

**Study Protocol**

Sponsor:

GlaxoSmithKline BiologicalsRue de l'Institut 89,
1330 Rixensart, Belgium**eTrack study number and
Abbreviated Title**

200150 (EPI-RSV-005 BOD)

Date of protocol

Final Version 1: 27 June 2013

Date of Amendment

Amendment 1 Final: 27 November 2013

Amendment 2 Final: 06 July 2016

Amendment 3 Final: 15 December 2017**Title**

A prospective, epidemiological study to assess the disease burden of RSV associated, suspected LRTIs from 0 to 2 years and population attributable risk percent of RSV LRTI on the development of recurrent wheeze and asthma from 0 to 6 years

Detailed Title

A prospective, epidemiological, interventional, multi-country based, cohort study to assess the disease burden of respiratory syncytial virus (RSV) associated, suspected lower respiratory tract infections (LRTIs) in newborns, from birth up to 2 years of age and population attributable risk percent of RSV LRTI on the development of wheeze and asthma from birth up to 6 years of age.

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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 14.0

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Protocol Amendment 3 Sponsor Signatory Approval

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Date of Amendment	<i>Amendment 3 Final: 15 December 2017</i>
Detailed Title	A prospective, epidemiological, interventional, multi-country based, cohort study to assess the disease burden of respiratory syncytial virus (RSV) associated, suspected lower respiratory tract infections (LRTIs) in newborns, from birth up to 2 years of age and population attributable risk percent of RSV LRTI on the development of wheeze and asthma from birth up to 6 years of age.
Sponsor signatory (Amended 15-DEC-2017)	Amanda Leach Clinical and Epidemiology R&D Project Leader RSV Program, GSK Biologicals

Signature

Date

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Protocol Amendment 3 Rationale**Amendment number:** Amendment 3**Rationale/background for changes:**

The protocol has been amended to reflect the following changes and provide the following clarifications / corrections.

The *Bisgaard* and *Jackson* asthma case definitions were adapted so as to integrate asthma treatment advances while preserving the case definitions' core intent.

The International Study of Asthma and Allergies in Childhood (ISAAC) asthma case definitions, which facilitate a wider range of international results comparisons, were added.

Throughout the document, text has been consolidated and clarified for consistency with these changes and to better facilitate uniform, harmonized implementation of the protocol. Beginning with Section 2.0, text pertaining to the primary study has been shaded in grey to differentiate it from text pertaining to the extension study. Some details regarding (a) recruitment into the primary study and (b) primary study visits and procedures have been moved from the body of the document to newly created Appendices.

Text that has been added or moved appears in ***boldface italics***. A detailed summary of changes (including deletions) appears in Appendix G.

Protocol Amendment 3 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, with the terms of the study agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) or other applicable guidelines and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject's parent(s)/legally acceptable representative(s) (LARs).
- 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.

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Investigator name

Signature

Date

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Sponsor Information

Sponsor

GlaxoSmithKline Biologicals

Rue de l'Institut 89,

1330 Rixensart, Belgium

Sponsor Medical Expert for the Study

Refer to the local study contact information document.

Sponsor Study Monitor

Refer to the local study contact information document.

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

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section [7.3.2](#).

SYNOPSIS**Detailed Title**

A prospective, epidemiological, interventional, multi-country based, cohort study to assess the disease burden of respiratory syncytial virus (RSV) associated, suspected lower respiratory tract infections (LRTIs) in newborns, from birth up to 2 years of age and population attributable risk percent of RSV LRTI on the development of wheeze and asthma from birth up to 6 years of age.

Rationale for the study (*Amended 15-DEC-2017*)

This study aims to assess the incidence and associated healthcare utilization of RSV-associated, suspected LRTI in a general population of infants from birth up to 2 years of age, and also to assess the accuracy of a newly developed LRTI case definition and severity scale, compared to two existing scales developed by *the* World Health Organization (WHO) [*Modjarrad, 2016*] and Nokes, *2008*.

This study will also assess the extent to which RSV LRTI in early life (up to 2 years of age) accounts for the total burden of wheeze and asthma up to the sixth birthday, and test the performance of 3 asthma case definitions – those of Bisgaard, Jackson and International Study of Asthma and Allergies in Childhood (ISAAC).

The LRTI case definition and severity scale to be used in this study has been developed as a consensus model derived from existing definitions in the literature, and existing guidelines by GlaxoSmithKline (GSK) clinical staff, taking into account the advice of a panel of international RSV experts.

Considering asthma case definitions, this study anticipates that one (Bisgaard) will be more specific and another (Jackson) more sensitive. Although it is likely that the third symptom-based case definition (ISAAC) will be the most sensitive and least specific of all three definitions, its incorporation will facilitate wider international results comparisons.

If, in future, a maternal and/or pediatric RSV vaccine is introduced, results of this study may be used to evaluate post-vaccination incidence reduction (in RSV LRTI and/or wheeze and asthma) and the vaccine's potential public health impact.

**Objectives for the
Primary study
(Amended 15-DEC-
2017)**

Co-Primary

In a cohort of infants followed-up from birth up to 2 years of age:

- To determine the total health burden* of RSV-associated RTI with a suspicion of involvement of the lower respiratory tract.

**Health burden refers to burden of the disease imposed on the study population in terms of incidence of the disease and associated healthcare utilization in any healthcare setting.*

- To assess the performance of the LRTI case definition and severity scale for RSV associated cases.

Secondary

In a cohort of infants followed-up from birth up to 2 years of age:

- To determine the total health burden of non-RSV-associated RTI with a suspicion of involvement of the lower respiratory tract.
- To evaluate the association between RSV-associated LRTI, RSV-associated severe LRTI and RSV neutralizing antibodies in the baseline cord blood samples collected from subjects, at birth.
- To determine the prevalence of RSV infection, at 2, 4, 6, 12, 18 and 24 months in a randomly selected sub-cohort of subjects by assessing antibodies in serum.
- To assess the between calendar year variability in the incidence rates of RSV-associated LRTI.

Tertiary

In a cohort of infants followed-up from birth up to 2 years of age:

- To explore the association of co-infections with the incidence of RSV-associated LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ respiratory viral panel (RVP) fast assay.
- To explore the association of RSV and other respiratory viruses with the incidence of LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.

- To explore the association of RSV viral load with the incidence of RSV-associated RTI, LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale).
- To explore the impact of changes to the symptoms and threshold levels of symptoms in the LRTI case definition and severity scale.
- To assess the impact of potential RSV risk factors (e.g. complications at birth, family history of respiratory disease, living environment and household composition, breast feeding, passive smoking, day care attendance) on the incidence and severity of RSV-associated LRTI.
- To explore the impact of variations in cord-blood sample collection variables on the stability of test results.
- To assess the natural decay of maternal antibody levels using a single follow-up blood sample collected from a randomly selected sub-cohort of subjects at 2, 4, 6, 12, 18 and 24 months.

Objectives for the extension study (Amended 15-DEC-2017)

Primary: None

Secondary:

In a cohort of infants followed-up from birth up to 6 years of age:

- To determine the population attributable risk of recurrent wheeze and asthma from birth to sixth birthday associated with RSV clinical LRTI in the first two years of life.
- To determine the population attributable risk percent of recurrent wheeze and asthma from birth to sixth birthday associated with RSV clinical LRTI in the first two years of life.

Tertiary

In a cohort of infants followed-up from birth up to 6 years of age:

- To explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma.
- To explore the performance of asthma case definitions.

Study design**(Amended 15-DEC-2017)**

- Type of design: Prospective, epidemiological, interventional, multi-country, cohort study.
- Study population: A cohort of approximately 2400 infants will be included in the study at birth and followed-up to 2 years of age. **A maximum of 2400** children will be included in the extension and followed to 6 years of age.
- Type of study: self-contained.
- Data collection: Electronic case report form (eCRF).
- Sampling Schedule (**Primary study only**):
 - **Cord** blood samples collected from all the subjects participating in the study, at birth.
 - Nasal swabs collected from subjects with potential LRTI symptoms i.e. RTI symptoms with any signs of difficulty in breathing.
 - Serum collected from the first 2000 enrolled subjects, **excluding** those born at a gestational age of less than 36 weeks. **Refer to** Synopsis Table 1.

Synopsis Table 1 Blood sample collection (Primary Study only)

Time point	Seroprevalence sub-cohort: Number of allocated subjects*
2 months	200
4 months	200
6 months	250
12 months	300
18 months	450
24 months	600
Total	2000

Note: Only one blood sample will be withdrawn from each subject in this sub-cohort.

*Number of subjects selected per time point is calculated based on the required number of subjects for adequate analysis considering an accumulating drop-out of subjects. Actual numbers might be less due to drop-outs and exclusion of subjects with gestational age < 36 weeks.

- **Duration of the study: Approximately 2 years for each subject enrolled in the primary study and an additional (approximately) 3 to 4 years for each subject enrolled in the extension study.**
 - **Epoch 1 (Primary Study): Begins at birth (Visit 1) and ends at the age of approximately 2 years (Visit or contact at 2 years).**
 - **Epoch 2 (Extension Study): Begins with the first contact for the extension study at approximately age 2 years; ends with the contact at approximately the sixth birthday**

Synopsis Table 2 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch 1	Epoch 2
Primary	Approximately 2400	0 days - 2 years	x	
Extension	Maximum 2400	Approximately 2 years to 6 years of age		x

Surveillance Plan:

- ***Surveillance for RSV Lower Respiratory Tract Infection (RSV LRTI), and wheeze during the Primary Study period.***
- ***Surveillance for wheeze and asthma during the extension study period.***

Safety Follow-up:

Study Procedure related Serious Adverse Events (SAEs) to be reported throughout the study.

Discussion of study design (Amended 15-DEC-2017)

The multi-country design of both the primary and extension studies will provide robust, multinational estimates of the incidence of RSV-associated LRTI during the first two years of life and assess the amount of wheeze and asthma that may be attributable to those early RSV-LRTI infections.

The Primary study will determine the incidence and associated healthcare utilization of RSV-associated LRTIs in infants from birth up to **the age of** 2 years, **and assess** the performance of a new LRTI case definition and severity scale. Using a cohort representing the general population of newborn infants, the study is designed to identify RSV-associated LRTI cases at any severity, and will not be limited to those that require hospitalization and/or other essential medical care. This will provide a comprehensive and accurate measure of the overall burden of disease, whereas many previous case-control or hospital-based cohort studies were limited to the hospitalized cases of RSV-associated LRTI.

The association of other respiratory viruses with RSV-associated LRTI and/or severe LRTI, and the impact of potential RSV risk factors on the incidence and severity of RSV-associated LRTI will also be explored. In order to assess and adjust for other potential confounders in the various analyses, data on the subjects' demography and lifestyle factors will be collected at baseline, and followed up quarterly for changes, during the regular follow-up contacts.

At 2nd birthday (on completion of primary study or as soon as possible thereafter), surveillance for RSV LRTI will be stopped. ***Consenting, eligible subjects from participating study centers will be enrolled in the study extension and followed for the occurrence of wheeze and asthma. This will permit an assessment of the proportion of wheeze and asthma from birth until the sixth birthday that may be attributable to RSV LRTI and RSV infection in the first two years of life. It will also test the performance of the different asthma case definitions.***

Number of subjects
(Amended 15-DEC-2017)

Target enrollment for the primary study will be 2400 subjects to obtain approximately 1920 fully evaluable subjects at primary study completion.

Target enrollment for extension period will be maximum 2400 subjects (all subjects participating in the primary study, ***meeting*** all inclusion ***and no*** exclusion criteria ***for the extension***).

Endpoints for the Primary Study
(Amended 15-DEC-2017)

Co-Primary

- Occurrence of RSV as confirmed by RT-qPCR.
- Occurrence of different types of healthcare utilization*.

**Healthcare utilization includes primary, secondary and tertiary care settings such as self-care with over-the-counter [OTC] drugs, general practitioner (GP) visits, emergency room (ER) visits, hospital visits, etc.*

- Occurrence of LRTI/severe LRTI as classified by the LRTI case definition and severity scale.
- Occurrence of LRTI/severe LRTI as classified by the existing comparator LRTI case definition by WHO and by Nokes *et al.*

Secondary

- Levels of RSV neutralizing antibodies in the cord blood samples collected at birth.
- Levels of RSV neutralizing antibodies in the blood samples collected at 2, 4, 6, 12, 18 and 24 months.
- Occurrence of LRTI/severe LRTI cases as classified by the LRTI case definition and severity scale, in the subgroups of subjects recruited from Months 1-6 and 13-18.

Tertiary

- Occurrence of RSV and other respiratory viruses as confirmed by xTAG™ RVP Fast assay:
 - RSV
 - Influenza A, including subtypes H1 and H3
 - Influenza B
 - Parainfluenza virus type 1, 2, 3, and 4
 - Human Metapneumovirus
 - Rhinovirus
 - Adenovirus
 - Bocavirus
 - Coronavirus - 229E, OC43, NL63, HKU1
- RSV viral load as determined by RSV RT-qPCR.
- Occurrence of any symptom identified in cases of potential LRTI.
- Cord-blood sample collection variables including collection and storage times and temperatures.
- Occurrence of potential risk factors including complications at birth, family history of respiratory disease, living environment and household composition, breastfeeding, passive smoking and day-care attendance.

***Endpoints for
the extension
study***

***(Amended 15-
DEC-2017)***

Primary: None

Secondary:

- Occurrence of medically attended wheeze
- Occurrence of wheeze or asthma requiring hospital admission.
- ***Use of*** prescription medications for wheeze or asthma.

Tertiary:

- Number of children with asthma (case definition 1) at age 2, 3, 4, 5, and 6 years of age
- Number of children with asthma (case definition 2) at age 2, 3, 4, 5 and 6 years of age.
- ***Number of children with asthma (case definition 3) at 3, 4, 5 and 6 years of age.***
- Number of children with recurrent wheeze.

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LIST OF ABBREVIATIONS

ATP:	According-To-Protocol
CI:	Confidence Interval
CP:	Concept Protocol
DE:	Design Effect
eCRF:	electronic Case Report Form
ER:	Emergency Room
FN:	False Negative
FP:	False Positive
GCP:	Good Clinical Practice
GP:	General Practitioner
GSK:	GlaxoSmithKline
HIV:	Human Immunodeficiency Virus
ICF:	Informed Consent Form
ICH:	International Conference on Harmonization
IEC:	Independent Ethics Committee
ILI:	Influenza-like Illness
IRB:	Institutional Review Board
LAR:	Legally Acceptable Representative
LRTI:	Lower Respiratory Tract Infection
mL:	Milliliter
NH:	Northern Hemisphere
NPV:	Negative Predictive Value
OTC:	Over-The-Counter
P:	Percentile
PCR:	Polymerase Chain Reaction
PPV:	Positive Predictive Value
RDE:	Remote Data Entry
ROC:	Receiver Operating Characteristics
RSV:	Respiratory Syncytial Virus
RR:	Respiratory Rate
RTI:	Respiratory Tract Infection
RT-qPCR:	Reverse transcription-quantitative real time polymerase chain reaction

RVP:	Respiratory Viral Panel
SAP	Statistical Analysis Plan
SAE:	Serious Adverse Events
SDV:	Source Document Verification
SaO2:	Blood Oxygen Saturation
SBIR:	Randomization System on Internet
SH:	Southern Hemisphere
SPM:	Study Procedure Manual
TN:	True Positive
TP:	True Negative
URTI:	Upper Respiratory Tract Infection
US:	United States
WHO:	World Health Organization

GLOSSARY OF TERMS (Amended 15-DEC-2017)

According-To-Protocol (ATP) cohort:	This cohort will include all cases enrolled in the study who meet the criteria defined in the protocol for the considered analysis.
<i>Active Surveillance:</i>	<i>A contact with the subject's parent/LAR, initiated by the study physician or by a member of the study staff for the purpose of identifying an event of interest.</i>
Child in care:	A child who has been placed under the control or protection of an agency, organization, 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:	Data from which personal identifier information has been removed and replaced by a key. These data are not anonymised since a decode listing exists and it is therefore possible to identify the patient under certain circumstances by an authorised or legally appointed third party data custodian, or by the original holder of the data.
Cohort study:	A form of epidemiological study where subjects in a study population are classified according to their exposure status/disease and followed over time (prospective/ retrospective) to ascertain the outcome(s).
Commitment:	Agreement made with Regulatory Authorities as specific condition of regulatory approval and authorization, either made at the time of product approval or during the lifecycle of the approved product.
Eligible:	Qualified for enrollment into the study based upon strict adherence to inclusion/exclusion criteria.
Epidemiological study:	An observational or interventional study without administration of medicinal product(s) as described in a research protocol.
Epoch:	An epoch is a self-contained set of consecutive time points or a single time point from a single protocol. Self-contained means that data collected for all subjects at all time points within that epoch allows to draw a complete conclusion. Typical examples of epochs are retrospective data collection and prospective data collection, etc.

eTrack:	GSK Biologicals' tracking tool for clinical/epidemiological trials.
Exercise	Any moderate or vigorous physical activity performed by the child
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 9.4 for details on criteria for evaluability).
Health Burden:	Burden of the disease imposed on the study population in terms of incidence of the disease and associated healthcare utilization in any healthcare setting.
Healthcare settings (Healthcare Utilization):	Primary, secondary and tertiary care settings such as self-care with over-the-counter [OTC] drugs, general practitioner (GP) visits, emergency room (ER) visits, hospital visits, etc.
Interventional Human Subject Research:	Studies in which participants are administered medical care, medicinal products and/or medical/scientific procedures as described in a research protocol.
<i>Passive Surveillance:</i>	<i>A contact initiated by the subject's parent/LAR, notifying the study physician / site staff of the occurrence of an event of interest.</i>
Population Attributable Risk:	Population attributable risk is the portion of the incidence of a disease in the population (exposed and unexposed) that is due to exposure. It is the incidence of a disease in the population that would be eliminated if exposure were eliminated.
Population Attributable Risk Percent:	Population attributable risk percent is the percent of the incidence of a disease in the population (exposed and unexposed) that is due to exposure. It is the percent of the incidence of a disease in the population that would be eliminated if exposure were eliminated.
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study objectives.
Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organization of a study. The protocol usually also gives the background and rationale for the study, but these could be provided in other protocol referenced documents.
Self-contained study:	Study with objectives not linked to the data of another study.

Seroprevalence:	The total number of cases within the study population at a specific time that test positive for the disease based on blood serum specimens.
Site Monitor:	An individual assigned by the Sponsor who is responsible for assuring the proper conduct of epidemiological studies at one or more investigational sites.
Study population:	Sample of population of interest.
Sub-cohort:	A subgroup of the total cohort of study subjects for whom the planned study procedures are different from those planned for the other study subjects.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the epidemiological study or a person about whom some medical information has been recorded in a database.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Surveillance:	The ongoing systematic collection, collation, analysis, and interpretation of descriptive epidemiological health data on a specific disease. Surveillance can monitor incidence and/or prevalence, and/or inform about when and where health problems are occurring and who is affected.
xTAG:	xTAG is an universal tag system that allows easy optimization, development and expansion of molecular diagnostic assays

1. INTRODUCTION

1.1. Background (Amended 15-DEC-2017)

1.1.1. RSV

Respiratory syncytial virus (RSV) is an important cause of respiratory diseases. RSV is a highly contagious seasonal pathogen that is transmitted from person-to-person via direct or close contact with contaminated secretions, including respiratory droplets or fomites [Canducci, 2008]. RSV belongs to the family *Paramyxoviridae* and is one of the only two human pneumoviruses that have been currently recognized (the other being metapneumovirus). There are two major genetic RSV subtypes, A and B. These subtypes co-circulate, and the predominance of one over the other varies by year and geographic location [Graham, 2011].

RSV is found across the globe with incidences varying between countries and regions and even within a country or a region over multiple seasons. In areas of the inner tropics with perennial high rainfall, RSV seasonality is less clear-cut. Countries close to the equator have a distinct pattern of RSV transmission, with most cases appearing in one half of the year, but not in the other. But neither temperature nor rainfall appears to be the main determinant of the timing of these outbreaks [Weber, 1998]. Seasonal epidemics of RSV occur most frequently in the cold season in regions with temperate and Mediterranean climates and in the rainy season in tropical countries with seasonal rainfall. Roughly the RSV season runs from October to March in the temperate areas of the Northern Hemisphere (NH) and from May to September in the Southern Hemisphere (SH). But the exact timing of RSV circulation varies by geographic location and also by year [Mullins, 2003; Weber, 1998; Law, 2002].

RSV infections result in high rates of hospitalization in infants, a large disease burden among infants and young children, and excess mortality in the elderly [Graham, 2011]. RSV infects 50-70% of infants during the first year of life and essentially all children will have had an RSV infection by their third birthday [Hall, 2004]. In the early months of life, infants may have protection against RSV through the maternal antibodies transferred to them during pregnancy. High titers of maternally derived RSV-neutralizing antibodies [Roca, 2002] have been shown to be inversely associated with the incidence of RSV associated-acute LRTI during the first 6 months of life, whereas incomplete transfer of maternally derived RSV-neutralizing antibodies has been implicated in the increased risk of severe RSV infection in preterm infants [de Sierra, 1993]. Previous infection with RSV does not prevent subsequent infections. Therefore, re-infection with RSV occurs throughout an individual's lifetime and is common in all age groups [Simoes, 1999; Krilov, 2011].

Globally, it is estimated that in children below 5 years of age, RSV causes >30 million acute lower respiratory infections (LRTI) each year resulting in more than 3 million hospitalizations, making it the most common cause of hospitalization for LRTI or all cause from any single pathogen in this age group [Nair, 2010]. RSV is the most common cause of bronchiolitis and pneumonia in children less than 1 year of age worldwide, with

highest risk in children less than 6 months of age [Nair, 2010; Langley, 2011]. A United States (US) based study conducted in children less than 18 years of age, found RSV in 58% of the hospitalizations for LRTI compared to parainfluenza virus 1-3 in 17%, influenza A/B in 21%, adenovirus in 2% and rhinovirus in 2% [Henrickson, 2004]. On a community level, a US day-care based study assessing the causes of respiratory tract infection (RTI) in children less than 1 year of age found that RSV infection was present in 11% of the cases, compared to rhinovirus in 35%, adenovirus in 22%, human coronavirus in 15%, parainfluenzavirus in 12%, human meta-pneumovirus in 4% and influenza A in 2% [Fairchok, 2010]. This difference between community and hospital proportions of RSV related infections indicates the propensity of RSV to cause more serious cases of RTI, mainly LRTI, compared to other viruses.

Viral co-infections with RSV are also common. Evidence about the severity of co-infections compared to single viral infections is conflicting, as the severity of co-infection may depend on the pathogens involved [Fairchok, 2010]. In a study conducted on dual respiratory infections it was concluded that dual viral RTIs had higher hospitalization rates than single infections [Drews, 1997]. Some examples of viruses which may exist as co-pathogens with RSV include, human Metapneumoviruses, human Bocaviruses and human Coronaviruses [Canducci, 2008].

Currently available estimates of RSV disease burden are most often centred on healthcare usage, particularly RSV-associated hospitalizations. Also, there is no widely supported guideline from regulatory authorities or transnational expert bodies on a case definition of RSV-associated LRTI in children. As a result, prospective, cohort-based epidemiological studies using a consistent case definition for RSV-associated LRTI are rare and there is no obvious case definition to be used uniformly as the outcome measure in clinical trials. Although the hospitalized cases represent the most severe cases of RSV disease, variation between medical practice policies, quality of the healthcare system and access to adequate healthcare can all influence whether a subject with RSV-associated LRTI is hospitalized or not. Therefore, hospitalization is not an ideal determinant of the disease severity and a more objective scoring system for the disease severity is needed. Several studies have attempted to develop a severity score or a model, but this has not lead to a validated model, accepted as the universal gold standard [Nokes, 2008].

The World Health Organization (WHO) has recently updated its RSV-associated LRTI case definition [Modjarrad, 2016]. The latter includes clinical features considered to be objective, easily standardized, generalizable across settings, and generally accepted markers of severe or very severe RSV disease.

1.1.2. Asthma

Infant respiratory viral infection and childhood asthma are the most common acute and chronic diseases of childhood, respectively. Common clinical features and links between these diseases have long been recognized, with early-life RSV LRTIs being strongly associated with increased asthma risk.

Although asthma is probably a heterogeneous disease or syndrome, three factors and/or events consistently emerge for their ability to significantly influence asthma inception in the first decade of life: immune response aberrations, which appear to be defined best by the concept of cytokine dysregulation; LRTIs, in particular RSV; and some form of gene–environment interaction that needs to occur at a critical time-period in the development of the immune system or the lung [Lemanske, 2002].

Population-level contribution of RSV LRTIs is best demonstrated by the population-attributable risk for asthma after these early-life LRTIs. Among infants, the cumulative incidence of LRTI is approximately 18 to 32% in the first year of life and approximately 9 to 17% in the second year of life. Among infants with LRTI, the prevalence of RSV can be as high as 80%, especially in the first 3 months of life. Thus, the phenotype of asthma after infant RSV LRTI accounts for up to 31% of early childhood asthma, with a population-attributable risk estimated at about 13% in several diverse populations [Feldman, 2015].

Numerous longitudinal studies have demonstrated this first important line of evidence that RSV LRTIs precede the development of asthma. Several birth cohort studies also demonstrate a dose–response relationship between infant respiratory viral infection severity and asthma risk, with increasing infant infection severity associated with greater childhood asthma risk and asthma severity [Feldman, 2015].

The Childhood Origins of Asthma (COAST) study was developed to determine and define the importance of these three factors on asthma pathogenesis and to test different asthma case classifications [Lemanske, 2002; Lemanske, 2005; Bisgaard, 2011; Jackson, 2008; Jackson, 2012; Bonnelykke, 2015]. These studies have used similar albeit slightly different case definitions on asthma diagnosis. The International Study of Asthma and Allergies in Childhood (ISAAC) takes a slightly different parent-based questionnaire approach toward assessing asthma prevalence in young children.

These definitions are widely used in epidemiological literature of asthma etiology in children. The Bisgaard definition [Bisgaard, 2011] has multiple criteria that are assessed by the physician from records. Therefore the advantage of this definition is that the same objective criteria are applied to ascertain each case of asthma. However, its application may be challenging in some settings if routine medical records do not fully capture the necessary information in source. The Jackson definition [Jackson, 2008] includes the criteria of health care provider (HCP) diagnosis of asthma and prescription of β_2 agonists. This definition is therefore influenced by any differences in local practice of asthma diagnosis and management of wheeze. However, its advantage is that it captures the reality of diagnosis and treatment. ISAAC uses simple, robust, primarily symptom-based questions that are administered solely to the parents. ISAAC's strength is that its definitions are not affected by differences in local asthma treatment practices; however, its lack of clinical case confirmation may decrease its precision. The ISAAC questionnaire has been used to assess pediatric asthma prevalence in at least 42 countries across the globe [Pearce, 2007].

1.2. Rationale for the study (Amended 15-DEC-2017)

This study aims to assess the incidence and associated healthcare utilization of RSV-associated, suspected LRTI in a general population of infants from birth up to 2 years of age, and also to assess the accuracy of a newly developed LRTI case definition and severity scale, compared to two existing scales developed by *the* World Health Organization (WHO) [[Modjarrad, 2016](#)] and [[Nokes, 2008](#)].

This study will also assess the extent to which RSV LRTI in early life (up to 2 years of age) accounts for the total burden of wheeze and asthma up to the sixth birthday, and test the performance of 3 asthma case definitions – those of Bisgaard, Jackson, and ISAAC.

The LRTI case definition and severity scale to be used in this study has been developed as a consensus model derived from existing definitions in the literature, and existing guidelines by GlaxoSmithKline (GSK) clinical staff, taking into account the advice of a panel of international RSV experts.

Considering asthma case definitions, this study anticipates that Bisgaard will be more specific and Jackson more sensitive. Although it is likely that the ISAAC symptom-based case definitions will be the most sensitive and least specific of all three definitions [[Yang, 2011](#)], its incorporation will facilitate wider international results comparisons. Hence, this study will assess the performance of *the different* case definitions since this will help planning for phase 3 clinical trials of RSV vaccines.

If, in future, a maternal and/or pediatric RSV vaccine is introduced, results of this study may be used to evaluate post-vaccination incidence reduction (in RSV LRTI and/or wheeze and asthma) and the vaccine's potential public health impact.

2. OBJECTIVES (AMENDED 15-DEC-2017)

2.1. Objectives for the Primary Study (first 2 years)

2.1.1. Co-Primary objectives

In a cohort of infants followed-up from birth up to 2 years of age:

- To determine the total health burden* of RSV-associated RTI with a suspicion of involvement of the lower respiratory tract..

**Health burden refers to burden of the disease imposed on the study population in terms of incidence of the disease and associated healthcare utilization in any healthcare setting.*

- To assess the performance of the LRTI case definition and severity scale for RSV associated cases.

Refer to Section 4 for the list of clinical parameters included under each LRTI case definition under LRTI case definitions for data analysis.

Refer to Section 9.1.1 for the definition of the co-primary endpoints.

2.1.2. Secondary objectives

In a cohort of infants followed-up from birth up to 2 years of age;

- To determine the total health burden of non-RSV-associated RTI with a suspicion of involvement of the lower respiratory tract.
- To evaluate the association between RSV-associated LRTI, RSV-associated severe LRTI and RSV neutralizing antibodies in the baseline cord blood samples collected from subjects, at birth.
- To determine the prevalence of RSV infection, at 2, 4, 6, 12, 18 and 24 months in a randomly selected sub-cohort of subjects by assessing antibodies in serum.
- To assess the between calendar year variability in the incidence rates of RSV-associated LRTI.

Refer to Section 9.1.2 for the definition of the secondary endpoints *for the primary study*.

2.1.3. Tertiary objectives

In a cohort of infants followed-up from birth up to 2 years of age:

- To explore the association of co-infections with the incidence of RSV-associated LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ respiratory viral panel (RVP) fast assay.
- To explore the association of RSV and other respiratory viruses with the incidence of LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.
- To explore the association of RSV viral load with the incidence of RSV-associated RTI, LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale).
- To explore the impact of changes to the symptoms and threshold levels of symptoms in the LRTI case definition and severity scale.
- To assess the impact of potential RSV risk factors (e.g. complications at birth, family history of respiratory disease, living environment and household composition, breast feeding, passive smoking, day care attendance) on the incidence and severity of RSV-associated LRTI.
- To explore the impact of variations in cord-blood sample collection variables on the stability of test results.
- To assess the natural decay of maternal antibody levels using a single follow-up blood sample collected from a randomly selected sub-cohort of subjects at 2, 4, 6, 12, 18 and 24 months.

Refer to Section 9.1.3 for the definition of the tertiary endpoints *for the primary study*.

2.2. Objectives for the Extension study (Approximately 2 years of age to 6th birthday)

2.2.1. Primary objectives

None

2.2.2. Secondary objectives

In a cohort of infants followed-up from birth up to 6 years of age:

- To determine the population attributable risk of recurrent wheeze and asthma from birth to sixth birthday associated with RSV clinical LRTI in the first two years of life.
- To determine the population attributable risk percent of recurrent wheeze and asthma from birth to sixth birthday associated with RSV clinical LRTI in the first two years of life.

Refer to Section 9.2.2 for the definition of the secondary endpoints for the extension study.

2.2.3. Tertiary objectives

In a cohort of infants followed-up from birth up to 6 years of age:

- To explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma.
- To explore the performance of asthma case definitions.

Refer to Section 9.2.3 for the definition of the tertiary endpoints for the extension study.

3. STUDY DESIGN OVERVIEW (AMENDED 15-DEC-2017)

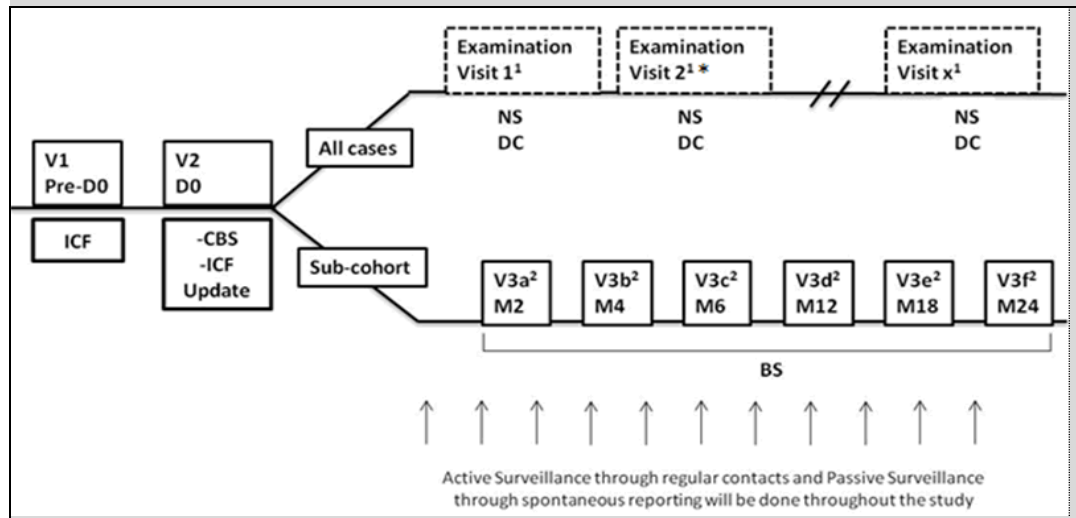
3.1. Design Summary

In the primary study, subjects were to be followed-up from birth up to the age of 2 years for RSV LRTI. *Episodes of wheeze and diagnoses of asthma were also documented. Refer to Figure 1.*

In order to determine the population attributable risk and population attributable risk percent of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life, and to explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma, the study will be extended and subjects will be followed-up from the age of 2 years up to 6 years to detect episodes of wheeze and diagnoses of asthma.* *Refer to Figure 2.*

*Note: The procedures related to primary study surveillance of RSV LRTI will not be extended into the extension period. The extension period will survey for occurrence of wheeze and asthma.

