Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
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eTrack study number and Abbreviated Title	201112 (EPI-NTHI-001 BOD APA)
Date of protocol	Final Version 1: 02 March 2014
Date of protocol amendment	Amendment 1 Final: 13 February 2017 Amendment 2 Final: 20 October 2017 Amendment 3 Final: 19 October 2018
Title	Occurrence of potential bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in Asia Pacific.
Detailed Title	A prospective, epidemiological, multi-country, cohort study to assess the occurrence of potential bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in moderate to very severe COPD patients, in Asia Pacific.
Co-ordinating author	PPD , Scientific Writer
Contributing authors	• PPD , Epidemiologist • PPD , Clinical Research and Development Lead • PPD , Clinical Research and Development Lead • PPD Senior Epidemiology Expert • PPD , Vaccine Development Leader • PPD Biostatistician • PPD Lead statistician • PPD Project Delivery Lead • PPD Study Delivery Lead • PPD Study Delivery Manager, (External Consultant [Freelance]for GSK Biologicals) • PPD Senior Manager, Oversight Data Management

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Contributing authors	• PPD Study Data Manager • PPD Vaccine Supply Coordinator (CVO Europe, for GSK Biologicals) • PPD Clinical Readout Team Leader, Clinical Laboratory Sciences • PPD Study Manager, GVCL (Business & Decision Life sciences, for GSK Biologicals)/PPD Study Manager, Clinical Laboratory Sciences • PPD Global Regulatory Lead and PPD Global Regulatory Affairs representative • PPD and PPD PPD and PPD PPD and PPD Clinical Safety Representatives • PPD Expert Scientist, Clinical Laboratory Sciences • PPD Study Data Manager (Business & Decision Life Sciences, for GSK Biologicals) • Dr. PPD, University of Sydney Head, Respiratory Trials, Contributing author from The George Institute for Global Health, Australia

GSK Biologicals' Protocol DS v15.0

Protocol Amendment 3 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	201112 (EPI-NTHI-001 BOD APA)
Date of protocol amendment	Amendment 3 Final: 19 October 2018
Detailed Title	A prospective, epidemiological, multi-country, cohort study to assess the occurrence of potential bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in moderate to very severe COPD patients, in Asia Pacific.
Sponsor signatory	Ashwani Kumar Arora Clinical and Epidemiology Project Lead
Signature	_____
Date	_____

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Protocol Amendment 3 Rationale

Amendment number: Amendment 3

Rationale/background for changes:

The protocol has been amended to implement the following changes:

- Removal of all STGG (Skim Milk-Tryptone-Glucose-Glycerol) wording throughout the protocol. STGG product not being available, it has been decided to replace it with cryobeads for storage of bacterial isolates. PCR testing on samples stored in cryobeads was shown to be as efficient as for samples stored in STGG. Besides, this decision has been taken for alignment with other COPD studies, where STGG solutions are no longer used.

- Clarification of technique used for target identification. For the primary endpoint, various viral pathogens will be identified but not quantified. Along this line, the wording "and by HRV quantitative RT-PCR" has been removed from primary endpoint to avoid confusion as HRV viral load data will be reported as part of the tertiary endpoints.

- Clarification in exclusion criteria section (4.3). Administration of antibiotics before study entry, where study entry is meant to be Visit 1.

- Clarification in study procedure section (6.3) and in the section for recording current medication (6.4.2.10) during scheduled visits, where it has been specified that recording of any current medication is applicable for any disease not only COPD-related. Moreover, a footnote referring to Table 10 "Reporting period for SAE and AEs leading to withdrawal" has been added for further clarification, both in Table 4 and 5.

- Addition of a section (actual 6.4.2.6) for Pregnancy test for Visit 1, Visit 2 and Visit 3. A Pregnancy test section for Screening Visit was included during Protocol Amendment 1: to be consistent, same wording has been included for the subsequent visits.

- Clarification of reporting of severe AECOPDs. Two sentences have been added in sections 7.1.1 and 7.2.2.1 for making clear that severe AECOPD events are not to be reported as SAE as they are meant as disease-related outcomes.

- Rewording in the table concerning reporting period for SAE and AEs leading to withdrawal for better clarification (Table 10).

- Addition of a new outsourced laboratory (DDL).

- To correct some typographical errors.

Protocol Amendment 3 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, with the terms of the study agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) or other applicable guidelines and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	201112 (EPI-NTHI-001 BOD APA)
Date of protocol amendment	Amendment 3 Final: 19 October 2018
Detailed Title	A prospective, epidemiological, multi-country, cohort study to assess the occurrence of potential bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in moderate to very severe COPD patients, in Asia Pacific.
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut 89
1330 Rixensart, Belgium

Sponsor Medical Expert for the Study

Refer to the local study contact information document.

Sponsor Study Monitor

Refer to the local study contact information document.

Study Contact for Reporting of a Serious Adverse Event (SAE)

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 7.3.2.

Study Contact for Reporting SAEs: refer to the local study contact information document.

SYNOPSIS

Detailed Title

A prospective, epidemiological, multi-country, cohort study to assess the occurrence of potential bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in moderate to very severe COPD patients, in Asia Pacific.

Objectives

Primary

To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients and during AECOPD, using respectively bacteriological methods and viral PCR, over the course of 1 year.

Secondary

- To evaluate the concordance between PCR and bacteriological methods data in sputum for potential bacterial pathogens.
- To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients, by GOLD grade.
- To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum during AECOPD, by severity of AECOPD.
- To estimate the incidence rate of all-cause AECOPD, overall and by GOLD grade.
- To describe the severity and duration of all-cause AECOPD, overall and by GOLD grade.
- To assess the impact of all-cause AECOPD on HRQOL.
- To assess the impact of all-cause AECOPD on lung function.
- To assess the impact of all-cause AECOPD on healthcare utilisation.

Tertiary

- To assess the use of EXACT-PRO for determining the end date of AECOPD.
- To evaluate the load of bacterial and viral pathogens in stable COPD and during AECOPD.
- To collect biological specimens for future respiratory disease-related testing:
  - Aliquots of sputum samples for assay development and microbiome analysis at stable visits and during exacerbation.
  - Blood sampling for potential identification/quantification of biomarkers at Visit 1 and Visit 3.

Rationale for the study

Because the infectious aetiology of AECOPD has been suggested to vary according to geographical region, the primary purpose of this study (which will be conducted in several countries in Asia Pacific) is to evaluate the occurrence of potential bacterial and viral pathogens in the sputum of stable COPD patients and at the time of AECOPD. Given the increasing and projected burden of COPD in the Asia Pacific region, this study will also evaluate the frequency, severity and duration of AECOPD, as well as the impact of AECOPD on health-related quality of life (HRQOL), healthcare utilisation and lung function.

In addition, both PCR and bacteriological methods will be used for characterisation (quantification in some cases) of bacteria in sputum. To date, identification of bacteria and assessment of bacterial load is done in the vast majority of cases by culture. However, the few studies that have compared culture with PCR demonstrated that PCR is more discriminatory at detecting typical airway bacteria [Curran, 2007; Singh 2014]. Moreover, in a multi-centre study, PCR has the advantage that it can be performed on stored sputum samples, and can therefore be done centrally, whereas culture has to be performed on fresh sputum samples, which can lead to variable results due to variability in testing methods between laboratories.

Study design

- Type of design: Prospective, epidemiological, interventional, multi-country, cohort study.
- Study population: A stable cohort of approximately 200 moderate to very severe COPD patients with at least 1 documented moderate or severe AECOPD in the year before enrolment.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF) and electronic diary cards.
- Primary completion Date: Visit 3 (Month 12). Refer to glossary of terms for the definition of PCD.
- End of Study (EoS): Last testing results released of samples collected at Visit 3.
- Duration of the study: The study will last approximately 1 year for each subject.
- Note: Screening visit should ideally occur not more than 6 weeks before visit 1.
- **Epoch 001**: Prospective data collection starting at Screening Visit (Pre-Month 0) and ending at Visit 3 (Month 12).

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch
			Epoch 001
Prospective	Approx 200	≥ 40 years	X

Discussion of study design

The primary objective of this study is to prospectively investigate the prevalence and distribution of bacteria and viruses isolated from sputum samples from moderate to very severe COPD patients and at the time of AECOPD, in Asian populations. In order to increase the chance that the patients will exacerbate during the study, only patients with a documented history of at least 1 moderate or severe AECOPD in the previous year will be recruited, as this is known to be the best predictor of AECOPD [Hurst, 2010]. Therefore, this study will permit an estimation of incidence of AECOPD in a COPD population at increased risk of exacerbation. It will not provide a true population-based incidence of AECOPD.

In order to study factors affecting disease progression in Asia Pacific, prospective data from a relatively large cohort of COPD patients is essential. The use of electronic Diary Cards to detect changes in the respiratory symptoms that define an AECOPD, the standardised questionnaires and spirometry

assessments will allow robust conclusions to be drawn about the effects of exacerbations on decline in lung function and HRQOL.

EXACT PRO will be used to assess breathlessness, cough and presence of sputum, chest symptoms, difficulty bringing up sputum, feeling tired or weak, sleep disturbance, and feeling scared or worried about their condition. Advantages to this approach include standardised data, reduced recall bias, and the potential to identify events and determine resolution based on a predefined scoring algorithm [Leidy, 2011]. Standardised questionnaires completed during study visits include the SGRQ-C and the CAT. The SGRQ-C is designed to assess HRQOL, current health and does not specify a recall period. The CAT is a simple, short, patient-completed instrument to assess HRQOL and symptom burden in patients with COPD. The CAT has good internal consistency and test-retest reliability, correlates strongly with the SGRQ-C, and is able to distinguish between stable patients and those undergoing an AECOPD [Jones, 2012].

Number of subjects

The target is to enrol approximately 200 eligible moderate to very severe COPD patients.

Endpoints

Primary (Amended on 19 October 2018)

- Occurrence of potential bacterial and viral pathogens in sputum of stable COPD patients and during AECOPD, over the course of 1 year:
  - Bacterial pathogens, as identified by bacteriological methods, including (but not necessarily limited to) *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*.
  - Viral pathogens, as identified by PCR, including (but not necessarily limited to) RSV, parainfluenza virus, enterovirus/ HRV, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus.

Secondary

- Occurrence of potential bacterial pathogens in sputum of stable COPD patients and during AECOPD, as measured by real-time qualitative PCR/ quantitative PCR and compared to data from bacteriological methods, over the course of 1 year:
  - Including (but not necessarily limited to) *H. influenzae*, *M. catarrhalis, S. pneumoniae, S. aureus* and *P. aeruginosa*.
- Occurrence of potential bacterial and viral pathogens (overall and by species) in sputum of stable COPD patients by GOLD grade, over the course of 1 year.
- Occurrence of potential bacterial and viral pathogens (overall and by species) in sputum during AECOPD by severity of AECOPD, over the course of 1 year.
- Incident rate (per subject per year) of AECOPD, overall and by GOLD grade, over the course of 1 year.
- Severity of AECOPD, overall and by GOLD grade, over the course of 1 year.
- Duration of AECOPD, overall and by AECOPD severity, over the course of 1 year.
- CAT score in stable COPD patients and during AECOPD, over the course of 1 year.
- SGRQ-C score in stable COPD patients, over the course of 1 year.
- FEV_1% of predicted normal value in stable COPD patients, at Pre-Month 0 and Month 12.
- Healthcare utilisation, over the course of 1 year.

Tertiary

- EXACT-PRO scores in stable COPD patients and during AECOPD, over the course of 1 year.
- Bacterial load measured by both culture and PCR in COPD and during AECOPD.
- HRV load measured by PCR in COPD and during AECOPD.

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LIST OF ABBREVIATIONS (Amended 19 October 2018)

A. baumannii	*Acinetobacter baumannii*
AE	Adverse Event
AECOPD	Acute exacerbation of COPD
ATS-DLD-78A	American Thoracic Society-Division of Lung Diseases-78A
CAT	COPD assessment test
CI	Confidence Interval
CLS	Clinical Laboratory Sciences
COPD	Chronic Obstructive Pulmonary Disease
DTT	Dithiothreitol
eCRF	electronic Case Report Form
EoS	End of Study
ER	Emergency Room
EXACT-PRO	Exacerbations of Chronic Pulmonary Disease Tool - Patient Reported Outcome
FAS	Full Analysis Set
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GCP	Good clinical practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GP	General Practitioner
GSK	GlaxoSmithKline
H. influenzae	*Haemophilus influenzae*
HRQOL	Health-related quality of life
HRV	Human Rhinovirus

ICD	International Classification of Diseases
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
K. pneumoniae	*Klebsiella pneumoniae*
LAR	Legally Acceptable Representative
M. catarrhalis	*Moraxella catarrhalis*
OTC	Over-the-counter
P. aeruginosa	*Pseudomonas aeruginosa*
PCD	Primary Completion Date
PCR	Polymerase chain reaction
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription polymerase chain reaction
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*
SAE	Serious Adverse Event
SGRQ-C	St. George's Respiratory Questionnaire for COPD patients
SPM	Study Procedures Manual
USA	United States of America

GLOSSARY OF TERMS

Adverse event:	Any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.
Anonymised data:	Information about an individual that GSK or a third party cannot reasonably attribute to the individual, or could only attribute to the individual by expending a disproportionate amount of time, effort or expense (e.g. de-identified or aggregated information). For the purpose of this policy, Key-Coded personally identifiable information shall not be considered Anonymised Information
Current smoker	A person who is currently smoking or who stopped smoking within the past 6 months.
Cohort study:	A form of epidemiological study where subjects in a study population are classified according to their exposure status/disease and followed over time (prospective/ retrospective) to ascertain the outcome(s).
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epidemiological study:	An observational or interventional study without administration of medicinal product(s) as described in a research protocol.

End of Study

(Synonym of End of Trial)

For studies without collection of human biologicals samples or imaging data EoS is the Last Subject Last Visit (LSLV).

For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV.

Epoch:

An epoch is a set of consecutive timepoints or a single timepoint from a single protocol. Epochs are defined to support a main purpose which either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the timepoints included in an epoch must be sufficient to fulfil the purpose of the epoch.

Typical examples of epochs are screening immunogenicity follow-up, safety follow-up, ESFU, follow-up.

eTrack:

GSK Biologicals' tracking tool for clinical/ epidemiological trials.

Evaluable:

Meeting all eligibility criteria, complying with the procedures defined in the protocol (see Section 9.3 for details on criteria for evaluability).

Former smoker

A person who stopped smoking for at least 6 months.

GOLD grade: (classification of severity of airflow limitation in COPD patients)

The spirometric classification of airflow limitation in COPD patients is based on post-bronchodilator FEV_1 and can be divided into four GOLD grades [GOLD, 2013]:

GOLD grade	In patients with FEV_1/ FVC < 0.70:
GOLD 1: Mild	$FEV_1 \geq 80\%$ predicted
GOLD 2: Moderate	$50\% \leq FEV_1 < 80\%$ predicted
GOLD 3: Severe	$30\% \leq FEV_1 < 50\%$ predicted
GOLD 4: Very Severe	$FEV_1 < 30\%$ predicted

Interventional Human Subject Research:	Studies in which participants are administered medical care, medicinal products and/or medical/scientific procedures as described in a research protocol.
Non-interventional (observational) Human Subject Research:	Studies where medicinal products, should they be administered, are prescribed in normal (routine) medical practice. No medical care or medical/scientific procedures as required in a research protocol are administered to participants except as part of routine medical care.
Pack-years of smoking:	Pack-years is a quantification of cigarette smoking, a way to measure the total amount a person has smoked in the course of his/ her lifetime. The number of pack-years is calculated as follows: (average number of *cigarettes* smoked per day x number of years smoked)/20 *E.g.* a smoking history of 10 pack-years means having smoked 20 cigarettes per day for 10 years, or having smoked 10 cigarettes per day for 20 years. *Note: For the purpose of this study, pipe and/or cigar use should not be used to calculate pack year history.*
Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organisation of a study. The protocol usually also gives the background and rationale for the study, but these could be provided in other protocol referenced documents.
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study objectives.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial/ pharmaco-epidemiological study was concluded according to the pre-specified protocol or was terminated**.**

Self-contained study:	Study with objectives not linked to the data of another study.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring the proper conduct of epidemiological studies at one or more investigational sites.
Study population:	Sample of population of interest.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the epidemiological study or a person about whom some medical information has been recorded in a database.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.

TRADEMARKS

The following trademarks are used in the present protocol.

Trademarks of the GSK group of companies	Generic description
COPD Assessment test (CAT)	Questionnaire to measure the impact of COPD on wellbeing and daily life
EXACT-PRO	Exacerbations of Chronic Pulmonary Disease Tool - Patient Reported Outcome
SGRQ-C	St. George's Respiratory Questionnaire for COPD patients

1. INTRODUCTION

1.1. Background

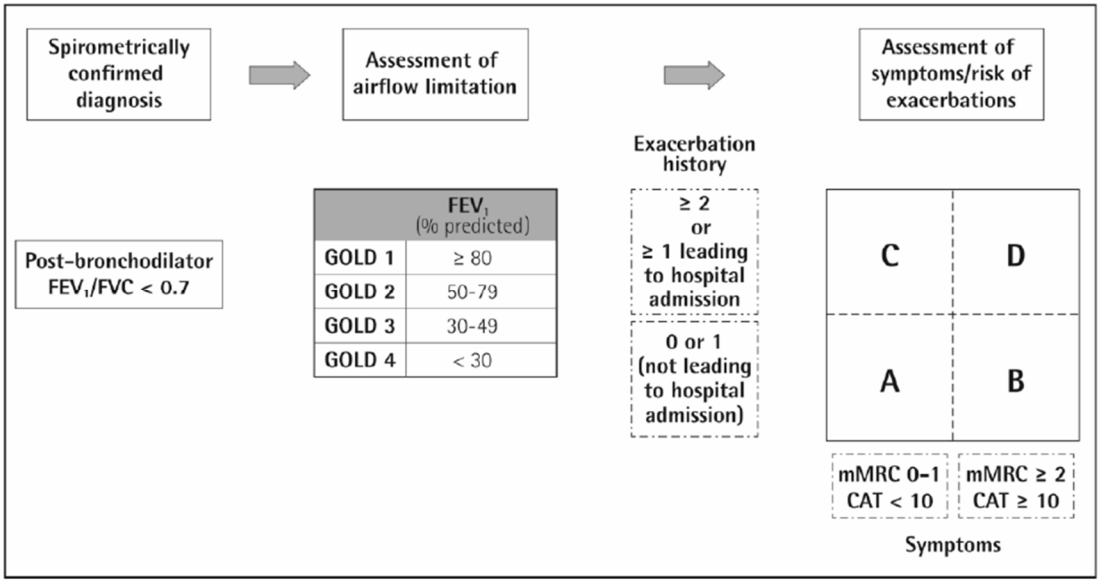
Chronic Obstructive Pulmonary Disease (COPD) is a common preventable and treatable disease characterised by persistent airflow obstruction which is not fully reversible and which is usually progressive in the long term. The classification of COPD severity is done based on the patient's spirometric classification according to [GOLD, 2013]. The airflow limitation in COPD patients can be classified into GOLD grades as shown in Table 1 [GOLD, 2013]. In this study, additional parameters are going to be collected in order to allow for a combined assessment that could be used for future evaluation, as detailed in Figure 1 [GOLD, 2017].

Table 1 Classification of severity of airflow limitation in COPD (based on post-bronchodilator FEV_1)

GOLD grade	In patients with FEV_1/ FVC < 0.70:
GOLD 1: Mild	$FEV_1 \geq 80\%$ predicted
GOLD 2: Moderate	$50\% \leq FEV_1 < 80\%$ predicted
GOLD 3: Severe	$30\% \leq FEV_1 < 50\%$ predicted
GOLD 4: Very Severe	$FEV_1 < 30\%$ predicted

GOLD = Global Initiative for Chronic Obstructive Lung Disease; **FEV_1** = forced expiratory volume in 1 second; **FVC** = forced vital capacity

Figure 1 The refined ABCD assessment tool



GOLD = Global Initiative for Chronic Obstructive Lung Disease; CAT = COPD assessment test; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; mMRC = Modified British Medical Research Council Questionnaire

In addition to the airflow limitation, number of exacerbation per year and number of hospitalizations per year and CAT score will be collected at the enrollment in order to allow for the classification according to the [GOLD, 2017]:

- Patient Group A – Low Risk, Less Symptoms Typically GOLD 1 or GOLD 2 (Mild or Moderate airflow limitation); and/or 0-1 exacerbation per year and no hospitalization for exacerbation; and CAT score < 10 or mMRC grade 0-1
- Patient Group B – Low Risk, More Symptoms Typically GOLD 1 or GOLD 2 (Mild or Moderate airflow limitation); and/or 0-1 exacerbation per year and no hospitalization for exacerbation; and CAT score ≥ 10 or mMRC grade ≥ 2
- Patient Group C – High Risk, Less Symptoms Typically GOLD 3 or GOLD 4 (Severe or Very Severe airflow limitation); and/or ≥ 2 exacerbations per year or ≥ 1 with hospitalization for exacerbation; and CAT score < 10 or mMRC grade 0-1
- Patient Group D – High Risk, More Symptoms Typically GOLD 3 or GOLD 4 (Severe or Very Severe airflow limitation); and/or ≥ 2 exacerbations per year or ≥ 1 with hospitalization for exacerbation; and CAT score ≥ 10 or mMRC grade ≥ 2.

