Study Protocol
Sponsor:
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eTrack study number and Abbreviated Title	115813 (EPI-STREP-064 BOD ES)
Date of protocol	Final: 25 May 2012
Date of protocol amendment	Amendment 1 Final: 08 March 2013 Amendment 2 Final: 05 October 2015
Title	Identification and characterisation of bacteria in the lower airways of children aged ≥ 6 months to < 6 years with suspected lower respiratory tract infections in Spain.
Detailed Title	A multi-centre, hospital-based, cross-sectional epidemiology study to identify and characterise bacteria in the lower airways of children aged ≥ 6 months to < 6 years with suspected chronic lower respiratory tract infections (LRTIs) in Spain.
Co-ordinating author	PPD, Scientific Writer
Contributing authors (Amended 05 October 2015)	• PPD ***, Senior Manager, Epidemiology*** • PPD, Senior Manager, Epidemiology • PPD, Epidemiologist, Cromsource for GSK Biologicals • PPD, Epidemiologist, freelance for GSK Biologicals • PPD ***, Study Statistician, freelance for GSK Biologicals*** • PPD, Project Statistician • PPD ***, Study Delivery Lead*** • PPD ***, Study Delivery Manager*** • PPD, Global Study Manager (Consultant, Harrison Clinical Research) • PPD, Global Study Manager, Valesta for GSK Biologicals

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GSK Biologicals' protocol template for observational studies and interventional studies without administration of medicinal products as described in a research protocol based on the Protocol Document Standard version 13.2

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	115813 (EPI-STREP-064 BOD ES)
Date of protocol amendment	Amendment 2 Final: 05 October 2015
Detailed Title	A multi-centre, hospital-based, cross-sectional epidemiology study to identify and characterise bacteria in the lower airways of children aged ≥ 6 months to < 6 years with suspected chronic lower respiratory tract infections (LRTIs) in Spain.
Sponsor signatory (Amended 05 October 2015)	Laurence Baril Director, ***Head of Global*** Epidemiology GlaxoSmithKline Biologicals
Signature	_____
Date	_____

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Protocol Amendment 2 Rationale

Amendment number: Amendment 2

Rationale/background for changes:

The EPI-STREP-064 BOD ES protocol has been updated for the following reasons:

- It has been clarified that:
  - the antibiotic susceptibility profile (including penicillin, erythromycin, azithromycin, tetracycline, levofloxacin, trimethoprim/ sulfamethoxazole, amoxicillin/ clavulanate) will be determined for *Haemophilus influenzae (H. influenzae), Streptococcus pneumoniae* (*S. pneumoniae)* and *Moraxella catarrhalis (M. catarrhalis)* identified not only from bronchoalveolar lavage (BAL) fluid but also from nasopharyngeal swab samples;
  - the beta-lactamase test will be performed for *H. influenzae* and *M. catarrhalis* identified not only from BAL fluid but also from nasopharyngeal swab samples;
  - ampicillin resistance testing will be performed for beta-lactamase negative *H. influenzae* from BAL fluid and from nasopharyngeal swab samples.
- In addition, inconsistencies between the secondary endpoints in the synopsis and the secondary endpoints in the body of the protocol amendment 1 have been clarified.

Other changes have been made for simplification, clarification or consistency.

Protocol Amendment 2 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline Biologicals (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 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 authorised 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	115813 (EPI-STREP-064 BOD ES)
Date of protocol amendment	Amendment 2 Final: 05 October 2015
Detailed Title	A multi-centre, hospital-based, cross-sectional epidemiology study to identify and characterise bacteria in the lower airways of children aged ≥ 6 months to < 6 years with suspected chronic lower respiratory tract infections (LRTIs) in Spain.
Investigator name	_____
Signature	_____
Date	_____

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SYNOPSIS

Detailed Title	A multi-centre, hospital-based, cross-sectional epidemiology study to identify and characterise bacteria in the lower airways of children aged ≥ 6 months to < 6 years with suspected chronic lower respiratory tract infections (LRTIs) in Spain.
Rationale for the study (Amended 05 October 2015)	The present study aims to identify and characterise bacteria present in the lower airways of children with suspected chronic LRTIs and for whom ***bronchoalveolar lavage (***BAL***)*** is indicated by the clinician. BAL is generally indicated in cases of unresolved persistent or recurrent respiratory symptoms. Bronchoscopic exploration of the airways along with cytology and microbiology results and analysis of other constituents of the BAL fluid can provide meaningful information for clinical management. This study may provide a better understanding of the aetiology of chronic and recurrent chronic LRTIs in children for whom a BAL sampling procedure was indicated. The ultimate aim is to improve treatment recommendations and determine whether there are potential prevention strategies including vaccination. In addition, it would be valuable to identify a reliable, less invasive procedure than BAL to describe the bacterial aetiology of chronic LRTIs. Linking evidence from BAL culture to nasopharyngeal swabs, combined with additional laboratory information (since nasopharyngeal swabs alone may not be specific enough) may allow for an evaluation of new approaches to estimate aetiology. If this could be validated, *i.e.* if a predictor of BAL culture results could be identified, then it may be possible to determine the aetiology of other, non-chronic or non-recurrent presentations of LRTIs for which an invasive procedure such as bronchoscopy would not be indicated to guide treatment and clinical management.
Objectives (Amended 05 October 2015)	**Primary** • To characterise the bacterial aetiology of BAL fluid samples in subjects visiting the hospital with suspected chronic LRTIs. **Secondary** In a hospital setting and among subjects with suspected chronic LRTIs: • To describe the bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* detected by quantitative culture and by molecular techniques (PCR) in the BAL

fluid.

- To describe the presence of other bacterial pathogens detected by qualitative culture in the BAL fluid.
- To describe the bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* detected by quantitative culture and by molecular techniques (PCR) in the nasopharyngeal swab samples.
- To describe the presence of other bacterial pathogens detected by qualitative culture in the nasopharyngeal swab samples.
- To describe colonisation of the upper airways by characterising *H. influenzae*, *S. pneumoniae, M. catarrhalis* and other pathogens in nasopharyngeal swab samples.
- To determine the serotypes of *H. influenzae* and *S. pneumoniae* identified from BAL fluid samples.
- To determine the serotypes of *H. influenzae* and *S. pneumoniae* identified from nasopharyngeal swab samples.
- To determine the antibiotic susceptibility profile for *H. influenzae, S. pneumoniae* and *M. catarrhalis* identified from BAL fluid ***and nasopharyngeal swab*** samples.
- To describe, according to microbiological results observed:
  - Age and gender
  - Clinical symptoms and radiological evidence
  - Pneumococcal conjugate vaccine, *H. influenzae* type b vaccine and influenza vaccine status
  - Medical history and co-morbidities
  - Information on feeding and day care practice, environmental exposure and smoking environment
  - History of antibiotic use in the past six months as well as other treatments for chronic lower respiratory disease
  - Laboratory results [including white blood cell counts, C-Reactive Protein (CRP) level and erythrocyte sedimentation rate, if available].

Study design

- Type of design: Epidemiological, interventional, multi-centre, hospital-based, cross-sectional study with prospective subject recruitment in Spain.

- Study population: Subjects aged ≥ 6 months to < 6 years visiting the study hospital with suspected chronic LRTI and for whom a BAL sample will be collected as per the recommendation of the clinician.
- Written informed consent will be obtained from the parent(s)/ legally acceptable representatives [LAR(s)] of those subjects who fulfil the inclusion/ exclusion criteria. Subject numbers will be assigned sequentially to subjects whose parent(s)/ LAR(s) consent for their participation in the study.
- Parent(s)/ LAR(s) of the subjects will be interviewed to collect data on demographics (e.g. age, gender), clinical characteristics (including medical history, chronic LRTI symptoms), vaccination history, feeding and day care practice, environmental exposure and smoking environment, radiological and laboratory results, etc. Data on physical examination will also be collected from the medical record of the subject. If results of blood testing are available from routine practice, then data on white blood cell counts, CRP level and erythrocyte sedimentation rate, etc will be recorded in the electronic Case Report Form (eCRF).
- BAL fluid collection will follow routine procedures and will only be performed in children with a medical indication for this procedure. The first aliquot of BAL fluid will be used for local testing and case management, and the second aliquot of BAL fluid obtained will be sent to the GlaxoSmithKline (GSK) designated laboratory for microbiological analysis.
- Nasopharyngeal swabs will be collected as part of this study. Collected samples will also be sent to the GSK designated laboratory for microbiological analysis.
- Type of study: This will be a self-contained study.
- Data collection: Remote Data Entry (RDE).
- Duration of the study: For each subject, there will be a single study visit. The duration of the study will be approximately 2 years
  - Epoch 001: Prospective data collection.

