically confirmed either by the isolation and identification of *E. coli* from blood or any other sterile body sites, or by the isolation and identification of *E. coli* from urine in a patient with urosepsis and no other identifiable source of infection. The definition is based on the case definition of invasive bacterial disease from the Active Bacterial Core Surveillance in the United States, a collaboration between the Centers for Disease Control and Prevention and several state health departments and universities participating in the Emerging Infections Program network.^{33,38,57,60}

Although IED affects all ages, adults aged ≥ 60 years have an increased risk of developing IED, including bacteremia and sepsis, which can be community-acquired, hospital-acquired or healthcare-associated. In a retrospective population-based study in the United States from 1998 to

2007, the incidence of *E. coli* bloodstream infections was shown to increase markedly with age from 60 years onwards. The incidence rates in adults aged ≥ 60 years were estimated to be between 136 to 152/100,000 person-years; while by age stratum, incidence rates were estimated at 100/100,000 person-years in adults aged 60 to 79 years, and 300/100,000 person-years in adults aged ≥ 80 years.¹ In another study, most cases were community-acquired (59.4%); the remainder were healthcare-associated (31.7%) or hospital-acquired (8.9%). The urinary tract was found to be the most common primary source of infection (79.8%), followed by the gastrointestinal tract (8.7%), the respiratory tract (3.0%) and other body sites (1.3%).² In a retrospective study in England from April 2012 to March 2014, the most common underlying cause of bacteremia was infection of the genitourinary tract (41.1%; 27,328/66,512), of which 98.4% (26,891/27,328) were urinary tract infections (UTIs).⁸ Another United States (US) population-based cohort study showed that the incidence of community-acquired ExPEC bloodstream infections was 150/100,000 person-years in adults aged ≥ 65 years (1998-2001), and 452/100,000 person-years in adults aged ≥ 85 years.³⁰ According to data extracted from the Public Health England voluntary surveillance database for bacteremia caused by *E. coli* between 2010 and 2014 in England, Wales, and Northern Ireland, the highest incidence rate of *E. coli* bacteremia was in patients aged ≥ 75 years (350.4/100,000 person-years), followed by patients aged 65 to 74 years (120.1/100,000 person-years).⁵³

Data from electronic medical records retrieved from the Kaiser Permanente Northwest (KPNW) in the United States and the Clinical Practice Research Database (CPRD) in combination with the Hospital Episode Statistics (HES) database in the United Kingdom, were evaluated to determine the incidence rate of IED and the prevalence of selected IED risk factors.⁶⁵ The trends in the US and United Kingdom data were remarkably consistent despite inherent differences in the databases. The sources of data for the databases relied upon coded medical diagnoses to identify IED cases and related risk factors, but they differed in their coding systems as well as the availability of laboratory information as a source for identifying IED, which was only available for the KPNW database. The incidence of IED increased markedly with age in both databases. The age group evaluated in both databases (aged ≥ 60 to ≤ 84 years) for incidence of IED constitutes the majority of the subjects who will develop IED. The overall incidence of IED among subjects aged ≥ 60 to ≤ 84 years was 274/100,000 person-years in the KPNW database and 95/100,000 person-years in the CPRD. The incidence of IED in relation to the presence of a range of potential risk factors was investigated. In stepwise multivariate regression models, the highest adjusted incidence rate ratio was observed in adults with a history of UTI. In both databases, subjects aged ≥ 60 to ≤ 84 years with a history of UTI had at least a 6-fold higher incidence of IED compared to subjects without a history of any UTI, suggesting that UTI is the most important independent risk factor for IED in this age group. To investigate whether elderly subjects with a recent history of UTI would be a feasible target population for the Phase 3 study, all subjects aged ≥ 60 to ≤ 84 years in the database on a specific date were entered in a mock study cohort and a 2-year follow-up was simulated. Subjects were excluded if they had any underlying comorbidities that would lead to exclusion from an actual clinical study (ie, organ transplant, malignancy, renal dialysis, chronic liver disease, dementia, and cachexia). This analysis showed that during the 2-year follow-up period in this mock study cohort, the incidence of IED in subjects with a UTI in the previous 2 years was 5.0-fold higher than subjects with no recent UTI history in the KPNW

database (768.0 versus 153.3 per 100,000 person-years, respectively) and 4.1-fold higher in the CPRD database (218.8 versus 53.5 per 100,000 person-years, respectively). The prevalence of any UTI within the past 2 years was 7.5% in the KPNW database and 6.2% in the CPRD.⁶⁵ In subjects with a UTI in the past 5 or 10 years, the incidence of IED was 595.3 per 100,000 person-years and 508.9 per 100,000 person-years, respectively, in the KPNW database and 181.8 per 100,000 person-years and 150.4 per 100,000 person-years, respectively, in the CPRD database. In the present study VAC52416BAC3001, the initially assumed incidence of IED (with microbiological confirmation from blood, sterile sites, or urine) in participants with a UTI in the previous 2 years for sample size calculations was 475 per 100,000 person-years. With an assumed vaccine serotype coverage rate of 65%, a yearly vaccine serotype-specific incidence rate of 300/100,000 person-years was assumed among participants who received placebo. For the primary endpoint of IED with microbiological confirmation from blood or sterile sites, a yearly vaccine serotype-specific incidence rate of 170/100,000 person years will be assumed among participants who received placebo. This expected incidence is based on the proportion of IED cases with microbiological confirmation from blood, and the assumption of an increased incidence rate among participants with at least one additional risk factor for IED, beyond a history of UTI in the past 2 years, who have been enrolled since Protocol Amendment 7.

The data described above from high quality medical record databases in different countries showed recent (2-year) history of UTI, a common condition that affects healthy adults in all age groups, to be the most important risk factor for developing IED.⁶⁵ The elevated risk of IED and high prevalence of UTIs make the participants with a history of UTI in the past 2 years an optimal target population for evaluating the vaccine efficacy (VE) of ExPEC9V in preventing IED, ie, evaluating the degree to which ExPEC9V vaccination reduces the probability that a participant might experience IED.

At present, there is no vaccine available to prevent IED. ExPEC9V is being developed to prevent IED in adults 60 years of age and older. The serotypes comprising the ExPEC9V vaccine (O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75) were selected to address invasive disease caused by the majority of clinically relevant ExPEC strains that also represent the majority ExPEC isolates causing antimicrobial-resistant IED, including ST131.

2.1.1. ExPEC9V

The ExPEC9V vaccine is based on *E. coli* O-antigen PS structures that are part of the lipopolysaccharide located at the surface of the gram-negative bacterial outer membrane. These highly immunogenic surface structures induce O-antigen specific antibodies capable of inducing bacterial killing by opsonophagocytosis. Based on prevalence and antibiotic resistance data collected in *E. coli* surveillance studies conducted in the United States and several European countries, the following nine O-serotypes, responsible for approximately 65% of bloodstream infections caused by ExPEC, were selected for inclusion in ExPEC9V: O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75.^{9,19}

The conjugation of the capsular polysaccharide to a protein carrier promotes a T cell-dependent immune response with the potential to elicit a more robust and durable immune response and to

enable induction of immune memory.⁴⁵ As the mechanism of action of conjugate vaccines in the prevention of invasive disease is not expected to be affected by antibiotic resistance mechanisms, ie, resistance mechanisms are not linked to O-PS structures, the sponsor has no reason to expect a difference in outcome of efficacy between antimicrobial-resistant and susceptible O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75 serotypes.

2.1.2. Medical Resource Utilization and Health Economics Data Collection

In the United States, over 970,000 sepsis cases (all causes) are admitted annually, a number which continues to increase every year.⁵¹ In 2013, sepsis accounted for 3.6% of hospital stays and for more than \$24 billion in hospital expenses. At approximately \$18,244 per hospitalization, the cost of sepsis management is the most expensive condition treated in US hospitals.⁵¹ Despite good standard of care and antibiotic therapy, the case fatality of IED is high in the developed world with a case fatality rate of 8.0% in the 60-84 year-old age group and 13.3% in the ≥ 80 year-old age group.⁶⁵ Similar results were observed in the CPRD database and were reported by Bonten et al who estimated a pooled case fatality of approximately 12.4% (95% CI, 10.7-14.3%; range, 2.8-28.5%) for *E. coli* bacteremia.^{7,65}

In the United States, UTIs account for approximately 0.9% of all outpatient and emergency room visits and 8% of all hospitalizations, resulting in an annual cost of \$2.3 billion.⁵² Hospital admissions for UTIs have increased significantly over the past years, indicating the growing burden and healthcare-related costs due to ExPEC.⁵²

Overall, the costs related to ExPEC-associated conditions are significant. Vaccination with ExPEC9V versus placebo may result in lower medical resource utilization (MRU), therefore comparison of MRU will be done across intervention groups. Refer to Section 8.6, Medical Resource Utilization and Health Economics, for further details.

2.2. Background

Nonclinical Studies

In a nonclinical immunogenicity study in New Zealand white (NZW) rabbits (TV-TEC-179827), 3 intramuscular (IM) injections with ExPEC9V, 10-valent extraintestinal pathogenic *Escherichia coli* vaccine (ExPEC10V), or saline control were administered 2 weeks apart. ExPEC9V and ExPEC10V were shown to be immunogenic in this study.

A 4-week intermittent repeat-dose toxicity and local tolerance study (TOX13465, Good Laboratory Practice study), with ExPEC10V was conducted in NZW rabbits. ExPEC10V was administered via IM injection once every 2 weeks, with a total of 3 IM injections. ExPEC10V was well tolerated in this study. All vaccine-related effects noted were consistent with a normal immunologic response to the vaccine and were not considered to be adverse.

For further details on the nonclinical immunogenicity and toxicology studies with ExPEC9V and/or ExPEC10V, refer to the latest version of the ExPEC10V/ExPEC9V IB.²⁹

Clinical Studies

ExPEC9V is being developed based on the sponsor's preceding clinical experience with ExPEC10V and ExPEC4V, an earlier vaccine candidate which comprised a subset of 4 of the O-antigen conjugates (O1A, O2, O6A, and O25B) also found in ExPEC9V.

The ExPEC4V vaccine has been evaluated in 2 completed Phase 1 clinical studies (GVXN EC-4V and 63871860BAC1001), and 2 completed Phase 2 clinical studies (63871860BAC2001 and 63871860BAC2003). Based on the results from these studies, ExPEC4V was well tolerated by the study participants and no vaccine-related safety signals were observed at doses up to 16 µg PS per serotype (O1A, O2, O6A, and O25B). Most adverse events (AEs) were Grade 1 and 2; very few Grade 3 AEs were reported. Late onset solicited local AEs (time to onset ≥ 6 days) were observed mainly with the higher doses of ExPEC4V. In each study, the ExPEC4V vaccine was shown to be immunogenic against all vaccine serotypes (O1A, O2, O6A, and O25B), demonstrating a dose-dependent vaccine immune response and O-antigen specific immunoglobulin G (IgG) titer increases, as measured by an enzyme-linked immunosorbent assay (ELISA). Functional activity of the antibodies was demonstrated with an ExPEC4V-optimized opsonophagocytic killing assay (OPA). Immunogenicity analysis from study 63871860BAC2001 has demonstrated durability of the immune response through 3 years after vaccination with ExPEC4V.⁶⁴ For further details of the safety/immunogenicity of ExPEC4V, refer to Section 4.3 and to the latest version of the ExPEC10V/ExPEC9V IB.²⁹ ExPEC9V includes nine O-serotypes and will increase coverage from 50% (ExPEC4V) to approximately 65% of bloodstream infections caused by ExPEC in adults aged 60 years or older.

One study with ExPEC10V is currently ongoing (VAC52416BAC1001) and 1 study with ExPEC10V is completed (VAC52416BAC1002). Study VAC52416BAC1001 is a randomized, multicenter, first-in-human Phase 1/2a study to evaluate the safety, reactogenicity, and immunogenicity of 3 different dose levels of ExPEC10V in 2 cohorts. Study VAC52416BAC1002 is a randomized, double-blind, single-center Phase 1 study to evaluate the safety, reactogenicity, and immunogenicity of different doses of ExPEC10V in Japanese adults aged 60 to 85 years. In this study, 24 participants were enrolled, of whom 16 were vaccinated with ExPEC10V.

VAC52416BAC1001 assesses the safety, reactogenicity, and immunogenicity of 3 different doses of ExPEC10V (serotypes O1A, O2, O4, O6A, O8, O15, O16, O18A, O25B, and O75) and to select an optimal dose of ExPEC10V for further clinical development. This study includes 2 cohorts. In Cohort 1, 416 participants aged ≥ 60 to ≤ 85 years in stable health received study vaccination (ExPEC10V [low, medium, or high dose], ExPEC4V, or Prevnar 13[®]). The 3 dose levels of ExPEC10V were evaluated in approximately 100 participants per dose level. Cohort 1 also included reference groups who received ExPEC4V or Prevnar 13[®] (approximately 52 participants per group). Results from Cohort 1 indicated that total IgG titers increased significantly for all ExPEC10V doses. A 2-fold or greater increase in IgG titers, for all serotypes except O8 and O75, was observed for at least 82% of participants who received the high dose. Multiplex opsonophagocytic killing assay (MOPA) results were generally consistent with electrochemiluminescent (ECL) data: increased functional antibody titers were observed on Day 15 for all ExPEC10V serotypes and dose groups, with the exception of serotype O8. The

MOPA data for serotype O8 were further investigated and a functional antibody response for this serotype could not be demonstrated in the qualified MOPA assay used. This prompted the sponsor to investigate a new formulation (ExPEC9V), excluding the O8 serotype, in the present VAC52416BAC3001 Phase 3 study. Removal of the O8 serotype is expected to result in a change of ExPEC9V vaccine O-serotype coverage from approximately 70% to approximately 65% of ExPEC-associated bloodstream infections in adults aged ≥ 60 years. Safety and immunogenicity data will be collected during the observer-blind follow-up period through Day 181 post-vaccination. Only participants from the selected ExPEC10V dose level and the Prevnar 13[®] group will progress to the follow-up period from Day 181 through 3 years (Day 1096) post-vaccination.

In Cohort 2 of study VAC52416BAC1001, approximately 420 participants aged ≥ 60 years in stable health with a history of UTI in the past 5 years were enrolled. Based on the acceptable safety profile of all 3 ExPEC10V formulations evaluated in Cohort 1 and the results of the immunogenicity dose selection algorithm, the high dose is being further evaluated in Cohort 2 of study VAC52416BAC1001. The high dose of ExPEC10V is being administered to 280 participants, with the remaining 140 participants receiving a placebo.

Safety results from study VAC52416BAC1001 (including preliminary results for Cohort 2) showed that the most frequent local solicited AE was pain/tenderness and the most frequently reported systemic AEs were myalgia, headache, and fatigue. While several of the observed solicited AEs occurred early, a trend towards late onset of local solicited AEs was observed in the VAC52416BAC1001 participants. Injection site erythema and swelling were the most common late onset events (time to first onset > 5 days after vaccination) in participants from the combined ExPEC10V groups in both Cohorts 1 and 2. A trend towards increased local reactogenicity with higher doses of ExPEC10V was observed in Cohort 1. The most frequent unsolicited AE assessed as related to the vaccine was injection site pruritus, reported by $< 3\%$ of the participants from the combined ExPEC10V groups in both Cohorts 1 and 2.

In Japanese participants (study VAC52416BAC1002), the reactogenicity profile of the medium and high doses was generally comparable to the reactogenicity profile in study VAC52416BAC1001.

The present Phase 3 study VAC52416BAC3001 will investigate the efficacy, safety, reactogenicity, and immunogenicity of the selected dose of ExPEC9V in medically stable adults aged ≥ 60 years with a history of UTI in the past 2 years. In the Phase 1/2a study VAC52416BAC1001, the precursor vaccine ExPEC10V was studied, VAC52416BAC3001 will be the first study in which ExPEC9V will be administered to humans.

In May 2022, an Independent Data Monitoring Committee (IDMC) meeting was held to review safety information from the first 500 randomized participants in study VAC52416BAC3001 who had completed the Day 30 visit (or discontinued earlier). Following this safety review, the IDMC recommended that the study continue unmodified until the next scheduled meeting. Thereafter, the IDMC has performed a safety review every 6 months.

2.3. Benefit-risk Assessment

More detailed information about the known and expected benefits and risks of ExPEC9V may be found in the ExPEC10V/ExPEC9V IB.²⁹

2.3.1. Risks Related to Study Participation

The following potential risks for ExPEC9V will be monitored during the study and are specified in the protocol:

General Risks Related to Vaccination

In general, IM injection may cause local itching, warmth, pain, tenderness, erythema/redness, induration, swelling, arm discomfort, or bruising of the skin. Additionally, participants may exhibit general signs and symptoms associated with injection of a vaccine and/or placebo, including fever, chills, rash, myalgia, nausea/vomiting, headache, dizziness, arthralgia, general itching, and fatigue. These side effects will be monitored but are generally short-term and do not require treatment.

Syncope can occur in association with administration of injectable vaccines. Syncope can be accompanied by falls. Procedures should be in place to avoid falling injury. If syncope develops, participants should be observed until the symptoms resolve. Fear of injection might lead to fainting and fast breathing.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, urticaria, or even anaphylaxis. Severe reactions are rare. Participants with a known allergy, or history of anaphylaxis or other serious adverse reactions to vaccines or their excipients (including specifically the excipients of the study vaccine) will be excluded from the study.

Pregnancy and Birth Control

The effect of this vaccine on a fetus or nursing baby are unknown. This study will enroll postmenopausal or permanently sterile female participants (as defined in Section 10.5, Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information) who are not intending to conceive by any methods. Therefore, information on birth control methods will not be collected.

Risks from Blood Draws

Blood draws may cause pain, tenderness, bruising, bleeding, dizziness, vaso-vagal response, syncope, and, rarely, infection at the site where the blood is taken.

Reactogenicity

Based on 2 completed Phase 1 studies and 2 completed Phase 2 studies in the ExPEC4V development program, most solicited (local and systemic) AEs started on Days 1 to 3 post-vaccination. Headache and fatigue/tiredness were the most frequent solicited events, reported as early onset events, with a median time of onset of 2 and 3 days, respectively. Late onset solicited (mainly local) AEs were observed starting Days 6 to 8 post-vaccination. Pain/tenderness

represented a majority of the late onset AEs and were Grade 1 in severity. There was a tendency for higher incidence of late onset AEs with higher ExPEC4V doses.

Late onset solicited AEs were also observed in ExPEC10V development program. The median time to first onset of local solicited AEs was 2 to 2.5 days after vaccination for those in the ExPEC10V dose groups. The most frequent solicited events, pain/tenderness, fatigue, myalgia, and headache occurred mostly as early onset events. Injection site erythema and swelling occurred as late onset events (time to first onset >5 days after vaccination) in the majority of participants from the combined ExPEC10V groups. In the combined ExPEC10V groups, 8 (2.6%) participants experienced late onset AEs with grade 3 severity. A trend towards increased local reactogenicity with higher doses of ExPEC10V was observed.

Unknown Risks

There may be other risks that are not known. If any significant new risks are identified, the principal investigator and participants will be informed.

2.3.2. Benefits of Study Participation

Participants may benefit from clinical testing and physical examination. Participants may benefit from the knowledge that they may aid in the development of a vaccine for the prevention of IED.

The clinical benefits of ExPEC9V have yet to be established. Currently, there are no effective vaccines for the prevention of IED and no efficacy can be concluded from current data. The overall benefit and risk balance for individual participants thus cannot be ascertained. Participants must be informed that this vaccine has not yet been proven to be effective, and it should be assumed that it is not the case until clinical studies are conducted to demonstrate its effectiveness.

2.3.3. Benefit-risk Assessment of Study Participation

Based on the available data and proposed safety measures, the overall benefit-risk assessment for this clinical study is considered acceptable for the following reasons:

- Only participants who meet all inclusion criteria and none of the exclusion criteria (specified in Section 5, Study Population) will be allowed to participate in this study. The selection criteria include adequate provisions to minimize the risk and protect the well-being of participants in the study.
- Safety will be closely monitored throughout the study:
 - In general, safety evaluations will be performed at scheduled visits during the study, as indicated in Section 1.3, Schedule of Activities.
 - After vaccination, all participants will remain at the study site for at least 30 minutes and will be closely observed by study staff. Necessary emergency equipment and medications must be available in the clinic to treat severe allergic reactions.
 - The investigator or the designee will document unsolicited AEs as indicated in Section 8.3, Adverse Events, Serious Adverse Events, and Other Safety Reporting and Section 10.4, Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

- For participants in the Safety Subset:
 - Participants will use an electronic diary (eDiary) to document solicited signs and symptoms. Details are provided in Section 8.3, Adverse Events, Serious Adverse Events, and Other Safety Reporting.
 - Any clinically significant abnormalities (including those persisting at the end of the study/early withdrawal) reported based on the criteria described in Section 8.3 will be followed by the investigator until resolution or until clinically stable.
- Several safety measures are included in this protocol to minimize the potential risk to participants, including the following:
 - The IDMC will review safety data at regular intervals as specified in the IDMC Charter.

More detailed information about the known and expected benefits and risks of ExPEC9V may be found in the ExPEC10V/ExPEC9V IB.²⁹

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood or other sterile sites, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75	First IED event^h, with microbiological confirmation from blood or other sterile sitesⁱ, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75
Secondary	
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes^j	First IED event^{h,k}, with microbiological confirmation from blood, other sterile sitesⁱ, or urine, caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of all IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes^j	All IEDs^k (including multiple IEDs per participant) caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first hospitalized IED event caused by ExPEC9V O-serotypes^j	First hospitalized IED event^k caused by ExPEC9V O-serotypes

^h Refer to Section 8.1.2 for the IED clinical case definition.

ⁱ Refer to Section 10.15, Appendix 15 for the sterile sites' definition.

^j Key secondary efficacy objectives, part of confirmatory testing strategy.

^k Indicated secondary endpoints related to IEDs will include events with *E. coli* microbiological confirmation from blood, other sterile sites, or urine.

Objectives	Endpoints
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes^j	First IED event^k meeting criteria for sepsis caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first bacteremic (ie, microbiological confirmation from blood) IED event caused by ExPEC9V O-serotypes^j	First bacteremic IED event caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first pyelonephritis event caused by ExPEC9V O-serotypes^j	First pyelonephritis event caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first UTI event caused by ExPEC9V O-serotypes^j	First UTI event^l caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of all UTIs (including multiple UTIs per participant) caused by ExPEC9V O-serotypes^j	All UTIs^l (including multiple UTIs per participant) caused by ExPEC9V O-serotypes
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event caused by <i>E. coli</i>^j	First IED event^k caused by <i>E. coli</i>
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first pyelonephritis event caused by <i>E. coli</i>^j	First pyelonephritis event caused by <i>E. coli</i>
To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of the first UTI event caused by <i>E. coli</i>^j	First UTI event^l caused by <i>E. coli</i>
To evaluate the immunogenicity of ExPEC9V in the Immunogenicity Subset	Antibody titers to vaccine O-serotype antigens and EPA in the Immunogenicity Subset, as determined by multiplex electrochemiluminescent (ECL)-based immunoassay and antibody titers to vaccine O-serotype antigens, as determined by multiplex opsonophagocytic killing assay (MOPA) on Day 1 (pre-vaccination), Day 30, Day 181, Year 1, Year 2, Year 3, and Year 4

^l UTI and ABP events that occur after implementation of Protocol Amendment 8 will no longer be included in analyses.

Objectives	Endpoints
To evaluate the safety and reactogenicity of ExPEC9V	- Solicited local and systemic AEs (collected until 14 days post-vaccination [from Day 1 to Day 15] in the Safety Subset) - Unsolicited AEs (collected until 29 days post-vaccination [from Day 1 to Day 30] in all participants) - Serious adverse events (SAEs)^m in all participants
- To evaluate the preservation of health status and health-related quality of life (HRQoL) of ExPEC9V compared to placebo as measured by the Short Form-36 (SF-36) survey and the 5-level EuroQol 5-dimension questionnaire (EQ-5D-5L) descriptive system - To evaluate the impact of IED, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL as measured by the SF-36 and EQ-5D-5L - To evaluate the impact of pyelonephritis, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL as measured by the SF-36 and the EQ-5D-5L - To evaluate the impact of UTI^l, caused by ExPEC9V O-serotypes, on physical and mental health, and overall HRQoL, as measured by the SF-36 and EQ-5D-5L	SF-36 and EQ-5D-5L responses at scheduled timepoints
To assess the degree of frailty in participants who received ExPEC9V versus placebo at baseline, Year 1, Year 2, Year 3, Year 4, and at the time of an IED	Frailty Index as a measure of frailty at baseline, Year 1, Year 2, Year 3, Year 4, and at the time of an IED^k
To determine the MRU for IED, UTI, and acute bacterial prostatitis (ABP) events caused by ExPEC9V O-serotypes	- MRU for IED events^k caused by ExPEC9V O-serotypes - MRU for UTI events^l (Immunogenicity Subset only) and ABP events^l (Immunogenicity Subset only) caused by ExPEC9V O-serotypes - Hospitalization and length of stay in the hospital, including ICU hospitalization and ICU length of stay, for IED^k, UTI^l, or ABP events^l caused by ExPEC9V O-serotypes

^m All SAEs until the end of Day 181 and only SAEs related to study vaccine, study procedures, or non-investigational sponsor products from Day 182 until the end of the study. Additional SAEs may be reported per local regulatory requirements.

Objectives	Endpoints
To determine the mortality associated with IED	IED-related^k and all-cause mortality
Exploratory	
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event caused by ExPEC9V O-serotypes resistant to ≥ 1, ≥ 2, and ≥ 3 antibiotic classes	First IED event caused by ExPEC9V O-serotypes showing resistance to ≥ 1, ≥ 2, and ≥ 3 antibiotic classes in the microbiological profile
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of first uncomplicated, complicated, and recurrent UTI events caused by ExPEC9V O-serotypes	- First uncomplicated UTI event^l caused by ExPEC9V O-serotypes - First complicated UTI event^l caused by ExPEC9V O-serotypes - First recurrent UTI (rUTI) event^l caused by ExPEC9V O-serotypes - First recurrent uncomplicated UTI event^l caused by ExPEC9V O-serotypes
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first ABP event caused by ExPEC9V O-serotypes	First ABP event^l caused by ExPEC9V O-serotypes
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first ABP event caused by <i>E. coli</i>	First ABP event^l caused by <i>E. coli</i>
To evaluate antibody titers to vaccine O-serotype antigens and EPA in participants with IED	Antibody titers to vaccine O-serotype antigens and EPA, as determined by multiplex ECL-based immunoassay and antibody titers to vaccine O-serotype antigens, as determined by MOPA assays in participants with IED, with sera obtained at the time of diagnosis of the suspected IED
To evaluate the severity of IED	Severity of IED (presence of sepsis or septic shock) in participants with IED
To identify a correlate of protection for the vaccine-mediated immune response associated with VE	Antibody titers for ExPEC9V determined by multiplex ECL-based immunoassay and MOPA assays at Day 1 pre-vaccination, Day 30 and at the time of diagnosis of the suspected IED (all participants) or at the time of a UTI or ABP event^l (Immunogenicity Subset only), in association with VE
To evaluate the efficacy of ExPEC9V compared to placebo in the prevention of the first invasive disease event caused by <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) and the first <i>P. aeruginosa</i> UTI/ABP event	- First invasive disease event caused by <i>P. aeruginosa</i> - First <i>P. aeruginosa</i> UTI event^l - First <i>P. aeruginosa</i> ABP event^l

Refer to Section 8, Study Assessments and Procedures for evaluations related to endpoints.

HYPOTHESES

For the primary objective, the following null and alternative hypotheses will be used:

$$H_0: VE \leq 20\%$$

versus

$$H_a: VE > 20\%$$

For the key secondary objectives related to IED, the following null and alternative hypotheses will be used:

$$H_0: VE \leq 0\%$$

versus

$$H_a: VE > 0\%$$

For the key secondary objectives related to pyelonephritis and UTI, the following null and alternative hypotheses will be used:

$$H_0: VE \leq 10\%$$

versus

$$H_a: VE > 10\%$$

Further details on statistical analyses to address the primary and key secondary objectives are provided in Section 9.4.1, Efficacy Analyses.

4. STUDY DESIGN

4.1. Overall Design

This is a randomized, double-blind, placebo-controlled, parallel-group, multicenter, interventional Phase 3 study to be conducted in approximately 19,800 medically stable adults aged ≥ 60 years and with a history of UTI in the past 2 years.

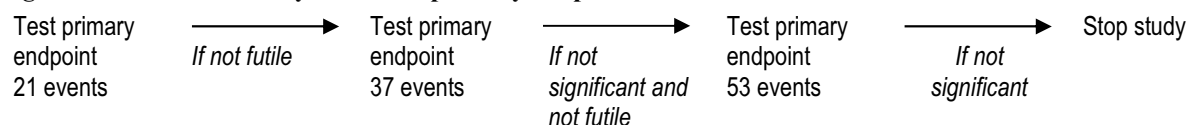
This study incorporates an inferentially seamless group-sequential design [Figure 3](#).

The target number of participants to be enrolled is approximately 19,800. Note that over enrollment may be allowed if required by local health authorities for the purpose of local health authority consideration. When the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, an immunogenicity interim analysis will be conducted and the sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation). Enrollment will not be paused during this immunogenicity interim analysis. The purpose of this immunogenicity interim analysis is to obtain an initial result of the immune response, no decision will be taken on the remainder of the study and therefore no type I error adjustment is required. In addition, once the first 500 randomized participants in the study (any participant, irrespective of

enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, the IDMC will review unblinded safety data. In case of serious safety concerns during this IDMC safety review, the IDMC may recommend the sponsor to stop the study. The safety data will include SAEs and unsolicited AEs from all participants and solicited AEs from participants in the Safety Subset. According to the ratio of the Safety Subset versus the initial total sample size of the study (4,000/18,556=21.6%), approximately 108 participants are targeted in the Safety Subset among the first 500 randomized participants (Section 6.3). Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety review will be conducted at least every 6 months. When 21 of 53 (40%) vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sitesⁿ) have been observed, an interim analysis for the primary endpoint (futility) will be performed (Figure 2). Based on the outcome of this interim analysis, the IDMC may recommend stopping enrollment of participants and stopping the study (in case of futility) or to continue the study. In the event of the study being stopped for futility, all participants will be followed up for at least 6 months post-vaccination. The futility analysis will be based on the Go/No Go approach. In addition, if the event rate is lower or higher than expected, a blinded sample size re-estimation (ie, based on the event rate combining all participants from both the ExPEC9V and placebo groups) may be performed by the sponsor at the time of this interim analysis; however, the sample size will not be increased by more than 50%.

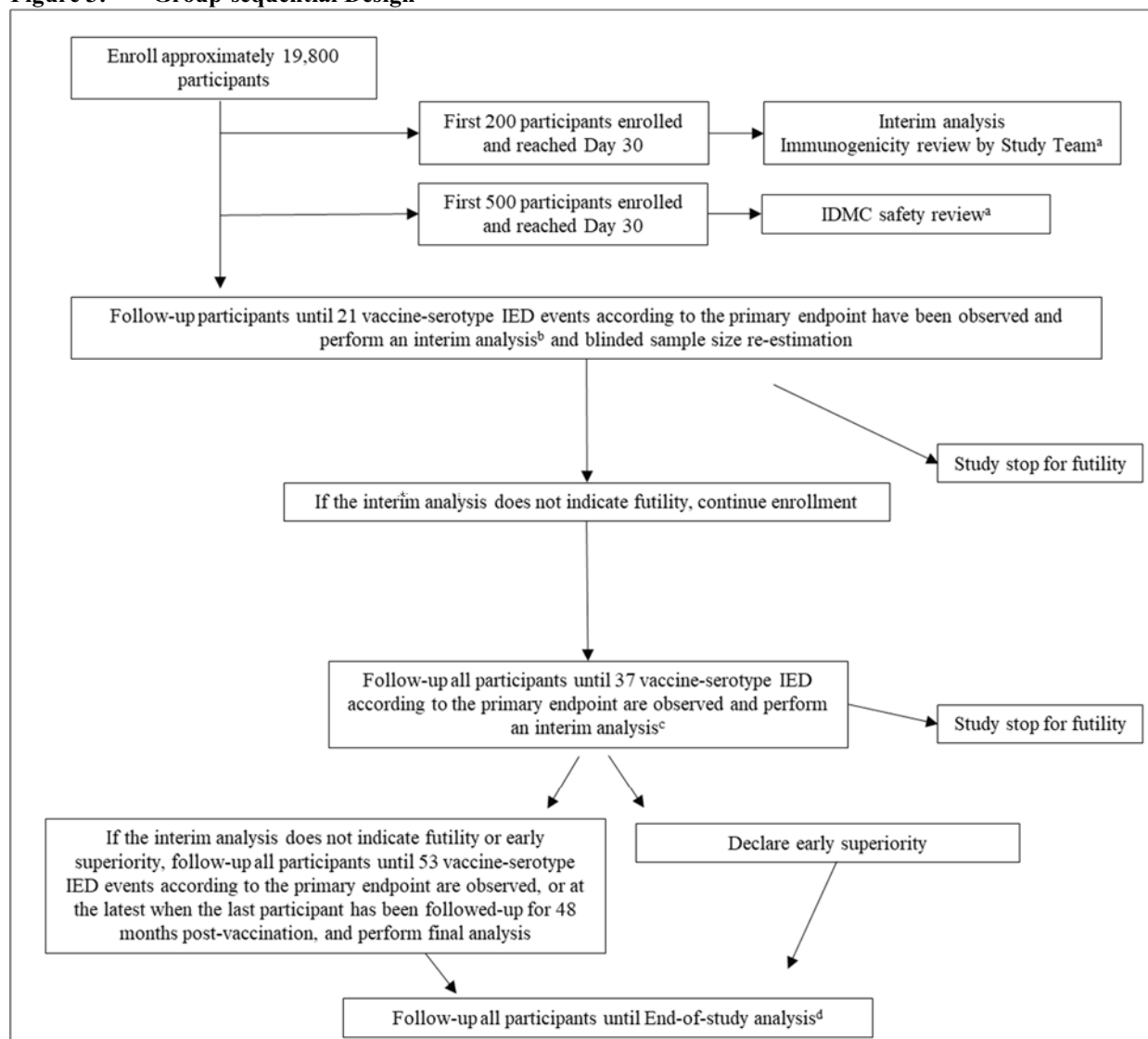
A second interim analysis for the primary endpoint (efficacy/futility) will be conducted when 37 of 53 (70%) vaccine-serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed. As with the first interim analysis, an independent statistician will perform the second interim analysis and will present the results to the IDMC. The final analysis for the primary endpoint will occur when 53 vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed in the study or at the latest when the last participant has been followed up for 48 months post-vaccination.

Figure 2: Interim analyses for the primary endpoint



The efficacy analyses for the primary endpoint will use the α -spending approach. For all the interim analyses, an independent statistician will perform the interim analyses and will present the results to the IDMC (at the immunogenicity interim analysis the independent statistician will also prepare the immunogenicity tables for the study team). For further details on the interim analyses, refer to Section 9.5.1.

ⁿ Refer to Section 10.15, Appendix 15 for the sterile sites' definition.

Figure 3: Group-sequential Design

AE=adverse event; ExPEC9V=9-valent extraintestinal pathogenic *Escherichia coli* vaccine; IDMC=Independent Data Monitoring Committee; IED=invasive *Escherichia coli* disease; SAE=serious adverse event; VE=vaccine efficacy.

^a When the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, an immunogenicity interim analysis will be conducted and the sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation). In addition, once the first 500 randomized participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3), or discontinued earlier, the IDMC will review unblinded safety data. The safety data will include SAEs and unsolicited AEs from all participants and solicited AEs from participants in the Safety Subset. Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety review will be conducted at least every 6 months.

^b At the first interim analysis of the primary endpoint (when 21 events have been observed), the study might be stopped for futility if the VE (point estimate) is $\leq 25.0\%$ (≥ 9 events in the ExPEC9V group; non-binding futility).

^c At the second interim analysis of the primary endpoint (when 37 events have been observed), early superiority may be declared if the VE (point estimate) is $\geq 72.4\%$ (≤ 8 events in the ExPEC9V group). In the unlikely event that there are ≥ 17 events in the ExPEC9V group at the second interim analysis of the primary endpoint, the study might be stopped for futility.

^d End-of-study analysis will include, safety and immunogenicity data collected at Year 1 (Day 366), Year 2 (Day 731), Year 3 (Day 1,096), and Year 4 (Day 1,461) after vaccination.

A diagram of the study design is provided in Section 1.2, Schema. The study consists of screening, randomization, and vaccination for each participant on Day 1 (selected screening procedures may be performed up to 8 days prior to vaccination on Day 1). Each participant will be followed for a maximum of approximately 48 months (1,461 days; 4 years) post-vaccination. At screening/Day 1, demographic data, Short Physical Performance Battery (SPPB) score, and medical conditions of interest will be collected. All participants will be enrolled and randomized in parallel in a 1:1 ratio to either ExPEC9V or placebo and receive the study vaccine on Day 1. Participants will be stratified at randomization according to their age (≥ 60 to < 65 years of age versus ≥ 65 to < 75 years of age versus ≥ 75 years of age) and according to the presence of at least one risk factor for complicated UTIs (cUTIs) at baseline (see complicating factors in cUTI case definition in Section 8.1.4 and of risk factors for cUTI in Section 10.16). A maximum of 20% of the study population will be enrolled in the ≥ 60 to < 65 years of age stratum. Efforts will be made to ensure good representation in terms of race, ethnicity, and gender. The sponsor expects to see the same proportional distribution of diversity during recruitment as in each site's patient database.