Figure 1 Study design diagram for the primary study (from birth up to the age of 2 years for detection of RSV LRTI)



V = Visit; D = Day; M = Month

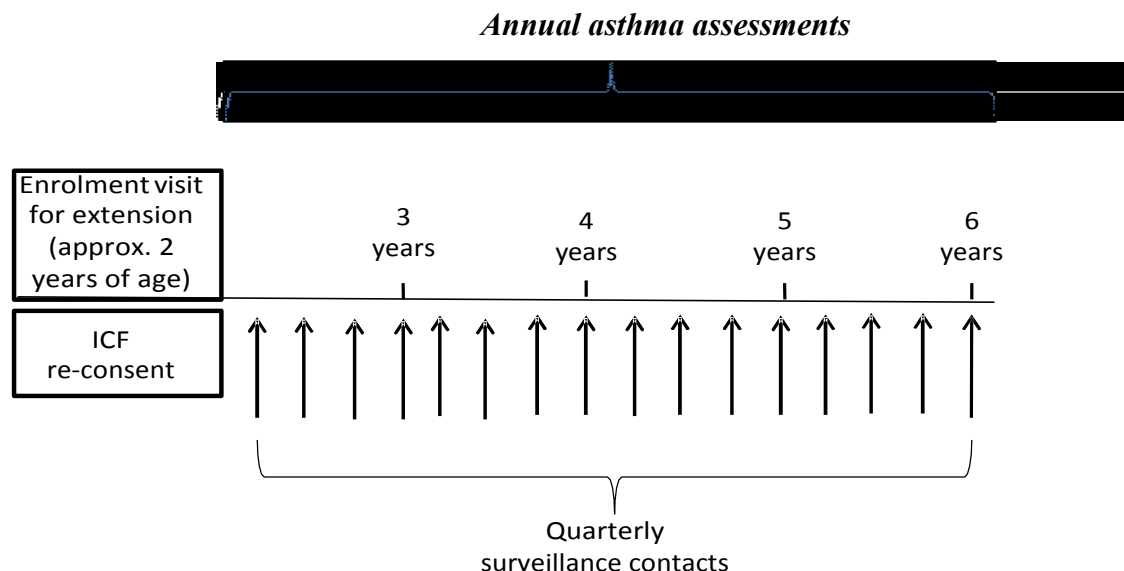
ICF = Informed Consent Form; CBS = Cord Blood Sample; NS = Nasal Swab; DC = Diary Card; BS = Blood Sample

¹ An examination visit (off-site/site) is to occur for new or worsened, potential LRTI case as identified during active or passive follow-up contact. The examination visit needs to occur as soon as possible and no later than 72 hours after the potential LRTI was identified during a follow-up contact. Each subject may have none, single or multiple examination visits over the course of the study.

² A sub-cohort of subjects will be randomized for a single blood draw to occur at one of the six possible time points and will have a Visit 3 corresponding to this time point (if still participating at that time); either V3a at 2, V3b at 4, V3c at 6, V3d at 12, V3e at 18 or V3f at 24 months.

* All follow up procedures associated with V2 (e.g. ICF) must be performed within 5 working days after birth.

Figure 2 Study design diagram for the extension (from the age of approximately 2 years up to the 6th birthday) for the detection of episodes of wheeze and diagnosis of asthma



Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6.3), are essential and required for study conduct.

- Type of design: Prospective, epidemiological, interventional, multi-country, cohort study.
- Study population: A cohort of approximately 2400 infants will be included in the study at birth and followed-up up to 2 years of age. ***A maximum of*** 2400 children will be included in the extension and followed up to 6 years of age.
- Type of study: Self-contained.
- Data collection: Electronic case report form (eCRF).
- Biological samples (***Primary Study only***):
 - ***Cord Blood collected from all participating subjects, at birth.***
 - ***Nasal swabs collected from subjects with potential LRTI.***
 - Serum collected from a ***sub-cohort of subjects, excluding*** those born at a gestational age of less than 36 weeks. Sampling time points for this sub-cohort will be randomly allocated as specified in [Table 1](#).

Table 1 Blood sample collection

Time point	Seroprevalence sub-cohort Number of allocated subjects*
2 months	200
4 months	200
6 months	250
12 months	300
18 months	450
24 months	600
Total	2000

Note: Only one blood sample will be withdrawn from each subject in this sub-cohort.

*Number of subjects selected per time point is calculated based on the required number of subjects for adequate analysis considering an accumulating drop-out of subjects. Actual numbers might be less due to drop-outs and exclusion of subjects with gestational age <36 weeks.

- ***Duration of the study: Approximately 2 years for each participant enrolled in the primary study and an additional (approximately) 3 to 4 years for each participant enrolled in the extension study.***
 - Epoch 1 (Primary Study): Begins at birth (Visit 1) and ends at the age of approximately 2 years (Visit or contact at 2 years).
 - ***Epoch 2 (Extension Study): Begins with the first contact for the extension study (at approximately age 2 years); ends with the contact at approximately the sixth birthday. Table 2 presents the study groups and epochs foreseen in the study.***

Table 2 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch 1	Epoch 2
Primary	Approximately 2400	0 days - 2 years	x	
Extension	Maximum 2400	Approximately 2 years to 6 years		x

* Data collection will also occur from medical charts retrospectively for those who have a gap period between the end of the primary study and providing re-consent for the extension.

Surveillance Plan:

- ***Surveillance for RSV Lower Respiratory Tract Infection (RSV LRTI), wheeze and asthma during the Primary Study period.****
- Surveillance for wheeze and asthma during the extension study period.

**** Note that discussion of additional primary study visit details has been moved to (new) APPENDIX D. Discussion of details regarding Surveillance for RSV Lower Respiratory Tract Infection (RSV LRTI), wheeze and asthma during the primary study period have been moved to (new) APPENDIX E.***

Safety Follow-up:

- ***Study procedure related Serious Adverse Events (SAEs) to be reported throughout the study.***

3.2. Discussion of study design

The multi-country design of both the primary and extension studies will provide robust, multinational estimates of the incidence of RSV-associated LRTI during the first two years of life and assess the amount of wheeze and asthma that may be attributable to those early RSV-LRTI infections.

3.2.1. Primary Study

The Primary study will determine the incidence and associated healthcare utilization of RSV-associated LRTIs in infants from birth up to the age of 2 years, **and assess** the performance of a new LRTI case definition and severity scale. Using a cohort representing the general population of newborn infants, the study is designed to identify RSV-associated LRTI cases at any severity, and will not be limited to those that require hospitalization and/or other essential medical care. This will provide a comprehensive and accurate measure of the overall burden of disease, whereas many previous case-control or hospital-based cohort studies were limited to the hospitalized cases of RSV-associated LRTI.

The association of other respiratory viruses with RSV-associated LRTI and/or severe LRTI, and the impact of potential RSV risk factors on the incidence and severity of RSV-associated LRTI will also be explored. In order to assess and adjust for other potential confounders in the various analyses, data on the subjects' demography and lifestyle factors will be collected at baseline, and followed up quarterly for changes, during the regular follow-up contacts.

3.2.2. Extension Study

At 2nd birthday (on completion of primary study or as soon as possible thereafter), surveillance for RSV LRTI will be stopped. ***Consenting, eligible subjects from participating study centers will be enrolled*** in the study extension ***and followed*** for the occurrence of wheeze and asthma (***applying 3 different asthma case definitions***). ***This will permit an assessment of the proportion of*** wheeze and asthma from birth until the sixth birthday ***that may*** be attributable to RSV LRTI and RSV infection in the first two years of life. It will also test the performance of the ***different*** asthma case definitions (Table 5).

4. CASE DEFINITIONS (AMENDED 15-DEC-2017)

4.1. Primary Study

DEFINITIONS FOR LRTI SURVEILLANCE:

During the surveillance for LRTI, a case is defined as;

A child suffering from a potential LRTI.

Potential LRTI:

Suspected involvement of the lower respiratory tract if a child with clinical signs of RTI, defined as either a runny nose, blocked nose or cough, is believed to have difficulties in breathing in the opinion of the investigator/study staff, based on the symptoms reported by the parent(s)/LAR(s) during the surveillance phone contacts. Difficulty in breathing as suspected by the investigator/study staff through reported symptoms will then be objectively confirmed by the investigator/study staff by means of pulse oximetry and RR at an examination visit. If at least one of these parameters is outside of the pre-defined normal range (Refer to [Table 3](#)), the case will be considered as an LRTI for the surveillance part of the study.

A subject is considered to have difficulty in breathing if he/she shows signs of:

- Wheezing or stridor
- Tachypnea
- Flaring (of nostrils)
- Chest indrawings
- Apnea

Table 3 Normal ranges for RR and SaO2

RR	SaO2
$\leq 60/\text{min}$ for a child below 2 months of age $\leq 50/\text{min}$ for a child aged 2 - 11 months $\leq 40/\text{min}$ for a child aged 12 - 24 months	$\geq 95\%$, independent of the age of the child

RR= Respiratory Rate; SaO2= Blood Oxygen Saturation; min = minutes

During the surveillance for LRTI, any potential LRTI that is not found to meet the criteria for an LRTI may need a subsequent objective assessment by the investigator/study staff.

Note: The investigator/study staff will do an initial assessment of a case reported for the examination visit as either RTI or LRTI, only to determine further study activities. The final classification of all the cases reported as either, RTI, LRTI or severe LRTI will be done during statistical analyses as per the definitions given below.

CASE DEFINITIONS FOR DATA ANALYSIS:**LRTI case definition and severity scale**

During the analysis of the study on the full dataset available for each case, all cases identified during the surveillance for LRTI will be definitively classified as either RTI, LRTI or severe LRTI according to the LRTI case definition and severity scale described in [Table 4](#), and also according to the classification of WHO and the classification used by Nokes *et al* [[Modjarrad](#), 2016; [Nokes](#), 2004] (see below).

Table 4 LRTI case definition and severity scale

RTI	LRTI	severe LRTI
Runny nose, OR Blocked nose, OR Cough	Child with RTI AND SaO ₂ < 95%*, OR RR increase: > 60/min < 2m of age > 50/min 2-11m of age > 40/min 12-24m of age	Child with LRTI AND SaO ₂ < 92%*, OR Difficulty breathing leading to: Irritability/agitation, OR Lethargy/sleepiness, OR Severe chest indrawing, OR Reduced/no vocalization, OR Apnoea > 20 sec, OR Cyanosis, OR Stop feeding well/dehydration

* Measured by oximeter. RR= Respiratory Rate; SaO₂= Blood Oxygen Saturation; m = months.

RTI = Respiratory Tract Infections; LRTI = Lower Respiratory Tract Infections

Case definition of LRTI and severe LRTI by Nokes et al.

LRTI is diagnosed when a child has a history of acute cough or difficulty in breathing and ≥ 1 of the following:

- Fast breathing for age (≥ 60 breaths/minimum if the child is < 2 months old or ≥ 50 breaths/minimum if the child is 2–11 months old)
- Indrawing, or
- Low oxygen saturation (< 90%) by pulse oxymetry or inability to feed (prostration or unconsciousness), when accompanied by a clinical diagnosis of LRTI or bronchiolitis.

Severe LRTI was diagnosed when a child had LRTI meeting second and/or third bullet above.

Case definition of LRTI by WHO (2015) [[Modjarrad, 2016](#)]

LRTI is diagnosed when a child <5 years presents with cough and/or difficulty in breathing has the following symptoms:

- Fast breathing, (> 60 per minute in a child < 2 months of age, > 50 per minute in a child 2 to 11 months of age and; > 40 per minute in a child 12 to 59 months of age), or
- Oxygen saturation < 95% by pulse oximetry.

Severe LRTI is diagnosed when a child has LRTI and 1 or more of the following features of severe disease:

- Oxygen saturation < 93% by pulse oximetry.
- Lower chest wall in-drawing.

RSV infection:

Detection of RSV by reverse transcription-quantitative real time polymerase chain reaction (RT-qPCR) in swab sample collected within 72 hours of first identification of a potential LRTI by the investigator/study staff during an active or passive surveillance study contact.

Start date of the LRTI case:

The start date of the case is defined as the point at which the LRTI was first identified as a potential LRTI by the investigator/study staff during an active or passive surveillance study contact, and if the LRTI is subsequently confirmed by the investigator/study staff (according to the LRTI case definition and severity scale for surveillance) during the examination visit (off-site/site).

End date of the LRTI case:

End date of the case is defined as the point at which the subject is considered symptom-free of difficulty in breathing, based on the data provided by the parent(s)/LAR(s) in the diary card and any additional data obtained from healthcare providers involved in management of the case.

New case of LRTI:

A new, single case of LRTI is any occurrence of an LRTI case meeting the LRTI case definition and severity scale with an interval of at least 7 symptom free days since the last episode of LRTI that was diagnosed.

4.2. *Extension Study*

[Table 5](#) presents the case definitions of wheeze and asthma. *The Bisgaard and Jackson case definitions have been updated so as to better account for advances in asthma treatment options while still preserving the case definitions' core intent.*

Table 5 Case definitions of wheeze and asthma (Amended 15-DEC-2017)

	Definition	Reference
Episode of reported wheeze	Parents report a whistling sound associated with labored breathing	NA
Medically attended wheeze	An episode of health care provider confirmed wheeze	NA
Wheeze or asthma requiring hospital admission	An episode of health care provider confirmed wheeze requiring hospital admission	NA
Recurrent wheeze	This will be defined as four or more episodes of wheeze in one year. These episodes may be parent reported wheeze or health care provider confirmed wheeze.	[Morgan 2005]
Asthma case definition 1	Asthma will be defined at annual review based on the documented presence of one or more of the following characteristics in the previous year: (1) provider diagnosis of asthma (2) Use of a short acting beta agonist (e.g., albuterol) for coughing or wheezing episodes as prescribed by a health care provider (3) use of a daily controller medication (4) step-up plan including use of a short acting beta agonist (e.g., albuterol) or short-term use of inhaled corticosteroids during illness (5) use of oral and/or systemic steroids for asthma exacerbation	Based on [Jackson, 2008]
Asthma case definition 2	Asthma will be defined at annual review based on parent interview and review of medical records with the presence of all of the following: (1) recurrent episodes of troublesome lung symptoms (wheeze or whistling sounds, breathlessness, or recurrent troublesome cough severely affecting the well-being of the infant occurring with a frequency of 5 episodes within 6 months period or 4 weeks of consecutive symptoms); (2) symptoms typical of asthma (recently proposed to be termed multitrigger wheeze; e.g., exercise-induced symptoms, prolonged nocturnal cough, recurrent cough outside the common cold, and symptoms causing waking at night); (3) intermittent rescue use of inhaled β_2 -agonist; (4) symptoms controlled by daily inhaled corticosteroids	Based on [Bisgaard, 2011]
Asthma case definition 3	In the past 12 months: four or more episodes of wheeze AND either one or more nights of sleep disturbance from wheeze per week, or wheeze that was severe enough to limit the child's speech to only one or two words at a time between breaths	Based on [Wong 2013]

5. STUDY POPULATION (AMENDED 15-DEC-2017)

5.1. Number of subjects/centres

Target enrollment for the primary study will be 2400 subjects to obtain approximately 1920 fully evaluable subjects at primary study completion. Target enrollment for extension period will be *a maximum of* 2400 subjects. (*All subjects participating in the primary study **whose parent(s)/LAR(s) give re-consent for the extension period and who fulfill all relevant inclusion/exclusion criteria may be followed in the extension.***)

5.2. Sub-cohorts

The primary study includes a sub-cohort for blood sampling. Refer to [Table 6](#).

Table 6 Sub-cohort

Sub-cohort name	Description	Estimated number of subjects
Seroprevalence	Blood sample (3.5 mL) collection at a randomly* allocated time point (2, 4, 6, 12, 18, 24 months)	2400

mL = milliliter

*Randomization will be performed using randomization system on internet (SBIR).

The overview of the recruitment plan has been moved to [APPENDIX C](#).

5.3. Inclusion criteria for enrolment

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

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

5.3.1. Primary study (from birth up to the age of 2 years)

Before birth:

- Subject whose parent(s)/LAR(s), in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., sample collection, availability for examination visits).
- Written informed consent (*or witnessed thumb printed consent in case of an illiterate subject*) (including consent to obtain a cord blood sample at birth) obtained from the parent(s)/LAR(s) of the subject

After Birth:

- Subject for whom updated and re-signed informed consent and confirmation of eligibility is available not later than 5 working days after birth.
- Cord blood sample collection of at least 3 mL, at birth.

5.3.2. Extension Study (from the age of approximately 2 years up to the 6th birthday)

- *Subject is enrolled at a study site that is participating in the extension period follow-up.*
- Subject whose parent(s)/LAR(s), in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., availability for quarterly contacts).
- Written informed consent (*or witnessed thumb printed consent in case of an illiterate subject*) obtained from the parent(s)/LAR(s) of the subject.
- Previous participation in the primary study (from birth up to the age of 2 years).

5.4. Exclusion criteria for enrollment

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

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

5.4.1. Primary study (from birth up to the age of 2 years)

Before birth:

- Subject expected to become Child in care

Please refer to the [GLOSSARY OF TERMS](#) for the definition of child in care.

- Subjects whose parent(s)/LAR(s) are below the age of 18 or the legal consenting age of the respective country if this is higher.

After Birth:

- Child in care.

Please refer to the [GLOSSARY OF TERMS](#) for the definition of child in care.

- Newborn with a gestational age of less than 28 weeks.
- Subjects with any congenital condition that will require an expected postnatal stay in hospital of more than 12 consecutive weeks.
- Subjects with major congenital defects or serious chronic illness limiting life expectancy to less than 5 years.
- Subjects with any confirmed or suspected immunosuppressive or immunodeficient condition (including positive infection with human immunodeficiency virus [HIV]), based on medical history, physical examination or positive test result.

5.4.2. Extension Study (from the age of approximately 2 years up to the 6th birthday)

- Child in care.

6. CONDUCT OF THE STUDY**6.1. Regulatory and ethical considerations, including the informed consent process**

The study will be conducted in accordance with the International Conference on Harmonization (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 Harmonized 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 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 Biologicals will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written (or witnessed thumb printed consent in case of an illiterate subject) informed consent must be obtained from each subject's parent(s)/LAR(s) or the impartial witness, as appropriate, prior to participation in the study.

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

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the

investigator with the assistance of the Sponsor’s representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

6.2. Subject identification

Subject numbers will be assigned sequentially to subjects whose parent(s)/LAR(s) have consented for their child to be included in the study, according to the range of subject numbers allocated to each study center.

6.3. 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.

6.4. Outline of study procedures (Amended 15-DEC-2017)

6.4.1. Primary Study (birth to 2 years of age)

Table 7 presents the list of study procedures pertaining to the primary study (from birth up to the age of 2 years).

Table 7 List of study procedures for the primary study (from birth up to the age of 2 years)

Epoch 1	Prospective data collection				
Visit/Contact	Visit 1	Visit 2	Visit 3	Surveillance contact ¹	Examination visit ²
Time points	Pre-Day 0	Post-Day 0 ⁷	As randomized ⁴	Regular ⁵	Unscheduled
Informed Consent	•				
Check inclusion/exclusion criteria applicable before birth	•				
Subject number allocation and registration in SBIR-Screening	•				
Collection of expected birth date	•				
Collection of cord blood sample (up to approximately 10 mL)		•			
Check inclusion/exclusion criteria applicable after birth and re-sign-off ICF		•			
Collection of family medical history, demographic and lifestyle characteristics		•			
Collection of data on child at birth and delivery (incl. medical care provided immediately after birth)		•			
Registration in SBIR-enrollment		•			
Distribution of diary cards to record symptoms and healthcare utilization (as needed)		0	0		x
Obtain blood sample at randomized time point (approximately 3.5 mL) from a sub-cohort of subjects			•		
Ascertain RTI symptoms and potential difficulty in breathing and assessment of need for examination visit (off-site/site)				•	
Recording of data on changes to baseline medical, demographic and lifestyle characteristics data E.g. Chronic diseases and changes to living conditions				• (quarterly)	
Examination during (off-site/site) visit and collection of all disease symptoms (including RR and pulse oximetry)					•
Data collection on, disease course and healthcare utilization before and during the examination visit (off-site/site) ³					•
Collection of nasal swab samples					•
Return of diary cards at completion or at case resolution ⁶				0	
Confirmation and expansion of data on healthcare utilization as recorded in the diary card occurring after the examination visit (off-site/site) ³				•	
Recording of SAEs related to study procedure throughout study period		•	•	•	•

Epoch 1	Prospective data collection				
Visit/Contact	Visit 1	Visit 2	Visit 3	Surveillance contact ¹	Examination visit ²
Time points	Pre-Day 0	Post-Day 0 ⁷	As randomized ⁴	Regular ⁵	Unscheduled
Diary card transcription into eCRF				•	•
End of primary study after 2 years follow-up				•	

• 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.

SBIR = Randomization System on Internet; mL = milliliter; ICF = Informed Consent Form

RTI = Respiratory Tract Infection; RR = Respiratory Rate

eCRF = electronic Case Report Form; SAE = Serious Adverse Events

¹The surveillance contact consists of the following:

Active surveillance: Follow-up contacts will be made by the investigator/study staff with the parent(s)/LAR(s) of the subjects at predefined intervals (to be confirmed based on the local settings) from Visit 2 up to study conclusion.

Passive surveillance: Follow-up contacts made by the parent(s)/LAR(s) with the study investigator/study staff in case of development of RTI symptoms or increase in severity of existing symptoms after the previous reporting of the initial symptoms.

²The examination visit (off-site/site) will be applicable only for subjects with a suspicion of LRTI.

³Whenever required healthcare utilization data should be completed or confirmed with the healthcare provider involved in the management of the case.

⁴Selected subjects will be randomized to the time point at which a single blood sample needs to be collected; 2, 4, 6, 12, 18 or 24 months. The sample can be collected between the intervals of ± 1 week. E.g. Month 2 ± 1 week, Month 4 ± 1 week. Randomization will be done using a second phase application of SBIR.

⁵Refer to [APPENDIX E](#) for the active contact schedule.

⁶Diary card can be collected by mail or in person depending on local best practice.

⁷Visit 2 can take place anytime within 5 working days after birth.

Refer to Section 6.5 for additional details.

6.4.2. Extension Study (approximately 2 years of age up to the 6th birthday)

Table 8 presents the list of procedures for the extension study.

Note that although extension study enrolment ideally occurs at the time of the subjects' second birthdays, enrolment into, and first contact for, the extension study may take place at any time between the end of the primary study and the subject's 6th birthday.

After the first extension study contact, subjects will be contacted around the time of each subject's birthday and (approximately) quarterly during the intervals between (a) first contact and next birthday; (b) subsequent birthdays.

Site staff may contact subjects more frequently than indicated in [Table 8](#) if **consistent with the language of the local ICF** and considered necessary by the study physician. Parents will be encouraged to seek a clinical assessment of their child in between quarterly contacts and/or during quarterly contacts whenever their child develops wheezing and/or breathing difficulties.

Refer to Section 6.6 and the SPM for additional details.

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Table 8 List of extension study procedures (from the age of approximately 2 years up to the 6th birthday) (Amended 15-DEC-2017)

Epoch 2	Data collection		
Visit/Contact	First Contact ^a	Quarterly Contact ^{b, c}	Annual contact ^{c, d}
Time points	Extension study enrolment		Around the 2 ^d , 3 rd , 4 th , 5 th , and 6 th birthdays (+ 3 months)
Perform / document results of these procedures...			
Informed consent for extension	•		
Check inclusion/exclusion criteria for extension	•		
Collect demographic data for extension	•		
Collect data on potential symptoms and risk factors for wheeze and asthma	•	• (applies to Version 1.0 of the quarterly contact script)	• ^d
Record episodes of wheeze, asthma, and troublesome lungs	•	•	• ^d
Collect data about medication used to treat asthma, wheeze, or troublesome lung symptoms	•	•	• ^d
Review / assess information about asthma diagnoses and medication use based on medical record abstraction and subject information over approximately the past 12 months or longer	• ^e		• ^e
Interim analysis after 4th birthday; Final analysis after 6th birthday			•
Investigator sign-off on eCRF before analysis			• ^f
Study conclusion for extension			• ^g
... and enter data into these eCRF modules			
	• First Contact • Annual ^a	• Quarterly	• Quarterly • Annual

• indicates a study procedure that requires documentation in the individual eCRF.

^a At the time of first contact, if delayed, all missed annual assessments of asthma will be completed retrospectively by the Investigator.

^b **At approximately 3, 6 and 9 months (+/- 1.5 months), between consecutive birthdays. There should be at least one month between each Quarterly Contact**

^c **Both Quarterly parent/LAR interview AND Annual investigator review/assessment data to be collected and reported.**

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^d **To accurately complete investigator-targeted asthma questions, up-to-date information about all asthma, wheeze, and troublesome lung episodes as well as** a review of any and all potential asthma medications that were taken by the subject **will be** needed for the accurate completion of the investigator **assessment. Refer to Section 6.6.4.2.**

^e The investigator will supplement information from parents with review of routine medical records/ consultation with routine health care provider(s), as appropriate.

^f **After completion of 4th birthday annual contact (for interim analysis), and at study conclusion**

^g Study conclusion for the extension will be at the last contact, at child's 6th birthday.

6.5. Detailed description of study procedures for the Primary Study period (from birth up to 2 years of age)

6.5.1. Informed consent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before study participation. Refer to Section 6.1 for the requirements on how to obtain informed consent.

- In case of anticipated twins (or *other multiple births*), an ICF needs to be signed for each expected child before the start of delivery.
- In case of unexpected twins (or *other multiple births*), only the children for whom an ICF has been signed before birth can be enrolled. This should occur as per the birth order.

6.5.2. Check inclusion and exclusion criteria applicable before birth

Check all applicable inclusion and exclusion criteria applicable before birth as described in Sections 5.3.1 and 5.4.1 before enrollment. A pre-selected child who does not meet the eligibility criteria should be considered a screening failure.

6.5.3. Subject number allocation and registration in SBIR-Screening

Subject numbers will be assigned sequentially to subjects whose parent(s)/LAR(s) have consented for their child participation in the study, according to the range of subject numbers allocated to each study center.

- In case of anticipated twins (or *other multiple births*), a subject number will be assigned sequentially to each child for whom the consent has been obtained, as per the birth order, where the first born will be associated with the lowest subject number, the second born with the successive subject number, etc.

After obtaining the ICF and confirming eligibility, the subject needs to be entered in to the SBIR screening module. SBIR will control the recruitment rate as per an allocated target.

6.5.4. Collection of expected birth date

Record the expected delivery date as required in the subject's eCRF.

6.5.5. Collection of cord blood sample

Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

A volume of up to approximately 10 mL of whole cord blood sample will be collected from all subjects at birth, which should yield up to approximately 3 mL of serum. In case

less than 3 mL of whole blood is collected, the subject will be considered a screening failure and the sample collected needs to be discarded according to local procedure.

Details of the sample collection such as duration of processing steps and storage conditions need to be collected and recorded in the eCRF in order to allow adjustment for these variables in the primary analyses and to explore the impact of collection variables on the measured antibody titer.

Cord blood samples collected will provide RSV- antibody levels in the child, at birth.

6.5.6. Check inclusion and exclusion criteria applicable after birth and re-sign-off the ICF

Check all applicable inclusion and exclusion criteria applicable after birth as described in Sections 5.3.1 and 5.4.1 before enrollment.

The informed consent should be updated with relevant personal identifiers as applicable by local requirements. The ICF should be re-signed off by the investigator/study staff and subject's parent(s)/LAR(s) and witness (when required) not later than 5 working days after the birth of the subject.

Any pre-selected subject not meeting the eligibility criteria should be considered a screening failure, and the cord blood sample collected should be discarded.

6.5.7. Collection of medical history, demographic and lifestyle characteristics

Obtain the family medical history, demographic and lifestyle characteristics of the subject as required by the eCRF, by interviewing parent(s)/LAR(s) and/or reviewing the medical records and record data collected in the subject's eCRF. This includes family history of respiratory disease, living environment and household composition, breastfeeding, passive smoking and day-care attendance.

6.5.8. Collection of data on child at birth and delivery

Information about the delivery and the newborn (including medical care provided immediately after birth and planned to be provided subsequently) should be collected from the parents/LAR and completed and/or confirmed from an original source accessed through a person involved in the delivery of the subject or from any authorized person representing the healthcare organization that is in charge of the source and the collected data should be recorded in the subject's eCRF.

6.5.9. Registration in SBIR-enrollment

After obtaining the updated ICF and confirming eligibility, the subject needs to be entered in to the SBIR enrollment module. SBIR will randomize the subject to a time point for blood sample collection if the subject is part of the sub-cohort the parent(s)/LAR(s) should be informed about this allocation.

6.5.10. Distribution of diary cards to record symptoms and healthcare utilization

The investigator/study staff will provide diary cards to the subject's parent(s)/LAR(s) as needed to record relevant symptoms and healthcare system utilization during the course of the case. The diary card provided needs to be filled by the parent(s)/LAR(s) only in case the investigator/study staff concludes that their child has developed an RTI with suspicion of difficulty breathing, leading to an examination visit (off-site/site) during the study follow-up period.

6.5.11. Blood sampling

Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

A volume of approximately 3.5 mL of whole blood sample will be collected from a sub-cohort of subjects at randomly allocated time points to obtain approximately 1 mL of serum.

Selected subjects will be randomized to the time point 2, 4, 6, 12, 18 or 24 months at which a single blood sample should be collected from them. The sample should be collected between the intervals of ± 1 week e.g. Month 2 ± 1 week, Month 4 ± 1 week in order for the subjects to be included in the analyses. Subjects born at a gestational age of less than 36 weeks will be excluded from this blood sampling. Randomization will be done using a second phase application of SBIR.

Blood samples collected will be used to assess RSV antibody levels in the subjects.

6.5.12. Ascertain RTI symptoms and potential difficulty in breathing and assessment of need for examination visit (off-site/site)

During each active or passive follow-up contact, the investigator/study staff should confirm with the parent(s)/LAR(s) of the subject, if the subject developed new RTI symptoms (cough, runny nose, blocked nose), and any signs of difficulty in breathing (during and between contacts). In case there is any current sign or suspicion of difficulty in breathing in a child with symptom(s) of RTI at the time of the contact, the investigator/study staff should instruct the parent(s)/LAR(s) to start completing the diary card and schedule an examination visit (off-site/site) as soon as possible, but no later than 72 hours after the contact. Performing the visit as soon as possible is important to assess the need for medical care, to ensure that early symptoms are captured and also to collect an optimal biological sample for detection of RSV. Active surveillance contacts required by the surveillance schedule and each passive surveillance contact, whether or not it triggers an examination visit, will be recorded in the site's source documentation and in the eCRF. Parent(s)/LAR(s) will be instructed to contact the study team as soon as possible if the severity of the already existing symptoms in the child increases, or if the child who did not have signs of difficulty breathing develops the symptom.

The active and passive surveillance contacts can also be made with person designated by the parent(s)/LAR(s) (e.g. grandparents, nanny) as long as the parent(s)/LAR(s) have approved the same. If needed, a field worker can also be assigned to support the parent(s)/LAR(s) and/or person designated by the parent(s)/LAR(s) in completing the diary card.

The investigator/study staff should also collect any further identifying data (e.g. official name, social security number) that becomes available for a subject during the course of the study at regular surveillance contacts, and record it in a supporting site document.

6.5.13. Recording of data on changes to baseline medical and demographic data

On a quarterly basis, the investigator/study staff should expand a regular surveillance contact by asking the parents/LARs for changes to the baseline medical, demographic and lifestyle characteristics data collected at Visit 2. This includes at least;

- diagnosis of chronic diseases
- medical events requiring hospitalization
- vaccinations received (on disease level, not full vaccination details)
- start/stop attendance of day-care and other changes in daily care
- start/stop breastfeeding
- changes to household composition and/or living conditions

This data will be recorded in a quarterly surveillance eCRF.

6.5.14. Examination during (off-site/site) visit and collection of all disease symptoms

The examination visit can be an on-site or off-site visit. An off-site visit is preferred in order to avoid influencing the pattern of healthcare utilization for the disease. Parent(s)/LAR(s) of the subjects can however be requested to bring their child to the study center for the site examination visit e.g. if this would be the normal standard of care to provide given the situation, if this will significantly expedite the timing of the visit, or if it is unlikely that the off-site examination visit can occur at all within the required timeframe. If the reported symptoms are already of a level of severity that urgent care is indicated, the parent(s)/LAR(s) should be redirected to the proper location to receive this care (e.g. ER) and an examination visit could be scheduled to take place there at a suitable time. During the examination visit (off-site/site), the investigator/study staff should objectively confirm the RTI and difficulty breathing experienced by the subject and if the symptoms meet the LRTI case definition for surveillance. Data should be collected on all the clinical symptoms present in the child including a measurement of the temperature, RR and pulse oximetry. The data collected should be recorded in the subject's eCRF.

For any case for which an examination visit is done (off-site/site), at least the following characteristic but not limited to, will be reported in the eCRF:

- Date of telephone contact.
- Date of visit.
- Start and end date of the case.
- Nasal swab sample number.
- Clinical symptoms (Eg: irritability, cyanosis) as assessed by the investigator/study staff during examination visit (off-site/site), and as subsequently assessed from the diary card, and/or confirmed with any healthcare provider involved, during disease course.
- SaO₂ as assessed by investigator/study staff during the examination visit (off-site/site), and as subsequently assessed by any healthcare provider involved, during disease course.
- Temperature as measured by the investigator/study staff during the examination visit (off-site/site), and as subsequently assessed from the diary card, and/or confirmed with any healthcare provider involved, during course of the disease.
- RR as assessed by investigator/study staff during the examination visit (off-site/site), and as subsequently assessed by any healthcare provider involved, during disease course.
- Healthcare utilization during disease course as assessed from the diary card and/or confirmed with any healthcare provider involved.

