Statistical Analysis Plan Amendment 2

Study ID: 207636

A prospective epidemiological study of women between 24-27 weeks of pregnancy to describe pregnancy outcomes and events of interest in the mother and neonate, as well as determine incidence in infants of RSV LRTI and RSV hospitalization

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GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A prospective epidemiological study of women between 24-27 weeks of pregnancy to describe pregnancy outcomes and events of interest in the mother and neonate, as well as determine incidence in infants of RSV LRTI and RSV hospitalization.
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LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Events of Special Interest
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CLS	Clinical Laboratory Sciences
COVID-19	Corona virus disease 2019
CSR	Clinical Study Report
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
ELISA	Enzyme-linked immunosorbent assay
EoS	End of Study
ES	Exposed Set
FAS	Full Analysis Set
FU	Follow-Up
GAIA	Global Alignment of Immunization Safety Assessment in pregnancy
GCP	Good Clinical Practice
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board

IU/ml	International units per milliliter
LAR	Legally Acceptable Representative
LL	Lower Limit of the confidence interval
LMP	Last Menstrual Period
LOD	Limit of Detection
LRTI	Lower Respiratory Tract Illness
LSLV	Last Subject Last Visit
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NB	Newborn
PCD	Primary Completion Date
PCR	Polymerase Chain Reaction
PD	Protocol Deviation
PPS	Per Protocol Set
RBC	Red Blood Cell
RR	Respiratory Rate
RSV	Respiratory Syncytial Virus
RTI	Respiratory Tract Illness
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SPM	Study Procedures Manual
SpO_2	Blood oxygen saturation as measured by pulse oximetry
SR	Study Report
T Domains	Trial Domains
TFL	Tables Figures and Listings
TOC	Table of Contents
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
8 May 2019	first version	Amendment 3 - 18 February 2019
27 November 2020	Amendment 1	Amendment 4 - 29 May 2020
24 September 2021	Amendment 2	Amendment 4 - 29 May 2020

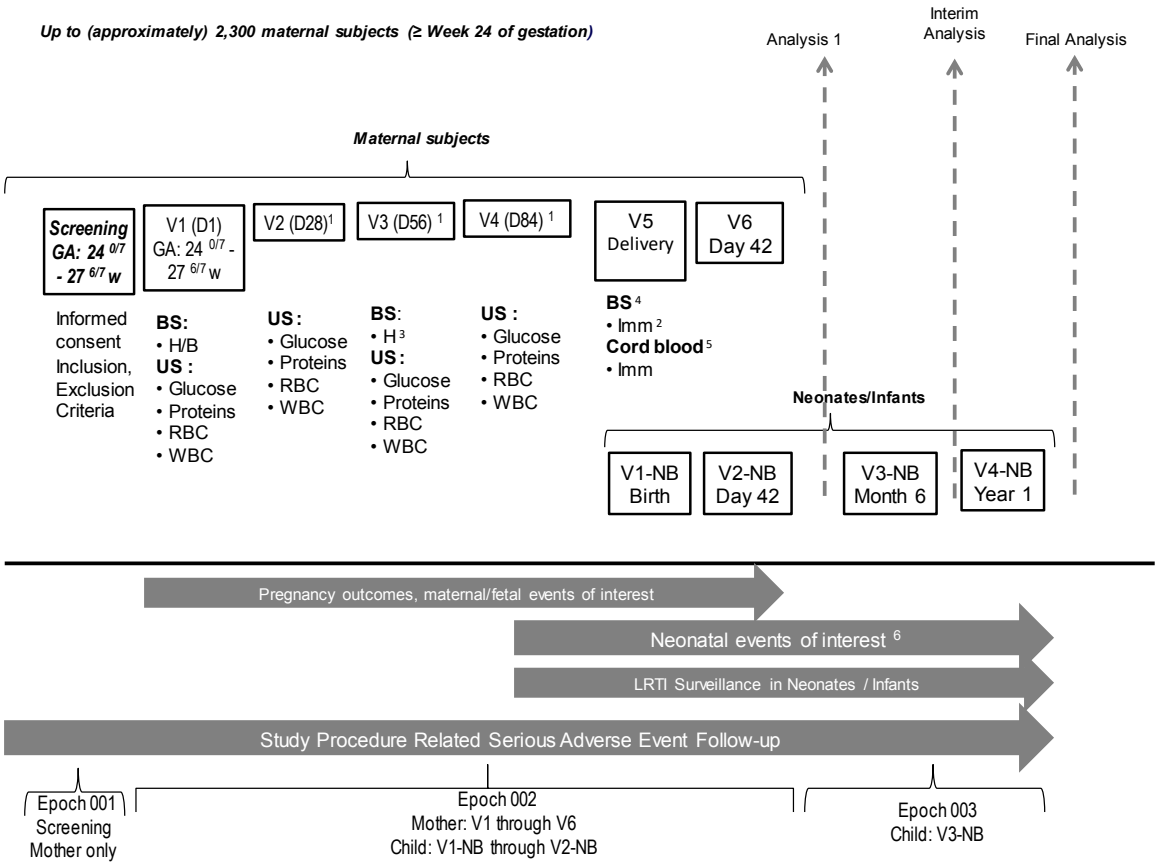
2. STUDY DESIGN

2.1. Summary

This is a prospective multi-country cohort study that aims to provide information for subsequent clinical trials for maternal immunization for respiratory syncytial virus (RSV). The study will provide background rates of maternal and neonatal events of interest, incidence rates of lower respiratory tract illnesses associated with RSV (RSV LRTI), and will determine the capacity of future sites for clinical trials. Approximately 2300 pregnant women from nine different countries will be enrolled during their third trimester of pregnancy and followed until 42 days after delivery. Their children will be followed from birth until one year of age. Epoch 1 of the study consists of the screening of maternal subjects. Epoch 2 includes visits 1-6 of the mother and visits 1-2 of the new born; during which time data on pregnancy outcomes and maternal and neonatal events of interest will be collected and RSV LRTI surveillance will begin (from visit 1 of the new born). Neonatal events of interest will only include events that occurred during the first 28 days of life, but data on these events can be collected up to one year of age. Maternal and cord blood samples will also be collected from the mother at delivery. Lastly, Epoch 3 will include RSV LRTI surveillance as well as continued recording of neonatal events of interest. During surveillance in Epoch 3, symptoms relevant to RSV illness will be recorded in diary cards, and RSV positivity will be confirmed via laboratory tests performed on nasal swabs collected during site visits.

Please note that when referring to epochs in the SAP, we are following the convention outlined in the protocol. Epochs in the CDISC T domains are named differently and are as follows: For maternal subjects: screening epoch and study procedures epoch. For infants: screening epoch and surveillance epoch (infants do not actually have a screening visit in this study but this is the naming standard used for CDISC T domains).

Figure 1 Study design



D = day; M = month; V = visit; W = week; Y = year. NB = newborn; GA = Gestational age; BS = blood sample; US = urine sample for dipstick testing; IMM = immune response; HB = hematology/biochemistry; RBC = red blood cell(s); WBC = white blood cell(s); RSV = respiratory syncytial virus; RTI = respiratory tract illness; LRTI = lower respiratory tract illness;

Refer to Table 1 for additional information about the analyses indicated above.

[1] If delivery occurs prematurely, skip to Visit 5 ("at delivery").

[2] At Delivery, RSV-A antibody titers for all women

[3] At V3, only hemoglobin testing.

[4] Allowed interval for blood sample collection begins with start of labor (e.g. when the mother arrives at the hospital to deliver and an intravenous line is inserted) and ends 72 hours after delivery.

[5] RSV-A antibodies in cord blood

[6] Neonatal events of interest occur (by definition) between 0 and 28 days after birth. They will be reported once site staff become aware of them (whether this occurs during the first 28 days after birth, or at a later time).

NOTE: Covid-19 cases identified within the surveillance framework of the study in maternal and infant subjects will also be recorded.

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

- **Type of design**: Prospective, epidemiological, interventional (without administration of medicinal products as described in a research protocol), multi-country, cohort study.

- **Study population**: The study will be conducted in multiple countries, in pregnant women and their infants.
- **Type of study**: self-contained.
- **Data collection**: Electronic Case Report Form (eCRF).

Table 1 Sampling schedule for maternal subjects

		Visit				
Sample	**Parameter(s) evaluated**	**1**	**2**	**3**	**4**	**Delivery**
Maternal **Blood**	Hematology/ biochemistry[1, 4]	x		x[3]		
	Antibody titer (RSV-A)					≤ 72 hours after delivery[5]
Cord Blood	Antibody titer (RSV-A)					x
Urine	Protein, glucose, RBC, WBC [2, 4]	x	x	x	x	

[1] To be performed preferentially by local healthcare providers, as per local practice. To be performed by the investigator/study staff ONLY if not done by the local healthcare provider within 2 weeks before the study visit.
[2] The investigator/site staff will perform a urine dipstick test using supplies provided by GSK.
[3] Hemoglobin only
[4] If results are abnormal, subjects will be referred per local standard of care.
[5] This sample may be collected from start of labor (e.g. when the mother arrives at the hospital to deliver and an intravenous line is inserted) through 72 hours after delivery.

- Sampling schedule for **neonates/infants**: Surveillance for RSV LRTI will be conducted in infants through the 12 month study period. During this 1-year post-birth surveillance period, for each RTI with suspicion of difficulty in breathing, or wheezing, or with parental concern, a nasal swab will be collected at a visit to assess potential RSV associated RTIs/LRTIs.
- Primary Completion Date (PCD): 42 days post-delivery/birth (i.e. Visit 6 for maternal subjects and Visit 2-NB for infant subjects) or last visit of Epoch 002.
- End of Study (EoS): Last testing results released of samples collected up to Visit 4-NB (assays related to primary and secondary endpoints).
- Duration of the study: Approximately 4.5 to 6 months for maternal subjects; approximately 1 year for infant subjects.
  - Epoch 001: Screening
  - Epoch 002: Primary starting at Visit 1 and ending 42 days post-delivery/birth (Visit 6 for maternal subjects and Visit 2-NB for infant subjects).*
  - Epoch 003: Follow-up of infants starting 43 days post-delivery/birth and ending at Visit 4-NB (1 year post-birth).

*Any safety and disease surveillance data collected after Visit 2-NB will be collected in Epoch 003.

Table 2 Study groups and epochs foreseen in the study

Study Groups	Number of **subjects** enrolled	Age (Min/Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
Maternal subjects	Up to ~ 2300	18 years - 45 years	x	x	
Infants	Up to ~ 2300	NA		x	x

Approximately 200 to 300 per country. To achieve the enrollment targets noted above, the number of pregnant women SCREENED in each country may exceed the country-specific enrolment goal.

- Surveillance for pregnancy outcomes and pregnancy-related events of interest that occur from Visit 1 up to 42 days after delivery (Visit 6).
- Surveillance for neonatal events of interest that occur from birth up to 28 days of age.
- Surveillance for RSV LRTI and RSV hospitalization in infants, from birth up to 1 year of age. Nasal swabs will be collected during surveillance visits to confirm RSV positivity.
- This study includes active and passive surveillance of RSV LRTI. In an active surveillance, site personnel will contact the subject's parent(s) / LAR(s) or their designate(s). Active contacts will occur at regular intervals (depending on RSV seasonality at the site) and will be scripted. In a Passive contact, the subject's parent(s) / LAR(s) or their designate(s) contact site personnel if their infant has any of the RTI symptoms (cough, runny nose, or blocked nose). Site personnel will use a script to guide data collection once a passive contact has been made. In each case, the site will use the decision tree (Figure 2 of protocol) to determine if a case assessment visit is needed (Occurrence of Surveillance contact flag and Surveillance Contact Report form in the eCRF). If the patient reported wheezing or difficulty breathing, the nurse will set up an appointment for the mother to bring her infant for follow up at the clinic within three days of the beginning of symptoms. Once the child has completed an assessment visit, site personnel will continue surveillance for the case episode. If the child's symptoms have deteriorated and the child is admitted to the hospital or needs oxygen therapy (Inpatient and Worsening flag in the eCRF), the physician will report worsening symptoms (Worsening form in the eCRF). Start dates will be recorded for cough, runny nose, wheezing, and difficulty breathing, but only end dates will be recorded for cough and difficulty breathing. If the infant experiences new bouts of cough or blocked nose after 7 days of her/his recovery from the last RTI symptoms, then a new case assessment form will be opened. Given that infants frequently experience runny nose, a case can be concluded even if the runny nose has not resolved.

2.2. Case Definitions

The case definitions are summarized in the following table: (see section 9.4 for detailed derivation of case definition).

Table 3 RTI/LRTI case definitions

RSV-RTI	(Runny nose, OR Blocked nose, OR Cough) AND Confirmed RSV infection [4]
RSV-LRTI	(History of cough OR difficulty in breathing [1]) AND (SpO_2 < 95% [2], OR RR increase [3]) AND Confirmed RSV infection [4]
RSV-severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 93%, OR lower chest wall in-drawing
RSV-very severe LRTI	Meeting the case definition of RSV-LRTI AND (SpO_2 < 90%, OR inability to feed OR failure to respond / unconscious)
RSV hospitalization	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]
All-cause RTI	Runny nose, OR Blocked nose, OR Cough
All-cause LRTI	(History of cough OR difficulty in breathing [1]) AND (SpO_2 < 95% 2, OR RR increase [3])

RTI = respiratory tract illness; LRTI = lower respiratory tract illness; RR = respiratory rate; SpO_2 = blood oxygen saturation by pulse oximetry.

[1] Based on history reported by parents/LARs and includes difficulty in breathing (e.g. showing signs of wheezing or stridor, tachypnoea, flaring [of nostrils], chest in-drawing, apnoea).

[2] For blood oxygen saturation (SpO_2), the lowest value monitored will be used. In high altitudes (>2500m), SpO_2 <92% for LRTI, <90% for severe LRTI, <87% for very severe LRTI.

[3] RR increase defined as:
> 60/minute (< 2 months of age)
> 50/minute (2 to < 12 months of age)
> 40/minute (12 to 24 months of age)

[4] RSV infection confirmed on nasal swab positive for RSV A or B by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[5] RSV sampling and testing from nasal swabs.

[6] Hospitalization is defined as admission for observation or treatment based on the judgement of a health care provider

Table 4 RTI/LRTI case definitions including site altitude

	Altitude	
	≤2500m	>2500m
RSV-RTI	Runny nose, OR Blocked nose, OR Cough AND Confirmed RSV infection [4]	Runny nose, OR Blocked nose, OR Cough AND Confirmed RSV infection [4]
RSV-LRTI	History of cough OR difficulty in breathing [1] AND SpO_2 < 95% [2], OR RR increase [3] AND Confirmed RSV infection [4]	History of cough OR difficulty in breathing [1] AND SpO_2 < 92% [2], OR RR increase [3] AND Confirmed RSV infection [4]
RSV-severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 93%, OR lower chest wall in-drawing	Meeting the case definition of RSV-LRTI AND SpO_2 < 90%, OR lower chest wall in-drawing
RSV-very severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 90%, OR inability to feed OR failure to respond / unconscious	Meeting the case definition of RSV-LRTI AND SpO_2 < 87%, OR inability to feed OR failure to respond / unconscious
RSV hospitalization	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]
All-cause RTI	Runny nose, OR Blocked nose, OR Cough	Runny nose, OR Blocked nose, OR Cough
All-cause LRTI	History of cough OR difficulty in breathing [1] AND SpO_2 < 95% 2, OR RR increase [3]	History of cough OR difficulty in breathing [1] AND SpO_2 < 92% 2, OR RR increase [3]

The site altitude is needed for some study locations to accurately derive the case definitions.

RTI = respiratory tract illness; LRTI = lower respiratory tract illness; RR = respiratory rate; SpO_2 = blood oxygen saturation by pulse oximetry.

[1] Based on history reported by parents/LARs and includes difficulty in breathing (e.g. showing signs of wheezing or stridor, tachypnoea, flaring [of nostrils], chest in-drawing, apnoea).

[2] For blood oxygen saturation (SpO_2), the lowest value monitored will be used.

[3] RR increase defined as:

> 60/minute (< 2 months of age)

> 50/minute (2 to < 12 months of age)

> 40/minute (12 to 24 months of age)

[4] RSV infection confirmed on nasal swab positive for RSV A or B by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[5] RSV sampling and testing from nasal swabs.

[6] Hospitalization is defined as admission for observation or treatment based on the judgement of a health care provider

Table 5 Alternative LRTI / Severe LRTI Case Definitions

	RSV confirmed	*Documented physical examination (PE) findings indicating lower respiratory tract involvement (at least one symptom)*	*Objective measures of clinical severity (at least one symptom)*
LRTI	*Confirmed RSV infection*	*Rhonchi*[1] *Rales*[1] *Crackles* *Wheeze*	*Increased respiratory rate (bpm)* *≥ 60 for < 2 mo* *≥ 50 for 2-6 mo*
			Hypoxemia: *SpO2 <95% at ≤1800 meters* *SpO2 <92% at > 1800 meters*
			New onset apnea
			Nasal flaring
			Retractions[2]
			Grunting
Severe LRTI	*Confirmed RSV infection*	*Rhonchi*[1] *Rales*[1] *Crackles* *Wheeze*	*Hypoxemia* *SpO2 <93% at ≤1800 meters* *SpO2 <90% at > 1800 meters*
			Acute hypoxic or ventilatory failure[3]
			Dehydration due to respiratory distress requiring IV hydration[4]
			Failure to respond or unconscious

[1]Term not listed in the eCRF page in this study.

[2] Intercostal recession or chest wall indrawing are considered synonymous with and will be used as alternatives to the term "retractions," which is not listed in the eCRF page for this study.

[3] Acute hypoxic or ventilatory failure is not listed in the eCRF page for this study. Instead, the presence of either respiratory support excluding mechanical ventilation OR requirement for mechanical ventilation or both will be used.

[4] Dehydration due to respiratory distress requiring IV hydration is not listed in the eCRF page for this study. Skin turgor > 2 seconds or administration of IV fluid therapy will be used instead.

3. OBJECTIVES/ENDPOINTS

3.1. Primary

3.1.1. Primary Objectives

In healthy pregnant women with uncomplicated pregnancies:

- To determine the frequencies of pregnancy outcomes.
- To determine the frequencies of pregnancy related events of interest from enrolment (Visit 1) through 42 days after delivery (Visit 6). (Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study.)

In all neonates live-born to women enrolled in the study:

- To determine the frequencies of neonatal events of interest.

Neonatal ***events*** of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.

3.1.2. Primary Endpoints

3.1.2.1. Pregnancy outcomes

These include:

- Live birth with no congenital anomalies,
- Live birth with congenital anomalies,
- Fetal death/stillbirth (loss at or after 22 weeks of gestation) with no congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Fetal death/still birth (loss at or after 22 weeks of gestation) with congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Elective/therapeutic termination with no congenital anomalies,
- Elective/therapeutic termination with congenital anomalies.

Of note, fetal death/stillbirth has multiple subcategories. For example, fetal death/stillbirth with no congenital anomalies is an outcome with two subcategories that include: 1) antepartum stillbirth; 2) intrapartum stillbirth. For each outcome, the investigator should select the applicable sub-category.

3.1.2.2. Pregnancy related events of interest

Pregnancy related events of interest from Visit 1 through Visit 6. Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study. They are listed below. Of note, some events of interest fall under a single category but have multiple subcategories. For example, hypertensive disorders of pregnancy is an event with three subcategories that includes: 1) gestational hypertension; 2) pre-eclampsia; and 3) pre-eclampsia with severe features (including eclampsia). For each event, the investigator should identify the event and select the applicable sub-category.

- Maternal death
- Hypertensive disorders of pregnancy:
  - Gestational hypertension,
  - Pre-eclampsia,
  - Pre-eclampsia with severe features (including eclampsia)
- Antenatal bleeding:
  - Morbidly adherent placenta
  - Placental abruption
  - Caesarean Scar Pregnancy
  - Uterine rupture
- Postpartum hemorrhage
- Fetal growth restriction
- Dysfunctional labor
  - first stage of labor
  - second stage of labor
- Gestational diabetes mellitus,
- Non- reassuring fetal status
- Pathways to preterm birth:
  - Premature preterm rupture of membranes,
  - Preterm labor,
  - Provider-initiated preterm birth.
- Chorioamnionitis
- Oligohydramnios
- Polyhydramnios

- Gestational Liver Disease:
  - Intrahepatic Cholestasis of Pregnancy (ICP)
  - Acute Fatty Liver of Pregnancy
- Maternal Sepsis
- Any other pregnancy related event considered by the investigator to be of concern

3.1.2.3. Neonatal events of interest

Neonatal events of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study. They include:

- Small for gestational age,
- Low birth weight including very low birth weight,
- Neonatal encephalopathy,
- Congenital microcephaly,
  - Postnatally diagnosed
  - Prenatally diagnosed
- Congenital anomalies,
  - Major external structural defects
  - Internal structural defects
  - Functional defects
- Neonatal death,
  - Neonatal death in a preterm live birth (gestational age>=28 to < 37 weeks)
  - Neonatal death in a term live birth
- Neonatal infections,
  - Blood stream infections
  - Meningitis
  - Respiratory infection
- Respiratory distress in the neonate,
- Preterm birth,
- Failure to thrive,
- Large for gestational age,
- Macrosomia,

Any other neonatal event considered by the investigator to be of concern

3.2. Secondary

3.2.1. Secondary Objectives

In healthy pregnant women with uncomplicated pregnancies at enrolment:

- To determine frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specified) from enrolment (Visit 1) through 42 days after delivery (Visit 6). (Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study.)
- To describe the distribution of RSV-A antibody titers in maternal blood at delivery.

In all neonates live-born to women enrolled in the study:

- To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified). Neonatal ***events*** of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.
- To describe the distribution of RSV-A antibody titers in cord blood at delivery.

In all neonates/infants live-born to women enrolled in the study, from birth through 1 year of age:

- To determine the incidence of all, of severe, and of very severe RSV-lower respiratory tract illnesses (LRTIs).
- To determine the incidence of RSV hospitalization.

3.2.2. Secondary Endpoints

- Pregnancy related events of interest from Visit 1 through Visit 6 (as defined in primary endpoints) for each GAIA level of diagnostic certainty (where applicable and feasible). GAIA levels are explained in detail in Appendix D of the study protocol.
- Neonatal events of interest from birth through 28 days of age (as defined in primary endpoints) for each GAIA level of diagnostic certainty (where applicable and feasible).

Of note, some events of interest fall under a single category but have multiple subcategories. For each event, the investigator should identify the event and select the applicable sub-category and the GAIA level of diagnostic certainty.

- RSV-A neutralizing antibody titres in maternal blood at delivery
- RSV-A neutralizing antibody titres in cord blood at delivery.
- Episode(s) of RSV-LRTI from birth up to 1 year of age.
- Episode(s) of RSV hospitalization from birth up to 1 year of age.

3.3. Tertiary

3.3.1. Tertiary objectives

- To describe co-infections of RSV-LRTI with other respiratory viruses in infants.
- To estimate the association of RSV-LRTI in neonates/infants and the level of RSV neutralizing antibodies in cord blood.
- ***To determine the incidence of LRTIs/ Severe LRTIs (using alternative case definitions)***
- To determine risk factors for pregnancy-related and neonatal events of interest
- If deemed necessary, to further characterize the immune responses to RSV and other infections in maternal subjects and infants (based on maternal serum and cord blood).

3.3.2. Tertiary endpoints

- Co-infections of RSV-LRTI with other respiratory viruses in infants, confirmed by PCR of nasal swabs in infants from birth up to 1 year of age:
  - Influenza A virus (Flu A)
  - Influenza B virus (Flu B)
  - Human Influenza A virus subtype H1 (Flu A-H1)
  - Human Influenza A virus subtype H3 (Flu A-H3)
  - Human Influenza A virus subtype H1pdm09 (Flu A-H1pdm09)
  - Human adenovirus (AdV)
  - Human metapneumovirus (MPV)
  - Human enterovirus (HEV)
  - Human parainfluenza virus 1 (PIV1)
  - Human parainfluenza virus 2 (PIV2)
  - Human parainfluenza virus 3 (PIV3)
  - Human parainfluenza virus 4 (PIV4)
  - Human bocavirus 1/2/3/4 (HBoV)
  - Human rhinovirus A/B/C (HRV)
  - Human coronavirus 229E (229E)
  - Human coronavirus NL63 (NL63)
  - Human coronavirus OC43 (OC43)
- ***Episodes of LRTIs/Severe LRTIs up to one year of age - using alternative case definitions***

- Potential risk factors for pregnancy related and neonatal events of interest.
- Any further exploratory characterization of immune responses to RSV and/or other respiratory tract infections or infections of relevance to pregnant women and their new-borns (based on maternal serum and cord blood). (***For example, levels of RSV-B neutralizing antibodies).***

4. ANALYSIS SETS

4.1. Definition

4.1.1. Exposed Sets (ES)

4.1.1.1. Pregnant women (mothers)

The ES will include all pregnant women (mothers) ***who signed a valid*** informed consent ***form.***

4.1.1.2. Neonates (infants)

Not applicable.

4.1.2. Enrolled Sets

4.1.2.1. Pregnant women (mothers)

The enrolled set will include all pregnant women (mothers) with a valid informed consent who completed Visit 1.

4.1.2.2. Neonates (infants)

The Enrolled Set will include all neonates/infants

- born to pregnant women in the enrolled set, and
- Who have a valid ICF signed by the mother/ parent(s) /LAR(s) (as appropriate per local regulations).

4.1.3. Per Protocol Set

4.1.3.1. Pregnant women (mothers)

The maternal PPS will include all pregnant women (mothers) ***in the enrolled set who*** meet 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).

4.1.3.2. Neonates (infants)

The infant PPS will include all neonates / infants

- ***born to pregnant women in the PPS and***
- ***who*** meet 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) who have at least one time point evaluation.

4.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each set.

4.2.1. Elimination from Maternal Exposed Set (ES)

Code 900 (invalid informed consent or fraudulent data) will be used for identifying pregnant women (mothers) eliminated from maternal ES.

4.2.2. Elimination from Infant Exposed Set (ES)

N/A

4.2.3. Elimination from Per-protocol analysis Set (PPS)

4.2.3.1. Maternal excluded subjects

A subject will be excluded from the maternal PPS analysis under the following conditions. (Brackets indicate the code from the eCRF for the indicated question. For questions where the code is the exact same as the question, only the code in brackets is provided).

Code	Condition under which the code is used
900	<u>Invalid informed consent or fraud data.</u> • Individuals who give written or witnessed/thumb printed informed consent after the study has been explained according to local regulatory requirements. – The informed consent given at screening should either include consent for both the mother's participation and participation of the infant after the infant's birth (if consistent with local regulations/guidelines), or consent for the mother's participation and expressed willingness to consider permitting the infant to take part after the infant has been born (if local regulations/guidelines require parent(s) to provide an additional informed consent after the infant's birth). – Both mother and father should consent if local regulations/guidelines require it.

Code	Condition under which the code is used
	• Individuals who consent to have cord blood collected at delivery for the purpose of the study.
2010	Protocol violation (inclusion/exclusion criteria). <u>Inclusion criteria for enrolment:</u> • If maternal subject marked as "No" for the following question in the Eligibility Check screen for maternal subjects [frmELIGIBILITYCHECK]: – "Did the subject meet all the entry criteria?" [Eligible]

4.2.3.2. Infant Excluded Subjects

A subject will be excluded from the infant PPS analysis under the following conditions

Code	Condition under which the code is used
900	<u>Invalid informed consent or fraud data.</u> • Infants who were in utero at the time maternal (and paternal, if required) informed consent was given, and who are live-born. • If local law requires it: Written or witnessed/thumb printed informed consent for study participation of the infant obtained from parent(s)/LAR(s) within 10 days of birth.
2010	Protocol violation (inclusion/exclusion criteria) <u>Inclusion criteria for enrolment:</u> • If infant marked as "No" for the following question in the ELIGIBILITY CHECK_NB screen [frmELIGIBILITYCHECK_NB]: – Did the subject meet all the entry criteria? [Eligible]

5. STATISTICAL ANALYSES

Note that data derivation rules and statistical methods are described in sections 9 and 10.1 and will not be repeated below.

The statistical analyses for each objective is divided into two sections. The first section presents the analysis plan exactly as it is written in the protocol. The second section, additional considerations, provides more details of the analysis including any changes that have been made to the analysis plan since the protocol. These changes from the planned analysis in the protocol will also be explained in section 8.

5.1. Demography

5.1.1. Analysis of demographics/baseline characteristics planned in the protocol

For the maternal PPS, analyses of demographic and baseline characteristics at Visit 1 will be described.

For the infant PPS, analyses of demographic characteristics at Visit 1-NB will be described. Analyses of lifestyle characteristics will be described at Visit 2 NB.

For Screening Failures, reasons for non-eligibility will be described.

Continuous variables will be summarized by providing the number of observations, mean, 95% confidence interval (CI), standard deviation (SD), median, and range. Categorical variables will be summarized by tabulating counts (N) and the percentage (%) of total subjects having the given characteristics, with missing data considered a separate category.

5.1.2. Additional Considerations

For all the descriptive analyses mentioned above, separate tables will be provided for mother and infant cohorts. The results will be summarized overall and by country.

For the maternal and infants exposed sets, only descriptive statistics for baseline characteristics (ex: age) will be provided because it is a requirement for clinicaltrials.gov. Descriptive statistics or all demographic and baseline characteristics will be provided for the maternal and infant PPS.

In addition to demographic and baseline characteristic mentioned in section 5.1.1, we will also provide descriptive statistics of lifestyle characteristics for the mother PPS. These include:

- Highest education level of the mother
- Household environment
- Household composition

- Mother smoking during current pregnancy
- Mother passive smoking exposure during current pregnancy
- Mother consuming alcohol during current pregnancy
- Method of cooking used during current pregnancy
- Mosquito control during current pregnancy
- Would subject have agreed to take part in a vaccine trial during this pregnancy

5.2. Analysis of primary objectives

The primary objectives analyses will be performed on the PPSs overall and possibly by region or other relevant grouping (the groupings are specific to each analysis). ***If there is a difference of 5% or more between the number of participants in the enrolled set and the PPS, primary objectives analyses on the enrolled set will also be performed.***

5.2.1. To determine the frequency of pregnancy outcomes in healthy pregnant women with uncomplicated pregnancies at time of enrollment.

5.2.1.1. Analysis of pregnancy outcomes planned in the protocol.

The number and percentage (with exact 95% CI) of women presenting the following outcomes: live birth, fetal death/stillbirth (antepartum or intrapartum), and elective/therapeutic termination will be reported for each event by presence or absence of congenital anomalies.

5.2.1.2. Additional considerations:

The following analysis will be done on the maternal PPS. The intervals of interest are Visit 1 to Visit 5 (delivery). Details of exact confidence intervals analysis are described in section 9.5.2. All frequencies will be reported overall, by country, and if applicable, by region. For example, if there are few subjects for the events of interest, tables by region will also be presented. Frequencies of pregnancy related events of interest will also be presented by age strata (18-34, 35-39, ≥40 years).

The number and percentage (with exact 95% CI) of women presenting the following outcomes will be reported:

- Live birth with no congenital anomalies,
- Live birth with congenital anomalies,
- Fetal death/stillbirth (loss at or after 22 weeks of gestation) with no congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth

- Fetal death/still birth (loss at or after 22 weeks of gestation) with congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Elective/therapeutic termination with no congenital anomalies,
- Elective/therapeutic termination with congenital anomalies

5.2.2. To determine the frequencies of pregnancy related events of interest in healthy pregnant women with uncomplicated pregnancies from visit 1 through visit 6.

Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study

5.2.2.1. Analysis of pregnancy related events of interest planned in the protocol

The number and percentage (with exact 95% CI) of pregnant women presenting with the following events of interest will be tabulated for each event within appropriate time windows. Missing data may be considered a separate category.

5.2.2.2. Additional Considerations:

This analysis will be done on the maternal PPS. For events of interest that include subcategories, the frequency of the main event of interest and of each event subcategory will be included in the analysis. The overall frequency (derived programmatically) will include mothers who present with at least one of the subcategory events. The intervals of interest are Visit 1 up to 42 days after Visit 5. All events reported during the entire study period will be included in this analysis.

Details of exact confidence intervals analysis are described in section 9.5.2.

All frequencies will be reported overall, by country, and if applicable, by region. Frequencies of pregnancy related events of interest will also be presented by age strata (18-34, 35-39, ≥40 years). The number and percentage of the following events will be reported:

- Maternal death
- Hypertensive disorders of pregnancy:
  - Gestational hypertension,
  - Pre-eclampsia,
  - Pre-eclampsia with severe features (including eclampsia)
- Antenatal bleeding:
  - Morbidly adherent placenta

  - Placental abruption
  - Cesarean Scar Pregnancy
  - Uterine rupture
- Postpartum hemorrhage
- Fetal growth restriction
- Dysfunctional labor
  - first stage of labor
  - second stage of labor
- Gestational diabetes mellitus,
- Non reassuring fetal status
- Pathways to preterm birth:
  - Premature preterm rupture of membranes,
  - Preterm labor,
  - Provider-initiated preterm birth.
- Chorioamnionitis
- Oligohydramnios
- Polyhydramnios
- Gestational Liver Disease:
  - Intrahepatic Cholestasis of Pregnancy (ICP)
  - Acute Fatty Liver of Pregnancy
- Maternal Sepsis
- Any other pregnancy related event considered by the investigator to be of concern

5.2.3. To determine the frequencies of neonatal events of interest in all neonates live-born to women enrolled in the study.

5.2.3.1. Analysis of neonatal related events of interest planned in the protocol

The number and percentage (with exact 95% CI) of neonates presenting the following events of interest will be tabulated for each event. Missing data may be considered a separate category.

5.2.3.2. Additional Considerations

This analysis will be done on the infant PPS, overall, by country, and if applicable, by region.

For events of interest that include subcategories, the frequency of the main event of interest and of each event subcategory will be included in the analysis. The overall frequency will include infants who present at least one of the subcategory events.

Details of exact confidence intervals analysis are described in section 9.5.2.