Events defined as IED or pyelonephritis will be collected for all participants according to study-specific procedures for the entire study duration (1,461 days; 4 years). As of Protocol Amendment 8, site study-specific procedures will not be performed for uncomplicated or complicated UTI or ABP events unless these events are suspected to be pyelonephritis. During study follow-up, participants are expected to inform the study site, as soon as possible, if they experience any signs or symptoms of UTI or ABP, or if they experience any new onset or worsening of symptoms that could be caused by a systemic infection. In addition, all participants will be questioned about past hospitalizations or medical events that could have been potential IEDs or pyelonephritis cases that were not captured by the site in real time. When implemented at a site level, for participants using study devices (provisioned or bring your own device), participant surveillance via eCOA will begin following the Day 30 visit and will continue until the study end or participant early withdrawal (registered as de-activation in the eCOA system). The eCOA surveillance instrument allows participants to confirm if they have sought urgent or non-routine medical care. Electronic COA surveillance does not apply to participants exclusively using interview mode. In addition to eCOA surveillance, throughout the study sites must communicate directly with all participants via telephone contact or other means of communication every 3 months $\pm$ 2 weeks after vaccination. During these direct contacts, sites should monitor participant eCOA surveillance adherence and review medical events that have occurred during the past interval. The sponsor has set up (semi) closed healthcare networks by identifying healthcare locations where a participant may go for treatment for an IED, pyelonephritis, UTI, or ABP. Collaborations between the clinical trial sites and relevant primary care physician offices, hospitals, and emergency rooms will be established proactively, prior to the start of recruitment, to ensure that relevant samples and clinical data (eg, laboratory and diagnostic procedures reports, discharge summaries) are collected and assessed per protocol. This measure is in addition to other extensive measures (eg, on-site and virtual visits, direct contact between the participant and site [via telephone or mobile application], participant wallet cards/necklace/bracelet [to ensure a participant is identified as a participant in a clinical study]) taken, to ensure that participants are identified by healthcare staff when presenting with symptoms. Upon all contacts, in addition to inquiring about development of IEDs or pyelonephritis, relevant safety information and MRU associated with medical encounters related

to IED (all participants) will be collected. As of Protocol Amendment 8, MRU data will no longer be collected for UTI or ABP events, including pyelonephritis. See Section 8.1, Efficacy Assessments, for further details.

For any participant who visits his/her healthcare provider and/or the study site with signs and symptoms of a suspected IED or pyelonephritis the investigator/trained healthcare professional will use the criteria in Section 8.1.1 for identification of suspected IED cases, Section 8.1.2 for the IED clinical case definition, and Section 8.1.3 for the pyelonephritis clinical case definition.

After vaccination, participants will remain under observation at the study site for at least 30 minutes for presence of any acute reactions. Additional safety assessments will be performed in a subset of approximately 4,000 participants (approximately 2,000 ExPEC9V participants and 2,000 placebo participants) from selected study sites who have given informed consent prior to randomization for additional safety assessments, referred to as the Safety Subset. Due to local requirements, over enrollment may occur for the Safety Subset. Participants in the Safety Subset will record solicited AEs in an eDiary for 14 days post-vaccination. Unsolicited AEs will be recorded for all participants through 29 days post-vaccination. See Section 8.2 and Section 8.3 for further details of safety assessments.

Additional blood samples will be collected from a subset of approximately 1,200 participants (approximately 600 ExPEC9V participants and 600 placebo participants) from selected study sites who have given informed consent prior to randomization for additional immunogenicity assessments, referred to as the Immunogenicity Subset. Due to local requirements, over enrollment may occur for the Immunogenicity Subset. Key immunogenicity assessments of collected sera will include the assessment of serum IgG antibody levels elicited by the vaccine against each of the 9 vaccine O-serotypes and the carrier protein EPA as measured by multiplex ECL-based immunoassay and vaccine O-serotype-specific functional serum antibodies as measured by MOPA. See Section 8.4 for further details of immunogenicity assessments.

An IDMC and Independent Endpoint Adjudication Committee (IEAC) will be commissioned for this study. Refer to Committees Structure in Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations for details.

4.2. Scientific Rationale for Study Design

Double-blind

Blinded study vaccination will be used to reduce potential bias during data collection and evaluation of efficacy, safety, and immunogenicity endpoints. There will be a designated unblinded pharmacist(s) and/or a qualified staff member(s) at the site with primary responsibility for study vaccine preparation; these study personnel will not participate in any other study evaluation. The vaccine administrator will be blinded and can perform other study evaluations.

Placebo Control

A placebo control will be used to establish the frequency and magnitude of changes in efficacy, safety, and immunogenicity endpoints that may occur in the absence of active intervention. The

use of placebo is justified by the fact that no suitable active control vaccine could be identified for this population.

Randomization

Randomization will be used to minimize bias in the assignment of participants to study vaccination groups, to increase the likelihood that known and unknown participant attributes (eg, demographic and baseline characteristics) are evenly balanced across study vaccination groups, and to enhance the validity of statistical comparisons across study vaccination groups.

Inferentially seamless group-sequential design

The study is designed using an inferentially seamless group-sequential design where the following interim analyses are planned:

- Cohort 1 and Cohort 2 of Phase 1/2a study VAC52416BAC1001 have studied the safety and immunogenicity profile of the ExPEC10V vaccine. Although it is not expected that the safety profile of the ExPEC9V vaccine will differ substantially from the safety profile of the ExPEC10V vaccine as the total antigen amount is the same, an IDMC safety review of the first 500 randomized participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) with safety data through Day 30 will be performed. The safety data will include SAEs and unsolicited AEs from all participants and solicited AEs from participants in the Safety Subset. Enrollment will be paused after randomization of the 500th participant until the IDMC issues a recommendation. Thereafter, an IDMC safety review will be conducted at least every 6 months.

In addition, when the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have been followed up through Day 30, the sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation) to confirm the immunogenicity profile of ExPEC9V. Upon confirmation of the immunogenicity profile the study will continue as planned. Enrollment will not be paused during this immunogenicity interim analysis. The purpose of this immunogenicity interim analysis is to obtain an initial result of the immune response, no decision will be taken on the remainder of the study and therefore no type I error adjustment is required. The 200 participants included in this interim analysis will continue their follow-up as the other participants and will be included in future futility and efficacy evaluations, hence the term “inferentially seamless”.

- When 21 of 53 (40%) vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed, a first interim analysis of the primary endpoint (futility) will be performed. Based on the outcome of this interim analysis, the IDMC may recommend stopping enrollment of participants and stopping the study (in case of futility) or to continue the study. In the event of the study being stopped for futility, all participants will be followed up for at least 6 months post-vaccination. The futility analysis will be based on the Go/No Go approach. By allowing the enrollment to stop in the event of futility, participants will be prevented from receiving an ineffective vaccine or a vaccine inferior to placebo.
- A second interim analysis of the primary endpoint (efficacy/futility) will be conducted when 37 of 53 (70%) vaccine serotype IED events according to the primary endpoint (with

microbiological confirmation from blood or other sterile sites) have been observed. At the time of this interim analysis early superiority may be declared.

- The final analysis of the primary endpoint will occur when 53 vaccine serotype IED events with microbiological confirmation from blood or other sterile sites have been observed.

Blinded sample size re-estimation

The assumption on the IED incidence used for the sample size calculation is based on several database mining studies and because of the uncertainty on this assumption, a blinded sample re-estimation is foreseen. If the event rate is lower or higher than expected, a blinded sample size re-estimation (ie, based on the event rate combining all participants from both the ExPEC9V and placebo groups) may be performed by the sponsor at the time of the first interim analysis of the primary endpoint (21 events).

Selection of Study Population

Data retrieved from existing databases (KPNW and CPRD) identified that subjects aged ≥ 60 years with a recent history of UTI are at an elevated risk of developing IED (Section 2.1, Study Rationale). The elevated risk of IED and high prevalence of UTIs make participants with a history of UTI in the past 2 years an optimal population for the evaluation of efficacy of the ExPEC9V in preventing IED. Therefore, medically stable adults aged ≥ 60 years with a history of UTI in the past 2 years are the target population in this Phase 3 study.

Immunogenicity responses and safety after vaccination with ExPEC9V are expected to be similar in the population aged ≥ 60 years with a recent history of UTI when compared to the population aged ≥ 60 years without a recent history of UTI. Cohort 1 of the ExPEC10V Phase 1/2a study (VAC52416BAC1001) has enrolled participants aged ≥ 60 to ≤ 85 years in stable health without consideration of their UTI history. Cohort 2 enrolled participants aged ≥ 60 years in stable health with a history of UTI in the past 5 years. Participants enrolled in the Phase 1/2a study may have an underlying illness such as hypertension, diabetes, ischemic heart disease, as long as their symptoms/signs are medically stable. The same population in terms of co-morbidities will be enrolled in this Phase 3 study.

Rationale for Duration of Collection of Solicited Adverse Events

As described in Section 2.3.1, late onset solicited AEs have been observed in clinical studies with ExPEC4V and in the ExPEC10V VAC52416BAC1001 study (Cohort 1). Therefore, the present Phase 3 study will further evaluate potential late onset reactogenicity by following solicited local and systemic AEs until Day 15 in the Safety Subset.

Medical Resource Utilization and Health Economics Data Collection

Overall, the costs related to ExPEC-associated conditions are significant (Section 2.1.2). Vaccination with ExPEC9V versus placebo may result in lower MRU; therefore, comparison of MRU will be done across intervention groups. Refer to Section 8.6, Medical Resource Utilization and Health Economics, for further details.

4.2.1. Study-specific Ethical Design Considerations

Potential participants will be fully informed of the risks and requirements of the study and, during the study, participants will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only participants who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be enrolled. Written consent/assent may be obtained through various sources (eg, paper or electronic such as eConsent, eSignature, or digital signature) as determined by regulations as well as study and/or participant preferences.

The primary ethical concern is the total blood volume to be collected, which is considered to be an acceptable amount of blood to be collected over this time period from the population in this study as it will not exceed the US Department of Health and Human Services (HHS) Office for Human Research Protections (OHRP) and US Food and Drug Administration (FDA) guidelines of 550 mL in any 8-week period.^{69,70}

See Section 2.3 for Benefit-risk Assessment.

4.3. Justification for Dose

The dose of ExPEC9V to be administered in the present study (Table 1) is based on the primary analysis of Cohort 1 of the ongoing ExPEC10V Phase 1/2a study (VAC52416BAC1001) (see Section 2.2), including immunogenicity data through Day 15 and all available safety data when all participants completed the Day 30 visit or discontinued earlier.

The 3 doses (low, medium, and high dose) of ExPEC10V, detailed below in Table 1, were developed based on the immunogenicity (ELISA and OPA) and safety results of a previous ExPEC4V Phase 2 study (63871860BAC2001), where 4:4:4:8 and 8:8:8:16 µg PS doses (per serotype doses for O1A, O2, O6A, and O25B, respectively) gave a robust immune response and reached a maximum or near-maximum immune response for all 4 serotypes on Day 15. One-year durability of the ExPEC4V antibody response, although demonstrated for both dose groups, was slightly improved for serotypes O1A and O6A in the 8:8:8:16 dose group. Thus, by extrapolation of the 4:4:4:8 and 8:8:8:16 ExPEC4V doses, the dose groups for the 10 vaccine serotypes in the ExPEC10V vaccine were as detailed in Table 1.

Table 1: Vaccine Dosages Used in the ExPEC Clinical Development

Study Vaccination Group	O1A (µg)	O2 (µg)	O4 (µg)	O6A (µg)	O8 (µg)	O15 (µg)	O16 (µg)	O18A (µg)	O25B (µg)	O75 (µg)	EPA (µg)	PS (Total) (µg)
ExPEC4V ^a	4	4	-	4	-	-	-	-	8	-	72 ^d	20
Low dose ExPEC10V ^b	4	4	4	4	4	4	4	4	8	4	159 ^d	44
Medium dose ExPEC10V ^b	8	4	4	8	4	4	4	4	16	4	217 ^d	60
High dose ExPEC10V ^b	8	8	8	8	8	8	8	8	16	8	319 ^d	88
ExPEC9V ^c	8	8	8	8	-	8	8	8	16	16	290 ^e	88

EPA=genetically detoxified form of exotoxin A derived from *Pseudomonas aeruginosa*; ExPEC4/9/10V=4/9/10-valent extraintestinal pathogenic *Escherichia coli* vaccine; PS=polysaccharide.

A: ExPEC4V consists of the O-antigen PSs of the ExPEC serotypes O1A, O2, O6A, and O25B separately bioconjugated to the EPA carrier protein.

B: ExPEC10V consists of the O-antigen PSs of the ExPEC serotypes O1A, O2, O4, O6A, O8, O15, O16, O18A, O25B, and O75 separately bioconjugated to the EPA carrier protein.

C: ExPEC9V consists of the O-antigen PSs of the ExPEC serotypes O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75 separately bioconjugated to the EPA carrier protein.

D: The EPA (µg) was calculated using a ratio of 0.276 for PS/EPA. However, the final EPA dose was confirmed at the release.

E: The maximum allowed EPA concentration in ExPEC9V is 382.5 µg/dose.

Based on the acceptable safety profile of all 3 ExPEC10V formulations evaluated and the results of the immunogenicity dose selection algorithm, the safety and immunogenicity profiles of the high dose have been confirmed in Cohort 2 of the VAC52416BAC1001 study.

MOPA data from VAC52416BAC1001 Cohort 1 showed that functional antibody titers were increased on Day 15 for all ExPEC10V serotypes and dose groups, with the exception of serotype O8. Although MOPA results for serotype O75 showed a distinct functional antibody response, titers were lower than for other responding serotypes suggesting the need for a further dose increase for O75. The failure to demonstrate presence of functional O8 antibodies moved the sponsor to investigate a new formulation (ExPEC9V) excluding this serotype. In addition, the dose level of the O75 PS antigen was increased (16 µg instead of 8 µg) to amplify the O75 vaccine-mediated antibody response (Table 1).

A single dose of study vaccine will be administered in the present study VAC52416BAC3001. Single-dose administration of ExPEC9V is based on the assumed durability of the immune response for at least 5 years, with possible extension to 10 years and a booster after 10 years post-vaccination. This assumption is proposed based on the long-term persistence of antibody responses induced by pneumococcal conjugate vaccine (PCV) and other glycoconjugate vaccines. Conjugation with a protein carrier improves the effectiveness of polysaccharide vaccines by inducing T-lymphocyte-dependent immunologic function and stimulating a long-lasting immune response, even if antibody titers decline over time.⁴⁶ In the CAPITA study assessing a single dose

of 13-valent PCV administered to immunocompetent community-dwelling adults aged ≥ 65 years who were naive to pneumococcal vaccines, PCV13 elicited total (IgG) and functional antibody responses that were significantly higher than baseline and the corresponding values in the placebo group through 2 years post-vaccination.⁶⁹ Over the 5 years of the same study, a single dose of PCV13 showed efficacy for all episodes of confirmed pneumococcal community-acquired pneumonia and all episodes of invasive pneumococcal disease.⁷⁶ Long-term immunogenicity has also been observed with a single dose of the quadrivalent meningococcal serogroups A, C, W, and Y tetanus toxoid conjugate vaccine in an open, randomized trial, in which at least 72% of vaccinated adolescents and adults had an immune response against these meningococcal serogroups that persisted for at least 5 years post-vaccination.⁶ ExPEC9V clinical development is based on preceding clinical experience with ExPEC4V, in which immunogenicity data from the Phase 2 study 63871860BAC2001 have demonstrated the durability of the immune response through at least 3 years post-vaccination.¹²

4.4. End of Study Definition

End of Study Definition

The end of study is considered as the last Year 4 assessment (Day 1,461) for the last participant. The final data from the study site will be sent to the sponsor (or designee) after completion of the final participant assessment at that study site, in the time frame specified in the Clinical Trial Agreement.

Participant Study Completion Definition

A participant will be considered to have completed the study if he or she has completed the 4-year follow-up period. A participant enrolled prior to Protocol Amendment 8 who is not reconsented on an updated ICF will be considered to have completed the study if he or she has completed the Year 3 (Day 1,096) visit.

5. STUDY POPULATION

Selected screening procedures may be performed up to 8 days prior to vaccination. If screening procedures are split over different days, eligibility criteria must be rechecked, pre-vaccination vital signs and pre-vaccination blood samples for immunogenicity should be collected on the day of vaccination (Day 1). Refer to Section 5.4, Screen Failures, for conditions under which the repeat of any screening procedures are allowed.

The inclusion and exclusion criteria for enrolling participants in this study are described in the following 2 subsections. If there is a question about these criteria, the investigator must consult with the appropriate sponsor representative and resolve any issues before enrolling a participant in the study. Protocol waivers are not allowed.

5.1. Inclusion Criteria

Each potential participant must satisfy all of the following criteria to be enrolled in the study:

1. Participant must sign an informed consent form (ICF) indicating that he or she understands the purpose, procedures, and potential risks and benefits of the study, and is willing to participate in the study.
2. Participant must be willing and able to adhere to the prohibitions and restrictions specified in this protocol.
3. Criterion modified per Amendment 4:
 - 3.1 Participant must be willing to share relevant medical information pertaining to medical history and to share medical records relevant to the medical events identified as suspected cases of IED, UTI, or ABP occurring during the study observation period.
4. Participant must be ≥ 60 years of age on the day of signing the ICF and is expected to be available for the duration of the study, with no current intention of moving away from a study site area or travelling for periods longer than 30 consecutive days during the course of the study.
5. Criterion modified per Amendment 3:
 - 5.1 Criterion modified per Amendment 4:
 - 5.2 Criterion modified per Amendment 5:
 - 5.3 Criterion modified per Amendment 6:
 - 5.4 Participant must have a history of UTI* in the past 2 years for which evidence of diagnosis** was verified by the investigator. In case of a recent history of UTI or ABP (acute bacterial prostatitis), the condition must have resolved >14 days*** prior to randomization.

*Note: this includes any uncomplicated, complicated, or recurrent UTIs that were diagnosed by a physician (or qualified healthcare provider) and that required antimicrobial therapy. History of isolated male accessory gland infections will not be considered as a history of UTI. See also Section 5.2, exclusion criteria 4.

**Note: the investigator must collect the relevant facts regarding UTI diagnosis made by a physician and a prescription of antibacterial treatment based on either the available medical records, or interview with the participant confirming the above details, or a combination of both. The interview must be documented in the medical records.

***Note: understood as resolution of UTI or ABP symptoms and conclusion of acute antimicrobial therapy.
6. Criterion modified per Amendment 1:
 - 6.1 Criterion modified per Amendment 5:
 - 6.2 Participant must be medically stable at the time of vaccination such that, according to the judgment of the investigator, hospitalization within the study period is not anticipated and the participant appears likely to be able to remain on study through the end of protocol-specified follow-up. A stable medical condition* is defined as disease not requiring significant change in therapy during the 6 weeks before enrollment and when hospitalization for worsening of the disease is not anticipated. Participants will be included on the basis of physical examination, medical history, and vital signs performed between ICF signature and vaccination.

*Note: Refer to Section 10.7 Appendix 7 for guidelines for stable infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV).

7. Before randomization, participants who were born female must be either (as defined in Section 10.5, Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information):
 - a. postmenopausal or permanently sterile, and
 - b. not intending to conceive by any methods.
8. Participant must be willing to provide verifiable identification, has means to be contacted and to contact the investigator during the study.
9. Criterion deleted per Amendment 5.
10. Criterion modified per Amendment 1:
 - 10.1 Criterion modified per Amendment 5:
 - 10.2 Participant and his/her designated caregiver (if applicable) must be able to read, understand, and complete questionnaires in the electronic clinical outcome assessment system (eCOA, ie, the electronic patient-reported outcomes [ePROs] and the eDiary). If the participant and caregiver are unable/unwilling to work with the eCOA system to complete the ePROs, participant or caregiver must agree to be available to be contacted by the site to complete all eCOA activities (ePROs) via site-assisted interview at the timepoints specified in the protocol. Participants in the Safety Subset must be willing and able to work with the eCOA system to complete the eDiary.
11. Criterion added per Amendment 7:
 - 11.1 Criterion modified per Amendment 8:
 - 11.2 Participant must have at least one additional risk factor for IED, beyond a history of UTI in the past 2 years. Additional risk factors for IED are defined as one or more of the following:
 - a. a history of urosepsis and/or *E. coli* bacteremia at any time prior to randomization, and/or
 - b. a history of inpatient hospitalization (for a medical/surgical cause) in the two years prior to randomization, and/or
 - c. presence at baseline of at least one risk factor for complicated UTI of any toxicity grade^{o,p}, and/or

^o Complicated UTI risk factors that must be present at baseline include: acute renal injury, benign prostatic hypertrophy (BPH), chronic kidney disease, cystostomy, hydronephrosis, medullary sponge kidney, nephrolithiasis, nephrostomy, nephrostomy tube placement, neurogenic bladder, obstructive uropathy, pelvic prolapse, renal transplant, ureteric stent, ureteric strictures, urethral strictures, urinary catheterization, urinary retention, urinary tract obstruction, urine incontinence, urolithiasis, urostomy, vesicoureteral reflux, and voiding dysfunction. The presence of any of these cUTI risk factors, irrespective of toxicity grading, is sufficient to meet the definition of at least one additional IED risk factor.

^p Refer to Appendix 10.16, Table 10 for information regarding the requisite timing of medical conditions for eligibility.

- d. a history of pyelonephritis of any toxicity grade that has resolved > 14 days prior to randomization (see also Inclusion Criterion 5)^P, and/or
- e. current or prior prostatic adenocarcinoma and/or tumors of the urinary tract of any toxicity grade (see also Exclusion Criterion 6)^P.

5.2. Exclusion Criteria

Any potential participant who meets any of the following criteria will be excluded from participating in the study:

1. Participant has a serious chronic disorder or significant cognitive impairment for which, in the opinion of the investigator, participation would not be in the best interest of the participant (eg, compromise well-being) or that could prevent, limit, or confound the protocol-specified assessments.
2. Participant has end-stage renal disease for which dialysis is required.
3. Criterion modified per Amendment 5:
 - 3.1. Participant has a contraindication to IM injections and blood draws eg, due to bleeding disorders or a history of difficult blood draws.
4. Participant has a clinically significant acute illness or temperature $\geq 38.0^{\circ}\text{C}$, preferably by oral temperature measurement, within 24 hours prior to the planned dose of study vaccine; enrollment at a later date is permitted (see Section 5.4).
5. Criterion deleted per Amendment 6.
6. Criterion modified per Amendment 1:
 - 6.1 Criterion modified per Amendment 3:
 - 6.2 Criterion modified per Amendment 5:
 - 6.3 Participant has a history of malignancy within 5 years before screening that does not include the following categories:
 - a. Participants with curatively treated squamous and basal cell carcinomas of the skin and carcinoma in situ of the cervix may be enrolled at the discretion of the investigator.
 - b. Participants with a diagnosis of localized prostate cancer may be enrolled at the discretion of the investigator if they completed treatment (observing Excl. 8.3c and Excl. 13; continuation of androgen deprivation therapy is allowed), or, if they remain under observation or active surveillance. Participants who underwent radical prostatectomy or radiotherapy may be enrolled at the discretion of the investigator if treatment has been completed 6 months prior to the planned administration of the study vaccine.
 - c. Participants with a history of other malignancy within 5 years, which is considered adequately treated with minimal risk of recurrence per the investigator's judgment, may be enrolled.
7. Criterion modified per Amendment 6:
 - 7.1 Participant has a known history of severe allergic reaction, anaphylaxis or other serious

adverse reactions to vaccines or vaccine excipients (including specifically the excipients of the study vaccine; refer to IB).²⁹

8. Criterion modified per Amendment 3:

8.1 Criterion modified per Amendment 4:

8.2 Criterion modified per Amendment 5:

8.3 Criterion modified per Amendment 6:

8.4 Participant with abnormal function of the immune system resulting from:

- a. Clinical conditions or their treatments expected to have an impact on the immune response elicited by the study vaccine. Participants with clinical conditions that are stable under treatment without the use of prohibited therapies (see exclusion criteria 8.3b and 8.3c) may be enrolled at the discretion of the investigator.
- b. Chronic or recurrent use of systemic corticosteroids within 3 months before administration of study vaccine and during the study. A substantially immunosuppressive steroid dose is considered to be ≥ 2 weeks of daily receipt of 20 mg or more of prednisone or equivalent.
Note: Ocular, topical, intra-articular, or inhaled steroids are allowed.
- c. Administration of antineoplastic and immunomodulating agents, (eg, cancer chemotherapeutic agents) or radiotherapy expected to have an impact on the immune response elicited by the study vaccine within 6 months before administration of study vaccine and during the study.

9. Criterion modified per Amendment 1:

9.1 Participant has a history of acute polyneuropathy (eg, Guillain-Barré syndrome) or chronic inflammatory demyelinating polyneuropathy.

10. Criterion modified per Amendment 4:

10.1 Participant has received any *E. coli*^q or ExPEC vaccine.

11. Criterion modified per Amendment 5:

11.1 Participant has received a hematopoietic stem cell transplant based on medical history, treatment with immunoglobulins within 2 months, apheresis therapies within 4 months, or blood products within 3 months prior to the planned administration of the study vaccine or has any plans to receive such treatment during the study.

Note: Given that not all immunoglobulins/monoclonal antibodies are expected to impact the vaccine-induced immune response, the investigator should contact the sponsor or delegate to discuss eligibility of participants on immunoglobulin treatment.

12. Criterion modified per Amendment 1:

12.1 Criterion modified per Amendment 5:

^q *E. coli* vaccines include, and are not limited to, immunoactive agents used for rUTI prophylaxis and containing *E. coli* immunogens, eg, inactivated whole bacterial lysates of *E. coli* (eg, Uromune[®]) or lyophilized preparation of *E. coli* membrane proteins (eg, Uro-vaxom[®]).

12.2 Participant has received or plans to receive:

- a. licensed live attenuated vaccines - within 28 days before or after planned administration of the study vaccination
- b. other licensed (not live) vaccines - within 14 days before or after planned administration of the study vaccination.
- c. vaccination with a vaccine authorized for emergency use (eg, EUA, CMA, or a similar program) is permitted when given at least 28 days before or after planned administration of the study vaccination.

Note: Enrollment outside of the above windows is permitted.

13. Participant has had major surgery (per the investigator's judgment) within 4 weeks before dosing or will not have recovered from surgery per the investigator's judgment at time of vaccination.

14. Criterion modified per Amendment 4:

14.1 Criterion modified per Amendment 6:

14.2 Participant has received an investigational drug or used an invasive investigational device within 30 days or received an investigational vaccine within 90 days before the planned administration of the study vaccine or is currently enrolled or plans to participate in another investigational study within 6 months after administration of the study vaccine.

Note: After sponsor approval, enrollment in the VAC52416BAC3001 study is permitted if a potential participant is in the long-term follow-up phase of another investigational study (ie, without further administration of an investigational study drug and without procedures that could interfere with the current study evaluations). Participation in an observational clinical study or in an investigational study from 6 months after administration of the study vaccine onwards is allowed with prior approval of the sponsor.

15. Participant is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator, or an employee of the sponsor or Clinical Research Organization (CRO).

16. Criterion modified per Amendment 1:

16.1 Criterion modified per Amendment 5:

16.2 Participant has chronic active hepatitis B or hepatitis C infection based on medical history.

Note: as per Inclusion Criterion 6, participant may have stable HBV or HCV infection. Refer to Section 10.7 Appendix 7 for guidelines for stable infection HBV or HCV.

17. Criterion modified per Amendment 1:

17.1 Criterion modified per Amendment 5:

17.2 Participant has evidence of HIV type 1 or type 2 infection by medical history.

Note: as per Inclusion Criterion 6 participant may have stable/well-controlled HIV. Refer to Section 10.7 Appendix 7 for guidelines for stable infection with HIV.

18. Participant has had major psychiatric illness or drug substance or alcohol abuse which in the investigator's opinion would compromise the participant's safety or compliance with the study procedures.
19. Participant cannot communicate reliably with the investigator.
20. Participant who, in the opinion of the investigator, is unlikely to adhere to the requirements of the study or is unlikely to complete the full course of vaccination and observation.

NOTE: Investigators must ensure that all study enrollment criteria have been met at screening. If a participant's clinical status changes (including any receipt of additional medical records) before the study vaccination is given such that he or she no longer meets all eligibility criteria, then the participant must be excluded from participation in the study. The required documentation to support meeting the enrollment criteria is described under Source Documents in Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations.

5.3. Lifestyle Considerations

Potential participants must be willing and able to adhere to the following lifestyle restrictions during the course of the study to be eligible for participation:

1. Refer to Section 6.8, Concomitant Therapy for details regarding prohibited and restricted therapy during the study.
2. Agree to follow all requirements that must be met during the study as noted in the Inclusion and Exclusion Criteria (Section 5.1 and Section 5.2, respectively).

5.4. Screen Failures

Participant Identification, Enrollment, and Screening Logs

The investigator agrees to complete a participant identification and enrollment log to permit easy identification of each participant during and after the study. This document will be reviewed by the sponsor study-site contact for completeness. This study will use IWRS. The investigator will not generate screening and enrollment logs directly from IWRS.

The participant identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure participant confidentiality, no copy will be made. All reports and communications relating to the study will identify participants by participant identification and age at initial informed consent. In cases where the participant is not randomized into the study, the date seen and age at initial informed consent will be used.

Rescreening

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened on one occasion. Rescreened participants must be assigned new participant numbers, undergo the informed consent process, and then restart a new screening process.

5.5. Criteria for Temporarily Delaying Enrollment

Not applicable for this study.

6. STUDY VACCINE AND CONCOMITANT THERAPY

6.1. Study Vaccine Administered

ExPEC9V (VAC52416): *E. coli* bioconjugate vaccine in phosphate buffered solution containing O-antigen PSs of ExPEC serotypes O1A, O2, O4, O6A, O15, O16, O18A, O25B, and O75 separately bioconjugated to the EPA carrier protein. A single 0.5 mL IM (deltoid) injection of ExPEC9V will be administered on Day 1. ExPEC9V will be supplied at a concentration of 176 µg PS/mL. The PS content of the dose to be tested in the present study is 88 µg/dose. The EPA concentration in the currently manufactured ExPEC9V clinical batches is 290 µg/dose. The maximal allowed EPA concentration in ExPEC9V is 382.5 µg/dose. The dose of ExPEC9V to be administered in the present study is detailed in [Table 1](#).

Placebo: Sodium chloride 0.9% w/v. A single 0.5 mL IM (deltoid) injection of placebo will be administered on Day 1. The placebo for this study will be supplied by the sponsor as a commercially available injectable solution of 9 mg/mL sodium chloride (0.9% w/v).

Study vaccine administration must be captured in the source documents and the electronic Case Report Form (eCRF).

ExPEC9V will be manufactured and provided under the responsibility of the sponsor. Refer to the IB for a list of excipients.²⁹

6.2. Preparation/Handling/Storage/Accountability

Preparation/Handling/Storage

The participants, clinical staff, investigators, and sponsor personnel will be blinded to study vaccination group allocation, except for the designated pharmacist(s) or qualified staff member(s) with primary responsibility for study vaccine preparation. These unblinded members will not be part of the team performing the evaluations. The vaccine administrator will be blinded and can perform other study evaluations.

Refer to the site investigational product procedures manual for additional guidance on study vaccine preparation, handling, and storage.

Accountability

The investigator is accountable for ensuring that all study vaccine received at the site is inventoried and accounted for throughout the study. The study vaccine administered to the participant must be documented on the vaccine accountability form. All study vaccines will be stored and disposed of according to the sponsor's instructions. Study-site personnel must not combine contents of the study vaccine containers.

Study vaccine must be handled in strict accordance with the protocol and as indicated on the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study vaccine must be available for verification by the sponsor's study site monitor during on-site monitoring visits. The return to the sponsor of unused study vaccine will be documented on the vaccine return form. When the study site is an authorized destruction unit and study vaccine supplies are destroyed on-site, this must also be documented on the vaccine return form.

Potentially hazardous materials containing hazardous liquids must be disposed of immediately in a safe manner and therefore will not be retained for vaccine accountability purposes.

Study vaccine must be dispensed under the supervision of the investigator or a qualified member of the study-site personnel, or by a hospital/clinic pharmacist. Study vaccine will be supplied only to participants participating in the study. Returned study vaccine must not be dispensed again, even to the same participant. Study vaccine may not be relabeled or reassigned for use by other participants. The investigator agrees neither to dispense the study vaccine from, nor store it at, any site other than the study sites agreed upon with the sponsor. Further guidance and information for the final disposition of unused study vaccine are provided in the site investigational product procedures manual.

6.3. Measures to Minimize Bias: Randomization and Blinding

Vaccine Allocation

Procedures for Randomization and Stratification

Central randomization will be implemented in this study. Participants will be randomly assigned to 1 of 2 study vaccination groups based on a computer-generated randomization schedule prepared before the study by or under the supervision of the sponsor.

The randomization will be stratified according to participant age (≥ 60 to < 65 years of age versus ≥ 65 to < 75 years of age versus ≥ 75 years of age) and according to the presence of at least one risk factor for cUTIs at baseline (ie, history of urinary retention, current indwelling urinary catheter, obstructive uropathy, or any relevant functional or anatomical abnormality, for more details see Section 8.1.4 for complicating factors in cUTI case definition and Section 10.16, Table 10, for risk factors for cUTI)) and done in blocks to ensure balance across arms. The following cap will be installed:

- The maximum number of participants aged ≥ 60 to < 65 years will be 3,960.

Safety Subset and Immunogenicity Subset participants will only be enrolled at a selection of sites. These sites will be carefully selected to represent a relevant proportion of the sites from the involved countries, regions, and sites' types and to include the widest possible demographic variety, in terms of race, ethnicity, and gender, for the target eligible population to be enrolled in the study. The sponsor expects to see the same proportional distribution of diversity during recruitment as in each site's patient database. All study participants randomized at these pre-selected sites will be enrolled into a single, pre-defined Subset at any given time. This will

eliminate, at the site level, any bias related to the willingness to consent to additional safety assessments or additional sampling. Following completion of a given subset, further enrollment in a non-subset group is allowed.

The same stratification rules will apply to the first 500 participants. According to the ratio of the Safety Subset versus the initial total sample size of the study ($4,000/18,556=21.6\%$), approximately 108 participants are targeted in the Safety Subset among the first 500 randomized participants. An additional effort will be made to include a reasonable proportion of participants in particular age groups. In line with the stratification rules for the general study population, the following cap will be installed for the first 500 participants:

- the maximum number of participants aged ≥ 60 to < 65 years will be 100

The interactive web response system (IWRS) will assign a unique intervention code, which will dictate the study vaccine assignment and matching study vaccine kit for the participant. The requestor must use his or her own user identification and personal identification number when contacting the IWRS and will then give the relevant participant details to uniquely identify the participant.

Blinding

The investigator will not be provided with randomization codes. The codes will be maintained within the IWRS, which has the functionality to allow the investigator to break the blind for an individual participant.

Data that may potentially unblind the intervention assignment (ie, different packaging of the study vaccines as well as preparation) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, clinical team, or others as appropriate until the time of database lock and unblinding.

The participants, study-site personnel, and investigators will be blinded to study vaccine group allocation throughout the study, except for the designated pharmacist(s) or qualified staff member(s) with primary responsibility for study vaccine preparation. These unblinded members will not be part of the team performing the evaluations. The vaccine administrator will be blinded and can perform other study evaluations. At the time of the interim analyses, the participants, investigator(s), and site staff will remain blinded to individual participants' study vaccination assignment. The sponsor will be blinded to study vaccine allocation until the database release of the final analysis of the primary endpoint. However, in case of positive efficacy results at an earlier interim analysis (ie, when the IDMC informs the sponsor committee that the superiority criteria have been met for the primary endpoint), the sponsor might be unblinded to start preparation of regulatory submissions. For blinding of study monitors, refer to Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations. In this case, the study will not be stopped, but follow-up of participants will be continued for efficacy, safety, and immunogenicity.

Under normal circumstances, the blind must not be broken until all participants have completed the study or discontinued earlier and the database is released. The investigator may, in an emergency, determine the identity of the intervention by contacting the IWRS. While the responsibility to break the intervention code in emergency situations resides solely with the investigator, it is recommended that the investigator contact the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its designee will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor must be informed as soon as possible. The date and reason for the unblinding must be documented by the IWRS, in the appropriate section of the eCRF and in the source document. The documentation received from the IWRS indicating the code break must be retained with the participant's source documents in a secure manner.

Participants who have had their intervention assignment unblinded should continue to return for scheduled evaluations.

In general, randomization codes will be disclosed fully only if the study is completed and the clinical database is closed. However, for the interim analyses, the randomization codes and, if required, the translation of randomization codes into intervention and control groups will be disclosed to those authorized and only for those participants included in the interim analysis.

Participants who withdraw will not be replaced.

6.4. Study Vaccination Compliance

Study vaccine will be administered as an IM injection by blinded qualified study-site personnel at the study site. Details of each administration will be recorded in the eCRF (including date and time of injection and deltoid used for injection).

6.5. Dose Modification

Not applicable.

6.6. Continued Access to Study Vaccination After the End of the Study

Participants who received placebo will be instructed that study vaccination will not be made available to them after they have completed the study.

6.7. Treatment of Overdose

For this study, any dose of ExPEC9V greater than the single administration (0.5 mL) of the study vaccine will be considered an overdose. The sponsor does not recommend specific intervention for an overdose.

In the event of an overdose, the investigator or treating physician should:

- Contact the Medical Monitor immediately.
- Closely monitor the participant for AE/SAE until resolution.
- Document the quantity of the excess dose as well as the duration of the overdosing in the eCRF.

- Report as a special reporting situation.

6.8. Concomitant Therapy

Relevant prestudy therapies (ie, non-steroidal anti-inflammatory drugs, corticosteroids, antihistamines, vaccinations, antivirals, and all antibacterials [including antibiotics and urinary tract antiseptics]) administered up to 30 days before dose of study vaccine must be recorded at screening.

Concomitant therapies associated with an SAE meeting the criteria outlined in Section 8.3.1 will be collected and recorded in the eCRF from ICF signature through the end of the study.

Concomitant medications associated with suspected IED or pyelonephritis episodes (per clinical case identification/definition criteria, see Section 8.1) will be recorded for all participants experiencing such an episode. As of Protocol Amendment 8, concomitant medications associated with treatment of suspected UTI or ABP episodes will no longer be recorded. Chronic systemic prophylactic use of antibacterials (including antibiotics and urinary tract antiseptics) (eg, for UTI prophylaxis) may be initiated during study participation, if medically indicated. Chronic use of antibacterials (including antibiotics and urinary tract antiseptics), for any indication, is to be recorded for the entire duration of the study.

Concomitant therapies associated with unsolicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 29 days after vaccination (Day 30) or until clinical resolution or stabilization. For participants in the Safety Subset, concomitant therapies associated with solicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 14 days after vaccination (Day 15) or until clinical resolution or stabilization.

Use of any experimental medication (including experimental vaccines other than the study vaccine) is not allowed within 6 months after the administration of the study vaccine. With prior approval of the sponsor, participation in an observational clinical study or in an investigational study is allowed no earlier than 6 months after administration of the study vaccine.

In order to avoid misinterpretation of adverse reactions and potential immune interference, vaccination with licensed live attenuated vaccines is prohibited within 28 days before or after the administration of the study vaccination. Vaccination with other licensed vaccines (not live) (eg, COVID-19, tetanus, hepatitis A, hepatitis B, rabies) is prohibited within 14 days before or after the administration of the study vaccine. If a vaccine is indicated in a post-exposure setting (eg, rabies or tetanus), it must take priority over the study vaccine.