The airflow limitation in COPD is associated with an enhanced and chronic inflammatory response to noxious particles and gases in the airways and the lungs [Hogg, 2004]. The most important environmental risk factor for COPD is tobacco smoking. However, long-term exposure to traffic pollution and biomass smoke are also important contributors. Indoor air pollution (generated largely by inefficient and poorly ventilated stoves burning biomass fuels such as wood, crop waste and dung, or coal) for instance accounts for the high prevalence of COPD among non-smoking women in parts of the Middle East, Africa and Asia [WHO, 2013].

COPD is the fifth leading cause of mortality worldwide, accounting for 6% of all deaths globally [WHO, 2015]. A recent study reported high overall morbidity and mortality rates due to COPD in the Asia Pacific region. The prevalence of moderate to severe COPD in adults aged 30 years or above was estimated to be 5.9% in South Korea, 5.4% in Taiwan, 4.7% in Australia and 3.5% in Hong Kong [Tan, 2009]. The overall estimated prevalence COPD in Asia-Pacific was estimated to be 6.2% with 19.1% having severe COPD [Lim, 2015]. The prevalence and burden of COPD are projected to continue to increase in the coming decades due to continued exposure to risk factors and the changing age structure of the world's population [Mathers, 2006]. In Asia, urbanisation is expected to play a major role in the increase of the number of prevalent cases of COPD between 2010 and 2020 [Tan, 2009]. In terms of the number of deaths, the burden of disease is higher in the Asia-Pacific region when compared to the industrialised western countries. It was estimated through mathematical modelling that 56.6 million people aged ≥ 30 years had moderate to severe COPD with a prevalence rate of 6.3% [Ko, 2008].

The morbidity and mortality of COPD is substantially contributed to by acute exacerbations of COPD (AECOPD). An AECOPD is defined as "a clinical diagnosis of exclusion, made when a patient with COPD experiences an acute worsening in respiratory symptoms (typically dyspnoea, cough, sputum quantity and/or sputum purulence), and in whom no alternative specific cause for that deterioration has been

identified by clinical examination and/or corroborative testing. The worsening in respiratory symptoms may or may not warrant a change in underlying therapy and the symptoms will typically resolve over a period of days to weeks" [ERS/ATS Draft Task Force Recommendations, Major Symposium, ERS Scientific Congress Barcelona 2013]. AECOPD are often associated with concurrent deteriorations in pulmonary function and increases in both local and systemic inflammation. Higher exacerbation rates have been related to a faster decline in lung function and are known to have a negative effect on the patient's quality of life. Moreover, frequent exacerbations are associated with significant mortality, particularly if they require hospitalisation. Between 40 - 60% of medical expenditure for COPD is a direct consequence of AECOPD [Cazzola, 2008]. The severity of AECOPD can be graded according to the intensity of medical intervention required (see Table 2) [Wedzicha, 2007].

Table 2 Classification of severity of AECOPD

Grade	Intensity of medical intervention
Mild	Controlled with an increase in dosage of regular medications
Moderate	Requires treatment with systemic corticosteroids and/ or antibiotics
Severe	Requires hospitalisation

Bacteria, viruses and environmental pollution are also thought to be important causes of AECOPD [Sapey, 2006]:

- The lungs are not sterile and are known to be colonised with different strains of bacteria [Charlson, 2011; Erb-Downward, 2011; Monsó, 1995]. It has been suggested that the bronchial bacterial load increases during AECOPD [Miravitlles, 2002; Wilkinson, 2006]. Based on a recent literature review, the most frequent bacterial pathogens identified during AECOPD in European countries and in the USA were *Haemophilus influenzae* (*H. influenzae*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Moraxella catarrhalis* (*M. catarrhalis*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) [Mapi Research Trust-literature review for GlaxoSmithKline (GSK) Biologicals]. However, it has been suggested that the infectious aetiology of AECOPD can vary according to geographical region. In Asia, a rise in infections caused by *P. aeruginosa* and the Gram-negatives *Klebsiella pneumoniae* (*K. pneumoniae*) and *Acinetobacter baumannii* (*A. baumannii*) has been reported in several countries [Hui, 2011; Ye, 2013]. In addition, the infectious aetiology of AECOPD has also been suggested to vary according to the severity of the illness, with *P. aeruginosa* being particularly common in patients with advanced disease [Domenech, 2013; Ko, 2007].
- The most commonly detected viruses during AECOPD are Human Rhinoviruses (HRV), RSV, coronaviruses, influenza, parainfluenza and adenoviruses [De Serres, 2009; Sapey, 2006]. It has also been shown that viruses can be present in the airways of stable COPD patients, suggesting that some respiratory viruses may cause persistent low-grade infection contributing to the pathogenesis of disease [Mohan, 2010].
- Mixed bacterial and viral co-infections occur in 8-58% of exacerbations [De Serres, 2009; Hutchinson, 2007; Papi, 2006; Perotin, 2013; Wilkinson, 2006].

AECOPD is sudden worsening of COPD symptoms. Many studies have attempted to measure the burden of bacterial or viral infections in patients with AECOPD, but very few have investigated the presence of mixed viral and bacterial infections both in stable COPD patients and during AECOPD.

1.2. Rationale for the study

Because the infectious aetiology of AECOPD has been suggested to vary according to geographical region, the primary purpose of this study (which will be conducted in several countries in Asia Pacific) is to evaluate the occurrence of potential bacterial and viral pathogens in the sputum of stable COPD patients and at the time of AECOPD. Given the increasing and projected burden of COPD in the Asia Pacific region, this study will also evaluate the frequency, severity and duration of AECOPD, as well as the impact of AECOPD on health-related quality of life (HRQOL), healthcare utilisation and lung function.

In addition, both PCR and bacteriological methods will be used for characterisation and (quantification in some cases) of bacteria in sputum. To date, identification of bacteria and assessment of bacterial load is done in the vast majority of cases by culture. However, the few studies that have compared culture with PCR demonstrated that PCR is more discriminatory at detecting typical airway bacteria [Curran, 2007;Singh, 2014]. Moreover, in a multi-centre study, PCR has the advantage that it can be performed on stored sputum samples, and can therefore be done centrally, whereas culture has to be performed on fresh sputum samples, which can lead to variable results due to variability in testing methods between laboratories.

2. OBJECTIVES

2.1. Primary objective

- To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients and during AECOPD, using respectively bacteriological methods and viral PCR, over the course of 1 year.

Refer to Section 9.1.1 for the definition of the primary endpoint.

2.2. Secondary objectives

- To evaluate the concordance between PCR and bacteriological methods data in sputum for potential bacterial pathogens.
- To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients, by GOLD grade.
- To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum during AECOPD, by severity of AECOPD.
- To estimate the incidence rate of all-cause AECOPD, overall and by GOLD grade.

- To describe the severity and duration of all-cause AECOPD, overall and by GOLD grade.
- To assess the impact of all-cause AECOPD on HRQOL.
- To assess the impact of all-cause AECOPD on lung function.
- To assess the impact of all-cause AECOPD on healthcare utilisation.

Refer to Section 9.1.2 for the definition of the secondary endpoints.

2.3. Tertiary objective(s)

- To assess the use of EXACT-PRO for determining the end date of AECOPD.
- To evaluate the load of bacterial and viral pathogen in stable COPD and during AECOPD.
- To collect biological specimens for future respiratory disease-related testing:
  - Aliquots of sputum samples for assay development and microbiome analysis at stable visits and during exacerbation.
  - Blood sampling for potential identification/quantification of biomarkers at Visit 1 and Visit 3.
  - Bacterial isolates for further strain characterisation.

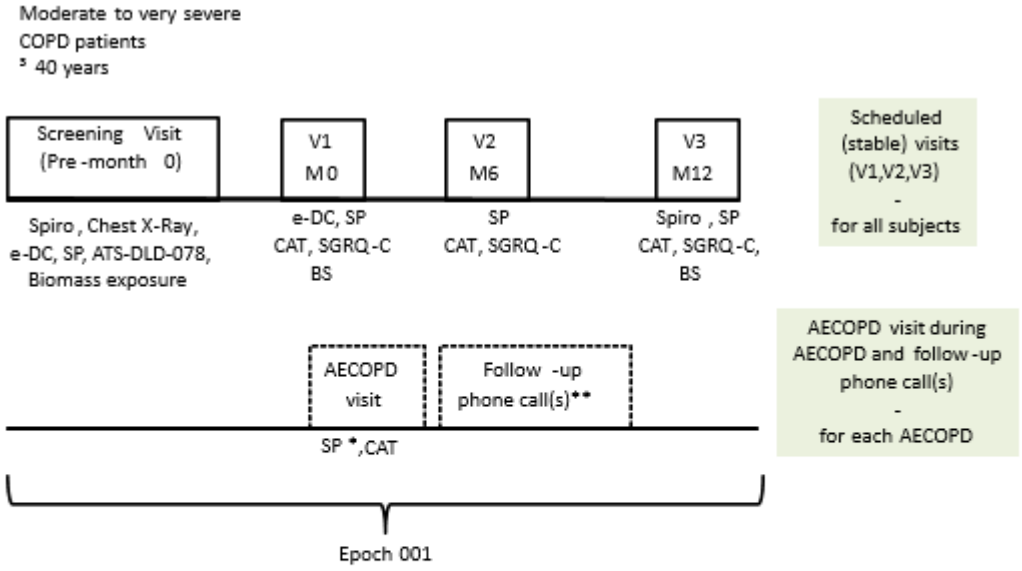
Refer to Section 9.1.3 for the definition of the tertiary endpoints.

3. STUDY DESIGN OVERVIEW

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6), are essential and required for study conduct.

Figure 2 presents an overview of the study design.

Figure 2 Study design overview



V = Visit; M = Month; SP = sputum sample; e-DC: Training and use of electronic Diary card; CAT = COPD assessment test; SGRQ-C = St. George's Respiratory Questionnaire for COPD patients; spiro = spirometry; BS = Blood Sampling; AECOPD = acute exacerbation of COPD; ATS-DLD-078 = American Thoracic Society and National Heart and Lung Institute-Division of Lung Disease Respiratory Questionnaire

* The sputum samples during AECOPD should preferably be obtained before administration of the first dose of antibiotics to treat the AECOPD (if applicable). Self collection of the sputum sample will be allowed in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place. This is not allowed at scheduled visits when subject should be in stable condition.

** Follow- up phone calls will take place at least every 2 weeks, until the AECOPD has resolved

Type of design: Prospective, epidemiological, interventional, multi-country, cohort study.

- Study population: A cohort of approximately 200 moderate to very severe COPD patients with at least 1 documented moderate or severe AECOPD in the year before enrolment.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF) and electronic diary cards.
- Primary completion Date: Visit 3 (Month 12). Refer to glossary of terms for the definition of PCD.
- End of Study (EoS): Last testing results released of samples collected at Visit 3.
- Duration of the study: The study will last approximately 1 year for each subject from screening visit up to study conclusion.

Note: Screening visit should ideally occur not more than 6 weeks before visit 1.

- **Epoch 001**: Prospective data collection starting at Screening Visit (Pre-month 0) and ending at Visit 3 (Month 12).

Table 3 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age	Epoch
			Epoch 001
Prospective	Approx 200	≥ 40 years	X

- **Study visits/contacts:**
  - Screening visit.
  - Three scheduled (stable) study visits occurring at 6 months intervals.
  - For each AECOPD: AECOPD visit (within 96 hours of the onset of the symptoms) and follow-up phone call(s) (at least every 2 weeks until the AECOPD has resolved). Follow-up phone contacts will define end of AECOPD.
- **COPD symptoms**

  Subjects will be asked to record their COPD symptoms in an electronic Diary Card on a daily basis:
  - Daily in the morning throughout the study: Morning symptoms questionnaire.
  - Daily at bedtime throughout the study: EXACT-PRO (EXAcerbations of Chronic Pulmonary Disease Tool - Patient Reported Outcome) questionnaire.
- **Biological samples:**
  - **Sputum samples** collected at site (spontaneous or induced, as per investigator judgement) will be collected at each study visit at the site (scheduled [stable] visits and AECOPD visits) if, in the opinion of the investigator, it is safe for the subject.

Or

  - **Sputum sample collected at subject's home (spontaneous).** Self-collection of the sputum sample will be allowed in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place. This is not allowed at scheduled visits when subject should be in stable condition.

*Note**:** The sputum samples collected during AECOPD should preferably be obtained before administration of the first dose of antibiotics to treat the AECOPD (if applicable).*

  - **Blood samples** for biomarker testing will be collected at Visit 1 (M0) and Visit 3 (M12).

- **HRQOL assessments**
  - Subjects will be asked to complete the COPD assessment Test (*CAT*) at each study visit (scheduled [stable] visits and AECOPD visits).
  - Subjects will be asked to complete the St. George's Respiratory Questionnaire for COPD patients (SGRQ-C) at each scheduled (stable) study visit (Visit 1 [Month 0], Visit 2 [Month 6] and at Visit 3 [Month 12]).
- Pre- and post-bronchodilator spirometry assessments will be done for all subjects at Screening Visit (pre-Month 0) and at Visit 3 (Month 12).

3.1. Discussion of study design

The primary objective of this study is to prospectively investigate the prevalence and distribution of bacteria and viruses isolated from sputum samples from moderate to very severe COPD patients and at the time of AECOPD, in Asian populations. In order to increase the chance that the patients will exacerbate during the study, only patients with a documented history of at least 1 moderate or severe AECOPD in the previous year will be recruited, as this is known to be the best predictor of AECOPD [Hurst, 2010]. Therefore, this study will permit an estimation of incidence of AECOPD in a COPD population at increased risk of exacerbation. It will not provide a true population-based incidence of AECOPD.

In order to study factors affecting disease progression in Asia Pacific, prospective data from a relatively large cohort of COPD patients is essential. The use of electronic Diary Cards to detect changes in the respiratory symptoms that define an AECOPD, the standardised questionnaires and spirometry assessments will allow robust conclusions to be drawn about the effects of exacerbations on decline in lung function and HRQOL. EXACT PRO will be used to assess breathlessness, cough and presence of sputum, chest symptoms, difficulty bringing up sputum, feeling tired or weak, sleep disturbance, and feeling scared or worried about their condition. Advantages to this approach include standardised data, reduced recall bias, and the potential to identify events and determine resolution based on a predefined scoring algorithm [Leidy, 2011]. Standardised questionnaires completed during study visits include the SGRQ-C and the CAT. The SGRQ-C is designed to assess HRQOL, current health and does not specify a recall period. The CAT is a simple, short, patient-completed instrument to assess HRQOL and symptom burden in patients with COPD. The CAT has good internal consistency and test-retest reliability, correlates strongly with the SGRQ-C, and is able to distinguish between stable patients and those undergoing an AECOPD [Jones, 2012].

3.1.1. Detection of AECOPD

Occurrence of potential AECOPD will be monitored by means of electronic Diary Cards which the subject will use to record his/ her morning symptoms on a daily basis. The electronic Diary Cards will be programmed as to detect potential AECOPD as follows (based on the Anthonisen criteria [Anthonisen, 1987]):

- Worsening of two or more of the following major symptoms for at least two consecutive days: dyspnoea, sputum volume, sputum purulence (colour), OR
- Worsening of any major symptom together with any of the following minor symptoms for at least two consecutive days: sore throat, colds (nasal discharge and/or nasal congestion), fever (oral temperature ≥ 37.5°C) without other cause, increased cough, increased wheeze.

Note that: The same two symptoms do not have to be present on both days as long as at least one major symptom is present on both days.

Each time a potential AECOPD is detected via the electronic Diary Card, the device will alert the subject to contact the study site, and at the same time an alert will be sent to the site so that the investigator or medically qualified individual contacts the subject to determine if the alert is an AECOPD or not, and if an AECOPD visit is warranted. In addition, the site should proactively follow-up all data received via the electronic Diary Card and contact the subject whenever deemed necessary.

During the contact with the subject, the investigator or medically qualified individual will determine whether the subject might actually be experiencing an AECOPD (e.g. notifications that can be explained solely by increased physical activity will not be considered):

- If the investigator or medically qualified individual concludes that the subject is not experiencing an AECOPD, this should be documented/ reported in the electronic Diary website (StudyWorks). Please refer to study procedures manual (SPM) for more details on how to perform this.
- If the investigator or medically qualified individual concludes that the subject may be experiencing an AECOPD, an AECOPD visit will be scheduled as soon as possible after the onset of AECOPD symptoms as recorded in the electronic Diary Card or confirmed by the subject (maximum 96 hours after onset of symptoms and, if applicable, preferably before starting treatment with antibiotics). The AECOPD onset date will be captured in the eCRF and additional information about severity will be also collected.
  - In case the AECOPD is confirmed but no AECOPD visit can take place, the site should record the information in the medical records subject files, and in the eCRF in medical records section and obtain all relevant information regarding the AECOPD (hospital record, medical record etc.) and record this in the eCRF.
- If the investigator concludes the subject is experiencing/continuing with the same event for which a visit has been performed already, an AECOPD visit should not take place. Medical treatment should be foreseen according to standard medical practice outside of the study.

During the AECOPD visit, the investigator will confirm the occurrence of the AECOPD based on clinical and medical judgement and based on the Anthonisen criteria and will record its date of onset. The end date of the AECOPD and its severity will be determined/ confirmed by the investigator/delegate during (a) follow-up phone call(s), which will take place at least every 2 weeks until the AECOPD has resolved.

If an AECOPD occurs at the time when one of the scheduled [stable] study visits is planned, it should be handled and recorded as an AECOPD visit, with all relevant AECOPD visit study procedures performed and, if possible, the stable study visit should be re-scheduled to a later date, when the subject is stable again and within the time window specified in the protocol.

The investigator/site must engage their best efforts to reach the patients, however, if the site does not succeed in contacting the patient, the reason should be recorded and an explanation given about what occurred. For example, the subject could be hospitalized or could visit a different physician (and in that case medical record should be obtained), or the subject is on holidays or not able to go the site.

3.1.1.1. Date of onset and end date of AECOPD

The date of onset is the first day (of at least 2 consecutive days) of worsening symptoms of COPD, as determined by the Investigator according to the Anthonisen criteria.

The end date should be based on when the investigator determines that the AECOPD symptoms have resolved. In determining this end date, consideration should be given to symptoms recorded in the electronic Diary Card and subject assessment during the phone calls.

Both start and end date of each confirmed AECOPD occurring from the screening visit to study conclusion will be recorded in the eCRF.

3.1.1.2. Guideline for assessing AECOPD that increase in severity

If an exacerbation starts off as mild, but becomes moderate or severe or starts off as moderate and becomes severe, the exacerbation should be captured as one exacerbation and classified by its highest level of severity.

4. STUDY POPULATION

This will be a multi-country study to be conducted in Asia Pacific.

4.1. Number of subjects/ centres

The target is to enrol approximately 200 eligible moderate to very severe COPD patients. Refer to Section 9.2 for a detailed description of the criteria used to estimate the sample size.

4.1.1. Recruitment of study centres

Centres will be selected for study participation based on the following criteria:

- Experience with patients with respiratory disorders.
- Able to perform microbiology testing. Please refer to SPM for details on the requirements to be met for microbiology testing.
- No limitations on the quantity of export of biological samples to GSK Biologicals or to designated laboratories.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardise the scientific integrity of the study and regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., completion of electronic Diary Card, sputum sampling, pre- and post-bronchodilator spirometry, return for follow-up visits).
- Written informed consent obtained from the subject.
- Male or female aged 40 years or older at the time of enrolment.
- Confirmed diagnosis of moderate to very severe COPD based on post-bronchodilator spirometry (i.e. forced expiratory volume in 1 second [FEV_1] over forced vital capacity [FVC] ratio [FEV_1/ FVC] < 0.7 and FEV_1 < 80% predicted [GOLD grades 2, 3 and 4].
- Stable COPD patient* with documented history** (e.g. medical record verification) of at least 1 moderate or severe AECOPD within the 12 months before study entry.

  ** Patient for whom the last episode of AECOPD is resolved for at least 30 days at the time of study entry.*

  **Note: A documented history of a COPD exacerbation (e.g., medical record verification) is a medical record of worsening COPD symptoms that required systemic/oral corticosteroids and/or antibiotics (for a moderate exacerbation) or

hospitalization (for a severe exacerbation). Prior use of antibiotics alone does not qualify as an exacerbation history unless the use was associated with treatment of worsening symptoms of COPD, such as increased dyspnea, sputum volume, or sputum purulence (color). Subject verbal reports are not acceptable.

Refer to the SPM for details on what is accepted as documented history of AECOPD. Refer to Table 2 for the definitions of moderate and severe AECOPD.

- Current or former tobacco smoker (cigarette) with a smoking history of ≥ 10 pack-years OR a subject exposed to biomass smoke for ≥ 20 years [Guoping Hu, 2010]

Refer to the glossary of terms for the definitions of pack-years, current and former smoker.

- Able to provide a sputum sample at Screening Visit.

4.3. Exclusion criteria for enrolment (Amended 19 October 2018)

Deviations from exclusion criteria are not allowed because they can potentially jeopardise the scientific integrity and regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Diagnosed with a respiratory disorder other than COPD (such as sarcoidosis, active tuberculosis or receiving tuberculosis treatment, clinically significant bronchiectasis, lung fibrosis, pulmonary embolism, pneumothorax, lung cancer diagnosed within the previous 5 years, current primary diagnosis of asthma in the opinion of the investigator), or chest X-ray revealing evidence of clinically significant abnormalities not believed to be due to the presence of COPD*. Subjects with allergic rhinitis do not need to be excluded and may be enrolled at the discretion of the investigator.

  * *A chest X-ray must be taken at Screening Visit, if no chest X-ray taken within the previous 3 months is available.*
- Diagnosis of α-1 antitrypsin deficiency as the underlying cause of COPD.
- Undergone or has had lung surgery 12 months before, or plans to have lung surgery 12 months after, study entry.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- Received chemotherapy within the 12 months before study entry.
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational vaccine/ product (pharmaceutical product or device).