Synopsis Table 1 Study group and epoch foreseen in the study

Study Group	Number of subjects	Age (Min/Max)	Epoch
Prospective	At least 150	≥ 6 months to < 6 years	Epoch 001

Discussion of study design

A cross-sectional study design is the simplest approach to describe the burden of disease, *i.e.* bacterial aetiology of chronic LRTI symptoms. BAL is routinely performed for diagnosis of infection in children with such chronic symptoms, and it is unlikely to be performed repeatedly for otherwise healthy children. Thus, extended follow-up would not be possible.

Considering the invasive nature of the BAL fluid sampling procedure, it has been decided to enrol only those subjects with suspected chronic LRTIs who have BAL procedure indicated by their clinician as part of the local routine diagnostic procedure. This should facilitate subject recruitment and increase participation rates. Nasopharyngeal swabs may not be systematically collected in routine practice. However, since the patient will be under sedation to undergo BAL, he/she will not be subject to additional discomfort or risk due to the swabbing procedure. Thus, it is not expected to affect the willingness of the parent(s)/ LAR(s) to allow their child's participation in this study.

Number of subjects

At least 150 subjects are planned to be enrolled in the study based on the sample size estimation.

Endpoints

(Amended 05 October 2015)

Primary

- Occurrence of *H. influenzae, S. pneumoniae, M. catarrhalis* and other bacteria in BAL fluid samples of subjects aged ≥ 6 months to < 6 years with suspected chronic LRTIs and an indication for BAL
  - *H. influenzae, S. pneumoniae* and *M. catarrhalis* confirmed by bacterial load >10^4 cfu/mL if present alone or 10^5 cfu/mL if present as co-infection.

Secondary

In a hospital setting and among subjects with suspected chronic LRTIs:

- ***Bacterial load of H. influenzae, S. pneumoniae and M. catarrhalis in the BAL fluid as determined by quantitative culture and PCR.***
- ***Description of other bacterial pathogens in the BAL fluid as determined by qualitative culture.***
- ***Bacterial load of H. influenzae, S. pneumoniae and M. catarrhalis in nasopharyngeal swab samples as determined by quantitative culture and PCR.***

- ***Description of other bacterial pathogens in nasopharyngeal swab samples as determined by qualitative culture.***
- ***Occurrence of H. influenzae, S. pneumoniae, M. catarrhalis and other pathogens detected by culture in the nasopharyngeal swab samples.***
- ***Occurrence of H. influenzae and S. pneumoniae serotypes identified from BAL fluid samples and nasopharyngeal swab samples.***
- ***Occurrence of H. influenzae, S. pneumoniae and M. catarrhalis minimum inhibitory concentration (MIC) values identified from BAL fluid and nasopharyngeal swab samples.***
- ***Demographic characteristics (age, gender, etc), clinical characteristics (including medical history, LRTI symptoms, treatment history, vaccination status, etc), radiological evidence and laboratory results (including white blood cell counts, CRP level and erythrocyte sedimentation rate, if available).***

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ATP	According-To-Protocol
BAL	Bronchoalveolar Lavage
CI	Confidence Interval
CRP	C-Reactive Protein
eCRF	electronic Case Report Form
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
H. haemolyticus	*Haemophilus haemolyticus*
H. influenzae	*Haemophilus influenzae*
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LAR	Legally Acceptable Representative
LRTIs	Lower Respiratory Tract Infections
M. catarrhalis	*Moraxella catarrhalis*
MIC	Minimum Inhibitory Concentration
RDE	Remote Data Entry
SAE	Serious Adverse Event
SDV	Source Document Verification
SPM	Study Procedures Manual
S. pneumoniae	*Streptococcus pneumoniae*

GLOSSARY OF TERMS

Child in care:	A child who has been placed under the control or protection of an agency, organisation, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.
Coded:	Information is associated with a subject number i.e. a code number. Coded information can only be linked back to the individual via a key code i.e. a listing of the research participants and their code. Within the pharmaceutical industry coding data is the usual mechanism used for protecting an individual's research data. The key code is kept secure, usually by the investigator, and GSK researchers cannot identify the research individual other than in exceptional and controlled circumstances.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epidemiology study:	An observational study or an interventional study without administration of medicinal product(s) as described in a research protocol.
Epoch:	An epoch is a well defined part of a protocol that covers a set of consecutive time points. Generally, an epoch is self-contained and allows to perform a data analysis to address some of the trial objectives (e.g. primary, booster, yearly follow-ups, retrospective data collection, prospective data collection).
eTrack:	GSK's tracking tool for clinical/epidemiology trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Section 7.3 for details on criteria for evaluability).
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 endpoints.

Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organisation of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of epidemiology 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 epidemiology study.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.

1. INTRODUCTION

1.1. Background

Lower respiratory tract infections (LRTIs) such as pneumonia and bronchitis, are important causes of childhood morbidity and mortality worldwide [Mulholland, 2007; Obaro, 2006; Rudan, 2004]. Determining the aetiology of LRTIs in children has always been a challenge due to the difficulty in obtaining a sample from the lungs. *Streptococcus pneumoniae* and *Haemophilus influenzae* are considered to be the main bacterial pathogens associated with lobar pneumonia, and their importance in disease was described in studies using transthoracic needle aspiration (lung tap), in which a sample was collected directly from the site of infection, *i.e.* the section of consolidation. Studies using this technique to describe non-consolidated infections such as broncho-pneumonia are less frequent. Lung taps are now rarely used because the potential risk to patients is considered unnecessary and LRTIs can be treated with empiric antibiotics [Hausdorff, 2008]. Thus, there have been attempts to use other less-invasive techniques for describing bacterial aetiology.

Among the non-invasive procedures, blood culture is the most widely used. It is routinely performed in patients hospitalised with moderate or severe pneumonia, but this procedure yields evidence of a pathogen in only 10-15% of the cases [Mccracken, 2000]. Moreover, since blood culture may not be performed for non-hospitalised cases, the role of un-encapsulated (non-typeable) *H. influenzae* strains in causing LRTIs may be underestimated [Shann, 1999].

Sputum samples, which are considered the best non-invasive alternative for detecting bacterial causes of LRTIs in adults, can rarely be collected in children because they often have difficulties producing sputum. Thus, the simplest approach of describing the aetiology of LRTI has been through the collection of nasopharyngeal samples in symptomatic children. Nevertheless, because the main bacteria associated with infection are also common colonisers of the nasopharynx, the specificity of such samples alone as a measurement of aetiology is considered low.

Another approach to obtaining a sample from the lower airways is bronchoalveolar lavage (BAL). In this procedure, a fiberoptic bronchoscope is introduced into a segmental or subsegmental bronchus until the airway is occluded proximally, and saline solution is instilled and subsequently aspirated. The fluid recovered can then be submitted for cytological and microbiological analysis. BAL is used as a diagnostic and research tool in adults with lung problems and in children and infants with underlying respiratory diseases [de Blic, 2000]. It is regarded as the best technique for microbacteriological surveillance of young children with cystic fibrosis, providing more reliable information than sampling of the upper airway with oropharyngeal/ nasopharyngeal swabs or nasal lavage [Brennan, 2008]. The main limitation of this procedure is in its invasive nature. As contamination from the upper respiratory tract flora during the procedure can sometimes occur, its capacity to describe LRTI aetiology may be challenged. Hence, a suitable cut-off should be used to avoid making spurious conclusions [Woodhead, 2005].

Recent studies reporting results from bacterial culture of BAL fluid in children with respiratory symptoms have highlighted the importance of *H. influenzae, S. pneumoniae,* and *Moraxella catarrhalis* in chronic or recurrent presentations of the disease (Table 1).

