Statistical analysis plan

An open label study to evaluate safety and immunogenicity of Ad26.ZEBOV booster dose in children previously vaccinated with the Ad.26ZEBOV and MVA-BN-Filo vaccine regimen

Protocol VAC52150EBL2011; Phase 2

Status: version 1.1

Date: 6 January 2022

Prepared by: LSHTM

TABLE OF CONTENTS

AMENDMENT HISTORY

Amendment 1

Version 1.1 Dated 6 January 2022:

- The interpretation of ELISA results for values above the upper limit of quantification (ULOQ) has been added under section 5.1.1.2 Handling of Missing/ Invalid Data
- The protocol version and date were updated in the reference section.

ABBREVIATIONS

AE	Adverse events
aMLV	Amphotropic murine leukemia virus
BMI	Body mass index
CDC	Center for Disease Control and Prevention
CI	Confidence interval
CRF	Case report form
CSR	Clinical study report
IDMC	Independent Data Monitoring Committee
DPS	Data programming specifications
EBOV GP	Ebola virus (formerly known as Zaire ebolavirus) Mayinga glycoprotein
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
EU/mL	Elisa units/mL
FAS	Full analysis set
FDA	Food and Drug Administration
IRE	Immediate Reportable Event
LLOQ	Lower limit of quantification
MeDRA	Medical Dictionary of Regulatory Activities
NSAIDs	Non-steroidal anti-inflammatory drugs
PP	Per protocol
PRNT	Plaque reduction neutralization test
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
ULOQ	Upper limit of quantification
VNA	Virus neutralization assay
WHO	World Health Organization

1. INTRODUCTION

This is the statistical analysis plan (SAP) for the phase 2 VAC52150EBL2011 study. This SAP will be applicable to the study results based on version 2.0 of the clinical protocol of 15 June 2021.

1.1. Trial Objectives

The primary objectives of the study are:

- To assess the safety and tolerability of a booster dose of Ad26.ZEBOV at a dose of 5×10^{10} viral particles (vp) in children previously vaccinated with Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval.
- To assess the vaccine-induced humoral immune response to the Ebola virus glycoprotein (EBOV GP), as measured by FANG ELISA, at 7 and 21 days following a booster dose of Ad26.ZEBOV at a dose of 5×10^{10} vp in children previously vaccinated with Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56 day interval.

1.2. Trial Design

This is a phase 2 open label study to evaluate the immune response to a booster dose of Ad26.ZEBOV administered to children who were previously vaccinated with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Only children who received Ad26.ZEBOV and MVA-BN-Filo regimen during their participation in VAC52150EBL3001 (EBOVAC-Salone) vaccine trial are eligible for enrolment in this study. Participants will be recruited in two age groups: children aged 4-11 years at the time of dose 1 vaccination and children aged 1-3 years at the time of dose 1 vaccination in the EBOVAC-Salone trial. Approximately 25 participants will be enrolled in each of these two age groups.

Participants will be followed up to 28 days after their booster vaccination.

1.3. Statistical Hypotheses for Trial Objectives

As this study is designed to provide descriptive information regarding safety and immunogenicity without formal treatment comparisons, no formal statistical hypothesis testing is planned.

1.4. Sample Size Justification

Approximately 50 participants are expected to be enrolled in this trial (approximately 25 for each age group). The sample size is a convenience sample and is not based on formal hypothesis testing considerations.

1.5. Randomization and Blinding

Randomisation

There will be no randomisation in this study with all participants receiving the booster dose of Ad26.ZEBOV vaccine.

Blinding

This is an open label study in which all the participants will receive the booster dose of Ad26.ZEBOV vaccine.

2. GENERAL ANALYSIS DEFINITIONS

Data will be shown by the study age groups (1-3 and 4-11 years). A booster baseline (or reference) value will be described as the value of the last available assessment performed prior to booster vaccination on Day 1.

Unless otherwise specified, the results of the statistical analysis will be presented by period, except for participant information sections. For immunogenicity, vital signs, and physical examination, data will be presented per time point as appropriate. Assessments over time will be reported for each period or visit (see Section 2.1).

- Categorical variables will be summarised using the following statistics: number of observations, percentages and Exact Clopper-Pearson 95% confidence interval (CI), as applicable.
- Continuous variables will be summarised using the following statistics as appropriate: number of observations, geometric mean, arithmetic mean (mean), 95% confidence interval (CI) for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum. In addition, reverse cumulative distribution plots will be used for exploratory analysis of antibody data.

Unless otherwise specified, listings will only be generated for final analysis.

2.1. Analysis Window and Analysis Periods

For descriptive statistics over time, assessments (regardless of the investigated parameter) will be allocated to an analysis visit based on the actual date, visit slotting will be done as specified below.

