PROMISE
Preparing for RSV Immunisation and Surveillance in Europe

innovative medicines initiative

101034339 – PROMISE

Preparing for RSV Immunisation and Surveillance in Europe

WP3 –Clinical validation studies

# D3.1 Design clinical protocol with WP4 partners to optimise patient recruitment and sampling for biomarker validation


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|---|---|
| **Lead contributor** | J.G. Wildenbeest (2 – UMCU) |
| | j.g.wildenbeest@umcutrecht.nl |
| **Other contributors** | L. Bont (2 - UMCU) |
| | J. Aerssens (19 – JPNV) |
| **Reviewers** | Matthew Snape (5 – UOXF); Deniz Oner (19 – JPNV); Federico Martinon Torres (13 – SERGAS) |


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# Definitions

- **Participants** of the PROMISE Consortium are referred to herein according to the following acronyms:
    1. **UEDIN**. The University of Edinburgh (United Kingdom)
    2. **UMCU**. Universitair Medisch Centrum Utrecht (Netherlands)
    3. **UA**. Universiteit Antwerpen (Belgium)
    4. **Imperial**. Imperial College of Science, Technology and Medicine (United Kingdom)
    5. **UOXF**. The Chancellor, Masters and Scholars of the University of Oxford (United Kingdom)
    6. **THL**. Terveyden Ja Hyvinvoinnin Laitos (Finland)
    7. **RIVM**. Rijksinstituut voor Volksgezondheid en Milieu (Netherlands)
    8. **NIVEL**. Stichting Nedelands Instituut voor Onderzoek van de Gezondheidzorg (Netherlands)
    9. **TUCH**. Varsinais-Suomen Sairaanhoitopiirin Kuntayhtymä (Finland)
    10. **TEAMIT**. TEAM IT Research, S.L. (Spain)
    11. **ReSViNET**. Stichting Resvinet (Netherlands)
    12. **SSI**. Statens Serum Institut (Denmark)
    13. **SERGAS**. Servizo Galego de Saúde (Spain)
    14. **PENTA**. Fondazione PENTA - For the treatment and care of children with HIV and related diseases - ONLUS (Italy)
    15. **FISABIO**. Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana (Spain)
    16. **MLU**. Martin-Luther-Universitaet Halle-Wittenberg (Germany)
    17. **SP**. Sanofi Pasteur, S.A. (France)
    18. **GSK**. GlaxoSmithKline Biologicals, S.A. (Belgium)
    19. **JANSSEN**. Janssen Pharmaceutica, N.V (Belgium)
    20. **Novavax**. Novavax Inc. (United States)
    21. **Pfizer**. Pfizer Limited (United Kingdom)
    22. **AZ**. AstraZeneca AB (Sweden)
- **Grant Agreement**. (Including its annexes and any amendments) The agreement signed between the beneficiaries of the action and the IMI2 JU for the undertaking of the PROMISE project (Grant Agreement No. 101034339).
- **Project**. The sum of all activities carried out in the framework of the Grant Agreement.
- **Work plan**. Schedule of tasks, deliverables, efforts, dates and responsibilities corresponding to the work to be carried out, as specified in Annex I to the Grant Agreement.
- **Consortium**. The PROMISE Consortium, comprising the above-mentioned participants.
- **Consortium Agreement**. The agreement concluded amongst PROMISE participants for the implementation of the Grant Agreement. The agreement shall not affect the parties' obligations to the Community and/or to one another arising from the Grant Agreement.

# Abbreviations

| Acronym / Abbreviation | Meaning |
|---|---|
| **ABR** | General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier) |
| **AE** | Adverse Event |
| **AR** | Adverse Reaction |
| **CA** | Competent Authority |
| **CCMO** | Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek |
| **CV** | Curriculum Vitae |
| **DSMB** | Data Safety Monitoring Board |
| **EU** | European Union |
| **EudraCT** | European drug regulatory affairs Clinical Trials |
| **GCP** | Good Clinical Practice |
| **GDPR** | General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG) |
| **IB** | Investigator's Brochure |
| **IC** | Informed Consent |
| **IMP** | Investigational Medicinal Product |
| **IMPD** | Investigational Medicinal Product Dossier |
| **METC** | Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC) |
| **RSV** | Respiratory Syncytial Virus |
| **(S)AE** | (Serious) Adverse Event |
| **SPC** | Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst |
| **Sponsor** | The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party. |
| **SUSAR** | Suspected Unexpected Serious Adverse Reaction |
| **UAVG** | Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG |
| **WMO** | Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen |

# Abstract/Summary

**Rationale:** Human respiratory syncytial virus (RSV) causes severe disease in young infants. Besides young age, prematurity and congenital cardiopulmonary disease are known risk factors for severe disease. However, most children admitted to the hospital with RSV are previously healthy. To identify those at risk for severe disease, biomarkers are needed. In the RESCEU case-control study we established biomarkers predictive of, or associated with, lower respiratory tract RSV infection and disease severity. In this follow-up study the detected biomarkers will be validated.

**Objective:**

To validate biomarkers that are associated with severe RSV infection and respiratory sequelae.

Study design: Prospective observational case-control study

**Study population:**

Previously healthy infants (<1 year of age) with a proven RSV infection in the following subgroups:

- Ventilated infants (n=40)
- Hospitalized, non-ventilated infants (n=40)
- Medically attended non-hospitalized infants (n=40)
- Healthy infants (n=40)

**Main study parameters/endpoints:** Validation of biomarkers that are associated with severe RSV infection and respiratory sequelae.