Table 1 Bacterial aetiology of BAL fluid culture from children with respiratory symptoms (Amended 05 October 2015)

Reference	Population	Number of samples	*H. influenzae*	*S. pneumoniae*	*M. catarrhalis*
[De Schutter, 2011]	Acute nonresponsive CAP (cut off ≥ ***10^4*** CFU/mL)	127	26%	6.3%	8.7%
	Recurrent CAP (cut off ≥ ***10^4*** CFU/mL)	123	51.2%	7.3%	21.1%
[Hare, 2010]	Bronchiectasis (cut off > ***10^4*** CFU/mL)	45	47%	18%	20%
[Marchant, 2006]	Chronic cough of > 3 weeks duration of unknown etiology	20	47%	35%	26%
[Saito, 2006]	Persistent bacterial bronchitis (persistent, wet cough for >1 month that resolves with appropriate antibiotic treatment)	19	26.3%	21%	42.1%
[Le Bourgeois, 2002]	Severe recurrent wheezy bronchitis unresponsive to inhaled steroids	30	30%	13.33%	10%
[Marguet, 1999]	5 groups: asthma chronic cough infantile wheeze cystic fibrosis control	72 (total) 12 9 23 10 10	 8.33% 33.33% 30.43% 20% 30%	 8.33% 22.22% 0.04% 10% -	 16.67% 11.11% 13.04% - -
[Schellhase, 1998]	Recurrent wheezing	27	3.7%	-	7.4%

CAP = community-acquired pneumonia

1.2. Rationale for the study

The present study aims to identify and characterise bacteria present in the lower airways of children with suspected chronic LRTIs and for whom BAL is indicated by the clinician. BAL is generally indicated in cases of unresolved persistent or recurrent respiratory symptoms. Bronchoscopic exploration of the airways along with cytology and microbiology results and analysis of other constituents of the BAL fluid can provide meaningful information for clinical management. This study may provide a better understanding of the aetiology of chronic and recurrent chronic LRTIs in children for whom a BAL sampling procedure was indicated. The ultimate aim is to improve treatment recommendations and determine whether there are potential prevention strategies including vaccination.

In addition, it would be valuable to identify a reliable, less invasive procedure than BAL to describe the bacterial aetiology of chronic LRTIs. Linking evidence from BAL culture to nasopharyngeal swabs, combined with additional laboratory information (since nasopharyngeal swabs alone may not be specific enough) may allow for an evaluation of new approaches to estimate aetiology. If this could be validated, *i.e.* if a predictor of BAL

culture results could be identified, then it may be possible to determine the aetiology of other, non-chronic or non-recurrent presentations of LRTIs for which an invasive procedure such as bronchoscopy would not be indicated to guide treatment and clinical management.

2. OBJECTIVES

2.1. Primary objective

- To characterise the bacterial aetiology of BAL fluid samples in subjects visiting the hospital with suspected chronic LRTIs.

Refer to Section 7.1.1 for the definition of the primary endpoint.

2.2. Secondary objectives

In a hospital setting and among subjects with suspected chronic LRTIs:

- To describe the bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* detected by quantitative culture and by molecular techniques (PCR) in the BAL fluid.
- To describe the presence of other bacterial pathogens detected by qualitative culture in the BAL fluid.
- To describe the bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* detected by quantitative culture and by molecular techniques (PCR) in the nasopharyngeal swab samples.
- To describe the presence of other bacterial pathogens detected by qualitative culture in the nasopharyngeal swab samples.
- To describe colonisation of the upper airways by characterising *H. influenzae*, *S. pneumoniae, M. catarrhalis* and other pathogens in nasopharyngeal swab samples.
- To determine the serotypes of *H. influenzae* and *S. pneumoniae* identified from BAL fluid samples.
- To determine the serotypes of *H. influenzae* and *S. pneumoniae* identified from nasopharyngeal swab samples.
- To determine the antibiotic susceptibility profile for *H. influenzae, S. pneumoniae* and *M. catarrhalis* identified from BAL fluid ***and nasopharyngeal swab*** samples. (Amended 05 October 2015)
- To describe, according to microbiological results observed:
  - Age and gender
  - Clinical symptoms and radiological evidence
  - Pneumococcal conjugate vaccine, *H. influenzae* type b vaccine and influenza vaccine status

- Medical history and co-morbidities
- Information on feeding and day care practice, environmental exposure and smoking environment
- History of antibiotic use in the past six months as well as other treatments for chronic lower respiratory disease
- Laboratory results [including white blood cell counts, C-Reactive Protein (CRP) level and erythrocyte sedimentation rate, if available].

Refer to Section 7.1.2 for the definition of the secondary endpoints.

3. STUDY DESIGN OVERVIEW

- Type of design: Epidemiological, interventional, multi-centre, hospital-based, cross-sectional study with prospective subject recruitment in Spain.
- Study population: Subjects aged ≥ 6 months to < 6 years visiting the study hospital with suspected chronic LRTI and for whom a BAL sample will be collected as per the recommendation of the clinician.
- Written informed consent will be obtained from the parent(s)/ legally acceptable representatives [LAR(s)] of those subjects who fulfil the inclusion/ exclusion criteria. Subject numbers will be assigned sequentially to subjects whose parent(s)/ LAR(s) consent for their participation in the study.
- Parent(s)/ LAR(s) of the subjects will be interviewed to collect data on demographics (e.g. age, gender), clinical characteristics (including medical history, chronic LRTI symptoms), vaccination history, feeding and day care practice, environmental exposure and smoking environment, radiological and laboratory results, etc. Data on physical examination will also be collected from the medical record of the subject. If results of blood testing are available from routine practice, then data on white blood cell counts, CRP level and erythrocyte sedimentation rate, etc will be recorded in the electronic Case Report Form (eCRF).
- BAL fluid collection will follow routine procedures and will only be performed in children with a medical indication for this procedure. The first aliquot of BAL fluid will be used for local testing and case management, and the second aliquot of BAL fluid obtained will be sent to the GlaxoSmithKline (GSK) designated laboratory for microbiological analysis.
- Nasopharyngeal swabs will be collected as part of this study. Collected samples will also be sent to the GSK designated laboratory for microbiological analysis.
- Type of study: This will be a self-contained study.
- Data collection: Remote Data Entry (RDE).
- Duration of the study: For each subject, there will be a single study visit. The duration of the study will be approximately 2 years
  - Epoch 001: Prospective data collection.

Table 2 presents the study group and epoch foreseen in the study.

Table 2 Study group and epoch foreseen in the study

Study Group	Number of subjects	Age (Min/Max)	Epoch
Prospec	At least 150	≥ 6 months to < 6 years	Epoch 001

3.1. Discussion of study design

A cross-sectional study design is the simplest approach to describe the burden of disease, *i.e.* bacterial aetiology of chronic LRTI symptoms. BAL is routinely performed for diagnosis of infection in children with such chronic symptoms, and it is unlikely to be performed repeatedly for otherwise healthy children. Thus, extended follow-up would not be possible.

Considering the invasive nature of the BAL fluid sampling procedure, it has been decided to enrol only those subjects with suspected chronic LRTIs who have BAL procedure indicated by their clinician as part of the local routine diagnostic procedure. This should facilitate subject recruitment and increase participation rates. Nasopharyngeal swabs may not be systematically collected in routine practice. However, since the patient will be under sedation to undergo BAL, he/she will not be subject to additional discomfort or risk due to the swabbing procedure. Thus, it is not expected to affect the willingness of the parent(s)/ LAR(s) to allow their child's participation in this study.

3.2. Case definitions

- **Suspected chronic LRTIs where BAL is indicated:**

  Based on clinical and/or radiological symptoms, suspected chronic LRTI will be defined as follows:

  – Persistent or recurrent respiratory signs/ symptoms that do not respond to usual treatment:

  i. *Wet cough* lasting > 4 weeks without additional associated symptoms

  ii. Recurrent (≥ 3 episodes/ year) or persistent *wheezing* (lasting > 3 months)

  iii. Persistent *pathologic auscultation* (apart from wheezing) (lasting > 4 weeks).

  AND/OR

  – Persistent (> 1 month) or recurrent (≥ 3 occurrences/ year) infiltrates/ atelectasis observed on chest radiograph.

  Episode of Wheezing: Being previously healthy, and now presenting pathological auscultation with wheezing during long expiration as the main feature.

  Evidence of infiltrate/ atelectasis: The presence of new radiological lung condensation, following radiological evidence of normalisation (between two occurrences).