Note:

Healthcare utilization (or healthcare setting availed) refers to self-care with OTC drugs, GP visits, ER visits, hospital visits, etc.

Data from healthcare providers (such as temperature, RR, SaO₂, symptoms and diagnosis) is to be regarded as a valuable, but optional extra, if this data is available and shared by the applicable healthcare provider.

A case eCRF needs to be created for each scheduled examination visit and data collected up to case resolution will be entered. Cases will be allocated with a unique case number that is a sequential number per subject.

6.5.15. Data collection on disease course and healthcare utilization before and during the examination visit (off-site/site)

Data on disease course recorded by the subject's parent(s)/LAR(s) in the diary card until the examination visit should be assessed and recorded by the investigator/study staff in the subject's eCRF.

Data provided by the parent(s)/LAR(s) in the diary card on healthcare utilization should be confirmed by direct contact of the investigator/study staff with the healthcare

providers involved in management of the case (e.g. GP, hospital staff), and recorded in the eCRF. Where possible, the data on healthcare utilization should be made more complete so as to best capture the healthcare utilization in the eCRF. Any clinical diagnoses for the respiratory infection (e.g. pneumonia, bronchiolitis) made by the contacted healthcare providers, should be collected and recorded in the eCRF as well as the symptoms assessed in support of this diagnosis (especially measured RR and SaO₂).

Note: In case of any discrepancies between healthcare utilization data recorded in the diary card and as provided (and supported with source documentation) by the HCPs, the data from the HCP will be used in the eCRF.

Data from healthcare providers (such as temperature, RR, SaO₂, symptoms and diagnosis) is to be regarded as a valuable, but optional extra, if this data is available and shared by the applicable healthcare provider.

6.5.16. Collection of nasal swab samples

- Nasal sampling: Cells and secretions from the nose will be collected by using sterile swabs for all the subjects who are reported for an examination visit (off-site/site) (this includes subjects with no confirmed LRTI).

Full details for obtaining nasal swabs are provided in the Module on Biospecimen Management in the SPM accompanying this protocol.

6.5.17. Return Diary card at completion or at case resolution

The diary card will be collected at completion.

- Regular completion is after the standard diary card duration of 14 days.
- If a surveillance case eCRF is 'closed' due to a subsequent examination visit for the same case, the diary card relating to the 'closed' eCRF is stopped prematurely and a new 14-day diary card is started (see also Section 3-examination visit).

If the subject is not free of the symptoms asked for in the diary card at the end of Day 14, the parent(s)/LAR(s) should continue to assess the symptoms until no more symptoms are present, and record in the diary card, the date at which no more symptoms were observed in the child.

6.5.18. Confirmation and expansion of data on healthcare utilization occurring after the examination visit (off-site/site) and collection of any further, relevant data on disease course with HCPs involved in subject care

Data on disease course recorded by the subject's parent(s)/LAR(s) in the diary card until diary card completion/case resolution should be assessed and recorded by the investigator/study staff in the subject's eCRF.

Data provided by the parent(s)/LAR(s) in the diary card on healthcare utilization should be confirmed by direct contact of the investigator/study staff with the healthcare providers involved in management of the case (e.g. GP, hospital staff), and recorded in the eCRF. Wherever possible, the data on healthcare utilization should be made more complete so as to best capture the healthcare utilization in the eCRF. Any clinical diagnoses for the respiratory infection (e.g. pneumonia, bronchiolitis) made by the contacted healthcare providers should be collected and recorded in the eCRF as well as the symptoms assessed in support of this diagnosis (especially measured RR and SaO₂).

Note: In case of any discrepancies between healthcare utilization data recorded in the diary card and as provided (and supported with source documentation) by the HCPs, the data from the HCP will be used in the eCRF.

Data from healthcare providers (such as temperature, RR, SaO₂, symptoms and diagnosis) is to be regarded as a valuable, but optional extra, if this data is available and shared by the applicable healthcare provider.

6.5.19. Recording of SAEs related to study procedure

- Refer to Section 7.2 for procedures for the investigator to record SAEs. Refer to Section 7.3 for guidelines on how to submit SAE reports to GSK Biologicals.
- 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.

6.5.20. Diary card transcription into eCRF

The investigator/study staff will transcribe the collected information into the eCRF in English.

6.5.21. End of primary study after 2 years follow-up

The investigator/study staff will:

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

6.6. Detailed description of Extension Study procedures

6.6.1. Informed consent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before participation in the extension. Refer to Section 6.1 for the requirements on how to obtain informed consent.

6.6.2. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria for the extension as described in Sections 5.3.2 and 5.4.2.

6.6.3. Collection of demographic data

Record gender *and* ethnicity in the subject's eCRF.

6.6.4. Potential risk factors for / occurrences of wheeze and asthma**6.6.4.1. Data collected from the parent(s)/LAR(s)**

The following data are to be collected by interviewing the parent(s)/LAR(s). Refer to the SPM for additional details

Genetic History

- ***Family*** history of asthma
- ***Family*** history of allergies

Child/subject variables of interest

- Past or current allergies
- Exposure to environmental/household or other risk factors for asthma
- Child's activity levels
- Potential signs/symptoms of asthma
- Episodes of wheeze, asthma, or troublesome lung symptoms since the end of the primary study and their associated care
- Medications taken to *prevent or* treat ***asthma, wheeze or troublesome*** lung symptoms

6.6.4.2. Investigator review and assessment

Investigators will review each subject's data to assess whether the subject met the protocol defined case definition(s) of asthma at the 2^d, 3^d, 4th, 5th and 6th birthdays. ***The assessments will be based on:***

- Review of information gathered at the first extension study contact with the subject's parent(s) / LAR(s) about past wheeze, asthma, and ***troublesome lung symptoms***, and the medications used to treat them.
- Review of information about any applicable primary study episodes of wheeze ***and troublesome lung symptoms***, and history of asthma medication ***use***.
- Review ***of the subject's*** routine medical records (***where these exist and are available***), as needed to verify/clarify study information.

- **Consultation** with *the* subject's health care provider(s), *as needed to verify/clarify study information.*

*Note that all information about all asthma, wheeze, and troublesome lung episodes as well as a review of any and all potential asthma medications that were taken by the subject **will be** needed for the accurate completion of the investigator **assessment**. (Refer to Section 4 for **case definitions** of asthma).*

To this end, quarterly contact information about asthma, wheeze, and troublesome lung episodes as well as the medications used to treat them should all be brought up-to-date at the time of each annual contact.

Refer to the SPM *for additional details*.

6.6.5. Medications for Wheeze or Asthma

Table 9 presents a list of medications for wheeze or asthma that should be identified during parent/LAR interviews and investigator review / assessment (Section 6.6.4), and recorded in the eCRF. These medications are usually prescribed by a health care provider.

Table 9 Medication(s) for wheeze or asthma (Amended 15-Dec-2017)

Oral steroids
Systemic (intramuscular or intravenous) steroids
Inhaled steroids (ICS)
Combination inhalers containing steroids and long-acting beta agonists
Long-acting beta agonists (LABA)
Short-acting beta agonists (SABA)
Leukotriene modifiers or receptor antagonists
Theophylline
Anti-IgE therapy (e.g., Omalizumab)

6.6.6. Recording of SAEs related to study procedures

Refer to Section 7.2 and Section 7.3. There is no biological sample collection during the extension study.

6.6.7. Study conclusion for the extension

The investigator/study staff will:

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

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

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

Any sample testing will be done in line with the consent of the individual subject's parent(s)/LAR(s).

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

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

6.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all samples (including serum 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 According-To-Protocol (ATP) analysis (See Section 9.4 for the definition of study cohorts/data sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals 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.

6.7.2. Biological samples

The following samples will be collected, as a part of the primary study:

- Cord blood sample (up to approximately 10 mL) at birth from all the subjects.
- Nasal swab samples within 72 hours from the first identification of a potential LRTI case by the investigator/study staff during an active or passive surveillance study contact.
- Single blood sample (approximately 3.5 mL) from a sub-cohort of subjects, at the time point allocated through randomization (2, 4, 6, 12, 18 or 24 months). Children who are born with a gestational age of less than 36 weeks will be excluded from this sub-cohort.

No additional samples will be collected during the extension period.

6.7.3. Laboratory assays

Please refer to [APPENDIX A](#) for a detailed description of the assays performed in the study. Please refer to [APPENDIX C](#) for the address of the clinical laboratories used for sample analysis.

The samples collected will be shipped to the study reference laboratory for testing.

The following assays will be performed at GSK Biologicals' laboratory or in a laboratory designated by GSK Biologicals using standardized and validated procedures.

For cord blood and sub-cohort blood samples:

- Serum neutralization assay to assess RSV antibody [[Table 10](#)].

Table 10 Antibody determination

System	Component	Method	Kit/ Manufacturer	Unit	Cut-off**	Laboratory
Serum	Anti-RSV-A	Neutralization	In house	ED60	8	GSK Biologicals* and/or designated laboratory
Serum	Anti-RSV-B	Neutralization	In house	ED60	6	GSK Biologicals* and/or designated laboratory

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

** Unit and cut-off for the RSV-A and/or RSV-B neutralization assay may be subject to change
RSV = Respiratory Syncytial Virus; ED = Effective Dose

For nasal swab samples:

- **RT-qPCR assay** for the detection of RSV and determination of RSV subtype (A/B).
- **xTAG™ RVP Fast assay** for the detection of the following 17 viruses and subtypes.
 - RSV
 - Influenza A, including subtypes H1 and H3
 - Influenza A H1N1 2009
 - Influenza B
 - Parainfluenza virus type 1, 2, 3, and 4
 - Human Metapneumovirus
 - Enterovirus/Rhinovirus
 - Adenovirus
 - Bocavirus
 - Coronavirus - 229E, OC43, NL63, HKU1

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

7. SAFETY

SAEs, which in the opinion of the investigator are related to the study procedures (e.g. blood sample and nasal swab sample collection), will be recorded throughout the study period.

The investigator or study 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 they/the subject manifest any signs or symptoms they perceive as serious/ of concern or indicating a change in their health status.

7.1. Safety definitions

7.1.1. Definition of an SAE

An SAE is any untoward medical occurrence 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 hospitalization or prolongation of an existing hospitalization,

NB: In general, hospitalization 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 hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether ‘hospitalization’ occurred or was necessary, the AE should be considered serious.

Hospitalization 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 hospitalization 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 ER or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

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

In absence of diagnosis, abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments that are judged by the

investigator to be clinically significant will be recorded as SAEs if they meet the definition of an SAE (refer to Section 7.1.1). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as 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.

7.2. Detecting and recording SAEs

7.2.1. Time periods for detecting and recording SAEs

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

In order to fulfil international reporting obligations, SAEs that are related to study participation (i.e., protocol-mandated procedures, invasive tests, a change from existing therapy) will be collected and recorded from the time when the subject parent(s)/ LAR(s) have consented their child's/children's participation in the study until she/he is discharged from the study.

7.2.2. Evaluation of SAEs

7.2.2.1. 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 (s) 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, date and time of onset, outcome, intensity and possible 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 the SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

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

7.2.2.2. Assessment of causality

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

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

If an event meets the criteria to be considered as ‘serious’ (see section 7.1.1), additional examinations/tests will be performed by the investigator in order to determine ALL possibly contributing factors to each SAE.

Possibly contributing factors include:

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

7.2.2.3. Assessment of outcomes

The investigator will assess the outcome of all SAEs recorded during the study as:

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

7.3. Reporting of SAEs

All SAEs occurring up to 30 days after the study procedure and considered related to study participation must be recorded on the SAE form, irrespective of intensity.

7.3.1. Prompt reporting of SAEs related to study participation

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

Table 11 Timeframes for submitting SAEs related to study participation

Type of event	Initial reports		Follow-up of relevant information on a previous report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to study participation	24 hours*	electronic expedited adverse event report	24 hours*	electronic expedited adverse event report

* Timeframe allowed after receipt or awareness of the information.

7.3.2. Contact information for reporting SAEs to GSK

Study Contact for Reporting SAEs
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs
<p>24/24 hour and 7/7 day availability:</p> <p>GSK Biologicals Clinical Safety & Pharmacovigilance</p> <p>Fax: +PPD or +PPD</p>

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

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

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

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

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

This back-up system should only be used if the electronic 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 designee) must complete the electronic SAE report

within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

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

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

7.3.5. Regulatory reporting requirements for SAEs

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

7.4. Follow-up of SAEs

7.4.1. Follow-up of SAEs

7.4.1.1. Follow-up during the study

After the initial SAE report, the investigator is required to proactively follow each subject and provide further relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs, refer to [Table 11](#)).

All SAEs documented at a previous visit/ contact and recorded as not recovered/ not resolved or recovering/ resolving will be reviewed at subsequent visits/ contacts until the end of the study.

7.4.1.2. Follow-up after the subject is discharged from the study

The investigator will follow-up subjects:

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

If the investigator receives additional relevant information on a previously reported SAE, he/ she will provide this information to GSK Biologicals using a paper SAE report.

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 recognized follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

8. SUBJECT COMPLETION AND WITHDRAWAL (AMENDED 15-DEC-2017)

8.1. Subject completion

A subject who is available for the concluding contact foreseen in the protocol *for the primary study* is considered to have completed the *primary* study.

A subject who is available for the concluding contact foreseen in the protocol for the extension study is considered to have completed the extension study.

8.2. Subject withdrawal

Subjects who are withdrawn *from the study* because of *study procedure related* SAEs must be clearly distinguished from subjects who are withdrawn for other reasons.

Investigators will follow subjects who are withdrawn *from the study* as *the* result of a *study procedure related* SAE until resolution of the event (see Section 7.4.1.2).

Withdrawals will not be replaced.

From an analysis perspective, a ‘withdrawal’ from the study refers to any subject who was not available for the concluding contact foreseen in the protocol.

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

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

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

- SAE.
- Non-serious AE.
- Protocol violation (specify).
- Consent withdrawal, not due to an AE*.

- Moved from the study area.
- Lost to follow-up**.
- Other (specify).

*In case a subject is withdrawn from the study because the subject's parent(s)/ LAR(s) has/have withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject's parent(s)/LAR(s), in the CRF.

****Details concerning the definition of "lost to follow up" are presented in the SPM.**

9. STATISTICAL METHODS (AMENDED 15-DEC-2017)

9.1. Endpoints for the *Primary Study* (first 2 years)

9.1.1. Co-Primary endpoints

- Occurrence of RSV as confirmed by RT-qPCR.
- Occurrence of different types of healthcare utilization*.

* *Healthcare utilization includes primary, secondary and tertiary care settings such as self-care with OTC drugs, GP visits, ER visits, hospital visits, etc.*

- Occurrence of LRTI/severe LRTI as classified by the LRTI case definition and severity scale.
- Occurrence of LRTI/severe LRTI as classified by the existing comparator LRTI case definition by WHO and by Nokes et al.

9.1.2. Secondary endpoints

- Levels of RSV neutralizing antibodies in the cord blood samples collected at birth.
- Levels of RSV neutralizing antibodies in the blood samples collected at 2, 4, 6, 12, 18 and 24 months.
- Occurrence of LRTI/severe LRTI cases as classified by the LRTI case definition and severity scale, in the subgroups of subjects recruited from Months 1-6 and 13-18.

9.1.3. Tertiary endpoints

- Occurrence of RSV and other respiratory viruses as confirmed by xTAG™ RVP Fast assay:

- Parainfluenza virus type 1, 2, 3, and 4
- Human Metapneumovirus
- Rhinovirus
- Adenovirus

- Bocavirus
- Coronavirus - 229E, OC43, NL63, HKU1
- RSV
- Influenza A, including subtypes H1 and H3
- Influenza B

- RSV viral load as determined by RSV RT-qPCR.
- Occurrence of any symptom identified in cases of potential LRTI.
- Cord-blood sample collection variables including collection and storage times and temperatures.
- Occurrence of potential risk factors including complications at birth, family history of respiratory disease, living environment and household composition, breastfeeding, passive smoking and day-care attendance.

9.2. *Endpoints for the Extension Study*

9.2.1. *Primary: none*

9.2.2. *Secondary:*

- Occurrence of medically attended wheeze
- Occurrence of wheeze or asthma requiring hospital admission.
- *Use of* prescription medications for wheeze or asthma.

9.2.3. *Tertiary*

- Number of children with asthma (case definition 1) at age 2, 3, 4, 5, and 6 years of age
- Number of children with asthma (case definition 2) at age 2, 3, 4, 5 and 6 years of age.
- *Number of children with asthma (case definition 3) at 3, 4, 5 and 6 years of age.*
- Number of children with recurrent wheeze.

9.3. Determination of sample size

Different RSV-associated LRTI hospitalization rates have been reported in industrialized countries. We observe incidence rates between 16.9 and 44.1 per 1000 children-years in the age group of infants below 6 months of age, between 5.1 and 14.2 per 1000 children-years in the age group of infants between 6-11 months and between 0.4 and 3.7 per 1000 children-years in the age group of children between 12-23 months [Table 12]. These rates are similar to the ones measured in several studies conducted in developing countries [Nair, 2010].

Table 12 RSV-associated LRTI hospitalization rates in industrialized countries (per 1000 children-years)

Source	Region	0-5 months	6-11 months	12-23 months
[Hall, 2009]	USA	16.9	5.1	2.7
[Holman, 2004]	USA	41.9	12.8	-
[Iwane, 2004]	NY/ Tennessee	18.5	7.4	0.4
[Boyce, 2000]	Tennessee	44.1	15.0	3.7
[Despande, 2003]	UK	36.0	11.3	-
[Vicente, 2003]	Spain	36.8	14.2	-

USA = United States of America

UK = United Kingdom

Table 13 shows the exact 95% confidence interval (CI) (Poisson exact distribution and normal approximation of the Poisson distribution accounting for a design effect of 4.18) for a range of expected incidence rates of RSV that we can reasonably estimate between 10 and 50 per 1000 children-years in the first year of birth. With an overall sample size of about 320 evaluable subjects by country during the first year of enrollment and with a follow-up period of 1 year after the last subject is recruited, the CI for an expected RSV-associated LRTI hospitalization rate of 20 cases per 1000 children-years is respectively [6.9; 40.8] for the exact Poisson distribution and [0; 51.7] for the normal approximation with design effect of 4.18.

Table 13 Precision for expected RSV-associated LRTI hospitalization rate during the first year of life (per 1000 children-years)

	Number of children-years	Number of cases	RSV-associated LRTI rate (per 1000 children-years)	95% CI Poisson Exact	95% CI Normal approx. with DE
First year of enrollment (analysis will be performed 1 year after the last subject is recruited)					
Country/region level	320	3	10	[1.9; 27.4]	[0; 32.4]
	320	6	20	[6.9; 40.8]	[0; 51.7]
	320	10	30	[13.9; 55.4]	[0; 68.8]
	320	13	40	[20.5; 67.5]	[0; 84.8]
	320	16	50	[28.6; 81.2]	[0; 100.1]
Overall	1280	13	10	[5.1; 16.9]	[0; 21.2]
	1280	26	20	[13.0; 29.3]	[4.2; 35.8]
	1280	38	30	[21.0; 40.7]	[10.6; 49.4]
	1280	51	40	[29.7; 52.4]	[17.6; 62.4]
	1280	64	50	[38.5; 63.8]	[25.0; 75.0]
Second year of enrollment (analysis will be performed 1 year after the last subject is recruited)					
Country/region level	160	2	10	[0.7; 40.1]	[0; 41.7]
	160	3	20	[3.9; 54.8]	[0; 64.8]
	160	5	30	[8.4; 68.5]	[0; 84.9]
	160	6	40	[13.8; 81.6]	[0; 103.4]
	160	8	50	[21.6; 98.5]	[0; 120.8]
Overall	640	6	10	[3.4; 20.4]	[0; 25.8]
	640	13	20	[10.2; 33.7]	[0; 42.4]
	640	19	30	[17.9; 46.4]	[2.6; 57.4]
	640	26	40	[25.9; 58.6]	[8.3; 71.7]
	640	32	50	[34.2; 70.6]	[14.6; 85.4]

CI = Confidence Interval; RSV = Respiratory Syncytial Virus;
LRTI = Lower Respiratory Tract Infection; DE = Design Effect

The design effect measures the increase in the standard error of the estimate due to the sampling design. The following formula will be used to estimate the design effect in the simulation [Bennett, 1991]:

$$DE = \frac{s^2_{CLUSTER SAMPLE}}{s^2_{SIMPLE RANDOM SAMPLE}}$$

With

$$s^2_{CLUSTER SAMPLE} = (c / \sum x_i)^2 (\sum y_i^2 - 2p \sum x_i y_i + p^2 \sum x_i^2) / [c(c - 1)]$$

$$s^2_{SIMPLE RANDOM SAMPLE} = p(1 - p)/n$$

Where, 'c' is the number of clusters, 'x_i', and 'y_i' are number of children-years and number of cases, respectively in the 'ith' cluster, 'n' is the total number of children-years and 'p' is the overall proportion $\sum y_i / \sum x_i$.

The intra-cluster correlation 'ρ' may then be estimated as:

$$\rho = (DE - 1) / [(\sum x_i / c) - 1]$$

Each simulation consists of creating 'c' clusters whose size and incidence vary around a given value. Then the above formula was applied to estimate the design effect in the simulation. Table 14 shows the median result of 1000 simulations (with 5th and 95th percentile). The design effect [Bennett, 1991] can be estimated as following once the intra-cluster correlation value is known or can be assumed:

$$DE = 1 + (b - 1) \rho$$

Where, ρ is the intra-cluster correlation and b is the average number of subjects sampled per cluster. According to the results of simulations, we could assume that the intra-cluster correlation of 0.02 (See Table 14 below) and considering 12 clusters of 160 subjects, we expect that the design effect will take a value of 4.18.

Table 14 Design effect calculated from 1000 simulations and for different hypothesis

Hypothesis of the simulation (Number of clusters; Overall number of children-years; incidence)	Results of 1000 simulations			
	Number of children-years by cluster (P5; Median; P95)	Proportion of cases by cluster (P5; Median; P95)	DE : Design Effect (P5; Median; P95)	ρ : intra-cluster correlation (P5; Median; P95)
9; 2400; 0.03	136; 266; 401	0; 0.028; 0.086	2.51; 6.46; 13.67	0.006; 0.021; 0.047
12; 2400; 0.03	100; 201; 300	0; 0.027; 0.087	2.57; 5.13; 9.49	0.008; 0.021; 0.042
15; 2400; 0.03	81; 160; 239	0; 0.027; 0.088	2.29; 4.34; 7.74	0.008; 0.021; 0.042
18; 2400; 0.03	68; 133; 199	0; 0.026; 0.091	2.08; 3.89; 6.69	0.009; 0.022; 0.043

P = Percentile

Few active studies reported the overall RSV-associated LRTI incidence rates (with or without hospitalization). According to the results observed in a birth cohort conducted in Kenya [Nokes, 2008] we expect that the RSV-associated LRTI rate will be 8 times greater than the hospitalization rate.

As the subjects may dropout from the study due to a variety of reasons, e.g. migration from the study area, the sample size has been increased to accommodate the same. Assuming a drop-out rate of about 20% during the course of follow-up approximately 400 subjects by country/region and 2400 subjects overall will need to be recruited.

9.4. Study cohorts/ data sets to be analysed

9.4.1. Screened cohort

The screened cohort will include all subjects screened for the study. All the aggregated information (anonymous) for these subjects will be collected in a logbook.

9.4.2. Analysis Cohorts for the Primary study (first 2 years)

9.4.2.1. Total enrolled cohort at Year 2

The total enrolled cohort will include all subjects enrolled in the *primary* study.

9.4.2.2. Per Protocol Set (PPS) cohort at Year 2

The *PPS* cohort will include all subjects *enrolled in the primary study* meeting all eligibility criteria up to the time of their censoring, either at completion *of epoch 1* or prematurely as drop-out (e.g. withdrawn consent, lost-to-follow-up, lack of compliance). *Additional details are provided in the Statistical Analysis Plan (SAP).*

9.4.2.3. Per Protocol Set (PPS) sub-cohort at Year 2

- The *PPS* sub-cohort *at Year 2* will include subjects *in the PPS cohort at Year 2* for whom a blood sample result will be available (at Month 2, 4, 6, 12, 18 or 24). Analyses will be done on the ATP sub-cohort from the total cohort defined in section 9.4.2 as well as from the ATP cohort defined in section 9.4.2.2.

9.4.3. Analysis Cohorts for the Extension Study

9.4.3.1. Total enrolled cohort for extension

The total enrolled cohort will include all subjects enrolled in the extension study.

9.4.3.2. PPS cohort for extension

The PPS cohort for the Extension study will include all subjects meeting all eligibility criteria up to the time of their censoring, either at study completion or prematurely as drop-out (e.g. withdrawn consent or lost-to-follow-up).

9.5. Derived and transformed data

Data transformations and derived variables will be described in the SAP. However, they may include the following:

- Age of the subject at time of the LRTI case will be expressed in months and will be computed as the difference between the start date of the LRTI case and the date of birth.
- Age of the subject at time of blood sample collection will be expressed in months and will be computed as the difference between the sample collection date and the date of birth.
- Age of the mother at the time of childbirth will be expressed in years and will be computed as the difference between the birth date of the subject and the birth date of the mother.
- Duration of the study for each subject will be computed as the difference between the date of last contact (i.e., active or passive surveillance contact or the date of the censoring) and the date of enrollment (date when ICF was first signed by the subject's parent[s]/LAR[s]).
- All potential LRTI cases identified during the surveillance period will be classified as RTI, LRTI or severe LRTI according to the LRTI case definition and severity scale (refer to Section 4). The cases will also be classified according to the classification of WHO and by Nokes *et al* [Modjarrad, 2016; Nokes, 2004]. First time, the classification will be done at visit level, i.e. according to the data collected at the initial visit and, if applicable, at each additional examination visit. Finally, a classification at case level will be done considering the most severe classification from all visits.

9.5.1. General considerations for statistical analyses

The primary analyses will at least be performed on the **PPS** cohort or sub-cohort. If more than 5% of the subjects are eliminated from the total enrolled cohort, then all analyses **may** also be performed on the total enrolled cohort in addition to the **PPS** cohort or sub-cohort.

When mentioned below in the upcoming analyses sections, analysis by subgroups **may** include:

- Age categories
- Hemisphere/Region/Country
- Birth on-season or off-season
- Collection time of swabs (72 hours/48 hours from the start date of the case (date of the call), 72 hours/48 hours from the reported onset of symptoms).

- For all incidence rate calculations, the 95% CI using the exact Poisson distribution will be estimated, as well the 95% CI using the normal approximation of the Poisson distribution, accounting for a design effect (after having derived the design effect from the real data).

9.6. Analysis of demographics

The distribution of subjects enrolled among the study sites *may* be tabulated overall, by hemisphere, *country, region, center* and/or by *other relevant grouping*. The demographic and lifestyle characteristics collected for the enrolled subjects may be summarized overall, by hemisphere, *country, region, center*, and/or *other relevant grouping*.

- Frequency tables will be generated *as appropriate*, for categorical *variables* such as center.
- Mean, median, standard error will be provided *as appropriate*, for continuous data such as age.

9.7. Analysis of Objectives for the Primary Study (birth to 2 years of age):

9.7.1. Co-primary objectives

- To determine the total health burden of RSV-associated RTI with a suspicion of involvement of the lower respiratory tract.**

Incidence rates will be calculated as the number of RSV-associated RTI with a suspicion of involvement of the lower respiratory tract over the number of children-years observations, overall and by subgroups.

Descriptive statistics of the different types of healthcare utilization from all healthcare settings will be provided (proportions, duration, etc.) among RSV-associated RTI with a suspicion of involvement of the lower respiratory tract, overall and by subgroups.

- To assess the performance of the LRTI case definition and severity scale for RSV-associated cases.**

Incidence rates will be calculated as the number of RSV-associated LRTI/severe LRTI as classified by the LRTI case definition and severity scale and also as classified by the comparator case definitions (WHO and Nokes, et al [Modjarrad, 2016; Nokes, 2004]), over the number of children-years observations, overall and by subgroups.

Descriptive statistics of the different types of healthcare utilization will be provided (proportions, duration, etc.) among RSV-associated LRTI/severe LRTI as classified by the LRTI case definition and severity scale and also as classified by the comparator case definitions (WHO and Nokes, et al [Modjarrad, 2016; Nokes, 2004]), overall and by subgroups.

Statistical analysis of agreement will be performed to compare the different case definitions of RSV-associated LRTI/severe LRTI and key healthcare utilization and health outcomes (the hospitalization status, (P) intensive care unit [ICU] attendance, clinical diagnosis of bronchiolitis or pneumonia, death) overall and by age categories.

Table 15 Comparison of two classifications for severe/Non-severe LRTI

RSV-associated cases		Classification of reference (severe/Non-severe)	
		Hospitalized	Not hospitalized
Alternative classification (severe / Non-severe)	Severe LRTI (LRTI case definition and severity scale)	True positive (TP)	False positive (FP)
	Non-severe LRTI (LRTI case definition and severity scale)	False negative (FN)	True Negative (TN)

2x2 contingency tables such as [Table 15](#) will be provided and the following measures of agreement (with 95% CI) will be calculated:

- Sensitivity: $TP / (TP + FN)$
- Specificity: $TN / (TN + FP)$
- Positive predictive value (PPV): $TP / (TP + FP)$
- Negative predictive value (NPV): $TN / (TN + FN)$
- Proportion of overall agreement, which is the proportion of cases similarly classified: $(TP + TN) / (TP + FP + FN + TN)$.
- Cohen's kappa coefficient. The magnitude of the kappa coefficient represents the proportion of agreement greater than that expected by chance.

The same analysis will at least be performed for all the following combinations (reference vs. alternative classification):

- Health/healthcare utilization outcome vs. LRTI case definition and severity scale.
- Health/healthcare utilization outcome vs. WHO case definition [[Modjarrad, 2016](#)].
- Health/healthcare utilization outcome vs. Nokes, et al case definition [[Nokes, 2004](#)].
- WHO case definition [[Modjarrad, 2016](#)] vs. LRTI case definition and severity scale.
- Nokes, et al case definition [[Nokes, 2004](#)] vs. LRTI case definition and severity scale.

9.7.2. Secondary objectives

- **To determine the total health burden of non-RSV-associated RTI with a suspicion of involvement of the lower respiratory tract.**

Incidence rates will be calculated as the number of non-RSV-associated RTI with a suspicion of involvement of the lower respiratory tract over the number of children-years observations, overall and by subgroups.

Descriptive statistics of the different types of healthcare utilization will be provided (proportions, duration, etc.) among non-RSV-associated RTI with a suspicion of involvement of the lower respiratory tract, overall and by subgroups.

- **To evaluate the association between RSV-associated LRTI, RSV-associated severe LRTI and RSV neutralizing antibodies in the baseline cord blood samples collected from subjects, at birth.**

The impact of the level of RSV neutralizing antibodies in the baseline cord blood samples on the risk of having a RSV-associated LRTI and RSV-associated severe LRTI will be assessed through a statistical model.

Incidence rates will be calculated overall and by level of antibodies in the cord blood sample, as the number RSV-associated LRTI/severe LRTI as classified by the case definition and severity scale over the number of children-years observations.

- **To determine the prevalence of RSV infection, at 2, 4, 6, 12, 18 and 24 months in a randomly selected sub-cohort of subjects by assessing antibodies in serum.**

The primary analysis will be based on ATP sub-cohort for this objective.

Infection by RSV will be assessed (with 95% CI) by time point as the proportion of samples with a positive score of antibody titer after adjusting for baseline antibody results over the total number of samples.

Scores will be determined based on the measured antibody titer vs. the expected antibody level at the time of sampling based on the individual baseline (cord blood) value and the average, modelled decay rate.

- **To assess the between calendar year variability in the incidence rates of RSV-associated LRTI.**

Incidence rates will be calculated as the number of RSV-associated LRTI/severe LRTI as classified by the case definition and severity scale, over the number of children-years observations, in the subgroups of subjects recruited from months 1-6 and from months 13-18.

9.7.3. Tertiary Objectives

- **To explore the association of co-infections with the incidence of RSV-associated LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.**

For each of the other respiratory viruses, incidence rates will be calculated as the number of RSV-associated LRTI/severe LRTI cases associated co-infected with the virus over the number of children-years observations.

- **To explore the association of RSV and other respiratory viruses with the incidence of LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.**

For each of the respiratory viruses, incidence rates will be calculated as the number of LRTI/severe LRTI cases associated with the virus over the number of children-years observations.

- **To explore the association of RSV viral load with the incidence of RSV-associated RTI, LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale).**

Descriptive analyses (mean, median, min, max) of viral load assessed by the RSV RT-qPCR of RSV RTI, RSV LRTI and severe LRTI cases will be tabulated.

- **To explore the impact of changes to the symptoms and threshold levels of symptoms in the LRTI case definition and severity scale.**

Frequency tables will summarize the proportion of RSV-associated LRTI/ severe LRTI cases associated with a list of symptoms and clinical parameters, overall and by age categories.

This will help us to determine potential areas of improvement of the LRTI case definition and severity scale. Incidence rates of RSV-associated LRTI/severe LRTI will be calculated for any proposal of modification of the LRTI case definition and severity scale.