**Nature and extent of the burden and risks associated with participation, benefit and group relatedness:** Blood, respiratory, buccal, urine and stool samples will be collected at the moment of medical attendance for RSV infection and 6-8 weeks after infection. Healthy controls will have only one timepoint (baseline) at which samples are collected. A questionnaire will be completed by the parents at the moment of RSV infection (baseline) followed by a diary for two weeks (14 consecutive days) for RSV positive children. A yearly questionnaire up to the age of 3 years old will be completed by the parents.

None of the study procedures is associated with any risk for serious complications. However, there is a minimal risk of minor complications due to study procedures (for example a nose bleed after a nose swab or bruise after blood sampling). This study is group related and can only be done in this patient group because severe RSV disease is mainly seen in infants and very rare in older children and adults.

Benefits of participating in the study: There are no particular benefits to participating in this study, apart from knowing that knowledge obtained from it may benefit other patients in the future.

# 1. Introduction and study design

## 1.1. Introduction

Human respiratory syncytial virus (RSV) causes severe disease in the very young, elderly and in high risk groups. Worldwide in 2015 there were an estimated 34 million cases of acute lower respiratory tract infection (ALRI), 3.4 million ALRI hospitalisations and 55,000 to 199,000 deaths associated with RSV in children <5 years old (1). Although RSV related mortality is rare in developed countries, the burden of RSV related hospitalization is substantial with an estimated yearly hospitalization rate of 0,8% in infants (2, 3). The majority of children admitted to the hospital with RSV are previously healthy. Although a younger age is a risk factor for severe disease in children without comorbidities, this cannot totally explain the difference in severity between young children of the same age. In addition, RSV infection in infancy is associated with recurrent wheezing episodes in the first year of life and beyond (4, 5). Given this substantial burden, there is an unmet need to identify the correlates of severe RSV disease for clinical management, classification of disease severity in clinical trials and identification of biomarkers for severe disease, which are currently lacking (6).

The RESCEU (Respiratory Syncytial virus Consortium in Europe) consortium, an IMI funded effort which brought together clinicians, epidemiologists, basic scientists, health economists, statisticians, public health professionals and industry from across Europe to answer key research questions relating RSV, conducted a biomarker discovery study in healthy young infants with RSV infection in 2 prospective studies, the RESCEU infant cohort study and the RESCEU infant case-control study (7, 8). First, possible biomarkers were identified by means of a systematic literature review (9). Biomarkers discovered in RESCEU include, but are not limited to: an array of RSV antibodies, including prefusion F protein (preF), postfusion F protein (postF) and neutralizing antibodies; gene expression profiles in infant whole blood at birth (susceptibility) as well as during disease (severity, prognosis). Results are currently being analysed and will be prepared for publication in the coming months.

A critical step in biomarker development is external clinical validation (10) as biomarkers are often identified in data-driven exploratory studies which increase the chance of false positive associations. Therefore, they need to be externally validated to minimise this risk and become acceptable for clinical implementation. This is also part of the regulatory requirements before introduction into routine care. In PROMISE we will establish a clinical study large enough to externally validate the biomarkers identified from RESCEU as well as those that are still being analysed. Gene expression profiles related to neutrophil degranulation as well as innate immunity and antiviral responses will be of specific interest for validation. The most promising candidates will be selected for verification, based on their biological relevance, statistical significance, and potential contribution to a clinical question (if the potential contribution is very small, candidates that did show statistically significance may not be developed further).

## 1.2. Objectives

Primary Objective:

- To validate biomarkers that are associated with severe RSV infection and respiratory sequelae.

Secondary Objective(s):

- To analyze dynamics of biomarkers in the upper and lower airways of children with RSV infection and compare these with blood
- To validate currently used case definitions and severity measures for RSV infection in infants

- To determine risk factors for severe RSV disease
- To determine long term sequelae of RSV infection in the first year of life

## 1.3. Study design

This will be a monocenter, prospective, observational case-control study conducted across 2 consecutive years in the Wilhelmina Children's hospital and surrounding general practitioner (GP) offices and general hospitals. This case-control study will validate discovered biomarkers related to RSV infection susceptibility and disease severity in the RESCEU case-control study and birth cohort study. In addition, dynamics of the same biomarkers will be analysed in the upper and lower airways to better understand the sources of these biomarker signals. For this a validation cohort of previously healthy infants with different severities of RSV infection will be used and compared to healthy infants.

The following subgroups will be studied:

- Ventilated infants (n=40)
- Hospitalized, non-ventilated infants (n=40)
- Medically attended non-hospitalized infants (n=40)
- Healthy infants (n=40)

Children will be recruited during two RSV seasons. If RSV is suspected but not tested as part of routine care a point of care test for RSV will be done. Samples will be collected within 96 hours after hospitalization or start of symptoms in infants who are not admitted and repeated after 6-8 weeks (convalescence). Healthy infants will be recruited year round. Collection of samples of healthy controls will be done once. There will be annual follow up by questionnaire for up to 3 years.

Blood, stool, buccal, urine and nasopharyngeal samples will be obtained from all RSV positive infants at moment of infection and in convalescence 6-8 weeks later. In ventilated RSV positive infants, bronchoalveolar lavage (BAL) and/or tracheal aspirate samples will be obtained in combination with routine clinical care. Healthy infants will have blood, stool, buccal, urine and nasopharyngeal samples only at enrolment (i.e. there will be no follow up sampling).