The number and percentage of the following events will be reported:

- Small for gestational age,
- Low birth weight including very low birth weight,
- Neonatal encephalopathy,
- Congenital microcephaly,
  - Postnatally diagnosed
  - Prenatally diagnosed
- Congenital anomalies,
  - Major external structural defects
  - Internal structural defects
  - Functional defects
- Neonatal death,
  - Neonatal death in a preterm live birth (gestational age>=28 to < 37 weeks)
  - Neonatal death in a term live birth
- Neonatal infections,
  - Blood stream infections
  - Meningitis
  - Respiratory infection
- Respiratory distress in the neonate,
- Preterm birth,
- Failure to thrive,
- Large for gestational age,
- Macrosomia,
- Any other neonatal event considered by the investigator to be of concern (specify, e.g. neurodevelopment delay)

5.3. Analysis of secondary objectives

The secondary objectives analyses will be performed on the PPSs (maternal or infant as applicable) overall and possibly by region or other relevant grouping. ***If there is difference of 5% or more between the number of participants in the enrolled set and the PPS, tertiary objectives analyses on the enrolled set will also be performed.***

5.3.1. To determine frequencies of fetal death/still birth according to GAIA levels of diagnostic certainty.

5.3.1.1. Analysis planned in the protocol

N/A

5.3.1.2. Additional considerations

This objective will not be performed.

5.3.2. To determine frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specified) in healthy pregnant women with uncomplicated pregnancies at time of enrolment.

5.3.2.1. Analysis of pregnancy related events of interest planned in the protocol.

To determine the frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specific) (APPENDIX D of protocol).

Considering all pregnant women followed from enrolment through Visit 6, the number and percentage of subjects with at least one maternal event of interest will be computed by GAIA levels of diagnostic certainty, with exact 95% CIs.

5.3.2.2. Additional considerations

This analysis will be done on the maternal PPS, overall and by country. If the frequencies of categories of events of interest are low, the results will also be presented by region.

Details of exact confidence intervals analysis are described in section 9.5.2.

Among the pregnancy related events of interest from Section 3.1.2.2, those with GAIA levels of diagnostic certainty will be presented in this analysis. For each event, the frequency of mothers with a specific diagnostic level of certainty will be reported. For events with subcategories, main events will not be presented since the levels of diagnostic certainty do not apply to the main categories because GAIA levels do not exist for the main categories.

Please note that GAIA levels are explained in detail in the Appendix D of the study protocol.

5.3.3. To describe the distribution of RSV-A antibody titres in maternal blood at delivery in healthy pregnant women with uncomplicated pregnancies at time of enrolment.

5.3.3.1. Analysis of RSV-A antibody titres in maternal blood collected at delivery planned in the protocol

To describe the distribution of RSV-A antibody titres in maternal blood at delivery.

- Geometric mean titres (GMTs) will be tabulated with 95% CI.
- Percentage of subjects above thresholds will be tabulated.

5.3.3.2. Additional considerations

This analysis will be done on the maternal PPS, overall and by country.

RSV-A neutralising antibodies will be obtained from maternal and cord blood collected at delivery. The frequency and percentage of subjects with titres above the threshold will be presented along with 95% CI. GMTs and corresponding 95% CI, minimum and maximum values will be reported.

5.3.4. To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified) in all neonates live-born to women enrolled in the study:

5.3.4.1. Analysis of neonatal events of interest planned in the protocol

To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified) (APPENDIX D of protocol). Neonatal events of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.

Considering all neonates the number and percentage of subjects with at least one neonatal event of interest will be computed by GAIA levels of diagnostic certainty, with exact 95% CI.

5.3.4.2. Additional considerations:

This analysis will be done on the infant PPS overall and by country. Among the neonatal events of interest identified in section 3.1.2.3, those with GAIA levels of diagnostic certainty will be presented in this analysis. For each event, the frequency of infants with a specific diagnostic level of certainty will be reported. For events with subcategories, main events will not be presented because GAIA levels do not exist for the main categories.

Please note that GAIA levels are explained in detail in Appendix D of the study protocol.

5.3.5. To describe the distribution of RSV-A antibody titres in cord blood at delivery in all neonates live-born to women enrolled in the study:

5.3.5.1. Analysis of RSV A antibody titres in cord blood planned in protocol

To describe the distribution of RSV-A antibody titres in cord blood at delivery

- Geometric mean titres (GMTs) will be tabulated with 95% CI.
- Percentage of subjects above thresholds will be tabulated.

5.3.5.2. Additional considerations:

A correction/elaboration from the analysis planned in protocol in section 5.3.5.1 is that we will be reporting on the percentage of subjects with GMTs above thresholds.

This analysis will be done on the Infant PPS, overall and by country.

RSV-A neutralising antibodies will be measured in the cord blood at delivery. The frequency and percentage of subjects with titres above the threshold will be presented along with 95% CI. GMTs and corresponding 95% CI, minimum and maximum values will be reported.

5.3.6. To determine the incidence of all, of severe, and of very severe RSV-lower respiratory tract illnesses (LRTIs) in all neonates/infants live-born to women enrolled in the study:

5.3.6.1. Analysis of RSV LRTI in all neonates/infants planned in protocol

Considering all infants followed from visit 1-NB to visit ***4***-NB, the percentage of subjects with at least one LRTI illness, exact 95% CI will be presented.

Incidence rates of LRTI illnesses will be calculated, with exact 95% CI.

Frequencies of repeat occurrences of LRTI will be tabulated.

5.3.6.2. Additional considerations

A correction from the analysis planned in protocol in section 5.3.6.1 is that we will be reporting on all cause LRTIs and RSV associated LRTIs.

This analysis will be based on the infant PPS and will be done on the first episode of all, of severe, and of very severe RSV LRTIs (Section 2.2).

Incidence analyses will be performed overall, by country and by age strata (0-2 months, 0-5 months, and 0-11 months). If applicable (in the case of low numbers of incidence), incidence analyses will also be presented by region (Section 9.3). Incidence rates will also be calculated in one-month intervals from birth to one year of age, please see the monthly infant age intervals in Section 9.3.

The analysis of RSV disease incidence is summarized in the table below showing endpoints and analytic methods, these include incidence rate, proportion affected, and incidence proportion.

Incidence rate is the number of first events per person time. Incidence proportion is the number of first RSV LRTI over population at risk during the specified time period (0-2 months, 0-5 months and 0-11 months). Proportion affected is the number of subjects with at least one episode of RSV LRTI at age interval over the total number of subjects at the start of the age interval.

For each of the RSV LRTI endpoints listed in Table 11, the 95% CI will be computed as described in section 9.5.2. Kaplan-Meier curves presenting the cumulative probability of the first episode will be displayed overall. The cumulative probability of presenting the first episode will be given with its 95% CI at the end of the follow-up.

Frequencies of repeat occurrences of RSV LRTI, severe LRTI and very sever LRTI will be tabulated.

Cumulative incidence will be presented from birth to one year of life by monthly intervals (Subgroups in section 9.3).

Table 6 Summary of RSV incidence analyses for RSV LRTI of infants from birth to 1 year (Visit 1-NB to visit 4-NB)

Endpoints	Incidence analysis method	Subgroup
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Incidence rates of first episode Cumulative probability of first episode (Kaplan Meier)	Overall, by country, and by age strata
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Proportion affected by all new episodes	Overall, by country, and by age strata
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Incidence proportion of first episode	Overall and by country within each age strata)

5.3.7. To determine the incidence of RSV hospitalization in all neonates/infants live-born to women enrolled in the study:

5.3.7.1. Analysis of RSV hospitalization planned in protocol

Considering all infants followed from visit 1-NB to visit 4-NB, the percentage of subjects with at least one RSV hospitalization, with exact 95% CI.

Incidence rates of RSV hospitalizations will be calculated, with exact 95% CI.

Frequencies of repeat occurrences of RSV hospitalization will be tabulated.

5.3.7.2. Additional considerations

This analysis will be performed on the infant PPS and the first episode of RSV hospitalization, overall and by country and by age strata. In additional to incidences of RSV hospitalization, we will also consider all cause LRTI hospitalizations.

A change from the planned analysis in section 5.3.7.1 is that only the first event of RSV hospitalization will be included in the analysis.

In addition, cumulative incidence, Kaplan-Meier curves, and proportion affected will be calculated.

Lastly, we will also present the frequencies of symptoms used in case definitions among those infants who are found to be RSV positive from nasal swab results. The symptoms that will be considered are:

- Runny nose
- Blocked nose
- Cough
- Wheezing
- Grunting
- Nasal flaring
- Intercostal recession
- Temperature ≥38 degrees Celsius
- Chest wall indrawing
- SpO_2 cutoff point for hypoxemia

5.4. Analysis of tertiary objectives

The following tertiary objectives analyses will be performed on infants within the PPS overall and possibly by region or other relevant grouping. ***If there is difference of 5% or more between the number of participants in the enrolled set and the PPS, tertiary objectives analyses on the enrolled set will also be performed.***

5.4.1. To describe co-infections of RSV-LRTI with other respiratory viruses in infants.

5.4.1.1. Analysis of respiratory viruses associated with RSV-LRTI as planned in protocol

Considering all infants followed from visit 1-NB to visit 4-NB, the percentage of subjects with the occurrence of RSV-LRTI and having other respiratory viral co- infection with another viral etiology identified by multiplex PCR, with exact 95% CI; classified by respiratory viruses.

5.4.1.2. Additional considerations

This analysis will be performed on the Infant PPS.

The number and percentage of cases for each respiratory virus will be identified for all RSV LRTI, severe RSV LRTI, and very severe RSV LRTI. For each respiratory virus infection, number and percentage will be summarized by single or co-infection status. The groups will include those who are:

*RSV positive (confirmed with nasal swabs)

*RSV positive (confirmed with nasal swabs) & positive with another infection

*RSV negative (confirmed with nasal swabs) & positive with another infection.

For cases of RSV LRTI, severe RSV LRTI and very severe RSV LRTI, the number and percentage of cases associated with at least one other respiratory virus will be described.

This analysis will be performed overall, by country, and by age strata (0-2 months, 0-5 months, and 0-11 months).

Other respiratory viruses causing infection:

- Influenza A virus (Flu A)
- Influenza B virus (Flu B)
- Human Influenza A virus subtype H1 (Flu A-H1)
- Human Influenza A virus subtype H3 (Flu A-H3)
- Human Influenza A virus subtype H1pdm09 (Flu A-H1pdm09)
- Human adenovirus (AdV)
- Human metapneumovirus (MPV)
- Human enterovirus (HEV)
- Human parainfluenza virus 1 (PIV1)
- Human parainfluenza virus 2 (PIV2)
- Human parainfluenza virus 3 (PIV3)
- Human parainfluenza virus 4 (PIV4)
- Human bocavirus 1/2/3/4 (HBoV)
- Human rhinovirus A/B/C (HRV)
- Human coronavirus 229E (229E)
- Human coronavirus NL63 (NL63)
- Human coronavirus OC43 (OC43)

Lastly, an additional analysis will be performed that considers the frequencies of clinical symptoms of two groups of infants: those are have RSV LRTI and those who have LRTI but are RSV negative.

The clinical symptoms that will be considered are:

- Runny nose
- Blocked nose
- Cough
- Wheezing
- Grunting
- Nasal flaring
- Intercostal recession
- Temperature ≥38 degrees Celsius
- Chest wall indrawing
- SpO_2 cutoff point for hypoxemia

5.4.2. To estimate the association of RSV-LRTI in neonates/infants and the level of RSV neutralizing antibodies in cord blood.

5.4.2.1. Analysis of association of RSV-LRTI in neonates and level of RSV neutralizing antibodies in cord blood as described in the protocol.

Correlation analysis will be done comparing the levels of RSV antibodies present in cord blood and the presence or absence of RSV-associated LRTI, RSV-associated severe LRTI, and RSV-associated very severe LRTI in infants.

5.4.2.2. Additional consideration.

When considering RSV antibodies in cord blood, we are only considering RSV-A antibodies at this time (however, in the future we might also consider RSV-B antibodies). When considering RSV positivity status in nasal swabs, we consider positivity for RSV-A or RSV-B.

This analysis will be performed on the Infant PPS and will be done on the first episode (not recurrent events) of RSV LRTIs occurring during the following age intervals: 0-2 months and 0-5 months.

Step 1: Descriptive analysis:

Descriptive analyses (seropositivity rate with 95% CIs, geometric mean, median, min, max) of RSV-A neutralizing antibodies in the baseline cord blood samples will be analysed in only those with RSV positive nasal swab samples.

Next, descriptive analyses (seropositivity rate with 95% CIs, geometric mean, median, min, max) of RSV-A neutralizing antibodies in the baseline cord blood samples will be presented by RSV LRTI, RSV severe LRTI, and RSV very severe LRTI case statuses.

In addition, if further exploration is desired, we may plot levels of RSV A vs RSV-B neutralizing antibodies in cord blood among RSV LRTI positive and negative infants to see if any patterns are observed.

Step 2: Cox models:

The impact of the level of RSV neutralizing antibodies in the baseline cord blood samples on the incidence of first event of RSV-LRTI, RSV-severe LRTI and RSV very severe LRTI separately will be assessed through Cox models.

Cox regression models will be performed for the univariate analyses to obtain unadjusted hazards ratios of the determinants of interest.

Next a multivariable Cox regression model will be performed to estimate the relative contribution of each potential risk factor adjusting for the simultaneous effects of the other covariates. The model will include: time-independent covariates (e.g. gender, etc.) and time-dependent covariates (age). Covariate selection will be done using backward elimination and statistical significance. Potential risk factors will be included in the multivariable models if univariate p-value will be less than 0.1 and the multivariable model will be performed only if the number of events in sufficient (at least 10 events per covariate). In addition, depending on the data, other models could be explored or the model could be simplified.

The following covariates in infants may be considered in the multivariable models:

- Age (continuous)
- Male sex (M/F)
- Small for gestational age (Continuous)
- Congenital anomalies with internal structural defects and/or with functional defects (Y/N)
- Household composition (number of people living in household) (continuous)
- How many children under age of 6 live in household (continuous)
- Cigarette smoking during pregnancy (Y/N)
- Born during RSV transmission season (Y/N)
- Alcohol consumption during this pregnancy (Y/N)
- Highest education level of mother (Y/N)

Step 2-a: RSV neutralizing antibodies classified as a binary variable: Seropositive and seronegative:

In a first step, the RSV-A neutralizing antibodies in the baseline cord blood samples will be introduced in the model as binary variable

Step 2-b: RSV neutralizing antibodies classified as semi-quantitative variable: Quartiles

In a second step, the RSV-A neutralizing antibodies levels in baseline cord blood will be introduced in the model using quartiles. And the Cox models will be performed as described in the previous paragraph (RSV-A neutralizing antibodies as quantitative variable).

Step 2-c: RSV neutralizing antibodies classified as continuous variable:

In a third step, the RSV neutralizing antibodies levels in baseline cord blood might be treated as continuous variable in Cox models to evaluate how each unit of neutralizing antibody level change impacts on the above clinical outcomes. The Cox models will be performed as described in the previous section (with log of RSV neutralizing antibodies as quantitative variable). For the subjects with antibody levels below the LOD, a value of LOD/2 will be imputed.

The model with the best fit using, log likelihood criteria, will be selected. This will be determined via backward elimination using statistical significance.

Note that depending on the results, a quantitative association between RSV-associated LRTI, RSV-associated severe LRTI, RSV very severe LRTI, and RSV neutralizing antibodies in the baseline cord blood samples could be further explored and described more in details in the main analysis.

Poisson regression models:

Multivariable Poisson regression of neutralizing RSV A cord blood antibodies estimating RSV LRTI incidence in the first year of life, controlling for covariates will be explored. The same risk factors as above will be test. Modelling will be done in a stepwise manner using backward elimination and statistical significance.

5.4.3. To determine the incidence of LRTIs/Severe LRTIs (using alternative case definitions)

5.4.3.1. Additional considerations

Repeat incidence analyses described in section 5.3.6 with alternative LRTIs/Severe LRTIs case definitions.

5.4.4. To determine risk factors for pregnancy related and neonatal events of interest.

5.4.4.1. Analysis of risk factors for pregnancy related and neonatal events of interest as described in the protocol.

- Both pregnancy related events of interest and neonatal events of interest will be described according to variables collected in the mother's clinical examination and obstetric risk factors. Frequency tables will be generated for categorical variables.
- Furthermore, multiple variable analyses (such as multiple logistic regression for binary outcomes and multiple Poisson/negative binomial and/or other appropriate models for count outcomes) will be conducted to adjust for covariates of interest, provided there are sufficient numbers of events of interest.

5.4.4.2. Additional considerations:

This analysis will be performed on the maternal and infant PPSs depending on whether the risk factors are for pregnancy-related events of interest or for neonatal events of interest.

There are currently 14 potential pregnancy related events of interest and 9 potential risk factors. Likewise, there are 12 potential neonatal events of interest and 13 potential risk factors. (These do not include GAIA subcategories).

Univariate modelling will be done to first determine which risk factors are associated with which event of interest.

For each potential risk factor,

- Frequency tables will be generated as appropriate, for categorical variables.
- Mean, median, standard error, minimum, maximum and number of missing values will be provided as appropriate, for continuous data.

For pairwise distributions

- Cross-tabulations (i.e. two-way frequency tables) will be generated as appropriate, for categorical-categorical pairs;
- Mean, median, standard error, minimum, maximum and number of missing values by category will be provided as appropriate, for continuous-categorical pairs;

Covariates with univariate p-value less than 0.1 will be included in the multivariable model. The number of covariates included in the model will depend on the number of events (at least 10 events per covariates).

Multiple logistic regression, multiple Poisson regression/negative binomial modelling will be used when appropriate only on risk factors and events of interest that were associated in the univariate analysis and provided there are sufficient numbers of events of interest.

5.4.4. If deemed necessary, to further characterize the immune responses to RSV and other infections in maternal subjects and infants (based on maternal serum and cord blood).

5.4.4.1. Analysis of immune responses to disease as planned in the protocol

- ***For example, for levels of neutralizing antibodies to RSV-B:***
  - GMTs/ GMCs may be tabulated with 95% CI.
  - Percentage of subjects above various thresholds, depending on the antibody tested, may be tabulated with exact 95% CI.

5.4.4.2. Additional Considerations:

N/A

5.5. COVID-19 Supplement

5.5.1. In addition to satisfying local reporting requirements for COVID-19 cases, maternal and infant COVID-19 cases identified within the existing framework of the study will be captured and reported on the COVID-19 eCRF for the study.

COVID-19 cases should be reported in the eCRF according to the WHO Case Definition [WHO, 2020]. Please refer to Section 11 using one of the following terms:

- Suspected COVID-19 infection
- Probably COVID-19 infection
- Confirmed COVID-19 infection

5.5.2. Additional Considerations

5.5.2.1. WHO Case Definition (March 20, 2020 Version):

Suspected case

a. A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), AND a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset;

   OR

b. A patient with any acute respiratory illness AND having been in contact (see definition of "contact" below) with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset;

   OR

c. A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

Probable case

a. A suspect case for whom testing for the COVID-19 virus is inconclusive (Inconclusive being the result of the test reported by the laboratory).

OR

b. A suspect case for whom testing could not be performed for any reason.

Confirmed case

A person with laboratory confirmation of COVID-19 infection, *irrespective of clinical signs and symptoms*.

A contact is a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:

1. Face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 minutes;
2. Direct physical contact with a probable or confirmed case;
3. Direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment;

   OR

4. Other situations as indicated by local risk assessments.

   Note: for confirmed asymptomatic cases, *the period of contact* is measured as the 2 days before through the 14 days after the date on which the sample was taken which led to confirmation.

5.5.2.2. Reporting COVID-19 cases—Maternal and Infant Subjects

Frequency of COVID-19 cases (suspected, probable, and confirmed) will be reported for mothers and infants.

Frequency of mothers and infants who had a COVID-19 diagnosis and test performed and number of subjects with positive, negative, and indeterminate results with be reported.

Incidence of COVID-19 confirmed cases will be reported for mothers.

6. ANALYSIS INTERPRETATION

Analyses will be descriptive with the aim to characterize different sub-groups of women. These sub-groups will be defined prior to analyses and will be related to e.g. risk factors for maternal and neonatal events of interest and endemic disease. These descriptive analyses should be interpreted with caution considering that there is no adjustment for multiplicity for these comparisons.

7. CONDUCT OF ANALYSES

All analyses will be done on cleaned data.

Analyses will be performed in a stepwise manner after all subjects in all countries have completed all study visits in Epoch 002 and again after completion of all study visits in Epoch 003.

Analyses of pregnancy outcomes, pregnancy related events of interest, and neonatal events of interest will be performed when all data up to 42 days post-delivery are available (Epoch 002). For these analyses a statistical report but no CSR will be prepared.

Final analyses will be performed when all data up to study end are available (Epoch 002 and Epoch 003). An integrated *CSR* including all available data will be written and made available to the investigator(s).

If the data for tertiary endpoints become available at a later stage, additional analysis/analyses will be performed. These data will be documented in annex(es) to the study report and made available to the investigators.

Additional analyses may be performed if deemed necessary to inform the design or implementation of future clinical trials.

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.

Interim analysis for RSV LRTIs

If deemed necessary, an interim analysis ***may*** be performed on RSV surveillance data as part of the secondary objective of determining incidence of RSV LRTI. This interim analysis ***may*** be performed to obtain preliminary information on the performance of the case definitions used for RSV LRTI, severe LRTI, and very severe LRTI (Table 3) and to assess levels of RSV neutralising antibody in maternal and cord blood at delivery. The interim analysis ***may*** occur after the database freeze at the time point when approximately 50% of infants will have completed up to 6 months of follow up during the surveillance period following birth (up to Visit 3-NB). ***If an interim analysis is performed,*** data cleaning plans will be scheduled as needed to supply data for interim analyses in a timely manner. This interim analysis ***may*** be performed on data that is as clean as possible. Preliminary results will be made available in a timely manner for use in the potential adjustment of the RSV LRTI case definitions to be used in pivotal clinical trials scheduled to begin shortly thereafter. The results pertaining to this analysis ***(if performed)*** will be purely descriptive, with no adjustment of type I error, and will be reported in an interim statistical report. No CSR will be prepared.

7.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)
Month 12	E1_01	CTRS Study report	Yes	Yes
Month 6 interim analysis-***may occur***	E1_02	Internal	Yes	No
Analysis of epoch 2	E1_03	Internal	Yes	No

Analysis ID will depend on whether interim and epoch 2 analysis will be run using the same DBF depending on availability of lab results.

8. CHANGES FROM PLANNED ANALYSES

Only the first episode within a given age interval is considered for the computation of incidence. The incidence of event is computed using three different calculation methods: incidence rate, proportion affected, and incidence proportion. See section 5.3.6 for details of calculation.

In analysing incidence of RSV LRTI and RSV Hospitalization, only the first episode of each will be considered.

A correction from the analysis planned in protocol in section 5.3.5.1 is that we will be reporting on all cause LRTIs and RSV associated LRTIs.

Analyses will be stratified by country and by age strata (mother or infant depending on the analysis).

In instances where there are low frequencies (either in events of interest or case definitions) results may be presented by region in addition to by country (see Section 9.3 for subgroups).

The cohort have been adjusted in Amendment 4 of the protocol. See section 4 in the SAP for details. Analyses will be conducted on PPS cohorts. If there is a difference of 5% or more between the number of participants in the enrolled set and the PPS, analyses will also be performed on enrolled sets.

India has been removed from the study and Bangladesh and Panama have been added.

Alternative LRTI/Severe LRTI case definitions have been added to the study and subsequent analyses.

The interim analyses is no longer planned and may only occurred if deemed necessary.

Considering the COVID19 pandemic, Primary analysis (all subjects have completed all study visit in Epoch 002) will be conducted using as clean as possible data based on the status of the data monitoring defined by the pre-defined proportion of Maternal Subjects Assigned to the SDV/SDR Scheme defined in the monitoring plan.

In addition to the pre-defined analysis, sensitivity analysis will be also conducted, as appropriate, based on the % of maternal and infant subjects reaching the threshold of 70% SDV/SDR scheme as defined in the monitoring plan."

9. NON-STANDARD DATA DERIVATION RULES AND STATISTICAL METHODS

9.1. Data Derivation

9.1.1. Maternal blood and cord blood

Antibody titres will be obtained from maternal blood and cord blood at delivery.The GMT/GMC and its 95% CI will be obtained by exponentiating the mean and its 95% CI of the log-transformed titres/concentrations. All CI computed will be two-sided 95% CI.

9.1.1.1. Assay cut-offs for serology results

A seronegative subject is a subject whose antibody titre is below the cut-off value of the assay. A seropositive subject is a subject whose antibody titre is greater than or equal to the cut-off value of the assay. The cut-off tests for immunogenicity evaluation will be as per following:

Table 7 Derivation rule for GMT calculation and seropositivity status with respect to RSV A and RSV B neutralizing antibody titers in maternal blood at delivery and cord blood

System	Component	Method	Unit	Cut-off *(LLOQ)	ULOQ
Serum	RSV-A Neutralising Antibody	NEU	ED60 and/or IU (international unit)	18 for ED60 56 for IU	ED60 : 123535 IU : 217400
Serum	RSV-B Neutralising Antibody	NEU	ED60 and/or IU (international unit)	30 for ED60 7 for IU	***N/A***

RSV = respiratory syncytial virus; ED60 = serum dilution inducing 60% inhibition in plaque forming units; IU/ml = International units/milliliter, IU= International units

Assay cut-off and unit might be subject to change before starting of testing (e.g. in case of requalification, revalidation or standardisation). In this case, this will be documented in the clinical report.

Assay	Raw result	Derivation for GMT calculation	Derivation of positivity status
RSV-A and RSV-B neutralizing antibody	<LOD	LOD/2	Negative
	≥LOD	Exact value	Positive

LOD=18 ED_{60}

9.1.2. Nasal swabs from infants during RSV LRTI episodes

The sponsor will analyze nasal swabs by quantitative reverse transcription polymerase chain reaction (RT-PCR) for the presence of RSV A/B. A positive (RSV A or B) test result constitutes a case of RSV infection, if viral load is greater than or equal to the cut-off value of the assay

Table 8 RSV positivity status in infants will be determined from viral loads from nasal swabs taken during infant visits. The infant will be considered RSV positive according to the following thresholds of copies/mL of RSV antibodies

Component	Method	Unit	Cut-off*
RSV A	RT-qPCR	Copies of RSV A RNA per mL	304 copies/ml
RSV B	RT-qPCR	Copies of RSV B RNA per mL	475 copies/ml

*Assay cut-off and unit might be subject to change before starting of testing (e.g. in case of requalification, revalidation or standardisation). In this case, this will be documented in the clinical report.

Assay	Raw result*	Derivation for GMT calculation	Derivation of positivity status
RSV-A and RSV-B	<LOD	LOD/2	Negative
	≥LOD	Exact value	Positive

*RSV-A : LOD=304 copies/ml
*RSV-B : LOD=475 copies/ml

9.1.3. Risk factors for pregnancy-related and neonatal events of interest

Table 9 Risk factors for pregnancy-related events of interest

Risk Factor	Categories
Prenatal smoking exposure during this pregnancy	Yes/No
Alcohol consumption during this pregnancy	Yes/No
BMI pre-pregnancy (kg/cm^2)	Continuous
Age of mother at delivery in current pregnancy(years)	18-34 ≥35
Subject currently lives in a country or region with Zika transmission	Yes/No
Subject has travelled to a country or region with Zika transmission since the beginning of their pregnancy	Yes/No
Subject travelled to country or region with Zika virus transmission	Yes/No
Predominant geographic ancestry	African Heritage/African American American Indian or Alaskan Native Asian-Central/South Asian Heritage Asian-East Asian Heritage Asian-Japanese Heritage Asian-South East Asian Heritage Native Hawaiian or Other Pacific Islander White Middle-eastern /North African Heritage White-Caucasian/ European Heritage Latino/Mestizo Other
Caesarean section in previous pregnancy	Yes/No
Highest education level of mother	High education Yes: Bachelor's degree or higher No: Less than bachelor's degree

Table 10 Risk factors for neonatal events of interest

Risk Factor	Categories
Prenatal smoking exposure during this pregnancy	Yes/No
Age of mother at delivery in current pregnancy(years)	18-34 ≥35
Alcohol consumption during this pregnancy	Yes/No
BMI pre-pregnancy (kg/cm^2)	Continuous
Gestational diabetes mellitus in current pregnancy	Yes/No
Gestational hypertension in current pregnancy	Yes/No
Fetal growth restriction	Y/N
Antenatal bleeding	Y/N
Dysfunctional labor	Y/N
Gender of new-born	Male/Female
Subject lives in or travelled to country or region with Zika virus infection during pregnancy	Yes/No
Predominant geographic ancestry	African Heritage/African American American Indian or Alaskan Native Asian-Central/South Asian Heritage Asian-East Asian Heritage Asian-Japanese Heritage Asian-South East Asian Heritage Native Hawaiian or Other Pacific Islander White Middle-eastern /North African Heritage White-Caucasian/ European Heritage Latino/Mestizo Other
Apgar at 5 minutes of age	0-3 inclusive 4-6 inclusive 7 or greater

9.1.4. Risk factors for RSV LRTI

- Age (continuous)
- Male sex (M/F)
- Small for gestational age (Continuous)
- Congenital anomalies with internal structural defects and/or with functional defects (Y/N)
- Household composition (number of people living in household) (continuous)
- How many children under age of 6 live in household (continuous)
- Cigarette smoking during pregnancy (Y/N)
- Born during RSV transmission season (Y/N)
- Alcohol consumption during this pregnancy (Y/N)
- Highest education level of mother (Y/N)

9.1.5. RSV Seasonality

Table 11 RSV Transmission seasons by country/region and centre

Country/region	Seasonality	Centre #
Thailand		PPD
Bangkok	June-October	
Chiang Mai	July-November	
Malaysia		
Selangor	Year round	
Kota Kinabalu	Year round	
Alor Setar	Year round	
Kuching	Year round	
Philippines		
Manila	Year round	
Cebu	Year round	
South Africa		
Parow, Western Cape	April-August	
Pretoria, Gauteng	February-June	
	February-August	
Panama		
David	May-January	
Panamá	May-January	
Chorrera	May-January	
Bangladesh		
Matlab	July-December	
Kamlapur	July-February	
Brazil		
Ribeirao Preto/SP	March-July	
Belo Horizonte/MG	March-July	
Nata/RN	March-July	
Argentina		
Buenos Ares	May-July	
Cordoba	May-July	
Mendoza	May-July	
Colombia		
Medellin	Mar-Jun & Sep-Nov	
Medellin	Year round	
Bogota	Year round	
Bogota	Mar-May	
Cali	April-Jun	
Villavicencio	Mar-Apr/May & Nov-Dec	
Mexico		
Monterrey	September-April	
Oaxaca	September-April	
Durango	September-April	

9.2. Data presentation

The following decimal description will be used for the analyses.

Table 12 Decimal points in analyses

Display Table	Parameters	Number of decimal digits
All summaries	% of frequency, including LL & UL of CI	1
All summaries	% frequency, including LL & UL of CI	1
All summaries	Mean, median, minimum, maximum	1
All summaries	SD	2
All summaries	*P*-value	3

9.3. Subgroup definitions

The following sub-group names will be used for statistical analyses:

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
Country		
1	TH	Subjects from Thailand
2	MY	Subjects from Malaysia
3	PH	Subjects from Philippines
4	SA	Subjects from South Africa
5	BD	Subjects from Bangladesh
6	PA	Subjects from Panama
7	BR	Subjects from Brazil
8	AR	Subjects from Argentina
9	CO	Subjects from Colombia
10	MX	Subjects from Mexico
Region		
Latin America	LatAM	Subjects from Brazil, Argentina, Colombia. Mexico, Panama
Asia Pacific	AsiaPac	Subjects from Thailand, Malaysia, Philippines, Bangladesh
South Africa	SA	Subjects from South Africa
Age category maternal subject at enrolment		
1	18-34	18-34 years of age
2	≥35	35 years of age or older
Age category infant (3 categories)		
1	0-2	Birth to one day before the 3rd month of life (0-30 days)
2	0-5	Birth to one day before the 6th month of life (0-180 days)
3	0-11	Birth one day before the 12th month of life (0-365 days)
Age category infant (One-month intervals)		
1	0	Birth to one day before the 1st month of life
2	1	First day of 1 month to one day before the 2nd month of life
3	2	First day of 2 months to one day before the 3rd month of life
4	3	First day of 3 months to one day before the 4th month of life
5	4	First day of 4 months to one day before the 5th month of life

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
6	5	First day of 5 months to one day before the 6th month of life
7	6	First day of 6 months to one day before the 7th month of life
8	7	First day of 7 months to one day before the 8th month of life
9	8	First day of 8 months to one day before the 9th month of life
10	9	First day of 9 months to one day before the 10th month of life
11	10	First day of 10 months to one day before the 11th month of life
12	11	First day of 11 months to one day before the 12th month of life

9.4. Case definitions

Please refer to section 2.1 for an explanation of how data is collected for surveillance in this study since that is the data that will be used for the derivation of case definitions.

Note that if a worsening visit(s) takes place for the same episode, then all symptoms collected from the initial visit and the worsening visit(s) are combined and counted under one episode, with the most severe level of the symptoms being used in the case definition derivation. For example, if SpO_2 is collected in a case assessment and a worsening visit for the same episode, then the lowest SpO_2 from any visit will be used for the case definition derivation. This is done because the most severe level of a symptom will not necessarily be in the worsening form, so multiple scenarios of visits and data collection can be accounted for. The earliest date of a reported symptom will be used as the start date of the episode. The latest date of a reported symptom will be used as the end date of episode.

If an infant comes in for a visit with new bouts of cough or blocked nose after 7 days of his/her latest end date of an RTI symptom, then a new case assessment form is opened and this is potentially the start of a new episode.

Variables from a Case Assessment Visit will be pulled from [frmASSESSMENT_INFO], [frmSYMPTOMS_RTI], and [frmVITALSIGNS_RTI].

Variables from a Worsening Visit will be pulled from [frmWORSENING_INFO] and [frmVITALSIGNS_WOR].