The number of days in the phase (*relday*) will be defined as:

relday = *visit day – reference day*+1 for visits on or after the reference day,

relday = *visit day – reference day*+1 for visits before the reference day,

where the reference day equals the date of vaccination.

All dates will be allocated to the following time points, based on *relday* (Table 1). A per protocol time interval is defined for immunogenicity analysis only. The windows are defined as per protocol.

Table 1: Visit windows

Analysis time point	Target day	Time interval Per Protocol Set (days)	Time interval Full Analysis Set (days)
Day 1 (Booster Baseline)*	1	≤ 1	≤ 1
Day 8 (7 days post booster dose)	8	[8-11]	[8-14]
Day 22 (21 days post booster dose)	22	[19-25]	[15-29]
Day 29 (28 days post booster dose)	29	NA	NA

NA: Not applicable, no immunogenicity assessment.

*For safety assessments, the date and time of the baseline assessment should fall before the date and time of booster vaccination

All assignments will be made in chronological order. Once an assessment is assigned to an analysis window it will no longer be used for a later window.

For adverse events, vital signs abnormalities and concomitant therapies, analysis periods will be constructed as follows:

Table 2: Period definitions

Analysis period	**Interval**	
	From	**To**
Screening	00:00 of the date of signing the informed consent	One minute prior to booster dose on Day 1
Post booster vaccination follow up (FU)	One minute after the end of post booster vaccination period	23:59 on the date of last contact (ie, study completion or early discontinuation)

2.2. Pooling of Data

Results will be pooled over sites.

2.3. Analysis Sets

2.3.1. Full Analysis Set

The full analysis set will include all participants with the study intervention administration documented. All safety analysis will be performed on the full analysis set. In case a significant number of subjects are excluded from per protocol set (≥ 10%), then the immunogenicity analysis conducted on the full analysis set will be presented as sensitivity analysis, to investigate the impact of major protocol deviations on immune response.

2.3.2. Per Protocol Immunogenicity Population

The per-protocol immunogenicity population will include all vaccinated participants for whom immunogenicity data are available, excluding participants with major protocol deviations expected to impact the immunogenicity outcomes. The primary immunogenicity analysis will be performed on the per protocol immunogenicity population.

2.4. Definition of Subgroups

Data will be shown by age groups (1-3 years and 4-11 years).

These subgroups will be used for showing the data for both safety analysis (full analysis set) and immunogenicity analysis (per protocol immunogenicity population).

3. CHANGES TO THE PLANNED ANALYSIS

No changes have been made to the planned analysis contained in the protocol.

INTERIM ANALYSIS AND DATA MONITORING COMMITTEE REVIEW

No interim analysis will be performed for this study.

An IDMC will not be appointed for this study. The safety of Ad26.ZEBOV has already been shown in children in previous studies. A booster dose of Ad26.ZEBOV was also shown to be safe in adults. Thus the role of the IDMC will be designated to an independent medical reviewer. The independent medical reviewer will be identified by the sponsor to review the accumulating safety data on an ongoing basis to ensure the continuing safety of the participants enrolled in this study.

The independent medical reviewer will be consulted periodically to review newly generated data. Ad hoc meetings may be requested via the sponsor if any of the pre-specified pausing rules for this study are met or in any situation that could affect the safety of the participants.

For more details, refer to section 10.7 of the protocol. [(1)]

4. PARTICIPANT INFORMATION

Participant information will be analysed based on the full analysis set.

4.1. Demographics and Baseline Characteristics

Demographic characteristics and screening/baseline characteristics (e.g. physical examination, medical history) will be tabulated and summarized with descriptive statistics.

The following demographic and baseline characteristics will be summarized:

- Sex (Male/ Female)
- Age (years)
- Race
- Ethnicity
- Height
- Weight
- Weight-for-age for children 2-5 years and 5-10 years according to WHO growing charts
- Height-for-age for children 2-5 years and 5-19 years according to WHO growing charts
- Weight-for-height for children 2-5 years according to WHO growing charts
- BMI-for-age for children 5-19 years according to WHO growing charts
- Time between vaccination with Ad26.ZEBOV/ MVA-BA-Filo regimen and Ad26.ZEBOV booster vaccination (Median time, Q1, Q3, and range)

The charts are available from the link below:

https://www.who.int/tools/child-growth-standards/standards/weight-for-age
https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/weight-for-age-5to10-years

https://www.who.int/tools/child-growth-standards/standards/length-height-for-age
https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/height-for-age

https://www.who.int/tools/child-growth-standards/standards/weight-for-length-height

https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age

4.1.1. Description of the procedure to determine Weight-for-Age percentile

Reference material in Excel format can be found at this link for boys and girls:

https://www.who.int/docs/default-source/child-growth/growth-reference-5-19-years/weight-for-age-(5-10-years)/wfa-girls-perc-who2007-exp.xlsx?sfvrsn=5c5825c4_4

https://www.who.int/docs/default-source/child-growth/growth-reference-5-19-years/weight-for-age-(5-10-years)/wfa-boys-perc-who2007-exp.xlsx?sfvrsn=97ab852c_4

.

