WHO STRATEGY FOR GLOBAL RESPIRATORY SYNCYTIAL VIRUS SURVEILLANCE PROJECT BASED ON THE INFLUENZA PLATFORM

-- Revised based on outcomes of the pilot phase --

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WHO Strategy for the Global Respiratory Syncytial Virus Surveillance based on Influenza Surveillance

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Contents

Abbreviations

ARI	Acute Respiratory Infection
CDC	Centers for Disease Control and Prevention, Atlanta
Ct	Cycle threshold
GIP	Global Influenza Programme
GISRS	Global Influenza Surveillance and Response System
ICD	International Classification of Diseases
ILI	Influenza-like Illness
NIC	National Influenza Centre
NICD	National Institute for Communicable Diseases
PHE	Public Health England
QCMD	Quality Control for Molecular Diagnostics
RSV	Respiratory Syncytial Virus
rRT-PCR	real-time reverse transcription polymerase chain reaction
SARI	Severe Acute Respiratory Infection
United Kingdom	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
WGS	Whole Genome Sequencing
WHO	World Health Organization

WHO regions:

AFR	African Region
AMR	Region of the Americas
EMR	Eastern Mediterranean Region
EUR	European Region
SEAR	South-East Asia Region
WPR	Western Pacific Region

1. Introduction

Respiratory syncytial virus (RSV) has long been recognized as an important respiratory pathogen that often causes severe disease and mortality, particularly in very young children but also in other age and at-risk groups. The global burden of RSV-associated acute lower respiratory infections is estimated at 33 million annually, resulting in more than 3 million hospitalizations and 59 600 in-hospital deaths in children aged under 5 years. In infants under 6 months, RSV-associated acute lower respiratory infections account for about 1.4 million hospitalizations and 27 300 in-hospital deaths [1]. Many countries have recognized the importance of this pathogen and have established surveillance of RSV in certain settings.

WHO has conducted global surveillance of influenza for more than 60 years through a network of laboratories known as the Global Influenza Surveillance and Response System (GISRS). This long-established, well-functioning platform offers a cost-effective opportunity to leverage existing capacity to conduct surveillance for RSV without disturbing ongoing influenza surveillance. While there are differences in the epidemiology of influenza and RSV disease, the requirements for surveillance overlap, including sentinel sites, specimen source, laboratory diagnostics and personnel. For both infections the risks among children are of special interest.

WHO is committed to developing a surveillance system for RSV using the GISRS platform. In the long term, global RSV surveillance will provide a continuous, comprehensive and updated understanding of the epidemiology of this virus and the diseases it causes. Of interest are seasonal variations in RSV disease patterns in different countries and geographical regions, as well as the health-care burden due to RSV disease. Most importantly, RSV surveillance will help identify at-risk groups that will benefit most from immunization once vaccines become available. The RSV surveillance platform has the potential to provide a platform to evaluate vaccine impact following RSV vaccine introduction. RSV surveillance data can be used by health officials and decision-makers for evidence-based policy making.

Over a period of 3 years (2016-18), with support from the Bill & Melinda Gates Foundation and countries, appropriate and feasible processes for RSV surveillance with GISRS were established and evaluated in the RSV surveillance pilot. To achieve this, WHO regional offices identified two or three countries to participate in the pilot in each of the six regions where influenza surveillance was already established. Each country assigned RSV focal point representatives for laboratory and epidemiological aspects, and activities were conducted at selected laboratories and sentinel sites. The pilot was designed not to affect existing national surveillance systems; however, it was expected that national systems may benefit from the experiences and results of the pilot. Fourteen countries successfully participated in the pilot to evaluate whether RSV surveillance could be built on the well-established influenza surveillance conducted by the GISRS.

Countries used the extended criteria (not requiring the existence of fever) to existing SARI and ILI case-definitions to collect respiratory specimens from patients in all age-groups for the detection of RSV. Specimens were tested using standardized PCR assays for RSV. Most laboratories used a validated rRT-PCR assay developed and distributed by the CDC Atlanta. Laboratories using in-house detection or commercial assays were required to participate along with the other laboratories in an external quality assurance assessment for the molecular detection of RSV. The WHO FluMart data platform was adapted to receive case-based RSV laboratory and clinical data. At a meeting in Bangkok in October 2018, the outcomes of the two years of the pilot were reviewed, experiences exchanged, and lessons learnt discussed. The important outcomes from the pilot were:

1. Establishment of reference protocols for RSV detection
2. Establishment of the WHO RSV EQA 2019 for detection and typing

3. Testing the feasibility of leveraging the GISRS for RSV surveillance with marginal incremental costs without any significant adverse impact on influenza surveillance
4. Evaluation of the extended SARI and ARI case definition for detection of RSV infection
5. Assessment of seasonality of RSV epidemics in the participating countries

The meeting concluded with the decision to continue RSV surveillance for a further three years with the expansion of surveillance to low and middle-income countries (LMICs).