## 1.4. Study population

### 1.4.1. Population (base)

The total study population consists of 160 (previously) healthy infants (<1 year of age). 120 infants with RSV infection will be recruited, who are divided in the following groups:

- Ventilated infants (n=40)
- Hospitalized, non-ventilated infants (n=40)
- Medically attended non-hospitalized infants (n=40)

In addition a group of healthy infants (n=40) without infection at moment of sampling will be recruited to validate whether the discovered biomarkers would also be able to distinguish between infants with and without infection.

All infants will be recruited at the Wilhelmina Children's hospital and surrounding GP offices and general hospitals during 2 consecutive RSV seasons.

### 1.4.2. Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Parents/legal guardians of infant are willing and able to give informed consent for participation in the study.
- Less than 12 months of age at enrolment.
- Hospitalized with (suspected) RSV infection for <96 hours at enrolment or within 96hrs of onset of illness (for those not admitted).*

* Not applicable for healthy control infants

### 1.4.3. Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- History of concurrent clinically significant medical illness (not directly attributable to RSV infection) including but not limited to, cardiovascular, respiratory, renal, gastrointestinal, haematology, neurology, endocrinology, immunology, musculoskeletal, oncological or congenital disorders, as judged by the investigator.

  Specifically excluded examples include, but are not limited to:

  - Known congenital or acquired immunosuppression
  - Bronchopulmonary dysplasia/chronic lung disease of infancy
  - Congenital heart disease
  - Down syndrome
- Prematurity, defined as gestational age <37 weeks at birth
- History of receipt of medication to treat RSV infection (e.g. ribavirin)
- Prior exposure to an RSV investigational vaccine or medication.
- History of receipt of immunoglobulin or monoclonal antibodies (including palivizumab).
- Use of steroids or montelukast within 7 days of enrolment in the study.
- Participation in another clinical study for an investigational drug within 12 weeks before entry into this study
- Parents not able to understand and communicate in the local language or English.

### 1.4.4. Sample size calculation

This study is a follow-up study of the RESCEU infant studies. Biomarker analysis of these samples is currently ongoing. Therefore, accurate sample size calculations are not yet possible. We will use the following considerations to select the sample size: For external validation of the biomarkers ideally a representative sample of patients independent of the development set will be used, large enough to detect potentially relevant differences between groups with sufficient precision, taking into account the observed differences between the groups in the previous studies, and the potential clinical relevance of identified differences. Given the observational case-control design, ideally the sample size would allow for adjustment for the pre-specified relevant demographic and clinical confounders. Repeated sampling within subjects will allow for estimation of within patient variation over time and may increase precision of between-group estimates. With 40 cases and 40 controls the chance is

high to verify a (true) biomarker with a reasonable combination of attributes, expressed in the fraction of cases expressing the biomarker and the separation between cases and controls (Figure 1). Therefore, the sample size of PROMISE is sufficient because we have even 3 populations of distinct severity with 40 children per group plus a healthy control population.

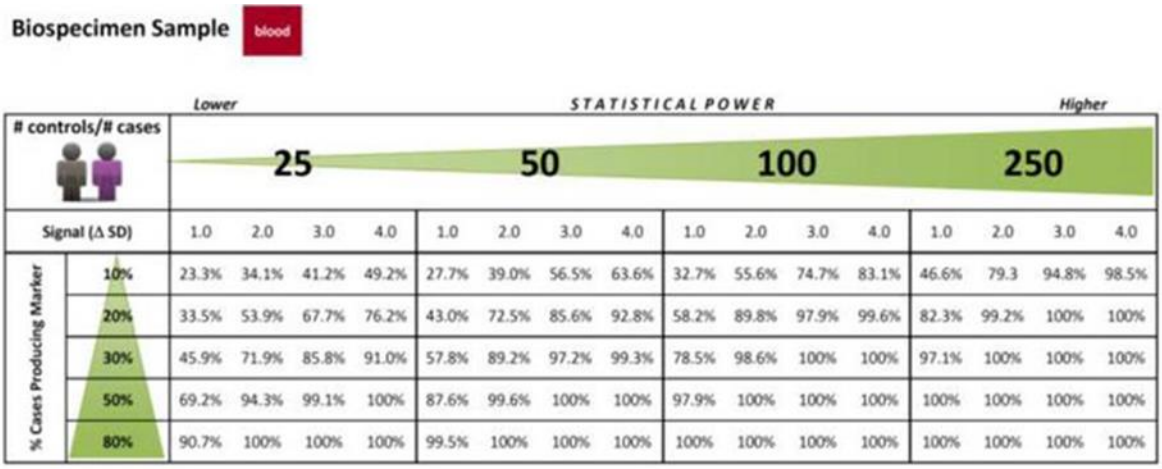


***Figure 1. Validation: Probability of Biomarkers Successfully Passing Verification Stage (12).***

# 2. Methods

## 2.1. Study parameters/endpoints

### 2.1.1. Main study parameter/endpoint

The primary endpoint is disease severity of RSV infection in the first year of life. The use of biomarkers that are associated with severe RSV infection and respiratory sequelae will be validated.