Consult https://www.who.int/growthref/computation.pdf for more information on the data contained in the Excel file and instructions on how to calculate the exact percentile.

First determine the age point to use. Age at the date of the corresponding weight assessments is to be used. Age is to be used at the half month point for the entire month; for example, 1.5 months represents 1.0-1.99 months or 1.0 month up to but not including 2.0 months of age. Using the appropriate chart (boys vs. girls), determine the values of L, M, and S to use.

4.2. Disposition Information

The number and percentage of participants screened, vaccinated and included in each analysis set will be tabulated.

Number and percentage of participants in the full analysis set that complete the study and those who are going to be discontinued together with reasons for discontinuation will be tabulated.

4.3. Protocol Deviations

All major protocol deviations will be tabulated by deviation category. Major protocol deviations that may have an influence on immune response will be flagged in a listing.

4.4. Medical History

Medical history will be listed.

4.5. Concomitant Medications

The analysis of concomitant therapies will be done using generic drug names.

Based on their start and stop date, concomitant therapies will be reported in each analysis period during which they were applied. For missing or partial start and/or stop dates (time and/or day and/or month and/or year) the following allocation rules will be applied:

- In case of partial start or stop dates, the concomitant therapy records will be allocated to periods using the available partial information, without imputations. If, for example, only month and year are available this will be compared to the month and the year of the periods, and concomitant therapy will be allocated to the period(s) where these date parts match.
- In case of a completely missing start date, the concomitant therapy will be considered as having started before the trial.
- In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

Concomitant therapies will be tabulated per period (including follow up periods). A listing of all concomitant therapies will be provided. All therapies taken within 30 days prior to signing the informed consent form will also be listed.

There will be special attention to analgesics/ antipyretics such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDS) and aspirin, administered until day 8 post-vaccination (including day of vaccination).

Remarks:

In addition to the date information, time information is considered to allocate concomitant therapies to periods, if available.

5. IMMUNOGENICITY

The primary analysis of immunogenicity will be done on the per protocol immunogenicity population (as defined in section 2.3).

5.1. Immunogenicity against EBOV GP

5.1.1. Humoral Immune response

5.1.1.1. Parameters

Humoral immune response as measured by the following assays will be analysed.

- **Binding antibody responses using Filovirus Animal Nonclinical Group (FANG) enzyme-linked immunosorbent assay (ELISA):** Quantification of antibodies binding to EBOV GP using ELISA units/mL (EU/mL).

In addition, the following will be defined for ELISA (EU/mL):

- **Sample interpretation:** a sample will be considered positive, if the value is above the assay-specific lower limit of quantification (LLOQ).
- **Responder**:
  - If sample interpretation is negative at baseline before dose 1 vaccination in EBL3001 but positive post-baseline and the post-baseline value is greater than 2.5 x LLOQ; OR
  - If sample interpretation is positive at both baseline before dose 1 vaccination in EBL3001 and post-baseline and there is a greater than 2.5-fold increase from baseline (2.5-fold increase on the original scale)

5.1.1.2. Handling of Missing/ Invalid Data

ELISA:

For ELISA binding antibody responses, values below the LLOQ will be imputed with LLOQ/2 EU/mL. For the calculation of fold increases, values of binding antibody responses below the LLOQ will be imputed with LLOQ EU/mL. The LLOQ is provided in the database.

Values above the upper limit of quantification (ULOQ) will be imputed as ULOQ EU/mL. This will be used for the calculation of fold increases. The ULOQ is provided in the database.

5.2. Immunogenicity Against Ad26 Vector

5.2.1. Humoral Immune Response

For immunogenicity against the Ad26 vector, a vector-specific reference value will be defined as the value closest but prior to the administration of the vector. The analysis of the immunogenicity against the vector will be performed only if the assay is available.

Sample interpretation: a sample will be considered positive, if the value is above the assay LLOQ.

The immune response values will be log_{10}-transformed before any further handling. Results will be presented on the log_{10}-scale, geometric mean titers and geometric mean increases with corresponding 95% CIS will be provided in the tables.

5.2.1.1. Handling Missing/ Invalid Data

For all outcomes (Ad26) values below the corresponding LLOQ will be imputed with half of the LLOQ (LLOQ/2).

5.3. Analysis

Summary statistics of observed values (on the log_{10} scale) will be presented for all immunogenicity parameters at each time point (Day 1, 8 and 22). Geometric mean concentrations with corresponding 95% CI and fold increase from pre-booster values will be calculated for ELISA antibody responses (EU/mL) on Day 8 and Day 22.