2. WHO RSV Surveillance – extension phase

The output from the pilot project establishes a solid RSV surveillance strategy with evidence-based standards and a tested mechanism for RSV surveillance based on the GISRS. The 3-year extension phase (Nov 2018 – Oct 2021) aims to consolidate the achievements of the original investment and proposes to (a) enhance the surveillance in infants and young children, (b) focus on the more severe disease requiring hospitalization, (c) expand virologic monitoring to differentiate virus types and to identify genetic groups, and (d) generate a better understanding of the seasonality, age groups at risk and disease burden among young children, particularly in LMICs representative in all WHO Regions.

Based on the key outcomes from the original pilot project, the extension phase (phase II) proposes to

1. Prioritize RSV surveillance in children less than two years of age
2. Focus on severe RSV disease that required hospitalization
3. Extend RSV surveillance with priority to Gavi-eligible countries and/or likely early recipient countries for RSV vaccines for deployment in national EPI programs
4. Implement methodologies to extrapolate RSV-associated hospitalization burden from routine surveillance
5. Develop considerations for ICD codes-based surveillance for RSV
6. Develop laboratory and surveillance guidance for RSV typing and genetic characterization
7. Enrich the genetic resource of RSV viruses in publicly accessible database(s), and
8. Extend the global RSV surveillance over at least three more seasons to better understand the epidemiology and global circulation of RSV strains.

Adjustments to Phase II include modifications of profiles of national and reference laboratory activities, and amendment in the sampling strategy (Table 1).

Table 1: Activities of participating countries and reference laboratories in Phase I and II

	Participating countries	Reference laboratories
Pilot Phase	• 14 countries • RSV detection all year round • 1000 specimens per year • all age-groups included	• standardized molecular detection of RSV in pilot countries • quality assurance of laboratory performance • technical support for countries • reagent support
Phase II	• 22 countries • RSV detection and typing all year round or if seasonality well defined then sampling during season • 400 specimens per year • age-restricted to <2-year-olds • periodic shipment of specimens to reference laboratories	• technical support for countries • standardized molecular detection and typing protocols • oversee preparation of the WHO RSV EQA 2019 • share protocols for sequencing between reference laboratories • perform genetic sequencing and analysis on specimens received from participating laboratories, and share results • select recent RSV isolates to create reference standards • establish genetic sequencing standards and information management • establish guidelines for selecting and sharing materials from participating laboratories

Countries in Phase II will work more closely with one of the reference laboratories of their choice for support in standardization of typing methodologies and sequencing of RSV viruses. Reference laboratories will take the lead in establishing sequencing protocols. In the first year of phase II, reference laboratories will establish protocols and a framework for sampling for sequence analysis using viruses collected during the pilot phase by countries (Table 2). In the second year, reference laboratories will complete the standardization of RSV sequencing protocols.

Table 2: Roles and responsibilities of laboratories participating in Phase II of RSV surveillance

Participating countries	Reference laboratories
• Participate in WHO RSV EQA 2019 • Test appropriate clinical specimens • Report results to FluMart regularly and timely • Report results of typing (RSV-A or RSV-B) • Send selected specimens to reference laboratories for genetic sequencing and virus isolation • Maintain internal quality assurance programs • Observe national regulations for sample and data sharing • Comply with national import and export restrictions	• Select materials for WHO RSV EQA 2019 • Exchange materials between reference laboratories and develop protocols for RSV typing and sequence characterization • Share sequence from selected viruses to compare informatics pipelines • Provide feedback results to countries • Work with EQA provider • Participate in WHO RSV EQA 2019 • Recommend nomenclature for RSV

This document presents WHO's strategy for leveraging the existing capacities of the GISRS network for RSV surveillance without compromising influenza surveillance. It is intended for use by the GISRS network, national influenza surveillance systems participating in the WHO global RSV surveillance, and international entities interested in RSV surveillance.

3. Objectives of the RSV surveillance

RSV surveillance is to be built on the GISRS platform; identifying ways in which existing surveillance criteria can be expanded to meet the needs for RSV surveillance without negatively affecting influenza surveillance. The surveillance will provide information on how well case definitions that do not require fever, capture RSV disease compared to those that require fever.