Vaccination with a vaccine authorized for emergency use (eg, EUA, CMA, or a similar program) is permitted when given at least 28 days before or after administration of the study vaccine.

Use of licensed *E. coli*^r vaccines is prohibited. If *E. coli* vaccines other than ExPEC9V/study vaccination are administered during the study period due to medical necessity, their use should be recorded and will be considered a protocol deviation. Participants who receive other *E. coli* vaccines will be excluded from the per protocol efficacy (PPE) population and the per protocol immunogenicity (PPI) population.

Use of licensed *E. coli* vaccines must always be recorded as concomitant therapy for the entire duration of the study.

Chronic or recurrent use of systemic corticosteroids^s, is prohibited during the study and within 3 months before the planned administration of the study vaccine. A substantially immunosuppressive steroid dose is considered to be ≥ 2 weeks of daily receipt of 20 mg or more of prednisone or equivalent. The definition of recurrent corticosteroid use is at the discretion of the investigator.

Administration of antineoplastic and immunomodulating agents or radiotherapy is prohibited during the study and within 6 months before the planned administration of the study vaccine. If any of these agents are indicated in a disease setting, these must take priority over the study vaccine.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered. All such instances will be recorded as a protocol deviation, but this will not exclude the concerned participant from further study participation, unless other conditions apply (see Section 7.2); however, the participant may be excluded from the PPE and/or PPI populations (see Section 9.3). Any medication (whether licensed or experimental) that is prescribed by a treating physician as a treatment of any condition, will have priority over protocol restrictions.

7. DISCONTINUATION OF STUDY VACCINATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Vaccination

As this is a single vaccination study, this section is not applicable.

^r *E. coli* vaccines include, and are not limited to, immunoactive agents used for rUTI prophylaxis and containing *E. coli* immunogens, eg, inactivated whole bacterial lysates of *E. coli* (eg, Uromune[®]) or lyophilized preparation of *E. coli* membrane proteins (eg, Uro-vaxom[®]).

^s Note: Ocular, topical, intra-articular or inhaled steroids are allowed.

7.2. Participant Discontinuation/Withdrawal From the Study

A participant will be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent for study participation
- Death
- Repeated failure to comply with protocol requirements
- Decision by the sponsor or the investigator to stop or cancel the study
- Decision by local regulatory authorities or IRB/IEC to stop or cancel the study

When a participant withdraws before study completion, the reason for withdrawal is to be documented in the eCRF and in the source document. Study vaccine assigned to the withdrawn participant may not be assigned to another participant. If the reason for withdrawal from the study is withdrawal of consent then no additional assessments are allowed.

Note: If a participant's clinical status changes during study follow-up, including new comorbidities and need for prohibited concomitant medications, such that he or she no longer meets the original eligibility criteria, or is unable to follow protocol procedures, this does NOT represent a reason for participant's withdrawal from the study (see also Section 6.8).

Withdrawal of Consent

A participant declining to return for scheduled visits does not necessarily constitute withdrawal of consent. Alternate follow-up mechanisms that the participant agreed to when signing the consent form apply as local regulations permit.

7.2.1. Withdrawal From the Use of Study Samples

Withdrawal From the Use of Study Samples

The participant may withdraw consent for use of study samples for research not required by the protocol (refer to Storage, Use, Transfer, and Retention of Data and Samples in Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations). In such a case, samples will be destroyed after they are no longer needed for the clinical study. Details of sample retention are presented in the ICF.

7.3. Lost to Follow-up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits/contacts and is unable to be contacted by the study site. A participant cannot be deemed lost to follow-up until all reasonable efforts made by the study-site personnel to contact the participant are deemed futile.

The following actions must be taken if a participant fails to return to the study site for a required study visit:

- The study-site personnel must attempt to contact the participant to reschedule the missed visit as soon as possible, to counsel the participant on the importance of maintaining the assigned visit schedule, to ascertain whether the participant wishes to or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every reasonable effort to regain contact with the participant (where possible, 3 telephone calls, e-mails, fax, and, if necessary, a certified letter to the participant's last known mailing address, or local equivalent methods). These contact attempts should be documented in the participant's medical records.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study.

Should a study site close, eg, for operational, financial, or other reasons, and the investigator cannot reach the participant to inform them, their contact information will be transferred to another study site.

8. STUDY ASSESSMENTS AND PROCEDURES

Overview

The [Schedule of Activities \(SoA\)](#) summarizes the frequency and timing of efficacy, safety, immunogenicity, frailty, HRQoL, and MRU measurements applicable to this study. The timings of the post-vaccination visits will be determined relative to the actual day of the vaccination. Unscheduled visits may be performed based on the investigator's clinical judgement and may include further evaluations, as needed.

The ePROs (SF-36 version 2 and the EQ-5D-5L) collected during screening should be completed on the visit date, prior to vaccination, preferably before any tests, procedures, or other consultations to prevent influencing of the participant. In case of IED assessment, the ePROs can be completed up to 7 days post IED confirmation.

If multiple assessments are scheduled for the same timepoint, procedures should preferably be performed in the following sequence: ePRO assessments (SF-36 version 2 followed by EQ-5D-5L), clinical assessments including laboratory and cultures, blood draws for immunogenicity, and MRU data collection.

The total blood volume to be collected will be approximately 70 mL from each participant in the Immunogenicity Subset, and 20 mL from each participant who is not in the Immunogenicity Subset. An additional 50 mL blood sample will be taken at selected sites in which case the total volume will be approximately 120 mL for each participant in the immunogenicity subset and 70 mL from each participant who is not in the Immunogenicity Subset. Additional blood samples will be collected for participants with suspected IED (see Section 1.3.2) or with suspected pyelonephritis (participants in the Immunogenicity Subset only; see Section 1.3.3). Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

MRU and health economics data will be collected. Refer to Section 8.6 for details.

Participants in the Safety Subset will be provided a thermometer (to measure body temperature), ruler (to measure local injection site reactions), and participant eDiary with instructions for the proper recording of body temperature and solicited local (injection site) and systemic signs and symptoms.

The eDiary given to participants in the Safety Subset for the recording of solicited AEs includes instructions on how to capture the data and grading scales to assess severity of the signs and symptoms. The study staff is responsible for providing appropriate training to the participant to avoid missing or incorrect data (refer to the Study Procedures Manual). The eDiary will be reviewed by the study personnel at visits indicated in Section 1.3, Schedule of Activities. If the eDiary review is missed, the eDiary will be reviewed during the following visit.

If a participant is too ill to record safety data in the eDiary or experiences any issue with the eDiary at any time, site staff or a caregiver can select the portion of the eDiary that allows for assisted completion and ask the participant the questions, read the response options and record their responses. Participant should continue to use the eDiary when the participant has recovered.

Visit Windows

The timings of the post-vaccination visits will be determined relative to the actual day of the vaccination. Refer to Section 1.3, Schedule of Activities for the visit windows.

Screening

Only medically stable participants without acute illness or fever and complying with the inclusion and exclusion criteria specified in Section 5 will be included into the study.

The investigator will provide detailed information on the study to the participants and will obtain written informed consent prior to each participant's participation in the study. All procedures described in Section 1.3, Schedule of Activities will only take place after written informed consent has been obtained.

Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the eCRF or laboratory requisition form. Refer to Section 1.3, Schedule of Activities for the timing and frequency of all sample collections.

Instructions for the collection, handling, storage, and shipment of samples are found in the laboratory manual that will be provided. Collection, handling, storage, and shipment of samples must be under the specified, and where applicable, controlled temperature conditions as indicated in the laboratory manual.

Study-specific Materials

The investigator will be provided with the following supplies:

- IB
- Study site investigational product and procedures manual, investigational product (ExPEC9V and placebo), Investigational Product Preparation Instructions
- Laboratory manual and laboratory kits
- Participant eDiaries and instructions for use / Instructions for installing ePRO on mobile devices, ePRO completion guides, and ePRO participant information sheets.
- IWRS Manual
- Sample ICF
- Rulers and thermometers
- Participant wallet cards. A bracelet or necklace may be provided to the participant (in addition to a participant wallet card) to identify the participant as part of the study.
- Participant instructions on when to contact study site (ie, symptoms, hospitalization etc)
- Electronic Data Capture (eDC) Manual / eCRF completion guidelines

8.1. Efficacy Assessments

The primary objective of the study will be to demonstrate:

- the efficacy of ExPEC9V compared to placebo in the prevention of the first IED event with microbiological confirmation from blood or other sterile sites caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75.

The participants will be advised to monitor for any signs or symptoms of suspected IED, UTI, or ABP and will be asked to contact their physicians and/or study site as soon as possible if they experience any signs or symptoms of IED, UTI, or ABP or if they are admitted to the hospital for suspected IED, UTI or ABP. Each participant or his/her designated caregiver will be asked to contact the study site in case of hospital admission and inform the study site about the reason for hospitalization. As part of the IED and pyelonephritis catchment strategy, sites are required to perform active surveillance through medical health records or by any other means.

During study follow-up, participants are expected to inform the study site, as soon as possible, if they experience any signs or symptoms of UTI or ABP, or if they experience any new onset or worsening of symptoms that could be caused by a systemic infection. In addition, all participants will be questioned about past hospitalizations or medical events that could have been potential IEDs or pyelonephritis cases that were not captured by the site in real time. When implemented at a site level, for participants using study devices (provisioned or bring your own device), participant surveillance via eCOA will begin following the Day 30 visit and continue until study end or participant early withdrawal (registered as de-activation in the eCOA system). The eCOA surveillance instrument allows participants to confirm if they have sought urgent or non-routine medical care. This eCOA surveillance method does not apply to participants exclusively using

interview mode. In addition to eCOA surveillance, throughout the study sites must communicate directly with all participants via telephone contact or other means of communication every 3 months $\pm$ 2 weeks after vaccination. During these direct contacts, sites should monitor participant eCOA surveillance adherence and review medical events that have occurred during the past interval. Events defined as IED (including invasive infection caused by *P. aeruginosa*) or pyelonephritis will be collected for all participants for the entire study duration (1,461 days; 4 years). For IED cases, MRU data over the last 3 months will be collected at IED-Day 1. In addition, MRU data will be collected on IED-Day 29, IED-Day 91, and IED-Day 366. As of Protocol Amendment 8, MRU data will no longer be collected for UTI and ABP events, including pyelonephritis.

For any participant who visits his/her healthcare provider and/or the study site with signs and symptoms of a suspected IED, UTI, or ABP the investigator/trained healthcare professional will assess whether the criteria shown in Section 8.1.1 (identification of a suspected clinical case of IED), Section 8.1.2 (clinical case definition for IED), Section 8.1.3 (clinical case definition for pyelonephritis), Section 8.1.4 (clinical case definition for UTI) and Section 8.1.5 (clinical case definition for ABP) are met. After the initial clinical evaluation, blood samples (if feasible) (or samples from other normally sterile body sites, if applicable) and urine samples will be collected for laboratory and microbiology testing (including culture) to confirm the presence of IED. Urine samples will be collected for laboratory and microbiology testing (including culture) to confirm the presence of pyelonephritis. Blood and urine samples will be collected prior to the administration of antibiotic treatment, if possible. If collection of the immunogenicity samples prior to administration of antibiotic treatment is not possible, the samples should be collected as soon as possible and within approximately 48 hours after a systemic infection is suspected by the investigator.

Information regarding each case of suspected systemic infection or pyelonephritis will be recorded in the eCRF. As of Protocol Amendment 8, uncomplicated and complicated UTI and ABP events will no longer be collected in the eCRF unless these events are suspected to be pyelonephritis. For events initially suspected to be an IED or pyelonephritis, even if not subsequently confirmed clinically by the site, at a minimum the following information will be recorded in the eCRF: signs and symptoms, collection of immunogenicity sample (if feasible), collection of relevant laboratory samples, and cultures and final diagnosis (including non-infectious conditions, if applicable). The events will be reported if they meet relevant criteria for AE reporting according to the protocol (Section 8.3 and Section 8.3.6).

The severity and outcome of IED cases will be assessed by recording whether the participant experienced sepsis or septic shock and by recording any adverse sequelae of IED (eg, residual organ impairment or accelerated progression of pre-existing chronic conditions).

Sepsis is defined as life-threatening organ dysfunction due to dysregulated host response to infection. A case of IED will be defined as meeting criteria for sepsis if there is a documented acute change in total Sequential Organ Failure Assessment (SOFA) score (Section 10.9 Appendix 9) of ≥ 2 points from baseline and deemed secondary to the IED.⁶⁰

Septic shock occurs in a subset of patients with sepsis and comprises an underlying circulatory and cellular/metabolic abnormality that is associated with increased mortality. A case of IED will be defined as leading to septic shock if the following criteria are documented:

- persisting hypotension requiring vasopressors to maintain a mean arterial pressure of 65 mm Hg or higher, and
- a serum lactate level greater than 2 mmol/L (18 mg/dL) despite adequate volume resuscitation.⁶⁰

The severity of IED cases, to confirm if they meet criteria for sepsis or septic shock, will be verified and confirmed by the IEAC.

8.1.1. Identification of Suspected IED Clinical Cases

The process for identification of a suspected IED case will immediately be started when a study participant acutely presents with new onset, or worsening, of the general signs and symptoms suggestive of a potential systemic infection: eg, malaise, asthenia, myalgia, fever, chills or shivering, nausea or vomiting, dyspnea, dizziness, or lightheadedness. A similar process is applicable to the participants who are already hospitalized due to an underlying condition or who recently underwent a surgical procedure.

The site will collect relevant source clinical data to ascertain a suspicion of an IED. The following clinical data should be assessed if there is suspicion of a systemic infection^{33,38,57,60}:

- presence of positive systemic inflammatory response syndrome (SIRS)⁵⁹ (Section 10.8 Appendix 8) or quick sequential organ failure assessment (qSOFA) score^{40,42,60} (Section 10.10 Appendix 10), or with an acute change in total SOFA score (Section 10.9 Appendix 9) ≥ 2 points, or
- reporting fever $>38.0^{\circ}\text{C}$ (100.4°F) or hypothermia: $<36.0^{\circ}\text{C}$ (96.8°F) (Note: intentionally different from fever assessments for reactogenicity as per Section 10.6, Appendix 6: Toxicity Tables), or
- at least 2 of the following clinical criteria:
 - tachycardia (heart rate [HR]): >90 beats/min,
 - tachypnea (respiratory rate [RR]): >20 breaths/min or partial pressure of arterial carbon dioxide (PaCO_2) <32 mm Hg,
 - altered mentation (Glasgow Coma Scale score) <15 ,
 - systolic blood pressure: ≤ 100 mm Hg,
 - laboratory values indicating an important bacterial infection and/or sepsis, including, but not limited to, white blood cell (WBC) count or immature bands (eg, platelets, prothrombin time, activated partial thromboplastin time, bilirubin, creatinine), or
 - signs and/or symptoms of UTI (eg, dysuria, flank pain, suprapubic pain, urgency, frequency, hematuria, pyuria).

If the treating physician is not part of the study-site personnel, information regarding any suspected

IED case will be collected retrospectively and assessed accordingly by the investigator. If the study site learns after the fact (eg, only at the regular contact with the participant) about a suspected systemic infection event, every effort should be made to collect relevant clinical information and samples, as required for endpoint assessment and as feasible.

- Information regarding each case of suspected systemic infection will be recorded in the eCRF. If IED or another systemic infection is not suspected after initial evaluation, at a minimum the following information will be recorded in the eCRF: signs and symptoms, collection of immunogenicity sample (if feasible), collection of relevant laboratory samples and cultures and final diagnosis (including non-infectious conditions, if applicable).

IED-Day 1, case evaluation:

If a systemic infection cannot be excluded, the data listed below will be collected (as available) at the hospital/study site (IED-Day 1) for an assessment of IED diagnosis by the investigator according to the clinical case definition. The following clinical and laboratory evaluations, which are relevant to the presence of a positive SIRS or an acute change in total SOFA score, are expected to be performed per standard of care and upon medical judgement of the treating physician:

- clinical data:
 - general signs and symptoms (reported by the patient),
 - body temperature,
 - heart rate,
 - respiratory rate,
 - Arterial blood gas: PaCO₂ and partial pressure of arterial oxygen (PaO₂) (if applicable per standard of care),
 - PaO₂/fraction of inspired oxygen (FiO₂) ratio (if applicable per standard of care),
 - Glasgow Coma Scale score (or altered mentation state),
 - blood pressure (and if administration of vasopressors is necessary),
 - record signs and/or symptoms of UTI (eg, dysuria, flank pain, suprapubic pain, urgency, frequency, hematuria, pyuria),
 - urine output (if applicable per standard of care).
- laboratory data:
 - hematology (WBC count with differential [absolute and %], hemoglobin, hematocrit, and platelets),
 - serum chemistry (glucose, creatinine, bilirubin, alanine aminotransferase, aspartate aminotransferase, procalcitonin, C-reactive protein, and lactate^t),
 - urinalysis (at minimum WBC count).

^t Recommended in case of severe course of systemic infection or any suspicion of a septic shock.

If above mentioned laboratory procedures are not collected per standard of care, these procedures are not mandatory. Additional laboratory results may be collected if the condition considerably worsened after the first contact, upon medical judgement by the treating physician.

The following procedures should be followed, as feasible, for all suspected systemic infections (as long as a systemic infection cannot be excluded). These procedures must be conducted as study procedures if not part of the standard of care:

- microbiology data: two or more sets of blood cultures are to be collected (if feasible), as well as cultures from any other sterile site and urine (as applicable per clinical case), if possible before initiating treatment including antibiotic(s). Additional blood cultures, as well as cultures from any other sterile site and urine, may need to be collected (as per standard of care) before initiation of any new antibiotic. Please refer to Section 10.13 for guidance on obtaining blood cultures. The *E. coli* or *P. aeruginosa* isolates (if retained and available) will be sent to the central laboratory when originating from cultures positive for *E. coli* or *P. aeruginosa*. This procedure is applicable independent of confirmation of IED. The central laboratory will conduct further characterization including reconfirmation of bacterial identification (*E. coli* and *P. aeruginosa*) and antibacterial susceptibility testing (*E. coli* only) as detailed in Section 8.5.
- If feasible, an immunogenicity blood sample should be taken for all suspected systemic infection cases, if possible before any treatment or antibiotic(s) is given. If sample collection prior to treatment initiation is not possible, the samples should be collected as soon as possible and within approximately 48 hours after a systemic infection is suspected by the investigator. The blood samples will be sent to the central laboratory for participants meeting the IED clinical case definition criteria (see Section 8.1.2), including participants who test positive for *P. aeruginosa*. Immunogenicity assessments will be performed as detailed in Section 8.4.

In addition, the following study-specific procedures should also be followed for all suspected systemic infections (as long as a systemic infection cannot be excluded):

- collection of information regarding medical conditions of interest and treatment received within the past 90 days,
- assessment of source of the infection and type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) (see Section 10.1 for definitions),
- assessment of HRQoL, and
- MRU data over the last 3 months.

Following collection of the data, the IED case definition criteria should be applied (see Section 8.1.2) to decide on the further study-specific procedures (see Section 1.3.2) (Figure 4). Follow-up of the suspected IED case should also be performed for cases for which confirmation of the IED diagnosis is still pending but for which the collected cultures are positive for *E. coli* or *P. aeruginosa*. The Medical Monitor should be contacted for further guidance.

For participants meeting the IED clinical case definition criteria (see Section 8.1.2), including participants who test positive for *P. aeruginosa*, and for participants with suspected IED with microbiological evidence, the following data will be collected and recorded in the eCRF if the procedures are performed per standard of care (see Section 1.3.2):

- additional laboratory results (including cultures) upon medical judgement by the treating physician; in case of changes in clinical severity of the event over time, the relevant worst clinical data recorded after IED-Day 1 (in particular data corresponding to SIRS and SOFA scores) should be recorded daily in the eCRF as unscheduled evaluations,
- any relevant results of diagnostic procedures (eg, computed tomography [CT] scan, or magnetic resonance imaging [MRI], and or/ultrasonography),
- treatment for IED (including antibiotics and/or any supportive therapy). Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures.

IED-Day 29, follow-up:

On Day 29 post-IED identification or on the day of resolution of IED signs and symptoms (whichever comes first), the following additional data should be collected for confirmed IED cases (per clinical case definition criteria or when IED cannot be excluded) as well as for cases of invasive disease due to *P. aeruginosa*:

- laboratory data (optional; it is recommended to repeat relevant procedures performed for IED diagnosis – this includes data already collected to assess recovery/discharge before actual Day 29):
 - hematology (WBC count with differential [absolute and %], hemoglobin, hematocrit, and platelets),
 - serum chemistry (glucose, creatinine, bilirubin, alanine aminotransferase, aspartate aminotransferase, procalcitonin, C-reactive protein, and lactate), and
 - urinalysis (at minimum WBC count),
- collection of relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care,
- relevant treatment for IED (including antibiotics and/or any supportive therapy),
- assessment of HRQoL,
- MRU data (MRU data between IED-Day 1 and IED-Day 29 to be collected)
- recovery status (outcome and complications of IED) and discharge from hospital.

IED-Day 91, Post-IED Follow-up:

On Day 91 post-IED identification or at the end of study (whichever comes first), the following data should be collected for confirmed IED cases (per clinical case definition criteria or when IED cannot be excluded) as well as for cases of invasive disease due to *P. aeruginosa*:

- assessment of HRQoL,
- MRU data (MRU data between IED-Day 29 and IED-Day 91 to be collected), and
- recovery status (outcome of IED).

The Day 91 visit may be conducted remotely (eg, via telephone call).

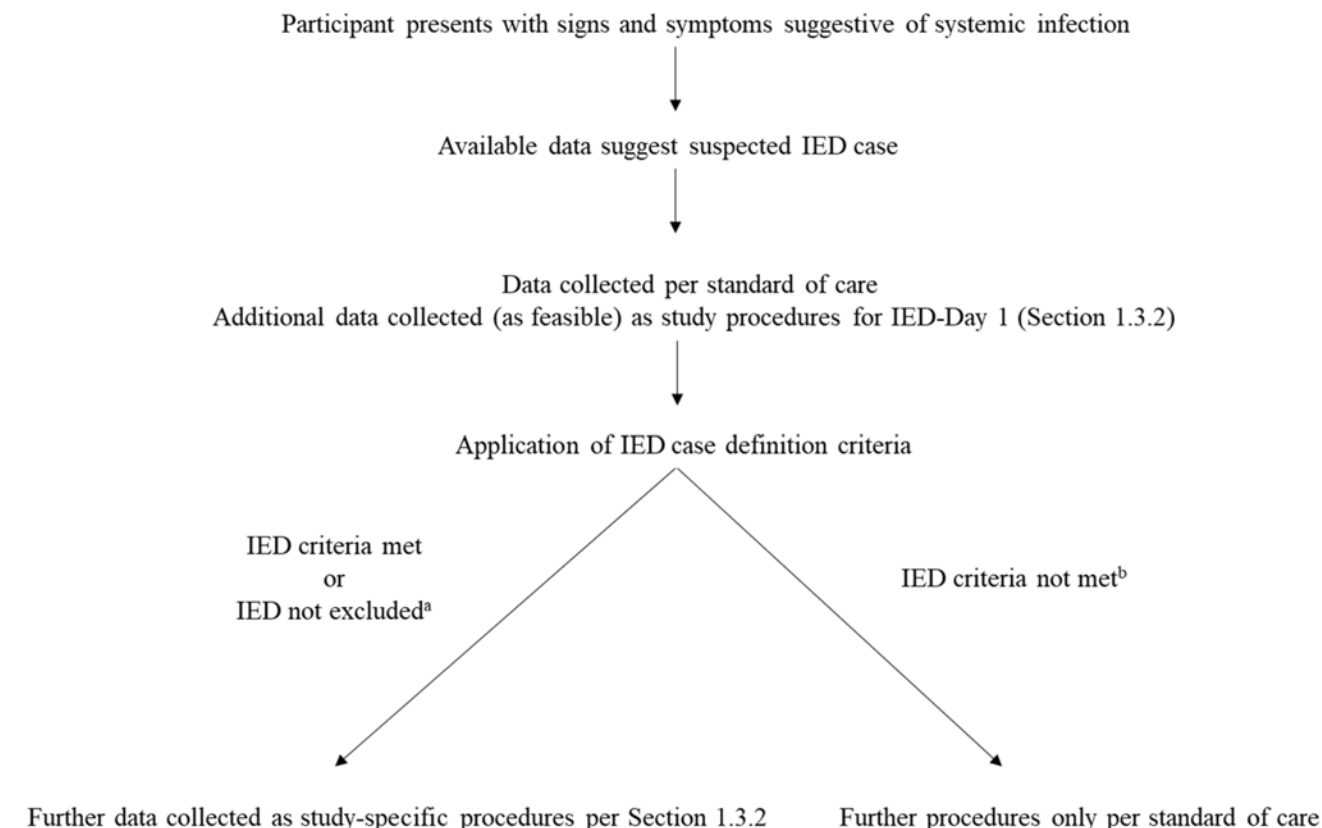
IED-Day 366, Post-IED Follow-up:

On Day 366 post-IED identification or at the end of study (whichever comes first), the following data should be collected for confirmed IED cases (per clinical case definition criteria or when IED cannot be excluded) as well as for cases of invasive disease due to *P. aeruginosa*:

- an immunogenicity blood sample should be taken. Immunogenicity assessments will be performed as detailed in Section 8.4.
- assessment of HRQoL,
- MRU data related to IED complications (MRU data between IED Day 91 and IED-Day 366 to be collected), and
- recovery status (outcome of IED).

Potential outcomes of IED include:

- recovery: resolution of IED signs and symptoms, and conclusion of antimicrobial therapy,
- IED ongoing,
- complications: pneumonia, heart dysfunction, lung dysfunction, brain dysfunction, kidney dysfunction, hepatic dysfunction, hypotension, hypoperfusion, disseminated intravascular coagulation, and neurological sequelae,
- Death.

Figure 4: Decision Tree for IED Clinical Cases

IED=invasive *Escherichia coli* disease.

^a Including cases of suspected IED for which confirmation of IED is pending but for which the collected cultures are positive for *E. coli* or *P. aeruginosa*.

^b In case of any doubts please consult the sponsor or its designee.

8.1.2. IED Clinical Case Definition

A participant will be considered to have IED if the below criteria are met and the diagnosis is confirmed by the IEAC. Data packages for evaluation by the IEAC will be sent for all cases which are considered as suspected IED (both those confirmed by the investigator as meeting the clinical case definition as well as those where IED cannot be excluded) and which include confirmation of presence of *E. coli* in relevant cultures (including cases of mixed pathogens). The cases of invasive disease due to *P. aeruginosa* will not be sent to the IEAC for confirmation of the diagnosis recorded by the investigator. The package will consist of all available clinical data collected by Day 29 post-IED identification or by the day of resolution of IED signs and symptoms (whichever comes first). During IEAC review, the IEAC reviewers need to decide if the collected data are pertinent to be included in the case definition evaluation for the same IED event.

An adult participant diagnosed with IED is any participant with ^{33,38,57,60}:

- signs and symptoms of systemic bacterial infection as indicated by the presence of any of the following:
 - temperature <36.0°C (96.8°F) or >38.0°C (100.4°F) (Note: intentionally different from fever assessments for reactogenicity as per Section 10.6, Appendix 6: Toxicity Tables),

- tachycardia (HR) >90 bpm,
 - tachypnea (RR) >20 breaths/minute or PaCO₂ <32 mm Hg,
 - WBC count <4 or >12 x 10⁹/L or ≥10% immature (band) forms,
- OR
- an acute change in total SOFA^{40,42,60} score of ≥2 points from baseline^u

AND

- microbiological confirmation by culture of:
 - *E. coli* in blood or in any other sterile site^v (eg, cerebrospinal fluid, pleura),
- AND/OR
- *E. coli* in urine (≥10⁵ colony-forming units [CFU]/mL) with UTI signs/symptoms^w and with no other identifiable site of infection and with an acute change in total SOFA score^{40,42,60} of ≥2 points from baseline^u.

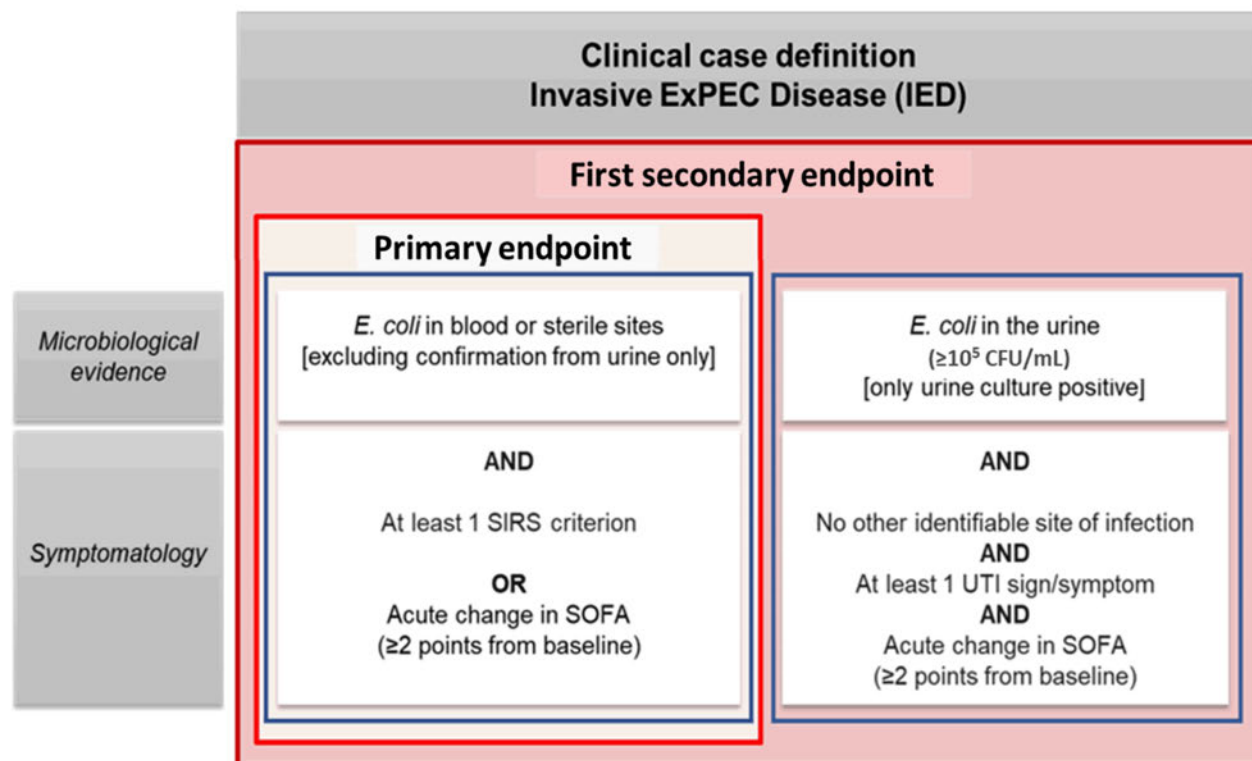
^u When a participant is assessed for the first time (eg, at the moment of emergency room evaluation or hospital admission), the baseline will be considered as 0, unless the medical history indicates otherwise. In case multiple SOFA scores are available before the onset of systemic infection, the acute change will be counted from the baseline score defined as the lowest score over the last 72 hours preceding the diagnosis of systemic infection, ie, the time when the first positive culture was obtained that is attributable to the same IED event.

^v Refer to Section 10.15, Appendix 15 for the sterile sites' definition.

^w At least one of the UTI signs/symptoms (Section 8.1.4) over the last 72 hours preceding the diagnosis of systemic infection.

A schematic diagram of the IED clinical case definition is depicted in [Figure 5](#).

Figure 5: Schematic Diagram of IED Clinical Cases



CFU=colony-forming units; *E. coli*=*Escherichia coli*; IED=invasive *Escherichia coli* disease; SIRS=systemic inflammatory response syndrome; SOFA=sequential organ failure assessment; UTI=urinary tract infection.

For the primary endpoint, only IED events with microbiological confirmation from blood or other sterile sites will be taken into account, excluding IED cases with microbiological confirmation from urine only.

Secondary endpoints related to IEDs (unless otherwise indicated per the Objectives and Endpoints table in Section 3) will include events with *E. coli* microbiological confirmation from blood, other sterile sites, or urine.

For the primary and secondary endpoints, with IED caused by any of the ExPEC9V O-serotypes, the O-serotyping has to be available from the central lab and the IED has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the secondary endpoints related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient. All cases with multiple pathogens in the culture results from blood or any other sterile site (see Section 10.15, Appendix 15: Sterile sites' definition) will be included in the efficacy analysis for the primary and secondary endpoints. For the secondary endpoints, including culture-confirmed IED and pyelonephritis cases, cases with multiple pathogens in the urine culture results will be evaluated by the IEAC and included only if *E. coli* is determined to be the primary causative pathogen. UTI and ABP cases with urine culture results with mixed pathogens (including *E. coli*) will be excluded from efficacy analyses but included in sensitivity analyses.

As an exploratory endpoint, participants meeting the above definition of IED (except the microbiological confirmation of *E. coli*) but with samples positive for *P. aeruginosa* will be reported to have invasive disease due to *P. aeruginosa*. No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens in the culture results, including *P. aeruginosa*, will not be considered for this exploratory endpoint.

8.1.3. Pyelonephritis Clinical Case Definition and Procedures

If a participant has contacted their healthcare provider and/or study site with signs and symptoms of pyelonephritis, the participant will be defined as having a confirmed pyelonephritis endpoint if the criteria outlined below are met^{20,67,68,75} and confirmed by the IEAC for cases of pyelonephritis. For cases of UTI see Section 8.1.4; for cases of ABP see Section 8.1.5.

Data packages for evaluation by the IEAC will be sent for all cases of *E. coli* confirmed pyelonephritis. Data will include all available clinical data collected by Day 29 post-pyelonephritis diagnosis or by the day of resolution of UTI signs and symptoms (whichever comes first). For pyelonephritis, the IEAC will review all the clinically diagnosed cases of pyelonephritis with microbiological confirmation of *E. coli* (regardless of meeting protocol criteria) as well as the cases that would be identified based on the protocol case definition. Only pyelonephritis cases confirmed by the IEAC will be used in the efficacy analysis.

An event is considered a confirmed pyelonephritis endpoint if an adult with documented pyuria (WBC count ≥ 10 cells/mm³ in non-centrifuged urine or ≥ 5 WBC per high-power field [WBC/HPF] [400x enlargement] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with at least 2 of the following signs and symptoms:

- fever (temperature $\geq 38.0^\circ\text{C}$ [as measured by the participant or health care professional]), or chills, or rigors, or warmth (warmth only if associated with fever), or
- flank pain, or
- tenderness in the costovertebral angle on physical examination, or
- nausea or vomiting.

If case identification criteria for IED are also met (see Section 8.1.1), data will be collected as presented in Section 1.3.2 and Section 8.1.2.

If the treating physician is not part of the study-site personnel, information regarding any pyelonephritis case will be collected retrospectively and assessed accordingly by the investigator. If the study site learns after the fact (eg, only at the regular contact with the participant) about a pyelonephritis event, every effort should be made to collect relevant clinical information and samples, as required for endpoint assessment and as feasible.

For the secondary endpoints with pyelonephritis caused by any of the ExPEC9V O-serotypes, the O-serotyping has to be available from the central lab and the pyelonephritis has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the secondary endpoints related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient. Cases with multiple pathogens in the urine culture results and with the

presence of *E. coli* will not be considered for the secondary endpoints. These cases will be included in sensitivity analyses.

The following procedures should be performed for cases with a clinical diagnosis of pyelonephritis (as per treating physician) as well as suspected pyelonephritis cases for which antibacterial treatment has been initiated:

UTI-Day 1, pyelonephritis case evaluation:

- clinical data collection of UTI signs and symptoms,
- laboratory data including dipstick urinalysis, urine microscopy, or urine flow cytometry in a mid-stream void urine specimen. Optionally: hematology test (WBC count and differential), CT scan, or MRI, and/or ultrasonography,

The following procedures should be followed for all pyelonephritis cases, these procedures must be conducted as study procedures if not part of the standard of care:

- urine microscopy or urine flow cytometry in a mid-stream void urine specimen (at minimum WBC count should be available). Note that this is a mandatory study procedure on Day 1. The quantitative results (number of cells per unit of volume or per high power field) of urinalysis (by flow cytometry or by microscopy) on Day 1 are required to verify the protocol case definition of pyelonephritis. Abnormal leukoesterase on dipstick results alone will not be sufficient evidence of pyuria to meet the pyelonephritis case definition. As new urinalysis assays may develop, these may be evaluated and accepted by the sponsor only if the provided results are quantified as number of white blood cells per unit of volume.
- microbiology data: urine cultures (from mid-stream urine) will be taken, if possible before initiating treatment including antibiotic(s). Patients with an indwelling catheter should have urine samples collected following the placement of a new catheter, or through aseptic techniques via a properly disinfected collection port if the indwelling catheter cannot be removed. Standard clinical practice should be followed for collection of cultures. In case of a more severe course of the disease, obtaining blood cultures should be considered, if possible before initiating treatment including antibiotic(s). Please refer to Section 10.13 for guidance on obtaining blood cultures. The *E. coli* or *P. aeruginosa* isolates (if retained and available) will be sent to the central laboratory when originating from cultures positive for *E. coli* or *P. aeruginosa* (for urine cultures only if $\geq 10^5$ CFU/mL). This procedure is applicable independent of confirmation of pyelonephritis. The central laboratory will conduct further characterization including reconfirmation of bacterial identification (*E. coli* and *P. aeruginosa*) and antibacterial susceptibility testing (*E. coli* only) as detailed in Section 8.5.

In addition, the following study-specific procedures should be followed for all clinically diagnosed pyelonephritis cases (regardless of microbiological confirmation) as well as suspected pyelonephritis cases for which antibacterial treatment has been initiated:

- If feasible, an immunogenicity blood sample (only for participants in the Immunogenicity Subset) should be taken at the time of pyelonephritis diagnosis, if possible before any treatment is given. If sample collection prior to treatment initiation is not possible, the samples should be collected as soon as possible and within approximately 48 hours after

pyelonephritis diagnosis. Immunogenicity assessments will be performed as detailed in Section 8.4.