- Administration of antibiotics within 1 month of study entry OR continuous administration of antibiotics (defined as more than 30 days in total) within 90 days before study entry*.

  **** Study entry is Visit 1. If this exclusion criteria is met during Screening Visit the patient can enter the study and this exclusion criteria will be reassessed during Visit 1.***

- Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to informed consent.
- Contraindication for spirometry testing (such as recent eye surgery, recent thoracic or abdominal surgery procedures, unstable cardiovascular status, recent myocardial infarction or pulmonary embolism).
- Psychiatric illness or any other condition that interferes with the ability to understand the study procedures.
- Pregnant female.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP) or other applicable guidelines, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

GSK will obtain favourable opinion/approval to conduct the study prior to a site initiating the study in that country or will document that neither a favourable opinion nor an approval to conduct the study is needed.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK Biologicals will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written informed consent must be obtained from each subject, as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the applicable ICH GCP or other applicable guidelines, and GSK Biologicals required elements. While it is strongly recommended that this model ICF be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

6. DETAILED STUDY PROCEDURES (AMENDED 19 OCTOBER 2018)

6.1. Subject identification

Subject numbers will be assigned sequentially to subjects consenting to participate, according to the range of subject numbers allocated to each study centre.

6.2. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying Study Procedures Manual (SPM). The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

6.3. Outline of study procedures

Table 4 presents the study procedures involved during scheduled [stable] visits.

Table 4 List of study procedures for scheduled visits

Epoch	Epoch 001			
Type of contact	Screening Visit	Visit 1	Visit 2	Visit 3
Time point	Pre-Month 0	Month 0	Month 6	Month 12
Informed consent[a]	●			
Check inclusion/exclusion criteria[a]	●	O		
Record demographic data[a]	●			
Record medical history	●			
Record history of AECOPD within the previous year[b]	●			
Record intercurrent comorbidities[c]	O	●	●	●
Record history of pneumococcal and influenza vaccination	O	●	●	●
Smoking exposure history (ATS-DLD-78A questionnaire)/ biomass exposure history (Biomass Exposure questionnaire)	O			
Smoking status	●	●	●	●
Physical examination including vital signs[h]	●	O	O	O
Urine Pregnancy Test	●	●	●	●
Measure/ record height and weight[d]	●			●
Pre- and post-bronchodilator spirometry[e]	●			●
Chest X-ray[f]	●			
Record subject's COPD status (stable, recovered or not recovered)		●	●	●
Record current medication		●	●	●
Record healthcare resource utilisation		●	●	●
Record additional treatments prescribed by primary and secondary care physicians		●	●	●
Train subject on use of electronic Diary Card and assign electronic Diary Card to the subject	O	O		
Return electronic Diary Card				O
Sputum sampling[g]	●	●	●	●

Epoch	Epoch 001			
Type of contact	**Screening Visit**	**Visit 1**	**Visit 2**	**Visit 3**
Time point	**Pre-Month 0**	**Month 0**	**Month 6**	**Month 12**
Blood samples for biomarkers		●		●
Record Adverse Events (AEs)* ^	●	●	●	●
Record serious adverse events (SAEs) ^	●	●	●	●
Screening conclusion	●			
Study conclusion				●
HRQOL questionnaires:				
CAT		O	O	O
SGRQ-C		O	O	O

● is used to indicate a study procedure that requires documentation in the individual eCRF.
O is used to indicate a study procedure that does not require documentation in the individual eCRF.
[a] For non-eligible subjects, only informed consent, inclusion/ exclusion criteria, demographic data and SAEs related to study participation that occurred after signing the informed consent pages need to be completed in the subject's eCRF. For other activities, 'not done' can be indicated.
[b] Record both documented and self-reported, non-documented AECOPD. Subjects need documentation for at least 1 moderate or severe AECOPD within the previous year to be eligible for study participation.
[c] Significant comorbidities include weight loss, cardiovascular disease, hypertension, gastro-oesophageal reflux disease, osteoporosis/ osteopenia, skeletal muscle wasting and dysfunction, anxiety/ depression and diabetes.
[d] To be recorded in the 'Physical examination' section of the eCRF.
[e] Only study certified site staff can perform spirometry assessment.
[f] Only if no chest X-ray is taken within the previous 3 months.
[g] Sputum can be collected spontaneously or can be induced, as per investigator judgement. Sputum sampling should only be done if, in the opinion of the investigator, it is safe for the subject.
[*] Non-serious AEs will be recorded in the eCRF and will not be entered into the safety database.
^ *Refer to Table 10 for description of which AE/SAEs are collected in this study*.
[h] Physical examination ***(Visit1-3)*** should be done only if necessary by the investigator or delegate.

Table 5 presents the list of study procedures for AECOPD visits.

Table 5 List of study procedures for AECOPD visits

Type of contact	AECOPD Visit	End of AECOPD phone call(s)
Time point	**within 96 hours of onset symptoms**	**at least every 2 weeks as of AECOPD visit until AECOPD has resolved[i]**
Record date of visit	●	
Physical examination[l]	●	
Urine Pregnancy Test	●	
Chest X-rays/pneumonia confirmation[j]	●	
Confirm AECOPD and record its start date	●	
Record current medication for ***AE***COPD	●	
Record healthcare resource utilisation	●	
Record additional COPD treatments prescribed by primary and secondary care physicians		●
Sputum sampling[k]	●	
HRQOL questionnaire:		
CAT	O	
Record AECOPD severity[m]	●	●
Record AECOPD end date		●
Record Adverse Events (AEs)*^	●	●
Record serious adverse events (SAEs)^	●	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
[i] End of AECOPD phone calls/ visits should be scheduled at least every 2 weeks, until the AECOPD has resolved. Only the last phone call will be recorded in the eCRF as the end of the AECOPD phone call. All intermediate calls should be recorded on source documentation.
[j] Only if clinically indicated to exclude another cause of worsening of symptoms (*e.g.* pneumonia). If a chest X-ray is clinically indicated but a chest X-ray/ CT scan has already been taken for that AECOPD as part of medical care before the study AECOPD visit takes places, the results of that chest X-ray/ CT scan can be used. All cases of pneumonia (including all signs and symptoms assessed to confirm pneumonia) should be documented in the eCRF.
[k] Sputum can be collected spontaneously or can be induced, as per investigator judgement. Sputum sampling should only be done if, in the opinion of the investigator, it is safe for the subject.
[l] Physical examination should be done only if necessary by the investigator or delegate.
[m] AECOPD severity grading as indicated in Table 2.
* Non-serious AEs will be recorded in the eCRF and will not be entered into the safety database.
^ *Refer to Table 10 for description of which AE/SAEs are collected in this study*.

Table 6 presents the intervals between study visits.

Table 6 Intervals between study visits

Interval	Optimal length of interval	Allowed interval[1]
Screening Visit (pre-Month 0)→ Visit 1	± 14 days	≤42 days before Visit 1
	Scheduled study visits	
Visit 1 (Month 0) → Visit 2 (Month 6)	182 days	175 - 203 days
Visit 2 (Month 6) →Visit 3 (Month 12)	182 days	175 - 203 days
AECOPD-driven study visit(s) and/ or phone contacts		
Onset AECOPD symptoms → AECOPD Visit	-	max 96 hours[2]

[1] If an AECOPD occurs at the time one of the scheduled study visits is planned, the stable study visit should be re-scheduled to a later date, when the subject is stable again and within the time window specified. In consultation with GSK, the concluding visit (Visit 3, Month 12) may however exceptionally be conducted outside of the allowed interval if necessary by the investigator.
[2] AECOPD visits will be scheduled as soon as possible after the onset of AECOPD symptoms as recorded in the electronic Diary Card or confirmed by the subject (maximum 96 hours after and, if applicable, preferably before starting treatment with antibiotics).

6.4. Detailed description of study procedures

6.4.1. Procedures during the Screening Visit

6.4.1.1. Informed consent

The signed informed consent of the subject must be obtained before study participation during the screening visit. Refer to Section 5.1 for the requirements on how to obtain informed consent.

6.4.1.2. Check inclusion and exclusion criteria

All applicable inclusion and exclusion criteria as described in Sections 4.2 and 4.3 will be checked before enrolment.

6.4.1.3. Record demographic data

Demographic data such as date of birth, gender, geographic ancestry will be recorded in the subject's eCRF.

6.4.1.4. Record medical history

The subject's medical history will be obtained by review of the subject's medical records. Prior to study participation, any pre-existing conditions or signs and/or symptoms present in a subject will be recorded**.**

6.4.1.5. Record AECOPD history within the previous year

Documented, self-reported and non-documented AECOPD should be recorded in the eCRF. However, for study participation the subject should have had at least one documented moderate or severe AECOPD episode within the previous year.

6.4.1.6. Smoking exposure history (ATS-DLD-78A questionnaire)/biomass exposure history (At screening visit only)

The subject will be asked to complete the smoking history questionnaire (which is a shortened version of the American Thoracic Society-Division of Lung Diseases-78A [ATS-DLD-78A] questionnaire) by himself/herself. The subject will have to provide information about his/ her smoking history, including duration (number of years) and number of cigarettes smoked. Subject years exposed to biomass will be assessed from information obtained on indoor exposure to fuels such as wood, crop waste and dung, or coal.

From the information obtained via the questionnaire, calculation of the pack-years will be done for inclusion into the study. Please refer to the SPM for details on how to calculate pack years using this questionnaire and for guidance on administration of the exposure history questionnaire.

6.4.1.7. Smoking status

The subject's smoking status (current or former smoker) will be recorded in the eCRF. Refer to the glossary of terms for the definitions of current and former smoker.

6.4.1.8. Physical examination

At the screening visit, a complete physical examination of the subject will be performed which will include vital signs after at least 10 minutes of rest (systolic/ diastolic blood pressure, heart rate, respiratory rate). Collected information will be recorded in the eCRF.

Treatment of any abnormality observed during a physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.4.1.9. Pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study procedure. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit. The test should be carried out before performing chest x-ray. If the test is positive, the chest x- ray should not be performed and the subject should be withdrawn from the study.

6.4.1.10. Measure/ record height and weight

The height and weight of the subject will be measured and recorded in the 'Physical examination' section of the eCRF.

6.4.1.11. Pre- and post-bronchodilator spirometry

Pre- and post-bronchodilator spirometry should be performed during the screening visit. Only study-certified staff can perform spirometry assessment for this study. Spirometry will be performed following the eResearchTechnology (ERT) instructions for use FlowScreen manual and following all safety requirements.

A good quality spirometry should be obtained, and will be confirmed by the spirometry provider. If during the Screening Visit a good quality spirometry was not obtained, the spirometry can be repeated, as per investigator's medical judgement. If a repeat spirometry is elected, the site should make all possible efforts to repeat the spirometry preferably within 7 days of the previous spirometry. The data will be directly transferred from the provider to GSK Biologicals.

Treatment of any abnormality observed during spirometry has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.4.1.12. Chest X-ray

Screening - Baseline test

A posterior to anterior (PA) chest X-ray must be performed at the screening visit if no chest X-ray/ CT scan is available within the last 3 months.

Subjects with evidence of clinically significant abnormalities not believed to be due to the presence of COPD will not be eligible for study participation.

6.4.1.13. Train subjects on the use of electronic Diary Card and assign electronic Diary Card to subject

- From screening visit to visit 1, subjects will be trained on how to use their electronic Diary Card. At visit 1, the investigator should evaluate whether or not the subject will be able to comply with the daily completion of the electronic Diary Card throughout the study. Compliance with electronic Diary Card completion implies that subject learns how to translate his/ her respiratory symptoms in answers to the questions as well as acquiring the technical expertise to use the device. Refer to the SPM for recommendations on what is considered adequate compliance.
- The site staff will follow electronic Diary Card completion closely and should provide timely input/guidance to ensure that the subject correctly completes the eDiary Card both AM & PM as per protocol.

In addition, site staff will pro-actively monitor electronic Diary Card compliance throughout the study and provide the necessary input to maintain compliance.

6.4.1.14. Sputum sampling (Amended 19 October 2018)

- Sputum samples will be collected during the screening visit if, in the opinion of the investigator, it is safe for the subject. If it is not safe, the sputum sample will not be collected and the subject will not be included in the study.
- Sputum samples can be either spontaneous or induced, as per investigator judgement. Internal standard operating procedures should be put in place to ensure proper sputum collection, sample tracking and subject safety at study collection site.
- At home, sputum collection will not be permitted.
- Collected sputum will be diluted in DTT, processed and cultured within 6hrs of collection for microbiology testing.
- Remaining DTT-sputum will be aliquoted as such. These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.

Refer to the SPM and the Central Laboratory Investigator Manual for more details and guidance on handling of sputum samples.

6.4.1.15. Recording of AE/SAEs

- Recording of AE/SAEs
- Refer to Section 7.2 for procedures for the investigator to record AE/SAEs. Refer to Section 7.3 for guidelines on how to submit SAE reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.4.1.16. Screening conclusion

The investigator will review all the data collected during the screening visit to ensure accuracy and completeness.

6.4.2. Procedures during Visit 1, Visit 2 and Visit 3

6.4.2.1. Check inclusion and exclusion criteria

At visit 1, all applicable inclusion and exclusion criteria as described in Sections 4.2 and 4.3 will be checked before enrolment.

6.4.2.2. Record intercurrent comorbidities

Significant comorbidities include, but is not limited to, weight loss, cardiovascular disease, hypertension, gastro-oesophageal reflux disease, osteoporosis/ osteopenia, skeletal muscle wasting and dysfunction, anxiety/ depression and diabetes.

6.4.2.3. Vaccination History

Record in the eCRF whether the subject received any influenza vaccination within the previous 12 months or has ever received any pneumococcal vaccination (including date of vaccination [as detailed as possible]).

6.4.2.4. Smoking status

The subject's smoking status (current or former smoker) will be recorded in the eCRF. Refer to the glossary of terms for the definitions of current and former smoker.

6.4.2.5. Physical examination

Physical examination at each study visit (Visit 1, Visit 2 and Visit 3) will be performed only if deemed necessary by the investigator or delegate.

6.4.2.6. Pregnancy test (Amended 19 October 2018)

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study procedure. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

6.4.2.7. Measure/ record height and weight

The height and weight of the subject will be measured at Visit 3 and recorded in the 'Physical examination' section of the eCRF.

6.4.2.8. Pre- and post-bronchodilator spirometry

Pre- and post-bronchodilator spirometry should be performed during Visit 3 as detailed in Table 4. Only study-certified staff can perform spirometry assessment for this study. Spirometry will be performed following the eResearchTechnology (ERT) instructions for use FlowScreen manual and following all safety requirements.

A good quality spirometry should be obtained, and will be confirmed by the spirometry provider. If during Visit 3 a good quality spirometry was not obtained, the spirometry can be repeated, as per investigator's medical judgement. If a repeat spirometry is elected, the site should make all possible efforts to repeat the spirometry preferably within 7 days of the previous spirometry. The data will be directly transferred from the provider to GSK Biologicals.

Treatment of any abnormality observed during spirometry has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.4.2.9. Record subject's COPD status

The subject's COPD status (stable/ recovered or not recovered) will be recorded in the eCRF.

6.4.2.10. Record current medication (Amended 19 October 2018)

- At each study visit/contact, the investigator should question the subject about any medications taken.
- All medications administered will be recorded in the eCRF.

6.4.2.11. Record healthcare resource utilisation

Healthcare use will be obtained through review of the subject's medical record (aided by subject self-reporting). Healthcare utilisation includes all unscheduled visits to a physician office, visits to urgent care, visits to emergency department, and hospitalizations. Healthcare use should be recorded in eDiary Card and reported in the

eCRF at study visit. Refer to the SPM for more details and guidance on recording of healthcare use.

6.4.2.12. Additional treatments prescribed by primary and secondary care physician

All treatments prescribed by the primary and secondary healthcare physician will be recorded in the eCRF.

6.4.2.13. Train subjects on the use of electronic Diary Card and assign electronic Diary Card to subject

- From screening visit to visit 1, subjects will be trained on how to use their electronic Diary Card. At visit 1, the investigator should evaluate whether or not the subject will be able to comply with the daily completion of the electronic Diary Card throughout the study. Compliance with electronic Diary Card completion implies that subject learns how to translate his/ her respiratory symptoms in answers to the questions as well as acquiring the technical expertise to use the device. Refer to the SPM for recommendations on what is considered adequate compliance.
- The site staff will follow electronic Diary Card completion closely and should provide timely input/guidance to ensure that the subject reaches the targeted learning curve.

In addition, site staff will pro-actively monitor electronic Diary Card compliance throughout the study and provide the necessary input to maintain compliance.

6.4.2.14. Return the electronic diary card

On the last visit (Visit 3), the site staff will pro-actively check with the subjects on whether he/she has returned the electronic Diary Card.

6.4.2.15. Sputum sampling (Amended 19 October 2018)

- Sputum samples will be collected during the scheduled visits, if in the opinion of the investigator, it is safe for the subject.
- Sputum samples can be either spontaneous or induced, as per investigator judgement. Internal standard operating procedures should be put in place to ensure proper sputum collection, sample tracking and subject safety at study collection site.
- At home, sputum collection will not be permitted.
- Collected sputum will be diluted in DTT, processed and cultured within 6hrs of collection for microbiology testing
- Remaining DTT-sputum will be aliquoted as such. These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.

Refer to the SPM and the Central Laboratory Investigator Manual for more details and guidance on handling of sputum samples.

6.4.2.16. Blood Sampling

- Blood samples for biomarker analysis will be taken at the scheduled visits; Visit 1 (M0) and Visit 3 (M12), including:
  - Hematology profile, including differential cell counts
    - Approximately 2.0 mL of whole blood will be collected for hematology assessment. These samples should be kept at room temperature and shipped on the day of collection, ambient.
  - Specific biomarkers will include serum hsCRP, CXCL10 (IP-10) and plasma fibrinogen, may include other biomarkers based on results of ongoing disease understanding research.
    - Approximately 8.5 mL of whole blood will be collected and processed to serum for hsCRP & CXCL10 (IP-10) assessment. After processing, these samples should be kept at -70/-80°C until shipment.
    - Approximately 4.5 mL of whole blood (Na Citrate) will be collected and processed to plasma for fibrinogen assessment. After processing, these samples should be kept at -70/-80°C until shipment.
    - Approximately 6.0 mL of whole blood (EDTA) will be collected and processed to plasma for other biomarker assessment. After processing, these samples should be kept at -70/-80°C until shipment.

Refer to the SPM and the Central Laboratory Investigator Manual for more details and guidance on the collection, handling, and processing of blood samples.

6.4.2.17. HRQOL questionnaires

- The subject will be asked to complete the HRQOL questionnaires by himself/herself, during specified study visits directly in the electronic Diary Card as detailed in Table 4 and Table 5.
- Refer to the SPM for more details and guidance on the HRQOL questionnaires.

6.4.2.18. Recording of AE/SAEs

- Refer to Section 7.2 for procedures for the investigator to record AE/SAEs. Refer to Section 7.3 for guidelines on how to submit SAE reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.4.2.19. Study conclusion

The investigator will:

- Review all the data collected to ensure accuracy and completeness
- Complete the Study Conclusion screen in the eCRF.

6.4.3. Procedures during AECOPD visit

6.4.3.1. Record date of visit

If an AECOPD occurs at of the scheduled study visits, the date of visit should be document in the eCRF.

6.4.3.2. Physical examination

Physical examination at the AECOPD visit will be performed only if deemed necessary by the investigator or delegate.

Treatment of any abnormality observed during a physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.4.3.3. Pregnancy Test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study procedure. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit. The test should be carried out before performing chest x-ray. If the test is positive, the chest x- ray should not be performed and the subject should be withdrawn from the study.

6.4.3.4. Chest X-rays/pneumonia confirmation

AECOPD visit test

A chest X-ray should be performed at the AECOPD visit if it is clinically indicated to exclude another cause of worsening of symptoms (*e.g.* new infiltrate for pneumonia cases).

All suspected pneumonias will require confirmation as defined by the presence of new infiltrate(s) on chest x-ray AND at least 2 of the following signs and symptoms:

- Increased cough,
- Increased sputum purulence (colour) or production,
- Auscultatory findings of adventitious sounds (e.g., egophony, bronchial breathing sounds, rales, etc),
- Dyspnea or tachypnea,
- Fever (oral temperature ≥ 37.5 °C),
- Elevated WBC (>10,000/mm3 or >15% immature forms),
- Hypoxemia (HbO2 saturation <88% or at least 2% lower than baseline value),

All incidences of pneumonia must be captured on the pneumonia page of the eCRF.

The investigators and site staff should remain vigilant for the possible development of pneumonia in patients with COPD as the clinical features of such infections overlap with the symptoms of COPD exacerbations. For all suspected cases of pneumonia, investigators are strongly encouraged to confirm the diagnosis (this includes obtaining a chest x-ray) and to initiate appropriate therapy as promptly as possible.

Please refer to SPM for details on what should be considered for pneumonia confirmation.

6.4.3.5. Confirm AECOPD and record start date

The investigator should confirm the AECOPD based on his/her medical judgement. The date of onset of AECOPD will be determined as detailed in Section 3.1.1.1 and will be documented in the eCRF.

6.4.3.6. Record current medication for AECOPD

- At each study visit/contact, the investigator should question the subject about any medications administered for treatment of AECOPD.
- All medications administered for treatment of AECOPD will be recorded in the eCRF.

6.4.3.7. Record healthcare resource utilisation

Healthcare use will be obtained through review of the subject's medical record (aided by subject self-reporting). Healthcare utilisation includes all unscheduled visits to a physician office, visits to urgent care, visits to emergency department, and hospitalizations. Healthcare use should be recorded in the eDiary Card and reported in the eCRF at study visit. Refer to the SPM for more details and guidance on recording of healthcare use.