- **To explore the impact of variations in cord-blood sample collection variables on the stability of test results.**

The measured maternal antibody levels will be assessed versus variability in sample collection variables. This involves assessment versus various continuous and categorical variables and an overall comparison between samples as classified 'according to recommended procedure' and 'not according to recommended procedure'.

The measured maternal antibody levels by duration of the processing step and overall process duration will be analysed graphically and by log-linear regression.

The measured maternal antibody levels by storage condition or tube type will be analysed graphically and by group statistics (mean, 95% CI). Group means will be compared by t-test to assess the statistical significance of differences.

The measured maternal antibody levels in samples collected 'according to recommended procedure' and 'not according to recommended procedure' will be analysed graphically and by group statistics (mean, 95% CI). Group means will be compared by t-test to assess the statistical significance of any difference.

- **To assess the impact of potential RSV risk factors (e.g. complications at birth, family history of respiratory disease, lifestyle characteristics and household composition, breast feeding, passive smoking, day care attendance) on the incidence and severity of RSV-associated LRTI.**

The incidence of any, severe, non-severe RSV-associated LRTI will be analysed using a Poisson regression model including potential risk factors.

- **To assess the natural decay of maternal antibody levels using a single follow-up blood sample collected from a randomly selected sub-cohort of subjects at 2, 4, 6, 12, 18 and 24 months.**

The primary analysis will be based on ATP sub-cohort for this objective.

The decay of maternal antibody levels over time will be analysed graphically and by a log-linear regression. A true, natural decay curve will be explored by stochastically reducing the sample to uninfected subjects only. The following steps will be taken to identify, confirmed and suspected infected subjects to eliminate these from the sample;

- Subjects with an RSV positive nasal swab during the study before time of sampling will be eliminated.
- A sensitivity analysis of the decay curve will be explored in sub-sample consisting of the subjects born 'off-season' as these have the lowest likelihood of having been infected.
- Subjects with an antibody titer at the sampling time that is more than 2-fold above the expected value based on their baseline (cord blood) value and the established decay curve will be considered to have been infected, eliminated and the decay curve refined.
- Step 3 is repeated up to 5 times until the most accurate decay curve is established.

9.8. Analysis of Objectives for the Extension study

9.8.1. Primary objectives: none

9.8.2. Secondary objectives

Data for secondary endpoints may be pooled across the primary and extension periods. Additional details are provided in the SAP.

9.8.2.1. To determine the population attributable risk of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life.

The incidence rate of medically attended wheeze, wheeze or asthma requiring hospital admission, and prescription of medications for wheeze or asthma may be calculated and stratified by country.

The population attributable risk *may be* calculated by subtracting the incidence rate in the subjects who did not have RSV clinical LRTI in the first two years of life from the incidence rate in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

Details will be provided in the SAP. An example follows.

The population attributable risk of medically attended wheeze may be calculated as:

Ip-Iu

Where Iu is the incidence rate of medically attended wheeze in subjects who did not have RSV clinical LRTI in the first two years of life, and Ip is the incidence rate of medically attended wheeze in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life. ***The above population attributable risk formula may be further adapted to best accommodate the data and their required analytic assumptions (e.g., a modified formula may be used for population attributable risk calculations involving recurrent outcomes).***

9.8.2.2. To determine the population attributable risk percent of wheeze and asthma from birth until sixth birthday due to RSV clinical LRTI in the first two years of life.

The population attributable risk percent together with 95% CI of medically attended wheeze, wheeze or asthma requiring ***hospital*** admission, and prescription of medications for wheeze and asthma ***may*** be calculated overall and by age strata (0-2 years, 2-4 years and 5-6 years), respectively.

The population attributable risk percent ***may be*** calculated by dividing the population attributable risk by the incidence rate in all the subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

For example, the population attributable risk percent of medically attended wheeze ***may*** be calculated as:

$$(Ip-Iu)/Ip*100$$

Where Iu is the incidence rate of medically attended wheeze in subjects who did not have RSV clinical LRTI in the first two years of life, and Ip is the incidence rate of medically attended wheeze in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life. ***As with the population attributable risk, the above formula may be adapted, as appropriate, to best suit the data.***

The detail for calculating the CI for both population attributable risks and population attributable risk ***percent*** will be provided in the ***SAP***.

9.8.3. Tertiary objectives

9.8.3.1. To explore the association of RSV clinical LRTI in the first two years of life and other potential risk factors for wheeze and asthma

Endpoints such as medically attended wheeze, wheeze or asthma requiring ***hospital*** admission, and prescription of medications for wheeze or asthma ***may be calculated as recurrent events and*** stratified by country.

Occurrence/recurrence rates will be calculated overall and by subgroups ***for each of the outcomes, as relevant.*** Multivariable Poisson regression ***or other appropriate*** models

including RSV clinical LRTI in the first two years of life and other potential risk factors such as, but not limited to, family history of asthma, and/or allergies, contact with pets, child history of allergy, eczema or atopic dermatitis, etc., will be explored, respectively.

9.8.3.2. To explore the performance of asthma case definitions.

The number and percentage of children with diagnosis of asthma (case definitions 1, 2, **and 3** will be described for each *applicable* age; the number and percentage of children with recurrent wheeze **at each applicable age** will be described overall and by country in frequency tables.

The risk of asthma at the age of 6 years using case definitions 1, 2 and 3 (occurring from 2nd to the 6th birthday) will be analyzed using *multivariable* logistic regression model including RSV clinical LRTI in the first two years of life and other potential risk factors listed above, respectively.

- Statistical analysis of agreement will be performed to compare *asthma* case definitions **1, 2, and 3 to each other**.
- Cohen's kappa (*or other appropriate*) coefficient **may be used for the comparisons**. The magnitude of the kappa coefficient represents the proportion of agreement greater than that expected by chance.

9.9. Interpretation of analyses

All analyses will be descriptive with the aim to characterise overall occurrences, patterns and potential differences between groups in the endpoints related to the objectives.

The tertiary objective 'To explore the impact of changes to the symptoms and threshold levels of symptoms in the LRTI case definition and severity scale' will be assessed if we detect potential areas of improvement of the LRTI case definition and severity scale, such as clearly under or overestimated incidence rates of LRTI/ severe LRTI compared to the rates in the literature or a lack of discriminative power between severe and non-severe cases. This decision will not be taken on specific pre-defined thresholds.

9.10. Conduct of analyses

Analytic plans may be modified as appropriate to accommodate final sample sizes and data distributions. Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

9.11. Sequence of analyses

An interim analysis will be performed after data lock at the end of recruitment (expected after 18 months) or in June 2015 whichever occurs first, on the data collected for the subjects recruited in the first year of the study (approximately 1600). This interim analysis will be performed to obtain preliminary indications of the results in very young infants. These preliminary results will be used for potential adjustment of the LRTI Case

Definition and Severity Scale before final analysis, and to provide data for other relevant decision making, as soon as possible.* The results pertaining to this analysis will be descriptively reported in *an* interim statistical report.

*Based on the preliminary results of the interim analyses it was decided that the incidence rate would be more robust if all subjects completed the primary study until 2 years of age.

Final analysis will be performed on all the data collected and cleaned in the study, at the end of the extension period which is expected to occur 7 years after study start. An integrated study report presenting the consolidated analysis for the entire study duration (primary and extension study period [as applicable]) will be written and made available to the investigators.

9.11.1. Statistical considerations for the primary study

Statistical analyses for the interim analysis will be performed on all the data collected and cleaned for the subjects recruited in the first year of the study, at the end of recruitment (expected after 18 months) or in June 2015 whichever occurs first.

The interim analysis will be purely descriptive, no adjustment of the type I error is foreseen.

9.11.2. Statistical considerations for the extension study

All statistical analyses pertaining to the *extension study* will be performed on data collected and cleaned for the subjects who re-consented for the extension.

An interim analysis for the extension study will be conducted near the midway point of extension study data collection, after all subjects have reached four years of age (and completed their annual contact, or passed the window for that contact). The interim analysis will be purely descriptive, no adjustment of the type I error is foreseen.

Final extension study analysis will be conducted after all subjects have reached six years of age (and completed their annual contact, or passed the window for that contact).

10. ADMINISTRATIVE MATTERS

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

10.1. 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 designee. In all cases, the investigator remains accountable for the study data.

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

10.2. Study monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst 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, any other study agreements, GCP and all applicable regulatory requirements.

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

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

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a 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 each visit.

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

10.3. Record retention

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

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. 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 a particular site, as dictated by ICH GCP or other applicable guidelines, any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

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

10.4. Quality assurance

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

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

Study information from this protocol will be posted on public registers before enrollment of subjects begins/before start of the analyses.

Interventional studies that do not evaluate vaccines/products are progressed for publication in the scientific literature when the results provide important scientific or medical knowledge.

10.6. Provision of study results to investigators

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

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

11. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

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APPENDIX A LABORATORY ASSAYS

Neutralization assay

The Serum Neutralization assay is a functional assay that measures the ability of serum antibodies to neutralise the cytopathic effects of RSV (for example, strains A and B) on the host cell line, hence RSV replication. In the first step of the assay, a specific quantity of a replication capable RSV virus and a defined dilution of serum are mixed and incubated. Each serum dilution is tested in duplicates. During the second step, the virus-serum reaction mixture is transferred on the host cells and only non-neutralized virus is able to infect and replicate in the host cells. This leads to the formation of a given number of plaque forming units (PFU) on the cell monolayer that can be detected using a fluorochrome-tagged anti-RSV antibody. The neutralising titer is determined by calculating the serum dilution inducing a 60% inhibition in PFUs compared to a cell monolayer infected with virus alone, without serum.

RT-qPCR assay

Briefly, RSV A and RSV B ribonucleic acids (RNAs) extracted from the nasal swabs are detected in a duplex PCR format using specific amplification primers and fluorescent probes designed in the RSV N gene, encoding the RSV nucleocapsid protein. The process involves nucleic acids extraction, conversion of RNA to complementary deoxyribonucleic acid (cDNA) by reverse transcription and detection by real- time PCR reaction using a calibration curve (absolute quantitation). The RSV viral load is reported as copies of RSV RNA per mL of sample.

xTAG™ RVP Fast assay

xTAG™ RVP Fast assay is a qualitative nucleic acid multiplex tests intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasal/nasopharyngeal swabs from individuals suspected of respiratory tract infections. A multiplexed PCR reaction is performed to amplify the regions of interest in the target human or infectious agent genes. The PCR reaction is treated to remove excess nucleotides and primers. The PCR reaction is then subjected to a primer extension step that is specific for the allele or the infectious agent that is being analyzed: Allele Specific Primer Extension (ASPE) or Target Specific Primer Extension (TSPE). The 5' end of the ASPE or TSPE primers is attached to an xTAG universal tag sequence. The 5' universal tag sequence is hybridized to the complementary anti-tag sequence coupled to a particular xMAP bead set. The hybridized beads are read by the xMAP system and results are analyzed by the data analysis software.

APPENDIX B CLINICAL LABORATORIES**Table 16 GSK Biologicals' laboratories**

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

Table 17 Outsourced laboratory

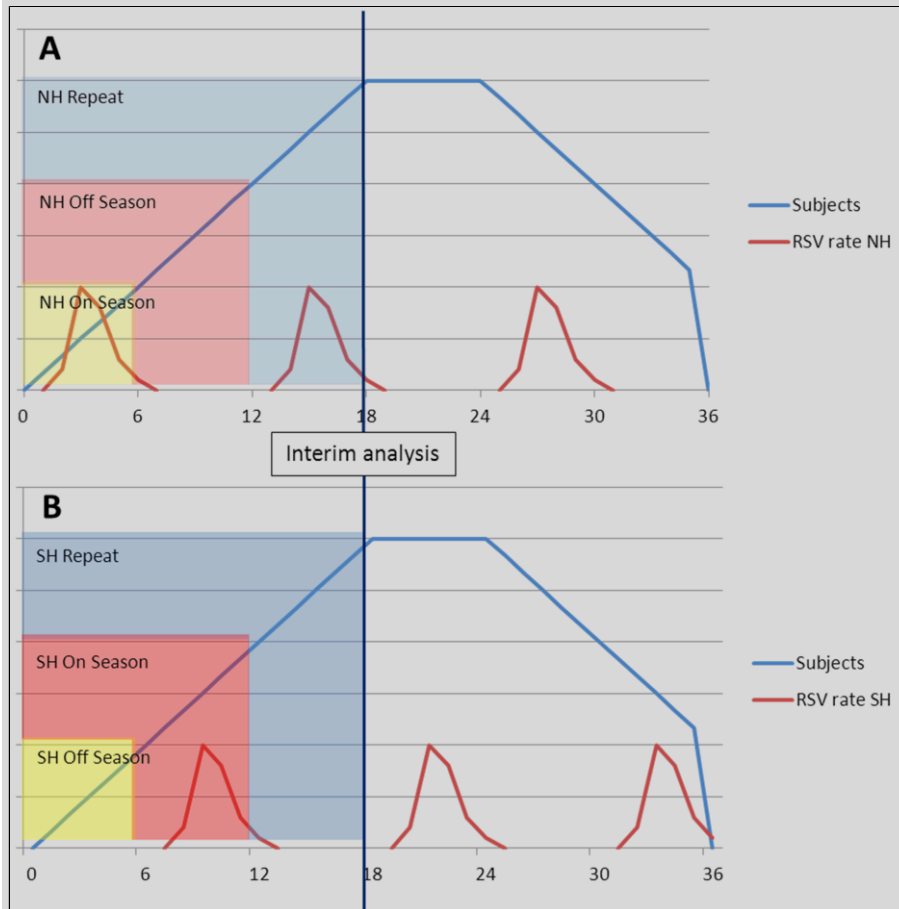
Laboratory	Address
NÉOMED-LABS Inc.	525, Cartier Ouest Laval, Quebec Canada H7V 3S8

APPENDIX C OVERVIEW OF RECRUITMENT PLAN (PRIMARY STUDY) (AMENDED 15-DEC-2017)

- *Subjects will be pre-selected to be enrolled in the study, by obtaining the informed consent from the parent(s)/LAR(s) during pregnancy, after 20 weeks of gestation and before the start of delivery, to include their child in the study at birth. On obtaining the consent, the subjects will be checked for inclusion and exclusion criteria applicable before birth. A pre-selected child not meeting the inclusion and exclusion criteria will be considered a screening failure.*
- *On receiving consent, subject numbers will be assigned sequentially to each subject, according to the range of subject numbers allocated to each study center.*
- *After birth, the final inclusion and exclusion criteria will be checked and the informed consent will be updated with, time and date of birth and any other potentially relevant identifiers as applicable by local requirements and re-signed. Any pre-selected subject not meeting the eligibility criteria will be considered a screening failure.*
- *Subject recruitment is intended to occur over a period 18 months or until the planned target enrollment has been met. Assuming no delays in recruitment, two-thirds of the subjects (1600) will be recruited in Year 1 and the remaining one-third of the subjects (800) in the first half of Year 2 of the study. In case of any delays in recruitment for the first two-thirds of the subjects, the study duration will be extended until all these subjects have completed follow-up to 2 years of age. Delays in the recruitment of the last one-third of the subjects will be absorbed by a shorter follow-up period for these subjects, unless the decision is taken after the interim analysis to extend the study to allow all subjects to complete 2 years of follow-up.*
- *The recruitment will be performed using a first application of SBIR where subjects will be entered on obtaining ICF and recruitment is controlled on the monthly recruitment rate. The aim is to attain a reasonably stable number of subjects per month over the full recruitment period in order to achieve an equal spread in the age of subjects at first exposure to an RSV season. Recruitment will be actively controlled through the SBIR system on country level according to agreed targets, actual recruitment rates, and further recruitment potential, so as to achieve a sufficient population on a regional level. With concurrent recruitment across the globe (with different seasonality) and an intended, steady recruitment over 18 months, the following population is to be included;*
 - *Subjects from NH during first 6 months*
 - *Subjects from SH during first 6 months*
 - *Subjects from NH during second 6 months*
 - *Subjects from SH during second 6 months*
 - *Subjects from NH during third 6 months; repeat of first 6 months NH*
 - *Subjects from SH during third 6 months; repeat of first 6 months SH*

- *In case recruitment is extended beyond 18 months, subjects recruited in the fourth 6 month period are a repeat to those recruited during the second 6 month period*

Figure 3 RSV seasons in Northern and Southern Hemispheres



NH=Northern hemisphere, SH=Southern Hemisphere. Figure assumes equal recruitment targets on both hemispheres (which is not a requirement for the study).

Figure A represents recruitment in the NH and assumes RSV seasons running from November to April relative to a currently estimated time point 0 in Nov 2013.

Figure B represents recruitment in the SH and assumed RSV seasons running from May to October relative to a currently estimated time point 0 in Nov 2013.

Recruitment on both Hemispheres will include a period of 'On Season' recruitment and a period of 'Off Season' recruitment and a third period of recruitment matching the first period.

An interim analysis data lock is scheduled after 1.5 years or in June 2015, whichever occurs first.

APPENDIX D ADDITIONAL INFORMATION REGARDING PRIMARY STUDY VISITS (AMENDED 15-DEC- 2017)

Study visit schedule related to the primary study (from birth up to the age of 2 years):

Visit 1 (screening visit):

- *Informed consent will be obtained by the investigator/study staff for each subject from the parent(s)/LAR(s) of the subjects during pregnancy, after at least 20 weeks of gestation and before the start of delivery, to include their child in the study, at birth.*
- *On obtaining the consent, one subject number will be assigned to each unborn child and recorded in the ICF and the copy of the ICF will be provided to the parent(s)/LAR(s). A sequential allocation of subject numbers will be assigned according to the range of subject numbers allocated to each study center.*
 - *In case of anticipated twins (or other multiple births), an ICF needs to be signed for each expected child before the start of delivery. A subject number will be assigned sequentially to each child as per the birth order, where the first born will be associated with the lowest subject number, the second born with the successive subject number, etc.*
 - *In case of unexpected twins (or other multiple births), only the children for whom an ICF was signed and subject number was assigned before birth can be enrolled. This should again occur as per the birth order.*
- *Eligibility criteria applicable before birth will be assessed. A pre-selected child who does not meet the eligibility criteria will be considered a screening failure.*
- *The expected delivery date will be collected as required by the eCRF.*
- *After obtaining the ICF and confirming eligibility, the subject needs to be entered into the randomization system on internet (SBIR) screening module. SBIR will control the recruitment rate as per an allocated target.*
- *The study site will contact the pregnant mother at their own discretion to monitor the status of the pregnancy.*

Visit 2 (enrollment visit commencing at birth):

- *During this visit, and before any study follow-up procedures are performed (no later than 5 working days after birth of the subject), the informed consent will be updated with relevant personal identifiers as applicable by local requirements and re-signed by the parent(s)/LAR(s), investigator and witness (when required). The investigator/study staff should also collect any further identifying data (e.g. official name, social security number) that becomes available for a subject during the course of the study, at regular surveillance contacts and record it in a supporting document.*

- *A baseline cord blood sample of up to approximately 10 mL will be collected at birth from all live born infants recruited at Visit 1 in the study.*
- *Eligibility of the infants for the study will be confirmed as per the inclusion and exclusion criteria that can be assessed only after birth. If the subject is found to be ineligible during this assessment, the cord blood sample that was collected will be discarded and the subject will be considered a screening failure. Eligibility (including the updated ICF) needs to be definitively confirmed not later than 5 working days after the birth or the subject will automatically become a screening failure. Eligibility also needs to be definitively confirmed before any other study procedure is performed.*

Refer to section 5.3 and 5.3.2 for details on the inclusion and exclusion criteria.

- *After obtaining the updated ICF and confirming eligibility, the subject needs to be entered in to the SBIR enrollment module. SBIR will randomize the subject to a time point for blood sample collection. If the subject is part of the sub-cohort, parent(s)/LAR(s) should be informed about this allocation.*
- *Information about the delivery and the newborn (including medical care provided immediately after birth and planned to be provided subsequently) will be collected by the study team from the parents/LAR, and if possible, completed and/or confirmed with an original source accessed through a person involved in the delivery of the subject or from any authorized person representing the healthcare organization that is in charge of the source.*
- *The parent(s)/LAR(s) will be interviewed to obtain information on the family medical history and demographic and lifestyle characteristics of the subject as required by the eCRF.*
- *Parent(s)/LAR(s) of the subjects will be instructed when to contact the investigator/study staff and will be provided with diary cards to record symptoms and healthcare utilization over the course of the disease when instructed to do so by the investigator/study staff. For details refer to ‘surveillance contacts (active and passive surveillance)’ below.*

Visit 3 (blood sample collection):

- *This visit will be applicable only for a sub-cohort of subjects. At the time point randomly allocated to each subject selected for a blood draw (2, 4, 6, 12, 18 or 24 months), an off-site or site visit needs to be scheduled and a single follow-up blood sample of 3.5 mL should be collected from the subjects between the intervals of ± 1 week, to be included in the analyses e.g. Month 2 ± 1 week, Month 4 ± 1 week. For details refer to [Table 1](#). Children who are born with a gestational age of less than 36 weeks will be excluded from this sub-cohort.*

APPENDIX E ADDITIONAL INFORMATION REGARDING LRTI SURVEILLANCE, INCLUDING ACTIVE SURVEILLANCE CONTACT SCHEDULE (PRIMARY STUDY) (AMENDED 15-DEC-2017)

Surveillance contacts (ACTIVE AND PASSIVE SURVEILLANCE)

In order to optimize the reporting procedures of suspected LRTI cases and to ensure that the maximum number of cases are timely identified and reported to the study sites, both active and passive surveillance will be conducted.

- *Passive surveillance by ‘self’-report of symptoms by parent(s)/LAR(s) of the subjects is key for timely identification of a potential LRTI case. Parent(s)/LAR(s) need to contact the investigator/study staff as soon as the child experiences new RTI symptoms (cough, runny nose, blocked nose), irrespective of the appearance of signs of difficulty in breathing, which will be determined by the investigator/study staff on contact.*
- *For the active surveillance, parent(s)/LAR(s) of all the subjects will be contacted by the investigator/study staff on a regular basis (active follow-up schedule will be confirmed based on the local settings and seasonality) to identify any potential LRTI case and to remind the parent(s)/LAR(s) of the subjects to report any new sign of RTI symptoms (cough, runny nose, blocked nose) as soon as possible themselves.*

Note: A child experiencing RTI symptoms (cough, runny nose, blocked nose) with any signs of difficulty in breathing will be considered a potential LRTI case.

During each active or passive follow-up contact, the investigator/study staff will:

- *Confirm with the parent(s)/LAR(s) of the subject, if the subject developed new RTI symptoms (cough, runny nose, blocked nose) and if he/she has developed any signs of difficulty in breathing (during and between contacts).*

- *In case there is any current sign or suspicion of difficulty in breathing in a child with symptom(s) of RTI at the time of the contact, the investigator/study staff will instruct the parent(s)/LAR(s) to start completing the diary card and will schedule an examination visit as soon as possible, but no later than 72 hours after the contact. Performing the visit as soon as possible is important to assess the need for medical care, to ensure that early symptoms are captured, and also to collect an optimal biological sample for the detection of RSV.*

The examination visit can be an on-site or off-site visit. An off-site visit is preferred in order to avoid influencing the pattern of healthcare utilization for the disease. Parent(s)/LAR(s) of the subjects can however be requested to bring their child to the study center for the site examination visit e.g. if this would be the normal standard of care to provide given the situation, if this will significantly expedite the timing of the visit, or if it is unlikely that the off-site examination visit can occur at

all within the required timeframe. If the reported symptoms are already of a level of severity that urgent care is indicated, the parent(s)/LAR(s) should be redirected to the proper location to receive this care (e.g. Emergency Room [ER]) and an examination visit could be scheduled to take place there at a suitable time.

- *The surveillance contact and the decision that an examination visit is indicated should be recorded in the eCRF. Refer to ‘examination visit’ for next steps.*
- *In case the symptoms do not include current signs of RTI and difficulty in breathing, this surveillance/episode will be recorded in the site’s source documentation and in the eCRF, but no examination visit will be scheduled. Parent(s)/LAR(s) will be instructed to contact the study team as soon as possible if the severity of the already existing symptoms in the child increases or if the child who did not have signs of difficulty breathing develops the symptom.*
- *If during the contact, the investigator/study staff schedules an examination visit (off-site/site), the parent(s)/LAR(s) are instructed to start completion of the diary card. Parent(s)/LAR(s) need to fill the diary card until Day 14 from the day the investigator/study staff have instructed them to initiate the diary card, unless the investigator/study staff informs them otherwise (e.g. in case of a second examination visit). If the symptoms last for more than 14 days the parent(s)/LAR(s) should continue to assess the symptoms until no more symptoms are present, and record in the diary card, the date at which no more symptoms were observed in the child.*
- *For any reported illness, the investigator/study staff should assess the need for any intervention and provide the same as part of regular healthcare or instruct/advise the parent(s)/LAR(s) where to obtain this care.*
- *The active and passive surveillance contacts and completion of diary card can also be made by/with person designated by the parent(s)/LAR(s) (e.g. grandparents, nanny) as long as the parent(s)/LAR(s) have approved the same. If needed, a field worker can also be assigned to support the parent(s)/LAR(s) and/or person designated by the parent(s)/LAR(s) in completing the diary card.*
- *On a quarterly basis, an active surveillance contact will be expanded to obtain updated information on the demographic, lifestyle and general medical characteristics of the subject as required by the eCRF.*

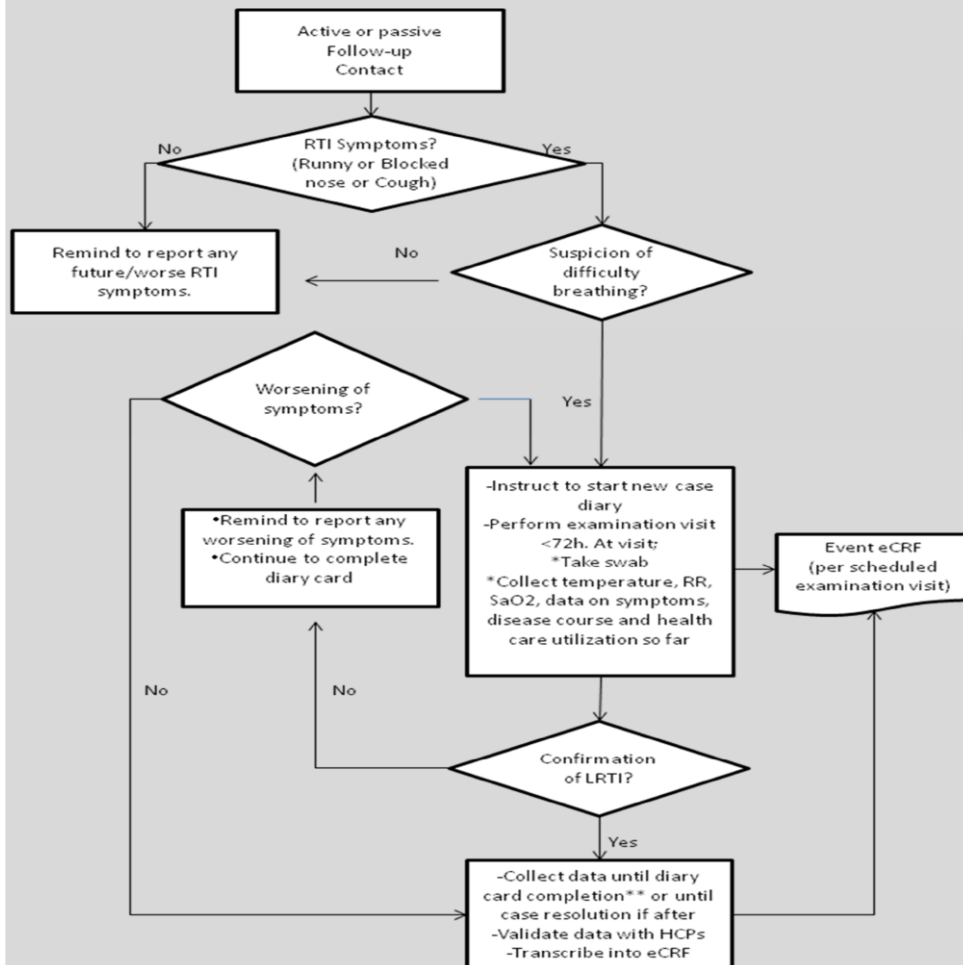
Refer to phone script provided along with the protocol for details.

Examination visit (off-site/site):

- *A case eCRF needs to be created for each scheduled examination visit and data collected up to case resolution will be entered. Examination visits will be allocated with a unique visit number that is a sequential number per subject.*
- *During the examination visit (off-site/site), the investigator/study staff will objectively confirm the RTI and difficulty breathing (i.e. involvement of lower*

respiratory tract) experienced by the subject. Data will be collected on all the clinical symptoms present in the child including a measurement of the respiratory rate (RR) and blood oxygen saturation [SaO2] by pulse oximetry and recorded in the eCRF. The investigator/study staff will consider whether the symptoms meet the LRTI case definition for surveillance. Refer Section 4 for details on symptoms to be recorded. If the symptoms are not (yet) considered to be an LRTI, a potential additional examination visit (off-site/site) in case of worsening of symptoms may be needed (Refer to Figure 4 for the decision model).

Figure 4 Decision tree for active and passive follow-up contact for the primary study



RTI = Respiratory Tract Infection; 72h = 72 hours; RR = Respiratory Rate; SaO2 = Blood Oxygen Saturation
eCRF = electronic Case Report Forms; LRTI = Lower Respiratory Tract Infections; HCP = Healthcare Provider

** Parent(s)/LAR(s) need fill in the diary card until Day 14 from the day the investigator/study staff have instructed them to initiate the diary card.

Note: The investigator/study staff will do an initial assessment of a case reported for the examination visit as either RTI or LRTI, only to determine further study activities. The final classification of all the cases reported as either RTI, LRTI or severe LRTI will be done during statistical analyses (as per the LRTI case definition and severity scale and comparative scales).

- *Nasal swabs will be collected for all the subjects who are reported for an examination visit (off-site/site) (this includes subjects with no confirmed LRTI).*
- *Data on disease course and healthcare utilization recorded by the subject's parent(s)/LAR(s) in the diary card until the examination visit will be reviewed and recorded by the investigator/study staff in the subject's eCRF.*
- *During subsequent regular surveillance contacts (active and passive) after an examination visit, the status and evolution of the case will be followed, and parent(s)/LAR(s) will be reminded to complete the diary card for the full period or until case resolution, if after. Data provided by the parent(s)/LAR(s) in the diary card on healthcare utilization should ideally be confirmed by direct contact of the investigator/study staff with the healthcare providers involved in management of the case (e.g. General Practitioner [GP], hospital staff) and recorded in the eCRF. Where possible, the data on healthcare utilization should be made more complete as to best capture the healthcare utilization in the eCRF. Any clinical diagnoses for the respiratory infection (e.g. pneumonia, bronchiolitis) made by the contacted healthcare providers should be collected and recorded in the eCRF as well as the symptoms assessed in support of this diagnosis (especially measured RR and SaO₂).*
- *A new diary card and case eCRF will be initiated for each scheduled examination visit. Any ongoing eCRF for that case from previous examination visits will be closed and further data recorded in the new case eCRF. If a surveillance case eCRF is 'closed' due to a subsequent examination visit for the same case, the diary card relating to the 'closed' eCRF is stopped prematurely and a new 14-day diary card is started (see also Section 3-examination visit).*
- *Collection of the completed diary cards will be done at resolution by mail or in person depending on local best practice.*

ACTIVE SURVEILLANCE CONTACT SCHEDULE to identify any potential LRTI case

Active surveillance is aimed at confirming that a case is/was not missed due to lack of reporting by parent(s)/LAR(s) and to remind parent(s)/LAR(s) to report cases of RTI symptoms as soon as possible themselves.

Active surveillance is allowed to occur through various media (e.g. text messaging, email, telephone, and in-person) based on local possibilities

- *Requirements for non-live media (e.g., text messaging and email) are that;*
 - *They meet GSK, local and international requirements with regards to the validity of the system used and data privacy and security.*

- *At least one live (e.g., telephone or in-person) contact occurs every quarter.*
- *An adequate response is obtained on non-live contact (e.g. return text message or return email). Any non-live contact that is not responded to after a maximum of 2 reminders within the course of 2 days should be followed up with a live contact.*

Active surveillance is to occur at a defined frequency dependent on the known RSV seasonality.

- *RSV has a typical winter/monsoon seasonality. The peak rates of RSV occur at different times on the Northern and Southern Hemisphere. The tropical regions are generally known to have little or no seasonality.*

For this reason, an active surveillance schedule will usually be applied based on the known or expected RSV seasonality for each study country/site.

- *12 annual contacts - One monthly contact in all the regions.*
- *At least 4 seasonal contacts – An intermediate contact in the four known/expected peak months, depending on the RSV seasons in the country/site. More intermediate contacts may be scheduled if deemed necessary by the investigator.*

Outside the scheduled active surveillance contacts, each country/site can employ additional activities to enforce active and passive surveillance at their discretion. These activities can be tailored to best meet the local situation (e.g. reminders, community consultation sessions, additional in-person subject support visits, etc.).

TIMING AND REQUIREMENTS FOR CONTACT

- *Active surveillance contacts are scheduled compared to the calendar week (Sat-Sun) of enrollment into the study. So regardless of the day of birth, the first Sunday after birth is Week 1 in the surveillance schedule.*
- *Seasonal contacts can occur anytime during the season they are scheduled to occur. A minimum of three attempts, occurring on at least two different working days should be made.*
- *Monthly contacts should occur in the first two weeks of the calendar month. A minimum of six attempts, occurring on at least four different working days spread over two subsequent weeks should be made to make contact for monthly surveillance contacts.*

DISCONTINUATION OF ACTIVE SURVEILLANCE

All reasonable efforts should be made to conduct all active surveillance contacts in line with the schedule mentioned above. However, subjects' parent(s)/LAR(s) of the subject may not be available for active surveillance for a period of time.