9.4.1. RSV RTI

The child will be reported positive for RSV RTI when he/she presents with at least one of the following symptoms: (data pulled from the Case Assessment Visit)

- Runny nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= ‘YES’) and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= ‘Yes’) and [frmSYMPTOMS_RTI] (fields[ASSESS_VIS]=’Yes’ and [sctSYMPTOMS_RTI]=[RUNNY]

- Blocked nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes') and [frmSYMPTOMS_RTI] (fields[ASSESS_VIS]='Yes' and [sctSYMPTOMS_RTI]=[BLOCKED]Cough reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')

AND confirmed RSV infection

9.4.2. RSV LRTI

The child will be reported positive for RSV LRTI when he/she presents with at least one of the following symptoms: (The data can be pulled from the Case Assessment Visit)

- Cough reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')
- Difficulty breathing reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING')

  AND at least one of following symptoms: **(The data can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)**
- SpO_2(<95% if altitude ≤2500m and <92% if altitude >2500m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

  OR
- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

  AND

Confirmed RSV infection from a nasal swab test.

9.4.2.1. Start date of RSV-LRTI

Start dates of RSV LRTI symptoms will be pulled from the Case Assessment Visit for at least one of the following: parental report of history of cough or difficult breathing. The start date of RSV LRTI is the earliest of the start dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'SRDAT')

  Or

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'SRDAT')

9.4.2.2. End date of RSV-LRTI

End dates of RSV LRTI symptoms will be pulled from the Case Assessment Visit and will be defined as the latest date of the end dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'ENDAT')

  Or

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'ENDAT')

9.4.3. RSV severe LRTI

RSV severe LRTI cases will be reported if the person meets the case definition of RSV LRTI

AND

(The data for below can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<93% if altitude ≤2500m and <90% if altitude >2500m) (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '93.0% or <90%')

  OR

- Lower chest wall indrawing reported during the physical examination. (field [sctCHEST_INDRAWING_RTI]=[INDRAWING]= 'YES') OR (field [sctCHEST_INDRAWING_WOR]=[INDRAWING]= 'YES')

9.4.3.1. RSV Severe LRTI start date

Start dates will be defined as the earliest date of cough or difficulty breathing as described in section 9.4.2.1.

9.4.3.2. RSV Severe LRTI end date

End dates will be defined as the latest date of cough or difficulty breathing as described in section 9.4.2.2.

9.4.4. RSV very severe LRTI

RSV very severe LRTI is defined as meeting the case definition of RSV LRTI

AND

(The data for below for SpO_2 can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<90% if altitude ≤2500m and <87% if altitude >2500m) (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '90.0% or <87%')

  OR

- Inability to feed reported in signs and symptoms. [sctSEVERITY_SIGNS_RTI]=[FEEDING]= 'Unable to feed')

  OR

- Failure to respond/unconscious reported in signs and symptoms. [sctSEVERITY_SIGNS_RTI]=[CONSCIOUS]= 'The child is unresponsive to all stimuli')

9.4.4.1. RSV very severe LRTI start date

Start dates will be defined as the earliest start date of cough or difficulty breathing as described in section 9.4.2.1.

9.4.4.2. RSV very severe LRTI end date

End dates will be defined as the latest end date of cough or difficulty breathing as described in section 9.4.2.2.

9.4.5. RSV Hospitalization

RSV hospitalization is determined if both the following criteria are met:

- Confirmed RSV infection with nasal swab test from central GSK lab.

  AND

- Hospitalization for acute medical condition determined in Inpatient and worsening flag form. Variables will be pulled from [frmINPATIENT_WORSE_FLG] (field [HOSPI_YN]= 'Yes').

9.4.5.1. RSV Hospitalization start dates

Hospitalization start date will be determined from the Inpatient Care form. Variables will be pulled from [frmINPATIENTCARE] (field [HOSP_SRDAT]=)

9.4.5.2. RSV Hospitalization end dates

Hospitalization end date will be determined from the Inpatient Care form. Variables will be pulled from [frmINPATIENTCARE] (field [HOSP_ENDAT]=)

9.4.6. All cause RTI

All cause RTI is reported if the child has one or more of the following symptoms:

- Runny nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'RUNNY')
- Blocked nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BLOCKED')
- COUGH reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')

9.4.6.1. All cause RTI start date

- Start dates of all cause RTI cases will be the earliest dates of cough, runny nose, or blocked nose. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and
- [sctSYMPTOMS_RTI]= 'Cough' and [cmpCOUGH_DT]= 'SRDAT'

  OR
- [sctSYMPTOMS_RTI]= 'Blocked' and [cmpBLOCKED_DT]= 'SRDAT'

  OR
- [sctSYMPTOMS_RTI]= 'RUNNY' and [cmpRUNNY_DT]= 'SRDAT')

9.4.6.2. All cause RTI end date

End dates of all cause RTI cases will be that lastest dates of Cough. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'Cough' and [cmpCOUGH_DT]= 'ENDAT')

9.4.7. All cause LRTI

All cause LRTI is reported if the child has one or more of the following:

- COUGH reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')
- Difficulty breathing reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING')

  AND

(The data for below can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<95% if altitude ≤2500m and <92% if altitude >2500m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

  OR
- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

9.4.7.1. All cause LRTI start dates

The start date of all cause LRTI with the earliest of the start dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'SRDAT')

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'SRDAT')

9.4.7.2. All cause LRTI end dates

The end date of all cause LRTI is the latest of the end dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'ENDAT')

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'ENDAT')

Figure 2 Derivation of RSV-LRTI events date and duration according to the the corresponding LRTI event

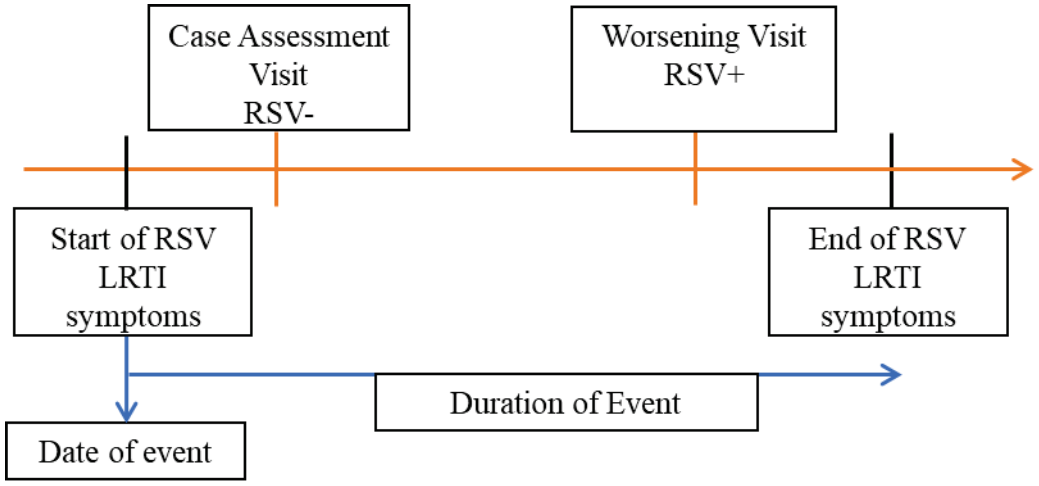


Figure 3 Derivation of RSV-Hospitalization events date and duration

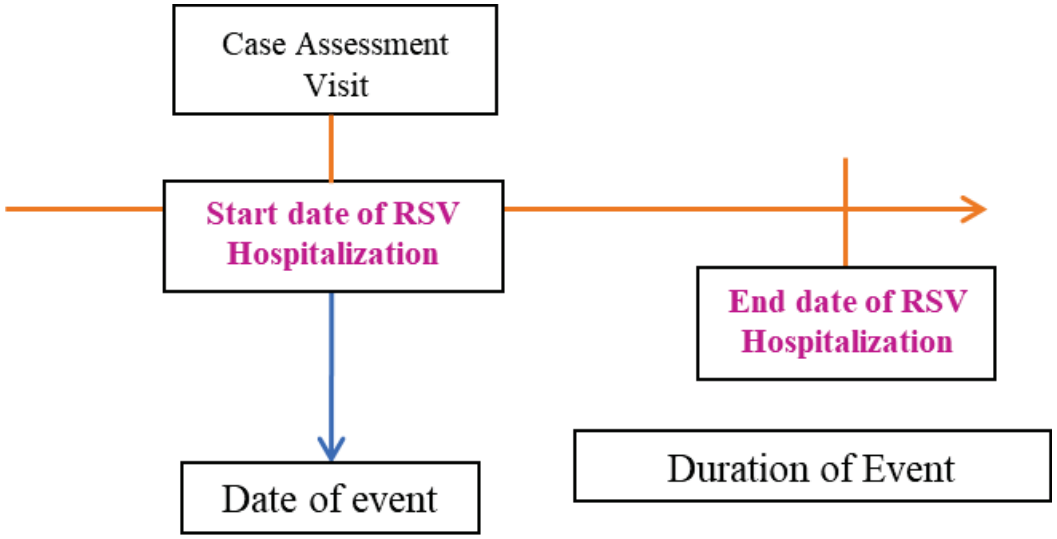
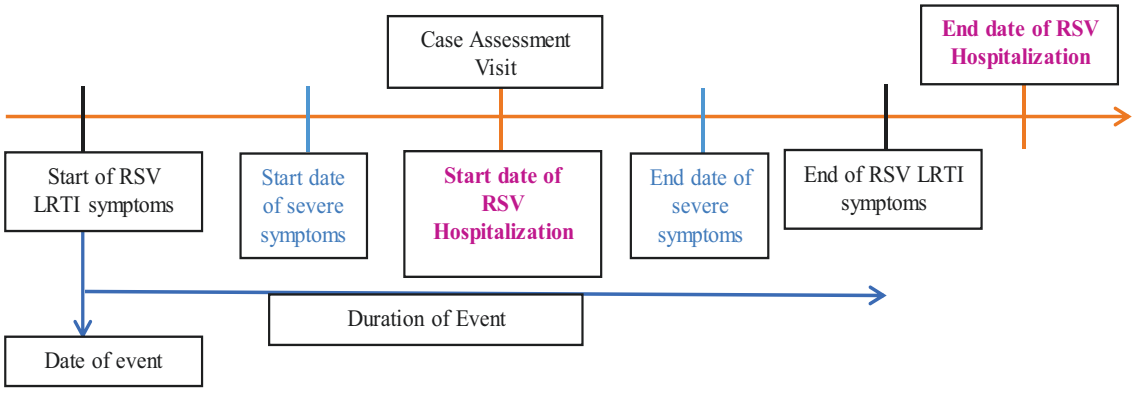


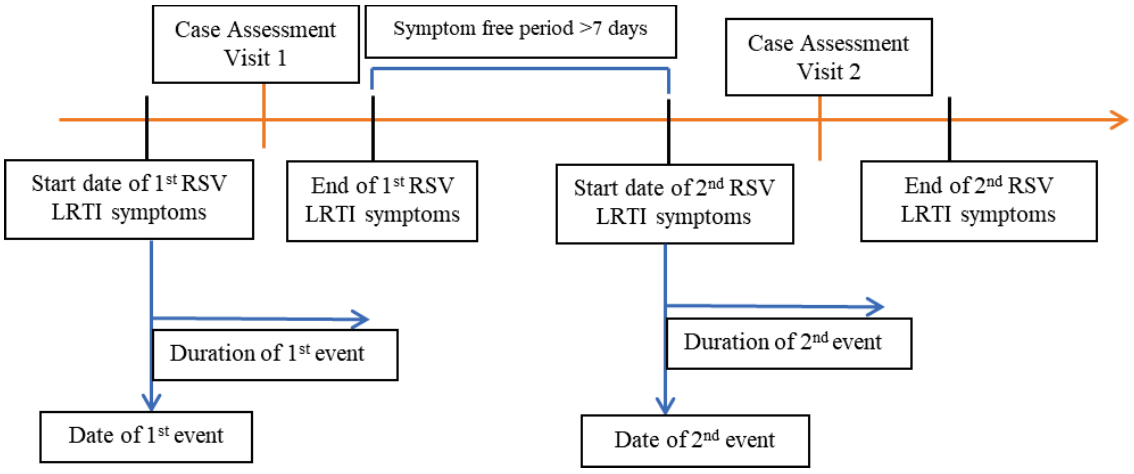
Figure 4 Derivation of RSV-LRTI hospitalization events date and duration according to the corresponding LRTI event



9.4.8. New RSV LRTI episode rule

A new episode of an event is a single case of RSV RTI, RSV LRTI, severe RSV LRTI, or hospitalization meeting the respective case definitions and severity scale with an interval of at least 7 symptom free days since the last episode that was diagnosed. (Note: persistent runny nose is an exception to the 7 symptom free days rule. A child may have persistent runny nose but still present with a new episode of RSV LRTI). If symptoms worsen within 7 days, the subject comes for a worsening visit.

Figure 5 Derivation of 2 RSV LRTI events date and duration with a symptom free period of at least 7 days



9.4.9. Symptoms

9.4.9.1. Increased respiratory rate

The subject will be considered to have increased respiratory rate if one of the following statements is reached:

- Respiratory rate (field [sctRESPIRATORYRATE_RTI])
- > 60/minute for subjects <2 months of age (fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])
- > 50/minute for subjects 2-11 months of age(fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])
- > 40/minute for subjects 12-24 months of age(fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])

Please note that respiratory rate is measured during the general and obstetric physical exam.

9.5. Statistical Methods

9.5.1. Computation of incidence rates

For each endpoint, the incidence rate (IR, number of episodes/endpoints per 100 person-years) will be calculated by dividing the number of subjects reporting the first episode over the follow-up period by the total person-year. A 95% CI will be computed using an exact method for a Poisson variable as described below.

The person-time at risk for an event of interest (RSV-LRTI or RSV hospitalization) will be calculated as the time between the date of birth and the end of the at-risk period or the earliest of the followings:

- Date of first diagnosis of event of interest (e.g. first episode of RSV-LRTI);
- Date when child reaches 1 year (or 6 months if analysis is done during the interim analysis);
- Date of death;
- Date of last follow-up in study.

Please note that in a case where a subject comes to an assessment visits, has LRTI symptoms, is not confirmed for RSV infection via a nasal swab (because either no nasal swab was taken or the lab yielded invalid results), then we will consider the subject as not have a laboratory-confirmed event. The Subject will therefore be censored at the event (n=0, T=from start of the follow-up period at risk up to event).

9.5.2. Exact confidence intervals (CIs)

The exact confidence interval within a group for an incidence rate (per 100 person-years):

To estimate the confidence limit of the incidence rate, the exact Poisson confidence limit will be used [Clopper, 1934]:

If *n* is the number of subjects presenting a given characteristic among these *Ny* subjects per year, the true incidence rate can be estimated by *(n/Ny)*100*. Its exact *(1-α)%* confidence interval is obtained from:

*CINV(α/2,2*n)/2/Ny*100* as the lower boundary

and

CINV((1-α/2),2(n+1))/2/Ny*100* as the upper boundary.

where *CINV(probability, degrees of freedom)* returns the inverse of the chi-squared probability distribution and α is the type I error rate.

9.5.3. Computation of proportion affected

Proportion affected will be computed as the number of subject who had at least one episode in the age interval divided by the total number of subjects at start of considered age strata. A 95% CI will be computed using an exact method for a Poisson variable.

9.5.4. Computation of incidence proportion

Incidence proportion will be computed as the number of subjects who had at least one episode in the age interval (monthly) divided by the number of subject at risk of event at the beginning of the age interval. A subject that has an event will no longer consider of at risk, i.e. subjects with event in 0 – 1 month are excluded from denominator for future monthly incidence calculation. A 95% CI will be computed using an exact method.

9.5.5. Cox models

All multivariable modelling will be done in a stepwise manner using backward elimination and statistical significance.

9.5.5.1. Univariate Models

Please see section 5.4.4.2. Univariate modelling will be done to first determine which risk factors are associated with which event of interest.

For each potential risk factor:

- Frequency tables will be generated as appropriate, for categorical variables.
- Mean, median, standard error, minimum, maximum and number of missing values will be provided as appropriate, for continuous data.

For pairwise distributions:

- Cross-tabulations (i.e. two-way frequency tables) will be generated as appropriate, for categorical-categorical pairs;
- Mean, median, standard error, minimum, maximum and number of missing values by category will be provided as appropriate, for continuous-categorical pairs;

9.5.5.2. Multivariable Models

Covariate selection will be done using statistical significance: the final multivariable regression models will include all potential risk factors with a simple regression model with p-value < 0.10. The number of covariates included in the models will depend on the number of events (at least 10 events per covariate). The models will be performed only if the number of events is sufficient (at least 10 events per covariate). In addition, depending on the data and the validity of the model assumptions, other models could be explored or the models could be simplified.

The results from the multivariable regression models will include hazard ratios (Cox regression models) and odds ratios (logistic and longitudinal logistic regression models) and their 95% CI.

9.5.5.3. Multiple Logistic Regression models

Multiple logistic regression models will be used to test the association between events of interest and risk factors. PROC LOGISTIC or PROC GENMOD can be used. What is tested will depend on how many people are observed with these outcome and risk factors and if they are associated in the univariate analysis.

9.5.6. Multiple Poisson/negative binomial models

PROC GENMOD can be used for multiple Poisson/negative binomial modelling.

10. ANNEXES

10.1. Business rules for standard data derivations and statistical methods

This section contains GSK Vaccines' standard rules for data display and derivation for clinical and epidemiological studies. These rules will be applied along with those detailed in section 9 (additional study-specific rules).

10.1.1. Age

Age of infant will be expressed in months and will be computed as the number of complete calendar months between the date of birth (DOB) and the date of event. For example:

DOB = 10JUN2017, Date of event = 09JUL2018 -> Age = 12 months

DOB = 10JUN2017, Date of event = 10JUL2018 -> Age = 13 months

Age of the mother at the time of childbirth will be expressed in years and will be computed as the number of complete calendar years between the birth date of the infant and the birth date of the mother. For example:

DOB = 10SEP1983, Date of vaccination = 09SEP2018 -> Age = 34 years

DOB = 10SEP1983, Date of vaccination = 10SEP2018 -> Age = 35 years

10.1.2. Handling of missing data

10.1.2.1. Dates

When partially completed dates (i.e. with missing day or month) are used in calculations, the following standard rules will be applied:

- A missing day will be replaced by 15
- A missing day and month will be replaced by June 30th.

10.1.2.2. Laboratory data

Missing laboratory results (including immunological data) will not be replaced.

10.1.2.2.1. Studies with paper diaries

For studies using paper diaries which have questions in the CRF indicating the presence or absence of solicited symptoms, the following rules are applicable.

Denominators for the summary of local (or general) solicited symptoms will be calculated using the number of subjects who respond "Yes" or "No" to the question concerning the occurrence of local (or general) symptoms.

When a specific symptom is marked as having not occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=N for the specified post-vaccination period for the symptom in question), all daily measurements will be imputed as Grade 0.

When a specific symptom is marked as having occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=Y for the specified post-vaccination period for the symptom in question), any missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

When the occurrence of a specific symptom is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-vaccination period for the symptom in question) but the group of symptoms (local or general) is marked as having occurred (i.e. SDTM CE.CEOCCUR=Y), all missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

The following table shows how subjects contribute to each category for a specific solicited symptom over the Day X to Day Y post-vaccination period:

Solicited symptom category	Subjects included in the calculation of the numerator
Any	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y or with the symptom marked as present and at least one missing daily recording between Day X and Day Y
At least grade 1	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y
At least grade 2	All subjects with at least one occurrence of the symptom at grade 2 or grade 3 between Day X and Day Y
At least grade 3	All subjects with at least one occurrence of the symptom at grade 3 between Day X and Day Y

10.1.3. Data derivation

10.1.3.1. Weight

Weight will be presented in kilograms. Weights reported in pounds will be converted as follows:

Weight in kilograms = Weight in pounds / 2.2

10.1.3.2. Height

Height will be presented in centimeters. Heights reported in feet and inches will be converted as follows:

Height in centimeters = Height in inches x 2.54

10.1.3.3. Body mass index (BMI)

BMI will be calculated as follows:

BMI = (Weight in kilograms) / (Height in meters)^2

10.1.3.4. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

Temperature (Celsius) = ((Temperature (Fahrenheit) - 32) x 5)/9

10.1.3.5. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
“NEG”, “-“, or “(-)”	cut-off/2
“POS”, “+”, or “(+)”	cut-off
“< value” and value is <= assay cut-off	cut-off/2
“< value” and value is > assay cut-off	value
“> value” and value is < assay cut-off	cut-off/2
“> value” and value is >= assay cut-off	value
“value” and value is < cut-off	cut-off/2
“value” and value is >= cut-off	value
All other cases	missing

10.1.3.6. Geometric mean titres (GMTs) and concentrations (GMCs)

Geometric Mean Titre (GMT) or Concentration (GMC) calculations are performed by taking the inverse logarithm of the mean of the log titre or concentration transformations. Antibody titres or concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMT/GMC calculation. The cut-off value is defined by the laboratory before the analysis and is described in the protocol.

10.1.3.7. Onset day

The onset day for an event (e.g. AE, medication, vaccination) is the number of days between the last study vaccination and the start date of the event. This is 1 for an event occurring on the same day as a vaccination (and reported as starting after vaccination).

10.1.3.8. Duration of events

The duration of an event with a start and end date will be the number of days between the start and end dates plus one day, i.e. an event that starts on 03MAR2018 and ends on 12MAR2018 has a duration of 10 days.

The duration of solicited events will be calculated as the sum of the individual days with the symptom reported at grade 1 or higher.

10.1.4. Display of decimals

10.1.4.1. Percentages

Percentages and their corresponding confidence limits will be displayed with:

- no decimals when there are fewer than 50 subjects in each tabulated group
- one decimal when there are at least 50 subjects in at least one tabulated group
  - Exceptions will be made for percentages that are not 0% or 100% but appear as 0% or 100% due to rounding. For these specific cases the number of decimals will be increased until the displayed value is no longer 0% or 100%. Examples are given in the following table.

n/N	Displayed percentage
10/45	22%
1/45	2%
10/55	18.2%
1/55	1.8%
1/300	0.3%
1/3000	0.03%
1/30000	0.003%
299/300	99.7%
2999/3000	99.97%
29999/30000	99.997%

- The display of additional decimals for values close to 0% or 100% will be applied only to point estimates and not confidence limits, which can be rounded and displayed as 0% or 100%.
- Values of exactly 0% or 100% will be presented with no decimals regardless of the number of subjects per tabulated group.

10.1.4.2. Differences in percentages

Differences in percentages and their corresponding confidence limits will be displayed with one more decimal than the maximum number used to display the individual percentages, for example the difference between two percentages displayed with one decimal will be displayed with two decimals.

10.1.4.3. Demographic/baseline characteristics statistics

The mean, median, and standard deviation for continuous baseline characteristics (height, weight, body mass index (BMI), pre-vaccination body temperature) will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maxima and minima of transformed height variables will be displayed with no decimals.

The maxima and minima of transformed weight variables will be displayed with no decimals with the exception of values are below 10kg where one decimal will be displayed.

The maximum and minima of transformed body temperatures will be displayed with one decimal.

10.1.4.4. Serological summary statistics

The number of decimals used when displaying geometric mean titers (GMT) or concentrations (GMC) and their confidence limits is shown in the following table:

GMT or GMC value	Number of decimals to display
<0.1	3
>=0.1 and <10	2
>=10 and <1000	1
>=1000	0

When multiple categories of GMT or GMC values are present in the same table, the number of decimals displayed should match that of the smallest category (i.e. the one with the higher number of decimals). For example, if GMT or GMC values of <0.1 appear in the same table as values of >=0.1 and <10, 3 decimals should be displayed for both.

GMT or GMC ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

10.1.5. Statistical methodology

10.1.5.1. Exact confidence intervals around proportions

The exact confidence intervals around within-group proportions are derived using the method of Clopper and Pearson [Clopper, 1934].

10.1.5.2. Standardized asymptotic confidence intervals around differences in proportions

The standardized asymptotic confidence intervals around differences in proportions are derived using the method of Mietinnen and Nurminen [Miettinen, 1985].

10.1.5.3. Adjusted GMT or GMC ratios

When between-group GMT or GMC ratios are computed and adjusted for two-level categorical co-variables, these co-variables should be included as dummy continuous variables in the SAS procedure.

10.2. TFL and/or TFL ToC

The TFL and the TFL ToC will be found in eTMF folder section:
PPD

11. REFERENCES

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934; 26:404-13.

Miettinen, O. S. and Nurminen, M. Comparative analysis of two rates. *Statistics in Medicine*, 1985; 4,213-226.

World Health Organization [WHO]. Global Surveillance for COVID-19 caused by human infection with COVID-19 virus: Interim Guidance. March 2020. Available at: https://apps.who.int/iris/rest/bitstreams/1272502/retrieve. Accessed 8 May 2020.

GlaxoSmithKline	**Statistical Analysis Plan Amendment 1**
Detailed Title:	A prospective epidemiological study of women between 24-27 weeks of pregnancy to describe pregnancy outcomes and events of interest in the mother and neonate, as well as determine incidence in infants of RSV LRTI and RSV hospitalization.
eTrack study number and Abbreviated Title	207636 (EPI-RSV-008 BOD)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Final: 08-May-2020
Date of Amendment	Final: 27-Nov-2020
Co-ordinating author:	PPD (Expert Epidemiology Biostatistician)
Reviewed by:	PPD (Clinical and Epidemiology Project Lead) PPD (Lead Epidemiologist) PPD (Lead Epidemiology Statistician) PPD (Lead Epidemiology Statistician) PPD (Lead Statistical Analyst) PPD (Lead Scientific Writer) PPD (Regulatory Affairs) PPD (SERM Physician) PPD (Public disclosure representative)
Approved by:	PPD (Lead Epidemiology Statistician)

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Co-ordinating author:	PPD (Expert Epidemiology Biostatistician)
Authors who contributed to previous versions of the SAP	PPD (Lead Epidemiology Statistician) PPD (Lead Statistical Analyst) PPD (Senior Scientific Writer) PPD (Regulatory Affairs) PPD (Public disclosure representative)

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

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LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Events of Special Interest
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CLS	Clinical Laboratory Sciences
COVID-19	Corona virus disease 2019
CSR	Clinical Study Report
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
ELISA	Enzyme-linked immunosorbent assay
EoS	End of Study
ES	Exposed Set
FAS	Full Analysis Set
FU	Follow-Up
GAIA	Global Alignment of Immunization Safety Assessment in pregnancy
GCP	Good Clinical Practice
GMT	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IU/ml	International units per milliliter
LAR	Legally Acceptable Representative
LL	Lower Limit of the confidence interval
LMP	Last Menstrual Period
LOD	Limit of Detection
LRTI	Lower Respiratory Tract Illness

LSLV	Last Subject Last Visit
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NB	Newborn
PCD	Primary Completion Date
PCR	Polymerase Chain Reaction
PD	Protocol Deviation
PPS	Per Protocol Set
RBC	Red Blood Cell
RR	Respiratory Rate
RSV	Respiratory Syncytial Virus
RTI	Respiratory Tract Illness
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SPM	Study Procedures Manual
SpO_2	Blood oxygen saturation as measured by pulse oximetry
SR	Study Report
T Domains	Trial Domains
TFL	Tables Figures and Listings
TOC	Table of Contents
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
08-MAY-2019	first version	Amendment 3 – 18-FEB-2019
27-NOV-2020	Amendment 1 Final	Amendment 4- 03-JUNE-2020

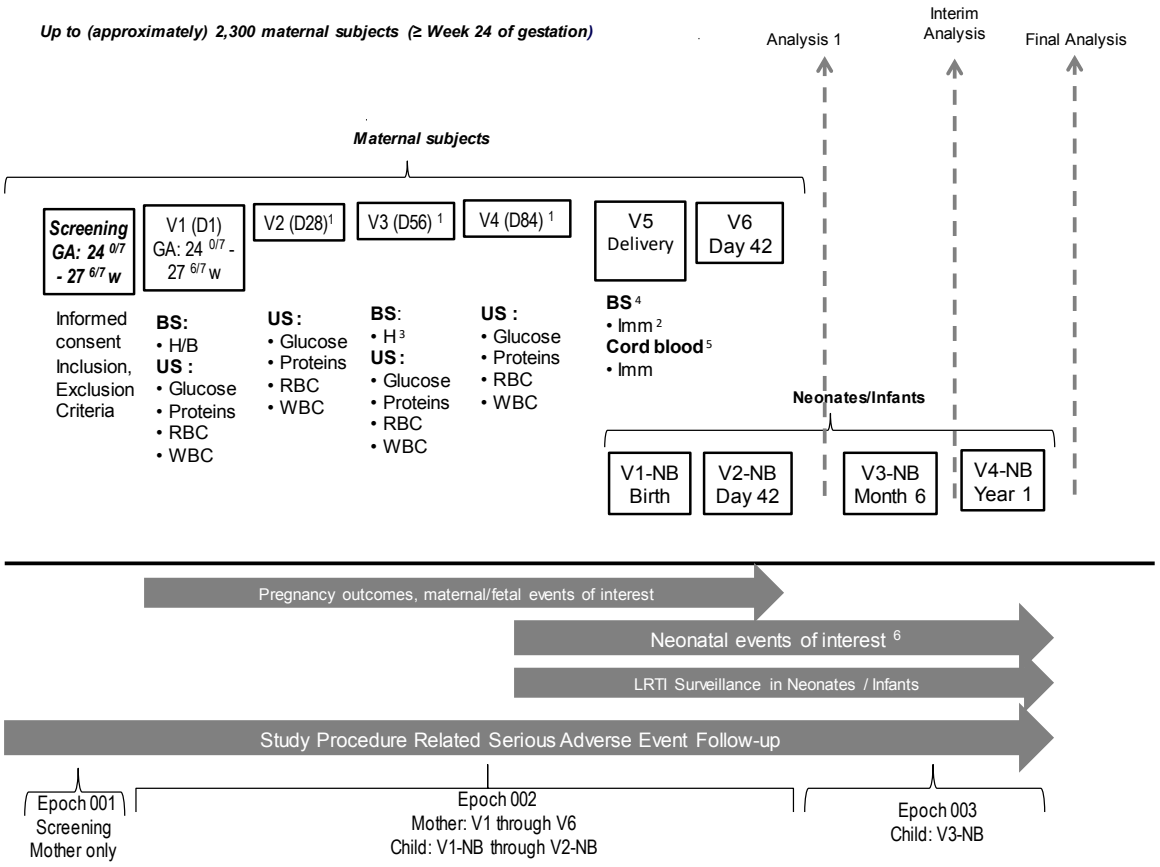
2. STUDY DESIGN

2.1. Summary

This is a prospective multi-country cohort study that aims to provide information for subsequent clinical trials for maternal immunization for respiratory syncytial virus (RSV). The study will provide background rates of maternal and neonatal events of interest, incidence rates of lower respiratory tract illnesses associated with RSV (RSV LRTI), and will determine the capacity of future sites for clinical trials. Approximately 2300 pregnant women from nine different countries will be enrolled during their third trimester of pregnancy and followed until 42 days after delivery. Their children will be followed from birth until one year of age. Epoch 1 of the study consists of the screening of maternal subjects. Epoch 2 includes visits 1-6 of the mother and visits 1-2 of the new born; during which time data on pregnancy outcomes and maternal and neonatal events of interest will be collected and RSV LRTI surveillance will begin (from visit 1 of the new born). Neonatal events of interest will only include events that occurred during the first 28 days of life, but data on these events can be collected up to one year of age. Maternal and cord blood samples will also be collected from the mother at delivery. Lastly, Epoch 3 will include RSV LRTI surveillance as well as continued recording of neonatal events of interest. During surveillance in Epoch 3, symptoms relevant to RSV illness will be recorded in diary cards, and RSV positivity will be confirmed via laboratory tests performed on nasal swabs collected during site visits.

Please note that when referring to epochs in the SAP, we are following the convention outlined in the protocol. Epochs in the CDISC T domains are named differently and are as follows: For maternal subjects: screening epoch and study procedures epoch. For infants: screening epoch and surveillance epoch (infants do not actually have a screening visit in this study but this is the naming standard used for CDISC T domains).

Figure 1 Study design



D = day; M = month; V = visit; W = week; Y = year. NB = newborn; GA = Gestational age; BS = blood sample; US = urine sample for dipstick testing; IMM = immune response; HB = hematology/biochemistry; RBC = red blood cell(s); WBC = white blood cell(s); RSV = respiratory syncytial virus; RTI = respiratory tract illness; LRTI = lower respiratory tract illness;

Refer to Section **Error! Reference source not found.** for additional information about the analyses indicated above.

[1] If delivery occurs prematurely, skip to Visit 5 ("at delivery").

[2] At Delivery, RSV-A antibody titers for all women

[3] At V3, only hemoglobin testing.

[4] Allowed interval for blood sample collection begins with start of labor (e.g. when the mother arrives at the hospital to deliver and an intravenous line is inserted) and ends 72 hours after delivery.

[5] RSV-A antibodies in cord blood

[6] Neonatal events of interest occur (by definition) between 0 and 28 days after birth. They will be reported once site staff become aware of them (whether this occurs during the first 28 days after birth, or at a later time).

NOTE: Covid-19 cases identified within the surveillance framework of the study in maternal and infant subjects will also be recorded.

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

- Type of design: Prospective, epidemiological, interventional (without administration of medicinal products as described in a research protocol), multi-country, cohort study.

- Study population: The study will be conducted in multiple countries, in pregnant women and their infants.
- Type of study: self-contained.
- Data collection: Electronic Case Report Form (eCRF).

Table 1 Sampling schedule for maternal subjects

		Visit				
Sample	**Parameter(s) evaluated**	**1**	**2**	**3**	**4**	**Delivery**
Maternal **Blood**	Hematology/ biochemistry[1, 4]	x		x[3]		
	Antibody titer (RSV-A)					≤ 72 hours after delivery[5]
Cord Blood	Antibody titer (RSV-A)					x
Urine	Protein, glucose, RBC, WBC [2, 4]	x	x	x	x	

[1] To be performed preferentially by local healthcare providers, as per local practice. To be performed by the investigator/study staff ONLY if not done by the local healthcare provider within 2 weeks before the study visit.
[2] The investigator/site staff will perform a urine dipstick test using supplies provided by GSK.
[3] Hemoglobin only
[4] If results are abnormal, subjects will be referred per local standard of care.
[5] This sample may be collected from start of labor (e.g. when the mother arrives at the hospital to deliver and an intravenous line is inserted) through 72 hours after delivery.