- collection of information regarding medical conditions of interest and treatment received within the past 90 days,
- assessment of type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) (see Section 10.1 for definitions),
- collection of information on treatment for pyelonephritis (including antibiotics and/or any supportive therapy; special diligence should be applied to record the time of antibiotic administration relative to collection of cultures),
- assessment of HRQoL, and

For all clinically diagnosed pyelonephritis cases as well as suspected pyelonephritis cases for which antibacterial treatment has been initiated, at a minimum the following data must be collected in the eCRF:

- signs and symptoms of UTI,
- data on cultures taken and causative pathogen,
- treatment for pyelonephritis (including antibiotics and/or any supportive therapy; special diligence should be applied to record the time of antibiotic administration relative to collection of cultures),
- data on the immunogenicity sample (Immunogenicity Subset only),
- final diagnosis

For pyelonephritis cases without microbiological confirmation of *E. coli* or *P. aeruginosa* no study-specific follow-up is foreseen.

UTI-Day 29, pyelonephritis follow-up:

On Day 29 post-pyelonephritis diagnosis or on the day of resolution of pyelonephritis signs and symptoms (whichever comes first), the following relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care will be collected and recorded in the eCRF for all clinically diagnosed pyelonephritis cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa* ($\geq 10^5$ CFU/mL):

- laboratory data: dipstick urinalysis, urine microscopy or urine flow cytometry in a mid-stream void specimen urine. Additional procedures may be applicable: hematology test (WBC count and differential), CT scan, or MRI, and/or ultrasonography,
- microbiology data: additional urine cultures (from mid-stream urine) are recommended to be taken if signs and symptoms of UTI persist.
- collection of additional data on treatment for pyelonephritis,
- assessment of HRQoL,
- collection of recovery status (outcome of pyelonephritis).

If the participant has recovered and laboratory testing is not required, the Day 29 visit may be conducted remotely (eg, via telephone call).

Potential outcomes of pyelonephritis:

- recovery: resolution of UTI signs and symptoms and conclusion of antimicrobial therapy,
- pyelonephritis ongoing,
- complications (eg, IED – UTI identified as source of infection). In case UTI is complicated by IED, the follow-up data are to be collected as for participants meeting the IED clinical case definition criteria (see Section 1.3.3),
- Death.

The next event of pyelonephritis may be described as:

- relapse: recurrent infection with an organism similar to the pretherapy isolate, usually following persistence of the organism in the genitourinary tract.
- reinfection: recurrent infection with a new organism.

8.1.4. UTI Clinical Case Definition

As of Protocol Amendment 8, site study-specific procedures will not be performed for uncomplicated or complicated UTI events. Participants will still be required to inform sites about events that could be potential UTI cases. Site study-specific procedures are still required for suspected pyelonephritis and IED events. Refer to Section 10.18, Appendix 18 for site study-specific procedures for suspected UTI events prior to Protocol Amendment 8.

An event is considered a confirmed UTI endpoint if an adult with documented pyuria (WBC count ≥ 10 cells/mm³ in non-centrifuged urine or ≥ 5 WBC per high-power field [WBC/HPF] [400x enlargement] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with one of the following definitions for signs and symptoms:

- for uncomplicated UTI, at least 2 of the following signs or symptoms:
 - dysuria,
 - urinary frequency,
 - urinary urgency, or
 - suprapubic pain.
- for complicated UTI (cUTI):
 - at least 2 of the following signs or symptoms:
 - fever (temperature $\geq 38.0^\circ\text{C}$ [as measured by the participant or health care professional]), or chills, or rigors, or warmth (warmth only if associated with fever),
 - dysuria, urinary frequency or urinary urgency,
 - lower abdominal pain or pelvic pain, or
 - nausea or vomiting,

AND,

- at least 1 of the following complicating factors:
 - history of urinary retention (in male participants),
 - current indwelling urinary catheter,
 - obstructive uropathy, or
 - any relevant functional or anatomical abnormality.
- for recurrent UTI (rUTI):
 - recurrence of symptomatic UTIs with a frequency of 2 UTIs in 6 months, or
 - recurrence of symptomatic UTIs with a frequency of at least 3 UTIs in 1 year.
- for recurrent uncomplicated UTI:
 - recurrence of uncomplicated UTIs with a frequency of 2 UTIs in 6 months, or
 - recurrence of uncomplicated UTIs with a frequency of at least 3 UTIs in 1 year.

For the secondary endpoints, with UTI caused by any of the ExPEC9V O-serotypes, the O-serotyping has to be available from the central lab and the UTI has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the secondary endpoints related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient. Cases with multiple pathogens in the urine culture results and with the presence of *E. coli* will not be considered for the secondary endpoints. These cases will be included in sensitivity analyses.

As an exploratory endpoint, participants meeting the above definition of UTI but with urine samples positive for *P. aeruginosa* ($\geq 10^5$ CFU/mL) will be reported to have *P. aeruginosa* UTI. No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens with the presence of *P. aeruginosa* will not be considered for this exploratory endpoint.

8.1.5. ABP Clinical Case Definition

As of Protocol Amendment 8, site study-specific procedures will not be performed for ABP events. Participants will still be required to inform sites about events that could be potential ABP cases. Site study-specific procedures are still required for suspected pyelonephritis and IED events. Refer to Section 10.18, Appendix 18 for site study-specific procedures for suspected ABP events prior to Protocol Amendment 8.

An event is considered a confirmed ABP endpoint if an adult male with documented pyuria (WBC count ≥ 10 cells/mm³ in non-centrifuged urine or ≥ 5 WBC/HPF [400x enlargement] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with fever (temperature $\geq 38.0^\circ\text{C}$ [as measured by the participant or health care professional]) and an acute onset of the following symptoms^{13,58,74}:

- irritative voiding symptoms:
 - dysuria,

- urinary frequency,
- urinary urgency,
- OR
- obstructive voiding symptoms:
 - hesitancy,
 - incomplete voiding,
 - straining to urinate,
 - weak stream,
- AND
- suprapubic, rectal, or perineal pain.

For the endpoint of ABP caused by any of the ExPEC9V O-serotypes, the O-serotyping has to be available from the central lab and the ABP event has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the exploratory endpoint related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient. Cases with multiple pathogens in the urine culture results and with the presence of *E. coli* will not be considered for the exploratory endpoint. These cases will be included in sensitivity analyses.

As an exploratory endpoint, participants meeting the above definition of ABP but with urine samples positive for *P. aeruginosa* ($\geq 10^5$ CFU/mL) will be reported to have *P. aeruginosa* ABP. No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens with the presence of *P. aeruginosa* will not be considered for this exploratory endpoint.

8.1.6. Patient-reported Outcomes

Electronic PRO instruments will be completed by all participants per the Schedule of Activities in Section 1.3.1. Health-related quality of life will also be assessed for participants with confirmed IED cases and participants meeting the clinical case definition for pyelonephritis per the Schedule of Activities in Section 1.3.2 and Section 1.3.3, respectively. Two ePRO instruments will be completed by participants in the study to measure HRQoL: the SF-36 version 2 (acute) and EQ-5D-5L. The SF-36 version 2 and EQ-5D-5L will be completed via the eCOA system at the timepoints indicated in Section 1.3, Schedule of Activities.

The SF-36 version 2 (acute) is a self-administered, 36-item questionnaire⁵⁴ with a recall period for all items of 1 week. The SF-36 version 2 includes 8 domains that measure physical functioning, role limitations due to physical health problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems and mental health. The 8 domains can be aggregated into 2 summary scales that reflect physical and mental health: a physical component summary and a mental component summary. Responses to all items are rated on a 3-, 5- or 6-point Likert scale. The SF-36 version 2 can generally be completed in 5 to 10 minutes.

The EQ-5D-5L descriptive system is a self-administered, standardized measure of health status in a wide range of health conditions and treatments.^{18,27,31} It provides a descriptive profile and a single

index value for health status that can be used in the clinical and economic evaluation of health care. The recall period for all items is ‘Today’. The EQ-5D-5L consists of the EuroQol 5-dimension (EQ-5D) descriptive system and the EQ visual analog scale (EQ-VAS). The EQ-5D descriptive system is comprised of 5 items across the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ-5D-5L uses a 5-point Likert response scale ranging from “No problems” to “Extreme problems”. The EQ-5D health states defined by the EQ-5D descriptive system can be converted into a single index value using country-specific value sets. The index value facilitates the calculation of quality-adjusted life years that are used to inform economic evaluations of health care interventions. The EQ-5D also includes an EQ-VAS that has endpoints labeled "best imaginable health state" and "worst imaginable health state" anchored at 100 and 0, respectively. Participants are asked to indicate how they rate their own health by indicating the point on the EQ-VAS which best represents their own health on that day. The EQ-5D-5L can generally be completed in 2 to 3 minutes.

Participants unable or unwilling to work with the eCOA system may opt for interview mode, available through the eCOA system. If participants opt for interview mode, it is strongly recommended that interview mode is used consistently throughout the study. Additionally, if the eCOA capture system is unavailable, agreement with the sponsor can be obtained for paper use by site staff via interview mode. Participant should continue to use the eCOA system once the system is available again.

If a participant is too ill to complete the ePROs, site staff or a caregiver can select the portion of the ePRO that allows for assisted completion and ask the participant the questions, read the response options and record their responses. Participant should continue to use the eCOA system when the participant has recovered.

The patient-reported outcome (PRO) instrument will be provided in the local language in accordance with local guidelines.

The PRO instrument will be available for regulators and for Independent Ethics Committees/Institutional Review Boards (IEC/IRB) submissions and will be provided separately in a companion manual with the instruments that will be submitted with the protocol.

The PRO and AE data will not be reconciled with each other. Best practices on the collection of ePRO data are provided in the ePRO guidelines.

8.1.7. Frailty Assessments

Frailty will be assessed at baseline based on the SPPB score (see Section 10.11, Appendix 11) and in addition, the Frailty Index will be calculated using participants self-reported information regarding health status (components of SF-36 version 2 and EQ-5D-5L), and baseline health conditions collected through medical conditions of interest.^{14,15,62} Frailty Index will also be assessed at Year 1, Year 2, Year 3, Year 4, and at the Early Exit visit, as well as at the time of IED case evaluation (Day 1), and at follow-up after IED (see Section 1.3 Schedule of Activities [SoA]).

The specific medical conditions of interest used for the calculation of the Frailty Index will include: diabetes mellitus, history of myocardial infarction, congestive heart failure, hypertension, history of cerebrovascular disease, chronic obstructive pulmonary disease, cancer, osteoarthritis or rheumatoid arthritis, gastric or duodenal ulcer, long-term disability or handicap, hearing problems, migraine, cataract, and glaucoma. Please refer to Section 10.16 for more details on the medical conditions of interest to be used for the Frailty Index.

A total Frailty Index, ranging from 0 to 43, will be calculated as the accumulation of the individual's deficits. Relevant medical conditions, as listed above, contribute a maximum score of 14 and the SF-36 survey and EQ-5D-5L contribute a maximum score of 25 and 4, respectively.¹⁴

In addition to the Frailty Index, the Charlson Comorbidity Index score will be calculated at the same time points as the Frailty Index. Calculations will be based on the data collected through the medical conditions of interest. Please refer to Section 10.16 for more details on the medical conditions of interest to be used for the Charlson Comorbidity Index score^{55,63}.

8.2. Safety Assessments

Details regarding the review of safety data by the IDMC are provided in Committees Structure in Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations.

AEs will be reported and followed by the investigator as specified in Section 8.3, Adverse Events, Serious Adverse Events, and Other Safety Reporting and Section 10.4, Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-Up, and Reporting.

Relevant pre-existing medical conditions until the time of vaccination should be recorded as medical history.

Any clinically relevant changes meeting the criteria for reporting described in Section 8.3 must be recorded on the Adverse Event section of the eCRF.

Any clinically significant abnormalities meeting the criteria for reporting described in Section 8.3 and persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable condition is reached.

The study will include the following evaluations of safety and reactogenicity according to the time points provided in Section 1.3, Schedule of Activities.

Safety Subset

Additional safety assessments will be carried out in a subset of approximately 4,000 participants (ie, approximately 2,000 participants in the ExPEC9V group and 2,000 participants in the placebo group) from selected study sites, referred to as the Safety Subset. Over enrollment may occur for the Safety Subset due to local requirements. Refer to Section 8.3 for further details.

8.2.1. Physical Examinations

A general physical examination including body weight and height will be carried out at screening

for all participants and it is at the investigator's discretion how detailed the examination (which body systems are to be covered) is deemed needed. At all other visits, a targeted (abbreviated and symptom-directed) examination will be performed for participants in the Safety Subset at the discretion of the investigator, based on clinically relevant issues, clinically relevant symptoms and medical history. The targeted physical examination may be repeated if deemed necessary by the investigator.

Physical examinations will be performed by the investigator or an appropriately trained and qualified delegate. Any clinically relevant abnormalities or changes in severity observed during the review of body systems should be documented in the eCRF as an AE (if pre-vaccination, then as medical history) based on the criteria described in Section 8.3.1.

8.2.2. Vital Signs

Vital signs are to be measured per the [Schedule of Activities \(SoA\)](#), ie, both before vaccination (and preferably before blood draws) and after the end of the 30-minute observation period (no later than 45 minutes after vaccination).

Temperature (oral route preferred), pulse/heart rate, respiratory rate, blood pressure will be assessed. Blood pressure and pulse/heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions (eg, television, cell phones).

Confirmatory vital signs measurement can be done if inconsistent with a prior measurement. If any clinically significant changes in vital signs are observed, they will be reported as an AE based on the criteria described in Section 8.3.1 and 8.3.2 and followed to resolution, or until clinically stable. If any clinically significant changes in vital signs are observed pre-vaccination, they will be reported as medical history.

Participants from the Safety Subset will utilize an eDiary to record body temperature measurements post-vaccination (see Section 8).

8.3. Adverse Events, Serious Adverse Events, and Other Safety Reporting

Timely, accurate, and complete reporting and analysis of safety information, including AEs, SAEs, and Product Quality Complaints (PQCs), from clinical studies are crucial for the protection of participants, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established Standard Operating Procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

Based on the criteria described in Section 8.3.1 and Section 8.3.2, AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally acceptable representative).

Further details on AEs, SAE, and PQCs can be found in Section 10.4, Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-Up, and Reporting.

8.3.1. Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All Adverse Events

For all participants, AEs and special reporting situations, whether serious or non-serious, that are related to study procedures or that are related to non-investigational sponsor products will be reported from the time a signed and dated ICF is obtained until the end of the study/early withdrawal.

Clinically relevant medical events not meeting the above criteria and occurring between signing of ICF and moment of first vaccination will be collected on the Medical Conditions of Interest eCRF page as pre-existing conditions.

For the Safety Subset, solicited AEs, collected through an eDiary, will be recorded from the time of vaccination until 14 days post-vaccination. All other unsolicited AEs and special reporting situations will be reported for all participants from the time of vaccination until 29 days post-vaccination.

For all participants, SAEs and AEs leading to discontinuation from the study (regardless of the causal relationship) are to be reported from the moment of first vaccination until completion of the participant's last study-related procedure, which may include contact for safety follow-up. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

All reported AEs will be followed until resolution or until clinically stable.

Serious Adverse Events

All SAEs will be collected for all participants from the administration of the study vaccine until the end of Day 181. Additionally, SAEs that are related to the study vaccine, study procedures or non-investigational sponsor products will be collected beyond Day 181 until the end of the study. These SAEs must be reported to the appropriate sponsor contact person by study-site personnel immediately, but no later than 24 hours of their knowledge of the event. Additional SAE reporting may be performed per local regulatory requirements.

Information regarding SAEs will be transmitted to the sponsor using the Serious Adverse Event Form and Safety Report Form of the eCRF, which must be completed and reviewed by a physician from the study site and transmitted to the sponsor immediately, but no later than within 24 hours of their knowledge of the event. The initial and follow-up reports of an SAE should be transmitted electronically or by facsimile (fax). Telephone reporting should be the exception and the reporter should be asked to complete the appropriate form(s) first.

Concomitant therapies associated with recorded SAEs will be collected and recorded in the eCRF.

Deaths will be recorded throughout the study and reported as SAEs according to the guidelines provided above.

8.3.2. Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

After vaccination, all participants will remain under observation at the study site for at least 30 minutes for presence of any acute reactions. For all participants, any SAEs and unsolicited AEs occurring in this observation period will be recorded in the eCRF. For participants in the Safety Subset, any solicited AEs will be documented in the eCRF by study-site personnel following the 30-minute observation period (no later than 45 minutes after vaccination).

Solicited local and systemic AEs, collected for participants in the Safety Subset only, are described below.

Solicited Adverse Events

Solicited AEs are used to assess the reactogenicity of the study vaccine and are predefined local (at injection site) and systemic events for which the participant is specifically questioned and which are noted by participants in their eDiary.

Participants in the Safety Subset will record solicited signs and symptoms in an eDiary for 14 days post-vaccination. Participants in the Safety Subset will be provided with an eDiary and instructions on how to complete the eDiary (see Overview in Section 8, Study Assessments and Procedures). Electronic diary information will be transferred from the eDiary source to the sponsor. After review and verbal discussion of the initial eDiary entries with the participant, the investigator will complete his/her own assessment in the relevant sections of the eCRF. Once a solicited sign or symptom from an eDiary is considered to be of severity Grade 1 or above, it will be recorded as a solicited AE on the Investigator Assessment of Reactogenicity Form and not on the Adverse Event Form.

For participants in the Safety Subset, concomitant therapies associated with solicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 14 days after vaccination (Day 15) or until clinical resolution or stabilization.

Solicited Injection Site (Local) Adverse Events

Participants in the Safety Subset will be asked to note in the eDiary occurrences of injection site pain/tenderness, erythema, and swelling at the study vaccine injection site daily for 14 days post-vaccination (day of vaccination and the subsequent 14 days). The extent (largest diameter) of any erythema and swelling should be measured (using the ruler supplied) and recorded daily. The case definitions for solicited injection site events can be found in the references.^{24,36}

Solicited Systemic Adverse Events

Participants in the Safety Subset will be instructed on how to record daily temperature using a thermometer provided for home use. Participants should record the temperature (oral route preferred) in the eDiary in the evening of the day of vaccination, and then daily for the next 14 days

approximately at the same time each day. If more than one measurement is made on any given day, the highest temperature of that day will be used in the eCRF.

Fever is defined as endogenous elevation of body temperature $\geq 38.0^{\circ}\text{C}$, as recorded in at least one measurement.⁴¹

Participants in the Safety Subset will also be instructed on how to note signs and symptoms in the eDiary on a daily basis for 14 days post-vaccination (day of vaccination and the subsequent 14 days), for the following events: fatigue, headache, nausea, myalgia.

If a solicited local or systemic AE has not resolved within 14 days after vaccination (Day 15), safety follow-up should be performed until the symptom resolves. The study-site personnel will be instructed to record the date of last symptoms and maximum severity until resolution in the source document and eCRF.

Unsolicited Adverse Events

Unsolicited AEs are all AEs for which the participant is not specifically questioned in the participant eDiary. Concomitant therapies associated with unsolicited AEs will be collected and recorded in the eCRF from the time of vaccine administration through 29 days after vaccination (Day 30) or until clinical resolution or stabilization.

8.3.3. Follow-up of Adverse Events and Serious Adverse Events

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and evaluations as medically indicated to elucidate the nature and causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

AEs and the special reporting situation of pregnancy will be followed by the investigator as specified in Section 10.4, Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

8.3.4. Regulatory Reporting Requirements for Serious Adverse Events

The sponsor assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The sponsor will also report to the investigator (and the head of the investigational institute where required) all suspected unexpected serious adverse reactions (SUSARs). The investigator (or sponsor where required) must report SUSARs to the appropriate IEC/IRB that approved the protocol unless otherwise required and documented by the IEC/IRB. A SUSAR will be reported to regulatory authorities unblinded. Participating investigators and IEC/IRB will receive a blinded SUSAR summary, unless otherwise specified.

8.3.5. Pregnancy

All initial reports of pregnancy in participants or partners of male participants must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (eg, spontaneous

abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and must be reported using the Serious Adverse Event Form.

Follow-up information regarding the outcome of the pregnancy for female participants who become pregnant, or where the pregnancy was the result of male participant and his partner will be retrieved, if possible.

8.3.6. Disease-related Events and Disease-related Outcomes not Qualifying as Adverse Events or Serious Adverse Events

All IED, pyelonephritis, and UTI/ABP events that meet the definition of an SAE will be reported as SAEs, regardless of whether they are protocol-specific assessments.

IED, pyelonephritis, and UTI/ABP events reported as SAEs during the course of the study, will be excluded from any SAE analyses in the clinical study report if they are subsequently found to be caused by *E. coli* or *P. aeruginosa* infection and confirmed to meet the clinical case definition for the primary or secondary efficacy endpoints. These events will be removed at the analysis level from the SAE listings and tables and presented separately in the clinical study report.

8.4. Immunogenicity Assessments

The “Immunogenicity Subset” refers to approximately 1,200 participants (approximately 600 ExPEC9V participants and 600 placebo participants) from selected study sites who have given informed consent prior to randomization for additional immunogenicity assessments. Immunogenicity assessments will also be performed for participants who experience IED (all participants) or pyelonephritis (participants in the Immunogenicity Subset only). As of Protocol Amendment 8, immunogenicity samples will no longer be collected for uncomplicated or complicated UTI or ABP events unless these events are suspected to be pyelonephritis.

For assessment of immunogenicity, serum IgG antibody levels elicited by the vaccine against each of the 9 vaccine O-serotypes and the carrier protein EPA will be measured by a validated multiplex ECL-based immunoassay, and vaccine O-serotype-specific functional serum antibodies will be measured by a validated MOPA. Blood samples will be collected from all participants on Day 1 (pre-vaccination) and Day 30 (29 days post-vaccination). Immunogenicity analyses on these samples will only be performed for participants in the Immunogenicity Subset. For immunogenicity determinations in participants who have a medical evaluation with signs or symptoms of IED (all participants) or pyelonephritis (Immunogenicity Subset only), additional blood samples should be taken before initiating treatment including antibiotic(s), if possible, and 1 year after IED identification or at the end of the study, whichever comes first. These blood samples will be analyzed to identify a correlate of protection for the vaccine-mediated immune response associated with VE. Additional blood samples will be collected on Days 181, 366, 731, 1,096, and 1,461 from participants in the Immunogenicity Subset.

Immunogenicity assessments will be performed at a central laboratory designated by the sponsor.

8.5. Microbiological Assessments

For suspected IED cases, blood cultures (or cultures from any other sterile site, if applicable) and, if feasible, urine cultures will be collected. For pyelonephritis cases, urine cultures will be collected. These cultures will be taken before initiating treatment including antibiotic(s) if possible. Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures. Standard clinical practice should be followed for collection of cultures.

Cultures from blood (or other normally sterile body sites) or urine will be used for isolate identification and antibiotic susceptibility tests by the local laboratory and according to local practice. The results will be used to guide treatment decisions. Culture will be processed at local laboratories according to the local standard procedures. Cultured *E. coli* and *P. aeruginosa* bacteria will be collected and stored, and representative bacterial colonies will be identified to the species level as per local laboratory procedures. If urine cultures are positive, quantification will be required for all identified bacteria (not only for *E. coli* and *P. aeruginosa*).

Isolates of *E. coli* or *P. aeruginosa* identified at the local microbiology laboratory (independent of the presence of mixed pathogens in the culture results) will be shipped to the central microbiology laboratory for further characterization as follows:

- For suspected pyelonephritis events, all isolates from urine quantified at $\geq 10^5$ CFU/mL.
- For suspected IED events, all isolates from urine (any quantification), blood and/or other sterile sites irrespective of bacterial quantification.

The central laboratory will assess the identification of *E. coli* or *P. aeruginosa* for each isolate, and all confirmed *E. coli* but not *P. aeruginosa* isolates will be subjected to antimicrobial susceptibility testing using broth microdilution by established European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical and Laboratory Standards Institute (CLSI) methodology. Interpretive criteria should follow either EUCAST or CLSI guidelines, with minimum inhibitory concentrations (MICs) being reported alongside interpretations of susceptible, intermediate, or resistant. The panel of antibacterial agents for susceptibility testing is presented in Section 10.14, Appendix 14: Antibacterial Agents for Susceptibility Testing.

E. coli isolates from IED, pyelonephritis, UTI, or ABP cases with a confirmed identification of *E. coli* in the central microbiology laboratory will be characterized by agglutination O-serotyping. The validated agglutination O-serotyping assay uses typing antisera specific to each of the nine O-serotypes for which the antigens are included in the candidate vaccine. For *E. coli* isolates from IED cases, agglutination will be performed twice and concordant O-serotype results will be reported as the specific ExPEC9V O-serotype or negative for all ExPEC9V typing antisera. In cases where the 2 initial agglutination results are discordant, agglutination will be performed a third time. If 2 out of 3 concordant results are not obtained, the isolate will be classified as non-typable. For *E. coli* isolates from pyelonephritis, UTI, or ABP cases, agglutination will be performed only once.

Whole genome sequencing may be performed to further characterize *E. coli* isolates. Genomic data will not be utilized for ExPEC9V O-serotype determination for primary and secondary efficacy analyses.

8.6. Medical Resource Utilization and Health Economics

MRU data associated with medical encounters related to IED will be collected in the eCRF by the investigators and study-site personnel for participants diagnosed with IED (all participants). As of Protocol Amendment 8, MRU data will no longer be collected for UTI or ABP events, including pyelonephritis. MRU data over the last 3 months will be collected on IED-Day 1. In addition, MRU data will be collected on IED/UTI/ABP-Day 29, IED-Day 91, and IED-Day 366. At these timepoints, the data collection window will be from the previous visit to the current visit (eg, at the IED-Day 29 visit/contact, MRU data between IED-Day 1 and IED-Day 29 will be collected). At IED-Day 366, only medical encounters related to IED complications will be collected. Protocol-mandated procedures, tests, and encounters are excluded. The data collected may be used to conduct economic analysis and will include the following:

- Number and duration of each hospitalization, including duration by wards (length of stay in the intensive care unit or other ward, total length of stay, etc).
- Outpatient and inpatient medical encounters and treatments, including physician/specialist or emergency room visits, tests and procedures, and medications.
- Number and duration of other medical care encounters, including surgeries (inpatient and outpatient procedures), and number and character of diagnostic and therapeutic tests and procedures.
- Indirect costs related to the value of time (patient time receiving care, family and informal caregiver) and lost productivity.
- Data collection for institutional care admission other than hospitals, including long-term facilities and rehabilitation facilities.

9. STATISTICAL CONSIDERATIONS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the efficacy, safety, and immunogenicity data is outlined below. Specific details will be provided in the Statistical Analysis Plan.

9.1. Statistical Hypotheses

For the primary objective, the following null and alternative hypotheses will be used:

H_0 : VE $\leq$ 20%

versus

H_a : VE >20%

For the key secondary objectives related to IED, the following null and alternative hypotheses will be used:

H_0 : VE $\leq$ 0%

versus

H_a : VE >0%

For the key secondary objectives related to pyelonephritis and UTI, the following null and alternative hypotheses will be used:

H_0 : VE $\leq$ 10%

versus

H_a : VE >10%

For further details, see Section 9.4.1. Efficacy Analyses.

9.2. Sample Size Determination

9.2.1. Efficacy

To meet the primary objective of the study, it is estimated that with 53 participants developing at least 1 IED event with microbiological confirmation in blood or other sterile sites caused by ExPEC9V serotypes, this objective has statistical power of approximately 90% to demonstrate pivotal efficacy for a vaccine with 70% VE at a 0.025 (1-sided) level of significance, considering a futility interim analysis after 21 events, an efficacy/futility interim analysis after 37 events and a final analysis after 53 events will be performed (note that the immunogenicity interim analysis will only include an immunogenicity review and will not impact the power of the study).

The study is designed to reject the null hypothesis that the true VE is $\leq$ 20% for the primary objective. Assuming a 10% yearly dropout rate and assuming a yearly vaccine serotype-specific incidence rate of 0.17% among participants who received placebo (see Section 2.1), and assuming that on average a participant will be followed up for 3.5 years, a total of approximately 19,800 participants (1:1 ExPEC9V:Placebo) will be needed for this study. See Section 10.12, Appendix 12: Statistical Methodology for Sample Size Calculations, for more details.

The estimate of 70% VE for ExPEC9V in the prevention of IED is based on a prior efficacy study of the pneumococcal conjugate vaccine, PCV13. In the CAPITA study assessing a single dose of PCV13 administered to immunocompetent community-dwelling adults aged $\geq$ 65 years who were naive to pneumococcal vaccines, VE of 75% was reported for the prevention of vaccine-type

invasive pneumococcal disease.⁶ Immunogenicity results from Phase 1 and Phase 2 clinical studies with ExPEC4V have demonstrated a robust, functional (opsonophagocytic killing) and durable antibody mediated vaccine response. Exemplified by the completed Phase 2 Estella study (63871860BAC2001), geometric mean titers (GMT) of total IgG antibody at Day 30 were between 5.5- and 22-fold higher than baseline, depending on the dose and serotype.¹² After 1 year, GMT values remained 2.6- and 7.6-fold above baseline, demonstrating durability of the ExPEC4V vaccine. These results are similar to those observed with PCV13, where Day 30 IgG geometric mean concentration (GMC) fold increases from baseline ranged from 3- to 13-fold, and 1 year GMC values were 1.6- to 6-fold above baseline.⁷²

9.2.2. Immunogenicity Subset

In this study, a total of approximately 1,200 participants will be evaluated for their immune response to ExPEC9V or placebo. In total, the subset sample size of 600 participants for the ExPEC9V vaccine and 600 participants for placebo will provide precision ranging from 0.032 to 0.044 (log₁₀ scale; 7.6%-10.7% on the original scale) in the 95% confidence intervals (CIs) for the Day 30 antibody geometric mean titers for the 9 serotypes determined by the multiplex ECL-based immunoassay, assuming a standard deviation ranging from 0.4 to 0.55. Using the MOPA, the precision range is 0.048 to 0.064 (log₁₀ scale; 11.7%-15.9% on the original scale), assuming a standard deviation ranging from 0.6 to 0.8. These estimations have been based on immunogenicity data from the ExPEC10V VAC52416BAC1001 study.¹²

9.2.3. Safety Subset

While mild-to-moderate vaccine reactions (administration injection-site, systemic responses) are expected, AEs that preclude further vaccine administration in other participants are not anticipated.

For unsolicited AEs and SAEs, which are captured in all participants, the observation of 0 reactions in the database would be associated with 95% confidence that the true rate is less than 0.03% based on 9,900 active study vaccine participants.

Table 2 shows the probabilities of observing at least 1 AE in 1 of the study vaccination groups at given true AE rates in the Safety Subset and in all participants.

Table 2: Probability of Observing at Least 1 Adverse Event at a Given True Adverse Event Rate

True Adverse Event Rate	Probability of Observing at Least 1 Adverse Event in N Participants	
	N=2,000	N=9,900
0.01%	18%	63%
0.05%	63%	99%
0.1%	86%	>99.9%
0.5%	>99.9%	>99.9%

N=number of participants.

Table 3 shows the 95% 2-sided CI and corresponding width for several observed incidences when using a Safety Subset of 2,000 active study vaccine participants. With a subset of 2,000 active study vaccine participants and an observed incidence of 50%, the width of the 95% CI is 4.4%.

Table 3: 95% 2-sided CI and Corresponding Width for Different Observed Incidence Rates when Using a Subset of 2,000 Participants/Arm

Observed Incidence	95% 2-sided CI	Width of the 95% 2-sided CI
5%	[4.1%;6.0%]	2.0%
10%	[8.7%;11.4%]	2.7%
20%	[18.3%;21.8%]	3.6%
30%	[28.0%;32.1%]	4.1%
40%	[37.8%;42.2%]	4.3%
50%	[47.8%;52.2%]	4.4%

CI=confidence interval.

9.3. Populations for Analyses

Vaccination assignment will follow the as-treated principle.

FAS: The full analysis set (FAS) will include all participants with documented vaccine administration, regardless of the occurrence of protocol deviations. The FAS is the primary safety population. The analysis of solicited AEs will be restricted to a subset of the FAS (ie, the Safety Subset).

PPE: The per protocol efficacy (PPE) population will include all randomized and vaccinated participants for whom efficacy data concerning endpoint measures are available excluding participants with major protocol deviations expecting to impact the efficacy outcomes. Participants will be excluded from the date of the major protocol deviation onwards. Participants who receive other *E. coli* vaccines will be excluded from the PPE population. The PPE population is the primary efficacy population.

PPI: The per protocol immunogenicity (PPI) population will include all randomized and vaccinated participants, who are part of the Immunogenicity Subset and for whom immunogenicity data are available, excluding participants with major protocol deviations expecting to impact the immunogenicity outcomes. Participants will be excluded from the date of the major protocol deviation onwards. Participants who receive other *E. coli* vaccines will be excluded from the PPI population. The PPI population is the primary immunogenicity population. For key tables, sensitivity immunogenicity analyses will also be performed on the FAS, including participants that are part of the Immunogenicity Subset for whom immunogenicity measures are available. Excluded samples might be taken into account as well in the sensitivity analysis.

9.4. Statistical Analyses

The Statistical Analysis Plan will be finalized prior to the first interim Database Lock, and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.4.1. Efficacy Analyses

9.4.1.1. Primary Endpoint

The primary analysis of the primary endpoint will evaluate:

- the number of participants with at least 1 IED event with microbiological confirmation in blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC serotypes O1, O2, O4, O6, O15, O16, O18, O25, and O75 with onset at least 29 days after vaccination (from Day 30) in the active vaccine (ExPEC9V) group compared to the placebo group in the PPE population.

The follow-up time will also be taken into account. For participants with an IED event, the follow-up time is defined as the time between vaccination and the occurrence of the first event. For participants without an IED event, it is the time between vaccination and last visit (or last contact for participants that discontinued the study). The null hypothesis of $VE \leq 20\%$ will be tested versus the alternative hypothesis $VE > 20\%$ for the primary endpoint.

If participants have multiple IED events during their follow-up period, the primary analysis of the primary endpoint will only take the first ExPEC9V IED with microbiological confirmation in blood or other sterile sites into account, excluding ExPEC9V IED events with microbiological confirmation in urine only.

A sensitivity analysis in the FAS population will evaluate the number of participants with at least 1 IED event caused by ExPEC9V serotypes with onset from the administration of the study vaccine.

VE will be estimated by 1 minus the ratio of the incidence rate of cases of IED in the ExPEC9V group compared to the placebo group. An exact Poisson regression, stratified for the stratification factors used for randomization, ie, age (≥ 60 to < 65 years of age versus ≥ 65 to < 75 years of age versus ≥ 75 years of age) and the presence of at least one risk factor for cUTIs at baseline (also see complicating factors in cUTI case definition in Section 8.1.4 and risk factors for cUTI in Section 10.16) will be fitted with the event rate, defined as the number of cases over the follow-up time (offset) as dependent variable and the study vaccination group as independent variable. The exact 2-sided $(1-2\alpha)\%$ CI of VE will be constructed for the interim and final analyses. The nominal $\alpha(s)$ will be defined at the interim and final analyses using the α -spending approach (see Section 9.5). If the lower limit of the CI is higher than 20%, this will suggest that VE for the primary endpoint has been demonstrated.

Additional supportive analyses will include an exact binomial CI, based on the PPE and the FAS set.

All cases with multiple pathogens in the culture results from blood or any other sterile site (see Section 10.15, Appendix 15: Sterile sites' definition) will be included in the efficacy analysis for the primary endpoint.

For the primary endpoint with IED caused by any of the ExPEC9V O-serotypes, the O-serotyping (see Section 8.5) has to be available from the central lab and the IED has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive.

9.4.1.2. Secondary Endpoints

A similar Poisson regression model as for the primary endpoint will be used for the analyses of the key secondary efficacy endpoints.

For the secondary endpoint where all IEDs (including multiple IEDs per participant) are considered, the follow-up time is defined for all participants as the time between vaccination and last visit or last contact for participants that discontinued the study. For the secondary endpoint where all UTIs (including multiple UTIs per participant) are considered, the follow-up time is defined for all participants as the time between vaccination and the implementation date of Protocol Amendment 8, last visit, or last contact for participants that discontinued the study, whichever comes first.

A sensitivity analysis in the FAS population will evaluate the number of participants with at least 1 IED event caused by ExPEC9V serotypes with onset from the administration of the study vaccine. This sensitivity analysis will be performed for the first secondary endpoint (first IED event, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes).

All cases with multiple pathogens in the culture results from blood or any other sterile site (see Section 10.15, Appendix 15: Sterile sites' definition) will be included in the efficacy analysis for the secondary endpoints. For the secondary efficacy endpoints, including culture-confirmed IED and pyelonephritis cases, cases with multiple pathogens in the urine culture results will be evaluated by the IEAC, and included only if *E. coli* is determined to be the primary causative pathogen. UTI and ABP cases with urine culture results with mixed pathogens (including *E. coli*) will be excluded from efficacy analyses but included in sensitivity analyses.

For the secondary endpoints, with IED, UTI, or ABP caused by any of the ExPEC9V O-serotypes, the O-serotyping (see Section 8.5) has to be available from the central lab and the IED, UTI, or ABP has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the secondary endpoints related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient.

A hierarchical testing strategy²⁶ will be applied to the following endpoints:

Primary:

1. First IED with microbiological confirmation from blood or other sterile sites, excluding IED cases with microbiological confirmation from urine only, caused by ExPEC9V O-serotypes

Secondary:

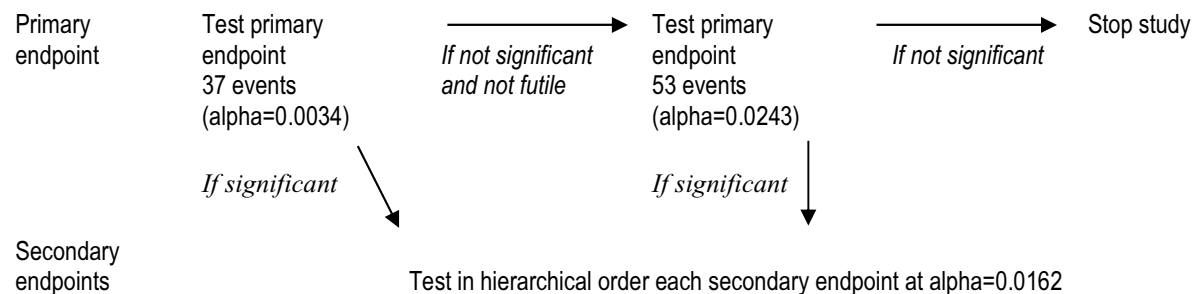
1. First IED, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes
2. All IEDs (including multiple IEDs per participant) caused by ExPEC9V O-serotypes
3. First hospitalized IED event caused by ExPEC9V O-serotypes
4. First IED event meeting criteria for sepsis caused by ExPEC9V O-serotypes
5. First bacteremic IED event caused by ExPEC9V O-serotypes
6. First pyelonephritis event caused by ExPEC9V O-serotypes
7. First UTI caused by ExPEC9V O-serotypes
8. All UTIs (including multiple UTIs per participant) caused by ExPEC9V O-serotypes
9. First IED event caused by *E. coli*
10. First pyelonephritis event caused by *E. coli*
11. First UTI caused by *E. coli*

In this testing strategy, the first key secondary endpoint (first IED event, with microbiological confirmation from blood, other sterile sites, or urine, caused by ExPEC9V O-serotypes) will only be tested when the primary endpoint shows statistical significance (at the interim or final analyses). If the first key secondary endpoint shows statistical significance, the other secondary endpoints will be tested according to the above hierarchical testing strategy.