- *During active contacts, investigator/study staff should request the parent(s)/LAR(s) of the subject if they will be unavailable for a scheduled period (e.g. due to holiday). If so, the active surveillance contacts can then be actively planned differently or cancelled compared to the regular schedule. No more than 2 consecutive active surveillance contacts and/or a period of more than 1 month can be cancelled.*
- *Active surveillance should be suspended after there has been no contact for more than 3 planned contacts and/or no contact for more than 2 months and a final attempt has been made by mail or a live visit. If the parent(s)/LAR(s) of the subject contact the investigator/study staff at a later point in time through passive surveillance, the case should be followed up, data should be collected at intermittent periods and active surveillance could be reinitiated.*

APPENDIX F AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

<p style="text-align: center;">GlaxoSmithKline Biologicals</p> <p style="text-align: center;">Vaccine Value & Health Science (VVHS)</p> <p style="text-align: center;">Protocol Amendment 1</p>	
eTrack study number and Abbreviated Title	200150 (EPI-RSV-005 BOD)
Amendment number:	1
Amendment date:	27 November 2013
Co-ordinating author:	PPD, Scientific writer
<p>Rationale/background for changes:</p> <p>In order to reduce study complexity and manage the global study budget the study design has been amended as follows.</p> <ul style="list-style-type: none"> The sample size has been reduced from 3,000 subjects to 2,400 subjects to balance recruitment of approximately 400 subjects in each geographic/climatic area. Reducing the sample size will affect the precision of the estimates obtained, but will be sufficient to meet the study objectives with a sample size of 2,400 subjects. The maximum number of active surveillance contacts has been fixed to 16 per year per subject. Reducing active surveillance impacts the probability to detect cases of LRTI. To balance the risk of missing cases of LRTI, the study sites will also perform passive surveillance. The study procedure manual will outline passive surveillance methods that could be implemented in country settings e.g. social media applications in developed countries versus regular (weekly) person-to-person community visits in less developed countries. In addition, some minor editorial changes have been made. 	

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, Discovery and Early Development***
- PPD, ~~Global Study~~ ***Delivery Manager***, Harrison Clinical Research Benelux n.v. a SynteractHCR, Inc Company for GlaxoSmithKline Biologicals
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- PPD, ***Project Delivery Lead***
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- PPD, ~~Project Manager, Global Study Management~~ ***Study Delivery Lead***
- PPD, ~~Lead Statistician~~ ***Senior Manager, Biometrics***

Synopsis (Tertiary objectives):

- ~~To explore the association of other respiratory viruses with the incidence of LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.~~
- *To explore the impact of variations in cord-blood sample collection variables on the stability of test results.*
- *To assess the natural decay of maternal antibody levels using a single follow-up blood sample collected from a randomly selected sub-cohort of subjects at 2, 4, 6, 12, 18 and 24 months.*

Synopsis and Section 3. (Study design overview):

Study population: A cohort of approximately ~~3000~~ **2400** infants will be included in the study at birth and followed-up to a maximum of 2 years of age.

- Subjects will be recruited and followed up through active and passive surveillance for a maximum of 2 years. The study involves the following visit and contact schedule:
 - Visit 1 (screening visit)
 - Visit 2 (enrollment visit, **commencing** at birth)
 - Assuming no delays in recruitment, two-thirds of the subjects (approximately ~~2000~~**1600**) will be recruited in Year 1 of the study and will be followed-up up to 2 years of age, resulting in a total study duration of 3 years. The remaining subjects (approximately ~~4000~~**800**) will be recruited in the first half of Year 2 of the study and will be followed up till the end of Year 3.

Table 2 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch 1
Prospective	Approximately 3000 2400	At birth-maximum of 2 years	x

Synopsis and Section 3.1 (Discussion of study design):

The first 2000 subjects (~~ideally recruited in the first year of recruitment~~) enrolled in the study will be randomized at recruitment to a time point at which a single blood sample will be collected from these subjects ***if they are still participating in the study at that time.***

Synopsis (Number of subjects):

Target enrollment will be ~~3000~~**2400** subjects to obtain approximately ~~2400~~**1920** fully evaluable subjects at study completion.

Section 5.1. Number of subjects/centers: Target enrollment will be ~~3000~~**2400** subjects to obtain approximately ~~2400~~**1920** fully evaluable subjects at study completion.

Subject recruitment is intended to occur over a period 18 months or until the planned target enrollment has been met. Assuming no delays in recruitment, two-thirds of the subjects (~~2000~~**1600**) will be recruited in Year 1 and the remaining subjects (~~4000~~**800**) in the first half of Year 2 of the study (See Figure 3).

Synopsis and Section 9.1.3 (Tertiary endpoints):

Cord-blood sample collection variables including collection and storage times and temperatures.

Section 2.3 Tertiary objectives:

To explore the impact of variations in cord-blood sample collection variables on the stability of test results.

Section 3 Study Design Overview:

Figure 1 Study design diagram:

Footnote added – “* *All follow up procedures associated with V2 (e.g. ICF) must be performed within 5 working days after birth.*”

Study visit schedule:**Visit 1 (screening visit)**

The study site will contact the pregnant mother at their own discretion to monitor the status of the pregnancy.

Visit 2 (enrollment visit commencing at birth):

Information about the delivery and the newborn (including medical care provided immediately after birth and planned to be provided subsequently) will be collected by the study team from ***the parents/LAR, and if possible, completed and/or confirmed with*** an original source accessed through a person involved in the delivery of the subject or from any authorized person representing the healthcare organization that is in charge of the source.

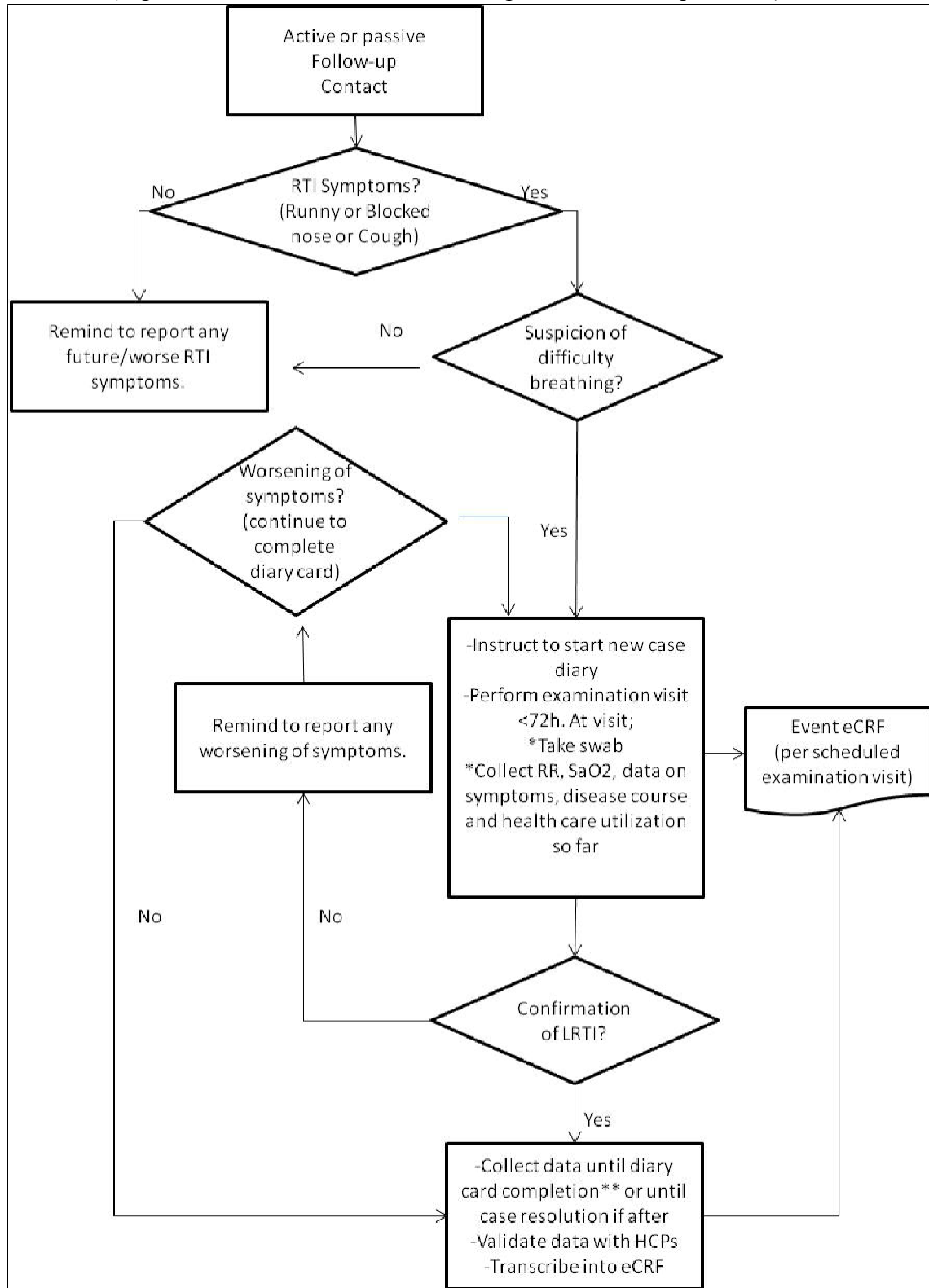
Data provided by the parent(s)/LAR(s) in the diary card on healthcare utilization should ***ideally*** be confirmed by direct contact of the investigator/study staff with the healthcare providers involved in management of the case (e.g. General Practitioner [GP], hospital staff) and recorded in the eCRF.

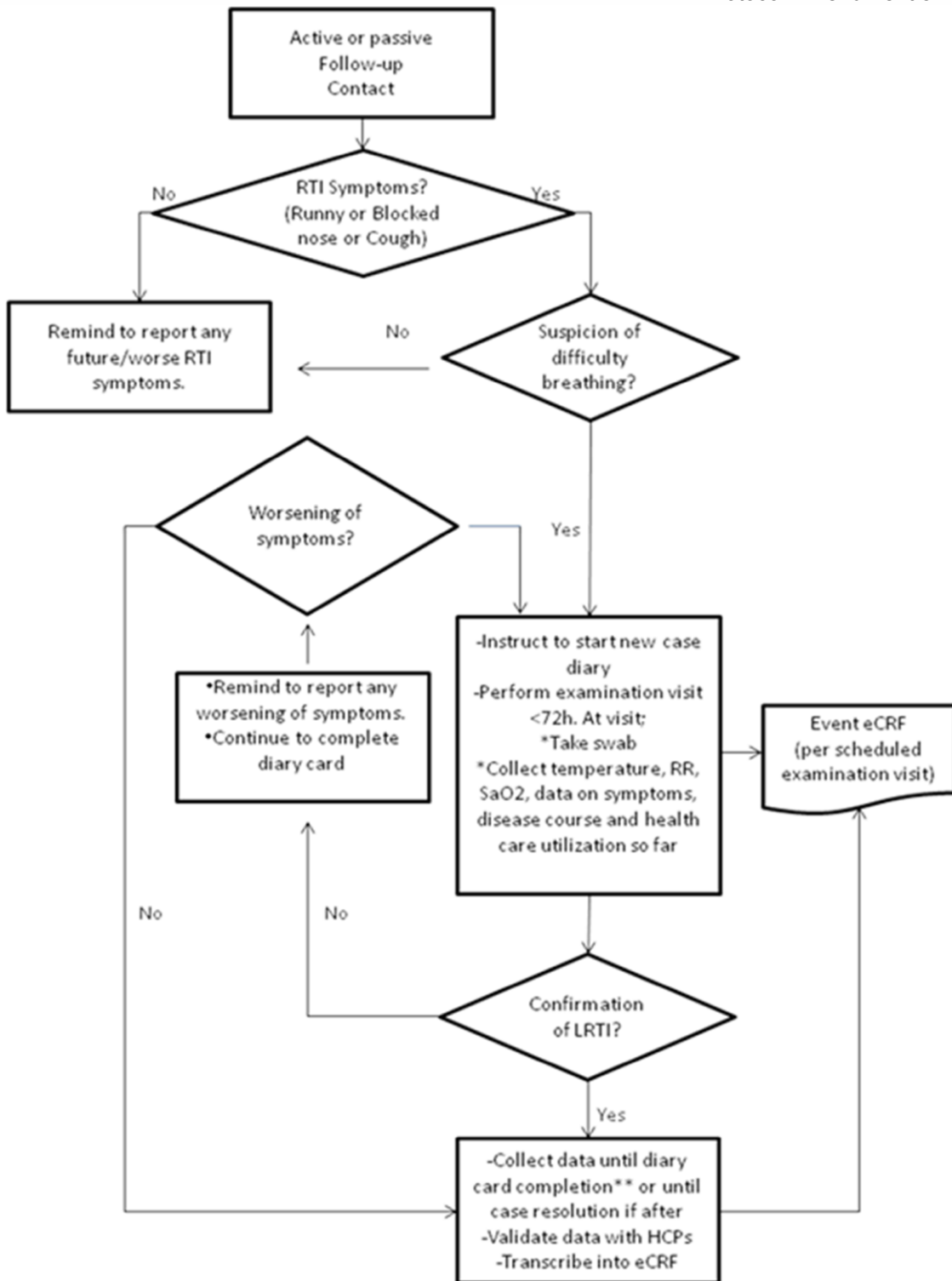
Refer to APPENDIX C for active and passive contact schedule.

Examination visit (off-site/site):

Data provided by the parent(s)/LAR(s) in the diary card on healthcare utilization should ***ideally*** be confirmed by direct contact of the investigator/study staff with the healthcare providers involved in management of the case (e.g. General Practitioner [GP], hospital staff) and recorded in the eCRF.

Section 3 (Figure 2 Decision tree for active and passive follow-up contact)





Section 3.1:

Proper passive surveillance is important in order to identify potential cases as early as possible. The active surveillance is mostly aimed at reinforcing the passive surveillance as the chance of picking up a (developing) case at the exact time of the active surveillance contact is limited.

In between scheduled active surveillance contacts, the study staff can employ activities to solicit passive surveillance according to local practices, e.g. reminder emails or text messages up to regular community centre visits.

*Where possible, the study team will confirm **and expand** relevant healthcare utilization with the healthcare provider involved in the management of the case.*

Section 4 Case definition:

- ~~A~~apnea

Section 5.2 Inclusion criteria for enrolment:

- Subject for whom updated and re-signed informed consent and confirmation of eligibility is available not later than 5 **working** days₇ after birth.
- ~~New born male or female.~~
- ~~Cord B~~blood ~~cord~~ sample collection of at least 3 mL, at birth.

Section 5.3:

Subjects with any confirmed or suspected immunosuppressive or immunodeficient condition **at time of enrollment** (including positive infection with human immunodeficiency virus [HIV]), based on medical history, physical examination or positive test result.

Section 6.4:

Table 6 List of study procedures

Only the amended rows have been added here:

Time points	Pre - Day 0	At birth Post - Day 0 [†]	As randomized [†]	Regular ^{&}	Unscheduled
Examination during (off-site/site) visit and collection of all disease symptoms (including temperature , RR and pulse oximetry)					•

[†] *Visit 2 can take place anytime within 5 working days after birth.*

Section 6.5.5:

In case less than 3 mL of whole blood is collected, the subject will be considered a screening failure and the sample collected needs to be discarded according to local procedure.

Details of the sample collection such as duration of processing steps and storage conditions need to be collected and recorded in the eCRF in order to allow adjustment

for these variables in the primary analyses and to explore the impact of collection variables on the measured antibody titer.

Section 6.5.8:

Information about the delivery and the newborn (including medical care provided immediately after birth and planned to be provided subsequently) should be collected ***from the parents/LAR and completed and/or confirmed*** from an original source accessed through a person involved in the delivery of the subject or from any authorized person representing the healthcare organization that is in charge of the source and the collected data should be recorded in the subject's eCRF.

Section 6.5.12:

~~Each a~~ ***Active surveillance contacts required by the surveillance schedule and each*** passive surveillance contact, whether or not it triggers an examination visit, will be recorded in the site's source documentation and in the eCRF.

Section 6.5.13:

On a quarterly basis, the investigator/study staff should expand a regular surveillance contact by asking ***the parents/LARs*** for changes to the baseline medical, demographic and lifestyle characteristics data collected at Visit 2.

- vaccinations received (***on disease level, not full vaccination details***)

Section 6.5.14:

Data should be collected on all the clinical symptoms present in the child including a measurement of the ***temperature***, RR and pulse oximetry.

Temperature as measured by the investigator/study staff during the examination visit (off-site/site), and as subsequently assessed from the diary card, and/or confirmed with any healthcare provider involved, during course of the disease.

Note:

~~H~~***Healthcare utilization (or healthcare setting availed) refers to self-care with OTC drugs, GP visits, ER visits, hospital visits, etc.***

Data from healthcare providers (such as RR, SaO2, symptoms and diagnosis) is to be regarded as a valuable, but optional extra, if this data is available and shared by the applicable healthcare provider.

Section 6.5.15 and Section 6.5.18 (Note):

Data from healthcare providers (such as RR, SaO2, symptoms and diagnosis) is to be regarded as a valuable, but optional extra, if this data is available and shared by the applicable healthcare provider.

Section 9.2:

Table 10 shows the exact 95% confidence interval (CI) (Poisson exact distribution and normal approximation of the Poisson distribution accounting for a design effect ***of 4.18***) for a range of expected incidence rates of RSV that we can reasonably estimate between 10 and 50 per 1000 children-years in the first year of birth. With an overall sample size of about ~~400320~~ ***400320*** evaluable subjects by country during the first year of enrolment and with a

follow-up period of 1 year after the last subject is recruited, the CI for an expected RSV-associated LRTI hospitalization rate of 20 cases per 1000 children-years is respectively [8.66.9; 39.440.8] for the exact Poisson distribution and [0; 53.751.7] for the normal approximation with design effect of 4.18.

Table 10 Precision for expected RSV-associated LRTI hospitalization rate during the first year of life (per 1000 children-years)

	Number of children-years	Number of cases	RSV-associated LRTI rate (per 1000 children-years)	95% CI Poisson Exact	95% CI Normal approx. with DE
First year of enrolment (analysis will be performed 1 year after the last subject is recruited)					
Country/region level	400320	43	10	[2.7; 25.6] [1.9; 27.4]	[0.0; 32.2] [0; 32.4]
	400320	86	20	[8.6; 39.4] [6.9; 40.8]	[0.0; 51.4] [0; 51.7]
	400320	1210	30	[15.5; 52.4] [13.9; 55.4]	[0.0; 68.4] [0; 68.8]
	400320	1613	40	[22.9; 65.0] [20.5; 67.5]	[0.0; 84.4] [0; 84.8]
	400320	2016	50	[30.5; 77.2] [28.6; 81.2]	[0.4; 99.6] [0; 100.1]
Overall	16001280	1613	10	[5.7; 16.2] [5.1; 16.9]	[0.0; 21.1] [0; 21.2]
	16001280	3226	20	[13.7; 28.2] [13.0; 29.3]	[4.3; 35.7] [4.2; 35.8]
	16001280	4838	30	[22.1; 39.8] [21.0; 40.7]	[10.8; 49.2] [10.6; 49.4]
	16001280	6451	40	[30.8; 51.1] [29.7; 52.4]	[17.8; 62.2] [17.6; 62.4]
	16001280	8064	50	[39.6; 62.2] [38.5; 63.8]	[25.2; 74.8] [25.0; 75.0]
Second year of enrolment (analysis will be performed 1 year after the last subject is recruited)					
Country/region level	200160	2	10	[1.2; 36.1] [0.7; 40.1]	[0.0; 41.4] [0; 41.7]
	200160	43	20	[5.4; 51.2] [3.9; 54.8]	[0.0; 64.4] [0; 64.8]
	200160	65	30	[11.0; 65.3] [8.4; 68.5]	[0.0; 84.4] [0; 84.9]
	200160	86	40	[17.3; 78.8] [13.8; 81.6]	[0.0; 102.8] [0; 103.4]
	200160	108	50	[24.0; 92.0] [21.6; 98.5]	[0.0; 120.2] [0; 120.8]
Overall	800640	86	10	[4.3; 19.7] [3.4; 20.4]	[0.0; 25.7] [0; 25.8]
	800640	1613	20	[11.4; 32.5] [10.2; 33.7]	[0.0; 42.2] [0; 42.4]
	800640	2419	30	[19.2; 44.6] [17.9; 46.4]	[2.8; 57.2] [2.6; 57.4]
	800640	3226	40	[27.4; 56.5] [25.9; 58.6]	[8.6; 71.4] [8.3; 71.7]
	800640	4032	50	[41.5; 59.8] [34.2; 70.6]	[14.9; 85.1] [14.6; 85.4]

CI = Confidence Interval; RSV = Respiratory Syncytial Virus;
LRTI = Lower Respiratory Tract Infection; DE = Design Effect

The design effect measures the increase in the standard error of the estimate due to the sampling design. The following formula will be used to estimate the design effect *in the simulation* [Bennett, 1991]

Each simulation consists of creating ‘c’ clusters whose size and incidence vary around a given value. Then the above formula was applied to estimate the design effect *in the simulation*. Table 11 shows the median result of 1000 simulations (with 5th and 95th percentile). *The design effect [Bennett, 1991] can be estimated as following once the intra-cluster correlation value is known or can be assumed:*

$$DE = 1 + (b - 1) \rho$$

Where, ρ is the intra-cluster correlation and b is the average number of subjects sampled per cluster. According to the results of simulations, *we could assume that the intra-cluster correlation of 0.02 (See Table 11 below)* and considering 12 clusters of ~~200160~~ subjects, we expect that the design effect will take a value *of 4.18*~~between 2.57 and 9.49. A median value of 5.13 was used for the CIs described in Table 9.~~

Assuming a drop-out rate of about 20% during the course of follow-up approximately ~~6400~~ subjects by country/region and ~~3000~~**2400** subjects overall will need to be recruited.

Section 9.3.3 ATP cohort:

The ATP cohort will include all subjects meeting all eligibility criteria up to the time of their censoring, either at study completion or prematurely as drop-out (e.g. withdrawn consent, lost-to-follow-up, lack of compliance).

Section 9.8 Analysis of tertiary objectives:

- *To explore the impact of variations in cord-blood sample collection variables on the stability of test results.*

The measured maternal antibody levels will be assessed versus variability in sample collection variables. This involves assessment versus various continuous and categorical variables and an overall comparison between samples as classified ‘according to recommended procedure’ and ‘not according to recommended procedure’.

The measured maternal antibody levels by duration of the processing step and overall process duration will be analysed graphically and by log-linear regression.

The measured maternal antibody levels by storage condition or tube type will be analysed graphically and by group statistics (mean, 95% CI). Group means will be compared by t-test to assess the statistical significance of differences.

The measured maternal antibody levels in samples collected ‘according to recommended procedure’ and ‘not according to recommended procedure’ will be analysed graphically and by group statistics (mean, 95% CI). Group means will be compared by t-test to assess the statistical significance of any difference.

Section 9.9 Interpretation of analyses:

The ~~second~~ tertiary objective ***'To explore the impact of changes to the symptoms and threshold levels of symptoms in the LRTI case definition and severity scale'*** will be assessed if we detect potential areas of improvement of the LRTI case definition and severity scale, such as clearly under or overestimated incidence rates of LRTI/ severe LRTI compared to the rates in the literature or a lack of discriminative power between severe and non-severe cases.

Section 9.10.1 Sequence of analyses:

An interim analysis will ***be*** performed after data lock at the end of recruitment (expected after 18 months) or in June 2015 whichever occurs first, on the data collected for the subjects recruited in the first year of the study (approximately ~~2000~~**1600**).

APPENDIX C Active surveillance contact schedule:

- Requirements for the ***non-live*** media chosen are that;
 - At least one ~~in three subsequent~~ ***live*** contacts ~~is done by phone~~***occurs every quarter.***
 - An adequate response is obtained on non-live contact (e.g. return text message or return email). Any non-live contact that is not responded to ***after a maximum of 2 reminders*** within ***the course of 2 + days*** should be followed up with a ~~phone call~~***live contact.***

RSV has a typical winter/***monsoon*** seasonality.

For this ~~e-above mentioned~~ reasons, ***an*** active surveillance ***schedule will be applied based on the known or expected RSV seasonality for each study country/site*** ~~is to occur according to the following schedule per geographic area (each study site will be allocated to a schedule based on geography and known data on seasonality pattern);~~

- ***12 annual contacts - One monthly contact in all the regions.***
- ***4 seasonal contacts – An intermediate contact in the four known/expected peak months, depending on the RSV seasons in the country/site.***
- ***Outside the scheduled active surveillance contacts, each country/site can employ additional activities to enforce passive surveillance at their discretion. These activities can be tailored to best meet the local situation (e.g. reminders, community consultation sessions etc.).***
- Northern Hemisphere
 - Every two weeks in October
 - Every week in November to February
 - Every two weeks in March
 - Every month in April to September
- Southern Hemisphere

- ~~Every two weeks in May~~
- ~~Every week in June to August~~
- ~~Every two weeks in September~~
- ~~Every month in October to April~~
- ~~Tropical region-~~
~~Every two weeks all year~~

~~Weekly and two weekly surveillance~~ *Seasonal contacts* can occur anytime during the ~~week~~ *season* they are scheduled to occur. A minimum of three attempts, occurring on at least two different working days should be made ~~to make contact for weekly and two-weekly surveillance contacts.~~

Active surveillance should be suspended after there has been no contact for more than 3 planned contacts and/or no contact for more than 2 months and a final attempt has been made by mail or *a live visit*.

GlaxoSmithKline Biologicals Vaccine Value & Health Science (VVHS) Protocol Amendment 2	
eTrack study number and Abbreviated Title	200150 (EPI-RSV-005 BOD)
Amendment number:	Amendment 2
Amendment date:	06 July 2016
Co-ordinating author:	PPD [REDACTED], Scientific writer
Rationale/background for changes: <p>Infant respiratory viral infection and childhood asthma are the most common acute and chronic diseases of childhood, respectively. Common clinical features and links between these diseases have long been recognized, with early-life respiratory syncytial virus (RSV) lower respiratory tract infections (LRTIs) being strongly associated with increased asthma risk with no direct link to causality.</p> <p>World Health Organization (WHO) has recently updated its RSV-associated LRTI case definition. The latter includes clinical features considered to be objective, easily standardized, generalizable across settings, and generally accepted markers of severe or very severe RSV disease.</p> <p>Although asthma is probably a heterogeneous disease or syndrome, three factors and/or events consistently emerge for their ability to significantly influence asthma inception in the first decade of life: immune response aberrations, which appear to be defined best by the concept of cytokine dysregulation; LRTIs, in particular RSV; and some form of gene–environment interaction that needs to occur at a critical time-period in the development of the immune system or the lung [Lemanske, 2002].</p>	

The Childhood Origins of Asthma (COAST) study was developed to determine and define the importance of these three factors on asthma pathogenesis and to test different asthma case classifications [Lemanske, 2002, Lemanske, 2005, Bisgaard, 2011, Jackson, 2008, Jackson, 2012, Bønnelykke, 2015]. These studies have used similar albeit slightly different case definitions on asthma diagnosis. These definitions are widely used in epidemiological literature of asthma etiology in children. The Bisgaard definition [Bisgaard, 2011] has multiple criteria that are assessed by the physician from records. Therefore the advantage of this definition is that the same objective criteria are applied to ascertain each case of asthma. However, its application may be challenging in some settings if routine medical records do not fully capture the necessary information in source. The Jackson definition [Jackson, 2008] includes the criteria of HCP diagnosis of asthma and prescription of β_2 agonists. This definition is therefore influenced by any differences in local practice of asthma diagnosis and management of wheeze. However, its advantage is that it captures the reality of diagnosis and treatment. This study anticipates that Bisgaard will be more specific and Jackson more sensitive. Hence, this study will assess the performance of these two case definitions since this will help planning for phase 3 clinical trials of RSV vaccines.

Numerous longitudinal studies have demonstrated this first important line of evidence that RSV LRTIs precede the development of asthma. Several birth cohort studies also demonstrate a dose–response relationship between infant respiratory viral infection severity and asthma risk, with increasing infant infection severity associated with greater childhood asthma risk and asthma severity [Feldman, 2015].

Population-level contribution of RSV LRTIs is best demonstrated by the population-attributable risk for asthma after these early-life LRTIs. Among infants, the prevalence of LRTI is approximately 18 to 32% in the first year of life and approximately 9 to 17% in the second year of life.

Among infants with LRTI, the prevalence of RSV can be as high as 80%, especially in the first 3 months of life. Thus, the phenotype of asthma after infant RSV LRTI accounts for up to 31% of early childhood asthma, with a population-attributable risk estimated at about 13% in several diverse populations [Feldman, 2015].

This cohort study provides us an opportunity to evaluate a potential association and the disease burden in the population due to RSV LRTI (exposure) early in life and the development of recurrent wheeze and asthma later in life (outcome). Derived calculations of incidence of recurrent wheeze and asthma among RSV LRTI positive and negative subjects and the calculation of population attributable risk percent will be able to potentially demonstrate a measurement of potential public health impact if a successful maternal and/or pediatric vaccine introduced and exposure (RSV) eliminated/reduced.

Hence, the protocol is being amended to reflect the following changes:

- All subjects enrolled in the study will now be followed up from 2 years of age up to 6 years of age to capture episodes of wheeze and the diagnosis of asthma.
- Additional secondary and tertiary objectives are being included in order to support exploratory analysis of the extent the early life experience of RSV LRTI is accounting for the total burden of wheeze and asthma from birth up to 6 years of age.
- To perform an exploratory analysis and test of asthma case definitions and case classification performance based on well defined and commonly used case definitions from epidemiological studies.
- To update LRTI case definitions and severity scale (according to WHO, 2015 [Modjarrad, 2016]).
- Information from this study will now be used to infer the potential benefit in terms of disease burden reduction and health care utilization of successful implementation of vaccination against RSV and measurement of potential public health impact and potential incidence reduction if a maternal and/or pediatric vaccine introduced and exposure (RSV) eliminated.
- Overall, the new objectives aim to support the fact that successful maternal and paediatric vaccines that would be introduced would provide protection through the first two years of life against RSV LRTI and thus would reduce the burden of recurrent wheeze and asthma associated RSV from birth until the sixth birthday.

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

Title page:

Title:

A prospective, epidemiological study to assess the disease burden of ~~respiratory syncytial virus (RSV) associated, suspected lower respiratory tract infections (LRTIs) in newborns, from birth up to 2 years of age~~ ***from 0 to 2 years and population attributable risk percent of RSV LRTI on the development of recurrent wheeze and asthma from 0 to 6 years.***

Detailed title:

A prospective, epidemiological, interventional, multi-country based, cohort study to assess the disease burden of respiratory syncytial virus (RSV) associated, suspected lower respiratory tract infections (LRTIs) in newborns, from birth up to 2 years of age ***and population attributable risk percent of RSV LRTI on the development of recurrent wheeze and asthma from birth up to 6 years of age.***

List of contributing authors:

- PPD [REDACTED], **Senior Epidemiology Lead**
- PPD [REDACTED], ~~Global Vaccines Clinical Laboratory, Laboratory Project Manager~~
Clinical Read-Out Team Leader
- PPD [REDACTED], **Project Delivery Lead**
- PPD [REDACTED], **Clinical Laboratory Sciences Study Manager**
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- PPD [REDACTED]; **Project Data Manager**
- PPD [REDACTED], **Project Data Manager**
- PPD [REDACTED], **Study Data Manager**

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Protocol Amendment 2 Sponsor Signatory Approval:

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Director,

~~VVHS Epidemiology, GSK Biologicals~~

Amanda Leach

Clinical and Epidemiology R&D Project Leader

RSV Program, GSK Biologicals

Glossary of terms:

Exercise: Any moderate or vigorous physical activity performed by the child.

Population Attributable Risk: Population attributable risk is the portion of the incidence of a disease in the population (exposed and unexposed) that is due to exposure. It is the incidence of a disease in the population that would be eliminated if exposure were eliminated.

Population Attributable Risk Percent: Population attributable risk percent is the percent of the incidence of a disease in the population (exposed and unexposed) that is due to exposure. It is the percent of the incidence of a disease in the population that would be eliminated if exposure were eliminated.

Synopsis and section 1.2 Rationale for the study

Infant respiratory viral infection and childhood asthma are the most common acute and chronic diseases of childhood, respectively. Common clinical features and links between these diseases have long been recognized, with early-life RSV LRTIs being strongly associated with increased asthma risk.

World Health Organization (WHO) has recently updated its RSV-associated LRTI case definition [Modjarrad, 2016]. The latter includes clinical features considered to be objective, easily standardized, generalizable across settings, and generally accepted markers of severe or very severe RSV disease.

Although asthma is probably a heterogeneous disease or syndrome, three factors and/or events consistently emerge for their ability to significantly influence asthma inception in the first decade of life: immune response aberrations, which appear to be defined best by the concept of cytokine dysregulation; LRTIs, in particular RSV; and some form of gene–environment interaction that needs to occur at a critical time-period in the development of the immune system or the lung [Lemanske, 2002].