6.4.3.8. Additional COPD treatments prescribed by primary and secondary care physician

All treatments prescribed by the primary and secondary healthcare physician will be recorded in the eCRF.

6.4.3.9. Sputum sampling (Amended 19 October 2018)

- Sputum samples during AECOPD should preferably be obtained before administration of the first dose of antibiotics to treat the AECOPD (if applicable). Sputum can be collected spontaneously or can be induced, as per investigator judgement. Sputum sampling should only be done if, in the opinion of the investigator, it is safe for the subject.

- Sputum sample collected at subject's home (spontaneous): Self-collection of the sputum sample will be allowed in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place. This is not allowed at scheduled visits when subject should be in stable condition.
- Collected sputum will be diluted in DTT, processed and cultured within 6hrs of collection for microbiology testing.
- Remaining DTT-sputum will be aliquoted as such. These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.

Refer to the SPM and the Central Laboratory Investigator Manual for more details and guidance on handling of sputum samples.

6.4.3.10. HRQOL questionnaires

- The subject will be asked to complete the HRQOL questionnaire (CAT only) by himself/herself directly in the electronic Diary Card, during the AECOPD visit as detailed in Table 5.
- Refer to the SPM for more details and guidance on the HRQOL questionnaires.

6.4.3.11. Record AECOPD severity and AECOPD end date

The severity and end date of each confirmed AECOPD will be recorded in the eCRF. The AECOPD severity will be assessed using the AECOPD severity scale (refer Table 2) and the AECOPD end date will be determined as detailed in Section 3.1.1.1 and Section 3.1.1.2.

6.4.3.12. Recording of AE/SAEs

- Refer to Section 7.2 for procedures for the investigator to record AE/SAEs. Refer to Section 7.3 for guidelines on how to submit SAE reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.4.4. Sampling

6.4.4.1. Sputum Sampling

- Sputum sampling: Refer to section 6.4 for description of the sampling procedure.

Full details for obtaining sputum samples are provided in the Module on Biospecimen Management in the SPM and in the Central Laboratory Investigator Manual accompanying this protocol.

6.4.4.2. Blood Sampling

Blood sampling: Refer to section 6.4 for the description of sampling procedure.
Full details for the collection, handling, and processing of blood samples are provided in the Module on Biospecimen Management in the SPM and in the Central Laboratory Investigator Manual accompanying this protocol.

6.4.5. Health Related Quality of Life questionnaires

Refer to section 6.4.2 for description of the Quality of Life (QoL) questionnaire. The subject will be asked to complete the HRQOL questionnaires by himself/herself, during specified study visits directly in the electronic Diary Card.

6.4.6. Recording of AEs/SAEs

- Refer sections 6.4.1.15, 6.4.2.18 and 6.4.3.12 for recording of AEs/SAEs.

6.4.7. Study conclusion

Refer section 6.4.2.19 for study conclusion.

6.5. Biological sample handling and analysis

Please refer to the SPM and the Central Laboratory Investigator Manual for details of biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subjects but will be coded with the identification number for the subject (subject number).

- Collected samples will be used for protocol mandated research. In addition, these samples may be used to perform research related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects in countries where this is allowed will be invited to give another specific consent to allow GSK or a contracted partner to use the samples for future research including development of tests and their quality assurance. Future research will be subject to the laws and regulations in the respective countries and will only be performed once an independent Ethics Committee or Review Board has approved this research.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject.

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit/contact), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

6.5.1. Use of specified study materials

When materials are provided by GSK Biologicals or the central laboratory, it is MANDATORY that all samples be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the analysis. The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals or the central laboratory does not provide material for collecting and storing samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM and Central Laboratory investigator manual.

6.5.2. Biological samples

Table 7 present the biological samples that will be collected for this study.

Table 7 Biological samples

Sample type	Quantity	Unit	Timepoints	Sub-cohort
Fresh Sputum	Maximum possible	ml	Screening visit (Pre-Month 0)	All screened subjects
			• Visit 1 (Month 0) • Visit 2 (Month 6) • Visit 3 (Month 12)	All enrolled subjects
			• AECOPD visit (within 96 hours of onset of symptoms)	
Blood for biomarkers	21	ml	• Visit 1 (Month 0) • Visit 3 (Month 12)	All enrolled subjects

6.5.3. Laboratory assays (Amended 19 October 2018)

Please refer to APPENDIX A for the address of the clinical laboratories used for sample analysis.

The quality of sputum samples will be assessed at the investigator's institution and/ or at a laboratory designated by GSK Biologicals by gram staining.

Standard bacteriological methods (and semi-quantitative counts) will be performed on fresh sputum samples at the investigator's institution and/or at a laboratory designated by GSK Biologicals. Identification of potential bacterial pathogens will be performed according to agreed identification methods (potential pathogens including, but not necessarily limited to, *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*). All results should be entered in the eCRF.

Further bacterial characterization for *H. influenzae* isolates: Identified *H. influenzae* isolates should be collected and stored in the investigator's institution and will undergo further species confirmation (i.e. confirmed *H. influenzae)* and when possible, further differentiation (i.e., Hi/NTHi**)** at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals using molecular techniques such as PCR. Identified *H. influenzae* isolates might undergo further testing at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals' using other molecular techniques such as PCR and sequencing.

Identified *M. catarrhalis* isolates should be collected and stored in the investigator's institution and might undergo further species confirmation (and when possible, further differentiation) at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals using molecular techniques such as PCR and sequencing.

Identified *P. aeruginosa* isolates should be collected and stored in the investigator's institution and might potentially undergo further testing at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals.

Further bacterial pathogens identification (including, but not necessarily limited to, *H. influenzae, S. pneumoniae*, *M. catarrhalis*, *S. pyogenes, S. aureus* and *P. aeruginosa*) and quantification (for *H. influenzae, S. pneumoniae, M. catarrhalis*) on stored sputum samples will be performed at GSK Biologicals' laboratory or a laboratory designated by GSK Biologicals using qualitative PCR and/or quantitative PCR.

Viral pathogens identification (including, but not necessarily limited to, RSV, parainfluenza virus, enterovirus/ HRV, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus) on stored sputum samples will be performed at GSK Biologicals' laboratory or a laboratory designated by GSK Biologicals using multiplex reverse transcription polymerase chain reaction (RT-PCR).

In addition, some respiratory viral pathogens (such as HRV) will be quantified in stored sputum samples (or subset) using RT-PCR at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals.

Table 8 present the methods that will be used for identification of bacterial and viral pathogens.

Table 8 Microbiology

System	Component	Method	Scale	Laboratory
Quality assessment				
Fresh sputum	Please refer to SPM and/or Central Laboratory Manual for components tested	Gram staining	Qualitative/ semi-quantitative	Investigator institution or GSK designated laboratory
Bacterial pathogen identification				
Fresh sputum	***R***espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*)	Standard bacteriological culture and standard identification methods including semi-quantitative culture	Qualitative Semi-quantitative	Investigator's institution and/or at a laboratory designated by GSK Biologicals
H. influenzae isolates of positive *H. influenzae* culture plates	***H. influenzae*** species confirmation and when possible Hi/NTHi differentiation Sample collection for potential further characterisation	Molecular techniques such as differentiation PCR	Qualitative	GSK Biologicals laboratory* or designated laboratory
Stored sputum	***R***espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus* and *P. aeruginosa, Streptococcus pyogenes*))	Multiplex PCR	Qualitative and/ or quant itative	GSK Biologicals laboratory * or designated laboratory
Viral pathogen identification				
Stored sputum	Respiratory viral pathogens (including RSV, parainfluenza virus, enterovirus/ HRV, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus)	Multiplex-PCR	Qualitative	GSK Biologicals laboratory * or designated laboratory
	Respiratory viral pathogens (such as HRV)	RT-PCR	Quantitative	GSK Biologicals laboratory* or designated laboratory

* GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Wavre, Belgium; Rixensart, Belgium; Marburg, Germany.

Stored frozen sputum samples (in dithiothreitol [DTT]) might be used for assay development, such as assays for diagnostic purpose or for microbiome analysis and/or for assay validation/characterization purpose and/or quality control.

Additional testing on stored sputum samples (such as, but not limited to, *K. pneumoniae* and *A. baumannii* PCR detection, quantitative PCR for other bacterial and/or viral pathogens, further characterisation of virus such as rhinovirus typing, pathogen genome/gene sequencing, pathogen quantitative/qualitative serotype-specific PCR, multilocus sequence typing, microarray typing, 16sRNA analysis) or on *H. influenzae, M. catarrhalis and P. aeruginosa* bacterial isolates (such as agglutination assays, further strain characterisation using new molecular biology tools) may be done during the study or after study completion, should these data be required for accurate interpretation of the study data and/ or for further research related to respiratory diseases and/ or should such test(s) become available at GSK Biologicals' laboratory or a laboratory designated by GSK.

Table 9 present the planned assays on serological samples.

Table 9 Planned assays on serological samples

Sample type	Component	Method	Scale	Laboratory
Plasma	Fibrinogen	Per contract laboratory's procedures	Quantitative	GSK Biologicals or GSK designated lab
Serum	hsCRP	Per contract laboratory's procedures	Quantitative	
Serum	CXCL10 (IP-10)	Per contract laboratory's procedures	Quantitative	
Whole blood	Leukocytes (White Blood Cells)	Per contract laboratory's procedures	Quantitative	
	Neutrophils *	Per contract laboratory's procedures	Quantitative	
	Lymphocytes *	Per contract laboratory's procedures	Quantitative	
	Eosinophils *	Per contract laboratory's procedures	Quantitative	
	Basophils *	Per contract laboratory's procedures	Quantitative	
	Monocytes *	Per contract laboratory's procedures	Quantitative	
	Erythrocyte (Red Blood Cells)	Per contract laboratory's procedures	Quantitative	
	Haemoglobin	Per contract laboratory's procedures	Quantitative	
	Platelets	Per contract laboratory's procedures	Quantitative	

*For white blood cell differential counts

Additional testing for biomarkers on stored serum/plasma may be done during the study or after study completion, should these data be required for accurate interpretation of the study data and/ or for further research related to respiratory diseases available at GSK Biologicals' laboratory or a laboratory designated by GSK.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

7. SAFETY

The investigator or site staff is/are responsible during the study for the detection and documentation of events meeting the criteria and definition of an SAE as provided in this protocol.

Each subject will be instructed to contact the investigator immediately should they/the subject manifest any signs or symptoms they perceive as serious.

7.1. Safety definitions

7.1.1. Definition of an adverse event (Amended 19 October 2018)

An AE is any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure.

Examples of an AE include

- Signs, symptoms, or the clinical sequelae of a suspected interaction.

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (e.g., social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- ***Occurrence of a severe AECOPD, unless in the opinion of the Investigator this is related to study procedure.***

7.1.2. Definition of a serious adverse event

An SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term ‘life-threatening’ in the definition of ‘serious’ refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalisation or prolongation of an existing hospitalisation,

Note: In general, hospitalisation signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting.

Complications that occur during hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an SAE.

d. Results in disability/incapacity,

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza like illness, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

or

e. Is a congenital anomaly/birth defect in the offspring of a study subject

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation.

7.1.3. Clinical laboratory parameters and other abnormal assessments qualifying as AEs or SAEs

In absence of diagnosis, abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE or an SAE (refer to Sections 7.1.1 and 7.1.2). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.2. Detecting and recording AEs, SAEs

7.2.1. Time periods for detecting and recording AEs and SAEs

AEs and SAEs related to study participation will be collected and recorded from the time of the first study visit until the subject is discharged from the study.

In order to fulfil international reporting obligations, SAEs that are related to study participation (i.e., protocol-mandated procedures, invasive tests, a change from existing therapy) will be collected and recorded from the time the subject consents to participate in the study/study start until she/he is discharged from the study.

AEs leading to withdrawal from the study, will be collected and recorded from the time of the first study visit until the subject is discharged from the study.

An overview of the protocol-required reporting periods for SAEs and AEs leading to withdrawal is given in Table 10.

Table 10 Reporting period for SAE and AEs leading to withdrawal (Amended 19 October 2018)

Study activity	Screening Visit		Visit 1		Visit 2		Visit 3		AECOPD Visit		Study Conclusion
	Pre-Month 0		M 0		M 6		M 12		Within 96 hours of onset symptoms		
AEs/SAEs related to study participation*											
AEs/*SAEs* leading to withdrawal from the study											

Study participation: Specific procedures required by study participation.

7.2.2. Evaluation of SAEs (Amended 19 October 2018)

7.2.2.1. Active questioning to detect SAEs

Each subject/ will be instructed to contact the investigator immediately should the subject manifest any signs and symptoms (s)he perceives/ they perceive as serious. ***However, an occurrence of severe AECOPD is not defined as an SAE and therefore does not need to be reported as such, unless in the opinion of the Investigator this is related to a study procedure.***

All SAEs either observed by the investigator or his/ her staff or reported by the subject spontaneously or in response to a direct question will be evaluated by the investigator. The nature of each event, date and time of onset, outcome, intensity and possible relationship to the study procedures should be established.

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding the AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/ or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

7.2.2.2. Assessment of causality

The investigator should assess the causality of each SAE. The investigator will use clinical judgement to determine the relationship between the SAEs and study participation. Alternative causes, such as natural history of the underlying diseases, other concomitant therapy and other risk factors will be considered and investigated.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly.

If an event meets the criteria to be considered as 'serious' (see section 7.1.2), additional examinations/tests will be performed by the investigator in order to determine ALL possibly contributing factors to each SAE.

Possibly contributing factors include:

- Medical history.
- Concomitant medication.
- Protocol required procedure.
- Other procedure not required by the protocol.

7.2.2.3. Assessment of outcomes

The investigator will assess the outcome of all AEs (including SAEs) recorded during the study as:

- Recovered/ resolved.
- Recovering/ resolving.
- Not recovered/ not resolved.
- Recovered with sequelae/ resolved with sequelae.
- Fatal (SAEs only).

7.3. Reporting of SAEs

7.3.1. Prompt reporting of SAEs related to study participation to GSK

SAEs that occur in the time period defined in Section 7.2.1 will be reported promptly to GSK within the timeframes described in Table 11 once the investigator determines that the event meets the protocol definition of an SAE.

Table 11 Timeframes for submitting SAEs related to study participation to GSK

Type of event	Initial reports		Follow-up of relevant information on a previous report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to study participation	24 hours*	Electronic/ Expedited Adverse Event Report	24 hours*	Electronic/ Expedited Adverse Event Report

* Timeframe allowed after receipt or awareness of the information.

7.3.2. Contact information for reporting SAEs to GSK

Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD or PPD

7.3.3. Completion and transmission of SAEs reports related to study participation to GSK

Once an investigator becomes aware that an SAE has occurred in a study subject, the investigator (or designee) must complete the information in the electronic Expedited Adverse Event Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding an SAE, the report should still be completed within 24 hours. Once additional information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

7.3.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designee) must complete, then date and sign a paper Expedited Adverse Event Report and fax it to the GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designee) must complete the electronic Expedited Adverse Event Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic reporting system.

7.3.4. Updating of SAE after freezing of the subject's eCRF

When SAE information is received after freezing of the subject's eCRF, new or updated information should be recorded on a paper Expedited Adverse Event Report, with all changes signed and dated by the investigator. The updated report should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (see the Sponsor Information) within the designated reporting time frames specified in Table 11.

Note: For studies using INFORM, freezing of the subject's eCRF would mean removal of write access in the subject's eCRF.

7.3.5. Regulatory reporting requirements for SAEs

The investigator will promptly report all SAEs to GSK Biologicals in accordance with the procedures detailed in Section 7.3.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under epidemiological investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

7.4. Treatment of AEs

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of a SAE (that are required to be reported as per protocol should be recorded in the Expedited Adverse Event Report of the subject's eCRF.

8. SUBJECT COMPLETION AND WITHDRAWAL

8.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

8.2. Subject withdrawal

Subjects who are withdrawn because of AEs or SAEs related to study participation must be clearly distinguished from subjects who are withdrawn for other reasons.

From an analysis perspective, a 'withdrawal' from the study refers to any subject who did not come back for the concluding contact foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up. The mode of contact will be by telephone. Three phone contacts will be made with an interval of one week between each phone call.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- SAE.
- Non-serious AE.
- Protocol violation (specify).
- Consent withdrawal, not due to an AE*.
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

*In case a subject is withdrawn from the study because he/she has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject in the eCRF.

9. STATISTICAL METHODS

9.1. Endpoints

9.1.1. Primary endpoint (Amended 19 October 2018)

- Occurrence of potential bacterial and viral pathogens in sputum of stable COPD patients and during AECOPD, over the course of 1 year:
  - Bacterial pathogens, as identified by bacteriological methods, including (but not necessarily limited to) *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*.
  - Viral pathogens, as identified by PCR, including (but not necessarily limited to) RSV, parainfluenza virus, enterovirus/ HRV, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus.

9.1.2. Secondary endpoints

- Occurrence of potential bacterial pathogens in sputum of stable COPD patients and during AECOPD, as measured by real-time qualitative PCR/ quantitative PCR and compared to data from bacteriological methods, over the course of 1 year:
  - Including (but not necessarily limited to) *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus* and *P. aeruginosa*.
- Occurrence of potential bacterial and viral pathogens (overall and by species) in sputum of stable COPD patients by GOLD grade, over the course of 1 year.
- Occurrence of potential bacterial and viral pathogens (overall and by species) in sputum during AECOPD by severity of AECOPD, over the course of 1 year.
- Incident rate (per subject per year) of AECOPD, overall and by GOLD grade, over the course of 1 year.
- Severity of AECOPD, overall and by GOLD grade, over the course of 1 year.
- Duration of AECOPD, overall and by AECOPD severity, over the course of 1 year.
- CAT score in stable COPD patients and during AECOPD, over the course of 1 year.
- SGRQ-C score in stable COPD patients, over the course of 1 year.
- FEV_1% of predicted normal value in stable COPD patients, at Pre-Month 0 and Month 12.
- Healthcare utilisation, over the course of 1 year.

9.1.3. Tertiary endpoints

- EXACT-PRO scores in stable COPD patients and during AECOPD, over the course of 1 year.
- Bacterial load measured by both culture and PCR in COPD and during AECOPD.
- HRV load measured by PCR in COPD and during AECOPD.

9.2. Determination of sample size

The primary objective of this study is to describe the proportion of bacterial and viral pathogens (overall and by species) detected in sputum in stable COPD and during AECOPD.

Assuming around 90% of the All Enrolled Set participated in the study, on average 1 AECOPD visit and 80% of the above population set can provide an evaluable sputum sample at AECOPD, for 240, 200, 160, and 50 enrolled subjects, one may expect around 180, 140, 120, 40 sputum samples, respectively. Assuming that results from these sputum samples are independent and that the between country variability is much smaller than the within country variability, we would have the following 95% exact confidence interval for different incidences in sputum samples from exacerbations (see Table 12).

Table 12 Exact 95% confidence intervals of the percentage of occurrence of a specific bacterium from sputum collected during AECOPD

	Number of evaluable sputum samples during AECOPD			
%	N=180	N=140	N=120	N=40
5	[2.3 ; 9.3]	[2.0 ; 10.0]	[1.9 ; 10.6]	[0.6 ; 16.9]
10	[6.0 ; 15.3]	[5.6 ; 16.2]	[5.3 ; 16.8]	[2.8 ; 23.7]
20	[14.4 ; 26.6]	[13.7 ; 27.6]	[13.3 ; 28.3]	[9.1 ; 35.6]
30	[23.4 ; 37.3]	[22.6 ; 38.3]	[22.0 ; 39.0]	[16.6 ; 46.5]
40	[32.8 ; 47.6]	[31.8 ; 48.6]	[31.2 ; 49.3]	[24.9 ; 56.7]
50	[42.5 ; 57.5]	[41.4 ; 58.6]	[40.7 ; 59.3]	[33.8 ; 66.2]

N = total number of evaluable sputum samples during AECOPD.

One of the secondary objectives is to estimate the incidence rate of all-cause AECOPDs in the study population.

Assuming around 80% of the all enrolled set completed the study, for 240, 200, 160 and 50 subjects enrolled, one would have at least 190, 160, 130 and 40 subjects evaluable respectively. Assuming further that around 80% of the subjects can provide an evaluable sputum sample at AECOPD, this would lead to around 150, 130, 100, 30 subjects (see Table 13).