- Sampling schedule for **neonates/infants**: Surveillance for RSV LRTI will be conducted in infants through the 12 month study period. During this 1-year post-birth surveillance period, for each RTI with suspicion of difficulty in breathing, or wheezing, or with parental concern, a nasal swab will be collected at a visit to assess potential RSV associated RTIs/LRTIs.
- Primary Completion Date (PCD): 42 days post-delivery/birth (i.e. Visit 6 for maternal subjects and Visit 2-NB for infant subjects) or last visit of Epoch 002.
- End of Study (EoS): Last testing results released of samples collected up to Visit 4-NB (assays related to primary and secondary endpoints).
- Duration of the study: Approximately 4.5 to 6 months for maternal subjects; approximately 1 year for infant subjects.
  - Epoch 001: Screening
  - Epoch 002: Primary starting at Visit 1 and ending 42 days post-delivery/birth (Visit 6 for maternal subjects and Visit 2-NB for infant subjects).*
  - Epoch 003: Follow-up of infants starting 43 days post-delivery/birth and ending at Visit 4-NB (1 year post-birth).

*Any safety and disease surveillance data collected after Visit 2-NB will be collected in Epoch 003.

Table 2 Study groups and epochs foreseen in the study

Study Groups	Number of **subjects** enrolled	Age (Min/Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
Maternal subjects	Up to ~ 2300	18 years - 45 years	x	x	
Infants	Up to ~ 2300	NA		x	x

Approximately 200 to 300 per country. To achieve the enrollment targets noted above, the number of pregnant women SCREENED in each country may exceed the country-specific enrolment goal.

- Surveillance for pregnancy outcomes and pregnancy-related events of interest that occur from Visit 1 up to 42 days after delivery (Visit 6).
- Surveillance for neonatal events of interest that occur from birth up to 28 days of age.
- Surveillance for RSV LRTI and RSV hospitalization in infants, from birth up to 1 year of age. Nasal swabs will be collected during surveillance visits to confirm RSV positivity.
- This study includes active and passive surveillance of RSV LRTI. In an active surveillance, site personnel will contact the subject's parent(s) / LAR(s) or their designate(s). Active contacts will occur at regular intervals (depending on RSV seasonality at the site) and will be scripted. In a Passive contact, the subject's parent(s) / LAR(s) or their designate(s) contact site personnel if their infant has any of the RTI symptoms (cough, runny nose, or blocked nose). Site personnel will use a script to guide data collection once a passive contact has been made. In each case, the site will use the decision tree (Figure 2 of protocol) to determine if a case assessment visit is needed (Occurrence of Surveillance contact flag and Surveillance Contact Report form in the eCRF). If the patient reported wheezing or difficulty breathing, the nurse will set up an appointment for the mother to bring her infant for follow up at the clinic within three days of the beginning of symptoms. Once the child has completed an assessment visit, site personnel will continue surveillance for the case episode. If the child's symptoms have deteriorated and the child is admitted to the hospital or needs oxygen therapy (Inpatient and Worsening flag in the eCRF), the physician will report worsening symptoms (Worsening form in the eCRF). Start dates will be recorded for cough, runny nose, wheezing, and difficulty breathing, but only end dates will be recorded for cough and difficulty breathing. If the infant experiences new bouts of cough or blocked nose after 7 days of her/his recovery from the last RTI symptoms, then a new case assessment form will be opened. Given that infants frequently experience runny nose, a case can be concluded even if the runny nose has not resolved.

2.2. Case Definitions

The case definitions are summarized in the following table:

Table 3 RTI/LRTI case definitions

RSV-RTI	(Runny nose, OR Blocked nose, OR Cough) AND Confirmed RSV infection [4]
RSV-LRTI	(History of cough OR difficulty in breathing [1]) AND (SpO_2 < 95% [2], OR RR increase [3]) AND Confirmed RSV infection [4]
RSV-severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 93%, OR lower chest wall in-drawing
RSV-very severe LRTI	Meeting the case definition of RSV-LRTI AND (SpO_2 < 90%, OR inability to feed OR failure to respond / unconscious)
RSV hospitalization	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]
All-cause RTI	Runny nose, OR Blocked nose, OR Cough
All-cause LRTI	(History of cough OR difficulty in breathing [1]) AND (SpO_2 < 95% 2, OR RR increase [3])

RTI = respiratory tract illness; LRTI = lower respiratory tract illness; RR = respiratory rate; SpO_2 = blood oxygen saturation by pulse oximetry.

[1] Based on history reported by parents/LARs and includes difficulty in breathing (e.g. showing signs of wheezing or stridor, tachypnoea, flaring [of nostrils], chest in-drawing, apnoea).

[2] For blood oxygen saturation (SpO_2), the lowest value monitored will be used. In high altitudes (>2500m), SpO_2 <92% for LRTI, <90% for severe LRTI, <87% for very severe LRTI.

[3] RR increase defined as:
> 60/minute (< 2 months of age)
> 50/minute (2 to < 12 months of age)
> 40/minute (12 to 24 months of age)

[4] RSV infection confirmed on nasal swab positive for RSV A or B by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[5] RSV sampling and testing from nasal swabs.

[6] Hospitalization is defined as admission for observation or treatment based on the judgement of a health care provider

Table 4 RTI/LRTI case definitions including site altitude

	Altitude	
	≤2500m	>2500m
RSV-RTI	Runny nose, OR Blocked nose, OR Cough AND Confirmed RSV infection [4]	Runny nose, OR Blocked nose, OR Cough AND Confirmed RSV infection [4]
RSV-LRTI	History of cough OR difficulty in breathing [1] AND SpO_2 < 95% [2], OR RR increase [3] AND Confirmed RSV infection [4]	History of cough OR difficulty in breathing [1] AND SpO_2 < 92% [2], OR RR increase [3] AND Confirmed RSV infection [4]
RSV-severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 93%, OR lower chest wall in-drawing	Meeting the case definition of RSV-LRTI AND SpO_2 < 90%, OR lower chest wall in-drawing
RSV-very severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 90%, OR inability to feed OR failure to respond / unconscious	Meeting the case definition of RSV-LRTI AND SpO_2 < 87%, OR inability to feed OR failure to respond / unconscious
RSV hospitalization	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]
All-cause RTI	Runny nose, OR Blocked nose, OR Cough	Runny nose, OR Blocked nose, OR Cough
All-cause LRTI	History of cough OR difficulty in breathing [1] AND SpO_2 < 95% 2, OR RR increase [3]	History of cough OR difficulty in breathing [1] AND SpO_2 < 92% 2, OR RR increase [3]

The site altitude is needed for some study locations to accurately derive the case definitions.

RTI = respiratory tract illness; LRTI = lower respiratory tract illness; RR = respiratory rate; SpO_2 = blood oxygen saturation by pulse oximetry.

[1] Based on history reported by parents/LARs and includes difficulty in breathing (e.g. showing signs of wheezing or stridor, tachypnoea, flaring [of nostrils], chest in-drawing, apnoea).

[2] For blood oxygen saturation (SpO_2), the lowest value monitored will be used.

[3] RR increase defined as:
- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

[4] RSV infection confirmed on nasal swab positive for RSV A or B by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[5] RSV sampling and testing from nasal swabs.

[6] Hospitalization is defined as admission for observation or treatment based on the judgement of a health care provider

Table 5 Alternative LRTI / Severe LRTI Case Definitions

	RSV confirmed	Documented physical examination (PE) findings indicating lower respiratory tract involvement (at least one symptom)	Objective measures of clinical severity (at least one symptom)
LRTI	Confirmed RSV infection	Rhonchi[1] Rales[1] Crackles Wheeze	Increased respiratory rate (bpm) ≥ 60 for < 2 mo ≥ 50 for 2-6 mo
			Hypoxemia: SpO2 <95% at ≤1800 meters SpO2 <92% at > 1800 meters
			New onset apnea
			Nasal flaring
			Retractions[2]
			Grunting
Severe LRTI	Confirmed RSV infection	Rhonchi[1] Rales[1] Crackles Wheeze	Hypoxemia SpO2 <93% at ≤1800 meters SpO2 <90% at > 1800 meters
			Acute hypoxic or ventilatory failure[3]
			Dehydration due to respiratory distress requiring IV hydration[4]
			Failure to respond or unconscious

[1]Term not listed in the eCRF page in this study.

[2] Intercostal recession or chest wall indrawing are considered synonymous with and will be used as alternatives to the term “retractions,” which is not listed in the eCRF page for this study.

[3] Acute hypoxic or ventilatory failure is not listed in the eCRF page for this study. Instead, the presence of either respiratory support excluding mechanical ventilation OR requirement for mechanical ventilation or both will be used.

[4] Dehydration due to respiratory distress requiring IV hydration is not listed in the eCRF page for this study. Skin turgor > 2 seconds or administration of IV fluid therapy will be used instead.

3. OBJECTIVES/ENDPOINTS

3.1. Primary

3.1.1. Primary Objectives

In healthy pregnant women with uncomplicated pregnancies:

- To determine the frequencies of pregnancy outcomes.
- To determine the frequencies of pregnancy related events of interest from enrolment (Visit 1) through 42 days after delivery (Visit 6). (Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study.)

In all neonates live-born to women enrolled in the study:

- To determine the frequencies of neonatal events of interest.

Neonatal ***events*** of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.

3.1.2. Primary Endpoints

3.1.2.1. Pregnancy outcomes

These include:

- Live birth with no congenital anomalies,
- Live birth with congenital anomalies,
- Fetal death/stillbirth (loss at or after 22 weeks of gestation) with no congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Fetal death/still birth (loss at or after 22 weeks of gestation) with congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Elective/therapeutic termination with no congenital anomalies,
- Elective/therapeutic termination with congenital anomalies.

Of note, fetal death/stillbirth has multiple subcategories. For example, fetal death/stillbirth with no congenital anomalies is an outcome with two subcategories that include: 1) antepartum stillbirth; 2) intrapartum stillbirth. For each outcome, the investigator should select the applicable sub-category.

3.1.2.2. Pregnancy related events of interest

Pregnancy related events of interest from Visit 1 through Visit 6. Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study. They are listed below. Of note, some events of interest fall under a single category but have multiple subcategories. For example, hypertensive disorders of pregnancy is an event with three subcategories that includes: 1) gestational hypertension; 2) pre-eclampsia; and 3) pre-eclampsia with severe features (including eclampsia). For each event, the investigator should identify the event and select the applicable sub-category.

- Maternal death
- Hypertensive disorders of pregnancy:
  - Gestational hypertension,
  - Pre-eclampsia,
  - Pre-eclampsia with severe features (including eclampsia)
- Antenatal bleeding:
  - Morbidly adherent placenta
  - Placental abruption
  - Caesarean Scar Pregnancy
  - Uterine rupture
- Postpartum hemorrhage
- Fetal growth restriction
- Dysfunctional labor
  - first stage of labor
  - second stage of labor
- Gestational diabetes mellitus,
- Non- reassuring fetal status
- Pathways to preterm birth:
  - Premature preterm rupture of membranes,
  - Preterm labor,
  - Provider-initiated preterm birth.
- Chorioamnionitis
- Oligohydramnios
- Polyhydramnios

- Gestational Liver Disease:
  - Intrahepatic Cholestasis of Pregnancy (ICP)
  - Acute Fatty Liver of Pregnancy
- Maternal Sepsis
- Any other pregnancy related event considered by the investigator to be of concern

3.1.2.3. Neonatal events of interest

Neonatal events of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study. They include:

- Small for gestational age,
- Low birth weight including very low birth weight,
- Neonatal encephalopathy,
- Congenital microcephaly,
  - Postnatally diagnosed
  - Prenatally diagnosed
- Congenital anomalies,
  - Major external structural defects
  - Internal structural defects
  - Functional defects
- Neonatal death,
  - Neonatal death in a preterm live birth (gestational age>=28 to < 37 weeks)
  - Neonatal death in a term live birth
- Neonatal infections,
  - Blood stream infections
  - Meningitis
  - Respiratory infection
- Respiratory distress in the neonate,
- Preterm birth,
- Failure to thrive,
- Large for gestational age,
- Macrosomia,

Any other neonatal event considered by the investigator to be of concern

3.2. Secondary

3.2.1. Secondary Objectives

In healthy pregnant women with uncomplicated pregnancies at enrolment:

- To determine frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specified) from enrolment (Visit 1) through 42 days after delivery (Visit 6). (Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study.)
- To describe the distribution of RSV-A antibody titers in maternal blood at delivery.

In all neonates live-born to women enrolled in the study:

- To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified). Neonatal ***events*** of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.
- To describe the distribution of RSV-A antibody titers in cord blood at delivery.

In all neonates/infants live-born to women enrolled in the study, from birth through 1 year of age:

- To determine the incidence of all, of severe, and of very severe RSV-lower respiratory tract illnesses (LRTIs).
- To determine the incidence of RSV hospitalization.

3.2.2. Secondary Endpoints

- Pregnancy related events of interest from Visit 1 through Visit 6 (as defined in primary endpoints) for each GAIA level of diagnostic certainty (where applicable and feasible). GAIA levels are explained in detail in Appendix D of the study protocol.
- Neonatal events of interest from birth through 28 days of age (as defined in primary endpoints) for each GAIA level of diagnostic certainty (where applicable and feasible).

Of note, some events of interest fall under a single category but have multiple subcategories. For each event, the investigator should identify the event and select the applicable sub-category and the GAIA level of diagnostic certainty.

- RSV-A neutralizing antibody titres in maternal blood at delivery
- RSV-A neutralizing antibody titres in cord blood at delivery.
- Episode(s) of RSV-LRTI from birth up to 1 year of age.
- Episode(s) of RSV hospitalization from birth up to 1 year of age.

3.3. Tertiary

3.3.1. Tertiary objectives

- To describe co-infections of RSV-LRTI with other respiratory viruses in infants.
- To estimate the association of RSV-LRTI in neonates/infants and the level of RSV neutralizing antibodies in cord blood.
- To determine the incidence of LRTIs/ Severe LRTIs (using alternative case definitions)
- To determine risk factors for pregnancy-related and neonatal events of interest
- If deemed necessary, to further characterize the immune responses to RSV and other infections in maternal subjects and infants (based on maternal serum and cord blood).

3.3.2. Tertiary endpoints

- Co-infections of RSV-LRTI with other respiratory viruses in infants, confirmed by PCR of nasal swabs in infants from birth up to 1 year of age:
  - Influenza A virus (Flu A)
  - Influenza B virus (Flu B)
  - Human Influenza A virus subtype H1 (Flu A-H1)
  - Human Influenza A virus subtype H3 (Flu A-H3)
  - Human Influenza A virus subtype H1pdm09 (Flu A-H1pdm09)
  - Human adenovirus (AdV)
  - Human metapneumovirus (MPV)
  - Human enterovirus (HEV)
  - Human parainfluenza virus 1 (PIV1)
  - Human parainfluenza virus 2 (PIV2)
  - Human parainfluenza virus 3 (PIV3)
  - Human parainfluenza virus 4 (PIV4)
  - Human bocavirus 1/2/3/4 (HBoV)
  - Human rhinovirus A/B/C (HRV)
  - Human coronavirus 229E (229E)
  - Human coronavirus NL63 (NL63)
  - Human coronavirus OC43 (OC43)
- Episodes of LRTIs/Severe LRTIs up to one year of age - using alternative case definitions

- Potential risk factors for pregnancy related and neonatal events of interest.
- Any further exploratory characterization of immune responses to RSV and/or other respiratory tract infections or infections of relevance to pregnant women and their new-borns (based on maternal serum and cord blood). (For example, levels of RSV-B neutralizing antibodies).

4. ANALYSIS SETS

4.1. Definition

4.1.1. Exposed Sets (ES)

4.1.1.1. Pregnant women (mothers)

The ES will include all pregnant women (mothers) who signed a valid informed consent form.

4.1.1.2. Neonates (infants)

Not applicable.

4.1.2. Enrolled Sets

4.1.2.1. Pregnant women (mothers)

The enrolled set will include all pregnant women (mothers) with a valid informed consent who completed Visit 1.

4.1.2.2. Neonates (infants)

The Enrolled Set will include all neonates/infants

- born to pregnant women in the enrolled set, and
- Who have a valid ICF signed by the mother/ parent(s) /LAR(s) (as appropriate per local regulations).

4.1.3. Per Protocol Set

4.1.3.1. Pregnant women (mothers)

The maternal PPS will include all pregnant women (mothers) in the enrolled set who meet 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).

4.1.3.2. Neonates (infants)

The infant PPS will include all neonates / infants

- ***born to pregnant women in the PPS and***
- ***who*** meet 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) who have at least one time point evaluation.

4.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each set.

4.2.1. Elimination from Maternal Exposed Set (ES)

Code 900 (invalid informed consent or fraudulent data) will be used for identifying pregnant women (mothers) eliminated from maternal ES.

4.2.2. Elimination from Infant Exposed Set (ES)

N/A

4.2.3. Elimination from Per-protocol analysis Set (PPS)

4.2.3.1. Maternal excluded subjects

A subject will be excluded from the maternal PPS analysis under the following conditions. (Brackets indicate the code from the eCRF for the indicated question. For questions where the code is the exact same as the question, only the code in brackets is provided).

Code	Condition under which the code is used
900	<u>Invalid informed consent or fraud data.</u> • Individuals who give written or witnessed/thumb printed informed consent after the study has been explained according to local regulatory requirements. – The informed consent given at screening should either include consent for both the mother's participation and participation of the infant after the infant's birth (if consistent with local regulations/guidelines), or consent for the mother's participation and expressed willingness to consider permitting the infant to take part after the infant has been born (if local regulations/guidelines require parent(s) to

	provide an additional informed consent after the infant's birth). – Both mother and father should consent if local regulations/guidelines require it. • Individuals who consent to have cord blood collected at delivery for the purpose of the study.
2010	Protocol violation (inclusion/exclusion criteria). <u>Inclusion criteria for enrolment:</u> • If maternal subject marked as "No" for the following question in the Eligibility Check screen for maternal subjects [frmELIGIBILITYCHECK]: – "Did the subject meet all the entry criteria?" [Eligible]

4.2.3.2. Infant Excluded Subjects

A subject will be excluded from the infant PPS analysis under the following conditions

Code	Condition under which the code is used
900	Infant enrolled in study without signed consent from parents •
2010	Protocol violation (inclusion/exclusion criteria) Inclusion criteria for enrolment: • Infants who were in utero at the time maternal (and paternal, if required) informed consent was given, and who are live-born. If local law requires it: Written or witnessed/thumb printed informed consent for study participation of the infant obtained from parent(s)/LAR(s) within 21 days of birth. • If infant marked as "No" for the following question in the ELIGIBILITY CHECK_NB screen [frmELIGIBILITYCHECK_NB]: – Did the subject meet all the entry criteria? [Eligible]

5. STATISTICAL ANALYSES

Note that data derivation rules and statistical methods are described in sections 9 and 10.1 and will not be repeated below.

The statistical analyses for each objective is divided into two sections. The first section presents the analysis plan exactly as it is written in the protocol. The second section, additional considerations, provides more details of the analysis including any changes that have been made to the analysis plan since the protocol. These changes from the planned analysis in the protocol will also be explained in section 8.

5.1. Demography

5.1.1. Analysis of demographics/baseline characteristics planned in the protocol

For the maternal PPS, analyses of demographic and baseline characteristics at Visit 1 will be described.

For the infant PPS, analyses of demographic characteristics at Visit 1-NB will be described. Analyses of lifestyle characteristics will be described at Visit 2 NB.

For Screening Failures, reasons for non-eligibility will be described.

Continuous variables will be summarized by providing the number of observations, mean, 95% confidence interval (CI), standard deviation (SD), median, and range. Categorical variables will be summarized by tabulating counts (N) and the percentage

(%) of total subjects having the given characteristics, with missing data considered a separate category.

5.1.2. Additional Considerations

For all the descriptive analyses mentioned above, separate tables will be provided for mother and infant cohorts. The results will be summarized overall and by country.

For the maternal exposed set and infant enrolled set, only descriptive statistics for baseline characteristics (ex: age) will be provided because it is a requirement for clinicaltrials.gov. Descriptive statistics or all demographic and baseline characteristics will be provided for the maternal and infant PPS.

In addition to demographic and baseline characteristic mentioned in section 5.1.1, we will also provide descriptive statistics of lifestyle characteristics for the mother PPS. These include:

- Highest education level of the mother
- Household environment
- Household composition
- Mother smoking during current pregnancy
- Mother passive smoking exposure during current pregnancy
- Mother consuming alcohol during current pregnancy
- Method of cooking used during current pregnancy
- Mosquito control during current pregnancy
- Would subject have agreed to take part in a vaccine trial during this pregnancy

5.2. Analysis of primary objectives

The primary objectives analyses will be performed on the PPSs overall and possibly by region or other relevant grouping (the groupings are specific to each analysis). If there is a difference of 5% or more between the number of participants in the enrolled set and the PPS, primary objectives analyses on the enrolled set will also be performed.

5.2.1. To determine the frequency of pregnancy outcomes in healthy pregnant women with uncomplicated pregnancies at time of enrollment.

5.2.1.1. Analysis of pregnancy outcomes planned in the protocol.

The number and percentage (with exact 95% CI) of women presenting the following outcomes: live birth, fetal death/stillbirth (antepartum or intrapartum), and elective/therapeutic termination will be reported for each event by presence or absence of congenital anomalies.

5.2.1.2. Additional considerations:

The following analysis will be done on the maternal PPS. The intervals of interest are Visit 1 to Visit 5 (delivery). Details of exact confidence intervals analysis are described in section 9.5.2. All frequencies will be reported overall, by country, and if applicable, by region. For example, if there are few subjects for the events of interest, tables by region will also be presented. Frequencies of pregnancy related events of interest will also be presented by age strata (18-34, 35-39, ≥40 years).

The number and percentage (with exact 95% CI) of women presenting the following outcomes will be reported:

- Live birth with no congenital anomalies,
- Live birth with congenital anomalies,
- Fetal death/stillbirth (loss at or after 22 weeks of gestation) with no congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Fetal death/still birth (loss at or after 22 weeks of gestation) with congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Elective/therapeutic termination with no congenital anomalies,
- Elective/therapeutic termination with congenital anomalies

5.2.2. To determine the frequencies of pregnancy related events of interest in healthy pregnant women with uncomplicated pregnancies from visit 1 through visit 6.

Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study

5.2.2.1. Analysis of pregnancy related events of interest planned in the protocol

The number and percentage (with exact 95% CI) of pregnant women presenting with the following events of interest will be tabulated for each event within appropriate time windows. Missing data may be considered a separate category.

5.2.2.2. Additional Considerations:

This analysis will be done on the maternal PPS. For events of interest that include subcategories, the frequency of the main event of interest and of each event subcategory will be included in the analysis. The overall frequency (derived programmatically) will include mothers who present with at least one of the subcategory events. The intervals of interest are Visit 1 up to 42 days after Visit 5. All events reported during the entire study period will be included in this analysis.

Details of exact confidence intervals analysis are described in section 9.5.2.

All frequencies will be reported overall, by country, and if applicable, by region. Frequencies of pregnancy related events of interest will also be presented by age strata (18-34, 35-39, ≥40 years). The number and percentage of the following events will be reported:

- Maternal death
- Hypertensive disorders of pregnancy:
  - Gestational hypertension,
  - Pre-eclampsia,
  - Pre-eclampsia with severe features (including eclampsia)
- Antenatal bleeding:
  - Morbidly adherent placenta
  - Placental abruption
  - Cesarean Scar Pregnancy
  - Uterine rupture
- Postpartum hemorrhage
- Fetal growth restriction
- Dysfunctional labor
  - first stage of labor
  - second stage of labor
- Gestational diabetes mellitus,
- Non reassuring fetal status
- Pathways to preterm birth:
  - Premature preterm rupture of membranes,
  - Preterm labor,
  - Provider-initiated preterm birth.
- Chorioamnionitis

- Oligohydramnios
- Polyhydramnios
- Gestational Liver Disease:
  - Intrahepatic Cholestasis of Pregnancy (ICP)
  - Acute Fatty Liver of Pregnancy
- Maternal Sepsis
- Any other pregnancy related event considered by the investigator to be of concern

5.2.3. To determine the frequencies of neonatal events of interest in all neonates live-born to women enrolled in the study.

5.2.3.1. Analysis of neonatal related events of interest planned in the protocol

The number and percentage (with exact 95% CI) of neonates presenting the following events of interest will be tabulated for each event. Missing data may be considered a separate category.

5.2.3.2. Additional Considerations

This analysis will be done on the infant PPS, overall, by country, and if applicable, by region.

For events of interest that include subcategories, the frequency of the main event of interest and of each event subcategory will be included in the analysis. The overall frequency will include infants who present at least one of the subcategory events.

Details of exact confidence intervals analysis are described in section 9.5.2.

The number and percentage of the following events will be reported:

- Small for gestational age,
- Low birth weight including very low birth weight,
- Neonatal encephalopathy,
- Congenital microcephaly,
  - Postnatally diagnosed
  - Prenatally diagnosed
- Congenital anomalies,
  - Major external structural defects
  - Internal structural defects
  - Functional defects
- Neonatal death,

  - Neonatal death in a preterm live birth (gestational age>=28 to < 37 weeks)
  - Neonatal death in a term live birth
- Neonatal infections,
  - Blood stream infections
  - Meningitis
  - Respiratory infection
- Respiratory distress in the neonate,
- Preterm birth,
- Failure to thrive,
- Large for gestational age,
- Macrosomia,
- Any other neonatal event considered by the investigator to be of concern (specify, e.g. neurodevelopment delay)

To support clinical studies, frequencies of major and minor congenital anomalies will be reported as they appear in the study if data is available in the form of MEDRA codes. The specific congenital anomalies are to be determined by the clinical studies and will follow those listed on the CDC website: https://www.cdc.gov/ncbddd/birthdefects/surveillancemanual/chapters/chapter-1/chapter1-4.html . The specific congenital anomalies will be presented according to their MedRA codes provided by data management.

5.3. Analysis of secondary objectives

The secondary objectives analyses will be performed on the PPSs (maternal or infant as applicable) overall and possibly by region or other relevant grouping. If there is difference of 5% or more between the number of participants in the enrolled set and the PPS, tertiary objectives analyses on the enrolled set will also be performed.

5.3.1. To determine frequencies of fetal death/still birth according to GAIA levels of diagnostic certainty.

5.3.1.1. Analysis planned in the protocol

N/A

5.3.1.2. Additional considerations

This objective will not be performed.

5.3.2. To determine frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specified) in healthy pregnant women with uncomplicated pregnancies at time of enrolment.

5.3.2.1. Analysis of pregnancy related events of interest planned in the protocol.

To determine the frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specific) (APPENDIX D of protocol).

Considering all pregnant women followed from enrolment through Visit 6, the number and percentage of subjects with at least one maternal event of interest will be computed by GAIA levels of diagnostic certainty, with exact 95% CIs.

5.3.2.2. Additional considerations

This analysis will be done on the maternal PPS, overall and by country. If the frequencies of categories of events of interest are low, the results will also be presented by region.

Details of exact confidence intervals analysis are described in section 9.5.2.

Among the pregnancy related events of interest from 4.1.2, those with GAIA levels of diagnostic certainty will be presented in this analysis. For each event, the frequency of mothers with a specific diagnostic level of certainty will be reported. For events with subcategories, main events will not be presented since the levels of diagnostic certainty do not apply to the main categories because GAIA levels do not exist for the main categories.

Please note that GAIA levels are explained in detail in the Appendix D of the study protocol.

5.3.3. To describe the distribution of RSV-A antibody titres in maternal blood at delivery in healthy pregnant women with uncomplicated pregnancies at time of enrolment.

5.3.3.1. Analysis of RSV-A antibody titres in maternal blood collected at delivery planned in the protocol

To describe the distribution of RSV-A antibody titres in maternal blood at delivery.

- Geometric mean titres (GMTs) will be tabulated with 95% CI.
- Percentage of subjects above thresholds will be tabulated.

5.3.3.2. Additional considerations

This analysis will be done on the maternal PPS, overall and by country.

RSV-A neutralising antibodies will be obtained from maternal and cord blood collected at delivery. The frequency and percentage of subjects with titres above the threshold will be presented along with 95% CI. GMTs and corresponding 95% CI, minimum and maximum values will be reported.

5.3.4. To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified) in all neonates live-born to women enrolled in the study:

5.3.4.1. Analysis of neonatal events of interest planned in the protocol

To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified) (APPENDIX D of protocol). Neonatal events of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.

Considering all neonates the number and percentage of subjects with at least one neonatal event of interest will be computed by GAIA levels of diagnostic certainty, with exact 95% CI.

5.3.4.2. Additional considerations:

This analysis will be done on the infant PPS overall and by country. Among the neonatal events of interest identified in section 3.1.2.3, those with GAIA levels of diagnostic certainty will be presented in this analysis. For each event, the frequency of infants with a specific diagnostic level of certainty will be reported. For events with subcategories, main events will not be presented because GAIA levels do not exist for the main categories.

Please note that GAIA levels are explained in detail in Appendix D of the study protocol.

5.3.5. To describe the distribution of RSV-A antibody titres in cord blood at delivery in all neonates live-born to women enrolled in the study:

5.3.5.1. Analysis of RSV A antibody titres in cord blood planned in protocol

To describe the distribution of RSV-A antibody titres in cord blood at delivery

- Geometric mean titres (GMTs) will be tabulated with 95% CI.
- Percentage of subjects above thresholds will be tabulated.

5.3.5.2. Additional considerations:

A correction/elaboration from the analysis planned in protocol in section 5.3.5.1 is that we will be reporting on the percentage of subjects with GMTs above thresholds.

This analysis will be done on the Infant PPS, overall and by country.

RSV-A neutralising antibodies will be measured in the cord blood at delivery. The frequency and percentage of subjects with titres above the threshold will be presented along with 95% CI. GMTs and corresponding 95% CI, minimum and maximum values will be reported.

5.3.6. To determine the incidence of all, of severe, and of very severe RSV-lower respiratory tract illnesses (LRTIs) in all neonates/infants live-born to women enrolled in the study:

5.3.6.1. Analysis of RSV LRTI in all neonates/infants planned in protocol

Considering all infants followed from visit 1-NB to visit ***4***-NB, the percentage of subjects with at least one LRTI illness, exact 95% CI will be presented.

Incidence rates of LRTI illnesses will be calculated, with exact 95% CI.

Frequencies of repeat occurrences of LRTI will be tabulated.

5.3.6.2. Additional considerations

A correction from the analysis planned in protocol in section 5.3.6.1 is that we will be reporting on all cause LRTIs and RSV associated LRTIs.

This analysis will be based on the infant PPS and will be done on the first episode of all, of severe, and of very severe RSV LRTIs (Section 2.2).

Incidence analyses will be performed overall, by country and by age strata (0-2 months, 0-5 months, and 0-11 months). If applicable (in the case of low numbers of incidence), incidence analyses will also be presented by region (Section 9.3). Incidence rates will also be calculated in one-month intervals from birth to one year of age, please see the monthly infant age intervals in Section 9.3.

The analysis of RSV disease incidence is summarized in the table below showing endpoints and analytic methods, these include incidence rate, proportion affected, and incidence proportion.

Incidence rate is the number of first events per person time. Incidence proportion is the number of first RSV LRTI over population at risk during the specified time period (0-2 months, 0-5 months and 0-11 months). Proportion affected is the number of subjects with at least one episode of RSV LRTI at age interval over the total number of subjects at the start of the age interval.

For each of the RSV LRTI endpoints listed in Table , the 95% CI will be computed as described in section 9.5.2. Kaplan-Meier curves presenting the cumulative probability of the first episode will be displayed overall. The cumulative probability of presenting the first episode will be given with its 95% CI at the end of the follow-up.

Frequencies of repeat occurrences of RSV LRTI, severe LRTI and very sever LRTI will be tabulated.

Cumulative incidence will be presented from birth to one year of life by monthly intervals (Subgroups in section 9.3).

Table 6 Summary of RSV incidence analyses for RSV LRTI of infants from birth to 1 year (Visit 1-NB to visit 4-NB)

Endpoints	Incidence analysis method	Subgroup
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Incidence rates of first episode Cumulative probability of first episode (Kaplan Meier)	Overall, by country, and by age strata
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Proportion affected by all new episodes	Overall, by country, and by age strata
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Incidence proportion of first episode	Overall and by country within each age strata)

5.3.7. To determine the incidence of RSV hospitalization in all neonates/infants live-born to women enrolled in the study:

5.3.7.1. Analysis of RSV hospitalization planned in protocol

Considering all infants followed from visit 1-NB to visit 4-NB, the percentage of subjects with at least one RSV hospitalization, with exact 95% CI.

Incidence rates of RSV hospitalizations will be calculated, with exact 95% CI.

Frequencies of repeat occurrences of RSV hospitalization will be tabulated.

5.3.7.2. Additional considerations

This analysis will be performed on the infant PPS and the first episode of RSV hospitalization, overall and by country and by age strata. In additional to incidences of RSV hospitalization, we will also consider all cause LRTI hospitalizations.

A change from the planned analysis in section 5.3.7.1 is that only the first event of RSV hospitalization will be included in the analysis.

In addition, cumulative incidence, Kaplan-Meier curves, and proportion affected will be calculated.

Lastly, we will also present the frequencies of symptoms used in case definitions among those infants who are found to be RSV positive from nasal swab results. The symptoms that will be considered are:

- Runny nose
- Blocked nose
- Cough
- Wheezing
- Grunting
- Nasal flaring
- Intercostal recession
- Temperature ≥38 degrees Celsius
- Chest wall indrawing
- SpO_2 cutoff point for hypoxemia

5.4. Analysis of tertiary objectives

The following tertiary objectives analyses will be performed on infants within the PPS overall and possibly by region or other relevant grouping. If there is difference of 5% or more between the number of participants in the enrolled set and the PPS, tertiary objectives analyses on the enrolled set will also be performed.

5.4.1. To describe co-infections of RSV-LRTI with other respiratory viruses in infants.

5.4.1.1. Analysis of respiratory viruses associated with RSV-LRTI as planned in protocol

Considering all infants followed from visit 1-NB to visit 4-NB, the percentage of subjects with the occurrence of RSV-LRTI and having other respiratory viral co- infection with another viral etiology identified by multiplex PCR, with exact 95% CI; classified by respiratory viruses.

5.4.1.2. Additional considerations

This analysis will be performed on the Infant PPS.