If the first secondary endpoint is tested, it will be tested using the Pocock boundary using a 1-sided significance level of 0.0162, regardless of whether this test occurs at an interim or final analysis. This testing strategy is graphically presented in [Figure 6](#).

If the lower limit of the $(1-2\alpha)\%$ CI for the first key secondary endpoint is above 0%, VE will have been demonstrated for this endpoint and the next key secondary endpoint will be tested using the same strategy. For the secondary endpoints related to pyelonephritis and UTI, a lower limit of the $(1-2\alpha)\%$ CI of 10% will be used. For more details on analyses, please refer to Section [9.5](#).

Applying the Pocock boundary to the key secondary endpoints is an optimal allocation of the significance level for the hierarchical testing strategy as the minimal significance level to be used at an unspecified timepoint is maximized.²⁶

Figure 6: Hierarchical Testing Strategy for Primary and Secondary Endpoints

The primary endpoint will use 1-sided significance levels 0.0034 and 0.0243 at the interim and the final analysis, respectively. The secondary endpoints will use 0.0162 as a 1-sided significance level irrespective of when they are tested; however, they will only be tested once the primary endpoint is significant.

The World Health Organization Drug Dictionary (WHO-DD) will be used to code antibiotic treatment for IED, UTI, or ABP.

Descriptive statistics will be used to compare the mortality and severity of IED between groups. Further details will be provided in the Statistical Analysis Plan.

9.4.2. Safety Analyses

No formal statistical testing of safety data is planned. The safety analysis will include the descriptive summary of solicited local AEs, solicited systemic AEs, unsolicited AEs, and SAEs. The overall frequencies per group as well as frequencies according to severity and duration will be calculated for solicited and unsolicited AEs. Safety analyses will be done on the FAS, which is the primary safety population. The analysis of solicited AEs will be restricted to a subset of the FAS (Safety Subset).

Adverse Events

The verbatim terms used in the eCRF by investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported AEs, and events-related eDiary information (solicited local at injection site and systemic) with onset at or after vaccination (ie, treatment-emergent adverse events) will be included in the analysis. For each AE, the percentage of participants who experience at least 1 occurrence of the given event will be summarized by vaccine group. Concomitant medications will be coded using the WHO-DD.

Solicited local (injection site) and systemic AEs will be summarized descriptively. The number and percentages of participants with at least one solicited local (injection site) or systemic AE will be presented. The frequencies by vaccine group as well as frequencies according to severity and duration will be described for solicited AEs. Frequencies of unsolicited AEs, separately for all and vaccination-related only, will be presented by System Organ Class and preferred term, while those of solicited AEs will be presented only by preferred term.

Summaries, listings, datasets, or participant narratives may be provided, as appropriate, for those participants who die, who discontinue treatment due to an AE, or who experience a severe or an

SAE, IED, pyelonephritis, and UTI/ABP events reported as SAEs during the course of the study will be excluded from any SAE analyses in the clinical study report if they are subsequently found to be caused by *E. coli* or *P. aeruginosa* infection and confirmed to meet the clinical case definition for the primary or secondary efficacy endpoints.

Vital Signs

Vital signs including temperature, pulse/heart rate, respiratory rate, and blood pressure (systolic and diastolic, sitting) will be summarized at baseline using descriptive statistics.

Physical Examinations

Physical examination findings will be summarized at baseline using descriptive statistics.

9.4.3. Immunogenicity Analyses

The primary immunogenicity analysis will be done on the PPI population.

For ExPEC9V serotype antibodies as measured by multiplex ECL-based immunoassay and MOPA, and EPA as measured by multiplex ECL-based immunoassay only, the following measures of immunogenicity will be evaluated and tabulated by the study vaccination groups, for all immunogenicity timepoints:

- proportion of participants with a ≥ 2 -fold and ≥ 4 -fold increase in serum antibody titers from Day 1 (pre-vaccination)
- geometric mean titer
- geometric mean of fold change from baseline, calculated from the post-baseline/baseline value.

Graphical representations of immunological parameters will be made as applicable.

For the 4-year follow-up period, descriptive summaries of immunogenicity will be presented for each serotype.

An attempt will be made to determine a serological correlate of protection for the ExPEC9V vaccine, based on an analysis of protection from IED and UTI/ABP, and antibody titers from vaccinated and placebo participants. Statistical modelling of the vaccine-mediated immunogenicity with clinical efficacy will be performed to provide an estimate of this correlate, if feasible. The ability to determine a correlate of protection will be dependent on the specifics of the efficacy and immune responses to the vaccine. The correlate of protection will be investigated using several methods, including among others the A:B model.¹⁰ Furthermore, the relationship between antibody titer data and VE will be presented graphically.^{4,25}

9.4.4. Medical Resource Utilization and Health Economics Analyses

MRU and health economics will be descriptively summarized by study vaccine group, including the number of hospitalizations and the length of stay in the hospital. MRU and health economics will be further detailed in a Statistical Analysis Plan.

9.4.5. Patient-reported Outcome Analyses

SF-36 data will be analyzed descriptively by domains and total scores (physical component summary, mental component summary) and summarized by study vaccination group, participants who had UTI, and participants who had IED. EQ-5D-5L data will be analyzed descriptively by dimension (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), single index value, and EQ-VAS, and summarized by study vaccination group, participants who had UTI, and participants who had IED. This will be further detailed in a Statistical Analysis Plan.

9.5. Planned Analyses

9.5.1. Interim Analyses

This study incorporates an inferentially seamless group-sequential design. The target number of participants to be enrolled is approximately 19,800. Note that over enrollment may be allowed if required by local health authorities for the purpose of local health authority consideration. When the first 200 vaccinated participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3) or have discontinued earlier, an immunogenicity interim analysis will be conducted and the sponsor will review summary statistics of the immunogenicity data by study vaccination group (at this stage, the sponsor will remain blinded to individual study vaccine allocation). Enrollment will not be paused during this immunogenicity interim analysis. When 21 of 53 (40%) vaccine serotype IED events according to the primary endpoint (with microbiological confirmation from blood or other sterile sites) have been observed, a first interim analysis for the primary endpoint (futility) will be performed. The futility analysis will be based on the Go/No Go approach. The study will continue to enroll the remaining participants if there is at least 80% probability that $VE > 0\%$. Futility will be declared if there is at least 90% probability that $VE < 70\%$. A second interim analysis for the primary endpoint (efficacy/futility) will be conducted when 37 of 53 (70%) vaccine serotype IED events according to the primary endpoint have been observed; this analysis will use the α -spending approach.

If the event rate is lower or higher than expected, a blinded sample size re-estimation may be performed by the sponsor at the time of the first interim analysis of the primary endpoint (21 events). At the time of this interim analysis and before unblinded data of the interim analysis is sent to the IDMC for efficacy evaluation, the study team will first perform the sample size re-estimation based on the incidence rate of the combined study vaccination groups (blinded, thus placebo and vaccine groups pooled and therefore no alpha adjustment is required).

If the incidence rate is lower than expected, the sample size will not be increased by more than 50%. At the time of the first interim analysis of the primary endpoint (21 events), the sponsor will decide whether to increase the sample size of the study and/or extend the follow-up period of participants in the study (eg, from 4 to 5 years).

For all the interim analyses, an independent statistician will perform the interim analyses and will present the results to the IDMC (at the immunogenicity interim analysis the independent statistician will also prepare the immunogenicity tables for the study team). Based on the outcome

of the first interim analysis of the primary endpoint, the IDMC may recommend stopping enrollment of participants and stopping the study (in case of futility) or to continue the study. In the event of the study being stopped for futility, all participants will be followed up for at least 6 months post-vaccination. If the recommendation from the IDMC is to continue the study, the results will not be shared with the sponsor by the IDMC. By allowing the enrollment to stop in the event of futility, participants will be prevented from receiving an ineffective vaccine or a vaccine inferior to placebo.

9.5.1.1. Interim Analyses for the Primary Endpoint

Details of the interim analyses for the primary endpoint, including operating characteristics, are presented in [Table 4](#).

Table 4: Criteria of Interim and Final Analyses for the Primary Endpoint

Analysis	Total planned events	Alpha (1-sided) ^b	Futility ^a			Efficacy				
			Cases in vaccine group	Prob under H ₀	Prob under H _a	Cases in vaccine group	VE	(1-2α)% CI	Prob under H ₀	Prob under H _a
IA1 (futility)	21 (40%)	-	≥9 (≤25.0% VE)	64.0%	3.5%	-				
IA2 (efficacy/futility)	37 (70%)	0.0034	≥17	5.1%	0.0%	≤8	≥72.4%	[20.1%-92.5%]	0.3%	50.8%
Final analysis	53 (100%)	0.0243				≤16	≥56.8%	[20.1%-77.6%]	2.0%	39.1%
										Global power 89.9%

CI=confidence interval; H₀=null hypothesis; H_a=alternative hypothesis; IA=interim analysis; NA=not applicable; Prob under H₀=probability to reach futility or efficacy under the null hypothesis (VE=20%); Prob under H_a=probability to reach futility or efficacy under the alternative hypothesis (VE=70%); VE=vaccine efficacy.

^a Go/No Go decision criteria: Go = P(VE > 0%) > 80%; No Go = P(VE < 70%) > 90%.

^b User-defined alpha-spending function.

At the first interim analysis (IA1; when 21 vaccine-serotype IED events according to the primary endpoint have been observed), Go/No Go criteria will be used for futility testing. The study might be stopped for futility if the VE (point estimate) is ≤25.0%, ie, ≥9 events in the ExPEC9V group (non-binding futility). At the first interim analysis of the primary endpoint, there is no possibility to declare early superiority of this primary endpoint. It is possible that when triggering this interim analysis, more events have occurred and might be included in the analysis; however, the Go/No Go criteria will not change.

The non-binding futility boundary for the first interim analysis of the primary endpoint has been set up based on Go/No Go criteria. If there is at least 90% probability that VE <70%, the IDMC may recommend termination of the study for futility. This will not affect the overall Type 1 error. The IDMC recommendations with regard to futility at the time of this interim analysis will be communicated to the sponsor or its designee(s) for further evaluation.

At the second interim analysis (IA2; when 37 vaccine-serotype IED events according to the primary endpoint have been observed), the nominal α will be fixed at 0.0034 (1-sided). Using this nominal α , superiority will be declared if ≤ 8 (out of 37) events are observed in the ExPEC9V group (ie, if the VE [point estimate] is $\geq 72.4\%$). In the unlikely event that there are ≥ 17 events in the ExPEC9V group at the time of the second interim analysis, the study might be stopped for futility. It is possible that when triggering this interim analysis, more events have occurred and might be included in the analysis, however, the nominal α will not change.

The Statistical Analysis Plan will describe the planned interim analyses in greater detail.

9.5.2. Final Analysis

Details of the final analysis for the primary endpoint are presented in Table 4. The final analysis for the primary endpoint will occur when 53 vaccine-serotype IED events according to the primary endpoint have been observed in the study or at the latest when the last participant has been followed up for 48 months post-vaccination.

If the final analysis is performed with 53 events and the second interim analysis with 37 events, the nominal 1-sided significance level of 0.0243 will be used (if the number of events is different from 37 at the second interim analysis or 53 at the final analysis, the final nominal α will depend on the observed number of events and will be determined using the α -spending approach) and superiority will be declared if ≤ 16 (out of 53) events (ie, if the VE [point estimate] is $\geq 56.8\%$) are observed in the ExPEC9V group.

Table 5 shows some operating characteristics for different true VE assumptions, including VE of 30% and 50%.

Table 5: Operating Characteristics Under Different True VE Assumptions

True VE	Full study	IA1 (21 events)		IA2 (37 events)		Final analysis (53 events)	
	Overall power	Superiority	Futility	Superiority	Futility	Success	Failure
0%	0.3%	0%	80.8%	0.0%	5.5%	0.2%	13.4%
10%	0.8%	0%	73.5%	0.1%	5.6%	0.7%	20.1%
20%	2.3%	0%	64.0%	0.3%	5.1%	2.0%	28.6%
30%	6.4%	0%	52.1%	1.0%	4.1%	5.4%	37.5%
40%	16.2%	0%	38.3%	3.1%	2.6%	13.1%	43.0%
50%	35.7%	0%	24.0%	8.7%	1.2%	27.0%	39.1%
60%	64.5%	0%	11.6%	22.9%	0.3%	41.6%	23.6%
70%	89.9%	0%	3.5%	50.8%	0.0%	39.1%	6.5%
80%	99.2%	0%	0.4%	84.9%	0.0%	14.4%	0.3%
90%	100.0%	0%	0.0%	99.5%	0.0%	0.5%	0.0%

IA=interim analysis; VE=vaccine efficacy.

9.5.3. End-of-study Analysis

End-of-study Analysis will include efficacy, safety, and immunogenicity data (multiplex ECL-based immunoassay and MOPA) collected at Year 1 (Day 366), Year 2 (Day 731), Year 3 (Day 1,096), and Year 4 (Day 1,461) after vaccination.

9.6. Data Monitoring Committee or Other Review Board

To maintain the integrity of the study, an IDMC will be established as noted in Committees Structure in Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations. The IDMC will be convened for safety, futility, and efficacy evaluation. All IDMC members will be external and independent of the sponsor, including at least one medical expert in the relevant therapeutic area and at least one statistician.

The IDMC will review unblinded safety data at regular intervals, as specified in the IDMC charter. The first unblinded IDMC safety review will take place when the first 500 randomized participants in the study (any participant, irrespective of enrollment in the Safety or Immunogenicity Subset) have completed the Day 30 visit (Visit 3) or discontinued earlier. Thereafter, an IDMC safety review will be conducted at least every 6 months. To maintain the blinding of the study to the clinical staff, investigators, and sponsor personnel, an independent statistician will perform the interim analyses and will present the results to the IDMC. A sponsor committee will be established for this study, which will be the point of contact between the IDMC and sponsor. After the review, the IDMC will make recommendations regarding the continuation of the study. The conclusions of the IDMC will be communicated to the investigators, the IEC/IRB, and the national regulatory authorities, as appropriate.

The IDMC responsibilities, authorities, and procedures will be provided in its charter.

In addition, an independent blinded EAC will be established as noted in Committees Structure in Section 10.3, Appendix 3: Regulatory, Ethical, and Study Oversight Considerations. The IEAC will convene at regular intervals to assess the *E. coli* culture-confirmed suspected IED and *E. coli* culture-confirmed pyelonephritis cases and the deaths attributable to IED or to other causes. Further details of the IEAC will be provided in the IEAC charter.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Abbreviations and Definitions

ABP	acute bacterial prostatitis
AE	adverse event
CFU	colony-forming units
CI	confidence interval
CMA	Conditional Marketing Authorization
COVID-19	coronavirus disease 2019
CPRD	Clinical Practice Research Database
cUTI	complicated urinary tract infection
CT	computed tomography
ECL	electrochemiluminescent
eCOA	electronic clinical outcome assessment
<i>E. coli</i>	<i>Escherichia coli</i>
eCRF	electronic case report form
eDC	electronic data capture
eDiary	electronic diary
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EPA	genetically detoxified form of exotoxin A derived from <i>Pseudomonas aeruginosa</i>
ePRO	electronic patient-reported outcome
EQ-5D	EuroQol 5-dimension
EQ-5D-5L	5-level EuroQol 5-dimension questionnaire
EQ-VAS	EQ visual analog scale
EUA	Emergency Use Authorization
ExPEC	extraintestinal pathogenic <i>Escherichia coli</i>
ExPEC4V	4-valent extraintestinal pathogenic <i>Escherichia coli</i> vaccine
ExPEC9V	9-valent extraintestinal pathogenic <i>Escherichia coli</i> vaccine
ExPEC10V	10-valent extraintestinal pathogenic <i>Escherichia coli</i> vaccine
FAS	full analysis set
FDA	(United States) Food and Drug Administration
FiO ₂	fraction of inspired oxygen
GCP	Good Clinical Practice
GMC	geometric mean concentration
GMT	geometric mean titer
HBV	hepatitis B virus
HCV	hepatitis C virus
HES	Hospital Episode Statistics
HHS	Health and Human Services
HIV	human immunodeficiency virus
HPF	high-power field
HRQoL	health-related quality of life
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
ICU	intensive care unit
IDMC	Independent Data Monitoring Committee
IEAC	Independent Endpoint Adjudication Committee
IEC	Independent Ethics Committee
IED	invasive <i>Escherichia coli</i> disease
IgG	immunoglobulin G
IM	intramuscular
IRB	Institutional Review Board
IWRS	interactive web response system

KPNW	Kaiser Permanente Northwest
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency
MOPA	multiplex opsonophagocytic killing assay
MRI	magnetic resonance imaging
MRU	medical resource utilization
NZW	New Zealand White
OHRP	Office for Human Research Protections
OPA	opsonophagocytic killing assay
PaCO ₂	partial pressure of arterial carbon dioxide
PaO ₂	partial pressure of arterial oxygen
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PCC	protocol clarification communication
PCV	pneumococcal conjugate vaccine
PPE	per protocol efficacy
PPI	per protocol immunogenicity
PQC	Product Quality Complaint
PRO	patient-reported outcome
PS(s)	polysaccharide(s)
qSOFA	quick sequential organ failure assessment
RR	respiratory rate
rUTI	recurrent UTI
SAE	serious adverse event
SF-36	Short Form-36
SIRS	systemic inflammatory response syndrome
SoA	Schedule of Activities
SOFA	sequential organ failure assessment
SPPB	Short Physical Performance Battery
SUSAR	suspected unexpected serious adverse reaction
ST	sequence type
US	United States
UTI	urinary tract infection
VE	vaccine efficacy
WBC	white blood cell
WHO-DD	World Health Organization Drug Dictionary

Definitions of Terms

ePRO	The electronic technology used to collect the patient-reported outcome data. PROs are reports that come directly from the participant without interpretation by clinician or anyone else.
Interview mode	Site-assisted interview where the responses are added to the ePRO device for participants who are unwilling or unable to use a device themselves.
eCOA	An umbrella term encompassing different types of outcome assessments such as ePRO and eDiary.
eDiary	The electronic technology used to record solicited signs and symptoms by the participants in the Safety Subset.
Electronic source system	Contains data traditionally maintained in a hospital or clinic record to document medical care or data recorded in a case report form as determined by the protocol. Data in this system may be considered source documentation.
Home health visit	A home visit by a healthcare professional in the event that the participant is unable to come to the site.
Remote visit	A visit for which collection of samples is not required and which will be conducted via telephone call.

Hospital-acquired	IED, UTI, and ABP episodes are classified as hospital-acquired if a first positive culture (blood, other sterile sites, or urine) occurs >48 hours after hospital admission, without evidence that the pathogen was already in the incubation phase, or within 48 hours of discharge from the hospital.⁵⁶
Healthcare-associated	IED episodes are defined as healthcare-associated if a first culture (blood, sterile sites, urine) is obtained <48 hours of hospital admission, or emergency department visit, and meeting at least one of the following criteria: 1. Visit to a hospital clinical or emergency department within the past 5 to 30 days before onset of the IED, 2. Hospitalization at an acute care hospital for 2 or more days within the past 90 days before onset of the IED. 3. Intravenous therapy, specialist nursing care at home, or dialysis in the past 30 days before onset of the IED, 4. Active cancer, 5. Resident status at a nursing home or long-term care facility.²³ ABP and UTI episodes are defined as healthcare-associated if positive urine cultures are obtained from outpatient clinics, general practice clinics, emergency departments, or within 48 hours of hospital admission and meeting the following criteria: 1. The participant has had an indwelling urinary catheter within the last 7 days 2. The infection occurs after urinary tract instrumentation in outpatient care, hospital, or emergency room setting that took place within last 7 days, and there is no evidence that the infection was present or incubating at the time of the procedure The participant has resident status at a nursing home or residential care facility.⁷³
Community-acquired	IED episodes are defined as community-acquired if a first culture (blood, sterile sites, or urine) obtained <48 hours after hospital admission and not meeting criteria for a healthcare-associated episode.²³ ABP and UTI episodes are defined as community-acquired if positive urine cultures are obtained from outpatient clinics, general practice clinics, emergency departments, or within 48 hours of hospital admission and when the case does not meet criteria for a healthcare-associated episode.²²

10.2. Appendix 2: Clinical Laboratory Tests

Not applicable

10.3. Appendix 3: Regulatory, Ethical, and Study Oversight Considerations

10.3.1. Regulatory and Ethical Considerations

The term “sponsor” refers to the sponsor or its designee, as applicable.

Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country- or territory-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of study participants are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

Protocol Clarification Communications

If text within a final approved protocol requires clarification (eg, current wording is unclear or ambiguous) that does not change any aspect of the current study conduct, a protocol clarification communication (PCC) may be prepared. The PCC Document will be communicated to the Investigational Site, Site Monitors, Local Trial Managers, Clinical Trial Managers, and/or Contract Research Organizations who will ensure that the PCC explanations are followed by the investigators.

The PCC Document may be shared by the sites with IECs/IRBs per local regulations.

The PCC Documents must NOT be used in place of protocol amendments, but the content of the PCC Document must be included in any future protocol amendments.

Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the participants, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involve only logistic or administrative aspects of the study, the IEC/IRB (where required) only needs to be notified.

In situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative listed in the Contact Information page(s), which will be provided as a separate document. Except in emergency situations, this contact must be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the eCRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country/territory, if applicable. A study may not be initiated until all local regulatory requirements are met.

Required Prestudy Documentation

The following documents must be provided to the sponsor before shipment of study vaccine to the study site:

- Protocol and amendment(s), if any, signed and dated by the principal investigator
- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC/IRB approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, participant compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorized designee.
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the study-site personnel is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification, if applicable
- Signed and dated statement of investigator (eg, Form FDA 1572), if applicable
- Documentation of investigator qualifications (eg, curriculum vitae)
- Completed investigator financial disclosure form from the principal investigator, where required
- Signed and dated clinical trial agreement, which includes the financial agreement
- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrollment of the first participant:

- Completed investigator financial disclosure forms from all subinvestigators
- Documentation of subinvestigator qualifications (eg, curriculum vitae)
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable
- Local laboratory documentation demonstrating competence and test reliability (eg, accreditation/license), if applicable

Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the participants)
- IB (or equivalent information) and amendments/addenda
- Sponsor-approved participant recruiting materials
- Information on compensation for study-related injuries or payment to participants for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for participants
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no consequences for participants, data or study conduct, unless required locally), the ICF, applicable recruiting materials, and participant compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data or study conduct)
- Revision(s) to ICF and any other written materials to be provided to participants
- If applicable, new or revised participant recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to participants for participation in the study, if applicable

- New edition(s) of the IB and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of AEs that are serious, unlisted/unexpected, and associated with the study vaccine
- New information that may adversely affect the safety of the participants or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the participants
- Report of deaths of participants under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this study, where required.

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

Country/Territory Selection

This study will only be conducted in those countries/territories where the intent is to launch or otherwise help ensure access to the developed product if the need for the product persists, unless explicitly addressed as a specific ethical consideration in Section 4.2.1, Study-Specific Ethical Design Considerations.

Other Ethical Considerations

For study-specific ethical design considerations, refer to Section 4.2.1., Study-Specific Ethical Design Considerations.

10.3.2. Financial Disclosure

Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information in accordance with local regulations to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

Refer to Required Prestudy Documentation (above) for details on financial disclosure.

10.3.3. Informed Consent Process

Each participant must give consent according to local requirements after the nature of the study has been fully explained. The ICF(s) must be signed before performance of any study-related activity. The ICF(s) that is/are used must be approved by both the sponsor and by the reviewing IEC/IRB and be in a language that the participant can read and understand. The informed consent must be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Informed consent may be obtained remotely, including at the participant's home. Refer to the Clinical Management Plan.

Before enrollment in the study, the investigator or an authorized member of the study-site personnel must explain to potential participants the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Participants will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the participant will receive. Finally, they will be told that the investigator will maintain a participant identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the participant, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the participant is authorizing such access. It also denotes that the participant agrees to allow his or her study physician to recontact the participant for the purpose of obtaining consent for additional safety evaluations, if needed.

The participant will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent must be appropriately recorded by means of the participant's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the participant.

Participants who are rescreened are required to sign a new ICF.

As described in Section 8.1.6, a caregiver may assist a participant who is unable to complete questionnaires in the eCOA (ie, ePRO and eDiary), by reading the questions aloud and recording the responses in the eCOA, according to the PRO Completion Guidelines, on the participant's behalf (using the caregiver's unique identifier and PIN). For this purpose, a caregiver consent form has been developed. Consent must be obtained according to local requirements and must be obtained from the caregiver before he or she is allowed to complete the eCOA on behalf of the participant. After having obtained the caregiver's consent, a copy of the consent form must be given to the caregiver. Of note, the caregiver is not intended to be a Legally Authorized Representative who can provide informed consent for study participation on behalf of the participant.

10.3.4. Data Protection

Privacy of Personal Data

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent obtained from the participant includes information about, and where required per applicable regulations, explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. The informed consent also provides information to address the lawful transfer of the data to other entities and to other countries/territories.

The participant has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete, or make requests concerning his or her personal data in accordance with applicable data protection law. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

In the event of a data security breach, the sponsor will apply measures to adequately manage and mitigate possible adverse effects taking into consideration the nature of the data security breach as necessary to address other obligations such as notifying appropriate authorities in accordance with applicable data protection law.

Exploratory research is not conducted under standards appropriate for the return of data to participants. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to participants or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

10.3.5. Storage, Use, Transfer, and Retention of Data and Samples

Study samples will be coded or anonymized at all times in accordance with the informed consent and will not be labeled with personal identifiers.

The investigator and study site will only store, use, transfer and retain data and study samples, including optional study samples, in accordance with the informed consent and applicable law, and in accordance with any separate written agreement with sponsor. Other than what is specified

in a separate written agreement with sponsor, study site and investigator shall not conduct or facilitate any research by a third party not required by the protocol (i) on participants if such research interferes with the conduct of the study or (ii) on samples collected from study participants during the study, including optional samples, if the research relates to ExPEC9V or (iii) on data collected from study participants during the study if the research relates to ExPEC9V.

The sponsor may store, use, transfer or retain the data and study samples, including optional study samples, for uses not specified by the protocol, including compatible research, in compliance with the informed consent and applicable law.

10.3.6. Committees Structure

Independent Data Monitoring Committee

An IDMC will be established to monitor data on an ongoing basis, to review interim data, to ensure the continuing safety of the participants enrolled in this study and for safety, futility, and efficacy evaluations. This committee will consist of at least one medical expert in the relevant therapeutic area and at least one statistician; committee membership responsibilities, authorities, and procedures will be documented in its charter. The committee will meet periodically to review interim data. After the review, the IDMC will make recommendations regarding the continuation of the study.

Independent Endpoint Adjudication Committee

The IEAC will convene at regular intervals to assess the *E. coli* culture-confirmed suspected IED and *E. coli* culture-confirmed pyelonephritis cases as well as the deaths attributable to IED or to other causes. Further details of the IEAC will be provided in the IEAC charter.

10.3.7. Use of Information and Publication

All information, including but not limited to information regarding ExPEC9V, supplied by the sponsor to the study site or investigator and not previously published, and any data or analysis generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The study site and investigator shall not use this information except in the performance of this study and shall not disclose this information to anyone except to persons involved in the study that need such information to assist in conducting the study, and then only on like terms of confidentiality and non-use.

The study site and investigator shall not publish study results except as required by law or as specified in a separate, written agreement between the sponsor and the study site or investigator.

The Sponsor will register the study and publish the study results in compliance with applicable law and may register the study or publish study results when not required.

Authorship of any peer-reviewed publications will be determined by mutual agreement in line with International Committee of Medical Journal Editors authorship guidelines.

In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and disclose the interim results of clinical studies as required by law. The disclosure of the study results will be performed after the end of study at the latest.

10.3.8. Data Quality Assurance

Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, and periodic monitoring visits by the sponsor. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for eCRF completion will be provided and reviewed with study-site personnel before the start of the study. The sponsor will review eCRF for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

10.3.9. Case Report Form Completion

Electronic case report forms (eCRFs) are prepared and provided by the sponsor for each participant in electronic format. All data relating to the study must be recorded in the eCRF. All eCRF entries, corrections, and alterations must be made by the investigator or authorized study-site personnel. The investigator must verify that all data entries in the eCRF are accurate and correct.

The study data will be transcribed by study-site personnel from the source documents onto an eCRF, if applicable. Study-specific data will be transmitted in a secure manner to the sponsor.

Data must be entered into the eCRF in English. The eCRF must be completed as soon as possible after a participant visit and the forms must be available for review at the next scheduled monitoring visit.

If necessary, queries will be generated in the eDC tool. If corrections to an eCRF are needed after the initial entry into the eCRF, this can be done in either of the following ways:

- Investigator and study-site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Sponsor or sponsor delegate can generate a query for resolution by the investigator and study-site personnel.

10.3.10. Source Documents

At a minimum, source documents consistent in the type and level of detail with that commonly recorded at the study site as a basis for standard medical care must be available for the following: participant identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all AEs and follow-up of AEs; concomitant medication; vaccine receipt/dispensing/return records; study vaccine administration information; and date of study completion and reason for withdrawal from the study, if applicable.

The author of an entry in the source documents must be identifiable. Given that PROs are reports of a patient's health condition that come directly from the patient, without interpretation by a clinician or anyone else, the responses to PRO measures entered by study participants into source records cannot be overridden by site staff or investigators.

Participant-completed assessments designated by the sponsor (ePRO instruments) will be recorded directly into an electronic device and will be considered source data. The participant's eDiary used to collect information regarding solicited signs and symptoms after vaccination will be considered source data.

Specific details required as source data for the study and source data collection methods may be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

The following data will be recorded directly into the eCRF or eCOA (ie, ePRO and eDiary) and will be considered source data:

- Race
- Blood pressure and pulse/heart rate
- Height and weight
- Details of physical examinations
- Participant completed scales and assessments
- Health economics data

An eSource system may be utilized, which contains data traditionally maintained in a hospital or clinic record to document medical care (eg, electronic source documents) as well as the clinical study-specific data fields as determined by the protocol. This data is electronically extracted for use by the sponsor. If eSource is utilized, references made to the eCRF in the protocol include the eSource system but information collected through eSource may not be limited to that found in the eCRF.

10.3.11. Monitoring

The sponsor will use a combination of monitoring techniques (central, remote, and/or on-site monitoring) to monitor this study.

Monitoring is based on risk control strategies including central (remote) monitoring of key study endpoints, establishing quality indicators to alert about potential issues with study data, on-site source data verification of critical data points, and on-site source data review for site processes. The detailed strategies for remote and on-site activities are defined through a protocol-based risk assessment process based on a final protocol.

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the study site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the eCRF with the source documents (eg, hospital/clinic/physician's office medical records). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study-site personnel.

Direct access to source documents (medical records) must be allowed for the purpose of verifying that the recorded data are consistent with the original source data. Findings from this review will be discussed with the study-site personnel. The sponsor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documents will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

In addition to on-site monitoring visits, remote contacts can occur. It is expected that during these remote contacts, study-site personnel will be available to provide an update on the progress of the study at the site.

Central monitoring will take place for data identified by the sponsor as requiring central review.

There will be independent monitoring of the pharmacy and preparation of study vaccines by an unblinded monitor (independent study vaccine monitor); regular monitors will be blinded.

10.3.12. On-site Audits

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection. Participant privacy must, however, be respected. The investigator and study-site personnel are responsible for being present and available for consultation during routinely scheduled study-site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a

regulatory submission. The investigator must immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

10.3.13. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all eCRF and all source documents that support the data collected from each participant, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

10.3.14. Study and Site Start and Closure

First Act of Recruitment

The first participant screened is considered the first act of recruitment and it becomes the study start date.

Study Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study vaccine development

10.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.4.1. Adverse Event Definitions and Classifications

Adverse Event

An AE is any untoward medical occurrence in a clinical study participant administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per ICH).

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: For time period of sponsor's AE collection, see All Adverse Events under Section 8.3.1, Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information.

Serious Adverse Event

An SAE based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
(The participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

*Medical and scientific judgment must be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected AE occurs for which there is evidence suggesting a causal relationship between the study vaccine and the event (eg, death from anaphylaxis), the event must be reported as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (eg, all-cause mortality).

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For ExPEC9V, the expectedness of an AE will be determined by whether or not it is listed in the IB.²⁹

10.4.2. Attribution Definitions**Assessment of Causality**

The causal relationship to study vaccine is assessed by the Investigator and documented in the medical records.

The assessment of causality must consider the following factors:

- Temporal relationship
- Clinical characteristics of event
- Pharmacological plausibility
- Confounding risk factors:
 - Concomitant medication
 - Underlying/concurrent disease
 - Family/social history
- Other considerations: Participant characteristics and past medical history, and quality of information

The following selection must be used to assess all AEs.

Related

There is a reasonable causal relationship between study vaccine administration and the AE.

Not Related

There is not a reasonable causal relationship between study vaccine administration and the AE.

By definition, all solicited adverse events at the injection site (local) will be considered related to the study vaccine administration.

10.4.3. Severity Criteria

All AEs and laboratory data will be coded for severity using a modified version of the FDA grading table, based on the version of September 2007,⁶⁶ included in Section 10.6, Appendix 6: Toxicity Tables.

For AEs not identified in the grading table, the following guidelines will be applied:

Grade 1	Mild	Symptoms causing no or minimal interference with usual social and functional activities
Grade 2	Moderate	Symptoms causing greater than minimal interference with usual social and functional activities
Grade 3	Severe	Symptoms causing inability to perform usual social and functional activities and requires medical intervention
Grade 4	Potentially life-threatening	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability OR ER visit or hospitalization

The investigator must use clinical judgment in assessing the severity of events not directly experienced by the participant (eg, laboratory abnormalities).

The severity of solicited signs and symptoms will be graded in the eDiary by the participant based on the severity assessment provided in the eDiary and then verified by the investigator using the toxicity grading scale in Section 10.6, Appendix 6: Toxicity Tables (Note: severity of the measured events will be derived from the diameter [for erythema and swelling] and the temperature measurements [for fever]).

10.4.4. Special Reporting Situations

Safety events of interest on a sponsor study vaccine in an interventional study that may require expedited reporting or safety evaluation include, but are not limited to:

- Overdose of a sponsor study vaccine
- Suspected abuse/misuse of a sponsor study vaccine
- Accidental or occupational exposure to a sponsor study vaccine
- Medication error, intercepted medication error, or potential medication error involving a Johnson & Johnson medicinal product (with or without patient exposure to the Johnson & Johnson medicinal product, eg, product name confusion, product label confusion, intercepted prescribing or dispensing errors)
- Reporting of participant pregnancy or participant partner(s) pregnancy

Participant-specific special reporting situations must be recorded in the eCRF. Any special reporting situation that meets the criteria of an SAE must be recorded on the SAE page of the eCRF.

10.4.5. Procedures

All Adverse Events

All recorded AEs, regardless of seriousness, severity, or presumed relationship to study vaccine, must be recorded using medical terminology in the source document and the eCRF. Whenever

possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the eCRF their opinion concerning the relationship of the AE to study vaccine. All measures required for AE management must be recorded in the source document and reported according to sponsor instructions.

For all studies with an outpatient phase, including open-label studies, the participant must be provided with a "wallet (study) card" and instructed to carry this card with them for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the participant is participating in a clinical study
- Investigator's name and 24-hour contact telephone number
- Local sponsor's name and 24-hour contact telephone number (for medical personnel only)
- Site number
- Participant number
- Any other information that is required to do an emergency breaking of the blind

Serious Adverse Events

All recorded SAEs that have not resolved by the end of the study, or that have not resolved upon the participant's discontinuation from the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study vaccine or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (participant or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as an SAE. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during participation in a study must be reported as an SAE, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or AE (eg, social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study (must be documented in the eCRF). Note: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered SAEs. Any AE that results in a prolongation of the originally planned hospitalization is to be reported as a new SAE.

The cause of death of a participant in a study, whether or not the event is expected or associated with the study vaccine, is considered an SAE.

Information regarding SAEs will be transmitted to the sponsor using an SAE reporting form and safety report form of the CRF, which must be completed and reviewed by a physician from the study site, and transmitted in a secure manner to the sponsor immediately, but no later than within 24 hours of their knowledge of the event. The initial and follow-up reports of an SAE should be transmitted in a secure manner electronically or by facsimile (fax). Telephone reporting should be the exception and the reporter should be asked to complete the appropriate form(s) first.

10.4.6. Product Quality Complaint Handling

Definition

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality, durability, reliability, or performance of a distributed product, including its labeling, drug delivery system, or package integrity. A PQC may have an impact on the safety and efficacy of the product. In addition, it includes any technical complaints, defined as any complaint that indicates a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product or the drug delivery system.

Procedures

All initial PQCs must be reported to the sponsor by the study-site personnel within 24 hours after being made aware of the event.

A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

10.4.7. Contacting Sponsor Regarding Safety, Including Product Quality

The names (and corresponding telephone numbers) of the individuals who must be contacted regarding safety issues, PQC, or questions regarding the study are listed in the Contact Information page(s), which will be provided as a separate document.

10.5. Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

Pregnancy information will be collected and reported as noted in Section 8.3.5, Pregnancy and Section 10.4, Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting.

Definitions

Participants Not of Childbearing Potential

- **postmenopausal**
A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
- **permanently sterile (for the purpose of this study)**
 - Permanent sterilization methods include hysterectomy, or bilateral salpingectomy, or bilateral oophorectomy.
 - Has congenital abnormalities resulting in sterility.