Table 13 95% confidence intervals of AECOPD incidence rates with different overdispersion values

			Φ = 1		Φ = 1.5		Φ = 2	
0.2 AECOPD/ subject/ year			95% CI*		95% CI*		95% CI*	
T	n	Value (n/T)	LL	UL	LL	UL	LL	UL
30	6	0.20	0.04	0.36	0.00	0.40	-0.03	0.43
100	20	0.20	0.11	0.29	0.09	0.31	0.08	0.32
130	26	0.20	0.12	0.28	0.11	0.29	0.09	0.31
150	30	0.20	0.13	0.27	0.11	0.29	0.10	0.30
1 AECOPD/ subject/ year			95% CI*		95% CI*		95% CI*	
T	n	Value (n/T)	LL	UL	LL	UL	LL	UL
40	40	1.00	0.69	1.31	0.62	1.38	0.56	1.44
130	130	1.00	0.83	1.17	0.79	1.21	0.76	1.24
160	160	1.00	0.85	1.15	0.81	1.19	0.78	1.22
190	190	1.00	0.86	1.14	0.83	1.17	0.80	1.20
2 AECOPD/ subject/ year			95% CI*		95% CI*		95% CI*	
T	n	Value (n/T)	LL	UL	LL	UL	LL	UL
40	80	2.00	1.56	2.44	1.46	2.54	1.38	2.62
130	260	2.00	1.76	2.24	1.70	2.30	1.66	2.34
160	320	2.00	1.78	2.22	1.73	2.27	1.69	2.31
190	380	2.00	1.80	2.20	1.75	2.25	1.72	2.28
3 AECOPD/ subject/ year			95% CI*		95% CI*		95% CI*	
T	n	Value (n/T)	LL	UL	LL	UL	LL	UL
40	120	3.00	2.46	3.54	2.34	3.66	2.24	3.76
130	390	3.00	2.70	3.30	2.64	3.36	2.58	3.42
160	480	3.00	2.73	3.27	2.67	3.33	2.62	3.38
190	570	3.00	2.75	3.25	2.70	3.30	2.65	3.35

* Normal approximation with variance = $\Phi\ n/T^2$, where Φ is the overdispersion factor for a Poisson distribution (calculations performed in Microsoft Excel).
T = Total number of evaluable subjects; **n** =Total number of exacerbations; **CI** = confidence interval; **LL** = lower limit; **UL** = upper limit.

9.3. Cohorts for Analyses

The following study cohorts will be evaluated.

9.3.1. All screened Set

The all screened set will include all screened patients.

9.3.2. All Enrolled Set

The all enrolled set will include all successfully screened subjects in the study.

9.3.3. Full Analysis Set

The Full Analysis Set (FAS) will include all enrolled patients except for those who discontinued the study during Visit 1.

Study objectives will be assessed on the FAS. The population set for each analysis will change according to the subjects evaluable for the specific endpoint.

Note: Subjects using antibiotics on a continual basis (defined as more than 1 month in total) will be allowed to continue study participation, but may be eliminated from the analyses.

9.4. Analysis of demographics

Demographic characteristics (age at enrolment, gender and geographical ancestry) and cohort description will be summarised using descriptive statistics:

- Frequency tables will be generated for categorical variable such as geographical ancestry.
- Mean, median, standard deviation, minimum and maximum will be provided for continuous data such as age.

The distribution of subjects enrolled among the study sites will be tabulated.

Withdrawal status will be summarised according to the reason for withdrawal. The number of withdrawn patients will be tabulated by study visit and overall.

All tables will also be generated by country.

9.5. Analysis of primary objective

The proportion of sputum samples obtained at each visit (confirmed stable visits[*] and AECOPD visits and positive for specific bacterial/ viral pathogens by bacteriological methods and PCR, respectively (overall and by bacterial/viral species) will be computed with 95% confidence intervals.

[*] *A confirmed stable visit will be defined as a scheduled study visit for which the investigator confirms in the eCRF that the subject is stable/ has recovered from a previous exacerbation.*

9.6. Analysis of secondary objectives

The proportion of sputum samples obtained at each visit (confirmed stable visits* and AECOPD visits [any severity, mild, moderate and severe]) and positive for specific bacterial pathogens by both bacteriological methods and PCR (overall and by bacterial species) will be computed with 95% confidence intervals.

> * *A confirmed stable visit will be defined as a scheduled study visit for which the investigator confirms in the eCRF that the subject is stable / has recovered from a previous exacerbation.*

The proportion of sputum samples obtained at each stable visit and positive for specific bacterial/viral pathogens by bacteriological methods and PCR, respectively (overall and by bacterial/ viral species) will be computed with 95% confidence intervals, by GOLD grade at enrolment.

The proportion of sputum samples obtained at each AECOPD visits and positive for specific bacterial/ viral pathogens by bacteriological methods and PCR, respectively (overall and by bacterial/viral species) will be computed with 95% confidence intervals, by AECOPD severity.

The proportion of sputum samples obtained at each confirmed stable/AECOPD visit with previous administration of antibiotics or not and positive for specific bacterial/ viral pathogens by bacteriological culture and PCR, respectively (overall and by bacterial/viral species) will be computed with 95% confidence intervals.

The following incidence rates will be computed, with 95% confidence intervals (CI):

- All-cause AECOPD.
- AECOPD having sputum containing bacterial pathogens found by PCR ***or*** by bacteriological methods or by both methods (overall and by, but not limited to, the following bacterial species: *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, and *P. aeruginosa*).

The 95% CI of the incidence rate will be computed using a model which accounts for repeated events. The Generalised linear model assuming the Negative Binomial distribution for the response variable with logarithm as link function, and the logarithm of time for follow-up as an offset variable will be used. The incidence rates described above will also be computed for mild, moderate severe AECOPD and by GOLD grade at enrolment and will be estimated by country.

The number of subjects that report at least 1 AECOPD will be tabulated and descriptive statistics (median, mean, range, standard deviation, first and third quartiles) on the number of days of AECOPD episodes will be presented, for any, mild, moderate and severe AECOPD.

Descriptive statistics (median, mean, range, standard deviation, first and third quartiles) on the *CAT* and SGRQ-C scores will be tabulated at each respective visit.

Summary statistics (mean, median, standard deviation, maximum and minimum) on post-bronchodilator FEV1% of predicted normal value will be tabulated at each respective visit.

Descriptive summaries on healthcare use will be provided.

9.7. Analysis of tertiary objectives

Descriptive statistics (median, mean, range, standard deviation, first and third quartiles) on the end date of AECOPD as provided by the investigator or estimated by EXACT-PRO (via a specific algorithm which will be detailed in the Statistical Analysis Plan) will be presented.

Descriptive summaries of the quantity of specific bacteria at each scheduled [stable] and exacerbation visit will be provided for both culture and PCR analysis.

Descriptive summaries of the quantity of specific viruses at each scheduled [stable] and exacerbation visit will be provided for PCR analysis.

A conditional logistic model will be fitted to estimate the odds ratio of being in a stable state *vs.* an exacerbation state given the presence or not of several bacterial pathogens found by bacteriological culture and by country. The same model will also be performed for bacterial and viral pathogens found by PCR.

9.8. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

9.8.1. Sequence of analyses

- An interim analysis of the bacterial primary objective will be performed when at least 40 AECOPD sputum samples are available. The rate of positive samples will be computed for Hi, NTHi and Mcat together and per pathogen. No study report will be written at this stage.
- A final analysis of all objectives will be performed after the last subject last visit of the entire study. A final study report will be written at this stage.

9.8.2. Statistical considerations for interim analyses

All analyses are exploratory and will be conducted on final data and therefore no statistical adjustment for interim analyses is required.

10. ADMINISTRATIVE MATTERS

To comply with ICH GCP or other applicable guidelines, administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, ownership, public disclosure requirements and publications must be met.

10.1. Electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

Once the database is archived and the clinical study report is complete and approved by all parties, each participating investigator will be provided with a CD-ROM of the final version of the data generated at his/her investigational site.

10.2. Study monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and

investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

10.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g., audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP or other applicable guidelines, any institutional requirements or applicable laws or regulations, or GSK standards/procedures otherwise, the minimum retention period will default to 25 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility and transfer of ownership of the records in the event the investigator leaves the site.

10.4. Quality assurance

To ensure compliance with GCP or other applicable guidelines and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

10.5. Posting of information on publicly available registers and publication policy

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required time-frame, in compliance with the current regulations.

Studies that do not evaluate vaccines/products are progressed for publication in the scientific literature when the results provide important scientific or medical knowledge or are relevant for patient care, and will be considered for disclosure on the GSK website and in publicly accessible regulatory registry(ies).

10.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK Biologicals site or other mutually-agreed location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

10.7. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non- GSK studies, to investigate further about the study product(s) and other product(s), and /or the disease/condition under investigation and related diseases and conditions.

11. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

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APPENDIX A CLINICAL LABORATORIES (Amended 19 October 2018)

Table 14 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Clinical Laboratory Sciences (CLS) Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Clinical Laboratory Sciences (CLS) Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 15 Outsourced Laboratories

Laboratory	Address
Q² Solutions Clinical Trials (US)	27027 Tourney Road, Suite 2E Valencia, CA 91355 USA
Q² Solutions (Singapore)	Tan Tock Seng Hospital Dept of Laboratory Medicine Level 2, Podium Block Tan Tock Seng Hospital 11 Jalan Tan Tock Seng Singapore 308433
DDL Diagnostic Laboratory B.V.	Fonteijnenburghlaan 7 Voorburg Netherland

APPENDIX B AMENDMENT TO THE PROTOCOL

GlaxoSmithKline Biologicals

Vaccine Value & Health Science (VVHS)

Protocol Amendment 1

eTrack study number and Abbreviated Title	201112 (EPI-NTHI-001 BOD APA)
Amendment number:	Amendment 1
Amendment date:	13 February 2017
Co-ordinating author:	PPD Scientific Writer

Rationale/background for changes:
The protocol amendment has been issued to implement the following changes:

- Alignment of the protocol to the updated GOLD consensus report of 2017 and the COPD fact sheet.
- Alignment of the study endpoints to the study objectives.
- Overall update based on the scientific and operational experience gained from the current COPD studies.
- To update the new literature references.
- To reflect the upgrade to new version for CRF, ICF, protocol, SPM and overall changes in the functions due to reorganization.
- To correct some typographical errors.
- Update of the GSK laboratory's name.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Title page:

Title	Occurrence of ***potential*** bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in Asia Pacific.

Detailed Title

A prospective, epidemiological, multi-country, cohort study to assess the occurrence of ***potential*** bacterial and viral pathogens in stable chronic obstructive pulmonary disease (COPD) and during acute exacerbations of COPD (AECOPD), in moderate to very severe COPD patients, in Asia Pacific.

Contributing authors

- PPD Epidemiologist
- PPD Clinical ***Research*** ~~Development~~ and ~~Research~~ ***Development*** Lead
- PPD Clinical ***Research*** ~~Development~~ and ~~Research~~ ***Development*** Lead
- PPD Biostatistician
- PPD Lead statistician
- PPD ***Project*** Delivery Lead
- PPD Study Delivery Lead
- PPD Study Delivery Manager, (External Consultant [Freelance]for GSK Biologicals)
- PPD ~~Study Data Manager (Business & Decision Life Sciences for GSK Biologicals)~~ ***Senior Manager, Oversight Data Management***
- PPD ***Study Data Manager***
- PPD Vaccine Supply Coordinator (CVO Europe, for GSK Biologicals)
- PPD ~~Project Manager, GVCL~~ ***Clinical Readout Team Leader***, ***Clinical Laboratory Sciences***
- PPD Study Manager, GVCL (Business & Decision Life sciences, for GSK Biologicals)/ PPD ***Study Manager***, ***Clinical Laboratory Sciences***
- PPD ***Global Regulatory Lead and*** PPD ~~Technical~~ **Global** Regulatory Affairs representative

- PPD and PPD PPD ***and*** PPD PPD ***and*** PPD Clinical Safety Representatives
- PPD ***Expert Scientist, Clinical Laboratory Sciences***
- PPD Study Data Manager (Business & Decision Life Sciences, for GSK Biologicals)
- Dr. PPD, University of Sydney Head, Respiratory Trials, Contributing author from The George Institute for Global Health, Australia

~~**Contributing author from The George Institute for Global Health, Australia**~~

- ~~Dr.~~ PPD ~~, University of Sydney Head, Respiratory Trials~~

PPD

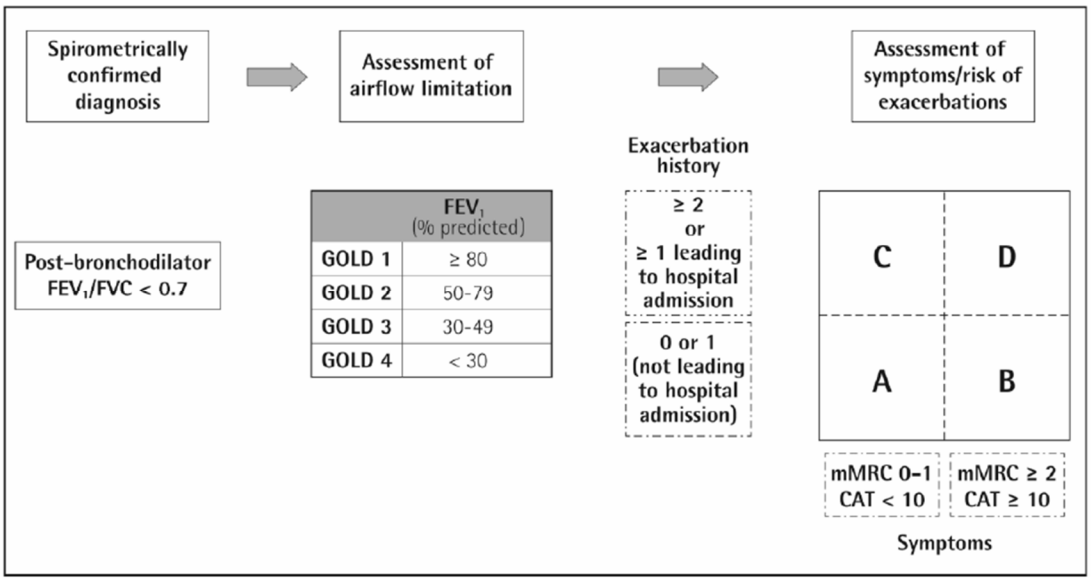
Trademarks

Trademarks of the GlaxoSmithKline group of companies	Generic description
EXACT-PRO®	***Exacerbations of Chronic Pulmonary Disease Tool - Patient Reported Outcome***
SGRQ-C	***St. George's Respiratory Questionnaire for COPD patients***

Background

Chronic Obstructive Pulmonary Disease (COPD) is a ***common preventable and treatable*** disease characterised by persistent airflow obstruction which is not fully reversible and which is usually progressive in the long term. ***The classification of COPD is done based on the patient's spirometric classification according to GOLD, 2013***. The airflow limitation in COPD patients can be classified into GOLD grades as shown in Table 1 [GOLD, 2013]**.** ***In this study, additional parameters are going to be collected in order to allow for a combined assessment that could be used for future evaluation, as detailed in*** *Figure 1* ***[GOLD, 2017].***

Figure 1 The refined ABCD assessment tool



GOLD = Global Initiative for Chronic Obstructive Lung Disease; CAT = COPD assessment test; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; mMRC = Modified British Medical Research Council Questionnaire

The classification of severity of airflow limitation in COPD patients is provided according to* GOLD*, 2017 as follows,

- ***Patient Group A – Low Risk, Less Symptoms Typically GOLD 1 or GOLD 2 (Mild or Moderate airflow limitation); and/or 0-1 exacerbation per year and no hospitalization for exacerbation; and CAT score < 10 or mMRC grade 0-1***
- ***Patient Group B – Low Risk, More Symptoms Typically GOLD 1 or GOLD 2 (Mild or Moderate airflow limitation); and/or 0-1 exacerbation per year and no hospitalization for exacerbation; and CAT score ≥ 10 or mMRC grade ≥ 2***
- ***Patient Group C – High Risk, Less Symptoms Typically GOLD 3 or GOLD 4 (Severe or Very Severe airflow limitation); and/or ≥ 2 exacerbations per year or ≥ 1 with hospitalization for exacerbation; and CAT score < 10 or mMRC grade 0-1***
- ***Patient Group D – High Risk, More Symptoms Typically GOLD 3 or GOLD 4 (Severe or Very Severe airflow limitation); and/or ≥ 2 exacerbations per year or ≥ 1 with hospitalization for exacerbation; and CAT score ≥ 10 or mMRC grade ≥ 2.***

COPD is the fifth leading cause of mortality worldwide, accounting for 6% of all deaths globally [WHO 2015]. A recent study reported high overall morbidity and mortality rates due to COPD in the Asia Pacific region. The prevalence of moderate to severe COPD in adults aged 30 years or above was estimated to be 5.9% in South Korea, 5.4% in Taiwan, 4.7% in Australia and 3.5% in Hong Kong [Tan, 2009]. ***The overall estimated prevalence COPD in Asia-Pacific was estimated to be 6.2% with 19.1% having severe COPD [Lim, 2015]***. The prevalence and burden of COPD are projected to continue to increase in the coming decades due to continued exposure to risk factors and the changing age structure of the world's population [Mathers, 2006]. In Asia,

urbanisation is expected to play a major role in the increase of the number of prevalent cases of COPD between 2010 and 2020 [Tan, 2009]. In terms of the number of deaths, the burden of disease is higher in the Asia-Pacific region when compared to the industrialised western countries. It was estimated through mathematical modelling that 56.6 million people aged ≥ 30 years had moderate to severe COPD with a prevalence rate of 6.3% [Ko, 2008].

Synopsis and Section 1.2 Rationale for the study

Because the infectious aetiology-of AECOPD has been suggested to vary according to geographical region, the primary purpose of this study (which will be conducted in several countries in Asia Pacific) is to evaluate the occurrence of bacterial and viral pathogens in the sputum of stable COPD patients and at the time of AECOPD. Given the increasing and projected burden of COPD in the Asia Pacific region, this study will also evaluate the frequency, severity and duration of AECOPD, as well as the impact of AECOPD on health-related quality of life (HRQOL), healthcare utilisation~~,~~ ***and*** lung function~~and on exercise capacity~~.

In addition, both PCR and bacteriological methods will be used for characterisation and ***(***quantification ***in some cases)*** of bacteria in sputum. To date, identification of bacteria and assessment of bacterial load is done in the vast majority of cases by culture. However, the few studies that have compared culture with PCR demonstrated that PCR is more discriminatory at detecting typical airway bacteria [Curran, 2007; ***Singh***, ***2014***]. Moreover, in a multi-centre study, PCR has the advantage that it can be performed on stored sputum samples, and can therefore be done centrally, whereas culture has to be performed on fresh sputum samples, which can lead to variable results ~~in case of~~ ***due to*** variability in testing methods between laboratories.

Synopsis and Section 2 Objectives

Primary objective

- ~~To estimate the proportion of bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients and during AECOPD.~~ ***To estimate the proportion of potential bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients and during AECOPD, using respectively bacteriological methods and viral PCR, over the course of 1 year.***

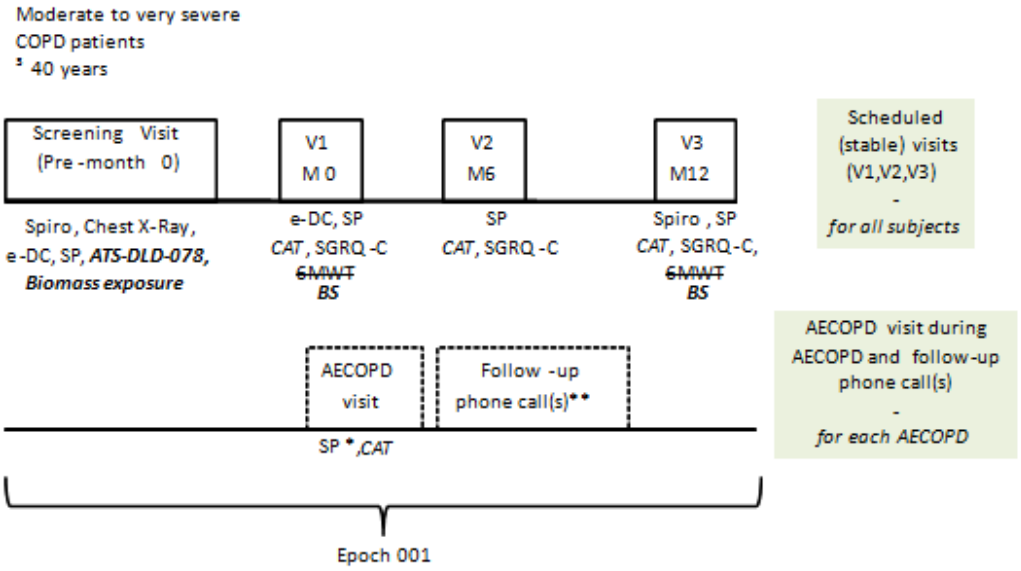
Secondary objectives

- To evaluate ***the concordance between*** PCR and ***bacteriological methods data***~~bacteriological culture with respect to their ability to characterise and ***(quantify; in some cases)*** bacterial pathogens in sputum~~ ***in sputum for potential bacterial pathogens***.
- To estimate the proportion of ***potential*** bacterial and viral pathogens (overall and by species) detected in sputum of stable COPD patients, by GOLD grade.
- To estimate the proportion of ***potential*** bacterial and viral pathogens (overall and by species) detected in sputum during AECOPD, by severity of AECOPD.
- ~~To assess the impact of all-cause AECOPD on exercise capacity~~.

Tertiary objectives

- ~~To estimate the difference in the presence of bacterial pathogens in sputum between stable state and AECOPD.~~
- ***To evaluate the load of bacterial and viral pathogen in stable COPD and during AECOPD.***
- ***To collect biological specimens for future respiratory disease-related testing:***
  - ***Aliquots of sputum samples for assay development and microbiome analysis at stable visits and during exacerbation.***
  - ***Blood sampling for potential biomarkers for identification/quantification of biomarkers.***
  - ***Bacterial isolates for further strain characterisation.***

Section 3 Study design overview



V = Visit; **M** = Month; **SP** = sputum sample; **e-DC:** Training and use of electronic Diary card; **CAT**= COPD assessment test; **SGRQ -C** = St. George's Respiratory Questionnaire for COPD patients; **spiro** = spirometry; ~~**6MWT** = 6 minute walk test~~; ***BS = Blood Sampling;*** **AECOPD** = acute exacerbation of COPD; = acute exacerbation of COPD; ***ATS-DLD-078 = American Thoracic Society and National Heart and Lung Institute-Division of Lung Disease Respiratory Questionnaire***

* The sputum samples during AECOPD should preferably be obtained before administration of the first dose of antibiotics to treat the AECOPD (if applicable). Self collection of the sputum sample will be allowed in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place. This is ~~not~~ allowed at scheduled visits when subject should be in stable condition.