### 2.1.2. Secondary study parameters/endpoints

1. Values of biomarkers in the upper and lower airways of children with RSV infection and comparison with the values of these biomarkers in blood
2. Performance of currently used case definitions and severity measures for RSV infection in infants
3. Risk factors for severe RSV disease
4. Long-term sequelae of RSV infection in the first year of life

## 2.2. Study procedures

### 2.2.1. Main study parameter/endpoint

Participants are recruited at the moment of acute respiratory infection in hospital, at the emergency department, or GP offices.

In hospital setting (inpatient and outpatient department, emergency department):

Potential participants for the study will be identified by clinical research staff. A member of the paediatric clinical research team will identify any potentially eligible participants by attending doctor's handovers, talking to the clinical staff in hospital departments and/or screening hospital computer systems. Potential participants are informed about the study by a member of the clinical team. If parents are interested in participating in the study, a member of the study team will provide verbal and written information about the study. Medical records will be screened to confirm eligibility for inclusion, either after full consent has been received or with verbal permission from the parent/guardian/legally authorised representative. If the potential participant's RSV status cannot be determined from the medical records, written consent will be sought to perform a bedside test to assess for eligibility.

In community setting (general practitioners office):

Potential participants for the study will be identified by the general practitioner during consultations. Potentially eligible and interested participants are referred to the clinical research staff by the general practitioner. A member of the research team will contact the parents/legal guardians of potentially candidates and will explain the study. If they are expressing an interest, written consent will be sought to perform an RSV point of care test to assess for eligibility.

Healthy control infants will be identified in hospital. Patients who are in hospital for minimal invasive elective surgery procedures which are not considered to have an effect on the general health of the infant (for example correction of inguinal herniation, orchidopexy, correction of polydactyly etc) or regular check-up which requires the drawing of blood are identified by attending doctor's handovers, talking to the clinical staff in hospital departments or screening hospital computer systems. Potential

participants are informed about the study by member of the clinical team. If parents are interested in participating in the study, a member of the study team will provide verbal and written information about the study. Medical records will be screened to confirm eligibility for inclusion, either after full consent has been received or with verbal permission from the parent/guardian/legally authorised representative.

### RSV point-of-care test

A molecular point-of-care test will be used for the quick identification of RSV in respiratory samples for eligibility to the study, when routine RSV diagnostics have not been performed.

### Baseline visit

If RSV infection is confirmed, information will be collected on participant's medical history, any clinical examination performed and any laboratory and radiological investigations that are undertaken as part of routine medical care, and recorded on a case report form (CRF). A standardised respiratory clinical severity score (ReSViNET scale, appendix A (13)) grading the severity of the RSV disease will be calculated at enrolment. Clinical and health economic and quality of life data will be collected. An ARTI diary will be completed for 2 weeks from enrolment (RSV positive infants only).

At enrolment sampling will be done as follows (see also Table 2).

- Respiratory viral flocked nasopharyngeal swab
- Synthetic absorption matrix [SAM] 2 nasal strips
- Nasopharyngeal bacterial swab
- Venous or capillary blood sample (maximum of ~0.8ml/kg)
- Urine sample (clean catch or from gauze in a nappy)
- Stool sample (from a "dirty" nappy)
- Buccal swab
- If the child is intubated and ventilated: BAL/tracheal aspirate

### Subsequent Visits

<u>One week after enrolment (healthy infants only):</u>
One week after enrolment parents of participants will be contacted to assess if their infant developed any illness since enrolment.

<u>Six to eight weeks (convalescent time point) (RSV positive infants only):</u>
At 7 weeks (+/- 1 week) after discharge from hospital/emergency department, or 7 weeks post onset of symptoms for participants not attending hospital, participants will have one follow up visit. This will be a home visit by the study staff. If the child has an ARTI at that moment the follow-up visit will be postponed until the ARTI symptoms have disappeared.
At this time the recent respiratory health will be assessed and the following samples will be taken (see also Table 1):

- Respiratory viral flocked nasopharyngeal swab
- Synthetic absorption matrix [SAM] 2 nasal strips
- Nasopharyngeal bacterial swab

- Venous or capillary blood sample (maximum of ~0.8ml/kg)
- Urine sample (clean catch or from gauze in a nappy)
- Stool sample (from a “dirty” nappy)
- Buccal swab

**Questionnaires**

Baseline

- At inclusion a questionnaire about pregnancy, perinatal course and potential risk factors for RSV-hospitalization and sequelae will be filled in

One year of age

- Assessment of respiratory symptoms by parental questionnaire at age 1 year through telephone contact/email/online
- Cost, resource use and Health-Related Quality of Life (HRQoL) data will be collected using questionnaires for caregivers

Two years of age

- Assessment of respiratory symptoms by parental questionnaire at age 2 years through telephone contact/email/online
- Cost, resource use and HRQoL data will be collected using questionnaires for caregivers

Three years of age

- Assessment of respiratory symptoms by parental questionnaire at age 3 years through telephone contact/email/online
- Cost, resource use and HRQoL data will be collected using questionnaires for caregivers

**Handling and storage of samples**

See table 1 for volume and frequency of sampling. Samples will be stored at the biobank of the UMCU. During the study, samples will be shipped to other laboratories for analysis. Some samples will be sent to laboratories in countries within the European Union (EU) and some samples may be sent to laboratories in countries outside the EU for analysis.