The number and percentage of cases for each respiratory virus will be identified for all RSV LRTI, severe RSV LRTI, and very severe RSV LRTI. For each respiratory virus infection, number and percentage will be summarized by single or co-infection status. The groups will include those who are:

*RSV positive (confirmed with nasal swabs)

*RSV positive (confirmed with nasal swabs) & positive with another infection

*RSV negative (confirmed with nasal swabs) & positive with another infection.

For cases of RSV LRTI, severe RSV LRTI and very severe RSV LRTI, the number and percentage of cases associated with at least one other respiratory virus will be described.

This analysis will be performed overall, by country, and by age strata (0-2 months, 0-5 months, and 0-11 months).

Other respiratory viruses causing infection:

- Influenza A virus (Flu A)
- Influenza B virus (Flu B)
- Human Influenza A virus subtype H1 (Flu A-H1)
- Human Influenza A virus subtype H3 (Flu A-H3)
- Human Influenza A virus subtype H1pdm09 (Flu A-H1pdm09)
- Human adenovirus (AdV)
- Human metapneumovirus (MPV)
- Human enterovirus (HEV)
- Human parainfluenza virus 1 (PIV1)
- Human parainfluenza virus 2 (PIV2)
- Human parainfluenza virus 3 (PIV3)
- Human parainfluenza virus 4 (PIV4)
- Human bocavirus 1/2/3/4 (HBoV)
- Human rhinovirus A/B/C (HRV)
- Human coronavirus 229E (229E)
- Human coronavirus NL63 (NL63)
- Human coronavirus OC43 (OC43)

Lastly, an additional analysis will be performed that considers the frequencies of clinical symptoms of two groups of infants: those are have RSV LRTI and those who have LRTI but are RSV negative.

The clinical symptoms that will be considered are:

- Runny nose
- Blocked nose
- Cough

- Wheezing
- Grunting
- Nasal flaring

- Intercostal recession
- Temperature ≥38 degrees Celsius
- Chest wall indrawing
- SpO_2 cutoff point for hypoxemia

5.4.2. To estimate the association of RSV-LRTI in neonates/infants and the level of RSV neutralizing antibodies in cord blood.

5.4.2.1. Analysis of association of RSV-LRTI in neonates and level of RSV neutralizing antibodies in cord blood as described in the protocol.

Correlation analysis will be done comparing the levels of RSV antibodies present in cord blood and the presence or absence of RSV-associated LRTI, RSV-associated severe LRTI, and RSV-associated very severe LRTI in infants.

5.4.2.2. Additional consideration.

When considering RSV antibodies in cord blood, we are only considering RSV-A antibodies at this time (however, in the future we might also consider RSV-B antibodies). When considering RSV positivity status in nasal swabs, we consider positivity for RSV-A or RSV-B.

This analysis will be performed on the Infant PPS and will be done on the first episode (not recurrent events) of RSV LRTIs occurring during the following age intervals: 0-2 months and 0-5 months.

Step 1: Descriptive analysis:

Descriptive analyses (seropositivity rate with 95% CIs, geometric mean, median, min, max) of RSV-A neutralizing antibodies in the baseline cord blood samples will be analysed in only those with RSV positive nasal swab samples.

Next, descriptive analyses (seropositivity rate with 95% CIs, geometric mean, median, min, max) of RSV-A neutralizing antibodies in the baseline cord blood samples will be presented by RSV LRTI, RSV severe LRTI, and RSV very severe LRTI case statuses.

In addition, if further exploration is desired, we may plot levels of RSV A vs RSV-B neutralizing antibodies in cord blood among RSV LRTI positive and negative infants to see if any patterns are observed.

Step 2: Cox models:

The impact of the level of RSV neutralizing antibodies in the baseline cord blood samples on the incidence of first event of RSV-LRTI, RSV-severe LRTI and RSV very severe LRTI separately will be assessed through Cox models.

Cox regression models will be performed for the univariate analyses to obtain unadjusted hazards ratios of the determinants of interest.

Next a multivariable Cox regression model will be performed to estimate the relative contribution of each potential risk factor adjusting for the simultaneous effects of the other covariates. The model will include: time-independent covariates (e.g. gender, etc.) and time-dependent covariates (age). Covariate selection will be done using backward elimination and statistical significance. Potential risk factors will be included in the multivariable models if univariate p-value will be less than 0.1 and the multivariable model will be performed only if the number of events in sufficient (at least 10 events per covariate). In addition, depending on the data, other models could be explored or the model could be simplified.

The following covariates in infants may be considered in the multivariable models:

- Age (continuous)
- Male sex (M/F)
- Small for gestational age (Continuous)
- Congenital anomalies with internal structural defects and/or with functional defects (Y/N)
- Household composition (number of people living in household) (continuous)
- How many children under age of 6 live in household (continuous)
- Cigarette smoking during pregnancy (Y/N)
- Born during RSV transmission season (Y/N)
- Alcohol consumption during this pregnancy (Y/N)
- Highest education level of mother (Y/N)

Step 2-a: RSV neutralizing antibodies classified as a binary variable: Seropositive and seronegative:

In a first step, the RSV-A neutralizing antibodies in the baseline cord blood samples will be introduced in the model as binary variable

Step 2-b: RSV neutralizing antibodies classified as semi-quantitative variable: Quartiles

In a second step, the RSV-A neutralizing antibodies levels in baseline cord blood will be introduced in the model using quartiles. And the Cox models will be performed as described in the previous paragraph (RSV-A neutralizing antibodies as quantitative variable).

Step 2-c: RSV neutralizing antibodies classified as continuous variable:

In a third step, the RSV neutralizing antibodies levels in baseline cord blood might be treated as continuous variable in Cox models to evaluate how each unit of neutralizing antibody level change impacts on the above clinical outcomes. The Cox models will be performed as described in the previous section (with log of RSV neutralizing antibodies as quantitative variable). For the subjects with antibody levels below the LOD, a value of LOD/2 will be imputed.

The model with the best fit using log likelihood criteria, will be selected. This will be determined via backward elimination using statistical significance.

Note that depending on the results, a quantitative association between RSV-associated LRTI, RSV-associated severe LRTI, RSV very severe LRTI, and RSV neutralizing antibodies in the baseline cord blood samples could be further explored and described more in details in the main analysis.

Poisson regression models:

Multivariable Poisson regression of neutralizing RSV A cord blood antibodies estimating RSV LRTI incidence in the first year of life, controlling for covariates will be explored. The same risk factors as above will be test. Modelling will be done in a stepwise manner using backward elimination and statistical significance.

5.4.3. To determine the incidence of LRTIs/Severe LRTIs (using alternative case definitions)

5.4.3.1. N/A

5.4.3.2. Additional considerations

Repeat incidence analyses described in section 5.3.6 with alternative LRTIs/Severe LRTIs case definitions.

5.4.4. To determine risk factors for pregnancy related and neonatal events of interest.

5.4.4.1. Analysis of risk factors for pregnancy related and neonatal events of interest as described in the protocol.

- Both pregnancy related events of interest and neonatal events of interest will be described according to variables collected in the mother's clinical examination and obstetric risk factors. Frequency tables will be generated for categorical variables.
- Furthermore, multiple variable analyses (such as multiple logistic regression for binary outcomes and multiple Poisson/negative binomial and/or other appropriate models for count outcomes) will be conducted to adjust for covariates of interest, provided there are sufficient numbers of events of interest.

5.4.4.2. Additional considerations:

This analysis will be performed on the maternal and infant PPSs depending on whether the risk factors are for pregnancy-related events of interest or for neonatal events of interest.

There are currently 14 potential pregnancy related events of interest and 9 potential risk factors. Likewise, there are 12 potential neonatal events of interest and 13 potential risk factors. (These do not include GAIA subcategories).

Univariate modelling will be done to first determine which risk factors are associated with which event of interest.

For each potential risk factor,

- Frequency tables will be generated as appropriate, for categorical variables.
- Mean, median, standard error, minimum, maximum and number of missing values will be provided as appropriate, for continuous data.

For pairwise distributions

- Cross-tabulations (i.e. two-way frequency tables) will be generated as appropriate, for categorical-categorical pairs;
- Mean, median, standard error, minimum, maximum and number of missing values by category will be provided as appropriate, for continuous-categorical pairs;

Covariates with univariate p-value less than 0.1 will be included in the multivariable model. The number of covariates included in the model will depend on the number of events (at least 10 events per covariates).

Multiple logistic regression, multiple Poisson regression/negative binomial modelling will be used when appropriate only on risk factors and events of interest that were associated in the univariate analysis and provided there are sufficient numbers of events of interest.

5.4.4. If deemed necessary, to further characterize the immune responses to RSV and other infections in maternal subjects and infants (based on maternal serum and cord blood).

5.4.4.1. Analysis of immune responses to disease as planned in the protocol

- ***For example, for levels of neutralizing antibodies to RSV-B:***
  - GMTs/ GMCs may be tabulated with 95% CI.
  - Percentage of subjects above various thresholds, depending on the antibody tested, may be tabulated with exact 95% CI.

5.4.4.2. Additional Considerations:

N/A

5.5. COVID-19 Supplement

5.5.1. Reporting COVID-19 cases—Maternal and Infant Subjects

In addition to satisfying local reporting requirements for COVID-19 cases, maternal and infant COVID-19 cases identified within the existing framework of the study will be captured and reported on the COVID-19 eCRF for the study.

COVID-19 cases should be reported in the eCRF according to the WHO Case Definition [WHO, 2020] using one of the following terms:

- Suspected COVID-19 infection
- Probable COVID-19 infection
- Confirmed COVID-19 infection

5.5.2. Additional Considerations

Cases of COVID-19 reported prior to the implementation of the COVID specific eCRF were will recorded according to suspected, probably, and confirmed.

5.5.2.1. WHO Case Definition (March 20, 2020 Version):

Suspected case

A. A patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath), AND a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset;

OR

B. A patient with any acute respiratory illness AND having been in contact (see definition of "contact" below) with a confirmed or probable COVID-19 case (see definition of contact) in the last 14 days prior to symptom onset;

OR

C. A patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath; AND requiring hospitalization) AND in the absence of an alternative diagnosis that fully explains the clinical presentation.

Probable case

A. A suspect case for whom testing for the COVID-19 virus is inconclusive (Inconclusive being the result of the test reported by the laboratory).

OR

B. A suspect case for whom testing could not be performed for any reason.

Confirmed case

A person with laboratory confirmation of COVID-19 infection, *irrespective of clinical signs and symptoms*.

A contact is a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:

1. Face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 minutes;
2. Direct physical contact with a probable or confirmed case;
3. Direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment; 2OR
4. Other situations as indicated by local risk assessments.

Note: for confirmed asymptomatic cases, *the period of contact* is measured as the 2 days before through the 14 days after the date on which the sample was taken which led to confirmation.

5.5.2.2. Reporting COVID-19 cases—Maternal and Infant Subjects

Frequency of COVID-19 cases (suspected, probable, and confirmed) will be reported for mothers and infants.

Frequency of mothers and infants who had a COVID-19 diagnosis and test performed and number of subjects with positive, negative, and indeterminate results will be reported.

Incidence of COVID-19 confirmed cases will be reported for mothers and babies.

6. ANALYSIS INTERPRETATION

Analyses will be descriptive with the aim to characterize different sub-groups of women. These sub-groups will be defined prior to analyses and will be related to e.g. risk factors for maternal and neonatal events of interest and endemic disease. These descriptive analyses should be interpreted with caution considering that there is no adjustment for multiplicity for these comparisons.

7. CONDUCT OF ANALYSES

All analyses will be done on cleaned data.

Analyses will be performed in a stepwise manner after all subjects in all countries have completed all study visits in Epoch 002 and again after completion of all study visits in Epoch 003.

Analyses of pregnancy outcomes, pregnancy related events of interest, and neonatal events of interest will be performed when all data up to 42 days post-delivery are available (Epoch 002). For these analyses a statistical report but no CSR will be prepared.

Final analyses will be performed when all data up to study end are available (Epoch 002 and Epoch 003). An integrated *CSR* including all available data will be written and made available to the investigator(s).

If the data for tertiary endpoints become available at a later stage, additional analysis/analyses will be performed. These data will be documented in annex(es) to the study report and made available to the investigators.

Additional analyses may be performed if deemed necessary to inform the design or implementation of future clinical trials.

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.

Interim analysis for RSV LRTIs

If deemed necessary, an interim analysis ***may*** be performed on RSV surveillance data as part of the secondary objective of determining incidence of RSV LRTI. This interim analysis ***may*** be performed to obtain preliminary information on the performance of the case definitions used for RSV LRTI, severe LRTI, and very severe LRTI (*Error! Reference source not found.*) and to assess levels of RSV neutralising antibody in maternal and cord blood at delivery. The interim analysis ***may*** occur after the database freeze at the time point when approximately 50% of infants will have completed up to 6 months of follow up during the surveillance period following birth (up to Visit 3-NB). ***If an interim analysis is performed,*** data cleaning plans will be scheduled as needed to supply data for interim analyses in a timely manner. This interim analysis ***may*** be performed on data that is as clean as possible. Preliminary results will be made available in a timely manner for use in the potential adjustment of the RSV LRTI case definitions to be used in pivotal clinical trials scheduled to begin shortly thereafter. The results

pertaining to this analysis ***(if performed)*** will be purely descriptive, with no adjustment of type I error, and will be reported in an interim statistical report. No CSR will be prepared.

7.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)
Month 12	E1_01	CTRS Study report	Yes	Yes
Month 6 interim analysis-***may occur***	E1_03	Internal	Yes	No
Analysis of epoch 2	E1_02	Internal	Yes	No

Analysis ID will depend on whether interim and epoch 2 analysis will be run using the same DBF depending on availability of lab results.

8. CHANGES FROM PLANNED ANALYSES

Only the first episode within a given age interval is considered for the computation of incidence. The incidence of event is computed using three different calculation methods: incidence rate, proportion affected, and incidence proportion. See section 5.3.6 for details of calculation.

In analysing incidence of RSV LRTI and RSV Hospitalization, only the first episode of each will be considered.

A correction from the analysis planned in protocol in section 5.3.5.1 is that we will be reporting on all cause LRTIs and RSV associated LRTIs.

Analyses will be stratified by country and by age strata (mother or infant depending on the analysis).

In instances where there are low frequencies (either in events of interest or case definitions) results may be presented by region in addition to by country (see Section 9.3 for subgroups).

Continuous variables will not report 95% confidence intervals.

The cohorts have been adjusted in Amendment 4 of the protocol. See section 4 in the SAP for details. Analyses will be conducted on PPS cohorts. If there is a difference of 5% or more between the number of participants in the enrolled set and the PPS, analyses will also be performed on enrolled sets.

India has been removed from the study and Bangladesh and Panama have been added.

Alternative LRTI/Severe LRTI case definitions have been added to the study and subsequent analyses.

The interim analyses is no longer planned and may only occurred if deemed necessary.

9. NON-STANDARD DATA DERIVATION RULES AND STATISTICAL METHODS

9.1. Data Derivation

9.1.1. Maternal blood and cord blood

Antibody titres will be obtained from maternal blood and cord blood at delivery. Geometric means and seropositivity status for RSV neutralising antibodies in maternal or cord blood samples will be derived according to the following table:

Table 7 Derivation rule for GMT calculation and seropositivity status with respect to RSV A neutralizing antibody titers in maternal blood at delivery and cord blood

Assay	Raw result	Derivation for GMT calculation	Derivation of positivity status
RSV-A neutralizing antibody	<LOD	LOD/2	Negative
	≥LOD	Exact value	Positive

LOD=18 ED_{60}

9.1.2. Nasal swabs from infants during RSV LRTI episodes

Table 8 RSV positivity status in infants will be determined from viral loads from nasal swabs taken during infant visits. The infant will be considered RSV positive according to the following thresholds of copies/mL of RSV antibodies:

Component	Method	Unit	LOD	Derivation for positivity status	Derivation for GM calculation
RSV A	RT-qPCR	Copies of RSV A RNA per mL	To be determined	Negative	LOD/2
	RT-qPCR	Copies of RSV A RNA per mL	To be determined	Positive	Exact value
RSV B	RT-qPCR	Copies of RSV B RNA per mL	To be determined	Negative	LOD/2
	RT-qPCR	Copies of RSV B RNA per mL	To be determined	Positive	Exact value

9.1.3. Risk factors for pregnancy-related and neonatal events of interest

Table 9 Risk factors for pregnancy-related events of interest

Risk Factor	Categories
Prenatal smoking exposure during this pregnancy	Yes/No
Alcohol consumption during this pregnancy	Yes/No
BMI pre-pregnancy (kg/cm^2)	Continuous
Age of mother at delivery in current pregnancy(years)	18-34 35-39 ≥40
Subject currently lives in a country or region with Zika transmission	Yes/No
Subject has travelled to a country or region with Zika transmission since the beginning of their pregnancy	Yes/No
Subject travelled to country or region with Zika virus transmission	Yes/No
Predominant geographic ancestry	African Heritage/African American American Indian or Alaskan Native Asian-Central/South Asian Heritage Asian-East Asian Heritage Asian-Japanese Heritage Asian-South East Asian Heritage Native Hawaiian or Other Pacific Islander White Middle-eastern /North African Heritage White-Caucasian/ European Heritage Latino/Mestizo Other
Caesarean section in previous pregnancy	Yes/No
Highest education level of mother	High education Yes: Bachelor's degree or higher No: Less than bachelor's degree

Table 10 Risk factors for neonatal events of interest

Risk Factor	Categories
Prenatal smoking exposure during this pregnancy	Yes/No
Age of mother at delivery in current pregnancy(years)	18-34 35-39 ≥40
Alcohol consumption during this pregnancy	Yes/No
BMI pre-pregnancy (kg/cm^2)	Continuous
Gestational diabetes mellitus in current pregnancy	Yes/No
Gestational hypertension in current pregnancy	Yes/No
Fetal growth restriction	Y/N
Antenatal bleeding	Y/N
Dysfunctional labor	Y/N
Gender of new-born	Male/Female
Subject lives in or travelled to country or region with Zika virus infection during pregnancy	Yes/No
Predominant geographic ancestry	African Heritage/African American American Indian or Alaskan Native Asian-Central/South Asian Heritage Asian-East Asian Heritage Asian-Japanese Heritage Asian-South East Asian Heritage Native Hawaiian or Other Pacific Islander White Middle-eastern /North African Heritage White-Caucasian/ European Heritage Latino/Mestizo Other
Apgar at 5 minutes of age	0-3 inclusive 4-6 inclusive 7 or greater

9.1.4. Risk factors for RSV LRTI

- Age (continuous)
- Male sex (M/F)
- Small for gestational age (Continuous)
- Congenital anomalies with internal structural defects and/or with functional defects (Y/N)
- Household composition (number of people living in household) (continuous)
- How many children under age of 6 live in household (continuous)
- Cigarette smoking during pregnancy (Y/N)
- Born during RSV transmission season (Y/N)
- Alcohol consumption during this pregnancy (Y/N)
- Highest education level of mother (Y/N)

9.1.5. RSV Seasonality

Table 11 RSV Transmission seasons by country/region and centre

Country/region	Seasonality	Centre #
Thailand		PPD
Bangkok	June-October	
Chiang Mai	July-November	
Malaysia		
Selangor	Year round	
Kota Kinabalu	Year round	
Alor Setar	Year round	
Kuching	Year round	
Philippines		
Manila	Year round	
Cebu	Year round	
South Africa		
Parow, Western Cape	April-August	
Pretoria, Gauteng	February-June	
	February-August	
Panama		
David	May-January	
Panamá	May-January	
Chorrera	May-January	
Bangladesh		
Matlab	July-December	
Kamlapur	July-February	
Brazil		
Ribeirao Preto/SP	March-July	
Belo Horizonte/MG	March-July	
Nata/RN	March-July	
Argentina		
Buenos Ares	May-July	
Cordoba	May-July	
Mendoza	May-July	
Colombia		
Medellin	Mar-Jun & Sep-Nov	
Medellin	Year round	
Bogota	Year round	
Bogota	Mar-May	
Cali	April-Jun	
Villavicencio	Mar-Apr/May & Nov-Dec	
Mexico		
Monterrey	September-April	
Oaxaca	September-April	

Durango	September-April	PPD

9.2. Data presentation

The following decimal description will be used for the analyses.

Table 12 Decimal points in analyses

Display Table	Parameters	Number of decimal digits
All summaries	% of frequency, including LL & UL of CI	1
All summaries	% frequency, including LL & UL of CI	1
All summaries	Mean, median, minimum, maximum	1
All summaries	SD	2
All summaries	*P*-value	3

9.3. Subgroup definitions

The following sub-group names will be used for statistical analyses:

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
Country		
1	TH	Subjects from Thailand
2	MY	Subjects from Malaysia
3	PH	Subjects from Philippines
4	SA	Subjects from South Africa
5	BD	Subjects from Bangladesh
6	PA	Subjects from Panama
7	BR	Subjects from Brazil
8	AR	Subjects from Argentina
9	CO	Subjects from Colombia
10	MX	Subjects from Mexico
Region		
Latin America	LatAM	Subjects from Brazil, Argentina, Colombia. Mexico, Panama
Asia Pacific	AsiaPac	Subjects from Thailand, Malaysia, Philippines, Bangladesh
South Africa	SA	Subjects from South Africa
Age category maternal subject at enrolment		
1	18-34	18-34 years of age
2	35-39	35-39 years of age
	>=40	40 years of age and older
Age category infant (3 categories)		
1	0-2	Birth to one day before the 3rd month of life (up to 3 months0-9)
2	0-5	Birth to one day before the 6th month of life (up to 6 months)
3	0-11	Birth one day before the 12th month of life (up to 12 months)
Age category infant (One-month intervals)		
1	0	Birth to one day before the 1st month of life
2	1	First day of 1 month to one day before the 2nd month of life
3	2	First day of 2 months to one day before the 3rd month of life

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
4	3	First day of 3 months to one day before the 4th month of life
5	4	First day of 4 months to one day before the 5th month of life
6	5	First day of 5 months to one day before the 6th month of life
7	6	First day of 6 months to one day before the 7th month of life
8	7	First day of 7 months to one day before the 8th month of life
9	8	First day of 8 months to one day before the 9th month of life
10	9	First day of 9 months to one day before the 10th month of life
11	10	First day of 10 months to one day before the 11th month of life
12	11	First day of 11 months to one day before the 12th month of life

9.4. Case definitions

Please refer to section 2.1 for an explanation of how data is collected for surveillance in this study since that is the data that will be used for the derivation of case definitions.

Note that if a worsening visit(s) takes place for the same episode, then all symptoms collected from the initial visit and the worsening visit(s) are combined and counted under one episode, with the most severe level of the symptoms being used in the case definition derivation. For example, if SpO_2 is collected in a case assessment and a worsening visit for the same episode, then the lowest SpO_2 from any visit will be used for the case definition derivation. This is done because the most severe level of a symptom will not necessarily be in the worsening form, so multiple scenarios of visits and data collection can be accounted for. The earliest date of a reported symptom will be used as the start date of the episode. The latest date of a reported symptom will be used as the end date of episode.

If an infant comes in for a visit with new bouts of cough or blocked nose after 7 days of his/her latest end date of an RTI symptom, then a new case assessment form is opened and this is potentially the start of a new episode.

Variables from a Case Assessment Visit will be pulled from [frmASSESSMENT_INFO], [frmSYMPTOMS_RTI], and [frmVITALSIGNS_RTI].

Variables from a Worsening Visit will be pulled from [frmWORSENING_INFO] and [frmVITALSIGNS_WOR].

9.4.1. RSV RTI

The child will be reported positive for RSV RTI when he/she presents with at least one of the following symptoms: (data pulled from the Case Assessment Visit)

- Runny nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= ‘YES’) and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= ‘Yes’) and [frmSYMPTOMS_RTI] (fields[ASSESS_VIS]=’Yes’ and [sctSYMPTOMS_RTI]=[RUNNY]

- Blocked nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes') and [frmSYMPTOMS_RTI] (fields[ASSESS_VIS]='Yes' and [sctSYMPTOMS_RTI]=[BLOCKED]Cough reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')

AND confirmed RSV infection

9.4.2. RSV LRTI

The child will be reported positive for RSV LRTI when he/she presents with at least one of the following symptoms: (The data can be pulled from the Case Assessment Visit)

- Cough reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')
- Difficulty breathing reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING')

AND at least one of following symptoms: **(The data can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)**

- SpO_2(<95% if altitude ≤2500m and <92% if altitude >2500m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

OR

- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

AND

Confirmed RSV infection from a nasal swab test.

9.4.2.1. Start date of RSV-LRTI

Start dates of RSV LRTI symptoms will be pulled from the Case Assessment Visit for at least one of the following: parental report of history of cough or difficult breathing. The start date of RSV LRTI is the earliest of the start dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'SRDAT')

Or

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'SRDAT')

9.4.2.2. End date of RSV-LRTI

End dates of RSV LRTI symptoms will be pulled from the Case Assessment Visit and will be defined as the latest date of the end dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'ENDAT')

Or

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'ENDAT')

9.4.3. RSV severe LRTI

RSV severe LRTI cases will be reported if the person meets the case definition of RSV LRTI

AND

(The data for below can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<93% if altitude ≤2500m and <90% if altitude >2500m) (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '93.0% or <90%')

  OR

- Lower chest wall indrawing reported during the physical examination. (field [sctCHEST_INDRAWING_RTI]=[INDRAWING]= 'YES') OR (field [sctCHEST_INDRAWING_WOR]=[INDRAWING]= 'YES')

9.4.3.1. RSV Severe LRTI start date

Start dates will be defined as the earliest date of cough or difficulty breathing as described in section 9.4.2.1.

9.4.3.2. RSV Severe LRTI end date

End dates will be defined as the latest date of cough or difficulty breathing as described in section 9.4.2.2.

9.4.4. RSV very severe LRTI

RSV very severe LRTI is defined as meeting the case definition of RSV LRTI

AND

(The data for below for SpO_2 can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<90% if altitude ≤2500m and <87% if altitude >2500m) (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '90.0% or <87%')

  OR

- Inability to feed reported in signs and symptoms. [sctSEVERITY_SIGNS_RTI]=[FEEDING]= 'Unable to feed')

  OR

- Failure to respond/unconscious reported in signs and symptoms. [sctSEVERITY_SIGNS_RTI]=[CONSCIOUS]= 'The child is unresponsive to all stimuli')

9.4.4.1. RSV very severe LRTI start date

Start dates will be defined as the earliest start date of cough or difficulty breathing as described in section 9.4.2.1.

9.4.4.2. RSV very severe LRTI end date

End dates will be defined as the latest end date of cough or difficulty breathing as described in section 9.4.2.2.

9.4.5. RSV Hospitalization

RSV hospitalization is determined if both the following criteria are met:

- Confirmed RSV infection with nasal swab test from central GSK lab.

AND

- Hospitalization for acute medical condition determined in Inpatient and worsening flag form. Variables will be pulled from [frmINPATIENT_WORSE_FLG] (field [HOSPI_YN]= 'Yes').

9.4.5.1. RSV Hospitalization start dates

Hospitalization start date will be determined from the Inpatient Care form. Variables will be pulled from [frmINPATIENTCARE] (field [HOSP_SRDAT]=)

9.4.5.2. RSV Hospitalization end dates

Hospitalization end date will be determined from the Inpatient Care form. Variables will be pulled from [frmINPATIENTCARE] (field [HOSP_ENDAT]=)

9.4.6. All cause RTI

All cause RTI is reported if the child has one or more of the following symptoms:

- Runny nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'RUNNY')
- Blocked nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BLOCKED')
- COUGH reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')

9.4.6.1. All cause RTI start date

- Start dates of all cause RTI cases will be the earliest dates of cough, runny nose, or blocked nose. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and
- [sctSYMPTOMS_RTI]= 'Cough' and [cmpCOUGH_DT]= 'SRDAT'

  OR
- [sctSYMPTOMS_RTI]= 'Blocked' and [cmpBLOCKED_DT]= 'SRDAT'

  OR
- [sctSYMPTOMS_RTI]= 'RUNNY' and [cmpRUNNY_DT]= 'SRDAT')

9.4.6.2. All cause RTI end date

End dates of all cause RTI cases will be that lastest dates of Cough. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'Cough' and [cmpCOUGH_DT]= 'ENDAT')

9.4.7. All cause LRTI

All cause LRTI is reported if the child has one or more of the following:

- COUGH reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')
- Difficulty breathing reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING')

AND

(The data for below can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<95% if altitude ≤2500m and <92% if altitude >2500m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

OR

- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

9.4.7.1. All cause LRTI start dates

The start date of all cause LRTI with the earliest of the start dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'SRDAT')

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'SRDAT')

9.4.7.2. All cause LRTI end dates

The end date of all cause LRTI is the latest of the end dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'ENDAT')

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'ENDAT')

9.4.8. Alternative LRTI

The child will be reported positive for Alternative LRTI when he/she presents with at least one of the following symptoms: (The data can be pulled from the Case Assessment Visit under signs and symptoms).

- Crackles reported during the case assessment visit. Variables will be pulled from [frmSIGNSANDSYMPTOMS] [sctAUSCULTATION_RTI] [CRACKLES_AUSC]= "Y")
- OR
- wheeze reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'wheezing')

AND at least one of following symptoms: **(The data can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)**

- SpO_2(<95% if altitude ≤1800m and <92% if altitude >1800m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

OR

- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to 6 months of age)

OR

New onset apnea Variable pulled from

[frmSIGNSANDSYMPTOMS] [sctSeverity_SIGNS_RTI]

OR

Nasal flaring

[frmSIGNSANDSYMPTOMS_WOR] [sctRESPIRATORY_SIGNS_RTI]

OR Intercostal recession (in place of ratractions)

[frmSIGNSANDSYMPTOMS_WOR] [sctRESPIRATORY_SIGNS_RTI]

OR Grunting

[frmSIGNSANDSYMPTOMS_WOR] [sctRESPIRATORY_SIGNS_RTI]

AND

Confirmed RSV infection from a nasal swab test.

9.4.9. Severe LRTI

Alternative LRTI is defined as meeting the case definition for Alternative LRTI and

Respiratory support excluding mechanical ventilation or requirement for mechanical ventilation or both. Variables pulled from [frmINPATIENTCARE] [sctINPATIENTCARE] [RESP_SUPPORT] or [VENTILATION]

OR

Skin turgor. Variable pulled from [frmSIGNSANDSYMPTOMS_WOR] [sctSEVERITY_SIGNS_RTI] [TURGOR]

Figure 2 **Derivation of RSV-LRTI events date and duration according to the the corresponding LRTI event**

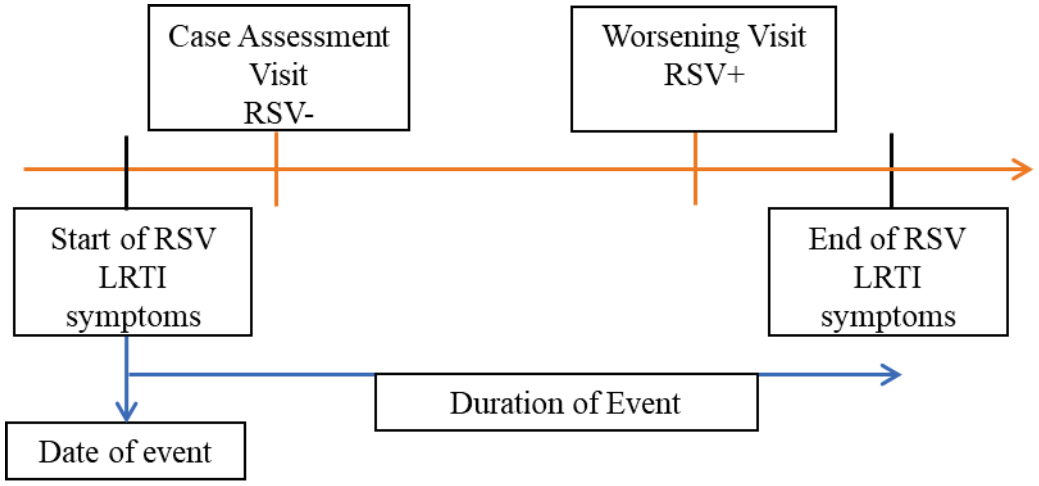


Figure 3 **Derivation of RSV-Hospitalization events date and duration**

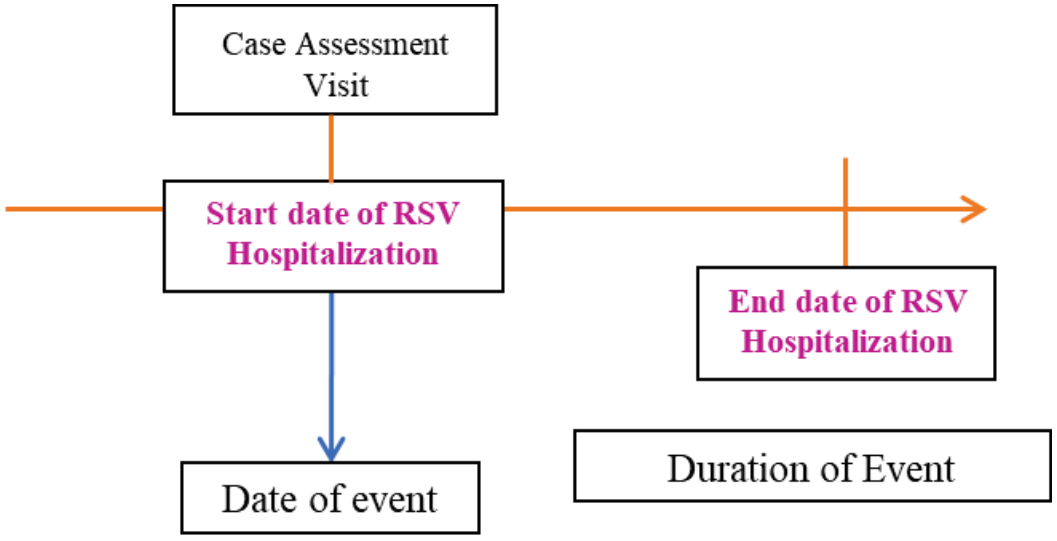
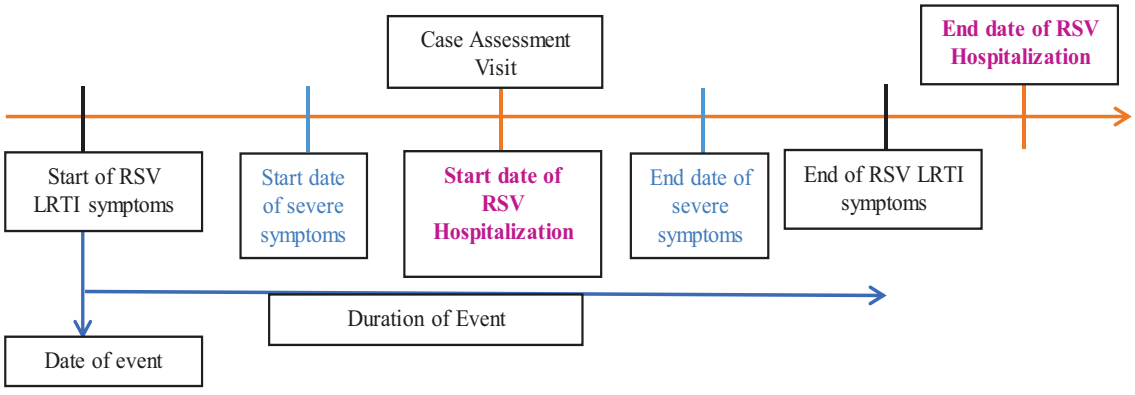


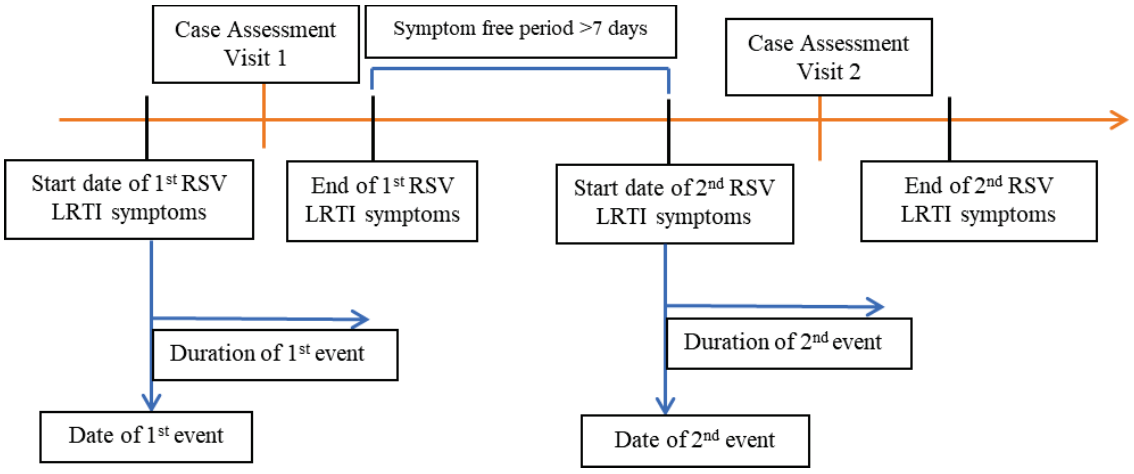
Figure 4 **Derivation of RSV-LRTI hospitalization events date and duration according to the corresponding LRTI event**



9.4.10. New RSV LRTI episode rule

A new episode of an event is a single case of RSV RTI, RSV LRTI, severe RSV LRTI, or hospitalization meeting the respective case definitions and severity scale with an interval of at least 7 symptom free days since the last episode that was diagnosed. (Note: persistent runny nose is an exception to the 7 symptom free days rule. A child may have persistent runny nose but still present with a new episode of RSV LRTI). If symptoms worsen within 7 days, the subject comes for a worsening visit.