** Follow up phone calls will take place at least every 2 weeks, until the AECOPD has resolved

Note: Screening visit should ideally **occur** not **more than** ~~be longer than~~ 6 weeks **before visit 1** .**Study visits/contacts:**

- Screening visit.
- ~~6-monthly~~ ***Three*** scheduled **(**stable) study visits ***occurring at 6 month intervals***.
- For each AECOPD: AECOPD visit (within 96 hours of the onset of the symptoms) and follow-up phone call(s) (at least every 2 weeks until the AECOPD has resolved). Follow-up phone contacts will define end of AECOPD.

- **Biological samples:**
  - ***Blood samples for biomarker testing will be collected at Visit 1 (M0), Visit 3 (M12).***

Synopsis and Section 3.1 Discussion of study design

The primary objective of this study is to prospectively investigate the prevalence and distribution of bacteria and viruses isolated from sputum samples from ***moderate to very severe*** ~~stable~~ COPD patients ~~(with moderate-very severe COPD)~~ and at the time of AECOPD, in Asian populations. In order to increase the chance that the patients will exacerbate during the study, only patients with a documented history of at least 1 moderate or severe AECOPD in the previous year will be recruited, as this is known to be the best predictor of AECOPD [Hurst, 2010]. Therefore, this study will permit an estimation of incidence of AECOPD in a COPD population at increased risk of exacerbation. It will not provide a true population-based incidence of AECOPD.

In order to study factors affecting disease progression in Asia Pacific, prospective data from a relatively large cohort of COPD patients is essential. The use of electronic Diary Cards to detect changes in the respiratory symptoms that define an AECOPD, the standardised questionnaires ***and*** spirometry assessments~~, and exercise capacity tests~~ will allow robust conclusions to be drawn about the effects of exacerbations on decline in lung function and HRQOL. ~~Furthermore, the completeness of vital status information will allow to ***determine the*** effects of exacerbations and hospitalizations on mortality to be examined with confidence~~.

EXACT PRO will be used to assess breathlessness, cough and ***presence of*** sputum, chest symptoms, difficulty bringing up sputum, feeling tired or weak, sleep disturbance, and feeling scared or worried about their condition. Advantages to this approach include standardised data, reduced recall bias, and the potential to identify events and determine resolution based on a predefined scoring algorithm [Leidy, 2011]. Standardised questionnaires completed during study visits include the SGRQ-C and the CAT. The SGRQ-C is designed to assess ***HRQOL***, current health and does not specify a recall period. The *CAT* is a simple, short, patient-completed instrument to assess HRQOL and symptom burden in patients with COPD. The *CAT* has good internal consistency and test-retest reliability, correlates strongly with the SGRQ-C, and is able to distinguish between stable patients and those undergoing an AECOPD [Jones, 2012].

Section 3.1.1 Detection of AECOPD

Note that:

- ~~*Any symptom occurring on at least 5 consecutive days prior to a potential exacerbation will not be taken into account to define an exacerbation.*~~

Each time a potential AECOPD is detected via the electronic Diary Card, the device will alert the subject to contact the study site, and at the same time an alert will be sent to the site so that the site staff contacts the subject to ***determine if the alert is an AECOPD or not, and if*** ~~check whether~~ an AECOPD visit is warranted. In addition, the site should

proactively follow-up all data received via the electronic Diary Card and contact the subject whenever deemed necessary.

During the contact with the subject, the site will determine whether the subject might actually be experiencing an AECOPD (*e.g.* notifications that can be explained solely by increased physical activity will not be considered):

- If the site concludes that the subject is not experiencing an AECOPD, this should be documented/ reported ***in the eCRF***. Please refer to study procedures manual (SPM) for more details on how to perform this.
- If the site concludes that the subject may be experiencing an AECOPD, an AECOPD visit will be scheduled as soon as possible after the onset of AECOPD symptoms as recorded in the electronic Diary Card or confirmed by the subject (maximum 96 hours after onset of symptoms and, if applicable, preferably before starting treatment with antibiotics). ***The AECOPD onset date will be captured in the eCRF and additional information about severity will be also collected.***
  - ***In case the AECOPD is confirmed but no AECOPD visit can take place, the site should record the information in the medical records subject files, and in the CRF in medical records section and obtain all relevant information regarding the AECOPD(hospital record, medical record etc) and record this in the eCRF.***

During the AECOPD visit, the investigator will confirm the occurrence of the AECOPD based on clinical and medical judgement ***and based on the Anthonisen criteria***~~,~~ and will record its date of onset. The end date of the AECOPD and its severity will be determined/ confirmed by the investigator***/delegate*** during (a) follow-up phone call(s), which will take place at least every 2 weeks until the AECOPD has resolved.

If an AECOPD occurs at the time when one of the scheduled [stable] study visits is planned, it should be handled and recorded as an AECOPD visit, with all relevant ***AECOPD visit*** study procedures performed and, if possible, the stable study visit should be re-scheduled to a later date, when the subject is stable again and within the time window specified in the protocol. ~~However, in exceptional cases, in consultation with GSK Biologicals, the concluding visit (Visit 3, Month 12) may be exceptionally conducted outside of the allowed interval, if deemed necessary by the investigator. The reason for this should be documented in the eCRF.~~

The investigator/site must engage their best efforts to reach the patients, however, if the site does not succeed in contacting the patient, the reason should be recorded and an explanation given about what occurred. For example, the subject could be hospitalized or could visit a different physician (and in that case medical record should be obtained), or the subject is on holidays or not able to go the site.

Section 3.1.1.1 Date of onset and end date of AECOPD

The date of onset is the first day (of at least 2 consecutive days) of worsening symptoms of COPD, ***as determined by the Investigator according to the Anthonisen criteria.***

The end date should be based on when the investigator determines that the AECOPD symptoms have resolved. In determining this end date, consideration should be given to symptoms recorded in the electronic Diary Card and subject assessment ***during the phone calls.***

Section 4.1 Number of subjects/centres

The target is to enrol approximately 200 eligible moderate to very severe COPD patients. ~~All subjects should be recruited within the period of 1 year.~~

Section 4.2 Inclusion criteria for enrolment

- Confirmed diagnosis of moderate to very severe COPD based on post-bronchodilator spirometry (*i.e.* forced expiratory volume in 1 second [FEV_1] over forced vital capacity [FVC] ratio [FEV_1/ FVC] < 0.7 ~~**AND**~~ ***and*** FEV_1 < 80% predicted [GOLD grades 2, 3 and 4].
- Stable COPD patient* with documented history** (e.g. medical record verification) of at least 1 moderate or severe AECOPD within the 12 months before study entry.

  ** Patient for whom the last episode of AECOPD is resolved for at least 30 days at the time of study entry.*****Note: A documented history of a COPD exacerbation (e.g., medical record verification) is a medical record of worsening COPD symptoms that required systemic/oral corticosteroids and/or antibiotics (for a moderate exacerbation) or hospitalization (for a severe exacerbation). Prior use of antibiotics alone does not qualify as an exacerbation history unless the use was associated with treatment of worsening symptoms of COPD, such as increased dyspnea, sputum volume, or sputum purulence (color). Subject verbal reports are not acceptable.***
- Current or former ***tobacco*** smoker ***(cigarette)*** with a smoking history of ≥ 10 pack-years OR a subject exposed to biomass smoke for ≥ 20 years. [***Guoping Hu 2010***]

Section 4.3 Exclusion criteria for enrolment

- ~~Undergone or planning lung surgery within 12 months from study entry.~~ ***Undergone or has had lung surgery 12 months before, or plans to have lung surgery 12 months after, study entry.***
- Administration of antibiotics within 1 month of study entry OR continuous administration of antibiotics (defined as more than ~~1 month~~ ***30 days*** in total) within ~~the three months~~ ***90 days*** before study entry.
- ***Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to informed consent.***

- Contraindication for spirometry testing (such as recent eye surgery, recent thoracic or abdominal surgery procedures, unstable cardiovascular status, recent myocardial ~~infection~~ ***infarction*** or pulmonary embolism).
- ~~Mentally impaired patients.~~***Psychiatric illness or any other condition that interferes with the ability to understand the study procedures.***
- Pregnant female ~~with COPD~~.

Section 6.3 Outline of study procedures

Table 4 List of study procedures for scheduled ~~[stable]~~ visits

Epoch	Epoch 001			
Type of contact	**Screening Visit**	**Visit 1**	**Visit 2**	**Visit 3**
Time point	**Pre-Month 0**	**Month 0**	**Month 6**	**Month 12**
Smoking exposure history (ATS-DLD-78A questionnaire)/ biomass exposure history	~~●~~O			
Urine Pregnancy test	●			
Physical examination including vital signs	●	O	O	O
Record healthcare ***resource*** utilisation ~~for COPD~~				
Blood samples for biomarkers and haematology		●		●
HRQOL questionnaires:				
CAT		O	O	O
SGRQ-C		O	O	O
~~6MWT~~		~~●~~		~~●~~

● is used to indicate a study procedure that requires documentation in the individual eCRF.
O is used to indicate a study procedure that does not require documentation in the individual eCRF.
[a] For non-eligible subjects, only informed consent, inclusion/ exclusion criteria, demographic data and SAEs related to study participation that occurred after signing the informed consent pages need to be completed in the subject's eCRF. For other activities, 'not done' can be indicated.
[b] Record both documented and self-reported, non-documented AECOPD. Subjects need documentation for at least 1 moderate or severe AECOPD within the previous year to be eligible for study participation.
[c] Significant comorbidities include weight loss, cardiovascular disease, hypertension, gastro-oesophageal reflux disease, osteoporosis/ osteopenia, skeletal muscle wasting and dysfunction, anxiety/ depression and diabetes.
[d] To be recorded in the 'Physical examination' section of the eCRF.
[e] Only study certified site staff can perform spirometry assessment.
[f] Only if no chest X-ray is taken within the previous 3 months.
[g] Sputum can be collected spontaneously or can be induced, as per investigator judgement. Sputum sampling should only be done if, in the opinion of the investigator, it is safe for the subject.
[*] Non-serious AEs will be recorded in the eCRF and will not be entered into the safety database.

Table 5 List of study procedures for AECOPD visit

Type of contact Time point	AECOPD Visit within 96 hours of onset symptoms	End of AECOPD phone call(s) at least every 2 weeks as of AECOPD visit until AECOPD has resolved[h]
Record date of visit	●	
Physical examination	●	
Urine Pregnancy Test	●	
Chest X-rays/pneumonia confirmation[i]	●	
Confirm AECOPD and record its start date	●	
Record current medication for COPD	●	
Record healthcare ***resource*** utilisation	●	
Record additional COPD treatments prescribed by primary and secondary care physicians		●
Sputum sampling[j]	●	
HRQOL questionnaire:		
CAT	O	
Record AECOPD severity[k]	●	●
Record AECOPD end date		●
Record Adverse Events (AEs) related to study participation*	●	●
Record serious adverse events (SAEs)	●	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
~~O is used to indicate a study procedure that does not require documentation in the individual eCRF.~~
[h] End of AECOPD phone calls/ visits should be scheduled at least every 2 weeks, until the AECOPD has resolved.
[i] Only the last phone call will be recorded in the eCRF as the end of the AECOPD phone call. All intermediate calls should be recorded on source documentation.
[j] [j] ***Physical examination should be done only if necessary.***
[i] Only if clinically indicated to exclude another cause of worsening of symptoms (*e.g.* pneumonia). If a chest X-ray is clinically indicated but a chest X-ray/ CT scan has already been taken for that AECOPD as part of medical care before the study AECOPD visit takes places, the results of that chest X-ray/ CT scan can be used. All cases of pneumonia (including all signs and symptoms assessed to confirm pneumonia) should be documented in the eCRF.
[j] Sputum can be collected spontaneously or can be induced, as per investigator judgement. Sputum sampling should only be done if, in the opinion of the investigator, it is safe for the subject.
[k] AECOPD severity grading as indicated in Table 1.
* Non-serious AEs will be recorded in the eCRF and will not be entered into the safety database.

Table 6 Intervals between study visits

Interval	Optimal length of interval	Allowed interval[1]
	~~*Screening Visit*~~	
Screening Visit (pre-Month 0)→ Visit 1	± 14 days	≤42 days before Visit 1
	Scheduled study visits	
Visit 1 (Month 0) → Visit 2 (Month 6)	182 days	175 - 203 days
Visit 2 (Month 6) →Visit 3 (Month 12)	182 days	175 - 203 days
AECOPD-driven study visit(s) and/ or phone contacts		
Onset AECOPD symptoms → AECOPD Visit	-	max 96 hours[2]

[1] If an AECOPD occurs at the time one of the scheduled ~~[stable]~~ study visits is planned, the stable study visit should be re-scheduled to a later date, when the subject is stable again and within the time window specified. In consultation with GSK, the concluding visit (Visit 3, Month 12) may however exceptionally be conducted outside of the allowed interval if necessary by the investigator.
[2] AECOPD visits will be scheduled as soon as possible after the onset of AECOPD symptoms as recorded in the electronic Diary Card or confirmed by the subject (maximum 96 hours after and, if applicable, preferably before starting treatment with antibiotics).

Section 6.4.1.5 Record AECOPD history within the previous year

Documented, self-reported and non-documented AECOPD should be recorded in the eCRF. However, for study participation the subject should have had at least one ***documented*** moderate or severe AECOPD episode within the previous year.

Section 6.4.1.6 Smoking exposure history (ATS-DLD-78A questionnaire)/biomass exposure history (At ~~Screening~~ *screening* visit ~~ONLY~~ *only*)

The subject will be asked to complete the smoking history questionnaire (which is a shortened version of the American Thoracic Society-Division of Lung Diseases-78A [ATS-DLD-78A] questionnaire) by himself/herself. ~~This questionnaire will be provided in paper.~~ The subject will have to provide information about his/ her smoking history, including duration (number of years) and number of cigarettes smoked. Subject years exposed to biomass will be assessed from information obtained on indoor exposure to fuels such as wood, crop waste and dung, or coal.

Section 6.4.1.9 Pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study procedure. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit. The test should be carried out before performing chest x-ray. If the test is positive, the chest x- ray should not be performed and the subject should be withdrawn from the study.

Section 6.4.1.13 Train subjects on the use of electronic Diary Card and assign electronic dairy card to subject

- The site staff will follow electronic Diary Card completion closely and should provide timely input/guidance to ensure that the subject ***correctly completes the eDiary Card both AM & PM as per protocol*** ~~reaches the targeted learning curve~~.

Section 6.4.1.14 Sputum sampling

- ~~**Sputum sample collected at subject's home (spontaneous):**~~***At home, sputum collection will not be permitted. However,*** ~~**S**~~***self***-collection of the sputum sample will be allowed ***only*** in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place.
- ***Collected sputum will be diluted in DTT, processed and cultured within 6hrs of collection for microbiology testing***
- ***Remaining DTT-sputum will be aliquoted as such and/or further diluted in STGG . These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.***

Refer to the SPM and the ***Central Laboratory Investigator Manual*** for more details and guidance on handling of sputum samples.

Section 6.4.2.1 Check inclusion and exclusion criteria

At visit 1, all applicable inclusion and exclusion criteria as described in Sections* 4.2 *and* 4.3 *will be checked before enrolment.

Section 6.4.2.10 Record healthcare utilisation ~~for COPD~~

Healthcare use ~~for each COPD patient~~ will be obtained through review of the subject's medical record (aided by subject self-reporting). ~~Healthcare utilisation includes primary, secondary and tertiary care settings such as self-care with over-the-counter [OTC] drugs, general practitioner (GP) visits, emergency room (ER) visits, and hospital visits.~~ ***Healthcare utilisation includes all unscheduled visits to a physician office, visits to urgent care, visits to emergency department, and hospitalizations***. Healthcare use ~~for the COPD~~ should be entered in the eCRF. Refer to the SPM for more details and guidance on recording of healthcare use.

Section 6.4.2.14 Sputum Sampling

- Sputum samples will be collected during the scheduled ~~stable~~ visits, if in the opinion of the investigator, it is safe for the subject.
- ***Collected sputum will be diluted in DTT, processed and cultured within 6hrs of collection for microbiology testing.***
- ***Remaining DTT-sputum will be aliquoted as such and/or further diluted in STGG . These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.***

Refer to the SPM **and the** ***Central Laboratory Investigator Manual*** for more details and guidance on handling of sputum samples.

Section 6.4.2.15 Blood Sampling

- ***Blood samples for biomarker analysis will be taken at the scheduled visits; Visit 1(M0) and Visit 3 (M12), including:***
  - ***Hematology profile, including differential cell counts***
    - ***Approximately 2.0 mL of whole blood will be collected for hematology assessment. These samples should be kept at room temperature and shipped on the day of collection, ambient.***
  - ***Specific biomarkers will include serum hsCRP, CXCL10 (IP-10) and plasma fibrinogen, may include other biomarkers based on results of ongoing disease understanding research.***
    - ***Approximately 8.5 mL of whole blood will be collected and processed to serum for hsCRP & CXCL10 (IP-10) assessment. After processing, these samples should be kept at -70/-80°C until shipment.***
    - ***Approximately 4.5 mL of whole blood (Na Citrate) will be collected and processed to plasma for fibrinogen assessment. After processing, these samples should be kept at -70/-80°C until shipment.***

- ***Approximately 6.0 mL of whole blood (EDTA) will be collected and processed to plasma for other biomarker assessment. After processing, these samples should be kept at -70/-80°C until shipment.***

Refer to the SPM and the Central Laboratory Investigator Manual for more details and guidance on the collection, handling, and processing of blood samples.

Sections 6.4.2.16MWT

- ~~The subject should perform the supervised, standardised 6MWT at Visit 1 and Visit 3 as presented in Table 4. The result of the test will be recorded in the eCRF.~~
- ~~Refer to the SPM for more details and guidance on the 6MWT.~~

Sections 6.4.3.3 Pregnancy Test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study procedure. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit. The test should be carried out before performing chest x-ray. If the test is positive, the chest x- ray should not be performed and the subject should be withdrawn from the study.

Section 6.4.3.7 Record healthcare utilisation ~~for AECOPD~~

Healthcare use ~~for each AECOPD~~ will be obtained through review of the subject's medical record (aided by subject self-reporting). Healthcare utilisation includes ~~primary, secondary and tertiary care settings such as self-care with over-the-counter [OTC] drugs, general practitioner (GP) visits, emergency room (ER) visits, and hospital visits.~~ ***all unscheduled visits to a physician office, visits to urgent care, visits to emergency department, and hospitalizations***. Healthcare use ~~for the AECOPD~~ should be entered in the eCRF. Refer to the SPM for more details and guidance on recording of healthcare use.

Section 6.4.3.9 Sputum sampling

- ***Collected sputum will be diluted in DTT, processed and cultured within 6hrs of collection for microbiology testing***
- ***Remaining DTT-sputum will be aliquoted as such and/or further diluted in STGG . These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.***

Refer to the SPM **and the *Central Laboratory Investigator Manual*** for more details and guidance on handling of sputum samples.

Section 6.4.4.1 Sputum sampling

Full details for obtaining sputum samples are provided in the Module on Biospecimen Management in the SPM and in the Central Laboratory Investigator Manual accompanying this protocol.

Section 6.5 Biological sample handling and analysis

Please refer to the SPM ***and the Central Laboratory Investigator Manual*** for details of biospecimen management (handling, storage and shipment).

Section 6.5.1 Use of specified study materials

When materials are provided by GSK Biologicals ***or the central laboratory***, it is MANDATORY that all samples be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the PP analysis (See Section 9.3 for the definition of study cohorts/data sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals ***or the central laboratory*** does not provide material for collecting and storing samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM ***and Central Laboratory investigator manual***.

Section 6.5.2 Biological samples

Table 7 Biological Samples

Sample type	Quantity	Unit	Time point				
			Screening Visit	Visit 1	Visit 2	Visit 3	AECOPD Visit
Fresh~~/stored~~ sputum	Maximum possible	mL	Pre-Month 0	Month 0	Month 6	Month 12	Within 96 hours of onset of symptoms
Blood for biomarkers	***21***	***mL***		***Month 0***		***Month 12***	

Section 6.5.3 Laboratory assays

The quality of sputum samples will be assessed at the investigator's institution and/ or at a laboratory designated by GSK Biologicals by gram staining.

Standard bacteriological ***method*~~s~~*~~culture~~** ***(and semi-quantitative counts)*** will be performed on fresh sputum samples at the investigator's institution ***and/or at a laboratory designated by GSK Biologicals***. Identification of potential bacterial pathogens will be performed according to agreed identification methods (potential pathogens including, but not necessarily limited to, *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and and *A. baumannii*). All results should be entered in the eCRF.

Further bacterial characterization for *H. influenzae* isolates*:*Identified *H. influenzae* isolates should be collected ***and stored*** in the investigator's institution and will undergo further species confirmation ***((i.e. H. influenzae/ H. haemolyticus and when possible, further differentiation (i.e., Hi/NTHi)*** at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals using molecular techniques such as *H. influenzae*/ *Haemophilus haemolyticus* (*H. haemolyticus*) differentiation PCR. ***Identified H.***

influenzae isolates might undergo further testing at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals' using molecular techniques such as PCR and sequencing.

Identified M. catarrhalis isolates should be collected and stored in the investigator's institution and might undergo further species confirmation (and when possible, further differentiation) at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals using molecular techniques such as PCR and sequencing.

Identified P. aeruginosa isolates should be collected and stored in the investigator's institution for future testing at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals.