10.6. Appendix 6: Toxicity Tables

Adapted from the FDA Guidance document “Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials” (September 2007).⁶⁶

A: Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain/Tenderness [#]	Aware of symptoms but easily tolerated; Does not interfere with activity; Discomfort only to touch	Notable symptoms; Requires modification in activity or use of medications; Discomfort with movement	Incapacitating symptoms; Inability to do work, school, or usual activities; Use of narcotic pain reliever	Hospitalization; Pain/tenderness causing inability to perform basic self-care function
Erythema [#]	25 – 50 mm	51 – 100 mm	> 100 mm	Hospitalization; Necrosis or exfoliative dermatitis
Swelling [#]	25 – 50 mm	51 – 100 mm	> 100 mm	Hospitalization; Necrosis

[#] Revised by the sponsor.

Vital Signs *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F)**	38.0 - 38.4 100.4 - 101.1	38.5 - 38.9 101.2 - 102.0	39.0 - 40.0 102.1 - 104.0	> 40 > 104.0
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia [#]
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia [#]
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension [#]
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension [#]
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	Hospitalization for hypotensive shock [#]
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Participant should be at rest for all vital sign measurements.

** For oral temperature: no recent hot or cold beverages or smoking.

*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy participant populations, for example, conditioned athletes.

[#] Revised by the sponsor.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Vomiting [#]	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	Hospitalization; Hypotensive shock
Nausea [#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities	Hospitalization; Inability to perform basic self-care functions
Diarrhea [#]	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800 gms/24 hours or oral rehydration necessary	Hospitalization; Hypotensive shock OR IV fluid replacement indicated
Headache [#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions
Fatigue [#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions
Myalgia [#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions

[#] Revised by the sponsor.

Systemic Illness	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Hospitalization [#]

[#] Revised by the sponsor.

B: Tables for Laboratory Abnormalities

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen (BUN) mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as potentially life-threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mEq/L) should be recorded as a Grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.

***ULN is the upper limit of the normal range.

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm ³	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm ³	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm ³	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm ³	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm ³	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm ³	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** ULN is the upper limit of the normal range.

Urine *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high-power field (RBC/HPF)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

10.7. Appendix 7: Specific Criteria for the Inclusion of Potential Participants with Chronic Stable HIV, HCV, or HBV Infection

The following guidelines should be used to consider for inclusion potential participants with chronic stable HIV, HCV, or HBV infection. If a potential participant does not have relevant laboratory results pertaining to HIV infection in his/her medical records from the last 6 months before screening, they will be instructed to go to their local health care provider and obtain the necessary data for potential entry into the trial. Potential participants will not be screened for laboratory evidence of HIV, HCV, or HBV infection. For a potential participant with a medical history of HCV or HBV infection, the below guidelines regarding stable infection are intended to inform the investigator's judgment of eligibility, but the availability of all the listed laboratory data in the medical records is not mandatory.

1. Stable/well-controlled HIV infection includes:

- a. CD4 cell count ≥ 300 cells/ μ L.
- b. HIV viral load < 50 copies/mL.
- c. Participant must be on stable anti-retroviral treatment (ART) for 6 months (unless the change is due to tolerability, in which case the regimen can be for only the previous 3 months; changes in formulation are allowed; nationwide guidelines that require transition from one ART regimen to another are allowed) and the participant must be willing to continue his/her ART throughout the study as directed by his/her local physician.

Note: Participants with ongoing and progressive comorbidities associated with HIV infection will be excluded but comorbidities associated with HIV infection that have been clinically stable for the past 6 months are not an exclusion criterion.

Laboratory methods for confirming a diagnosis of HIV infection are: Any evidence (historic or current) from medical records, such as ELISA with confirmation with Western Blot or PCR, or of a detectable viral load (country-specific regulatory approved tests).

2. Stable known HCV infection:

History of chronic HCV with evidence of sustained virological response (defined as undetectable HCV RNA) for ≥ 12 weeks following HCV treatment or without evidence of HCV RNA viremia (undetectable HCV viral load).

3. Stable known HBV infection:

May be defined as presence of hepatitis B surface antigen for ≥ 6 months and:

- a. HBeAg negative, anti-HBe positive, and/or
- b. Serum HBV DNA $< 2,000$ IU/mL; and
- c. No other findings indicating active inflammatory process in liver.

10.8. Appendix 8: Systemic Inflammatory Response Syndrome Score

The SIRS criteria are provided below in [Table 6](#).

Table 6: Systemic Inflammatory Response Syndrome Criteria

Criteria	
Heart rate	>90 beats/min (tachycardia)
Respiratory rate	> 20 breaths/min (tachypnoea) or PaCO ₂ <32 mm Hg
Temperature	>38°C or <36°C
Abnormal WBC OR bandemia	<4x10 ⁹ /L or >12x10 ⁹ /L (<4,000/mm ³ or > 12,000/mm ³) or ≥10% immature (band) forms

PaCO₂=partial pressure of arterial carbon dioxide; WBC=white blood cells.

The SIRS criteria are met if at least two of the four criteria are met (= positive SIRS for the purpose of identification of a suspected systemic infection [note the difference from the IED clinical case definition]). The clinically relevant SIRS scores (in particular in case of changes in clinical severity) are to be recorded for each day for the cases of suspected systemic infection. For participants meeting the IED clinical case definition criteria (see Section [8.1.2](#)), including participants who test positive for *P. aeruginosa*, and for participants with suspected IED with microbiological evidence, the SIRS scores will be recorded in the eCRF (as unscheduled evaluations after IED-Day 1).

10.9. Appendix 9: Sequential Organ Failure Assessment Score

The SOFA score criteria are provided below in [Table 7](#).

Table 7: Sequential Organ Failure Assessment Score

Organ system	SOFA score				
	0	1	2	3	4
<i>Respiratory</i> PaO ₂ /FiO ₂ [mm Hg (kPa)]	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support*	<100 (13.3) with respiratory support*
<i>Central nervous system</i> Glasgow coma scale**	15	13-14	10-12	6-9	<6
<i>Cardiovascular system</i>	MAP ≥70 mm Hg	MAP <70 mm Hg	dopamine ≤5 OR dobutamine (any dose)***	dopamine 5.1 - 15 OR epinephrine ≤0.1 OR norepinephrine ≤0.1* **	dopamine >15 OR epinephrine >0.1 OR norepinephrine >0.1* **
<i>Liver</i> Bilirubin (mg/dl) [μmol/L]	<1.2 (<20)	1.2–1.9 (20–32)	2.0–5.9 (33–101)	6.0–11.9 (102–204)**	≥12.0 (>204)**
<i>Coagulation</i> Platelets×10 ³ /μ	≥150	<150	<100	<50	<20
<i>Renal</i> Creatinine (mg/dl) [μmol/L] (OR urine output)	<1.2 [<110]	1.2–1.9 [110–170]	2.0–3.4 [171–299]	3.5–4.9 [300–440] (OR <500 ml/day)	≥5.0 [>440] (OR <200 ml/day)

FiO₂=fraction of inspired oxygen; MAP=mean arterial pressure; PaO₂=partial pressure of arterial oxygen.

MAP is calculated as: ((2 X diastolic blood pressure) + systolic blood pressure)/3

*On mechanical ventilation, including continuous positive airway pressure.

**Glasgow Come Scale scores range from 3 to 15. The higher the score, the better the neurological function.

***Catecholamine doses are given as μg/kg/min for at least 1 hour.

The SOFA criteria for sepsis are met when an acute change in total SOFA score of ≥2 points from baseline can be demonstrated. When a participant is assessed for the first time (eg, at the moment of emergency room evaluation or hospital admission), the baseline will be considered as 0, unless the medical history indicates otherwise. In case multiple SOFA scores are available before the onset of systemic infection, the acute change will be counted from the baseline score defined as the lowest score over the last 72 hours preceding the diagnosis of systemic infection, ie, the time when the first positive culture was obtained that is attributable to the same IED event.

The SOFA score grades abnormality by organ system and accounts for clinical interventions. However, laboratory variables, namely, PaO₂, platelet count, creatinine level, and bilirubin level, are needed for full computation. A higher SOFA score is associated with an increased probability of mortality.

The clinically relevant SOFA scores (in particular in case of changes in clinical severity) are to be recorded for each day for the cases of suspected systemic infection. For participants meeting the IED clinical case definition criteria (see Section 8.1.2) including participants who test positive for

P. aeruginosa, and for participants with suspected IED with microbiological evidence, the SOFA scores will be recorded in the eCRF (as unscheduled evaluations after IED-Day 1). It should be taken into consideration that not all parameters for SOFA score might be available or reliably assessed in some particular clinical situations, eg, if participants are sedated, the Glasgow Coma Scale cannot be assessed. The relevant clinical case information should be recorded in the eCRF for information of IEAC.

10.10. Appendix 10: Quick Sequential Organ Failure Assessment Score

The qSOFA score criteria are provided in [Table 8](#).

Table 8: Quick Sequential Organ Failure Assessment Criteria

Criteria		Points
Systolic blood pressure	≤100 mm Hg	1
Respiratory rate	≥22 breaths/min	1
Altered mentation	Altered mentation (or Glasgow Coma Scale <15)	1

This score is a modified version of the SOFA. The qSOFA consists of only three components that are each allocated one point ([Table 8](#)). A qSOFA score of ≥ 2 points is indicative of organ dysfunction. The clinically relevant qSOFA scores (in particular in case of changes in clinical severity) are to be recorded for each day for the cases of suspected systemic infection. For participants meeting the IED clinical case definition criteria (see [Section 8.1.2](#)), including participants who test positive for *P. aeruginosa*, and for participants with suspected IED with microbiological evidence, the qSOFA scores will be recorded in the eCRF (as unscheduled evaluations after IED-Day 1).

10.11. Appendix 11: Short Physical Performance Battery

Note: This appendix provides a representative example of instructions to perform the test that will be used in this study. The site should always use the most recently provided version of these instructions.

SHORT PHYSICAL PERFORMANCE BATTERY PROTOCOL AND SCORE SHEET

1. BALANCE TESTS

The participant must be able to stand unassisted without the use of a cane or walker. You may help the participant to get up.

Now let's begin the evaluation. I would now like you to try to move your body in different movements. I will first describe and show each movement to you. Then I'd like you to try to do it. If you cannot do a particular movement, or if you feel it would be unsafe to try to do it, tell me and we'll move on to the next one. Let me emphasize that I do not want you to try to do any exercise that you feel might be unsafe.

Do you have any questions before we begin?

A. Side-by-Side Stand

1. *Now I will show you the first movement.*
2. (Demonstrate) *I want you to try to stand with your feet together, side-by-side, for about 10 seconds.*
3. *You may use your arms, bend your knees, or move your body to maintain your balance, but try not to move your feet. Try to hold this position until I tell you to stop.*
4. Stand next to the participant to help him/her into the side-by-side position.
5. Supply just enough support to the participant's arm to prevent loss of balance.
6. When the participant has his/her feet together, ask **"Are you ready?"**
7. Then let go and begin timing as you say, **"Ready, begin."**
8. Stop the stopwatch and say **"Stop"** after 10 seconds or when the participant steps out of position or grabs your arm.
9. If participant is unable to hold the position for 10 seconds, record result and go to the gait speed test.

B. Semi-Tandem Stand

1. ***Now I will show you the second movement.***
2. (Demonstrate) ***Now I want you to try to stand with the side of the heel of one foot touching the big toe of the other foot for about 10 seconds. You may put either foot in front, whichever is more comfortable for you.***
3. ***You may use your arms, bend your knees, or move your body to maintain your balance, but try not to move your feet. Try to hold this position until I tell you to stop.***
4. Stand next to the participant to help him/her into the semi-tandem position
5. Supply just enough support to the participant's arm to prevent loss of balance.
6. When the participant has his/her feet together, ask ***"Are you ready?"***
7. Then let go and begin timing as you say ***"Ready, begin."***
8. Stop the stopwatch and say ***"Stop"*** after 10 seconds or when the participant steps out of position or grabs your arm.
9. If participant is unable to hold the position for 10 seconds, record result and go to the gait speed test.

C. Tandem Stand

1. ***Now I will show you the third movement.***
2. (Demonstrate) ***Now I want you to try to stand with the heel of one foot in front of and touching the toes of the other foot for about 10 seconds. You may put either foot in front, whichever is more comfortable for you.***
3. ***You may use your arms, bend your knees, or move your body to maintain your balance, but try not to move your feet. Try to hold this position until I tell you to stop.***
4. Stand next to the participant to help him/her into the tandem position.
5. Supply just enough support to the participant's arm to prevent loss of balance.
6. When the participant has his/her feet together, ask ***"Are you ready?"***
7. Then let go and begin timing as you say, ***"Ready, begin."***
8. Stop the stopwatch and say ***"Stop"*** after 10 seconds or when the participant steps out of position or grabs your arm.

SCORING:**A. Side-by-side-stand**Held for 10 sec ☐ 1 pointNot held for 10 sec ☐ 0 pointsNot attempted ☐ 0 points**If 0 points, end Balance Tests**Number of seconds held if
less than 10 sec: _____. __ sec*If participant did not attempt test or failed, circle why:*

Tried but unable 1

Participant could not hold position unassisted 2

Not attempted, you felt unsafe 3

Not attempted, participant felt unsafe 4

Participant unable to understand
instructions 5

Other (specify) _____ 6

Participant refused 7

B. Semi-Tandem StandHeld for 10 sec ☐ 1 pointNot held for 10 sec ☐ 0 pointsNot attempted ☐ 0 points**If 0 points, end Balance Tests**

Number of seconds held if less than 10 sec: _____. __ sec

C. Tandem StandHeld for 10 sec ☐ 2 pointsHeld for 3 to 9.99 sec ☐ 1 pointHeld for < than 3 sec ☐ 0 pointsNot attempted ☐ 0 points (circle reason above)

Number of seconds held if less than 10 sec: _____. __ sec

D. Total Balance Tests score _____ (sum points)**Comments:**

2. GAIT SPEED TEST

Now I am going to observe how you normally walk. If you use a cane or other walking aid and you feel you need it to walk a short distance, then you may use it.

A. First Gait Speed Test

1. ***This is our walking course. I want you to walk to the other end of the course at your usual speed, just as if you were walking down the street to go to the store.***
2. Demonstrate the walk for the participant.
3. ***Walk all the way past the other end of the tape before you stop. I will walk with you. Do you feel this would be safe?***
4. Have the participant stand with both feet touching the starting line.
5. ***When I want you to start, I will say: "Ready, begin."*** When the participant acknowledges this instruction say: ***"Ready, begin."***
6. Press the start/stop button to start the stopwatch as the participant begins walking.
7. Walk behind and to the side of the participant.
8. Stop timing when one of the participant's feet is completely across the end line.

B. Second Gait Speed Test

1. ***Now I want you to repeat the walk. Remember to walk at your usual pace, and go all the way past the other end of the course.***
2. Have the participant stand with both feet touching the starting line.
3. ***When I want you to start, I will say: "Ready, begin."*** When the participant acknowledges this instruction say: ***"Ready, begin."***
4. Press the start/stop button to start the stopwatch as the participant begins walking.
5. Walk behind and to the side of the participant.
6. Stop timing when one of the participant's feet is completely across the end line.

GAIT SPEED TEST SCORING:

Length of walk test course: Four meters ☐ Three meters ☐

A. Time for First Gait Speed Test (sec)

1. Time for 3 or 4 meters __. __. __ sec
2. If participant did not attempt test or failed, circle why:
Tried but unable 1
Participant could not walk unassisted 2
Not attempted, you felt unsafe 3
Not attempted, participant felt unsafe 4
Participant unable to understand instructions 5
Other (Specify) _____ 6
Participant refused 7
Complete score sheet and go to chair stand test
3. Aids for first walk.....None ☐ Cane ☐ Other ☐

Comments:

B. Time for Second Gait Speed Test (sec)

1. Time for 3 or 4 meters . sec
2. If participant did not attempt test or failed, circle why:
Tried but unable 1
Participant could not walk unassisted 2
Not attempted, you felt unsafe 3
Not attempted, participant felt unsafe 4
Participant unable to understand instructions 5
Other (Specify) _____ 6
Participant refused 7
Complete score sheet and go to chair stand test
3. Aids for second walk.....None ☐ Cane ☐ Other ☐

What is the time for the faster of the two walks? Record
the shorter of the two times __. __. __ sec
[If only 1 walk done, record that time] __. __. __ sec

If the participant was unable to do the walk: ☐ 0 points

For 4-Meter Walk:

- If time is more than 8.70 sec: ☐ **1 point**
If time is 6.21 to 8.70 sec: ☐ **2 points**
If time is 4.82 to 6.20 sec: ☐ **3 points**
If time is less than 4.82 sec: ☐ **4 points**

For 3-Meter Walk:

- If time is more than 6.52 sec: ☐ **1 point**
If time is 4.66 to 6.52 sec: ☐ **2 points**
If time is 3.62 to 4.65 sec: ☐ **3 points**
If time is less than 3.62 sec: ☐ **4 points**

3. CHAIR STAND TEST**Single Chair Stand**

1. ***Let's do the last movement test. Do you think it would be safe for you to try to stand up from a chair without using your arms?***
2. ***The next test measures the strength in your legs.***
3. (Demonstrate and explain the procedure.) ***First, fold your arms across your chest and sit so that your feet are on the floor; then stand up keeping your arms folded across your chest.***
4. ***Please stand up keeping your arms folded across your chest.*** (Record result).
5. If participant cannot rise without using arms, say ***"Okay, try to stand up using your arms."*** This is the end of their test. Record result and go to the scoring page.

Repeated Chair Stands

1. ***Do you think it would be safe for you to try to stand up from a chair five times without using your arms?***
2. (Demonstrate and explain the procedure): ***Please stand up straight as QUICKLY as you can five times, without stopping in between. After standing up each time, sit down and then stand up again. Keep your arms folded across your chest. I'll be timing you with a stopwatch.***
3. When the participant is properly seated, say: ***"Ready? Stand"*** and begin timing.
4. Count out loud as the participant arises each time, up to five times.
5. Stop if participant becomes tired or short of breath during repeated chair stands.
6. Stop the stopwatch when he/she has straightened up completely for the fifth time.
7. Also stop:
 - If participant uses his/her arms
 - After 1 minute, if participant has not completed rises
 - At your discretion, if concerned for participant's safety

8. If the participant stops and appears to be fatigued before completing the five stands, confirm this by asking ***“Can you continue?”***
9. If participant says “Yes,” continue timing. If participant says “No,” stop and reset the stopwatch.

SCORING

Single Chair Stand Test

- | | | |
|---|--|---------------------------------------|
| A. Safe to stand without help | Yes
☐ | No
☐ |
| B. Results: | | |
| Participant stood without using arms | ☐ | → Go to Repeated Chair Stand Test |
| Participant used arms to stand | ☐ | → End test; score as 0 points |
| Test not completed | ☐ | → End test; score as 0 points |
| C. If participant did not attempt test or failed, circle why: | | |
| Tried but unable | 1 | |
| Participant could not stand unassisted | 2 | |
| Not attempted, you felt unsafe | 3 | |
| Not attempted, participant felt unsafe | 4 | |
| Participant unable to understand instructions | 5 | |
| Other (Specify) _____ | 6 | |
| Participant refused | 7 | |

Repeated Chair Stand Test

A. Safe to stand five times

Yes **No**

☐ ☐

B. If five stands done successfully, record time in seconds.

Time to complete five stands __. __. __ sec

C. If participant did not attempt test or failed, circle why:

Tried but unable	1
Participant could not stand unassisted	2
Not attempted, you felt unsafe	3
Not attempted, participant felt unsafe	4
Participant unable to understand instructions	5
Other (Specify) _____	6
Participant refused	7

Scoring the Repeated Chair Test

Participant unable to complete 5 chair stands or completes stands in >60 sec:	☐ 0 points
If chair stand time is 16.70 sec or more:	☐ 1 points
If chair stand time is 13.70 to 16.69 sec:	☐ 2 points
If chair stand time is 11.20 to 13.69 sec:	☐ 3 points
If chair stand time is 11.19 sec or less:	☐ 4 points

Study ID _____ Date _____ Tester Initials _____

Scoring for Complete Short Physical Performance Battery**Test Scores**

Total Balance Test score _____ points

Gait Speed Test score _____ points

Chair Stand Test score _____ points

Total Score _____ points (sum of points above)

10.12. Appendix 12: Statistical Methodology for Sample Size Calculations

VE will be estimated by 1 minus the ratio of the incidence rate of cases of IED in the ExPEC9V group compared to the placebo group.

VE can be estimated by:

$$VE = 1 - \frac{n_1/N_1}{n_2/N_2} = 1 - \frac{n_1}{r * n_2}$$

Where:

n_1 = number of cases in the vaccinated group

N_1 = follow-up time in the vaccinated group

n_2 = number of cases in the control group

N_2 = follow-up time in the control group

$r = N_1/N_2$.

Conditionally to the total number of cases $n = n_1 + n_2$ and r , let p denote the proportion of cases in the vaccine group. Then, VE can be rewritten as:

$$VE = 1 - \frac{n_1}{n} * \frac{n}{r * (n - n_1)} = 1 - \frac{p}{r * (1 - p)}$$

Further, note that n_1 is binomially distributed $B(n, p)$. Therefore, there is a monotonic link between VE and p , the proportion of cases in the vaccine group among the total cases.

The CI for VE can then be derived from the exact CI for p based on the exact Poisson regression.^{17,49}

This methodology has already been used in other Phase 3 VE studies.³⁷

Sample size calculation

To determine the number of events that is needed, standard software, like PASS 15 can then be used.

The null hypothesis $VE \leq 20\%$ can be translated into $H_0: p \geq 0.4444$ (using the above formula).

For the power calculations of the primary endpoint, the $VE=70\%$ translates into $p=0.2308$. Hence, using PASS 15 (Tests for one proportion), it can be seen that 53 events are needed to reach a statistical power of at least 90% using a 1-sided 0.025 significance level.

Power	n	Proportion Given H0 P0	Proportion Given H1 P1	Difference P1-P0	Target Alpha	Actual Alpha	Reject H0 If R ≤
0,91459	53	0,4444	0,2308	-0,2136	0,0250	0,0242	16

The number of participants can then be determined taking into account the incidence rate in the placebo group (~0.17%), the incidence rate in the vaccine group (~0.051%, assuming 70% VE) and yearly dropout rate (10%). The average follow-up for a participant was assumed to be 3.5 years.

$$N = 53 / [(0.0017 + 0.0017 * (1 - 0.7)) / 2] / (0.90^{3.5}) / 3.5 \sim 19,800.$$

Hence, approximately 19,800 participants are planned to be enrolled into the study.

10.13. Appendix 13: Obtaining Blood Cultures

In all patients with suspected sepsis, two or more sets (aerobic and anaerobic) of blood cultures are to be collected (if feasible) before initiation of any new antimicrobial. Required blood cultures may be drawn together on the same occasion.

Timing

Cultures should be obtained before initiation of antimicrobial therapy from all sites considered to be potential sources of infection if it results in no substantial delay (ie, within 45 minutes) in the administration of fluids and start of antimicrobials.⁴⁸

Volume

A minimum of two blood cultures consisting of 20-30 ml per culture (ideally one peripheral draw and one drawn through the catheter most suspicious of being infected [if line sepsis is suspected]) when a septic patient is first evaluated.⁴⁸

Sources of Infection

If history or clinical examination clearly indicates a specific anatomic site of infection, cultures of other sites (apart from blood and that site) are generally unnecessary. In potentially septic patients with an intravascular catheter (in place >48 hours) in whom a site of infection is not clinically apparent, or a suspicion of intravascular catheter-associated infection exists, at least one blood culture set should be obtained from the catheter (along with simultaneous peripheral blood cultures). In patients without a suspicion of catheter-associated infection and in whom another clinical infection site is suspected, at least one blood culture (of the two or more that are required) should be obtained peripherally.⁴⁸ Options include:

- all cultures drawn peripherally via venipuncture,
- cultures drawn through each separate intravascular device but not through multiple lumens of the same intravascular catheter, or
- cultures drawn through multiple lumens in an intravascular device.

10.14. Appendix 14: Antibacterial Agents for Susceptibility Testing

The following antibiotic panel will be used to screen isolates for antimicrobial resistance:

Amoxicillin
Amoxicillin/Clavulanate
Aztreonam
Cefotaxime
Ceftazidime
Ceftazidime/avibactam
Ceftolozane/tazobactam
Cefepime
Piperacillin/Tazobactam
Cefiderocol
Imipenem
Meropenem
Colistin
Amikacin
Gentamicin
Ciprofloxacin
Doxycycline
Tigecycline
Trimethoprim/sulfamethoxazole
Nitrofurantoin
Plazomicin

10.15. Appendix 15: Sterile Sites' Definition

Normally sterile sites:

- Blood
- Cerebrospinal fluid
- Pleural fluid (ie, chest fluid, thoracentesis fluid)
- Peritoneal fluid (ie, abdominal fluid, ascites)
- Pericardial fluid
- Bone, including bone marrow
- Joint fluid (ie, synovial fluid, fluid, needle aspirate, or culture of any specific joint such as knee, ankle, elbow, hip, wrist)
- Muscle, fascia, or tendon
- Internal body sites (ie, lymph node, brain, heart, liver, spleen, vitreous fluid, kidney, pancreas, ovary, vascular tissue)
- Bronchoalveolar lavage fluid

For the definition of normally sterile sites, the following specimen sources are excluded:

- Abscess
- Wound
- Gallbladder, bile, biliary tract
- Urinary tract
- Respiratory tract (unless it was obtained by bronchoalveolar lavage)

10.16. Appendix 16: Medical Conditions of Interest for the Charlson Comorbidity Index, the Frailty Index, Additional IED Risk Factors, and the Risk Factors for Complicated UTI

The medical conditions of interest and medical conditions required for assessment of the Charlson Comorbidity Index and the Frailty Index are presented by organ system in [Table 9](#).

The medical conditions of interest considered for eligibility as additional IED risk factors (with their requisite timing relative to randomization) and considered risk factors for complicated UTI (with their requisite toxicity grade for stratification purposes) are presented in [Table 10](#).

Table 9: Medical Conditions of Interest, Medical Conditions of Interest for the Charlson Comorbidity Index, and Frailty Index

Organ system	Medical conditions of interest	CCI term (weight)	Medical conditions of interest for Frailty Index
Urinary tract disorders	Medullary sponge kidney	Renal disease (1)	
	Acute Renal Injury	Renal disease (1)	
	Chronic kidney disease	Renal disease (1)	
	Hydronephrosis	Renal disease (1)	
	Neurogenic Bladder		
	Obstructive Uropathy		
	Urinary fistula		
	Ureteric strictures		
	Urethral Strictures		
	Urinary Retention		
	Urinary Tract Obstruction		
	Urine incontinence		
	Urolithiasis		
	Vesicoureteral Reflux		
	Voiding dysfunction		
	Interstitial cystitis		
	Chronic prostatitis		
	Benign Prostatic Hypertrophy		
	Acute prostatitis		
	Pelvic prolapse		
	Pyelonephritis		
	Nephrolithiasis		
Surgical and medical procedures	Cystostomy		
	Nephrostomy		
	Urostomy		
	Nephrostomy tube placement		
	Renal transplant	Renal disease (1)	
	Ureteric stent		
	Urinary catheterization		
	Urological intervention		
Respiratory disorders	COPD or other chronic pulmonary disease	Chronic pulmonary disease (1)	Has chronic bronchitis or emphysema
Nervous system disorders	Dementia	Dementia (2)	
	Migraine		Has migraine headache
	Mental disorder		
	Hearing problems		Has hearing problem
Malignancies	Prostatic Adenocarcinoma		Has cancer

Table 9: Medical Conditions of Interest, Medical Conditions of Interest for the Charlson Comorbidity Index, and Frailty Index

Organ system	Medical conditions of interest	CCI term (weight)	Medical conditions of interest for Frailty Index
	Tumours of the urinary tract	Any malignancy including leukemia and lymphoma (2)	Has cancer
	Any malignancy, including lymphoma and leukemia, except malignant neoplasm of skin, tumors of urinary tract and prostatic adenocarcinoma	Any malignancy including leukemia and lymphoma (2)	Has cancer
	Metastatic solid tumour	Metastatic solid tumor (6)	Has cancer
Musculoskeletal and connective tissue disorders	Arthritis	If toxicity grade ≥ 3 Rheumatologic disease (1)	Has arthritis or rheumatism
Metabolic disorders	Diabetes mellitus	If toxicity grade ≥ 2 Diabetes with chronic complications (1)	Has diabetes mellitus
Infections	Urosepsis		
	<i>E. coli</i> bacteremia		
	HBV infection	If toxicity grade ≤ 2 Mild liver disease (2)	
	HCV infection	If toxicity grade ≤ 2 Mild liver disease (2)	
	HIV infection	AIDS/HIV (4)	
Immune system disorders	Anaphylactic and anaphylactoid responses		
	Cachexia		
	Long term Disability or Handicap; Hemiplegia or paraplegia; Immobility	Hemiplegia or paraplegia (2)	Has long-term disability or handicaps
	Major organ transplantation		
Gastrointestinal disorders	Anorectal Conditions		
	Fecal Incontinency		
	Peptic ulcer		Has stomach or intestinal ulcers
	Enterovesical fistula		
	Cholangitis	If toxicity grade ≤ 2 Mild liver disease (2)	
	Cholelithiasis	If toxicity grade ≤ 2 Mild liver disease (2)	
	Chronic Liver Disease	If toxicity grade ≤ 2 Mild liver disease (2)	
		If toxicity grade > 2 Moderate or severe liver disease (4)	
Eye disorders	Cataract		Has cataract
	Glaucoma		Has glaucoma
Cardiovascular disorders	Congestive Heart Failure	Congestive Heart Failure (2)	Has heart failure
	Cerebrovascular accident		Suffers from the effect of stroke

Table 9: Medical Conditions of Interest, Medical Conditions of Interest for the Charlson Comorbidity Index, and Frailty Index

Organ system	Medical conditions of interest	CCI term (weight)	Medical conditions of interest for Frailty Index
	Central venous catheterization		
	Peripheral vascular disease		
	Hypertension		Has high blood pressure
	Myocardial infarction		Has history of myocardial infarction

The medical conditions of interest considered for eligibility as additional IED risk factors or risk factors for complicated UTI are presented in [Table 10](#).

For eligibility purposes under Inclusion Criterion 11, select medical conditions of any toxicity grade qualify as additional IED risk factors for study eligibility; however, they are required to have occurred within a specific timing relative to randomization as indicated in the middle column of [Table 10](#).

For stratification purposes at randomization, select medical conditions are only considered complicated UTI risk factors when they are present at a toxicity grading of 2 or above, as indicated in the rightmost column of [Table 10](#). Refer to [Table 11](#) for guidelines on toxicity grading.

Table 10: Medical Conditions Considered Complicated UTI (cUTI) Risk Factors for Inclusion Criteria 11 AND Requisite Toxicity Grading for Signant Health/IWRS Stratification

Medical Conditions	Timing of Medical Condition Relative to Randomization for Consideration as a cUTI Risk Factor for Inclusion Criterion 11	Toxicity Grading Required for Answering "yes" to the Signant Health/IWRS Stratification Question for cUTI Risk Factor Presence
Medullary sponge kidney	Present at baseline	If toxicity grade ≥ 2
Acute Renal Injury	Present at baseline, must be eligible per Inclusion Criterion 6 and Exclusion Criterion 4	If toxicity grade ≥ 2
Chronic kidney disease	Present at baseline	If toxicity grade ≥ 2
Hydronephrosis	Present at baseline	Any toxicity grade
Neurogenic Bladder	Present at baseline	If toxicity grade ≥ 2
Obstructive Uropathy	Present at baseline	Any toxicity grade
Ureteric strictures	Present at baseline	If toxicity grade ≥ 2
Urethral Strictures	Present at baseline	If toxicity grade ≥ 2
Urinary Retention	Present at baseline	Any toxicity grade
Urinary Tract Obstruction	Present at baseline	If toxicity grade ≥ 2
Urine incontinence	Present at baseline	If toxicity grade ≥ 2
Urolithiasis	Present at baseline	If toxicity grade ≥ 2
Vesicoureteral Reflux	Present at baseline	If toxicity grade ≥ 2
Voiding dysfunction	Present at baseline	If toxicity grade ≥ 2
Benign Prostatic Hypertrophy	Present at baseline	If toxicity grade ≥ 2
Pelvic prolapse	Present at baseline	If toxicity grade ≥ 2
Cystostomy	Present at baseline	Any toxicity grade
Nephrostomy	Present at baseline	Any toxicity grade
Urostomy	Present at baseline	Any toxicity grade

Table 10: Medical Conditions Considered Complicated UTI (cUTI) Risk Factors for Inclusion Criteria 11 AND Requisite Toxicity Grading for Signant Health/IWRS Stratification

Medical Conditions	Timing of Medical Condition Relative to Randomization for Consideration as a cUTI Risk Factor for Inclusion Criterion 11	Toxicity Grading Required for Answering "yes" to the Signant Health/IWRS Stratification Question for cUTI Risk Factor Presence
Nephrostomy tube placement	Present at baseline	Any toxicity grade
Renal transplant	Present at baseline, must be eligible per Exclusion Criterion 8	Any toxicity grade
Ureteric stent	Present at baseline	Any toxicity grade
Urinary catheterization	Present at baseline	Any toxicity grade
Nephrolithiasis	Present at baseline	If toxicity grade ≥ 2
Prostatic Adenocarcinoma	Current or prior, must be eligible per Exclusion Criterion 6	If toxicity grade ≥ 2
Tumours of the urinary tract	Current or prior, must be eligible per Exclusion Criterion 6	If toxicity grade ≥ 2
History of pyelonephritis	>14 days prior to randomization	If toxicity grade ≥ 2

Table 11: General Guidelines for Toxicity Grading

The following general guidelines will be applied (modified from FDA toxicity grading table based on Renal and Urinary Conditions of the CTCAE v5.0⁵⁰) for medical conditions of interest:

Grade 1	Mild	Aware of symptoms but easily tolerated; Does not interfere with activity; clinical or diagnostic observations only; intervention NOT indicated.
Grade 2	Moderate	Notable symptoms; Requires modification in activity or use of medications; (minimal, local or noninvasive intervention indicated).
Grade 3	Severe	Incapacitating symptoms; Inability to do work, or usual activities; hospitalization or prolongation of existing hospitalization indicated.
Grade 4	Potentially life-threatening	Life-threatening consequences; urgent intervention indicated.

10.17. Appendix 17: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

Amendment 7 (17 November 2023)

Overall Rationale for the Amendment: The main purpose of the amendment is to add an inclusion criterion that will require participants to have at least one additional risk factor for invasive *E. coli* disease (IED), beyond a history of UTI in the past 2 years. To date, the observed incidence of IED cases in the study has been lower than expected. With this change, the study population will include more individuals at higher risk of IED, thereby increasing the incidence of IED events in the study.

Additional risk factors for IED are defined as one or more of the following: 1) a history of urosepsis and/or *E. coli* bacteremia at any time prior to randomization, 2) a history of inpatient hospitalization (for a medical/surgical cause) in the two years prior to randomization, and/or 3) presence at baseline of at least one risk factor for complicated UTI of any toxicity grade. Recurrence of urosepsis and gram-negative bacteremia has been estimated to occur in approximately 2 to 10% of cases.^{3, 5, 28, 34, 35} Accordingly, history of urosepsis and/or *E. coli* bacteremia has been included among IED risk factors to capture during the study these recurrent bacteremic events, many of which will be IEDs. A recent meta-analysis found an association between prior hospitalization and increased risk of drug-resistant *E. coli*.⁷⁸ As antibiotic-resistant bacteremia has been associated with increased risk of recurrent bacteremia⁷⁷, the sponsor believes that enriching the population for individuals with prior hospitalization will increase the IED events in the study. Risk factors for complicated UTI, which includes functional and anatomical abnormalities of the urinary tract, are noted in a pre-existing protocol-defined list (Table 11) that is used as a stratification factor in E.mbrace. The diagnoses included on this list derive from FDA guidance on complicated urinary tract infections (US Department of Health and Human Services, Food and Drug Administration, Center for Drugs Evaluation and Research. May 2018. Guidance for Industry. Complicated Urinary Tract Infections: Developing Drugs for Treatment). Many of these complicated UTI risk factors have been previously identified as risk factors for blood stream infections,^{11, 30, 43} thus inclusion of participants with one or more of these medical conditions is expected to increase the risk of IEDs.

Secondly, an update was made to the serotyping algorithm for cases in which there are discordant results noted between the agglutination assay and whole genome sequencing (WGS) on repeat testing. Isolates that are characterized on repeat testing as 9V serotype by agglutination, but as non-9V serotype by WGS will now be classified as 9V serotype instead of non-typeable.

The changes made to the clinical protocol VAC52416BAC3001 as part of Protocol Amendment 7 are listed below, including the rationale of each change and a list of all applicable sections.

Section Number and Name	Description of Change	Brief Rationale
5.1 Inclusion Criteria, Criterion 11	An inclusion criterion has been added to require at least one additional risk factor for IED, beyond a history of UTI in the past 2 years.	To increase the incidence of IED events in the study.
8.1.1 Identification of Suspected IED Clinical Cases 8.5 Microbiological Assessments	An update was made to clarify that for suspected IED cases all <i>E. coli</i> isolates identified in urine should be sent to the central lab for further	To provide more comprehensive microbiologic assessments of suspected IED events.

Section Number and Name	Description of Change	Brief Rationale
	characterization, irrespective of quantification.	
8.5 Microbiological Assessments 10.15 Appendix 15: Decision Tree for Isolate Characterization	An update was made to the serotyping algorithm for cases in which there are discordant results noted between the agglutination assay and whole genome sequencing (WGS) on repeat testing.	This update was made following a health authority request.
5.4 Screen Failures	Wording was added to indicate that the investigator will not generate screening and enrollment logs directly from IWRS.	To align with the sponsor's most recent template update.
10.17 Appendix 17: Medical Conditions of Interest for the Charlson Comorbidity Index, the Frailty Index, and the Risk Factors for Complicated UTI	<i>E. coli</i> bacteremia was added as a medical condition of interest.	To add precision to the list of conditions of interest representing risk factors for IED.
Throughout the protocol	Minor changes to make the protocol consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.

Amendment 6 (20 July 2023)

Overall Rationale for the Amendment: The main purpose of this amendment is to remove exclusion criterion 5 to allow enrollment of participants on antimicrobial prophylaxis of UTI. The sponsor is of the opinion that these participants are at least at a similar risk of IED as participants with a history of UTI without such prophylaxis. In addition, changes have been made to the active surveillance.

The changes made to the clinical protocol VAC52416BAC3001 as part of Protocol Amendment 6 are listed below, including the rationale of each change and a list of all applicable sections.