- Sample handling and processing
- Serum: Blood will be drawn into serum collection tubes containing a clot activator and refrigerated between 2°C - 8°C for a maximum of 24 hours. Tubes will be centrifuged at 3000 rpm, 4$^{o}$C for 10 minutes, serum extracted and stored at -80$^{o}$C for later analysis.
- Whole blood: Whole blood will be collected in Natrium Heparin tubes and have appropriate buffers added and then be spun down and stored at -80C for later analysis.
- Functional Genomics: Whole blood will be added to Paxgene tubes containing a RNA stabilization reagent and stored at -80$^{o}$C. Subsequently, RNA will be extracted and gene transcription determined using next generation sequencing approaches.
- Respiratory samples: A bedside RSV test (nasopharyngeal swab) will be carried out prior to enrolment (if no RSV test was done as part of routine clinical care). After enrolment nasopharyngeal swabs will be collected, aliquoted directly into storage tubes and stored at -80$^{o}$C for later analysis.

- Synthetic absorption matrix [SAM] nasal strips will be collected and stored at -70 to -80°C for later analysis.
- Urine: 3-5 ml of urine will be collected and stored at -70 to -80°C for later analysis.
- Stool: 2-5 ml of stool will be collected and stored at -80ºC for later analysis.
- Buccal swab: a buccal swab will be collected and stored at -70 to -80°C for later analysis.

Prioritisation may be required for some of the blood tests (but not other samples) as a result of limited sample volume. We will prioritise serological and transcriptomic analysis but will store whole blood whenever sample volume permits.

All samples will be stored in the UMCU Central Biobank. Participants are being asked separately in the PIF/IC-form whether they give permission for storage of the samples in the biobank and usage of the samples for further research about viral and airway infections according to the biobank regulations. Samples will be stored in the biobank indefinitely.

***Table 1. Overview of sampling materials and volumes.***

| Moment of sampling | Sample | Volume | Analysis (minimum amount) |
|---|---|---|---|
| **At inclusion/baseline visit** | Serum (venous/capillary) | 0.5-2 ml | RSV serology (350-400 µl)<br>Metabolomics (500 µl)<br>Proteome (100 µl)* |
| | Paxgene (venous/capillary) | 0.2-0.5 ml | Transcriptome (200 µl)* |
| | Whole blood (venous/capillary) | 1-2 ml | Cellular immunology* |
| | Stool | 2-5 ml | Microbiome<br>Metagenomics/Metabolomics |
| | Synthetic absorption matrix [SAM] nasal strip | n/a | Cellular immunology<br>RNA analysis<br>Metagenomics/Metabolomics<br>Mucosal antibodies |
| | Nasopharyngeal viral swab | 3 aliquots of 900 microliter | RSV viral (deep) sequence analysis<br>Multiplex viral PCR |
| | Nasopharyngeal bacterial swab | 4-5 aliquots of 200 microliter | Airway microbiome |
| | Buccal swab | 1 swab | DNA/GWAS |
| | Urine | 3-5 ml | Metabolomics |
| **If ventilated** | Broncheo-alveolar aspirate | | Cellular immunology<br>RNA analysis |

| | | | |
|---|---|---|---|
| Daily if hospitalized | Nasopharyngeal viral swab | 2 aliquots of 1500 microliter | RSV viral load |
| **6-8 weeks after RSV RTI (RSV positive infants only)** | Serum (venous/capillary) | 1-2 ml | RSV serology (350-400 µl)<br>Metabolomics (500 µl)<br>Proteome (100 µl)* |
| | Paxgene (venous/capillary) | 0.2-0.5 ml | Transcriptome (200 µl)* |
| | Whole blood (venous/capillary) | 1-2 ml | Cellular immunology* |
| | Stool | 2-5 ml | Microbiome<br>Metagenomics/Metabolomics |
| | Synthetic absorption matrix [SAM] nasal strip | n/a | Cellular immunology<br>RNA analysis<br>Metagenomics/Metabolomics<br>Mucosal antibodies |
| | Nasopharyngeal viral swab | 3 aliquots of 900 microliter | RSV viral (deep) sequence analysis<br>Multiplex viral PCR |
| | Nasopharyngeal bacterial swab | 4-5 aliquots of 200 microliter | Airway microbiome<br>Airway transcriptome |
| | Buccal swab | 1 swab | DNA/GWAS |
| | Urine | 3-5 ml | Metabolomics |

* and additional RSV-related biomarkers

## 2.3. Withdrawal of individual study subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

### 2.3.1. Specific criteria for withdrawal

In addition, the investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)

- Significant protocol deviation
- Withdrawal of Consent
- Loss to follow up

## 2.4. Replacement of individual subjects after withdrawal

Withdrawn participants will be replaced depending on the moment of withdrawal.

## 2.5. Follow up of subjects withdrawn from treatment

If the parent/guardian/legally authorised representative withdraws consent, we will analyse any previously collected sample and include their data in further analyses unless they state otherwise.

## 2.6. Premature termination of the study

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

# 3. Safety reporting

# AEs, SAEs and SUSARs

## 3.1. Temporary halt for reasons of subject safety

Not applicable.

## 3.2. AEs, SAEs and SUSARs

### 3.2.1. Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. Because this is a non-interventional, low risk, observational study only SAEs will be registered. Expected AEs directly related to one of the interventions (for example a nose bleed after a nasopharyngeal swab or bruise after a blood test) will not be registered.

### 3.2.2. Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Only SAEs directly related to any of the sampling interventions (vene or capillary puncture, collection of buccal and/or nasopharyngeal swabs) will be registered as this is a non-interventional, low risk, observational study.