Figure 5 Derivation of 2 RSV LRTI events date and duration with a symptom free period of at least 7 days



9.4.11. Symptoms

9.4.11.1. Increased respiratory rate

The subject will be considered to have increased respiratory rate if one of the following statements is reached:

- Respiratory rate (field [sctRESPIRATORYRATE_RTI])
- > 60/minute for subjects <2 months of age (fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])
- > 50/minute for subjects 2-11 months of age(fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])
- > 40/minute for subjects 12-24 months of age(fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])

Please note that respiratory rate is measured during the general and obstetric physical exam.

9.5. Statistical Methods

9.5.1. Computation of incidence rates

For each endpoint, the incidence rate (IR, number of episodes/endpoints per 100 person-years) will be calculated by dividing the number of subjects reporting the first episode over the follow-up period by the total person-year. A 95% CI will be computed using an exact method for a Poisson variable as described below.

The person-time at risk for an event of interest (RSV-LRTI or RSV hospitalization) will be calculated as the time between the date of birth and the end of the at-risk period or the earliest of the followings:

- Date of first diagnosis of event of interest (e.g. first episode of RSV-LRTI);
- Date when child reaches 1 year (or 6 months if analysis is done during the interim analysis);
- Date of death;
- Date of last follow-up in study.

Please note that in a case where a subject comes to an assessment visits, has LRTI symptoms, is not confirmed for RSV infection via a nasal swab (because either no nasal swab was taken or the lab yielded invalid results), then we will consider the subject as not have a laboratory-confirmed event. The Subject will therefore be censored at the event (n=0, T=from start of the follow-up period at risk up to event).

9.5.2. Exact confidence intervals (CIs)

The exact confidence interval within a group for an incidence rate (per 100 person-years):

To estimate the confidence limit of the incidence rate, the exact Poisson confidence limit will be used [Clopper, 1934]:

If *n* is the number of subjects presenting a given characteristic among these *Ny* subjects per year, the true incidence rate can be estimated by *(n/Ny)*100*. Its exact *(1-α)%* confidence interval is obtained from:

*CINV(α/2,2*n)/2/Ny*100* as the lower boundary

and

CINV((1-α/2),2(n+1))/2/Ny*100* as the upper boundary.

where *CINV(probability, degrees of freedom)* returns the inverse of the chi-squared probability distribution and α is the type I error rate.

9.5.3. Computation of proportion affected

Proportion affected will be computed as the number of subject who had at least one episode in the age interval divided by the total number of subjects at start of considered age strata. A 95% CI will be computed using an exact method for a Poisson variable.

9.5.4. Computation of incidence proportion

Incidence proportion will be computed as the number of subjects who had at least one episode in the age interval (monthly) divided by the number of subject at risk of event at the beginning of the age interval. A subject that has an event will no longer consider of at risk, i.e. subjects with event in 0 – 1 month are excluded from denominator for future monthly incidence calculation. A 95% CI will be computed using an exact method.

9.5.5. Cox models

All multivariable modelling will be done in a stepwise manner using backward elimination and statistical significance.

9.5.5.1. Univariate Models

Please see section 5.4.4.2. Univariate modelling will be done to first determine which risk factors are associated with which event of interest.

For each potential risk factor:

- Frequency tables will be generated as appropriate, for categorical variables.
- Mean, median, standard error, minimum, maximum and number of missing values will be provided as appropriate, for continuous data.

For pairwise distributions:

- Cross-tabulations (i.e. two-way frequency tables) will be generated as appropriate, for categorical-categorical pairs;
- Mean, median, standard error, minimum, maximum and number of missing values by category will be provided as appropriate, for continuous-categorical pairs;

9.5.5.2. Multivariable Models

Covariate selection will be done using statistical significance: the final multivariable regression models will include all potential risk factors with a simple regression model with p-value < 0.10. The number of covariates included in the models will depend on the number of events (at least 10 events per covariate). The models will be performed only if the number of events is sufficient (at least 10 events per covariate). In addition, depending on the data and the validity of the model assumptions, other models could be explored or the models could be simplified.

The results from the multivariable regression models will include hazard ratios (Cox regression models) and odds ratios (logistic and longitudinal logistic regression models) and their 95% CI.

9.5.5.3. Multiple Logistic Regression models

Multiple logistic regression models will be used to test the association between events of interest and risk factors. PROC LOGISTIC or PROC GENMOD can be used. What is tested will depend on how many people are observed with these outcome and risk factors and if they are associated in the univariate analysis.

9.5.6. Multiple Poisson/negative binomial models

PROC GENMOD can be used for multiple Poisson/negative binomial modelling.

10. ANNEXES

10.1. Business rules for standard data derivations and statistical methods

This section contains GSK Vaccines' standard rules for data display and derivation for clinical and epidemiological studies. These rules will be applied along with those detailed in section 9 (additional study-specific rules).

10.1.1. Age

Age of infant will be expressed in months and will be computed as the number of complete calendar months between the date of birth (DOB) and the date of event. For example:

DOB = 10JUN2017, Date of event = 09JUL2018 -> Age = 12 months

DOB = 10JUN2017, Date of event = 10JUL2018 -> Age = 13 months

Age of the mother at the time of childbirth will be expressed in years and will be computed as the number of complete calendar years between the birth date of the infant and the birth date of the mother. For example:

DOB = 10SEP1983, Date of V1 = 09SEP2018 -> Age = 34 years

DOB = 10SEP1983, Date of V1 = 10SEP2018 -> Age = 35 years

10.1.2. Handling of missing data

10.1.2.1. Dates

When partially completed dates (i.e. with missing day or month) are used in calculations, the following standard rules will be applied:

- A missing day will be replaced by 15
- A missing day and month will be replaced by June 30th.

10.1.2.2. Laboratory data

Missing laboratory results (including immunological data) will not be replaced.

10.1.2.2.1. Studies with paper diaries

For studies using paper diaries which have questions in the CRF indicating the presence or absence of solicited symptoms, the following rules are applicable.

Denominators for the summary of local (or general) solicited symptoms will be calculated using the number of subjects who respond "Yes" or "No" to the question concerning the occurrence of local (or general) symptoms.

When a specific symptom is marked as having not occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=N for the specified post-vaccination period for the symptom in question), all daily measurements will be imputed as Grade 0.

When a specific symptom is marked as having occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=Y for the specified post-vaccination period for the symptom in question), any missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

When the occurrence of a specific symptom is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-vaccination period for the symptom in question) but the group of symptoms (local or general) is marked as having occurred (i.e. SDTM CE.CEOCCUR=Y), all missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

The following table shows how subjects contribute to each category for a specific solicited symptom over the Day X to Day Y post-vaccination period:

Solicited symptom category	Subjects included in the calculation of the numerator
Any	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y or with the symptom marked as present and at least one missing daily recording between Day X and Day Y
At least grade 1	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y
At least grade 2	All subjects with at least one occurrence of the symptom at grade 2 or grade 3 between Day X and Day Y
At least grade 3	All subjects with at least one occurrence of the symptom at grade 3 between Day X and Day Y

10.1.3. Data derivation

10.1.3.1. Weight

Weight will be presented in kilograms. Weights reported in pounds will be converted as follows:

Weight in kilograms = Weight in pounds / 2.2

10.1.3.2. Height

Height will be presented in centimeters. Heights reported in feet and inches will be converted as follows:

Height in centimeters = Height in inches x 2.54

10.1.3.3. Body mass index (BMI)

BMI will be calculated as follows:

BMI = (Weight in kilograms) / (Height in meters)^2

10.1.3.4. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

Temperature (Celsius) = ((Temperature (Fahrenheit) - 32) x 5)/9

10.1.3.5. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
“NEG”, “-“, or “(-)”	cut-off/2
“POS”, “+”, or “(+)”	cut-off
“< value” and value is <= assay cut-off	cut-off/2
“< value” and value is > assay cut-off	value
“> value” and value is < assay cut-off	cut-off/2
“> value” and value is >= assay cut-off	value
“value” and value is < cut-off	cut-off/2
“value” and value is >= cut-off	value
All other cases	missing

10.1.3.6. Geometric mean titres (GMTs) and concentrations (GMCs)

Geometric Mean Titre (GMT) or Concentration (GMC) calculations are performed by taking the inverse logarithm of the mean of the log titre or concentration transformations. Antibody titres or concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMT/GMC calculation. The cut-off value is defined by the laboratory before the analysis and is described in the protocol.

10.1.3.7. Onset day

The onset day for an event (e.g. new RSV LRTI case) is the earliest date for the reported symptom.

10.1.3.8. Duration of events

The duration of an event with a start and end date will be the number of days between the start and end dates plus one day, i.e. an event that starts on 03MAR2018 and ends on 12MAR2018 has a duration of 10 days.

10.1.4. Display of decimals

10.1.4.1. Percentages

Percentages and their corresponding confidence limits will be displayed with:

- no decimals when there are fewer than 50 subjects in each tabulated group
- one decimal when there are at least 50 subjects in at least one tabulated group
  - Exceptions will be made for percentages that are not 0% or 100% but appear as 0% or 100% due to rounding. For these specific cases the number of decimals will be increased until the displayed value is no longer 0% or 100%. Examples are given in the following table.

n/N	Displayed percentage
10/45	22%
1/45	2%
10/55	18.2%
1/55	1.8%
1/300	0.3%
1/3000	0.03%
1/30000	0.003%
299/300	99.7%
2999/3000	99.97%
29999/30000	99.997%

- The display of additional decimals for values close to 0% or 100% will be applied only to point estimates and not confidence limits, which can be rounded and displayed as 0% or 100%.
- Values of exactly 0% or 100% will be presented with no decimals regardless of the number of subjects per tabulated group.

10.1.4.2. Differences in percentages

Differences in percentages and their corresponding confidence limits will be displayed with one more decimal than the maximum number used to display the individual percentages, for example the difference between two percentages displayed with one decimal will be displayed with two decimals.

10.1.4.3. Demographic/baseline characteristics statistics

The mean, median, and standard deviation for continuous baseline characteristics (height, weight, body mass index (BMI)will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maxima and minima of transformed height variables will be displayed with no decimals.

The maxima and minima of transformed weight variables will be displayed with no decimals with the exception of values are below 10kg where one decimal will be displayed.

The maximum and minima of transformed body temperatures will be displayed with one decimal.

10.1.4.4. Serological summary statistics

The number of decimals used when displaying geometric mean titers (GMT) or concentrations (GMC) and their confidence limits is shown in the following table:

GMT or GMC value	Number of decimals to display
<0.1	3
>=0.1 and <10	2
>=10 and <1000	1
>=1000	0

When multiple categories of GMT or GMC values are present in the same table, the number of decimals displayed should match that of the smallest category (i.e. the one with the higher number of decimals). For example, if GMT or GMC values of <0.1 appear in the same table as values of >=0.1 and <10, 3 decimals should be displayed for both.

GMT or GMC ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

10.1.5. Statistical methodology

10.1.5.1. Exact confidence intervals around proportions

The exact confidence intervals around within-group proportions are derived using the method of Clopper and Pearson [Clopper, 1934].

10.1.5.2. Standardized asymptotic confidence intervals around differences in proportions

The standardized asymptotic confidence intervals around differences in proportions are derived using the method of Mietinnen and Nurminen [Miettinen, 1985].

10.1.5.3. Adjusted GMT or GMC ratios

When between-group GMT or GMC ratios are computed and adjusted for two-level categorical co-variables, these co-variables should be included as dummy continuous variables in the SAS procedure.

10.2. TFL and/or TFL ToC

The TFL and the TFL ToC will be found in eTMF folder section:
PPD

11. REFERENCES

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934;26:404-13.

Miettinen, O. S. and Nurminen, M. Comparative analysis of two rates. Statistics in Medicine, 1985;4,213-226.

GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A prospective epidemiological study of women between 24-27 weeks of pregnancy to describe pregnancy outcomes and events of interest in the mother and neonate, as well as determine incidence in infants of RSV LRTI and RSV hospitalization.
eTrack study number and Abbreviated Title	207636 (EPI-RSV-008 BOD)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Final: 08-May-2019
Co-ordinating author:	PPD (Expert Epidemiology Biostatistician)
Reviewed by:	PPD (Clinical and Epidemiology Project Lead) PPD (Lead Epidemiologist) PPD (Lead Epidemiology Statistician) PPD (Lead Epidemiology Statistician) PPD (Lead Statistical Analyst) PPD (Lead Scientific Writer) PPD (Senior Scientific Writer) PPD (Stat Peer Reviewer) PPD (Regulatory Affairs) PPD (SERM Physician) PPD (Public disclosure representative)
Approved by:	PPD (Clinical and Epidemiology Project Lead) PPD (Lead Epidemiology Statistician) PPD (Expert Epidemiology Biostatistician) PPD (Lead Scientific Writer) PPD (Lead Statistical Analyst)

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

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LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Events of Special Interest
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CLS	Clinical Laboratory Sciences
CSR	Clinical Study Report
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
ELISA	Enzyme-linked immunosorbent assay
EoS	End of Study
ES	Exposed Set
FAS	Full Analysis Set
FU	Follow-Up
GAIA	Global Alignment of Immunization Safety Assessment in pregnancy
GCP	Good Clinical Practice
GMT	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IU/ml	International units per milliliter
LAR	Legally Acceptable Representative
LL	Lower Limit of the confidence interval
LMP	Last Menstrual Period
LOD	Limit of Detection
LRTI	Lower Respiratory Tract Illness
LSLV	Last Subject Last Visit

MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NB	Newborn
PCD	Primary Completion Date
PCR	Polymerase Chain Reaction
PD	Protocol Deviation
PPS	Per Protocol Set
RBC	Red Blood Cell
RR	Respiratory Rate
RSV	Respiratory Syncytial Virus
RTI	Respiratory Tract Illness
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SPM	Study Procedures Manual
SpO_2	Blood oxygen saturation as measured by pulse oximetry
SR	Study Report
T Domains	Trial Domains
TFL	Tables Figures and Listings
TOC	Table of Contents
UL	Upper Limit of the confidence interval

1. DOCUMENT HISTORY

Date	Description	Protocol Version
08-MAY-2019	first version	Amendment 3 – 18-FEB-2019

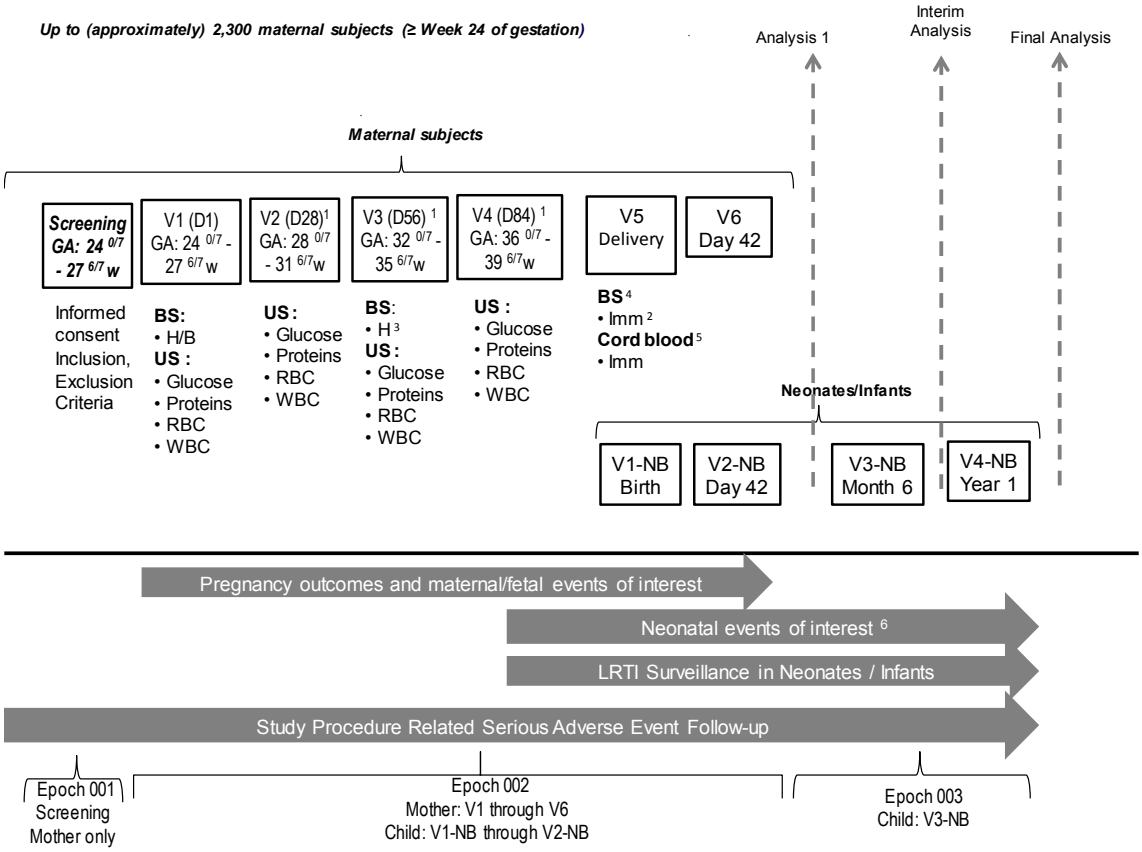
2. STUDY DESIGN

2.1. Summary

This is a prospective multi-country cohort study that aims to provide information for subsequent clinical trials for maternal immunization for respiratory syncytial virus (RSV). The study will provide background rates of maternal and neonatal events of interest, incidence rates of lower respiratory tract illnesses associated with RSV (RSV LRTI), and will determine the capacity of future sites for clinical trials. Approximately 2300 pregnant women from nine different countries will be enrolled during their third trimester of pregnancy and followed until 42 days after delivery. Their children will be followed from birth until one year of age. Epoch 1 of the study consists of the screening of maternal subjects. Epoch 2 includes visits 1-6 of the mother and visits 1-2 of the new born; during which time data on pregnancy outcomes and maternal and neonatal events of interest will be collected and RSV LRTI surveillance will begin (from visit 1 of the new born). Neonatal events of interest will only include events that occurred during the first 28 days of life, but data on these events can be collected up to one year of age. Maternal and cord blood samples will also be collected from the mother at delivery. Lastly, Epoch 3 will include RSV LRTI surveillance as well as continued recording of neonatal events of interest. During surveillance in Epoch 3, symptoms relevant to RSV illness will be recorded in diary cards, and RSV positivity will be confirmed via laboratory tests performed on nasal swabs collected during site visits.

Please note that when referring to epochs in the SAP, we are following the convention outlined in the protocol. Epochs in the CDISC T domains are named differently and are as follows: For maternal subjects: screening epoch and study procedures epoch. For infants: screening epoch and surveillance epoch (infants do not actually have a screening visit in this study but this is the naming standard used for CDISC T domains).

Figure 1 Study design



D = day; M = month; V = visit; W = week; Y = year. NB = new born; GA = Gestational age; BS = blood sample; US = urine sample for dipstick testing; IMM = immune response; HB = haematology/biochemistry; RBC = red blood cell(s); WBC = white blood cell(s); RSV = respiratory syncytial virus; RTI = respiratory tract illness; LRTI = lower respiratory tract illness;

1 If delivery occurs prematurely, skip to Visit 5 ("at delivery").
2 At Delivery, RSV-A antibody titres for all women
3 At V3, only haemoglobin testing.
4 Allowed intervals for blood sample collection begins with start of labor (e.g. when the mother arrives at the hospital to deliver and an intravenous line is inserted) and ends 72 hours after delivery.
5 RSV-A antibodies in cord blood
6 Neonatal events of interest occur (by definition) between 0 and 28 days after birth. They will be reported once site staff become aware of them (whether this occurs during the first 28 days after birth, or at a later time).

Type of design: Prospective, epidemiological, interventional (without administration of medicinal products as described in a research protocol), multi-country, cohort study.

Study population: The study will be conducted in multiple countries, in pregnant women and their infants.

Type of study: self-contained.

Data collection: Electronic Case Report Form (eCRF).

Table 1 Sampling schedule for maternal subjects

		Visit				
Sample	**Parameter(s) evaluated**	**1**	**2**	**3**	**4**	**Delivery**
Maternal Blood	Hematology/ biochemistry[1, 4]	x		x[3]		
	Antibody titre (RSV-A)					≤ 72 hours after delivery[5]
Cord Blood	Antibody titre (RSV-A)					x
Urine	Protein, glucose, RBC, WBC [2, 4]	x	x	x	x	

[1] To be performed preferentially by local healthcare providers, as per local practice. To be performed by the investigator/study staff ONLY if not done by the local healthcare provider within 2 weeks before the study visit.
[2] The investigator/site staff will perform a urine dipstick test using supplies provided by GSK.
[3] Hemoglobin only
[4] If results are abnormal, subjects will be referred per local standard of care.
[5] This sample may be collected from start of labor (e.g. when the mother arrives at the hospital to deliver and an intravenous line is inserted) through 72 hours after delivery.

- Sampling schedule for neonates/infants: Surveillance for RSV LRTI will be conducted in infants through the 12-month study period. During this 1-year post-birth surveillance period, for each RTI with history of cough, suspicion of difficulty in breathing, or wheezing, or with parental concern, a nasal swab will be collected at case assessment visit to assess potential RSV associated RTIs/LRTIs.
- Primary Completion Date (PCD): 42 days post-delivery/birth (i.e. Visit 6 for maternal subjects and Visit 2-NB for infant subjects) or last visit of Epoch 002.
- End of Study (EoS): Last testing results released of samples collected up to Visit 4-NB (assays related to primary and secondary endpoints).
- Duration of the study: Approximately 4.5 to 6 months for maternal subjects; approximately 1 year for infant subjects.
  - Epoch 001: Screening
  - Epoch 002: Primary starting at Visit 1 and ending 42 days post-delivery/birth (Visit 6 for maternal subjects and Visit 2-NB for infant subjects).*
  - Epoch 003: Follow-up of infants starting 43 days post-delivery/birth and ending at Visit 4-NB (1-year post-birth).

*Any safety and disease surveillance data collected after Visit 2-NB will be collected in Epoch 003.

Table 2 Study groups and epochs foreseen in the study

Study Groups	**Number of subjects enrolled**	**Age (Min/Max)**	**Epochs**		
			Epoch 001	**Epoch 002**	**Epoch 003**
Maternal subjects	Up to ~ 2300	18 years - 45 years	x	x	
Infants	Up to ~2300	NA		x	x

Approximately 200 to 300 per country. To achieve the enrolment targets noted above, the number of pregnant women SCREENED in each country may exceed the country-specific enrolment goal.

- Surveillance for pregnancy outcomes and pregnancy-related events of interest that occur from Visit 1 up to 42 days after delivery (Visit 6).
- Surveillance for neonatal events of interest that occur from birth up to 28 days of age.
- Surveillance for RSV LRTI and RSV hospitalization in infants, from birth up to 1 year of age. Nasal swabs will be collected during surveillance visits to confirm RSV positivity.
- This study includes active and passive surveillance of RSV LRTI. In an active surveillance, site personnel will contact the subject's parent(s) / LAR(s) or their designate(s). Active contacts will occur at regular intervals (depending on RSV seasonality at the site) and will be scripted. In a Passive contact, the subject's parent(s) / LAR(s) or their designate(s) contact site personnel if their infant has any of the RTI symptoms (cough, runny nose, or blocked nose). Site personnel will use a script to guide data collection once a passive contact has been made. In each case, the site will use the decision tree (Figure 2 of protocol) to determine if a case assessment visit is needed (Occurrence of Surveillance contact flag and Surveillance Contact Report form in the eCRF). If the patient reported wheezing or difficulty breathing, the nurse will set up an appointment for the mother to bring her infant for follow up at the clinic within three days of the beginning of symptoms. Once the child has completed an assessment visit, site personnel will continue surveillance for the case episode. If the child's symptoms have deteriorated and the child is admitted to the hospital or needs oxygen therapy (Inpatient and Worsening flag in the eCRF), the physician will report worsening symptoms (Worsening form in the eCRF). Start dates will be recorded for cough, runny nose, wheezing, and difficulty breathing, but only end dates will be recorded for cough and difficulty breathing. If the infant experiences new bouts of cough or blocked nose after 7 days of her/his recovery from the last RTI symptoms, then a new case assessment form will be opened. Given that infants frequently experience runny nose, a case can be concluded even if the runny nose has not resolved.

2.2. Case Definitions

The case definitions are summarized in the following table: (see section 9.4 for detailed derivation of case definition).

Table 3 RTI/LRTI case definitions

RSV-RTI	(Runny nose, OR Blocked nose, OR Cough) AND Confirmed RSV infection [4]
RSV-LRTI	(History of cough OR difficulty in breathing [1]) AND (SpO_2 < 95% [2], OR RR increase [3]) AND Confirmed RSV infection [4]
RSV-severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 93%, OR lower chest wall in-drawing
RSV-very severe LRTI	Meeting the case definition of RSV-LRTI AND (SpO_2 < 90%, OR inability to feed OR failure to respond / unconscious)
RSV hospitalization	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]
All-cause RTI	Runny nose, OR Blocked nose, OR Cough
All-cause LRTI	(History of cough OR difficulty in breathing [1]) AND (SpO_2 < 95% 2, OR RR increase [3])

RTI = respiratory tract illness; LRTI = lower respiratory tract illness; RR = respiratory rate; SpO_2 = blood oxygen saturation by pulse oximetry.

[1] Based on history reported by parents/LARs and includes difficulty in breathing (e.g. showing signs of wheezing or stridor, tachypnoea, flaring [of nostrils], chest in-drawing, apnoea).

[2] For blood oxygen saturation (SpO_2), the lowest value monitored will be used. In high altitudes (>2500m), SpO_2 <92% for LRTI, <90% for severe LRTI, <87% for very severe LRTI.

[3] RR increase defined as:
> 60/minute (< 2 months of age)
> 50/minute (2 to < 12 months of age)
> 40/minute (12 to 24 months of age)

[4] RSV infection confirmed on nasal swab positive for RSV A or B by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[5] RSV sampling and testing from nasal swabs.

[6] Hospitalization is defined as admission for observation or treatment based on the judgement of a health care provider

Table 4 RTI/LRTI case definitions including site altitude

	Altitude	
	≤2500m	>2500m
RSV-RTI	Runny nose, OR Blocked nose, OR Cough AND Confirmed RSV infection [4]	Runny nose, OR Blocked nose, OR Cough AND Confirmed RSV infection [4]
RSV-LRTI	History of cough OR difficulty in breathing [1] AND SpO_2 < 95% [2], OR RR increase [3] AND Confirmed RSV infection [4]	History of cough OR difficulty in breathing [1] AND SpO_2 < 92% [2], OR RR increase [3] AND Confirmed RSV infection [4]
RSV-severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 93%, OR lower chest wall in-drawing	Meeting the case definition of RSV-LRTI AND SpO_2 < 90%, OR lower chest wall in-drawing
RSV-very severe LRTI	Meeting the case definition of RSV-LRTI AND SpO_2 < 90%, OR inability to feed OR failure to respond / unconscious	Meeting the case definition of RSV-LRTI AND SpO_2 < 87%, OR inability to feed OR failure to respond / unconscious
RSV hospitalization	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]	Confirmed RSV infection [5] AND Hospitalized for acute medical condition [6]
All-cause RTI	Runny nose, OR Blocked nose, OR Cough	Runny nose, OR Blocked nose, OR Cough
All-cause LRTI	History of cough OR difficulty in breathing [1] AND SpO_2 < 95% 2, OR RR increase [3]	History of cough OR difficulty in breathing [1] AND SpO_2 < 92% 2, OR RR increase [3]

The site altitude is needed for some study locations to accurately derive the case definitions.

RTI = respiratory tract illness; LRTI = lower respiratory tract illness; RR = respiratory rate; SpO_2 = blood oxygen saturation by pulse oximetry.

[1] Based on history reported by parents/LARs and includes difficulty in breathing (e.g. showing signs of wheezing or stridor, tachypnoea, flaring [of nostrils], chest in-drawing, apnoea).

[2] For blood oxygen saturation (SpO_2), the lowest value monitored will be used.

[3] RR increase defined as:
- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

[4] RSV infection confirmed on nasal swab positive for RSV A or B by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[5] RSV sampling and testing from nasal swabs.

[6] Hospitalization is defined as admission for observation or treatment based on the judgement of a health care provider

3. OBJECTIVES/ENDPOINTS

3.1. Primary

3.1.1. Primary Objectives

In healthy pregnant women with uncomplicated pregnancies:

- To determine the frequencies of pregnancy outcomes.
- To determine the frequencies of pregnancy related events of interest from enrolment (Visit 1) through 42 days after delivery (Visit 6). (Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study.)

In all neonates live-born to women enrolled in the study:

- To determine the frequencies of neonatal events of interest.

Neonatal ***events*** of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.

3.1.2. Primary Endpoints

3.1.2.1. Pregnancy outcomes

These include:

- Live birth with no congenital anomalies,
- Live birth with congenital anomalies,
- Fetal death/stillbirth (loss at or after 22 weeks of gestation) with no congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Fetal death/still birth (loss at or after 22 weeks of gestation) with congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Elective/therapeutic termination with no congenital anomalies,
- Elective/therapeutic termination with congenital anomalies.

Of note, fetal death/stillbirth has multiple subcategories. For example, fetal death/stillbirth with no congenital anomalies is an outcome with two subcategories that include: 1) antepartum stillbirth; 2) intrapartum stillbirth. For each outcome, the investigator should select the applicable sub-category.

3.1.2.2. Pregnancy related events of interest

Pregnancy related events of interest from Visit 1 through Visit 6. Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study. They are listed below. Of note, some events of interest fall under a single category but have multiple subcategories. For example, hypertensive disorders of pregnancy is an event with three subcategories that includes: 1) gestational hypertension; 2) pre-eclampsia; and 3) pre-eclampsia with severe features (including eclampsia). For each event, the investigator should identify the event and select the applicable sub-category.