Further bacterial pathogen identification (including, but not necessarily limited to, *H. influenzae, S. pneumoniae*, *M. catarrhalis*, ***S. pyogenes,*** *S. aureus* and *P. aeruginosa*) ***and quantification (for H. influenzae, S. pneumoniae, M. catarrhalis)*** on stored sputum samples will be performed at GSK Biologicals' laboratory or a laboratory designated by GSK Biologicals using ~~multiplex~~ qualitative PCR and/or quantitative PCR.

Viral pathogen identification (including, but not necessarily limited to, RSV, parainfluenza virus, enterovirus/ rhinovirus, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus) on stored sputum samples will be performed at GSK Biologicals' laboratory or a laboratory designated by GSK Biologicals using multiplex reverse transcription polymerase chain reaction (RT-PCR).

In addition, some respiratory viral pathogens (such as rhinovirus) may be quantified in stored sputum samples using RT-PCR at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals.

Table 8 Microbiology

System	Component	Method	Scale	Laboratory
Quality assessment				
Fresh sputum	***Please refer to SPM and/or Central Laboratory Manual for components tested***	***Gram staining***	***Qualitative/ semi-quantitative***	***Investigator institution or GSK designated laboratory***
Bacterial pathogen identification				
Fresh sputum	*R*espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*)	Standard bacteriological culture and standard identification methods including semi-quantitative culture	Qualitative Semi-quantitative	Investigator's institution ***and/or at a laboratory designated by GSK Biologicals***
H. influenzae isolates of positive *H. influenzae* culture plates	*H. influenzae*/ *H.haemolyticus* differentiation	Molecular techniques such as differentiation PCR	Qualitative	GSK Biologicals laboratory* or designated laboratory
M. catarrhalis isolates of positive M. catarrhalis culture plates	***Sample collection for isolate characterisation***	***Molecular techniques***	***Qualitative***	***GSK Biologicals laboratory* or designated laboratory***
P. aeruginosa isolates of positive P. aeruginosa culture plates	***Sample collection for isolate characterisation***	***Molecular techniques***	***Qualitative***	***GSK Biologicals laboratory* or designated laboratory***
Stored sputum	*R*espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus* and *P. aeruginosa, Streptococcus pyogenes*)	Multiplex ~~qualitative PCR and/ or quantitative~~ ***PCR***	Qualitative and/ or quantitative	GSK Biologicals laboratory * or designated laboratory
Viral pathogen identification				
Stored sputum	Respiratory viral pathogens (including RSV, parainfluenza virus, enterovirus/ rhinovirus, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus)	Multiplex PCR	Qualitative and/or quantitative	GSK Biologicals laboratory * or designated laboratory
	Respiratory viral pathogens (such as rhinovirus)	***RT-PCR***	***Quantitative***	***GSK Biologicals laboratory* or designated laboratory***

* GSK Biologicals laboratory refers to the ***Clinical Laboratory Sciences (CLS)*** ~~Global Vaccines Clinical Laboratories (GVCL)~~ in Wavre, Belgium; Rixensart, Belgium.

Stored frozen sputum samples (either in dithiothreitol [DTT] ***and/***or in DTT supplemented with skim milk, tryptone, glucose, and glycerine [STGG] medium) might be used for assay development, such as assays for diagnostic purpose or for microbiome analysis and/or for assay validation/characterization purpose ***and/or quality control.***

Additional testing on stored sputum samples (such as, but not limited to, *K. pneumoniae* and *A. baumannii* PCR detection, quantitative PCR for other bacterial and/or viral pathogens, further characterisation of virus ***such as rhinovirus typing***, pathogen genome/gene sequencing, quantitative***/qualitative*** serotype-specific PCR, multilocus sequence typing, microarray typing, 16sRNA analysis) or on ***H. influenzae, M. catarrhalis and P. aeruginosa*** ~~*H. influenzae*~~ isolates (such as further strain characterisation using new molecular biology tools) may be done during the study or after study completion, should these data be required for accurate interpretation of the study data and/ or for further research related to ***respiratory diseases*** ~~COPD~~ and/ or should such test(s) become available at GSK Biologicals' laboratory or a laboratory designated by GSK.

Table 9 Planned assays on serological samples

Sample type	*Component*	*Method*	*Scale*	*Laboratory*
Plasma	*Fibrinogen*	*Per contract laboratory's procedures*	*Quantitative*	*GSK Biologicals or GSK designated lab*
Serum	*hsCRP*	*Per contract laboratory's procedures*	*Quantitative*	
Serum	*CXCL10 (IP-10)*	*Per contract laboratory's procedures*	*Quantitative*	
Whole blood	*Leukocytes (White Blood Cells)*	*Per contract laboratory's procedures*	*Quantitative*	
	*Neutrophils **	*Per contract laboratory's procedures*	*Quantitative*	
	*Lymphocytes **	*Per contract laboratory's procedures*	*Quantitative*	
	*Eosinophils **	*Per contract laboratory's procedures*	*Quantitative*	
	*Basophils **	*Per contract laboratory's procedures*	*Quantitative*	
	*Monocytes **	*Per contract laboratory's procedures*	*Quantitative*	
	Erythrocyte (Red Blood Cells)	*Per contract laboratory's procedures*	*Quantitative*	
	Haemoglobin	*Per contract laboratory's procedures*	*Quantitative*	
	Platelets	*Per contract laboratory's procedures*	*Quantitative*	

*For white blood cell differential counts

Additional testing for biomarkers on stored serum/plasma may be done during the study or after study completion, should these data be required for accurate interpretation of the study data and/ or for further research related to respiratory diseases available at GSK Biologicals' laboratory or a laboratory designated by GSK.

Section 7.2.1 Time periods for detecting and recording AEs and SAEs

AEs and SAEs related to study participation will be collected and recorded from the time of the first study visit until the subject is discharged from the study.

AEs leading to withdrawal from the study will be collected and recorded from the time of the first study visit until the subject is discharged from the study.

An overview of the protocol-required reporting periods for SAEs ***and AEs leading to withdrawal*** is given in Table 9.

Table 10 Reporting period for SAE

Study activity	Screening Visit		Visit 1		Visit 2		Visit 3		AECOPD Visit		Study Conclusion
	Pre-Month 0		M 0		M 6		M 12		Within 96 hours of onset symptoms		
AEs~~/SAEs~~ leading to withdrawal from the study											

Synopsis and Section 9 Statistical endpoints

Primary endpoint

- Occurrence of ***potential*** bacterial and viral pathogens in sputum of stable COPD patients and during AECOPD, over the course of 1 year:
  - Bacterial pathogens, as identified by bacteriological ~~culture~~ ***methods***, including (but not necessarily limited to) *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*.
  - Viral pathogens, as identified by ~~multiplex RT~~ PCR, including (but not necessarily limited to) RSV, parainfluenza virus, enterovirus/ rhinovirus, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus ***and by rhinovirus quantitative RT-PCR***.

Secondary endpoints

- Occurrence of ***potential*** bacterial pathogens in sputum of stable COPD patients and during AECOPD, as measured by ~~multiplex~~ ***real-time*** qualitative PCR/ quantitative PCR ***and compared to data from bacteriological methods***, over the course of 1 year:
  - Including (but not necessarily limited to) H. influenzae, M. catarrhalis, S. pneumoniae, S. aureus and P. aeruginosa.
- ***Occurrence of potential bacterial and viral pathogens (overall and by species) in sputum of stable COPD patients by GOLD grade, over the course of 1 year.***
- ***Occurrence of potential bacterial and viral pathogens (overall and by species) in sputum during AECOPD by severity of AECOPD, over the course of 1 year.***
- ~~Number~~ ***Incident rate (per subject per year)*** of AECOPD, ***overall and by GOLD grade***, over the course of 1 year.

- Severity of AECOPD, ***overall and by GOLD grade,*** over the course of 1 year.
- Duration of AECOPD, ***overall and by AECOPD severity,*** over the course of 1 year.
- FEV_1% of predicted normal value in stable COPD patients, at ***Pre-***Month 0 and Month 12.
- Healthcare utilisation ~~for COPD~~, over the course of 1 year.
- **Tertiary endpoints**
- ***Bacterial load measured by both culture and PCR in COPD and during AECOPD.***
- ***Viral load measured by PCR in COPD and during AECOPD.***

Section 9.5 Analysis of primary objective

The proportion of sputum samples obtained at each visit (confirmed stable visits* and AECOPD visits ~~[any severity, mild, moderate and severe])~~ and positive for specific bacterial/ viral pathogens by bacteriological ~~culture~~ ***methods*** and PCR, respectively (overall and by bacterial/viral species) will be computed with 95% confidence intervals.

> * *A confirmed stable visit will be defined as a scheduled ~~[stable]~~ study visit for which the investigator confirms in the eCRF that the subject is stable/ has recovered from a previous exacerbation.*

Section 9.6 Analysis of secondary objectives

The proportion of sputum samples obtained at each visit (confirmed stable visits* and AECOPD visits [any severity, mild, moderate and severe]) and positive for specific bacterial pathogens by ***both bacteriological methods and*** PCR (overall and by bacterial species) will be computed with 95% confidence intervals.

> * *A confirmed stable visit will be defined as a scheduled ~~[stable]~~ study visit for which the investigator confirms in the eCRF that the subject is stable / has recovered from a previous exacerbation.*

The proportion of sputum samples obtained at each stable visit and positive for specific bacterial/viral pathogens by bacteriological methods and PCR, respectively (overall and by bacterial/ viral species) will be computed with 95% confidence intervals, by GOLD grade at enrolment.

The proportion of sputum samples obtained at each AECOPD visits and positive for specific bacterial/ viral pathogens by bacteriological methods and PCR, respectively (overall and by bacterial/viral species) will be computed with 95% confidence intervals, by AECOPD severity.

~~These proportions will be also presented by GOLD grade at enrolment.~~

The following incidence rates will be computed, with 95% confidence intervals (CI):

- All-cause AECOPD.
- AECOPD having sputum containing bacterial pathogens found by PCR ~~and~~ ***or*** by ***bacteriological methods or by both methods*** overall and by, but not limited to, the following bacterial species: *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, and *P. aeruginosa*).

The 95% CI of the incidence rate will be computed using a model which accounts for repeated events. The Generalised linear model assuming the Negative Binomial distribution for the response variable with logarithm as link function, and the logarithm of time for follow-up as an offset variable will be used. The incidence rates described above will also be computed for ***mild***, moderate ~~AECOPD and for~~ severe AECOPD and by GOLD grade at enrolment and will be estimated by country.

Descriptive statistics (median, mean, range, standard deviation, first and third quartiles) on the *CAT* ***and*** SGRQ-C scores will be tabulated at each respective visit.

Descriptive summaries on healthcare use for ~~COPD~~ will be provided.

Section 9.6 Analysis of tertiary objectives

Descriptive summaries of the quantity of specific bacteria at each scheduled [stable] and exacerbation visit will be provided for both culture and PCR analysis.

Descriptive summaries of the quantity of specific viruses at each scheduled [stable] and exacerbation visit will be provided for PCR analysis.

Section 9.8.1 Sequence of analyses

- An interim analysis of the bacterial primary objective will be performed ***when*** ~~by the time of country allocation for the Phase 2 vaccine trial (provided that~~ at least ***40***~~50~~ AECOPD sputum samples are available~~)~~. ***The rate of positive samples will be computed for Hi, NTHi and Mcat together and per pathogen.*** No study report will be written at this stage.
- A final analysis of all objectives will be performed after the last subject last visit of the entire study. A final study report will be written at this stage.

Section 12 References

Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of Chronic Obstructive Pulmonary Disease, updated 2017. Available from: http://goldcopd.org/gold-2017-global-strategy-diagnosis-management-prevention-copd/

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Lim Sam, Lam Chi-Leung David et al. Impact of chronic obstructive pulmonary disease (COPD) in the Asia-Pacific region: the EPIC Asia population-based survey. Asia Pacific Family Medicine. 2015; 14:4

Singh Richa, Mackay AJ et al. Inflammatory thresholds and the species-specific effects of colonising bacteria in stable chronic obstructive pulmonary disease. Respiratory Research. 2014; 15: 114

World Health Organization (WHO). Chronic obstructive pulmonary disease (COPD) Fact Sheet. 2015. Available from: http://www.who.int/mediacentre/factsheets/fs315/en/. Accessed: 13 February 2017

Appendix A Clinical Laboratories

Table 14 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals ***Clinical Laboratory Sciences (CLS)***, ~~Global Vaccine Clinical Laboratory~~, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals ***Clinical Laboratory Sciences (CLS)***, ~~Global Vaccine Clinical Laboratory~~, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 15 Outsourced Laboratories

Laboratory	Address
Q² Solutions Clinical Trials (US)	***27027 Tourney Road, Suite 2E*** ***Valencia, CA 91355*** ***USA***
Q² Solutions (Singapore)	***Tan Tock Seng Hospital*** ***Dept of Laboratory Medicine*** ***Level 2, Podium Block*** ***Tan Tock Seng Hospital*** ***11 Jalan Tan Tock Seng*** ***Singapore 308433***

GlaxoSmithKline Biologicals	
Vaccine Value & Health Science (VVHS)	
Protocol Amendment 2	
eTrack study number and Abbreviated Title	201112 (EPI-NTHI-001 BOD APA)
Amendment number:	Amendment 2
Amendment date:	20 October 2017
Co-ordinating author:	PPD Scientific Writer

Rationale/background for changes:
The protocol has been amended to implement the following changes:

- The study procedure with respect to pre- and post-bronchodilator spirometry during the screening visit has been amended in order to clarify that if a good quality spirometry was not obtained, the test can be repeated (preferably within 7 days of the previous spirometry) as per investigator's medical judgement.
- Update of the laboratory section to better clarify that:
  - The PCR on *H. influenzae* isolate samples allows discrimination of Hi from non-Hi & identification of NTHi. Furthermore, reference to *H. haemolyticus* has been deleted from the protocol as the PCR that will be performed on bacterial Hi isolates is designed to identify *H. influenzae* and not *H. haemolyticus*.
- The cohorts for analyses have been updated as the PP analysis is not applicable to this specific study design, as all patients will be included in the analysis. Furthermore, according to ICH E9 recommendation a Full Analysis Set (FAS), All Screened set and All Enrolled Set have been added.
- To correct some typographical errors.

Removal of the ® and ™ symbols in the document and simplification of the copyright statement as per GSK Legal Global Trade Marks (LGTM) department's recommendation.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Contributing authors

- PPD and PPD
  PPD and PPD
  PPD and PPD,
  Clinical Safety Representatives

List of Abbreviations

FAS	***Full Analysis Set***
DTT	***Dithiothreitol***
HRV	***Human Rhinovirus***
STGG	***Skim Milk-Tryptone-Glucose-Glycerol***
~~***H. haemolyticus***~~	~~*Haemophilus haemolyticus*~~
~~**PP**~~	~~**Per Protocol**~~

Glossary of terms

Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, ~~and, therefore, included in the per protocol (PP) analysis~~ (see Section 9.3 for details on criteria or evaluability).

Trademarks

~~Note: In the body of the protocol (including the synopsis), the names of the products will be written without the superscript symbol ™ or ® and in *italics*.~~

Trademarks of the ~~GlaxoSmithKline~~ GSK group of companies	Generic description
COPD Assessment test™ (CAT)	Questionnaire to measure the impact of COPD on wellbeing and daily life
EXACT-PRO®	Exacerbations of Chronic Pulmonary Disease Tool - Patient Reported Outcome
SGRQ-C	St. George's Respiratory Questionnaire for COPD patients

Synopsis and Section 1.2 Rationale for the study

Because the infectious aetiology of AECOPD has been suggested to vary according to geographical region, the primary purpose of this study (which will be conducted in several countries in Asia Pacific) is to evaluate the occurrence of ***potential*** bacterial and viral pathogens in the sputum of stable COPD patients and at the time of AECOPD.

Given the increasing and projected burden of COPD in the Asia Pacific region, this study will also evaluate the frequency, severity and duration of AECOPD, as well as the impact of AECOPD on health-related quality of life (HRQOL), healthcare utilisation and lung function.

Synopsis and Section 3 Study design overview

- **COPD symptoms**

  Subjects will be asked to record their COPD symptoms in an electronic Diary Card on a daily basis:

  – Daily in the morning throughout the study: Morning symptoms ***questionnaire.***

  – Daily at bedtime throughout the study: EXACT-PRO (EXAcerbations of Chronic Pulmonary Disease Tool - Patient Reported Outcome) questionnaire.

- **Biological samples:**

  – **Sputum samples** collected at site (spontaneous or induced, as per investigator judgement) will be collected at each study visit at the site (scheduled [stable] visits and AECOPD visits) if, in the opinion of the investigator, it is safe for the subject.

Synopsis and Section 2.3 Tertiary objectives

Blood sampling for potential ~~biomarkers for~~ identification/quantification of biomarkers at Visit 1 and Visit 3.

Section 3.1.1 Detection of AECOPD

Each time a potential AECOPD is detected via the electronic Diary Card, the device will alert the subject to contact the study site, and at the same time an alert will be sent to the site so that the ~~site staff~~ ***investigator or medically qualified individual*** contacts the subject to determine if the alert is an AECOPD or not, and if an AECOPD visit is warranted. In addition, the site should proactively follow-up all data received via the electronic Diary Card and contact the subject whenever deemed necessary.

During the contact with the subject, the ~~site~~ ***investigator or medically qualified individual*** will determine whether the subject might actually be experiencing an AECOPD (e.g. notifications that can be explained solely by increased physical activity will not be considered):

- If the ***investigator or medically qualified individual*** ~~site~~ concludes that the subject is not experiencing an AECOPD, this should be documented/ reported ~~in the eCRF~~ ***in the electronic Diary website (StudyWorks)***. Please refer to study procedures manual (SPM) for more details on how to perform this.
- If the ***investigator or medically qualified individual*** ~~site~~ concludes that the subject may be experiencing an AECOPD, an AECOPD visit will be scheduled as soon as possible after the onset of AECOPD symptoms as recorded in the electronic Diary Card or confirmed by the subject (maximum 96 hours after onset of symptoms and, if applicable, preferably before starting treatment with antibiotics). The AECOPD onset date will be captured in the eCRF and additional information about severity will be also collected.

Section 5.1 Regulatory and ethical considerations, including the informed consent process

Freely given and written ~~or witnessed, thumb-printed~~ informed consent must be obtained from each subject, as appropriate, prior to participation in the study.

Section 6.3 Outline of study procedures

Table 16 List of study procedures for scheduled visits

Epoch	Epoch 001			
Type of contact	Screening Visit	Visit 1	Visit 2	Visit 3
Time point	Pre-Month 0	Month 0	Month 6	Month 12
Smoking exposure history (ATS-DLD-78A questionnaire)/ biomass exposure history ***(Biomass Exposure questionnaire)***	O			
Record additional ~~COPD~~ treatments prescribed by primary and secondary care physicians		●	●	●
Record Adverse Events (AEs) related to study participation*	●	●	●	●
Record serious adverse events (SAEs) ***related to study participation***	●	●	●	●

[h] ***Physical examination should be done only if necessary by the investigator or delegate.***

Table 5 List of study procedures for AECOPD visits

Type of contact	AECOPD Visit	End of AECOPD phone call(s)
Time point	within 96 hours of onset symptoms	at least every 2 weeks as of AECOPD visit until AECOPD has resolved[i]
Record date of visit	●	
Physical examination[l]	●	
Urine Pregnancy Test	●	

[l] Physical examination should be done only if necessary ***by the investigator or delegate.***

Section 6.4.1.1 Informed consent

The signed~~/thumb printed~~ informed consent of the subject must be obtained before study participation during the screening visit. Refer to Section 5.1 for the requirements on how to obtain informed consent ~~and assent, as appropriate.~~

Section 6.4.1.11 Pre and post-bronchodilator spirometry

Pre- and post-bronchodilator spirometry should be performed during the screening visit. Only study-certified staff can perform spirometry assessment for this study. Spirometry will be performed using ***following the eResearchTechnology (ERT) instructions for use FlowScreen manual*** ~~techniques that meet published standards [ATS/ERS, 2005]~~ and following all safety requirements.

A good quality spirometry should be obtained, and will be confirmed by the spirometry provider. If during the Screening Visit a good quality spirometry was not obtained, the spirometry can be repeated, as per investigator's medical judgement. If a repeat spirometry is elected, the site should make all possible efforts to repeat the spirometry

preferably within 7 days of the previous spirometry. The data will be directly transferred from the provider to GSK Biologicals.

Section 6.4.1.14 Sputum sampling

Sputum samples will be collected during the screening visit if, in the opinion of the investigator, it is safe for the subject. ***If it is not safe, the sputum sample will not be collected and the subject will not be included in the study.***

- At home, sputum collection will not be permitted. ~~However, self-collection of the sputum sample will be allowed only in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place.~~

Section 6.4.2.7 Pre- and post-bronchodilator spirometry

Pre- and post-bronchodilator spirometry should be performed during Visit 3 as detailed in Table 4. Only study-certified staff can perform spirometry assessment for this study. Spirometry will be performed ~~using techniques that meet published standards [ATS/ERS, 2005~~] ***following the eResearchTechnology (ERT) instructions for use FlowScreen manual*** and following all safety requirements. The data will be directly transferred from the provider to GSK Biologicals.

Section 6.4.2.7 Pre- and post-bronchodilator spirometry

Pre- and post-bronchodilator spirometry should be performed during Visit 3 as detailed in Table 4. Only study-certified staff can perform spirometry assessment for this study. Spirometry will be performed ***following the eResearchTechnology (ERT) instructions for use FlowScreen manual*** and following all safety requirements.