The investigator will report all SAEs directly related to any of the sampling interventions to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

## 3.3. Annual safety report

Not applicable.

## 3.4. Follow up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

## 3.5. Data safety monitoring board (DSMB)

Not DSMB is needed.

# 4. Statistical analysis

Statistical analysis will be performed by a specialized bioinformatics team.

## 4.1. Primary study parameter(s)

The primary endpoint is disease severity of RSV infection in the first year of life. The use of biomarkers that are associated with severe RSV infection and respiratory sequelae will be validated. RSV disease severity will be categorized in the following categories: hospitalized non-ventilated, hospitalized ventilated (PICU admitted) and non-hospitalized infants with RSV. If an infant was first recruited in the non-hospitalized group and is admitted during the course of RSV disease, the infant will be changed to the hospitalized non-ventilated or ventilated (PICU admission) group In addition a control group of healthy controls will be used.

Large data sets are necessary for integrative computational analyses in order to identify profiles associated with disease severity. These data will come in a wide variety of formats depending on the sample being tested and the test being undertaken. In some cases analyses will be undertaken using standard techniques while others may require novel techniques. We will work closely together with other partners in the consortium.

## 4.2. Secondary study parameter(s)

1. Values of biomarkers in the upper and lower airways of children with RSV infection and comparison with the values of these biomarkers in blood.

   Values of biomarkers for (severe) RSV infection will be compared between upper and lower airways (in ventilated infants) and blood (all infants). Results will be compared within individual children and between children of different symptom severity categories (hospitalized non-ventilated, hospitalized ventilated and non-hospitalized infants with RSV infection).

2. Performance of currently used case definitions and severity measures for RSV infection in infants.

   Clinical data about symptom severity of the participating infants will be compared with existing case definitions and severity measures. The performance of these case definitions and severity measures will be described as percentage agreement and percentage missed or incorrect diagnosis. In addition a new, simplified severity score (which will be developed within PROMISE and will be based on an extensive literature review and data obtained from the RESCEU infant studies) will be validated in this study.

3. Risk factors for severe RSV disease

   Risk factors for severe RSV disease will be determined using demographic parameters, clinical parameters and outcome and laboratory test results of participating infants. Results will be displayed as categorical data with percentages or continuous variables with mean (+/-SD) and/or median (interquartile range). Comparisons between groups of different severity (hospitalized non-ventilated, hospitalized ventilated and non-hospitalized infants with RSV) and healthy controls will be performed using chi-square for categorical variables, Student-t-test for normally distributed continuous variables or Mann-Whitney U test for not normally distributed continuous variables. Multivariate regression analysis will be performed to analyse multiple risk factors for severe RSV disease.

4. Long-term sequelae of RSV infection in the first year of life

Long-term sequelae (amongst others recurrent wheeze) will be determined using the yearly questionnaires. Results will be displayed as categorical data with percentages or continuous variables with mean (+/-SD) and/or median (interquartile range). Comparisons between groups of different severity (hospitalized non-ventilated, hospitalized ventilated and non-hospitalized infants with RSV) will be performed using chi-square for categorical variables, Student-t-test for normally distributed continuous variables or Mann-Whitney U test for not normally distributed continuous variables.

# 5. Ethical considerations

## 5.1. Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

The investigator will explain the nature of the study and will inform the parents/legal representative of the subject that participation is voluntary and that the subject can withdraw or be withdrawn from the study at any time. Written informed consent will be obtained from parents/legal representative of each subject prior to any study procedure. A copy of the signed consent form will be given to the parents /legal representative of the subject and the original will be maintained by the research team.

## 5.2. Recruitment and consent

In hospital setting (inpatient and outpatient department, emergency department): Potential participants for the study will be identified by clinical research staff. A member of the paediatric clinical research team will identify any potentially eligible participants by attending doctor's handovers, talking to the clinical staff in hospital departments or screening hospital computer systems. Potential participants will be contacted by a member of the clinical team. If parents are interested in participating in the study, a member of the study team will provide verbal and written information about the study. Medical records will be screened to confirm eligibility for inclusion, either after full consent has been received or with verbal permission from the parent/guardian/legally authorised representative. If the potential participant's RSV status cannot be determined from the medical records, written consent will be sought to perform a bedside test to assess for eligibility. After RSV is confirmed, informed consent from both parents/legal guardians is requested before entering the study.

In community setting (general practitioners office): Potential participants for the study will be identified by the general practitioner during consultations. Potentially eligible and interested participants are referred to the clinical research staff by the general practitioner. Parents/legal guardians of potentially candidates will receive an explanation about the study. If they are expressing an interest, written consent will be sought to perform a RSV point of care test to assess for eligibility. After RSV is confirmed, informed consent from both parents/legal guardians is requested before entering the study.

As mentioned above, a pre-study informed consent is asked to perform the RSV point of care test in order to see whether a potential participant is suitable to be included. However, there could be situations where only one of the parents is present at the moment of presentation (e.g. at the GP office or at the emergency department). In this case, the parent/caretaker who is present will be asked to contact the other parent/caretaker in order to obtain verbal consent for performing the point of care test for RSV. Subsequently, the RSV test is performed and the consent form, signed by the one parent who is present and the researcher will be given to the parent in order to sign by the parent/caretaker who is not present. The fully signed form is thereafter returned to the researcher by means of a return envelope. This method is not preferred but has been chosen to make the recruitment process feasible in case only one parent is present.