The Childhood Origins of Asthma (COAST) study was developed to determine and define the importance of these three factors on asthma pathogenesis and to test different asthma case classifications [Lemanske, 2002; Lemanske, 2005; Bisgaard, 2011; Jackson, 2008; Jackson, 2012; Bonnelykke, 2015]. These studies have used similar albeit slightly different case definitions on asthma diagnosis. These definitions are widely used in epidemiological literature of asthma etiology in children. The Bisgaard definition [Bisgaard, 2011] has multiple criteria that are assessed by the physician from records. Therefore the advantage of this definition is that the same objective criteria are applied to ascertain each case of asthma. However, its application may be challenging in some settings if routine medical records do not fully capture the necessary information in source. The Jackson definition [Jackson, 2008] includes the criteria of HCP diagnosis of asthma and prescription of β_2 agonists. This definition is therefore influenced by any differences in local practice of asthma diagnosis and management of wheeze. However, its advantage is that it captures the reality of diagnosis and treatment. This study anticipates that Bisgaard will be more specific and Jackson more sensitive. Hence, this study will assess the performance of these two case definitions since this will help planning for phase 3 clinical trials of RSV vaccines.

Numerous longitudinal studies have demonstrated this first important line of evidence that RSV LRTIs precede the development of asthma. Several birth cohort studies also demonstrate a dose–response relationship between infant respiratory viral infection severity and asthma risk, with increasing infant infection severity associated with greater childhood asthma risk and asthma severity [Feldman, 2015].

Population-level contribution of RSV LRTIs is best demonstrated by the population-attributable risk for asthma after these early-life LRTIs. Among infants, the prevalence of LRTI is approximately 18 to 32% in the first year of life and approximately 9 to 17% in the second year of life. Among infants with LRTI, the prevalence of RSV can be as high as 80%, especially in the first 3 months of life. Thus, the phenotype of asthma after infant RSV LRTI accounts for up to 31% of early childhood asthma, with a population-attributable risk estimated at about 13% in several diverse populations [Feldman, 2015].

This cohort provides us an opportunity and information to evaluate a potential association between RSV LRTI (exposure) early in life and the development of recurrent wheeze and asthma later in life (outcome). In other words, this study will assess to what extent this early life experience of RSV LRTI is accounting for the total burden of wheeze and asthma from birth until sixth birthday. Moreover, this study will also test the performance of asthma case definition. This study will be able to potentially demonstrate a measurement of potential public health impact and potential

incidence reduction if a successful maternal and/or pediatric vaccine introduced and exposure (RSV) eliminated/reduced.

Synopsis and section 2.1 Co-Primary objectives

In a cohort of infants followed-up from birth up to ~~a maximum~~ of 2 years of age:

- To determine the total health burden* of RSV-associated RTI with a suspicion of involvement of the lower respiratory tract.

Synopsis and section 2.2 Secondary objectives:

In a cohort of infants followed-up from birth up to ~~a maximum~~ of 2 years of age;

- To evaluate the association between RSV-associated LRTI, ***RSV-associated severe LRTI*** and RSV neutralizing antibodies in the baseline cord blood samples collected from subjects, at birth.
- To determine the prevalence of RSV ~~A and B~~ infection, at 2, 4, 6, 12, 18 and 24 months in a randomly selected sub-cohort of subjects by assessing antibodies in serum.

Objectives pertaining to the amendment related to wheeze and asthma:

In a cohort of infants followed-up from birth up to 6 years of age:

- *To determine the population attributable risk of wheeze and asthma from birth to sixth birthday associated with RSV clinical LRTI in the first two years of life.*
- *To determine the population attributable risk percent of wheeze and asthma from birth until sixth birthday associated with RSV clinical LRTI in the first two years of life.*

Synopsis and section 2.3 Tertiary objectives:

- To explore the association of ***RSV and*** other respiratory viruses with the incidence of LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.
- *To explore the association of RSV viral load with the incidence of RSV-associated RTI, LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale).*

Objective pertaining to the amendment related to wheeze and asthma

In a cohort of infants followed-up from birth up to 6 years of age:

- *To explore the association of RSV clinical LRTI in the first two years of life and other potential risk factors for wheeze and asthma.*
- *To explore the performance of asthma case definitions.*

Synopsis and section 3 Study design overview:

*In the primary study subjects were to be followed-up from birth up to the age of 2 years for RSV LRTI. In order to determine the population attributable risk and population attributable risk percent of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life, and to explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma, the study will be extended and subjects will be followed-up from the age of 2 years up to 6 years to detect episodes of wheeze and diagnoses of asthma.**

**Note: The procedures related to primary study (surveillance of RSV LRTI) will not be extended into the extension period. The extension period will survey for occurrence of wheeze and asthma. Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8).*

Epidemiological cohort studies have some limitations. It is possible that study dropouts might occur leading to differential lost to follow up and bias study. Moreover, it is possible that a selection bias might occur. Factors pertaining to enrollment of subjects into this prospective cohort study would not introduce selection bias. In order to introduce bias, selection has to be related to both exposure and outcome. But in this study, subjects are enrolled in prospective cohort before they experience the outcome of interest.

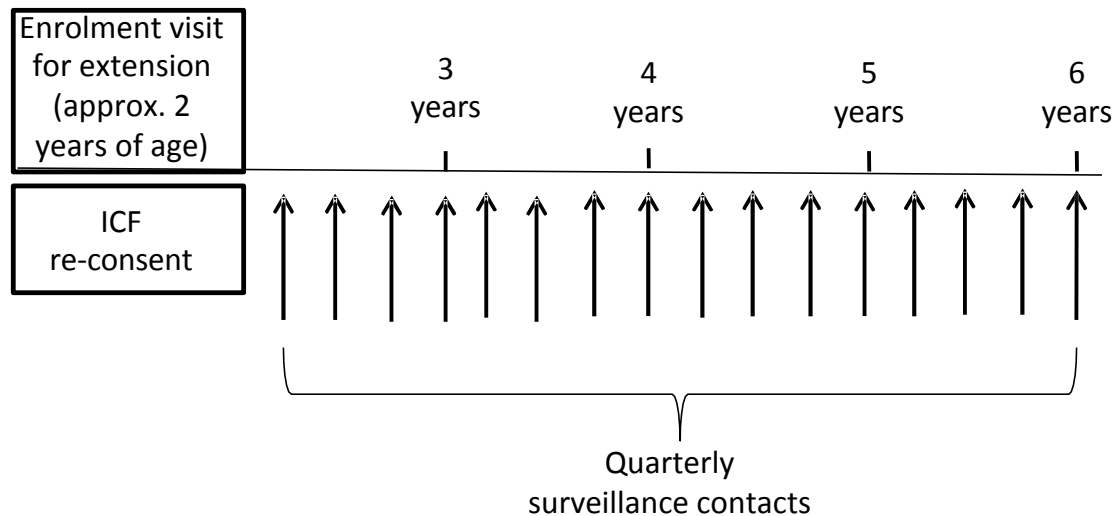
Another potential limitation of this study is the occurrence of confounding especially with potential risk factors. A possible alternative would be to match within a cohort study. But this can reduce the efficiency of a cohort study even when there is no sample-size reduction and even when the matching variable is a confounder. Furthermore, albeit cohort matching prevents confounding by the matched variable, it is not considered an advantage of matched designs since in an unmatched study confounding by the covariates can be removed by analytical methods. Hence, in order to deal with the confounding factors stratification analysis will be used for each confounder. Multivariate logistic regression will also be used to adjust to confounders after selection from the univariate logistic regression. All possible confounding factors such as gender, geographic ancestry, pets, parental smoking and previous maternal or siblings history of asthma will be analyzed first in stratification and then in logistic regression (univariate and multivariate).

Figure 1 shows the study diagram for the primary study (from birth up to the age of 2 years for detection of RSV LRTI).

Figure 2 describes the extension (from the age of approximately 2 years up to the age of 6 years for the detection of episodes of wheeze and diagnosis of asthma).

Figure 1 Study design diagram for the primary study (from birth up to the age of 2 years for detection of RSV LRTI)

Figure 2 Study design diagram for the extension (from the age of approximately 2 years up to the age of 6 years) for the detection of episodes of wheeze and diagnosis of asthma



- Parent(s)/legally acceptable representative(s) (LARs) of the subject will be contacted during the pregnancy in order to be informed about the study and to request consent for their child's participation in the **primary** study.
 - The parent(s)/LAR(s) will be requested to provide consent during pregnancy, after at least 20 weeks gestation, to enrol their child into the study, at birth.
 - The child will be checked for eligibility criteria applicable before birth.
 - The remaining eligibility criteria applicable after birth will be assessed as soon as possible, and the child's participation in the study will be confirmed through an updated and resigned informed consent form (ICF) (including any other potentially relevant identifiers as applicable by local requirements) , not later than 5 working days after the birth of the subject.

At the end of primary study (from birth up to the age of 2 years), parent(s)/LARs) will be asked for re-consent for their child's participation in the extension (from approximately 2 years of age up to 6 years of age), pertaining to the amendment related to wheeze and asthma.

- Study population: A cohort of approximately 2400 infants will be included in the study at birth and followed-up **up to a maximum of 2 years of age. Maximum 2400 children will be included in the extension and followed-up up to 6 years of age.**

- Biological samples:
 - Serum derived from up to approximately 10 milliliters (mL) of cord blood samples collected from all the subjects participating in the study, at birth.
 - Nasal swabs collected from subjects with potential LRTI symptoms i.e. RTI symptoms with any signs of difficulty in breathing.
 - Serum derived from a single blood sample of approximately 3.5 mL collected from a sub-cohort of subjects. This sub-cohort consists of the first 2000 enrolled subjects, from which those born at a gestational age of less than 36 weeks will be excluded from this blood sampling. Sampling time points for this sub-cohort will be randomly allocated as specified in Table 1.
 - ***No biological samples will be collected during the extension (from approximately 2 years of age up to 6 years of age).***
- Duration of the study: The entire duration of the ***primary*** study will be approximately 3 years.
 - Assuming no delays in recruitment, two-thirds of the subjects (approximately 1600) will be recruited in Year 1 of the study and will be followed-up up to 2 years of age, resulting in a total ***primary*** study duration of 3 years. The remaining subjects (approximately 800) will be recruited in the first half of Year 2 of the study and will be followed up till the end of Year 3. The follow-up period for subjects recruited in Year 2 will thus be 1.5 to 2 years.* Refer to Figure 4 for more details.
 - In case of any delays in recruitment for the first two-thirds of the subjects in the study, the study duration will be extended until all these subjects have completed follow-up up to 2 years of age. ~~Delays in the recruitment of the last one-third of the subjects will be absorbed by a shorter follow-up time for these subjects.~~
 - Based on the ***preliminary*** results of the interim analyses including any delays incurred in the recruitment at that time, a decision will be taken whether to complete the study as currently described in this protocol, or to extend study completion to allow all subjects to complete 2 years of follow-up.*
 - ****Note: Based on the preliminary results of the interim analyses it was decided that the incidence rate would be more robust if all subjects completed the study as described above.***

The duration of the extension period will be approximately 4 years. Subjects will be followed-up from approximately 2 years of age up to 6 years of age.

- Epoch 1: Prospective data collection starting at birth up to a maximum of 2 years of age ***on episodes of RSV LRTI.****
- ***Epoch 2: Prospective and retrospective data collection starting at the age of approximately 2 years and up to 6 years of age on episodes of wheeze and asthma.***

**Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8).*

Table 2 presents the study groups and epochs foreseen in the study.

Table 2 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min/Max)	Epoch 1	Epoch 2
ProspectivePrimary	Approximately 2400	At birth - maximum of 2 years	x	
Extension	Maximum 2400	Approximately 2 years to 6 years of age		x

** Data collection will also occur from medical charts retrospectively for those who have a gap period between the end of the primary study and providing re-consent for the extension.*

- Serious adverse events (SAEs) related to study procedures (E.g. blood sample and nasal swab sample collection) as determined by the investigator will be collected throughout the *primary* study period.

Study visit schedule related to the primary study (from birth up to the age of 2 years):

Surveillance contacts (active and passive surveillance) [Epoch 1]:

Examination visit (off-site/site):

Study visit/contact schedule related to the extension (from the age of approximately 2 years up to the age of 6 years) [Epoch 2]:

Enrollment visit for extension (at 2nd birthday [on completion of primary study or as soon as possible thereafter]):

- *Eligibility of the children for the study will be confirmed as per the inclusion and exclusion criteria for the extension. Refer to Sections 5.4 and 5.5 for details on the inclusion and exclusion criteria for the extension.*
- *The parent(s)/LAR(s) will asked for re-consent for their child's participation in the extension (from approximately 2 years of age up to 6 years of age), pertaining to the amendment related to wheeze and asthma.*
- *The parent(s)/LAR(s) will be interviewed to obtain complementary information on demographic characteristics of the subject. They will also be interviewed to obtain information on potential risk factors for wheeze and asthma, and on data about wheeze and asthma. In addition, the investigator will assess whether a child meets the protocol defined criteria of asthma based on information of parents and review of routine medical records /consultation with routine health care provider.*
- *Data collection will also occur from medical charts retrospectively for those who have already completed 2 years of life prior to providing re-consent for the extension (for any gap period between end of primary study and start of extension period).*

Quarterly surveillance contacts:

- *The parent(s)/LAR(s) will be interviewed to obtain information on potential risk factors for wheeze and asthma, and on data about wheeze and asthma. The investigator will supplement information from parents with review of routine medical records /consultation with routine health care provider.*
- *In addition, the investigator will on annual basis assess whether a child meets the protocol defined criteria of asthma based on information of parents and review of routine medical records /consultation with routine health care provider.*

Refer to Section 6.6 for details on procedures related to the extension.

Figure 3 **Decision tree for active and passive follow-up contact for the primary study**

Synopsis and section 3.1 Discussion of the study design

The association of other respiratory viruses with RSV-associated LRTI and/or severe LRTI, and the impact of potential RSV risk factors on the incidence and severity of RSV-associated LRTI will also be explored. ~~The influence of other respiratory viruses in co-infections will be assessed by testing selected samples for the occurrence of other viruses after the primary analyses are completed.~~ In order to assess and adjust for other potential confounders in the various analyses, data on the subjects' demography and lifestyle factors will be collected at baseline, and followed up quarterly for changes, during the regular follow-up contacts.

The subjects enrolled in the primary study will be followed up for episodes of RSV LRTI from 0 to 2 years of age. At 2nd birthday (on completion of primary study or as soon as possible thereafter), the surveillance for RSV LRTI will be stopped and the subjects will be re-consented for study extension during which they will be followed up for the occurrence of wheeze and asthma. Thus, this study also helps to assess how much of wheeze and asthma and its healthcare utilization from birth until the sixth birthday can be attributable to RSV clinical LRTI and RSV infection in the first two years of life. Any information pertaining to a gap period between completion of primary study and consent to the extension will be captured retrospectively by examination of health records, discussion with health care provider and parental interview.

**Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8).*

This cohort provides us an opportunity and information to evaluate a potential association between RSV LRTI (exposure) early in life and the development of recurrent wheeze and asthma later in life (outcome). In other words, this study will assess to what extent this early life experience of RSV LRTI is accounting for the total burden of wheeze and asthma from birth until sixth birthday. Moreover, this study will also test the performance of asthma case definition. This study will be able to potentially demonstrate a measurement of potential public health impact and potential

incidence reduction if a successful maternal and/or pediatric vaccine introduced and exposure (RSV) eliminated/reduced.

Section 4 Case definition

LRTI case definition and severity scale

During the analysis of the study on the full dataset available for each case, all cases identified during the surveillance for LRTI will be definitively classified as either RTI, LRTI or severe LRTI according to the LRTI case definition and severity scale described in Table 4, and also according to the classification of WHO and the classification used by Nokes *et al* [Wright, 2010; **Modjarrad, 2016**; Nokes, 2004] (see below).

Case definition of LRTI by WHO (2001).

~~LRTI is diagnosed when a child <5 years present with cough and/or difficulty in breathing has the following symptom:~~

~~In children <2 months of age:~~

- ~~• Fast breathing, (> 60 per minute in a child < 2 months of age), or~~
- ~~• Severe chest indrawing, or~~
- ~~• Stridor in a calm child, or~~
- ~~• Wheezing, or~~
- ~~• Apnea~~

~~In children 2–59 months of age:~~

- ~~• Fast breathing, (> 50 per minute in a child 2 to 11 months of age and; > 40 per minute in a child 12 to 59 months of age) or~~
- ~~• Chest indrawing, or~~
- ~~• Stridor in a calm child, or~~
- ~~• Wheezing, or~~
- ~~• Apnea~~

Case definition of LRTI by WHO (2015) [Modjarrad, 2016]

LRTI is diagnosed when a child <5 years present with cough and/or difficulty in breathing has the following symptoms:

- *Fast breathing, (> 60 per minute in a child < 2 months of age, > 50 per minute in a child 2 to 11 months of age and; > 40 per minute in a child 12 to 59 months of age), or***
- *Oxygen saturation < 95% by pulse oximetry.***

Severe LRTI is diagnosed when a child has LRTI and 1 or more of the following features of severe disease:

- *Oxygen saturation < 93% by pulse oximetry.*
- *Lower chest wall in-drawing.*

Case definition of asthma and recurrent wheeze pertaining to the amendment:

Table 5 presents the case definitions of asthma and recurrent wheeze.

Table 5 Case definitions of wheeze and asthma

	Definition	Reference
Episode of reported wheeze	Parents report a whistling or stridor sound associated with labored breathing	NA
Episode of confirmed wheeze	Healthcare provider confirmed wheeze (by chest auscultation with stethoscope) associated with labored breathing	NA
Recurrent wheeze	Four or more episodes of wheeze in one calendar year. These may be parent reported wheeze or health care provider confirmed wheeze	[Morgan, 2005]
Medically attended wheeze	An episode of health care provider confirmed wheeze	NA
Wheeze or asthma requiring admission	An episode of health care provider confirmed wheeze requiring admission	NA
Prescription of medication for wheeze or asthma	Oral steroids Intravenous steroids Inhaled steroids (ICS) Leukotriene modifiers or receptor antagonists Long-acting beta agonists (LABA) Short-acting beta agonists (SABA) Theophylline Combination inhalers that contain steroids and long-acting beta agonists Ipratropium (atrovent) Omalizumab	NA
Asthma case definition 1	Asthma will be defined at the annual review based on the documented presence of one or more of the following characteristics in the previous year: (1) provider diagnosis of asthma (2) use of albuterol for coughing or wheezing episodes (prescribed by physician) (3) use of a daily controller medication (4) step-up plan including use of albuterol or short-term use of inhaled corticosteroids during illness (5) use of prednisone for asthma exacerbation	[Jackson, 2008]
Asthma case definition 2	Asthma will be defined at the annual review based on parent interview and review of medical records with the presence of all of the following: (1) recurrent episodes of troublesome lung symptoms (wheeze or whistling sounds,	[Bisgaard, 2011]

	Definition	Reference
	<p><i>breathlessness, or recurrent troublesome cough severely affecting the well-being of the infant occurring with a frequency of 5 episodes within 6 months period or 4 weeks of consecutive symptoms);</i></p> <p><i>(2) symptoms typical of asthma (recently proposed to be termed multitrigger wheeze; e.g., exercise-induced symptoms, prolonged nocturnal cough, recurrent cough outside the common cold, and symptoms causing wakening at night);</i></p> <p><i>(3) intermittent rescue use of inhaled β_2-agonist;</i></p> <p><i>(4) response to a 3-month course of inhaled corticosteroids of 400 $\mu\text{g}/\text{d}$ and relapse when stopping treatment. Relapse was treated with a defined course of 6 months of 400 $\mu\text{g}/\text{d}$ inhaled corticosteroids and subsequent relapses by further 12-month courses only to be prolonged after documented relapse</i></p>	

Synopsis and section 5.1 Number of subjects/centres

Target enrollment *for the primary study* will be 2400 subjects to obtain approximately 1920 fully evaluable subjects at *primary study* completion. *Target enrollment for extension period will be maximum 2400 subjects (all subjects participating in the primary study, fulfilling all inclusion/exclusion criteria pertaining to the amendment related to wheeze and asthma and whose parent(s)/LAR(s) will give re-consent for the extension period).*

Section 5.2 Inclusion criteria for enrollment *in primary study (from birth up to the age of 2 years)*

Section 5.3 Exclusion criteria for enrollment *in primary study (from birth up to the age of 2 years)*

Section 5.4 *Inclusion criteria for extension period (from the age of approximately 2 years up to the age of 6 years)*

- *Subject whose parent(s)/LAR(s), in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., availability for quarterly contacts).*
- *Written informed consent obtained from the parent(s)/LAR(s) of the subject.*
- *Previous participation in the primary study (from birth up to the age of 2 years).*

Section 5.4 *Exclusion criteria for extension period (from the age of approximately 2 years up to the age of 6 years)*

- *Child in care.*

Section 6.3 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.

~~Collection and recording of data by the investigator.~~

Section 6.4 Outline of study procedures

*In the primary study subjects were to be followed-up from birth up to the age of 2 years for RSV LRTI. In order to determine the population attributable risk and population attributable risk percent of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life, and to explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma the study will be extended and subjects will be followed-up up to the age of 6 years.**

**Note: The procedures related to primary study (surveillance of RSV LRTI) will not be extended into the extension period. The extension period will survey for occurrence of wheeze and asthma. Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8).*

During the extension subjects will be followed up through quarterly contacts. Parents will be encouraged to seek a clinical assessment of their child in between quarterly contacts and/or during quarterly contacts whenever their child develops wheezing and/or breathing difficulties.

Table 7 presents the list of study procedures *pertaining to the primary study (from birth up to the age of 2 years)*.

Table 8 presents the list of study procedures pertaining to the extension (from the age of approximately 2 years up to the age of 6 years).

Table 7 List of study procedures *for the primary study (from birth up to the age of 2 years)*

Epoch-1	Prospective data collection				
Visit/Contact	Visit 1	Visit 2	Visit 3	Surveillance contact *	Examination visit **
Time points	Pre-Day 0	Post-Day 0 †	As randomized ‡ §	Regular & a	Unscheduled
Informed Consent	•				
Check inclusion/exclusion criteria applicable before birth	•				
Subject number allocation and registration in SBIR-Screening	•				
Collection of expected birth date	•				
Collection of cord blood sample (up to approximately 10 mL)		•			
Check inclusion/exclusion criteria applicable after birth and re-sign-off ICF		•			
Collection of family medical history, demographic and lifestyle characteristics		•			
Collection of data on child at birth and delivery (incl. medical care provided immediately after birth)		•			
Registration in SBIR-enrollment		•			
Distribution of diary cards to record symptoms and healthcare utilization (as needed)		0	0		0
Obtain blood sample at randomized time point (approximately 3.5 mL) from a sub-cohort of subjects			•		
Ascertain RTI symptoms and potential difficulty in breathing and assessment of need for examination visit (off-site/site)				•	
Recording of data on changes to baseline medical, demographic and lifestyle characteristics data E.g. Chronic diseases and changes to living conditions				• (quarterly)	
Examination during (off-site/site) visit and collection of all disease symptoms (including RR and pulse oximetry)					•
Data collection on, disease course and healthcare utilization before and during the examination visit (off-site/site) *** #					•
Collection of nasal swab samples					•
Return of diary cards at completion or at case resolution △ ‡				0	
Confirmation and expansion of data on healthcare utilization as recorded in the diary card occurring after the examination visit (off-site/site) *** #				•	

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Epoch-1	Prospective data collection				
Visit/Contact	Visit 1	Visit 2	Visit 3	Surveillance contact *	Examination visit **
Time points	Pre-Day 0	Post-Day 0 †	As randomized ^{‡ §}	Regular ^{& a}	Unscheduled
Recording of SAEs related to study procedure throughout the study period		•	•	•	•
Diary card transcription into eCRF				•	•
Study conclusion after 2 years follow-up or end of study End of primary study after 2 years follow-up				•	

• 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.

SBIR = Randomization System on Internet; mL = milliliter; ICF = Informed Consent Form

RTI = Respiratory Tract Infection; RR = Respiratory Rate

eCRF = electronic Case Report Form; SAE = Serious Adverse Events

*The surveillance contact consists of the following:

Active surveillance: Follow-up contacts will be made by the investigator/study staff with the parent(s)/LAR(s) of the subjects at predefined intervals (to be confirmed based on the local settings) from Visit 2 up to study conclusion.

Passive surveillance: Follow-up contacts made by the parent(s)/LAR(s) with the study investigator/study staff in case of development of RTI symptoms or increase in severity of existing symptoms after the previous reporting of the initial symptoms.

** The examination visit (off-site/site) will be applicable only for subjects with a suspicion of LRTI.

*** # Whenever required healthcare utilization data should be completed or confirmed with the healthcare provider involved in the management of the case.

^{‡ §} Selected subjects will be randomized to the time point at which a single blood sample needs to be collected; 2, 4, 6, 12, 18 or 24 months. The sample can be collected between the intervals of ± 1 week. E.g. Month 2 ± 1 week, Month 4 ± 1 week. Randomization will be done using a second phase application of SBIR.

^{& a} Refer to APPENDIX C for the active and passive of contact schedule.

^{^ ‡} Diary card can be collected by mail or in person depending on local best practice.

[†] Visit 2 can take place anytime within 5 working days after birth.

Table 8 *List of study procedures pertaining to the amendment related to wheeze and asthma (from the age of approximately 2 years up to the age of 6 years)*

Epoch-2	Data collection	
Visit/Contact	Extension enrollment visit	Surveillance for wheeze and asthma ¹
Time points	Approximately 2 years of age	Quarterly
Informed consent for extension	●	
Check inclusion/exclusion criteria for extension	●	
Collection of demographic data for extension	●	
Collection of data on potential risk factors for wheeze and asthma ⁴	●	●
Collection of data on wheeze and asthma ^{4, 5}	●	●
Classification of physician diagnosis of asthma	●	● ²
Study conclusion for extension		● ³

● 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.

¹ Surveillance contacts will happen quarterly, from approximately 2 years of age up to the age of 6 years.

² This will be done annually, i.e. at children's 3rd, 4th, 5th and 6th birthday.

³ Study conclusion for the extension will be at the last quarterly surveillance contact, at children's 6th birthday.

⁴ If there is a gap between the primary study and the extension period, the retrospective data pertaining to wheeze and asthma will be collected for the gap period.

⁵ The investigator will supplement information from parents with review of routine medical records/consultation with routine health care provider.

Section 6.5 Detailed description of study procedures *pertaining to the primary study (from birth up to the age of 2 years)*

Section 6.5.5 Collection of cord blood sample

Cord blood samples collected will provide RSV ~~A and B~~ antibody levels in the child, at birth.

Section 6.5.11 Blood sampling

Blood samples collected will be used to assess RSV ~~A and B~~ antibody levels in the subjects.

Section 6.5.21 Study conclusion ~~End of primary study~~ after 2 years follow-up

Section 6.6 Detailed description of study procedures for extension period (from the age of approximately 2 years up to the age of 6 years)

6.6.1 Informed consent

The signed/witnessed/thumb printed informed consent of the subject's parent(s)/LAR(s) must be obtained before participation in the extension. Refer to Section 6.1 for the requirements on how to obtain informed consent.

6.6.2 Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria for the extension as described in Sections 5.4 and 5.5.

6.6.3 Collection of demographic data

Record gender, geographic ancestry, ethnicity in the subject's eCRF.

6.6.4 Collection of data on potential risk factors for wheeze and asthma

The parent(s)/LAR(s) will be interviewed to obtain information on potential risk factors for wheeze and asthma. The investigator will supplement information from parents with review of routine medical records /consultation with routine health care provider.

If there is a gap between the primary study and the extension period, the retrospective data pertaining to potential risk factors for wheeze and asthma will be collected for the gap period.

At the first contact of the extension the following variables will be collected:

- *Maternal/parental exposure variables of interest (will also be collected annually)*
 - *maternal history of asthma hospitalization (yes/no);*
 - *family history of atopy or food allergy or asthma (yes/no).*
- *Children exposure variables of interest*
 - *living or recurrent contact with pets (e.g. dogs, cats) (yes/no) (will also be collected quarterly);*
 - *child history or diagnosis of atopy (yes/no) or allergy (yes/no) or eczema (yes/no) or atopic dermatitis (yes/no) or food allergy (yes/no) (will also be collected annually);*
 - *siblings history or diagnosis of atopy (yes/no) or allergy (yes/no) or eczema (yes/no) or atopic dermatitis (yes/no) or food allergy (yes/no) or asthma (yes/no) (will also be collected annually);*
 - *enrollment in school (yes/no) (will also be collected quarterly);*
 - *left school (yes/no);*
 - *baby or child exercise (yes/no or how many days of the week) (will also be collected quarterly).*

6.6.5 Collection of data on wheeze and asthma

The parent(s)/LAR(s) will be interviewed at the first contact of the extension and contacted quarterly to solicit morbidity associated with wheeze or asthma, with the questions since the last contact: Did your child have at least one episode of wheezing? Did your child take prescription medicines for wheeze or asthma? Has your child experienced troublesome symptoms at night of cough or wheezing or heavy breathing? The investigator will supplement information from parents with review of routine medical records /consultation with routine health care provider. Refer to the SPM for a more detailed set of questions.

Data collection will also occur from medical charts retrospectively for those who have already completed 2 years of life prior to providing re-consent for the extension.

6.6.6 Classification of physician diagnosis of asthma

The investigator will on an annual basis assess whether a child meets the protocol defined case definition of asthma based on information of parents and review of routine medical records /consultation with routine health care provider. (Refer to Section 4 for definition of asthma).

6.6.7 Study conclusion for the extension

The investigator/study staff will:

- *review all the data collected to ensure accuracy and completeness.*
- *complete the Study Conclusion screen in the eCRF.*

Section 6.7.2 Biological samples

The following samples will be collected, as a part of the **primary** study:

- Cord blood sample (up to approximately 10 mL) at birth from all the subjects.
- Nasal swab samples within 72 hours from the first identification of a potential LRTI case by the investigator/study staff during an active or passive surveillance study contact.
- Single blood sample (approximately 3.5 mL) from a sub-cohort of subjects, at the time point allocated through randomization (2, 4, 6, 12, 18 or 24 months). Children who are born with a gestational age of less than 36 weeks will be excluded from this sub-cohort.

No additional samples will be collected during the extension period

Section 6.7.3 Laboratory assays

For cord blood and sub-cohort blood samples:

- Serum neutralization assay to assess RSV-A and B antibody [Table 9].

Table 9 Antibody determination

System	Component	Method	Kit/ Manufacturer	Unit	Cut-off**	Laboratory
Serum	Anti-RSV-A	Neutralization	In house	TBD ED ₆₀	TBD 8	GSK Biologicals* and/or designated laboratory
Serum	Anti-RSV-B	Neutralization	In house	TBD ED ₆₀	TBD 6	GSK Biologicals* and/or designated laboratory

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

** **Unit and cut-off for the RSV-A and/or RSV-B neutralization assay may be subject to change**
RSV = Respiratory Syncytial Virus; TBD = To be defined; RR = Respiratory Rate; **ED = Effective Dose**

For nasal swab samples:

- **RT-qPCR assay** for the detection of RSV and determination of RSV subtype (A/B).
- **xTAG™ RVP Fast assay** for the detection of the following 17 viruses and subtypes ~~in selected nasal swabs.~~
 - RSV
 - Influenza A, including subtypes H1 and H3
 - ***Influenza A H1N1 2009***
 - Influenza B
 - Parainfluenza virus type 1, 2, 3, and 4
 - Human Metapneumovirus
 - ***Enterovirus/Rhinovirus***
 - Adenovirus
 - Bocavirus
 - Coronavirus - 229E, OC43, NL63, HKU1

Synopsis and section 9.1.2 Secondary endpoints

- Levels of RSV ~~A and B~~ neutralizing antibodies in the cord blood samples collected at birth.
- Levels of RSV ~~A and B~~ neutralizing antibodies in the blood samples collected at 2, 4, 6, 12, 18 and 24 months.

Endpoints pertaining to the amendment related to wheeze and asthma as secondary endpoints:

- ***Occurrence of medically attended wheeze.***
- ***Occurrence of wheeze or asthma requiring admission.***
- ***Occurrence of prescription of medication for wheeze or asthma.***

Synopsis and section 9.1.3 Tertiary endpoints

- Occurrence of **RSV and** other respiratory viruses as confirmed by xTAG™ RVP Fast assay:
 - RSV
 - Influenza A, including subtypes H1 and H3
 - Influenza B
 - Parainfluenza virus type 1, 2, 3, and 4
 - Human Metapneumovirus
 - Rhinovirus
 - Adenovirus
 - Bocavirus
 - Coronavirus - 229E, OC43, NL63, HKU1
- **RSV viral load as determined by RSV RT-qPCR**
- Cord-blood sample collection variables including collection and storage times **and** temperatures.

Endpoints pertaining to the amendment related to wheeze and asthma as tertiary endpoints:

- ***Number of children with diagnosis of asthma (case definition 1) at age 2, 3, 4, 5, and 6 years of age.***
- ***Number of children with diagnosis of asthma (case definition 2) at age 2, 3, 4, 5, and 6 years of age.***
- ***Number of children with recurrent wheeze.***

Section 9.3.5 Total enrolled cohort pertaining to the amendment related to wheeze and asthma

The total enrolled cohort for the amendment related to wheeze and asthma will include all subjects enrolled in the extension study.

Section 9.4 Derived and transformed data

- All potential LRTI cases identified during the surveillance period will be classified as RTI, LRTI or severe LRTI according to the LRTI case definition and severity scale (refer section 4). The cases will also be classified according to the classification of WHO and by Nokes *et al* [Wright, 2010; **Modjarrad, 2016**; Nokes, 2004]. First time, the classification will be done at visit level, i.e. according to the data collected at the initial visit and, if applicable, at each additional examination visit. Finally, a classification at case level will be done considering the most severe classification from all visits.

Section 9.6 Analysis of co-primary objectives

- **To assess the performance of the LRTI case definition and severity scale for RSV-associated cases.**

Incidence rates will be calculated as the number of RSV-associated LRTI/severe LRTI as classified by the LRTI case definition and severity scale and also as classified by the comparator case definitions (WHO and Nokes, et al [Wright, 2010 *Modjarrad, 2016*; Nokes, 2004]), over the number of children-years observations, overall and by subgroups.