- Maternal death
- Hypertensive disorders of pregnancy:
  - Gestational hypertension,
  - Pre-eclampsia,
  - Pre-eclampsia with severe features (including eclampsia)
- Antenatal bleeding:
  - Morbidly adherent placenta
  - Placental abruption
  - Caesarean Scar Pregnancy
  - Uterine rupture
- Postpartum hemorrhage
- Fetal growth restriction
- Dysfunctional labor
  - first stage of labor
  - second stage of labor
- Gestational diabetes mellitus,
- Non- reassuring fetal status
- Pathways to preterm birth:
  - Premature preterm rupture of membranes,
  - Preterm labor,
  - Provider-initiated preterm birth.
- Chorioamnionitis
- Oligohydramnios
- Polyhydramnios

- Gestational Liver Disease:
  - Intrahepatic Cholestasis of Pregnancy (ICP)
  - Acute Fatty Liver of Pregnancy
- Maternal Sepsis
- Any other pregnancy related event considered by the investigator to be of concern

3.1.2.3. Neonatal events of interest

Neonatal events of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study. They include:

- Small for gestational age,
- Low birth weight including very low birth weight,
- Neonatal encephalopathy,
- Congenital microcephaly,
  - Postnatally diagnosed
  - Prenatally diagnosed
- Congenital anomalies,
  - Major external structural defects
  - Internal structural defects
  - Functional defects
- Neonatal death,
  - Neonatal death in a preterm live birth (gestational age>=28 to < 37 weeks)
  - Neonatal death in a term live birth
- Neonatal infections,
  - Blood stream infections
  - Meningitis
  - Respiratory infection
- Respiratory distress in the neonate,
- Preterm birth,
- Failure to thrive,
- Large for gestational age,
- Macrosomia,

Any other neonatal event considered by the investigator to be of concern

3.2. Secondary

3.2.1. Secondary Objectives

In healthy pregnant women with uncomplicated pregnancies at enrolment:

- To determine frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specified) from enrolment (Visit 1) through 42 days after delivery (Visit 6). (Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study.)
- To describe the distribution of RSV-A antibody titers in maternal blood at delivery.

In all neonates live-born to women enrolled in the study:

- To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified). Neonatal ***events*** of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.
- To describe the distribution of RSV-A antibody titers in cord blood at delivery.

In all neonates/infants live-born to women enrolled in the study, from birth through 1 year of age:

- To determine the incidence of all, of severe, and of very severe RSV-lower respiratory tract illnesses (LRTIs).
- To determine the incidence of RSV hospitalization.

3.2.2. Secondary Endpoints

- Pregnancy related events of interest from Visit 1 through Visit 6 (as defined in primary endpoints) for each GAIA level of diagnostic certainty (where applicable and feasible). GAIA levels are explained in detail in Appendix D of the study protocol.
- Neonatal events of interest from birth through 28 days of age (as defined in primary endpoints) for each GAIA level of diagnostic certainty (where applicable and feasible).

Of note, some events of interest fall under a single category but have multiple subcategories. For each event, the investigator should identify the event and select the applicable sub-category and the GAIA level of diagnostic certainty.

- RSV-A neutralizing antibody titres in maternal blood at delivery
- RSV-A neutralizing antibody titres in cord blood at delivery.
- Episode(s) of RSV-LRTI from birth up to 1 year of age.
- Episode(s) of RSV hospitalization from birth up to 1 year of age.

3.3. Tertiary

3.3.1. Tertiary objectives

- To describe co-infections of RSV-LRTI with other respiratory viruses in infants.
- To estimate the association of RSV-LRTI in neonates/infants and the level of RSV neutralizing antibodies in cord blood.
- To determine risk factors for pregnancy-related and neonatal events of interest.
- If deemed necessary, to further characterize the immune responses to RSV and other infections in maternal subjects and infants (based on maternal serum and cord blood).

3.3.2. Tertiary endpoints

- Co-infections of RSV-LRTI with other respiratory viruses in infants, confirmed by PCR of nasal swabs in infants from birth up to 1 year of age:
  - Influenza A virus (Flu A)
  - Influenza B virus (Flu B)
  - Human Influenza A virus subtype H1 (Flu A-H1)
  - Human Influenza A virus subtype H3 (Flu A-H3)
  - Human Influenza A virus subtype H1pdm09 (Flu A-H1pdm09)
  - Human adenovirus (AdV)
  - Human metapneumovirus (MPV)
  - Human enterovirus (HEV)
  - Human parainfluenza virus 1 (PIV1)
  - Human parainfluenza virus 2 (PIV2)
  - Human parainfluenza virus 3 (PIV3)
  - Human parainfluenza virus 4 (PIV4)
  - Human bocavirus 1/2/3/4 (HBoV)
  - Human rhinovirus A/B/C (HRV)
  - Human coronavirus 229E (229E)
  - Human coronavirus NL63 (NL63)
  - Human coronavirus OC43 (OC43)
- Potential risk factors for pregnancy related and neonatal events of interest.

Any further exploratory characterization of immune responses to RSV and/or other respiratory tract infections or infections of relevance to pregnant women and their new-borns (based on maternal serum and cord blood).

4. ANALYSIS SETS

4.1. Definition

4.1.1. Exposed Sets (ES)

4.1.1.1. Maternal Exposed Set (Maternal ES)

The ES will include all pregnancy women (mothers) with a valid informed consent.

4.1.1.2. Infant Exposed Set (Infant ES)

The ES will include all study eligible neonates/infants with a valid ICF signed by the mother/ parent(s) /LAR(s) (as appropriate per local regulations).

4.1.2. Per Protocol Sets (PPS)

4.1.2.1. Maternal Subjects (mothers) PPS

The maternal subjects PPS will include all pregnant women (mothers) 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).

4.1.2.2. Neonates/Infants PPS

The infant PPS will include all enrolled neonates / infants 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) who have at least one time point evaluation.

4.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each set.

4.2.1. Elimination from Maternal Exposed Set (ES)

Code 900 (invalid informed consent or fraudulent data) will be used for identifying pregnant women (mothers) eliminated from maternal ES.

4.2.2. Elimination from Infant Exposed Set (ES)

Code 900 (invalid informed consent or fraudulent data) will be used for identifying neonates/infants (from mothers that have consented) eliminated from infant ES. Infants must already have mothers with valid ICF from epoch 001.

4.2.3. Elimination from Per-protocol analysis Set (PPS)

4.2.3.1. Maternal excluded subjects

A subject will be excluded from the maternal PPS analysis under the following conditions. (Brackets indicate the code from the eCRF for the indicated question. For questions where the code is the exact same as the question, only the code in brackets is provided).

Code	Condition under which the code is used
900	Invalid informed consent or fraud data. • Individuals who give written or witnessed/thumb printed informed consent after the study has been explained according to local regulatory requirements. – The informed consent given at screening should either include consent for both the mother's participation and participation of the infant after the infant's birth (if consistent with local regulations/guidelines), or consent for the mother's participation and expressed willingness to consider permitting the infant to take part after the infant has been born (if local regulations/guidelines require parent(s) to provide an additional informed consent after the infant's birth). – Both mother and father should consent if local regulations/guidelines require it. • Individuals who consent to have cord blood collected at delivery for the purpose of the study.
2010	Protocol violation (inclusion/exclusion criteria). Inclusion criteria for enrolment: • If maternal subject marked as "No" for the following question in the Eligibility Check screen for maternal subjects [frmELIGIBILITYCHECK]: – "Did the subject meet all the entry criteria?" [Eligible]

4.2.3.2. Infant Excluded Subjects

A subject will be excluded from the infant PPS analysis under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data. • Infants who were in utero at the time maternal (and paternal, if required) informed consent was given, and who are live-born. • If local law requires it: Written or witnessed/thumb printed informed consent for study participation of the infant obtained from parent(s)/LAR(s) within 10 days of birth.
2010	Protocol violation (inclusion/exclusion criteria) Inclusion criteria for enrolment: • If infant marked as "No" for the following question in the ELIGIBILITY CHECK_NB screen [frmELIGIBILITYCHECK_NB]: – Did the subject meet all the entry criteria? [Eligible]

5. STATISTICAL ANALYSES

Note that data derivation rules and statistical methods are described in sections 9 and 10.1 and will not be repeated below.

The statistical analyses for each objective is divided into two sections. The first section presents the analysis plan exactly as it is written in the protocol. The second section, additional considerations, provides more details of the analysis including any changes that have been made to the analysis plan since the protocol. These changes from the planned analysis in the protocol will also be explained in section 8.

5.1. Demography

5.1.1. Analysis of demographics/baseline characteristics planned in the protocol

For the maternal PPS, analyses of demographic and baseline characteristics at Visit 1 will be described.

For the infant PPS, analyses of demographic characteristics at Visit 1-NB will be described. Analyses of lifestyle characteristics will be described at Visit 2 NB.

For Screening Failures, reasons for non-eligibility will be described.

Continuous variables will be summarized by providing the number of observations, mean, 95% confidence interval (CI), standard deviation (SD), median, and range. Categorical variables will be summarized by tabulating counts (N) and the percentage (%) of total subjects having the given characteristics, with missing data considered a separate category.

5.1.2. Additional Considerations

For all the descriptive analyses mentioned above, separate tables will be provided for mother and infant cohorts. The results will be summarized overall and by country.

For the maternal and infants exposed sets, only descriptive statistics for baseline characteristics (ex: age) will be provided because it is a requirement for clinicaltrials.gov. Descriptive statistics or all demographic and baseline characteristics will be provided for the maternal and infant PPS.

In addition to demographic and baseline characteristic mentioned in section 5.1.1, we will also provide descriptive statistics of lifestyle characteristics for the mother PPS. These include:

- Highest education level of the mother
- Household environment
- Household composition
- Mother smoking during current pregnancy
- Mother passive smoking exposure during current pregnancy
- Mother consuming alcohol during current pregnancy
- Method of cooking used during current pregnancy
- Mosquito control during current pregnancy
- Would subject have agreed to take part in a vaccine trial during this pregnancy

5.2. Analysis of primary objectives

The primary objectives analyses will be performed on the PPSs overall and possibly by region or other relevant grouping (the groupings are specific to each analysis).

5.2.1. To determine the frequency of pregnancy outcomes in healthy pregnant women with uncomplicated pregnancies at time of enrollment.

5.2.1.1. Analysis of pregnancy outcomes planned in the protocol.

The number and percentage (with exact 95% CI) of women presenting the following outcomes: live birth, fetal death/stillbirth (antepartum or intrapartum), and elective/therapeutic termination will be reported for each event by presence or absence of congenital anomalies.

5.2.1.2. Additional considerations:

The following analysis will be done on the maternal PPS. The intervals of interest are Visit 1 to Visit 5 (delivery). Details of exact confidence intervals analysis are described in section 9.5.2. All frequencies will be reported overall, by country, and if applicable, by region. For example, if there are few subjects for the events of interest, tables by region will also be presented. Frequencies of pregnancy related events of interest will also be presented by age strata (18-<35 and >=35 years).

The number and percentage (with exact 95% CI) of women presenting the following outcomes will be reported:

- Live birth with no congenital anomalies,
- Live birth with congenital anomalies,
- Fetal death/stillbirth (loss at or after 22 weeks of gestation) with no congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Fetal death/still birth (loss at or after 22 weeks of gestation) with congenital anomalies,
  - Antepartum stillbirth
  - Intrapartum stillbirth
- Elective/therapeutic termination with no congenital anomalies,
- Elective/therapeutic termination with congenital anomalies

5.2.2. To determine the frequencies of pregnancy related events of interest in healthy pregnant women with uncomplicated pregnancies from visit 1 through visit 6.

Although pregnancy related events of interest occur within the first 42 days after delivery they may only be detected later, and are to be reported throughout the study

5.2.2.1. Analysis of pregnancy related events of interest planned in the protocol

The number and percentage (with exact 95% CI) of pregnant women presenting with the following events of interest will be tabulated for each event within appropriate time windows. Missing data may be considered a separate category.

5.2.2.2. Additional Considerations:

This analysis will be done on the maternal PPS.

For events of interest that include subcategories, the frequency of the main event of interest and of each event subcategory will be included in the analysis. The overall frequency (derived programmatically) will include mothers who present with at least one of the subcategory events. The intervals of interest are Visit 1 up to 42 days after Visit 5. All events reported during the entire study period will be included in this analysis.

Details of exact confidence intervals analysis are described in section 9.5.2.

All frequencies will be reported overall, by country, and if applicable, by region. Frequencies of pregnancy related events of interest will also be presented by age strata (18-<35 and >=35 years).

The number and percentage of the following events will be reported:

- Maternal death
- Hypertensive disorders of pregnancy:
  - Gestational hypertension,
  - Pre-eclampsia,
  - Pre-eclampsia with severe features (including eclampsia)
- Antenatal bleeding:
  - Morbidly adherent placenta
  - Placental abruption
  - Cesarean Scar Pregnancy
  - Uterine rupture
- Postpartum hemorrhage
- Fetal growth restriction
- Dysfunctional labor
  - first stage of labor
  - second stage of labor
- Gestational diabetes mellitus,
- Non reassuring fetal status
- Pathways to preterm birth:
  - Premature preterm rupture of membranes,
  - Preterm labor,
  - Provider-initiated preterm birth.

- Chorioamnionitis
- Oligohydramnios
- Polyhydramnios
- Gestational Liver Disease:
  - Intrahepatic Cholestasis of Pregnancy (ICP)
  - Acute Fatty Liver of Pregnancy
- Maternal Sepsis
- Any other pregnancy related event considered by the investigator to be of concern

5.2.3. To determine the frequencies of neonatal events of interest in all neonates live-born to women enrolled in the study.

5.2.3.1. Analysis of neonatal related events of interest planned in the protocol

The number and percentage (with exact 95% CI) of neonates presenting the following events of interest will be tabulated for each event. Missing data may be considered a separate category.

5.2.3.2. Additional Considerations

This analysis will be done on the infant PPS, overall, by country, and if applicable, by region.

For events of interest that include subcategories, the frequency of the main event of interest and of each event subcategory will be included in the analysis. The overall frequency will include infants who present at least one of the subcategory events.

Details of exact confidence intervals analysis are described in section 9.5.2.

The number and percentage of the following events will be reported:

- Small for gestational age,
- Low birth weight including very low birth weight,
- Neonatal encephalopathy,
- Congenital microcephaly,
  - Postnatally diagnosed
  - Prenatally diagnosed
- Congenital anomalies,
  - Major external structural defects
  - Internal structural defects
  - Functional defects

- Neonatal death,
  - Neonatal death in a preterm live birth (gestational age>=28 to < 37 weeks)
  - Neonatal death in a term live birth
- Neonatal infections,
  - Blood stream infections
  - Meningitis
  - Respiratory infection
- Respiratory distress in the neonate,
- Preterm birth,
- Failure to thrive,
- Large for gestational age,
- Macrosomia,
- Any other neonatal event considered by the investigator to be of concern (specify, e.g. neurodevelopment delay)

5.3. Analysis of secondary objectives

The secondary objectives analyses will be performed on the PPSs (maternal or infant as applicable) overall and possibly by region or other relevant grouping.

5.3.1. To determine frequencies of fetal death/still birth according to GAIA levels of diagnostic certainty.

5.3.1.1. Analysis planned in the protocol

N/A

5.3.1.2. Additional considerations

This objective will not be performed.

5.3.2. To determine frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specified) in healthy pregnant women with uncomplicated pregnancies at time of enrolment.

5.3.2.1. Analysis of pregnancy related events of interest planned in the protocol.

To determine the frequencies of pregnancy related events of interest according to GAIA levels of diagnostic certainty (where these are specific) (APPENDIX D of protocol).

Considering all pregnant women followed from enrolment through Visit 6, the number and percentage of subjects with at least one maternal event of interest will be computed by GAIA levels of diagnostic certainty, with exact 95% CIs.

5.3.2.2. Additional considerations

This analysis will be done on the maternal PPS, overall and by country. If the frequencies of categories of events of interest are low, the results will also be presented by region.

Details of exact confidence intervals analysis are described in section 9.5.2.

Among the pregnancy related events of interest from 4.1.2, those with GAIA levels of diagnostic certainty will be presented in this analysis. For each event, the frequency of mothers with a specific diagnostic level of certainty will be reported. For events with subcategories, main events will not be presented since the levels of diagnostic certainty do not apply to the main categories because GAIA levels do not exist for the main categories.

Please note that GAIA levels are explained in detail in the Appendix D of the study protocol.

5.3.3. To describe the distribution of RSV-A antibody titres in maternal blood at delivery in healthy pregnant women with uncomplicated pregnancies at time of enrolment.

5.3.3.1. Analysis of RSV-A antibody titres in maternal blood collected at delivery planned in the protocol

To describe the distribution of RSV-A antibody titres in maternal blood at delivery.

- Geometric mean titres (GMTs) will be tabulated with 95% CI.
- Percentage of subjects above thresholds will be tabulated.

5.3.3.2. Additional considerations

This analysis will be done on the maternal PPS, overall and by country.

RSV-A neutralising antibodies will be obtained from maternal and cord blood collected at delivery. The frequency and percentage of subjects with titres above the threshold will be presented along with 95% CI. GMTs and corresponding 95% CI, minimum and maximum values will be reported.

5.3.4. To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified) in all neonates live-born to women enrolled in the study:

5.3.4.1. Analysis of neonatal events of interest planned in the protocol

To determine frequencies of neonatal events of interest according to GAIA levels of diagnostic certainty (where these are specified) (APPENDIX D of protocol). Neonatal events of interest occur within the first 28 days after birth, but may only be detected later and are to be reported throughout the study.

Considering all neonates the number and percentage of subjects with at least one neonatal event of interest will be computed by GAIA levels of diagnostic certainty, with exact 95% CI.

5.3.4.2. Additional considerations:

This analysis will be done on the infant PPS overall and by country. Among the neonatal events of interest identified in section 3.1.2.3, those with GAIA levels of diagnostic certainty will be presented in this analysis. For each event, the frequency of infants with a specific diagnostic level of certainty will be reported. For events with subcategories, main events will not be presented because GAIA levels do not exist for the main categories.

Please note that GAIA levels are explained in detail in Appendix D of the study protocol.

5.3.5. To describe the distribution of RSV-A antibody titres in cord blood at delivery in all neonates live-born to women enrolled in the study:

5.3.5.1. Analysis of RSV A antibody titres in cord blood planned in protocol

To describe the distribution of RSV-A antibody titres in cord blood at delivery

- Geometric mean titres (GMTs) will be tabulated with 95% CI.
- Percentage of subjects above thresholds will be tabulated.

5.3.5.2. Additional considerations:

A correction/elaboration from the analysis planned in protocol in section 5.3.5.1 is that we will be reporting on the percentage of subjects with GMTs above thresholds.

This analysis will be done on the Infant PPS, overall and by country.

RSV-A neutralising antibodies will be measured in the cord blood at delivery. The frequency and percentage of subjects with titres above the threshold will be presented along with 95% CI. GMTs and corresponding 95% CI, minimum and maximum values will be reported.

5.3.6. To determine the incidence of all, of severe, and of very severe RSV-lower respiratory tract illnesses (LRTIs) in all neonates/infants live-born to women enrolled in the study:

5.3.6.1. Analysis of RSV LRTI in all neonates/infants planned in protocol

Considering all infants followed from visit 1-NB to visit ***4***-NB, the percentage of subjects with at least one LRTI illness, exact 95% CI will be presented.

Incidence rates of LRTI illnesses will be calculated, with exact 95% CI.

Frequencies of repeat occurrences of LRTI will be tabulated.

5.3.6.2. Additional considerations

A correction from the analysis planned in protocol in section 5.3.6.1 is that we will be reporting on all cause LRTIs and RSV associated LRTIs.

This analysis will be based on the infant PPS and will be done on the first episode of all, of severe, and of very severe RSV LRTIs (Section 2.2).

Incidence analyses will be performed overall, by country and by age strata (0-2 months, 0-5 months, and 0-11 months). If applicable (in the case of low numbers of incidence), incidence analyses will also be presented by region (Section 9.3). Incidence rates will also be calculated in one-month intervals from birth to one year of age, please see the monthly infant age intervals in Section 9.3.

The analysis of RSV disease incidence is summarized in the table below showing endpoints and analytic methods, these include incidence rate, proportion affected, and incidence proportion.

Incidence rate is the number of first events per person time. Incidence proportion is the number of first RSV LRTI over population at risk during the specified time period (0-2 months, 0-5 months and 0-11 months). Proportion affected is the number of subjects with at least one episode of RSV LRTI at age interval over the total number of subjects at the start of the age interval.

For each of the RSV LRTI endpoints listed in Table 11, the 95% CI will be computed as described in section 9.5.2. Kaplan-Meier curves presenting the cumulative probability of the first episode will be displayed overall. The cumulative probability of presenting the first episode will be given with its 95% CI at the end of the follow-up.

Frequencies of repeat occurrences of RSV LRTI, severe LRTI and very sever LRTI will be tabulated.

Cumulative incidence will be presented from birth to one year of life by monthly intervals (Subgroups in section 9.3).

Table 5 Summary of RSV incidence analyses for RSV LRTI of infants from birth to 1 year (Visit 1-NB to visit 4-NB)

Endpoints	Incidence analysis method	Subgroup
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Incidence rates of first episode Cumulative probability of first episode (Kaplan Meier)	Overall, by country, and by age strata
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Proportion affected by all new episodes	Overall, by country, and by age strata
All RSV LRTI Severe RSV LRTI Very Severe RSV LRTI	Incidence proportion of first episode	Overall and by country within each age strata)

5.3.7. To determine the incidence of RSV hospitalization in all neonates/infants live-born to women enrolled in the study:

5.3.7.1. Analysis of RSV hospitalization planned in protocol

Considering all infants followed from visit 1-NB to visit 4-NB, the percentage of subjects with at least one RSV hospitalization, with exact 95% CI.

Incidence rates of RSV hospitalizations will be calculated, with exact 95% CI.

Frequencies of repeat occurrences of RSV hospitalization will be tabulated.

5.3.7.2. Additional considerations

This analysis will be performed on the infant PPS and the first episode of RSV hospitalization, overall and by country and by age strata. In additional to incidences of RSV hospitalization, we will also consider all cause LRTI hospitalizations.

A change from the planned analysis in section 5.3.7.1 is that only the first event of RSV hospitalization will be included in the analysis.

In addition, cumulative incidence, Kaplan-Meier curves, and proportion affected will be calculated.

Lastly, we will also present the frequencies of symptoms used in case definitions among those infants who are found to be RSV positive from nasal swab results. The symptoms that will be considered are:

- Runny nose
- Blocked nose
- Cough
- Wheezing
- Grunting
- Nasal flaring
- Intercostal recession
- Temperature ≥38 degrees Celsius
- Chest wall indrawing
- SpO_2 cutoff point for hypoxemia

5.4. Analysis of tertiary objectives

The following tertiary objectives analyses will be performed on infants within the PPS overall and possibly by region or other relevant grouping.

5.4.1. To describe co-infections of RSV-LRTI with other respiratory viruses in infants.

5.4.1.1. Analysis of respiratory viruses associated with RSV-LRTI as planned in protocol

Considering all infants followed from visit 1-NB to visit 4-NB, the percentage of subjects with the occurrence of RSV-LRTI and having other respiratory viral co- infection with another viral etiology identified by multiplex PCR, with exact 95% CI; classified by respiratory viruses.

5.4.1.2. Additional considerations

This analysis will be performed on the Infant PPS.

The number and percentage of cases for each respiratory virus will be identified for all RSV LRTI, severe RSV LRTI, and very severe RSV LRTI. For each respiratory virus infection, number and percentage will be summarized by single or co-infection status. The groups will include those who are:

*RSV positive (confirmed with nasal swabs)

*RSV positive (confirmed with nasal swabs) & positive with another infection

*RSV negative (confirmed with nasal swabs) & positive with another infection.

For cases of RSV LRTI, severe RSV LRTI and very severe RSV LRTI, the number and percentage of cases associated with at least one other respiratory virus will be described.

This analysis will be performed overall, by country, and by age strata (0-2 months, 0-5 months, and 0-11 months).

Other respiratory viruses causing infection:

- Influenza A virus (Flu A)
- Influenza B virus (Flu B)
- Human Influenza A virus subtype H1 (Flu A-H1)
- Human Influenza A virus subtype H3 (Flu A-H3)
- Human Influenza A virus subtype H1pdm09 (Flu A-H1pdm09)
- Human adenovirus (AdV)
- Human metapneumovirus (MPV)
- Human enterovirus (HEV)
- Human parainfluenza virus 1 (PIV1)
- Human parainfluenza virus 2 (PIV2)
- Human parainfluenza virus 3 (PIV3)
- Human parainfluenza virus 4 (PIV4)
- Human bocavirus 1/2/3/4 (HBoV)
- Human rhinovirus A/B/C (HRV)
- Human coronavirus 229E (229E)
- Human coronavirus NL63 (NL63)
- Human coronavirus OC43 (OC43)

Lastly, an additional analysis will be performed that considers the frequencies of clinical symptoms of two groups of infants: those are have RSV LRTI and those who have LRTI but are RSV negative.

The clinical symptoms that will be considered are:

- Runny nose
- Blocked nose
- Cough
- Wheezing
- Grunting
- Nasal flaring

- Intercostal recession
- Temperature ≥38 degrees Celsius
- Chest wall indrawing
- SpO_2 cutoff point for hypoxemia

5.4.2. To estimate the association of RSV-LRTI in neonates/infants and the level of RSV neutralizing antibodies in cord blood.

5.4.2.1. Analysis of association of RSV-LRTI in neonates and level of RSV neutralizing antibodies in cord blood as described in the protocol.

Correlation analysis will be done comparing the levels of RSV antibodies present in cord blood and the presence or absence of RSV-associated LRTI, RSV-associated severe LRTI, and RSV-associated very severe LRTI in infants.

5.4.2.2. Additional consideration.

When considering RSV antibodies in cord blood, we are only considering RSV-A antibodies at this time (however, in the future we might also consider RSV-B antibodies). When considering RSV positivity status in nasal swabs, we consider positivity for RSV-A or RSV-B.

This analysis will be performed on the Infant PPS and will be done on the first episode (not recurrent events) of RSV LRTIs occurring during the following age intervals: 0-2 months and 0-5 months.

Step 1: Descriptive analysis:

Descriptive analyses (seropositivity rate with 95% CIs, geometric mean, median, min, max) of RSV-A neutralizing antibodies in the baseline cord blood samples will be analysed in only those with RSV positive nasal swab samples.

Next, descriptive analyses (seropositivity rate with 95% CIs, geometric mean, median, min, max) of RSV-A neutralizing antibodies in the baseline cord blood samples will be presented by RSV LRTI, RSV severe LRTI, and RSV very severe LRTI case statuses.

In addition, if further exploration is desired, we may plot levels of RSV A vs RSV-B neutralizing antibodies in cord blood among RSV LRTI positive and negative infants to see if any patterns are observed.

Step 2: Cox models:

The impact of the level of RSV neutralizing antibodies in the baseline cord blood samples on the incidence of first event of RSV-LRTI, RSV-severe LRTI and RSV very severe LRTI separately will be assessed through Cox models.

Cox regression models will be performed for the univariate analyses to obtain unadjusted hazards ratios of the determinants of interest.

Next a multivariable Cox regression model will be performed to estimate the relative contribution of each potential risk factor adjusting for the simultaneous effects of the other covariates. The model will include: time-independent covariates (e.g. gender, etc.) and time-dependent covariates (age). Covariate selection will be done using backward elimination and statistical significance. Potential risk factors will be included in the multivariable models if univariate p-value will be less than 0.1 and the multivariable model will be performed only if the number of events in sufficient (at least 10 events per covariate). In addition, depending on the data, other models could be explored or the model could be simplified.

The following covariates in infants may be considered in the multivariable models:

- Age (continuous)
- Male sex (M/F)
- Small for gestational age (Continuous)
- Congenital anomalies with internal structural defects and/or with functional defects (Y/N)
- Household composition (number of people living in household) (continuous)
- How many children under age of 6 live in household (continuous)
- Cigarette smoking during pregnancy (Y/N)
- Born during RSV transmission season (Y/N)
- Alcohol consumption during this pregnancy (Y/N)
- Highest education level of mother (Y/N)

Step 2-a: RSV neutralizing antibodies classified as a binary variable: Seropositive and seronegative:

In a first step, the RSV-A neutralizing antibodies in the baseline cord blood samples will be introduced in the model as binary variable

Step 2-b: RSV neutralizing antibodies classified as semi-quantitative variable: Quartiles

In a second step, the RSV-A neutralizing antibodies levels in baseline cord blood will be introduced in the model using quartiles. And the Cox models will be performed as described in the previous paragraph (RSV-A neutralizing antibodies as quantitative variable).

Step 2-c: RSV neutralizing antibodies classified as continuous variable:

In a third step, the RSV neutralizing antibodies levels in baseline cord blood might be treated as continuous variable in Cox models to evaluate how each unit of neutralizing antibody level change impacts on the above clinical outcomes. The Cox models will be performed as described in the previous section (with log of RSV neutralizing antibodies as quantitative variable). For the subjects with antibody levels below the LOD, a value of LOD/2 will be imputed.

The model with the best fit using, log likelihood criteria, will be selected. This will be determined via backward elimination using statistical significance.

Note that depending on the results, a quantitative association between RSV-associated LRTI, RSV-associated severe LRTI, RSV very severe LRTI, and RSV neutralizing antibodies in the baseline cord blood samples could be further explored and described more in details in the main analysis.

Poisson regression models:

Multivariable Poisson regression of neutralizing RSV A cord blood antibodies estimating RSV LRTI incidence in the first year of life, controlling for covariates will be explored. The same risk factors as above will be test. Modelling will be done in a stepwise manner using backward elimination and statistical significance.

5.4.3. To determine risk factors for pregnancy related and neonatal events of interest.

5.4.3.1. Analysis of risk factors for pregnancy related and neonatal events of interest as described in the protocol.

- Both pregnancy related events of interest and neonatal events of interest will be described according to variables collected in the mother's clinical examination and obstetric risk factors. Frequency tables will be generated for categorical variables.
- Furthermore, multiple variable analyses (such as multiple logistic regression for binary outcomes and multiple Poisson/negative binomial and/or other appropriate models for count outcomes) will be conducted to adjust for covariates of interest, provided there are sufficient numbers of events of interest.

5.4.3.2. Additional considerations:

This analysis will be performed on the maternal and infant PPSs depending on whether the risk factors are for pregnancy-related events of interest or for neonatal events of interest.

There are currently 14 potential pregnancy related events of interest and 9 potential risk factors. Likewise, there are 12 potential neonatal events of interest and 13 potential risk factors. (These do not include GAIA subcategories).

Univariate modelling will be done to first determine which risk factors are associated with which event of interest.

For each potential risk factor,

- Frequency tables will be generated as appropriate, for categorical variables.
- Mean, median, standard error, minimum, maximum and number of missing values will be provided as appropriate, for continuous data.

For pairwise distributions

- Cross-tabulations (i.e. two-way frequency tables) will be generated as appropriate, for categorical-categorical pairs;
- Mean, median, standard error, minimum, maximum and number of missing values by category will be provided as appropriate, for continuous-categorical pairs;

Covariates with univariate p-value less than 0.1 will be included in the multivariable model. The number of covariates included in the model will depend on the number of events (at least 10 events per covariates).

Multiple logistic regression, multiple Poisson regression/negative binomial modelling will be used when appropriate only on risk factors and events of interest that were associated in the univariate analysis and provided there are sufficient numbers of events of interest.

5.4.4. If deemed necessary, to further characterize the immune responses to RSV and other infections in maternal subjects and infants (based on maternal serum and cord blood).

5.4.4.1. Analysis of immune responses to disease as planned in the protocol

For levels of antibodies such as but not limited to RSV-B:

- GMTs/ GMCs may be tabulated with 95% CI.
- Percentage of subjects above various thresholds, depending on the antibody tested, may be tabulated with exact 95% CI.

5.4.4.2. Additional Considerations:

N/A

6. ANALYSIS INTERPRETATION

Analyses will be descriptive with the aim to characterize different sub-groups of women. These sub-groups will be defined prior to analyses and will be related to e.g. risk factors for maternal and neonatal events of interest and endemic disease. These descriptive analyses should be interpreted with caution considering that there is no adjustment for multiplicity for these comparisons.

7. CONDUCT OF ANALYSES

All analyses will be done on cleaned data.

Analyses will be performed in a stepwise manner after all countries in a region have completed all study visits in Epoch 002 and again after completion of all study visits in Epoch 003.

Analyses of pregnancy outcomes, pregnancy related events of interest, and neonatal events of interest will be performed when all data up to 42 days post-delivery are available (Epoch 002).

Final analyses will be performed when all data up to study end are available (Epoch 002 and Epoch 003).

Additional analyses will be performed if deemed necessary to inform the design or implementation of future clinical trials.

Interim analyses at Month 6 (Visit 3-NB)

An interim analysis will be performed on RSV surveillance data as part of the secondary objective of determining incidence of RSV LRTI. This interim analysis will be performed to obtain preliminary information on the performance of the case definitions used for RSV LRTI, severe LRTI, and very severe LRTI (Table 3) and to assess levels of RSV-A neutralising antibody in maternal and cord blood at delivery. The interim analysis will occur after the database freeze at the time point when approximately 50% of infants will have completed up to 6 months of follow up during the surveillance period following birth. Data cleaning plans will be scheduled as needed to supply data for interim analyses in a timely manner. This interim analysis will be performed on data that is as clean as possible. Preliminary results will be made available in a timely manner for use in the potential adjustment of the RSV LRTI case definitions to be used in pivotal clinical trials scheduled to begin shortly thereafter. The results pertaining to this analysis will be purely descriptive, with no adjustment of type I error, and will be reported in an interim statistical report.

7.1. Sequence of analyses

Description	Analysis ID	Disclosure Purpose (CTRS=public posting, SR=study report, internal)	Dry run review needed (Y/N)	*Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)
Month 12	E1_01	CTRS Study report	Yes	Yes
Month 6 interim analysis	E1_02	Internal	Yes	No
Analysis of epoch 2	E1_03	Internal	Yes	No

Analysis ID will depend on whether interim and epoch 2 analysis will be run using the same DBF depending on availability of lab results.

8. CHANGES FROM PLANNED ANALYSES

Only the first episode within a given age interval is considered for the computation of incidence. The incidence of event is computed using three different calculation methods: incidence rate, proportion affected, and incidence proportion. See section 5.3.6 for details of calculation.

In analysing incidence of RSV LRTI and RSV Hospitalization, only the first episode of each will be considered.

A correction from the analysis planned in protocol in section 5.3.5.1 is that we will be reporting on all cause LRTIs and RSV associated LRTIs.

Analyses will be stratified by country and by age strata (mother or infant depending on the analysis).

In instances where there are low frequencies (either in events of interest or case definitions) results may be presented by region in addition to by country (see Section 9.3 for subgroups).