**Healthy controls**

Infants who are in hospital for minimal invasive elective surgery procedures which are not considered to have an effect on the general health of the infant (for example correction of inguinal herniation, orchidopexy, correction of polydactyly etc) or regular check-up which requires the drawing of blood will be asked to participate in the study.

**Informed consent**

Both parents/guardians/legally authorised representatives must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed. Written and verbal versions of the Participant Information and Informed Consent will be presented to the parent/guardian/legally authorised representative detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the parent/guardian/legally authorised representative is free to withdraw the infant from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The parent/guardian/legally authorised representative will be allowed as long as needed to consider the information, and the opportunity to question the investigator, their doctor or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of parent/guardian/legally authorised representative dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the parent/guardian/legally authorised representative and a copy inserted in the medical records (if attending a hospital). The original signed form will be retained at the study site. As mentioned above, an alternative method of asking for consent for the point-of-care test has been chosen in case only one parent/caretaker is present at the moment of presentation.

## 5.3. Objection by minors or incapacitated persons

Subjects or their legal guardians can object to any procedure related to the study at any time for any reason and participation will be terminated. According to the behavioural code guidelines of the Dutch Society of Pediatrics (NVK 2001), in case of minors or incapacitated subjects, any physical signs interpreted by the investigator or legal guardian as an objection to a procedure will terminate participation.

## 5.4. Benefits and risk assessment, group relatedness

There are no risks of participating in the study. Blood and respiratory sampling can be associated with minor local effects, for example, discomfort, bruising or nose bleeds. There are no risks associated with collection of urine or stool samples.

The risks and burdens of sampling are outlined below:

- Venepuncture/capillary blood sampling: A blood sample will be taken on one (healthy control group) or two (RSV positive participants) occasions. If the first attempt is unsuccessful any further attempt will be made if the parent/legal representative gives verbal consent for this. Drawing blood can be moderately painful. This will be performed by trained personnel. If possible, blood sampling for the study will be combined with routine clinical blood sampling. The risk of complications is negligible. Bruising may be seen.
- Nasopharyngeal swab: A small swab will be introduced into the nose towards the nasopharynx and some mucus will be collected. The procedure can cause a brief moment of discomfort, however, the duration of this procedure is less than 10 seconds and the swab is very soft. This procedure will be performed by trained personnel. Minor complications (like a nose bleed) have been described, but are uncommon.
- Synthetic absorption matrix [SAM] nasal strip: Nasal absorption is performed by manoeuvering a strip of synthetic absorptive matrices (SAM) up the lumen of the nostril, avoiding rubbing against

the nasal mucosa. The outside of the nose is then pressed with a finger to cause apposition of the SAM against the mucosa. The procedure may tickle slightly but is painless. Absorption will be performed for 30 seconds. No complications have been described.

- Stool sample: a stool sample will be collected from nappies. This will cause no discomfort.
- Urine sample: a urine sample will be collected by putting cotton wool/gauze in the nappy or from a urine bag. If the child has urinated the wet cotton wool/gauze will be squeezed into a tube. If a urine bag is used there can be mild discomfort or skin irritation when removing the bag.
- Buccal swab: a swab will be introduced into the mouth and will be gently rubbed and rotated against the inside of the cheek for ~30 seconds. This is a non-invasive technique. The procedure can cause a brief moment of discomfort, however, the duration of this procedure is short. It will be performed by trained personnel. No complications have been described.

The testing of samples is intended solely for research and not diagnostic purposes and therefore is not a substitute for a clinical appointment. Analysis of samples may not be done in a timely fashion to be useful clinically. In the case of an incidental finding of a possible abnormality, the results will be discussed with the clinical team at the site where the participant was recruited. Where the participant's ongoing care is in a local hospital not participating in the study the investigator will inform the appropriate clinical team. The clinical team will discuss implications with the parent/guardian/legally authorised representative and further investigations will be arranged as necessary.

This study is group related and can only be done in this patient group because severe RSV disease is commonly seen in infants and very rare in older children and adults. It is therefore necessary to perform this in subjects belonging to these groups.

Benefits of participating in the study: There are no particular benefits to participating in this study, apart from knowing knowledge obtained from it may benefit other patients in the future.

## 5.5. Compensation for injury

Due to the type of study, observational with non-invasive diagnostic procedures without complications (as previously described), no adverse or serious adverse events are to be expected and participating in the study is with minimal risks. Therefore, we request dispensation from the statutory obligation to provide insurance.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

# 6. Administrative aspects, monitoring and publication

## 6.1. Handling and storage of data and documents

Data will be stored in a cloud-based database. Data will be anonymized before they enter the database. Each subject will receive a unique identification number, which cannot be traced back directly to the subject. The study team will keep a subject identification code list to trace data to an individual subject, if necessary. Data will be kept 15 years. The handling of personal data will be in compliance with the Dutch Personal Data Protection Act.

A GCP compliant electronic data capture (EDC) system will be used to guarantee a correct, complete and consistent data collection. Web-based case report forms will be developed and implemented on the EDC system. By using comprehensive data validation checks within these forms, only data of high quality can be submitted to the study database. The forms, integrated into the EDC system, can easily be accessed by a standard web browser.