Primary objectives of the RSV surveillance are to:

1. establish the feasibility of RSV surveillance built on the GISRS platform for future global expansion
2. evaluate the performance of the case definition for surveillance of RSV in different age groups among those aged <2 years
3. ascertain RSV seasonality patterns in different countries and geographical regions
4. improve knowledge of the RSV-associated hospitalization burden
5. determine age and at-risk groups among those aged <2 years, for severe RSV disease
6. build evidence that would enable countries to make informed policy decisions around introduction of RSV preventive products such as vaccines and monoclonal antibodies

Secondary objectives of the RSV surveillance are to:

1. standardize laboratory procedures for RSV detection, typing and develop updated external quality assurance for the molecular detection and typing of RSV
2. develop a strategy for genetic characterization of RSV
3. establish FluMart as a platform for reporting RSV data
4. enrich the genetic and bioinformatics resource of RSV in publicly accessible database(s)

5. report surveillance statistics to raise awareness and provide evidence to inform global and national health policy decisions
6. assess additional costs incurred through the implementation of RSV surveillance (including additional clinical, epidemiological and laboratory costs)
7. assess the performance of proposed sampling strategies for RSV detection
8. define the role of RSV reference laboratories within a global RSV surveillance program
9. document the level of GISRS staff acceptance of additional procedures and reports, and of potential negative impacts on existing influenza surveillance
10. contribute to the development of a future platform for a broader respiratory virus surveillance, and
11. provide a potential platform for special studies such as:
    a. disease burden studies in different age and at-risk groups
    b. vaccine studies (including vaccine impact studies and studies evaluating any changes in age incidence after introduction of vaccines)
    c. cost effectiveness of vaccines and other interventions, and
    d. studies of the spatial-temporal evolution of RSV strains by type and genotype, and the possible relationship between evolution of strains and vaccine effectiveness

The RSV surveillance is not expected to provide:

1. diagnostic services
2. population-based estimates of RSV disease burden
3. data on economic burden due to RSV disease, and
4. data that will give a complete and detailed clinical description of RSV disease in all age and at-risk groups

4. Countries participating in the RSV surveillance

The WHO GIP and the six WHO regional offices jointly invited two to three countries in each WHO region to participate in the RSV surveillance pilot. The countries have a functioning sentinel influenza surveillance system and National Influenza Center (NIC) for GISRS. The countries perform influenza surveillance based on a well-structured sentinel surveillance network, regularly share influenza data with WHO and have included various RSV detection protocols in their routine activities. Several additional countries will be invited to participate in the extension phase. The selection criteria for countries are:

1. Well-functioning sentinel surveillance for influenza
2. National Influenza Center with existing capacity for molecular diagnostics
3. Regular sharing of SARI / ILI data on FluNet and FluID
4. Regular sharing of influenza virus isolates / respiratory specimens with WHO Collaborating Centers for genetic and antigenic characterization
5. Eligible to receive vaccine support from Gavi Alliance
6. Geographic coverage of the region
7. Well-functioning and engaged programs for delivering antenatal care and Expanded Program for Immunization
8. Ministry of Health approval and commitment for participating in the RSV surveillance

In addition, other interested and well-resourced countries outside of the above criteria, could potentially join and contribute to the network with their own resources. The contact information and list of participating countries are given in Appendix A and Appendix B respectively. In addition, four reference laboratories with extensive

experience in RSV diagnostics and research have agreed to assume a reference function and provide support to the national laboratories. The four reference laboratories are:

1. The Centers for Disease Control and Prevention (CDC), Atlanta
2. Public Health England (PHE), London
3. National Institute for Communicable Diseases (NICD), Johannesburg, and
4. Victorian Infectious Diseases Reference laboratory (VIDRL), Melbourne

5. Case definitions for RSV surveillance

The SARI case definition [2] does not meet optimal criteria for RSV surveillance. SARI case definitions require that the patient has a fever of 38 °C or more when seen by a health-care worker, or a history of fever during the previous 10 days. Children with RSV infections often present without fever or history of fever. Thus, the case definition needs to be expanded beyond SARI to include cases that do not have fever or a history of fever. Hospital-based RSV surveillance will use the extended definition of severe acute respiratory infection (SARI) (Table 3).