A linear mixed model with the unstructured variance-covariance and unstructured mean (i.e. including time as a categorical variable) will be used to estimate the geometric mean concentrations and the associated 95% CI at these time points.

Humoral immune response will be graphically presented using regimen profile plots using also data from the parent trial EBL3001.

Reverse cumulative distribution plots will be used to display all the data for each immunogenicity parameter (on the log_{10} scale) against the percent of participants having at least that level of the parameter. The plots will be used to visually assess a value's rank among all other values and to simplify comparison of immunogenicity parameter distribution by age group.

Percentage of responders will be presented at different time points.

6. SAFETY

No formal statistical testing of safety data is planned. Safety data analysis will be performed on the full analysis set.

Safety data will be analysed descriptively (including 95% CI, if applicable). Baseline for all safety parameters will be defined as the last value before the booster dose.

The safety and tolerability data will include the following:

Solicited local and systemic adverse events (reactogenicity) until 7 days post vaccination.

Unsolicited adverse events, from provision of informed consent onwards until 28 days after booster vaccination.

Serious adverse events (SAEs) until the end of the study (Day 29).

6.1. Adverse events

The analysis of adverse events will be done using the solicited adverse event terms pre-specified in the diary card.

All reported adverse events (solicited local, solicited systemic and unsolicited) after the booster vaccination will be attributed to analysis period as defined in Attachment 1. For each adverse event, the number and percent of subjects who experience at least one occurrence of the given event will be summarized. Summaries, listings, datasets and /or participant narratives may be provided as appropriate, for those participants who die, discontinue study vaccinations due to an adverse event, or experience a severe or serious adverse event.

6.1.1. Definitions

Solicited AEs are precisely defined events (local and systemic) that participants are specifically asked about and which are noted by participants or study staff in the diary. All the other AEs are considered unsolicited. Please refer Clinical Protocol [(1)], Section 11 for further details.

Causality

Solicited AEs will be considered as related to the study vaccine. Unsolicited AEs will be considered to be related to the use of the study vaccine if attribution is possibly, probably or very likely. An AE will be considered not related to the use of the study vaccine if the attribution is not related or doubtful. Please refer to Clinical Protocol [(1)], Section 11.1.2 for further details.

Severity

The severity of AEs is classified as mild, moderate, severe or potentially life threatening. Please refer to CTP [(1)], section 11.1.3 for further details. Solicited events that are graded less than mild, are not considered as AE.

For induration/swelling both the diameter and grading as reported by the investigator are reported in the eCRF. Diameter will be used to derive toxicity grading. The highest grading should be used when both the diameter grading and the investigator-reported grading are available.

6.1.1.1. Solicited Local (Injection Site) Reactions

The analysis of solicited AEs will include:

- Erythema (redness of skin)
- Swelling/ Induration
- Pain/Tenderness
- Itching at the injection site

6.1.1.2. Solicited Systemic Adverse Events

The analysis of systemic solicited adverse events will include:

- Fatigue/tiredness
- Headache
- Nausea/vomiting
- Myalgia (muscle pain)
- Arthralgia (joint pain)
- Fever (defined as body temperature of 38^0 or higher)

Adverse Events of Special Interest

AESIs (including potential AESIs) are significant AEs that are judged to be of special interest because of clinical importance, known or suspected effects of similar vaccines, or based on nonclinical signals.

- Thrombosis with Thrombocytopenia Syndrome (TTS)
- Thrombotic events: suspected deep vessel venous or arterial thrombotic events as detailed in Appendix 2

  and/or

- Symptomatic thrombocytopenia, defined as platelet count below LLN for the testing lab

6.1.2. Imputation

Missing data will not be imputed. If relationship of AEs to the study vaccine could not be derived (i.e. missing or unknown) it will be considered as unknown, for analysis purposes. Solicited local AEs will be considered as related with the use of study vaccine. Missing relationships will not be imputed.

Period allocation of AEs is defined in Attachment 1.

6.1.3. Analysis

AEs (solicited and unsolicited) will be tabulated by presenting the number and percentage of participants having at least one of the observed AEs.

The unsolicited AEs will be summarised by System Organ Classification (SOC) and Preferred Term (PT). The solicited AEs will be summarized by class (local, systemic) and PT. For solicited as well as unsolicited AEs, tables focussing on severity and relatedness to vaccine will be created.

SAEs, AEs with fatal outcomes, AEs leading to permanent discontinuation of vaccine, Grade 3 AEs and AESIs will be listed. Tables, summarizing all those parameters will also be created.