Descriptive statistics of the different types of healthcare utilization will be provided (proportions, duration, etc.) among RSV-associated LRTI/severe LRTI as classified by the LRTI case definition and severity scale and also as classified by the comparator case definitions (WHO and Nokes, et al [Wright, 2010 *Modjarrad, 2016*; Nokes, 2004]), overall and by subgroups.

Section 9.7 Analysis of secondary objectives

Descriptive statistics of the different types of healthcare utilization will be provided (proportions, duration, etc.) among non-RSV-associated RTI with a suspicion of involvement of the lower respiratory tract, overall and by subgroups.

- **To evaluate the association between RSV-associated LRTI, *RSV-associated severe LRTI* and RSV neutralizing antibodies in the baseline cord blood samples collected from subjects, at birth.**

The impact of the level of RSV neutralizing antibodies in the baseline cord blood samples on the risk of having a RSV-associated LRTI and *RSV-associated severe LRTI* will be assessed through a statistical model.

- **To determine the prevalence of RSV A and B infection, at 2, 4, 6, 12, 18 and 24 months in a randomly selected sub-cohort of subjects by assessing antibodies in serum.**
- ***To determine the population attributable risk of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life.***

The population attributable risk together with 95% CI of medically attended wheeze, wheeze or asthma requiring admission, prescription of medications for wheeze or asthma will be calculated overall and by age strata (0-2 years, 2-4 years and 5-6 years) and by country, respectively.

The population attributable risk is calculated by subtracting the incidence rate in the subjects who did not have RSV clinical LRTI in the first two years of life from the incidence rate in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

For example, the population attributable risk of medically attended wheeze will be calculated as

$$Ip - Iu$$

Where Iu is the incidence rate of medically attended wheeze in subjects who did not have RSV clinical LRTI in the first two years of life, and Ip is the incidence rate of medically attended wheeze in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

- *To determine the population attributable risk percent of wheeze and asthma from birth until sixth birthday due to RSV clinical LRTI in the first two years of life.*

The population attributable risk percent together with 95% CI of medically attended wheeze, wheeze or asthma requiring admission, and prescription of medications for wheeze and asthma will be calculated overall and by age strata (0-2 years, 2-4 years and 5-6 years), respectively.

The population attributable risk percent is calculated by dividing the population attributable risk by the incidence rate in all the subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

For example, the population attributable risk percent of medically attended wheeze will be calculated as

$$(Ip - Iu) / Ip * 100$$

Where Iu is the incidence rate of medically attended wheeze in subjects who did not have RSV clinical LRTI in the first two years of life, and Ip is the incidence rate of medically attended wheeze in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

The population attributable risk and population attributable risk percent will be stratified by country and other confounding factors as well such as, but not limited to family and siblings, history of asthma, atopy or food allergy, living or recurrent contact with pets, child history or diagnosis of allergy, atopy, eczema or atopic dermatitis, etc. The detail for calculating the CI for both population attributable risk and population attributable risk percent will be provided in the statistical analysis plan.

Section 9.8 Analysis of tertiary objectives

- **To explore the association of RSV and other respiratory viruses with the incidence of LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale) using xTAG™ RVP Fast assay.**

For each of the other respiratory viruses, incidence rates will be calculated as the number of LRTI/severe LRTI cases associated with the virus over the number of children-years observations.

- *To explore the association of RSV viral load with the incidence of RSV-associated RTI, LRTI and/or severe LRTI (as determined by the LRTI case definition and severity scale).*

Descriptive analyses (mean, median, min, max) of viral load assessed by the RSV RT-qPCR of RSV RTI, RSV LRTI and severe LRTI cases will be tabulated.

- To assess the natural decay of maternal antibody levels using a single follow-up blood sample collected from a randomly selected sub-cohort of subjects at 2, 4, 6, 12, 18 and 24 months.

The decay of maternal antibody levels over time will be analysed graphically and by a log-linear regression. A true, natural decay curve will be explored by stochastically reducing the sample to uninfected subjects only. The following steps will be taken to identify, confirmed and suspected infected subjects to eliminate these from the sample;

- Subjects with an RSV positive sample ~~nasal swab~~ during the study before time of sampling will be eliminated.
- *To explore the association of RSV clinical LRTI in the first two years of life and other potential risk factors for wheeze and asthma.*

The incidence rate of medically attended wheeze, wheeze or asthma requiring admission, and prescription of medications for wheeze or asthma will be stratified by country.

Besides, multivariate Poisson regression model including RSV clinical LRTI in the first two years of life (Yes/No) and other potential risk factors such as, but not limited to, family and siblings, history of asthma, atopy or food allergy, living or recurrent contact with pets, child history or diagnosis of allergy, atopy, eczema or atopic dermatitis, etc, will be explored, respectively.

The number and percentage of children with diagnosis of asthma (case definition 1 and 2) will be described for each age (2, 3, 4, 5 and 6 years); the number and percentage of children with recurrent wheeze from age 2-3, 3-4, 4-5 and 5-6 will be described overall and by country listed above in frequency tables.

The risk of physician diagnosed asthma at the age of 6 years using case definition 1 and case definition 2 and recurrent wheeze (occurring from 2nd to the 6th birthday) will be analyzed using multivariate logistic regression model including RSV clinical LRTI in the first two years of life (Yes/No) and other potential risk factors listed above, respectively.

- *To explore the performance of asthma case definitions.*

Statistical analysis of agreement will be performed to compare the two case definitions of asthma. A 2x2 contingency table similar as for one of the co-primary objective will be provided and the following measures of agreement (with 95% CI) will be calculated:

- ***Sensitivity: $TP / (TP + FN)$***
- ***Specificity: $TN / (TN + FP)$***
- ***Positive predictive value (PPV): $TP / (TP + FP)$***
- ***Negative predictive value (NPV): $TN / (TN + FN)$***
- ***Proportion of overall agreement, which is the proportion of cases similarly classified: $(TP + TN) / (TP + FP + FN + TN)$.***
- ***Cohen's kappa coefficient. The magnitude of the kappa coefficient represents the proportion of agreement greater than that expected by chance.***

Data for endpoints will be pooled across primary and extension period as follows:

- ***Medically attended wheeze, in the primary phase corresponds to episodes of wheeze documented at an examination visit initiated by monthly contacts with parents and in the extension phase by a history from parents solicited by contact every 3 months, which may be supplemented by review of routine medical records or contact with health care provider.***
- ***Wheeze or asthma requiring admission in the primary phase corresponds to episodes of wheeze documented at an examination visit that were associated with admission to hospital and in the extension phase by a history from parents, which may be supplemented by review of routine medical records or contact with health care provider.***
- ***Prescription of medications for wheeze or asthma in the primary phase will be extracted from all treatments that were recorded during the examination visits and in the extension phase by specific request for relevant medication from parents, which may be supplemented by review of routine medical records or contact with health care provider.***
- ***Diagnosis of asthma according to case definition 1 and 2 will not be assessed during the primary phase and will be assessed for the first time at the first contact of the extension period and annually thereafter until study conclusion.***
- ***Diagnosis of recurrent wheeze as 4 or more episodes in one calendar year, will be assessed in the primary phase based on the episodes of wheeze documented at an examination visit initiated by monthly contacts with parents and in the extension phase by a history from parents solicited by contact every 3 months, which may be supplemented by review of routine medical records or contact with health care provider.***

Section 9.10.1 Sequence of analyses

An interim analysis will be performed after data lock at the end of recruitment (expected after 18 months) or in June 2015 whichever occurs first, on the data collected for the subjects recruited in the first year of the study (approximately 1600). This interim analysis will be performed to obtain preliminary indications of the results in very young infants. These preliminary results will be used for potential adjustment of the LRTI Case Definition and Severity Scale before final analysis, to assess the need to ***extend*** the ***primary*** study to allow all subjects to complete 2 years of follow-up and to provide data

for other relevant decision making, as soon as possible.* The results pertaining to this analysis will be descriptively reported in the interim *statistical* report.

****Based on the preliminary results of the interim analyses it was decided that the incidence rate would be more robust if all subjects completed the primary study until 2 years of age.***

Final analysis will be performed on all the data collected and cleaned in the study, at the end of the *extension* period which is expected to occur 7 years after study start. *An integrated study report* presenting the consolidated analysis for the entire study duration (*primary and extension study period [as applicable]*) will be written *and made available to the investigators.*

Section 9.10.3 *Statistical considerations pertaining to the amendment*

Statistical analyses pertaining to the amendment will be performed on all the data collected and cleaned for the subjects who re-consented for the extension. For these analyses data from birth up to the age of 6 years will be used.

There will be no interim analysis pertaining to the amendment.

Section 12 References

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Bisgaard H, et al. Endotyping early childhood asthma by quantitative symptom assessment. J Allergy Clin Immunol. 2011; 127(5):1155-64.e2.

Bønnelykke K, et al. Association between respiratory infections in early life and later asthma is independent of virus type. J Allergy Clin Immunol. 2015; 136(1):81-86.e4.

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Appendix A LABORATORY ASSAYS

The Serum Neutralization assay is a functional assay that measures the ability of serum antibodies to neutralise the cytopathic effects of RSV (for example, strains A and B) on the host cell line, hence RSV replication. In the first step of the assay, a specific quantity of a replication capable RSV virus and a defined dilution of serum are mixed and incubated. Each serum dilution is tested in duplicates ~~or triplicates~~.

RT-qPCR assay

Briefly, RSV A and RSV B ribonucleic acids (RNAs) extracted from the nasal swabs are detected in a duplex PCR format using specific amplification primers and fluorescent probes designed in the RSV N gene, encoding the RSV nucleocapsid protein. The process involves nucleic acids extraction, conversion of RNA to complementary deoxyribonucleic acid (cDNA) by reverse transcription and detection by real-time PCR reaction using a calibration curve (absolute quantitation). The RSV viral load is reported as copies of RSV RNA per mL of sample.

~~Based on genetic and antigenic variations in the structural proteins, RSV is classified in two subgroups, A and B. In order to identify and quantify RSV A and RSV B nucleic acids in various respiratory specimens, such as nasal and throat swab samples, a single-step quantitative RT-qPCR assay has been developed. Two sets of primers' pairs and probe have been designed in the N gene encoding the nucleocapsid protein: one targeting RSV A and the other targeting RSV B.~~

~~Viral RNA from 200 µL of the clinical sample is extracted using the MagNA Pure LC Instrument and MagNA Pure LC Total Nucleic Acid Isolation Kit. Purified RNA is eluted in a final volume of 50 µL. RNA from the clinical sample is amplified and detected using the specific primers and probes for RSV A and RSV B RNA with the help of ABI PRISM 7900 HT Sequence Detection System 96-Well Block Module (Applied Biosystems). Viral load values are quantified and the sample is considered positive when the measured viral load is equal to or above the assay cut-off. Several controls are included at the extraction step to monitor the efficiency of the extraction and the potential contamination during the extraction run. In addition, RT-qPCR positive controls corresponding to a standard curve obtained by dilution of a mix of RSV A and RSV B synthetic RNA from 10⁶ to 10² copies/RT-qPCR are added to the RT-qPCR step. Two~~

~~water controls are used for each RT-qPCR run (per 96 well plate) as RT-qPCR negative controls. These controls are used to monitor any potential contamination during the RT-qPCR run.~~

Appendix B CLINICAL LABORATORIES

Table 14 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Global Vaccine Clinical Laboratory <i>Clinical Laboratory Sciences</i> , Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Global Vaccine Clinical Laboratory, North America – Laval	Biospecimen Reception – Clinical Serology 525 Cartier blvd West Laval Quebec Canada H7V 3S8
GSK Biologicals Global Vaccine Clinical Laboratory <i>Clinical Laboratory Sciences</i> , Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 15 Outsourced laboratory

Laboratory	Address
<i>NEOMED-LABS Inc.</i>	<i>525, Cartier Ouest Laval, Quebec Canada H7V 3S8</i>

Appendix C ACTIVE SURVEILLANCE CONTACT SCHEDULE *TO IDENTIFY ANY POTENTIAL LRTI CASE*

GlaxoSmithKline Biologicals Vaccine Value & Health Science (VVHS) Protocol Amendment 3	
eTrack study number and Abbreviated Title	200150 (EPI-RSV-005 BOD)
Amendment number:	Amendment 3
Amendment date:	15-DEC-2017
Co-ordinating author:	PPD, Lead Science Writer
Rationale/background for changes: <p>The protocol has been amended to reflect the following changes and provide the following clarifications / corrections.</p> <p>The <i>Bisgaard</i> and <i>Jackson</i> asthma case definitions were adapted so as to integrate asthma treatment advances while preserving the case definitions' core intent.</p> <p>The International Study of Asthma and Allergies in Childhood (ISAAC) asthma case definitions, which facilitate a wider range of international results comparisons, were added.</p> <p>Throughout the document, text has been consolidated and clarified for consistency with these changes and to better facilitate uniform, harmonized implementation of the protocol. Beginning with Section 2.0, text pertaining to the primary study has been shaded in grey to differentiate it from text pertaining to the extension study. Some details regarding (a) recruitment into the primary study and (b) primary study visits and procedures have been moved from the body of the document to newly created Appendices.</p>	

Amended text has been included in ***bold italics*** and deleted text in ~~strikethrough~~ in the following sections. The grey shading indicated above appears only in Sections 2.0 through 12.0; it is not reproduced in the detailed summary of changes that follows.

Contributing authors:

The list has been re-organized to group all past and present contributing authors by role. The following additions to the list of contributing authors have been made:

- PPD [REDACTED], *RSV Epidemiologist*
- PPD [REDACTED], *Clinical Research & Development Lead*
- PPD [REDACTED], *Clinical and Epidemiology Project Lead*
- PPD [REDACTED], *Project Delivery Lead*
- PPD [REDACTED], *Study Delivery Lead*
- PPD [REDACTED], *Lead EPI Statistician*
- PPD [REDACTED], *Study statistician*
- PPD [REDACTED], *Oversight Data Manager*
- PPD [REDACTED], *Study Data Manager, TCS for GSK Biologicals*
- PPD [REDACTED], *Study Data Manager, TCS for GSK Biologicals*
- PPD [REDACTED], *Clinical Read-Out Team Leader*

List of Abbreviations

SAP *Statistical Analysis Plan*

Glossary of Terms

Active Surveillance *A contact with the subject's parent/LAR, initiated by the study physician or by a member of the study staff for the purpose of identifying an event of interest.*

Passive Surveillance *A contact initiated by the subject's parent/LAR, notifying the study physician / site staff of the occurrence of an event of interest.*

Section 1.1 Background

1.1.1 RSV

This study aims to assess the incidence and associated healthcare utilization of RSV-associated, suspected LRTI in a general population of infants from birth up to 2-years of age, and also to assess the accuracy of a newly developed LRTI case definition and severity scale compared to two existing scales developed by World Health Organization (WHO) and Nokes *et al.* *The World Health Organization (WHO) has recently updated its RSV-associated LRTI case definition [Modjarrad, 2016]. The latter includes clinical features considered to be objective, easily standardized, generalizable across settings, and generally accepted markers of severe or very severe RSV disease.*

1.1.2 Asthma

The LRTI case definition and severity scale has been developed as a consensus model derived from existing definitions in the literature, and existing guidelines by

GlaxoSmithKline (GSK) clinical staff, taking into account the advice of a panel of international RSV experts. Suspected cases of LRTI will be classified into RTI, LRTI or severe LRTI based on the data collected in the study, according to the newly developed LRTI case definition and severity scale. The presence of RSV in potential LRTI cases will be confirmed by polymerase chain reaction (PCR) on nasal swabs. Following the confirmation of RSV, the performance of LRTI case definition and severity scale will be determined based on the resulting incidence of LRTI and severe LRTI and their association with healthcare utilization. Full symptom data over the course of the disease for all suspected LRTI cases will be collected before positive identification of RSV by nasal swab testing. With this symptom data, the LRTI case definition and severity scale will be further explored to determine the clinical symptoms which better describe an LRTI and severe LRTI case and the LRTI case definition and severity scale will be updated if this improves its performance.

Infant respiratory viral infection and childhood asthma are the most common acute and chronic diseases of childhood, respectively. Common clinical features and links between these diseases have long been recognized, with early-life RSV LRTIs being strongly associated with increased asthma risk.

World Health Organization (WHO) has recently updated its RSV-associated LRTI case definition [Modjarrad, 2016]. The latter includes clinical features considered to be objective, easily standardized, generalizable across settings, and generally accepted markers of severe or very severe RSV disease.

Although asthma is probably a heterogeneous disease or syndrome, three factors and/or events consistently emerge for their ability to significantly influence asthma inception in the first decade of life: immune response aberrations, which appear to be defined best by the concept of cytokine dysregulation; LRTIs, in particular RSV; and some form of gene-environment interaction that needs to occur at a critical time-period in the development of the immune system or the lung [Lemanske, 2002].

Population-level contribution of RSV LRTIs is best demonstrated by the population-attributable risk for asthma after these early-life LRTIs. Among infants, the prevalence cumulative incidence of LRTI is approximately 18 to 32% in the first year of life and approximately 9 to 17% in the second year of life. Among infants with LRTI, the prevalence of RSV can be as high as 80%, especially in the first 3 months of life. Thus, the phenotype of asthma after infant RSV LRTI accounts for up to 31% of early childhood asthma, with a population-attributable risk estimated at about 13% in several diverse populations [Feldman, 2015].

Numerous longitudinal studies have demonstrated this first important line of evidence that RSV LRTIs precede the development of asthma. Several birth cohort studies also demonstrate a dose-response relationship between infant respiratory viral infection severity and asthma risk, with increasing infant infection severity associated with greater childhood asthma risk and asthma severity [Feldman, 2015].

The Childhood Origins of Asthma (COAST) study was developed to determine and define the importance of these three factors on asthma pathogenesis and to test different asthma case classifications [Lemanske, 2002; Lemanske, 2005; Bisgaard,

2011; Jackson, 2008; Jackson, 2012; Bønnelykke, 2015]. These studies have used similar albeit slightly different case definitions on asthma diagnosis. The International Study of Asthma and Allergies in Childhood (ISAAC) takes a slightly different parent-based questionnaire approach toward assessing asthma prevalence in young children.

These definitions are widely used in epidemiological literature of asthma etiology in children. The Bisgaard definition [Bisgaard, 2011] has multiple criteria that are assessed by the physician from records. Therefore the advantage of this definition is that the same objective criteria are applied to ascertain each case of asthma. However, its application may be challenging in some settings if routine medical records do not fully capture the necessary information in source. The Jackson definition [Jackson, 2008] includes the criteria of health care provider (HCP) diagnosis of asthma and prescription of β_2 agonists. This definition is therefore influenced by any differences in local practice of asthma diagnosis and management of wheeze. However, its advantage is that it captures the reality of diagnosis and treatment. This study anticipates that Bisgaard will be more specific and Jackson more sensitive. Hence, this study will assess the performance of these two case definitions since this will help planning for phase 3 clinical trials of RSV vaccines. ISAAC uses simple, robust, primarily symptom-based questions that are administered solely to the parents. ISAAC's strength is that its definitions are not affected by differences in local asthma treatment practices; however, its lack of clinical case confirmation may decrease its precision. The ISAAC questionnaire has been used to assess pediatric asthma prevalence in at least 42 countries across the globe [Pearce, 2007].

Numerous longitudinal studies have demonstrated this first important line of evidence that RSV LRTIs precede the development of asthma. Several birth cohort studies also demonstrate a dose-response relationship between infant respiratory viral infection severity and asthma risk, with increasing infant infection severity associated with greater childhood asthma risk and asthma severity [Feldman, 2015].

Population-level contribution of RSV LRTIs is best demonstrated by the population-attributable risk for asthma after these early-life LRTIs. Among infants, the prevalence of LRTI is approximately 18 to 32% in the first year of life and approximately 9 to 17% in the second year of life. Among infants with LRTI, the prevalence of RSV can be as high as 80%, especially in the first 3 months of life. Thus, the phenotype of asthma after infant RSV LRTI accounts for up to 31% of early childhood asthma, with a population-attributable risk estimated at about 13% in several diverse populations [Feldman, 2015].

Section 1.2 and Synopsis Rationale for the Study:

Paragraphs 1 and 2:

This study aims to assess the incidence and associated healthcare utilization of RSV-associated, suspected LRTI in a general population of infants from birth up to 2 years of age, and also to assess the accuracy of a newly developed LRTI case definition and severity scale compared to two existing scales developed by *the* World Health Organization (WHO) [Modjarrad, 2016] and Nokes, 2008.

This study will also assess the extent to which RSV LRTI in early life (up to 2 years of age) accounts for the total burden of wheeze and asthma up to the sixth birthday, and test the performance of 3 asthma case definitions – those of Bisgaard, Jackson, and ISAAC.

Paragraph 4:

Considering asthma case definitions, this study anticipates that Bisgaard will be more specific and Jackson more sensitive. Although it is likely that the ISAAC symptom-based case definitions will be the most sensitive and least specific of all three definitions [Yang 2011], its incorporation will facilitate wider international results comparisons. Hence, this study will assess the performance of *the different* case definitions since this will help planning for phase 3 clinical trials of RSV vaccines.

Paragraph 5:

If, in future, a maternal and/or pediatric RSV vaccine is introduced, results of this study may be used to evaluate post-vaccination incidence reduction (in RSV LRTI and/or wheeze and asthma) and the vaccine's potential public health impact.

Objectives, Section 2.0 and Synopsis:

Objectives for the primary study are now presented in **Section 2.1**; objectives for the extension study are now presented in **Section 2.2**.

Cross references to definitions of endpoints have been updated.

Study Design, Section 3.0 and Synopsis

The detailed discussions of the Study Visit Schedule and of Procedures for LRTI assessment in this section have been moved to *(new) Appendices D and E*.

Design (Section 3.0 and Synopsis)

3.1 Design Summary

In the primary study subjects were to be followed-up from birth up to the age of 2 years for RSV LRTI, *Episodes of wheeze and diagnoses of asthma were also documented. Refer to Figure 1.*

In order to determine the population attributable risk and population attributable risk percent of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life, and to explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma, the study will be extended and subjects will be followed-up from the age of 2 years up to 6 years to detect episodes of wheeze and diagnoses of asthma.* **Refer to Figure 2.**

*Note: The procedures related to primary study surveillance of RSV LRTI will not be extended into the extension period. The extension period will survey for occurrence of wheeze and asthma. ~~Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8.3).~~

~~Epidemiological cohort studies have some limitations. It is possible that study dropouts might occur leading to differential lost to follow up and bias study. Moreover, it is possible that a selection bias might occur. Factors pertaining to enrollment of subjects into this prospective cohort study would not introduce selection bias. In order to introduce bias, selection has to be related to both exposure and outcome. But in this study, subjects are enrolled in prospective cohort before they experience the outcome of interest.~~

~~Another potential limitation of this study is the occurrence of confounding especially with potential risk factors. A possible alternative would be to match within a cohort study. But this can reduce the efficiency of a cohort study even when there is no sample-size reduction and even when the matching variable is a confounder. Furthermore, albeit cohort matching prevents confounding by the matched variable, it is not considered an advantage of matched designs since in an unmatched study confounding by the covariates can be removed by analytical methods. Hence, in order to deal with the confounding factors stratification analysis will be used for each confounder. Multivariate logistic regression will also be used to adjust to confounders after selection from the univariate logistic regression. All possible confounding factors such as gender, geographic ancestry, pets, parental smoking and previous maternal or siblings history of asthma will be analyzed first in stratification and then in logistic regression (univariate and multivariate).~~

~~Figure 1 shows the study diagram for the primary study (from birth up to the age of 2 years for detection of RSV LRTI).~~

~~Figure 2 describes the extension (from the age of approximately 2 years up to the age of 6 years for the detection of episodes of wheeze and diagnosis of asthma).~~

In Figure 2, the title has been changed as follows:

Study design diagram for the extension (from the age of approximately 2 years up to the **age of 6 years 6th birthday**) for the detection of episodes of wheeze and diagnosis of asthma

Annual asthma assessments

The following text has been revised:

- ~~Parent(s)/legally acceptable representative(s) (LARs) of the subject will be contacted during the pregnancy in order to be informed about the study and to request consent for their child's participation in the **primary** study.~~
 - ~~The parent(s)/LAR(s) will be requested to provide consent during pregnancy, after at least 20 weeks gestation, to enrol their child into the study, at birth.~~

- The child will be checked for eligibility criteria applicable before birth.
- The remaining eligibility criteria applicable after birth will be assessed as soon as possible, and the child's participation in the study will be confirmed through an updated and resigned informed consent form (ICF) (including any other potentially relevant identifiers as applicable by local requirements), not later than 5 working days after the birth of the subject.

~~*At the end of primary study (from birth up to the age of 2 years), parent(s)/LARs will be asked for re-consent for their child's participation in the extension (from approximately 2 years of age up to 6 years of age), pertaining to the amendment related to wheeze and asthma.*~~

- Study population: A cohort of approximately 2400 infants will be included in the study at birth and followed-up up to 2 years of age. ~~Maximum~~ **A maximum of** 2400 children will be included in the extension and followed up to 6 years of age.
- Type of study: Self-contained.
- Data collection: Electronic case report form (eCRF).
- Biological samples (**Primary Study only**):
 - ~~Serum derived from up to approximately 10 milliliters (mL) of cord blood samples~~ **Cord Blood** collected from all the subjects participating in the study ~~subjects~~, at birth.
 - Nasal swabs collected from subjects with potential LRTI symptoms i.e. RTI symptoms with any signs of difficulty in breathing.
 - ~~Serum derived from a single blood sample of approximately 3.5 mL~~ **Serum** collected from a sub-cohort of subjects. This sub-cohort consists of the first 2000 enrolled subjects, from which **excluding** those born at a gestational age of less than 36 weeks will be excluded from this blood sampling. Sampling time points for this sub-cohort will be randomly allocated as specified in Table 1.
 - ~~No biological samples will be collected during the extension (from approximately 2 years of age up to 6 years of age).~~
- **Duration of the study:** The entire duration of **Approximately 2 years for each participant enrolled in the primary study** will be ~~and an additional (approximately) 3 years~~ **to 4 years for each participant enrolled in the extension study.**
 - Assuming no delays in recruitment, two-thirds of the subjects (approximately 1600) will be recruited in Year 1 of the study and will be followed-up up to 2 years of age, resulting in a total ~~primary~~ study duration of 3 years. The remaining subjects (approximately 800) will be recruited in the first half of Year 2 of the study and will be followed-up till the end of Year 3. The follow-up period for subjects recruited in Year 2 will thus be 1.5 to 2 years.* Refer to Figure 1 for more details.
 - In case of any delays in recruitment for the first two-thirds of the subjects in the study, the study duration will be extended until all these subjects have completed follow-up up to 2 years of age.

- Based on the ~~preliminary~~ results of the interim analyses including any delays incurred in the recruitment at that time, a decision will be taken whether to complete the study as currently described in this protocol, or to extend study completion to allow all subjects to complete 2 years of follow-up.*
- ~~*Note: Based on the preliminary results of the interim analyses it was decided that the incidence rate would be more robust if all subjects completed the primary study until 2 years of age.~~

~~The duration of the extension period will be approximately 4 years. Subjects will be followed up from approximately 2 years of age up to 6 years of age.~~

- ~~Epoch 1: Prospective data collection starting at birth up to 2 years of age on episodes of RSV LRTI.*~~
- ~~Epoch 2: Prospective data collection starting at the age of approximately 2 years and up to 6 years of age on episodes of wheeze and asthma.~~
~~* Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8.3).~~
 - **Epoch 1 (Primary Study): Begins at birth (Visit 1) and ends at the age of approximately 2 years (Visit or contact at 2 years).**
 - **Epoch 2 (Extension Study): Begins with the first contact for the extension study (at approximately age 2 years; ends with the contact at approximately the sixth birthday Table 2 presents the study groups and epochs foreseen in the study.**

Surveillance Plan:

- **Surveillance for RSV Lower Respiratory Tract Infection (RSV LRTI), wheeze and asthma during the Primary Study period.***
- Surveillance for wheeze and asthma during the extension study period.

*** Note that discussion of additional primary study visit details has been moved to (new) Appendix D. Discussion of details regarding Surveillance for RSV Lower Respiratory Tract Infection (RSV LRTI), wheeze and asthma during the primary study period have been moved to (new) Appendix E.**

Safety Follow-up:

- **Study procedure related Serious Adverse Events (SAEs) to be reported throughout the study.**

Design Discussion

Discussion of Study Design

~~This~~ **The multi-country design of both the primary and extension studies will provide multinational estimates of the incidence of RSV-associated LRTI during the first two**

years of life and assess the amount of wheeze and asthma that may be attributable to those early RSV-LRTI infections.

3.2.1 Primary Study

The Primary study is being conducted to will determine the incidence and associated utilization of RSV-associated LRTIs in infants from birth up to the age of 2 years, and also to assess the performance of a new LRTI case definition and severity scale.

Subjects will be identified for participation in the study during the pregnancy after at least 20 weeks gestation and before the start of the delivery. This will be done to ensure that the parent(s)/LAR(s) have sufficient time to consider and provide informed consent before the subject is born and to obtain a baseline blood cord sample from each subject, at birth. Using this sample, the level of natural protection conferred by the mother to the child at birth in the form of maternal antibodies will be assessed and prospectively related to subsequent risk of RSV-associated LRTI.

In order to identify all cases of LRTI that occur on a community level, the cohort of infants recruited at birth will be followed up by active and passive surveillance for respiratory symptoms and potential LRTI, up to maximum 2 years of age. For the passive surveillance, parent(s)/LAR(s) of the subjects will be instructed to report new RTI symptoms of cough or a runny or blocked nose as soon as possible to the investigator/study staff. Proper passive surveillance is important in order to identify potential cases as early as possible. The active surveillance is mostly aimed at reinforcing the passive surveillance as the chance of picking up a (developing) case at the exact time of the active surveillance contact is limited. Active surveillance, at a frequency dependent of the RSV seasonality, involves regular contact of the investigator/study staff with the parent(s)/LAR(s) of the subjects to report any new sign of RTI symptoms (cough, runny nose, blocked nose) as soon as possible themselves. In between scheduled active surveillance contacts, the study staff can employ activities to solicit passive surveillance according to local practices, e.g. reminder emails or text messages up to regular community centre visits.

After identification of a potential case of LRTI based on all reported symptoms, an examination visit will be scheduled as early as possible in the disease progression (within 72 hours after initial identification of a potential LRTI case by the investigator/study staff during a follow-up contact, based on reported symptoms). During this visit, data (including RR and SaO₂) will be collected on these cases through an initial assessment by the study team during examination visit (off-site/site) and through a diary card. Nasal swabs will be taken by the investigator/study staff during the examination visit (off-site/site), and will be used to positively identify cases of RSV infection.

*During the examination visit (off-site/site), an initial assessment of the presence of LRTI according to the **LRTI case definition for surveillance** will be made in order to assess the need for a potential subsequent examination visit if the case is found not to be an LRTI.*

RSV-associated healthcare resource utilization for RTI with a suspicion of difficulty breathing will be collected. Parent(s)/LAR(s) will be asked to report any care availed (e.g. self-care with over-the-counter [OTC] medication, GP visits, ER visits, hospital visits, etc.) via the diary card. Where possible, the study team will confirm and expand

~~relevant healthcare utilization with the healthcare provider involved in the management of the case. Healthcare resource utilization is expected to vary between countries, regions and/or sites due to the local variations in policy and healthcare.~~

The association of other respiratory viruses with RSV-associated LRTI and/or severe LRTI, and the impact of potential RSV risk factors on the incidence and severity of RSV-associated LRTI will also be explored. In order to assess and adjust for other potential confounders in the various analyses, data on the subjects' demography and lifestyle factors will be collected at baseline, and followed up quarterly for changes, during the regular follow-up contacts.

~~The study has been designed to target a multi-national population by including multiple countries across the globe. Including infants from multiple parts of the world into this single study will provide an overall estimate of the incidence of RSV-associated LRTI, and will indicate potential variations between countries and regions. Data on known and suspected risk factors of RSV and/or LRTI will be collected to perform exploratory analyses of the association between risk factors and disease.~~

~~Recruitment into the study will be controlled as much as possible to occur at a stable rate using SBIR, so as to achieve a population that is distributed evenly according to their age at first exposure to an RSV season. In practice, some subjects will be recruited just at the beginning of a season and be exposed immediately for several months, whereas others will be recruited just at the end of a season and thus be at least 6 months old before the next season starts. In order to assess the impact of fluctuations in the occurrence of RSV between consecutive seasons, the recruitment period will at least be one and a half years so that the recruitment after the first year yields a population of subjects with a similar age at exposure to an RSV season as the subjects recruited in the same time period, 1 year earlier.~~

~~The first 2000 subjects enrolled in the study will be randomized at recruitment to a time point at which a single blood sample will be collected from these subjects if they are still participating in the study at that time. This can be either at 2, 4, 6, 12, 18 or 24 months old. The serum of these samples will be tested for RSV A/B neutralizing antibodies. Adjusted for the baseline level of maternal antibodies, this will provide point prevalence estimates over time that will indicate the rate of any RSV infection in the study population, including the cases that did not develop into an LRTI. In addition, especially the samples at earlier time points will indicate the duration of persistence of maternal antibodies.~~

3.2.2 Extension Study

At 2nd birthday (on completion of primary study or as soon as possible thereafter), ~~the~~ **the** surveillance for RSV LRTI will be stopped ~~and the~~ **Consenting, eligible subjects from participating study centers will be re-consented for enrolled** in the study extension **and followed up** for the occurrence of wheeze and asthma. * ~~Thus, this study also helps to assess how much~~ **This will permit an assessment of the proportion of** wheeze and asthma from birth until the sixth birthday ~~can that may~~ **be attributable to RSV clinical** LRTI and RSV infection in the first two years of life. ~~Any information pertaining to a gap period between completion of primary study and consent to the extension will be~~

~~captured retrospectively by examination of health records, discussion with health care provider and parental interview.~~

~~*Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8.3).~~

~~It This cohort provides us an opportunity and information to evaluate a potential association between RSV LRTI (exposure) early in life and the development of recurrent wheeze and asthma later in life (outcome). In other words, this study will assess to what extent this early life experience of RSV LRTI is accounting for the total burden of wheeze and asthma from birth until sixth birthday. Moreover, this study will also test the performance of asthma case definitions. This study will be able to potentially demonstrate a measurement of potential public health impact and potential incidence reduction if a successful maternal and/or pediatric vaccine introduced and exposure (RSV) eliminated/reduced the different asthma case definitions (Table 5),~~

Section 4, Case Definitions

Case definitions for the primary study are now presented in *Section 4.1*.