ARI – Acute Respiratory Infection; ILI – Influenza-like Illness; RSV – Respiratory Syncytial Virus; SARI – Severe Acute Respiratory Infection;

A significant fraction (often >50%) of young children and elderly patients infected with RSV present without fever [3-5]. Therefore, inpatient RSV surveillance will include patients, with or without fever (reported or measured), who otherwise meet the SARI case definition. RSV infection commonly presents with other signs in the 0–<6 months age group. Therefore, hospital-based inpatient RSV surveillance in children aged 0–<6 months will additionally include those who present with apnea or sepsis (or both):

Countries implementing ARI surveillance may continue to use the ARI case definition for clinic-based outpatient RSV surveillance. However, countries implementing influenza-like illness (ILI) surveillance may use an extended ILI case definition in place of the ARI case definition for clinic-based outpatient RSV surveillance, to also include those without fever.

Table 3: Case definition for RSV surveillance

	RSV	Influenza
In-patient	***Extended SARI*** - severe (overnight, or more than 24 hours of, hospitalization) AND - acute (onset within past 10 days) AND - respiratory infection (cough or shortness of breath) In infants less than 6 months age, also include - Apnoea (temporary cessation of breathing from any cause) - Sepsis - fever more than 37.5 ^{o}C or hypothermia (body temperature less than 35 ^{o}C) AND - shock (lethargy, fast breathing, cold skin, prolonged capillary refill or fast weak pulse) AND - seriously ill without apparent cause	***SARI*** - severe (hospitalization) AND - acute (onset within past 10 days) AND - history of fever or measured fever of 38 ^{o}C or more AND - respiratory infection (cough or shortness of breath)
Out-patient	***ARI*** - acute (onset within past 10 days) AND - respiratory infection (at least one of cough, sore throat, shortness of breath or runny nose) ***Extended ILI*** - acute (onset within past 10 days) AND - respiratory infection (cough)	***ILI*** - onset within past 10 days AND - measured fever of 38 ^{o}C or more, AND - cough

Countries should consider prioritizing hospital-based inpatient RSV surveillance sites, to target more severe forms of RSV disease.

6. Selection of sentinel hospitals

There is no fixed number of sentinel hospital sites for a country. The aim is to select at least as many sentinel hospitals that will yield the required sample size for surveillance. The more important considerations are that the sentinel hospitals are reasonably representative of the hospitals in the country, and the data collected are of good quality. The underlying principles to consider for selection of sentinel hospitals are [2]:

1. Feasibility, representativeness, commitment and sustainability
2. In general, start with fewer sites that are expected to yield the required sample size among the target age group i.e. children less than 2 years, and expand as necessary only if they function well. It is important to not establish more sites than can be effectively managed, monitored and sustained
3. In general, prefer quality of data over large numbers. Small amounts of good quality data are better than large amounts of poor-quality data

The feasibility and sustainability of a sentinel surveillance site will depend on identification of a champion and leadership motivation, perceived public health benefit and commitment to implement and sustain surveillance with existing resources. Sentinel hospitals will need to develop or leverage the existing influenza surveillance systems for the collection, storage and transport of clinical specimens to the RSV designated national public health laboratory for RSV detection and typing. Sentinel hospitals should have well maintained, up-to-date and complete hospital administrative registers and systems for collecting and regular reporting of clinical data including necessary data management and communications systems.

For inpatient RSV surveillance, a sentinel hospital should ideally serve patients representative of the target age group (<2 years) and all socio-economic groups in the population it serves. Countries may opt to choose either secondary or tertiary care hospitals or a mix of both, as sentinel hospitals. In general, a secondary-care hospital is more likely to serve a well-defined catchment population and patients are more likely to be representative of the general population it serves. Whereas a specialty or tertiary-care referral hospital may serve an undefined catchment population but capture more severe forms of RSV infection. Pediatric wards, pediatric and neonatal intensive care units, emergency rooms in hospitals, as relevant, should be included for inpatient surveillance. For countries with a wide latitudinal spread and significantly different climate zones or socio-economic characteristics, multiple sentinel hospitals may be selected to represent these areas.

7. Case selection and sampling strategy

RSV surveillance should primarily aim to capture severe RSV infections requiring hospitalization. The reason for this is that once RSV vaccines and monoclonal antibodies become available and accessible, their use will primarily be aimed at preventing severe cases and RSV-associated hospitalization.

The burden of severe RSV disease is high in children less than 2 years, and particularly so in infants during their first 3 months of life. Surveillance should be focussed among children less than 2 years, an age group that has often not been well covered by influenza surveillance. Since this is a key aspect of the health-care burden for RSV, it is important to ensure that cases are sampled in this age group. In some settings, ensuring that surveillance covers very young children may require new surveillance sites to be set up in pediatric hospitals. Even in settings where new surveillance sites are not required, it may be necessary to give increased priority to surveillance in this age group.