For the solicited local and systemic AEs, the duration and time to onset of the events will also be summarized. If a participant experience more than one occurrence of a solicited event, the maximum duration of the events will be used. The time to first onset is defined as:

[*date of first onset – reference date+1*]

The reference date is the start date of each vaccination period. Duration and time to onset of AEs will be expressed in days.

Summaries, listings, datasets, and/or participant narratives may be provided as appropriate, for those participants who die, or experience a SAE.

The analysis of AEs will be based on MeDRA coded terms as provided in the clinical database.

A listing of all SAEs during the study period will be provided. A listing of all AESIs (if flagged in the database) will be presented as well.

6.2. Clinical Laboratory Tests

In case a laboratory result is *censored* (no numeric value is available, but only a verbatim term) then a numeric value will be imputed by a value exceeding the cut-off value with one unit. (<x: subtract 1 unit from x, >x: add 1 unit to x)

Toxicity grades will be determined according to the scales in Attachment 2 and 3. DMID Paediatric toxicity scale will be used. In case no toxicity grades are defined for a test, the abnormalities (below/ above normal range) will be used. In determining toxicity grades, the following rules will be applied:

Highest grades/worst abnormalities are determined over the entire period.

The abnormalities “abnormally low” and “abnormally high” are considered equally important and both abnormalities are shown in tables. (This mean that the sum of percentages can exceed 100%)

If a laboratory value falls within the grading as specified in the grading table but also within the local laboratory normal limits, the value is considered as normal or Grade 0.

Laboratory results falling between the grading scales will be allocated to the adjacent worst case grade (because the scale for some parameters in the grading table is not continuous as there may be zones where toxicity grades definitions do not exist)

Definition emerging abnormalities following vaccination: An abnormality (toxicity grade or abnormality based on normal ranges) will be considered as emerging in a particular period if it is worse than baseline value. A shift from “abnormally low” at baseline to “abnormally high” post baseline or vice versa is also emerging.

6.2.1. Analysis Methodology

Laboratory data will be analysed based on the full analysis set. The data will be summarized by the type of laboratory test.

Unless specified otherwise, percentages are calculated versus the number of participants in the analysis set with non-missing data for the parameter, period (if applicable) and vaccination group under evaluation.

Tabulation of the highest emerging graded abnormalities per period and overall will be provided. Special attention will be given to participants who develop Grade 3 toxicities. For tests that have no grading, tabulations of the worst emerging abnormalities (below/above) will be performed.

6.3. Vital Signs and Physical Examination Findings

Vital sign abnormalities will be determined in accordance with the DMID Vital Signs Toxicity Grading (attachment 2.3.) for adults and adolescents. The worst abnormalities (following vaccination) of vital signs will be tabulated (i.e., showing number and percentage) and listed.

For children, vital signs will be summarized and listed without grading. This summary will be presented per time point and showing the mean, standard deviation, median, Q1, Q3, minimum and maximum. Post-baseline assessments will be allocated to an analysis visit based on the eCRF visit; no visit slotting will be done. Scheduled visits will be used whenever available. If only unscheduled visits are available for a time point, the one closest to the scheduled visit will be used (in case of equidistance, the latest assessment will be selected). The baseline is the last available assessment prior to first vaccination.

It is important to note that a full physical examination is only conducted at screening. At other visits, a brief, symptom-directed examination (including body length/height and weight in

children aged 12 months up to and including 24 months at the time of the boost vaccination) will be performed based on any clinically relevant issues, clinically relevant symptoms and medical history. Therefore, only a listing of participants with worst physical examination findings (i.e., abnormalities) following vaccination will be provided.

REFERENCES

1. Clinical Protocol VAC52150EBL2011: Version 2.0. An open label study to evaluate safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen (15 June 2021).

ATTACHMENTS

1. PERIODIC ALLOCATION OF ADVERSE EVENTS

Solicited AEs are always allocated to the Post boost vaccination period. Unsolicited AEs are allocated to the different periods according to the following rules:

Step 1: Allocation of events to the periods:

Adverse events present in the SDTM database are allocated to periods based on their start date/time. If the start date/time of an event falls between (or on) the start and stop date/time of a period, the AE is attributed to that period (treatment-emergent principle).

Incomplete dates (i.e. time and/or day and/or month and/or year missing):

- In case of partial start or stop dates, the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, for the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event because of its assignment to multiple periods (see below example).
- In case of a completely missing end date (i.e., only for the calculation of duration):
    - In case the AE is not flagged as ongoing, the end date is considered as unknown, therefore the date will remain missing
    - In case the AE is flagged as ongoing the date is imputed by the cut-off date of the analysis for participants still ongoing in the study, and by the end date of the last period for participants who discontinued or completed the trial.