A good quality spirometry should be obtained, and will be confirmed by the spirometry provider. If during Visit 3 a good quality spirometry was not obtained, the spirometry can be repeated, as per investigator's medical judgement. If a repeat spirometry is elected, the site should make all possible efforts to repeat the spirometry preferably within 7 days of the previous spirometry. The data will be directly transferred from the provider to GSK Biologicals.

Section 6.4.2.10 Record healthcare resource utilisation

Healthcare use will be obtained through review of the subject's medical record (aided by subject self-reporting). Healthcare utilisation includes all unscheduled visits to a physician office, visits to urgent care, visits to emergency department, and hospitalizations. Healthcare use should be ***recorded in eDiary Card and reported in the eCRF at study visit*** ~~entered in the eCRF~~. Refer to the SPM for more details and guidance on recording of healthcare use.

Section 6.4.2.11 Additional ~~COPD~~ treatments prescribed by primary and secondary care physician

Section 6.4.2.14 Sputum sampling

- ***At home, sputum collection will not be permitted.***
- ~~Sputum sample collected at subject's home (spontaneous): Self-collection of the sputum sample will be allowed in specific cases, where the first dose of antibiotics absolutely needs to be taken before an AECOPD visit can take place. This is not allowed at scheduled visits when subject should be in stable condition.~~
- Remaining DTT-sputum will be aliquoted as such and/or ***when possible*** further diluted in STGG. These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.

Section 6.4.2.16 HRQOL questionnaires

- The subject will be asked to complete the ~~questionnaires on~~ HRQOL questionnaires by himself/herself, during specified study visits ***directly in the electronic Diary Card*** as detailed in Table 4 and Table 5.

Section 6.4.3.4 Chest X-rays/pneumonia confirmation

- Fever (oral temperature ~~≥~~> 37.5 °C),

All incidences of pneumonia must be captured on the pneumonia page of the eCRF.
~~All pneumonias must be captured on the AE/SAE page of the eCRF and on the pneumonia page of the eCRF.~~
The investigators and site staff should remain vigilant for the possible development of pneumonia in patients with COPD as the clinical features of such infections overlap with the symptoms of COPD exacerbations. For all suspected cases of pneumonia, investigators are strongly encouraged to confirm the diagnosis (this includes obtaining a chest x-ray) and to initiate appropriate therapy as promptly as possible. ~~All diagnoses of pneumonia (radiographically confirmed or unconfirmed) must be reported as an AE or SAE (if applicable) as detailed in Section 7.2 and Section 7.3.~~

Section 6.4.3.7 Record healthcare resource utilisation

Healthcare use will be obtained through review of the subject's medical record (aided by subject self-reporting). Healthcare utilisation includes all unscheduled visits to a physician office, visits to urgent care, visits to emergency department, and hospitalizations. Healthcare use should be ***recorded in the eDiary Card and reported in the eCRF at study visit*** ~~entered in the eCRF~~. Refer to the SPM for more details and guidance on recording of healthcare use.

Section 6.4.3.10 HRQOL questionnaires

- The subject will be asked to complete the HRQOL questionnaire (CAT only) by himself/herself ***directly in the electronic Diary Card***, during the AECOPD visit as detailed in Table 5.

Section 6.4.5 Health related quality of life questionnaires
Refer to section 6.4.2 for description of the Quality of Life (QoL) questionnaire. ***The subject will be asked to complete the HRQOL questionnaires by himself/herself, during specified study visits directly in the electronic Diary Card.***

~~The investigator will provide the Quality of Life (QoL) questionnaire to the subject to record his/her condition until the next visit. The subjects will be instructed to return the completed QoL questionnaire to the investigator at the next visit/contact.~~

~~Collection and verification of the completed diary card/QoL questionnaire will take place during discussion with the subject at the subsequent visit. Any unreturned diary cards/QoL questionnaires will be solicited from the subjects through telephone call(s) or any other convenient procedure. The investigator will transcribe the collected information into the eCRF in English.~~

Section 6.5.1 Use of specified study materials

When materials are provided by GSK Biologicals ***or the central laboratory***, it is MANDATORY that all samples be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ~~per protocol~~ analysis ~~(See Section 9.3 for the definition of study cohorts/data sets to be analysed).~~ The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals or the central laboratory does not provide material for collecting and storing samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM and Central Laboratory investigator manual.

Section 6.5.3 Laboratory assays

Further bacterial characterization for *H. influenzae* isolates: Identified *H. influenzae* isolates should be collected and stored in the investigator's institution and will undergo further species confirmation (i.e. ***confirmed*** *H. influenzae~~/ H. haemolyticus~~)* and when possible, further differentiation (i.e., Hi/NTHi**)** at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals using molecular techniques such as ~~*H. influenzae/ Haemophilus haemolyticus* (*H. haemolyticus*)~~ differentiation PCR. Identified *H. influenzae* isolates might undergo further testing at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals' using ***other*** molecular techniques such as PCR and sequencing.

Identified *P. aeruginosa* isolates should be collected and stored in the investigator's institution ***and might potentially undergo further*** ~~for future~~ testing at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals.

In addition, some respiratory viral pathogens (such as ***HRV***~~rhinovirus~~) ~~may~~ ***will*** be quantified in stored sputum samples (***or subset***) using RT-PCR at GSK Biologicals' laboratory or at a laboratory designated by GSK Biologicals.

Table 8 Microbiology

System	Component	Method	Scale	Laboratory
Quality assessment				
Fresh sputum	Please refer to SPM and/or Central Laboratory Manual for components tested	Gram staining	Qualitative/ semi-quantitative	Investigator institution or GSK designated laboratory
Bacterial pathogen identification				
Fresh sputum	*R*espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*)	Standard bacteriological culture and standard identification methods including semi-quantitative culture	Qualitative Semi-quantitative	Investigator's institution ***and/or at a laboratory designated by GSK Biologicals***
H. influenzae isolates of positive *H. influenzae* culture plates	***H. influenzae species confirmation and when possible Hi/NTHi* differentiation** ***Sample collection for potential further characterisation*** ~~*H. influenzae/ H.haemolyticus* differentiation~~	Molecular techniques such as differentiation PCR	Qualitative	GSK Biologicals laboratory* or designated laboratory
~~***M. catarrhalis*** isolates of positive ***M. catarrhalis*** culture plates~~	~~Sample collection for isolate characterisation~~	~~***Molecular techniques***~~	~~***Qualitative***~~	~~***GSK Biologicals laboratory* or designated laboratory***~~
~~***P. aeruginosa*** isolates of positive ***Paeruginosa*** culture plates~~	~~Sample collection for isolate characterisation~~	~~***Molecular techniques***~~	~~***Qualitative***~~	~~***GSK Biologicals laboratory* or designated laboratory***~~
Viral pathogen identification				
Stored sputum	Respiratory viral pathogens (including RSV, parainfluenza virus, enterovirus/ ***HRV***~~rhinovirus~~, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus)	Multiplex-PCR	Qualitative ~~and/or quantitative~~	GSK Biologicals laboratory * or designated laboratory

* GSK Biologicals laboratory refers to the ***Clinical Laboratory Sciences (CLS)*** in Wavre, Belgium; Rixensart, Belgium; ***Marburg, Germany***.

Stored frozen sputum samples (~~either~~ in dithiothreitol [DTT] ~~and/or in DTT supplemented with skim milk, tryptone, glucose, and glycerine [STGG] medium~~) might be used for assay development, such as assays for diagnostic purpose or for microbiome analysis and/or for assay validation/characterization purpose and/or quality control.

Stored frozen sputum samples (in DTT supplemented with skim milk, tryptone, glucose, and glycerine [STGG] medium) might be collected when possible for potential assay development/validation purpose.

Additional testing on stored sputum samples (such as, but not limited to, *K. pneumoniae* and *A. baumannii* PCR detection, quantitative PCR for other bacterial and/or viral pathogens, further characterisation of virus such as rhinovirus typing, pathogen genome/gene sequencing, ***pathogen*** quantitative/qualitative serotype-specific PCR, multilocus sequence typing, microarray typing, 16sRNA analysis) or on *H. influenzae, M. catarrhalis and P. aeruginosa* ***bacterial*** isolates (such as ***agglutination assays***, further strain characterisation using new molecular biology tools) may be done during the study or after study completion, should these data be required for accurate interpretation of the study data and/ or for further research related to respiratory diseases and/ or should such test(s) become available at GSK Biologicals' laboratory or a laboratory designated by GSK.

Section 7.2.1 Time periods for detecting and recording AEs and SAEs

Table 10 Reporting period for SAE and AEs leading to withdrawal

Study activity	Screening Visit		Visit 1		Visit 2		Visit 3		AECOPD Visit		Study Conclusion
	Pre-Month 0		M 0		M 6		M 12		Within 96 hours of onset symptoms		
AEs/SAEs related to study participation*											
AEs leading to withdrawal from the study											

Study participation: Specific procedures required by study participation.

Synopsis and Section 9.1.3

- ***Human Rhinovirus (HRV)*** ~~Viral~~ load measured by PCR in COPD and during AECOPD.

Section 9.2 Determination of sample size

Assuming around 90% of the ***All Enrolled Set participated in the study*** ~~population is in the PP cohort~~, on average 1 AECOPD visit ~~per subject~~ and 80% of the ***above population set***~~subjects in the PP cohort~~ can provide ***an evaluable*** sputum sample at AECOPD, for 240, 200, 160, and 50 enrolled subjects, one may expect around 180, 140, 120, 40 sputum samples ~~in the exacerbation category of the PP analysis~~, respectively. Assuming that results from these sputum samples are independent and that the between country variability is much smaller than the within country variability, we would have the following 95% exact confidence interval for different incidences in sputum samples from exacerbations (see Table 12).

Assuming around ***80***~~90~~% of the ***all enrolled set*** ~~population is in the PP cohort, from which around 90% of the subjects~~ completed ***the study*** ~~the one year follow up~~, for 240, 200, 160 and 50 subjects enrolled, one would have at least 190, 160, 130 and 40 subjects evaluable respectively. Assuming further that around 80% of the subjects can provide ***an evaluable*** sputum sample at AECOPD, this would lead to around 150, 130, 100, 30 subjects (see Table 13).

Section 9.3.1 ***All screened set***

The all screened set will include all screened patients.

Section 9.3.2 ~~Total~~ All Enrolled ~~cohort~~ Set

The all enrolled set will include all successfully screened subjects in the study ~~The total enrolled cohort will include all subjects enrolled in the study.~~

Section 9.3.3 ***Full Analysis Set***

The Full Analysis Set (FAS) will include all enrolled patients except for those who discontinued the study during Visit 1.

Study objectives will be assessed on the FAS. The population set for each analysis will change according to the subjects evaluable for the specific endpoint.

~~The PP cohort will include all evaluable subjects (*i.e.* those meeting all eligibility criteria and complying with procedures described in the protocol) who did not receive a concomitant medication/ vaccination leading to elimination from the PP cohort.~~

~~Missed study visits and intervals between study visits will not be considered as criteria for exclusion from the PP cohort.~~

Note: Subjects using antibiotics on a continual basis (defined as more than 1 month in total) will be allowed to continue study participation, but may be eliminated from the ~~per protocol (PP) cohort~~ analyses.

Section 9.4 Analysis of demographics

Withdrawal status will be summarised according to the reason for withdrawal. The number of withdrawn patients will be tabulated by study visit and overall.

~~Withdrawal status will be summarised using descriptive statistics:~~

- ~~The numbers of withdrawn subjects will be tabulated according to the reason for withdrawal.~~
- ~~The number of subjects enrolled into the study as well as the number of subjects excluded from PP analyses will be tabulated.~~

Section 12 References

~~M.R. Miller, J. Hankinson, V. Brusasco *et al*. Standardisation of spirometry. *Eur Respir J*. 2005;26:319-38. Series ‘‘ATS/ERS task force: standardisation of lung function testing’’.~~

GlaxoSmithKline Biologicals	
Vaccine Value & Health Science (VVHS)	
Protocol Amendment 3	
eTrack study number and Abbreviated Title	201112 (EPI-NTHI-001 BOD APA)
Amendment number:	Amendment 3
Amendment date:	19 October 2018
Co-ordinating author:	PPD Scientific Writer

Rationale/background for changes:

- Removal of all STGG (Skim Milk-Tryptone-Glucose-Glycerol) wording throughout the protocol. STGG product not being available, it has been decided to replace it with cryobeads for storage of bacterial isolates. PCR testing on samples stored in cryobeads was shown to be as efficient as for samples stored in STGG. Besides, this decision has been taken for alignment with other COPD studies, where STGG solutions are no longer used.

- Clarification of technique used for target identification. For the primary endpoint, various viral pathogens will be identified but not quantified. Along this line, the wording "and by HRV quantitative RT-PCR" has been removed from primary endpoint to avoid confusion as HRV viral load data will be reported as part of the tertiary endpoints.

- Clarification in exclusion criteria section (4.3). Administration of antibiotics before study entry, where study entry is meant to be Visit 1.

- Clarification in study procedure section (6.3) and in the section for recording current medication (6.4.2.10) during scheduled visits, where it has been specified that recording of any current medication is applicable for any disease not only COPD-related. Moreover, a footnote referring to Table 10 "Reporting period for SAE and AEs leading to withdrawal" has been added for further clarification, both in Table 4 and 5.

- Addition of a section (actual 6.4.2.6) for Pregnancy test for Visit 1, Visit 2 and Visit 3. A Pregnancy test section for Screening Visit was included during Protocol Amendment 1: to be consistent, same wording has been included for the subsequent visits.

- Clarification of reporting of severe AECOPDs. Two sentences have been added in sections 7.1.1 and 7.2.2.1 for making clear that severe AECOPD events are not to be reported as SAE as they are meant as disease-related outcomes.
- Rewording in the table concerning reporting period for SAE and AEs leading to withdrawal for better clarification (Table 10).
- Addition of a new outsourced laboratory (DDL).
- To correct some typographical errors.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

List of Abbreviations

~~STGG~~	***~~Skim Milk-Tryptone-Glucose-Glycerol~~***

Section 4.3 Exclusion criteria for enrolment

- Administration of antibiotics within 1 month of study entry OR continuous administration of antibiotics (defined as more than 30 days in total) within 90 days before study entry.

  * Study entry is Visit 1. If this exclusion criteria is met during Screening Visit the patient can enter the study and this exclusion criteria will be reassessed during Visit 1.

Section 6.3 Outline of study procedures

Table 4 List of study procedures for scheduled visits

Epoch	Epoch 001			
Type of contact	**Screening Visit**	**Visit 1**	**Visit 2**	**Visit 3**
Time point	**Pre-Month 0**	**Month 0**	**Month 6**	**Month 12**
Informed consent[a]	●			
Check inclusion/exclusion criteria[a]	●	O		
Record demographic data[a]	●			
Record medical history	●			
Record history of AECOPD within the previous year[b]	●			
Record intercurrent comorbidities[c]	O	●	●	●
Record history of pneumococcal and influenza vaccination	O	●	●	●
Smoking exposure history (ATS-DLD-78A questionnaire)/ biomass exposure history (Biomass Exposure questionnaire)	O			
Smoking status	●	●	●	●
Physical examination including vital signs[h]	●	O	O	O
Urine Pregnancy Test	●	●	●	●
Measure/ record height and weight[d]	●			●
Pre- and post-bronchodilator spirometry[e]	●			●
Chest X-ray[f]	●			
Record subject's COPD status (stable, recovered or not recovered)		●	●	●
Record current medication ~~for COPD~~		●	●	●
Record healthcare resource utilisation		●	●	●
Record additional treatments prescribed by primary and secondary care physicians		●	●	●
Train subject on use of electronic Diary Card and assign electronic Diary Card to the subject	O	O		
Return electronic Diary Card				O
Sputum sampling[g]	●	●	●	●
Blood samples for biomarkers		●		●
Record Adverse Events (AEs)*^ ~~related to study participation~~	●	●	●	●
Record serious adverse events (SAEs)^ ~~related to study participation~~	●	●	●	●
Screening conclusion	●			
Study conclusion				●
HRQOL questionnaires:				
CAT		O	O	O
SGRQ-C		O	O	O

^ *Refer to Table 10 for description of which AE/SAEs are collected in this study*.

[h] Physical examination ***(Visit1-3)*** should be done only if necessary by the investigator or delegate.

Table 5 List of study procedures for AECOPD visits

Type of contact	AECOPD Visit	End of AECOPD phone call(s)
Time point	**within 96 hours of onset symptoms**	**at least every 2 weeks as of AECOPD visit until AECOPD has resolved[i]**
Record date of visit	●	
Physical examination[l]	●	
Urine Pregnancy Test	●	
Chest X-rays/pneumonia confirmation[j]	●	
Confirm AECOPD and record its start date	●	
Record current medication for ***AE***COPD	●	
Record healthcare resource utilisation	●	
Record additional COPD treatments prescribed by primary and secondary care physicians		●
Sputum sampling[k]	●	
HRQOL questionnaire:		
CAT	O	
Record AECOPD severity[m]	●	●
Record AECOPD end date		●
Record Adverse Events (AEs)*^ ~~related to study participation~~	●	●
Record serious adverse events (SAEs)^ ~~related to study participation~~	●	●

^ Refer to Table 10 for description of which AE/SAEs are collected in this study.

Section 6.4 Detailed description of study procedure

Sputucm sampling at sub-sections 6.4.1.14 – 6.4.2.15 – 6.4.3.9:
Remaining DTT-sputum will be aliquoted as such ~~and/or further diluted in STGG~~. These samples will be kept at -70/80°C until shipment to GSK or GSK designated lab for testing.

Section 6.4.2.6 Pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study procedure. Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

Section 6.4.2.10 Record current medication ~~for COPD~~

- At each study visit/contact, the investigator should question the subject about any medications taken. ~~**administered for treatment of COPD.**~~
- All medications administered ~~**for treatment of COPD**~~ will be recorded in the eCRF.

Section 6.5.3 Laboratory assays

Table 8 Microbiology

Further bacterial pathogens identification

Viral pathogens identification

System	Component	Method	Scale	Laboratory
Quality assessment				
Fresh sputum	Please refer to SPM and/or Central Laboratory Manual for components tested	Gram staining	Qualitative/ semi-quantitative	Investigator institution or GSK designated laboratory
Bacterial pathogen identification				
Fresh sputum	***R***espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*)	Standard bacteriological culture and standard identification methods including semi-quantitative culture	Qualitative Semi-quantitative	Investigator's institution and/or at a laboratory designated by GSK Biologicals
H. influenzae isolates of positive *H. influenzae* culture plates	***H. influenzae*** species confirmation and when possible Hi/NTHi differentiation Sample collection for potential further characterisation	Molecular techniques such as differentiation PCR	Qualitative	GSK Biologicals laboratory* or designated laboratory
Stored sputum	***R***espiratory bacterial pathogens (including *H. influenzae, S. pneumoniae, M. catarrhalis, S. aureus* and *P. aeruginosa, Streptococcus pyogenes*))	Multiplex PCR	Qualitative and/ or quant itative	GSK Biologicals laboratory * or designated laboratory
Viral pathogen identification				
Stored sputum	Respiratory viral pathogens (including RSV, parainfluenza virus, enterovirus/ HRV, metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus)	Multiplex-PCR	Qualitative	GSK Biologicals laboratory * or designated laboratory
	Respiratory viral pathogens (such as HRV)	RT-PCR	Quantitative	GSK Biologicals laboratory* or designated laboratory

~~*Stored frozen sputum samples (in DTT supplemented with skim milk, tryptone, glucose, and glycerine [STGG] medium) might be collected when possible for potential assay development/validation purpose.*~~

Synopsis Primary endpoints and Section 9.1.1 Primary endpoint

- Occurrence of potential bacterial and viral pathogens in sputum of stable COPD patients and during AECOPD, over the course of 1 year:
  - Bacterial pathogens, as identified by bacteriological methods, including (but not necessarily limited to) *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *A. baumannii*.
  - Viral pathogens, as identified by RT PCR, including (but not necessarily limited to) RSV, parainfluenza virus, enterovirus/ Human Rhinovirus (HRV), metapneumovirus, influenza virus, adenovirus, bocavirus and coronavirus~~ and by HRV quantitative RT-PCR~~.

Section 7.1.1 Definition of an adverse event

An AE is any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure.

Examples of an AE include

- Signs, symptoms, or the clinical sequelae of a suspected interaction.

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (e.g., social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- ***Occurrence of a severe AECOPD, unless in the opinion of the Investigator this is related to study procedure.***

Section 7.2.1 Time periods for detecting and recording AEs and SAEs – Table 10

Study activity	Screening Visit		Visit 1		Visit 2		Visit 3		AECOPD Visit		Study Conclusion
	Pre-Month 0		M 0		M 6		M 12		Within 96 hours of onset symptoms		
AEs/SAEs related to study participation*											
AEs/*SAEs* leading to withdrawal from the study											

Section 7.2.2.1 Active questioning to detect SAEs

Each subject/ will be instructed to contact the investigator immediately should the subject manifest any signs and symptoms (s)he perceives/ they perceive as serious. ***However, an occurrence of severe AECOPD is not defined as an SAE and therefore does not need to be reported as such, unless in the opinion of the Investigator this is related to a study procedure.***

Appendix A

Table 15 Outsourced Laboratories

Laboratory	Address
Q² Solutions Clinical Trials (US)	27027 Tourney Road, Suite 2E Valencia, CA 91355 USA
Q² Solutions (Singapore)	Tan Tock Seng Hospital Dept of Laboratory Medicine Level 2, Podium Block Tan Tock Seng Hospital 11 Jalan Tan Tock Seng Singapore 308433
DDL Diagnostic Laboratory B.V.	Fonteijnenburghlaan 7 Voorburg Netherland