4. STUDY POPULATION

4.1. Number of subjects/ centres

Overview of the recruitment plan:

- This study will be conducted in at least 5 hospitals in Spain.
- The study population will include subjects aged ≥ 6 months to < 6 years visiting the study hospital with suspected chronic LRTIs and for whom BAL sampling will be performed as per the recommendation of the clinician.
- Information on demography and medical history of the subject will be collected. BAL samples and nasopharyngeal swabs will be obtained for this study.
- At least 150 subjects are planned to be enrolled in the study based on the sample size estimation. Refer to section 7.2 for a detailed description of the criteria used in the estimation of sample size.
- The duration of the study will be approximately 2 years from the study initiation. There will be a single visit for each subject.
- A GSK study staff member or delegate will be responsible for monitoring the study.

4.2. Inclusion criteria for enrolment

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

- Subjects who the investigator believes that parent(s)/ LAR(s) can and will comply with the requirements of the protocol.
- A male or female subject aged ≥ 6 months to < 6 years at the time of enrolment.
- Subjects meet the case definition of suspected chronic LRTIs where BAL is indicated (as listed under Section 3.2).
- Subject's parent(s)/ LAR(s) agree to the collection of a nasopharyngeal swab from the subject.
- Written informed consent obtained from the parent(s)/ LAR(s) of the subject.

4.3. Exclusion criteria for enrolment

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:

- Known cystic fibrosis, immunosuppression, or other severe immunodeficiencies such as agammaglobulinaemia, T cell deficiency or Human Immunodeficiency Virus (HIV)/ Acquired Immune Deficiency Syndrome (AIDS), chemotherapy treatment, etc.

- Exacerbation of persistent respiratory symptoms (cough, wheezing, difficulty in breathing, etc) in the previous 2 weeks. Please refer to Section 3.2 for the definition of exacerbation of persistent respiratory symptoms.
- Antibiotic treatment in the 2 weeks prior to study entry.
- Concurrent participation in another study within 30 days prior to study entry or at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device).
- Subjects having previously participated in this study.
- Child in care. Please refer to the GLOSSARY OF TERMS for the definition of child in care.

A list of criteria that may eliminate subjects from according to protocol (ATP) analyses can be found in Section 7.3.

5. CONDUCT OF THE STUDY

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

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the International Conference on Harmonisation (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.

The study has been designed and will be conducted in accordance with the ICH Harmonised Tripartite Guideline for clinical investigation of medicinal products in the paediatric population (ICH E11) and all other applicable ethical guidelines.

GSK will obtain favourable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, 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's parent(s)/LAR(s) informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK 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's parent(s)/LAR(s), 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 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.

Prior to the BAL procedure, the investigator will also ask the subject's parent(s)/LAR(s) to sign a hospital ICF with the details of the BAL sampling procedure. This hospital ICF is independent of GSK and allows a parent to decide on routine clinical care as separate from study participation.

5.2. Subject identification

Subject numbers will be assigned sequentially to subjects who will be included in the study, according to the range of subject numbers allocated to each study centre.

5.3. 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.

5.4. Outline of study procedures

Table 3 presents the list of study procedures.

Table 3 List of study procedures

Epoch	Prospective data collection
Visit	**VISIT 1**
Timing	**Day 0**
Check inclusion and exclusion criteria	●
Informed consent and subject number attribution	●
Demographic data	●
Collection of data such as clinical characteristics (medical history, chronic LRTI symptoms), vaccination history, feeding and day care practice, environmental exposure and smoking environment	●
Physical examination data	●
Collection of radiological and laboratory results (if available)	●
Collection of BAL fluid samples that were obtained as per routine practice and nasopharyngeal swabs	●
Send BAL fluid and nasopharyngeal swab samples to designated laboratory for microbiological analysis	○
Reporting of SAEs related to the study procedure	●
Laboratory results transcription to database	○
Study conclusion	●
Collection of aggregated data from logbooks	●*

BAL: Bronchoalveolar lavage
● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
*Aggregated data will be documented in the aggregated logbook module on a monthly basis

5.5. Detailed description of study procedures

5.5.1. Collection of aggregated data from logbooks

A logbook will be maintained at the study hospitals in which aggregated data on the following will be recorded during the entire study period by year of age, on a monthly basis:

- Total number of children presenting to the hospital with an indication for BAL.
- Total number of children presenting to the hospital with an indication for BAL that may be attributable to chronic LRTI.
- Total number of children presenting to the hospital with suspected chronic LRTI who have an indication for BAL and are not enrolled due to applying exclusion criteria.
- Total number of children presenting to the hospital and not being enrolled in the study due to refusal of consent by their parent(s)/ LAR(s).

5.5.2. Procedures prior to study participation

5.5.2.1. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.5.2.2. Informed consent

Before performing any other study procedure, the signed informed consent of the subject's parent(s)/ LAR(s) needs to be obtained. Refer to Section 5.1 for the requirements on how to obtain informed consent.

5.5.3. Procedures during study participation

5.5.3.1. Subject number attribution

Once a subject meets the eligibility requirements and has been included into the study, a unique subject number will be assigned. This subject number will be used to uniquely identify all data collected on the subject for the study.

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

5.5.3.2. Collect demographic data

The parent(s)/ LAR(s) of the subject will be interviewed to collect demographic data such as age and gender.

Collected information needs to be recorded in the eCRF.

5.5.3.3. Clinical characteristics and other information

For all subjects aged ≥ 6 months to < 6 years visiting the study hospital with suspected chronic LRTIs, the following information will be recorded, but will not be limited to:

- Clinical characteristics (including medical history, chronic LRTI symptoms),
- Vaccination history,
- Feeding and day care practice,
- Environmental exposure and smoking environment.

5.5.3.4. Collect physical examination data

A physical examination of the subject will be performed as per routine practice, which will include assessment of body temperature, heart rate, respiratory rate after at least 10

minutes of rest, blood pressure and oxygen saturation; the results will be collected as part of this study.

Collected information needs to be recorded in the eCRF.

5.5.3.5. Radiological and laboratory results

For all subjects aged ≥ 6 months to < 6 years visiting the study hospital with suspected chronic LRTIs, radiological and laboratory results will be collected, if available. Findings from the chest X-ray, (if present) will also be recorded.

If results of blood testing are available from routine practice, then data on white blood cell counts, CRP level and erythrocyte sedimentation rate, etc will be recorded in the eCRF.

5.5.3.6. Collection of BAL fluid samples and nasopharyngeal swabs

Following routine BAL procedures at the hospital, the second aliquot of BAL fluid that is obtained will be sent to the GSK designated laboratory for microbiological analysis.

Nasopharyngeal swabs will be collected as part of this study. Collected samples will also be sent to the GSK designated laboratory for microbiological analysis.

5.5.3.7. Recording of Serious Adverse Events

- The subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

Refer to Section 6.2 for procedures for the Investigator to record serious adverse events (SAEs) that are related to study participation and to Section 6.3 for guidelines on how to report these SAEs to GSK Biologicals.

5.5.3.8. Study conclusion

The investigator will review the data collected to ensure accuracy and completeness and will complete the Study Conclusion screen in the eCRF.

5.6. Biological Sample handling and analysis

Please refer to the SPM 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 may be used in other assays, for test improvement or test development of analytical methods related to the disease under study to allow to achieve a more

reliable measurement. Under these circumstances, additional testing on the samples may be performed by GSK Biologicals outside the scope of this protocol.

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's parent(s)/ LAR(s).

Any human pharmacogenetic testing will require specific consent from the individual subject's parent(s)/ LAR(s) and the ethics committee approval. Any human immunodeficiency virus (HIV) testing will also require specific consent and ethics committee approval.

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 up to 15 years (counting from when the last subject performed the last study visit), 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.

For routine sample collection when coded sample is shipped to GSK and needs to be linked to coded subject data, (re-) consent from the subjects and/or IEC/IRB approval will be sought.

5.6.1. Use of specified study materials

When materials are provided by GSK Biologicals, 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 ATP analysis (See Section 7.3 for the definition of study cohorts to be evaluated). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals 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.

5.6.2. Biological samples

Table 4 presents the biological samples that will be collected for this study.