Examples:

Screening period: start date: 14JUN2021 - stop date: 28JUN2021

Post-boost vaccination period: start date: 28JUN2021 - stop date: 19JUL2021

1) Adverse event: start date: JUN2021- stop date: 15JUL2021

As the start date only has information about month and year, only this information will be used from the periods (i.e. assuming any day of Jun is possible) and therefore the AE will be assigned to the Screening Period as well as to the Post-boost vaccination Period.

2) Adverse event: start date: JUL2021- stop date 14JUL2021

As the AE starts after the Screening Period and after the start of the Post-boost vaccination period, it is only assigned to the Post-boost vaccination period.

Remarks:

- In addition to the date information, time information is considered to allocate AEs to periods, if available.
- The imputation of missing end dates of ongoing AEs will only be used to derive the duration of the event (i.e., to give an indication of the minimum duration). The imputed end dates will not be shown in the data listings.

Step 2: Combination of events:

Overlapping/consecutive events are defined as events in the same participant with the same preferred term which have at least 1 day overlap or for which the start date of an event is 1 day after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

1) If overlapping/consecutive events start in one of the following periods - Screening or Post boost dose FU (i.e. non-active periods) - followed by an AE in - Post-boost dose (active period) - they are allocated to their respective periods and are considered as separate events.
2) In case overlapping/consecutive events start within a single period, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, period, and total duration.
3) In case overlapping/consecutive events start in both an active period followed by a nonactive period, they are allocated to the active period only and are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, treatment period, and total duration.
4) In case an active period is followed by an active period, and the overlapping/consecutive events start in both periods, they are allocated to their respective period and are considered as separate AEs. The same rule applies for 2 non-active periods.

Remarks:

1) Events can only be combined into one and the same AE if their start and stop dates are known.
2) In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.
3) Time is not considered when determining overlap of events.
4) Solicited AEs will never be attributed to the Screening Period

Examples:

Screening period: start date: 14JUN2021 - stop date: 28JUN2021

Post-boost dose period: start date: 28JUN2021 - stop date: 26JUL2021
Post-boost dose FU period: start date: 27JUL2021 - stop date: 15AUG2021
Example for the above scenario 1
AE1: start date: 20JUN2021 - stop date: 10JUL2021
AE2: start date: 08JUL2021 - stop date: 18JUL2021
AE1 will be attributed to the screening period and AE2 to the Post-boost dose period.
Example for the above scenario 3
AE1: start date: 18JUL2021- stop date: 28JUL2021
AE2: start date: 28JUL2021- stop date: 08AUG2021
As AE1 starts in the active period (Post-boost dose) and overlaps with AE2 which starts in a non-active period (Post-boost dose FU), this AE is considered as a single AE in the AE analysis starting on 18JUL2021 and ending on 08AUG2021 and is attributed to the post-boost dose period.

2. Toxicity Grading Scale for Healthy Paediatric Participants (older than 3 months) Enrolled in Preventive Vaccine Clinical Trials

Adapted from: DMID° Pediatric Toxicity Tables (November 2007, draft). For adverse events not included in the tables below, refer to the severity criteria guidelines. Reference source not found. Local lab references take preference over the DMD table and the different grades. The abbreviations used in the following tables are: LLN: lower limit of normal; IV: intravenous; ULN: upper limit of normal.

LOCAL REACTIONS				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Tenderness	Mild discomfort to touch; minimal to no limitation of use of limb	Notable discomfort to touch; Greater than minimal limitation of use of limb	Significant discomfort at rest; Severe limitation of use of limb	Hospitalization or ER visit for treatment
Erythema	<10 mm	10-25 mm	26-50 mm	>50 mm or any grade 3 with hospitalization or ER visit for treatment
Swelling	<10 mm	10-25 mm	26-50 mm	>50 mm or any grade 3 with hospitalization or ER visit for treatment
HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hemoglobin for children greater than 3 months and less than 2 years of age	9.0-9.9 gm/dL	7.0-8.9 gm/dL	<7.0 gm/dL	Cardiac Failure secondary to Anemia

Hemoglobin for children greater than 2 years of age	10.0-10.9 gm/dL	7.0-9.9 gm/dL	<7.0 gm/dL	Cardiac Failure secondary to Anemia
Absolute Neutrophil Count	750-1200/mm3	400-749/mm3	250-399/mm3	<250/mm3
Platelets	75,000-99,999/mm3	50,000-74,999/mm3	25,000-49,999/mm3	<25,000/mm3
Prothrombin Time (PT)	1.1-1.2 x ULN	1.3-1.5 x ULN	1.6-3.0 x ULN	>3.0 x ULN
Partial Thromboplastin Time (PTT)	1.1-1.6 x ULN	1.7-2.3 x ULN	2.4-3.0 x ULN	>3.0 x ULN

GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Bilirubin (Fractionated bilirubin test must be performed when total bilirubin is elevated)				
Bilirubin for children greater than 3 months of age (when accompanied by any increase in other liver function test)	1.1- <1.25 x ULN	1.25- <1.5 x ULN	1.5-1.75 x ULN	>1.75 x ULN
Bilirubin for children greater than 3 months of age (when other liver functions are in the normal range)	1.1- <1.5 x ULN	1.5- <2.0 x ULN	2.0-3.0 x ULN	>3.0 x ULN
AST (SGOT)	1.1- <2.0 x ULN	2.0- <3.0 x ULN	3.0-8.0 x ULN	>8.0 x ULN
ALT (SGPT)	1.1- <2.0 x ULN	2.0- <3.0 x ULN	3.0-8.0 x ULN	>8.0 x ULN
GGT	1.1- <2.0 x ULN	2.0- <3.0 x ULN	3.0-8.0 x ULN	>8.0 x ULN
Pancreatic Amylase	1.1- 1.4 x ULN	1.5- 1.9 x ULN	2.0-3.0 x ULN	>3.0 x ULN
Uric Acid	7.5-9.9 mg/dL	10.0-12.4 mg/dL	12.5-15.0 mg/dL	>15.0 mg/dL
Loss of Appetite	Feeding minimally reduced	Feeding reduced by more than 50% of normal for the child	Refusing all feeds	No solid or liquid taken orally for in the last 24 hours; requires intravenous fluids
Diarrhea	Change in consistency of stools OR increase of 1-3 stools over baseline per 24-hour period	liquid/watery stools OR increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥7 stools over baseline per 24-hour period	Requires IV fluid resuscitation and electrolytes repletion OR hypotensive shock

Constipation	Slight change in consistency and/or frequency of stools	Hard, dry stools with a change in frequency	Intestinal obstruction accompanied with abdominal pain	Hospitalization; Severe abdominal distention and vomiting accompanied with severe abdominal pain
Vomiting	1 episode/ day (24h)	2-3 episodes per day (24h)	4-6 episodes per day (24h)	Greater than 6 episodes per day (24h) OR intractable vomiting

ELECTROLYTES				
	Grade 1	Grade 2	Grade 3	Grade 4
Creatinine				
3 Months – 2 Years of age	0.6-0.8 x ULN	0.9-1.1 x ULN	1.2-1.5 x ULN	> 1.5 x ULN
2 Years – 3 Years of age	0.7-1.0 x ULN	1.1-1.6 x ULN	1.7-2.0 x ULN	>2.0 x ULN
Hypernatremia	----	145-149 mEq/L	150-155 mEq/L	>155 mEq/L or abnormal sodium AND mental status changes
Hyponatremia	----	130-135 mEq/L	129-124 mEq/L	<124 mEq/L or abnormal sodium AND mental status changes
Hyperkalemia	5.0-5.9 mEq/L	6.0-6.4 mEq/L	6.5-7.0 mEq/L	>7.0 mEq/L or abnormal potassium AND cardiac arrhythmia
Hypokalemia	3.0-3.5 mEq/L	2.5-2.9 mEq/L	2.0-2.4 mEq/L	<2.0 mEq/L or abnormal potassium AND cardiac arrhythmia
Hypercalcemia	10.5-11.2 mg/dL	11.3-11.9 mg/dL	12.0-12.9 mg/dL	>13.0 mg/dL
Hypocalcemia	7.8-8.4 mg/dL	7.0-7.7 mg/dL	6.0-6.9 mg/dL	>6.0 mg/dL
Hypomagnesemia	1.2-1.4 mEq/L	0.9-1.1 mEq/L	0.6-0.8 mEq/L	<0.6 mEq/L or abnormal magnesium AND cardiac arrhythmia
hypoglycemia	55-65 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or adnormal glucose AND mental status changes
Hyperglycemia	116-159 mg/dL	160-249 mg/dL	250-400 mg/dL	>400 mg/dL or ketoacidosis
Proteinuria	Tr-1+ or <150 mg/day	2+ or 150-499 mg/day	3+ or 500-1000 mg/day	4+ or Nephrotic syndrome >1000 mg/day
Hematuria	Microscopic <25 cells/hpf	Microscopic >25 cells/hpf	Gross hematuria	Hospitalization; Lifethreatening consequences