Case definitions for the Extension Study are now presented in *Section 4.2*, and have been adjusted as follows:

Table 5 presents the case definitions of wheeze and asthma. *The Bisgaard and Jackson case definitions have been updated so as to better account for advances in asthma treatment options while still preserving the case definitions' core intent.*

Table 5 Case definitions of wheeze and asthma

	Definition	Reference
Episode of reported wheeze	Parents report a whistling or stridor sound associated with labored breathing	NA
Episode of confirmed wheeze	Healthcare provider confirmed wheeze (by chest auscultation with stethoscope) associated with labored breathing	NA
Recurrent wheeze	<i>This will be defined as</i> four or more episodes of wheeze in one calendar year. These may be parent reported wheeze or health care provider confirmed wheeze	<i>[Morgan, 2005]</i>
Medically attended wheeze	An episode of health care provider confirmed wheeze	NA
Wheeze or asthma requiring hospital admission	An episode of health care provider confirmed wheeze requiring hospital admission	NA
Prescription of medication for wheeze or asthma (entire row moved to Section 6.6)	Oral steroids Intravenous Systemic (intramuscular or intravenous) steroids Inhaled steroids (ICS) Combination inhalers containing steroids and long-acting beta agonists Long-acting beta agonists (LABA) Short-acting beta agonists (SABA) Leukotriene modifiers or receptor antagonists Theophylline Combination inhalers that contain steroids and long-	NA

	Definition	Reference
	acting beta agonists ipratropium Anti-IgE therapy (e.g., Omalizumab)	
Asthma case definition 1	Asthma will be defined at the annual review based on the documented presence of one or more of the following characteristics in the previous year: (1) provider diagnosis of asthma (2) Use of a short acting beta agonist (e.g., albuterol) for coughing or wheezing episodes (prescribed by a physician healthcare provider) (3) use of a daily controller medication (4) step-up plan including use of a short acting beta agonist (e.g., albuterol) or short-term use of inhaled corticosteroids during illness (5) use of prednisone oral and/or systemic steroids for asthma exacerbation	Based on [Jackson, 2008]
Asthma case definition 2	Asthma will be defined at the annual review based on parent interview and review of medical records with the presence of all of the following: (1) recurrent episodes of troublesome lung symptoms (wheeze or whistling sounds, breathlessness, or recurrent troublesome cough severely affecting the well-being of the infant occurring with a frequency of 5 episodes within 6 months period or 4 weeks of consecutive symptoms); (2) symptoms typical of asthma (recently proposed to be termed multitrigger wheeze; e.g., exercise-induced symptoms, prolonged nocturnal cough, recurrent cough outside the common cold, and symptoms causing waking at night); (3) intermittent rescue use of inhaled β_2 -agonist; (4) response to a 3-month course of inhaled corticosteroids of 400 μg/d and relapse when stopping treatment. Relapse was treated with a defined course of 6 months of 400 μg/d inhaled corticosteroids and subsequent relapses by further 12-month courses only to be prolonged after documented relapse (4) symptoms controlled by daily inhaled corticosteroids	Based on [Bisgaard, 2011]
Asthma case definition 3	<i>In the past 12 months: four or more episodes of wheeze AND either one or more nights of sleep disturbance from wheeze per week, or wheeze that was severe enough to limit the child's speech to only one or two words at a time between breaths</i>	Based on [Wong 2013]

Section 5, Study Population:

Target enrollment for the primary study will be 2400 subjects to obtain approximately 1920 fully evaluable subjects at primary study completion. Target enrollment for extension period will be **a maximum of 2400 subjects**. (**All** subjects participating in the primary study ~~fulfilling all inclusion/exclusion criteria pertaining to the amendment related to wheeze and asthma and whose parent(s)/LAR(s) will give re-consent for the extension period and who~~ fulfill all **relevant** inclusion/exclusion criteria **may be followed in the extension.**)

The primary study includes a sub-cohort for blood sampling. Refer to Table 6.

The overview of the recruitment plan has been moved to Appendix C.

Subsections for the primary study and the extension study have been created: **Section 5.3.1 and 5.3.2, Inclusion Criteria and Sections 5.4.1 and 5.4.2, Exclusion Criteria:**

In Section 5.3.1, the following adjustment has been made:

- Written informed consent (*or witnessed thumb printed consent in case of an illiterate subject*) (including consent to obtain a cord blood sample at birth) obtained from the parent(s)/LAR(s) of the subject

In Section 5.3.2, the following adjustments have been made:

- *Subject is enrolled at a study site that is participating in the extension period follow-up.*
- Written informed consent (*or witnessed thumb printed consent in case of an illiterate subject*) obtained from the parent(s)/LAR(s) of the subject.

Section 6.4, Outline of Study Procedures

Subsections for the primary study and the extension study have been created: **Section 6.4.1, Primary Study and 6.4.2, Extension study.**

Changes to 6.4.1 are as follows:

~~In the primary study subjects were to be followed up from birth up to the age of 2 years for RSV LRTI. In order to determine the population attributable risk and population attributable risk percent of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life, and to explore the association of RSV clinical LRTI in the first two years of life with other potential risk factors for wheeze and asthma the study will be extended and subjects will be followed up up to the age of 6 years.*~~

~~*Note: The procedures related to primary study (surveillance of RSV LRTI) will not be extended into the extension period. The extension period will survey for occurrence of wheeze and asthma. Data about wheeze and asthma were also collected in the primary study (documented at an examination visit or extracted from all treatments that were recorded during the examination visits, see Section 9.8.3).~~

~~During the extension subjects will be followed up through quarterly contacts. Parents will be encouraged to seek a clinical assessment of their child in between quarterly contacts and/or during quarterly contacts whenever their child develops wheezing and/or breathing difficulties.~~

In Table 7, footnote symbols have been adjusted and a cross reference has been added:

Refer to Section 6.5 for additional details.

Changes to 6.4.2 are as follows:

Extension Study (approximately 2 years of age up to the 6th birthday)

Table 8 presents the list of procedures for the extension study.

Note that although extension study enrolment ideally occurs at the time of the subjects' second birthdays, enrolment into, and first contact for, the extension study may take place at any time between the end of the primary study and the subject's 6th birthday.

After the first extension study contact, subjects will be contacted around the time of each subject's birthday and (approximately) quarterly during the intervals between (a) first contact and next birthday; (b) subsequent birthdays.

Site staff may contact subjects more frequently *than indicated in Table 8* if *consistent with the language of the local ICF and* considered necessary by the study physician. Parents will be encouraged to seek a clinical assessment of their child in between quarterly contacts and/or during quarterly contacts whenever their child develops wheezing and/or breathing difficulties.

Refer to Section 6.6 and the SPM for additional details.

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Table 8 List of **extension** study procedures pertaining to the amendment related to wheeze and asthma (from the age of approximately 2 years up to the ~~age of 6 years~~ **6th birthday**)

Epoch 2	Data collection		
Visit/Contact	First Contact ^a Extension enrolment visit	Quarterly Contact ^{b,c} Surveillance for wheeze and asthma	Annual Contact ^{c, d}
Time points	Approximately 2 years of age Extension study enrolment		Approximately 2 years of age Around the 3 rd , 4 th , 5 th , and 6 th birthdays (+ 2 months)
Perform/document results of these procedures			
Informed consent for extension	•		
Check inclusion/exclusion criteria for extension	•		
Collection of Collect demographic data for extension	•		
Collection of Collect data on potential symptoms and risk factors for wheeze and asthma ⁴	•	• (applies to Version 1.0 of the quarterly contact script)	•d
Collection Record episodes of wheeze and, asthma, and troublesome lungs	•	•	•d
Classification of physician diagnosis of asthma Collect data about medication used to treat asthma, wheeze, or troublesome lung symptoms	•	•	•d
Review / assess information about asthma diagnoses and medication use based on medical record abstraction and subject information over approximately the past 12 months or longer	• e		• e
Interim analysis after 4th birthday; Final analysis after 6th birthday			•
Investigator sign-off on eCRF before analysis			•f
Study conclusion for extension			•g
... and enter data into these eCRF modules			
	<ul style="list-style-type: none"> First Contact Annual 	<ul style="list-style-type: none"> Quarterly 	<ul style="list-style-type: none"> Quarterly Annual

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● is used to indicate **indicates** 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.

¹ Surveillance contacts will happen quarterly, from approximately 2 years of age up to the age of 6 years.

² This will be done annually, i.e. at children's 3rd, 4th, 5th and 6th birthday.

³ Study conclusion for the extension will be at the last quarterly surveillance contact, at children's 6th birthday.

⁴ If there is a gap between the primary study and the extension period, the retrospective data pertaining to wheeze and asthma will be collected for the gap period.

^a **At the time of first contact, if delayed, all missed annual assessments of asthma will be completed retrospectively by the Investigator.**

^b **At approximately 3, 6 and 9 months (+/- 1.5 months), between consecutive birthdays. There should be at least one month between each Quarterly Contact**

^c **Both Quarterly parent/LAR interview AND Annual investigator review/assessment data to be collected and reported.**

^d **To accurately complete investigator-targeted asthma questions, up-to-date information about all asthma, wheeze, and troublesome lung episodes as well as** a review of any and all potential asthma medications that were taken by the subject **will be** needed for the accurate completion of the investigator **assessment. Refer to Section 6.6.4.2.**

^e The investigator will supplement information from parents with review of routine medical records/ consultation with routine health care provider(s), as appropriate.

^f **After completion of 4th birthday annual contact (for interim analysis), and at study conclusion**

^g Study conclusion for the extension will be at the last contact, at child's 6th birthday.

Refer to Section 6.6 and the SPM for additional details.

Section 6.5, Detailed Description of Study Procedures – Primary Study Period

- In case of anticipated twins (or ~~multiplets~~ **other multiple births**), an ICF needs to be signed for each expected child before the start of delivery.
- In case of unexpected twins (or ~~multiplets~~ **other multiple births**), only the children for whom an ICF has been signed before birth can be enrolled. This should occur as per the birth order.
- In case of anticipated twins (or ~~multiplets~~ **other multiple births**), a subject number will be assigned sequentially to each child for whom the consent has been obtained, as per the birth order, where the first born will be associated with the lowest subject number, the second born with the successive subject number, etc.

Section 6.6, Detailed Description of *Extension* Study Procedures**Section 6.6.3:**

Record gender, ~~geographic ancestry~~, **and** ethnicity in the subject's eCRF.

Section 6.6.4: ~~Collection of data on potential~~ **Potential** risk factors for / **occurrences of** wheeze and asthma

6.6.4.1 Data collected from the parent(s)/LAR

~~The parent(s)/LAR(s) will be interviewed to obtain information on potential risk factors for wheeze and asthma. The investigator will supplement information from parents with review of routine medical records /consultation with routine health care provider.~~

~~If there is a gap between the primary study and the extension period, the retrospective data pertaining to potential risk factors for wheeze and asthma will be collected for the gap period.~~

~~At the first contact of the extension the following variables will~~ **Data collected by interviewing the parent(s)/LAR(s)**

- ~~Maternal/parental exposure variables of interest (will also be collected annually)~~
 - ~~maternal history of asthma hospitalization (yes/no);~~
 - ~~family history of atopy or food allergy or asthma (yes/no).~~
- ~~Children exposure variables of interest~~
 - ~~living or recurrent contact with pets (e.g. dogs, cats) (yes/no) (will also be collected quarterly);~~
 - ~~child history or diagnosis of atopy (yes/no) or allergy (yes/no) or eczema (yes/no) or atopic dermatitis (yes/no) or food allergy (yes/no) (will also be collected annually);~~

- ~~siblings history or diagnosis of atopy (yes/no) or allergy (yes/no) or eczema (yes/no) or atopic dermatitis (yes/no) or food allergy (yes/no) or asthma (yes/no) (will also be collected annually);~~
- ~~enrollment in school (yes/no) (will also be collected quarterly);~~
- ~~left school (yes/no);~~
- ~~baby or child exercise (yes/no or how many days of the week) (will also be collected quarterly);~~

~~Collection of data on wheeze and asthma~~

~~The parent(s) / LAR(s) will be interviewed *at* the first contact of the extension and contacted quarterly to solicit morbidity associated with wheeze or asthma, with the questions since the last contact: Did your child have at least one episode of wheezing? Did your child take prescription medicines for wheeze or asthma? Has your child experienced troublesome symptoms at night of cough or wheezing or heavy breathing? The investigator will supplement information from parents with review of routine medical records /consultation with routine health care provider. Refer to the SPM for a more detailed set of questions.~~

~~Data collection will also occur from medical charts retrospectively for those who have already completed 2 years of life prior to providing re-consent for the extension.~~

At each of these contacts, the data collected may include the following types of information. Refer to the SPM for additional details

Genetic History

- ***Family*** history of asthma
- ***Family*** history of allergies

Child/subject variables of interest

- Past or current allergies
- Exposure to environmental/household or other risk factors for asthma
- Child's activity levels
- Potential signs/symptoms of asthma
- Episodes of wheeze, asthma, or troublesome lung symptoms since the end of the primary study and their associated care
- Medications taken to ***prevent or treat asthma, wheeze or troublesome lung problems symptoms***

Section 6.6.4.2: Investigator review and assessment

Investigators will review each subject's data to assess whether a child meets the subject previously met the protocol defined case definition(s) of asthma at the 2^d, 3^d, 4th, 5th and 6th birthdays. This First Contact assessment will be based on:

- Review of information gathered at the first extension study contact with the subject's parent(s) / LAR(s) about past wheeze, asthma, and **troublesome** lung **symptoms-issues**, and the medications used to treat them.
- Review of information about any applicable primary study episodes of wheeze **and troublesome lung symptoms**, and history of asthma medications **use**.
- Review **of the subject's** routine medical records (**where these exist and are available**), **as needed to verify/clarify study information** of parents and
- **Consultation** with **the** subject's health care provider(s), **as needed to verify/clarify study information**.

*Note that all information about all asthma, wheeze, and troublesome lung episodes as well as a review of any and all potential asthma medications that were taken by the subject **will be** needed for the accurate completion of the investigator **assessment**. (Refer to Section 4 for ~~definition~~ **case definitions** of asthma).*

To this end, quarterly contact information about asthma, wheeze, and troublesome lung episodes as well as the medications used to treat them should all be brought up-to-date at the time of each annual contact.

Refer to the SPM *for additional details*.

Section 6.6.5: Medications for Wheeze or Asthma

Table 9 presents a list of medications for wheeze or asthma that should be identified during parent/LAR interviews and investigator review / assessment (Section 6.6.4), and recorded in the eCRF. These medications are usually prescribed by a health care provider.

Table 9 Medication(s) for wheeze or asthma (Amended 15-Dec-2017)

Oral steroids
Systemic (intramuscular or intravenous) steroids
Inhaled steroids (ICS)
Combination inhalers containing steroids and long-acting beta agonists
Long-acting beta agonists (LABA)
Short-acting beta agonists (SABA)
Leukotriene modifiers or receptor antagonists
Theophylline
Anti-IgE therapy (e.g., Omalizumab)

Section 6.6.6: Recording of SAEs related to study procedures

Refer to Section 7.2 and Section 7.3. There is no biological sample collection during the extension study.

Section 6.6.7: Study conclusion for the extension

The investigator/study staff will:

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

Section 8, Subject Completion and Withdrawal

A subject who is available for the concluding contact foreseen in the protocol *for the primary study* is considered to have completed the *primary* study.

A subject who is available for the concluding contact foreseen in the protocol for the extension study is considered to have completed the extension study.

Subjects who are withdrawn *from the study* because of ~~AEs or SAEs~~ **study procedure related** to study participation **SAEs** must be clearly distinguished from subjects who are withdrawn for other reasons. ~~The investigator~~ **Investigators** will follow subjects who are withdrawn *from the study* as the result of an **a study procedure related** SAE until resolution of the event (see Section 7.4.1.2).

~~Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up. E.g. Three attempted telephone contacts and/or a certified letter to the last known address, those subjects who do not return for scheduled visits or follow-up.~~

~~Subjects who are withdrawn from the study because of SAEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of an SAE until resolution of the event (see Section 7.4.1.2).~~

*****Details concerning the definition of “lost to follow up” are presented in the SPM.***

Section 9.0 Statistical Methods

Endpoints for the primary study are now presented in Section 9.1. Endpoints for the Extension study are now presented in Section 9.2

Section 9.1: Endpoints pertaining to the amendment related to wheeze and asthma as secondary endpoints:

- ~~Occurrence of medically attended wheeze.~~
- ~~Occurrence of wheeze or asthma requiring admission.~~
- ~~Use of prescription medications for wheeze or asthma.~~

Section 9.2: Endpoints pertaining to the amendment related to for Extension Study

Primary: none

Secondary:

- **Use of** prescription medication(s) for wheeze or asthma.

Tertiary

- Number of children with ~~diagnosis of~~ asthma (case definition 1) at age 2, 3, 4, 5, and 6 years of age
- Number of children with ~~diagnosis of~~ asthma (case definition 2) at age 2, 3, 4, 5 and 6 years of age.
- **Number of children with asthma (case definition 3) at 3, 4, 5 and 6 years of age.**
- Number of children with recurrent wheeze.

Study Cohorts

Analysis cohorts for the primary study are now presented in **Section 9.4.2** Analysis cohorts for the extension study are now presented in **Section 9.4.3**.

Section 9.2.4.1: Total enrolled cohort at Year 2

The total enrolled cohort will include all subjects enrolled in the **primary** study.

Section 9.2.4.2: Per Protocol Set (PPS) cohort at Year 2

The ATP **PPS** cohort will include all subjects **enrolled in the primary study** meeting all eligibility criteria up to the time of their censoring, either at study completion **of epoch 1** or prematurely as drop-out (e.g. withdrawn consent, lost-to-follow-up, lack of compliance). **Additional details are provided in the Statistical Analysis Plan (SAP).**

- ~~Lost-to-follow-up is defined as no contact by the subject's parent(s)/LAR(s) over the period of 3 planned contacts and/or 2 months and after a final attempt has been made by mail. Once this has been reached, the subject is censored at the time of last contact.~~
- ~~Lack of subject's compliance is defined as 2 consecutive missed cases by not reporting through passive surveillance or by not cooperating to perform an examination visit in the given timeframe.~~

Section 9.2.4.3: ATP Per Protocol Set (PPS) sub-cohort at Year 2

- The ATP **PPS** sub-cohort **at Year 2** will include subjects **in the PPS cohort at Year 2** for whom a blood sample result will be available (at Month 2, 4, 6, 12, 18 or 24). Analyses will be done on the ATP sub-cohort from the total cohort defined in section 9.4.2 as well as from the ATP cohort defined in section 9.4.2.2.

Section 9.4.3: Analysis Cohorts for Extension Study**Section 9.4.3.1: Total enrolled cohort pertaining to the amendment related to wheeze and asthma for extension**

The total enrolled cohort will include all subjects enrolled in the extension study.

Section 9.4.3.2: PPS cohort for extension

The PPS cohort for the Extension study will include all subjects meeting all eligibility criteria up to the amendment-related time of their censoring, either at study completion or prematurely as drop-out (e.g. withdrawn consent or lost-to- wheeze and asthma follow-up).

~~The total enrolled cohort for the amendment related to wheeze and asthma will include all subjects enrolled in the extension study.~~

Section 9.5: Derived and transformed data

Data transformations and derived variables will be described in the SAP. However, they may include the following:

Section 9.5.1: General considerations for statistical analyses

The primary analyses will at least be performed on the ATP **PPS** cohort or sub-cohort. If more than 5% of the subjects are eliminated from the total enrolled cohort, then all analyses ~~will~~ **may** also be performed on the total enrolled cohort in addition to the ATP **PPS** cohort or sub-cohort.

When mentioned below in the upcoming analyses sections, analysis by subgroups ~~will~~**may** include:

Analysis of demographics

The distribution of subjects enrolled among the study sites ~~will~~ **may** be tabulated ~~at least~~ overall, by hemisphere, **country, region, center** and/or by region ~~and/or~~ **other relevant grouping**. The demographic and lifestyle characteristics collected for the enrolled subjects ~~will~~ **may** be summarized ~~at least~~ overall, by hemisphere ~~and by~~, **country, region, center, and/or other relevant grouping**.

- Frequency tables will be generated **as appropriate**, for categorical ~~variable~~ **variables** such as center.
- Mean, median, standard error will be provided **as appropriate**, for continuous data such as age.

Analysis of Objectives for the primary study now appears in **Section 9.7**; Analysis of Objectives for the extension study now appears in **Section 9.8**.

Section 9.7 Analysis of ~~eo~~ Objectives for the Primary Study (birth to 2 years of age):

Section 9.7.3 Tertiary Objectives:

- ~~To determine the population attributable risk of wheeze and asthma from birth to sixth birthday due to RSV clinical LRTI in the first two years of life.~~

~~The population attributable risk together with 95% CI of medically attended wheeze, wheeze or asthma requiring admission, prescription of medications for wheeze or asthma will be calculated overall and by age strata (0-2 years, 2-4 years and 5-6 years) and by country, respectively.~~

~~The population attributable risk is calculated by subtracting the incidence rate in the subjects who did not have RSV clinical LRTI in the first two years of life from the incidence rate in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.~~

~~For example, the population attributable risk of medically attended wheeze will be calculated as~~

- ~~• To determine the population attributable risk percent of wheeze and asthma from birth until sixth birthday due to RSV clinical LRTI in the first two years of life.~~

~~The population attributable risk percent together with 95% CI of medically attended wheeze, wheeze or asthma requiring admission, and prescription of medications for wheeze and asthma will be calculated overall and by age strata (0-2 years, 2-4 years and 5-6 years), respectively.~~

~~The population attributable risk percent is calculated by dividing the population attributable risk by the incidence rate in all the subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.~~

~~For example, the population attributable risk percent of medically attended wheeze will be calculated as~~

~~The detail for calculating the CI for both population attributable risk and population attributable risk percent will be provided in the statistical analysis plan.~~

Section 9.8 Analysis of Objectives for the Extension study

Primary objectives: none

Secondary objectives:

Data for secondary endpoints may be pooled across the primary and extension periods. Additional details are provided in the SAP.

Section 9.8.2.1:

The incidence rate of medically attended wheeze, wheeze or asthma requiring hospital admission, and prescription of medications for wheeze or asthma may be calculated and stratified by country.

The population attributable risk ***may be*** calculated by subtracting the incidence rate in the subjects who did not have RSV clinical LRTI in the first two years of life from the incidence rate in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

Details will be provided in the SAP. An example follows.

The population attributable risk of medically attended wheeze may be calculated as:

$$Ip - Iu$$

Where Iu is the incidence rate of medically attended wheeze in subjects who did not have RSV clinical LRTI in the first two years of life, and Ip is the incidence rate of medically attended wheeze in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life. ***The above population attributable risk formula may be further adapted to best accommodate the data and their required analytic assumptions (e.g., a modified formula may be used for population attributable risk calculations involving recurrent outcomes).***

Section 9.8.2.2: To determine the population attributable risk percent of wheeze and asthma from birth until sixth birthday due to RSV clinical LRTI in the first two years of life.

The population attributable risk percent together with 95% CI of ***wheeze***, medically attended wheeze, ***recurrent wheeze***, wheeze or asthma requiring admission, and prescription of medications for wheeze and asthma ***may*** be calculated overall and by age strata (0-2 years, 2-4 years and 5-6 years), respectively.

The population attributable risk percent ***may be*** calculated by dividing the population attributable risk by the incidence rate in all the subjects including both who had and who did not have RSV clinical LRTI in the first two years of life.

For example, the population attributable risk percent of medically attended wheeze ***may*** be calculated as:

$$(Ip - Iu) / Ip * 100$$

Where Iu is the incidence rate of medically attended wheeze in subjects who did not have RSV clinical LRTI in the first two years of life, and Ip is the incidence rate of medically attended wheeze in all subjects including both who had and who did not have RSV clinical LRTI in the first two years of life. ***As with the population attributable risk, the above formula may be adapted, as appropriate, to best suit the data.***

The detail for calculating the CI for both population attributable risks and population attributable risk ***percent*** will be provided in the ***SAP***.

Section 9.8.3: Tertiary objectives

Section 9.8.3.1: To explore the association of RSV clinical LRTI in the first two years of life and other potential risk factors for wheeze and asthma

~~The incidence rate of Endpoints such as medically attended wheeze, recurrent wheeze or asthma requiring admission, and prescription of medications for wheeze or asthma will be requiring hospital admission, and prescription of medications for wheeze or asthma may be calculated as recurrent events and stratified by country.~~

~~Besides, multivariate Occurrence/recurrence rates will be calculated overall and by subgroups for each of the outcomes, as relevant. Multivariate Poisson regression model including RSV clinical LRTI in the first two years of life (Yes/No) and other potential risk factors such as, but not limited to, family and siblings history of asthma, atopy or food allergy, living and/or recurrent allergies, contact with pets, child history or diagnosis of allergy, atopy eczema or atopic dermatitis, etc., will be explored, respectively.~~

Section 9.8.3.2: To explore the performance of asthma case definitions.

The number and percentage of children with diagnosis of asthma (case definitions 1, 2, ~~and 3~~) will be described for each ~~applicable age (2, 3, 4, 5 and 6 years);~~ the number and percentage of children with recurrent wheeze ~~from at each applicable age 2-3, 3-4, 4-5 and 5-6~~ will be described overall and by country in frequency tables.

The risk of ~~physician diagnosed~~ asthma at the age of 6 years using case ~~definitions 1 and case definition, 2 and recurrent wheeze 3~~ (occurring from 2nd to the 6th birthday) will be analyzed using multivariate logistic regression model including RSV clinical LRTI in the first two years of life (~~Yes/No~~) and other potential risk factors listed above, respectively.

Statistical analysis of agreement will be performed to compare ~~the two case definitions of asthma. A 2x2 contingency table similar as for one of the co-primary objective will be provided and the following measures of agreement (with 95% CI) will be calculated: asthma case definitions 1, 2, and 3 to each other.~~

- Sensitivity: $TP / (TP + FN)$
- Specificity: $TN / (TN + FP)$
- Positive predictive value (PPV): $TP / (TP + FP)$
- Negative predictive value (NPV): $TN / (TN + FN)$
- ~~Proportion of overall agreement, which is the proportion of cases similarly classified: $(TP + TN) / (TP + FP + FN + TN)$.~~

Cohen's kappa (~~or other appropriate~~) coefficient ~~may be used for the comparisons~~. The magnitude of the kappa coefficient represents the proportion of agreement greater than that expected by chance.

Data for endpoints will be pooled across primary and extension period as follows:

- Medically attended wheeze, in the primary phase corresponds to episodes of wheeze documented at an examination visit initiated by monthly contacts with parents and in the extension phase by a history from parents solicited by contact every 3 months, which may be supplemented by review of routine medical records or contact with health care provider.
- Wheeze or asthma requiring admission in the primary phase corresponds to episodes of wheeze documented at an examination visit that were associated with admission to hospital and in the extension phase by a history from parents, which may be supplemented by review of routine medical records or contact with health care provider.
- Prescription of medications for wheeze or asthma in the primary phase will be extracted from all treatments that were recorded during the examination visits and in the extension phase by specific request for relevant medication from parents, which may be supplemented by review of routine medical records or contact with health care provider.
- Diagnosis of asthma according to case definition 1 and 2 will not be assessed during the primary phase and will be assessed for the first time at the first contact of the extension period and annually thereafter until study conclusion.
- Diagnosis of recurrent wheeze as 4 or more episodes in one calendar year, will be assessed in the primary phase based on the episodes of wheeze documented at an examination visit initiated by monthly contacts with parents and in the extension phase by a history from parents solicited by contact every 3 months, which may be supplemented by review of routine medical records or contact with health care provider.

Section 9.9: Interpretation of analyses

~~Analyses~~ **All analyses** will be descriptive with the aim to characterise overall occurrences, patterns and potential differences between groups in the endpoints related to the objectives. No hypothesis testing will be performed.

Section 9.10: Conduct of analyses

Analytic plans may be modified as appropriate to accommodate final sample sizes and data distributions. 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.

Section 9.10.1: Sequence of analyses

..... These preliminary results will be used for potential adjustment of the LRTI Case Definition and Severity Scale before final analysis, ~~to assess the need to extend the primary study to allow all subjects to complete 2 years of follow-up~~ and to provide data for other relevant decision making, as soon as possible.* The results pertaining to this analysis will be descriptively reported in the **an** interim statistical report.

Section 9.10.2: Statistical considerations for ~~interim analyses~~ *the primary study***Section 9.10.3:** Statistical considerations *for the extension study*

All Statistical analyses pertaining to the *extension study* will be performed on ~~all the~~ data collected and cleaned for the subjects who re-consented for the extension. ~~For these analyses data from birth up to the age of 6 years will be used.~~

An interim analysis for the study extension will be conducted near the midway point of study extension data collection, after all subjects have reached four years of age (and completed their annual contact, or passed the window for that contact). The interim analysis will be purely descriptive, no adjustment of the type I error is foreseen.

Final extension study analysis will be conducted after all subjects have reached six years of age (and completed their annual contact, or passed the window for that contact).

References (Synopsis and Section 12)

Pearce N, Ait-Khaled N, Beasley R, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax 2007;62:758-766.

Wong G, Brunekreef B, Ellwood P, et al. Cooking fuels and prevalence of asthma: a global analysis of phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). Lancet Respir Med. 2013; 1: 386–94.

Yang CL, To T, Foty RG, Stieb DM, Dell SD. Verifying a questionnaire diagnosis of asthma in children using health claims data. BMC Pulmonary Medicine. 2011;11:52. doi:10.1186/1471-2466-11-52.

APPENDIX C OVERVIEW OF RECRUITMENT PLAN (Primary Study)

The text now presented in Appendix C has been *moved* (from Section 4.0) but it has not been altered.

APPENDIX D Additional Information Regarding Primary Study Visits

The text now presented in Appendix D has been *moved* (from Section 3.0) but it has not been altered.

APPENDIX E Additional Information Regarding LRTI Surveillance, including Active Surveillance Contact Schedule (Primary Study)

The text now presented in Appendix E has been *moved* (from Section 3.0) and minor alterations have been made. The alterations are presented below.

ACTIVE SURVEILLANCE CONTACT SCHEDULE to identify any potential LRTI case

Active surveillance is aimed at confirming that a case is/was not missed due to lack of reporting by parent(s)/LAR(s) and to remind parent(s)/LAR(s) to report cases of RTI symptoms as soon as possible themselves. ~~Although active surveillance is an important aspect to ensure that all cases are identified timely, the number of active surveillance contacts should not be too high. Too many active surveillance contacts could put an unnecessary demand on the parent(s)/LAR(s) of the subjects and has been known to negatively impact long term compliance. In order to reduce the number of active surveillance contacts to an acceptable and valuable level, the following measures should be applied.~~

Active surveillance is allowed to occur through various media (e.g. text messaging, email and, telephone, and in-person) based on local possibilities

DISCONTINUATION OF ACTIVE SURVEILLANCE

Active surveillance should be suspended after there has been no contact for more than 3 planned contacts and/or no contact for more than 2 months and a final attempt has been made by mail or a live visit. If the parent(s)/LAR(s) of the subject contact the investigator/study staff at a later point in time through passive surveillance, the case should be followed up, data should be collected at intermittent period and active surveillance could be reinitiated. ~~These subjects will however no longer be included in the ATP cohort, therefore, all efforts should be made to avoid this.~~

Protocol Amendment 3 Sponsor Signatory Approval**eTrack study number and
Abbreviated Title**

200150 (EPI-RSV-005 BOD)

Date of Amendment*Amendment 3 Final: 15 December 2017***Detailed Title**

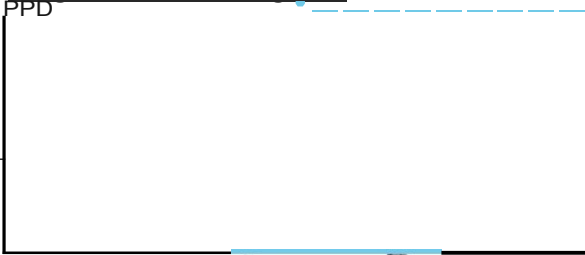
A prospective, epidemiological, interventional, multi-country based, cohort study to assess the disease burden of respiratory syncytial virus (RSV) associated, suspected lower respiratory tract infections (LRTIs) in newborns, from birth up to 2 years of age and population attributable risk percent of RSV LRTI on the development of wheeze and asthma from birth up to 6 years of age.

**Sponsor signatory (Amended
15-DEC-2017)**

Amanda Leach
Clinical and Epidemiology R&D Project Leader
RSV Program GSK Biologicals

Signature

PPD

**Date**

Dec 17

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