Table 4 Biological samples

Sample type	Quantity	Time point
BAL	At least 2 mL	Visit 1 Day 0
Nasopharyngeal swab	1 swab	

5.6.3. Laboratory assays

Laboratory assays to characterise BAL fluid and nasopharyngeal swab samples including the detection of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* will be performed by standard techniques at GSK Biologicals' laboratory or in a validated laboratory designated by GSK Biologicals using standardised and validated procedures (refer to Table 5).

Table 5 Laboratory assays (Amended 05 October 2015)

Pathogen/ Marker	Method	Scale		Sample type	
		Qualitative	Quantitative	BAL	NP
Identification and/ or quantification of bacteria					
S. pneumoniae	Culture		X	X	X
	Molecular techniques (PCR)		X		
H. influenzae	Culture		X	X	X
	Molecular techniques (PCR) ***(differentiation between Hi and Haemophilus haemolyticus)†***		X		
M. catarrhalis	Culture		X	X	X
	Molecular techniques (PCR)		X		
Other pathogens (if detected)	Culture	X		X	X
Serotyping of bacterial pathogens					
S. pneumoniae	Agglutination assay or molecular techniques	X		X	X
H. influenzae	Monovalent antisera	X		X	X
Antibiotic sensitivity : Including penicillin, erythromycin, azithromycin, tetracycline, levofloxacin, trimethoprim/ sulfamethoxazole, amoxicillin/ clavulanate ***susceptibility testing for H. influenzae, S. pneumoniae and M. catarrhalis isolated from BAL and NP swab samples;*** and additionally a beta-lactamase test for *H. influenzae* and *M. catarrhalis* ***isolated from BAL and NP swab samples***; ***and ampicillin resistance testing by broth microdilution for beta-lactamase negative H. influenzae isolated from BAL and NP swab samples.***					

†: PCR differentiation between Hi and *H. haemolyticus* will be performed on isolates from BAL/ NP swab samples that are *H. influenzae* positive

Collected samples will be used for purposes related to the quality assurance of data generated within the scope of this protocol, such as for maintenance of assays described in this protocol and comparison between analytical methods and/or laboratories.

6. SERIOUS ADVERSE EVENTS

The procedure of nasopharyngeal sampling will be performed on subjects enrolled in the study to collect nasopharyngeal swab samples.

Only SAEs related to the nasopharyngeal sampling procedure that occur until the subject is discharged will be recorded.

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's parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subject manifest any signs or symptoms they perceive as serious.

6.1. Safety definitions

6.1.1. Definition of an SAE

An SAE is any adverse event that:

a. Results in death,

b. Is life-threatening,

   NB: 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 existing hospitalisation,

   NB: 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,

   NB: 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.

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.

6.1.2. Clinical laboratory parameters and other abnormal assessments qualifying as SAEs

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 SAEs if they meet the definition of an SAE, as defined in Section 6.1.1. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as 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 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.

6.2. Detecting and recording SAEs

6.2.1. Time period for detecting and recording SAEs

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) or any fatal SAE will be collected and recorded from the time the parent(s)/ LAR(s) of the subject consents to allow participation in the study and will last until the subject is discharged.

6.2.2. Evaluation of SAEs

6.2.2.1. Active questioning to detect SAEs

Each subject's parent(s)/ LAR(s) will be instructed to contact the investigator immediately should the subject manifest any signs and symptoms they perceive as serious.

All SAEs either observed by the investigator or his/her staff or reported by the subject's parent(s)/ LAR(s) spontaneously or in response to a direct question will be evaluated by the investigator. The nature of each event, data and time (where appropriate) of onset, outcome, intensity and relationship to the study procedures should be established.

When an 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 an SAE on the eCRF or SAE Report screens as applicable. It is not acceptable for the investigator to send photocopies of the subject's medical records to GSK Biologicals instead of the appropriate completed SAE screens in 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 SAE and not the individual signs/symptoms.

6.2.2.2. Assessment of causality

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

There may be situations when an 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 to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE information accordingly.

If an event meets the criteria to be determined 'serious' (refer to Section 6.1.1), it will be examined by the investigator to the extent to enable determination of all contributing factors applicable to each SAE.

Possible contributing factors include:

- Medical history.
- Concomitant medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Other cause (specify).

6.2.2.3. Assessment of outcomes

Outcome of any SAE reported during the entire study will be assessed as:

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

6.3. Reporting of SAEs

All SAEs occurring until the subject is discharged and considered related to the nasopharyngeal sampling procedure must be recorded on the SAE screen.

6.3.1. Prompt reporting of SAEs related to a study procedure to GSK Biologicals

SAEs that occur in the time period defined in Section 6.2.1 will be reported promptly to GSK within the timeframes described in Table 6 once the investigator determines that the event meets the protocol definition of an SAE.

Table 6 Timeframes for submitting SAEs related to a study procedure to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to a study procedure	24 hours*	SAE screen	24 hours*	SAE screen

* Timeframe allowed after receipt or awareness of the information.

6.3.2. Contact information for reporting SAEs to GSK Biologicals

Please see the Sponsor Information Sheet for contact details.

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

6.3.3. Completion and transmission of SAE reports related to a study procedure to GSK Biologicals

Once an investigator becomes aware that an SAE has occurred in a study subject, the investigator (or designate) must complete the information in the SAE screens of the

eCRF WITHIN 24 HOURS. The SAE screens 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 SAE screens should still be completed within 24 hours. Once additional information is received, the SAE screens in the eCRF should be updated WITHIN 24 HOURS.

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

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

If the electronic SAE reporting system does not work, the investigator (or designate) must complete, then date and sign a SAE Report Form 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 SAE reporting system is not working and NOT if the system is slow. As soon as the electronic SAE reporting system is working again, the investigator (or designate) must complete the SAE screens in the eCRF within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

6.3.3.2. Updating of SAE information after freezing of the subject's eCRF

When additional information is received on a SAE after freezing of the subject's eCRF, new or updated information is to be recorded on a SAE Report Form, with all changes signed and dated by the investigator. The updated form should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) WITHIN 24 HOURS of receipt of the follow-up information.

6.4. Follow-up of SAEs

After the initial SAE report, the investigator is required to proactively follow each subject and provide further information on the subject's condition to GSK Biologicals.

All SAEs documented at the visit and designated as not recovered/not resolved or recovering/resolving will be reviewed until the end of the study.

Investigators will follow-up subjects:

- With SAEs until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relative to the subsequent course of such abnormalities noted for any subject must be made available to the Site Monitor.

GSK Biologicals may request that the investigator performs or arranges for the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognised follow-up period, GSK Biologicals will be provided with a copy of any available post-mortem findings, including histopathology.

7. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

7.1. Endpoints

7.1.1. Primary endpoint

- Occurrence of *H. influenzae, S. pneumoniae, M. catarrhalis* and other bacteria in BAL fluid samples of subjects aged ≥ 6 months to < 6 years with suspected chronic LRTIs and an indication for BAL
  - *H. influenzae, S. pneumoniae* and *M. catarrhalis* confirmed by bacterial load >10^4 cfu/mL if present alone or 10^5 cfu/mL if present as co-infection.

7.1.2. Secondary endpoints

In a hospital setting and among subjects aged ≥ 6 months to < 6 years with suspected chronic LRTIs and an indication for BAL:

- Bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* in the BAL fluid as determined by quantitative culture and PCR.
- Description of other bacterial pathogens in the BAL fluid as determined by qualitative culture.
- Bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* in nasopharyngeal swab samples as determined by quantitative culture and PCR.
- Description of other bacterial pathogens in nasopharyngeal swab samples as determined by qualitative culture.
- Occurrence of *H. influenzae*, *S. pneumoniae, M. catarrhalis* and other pathogens detected by culture in the nasopharyngeal swab samples.
- Occurrence of *H. influenzae* and *S. pneumoniae* serotypes identified from BAL fluid samples and nasopharyngeal swab samples.
- Occurrence of *H. influenzae, S. pneumoniae* and *M. catarrhalis* minimum inhibitory concentration (MIC) values identified from BAL fluid ***and nasopharyngeal swab*** samples. (Amended 05 October 2015)
- Demographic characteristics (age, gender, etc), clinical characteristics (including medical history, LRTI symptoms, treatment history, vaccination status, etc),