Section Number and Name	Description of Change	Brief Rationale
Title page 1.1 Synopsis	Added study EU CTR number.	To comply with EU CTR requirements and sponsor template.
1.1 Synopsis EFFICACY EVALUATIONS 1.3.1 Schedule of Activities – Assessments for All Participants 4.1 Overall Design 8.1 Efficacy Assessments	Changes have been made to the active surveillance. Participant surveillance via eCOA will begin following the Day 30 visit and continue until study end or participant early withdrawal.	To ensure study sites are able to capture medical events meeting the criteria for study efficacy endpoints in a timely matter.
1.1 Synopsis, Objectives and Endpoints 3 OBJECTIVES AND ENDPOINTS	The medical resource utilization endpoint was updated to include ICU hospitalization and ICU length of stay.	To clarify that ICU hospitalization and ICU length of stay will be collected as well.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis, Objectives and Endpoints, Statistical Methods 3 OBJECTIVES AND ENDPOINTS 9.4.1.2 Secondary Endpoints	‘Any ExPEC O-serotype’ has been reworded to ‘ <i>E. coli</i> ’.	To clarify that cases with <i>E. coli</i> , for which serotype data is not available will be included in efficacy analyses, where indicated.
1.3.1 Schedule of Activities – Assessments for All Participants	The Schedule of Activities has been updated to indicate that vital signs may be collected at the early exit visit. This is only applicable if the visit is done on-site.	To clarify what needs to be collected at the early exit visit and to indicate which assessments are not applicable in case the early exit visit is done via telephone call.
1.3.1 Schedule of Activities – Assessments for All Participants	‘Provision of the Participant Wallet Card’ has been added to the Schedule of Activities.	To align the protocol with the sponsor’s most recent protocol template.
1.3.1 Schedule of Activities – Assessments for All Participants	A 4-day window has been added for the ePRO assessment for all visit days except Day 1.	To provide flexibility in case the electronic system is not working on the actual visit day.
1.3.1 Schedule of Activities – Assessments for All Participants	A footnote in the Schedule of Activities has been updated to indicate that visits marked with a ‘●’ should preferably be completed on the day of vaccination.	Clarification.
1.3.1 Schedule of Activities – Assessments for All Participants	‘MOPA’ was deleted from the following assessment in the SoA: “Blood sampling for MOPA assay management (at selected sites only)”.	Deleted for accuracy samples may also be used for the management of other assays and not just MOPA.
1.3.1 Schedule of Activities – Assessments for All Participants 6.8 Concomitant Therapy	Wording was added to indicate that antibacterials (including antibiotics and urinary tract antiseptics) administered up to 30 days before the immunogenicity sampling must be recorded.	Antibacterials may affect the sample analysis and should therefore be recorded.
1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification	Pre-IED baseline SOFA scores are to be captured if available.	To establish a baseline for SOFA.
1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification	Footnote ‘n’ was updated to align with wording in Section 8.1.1.	Correction.
1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case	Wording was added to indicate that immunogenicity blood samples for	To acknowledge that obtaining those samples may be challenging in some circumstances, it has been

Section Number and Name	Description of Change	Brief Rationale
Identification 1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1 Efficacy Assessments 8.1.1 Identification of Suspected IED Clinical Cases 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition 8.4 Immunogenicity Assessments	suspected systemic infections, UTI, or ABP should be taken if feasible.	clarified that this study procedure related to an exploratory endpoint should be performed if feasible.
1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria	Wording was added to footnote K to indicate that quantitative urine white blood cell count should be available.	To clarify that at minimum quantitative urine white blood cell count should be available.
1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP Per Clinical Case Definition Criteria 8.1.3 UTI Clinical Case Definition	Wording was added regarding the use of dipstick results to determine whether a case meets the UTI case definition.	To clarify that abnormal leukesterase results alone are not sufficient evidence of pyuria to meet the UTI case definition.
5.1 Inclusion Criteria – Inclusion Criterion 5	Inclusion criterion 5 was updated and the following sentence: In case of prolonged antibacterial treatment (>3 weeks), the last dose must have been administered >60 days prior to randomization.	After the removal of Exclusion Criterion 5, the sentence regarding prolonged antibacterial treatment is no longer relevant.
5.2 Exclusion Criteria -Exclusion Criterion 5	Exclusion criterion 5 was deleted.	To allow enrollment of participants on antimicrobial prophylaxis of UTI as they are at least at a similar risk of IED as participants with a history of UTI without such prophylaxis.
5.2 Exclusion Criteria -Exclusion Criterion 7	Exclusion criterion 7 was reworded to indicate a participant should be excluded if the participant has a known history of severe allergic reactions.	Clarification.
5.2 Exclusion Criteria -Exclusion Criterion 8	Exclusion criterion 8 was updated to delete repetitive wording.	To delete repetitive wording.
5.2 Exclusion Criteria -Exclusion Criterion 14	Wording for exclusion criterion 14 was updated to align with Section 6.8.	To clarify that enrollment in the VAC52416BAC3001 study is permitted if a potential participant

Section Number and Name	Description of Change	Brief Rationale
		is in the long-term follow-up phase of another investigational study
6.8 Concomitant Therapy	Text regarding the use of experimental medication, participation in another study, and vaccination with licensed vaccines was reworded for better readability	Clarification.
6.8 Concomitant Therapy	Corrected the statement about the use of antineoplastic and immunomodulating agents or radiotherapy. Administration is prohibited during the study and within 6 months before the planned administration of the first dose of study vaccine.	Correction and clarification.
8.1.2 IED Clinical Case Definition	A sentence was added to indicate that for the first primary endpoint, IED events from both <i>E. coli</i> detected in blood or other sterile sites and from <i>E. coli</i> detected only in urine will be included.	To align with other Sections of the protocol where this information is already included.
8.1.2 IED Clinical Case Definition	Greater than or equal to sign, which had been omitted previously, was added to the quantification of immature bands.	Correction.
8.1.2 IED Clinical Case Definition	Correction to the definition of tachypnea that meets criteria for the primary endpoint was added.	To correct the definition of tachypnea indicative of systemic infection to be consistent with the consensus for SIRS criteria established by the American College of Chest Physicians and the Society of Critical Care Medicine (as listed in Section 10.8, Appendix 8: Systemic Inflammatory Response Syndrome Score), “or PaCO ₂ <32 mmHg” was added to address this prior omission. The sponsor is of the opinion that this change will not affect the number of cases meeting the criteria for the primary endpoints.
8.1.4 ABP Clinical Case Definition	Wording was added to describe that a temperature of temperature $\geq 38.0^{\circ}\text{C}$ is considered a fever.	To define the word ‘fever’.
8.1.2 IED Clinical Case Definition 8.1.3 UTI Clinical Case Definition	Text regarding the availability of serotyping data was rephrased for better readability.	Clarification.

Section Number and Name	Description of Change	Brief Rationale
8.1.4 ABP Clinical Case Definition 9.4.1.1 Primary Endpoint		
8.1.2 IED Clinical Case Definition 9.4.1.1 Primary Endpoint	Text regarding the cases to be used for the efficacy analyses was rephrased for better readability	Clarification.
8.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information	Wording was added to indicate that deaths will be reported as SAEs according to the general study guidelines for SAE reporting.	Clarification.
8.5 Microbiological Assessments 10.15 Appendix 15: Decision Tree for Isolate Characterization	The decision tree for isolate characterization and the corresponding text has been updated following a request from a health authority.	Upon health authority request.
9.5.1.1 Interim Analyses for the Fist Primary Endpoint	Footnote B was removed from Table 4.	Correction.
10.9 Appendix 9: Sequential Organ Failure Assessment Score	Corrections were made in Table 9.	Correction.
1.3.1 Schedule of Activities – Assessments for All Participants 2.3 Benefit-risk Assessment 10.14 Appendix 14: Guidance on Study Conduct During a Natural Disaster	COVID-19 specific requirements and the COVID-19 specific appendix (Appendix 14) have been removed.	COVID-19 specific language has been removed from the protocol as the pandemic has ended.
10.17 Appendix 17: Medical Conditions of Interest for the Charlson Comorbidity Index, the Frailty Index, and the Risk Factors for Complicated UTI	Pyelonephritis and nephrolithiasis were added as medical conditions of interest for risk factors for complicated UTI.	To add precision to the list of conditions of interest representing risk factors for cUTIs and IEDs.
10.19 Appendix 19: Country/Territory-specific Requirements	Appendix 19 has been removed.	A separate protocol will be generated for the EU countries to align with new EU CTR.
7.2 Participant Discontinuation/Withdrawal from the Study 8.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information 10.4.4 Special Reporting Situations 10.4.5 Procedures	Changes were implemented to align the VAC52416BAC3001 with the sponsor's current protocol template.	To implement changes from the sponsor's current protocol template.
Throughout the protocol	Minor changes to make the protocol consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.

Amendment 5 (2 December 2022)

Overall Rationale for the Amendment: The main purpose of this amendment is to update the study design to allow for continuous enrollment instead of staggered enrollment and to add another mode of ePRO data collection if a participant is unable or unwilling to work with the eCOA system.

The changes made to the clinical protocol VAC52416BAC3001 as part of Protocol Amendment 5 are listed below, including the rationale of each change and a list of all applicable sections.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis Overall Design 1.2 Schema 1.3.1 Schedule of Activities – Assessments for All Participants 4.1 Overall Design 4.2 Scientific Rationale for Study Design 4.4 End of Study Definition 6.3 Measures to Minimize Bias: Randomization and Blinding 8 Study Assessments and Procedures 8.2 Safety Assessments 8.4 Immunogenicity Assessments 9.2.2 Immunogenicity Subset 9.2.3 Safety Subset 9.5.1 Interim Analyses 9.5.1.1 Interim Analyses for the First Primary Endpoint	The study design has been updated to allow for continuous enrollment instead of staggered enrollment.	Initiation and ramp-up of sites and recruitment was more challenging than expected. Pausing the enrollment after getting the 9,278 randomized participants would disrupt the ongoing site activity. The sponsor believes that restarting recruitment after the pause would pose feasibility challenges and thus is now opting for continuous enrollment instead of staggered enrollment. The regular safety oversight by IDMC mitigates additional vaccine exposure before the first analysis for futility.
1.3.1 Schedule of Activities – Assessments for All Participants 1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8 Study Assessments and Procedures	Footnote 1 of the Schedule of Activities (Section 1.3.1) was updated to remove the sentence regarding the order of procedures when screening procedures are split over different days.	The requested order of screening procedures was not possible when split over different days.

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Schedule of Activities – Assessments for All Participants 1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 5.1 Inclusion Criteria 8 Study Assessments and Procedures 8.15 Patient-reported Outcomes 10.3.10 Source documents	Collecting and recording the ePRO assessments via interview mode, available through the eCOA system, has been added as an option for participants who are unable or unwilling to use the eCOA system on their own. For participants who elect to use the interview as their primary mode of data collection, it is recommended that this remains their primary mode throughout the duration of the clinical study.	To accommodate participants who are unable or unwilling to work with a digital system. The PRO questionnaires have documented equivalence to allow for both the patient-facing and interview modes to be used.
5.1 Inclusion Criteria	Inclusion criterion 9 has been removed.	This criterion is no longer applicable as a caregiver may assist the participant and the participant may opt for interview mode if the participant is unable or unwilling to use the eCOA system.
1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Wording was updated to indicate that a full urinalysis, using microscopy or urine flow cytometry, is a mandatory study procedure for UTI/ABP Day 1.	Clarification.
1.3.1 Schedule of Activities – Assessments for All Participants 1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 1.3.3 Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria	The Schedule of Activities was updated to indicate that scheduled visits which cannot be conducted in person at the study site may be performed as home health visit. In addition, decentralized sites may conduct the Day 1 visit as home health visit.	To allow home health visits when a participant is not able to visit the study site (eg, if a participant is sick). This is allowed for visits for which there are no study procedures requiring the in-person presence of the participant at the site.

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Schedule of Activities – Assessments for All Participants 8 Study Assessments and Procedures	Collection of an additional blood sample has been added to the Schedule of Activities. This blood sample will only be collected at selected sites and at the discretion of the PI.	An additional blood sample is required to generate reference samples to support MOPA assay management.
1.1 Synopsis Benefit-risk Assessment	Wording regarding the benefit-risk assessment has been added to the synopsis.	Update to the sponsor's most recent template.
2.2 Background	An update has been provided regarding the first IDMC review.	To provide an update on the first IDMC meeting.
1.1 Synopsis Number of Participants 1.1 Synopsis Safety Evaluations 4.1 Overall Design 8.2 Safety Assessments 9.5.1 Interim Analyses	A note has been added to indicate that over enrollment may occur due to local requirements.	To allow for over enrollment in the study by enrolling a specific number or proportion of participants coming from a given country/territory, as requested by local health authorities. This will only occur if global enrollment has been completed but locally required enrollment numbers are not fulfilled.
5.1 Inclusion Criteria	Inclusion criterion 5 was amended to indicate that a qualified healthcare provider may also make the diagnosis. In addition, text previously included in exclusion criterion 5 has been moved to inclusion criterion 5 as the sentence did not fit as part of the exclusion criterion.	The criterion was updated to use a more general term as the diagnosis is not always made by a physician (eg, an advanced nurse practitioner may make the diagnosis in the United States).
5.2 Exclusion Criteria	Exclusion criterion 3 has been modified to clarify what is meant with 'difficulty to perform blood draws'	Clarification.
5.2 Exclusion Criteria	Text has been added to exclusion criterion 5 to clarify what is meant with 'chronic use' of antibacterials.	Clarification.
5.2 Exclusion Criteria	Wording for exclusion criterion 6 has been updated from "not in the following categories" to "that does not include the following categories". In addition, wording was added to clarify that participants who underwent radical prostatectomy or radiotherapy may be enrolled at the discretion of the investigator if treatment has been completed 6 months prior to the planned administration of the study vaccine.	Health Authority request. Clarification.
5.2 Exclusion Criteria 6.8 Concomitant Therapy	Exclusion criterion 8 has been modified to shorten the washout period (from 6 months to 3 months) of systemic corticosteroids. In addition, changes were made to clarify what is considered to be a substantially immunosuppressive steroid dose and to align the criterion with the sponsor's most recent protocol template.	Upon reconsideration, the washout period was considered too long by the sponsor and the sponsor's protocol template was updated accordingly. Clarification and update to the sponsor's most recent template.

Section Number and Name	Description of Change	Brief Rationale
5.2 Exclusion Criteria	Exclusion criterion 11 was updated to allow a washout period of 3 months instead of 4 months for blood products. A note has been added to exclusion criterion 11 to indicate that the use of certain immunoglobulins/monoclonal antibodies (mAbs) may be allowed after sponsor approval.	This was adapted in the most recent sponsor template as the red blood cell cycle is 3 months. To allow participants to be enrolled if they use mAbs which don't impact the vaccine-induced immune response.
2.3 Benefit-risk Assessment 5.2 Exclusion Criteria	Exclusion criterion 12 was modified to indicate that vaccination with any vaccine authorized for emergency use (eg, EUA, CMA, or a similar program) (not limited to COVID-19 vaccines) is permitted when given at least 28 days before or after planned administration of the study vaccination.	With the amount of scientific data collected for the licensed COVID-19 vaccines, the criterion has been adapted to keep a 28-day window only for the vaccines authorized for emergency use. For the licensed COVID-19 vaccines, the standard windows will apply.
6.8 Concomitant Therapy	Redundant information was removed from this section.	Clarification.
5.1 Inclusion Criteria 5.2 Exclusion Criteria	The word 'criteria' was replaced for the term 'guidelines' in inclusion criterion 6 and exclusion criteria 16 and 17.	Correction.
1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 8.1.1 Identification of Suspected IED Clinical Cases	Wording has been added to clarify that follow-up of the suspected IED case should also be performed for cases for which confirmation of the IED diagnosis is still pending but for <u>which the collected cultures are positive for <i>E. coli</i> or <i>P. aeruginosa</i>.</u>	Clarification
8.1.3 UTI Clinical Case Definition	The signs and symptoms for complicated UTIs and pyelonephritis were updated to clarify that fever alone qualifies as symptom.	Clarification.
8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	A statement was added to clarify that a full urinalysis by microscopy or flow cytometry is a mandatory study procedure on UTI/ABP Day 1.	Clarification.
8 Study Assessments and Procedures 8.1.5 Patient-reported Outcomes	Text regarding the eDiary has been moved from Section 8.1.5 to Section 8 where the eDiary is discussed.	Clarification.
1.3.1 Schedule of Activities – Assessments for All Participants 1.3.2 Schedule of Activities – Assessments for Participants with Suspected IED per Clinical Case Identification 8.1.6 Frailty Assessments	The Charlson Comorbidity Index score will be calculated in addition to the Frailty Index.	To further characterize the study population.

Section Number and Name	Description of Change	Brief Rationale
8.3.6 Disease-related Events and Disease-related Outcomes not Qualifying as Adverse Events or Serious Adverse Events	The text was updated to remove non-serious AEs.	Non-serious AEs were removed from this section as these AEs are only reported from Day 1 to Day 30 and the per-protocol efficacy analysis will remove any events before Day 30. As such, there is little to no overlap between AEs and the clinical endpoints.
8.5 Microbiological Assessments 10.16 Appendix 16: Decision Tree for Isolate Characterization	Text regarding the O-serotype testing has been updated with additional testing using the O-agglutination assay per FDA advice.	To align with Health Authority feedback.
10.1 Appendix 1: Abbreviations and Definitions	New definitions were added.	Clarification.
10.7 Appendix 7: Specific Criteria for the Inclusion of Potential Participants with Chronic Stable HIV, HCV, or HBV Infection	The specific criteria for the inclusion of potential participants with chronic stable HIV have been updated.	To allow for alignment of the definition of stable HIV disease with the current approach, in different regions, to HIV diagnosis and management.
10.14 Appendix 14: Guidance on Study Conduct During a Natural Disaster	Wording has been added to clarify that symptoms of a COVID-19 episode should not be described separately as (S)AEs. However, complications or sequelae of the COVID-19 episode should be reported separately as (S)AEs. In addition, the appendix heading was modified.	Clarification.
10.18 Appendix 18: Medical Conditions of Interest for the Charlson Comorbidity Index, the Frailty Index, and the Risk Factors for Complicated UTI	An appendix has been added to indicate how the Charlson Comorbidity Index score, Frailty Index score, and the risk factors for complicated UTI will be derived from the medical conditions of interest.	For completeness.
10.19 Appendix 19: Country/Territory-specific Requirements	A new appendix has been added describing country/territory-specific requirements.	This appendix was added to create a consolidated protocol, per the new EU CTR requirements, to contain all country/territory-specific information.
Throughout the protocol	Minor changes to make the protocol consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.

Amendment 4 (02 June 2022)

Overall Rationale for the Amendment: The overall reason for the amendment is to implement a request from the Health Authorities to clarify the wording of the second primary endpoint and to implement protocol clarifications.

The changes made to the clinical protocol VAC52416BAC3001 as part of Protocol Amendment 4 are listed below, including the rationale of each change and a list of all applicable sections.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis Objectives and Endpoints 1.1 Synopsis Statistical Methods 3 Objectives and Endpoints 8.1.2 IED Clinical Case Definition 9.4.1.1 Primary Endpoint	The second primary endpoint was modified to clarify that invasive extraintestinal pathogenic <i>Escherichia coli</i> disease (IED) cases with microbiological confirmation from urine only are excluded.	To comply with the request from the Health Authorities to clarify the wording of the second primary endpoint.
1.1 Synopsis Objectives and Endpoints 3 Objectives and Endpoints 8.1.4 ABP Clinical Case Definition	An exploratory objective/endpoint was added to include efficacy evaluation of ExPEC9V in the prevention of the first acute bacterial prostatitis (ABP) event caused by any ExPEC O-serotype.	To include efficacy evaluation of ExPEC9V in the prevention of the first ABP event caused by any ExPEC O-serotype to have consistent evaluations for all the efficacy endpoints.
1.1 Synopsis Objectives and Endpoints 3 Objectives and Endpoints 8.1.4 ABP Clinical Case Definition	An exploratory objective/endpoint was modified to include efficacy evaluation of ExPEC9V in the prevention of the first <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>) ABP event.	To include efficacy evaluation of ExPEC9V in the prevention of the first <i>P. aeruginosa</i> ABP event to have consistent evaluations for all the efficacy endpoints.
8.1.2 IED Clinical Case Definition	Included sequential organ failure assessment (SOFA) criteria as an alternative to systemic inflammatory response syndrome (SIRS) criteria and updated the footnote for urinary tract infection (UTI) signs and symptoms for urosepsis cases. During Independent Endpoint Adjudication Committee (IEAC) review, the IEAC reviewers need to decide if the collected data are pertinent to be included in the case definition evaluation for the same IED event. Added a schematic diagram for visual representation of the IED clinical case definition.	SOFA criteria were added as an alternative to SIRS criteria to include in the IED definition cases meeting sepsis criteria when documentation of SIRS criteria may be lacking. To explain how the SOFA and UTI signs/symptoms are to be taken into account in the IED clinical case definition. To visualize and explain the IED clinical case definition.
8.1.2 IED Clinical Case Definition 10.9 Appendix 9: Sequential Organ Failure Assessment Score	Added that in case multiple SOFA scores are available before the onset of systemic infection, the acute change will be counted from the baseline score defined as the lowest score over the last 72 hours preceding the diagnosis of systemic infection.	To define the baseline SOFA score to be used for calculation of an acute change in case multiple SOFA scores are available.
5.1 Inclusion Criteria	Inclusion criterion 3 was modified to clarify the requirements regarding willingness to share relevant medical records and medical information.	To clarify that no specific medical records are required for medical history of UTIs.

Section Number and Name	Description of Change	Brief Rationale
5.1 Inclusion Criteria	Inclusion criterion 5.1 was updated to clarify that the investigator decides if the available documentation provides adequate evidence of the history of UTI. A clarifying note was added to explain ‘evidence of diagnosis’.	To clarify expectations with regards to the documentation of the medical history of UTI.
1.3.1 Part 1 and 2 5.2 Exclusion Criteria 6.8 Concomitant Therapy	Exclusion criterion 5 was modified from “chronic use of antimicrobials” to “chronic systemic use of antibacterials (including antibiotics and urinary tract antiseptics)”. This wording was modified throughout the protocol.	To clarify that chronic use of antimicrobials only includes systemic antibacterials and does not include, eg, antivirals, antifungals, antiparasitics.
5.2 Exclusion Criteria	Rephrased exclusion criterion 8.1 for clarification.	To avoid inclusion of participants with clinical conditions stable under treatment of prohibited therapies.
5.2 Exclusion Criteria	Footnote was added to exclusion criterion 10 to clarify what is considered an <i>Escherichia coli</i> (<i>E. coli</i>) vaccine.	To clarify which medicinal products are included in the protocol definition of an <i>E. coli</i> vaccine.
5.2 Exclusion Criteria	Added to exclusion criterion 14 that after sponsor approval, enrollment is permitted if a potential participant is in the long-term follow-up phase of an investigational study (ie, without further administration of an investigational study drug and without procedures that could interfere with the current study evaluations) and that participation in an investigational study from 6 months after administration of the study vaccine onwards is allowed with prior approval of the sponsor.	To adjust eligibility criteria for circumstances not having impact on the study evaluations.
1.3.1 Part 1 and 2 4.1 Overall Design 8.1 Efficacy Assessments	The Schedule of Activities (SoA) was modified to clarify that also in the beginning of the study a surveillance contact must be scheduled at a minimum every 3 months and that surveillance contacts must be documented and recorded in the electronic case report form (eCRF).	Clarification and instruction to document surveillance contacts in the eCRF.
1.3.3 Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Clarified that urinalysis and urine cultures should be performed as study procedures on UTI/ABP-Day 1, if not part of standard of care. If on Day 1 the urinalysis (or dipstick) does not confirm a UTI or ABP, the urine culture does not need to be performed. For the outcomes of recovery for UTI/ABP, negative urine cultures were removed. For the outcome of UTI, asymptomatic bacteriuria (with or without pyuria) was removed.	To clarify when urine culture is required on Day 1, to clarify that recovery of UTI/ABP will not require a confirmatory urine culture for all the cases, and to remove the potential UTI outcome of asymptomatic bacteriuria.
8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	The cut-off for the urine sediment microscopy results was added to the definition of pyuria, ie, ≥ 5 white blood cells per high-power field (WBC/HPF) (400x enlargement).	To update the definition of pyuria in case microscopy is performed on urine sediment as the protocol allows for either urine microscopy or urine flow cytometry for pyuria analysis.

Section Number and Name	Description of Change	Brief Rationale
8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Clarified that data from all clinically diagnosed UTI/ABP cases (regardless of microbiological confirmation) as well as cases of suspected UTI/ABP for which antibacterial treatment has been initiated must be collected and reported in the eCRF.	To clarify which cases of UTI/ABP need to be reported in the CRF.
1.3.3 Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Distinguished UTI/ABP cases meeting endpoint criteria from clinically diagnosed UTI/ABP cases and clarified which study-specific procedures to be followed when. Added that for pyelonephritis, the IEAC will review all the cases diagnosed by the investigator as pyelonephritis (regardless of meeting protocol criteria) as well as the cases that would be identified based on the protocol case definition. All pyelonephritis cases confirmed by the IEAC will be used in the efficacy analysis.	Clarification on UTI/ABP cases and its study-specific procedures.
1.3.2 Participants With Suspected IED per Clinical Case Definition 8.1.1 Identification of Suspected IED Clinical Cases	Clarified which study-specific procedures need to be performed when a systemic infection is suspected by the investigator (as long as a systemic infection cannot be excluded) and added that the follow-up of the suspected IED case should be performed as well for the cases for which confirmation of the IED diagnosis is still pending. The Medical Monitor should be contacted for further guidance.	Clarification on study-specific procedures to be followed when a systemic infection is suspected by the investigator.
1.3.2 Participants With Suspected IED per Clinical Case Definition 1.3.3 Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1.1 Identification of Suspected IED Clinical Cases 8.1.2 IED Clinical Case Definition 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	“Day of discharge from hospital” was changed to “Day of resolution (of IED signs and symptoms)”, and for alignment throughout the protocol “Day of recovery from UTI/ABP” was changed to “Day of resolution of UTI/ABP signs and symptoms”.	To align definitions of Day 29 with UTI/ABP endpoints to make sure the follow-up visit is performed at the earliest on the day of IED recovery, while discharge from the hospital may occur earlier.
8.1.1 Identification of Suspected IED Clinical Cases 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Added that <i>E. coli</i> or <i>P. aeruginosa</i> isolates (if retained and available) will be sent to the central laboratory when originating from cultures positive for <i>E. coli</i> or <i>P. aeruginosa</i> (for urine cultures only if $\geq 10^5$ CFU/mL) and that blood samples for immunogenicity assessments will be sent to the central laboratory for participants meeting the IED clinical case definition criteria (see Section 8.1.2), including participants who test positive for <i>P. aeruginosa</i> .	To clarify which samples for microbiological or immunogenicity assessments should be sent to the central laboratory.

Section Number and Name	Description of Change	Brief Rationale
1.3.2 Participants With Suspected IED per Clinical Case Definition 1.3.3 Participants with UTI (Including Pyelonephritis) 8.1 Efficacy Assessments 8.1.1 Identification of Suspected IED Clinical Cases 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Clarified that an immunogenicity sample is to be collected within approximately 48 hours after a systemic infection is suspected by the investigator or after UTI/ABP diagnosis.	To clarify when an immunogenicity sample needs to be collected if it could not be collected prior to treatment initiation.
1.1 Synopsis Objectives and Endpoints 1.1 Synopsis Efficacy Evaluations 1.1 Synopsis Statistical Methods 3 Objectives and Endpoints 8.1 Efficacy Assessments 8.1.2 IED Clinical Case Definition 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition 9.4.1.1 Primary Endpoint	Subserotypes A or B were removed from O-serotypes O1A, O6A, O18A, O25B that need to be available for the primary and secondary endpoints, and the ABP endpoint.	All isolates expressing ExPEC9V O-serotypes cannot be typed to the subserotype level with agglutination O-serotyping, and the subserotype also cannot be predicted from Wzx/Wzy DNA sequences. As these assays will not determine subserotype, only the O-serotype will be reported for characterized isolates.
1.1 Synopsis Objectives and Endpoints 1.3.1 Part 1 and 2 3 Objectives and Endpoints 4.2 Scientific Rationale for Study Design 4.2.1 Study-specific Ethical Design Considerations 8.7 Participant Medical Information Prior to, During, and After the Study (Real-world Data)	Addition of the utilization of tokenization and matching procedures to obtain medical data 5 years prior to enrollment of the participant until 5 years after the participant completed the study from consenting participants in the United States.	Participant medical data (electronic health records, claims, laboratory data from other care settings) prior to, during and following participation in the study (real-world data) is important to obtain in order to better understand the impact of prior medical history on the response to immunization and the impact of immunization on efficacy and duration of efficacy, or any other long-term outcome, as well as adverse events (AEs) that may occur during and after completion of the study.
10.7 Appendix 7 Specific Criteria for the Inclusion of Potential Participants with Chronic Stable HIV, HCV, or HBV Infection	Added that potential subjects will not be screened for laboratory evidence of hepatitis C virus (HCV) or hepatitis B virus (HBV) infection and that all laboratory data mentioned in the guidance is not required in the medical records to evaluate status of HCV or HBV infection.	Modified the guidance for the investigator to clarify the expected decision process for assessment of eligibility of patients with HCV and HBV infections.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis Overall Design 1.3.1 Part 1 and 2 4.1 Overall Design 5 Study Population	Added an 8-day window for pre-vaccination procedures and clarified that if screening procedures are split over different days, eligibility criteria must be rechecked, pre-vaccination vital signs and pre-vaccination blood samples for immunogenicity should be collected on the day of vaccination (Day 1) and that electronic patient-reported outcomes must be performed as first procedure of the first day.	To allow screening activities to be performed on a different day than randomization/vaccination.
1.3.1 Part 1 and 2	Addition of footnote to include collection of the participant's current living situation as part of demographics in the SoA.	To include the collection of the participant's current living situation in the eCRF.
1.3.1 Part 1 and 2 1.3.2 Participants With Suspected IED per Clinical Case Definition Study Assessments and Procedures 1.3.3 Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8 Study Assessments and Procedures 8.1.5 Patient-reported Outcomes 10.3.10 Source Documents	Added text throughout the protocol that, if the electronic clinical outcome assessment (eCOA) system is unavailable, eCOA data may be captured via for example, but not limited to, collection on paper by site staff via interviewer mode.	To clarify an alternative way of eCOA data capture if the eCOA system is unavailable.
1.3.1 Part 1 and 2 8.2.1 Physical Examinations	Clarified that no full physical examination is required at screening, but a general physical examination needs to be performed at the investigator's discretion.	To clarify it is at the investigator's discretion how detailed the physical examination is deemed needed at screening.
1.3.1 Part 1 and 2	Added to the SoA that solicited AE recording is continuous through Day 15.	To clarify that solicited AEs are recorded daily through Day 15.
1.3.1 Part 1 and 2 8.2.2 Vital Signs	Added that vital signs are to be measured before vaccination (and preferably before blood draws) AND after the end of the 30-minute observation period (no later than 45 minutes after vaccination).	To clarify that after the end of the 30-minute observation period after vaccination vital signs are to be measured in alignment with the SoA.
6.8 Concomitant Therapy 7.2 Participant Discontinuation/Withdrawal From the Study	Clarified that in case a participant is in need of prohibited therapies, the concerned participant should not be withdrawn from the study but may be excluded from the per protocol efficacy population and/or per protocol immunogenicity population, and that any medication (whether licensed or experimental) that is prescribed by a treating physician as a treatment of any condition, will have priority over protocol restrictions.	To clarify that if a participant is in need of prohibited concomitant medications, this does not represent a reason for participant's withdrawal from the study.

Section Number and Name	Description of Change	Brief Rationale
9.3 Populations for Analyses	Added that participants reported with a major protocol deviation impacting the efficacy/immunogenicity outcomes will be excluded from the per protocol efficacy and/or the per protocol immunogenicity populations from the date of the major protocol deviation onwards.	To clarify the time of exclusion from analysis populations when a participant is reported with a major protocol deviation that impacts the efficacy/immunogenicity outcomes.
10.15 Appendix 15: Antibacterial Agents for Susceptibility Testing	Added plazomicin to the antibiotic panel.	Plazomicin is a common antibiotic for the treatment of UTIs and was added for completeness based on its availability in central laboratories.
2.2 Background	Aligned status of the ExPEC10V studies with the current edition of the ExPEC10V/ExPEC9V Investigator's Brochure (IB).	To align with current edition of the ExPEC10V/ExPEC9V IB.
Throughout the protocol	Changes to implement clarifications from the sponsor's current protocol template.	To implement clarifications from the sponsor's current protocol template.
Throughout the protocol	Minor changes to make the protocol consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.

Amendment 3 (20 September 2021)

Overall Rationale for the Amendment: The overall reason for the amendment is to comply with the requirement of some Regulatory Authorities to expand the evaluation of the safety data in the first 500 randomized participants and to implement at the same time other requests from the Regulatory Authorities.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis Overall Design 1.2 Schema 4.1 Overall Design 4.2 Scientific Rationale for Study Design	Description of the details and type of data to be reviewed by the Independent Data Monitoring Committee (IDMC) have been updated. The safety data to be assessed by the IDMC once the first 500 randomized participants have completed the Day 30 visit (Visit 3), or discontinued earlier, will include serious adverse events (SAEs) and unsolicited adverse events (AEs) from all participants and solicited AEs from participants in the Safety Subset.	To comply with the request from the European Medicines Agency (EMA) and Food and Drug Administration (FDA) to expand the safety data in the first 500 randomized participants.
1.1 Synopsis Description of Vaccinations 6.1 Study Vaccine Administered	Text regarding the actual dose of polysaccharides (PSs) and carrier protein, a genetically detoxified form of exotoxin A derived from <i>Pseudomonas aeruginosa</i> (EPA), in ExPEC9V has been added to the protocol. The PS content of the dose to be tested in the present study is 88 µg/dose. The EPA concentration in the currently manufactured ExPEC9V clinical batches is 290 µg/dose. The maximum allowed EPA concentration in ExPEC9V is 382.5 µg/dose.	To comply with the requirements of the Medicines and Healthcare Products Regulatory Agency (MHRA) of including the actual PSs and EPA content in ExPEC9V.
4.3 Justification for Dose	Added text and Table 1 (new) to include the concentration of PSs and EPA in the low, medium, and high doses of ExPEC10V in study VAC52416BAC1001, and the concentration of PSs and EPA of ExPEC9V in study VAC52416BAC3001. All subsequent table numbers are updated.	To comply with the requirements of MHRA of updating the dose rationale.
6.3 Measures to Minimize Bias: Randomization and Blinding	Added text to describe the stratification rules for the first 500 participants. In addition, a cap of 100 participants aged ≥60 to <65 years will be installed for the first 500 participants.	To clarify the stratification rules as per request from EMA to expand safety data for the first 500 participants.
4.4 End of Study Definition	Adapted the end of study definition to a clear and unambiguous definition.	To comply with the request from the Danish Health Authority to update the end of study definition.
1.1 Synopsis Description of Vaccinations 6.3 Measures to Minimize Bias: Randomization and Blinding	Added text to describe that in case of positive efficacy results of the first primary endpoint, the study will not be stopped, but follow-up of participants will be continued for efficacy (interim and final analyses of the second primary endpoint and secondary endpoints), safety, and immunogenicity.	To comply with the request from EMA to clarify that the study will not stop after successful first primary endpoint.

Section Number and Name	Description of Change	Brief Rationale
8.1.2 IED Clinical Case Definition 9.4.1.1 Primary Endpoint	Rephrased text to indicate that cases with multiple pathogens in the urine culture results will not be considered for the primary and secondary endpoints if the systemic infection cannot be attributed only to <i>Escherichia coli</i> (<i>E. coli</i>), as per Independent Endpoint Adjudication Committee judgment. Cases with mixed pathogens and the presence of <i>E. coli</i> in urine cultures will be included in sensitivity analyses. All cases with multiple pathogens in the culture results from blood or any other sterile site will be included in the efficacy analysis for the primary and secondary endpoints.	To comply with the request from FDA to include in the efficacy analysis for the primary endpoints all cases of invasive extraintestinal pathogenic <i>E. coli</i> disease (IED) where <i>E. coli</i> is detected in blood or another sterile site, regardless of whether such sterile site cultures demonstrate the presence of additional pathogens.
1.1 Synopsis Objectives and Endpoints 1.1 Synopsis Overall Design 3 Objectives and Endpoints 4.1 Overall Design 8.1.2 IED Clinical Case Definition 8.5 Microbiological Assessments 10.17 Appendix 17: Sterile Sites' Definition	Added appendix with sterile sites' definition and referred to it in text.	To comply with the request from EMA and FDA to include a defined case definition of IED and <i>E. coli</i> detected in a sterile site.
1.1 Synopsis Overall Design 4.1 Overall Design	Added text to indicate that in case of serious safety concerns during the IDMC safety review once 500 randomized participants have completed the Day 30 visit (Visit 3), the IDMC may recommend the sponsor to stop the study.	To comply with the request from EMA to clarify what will be done in case serious safety concerns are raised during the IDMC safety review once 500 randomized participants have completed the Day 30 visit (Visit 3).
6.8 Concomitant Therapy 9.3 Populations for Analyses	Added text to indicate that participants who receive other <i>E. coli</i> vaccines will be excluded from the per protocol efficacy population and the per protocol immunogenicity population.	To comply with the request from FDA to exclude subjects who receive other <i>E. coli</i> vaccines from the per protocol efficacy population.
4.1 Overall Design 6.3 Measures to Minimize Bias: Randomization and Blinding 9.4.1.1. Primary endpoint	Rephrased text to indicate that the presence of at least one risk factor for complicated urinary tract infection (cUTI) (instead of risk factors for cUTIs) at baseline will be used to stratify the randomization.	To comply with the request from EMA to specify that randomization will be stratified according to the presence of at least one risk factor for cUTI at baseline.