The system meets all GCP guidelines for electronic data collection in terms of protecting data integrity and securing the information collected. This means, among other things, that users will get a role based access to the system after they have logged-in using their own username and password. The system will log all data entry steps with timestamps, update reasons and user information. The role based access to the system will avoid unauthorised data access and prevents that users perform actions that they are not allowed to do. Data from the EDC system will be transferred over the internet using secured data communication protocols. Data will be stored automatically and regularly back-ups will make sure that data never will be lost. Databases and web servers will be hosted in data centers that meet the highest possible security requirements.

Data will be shared within the PROMISE consortium with partners inside and outside the EU for analysis of results.

Human materials will be stored in the central biobank of UMC Utrecht. Materials will be anonymized before they enter the biobank with the same unique identification number, which cannot be traced back directly to the subject. Human materials will be stored indefinitely. Permission for storage and usage of the samples for further research about viral and airway infections according to the biobank regulations is asked separately in the PIF/IC. If no permission is given for storage in the biobank, samples will be stored for 15 years.

## 6.2. Monitoring and quality assurance

On-site monitoring will take place according to the NFU (Nederlandse Federatie van Universitaire Medisch Centra)-guideline “Kwaliteitsborging van mensgebonden onderzoek

2019” by the appointed monitor. This study is classified as negligible risk. Monitoring will take place to assure the quality and validity of the research data. The monitor will perform source data verification on the research data by comparing the data entered into the CRF with the available source documentation and other available documents. Source documents are defined as the patient’s hospital medical records, clinician notes, laboratory print outs, digital and hard copies of imaging, memos, electronic data etc.

The monitor will verify the following items: Patient flow (inclusion speed and dropout rate);

Informed consent forms (presence, dates, signatures); Informed consent process, Trial

Master File and Investigator Files (presence of all documents), in-/exclusion criteria (using source documents). After each control the monitor will send a written report to the sponsor (including a summary; quality assessment; summary of findings, deviations and shortcomings; possible solutions

to warrant compliance with the protocol; final conclusion).

## 6.3. Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion. All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

## 6.4. Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

## 6.5. Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

## 6.6. Public disclosure and publication policy

Results of this research will be disclosed unreservedly.

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# ANNEXES

## ANNEX I. ReSViNET score

| | Item | 0 points | 1 points | 2 points | 3 points |
|---|---|---|---|---|---|
| 1 | **Feeding intolerance** | **No** | **Mild** Decreased appetite and/or isolated vomits with cough. | **Partial** Frequent vomits with cough, rejected feed but able to tolerate fluids sufficiently to ensure hydration. | **Total** Oral intolerance or absolute rejection of oral feed, not able to guarantee adequate hydration orally. Required nasogastric and/or intravenous fluids |
| 2 | **Medical intervention** | **No** | **Basic** Nasal secretions aspiration, physical examination, trial of nebulized bronchodilators, antipyretics. | **Intermediate** Oxygen therapy required. Complementary exams were needed (chest X-rays, blood gases, hematimetry. . .). Maintained nebulized therapy with bronchodilators. | **High** Required respiratory support with positive pressure (either non-invasive in CPAP, BiPAP or high-flow O2; or invasive through endotracheal tube). |
| 3 | **Respiratory difficulty** | **No** | **Mild** Not in basal situation but does not appear severe. Wheezing only audible with stethoscope, good air entrance. If modified Wood Downes, Wang score or any other respiratory distress score is applied, it indicates mild severity. | **Moderate** Makes some extra respiratory effort (intercostal and/or tracheosternal retraction). Presented expiratory wheezing audible even without stethoscope, and air entrance may be decreased in localized areas. If modified Wood Downes, Wang score or any other respiratory distress score is applied, it indicates moderate severity. | **Severe** Respiratory effort is obvious. Inspiratory and expiratory wheezing and/or clearly decreased air entry. If modified Wood Downes, Wang score or any other respiratory distress score is applied, it indicates high severity. |
| 4 | **Respiratory frequency** | **Normal** < 2 m: 40–50 bpm 2–6 m: 35–45 bpm 6-12m: 30–40 bpm 12-24m:25–35 bpm 24-36m: 20–30 bpm | **Mild or occasional tachypnea** Presented episodes of tachypnea, well tolerated, limited in time by self-resolution or response to secretion aspiration or nebulization. | **Prolonged or recurrent tachypnea** Tachypnea persisted or recurred despite secretion aspiration and/or nebulization with bronchodilators. | **Severe alteration** Severe and sustained tachypnea. Very superficial and quick breath rate. Normal/low breath rate with obvious increased respiratory effort and/or mental status affected. Orientative rates of severe tachypnea: < 2 m: > 70 bpm 2–6 m: > 60 bpm 6-12m: >55 bpm 12-24m: >50 bpm 24-36m: >40 bpm |
| 5 | **Apnea** | **No** | | | **Yes** At least one episode of respiratory pause medically documented or strongly suggested through anamnesis. |
| 6 | **General Condition** | **Normal** | **Mild** Not in basal situation, child was mildly uncomfortable but does not appear to be in a severe condition, not impress of severity. Parents are not alarmed. Could wait in the waiting room or even stay at home. | **Moderate** Patient looks ill, and will need medical exam and eventually further complementary exams and/or therapy. Parents are concerned. Cannot wait in the waiting room. | **Severe** Agitated, apathetic, lethargic. No need of medical training to realize severity. Parents are very concerned. Immediate medical evaluation and/or intervention were required. |
| 7 | **Fever** | **No** | **Yes, mild** Central T < 38.5°C | **Yes, moderate** Central T > 38.5°C | |

(m = months)

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