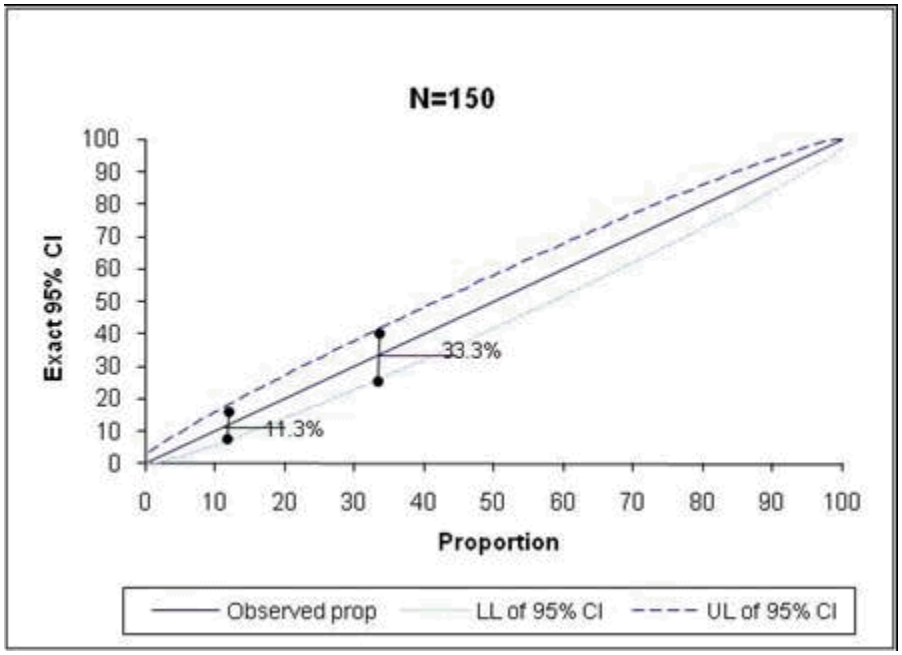
radiological evidence and laboratory results (including white blood cell counts, CRP level and erythrocyte sedimentation rate, if available).

7.2. Sample size consideration

The proportions of *S. pneumoniae* and *H. influenzae* among all BAL fluid samples collected is expected to be between 4% and 35%, and between 7% and 51%, respectively [Schellhase, 1998; Marguet, 1999; Marchant, 2006; De Schutter, 2011].

Figure 1 provides the precision that can be expected around an estimated proportion of *S. pneumoniae* and *H. influenzae* for a sample size of at least 150 subjects. For example, the exact 95% confidence interval (CI) for an observed proportion of 11.3% is 6.7; 17.5. The exact 95% CI for an observed proportion of 33.3% is 25.9; 41.5.

Figure 1 Illustration of the lower (LL) and upper (UL) limits of the exact 95%CI built around various observed proportions for a sample of 150 subjects



7.3. Study cohorts to be evaluated

7.3.1. Screened cohort

The screened cohort will include all subjects who visit the hospital with suspected chronic LRTIs and have an indication for BAL. All the information (anonymised) for these subjects will be collected in the aggregated logbook.

7.3.2. Total cohort

The total cohort will include all enrolled subjects.

7.3.3. According-To-Protocol cohort

The ATP cohort will include all evaluable subjects (i.e. those meeting all eligibility criteria and complying with the procedures defined in the protocol during the study) with BAL fluid sampling and/ or nasopharyngeal swab sampling results available.

A detailed, comprehensive list of reasons for elimination from ATP analyses will be established at the time of data cleaning.

7.4. Derived and transformed data

Age at time of enrolment in the study will be computed as the difference between the date of enrolment [date when the ICF was signed by the parent(s)/ LAR(s)] and the date of birth. The age will be expressed in months.

The age will be grouped into the following classes 6-11, 12-23, 24-35, 36-47, 48-59 and 60-71 months.

The duration of hospitalisation will be computed as the difference between the date of discharge and the date of admission +1.

The pneumococcal conjugate vaccination status will defined as:

- Vaccinated, if the subject either received at least two doses or received only one dose after one year of age;
- Unvaccinated, if the subject did not receive any dose or received only one dose within the first year of life;
- Unknown otherwise.

Temperature will be converted to axillary route temperature (if route is rectal or tympanic rectal, then value will be 0.5°C). If this derived value of temperature is ≥ 37.5°C, then the subject is considered to have fever.

7.5. 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.

7.5.1. Sequence of analyses

The analyses, as described in Sections 7.6.2 and 7.6.3, will be performed in one step, when all data, will be available and cleaned. These analyses and associated individual data will be presented in a final study report.

7.5.2. Statistical considerations for interim analyses

No interim analysis is planned for this study.

7.6. Statistical methods

Demographic, clinical and microbiological characteristics of subjects will be described using percentages for categorical variables and mean (standard deviation) or median (min-max) for continuous variables. The same variables will be presented descriptively, overall and by microbiological results.

7.6.1. Analysis of demographics/ baseline characteristics

The number of screened and enrolled subjects as well as the number excluded from ATP analyses will be presented. Demographic characteristics (age and gender) will be summarised using descriptive statistics.

7.6.2. Analysis of primary objective

The proportions and associated exact 2-sided 95% confidence intervals (CI) of *H. influenzae, S. pneumoniae, M. catarrhalis* and other bacteria confirmed by culture in BAL samples will be computed and presented for all study subjects. The denominators will be the number of tested subjects.

7.6.3. Analysis of secondary objectives

The distribution (with number of non-missing observations, geometric mean, standard deviation, median, minimum and maximum and number of missing observations) of the bacterial load of *H. influenzae*, *S. pneumoniae, M. catarrhalis* and other pathogens will be described according to the testing method (culture or PCR) and to the samples (BAL fluid sample or nasopharyngeal swab sample).

The serotype distribution (number and percentage) of *H. influenzae* ***and*** *S. pneumoniae* detected in BAL fluid will be presented in frequency tables.

The bacterial aetiology and serotype distribution of nasopharyngeal samples will be described along with that of BAL fluid samples.

The proportions and associated exact 2-sided 95% CI of *H. influenzae, S. pneumoniae, M. catarrhalis* and other bacterial pathogens confirmed by culture in nasopharyngeal swab will be computed for all study subjects.

For the antibiotic susceptibility, *H. influenzae, S. pneumoniae* ***and M. catarrhalis*** isolates (detected by culture of BAL fluid ***and nasopharyngeal swab***), classified as susceptible, intermediate or resistant (following ***Clinical and Laboratory Standards Institute*** guidelines ***[CLSI, 2015]***) will be tabulated by bacterial pathogen and antimicrobial agent.

(Amended 05 October 2015)

8. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications must be fulfilled.

8.1. Remote Data Entry instructions

Remote Data Entry (RDE), a validated computer application, 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 designate. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the clinical study report is complete and approved by all parties.

8.2. Monitoring by GSK Biologicals

Monitoring visits by a GSK Site Monitor are for the purpose of confirming that GSK Biologicals' sponsored studies are being conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with Good Clinical practice (GCP) and the applicable regulatory requirement(s) (verifying continuing compliance with the protocol, amendment(s), verifying that the site staff and facilities continue to be adequate to conduct the study).

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 an RDE review and a Source Document Verification (SDV). By SDV we understand verifying RDE 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 RDE. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the RDE will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For RDE, the monitor will mark completed and approved screens at the visit.

In accordance with applicable regulations, GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the eCRF entries will serve as the source document.

GSK will monitor the study to verify that, amongst others, 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 and any amendments, 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.

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.

8.3. Archiving of data at study sites

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data 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 for studies with an eCRF); however, caution needs to be exercised before such action is taken. The investigator must assure 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 there is an acceptable back-up of these reproductions and that an acceptable quality control process exists 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. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by ICH GCP any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

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

8.4. Audits

To ensure compliance with GCP 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.

8.5. Posting of information on public registers

Study information from this protocol will be posted on public registers (e.g. GSK Clinical Study Register, clinicaltrials.gov) before enrolment of subjects begins as applicable.

8.6. Ownership, confidentiality and publication

8.6.1. Ownership

All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) are the sole property of GSK.

All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of GSK, and are hereby assigned to GSK.

If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed between GSK and the study site, that contract's ownership provisions shall apply rather than this statement.