9. NON-STANDARD DATA DERIVATION RULES AND STATISTICAL METHODS

9.1. Data Derivation

9.1.1. Maternal blood and cord blood

Antibody titres will be obtained from maternal blood and cord blood at delivery. Geometric means and seropositivity status for RSV neutralising antibodies in maternal or cord blood samples will be derived according to the following table:

Table 6 Derivation rule for GMT calculation and seropositivity status with respect to RSV A neutralizing antibody titers in maternal blood at delivery and cord blood

Assay	Raw result	Derivation for GMT calculation	Derivation of positivity status
RSV-A neutralizing antibody	<LOD	LOD/2	Negative
	≥LOD	Exact value	Positive

LOD=18 ED_{60}

9.1.2. Nasal swabs from infants during RSV LRTI episodes

Table 7 RSV positivity status in infants will be determined from viral loads from nasal swabs taken during infant visits. The infant will be considered RSV positive according to the following thresholds of copies/mL of RSV antibodies:

Component	Method	Unit	LOD	Derivation for positivity status	Derivation for GM calculation
RSV A	RT-qPCR	Copies of RSV A RNA per mL	To be determined	Negative	LOD/2
	RT-qPCR	Copies of RSV A RNA per mL	To be determined	Positive	Exact value
RSV B	RT-qPCR	Copies of RSV B RNA per mL	To be determined	Negative	LOD/2
	RT-qPCR	Copies of RSV B RNA per mL	To be determined	Positive	Exact value

9.1.3. Risk factors for pregnancy-related and neonatal events of interest

Table 8 Risk factors for pregnancy-related events of interest

Risk Factor	Categories
Prenatal smoking exposure during this pregnancy	Yes/No
Alcohol consumption during this pregnancy	Yes/No
BMI pre-pregnancy (kg/cm^2)	Continuous
Age of mother at delivery in current pregnancy(years)	18-34 ≥35
Subject currently lives in a country or region with Zika transmission	Yes/No
Subject has travelled to a country or region with Zika transmission since the beginning of their pregnancy	Yes/No
Subject travelled to country or region with Zika virus transmission	Yes/No
Predominant geographic ancestry	African Heritage/African American American Indian or Alaskan Native Asian-Central/South Asian Heritage Asian-East Asian Heritage Asian-Japanese Heritage Asian-South East Asian Heritage Native Hawaiian or Other Pacific Islander White Middle-eastern /North African Heritage White-Caucasian/ European Heritage Latino/Mestizo Other
Caesarean section in previous pregnancy	Yes/No
Highest education level of mother	High education Yes: Bachelor's degree or higher No: Less than bachelor's degree

Table 9 Risk factors for neonatal events of interest

Risk Factor	Categories
Prenatal smoking exposure during this pregnancy	Yes/No
Age of mother at delivery in current pregnancy(years)	18-34 ≥35
Alcohol consumption during this pregnancy	Yes/No
BMI pre-pregnancy (kg/cm^2)	Continuous
Gestational diabetes mellitus in current pregnancy	Yes/No
Gestational hypertension in current pregnancy	Yes/No
Fetal growth restriction	Y/N
Antenatal bleeding	Y/N
Dysfunctional labor	Y/N
Gender of new-born	Male/Female
Subject lives in or travelled to country or region with Zika virus infection during pregnancy	Yes/No
Predominant geographic ancestry	African Heritage/African American American Indian or Alaskan Native Asian-Central/South Asian Heritage Asian-East Asian Heritage Asian-Japanese Heritage Asian-South East Asian Heritage Native Hawaiian or Other Pacific Islander White Middle-eastern /North African Heritage White-Caucasian/ European Heritage Latino/Mestizo Other
Apgar at 5 minutes of age	0-3 inclusive 4-6 inclusive 7 or greater

9.1.4. Risk factors for RSV LRTI

- Age (continuous)
- Male sex (M/F)
- Small for gestational age (Continuous)
- Congenital anomalies with internal structural defects and/or with functional defects (Y/N)
- Household composition (number of people living in household) (continuous)
- How many children under age of 6 live in household (continuous)
- Cigarette smoking during pregnancy (Y/N)
- Born during RSV transmission season (Y/N)
- Alcohol consumption during this pregnancy (Y/N)
- Highest education level of mother (Y/N)

9.1.5. RSV Seasonality

Table 10 RSV Transmission seasons by country/region and centre

Country/region	Seasonality	Centre #
Thailand		PPD
Bangkok	June-October	
Chiang Mai	July-November	
Malaysia		
Selangor	Year round	
Kota Kinabalu	Year round	
Alor Setar	Year round	
Kuching	Year round	
Philippines		
Manila	Year round	
Cebu	Year round	
South Africa		
Parow, Western Cape	April-August	
Pretoria, Gauteng	February-June	
India		
Pune	June-March	
Ballabgarh	Year round	
Chennai	September to March	
Vellore	September to March	
Brazil		
Ribeirao Preto/SP	March-July	
Belo Horizonte/MG	March-July	
Nata/RN	March-July	
Argentina		
Buenos Ares	May-July	
Cordoba	May-July	
Mendoza	May-July	
Colombia		
Medellin	Mar-Jun & Sep-Nov	
Medellin	Year round	
Bogota	Year round	
Bogota	Mar-May	
Cali	April-Jun	
Villavicencio	Mar-Apr/May & Nov-Dec	
Mexico		
Monterrey	September-April	
Oaxaca	September-April	
Durango	September-April	

9.2. Data presentation

The following decimal description will be used for the analyses.

Table 11 Decimal points in analyses

Display Table	Parameters	Number of decimal digits
All summaries	% of frequency, including LL & UL of CI	1
All summaries	% frequency, including LL & UL of CI	1
All summaries	Mean, median, minimum, maximum	1
All summaries	SD	2
All summaries	*P*-value	3

9.3. Subgroup definitions

The following sub-group names will be used for statistical analyses:

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
Country		
1	TH	Subjects from Thailand
2	MY	Subjects from Malaysia
3	PH	Subjects from Philippines
4	SA	Subjects from South Africa
5	IN	Subjects from India
6	BR	Subjects from Brazil
7	AR	Subjects from Argentina
8	CO	Subjects from Colombia
9	MX	Subjects from Mexico
Region		
Latin America	LatAM	Subjects from Brazil, Argentina, Colombia. Mexico
Asia Pacific	AsiaPac	Subjects from Thailand, Malaysia, Philippines, India
South Africa	SA	Subjects from South Africa
Age category maternal subject at enrolment		
1	18-34	18-34 years of age
2	≥35	35 years of age or older
Age category infant (3 categories)		
1	0-2	Birth to one day before the 3rd month of life (0-30 days)
2	0-5	Birth to one day before the 6th month of life (0-180 days)
3	0-11	Birth one day before the 12th month of life (0-365 days)
Age category infant (One-month intervals)		
1	0	Birth to one day before the 1st month of life
2	1	First day of 1 month to one day before the 2nd month of life
3	2	First day of 2 months to one day before the 3rd month of life
4	3	First day of 3 months to one day before the 4th month of life
5	4	First day of 4 months to one day before the 5th month of life
6	5	First day of 5 months to one day before the 6th month of life

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
7	6	First day of 6 months to one day before the 7th month of life
8	7	First day of 7 months to one day before the 8th month of life
9	8	First day of 8 months to one day before the 9th month of life
10	9	First day of 9 months to one day before the 10th month of life
11	10	First day of 10 months to one day before the 11th month of life
12	11	First day of 11 months to one day before the 12th month of life

9.4. Case definitions

Please refer to section 2.1 for an explanation of how data is collected for surveillance in this study since that is the data that will be used for the derivation of case definitions.

Note that if a worsening visit(s) takes place for the same episode, then all symptoms collected from the initial visit and the worsening visit(s) are combined and counted under one episode, with the most severe level of the symptoms being used in the case definition derivation. For example, if SpO_2 is collected in a case assessment and a worsening visit for the same episode, then the lowest SpO_2 from any visit will be used for the case definition derivation. This is done because the most severe level of a symptom will not necessarily be in the worsening form, so multiple scenarios of visits and data collection can be accounted for. The earliest date of a reported symptom will be used as the start date of the episode. The latest date of a reported symptom will be used as the end date of episode.

If an infant comes in for a visit with new bouts of cough or blocked nose after 7 days of his/her latest end date of an RTI symptom, then a new case assessment form is opened and this is potentially the start of a new episode.

Variables from a Case Assessment Visit will be pulled from [frmASSESSMENT_INFO], [frmSYMPTOMS_RTI], and [frmVITALSIGNS_RTI].

Variables from a Worsening Visit will be pulled from [frmWORSENING_INFO] and [frmVITALSIGNS_WOR].

9.4.1. RSV RTI

The child will be reported positive for RSV RTI when he/she presents with at least one of the following symptoms: (data pulled from the Case Assessment Visit)

- Runny nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= ‘YES’) and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= ‘Yes’) and [frmSYMPTOMS_RTI] (fields[ASSESS_VIS]=’Yes’ and [sctSYMPTOMS_RTI]=[RUNNY]
- Blocked nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= ‘YES’) and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= ‘Yes’) and [frmSYMPTOMS_RTI] (fields[ASSESS_VIS]=’Yes’ and [sctSYMPTOMS_RTI]=[BLOCKED]Cough

reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')

AND confirmed RSV infection

9.4.2. RSV LRTI

The child will be reported positive for RSV LRTI when he/she presents with at least one of the following symptoms: (The data can be pulled from the Case Assessment Visit)

- Cough reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')
- Difficulty breathing reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING')

AND at least one of following symptoms: **(The data can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)**

- SpO_2(<95% if altitude ≤2500m and <92% if altitude >2500m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

OR

- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

AND

Confirmed RSV infection from a nasal swab test.

9.4.2.1. Start date of RSV-LRTI

Start dates of RSV LRTI symptoms will be pulled from the Case Assessment Visit for at least one of the following: parental report of history of cough or difficult breathing. The start date of RSV LRTI is the earliest of the start dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'SRDAT')

Or

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'SRDAT')

9.4.2.2. End date of RSV-LRTI

End dates of RSV LRTI symptoms will be pulled from the Case Assessment Visit and will be defined as the latest date of the end dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'ENDAT')

Or

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'ENDAT')

9.4.3. RSV severe LRTI

RSV severe LRTI cases will be reported if the person meets the case definition of RSV LRTI

AND

(The data for below can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<93% if altitude ≤2500m and <90% if altitude >2500m) (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '93.0% or <90%')

  OR

- Lower chest wall indrawing reported during the physical examination. (field [sctCHEST_INDRAWING_RTI]=[INDRAWING]= 'YES') OR (field [sctCHEST_INDRAWING_WOR]=[INDRAWING]= 'YES')

9.4.3.1. RSV Severe LRTI start date

Start dates will be defined as the earliest date of cough or difficulty breathing as described in section 9.4.2.1.

9.4.3.2. RSV Severe LRTI end date

End dates will be defined as the latest date of cough or difficulty breathing as described in section 9.4.2.2.

9.4.4. RSV very severe LRTI

RSV very severe LRTI is defined as meeting the case definition of RSV LRTI

AND

(The data for below for SpO_2 can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<90% if altitude ≤2500m and <87% if altitude >2500m) (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '90.0% or <87%')

  OR

- Inability to feed reported in signs and symptoms. [sctSEVERITY_SIGNS_RTI]=[FEEDING]= 'Unable to feed')

  OR

- Failure to respond/unconscious reported in signs and symptoms. [sctSEVERITY_SIGNS_RTI]=[CONSCIOUS]= 'The child is unresponsive to all stimuli')

9.4.4.1. RSV very severe LRTI start date

Start dates will be defined as the earliest start date of cough or difficulty breathing as described in section 9.4.2.1.

9.4.4.2. RSV very severe LRTI end date

End dates will be defined as the latest end date of cough or difficulty breathing as described in section 9.4.2.2.

9.4.5. RSV Hospitalization

RSV hospitalization is determined if both the following criteria are met:

- Confirmed RSV infection with nasal swab test from central GSK lab.

AND

- Hospitalization for acute medical condition determined in Inpatient and worsening flag form. Variables will be pulled from [frmINPATIENT_WORSE_FLG] (field [HOSPI_YN]= 'Yes').

9.4.5.1. RSV Hospitalization start dates

Hospitalization start date will be determined from the Inpatient Care form. Variables will be pulled from [frmINPATIENTCARE] (field [HOSP_SRDAT]=)

9.4.5.2. RSV Hospitalization end dates

Hospitalization end date will be determined from the Inpatient Care form. Variables will be pulled from [frmINPATIENTCARE] (field [HOSP_ENDAT]=)

9.4.6. All cause RTI

All cause RTI is reported if the child has one or more of the following symptoms:

- Runny nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'RUNNY')
- Blocked nose reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BLOCKED')
- COUGH reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')

9.4.6.1. All cause RTI start date

- Start dates of all cause RTI cases will be the earliest dates of cough, runny nose, or blocked nose. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and
- [sctSYMPTOMS_RTI]= 'Cough' and [cmpCOUGH_DT]= 'SRDAT'

  OR
- [sctSYMPTOMS_RTI]= 'Blocked' and [cmpBLOCKED_DT]= 'SRDAT'

  OR
- [sctSYMPTOMS_RTI]= 'RUNNY' and [cmpRUNNY_DT]= 'SRDAT')

9.4.6.2. All cause RTI end date

End dates of all cause RTI cases will be that lastest dates of Cough. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'Cough' and [cmpCOUGH_DT]= 'ENDAT')

9.4.7. All cause LRTI

All cause LRTI is reported if the child has one or more of the following:

- COUGH reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH')
- Difficulty breathing reported during the case assessment visit. Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING')

AND

(The data for below can be pulled from the Case Assessment Visit OR Worsening Visits. If worsening visits took place for this episode, the most severe value of SpO_2 or RR will be used, whether it was observed in the Case Assessment Visit or the Worsening Visit.)

- SpO_2(<95% if altitude ≤2500m and <92% if altitude >2500m) during the physical examination of case assessment visit. (field [scOXYGEN_SATURATION_RTI] OR [scOXYGEN_SATURATION_WOR]=[SpO_2]< '95.0% or <92%')

OR

- RR increase (field [sctRESPIRATORYRATE_RTI]=[VSORRES_RESP] OR [sctRESPIRATORYRATE_WOR]= [VSORRES_RESP_WOR]

RR increase defined as:

- > 60/minute (< 2 months of age)
- > 50/minute (2 to < 12 months of age)
- > 40/minute (12 to 24 months of age)

9.4.7.1. All cause LRTI start dates

The start date of all cause LRTI with the earliest of the start dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'SRDAT')

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'SRDAT')

9.4.7.2. All cause LRTI end dates

The end date of all cause LRTI is the latest of the end dates of the following symptoms:

- Cough

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'COUGH' and [cmpCOUGH_DT]= 'ENDAT')

- Difficulty Breathing

  Variables will be pulled from [frmSURVEILLANCE_CONTACT] (field [ASSESS_VIS]= 'YES') and [frmSYMPTOMS_RTI] (fields [ASSESS_VIS]= 'Yes' and [sctSYMPTOMS_RTI]= 'BREATHING' and [cmpBREATHING_DT]= 'ENDAT')

Figure 2 **Derivation of RSV-LRTI events date and duration according to the the corresponding LRTI event**

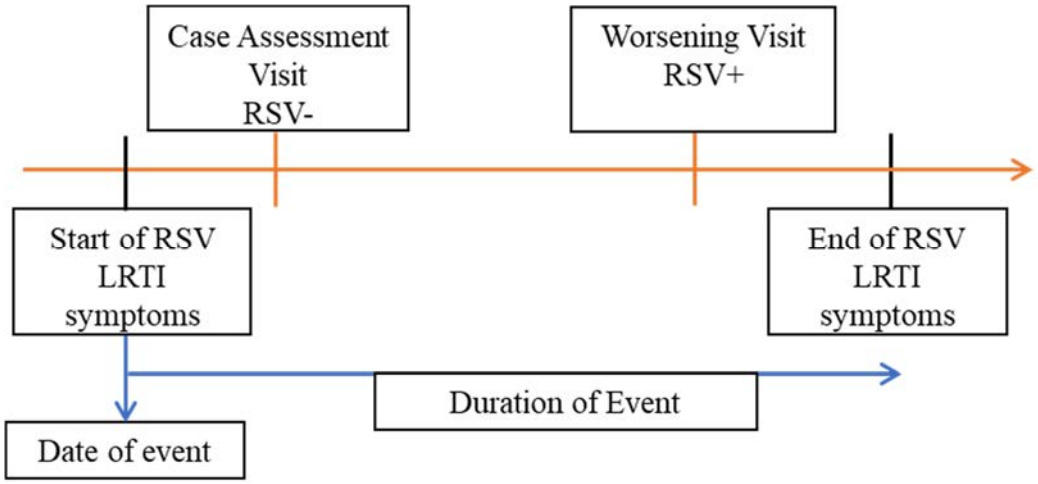


Figure 3 **Derivation of RSV-Hospitalization events date and duration**

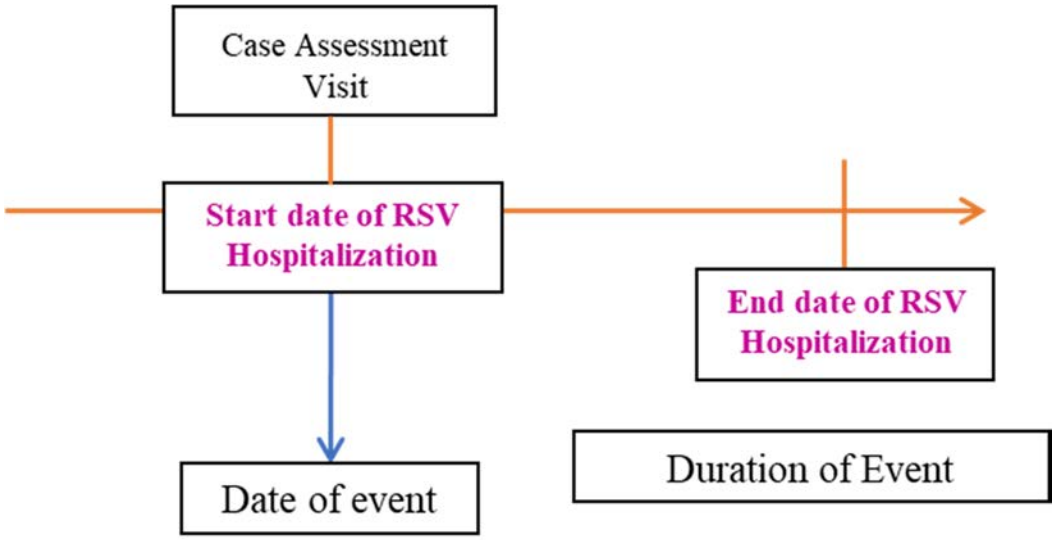
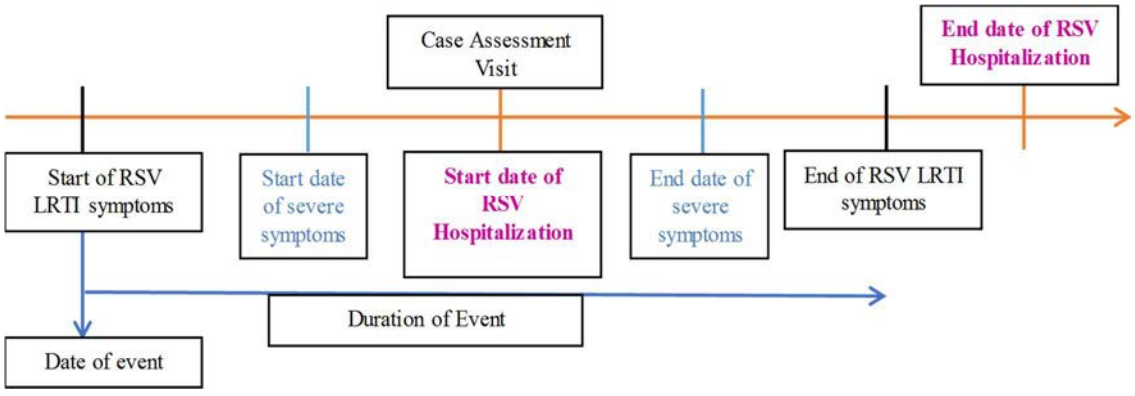


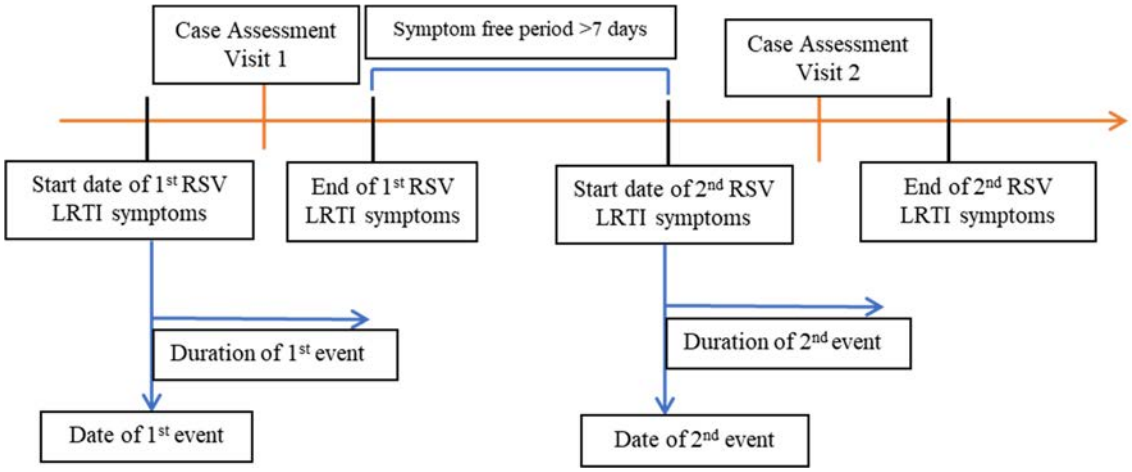
Figure 4 **Derivation of RSV-LRTI hospitalization events date and duration according to the corresponding LRTI event**



9.4.8. New RSV LRTI episode rule

A new episode of an event is a single case of RSV RTI, RSV LRTI, severe RSV LRTI, or hospitalization meeting the respective case definitions and severity scale with an interval of at least 7 symptom free days since the last episode that was diagnosed. (Note: persistent runny nose is an exception to the 7 symptom free days rule. A child may have persistent runny nose but still present with a new episode of RSV LRTI). If symptoms worsen within 7 days, the subject comes for a worsening visit.

Figure 5 Derivation of 2 RSV LRTI events date and duration with a symptom free period of at least 7 days



9.4.9. Symptoms

9.4.9.1. Increased respiratory rate

The subject will be considered to have increased respiratory rate if one of the following statements is reached:

- Respiratory rate (field [sctRESPIRATORYRATE_RTI])
- > 60/minute for subjects <2 months of age (fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])
- > 50/minute for subjects 2-11 months of age(fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])
- > 40/minute for subjects 12-24 months of age(fields [sctDEMOGRAPHY.itmDOB_RAW] and [rscRESPIRATORYRATE_RTI.VSORRES_RESP])

Please note that respiratory rate is measured during the general and obstetric physical exam.

9.5. Statistical Methods

9.5.1. Computation of incidence rates

For each endpoint, the incidence rate (IR, number of episodes/endpoints per 100 person-years) will be calculated by dividing the number of subjects reporting the first episode over the follow-up period by the total person-year. A 95% CI will be computed using an exact method for a Poisson variable as described below.

The person-time at risk for an event of interest (RSV-LRTI or RSV hospitalization) will be calculated as the time between the date of birth and the end of the at-risk period or the earliest of the followings:

- Date of first diagnosis of event of interest (e.g. first episode of RSV-LRTI);
- Date when child reaches 1 year (or 6 months if analysis is done during the interim analysis);
- Date of death;
- Date of last follow-up in study.

Please note that in a case where a subject comes to an assessment visits, has LRTI symptoms, is not confirmed for RSV infection via a nasal swab (because either no nasal swab was taken or the lab yielded invalid results), then we will consider the subject as not have a laboratory-confirmed event. The Subject will therefore be censored at the event (n=0, T=from start of the follow-up period at risk up to event).

9.5.2. Exact confidence intervals (CIs)

The exact confidence interval within a group for an incidence rate (per 100 person-years):

To estimate the confidence limit of the incidence rate, the exact Poisson confidence limit will be used [Clopper, 1934]:

If *n* is the number of subjects presenting a given characteristic among these *Ny* subjects per year, the true incidence rate can be estimated by *(n/Ny)*100*. Its exact *(1-α)%* confidence interval is obtained from:

*CINV(α/2,2*n)/2/Ny*100* as the lower boundary

and

CINV((1-α/2),2(n+1))/2/Ny*100* as the upper boundary.

where *CINV(probability, degrees of freedom)* returns the inverse of the chi-squared probability distribution and α is the type I error rate.

9.5.3. Computation of proportion affected

Proportion affected will be computed as the number of subject who had at least one episode in the age interval divided by the total number of subjects at start of considered age strata. A 95% CI will be computed using an exact method for a Poisson variable.

9.5.4. Computation of incidence proportion

Incidence proportion will be computed as the number of subjects who had at least one episode in the age interval (monthly) divided by the number of subject at risk of event at the beginning of the age interval. A subject that has an event will no longer consider of at risk, i.e. subjects with event in 0 – 1 month are excluded from denominator for future monthly incidence calculation. A 95% CI will be computed using an exact method.

9.5.5. Cox models

All multivariable modelling will be done in a stepwise manner using backward elimination and statistical significance.

9.5.5.1. Univariate Models

Please see section 5.4.3.2. Univariate modelling will be done to first determine which risk factors are associated with which event of interest.

For each potential risk factor:

- Frequency tables will be generated as appropriate, for categorical variables.
- Mean, median, standard error, minimum, maximum and number of missing values will be provided as appropriate, for continuous data.

For pairwise distributions:

- Cross-tabulations (i.e. two-way frequency tables) will be generated as appropriate, for categorical-categorical pairs;
- Mean, median, standard error, minimum, maximum and number of missing values by category will be provided as appropriate, for continuous-categorical pairs;

9.5.5.2. Multivariable Models

Covariate selection will be done using statistical significance: the final multivariable regression models will include all potential risk factors with a simple regression model with p-value < 0.10. The number of covariates included in the models will depend on the number of events (at least 10 events per covariate). The models will be performed only if the number of events is sufficient (at least 10 events per covariate). In addition, depending on the data and the validity of the model assumptions, other models could be explored or the models could be simplified.

The results from the multivariable regression models will include hazard ratios (Cox regression models) and odds ratios (logistic and longitudinal logistic regression models) and their 95% CI.

9.5.5.3. Multiple Logistic Regression models

Multiple logistic regression models will be used to test the association between events of interest and risk factors. PROC LOGISTIC or PROC GENMOD can be used. What is tested will depend on how many people are observed with these outcome and risk factors and if they are associated in the univariate analysis.

9.5.6. Multiple Poisson/negative binomial models

PROC GENMOD can be used for multiple Poisson/negative binomial modelling.

10. ANNEXES

10.1. Business rules for standard data derivations and statistical methods

This section contains GSK Vaccines' standard rules for data display and derivation for clinical and epidemiological studies. These rules will be applied along with those detailed in section 9 (additional study-specific rules).

10.1.1. Age

Age of infant will be expressed in months and will be computed as the number of complete calendar months between the date of birth (DOB) and the date of event. For example:

DOB = 10JUN2017, Date of event = 09JUL2018 -> Age = 12 months

DOB = 10JUN2017, Date of event = 10JUL2018 -> Age = 13 months

Age of the mother at the time of childbirth will be expressed in years and will be computed as the number of complete calendar years between the birth date of the infant and the birth date of the mother. For example:

DOB = 10SEP1983, Date of vaccination = 09SEP2018 -> Age = 34 years

DOB = 10SEP1983, Date of vaccination = 10SEP2018 -> Age = 35 years

10.1.2. Handling of missing data

10.1.2.1. Dates

When partially completed dates (i.e. with missing day or month) are used in calculations, the following standard rules will be applied:

- A missing day will be replaced by 15
- A missing day and month will be replaced by June 30th.

10.1.2.2. Laboratory data

Missing laboratory results (including immunological data) will not be replaced.

10.1.2.2.1. Studies with paper diaries

For studies using paper diaries which have questions in the CRF indicating the presence or absence of solicited symptoms, the following rules are applicable.

Denominators for the summary of local (or general) solicited symptoms will be calculated using the number of subjects who respond "Yes" or "No" to the question concerning the occurrence of local (or general) symptoms.

When a specific symptom is marked as having not occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=N for the specified post-vaccination period for the symptom in question), all daily measurements will be imputed as Grade 0.

When a specific symptom is marked as having occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=Y for the specified post-vaccination period for the symptom in question), any missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

When the occurrence of a specific symptom is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-vaccination period for the symptom in question) but the group of symptoms (local or general) is marked as having occurred (i.e. SDTM CE.CEOCCUR=Y), all missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the symptom summary tables.

The following table shows how subjects contribute to each category for a specific solicited symptom over the Day X to Day Y post-vaccination period:

Solicited symptom category	Subjects included in the calculation of the numerator
Any	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y or with the symptom marked as present and at least one missing daily recording between Day X and Day Y
At least grade 1	All subjects with at least one occurrence of the symptom at grade 1, grade 2, or grade 3 between Day X and Day Y
At least grade 2	All subjects with at least one occurrence of the symptom at grade 2 or grade 3 between Day X and Day Y
At least grade 3	All subjects with at least one occurrence of the symptom at grade 3 between Day X and Day Y

10.1.3. Data derivation

10.1.3.1. Weight

Weight will be presented in kilograms. Weights reported in pounds will be converted as follows:

Weight in kilograms = Weight in pounds / 2.2

10.1.3.2. Height

Height will be presented in centimeters. Heights reported in feet and inches will be converted as follows:

Height in centimeters = Height in inches x 2.54

10.1.3.3. Body mass index (BMI)

BMI will be calculated as follows:

BMI = (Weight in kilograms) / (Height in meters)^2

10.1.3.4. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

Temperature (Celsius) = ((Temperature (Fahrenheit) - 32) x 5)/9

10.1.3.5. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
“NEG”, “-“, or “(-)”	cut-off/2
“POS”, “+”, or “(+)”	cut-off
“< value” and value is <= assay cut-off	cut-off/2
“< value” and value is > assay cut-off	value
“> value” and value is < assay cut-off	cut-off/2
“> value” and value is >= assay cut-off	value
“value” and value is < cut-off	cut-off/2
“value” and value is >= cut-off	value
All other cases	missing

10.1.3.6. Geometric mean titres (GMTs) and concentrations (GMCs)

Geometric Mean Titre (GMT) or Concentration (GMC) calculations are performed by taking the inverse logarithm of the mean of the log titre or concentration transformations. Antibody titres or concentrations below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMT/GMC calculation. The cut-off value is defined by the laboratory before the analysis and is described in the protocol.

10.1.3.7. Onset day

The onset day for an event (e.g. AE, medication, vaccination) is the number of days between the last study vaccination and the start date of the event. This is 1 for an event occurring on the same day as a vaccination (and reported as starting after vaccination).

10.1.3.8. Duration of events

The duration of an event with a start and end date will be the number of days between the start and end dates plus one day, i.e. an event that starts on 03MAR2018 and ends on 12MAR2018 has a duration of 10 days.

The duration of solicited events will be calculated as the sum of the individual days with the symptom reported at grade 1 or higher.

10.1.4. Display of decimals

10.1.4.1. Percentages

Percentages and their corresponding confidence limits will be displayed with:

- no decimals when there are fewer than 50 subjects in each tabulated group
- one decimal when there are at least 50 subjects in at least one tabulated group
  - Exceptions will be made for percentages that are not 0% or 100% but appear as 0% or 100% due to rounding. For these specific cases the number of decimals will be increased until the displayed value is no longer 0% or 100%. Examples are given in the following table.

n/N	Displayed percentage
10/45	22%
1/45	2%
10/55	18.2%
1/55	1.8%
1/300	0.3%
1/3000	0.03%
1/30000	0.003%
299/300	99.7%
2999/3000	99.97%
29999/30000	99.997%

- The display of additional decimals for values close to 0% or 100% will be applied only to point estimates and not confidence limits, which can be rounded and displayed as 0% or 100%.
- Values of exactly 0% or 100% will be presented with no decimals regardless of the number of subjects per tabulated group.

10.1.4.2. Differences in percentages

Differences in percentages and their corresponding confidence limits will be displayed with one more decimal than the maximum number used to display the individual percentages, for example the difference between two percentages displayed with one decimal will be displayed with two decimals.

10.1.4.3. Demographic/baseline characteristics statistics

The mean, median, and standard deviation for continuous baseline characteristics (height, weight, body mass index (BMI), pre-vaccination body temperature) will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maxima and minima of transformed height variables will be displayed with no decimals.

The maxima and minima of transformed weight variables will be displayed with no decimals with the exception of values are below 10kg where one decimal will be displayed.

The maximum and minima of transformed body temperatures will be displayed with one decimal.

10.1.4.4. Serological summary statistics

The number of decimals used when displaying geometric mean titers (GMT) or concentrations (GMC) and their confidence limits is shown in the following table:

GMT or GMC value	Number of decimals to display
<0.1	3
>=0.1 and <10	2
>=10 and <1000	1
>=1000	0

When multiple categories of GMT or GMC values are present in the same table, the number of decimals displayed should match that of the smallest category (i.e. the one with the higher number of decimals). For example, if GMT or GMC values of <0.1 appear in the same table as values of >=0.1 and <10, 3 decimals should be displayed for both.

GMT or GMC ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

10.1.5. Statistical methodology

10.1.5.1. Exact confidence intervals around proportions

The exact confidence intervals around within-group proportions are derived using the method of Clopper and Pearson [Clopper, 1934].

10.1.5.2. Standardized asymptotic confidence intervals around differences in proportions

The standardized asymptotic confidence intervals around differences in proportions are derived using the method of Mietinnen and Nurminen [Miettinen, 1985].

10.1.5.3. Adjusted GMT or GMC ratios

When between-group GMT or GMC ratios are computed and adjusted for two-level categorical co-variables, these co-variables should be included as dummy continuous variables in the SAS procedure.

10.2. TFL and/or TFL ToC

The TFL and the TFL ToC will be found in eTMF folder section:

PPD

11. REFERENCES

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934;26:404-13.

Miettinen, O. S. and Nurminen, M. Comparative analysis of two rates. Statistics in Medicine, 1985;4,213-226.