NEUROLOGIC				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Irritability	Easily consolable; minimal or no interference with activity. Episodes of continuous crying <60 min.	Difficult to console. Episodes of continuous crying >60 min <120 min	Inconsolable, prevents daily activity. Episodes of continuous crying >120 min	Hospitalization or ER visit for treatment
Decreased Activity	Minimal decrease in alertness, minimal or no interference with activity	Some interference with activity, slightly subdued	unable to achieve normal level of alertness, lethargic	ER visit or hospitalization for treatment or life-threatening consequences
Neuropathy/ Lower Motor Neuropathy	----	Mild transient Paresthesia only	Persistent or progressive paresthesia, burning sensation in feet, or mild dysesthesia; no weakness; mild to moderate deep tendon reflex changes; no sensory loss	Onset of significant weakness, decrease or loss of DTRs, sensory loss in "stocking glove" distribution, radicular sensory loss, multiple cranial nerve involvement; bladder or bowel dysfunction, fasciculations, respiratory embarrassment from chest wall weakness.
Myopathy or Neuromuscular Junction Impairment	Normal or mild (<2 x ULN) CPK elevation	Mild proximal weakness and/or atrophy not affecting gross motor function. Mild myalgias, +/- mild CPK elevation (<2 x ULN)	Proximal muscle weakness and/or atrophy affecting motor function +/- CPK elevation; or severe myalgias with CPK >2 x ULN;	Onset of myasthenia-like symptoms (fatigable weakness with external, variable ophthalmoplegia and/or ptosis), or neuromuscular junction blockade (acute paralysis) symptoms
OTHER				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Fever/pyrexia	38.0 - 38.4 °C or 100.4 - 101.1 °F	38.5 - 38.9 °C or 101.2 - 102.0 °F	39.0 - 40.0 °C or 102.1 - 104.0 °F	Greater than 40 °C or 104.0 °F
Acute allergic reaction	Pruritus without Rash	Pruritic Rash	Mild Urticaria	Severe Urticaria Anaphylaxis, Angioedema
Stomatitis	Mild discomfort	Painful, difficulty swallowing, but able to eat and drink	Painful: unable to swallow solids	Painful: unable to swallow liquids; requires IV fluids
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

3. TRANSFORMING ON-SITE ASSESSMENTS AND DIARIES ASSESSMENTS OF SOLICITED AES INTO ANALYSIS FORMAT

When creating the analysis dataset for solicited AEs, solicited AEs (recorded by day) need to be converted into the format of unsolicited AEs (recorded by event).

All diary data will be considered, as well as any post-dose on-site assessment (scheduled as well as unscheduled) within 8 days after vaccination. For solicited local AEs for which a diameter is measured, the maximum of diameter derived grade and investigator severity will be used.

The start date of the AE will be considered as the date of first occurrence of the solicited AE (both local and systemic). If on subsequent day(s), the same grade is reported, the last reported date is used as the end date of the AE. A new record is created in case the grade of the event changes. If there is a time gap of at least one day between two (or more) occurrences of the particular solicited AE, then the second (and/or next) occurrence will be considered as a new AE. In case no data is reported for a day, this is analyzed as no event reported. If the on-site assessment differs in grade or relatedness (if collected) with the corresponding diary data, only the highest grade and highest level of relatedness per AE will be kept in the analysis database and used in the tables and listings. If relatedness is not collected for an on-site temperature assessment, then relatedness collected on the diary will be used.

The example below shows how the solicited AE should be converted into a format of unsolicited AEs:

Data from the Participant Diary
Participant: 0001
Solicited systemic AE: Headache

	On site assessment	DIARY DATA							
Solicited AE	Day 1 01Aug21	Day 1 01 Aug21	Day 2 02 Aug21	Day 3 03 Aug21	Day 4 04 Aug21	Day 5 05 Aug21	Day 6 06 Aug21	Day 7 07 Aug21	Day 8 08 Aug21
Grade	2	1	1	0	3	3	1	0	0
Relatedness	Doubtful	Probable							

The data should be converted and stored in the AE dataset as follows:

Participant No.	AE	Start Date (Char)	Stop Date (Char)	Severity	Relatedness	AEID	Duration
0001	Headache	01Aug21	02Aug21	2	Probable	1	6
0001	Headache	04Aug21	05JAug21	3	Probable	1	6
0001	Headache	06Aug21	06Aug21	1	Probable	1	6

If a solicited AE ends after day 8:

- The stop date of the event is the 'Date of Last day of symptom' as recorded in the eCRF and the 'Maximum severity' after Day 8 as recorded in the CRF. A separate record starting on day 9 is created for this, in case this severity deviates from the previous record.

Of note, to complete the start and end-date based on diary data, the date will be calculated based on the day the AE is reported relative to vaccination and not on the reported date. For example, if the vaccination is on 1st August 2021, and the AE starts on DAY 3, the start date will be set to the 3rd of August 2021 independent of the reported actual date. For the on-site assessments, the actual date as reported in the database will be used.

For the calculation of duration, the first and last day is used, even in case interruptions occurred in between by missing reporting days or 0 grade. In the above example, the 4 records contribute to the same AE, therefore AEID is set to the same value and the duration of the AE is set to 6 for all records.