8.6.2. Confidentiality

Documented evidence that a potential investigator is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

All information provided by GSK and all data and information generated by the site as part of the study (other than a subject's medical records) will be kept confidential by the investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (i) information which becomes publicly available through no fault of the investigator or site staff; (ii) information which it is necessary to

disclose in confidence to an IEC or IRB solely for the evaluation of the study; (iii) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (iv) study results which may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

8.6.3. Publication

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a 'Publication'), the investigator shall provide GSK with a copy of the proposed Publication and allow GSK a period to review the proposed Publication (at least twenty-one working days, or at least fifteen working days for abstracts/posters/presentations). Proposed Publications shall not include either GSK confidential information other than the study results or personal data on any subject, such as name or initials.

At GSK's request, the submission or other disclosure of a proposed Publication will be delayed a sufficient time to allow GSK to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract's publication provisions shall apply rather than this statement.

8.6.4. Provision of study results to investigators, posting to the clinical trials registers and publication

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 site or other mutually-agreeable location.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

9. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

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APPENDIX A AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals Clinical Research & Development **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	115813 (EPI-STREP-064 BOD ES)
Amendment number:	Amendment 1
Amendment date:	Amended: 08 March 2013
Co-ordinating author:	PPD, Scientific Writer

Rationale/background for changes:
It was initially intended to ship the collected bronchoalveolar lavage (BAL) fluid and nasopharyngeal swabs to the GSK designated laboratory (Instituto Valenciano de Microbiologia [IVAMI], Spain) within 4-6 hours of collection. For some sites, this is not feasible from the operational point of view due to conflicting hospital schedules and limited availability of daily flights between the two provinces participating in this study. One of the proposals was to bring in a technician from IVAMI for plating of samples within 4-6 hours, which again would not be cost-effective and may also create differences in test results between the centres, making it difficult to compare the results at the end of the study.

An alternative approach that has been decided is immediate freezing of the collected BAL fluid and nasopharyngeal swab samples between -70° to -80° C. As a result, the tertiary objective of comparing the bacterial load of the samples plated within 4-6 hours with delayed plating (22-26 hours and 46-50 hours) cannot be met.

Amended text has been included in *bold italics* in the following sections:

1. **Title page: List of contributing authors:**
   - PPD***, Study Data Manager***
   - PPD***, Laboratory Study Manager, Business and Decision for GSK Biologicals***
   - PPD***, Global Study Manager (Consultant, Harrison Clinical Research)***
   - PPD***, Local Study Coordinator***

2. **Sponsor signatory page**

PPD***, Director, Life cycle Management, Epidemiology, GlaxoSmithKline Biologicals***

PPD~~, GVD Director, Pediatric Vaccines Epidemiology, GlaxoSmithKline Biologicals~~

3. **Synopsis-Rationale for study and Section 1.2 (Rationale for the study):**

~~The study also provides an opportunity to understand more technical aspects of the BAL procedure, which might be useful for future studies. For instance, the effect of delayed plating of BAL fluid samples for bacterial culture has not been documented. Since the maximum time recommended before BAL sample processing is 4-6 hours, comparing bacterial load in BAL samples plated within the standard recommendation of 4-6 hours with delayed plating (22-26 hours and 46-50 hours) may provide updated logistical recommendations that could be helpful for future multi-centre studies.~~

4. **Synopsis –Tertiary objective and Section 2.3 (Tertiary objective) has been deleted.**

~~To compare the bacterial load detected by quantitative culture in a subset of up to 50% of the collected BAL samples plated within standard recommended 4-6 hours with delayed plating (22-26 hours and 46-50 hours).~~

~~Refer to Section 7.1.3 for the definition of the tertiary endpoint.~~

5. **Synopsis-Tertiary Endpoint and Section 7.1.3 (Tertiary Endpoint) has been deleted.**

~~Occurrence of bacteria as determined by quantitative culture in a subset of up to 50% of the collected BAL samples plated within standard recommended 4-6 hours and with delayed plating (22-26 hours and 46-50 hours).~~

6. **Section 5.6.2 – Biological Samples**

Table 4 Biological samples

Sample type	Quantity	Time point
BAL	~~Maximum possible~~ ***At least 2mL***	Visit 1 Day 0
Nasopharyngeal swab	1 swab	

7. **Section 5.6.3-Laboratory assays**

Table 5 Laboratory assays

Pathogen/ Marker	Method	Scale		~~Plating timelines~~	Sample type	
		Qualitative	Quantitative		BAL	NP
Identification and/ or quantification of bacteria						
S. pneumoniae	Culture	~~X~~	X	~~Within 4-6h after collection (Culture)~~	X	X
	Molecular techniques (PCR)	~~X~~	X			
	~~Culture~~	~~X~~	~~X~~	~~at 22-26h at 46-50h after first plating*~~	~~X~~	
H. influenzae	Culture	~~X~~	X	~~Within 4-6h after collection (Culture)~~	X	X
	Molecular techniques (PCR)	~~X~~	X			
	~~Culture~~	~~X~~	~~X~~	~~at 22-26h at 46-50h after first plating*~~	~~X~~	
M. catarrhalis	Culture	~~X~~	X	~~Within 4-6h after collection (Culture)~~	X	X
	Molecular techniques (PCR)	~~X~~	X			
	~~Culture~~	~~X~~	~~X~~	~~at 22-26h at 46-50h after first plating*~~	~~X~~	
Other pathogens (if detected)	Culture	X		~~Within 4-6h after collection~~	X	***X***
Serotyping of bacterial pathogens						
S. pneumoniae	Agglutination assay or molecular techniques	X			X	X
H. influenzae	Monovalent antisera and Molecular techniques (differentiation between Hi and *Haemophilus haemolyticus*)†	X ~~X~~			X	X
***Antibiotic sensitivity**:* Including penicillin, erythromycin, azithromycin, tetracycline, levofloxacin, trimethoprim/ sulfamethoxazole, amoxicillin/ clavulanate; and additionally a beta-lactamase test for *H. influenzae* and *M. catarrhalis*						

~~*: Delayed plating should be performed for every second BAL sample that is collected, as applicable~~

†: PCR differentiation between Hi and *H. haemolyticus* will be performed on isolates from BAL/ NP swab samples that are *H. influenzae* positive

: For BAL samples only

8. **Section 7.6 Statistical methods: Section 7.6.3 (Analysis of tertiary objective) has been deleted.**

~~The comparison of bacterial load will be done for at least 50% of the enrolled subjects using cross-tables for each bacteria and as per the plating time.~~

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 2

eTrack study number and Abbreviated Title	115813 (EPI-STREP-064 BOD ES)
Amendment number:	Amendment 2
Amendment date:	05 October 2015
Co-ordinating author:	PPD, Scientific Writer, Keyrus Biopharma for GSK Biologicals

Rationale/background for changes:
The EPI-STREP-064 BOD ES protocol has been updated for the following reasons:

- It has been clarified that:
  - the antibiotic susceptibility profile (including penicillin, erythromycin, azithromycin, tetracycline, levofloxacin, trimethoprim/ sulfamethoxazole, amoxicillin/ clavulanate) will be determined for *Haemophilus influenzae (H. influenzae), Streptococcus pneumoniae* (*S. pneumoniae)* and *Moraxella catarrhalis (M. catarrhalis)* identified not only from bronchoalveolar lavage (BAL) fluid but also from nasopharyngeal swab samples;
  - the beta-lactamase test will be performed for *H. influenzae* and *M. catarrhalis* identified not only from BAL fluid but also from nasopharyngeal swab samples;
  - ampicillin resistance testing will be performed for beta-lactamase negative *H. influenzae* from BAL fluid and from nasopharyngeal swab samples.
- In addition, inconsistencies between the secondary endpoints in the synopsis and the secondary endpoints in the body of the protocol amendment 1 have been clarified.

Other changes have been made for simplification, clarification or consistency.

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

Title page - List of contributing authors:

- PPD ***, Senior Manager, Epidemiology***
- PPD ***, Study Statistician, freelance for GSK Biologicals***
- PPD ***, Study Delivery Lead***
- PPD ***, Study Delivery Manager***
- PPD ***, Study Data Manager, TCS for GSK Biologicals***
- PPD ***, Safety Associate***

- PPD , ***GVCL Project Manager***
- PPD , ***GVCL Study Manager***
- PPD , ***Local Delivery Lead*** ~~Local Study Coordinator~~

Sponsor signatory page:

PPD , Director, ~~Life cycle Management~~, ***Head of Global*** Epidemiology, GlaxoSmithKline Biologicals

Synopsis – Rationale for the study:

The present study aims to identify and characterise bacteria present in the lower airways of children with suspected chronic LRTIs and for whom ***bronchoalveolar lavage (***BAL***)*** is indicated by the clinician.