Section Number and Name	Description of Change	Brief Rationale
1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3. Participants With UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1.1 Identification of Suspected IED Clinical Cases 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition 8.5 Microbiological Assessments	Added text to indicate that special diligence should be applied to record the time of antibiotic administration relative to collection of cultures.	To comply with the request from FDA to try to record the time of antibiotic administration relative to the collection of cultures.
5.1 Inclusion Criteria	Deleted the reference to the urinary tract infection (UTI) case definition in Section 8.1.3 in inclusion criterion 5.	To avoid confusion between the UTI case definition and the requirements for documentation of a history of UTI in inclusion criterion 5.
5.2 Exclusion Criteria	Rephrased text in exclusion criterion 6 (bullet a) to specify that participants with squamous and basal cell carcinomas of the skin and carcinoma in situ of the cervix may only be enrolled if they were curatively treated.	To avoid inclusion of participants with metastasized squamous cell carcinoma of the skin or other carcinomas that were not effectively treated.
5.2 Exclusion Criteria	Deleted “or systemic corticosteroids” from bullet c in exclusion criterion 8.	To avoid redundancy with bullet b in exclusion criterion 8 concerning the use of systemic corticosteroids.
6.8 Concomitant Therapy	Added text for instances in which prohibited therapies are administered: “All such instances will be recorded as protocol deviations, but these will not exclude the concerned participant from further study participation, unless other conditions apply (see Section 7.2).”	To clarify that participants who received prohibited therapies will not be excluded from the study unless other conditions apply.
1.3.2 Participants With Suspected IED per Clinical Case Identification 8.1.1 Identification of Suspected IED Clinical Cases	Added lactate to the serum chemistry panel in the assessment of IED cases.	To include serum lactate in the assessment of IED cases as this is a key parameter to evaluate the severity of the disease.
1.3.3 Participants With UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria	Deleted text indicating that frailty will be assessed at the time of UTI/ABP diagnosis based on the Frailty Index.	To align the data collection to assess frailty with the secondary objective to assess the degree of frailty in participants who received ExPEC9V versus placebo at baseline, Year 1, Year 2, Year 3, and at the time of an IED, and accompanying endpoint.

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Part 1 and Part 2 1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8 Study assessments and procedures 8.1.5 Patient-reported outcomes 10.3.3 Informed Consent Process 10.3.10 Source documents	Added text throughout the protocol that electronic clinical outcome assessment (eCOA) data include electronic patient-reported outcome (ePRO) and electronic diary (eDiary) data and that these data may be captured in an alternative way if the eCOA capture system is unavailable, upon agreement with the sponsor.	To clarify that eCOA data include ePRO and eDiary data and that these data may be captured in an alternative way if the eCOA capture system is unavailable, upon agreement with the sponsor.
1.3.1 Part 1 and Part 2 1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8 Study assessments and procedures	Adapted text to clarify the timing for completion of ePRO assessments.	To clarify the timing for completion of ePRO assessments.
1.3.2 Participants With Suspected IED per Clinical Case Identification 8.1.1 Identification of Suspected IED Clinical Cases 1.3.3 Participants With UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria 8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	The urinalysis tests required for IED case evaluation were changed from “white blood cell (WBC) count, red blood cell count, proteins, and gram staining” to “at minimum WBC count”. Added text to indicate that at minimum WBC count should be available for the urinalysis for the UTI/acute bacterial prostatitis (ABP) case evaluation on Day 1 and Day 29.	To specify that the only urinalysis parameter required for evaluation of UTI/ABP case definitions is WBC count.
8.1.3 UTI Clinical Case Definition 8.1.4 ABP Clinical Case Definition	Added text to indicate what data should be collected in the electronic case report form (eCRF) for UTI/ABP events without confirmed presence of either <i>E. coli</i> or <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>).	To clarify what data should be collected in the eCRF for UTI/ABP events without confirmed presence of either <i>E. coli</i> or <i>P. aeruginosa</i> .
8.3.5 Pregnancy	Deleted optional sponsor template text indicating that follow-up information regarding any postnatal sequelae in the infant will be retrieved, if possible.	To be consistent with the company process.

Section Number and Name	Description of Change	Brief Rationale
10.4.5 Procedures	Deleted text: “Suspected cases of IED, UTI, or ABP (that would meet the definition of an SAE based on ICH and EU Guidelines) are not to be reported as an SAE during the assessment/adjudication period. All confirmed cases of IED, UTI, or ABP which are not related to <i>E. coli</i> will then subsequently be reported as SAEs (if applicable).”	To remove an existing discrepancy in the protocol with Section 8.3.6.
10.14 Appendix 14: COVID-19 Appendix, Guidance on Study Conduct During the COVID-19 Pandemic	Added text to clarify how coronavirus disease 2019 (COVID-19) related events are to be reported during the study.	To clarify how COVID-19 related events are to be reported during the study.
10.15 Appendix 15: Antibacterial Agents for Susceptibility Testing	Removed pivmecillinam from the antibiotic panel.	The antibiotic is rarely used (except selected countries, indicated uniquely for uncomplicated UTI). Other antibiotics in the test system could be used as marker substances to indicate plasmid resistance or any other kind of resistance of the assessed bacterial strains.
Throughout the protocol	Changes to align the protocol text with the sponsor’s current protocol template.	To align the protocol text with the sponsor’s current protocol template.
Throughout the protocol	Minor changes to make the protocol consistent throughout. Minor grammatical, formatting, or spelling changes were made.	Minor errors and inconsistencies were noted.

Amendment 2 (19 May 2021)

Overall Rationale for the Amendment: The main purpose of this amendment is to clarify that patient reported outcome (PRO)/clinical outcome assessment (COA) data may be captured in an alternative way if the electronic PRO (ePRO)/electronic COA (eCOA) system is unavailable, upon agreement with the sponsor.

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Part 1 and Part 2 1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or Acute Bacterial Prostatitis (ABP) 8 STUDY ASSESSMENTS AND PROCEDURES 8.1.5 Patient-Reported Outcomes 10.2 Appendix 2: Regulatory, Ethical, and Study Oversight Considerations	Wording has been added to the protocol to clarify that PRO/COA data may be captured in an alternative way if the ePRO/eCOA capture system is unavailable.	To avoid loss of information if the ePRO/eCOA capture system is unavailable.

Amendment 1 (10 May 2021)

Overall Rationale for the Amendment: The main purpose of this amendment is to outline the procedures to be followed for invasive Extraintestinal Pathogenic *Escherichia coli* (ExPEC) disease (IED), urinary tract infection (UTI), and acute bacterial prostatitis (ABP) events and to update the procedures for medical resource utilization (MRU) data collection.

Section Number and Name	Description of Change	Brief Rationale
1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or Acute Bacterial Prostatitis per Clinical Case Definition Criteria 8.1.1 Identification of Suspected IED Clinical Cases 8.1.2 IED Clinical Case Definition Clinical Cases 8.1.3 UTI Clinical Case Definition 8.1.4 Acute Bacterial Prostatitis Clinical Case Definition	Descriptions of the procedures needed for IED, urinary tract infection (UTI), and acute bacterial prostatitis (ABP) case identification have been reworded for clarity and to specify which procedures are always expected as study-specific evaluations. The Schedule of Activities for participants with suspected IED and the Schedule of Activities for participants with UTI or ABP have been aligned accordingly.	Clarification and to specify which procedures are always expected as study-specific procedures.
1.1 Synopsis 1.3.1 Part 1 and Part 2 1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or Acute Bacterial Prostatitis per Clinical Case Definition Criteria 4.1 Overall Design 8.1.6 Frailty Assessments	The Multimorbidity Checklist has been removed from the Schedule of Activities. Medical conditions of interest, to be used for the Frailty Index Score calculation, will be collected.	There is an overlap in the conditions necessary to calculate the Frailty Index Score and the conditions collected through the relevant medical history. As such, filling out the separate Multimorbidity Checklist form is not required to collect medical conditions of interest.
1.3.2 Participants With Suspected IED per Clinical Case Identification 8.1 Efficacy Assessments 8.1.1 Identification of Suspected IED Clinical Cases 8.6 Medical Resource Utilization and Health Economics	An additional timepoint (IED-Day 91) has been added for the collection of medical resource utilization data (MRU), completion of electronic patient reported outcomes (ePROs) (to assess HRQoL), and documentation of the recovery status (outcome and complications of IED).	To allow for comparison of MRU for the same period of time (3 months) before and after IED identification and to collect ePROs and the outcome and complications of IED at a standardized timepoint (3 months post-IED identification), as early follow-up (IED-Day 29 or day of discharge) would not allow for this.

Section Number and Name	Description of Change	Brief Rationale
1.3.3 Participants With UTI (Including Pyelonephritis) or Acute Bacterial Prostatitis per Clinical Case Definition Criteria 8.1 Efficacy Assessments 8.1.3 UTI Clinical Case Definition 8.1.4 Acute Bacterial Prostatitis Clinical Case Definition 8.6 Medical Resource Utilization and Health Economics	Collection of medical resource utilization data on UTI/ABP-Day 1 has been removed from the Schedule of Activities.	Medical resource utilization data for UTI and ABP will only be collected on Day 29 as only MRU data related to the actual UTI/ABP event are of interest.
1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or Acute Bacterial Prostatitis per Clinical Case Definition Criteria	Sample collection for antibacterial susceptibility testing was removed from the list of activities. Antibacterial susceptibility testing will be performed for all the collected cultures at local laboratories per standard procedures. Isolates of <i>E. coli</i> will be sent to the central laboratory where antibacterial susceptibility testing will be performed.	A separate procedure is not required for antibacterial susceptibility testing as testing will be performed on all collected cultures.
1.3.2 Participants With Suspected IED per Clinical Case Identification 1.3.3 Participants With UTI (Including Pyelonephritis) or Acute Bacterial Prostatitis per Clinical Case Definition Criteria 8.1.1 Identification of Suspected IED Clinical Cases 8.1.3 UTI Clinical Case Definition 8.1.4 Acute Bacterial Prostatitis Clinical Case Definition 10.1 Appendix 1: Abbreviations and Definitions	‘Type of infection (community-acquired, hospital-acquired, or healthcare-associated infection)’ has been added as an assessment for IED, UTIs and ABP. Definitions were added for the terms ‘community-acquired’, ‘healthcare-associated’, and ‘hospital-acquired’. IED, UTI, and ABP cases will be assessed according to these definitions.	To allow for a distinction to be made between the different types of infections (community-acquired, hospital-acquired, or healthcare-associated).
1.1 Synopsis 3 OBJECTIVES AND ENDPOINTS	The following exploratory endpoint was added: First recurrent uncomplicated UTI event caused by ExPEC9V O-serotypes	To allow for separate evaluation of efficacy of the study vaccine in prevention of uncomplicated rUTIs.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 3 OBJECTIVES AND ENDPOINTS	Text (underlined and bold) has been added to the following secondary endpoint: To demonstrate the efficacy of ExPEC9V compared to placebo in the prevention of <u>the first</u> hospitalized IED event caused by ExPEC9V O-serotypes	To clarify that this secondary endpoint is related to the first hospitalized IED event.
1.1 Synopsis 3 OBJECTIVES AND ENDPOINTS	Text (underlined and bold) has been added to the following exploratory endpoint: Antibody titers for ExPEC9V determined by multiplex ECL-based immunoassay and MOPA assays at Day 1 pre-vaccination, Day 30 and at the time of diagnosis of the suspected IED <u>(all participants) or at the time of a UTI or ABP event (Immunogenicity Subset only)</u> , in association with vaccine efficacy	Antibody titers will also be determined at the time of a UTI or ABP event.
5.1 Inclusion Criteria	Inclusion criterion 6 has been reworded to indicate that a stable medical condition is defined as disease not requiring significant change in therapy during the 6 weeks before enrollment and when hospitalization for worsening of the disease is not anticipated.	The ‘6-weeks’ timeframe is only relevant for the change in therapy.
2 INTRODUCTION 2.2 Background 2.3.3 Benefit-Risk Assessment of Study Participation	References to the ExPEC9V investigator’s brochure (IB) have been updated and now refer to the ExPEC10V/ExPEC9V IB.	The ExPEC9V and ExPEC10V IBs have been combined. This is now reflected in the protocol.
1.1 Synopsis 4.1 Overall Design 6.3 Measures to Minimize Bias: Randomization and Blinding	Wording has been added to the protocol regarding the proportional distribution of diversity during recruitment.	To stress the importance of having a good demographic representation of the general population.
5.1 Inclusion Criteria 10.2 Appendix 2: Regulatory, Ethical, and Study Oversight Considerations	Wording in the protocol has been updated to indicate that the participant and his/her caregiver (if applicable) must be able to read.	The participant and the caregiver (if applicable) must be able to read to complete questionnaires in the electronic clinical outcome assessment (eCOA).
1.3.1 Part 1 and Part 2 6.8 Prestudy and Concomitant Therapy	Antiviral therapies have been added to the list of examples for the relevant prestudy specific therapies. Antibiotic therapies have been replaced by ‘all antimicrobials’	All antiviral and antimicrobial therapies are considered as relevant prestudy and concomitant therapies in the context of evaluation of suspected systemic infections.
1.3.1 Part 1 and Part 2	A footnote has been added to the Schedule of Activities to clarify that solicited adverse events (AEs) will be documented in the electronic case report form (eCRF) following the 30-minute observation period for participants in the Safety Subset.	Clarification

Section Number and Name	Description of Change	Brief Rationale
1.3.1 Part 1 and Part 2 2.3 Benefit-Risk Assessment 5.2 Exclusion Criteria 6.8 Prestudy and Concomitant Therapy	Wording has been added to clarify that vaccination for COVID-19 through any emergency use program (eg, Emergency Use Authorization, Conditional Marketing Authorization, or a similar program) is permitted.	Clarification.
2.3 Benefit-Risk Assessment	The benefit-risk section has been updated to include wording on the risk of coronavirus disease-2019 (COVID-19).	Participants are allowed to receive a vaccination authorized for emergency use to minimize the risk of COVID-19 infection.
10.7 Systemic Inflammatory Response Syndrome Score 10.8 Sequential Organ Failure Assessment Score 10.9 Quick Sequential Organ Failure Assessment Score	The systemic inflammatory response syndrome (SIRS) score, sequential organ failure assessment (SOFA), and the quick essential organ failure assessment score (qSOFA) have been added as appendices to the protocol.	To provide the SIRS, SOFA, and qSOFA scores in the protocol.
10.14 Appendix 14: Antibacterial Agents for Susceptibility Testing	Pivmecillinam and cefiderocol have been added to the list of antibacterial agents for susceptibility testing. Ceftaroline has been removed from the list.	The list of antibacterial agents for susceptibility testing has been extended to include additional antibiotics used for the treatment of UTIs.
2.2 Background	The background section has been updated. New clinical data from study VAC52416BAC1001 have been added to the protocol. Text regarding nonclinical studies conducted with ExPEC9V and ExPEC10V has been reworded and updated.	To provide the most recent clinical data from study VAC52416BAC1001. Clarification
10.13 Appendix 13: COVID-19 APPENDIX, GUIDANCE ON STUDY CONDUCT DURING THE COVID-19 PANDEMIC	Study-specific procedures for UTI and ABP have been added to the COVID-19 appendix.	To instruct the sites which study-specific procedures can be conducted by a home health nurse (if applicable).
1.1 Synopsis	Wording in the synopsis regarding the unblinding has been aligned with Section 6.3	Consistency
5.2 Exclusion Criteria	References to other inclusion/exclusion criteria were corrected for exclusion criteria 6, 16, and 17. In Exclusion Criteria 9, the word 'idiopathic' has been deleted and replaced by 'inflammatory'.	Correction
1.3.1 Part 1 and Part 2	The Year 3 Immunogenicity sample has been added to the Schedule of Activities and footnote 'u' has been aligned accordingly.	Correction.

Section Number and Name	Description of Change	Brief Rationale
Title page	The study name has been added to the title page. Prepared by line removed	For completeness. To align with internal guidelines on legal entity to be mentioned on title page.
Throughout the protocol	The term 'acute bacterial prostatitis' has been added throughout the protocol to differentiate between UTI and ABP. Minor errors and inconsistencies were corrected, and minor clarifications were added throughout the protocol.	Clarification Correction of minor errors and inconsistencies. Addition of minor clarifications.

10.18. Appendix 18: UTI and ABP Study-specific Procedures Prior to Protocol Amendment 8

As of Protocol Amendment 8, site study-specific procedures will not be performed for uncomplicated or complicated UTI events or ABP events. Site study-specific procedures for suspected UTI and ABP events prior to Protocol Amendment 8 are provided below for historical information purposes only. Site study-specific procedures are still required for suspected pyelonephritis (see Section 1.3.3) and IED events (see Section 1.3.2).

10.18.1. Schedule of Activities – Assessments for Participants with UTI (Including Pyelonephritis) or ABP per Clinical Case Definition Criteria

UTI/ABP ASSESSMENT ^a	UTI/ABP-Day 1: UTI/ABP Diagnosis ^{b, d}	Day 29 Post-UTI/ABP Diagnosis (UTI/ABP-Day 29±3d) ^{c, d, e}
Medical conditions of interest and pre-UTI therapy ^f	X	
ePRO instruments (SF-36 version 2 and EQ-5D-5L) ^g	X	X
Collection of UTI or ABP signs and symptoms ^{h, i}	X	X ⁿ
Type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) ^j	X	
Laboratory data ^k	X ^l	X ^o
Blood sample for immunogenicity assessments ^m	X	
Collection of urine samples for urinalysis and urine culture ⁿ	X	X ^o
Collection of information on treatment for UTI/ABP ^p	X	X
MRU ^q		X
Recovery status (outcome of UTI/ABP)		X

ABP=acute bacterial prostatitis; eCOA=electronic clinical outcome assessment; ePRO=patient-reported outcome(s); EQ-5D-5L=5-level EuroQol 5-dimension questionnaire; IED=invasive *Escherichia coli* disease; MRU=medical resource utilization; SF-36=Short Form-36; UTI=urinary tract infection.

Footnotes:

- If case identification criteria for IED are met (see Section 8.1.1), follow procedures per Section 1.3.2 for participants with suspected IED.
- Day 1 of UTI or ABP identification (UTI/ABP-Day 1) is the day on which a UTI or ABP are clinically diagnosed. As of this day, study procedures should be performed, and data should be collected for further evaluation of the UTI/ABP protocol case definition.
- Data collected on the day of resolution of UTI/ABP signs and symptoms or on UTI/ABP-Day 29 (whichever comes first).
- Scheduled visits which cannot be conducted in person at the study site may be conducted as home health visit. Prior sponsor approval is not required for these home health visits.
- If the participant has recovered and laboratory testing is not required, the Day 29 visit may be conducted remotely (eg, via telephone call).

- f. Medical conditions of interest, ie, conditions with increased risk for UTI and other relevant co-existing medical conditions (please refer to Section 10.16 for additional details). Any relevant treatment received in the last 90 days (ie, 3 months) prior to the UTI/ABP, any antibiotic use (prophylactic or therapeutic) within the prior 90 days (ie, 3 months). Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures.
- g. The ePROs (SF-36 version 2 and the EQ-5D-5L) should be completed on the visit date, preferably before any tests, procedures, or other consultations to prevent influencing of the participant. Alternative options are available for the collection of ePRO data if participants are unable or unwilling to work with the eCOA system or if the eCOA capture system is unavailable (please refer to Section 8.1.6 for further details). Best practices on the collection of ePRO data are provided in the ePRO guidelines.
- h. Identification of UTI/ABP (signs/symptoms and laboratory assessments), when the participant presents with UTI or ABP symptoms (for the clinical case definition of UTI/ABP see Section 8.1.4 and Section 8.1.5, respectively), as assessed by the investigator/trained healthcare provider. For all clinically diagnosed UTI/ABP cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa*, the participant will be followed up until 29 days post-UTI or ABP diagnosis or until resolution of UTI/ABP signs and symptoms (whichever comes first) to check the evolution of the infection condition and infection management.
- i. Signs and symptoms of ABP should be collected separately.
- j. Please see Section 10.1 for the definitions of community-acquired, hospital-acquired, and healthcare-associated infections.
- k. Laboratory procedures for UTI or ABP cases include dipstick urinalysis, urine microscopy, or urine flow cytometry in a mid-stream void specimen urine. **In case dipstick urinalysis yields abnormal/unclear results, it must be followed by urine microscopy or flow cytometry.** Additional procedures may be applicable in case of pyelonephritis or severe UTI: hematology test (red blood cell count, white blood cell count and differential), computed tomography scan, or magnetic resonance imaging, and/or ultrasonography. **If not part of the standard of care, urine microscopy or urine flow cytometry are to be performed at a local lab as study procedures** (at a minimum quantitative urine white blood cell/leukocyte count should be available. Abnormal leuk Esterase on dipstick results alone will not be sufficient evidence of pyuria to meet the UTI case definition).
- l. A full urinalysis (microscopy or flow cytometry) is a mandatory study procedure on Day 1 in order to verify the protocol case definition of UTI/ABP.
- m. Blood sample collection for immunogenicity assessments is only applicable for the Immunogenicity Subset. If feasible, an immunogenicity blood sample should be taken at the time of UTI or ABP diagnosis, if possible before any treatment or antibiotic(s) is given. If sample collection prior to treatment initiation is not possible, the samples should be collected as soon as possible and within approximately 48 hours after UTI/ABP diagnosis. Immunogenicity assessments will be performed as detailed in Section 8.4.
- n. Urine cultures (from mid-stream urine) will be taken, if possible before initiating treatment including antibiotic(s). Special diligence should be applied to record the time of antibiotic administration relative to collection of cultures. If not part of the standard of care, the urine culture is a mandatory study procedure on UTI/ABP Day 1. Standard clinical practice should be followed for collection of cultures. If on Day 1 the urinalysis (or dipstick) does not confirm a UTI or ABP, the urine culture does not need to be performed (see Section 10.18.2 and Section 10.18.3).
- o. Collection of relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care (see Section 10.18.2 and Section 10.18.3). These will be recorded as unscheduled evaluations after UTI/ABP-Day 1.
- p. Concomitant medications associated with UTI or ABP will be recorded for all participants experiencing such an episode.
- q. MRU is only applicable for participants in the Immunogenicity Subset. MRU data related to UTI/ABP will be collected on UTI/ABP-Day 29.

10.18.2. UTI Clinical Case Definition and Procedures

If a participant has contacted their healthcare provider and/or study site with signs and symptoms of a UTI, the participant will be defined as having a confirmed UTI endpoint if the criteria outlined below are met^{20,67,68,75} and confirmed by the IEAC for cases of pyelonephritis. For cases of ABP see Section 8.1.5.

Data packages for evaluation by the IEAC will be sent for all cases of *E. coli* confirmed pyelonephritis. Data will include all available clinical data collected by Day 29 post-UTI diagnosis or by the day of resolution of UTI signs and symptoms (whichever comes first). For pyelonephritis, the IEAC will review all the clinically diagnosed cases of pyelonephritis with microbiological confirmation of *E. coli* (regardless of meeting protocol criteria) as well as the cases that would be identified based on the protocol case definition. Only pyelonephritis cases confirmed by the IEAC will be used in the efficacy analysis.

An event is considered a confirmed UTI endpoint if an adult with documented pyuria (WBC count ≥ 10 cells/mm³ in non-centrifuged urine or ≥ 5 WBC per high-power field [WBC/HPF] [400x enlargement] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with one of the following definitions for signs and symptoms:

- for uncomplicated UTI, at least 2 of the following signs or symptoms:
 - dysuria,
 - urinary frequency,
 - urinary urgency, or
 - suprapubic pain.
- for complicated UTI (cUTI):
 - at least 2 of the following signs or symptoms:
 - fever (temperature $\geq 38.0^\circ\text{C}$ [as measured by the participant or health care professional]), or chills, or rigors, or warmth (warmth only if associated with fever),
 - dysuria, urinary frequency or urinary urgency,
 - lower abdominal pain or pelvic pain, or
 - nausea or vomiting,
 - AND,
 - at least 1 of the following complicating factors:
 - history of urinary retention (in male participants),
 - current indwelling urinary catheter,
 - obstructive uropathy, or
 - any relevant functional or anatomical abnormality.

- for pyelonephritis (regardless of underlying abnormality of the urinary tract) with at least 2 of the following symptoms:
 - fever (temperature $\geq 38.0^{\circ}\text{C}$ [as measured by the participant or health care professional]), or chills, or rigors, or warmth (warmth only if associated with fever),
 - flank pain, or
 - tenderness in the costovertebral angle on physical examination, or
 - nausea or vomiting.
- for recurrent UTI (rUTI):
 - recurrence of symptomatic UTIs with a frequency of 2 UTIs in 6 months, or
 - recurrence of symptomatic UTIs with a frequency of at least 3 UTIs in 1 year.
- for recurrent uncomplicated UTI:
 - recurrence of uncomplicated UTIs with a frequency of 2 UTIs in 6 months, or
 - recurrence of uncomplicated UTIs with a frequency of at least 3 UTIs in 1 year.

If case identification criteria for IED are also met (see Section 8.1.1), data will be collected as presented in Section 1.3.2 and Section 8.1.2.

If the treating physician is not part of the study-site personnel, information regarding any UTI case will be collected retrospectively and assessed accordingly by the investigator. If the study site learns about a UTI event after the fact (eg, only at the regular contact with the participant), every effort should be made to collect relevant clinical information and samples, as required for endpoint assessment and as feasible.

For the secondary endpoints, with UTI caused by any of the ExPEC9V O-serotypes, the O-serotyping has to be available from the central lab and the UTI has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the secondary endpoints related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient. Cases with multiple pathogens in the urine culture results and with the presence of *E. coli* will not be considered for the secondary endpoints. These cases will be included in sensitivity analyses.

As an exploratory endpoint, participants meeting the above definition of UTI but with urine samples positive for *P. aeruginosa* ($\geq 10^5$ CFU/mL) will be reported to have *P. aeruginosa* UTI. No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens with the presence of *P. aeruginosa* will not be considered for this exploratory endpoint.

The following procedures should be performed for cases with a clinical diagnosis of UTI (as per treating physician) as well as suspected UTI cases for which antibacterial treatment has been initiated:

UTI-Day 1, UTI case evaluation:

- clinical data collection of UTI signs and symptoms,
- laboratory data including dipstick urinalysis, urine microscopy, or urine flow cytometry in a mid-stream void urine specimen. Optionally, in particular in case of pyelonephritis or severe UTI: hematology test (red blood cell count, WBC count and differential), CT scan, or MRI, and/or ultrasonography,

The following procedures should be followed for all UTI cases, these procedures must be conducted as study procedures if not part of the standard of care:

- urine microscopy or urine flow cytometry in a mid-stream void urine specimen (at minimum WBC count should be available). Note that this is a mandatory study procedure on Day 1. The quantitative results (number of cells per unit of volume or per high power field) of urinalysis (by flow cytometry or by microscopy) on Day 1 are required to verify the protocol case definition of UTI. Abnormal leukoesterase on dipstick results alone will not be sufficient evidence of pyuria to meet the UTI case definition. As new urinalysis assays may develop, these may be evaluated and accepted by the sponsor only if the provided results are quantified as number of white blood cells per unit of volume.
- microbiology data: urine cultures (from mid-stream urine) will be taken, if possible before initiating treatment including antibiotic(s). Patients with an indwelling catheter should have urine samples collected following the placement of a new catheter, or through aseptic techniques via a properly disinfected collection port if the indwelling catheter cannot be removed. Standard clinical practice should be followed for collection of cultures. In case of a more severe course of the disease, obtaining blood cultures should be considered, if possible before initiating treatment including antibiotic(s). Please refer to Section 10.13 for guidance on obtaining blood cultures. The *E. coli* or *P. aeruginosa* isolates (if retained and available) will be sent to the central laboratory when originating from cultures positive for *E. coli* or *P. aeruginosa* (for urine cultures only if $\geq 10^5$ CFU/mL). This procedure is applicable independent of confirmation of UTI. The central laboratory will conduct further characterization including reconfirmation of bacterial identification (*E. coli* and *P. aeruginosa*) and antibacterial susceptibility testing (*E. coli* only) as detailed in Section 8.5.

In addition, the following study-specific procedures should be followed for all clinically diagnosed UTI cases (regardless of microbiological confirmation) as well as suspected UTI cases for which antibacterial treatment has been initiated:

- If feasible, an immunogenicity blood sample (only for participants in the Immunogenicity Subset) should be taken at the time of UTI diagnosis, if possible before any treatment is given. If sample collection prior to treatment initiation is not possible, the samples should be collected as soon as possible and within approximately 48 hours after UTI diagnosis. Immunogenicity assessments will be performed as detailed in Section 8.4.
- collection of information regarding medical conditions of interest and treatment received within the past 90 days,
- assessment of type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) (see Section 10.1 for definitions),

- collection of information on treatment for UTI (including antibiotics and/or any supportive therapy; special diligence should be applied to record the time of antibiotic administration relative to collection of cultures),
- assessment of HRQoL, and
- collection of MRU data (Immunogenicity Subset only).

For all clinically diagnosed UTI cases (regardless of microbiological confirmation) as well as suspected UTI cases for which antibacterial treatment has been initiated, at a minimum the following data must be collected in the eCRF:

- signs and symptoms of UTI,
- data on cultures taken and causative pathogen,
- treatment for UTI (including antibiotics and/or any supportive therapy; special diligence should be applied to record the time of antibiotic administration relative to collection of cultures),
- data on the immunogenicity sample (Immunogenicity Subset only),
- final diagnosis (including UTI type, if applicable),
- outcome.

For UTI cases without microbiological confirmation of *E. coli* or *P. aeruginosa* no study-specific follow-up is foreseen.

UTI-Day 29, follow-up:

On Day 29 post-UTI diagnosis or on the day of resolution of UTI signs and symptoms (whichever comes first), the following relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care will be collected and recorded in the eCRF for all clinically diagnosed UTI cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa* ($\geq 10^5$ CFU/mL):

- laboratory data: dipstick urinalysis, urine microscopy or urine flow cytometry in a mid-stream void specimen urine. In case of pyelonephritis or severe UTI, additional procedures may be applicable: hematology test (red blood cell count, WBC count and differential), CT scan, or MRI, and/or ultrasonography,
- microbiology data: additional urine cultures (from mid-stream urine) are recommended to be taken if signs and symptoms of UTI persist.

In addition, the following study-specific procedures should be followed for all clinically diagnosed UTI cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa*:

- collection of additional data on treatment for UTI,
- assessment of HRQoL,
- collection of MRU data (Immunogenicity Subset only) (MRU data between UTI-Day 1 and UTI-Day 29 to be collected), and

- collection of recovery status (outcome of UTI).

If the participant has recovered and laboratory testing is not required, the Day 29 visit may be conducted remotely (eg, via telephone call).

Potential outcomes of UTI:

- recovery: resolution of UTI signs and symptoms and conclusion of antimicrobial therapy,
- UTI ongoing,
- complications (eg, IED – UTI identified as source of infection). In case UTI is complicated by IED, the follow-up data are to be collected as for participants meeting the IED clinical case definition criteria (see Section 1.3.3),
- Death.

The next event of UTI may be described as:

- relapse: recurrent infection with an organism similar to the pretherapy isolate, usually following persistence of the organism in the genitourinary tract.
- reinfection: recurrent infection with a new organism.

10.18.3. ABP Clinical Case Definition and Procedures

If a participant has contacted their health care provider and/or study site with signs and symptoms of ABP, the participant will be defined as having a confirmed ABP endpoint if the below criteria are met.

An adult male with documented pyuria (WBC count ≥ 10 cells/mm³ in non-centrifuged urine or ≥ 5 WBC/HPF [400x enlargement] in urine sediment) and microbiological confirmation by culture of *E. coli* in urine ($\geq 10^5$ CFU/mL) presents with fever (temperature $\geq 38.0^\circ\text{C}$ [as measured by the participant or health care professional]) and an acute onset of the following symptoms^{13,58,74}:

- irritative voiding symptoms:
 - dysuria,
 - urinary frequency,
 - urinary urgency,OR
- obstructive voiding symptoms:
 - hesitancy,
 - incomplete voiding,
 - straining to urinate,
 - weak stream,AND
- suprapubic, rectal, or perineal pain.

If case identification criteria for IED are also met (see Section 8.1.1), data will be collected as presented in Section 1.3.2 and Section 8.1.2.

If the treating physician is not part of the study-site personnel, information regarding any ABP case will be collected retrospectively and assessed accordingly by the investigator. If the study site learns about an ABP event after the fact (eg, only at the regular contact with the participant), every effort should be made to collect relevant clinical information and samples, as required for endpoint assessment and as feasible.

For the endpoint of ABP caused by any of the ExPEC9V O-serotypes, the O-serotyping has to be available from the central lab and the ABP event has to be confirmed as being O1, O2, O4, O6, O15, O16, O18, O25, or O75 positive. For the exploratory endpoint related to *E. coli*, local lab confirmation of *E. coli* (without serotyping performed by the central lab) will be sufficient. Cases with multiple pathogens in the urine culture results and with the presence of *E. coli* will not be considered for the exploratory endpoint. These cases will be included in sensitivity analyses.

As an exploratory endpoint, participants meeting the above definition of ABP but with urine samples positive for *P. aeruginosa* ($\geq 10^5$ CFU/mL) will be reported to have *P. aeruginosa* ABP.

No serotyping will be performed for *P. aeruginosa*. Cases with mixed pathogens with the presence of *P. aeruginosa* will not be considered for this exploratory endpoint.

The following procedures should be performed for cases with a clinical diagnosis of ABP (and not meeting the case identification criteria for IED), on the day of ABP diagnosis (ABP-Day 1) and Day 29 post-ABP diagnosis or on the day of resolution of ABP signs and symptoms (whichever comes first) (see Section 1.3.3):

ABP-Day 1, case evaluation:

- clinical data collection of ABP signs and symptoms,
- laboratory data including dipstick urinalysis, urine microscopy or urine flow cytometry in a mid-stream void urine specimen. Optionally: hematology test (red blood cell count, WBC count, and differential), PSA, procalcitonin, C-reactive protein, and/or ultrasonography,

The following procedures should be followed for all ABP cases. These procedures must be conducted as study procedures if not part of the standard of care:

- urine microscopy or urine flow cytometry in a mid-stream void urine specimen (at minimum WBC count should be available). Note that this is a mandatory study procedure on Day 1. The quantitative results (number of cells per unit of volume or per high power field) of urinalysis (by flow cytometry or by microscopy) on Day 1 are required to verify the protocol case definition of ABP. Dipstick urinalysis will not provide the required information to meet the case definition. As new urinalysis assays may develop, these may be evaluated and accepted by the sponsor only if the provided results are quantified as number of cells per unit of volume.
- microbiology data: urine cultures (from mid-stream urine) will be taken, if possible before initiating treatment including antibiotic(s). In case of prostate abscess, puncture material should be received for microbiological evaluation. Standard clinical practice should be followed for collection of cultures. In case of a more severe course of the disease, obtaining repeated blood cultures should be considered, if possible before initiating treatment including antibiotic(s). Please refer to Section 10.13 for guidance on obtaining blood cultures. The *E. coli* or *P. aeruginosa* isolates (if retained and available) will be sent to the central laboratory when originating from cultures positive for *E. coli* or *P. aeruginosa* (for urine cultures only if $\geq 10^5$ CFU/mL). This procedure is applicable independent of confirmation of ABP. The central laboratory will conduct further characterization including reconfirmation of bacterial identification (*E. coli* and *P. aeruginosa*) and antibacterial susceptibility testing (*E. coli* only) as detailed in Section 8.5,

In addition, the following study-specific procedures should be followed for all clinically diagnosed ABP cases (regardless of microbiological confirmation) as well as suspected ABP cases for which antibacterial treatment has been initiated:

- If feasible, an immunogenicity blood sample (only for participants in the Immunogenicity Subset) should be taken at the time of ABP diagnosis, if possible before any treatment is given. If sample collection prior to treatment initiation is not possible, the samples should

be collected as soon as possible and within approximately 48 hours after ABP diagnosis. Immunogenicity assessments will be performed as detailed in Section 8.4,

- collection of information regarding medical conditions of interest and treatment received within the past 90 days (specifically cases that occurred following urological interventions will be recorded),
- assessment of type of infection (community-acquired, hospital-acquired, or healthcare-associated infection) (see Section 10.1 for definitions),
- collection of information on treatment for ABP (including antibiotics and/or any supportive therapy; special diligence should be applied to record the time of antibiotic administration relative to collection of cultures),
- assessment of HRQoL, and
- collection of MRU data (Immunogenicity Subset only).

For all clinically diagnosed ABP cases (regardless of microbiological confirmation) as well as suspected ABP cases for which antibacterial treatment has been initiated, at a minimum the following data must be collected in the eCRF:

- signs and symptoms of UTI/ABP,
- data on cultures taken and causative pathogen,
- treatment for ABP (including antibiotics and/or any supportive therapy; special diligence should be applied to record the time of antibiotic administration relative to collection of cultures),
- data on the immunogenicity sample (Immunogenicity Subset only),
- final diagnosis (including UTI type, if applicable)
- outcome.

For ABP cases without microbiological confirmation of *E. coli* or *P. aeruginosa* ($\geq 10^5$ CFU/mL) no study-specific follow-up is foreseen.

ABP-Day 29, follow-up:

On Day 29 post-ABP diagnosis or on the day of resolution of ABP signs and symptoms (whichever comes first), the following relevant additional clinical, laboratory, and microbiology data, based on the investigator's judgment and per standard of care will be collected and recorded in the eCRF for all clinically diagnosed ABP cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa* ($\geq 10^5$ CFU/mL):

- laboratory data: dipstick urinalysis, urine microscopy or urine flow cytometry in a mid-stream void specimen urine. Additional procedures may be applicable: expressed prostatic secretions (EPS), and post-massage urinalysis (VB3 - first 10 mL of voided urine after EPS), hematology test (red blood cell count, WBC count and differential), PSA, procalcitonin, C-reactive protein, and/or ultrasonography,
- microbiology data: additional urine cultures (from mid-stream urine) are recommended to be taken if signs and symptoms of ABP persist.

In addition, the following study-specific procedures should be followed for all clinically diagnosed ABP cases with microbiological confirmation of *E. coli* and/or *P. aeruginosa*:

- collection of additional data on treatment for ABP,
- assessment of HRQoL,
- collection of MRU data (Immunogenicity Subset only) (MRU data between ABP-Day 1 and ABP-Day 29 to be collected), and
- collection of the recovery status (outcome of ABP).

If the participant has recovered and laboratory testing is not required, the Day 29 visit may be conducted remotely (eg, via telephone call).

Potential outcomes of ABP:

- recovery: resolution of ABP signs and symptoms and conclusion of antimicrobial therapy,
- ABP ongoing,
- complications (eg, prostatic abscess, epididymitis, chronic prostatitis, IED – with prostatitis identified as a source of infection). In case ABP is complicated by IED, the follow-up data are to be collected as for participants meeting the IED clinical case definition criteria (see Section 1.3.3),
- Death.

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INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study intervention, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

Signature: _____ Date: _____

(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): PPD, MD

Institution: Janssen Research & Development

Signature: electronic signature appended at the end of the protocol Date: _____

(Day Month Year)

Note: If the address or telephone number of the investigator changes during the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

Signature

User	Date	Reason
PPD	09-Aug-2024 14:40:35 (GMT)	Document Approval