Synopsis – Secondary objectives and Section 2.2. Secondary objectives:

To determine the antibiotic susceptibility profile for *H. influenzae, S. pneumoniae* and *M. catarrhalis* identified from BAL fluid ***and nasopharyngeal swab*** samples.

Synopsis - Secondary endpoints:

In a hospital setting and among subjects with suspected chronic LRTIs:

- ~~To describe the bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* detected by quantitative culture and by molecular techniques (PCR) in the BAL fluid.~~
- ~~To describe the presence of other bacterial pathogens detected by qualitative culture in the BAL fluid.~~
- ~~To describe the bacterial load of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* detected by quantitative culture and by molecular techniques (PCR) in the nasopharyngeal swab samples.~~
- ~~To describe the presence of other bacterial pathogens detected by qualitative culture in the nasopharyngeal swab samples.~~
- ~~To describe colonisation of the upper airways by characterising *H. influenzae*, *S. pneumoniae, M. catarrhalis* and other pathogens in nasopharyngeal swab samples.~~
- ~~To determine the serotypes of *H. influenzae* and *S. pneumoniae* identified from BAL fluid samples.~~
- ~~To determine the serotypes of *H. influenzae* and *S. pneumoniae* identified from nasopharyngeal swab samples.~~
- ~~To determine the antibiotic susceptibility profile for *H. influenzae, S. pneumoniae* and *M. catarrhalis* identified from BAL fluid samples.~~

- ~~To describe, according to microbiological results observed:~~
  - ~~Age and gender~~
  - ~~Clinical symptoms and radiological evidence~~
  - ~~Pneumococcal conjugate vaccine, *H. influenzae* type b vaccine and influenza vaccine status~~
  - ~~Medical history and co-morbidities~~
  - ~~Information on feeding and day care practice, environmental exposure and smoking environment~~
  - ~~History of antibiotic use in the past six months as well as other treatments for chronic lower respiratory disease~~
  - ~~Laboratory results [including white blood cell counts, C-Reactive Protein (CRP) level and erythrocyte sedimentation rate, if available].~~
- ***Bacterial load of H. influenzae, S. pneumoniae and M. catarrhalis in the BAL fluid as determined by quantitative culture and PCR.***
- ***Description of other bacterial pathogens in the BAL fluid as determined by qualitative culture.***
- ***Bacterial load of H. influenzae, S. pneumoniae and M. catarrhalis in nasopharyngeal swab samples as determined by quantitative culture and PCR.***
- ***Description of other bacterial pathogens in nasopharyngeal swab samples as determined by qualitative culture.***
- ***Occurrence of H. influenzae, S. pneumoniae, M. catarrhalis and other pathogens detected by culture in the nasopharyngeal swab samples.***
- ***Occurrence of H. influenzae and S. pneumoniae serotypes identified from BAL fluid samples and nasopharyngeal swab samples.***
- ***Occurrence of H. influenzae, S. pneumoniae and M. catarrhalis minimum inhibitory concentration (MIC) values identified from BAL fluid and nasopharyngeal swab samples.***
- ***Demographic characteristics (age, gender, etc), clinical characteristics (including medical history, LRTI symptoms, treatment history, vaccination status, etc), radiological evidence and laboratory results (including white blood cell counts, CRP level and erythrocyte sedimentation rate, if available).***

Section 1.1. Background:

Table 1 Bacterial aetiology of BAL fluid culture from children with respiratory symptoms

Reference	Population	Number of samples	*H. influenzae*	*S. pneumoniae*	*M. catarrhalis*
[De Schutter, 2011]	Acute nonresponsive CAP (cut off ≥ ~~104~~ ***10^4*** CFU/mL)	127	26%	6.3%	8.7%
	Recurrent CAP (cut off ≥ ~~104~~ ***10^4*** CFU/mL)	123	51.2%	7.3%	21.1%
[Hare, 2010]	Bronchiectasis (cut off > ~~104~~ ***10^4*** CFU/mL)	45	47%	18%	20%
[Marchant, 2006]	Chronic cough of > 3 weeks duration of unknown etiology	20	47%	35%	26%
[Saito, 2006]	Persistent bacterial bronchitis (persistent, wet cough for >1 month that resolves with appropriate antibiotic treatment)	19	26.3%	21%	42.1%
[Le Bourgeois, 2002]	Severe recurrent wheezy bronchitis unresponsive to inhaled steroids	30	30%	13.33%	10%
[Marguet, 1999]	5 groups: asthma chronic cough infantile wheeze cystic fibrosis control	72 (total) 12 9 23 10 10	 8.33% 33.33% 30.43% 20% 30%	 8.33% 22.22% 0.04% 10% -	 16.67% 11.11% 13.04% - -
[Schellhase, 1998]	Recurrent wheezing	27	3.7%	-	7.4%

CAP = community-acquired pneumonia

Section 5.6.3. Laboratory assays:

Table 5 Laboratory analysis

Pathogen/ Marker	Method	Scale		Sample type	
		Qualitative	Quantitative	BAL	NP
Identification and/ or quantification of bacteria					
S. pneumoniae	Culture		X	X	X
	Molecular techniques (PCR)		X		
H. influenzae	Culture		X	X	X
	Molecular techniques (PCR) ***(differentiation between Hi and Haemophilus haemolyticus)†***		X		
M. catarrhalis	Culture		X	X	X
	Molecular techniques (PCR)		X		
Other pathogens (if detected)	Culture	X		X	X
Serotyping of bacterial pathogens					
S. pneumoniae	Agglutination assay or molecular techniques	X		X	X
H. influenzae	Monovalent antisera ~~and Molecular techniques (differentiation between Hi and Haemophilus haemolyticus)‡~~	X		X	X
Antibiotic sensitivity : Including penicillin, erythromycin, azithromycin, tetracycline, levofloxacin, trimethoprim/ sulfamethoxazole, amoxicillin/ clavulanate ***susceptibility testing for H. influenzae, S. pneumoniae and M. catarrhalis isolated from BAL and NP swab samples;*** and additionally a beta-lactamase test for *H. influenzae* and *M. catarrhalis* ***isolated from BAL and NP swab samples;*** ***and ampicillin resistance testing by broth microdilution for beta-lactamase negative H. influenzae isolated from BAL and NP swab samples.***					

†: PCR differentiation between Hi and *H. haemolyticus* will be performed on isolates from BAL/ NP swab samples that are *H. influenzae* positive

~~‡: For BAL samples only~~

Section 7.1.2. Secondary endpoints:

- Occurrence of *H. influenzae, S. pneumoniae* and *M. catarrhalis* minimum inhibitory concentration (MIC) values identified from BAL fluid ***and nasopharyngeal swab*** samples.

Section 7.6.3. Analysis of secondary objectives:

The serotype distribution (number and percentage) of *H. influenzae* ***and*** *S. pneumoniae* ~~and other bacterial isolates~~ detected in BAL fluid will be presented in frequency tables.

For the antibiotic susceptibility, *H. influenzae,* ~~and~~ *S. pneumoniae* ***and M. catarrhalis*** isolates (detected by culture of BAL fluid ***and nasopharyngeal swab***), classified as susceptible, intermediate or resistant (following ***Clinical and Laboratory Standards Institute*** ~~CLSI~~ guidelines ***[CLSI, 2015]***) will be tabulated by bacterial pathogen and antimicrobial agent.

Section 10. References:

Clinical and Laboratory Standards Institute (CLSI). M100-S25 Performance Standards for Antimicrobial Susceptibility Testing. January 2015.

Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	115813 (EPI-STREP-064 BOD ES)
Date of protocol amendment	Amendment 2 Final: 05 October 2015
Detailed Title	A multi-centre, hospital-based, cross-sectional epidemiology study to identify and characterise bacteria in the lower airways of children aged ≥ 6 months to < 6 years with suspected chronic lower respiratory tract infections (LRTIs) in Spain.
Sponsor signatory (Amended 05 October 2015)	Laurence Baril Director, ***Head of Global*** Epidemiology GlaxoSmithKline Biologicals
Signature	PPD
Date	9 October 2015

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