7.1 Age stratified sample size for RSV surveillance

Each country should aim to collect and test 400 specimens for RSV annually. A sample size of 400 would usually allow an overall RSV prevalence of 30% to be detected in children aged <2 years, with a 95% confidence interval of ± 5% [1].

The 400 specimens per year should constitute 100 in each of four age groups as follows:

- 0 – <3 months
- 3 – <6 months
- 6 – <12 months

[1] Sample size calculator – http://sampsize.sourceforge.net/iface/.

- 12 – <24 months

The laboratory focal point should monitor the submission of specimens on a weekly basis to ensure that the required sample sizes for each age group are met as above.

7.2 Case selection

The underlying principles [2] to consider for selection of patients from the sentinel hospitals are:

- Testing all probable RSV cases meeting the extended SARI case definition at a sentinel site may be beyond sample size requirement. A strategy should be clearly defined to select patients for testing that are representative of all potential eligible patients at the sentinel site meeting the case definition
- The sampling strategy may vary depending on the local context and surveillance practice in each country. Notwithstanding, selection of patients for testing should be based on some form of random sampling to minimize selection bias.
- Convenience or ad-hoc selection of patients can introduce significant bias and generate unreliable estimates of disease burden and should be avoided.

Place of recruitment

For inpatient surveillance, case selection and specimen collection should ideally occur at the place where the decision to admit the patient is made e.g. when a patient attends an outpatient clinic or an emergency room of a hospital before admission to the ward or intensive care unit. This is to maximize the possibility that community-acquired RSV infections are captured, and hospital-acquired RSV infections are excluded. If case selection of inpatients prior to admission is not practical, an acceptable approach would be to screen the hospital and ward admissions register for potential patients that meet the RSV case definition.

Day(s) of recruitment

If the number of daily admissions is low, hospital and ward registers may be screened daily for new admissions from the previous day, to identify potential cases for recruitment and collecting specimens. If the daily admission case-load for the hospital is high, then hospital and ward registers may be screened for new admissions from the previous day, only on certain pre-determined day/s of the week, to identify potential cases for recruitment and collecting specimens. An adjustment factor may need to be applied if screening happens on weekends or weekdays with atypical hospital admission load. In all events, patients who have been hospitalized for more than 24 hours should not be recruited to minimize the possibility of capturing hospital-acquired RSV infection.

Sampling approach

A true simple random sampling scheme is most representative but may not be feasible in surveillance settings. Admission logs may help in determining admission patterns. The most practical sampling scheme is likely to be a systematic random sampling that does not involve health-care provider decision to test patients (other than to determine eligibility for testing), and that covers different times of the day and different days of the week. Sentinel surveillance sites can adopt a sampling approach that is locally most suitable and feasible to conduct the RSV surveillance so that sampling quotas are reached. Some practical examples for selection of patients are illustrated in appendix C. The approach may vary from site to site; however, a few specific recommendations are relevant to all sites:

For countries with uncertain RSV seasonality

- Collect a total of 8 specimens per week all year-round as follows

- Collect 2 specimens each in 0 - <3m, 3 - <6m, 6 - <12m; and 12 - <24m, for a total of 8 specimens each week throughout the year

For countries with known RSV seasonality

- Collect a total of 12 specimens per week during the RSV season as follows
  - Define the start and end date of the RSV season based on prior seasonality evidence
  - Collect 3 specimens each in 0 - <3m, 3 - <6m, 6 - <12m; and 12 - <24m for a total of 12 specimens each week, at least 4 weeks prior to the start date of the RSV season
  - Continue collection for at least 4 weeks after the known end date of the RSV season and then stop collection

The RSV designated national public health laboratory focal point should monitor the specimens received from each sentinel site on a weekly basis and report back to the sentinel hospitals to adjust patient recruitment for each age group as necessary. The laboratory and epidemiology focal point should monitor the progress of patient recruitment to ensure that the sample size requirement is met for all the age groups for each calendar month and at the end of the calendar year and provide timely feedback to the sentinel hospitals to adjust the sampling strategy as necessary. The epidemiology focal point should monitor patient recruitment at sentinel hospitals and ensure that the cases are selected for RSV testing adhere to the sampling strategy.

Sampling strategies should not specifically exclude individuals belonging to special at-risk groups such as children with pre-existing illness. When the number of specimens submitted exceeds the quota for the week, the laboratory should maintain the weekly *average* testing, unless there are additional resources available to test the additional specimens (generally, resource support for reagents from WHO is limited to 400 tests annually). Each country should select its sampling strategy based on the local context and practice and resources available. However, whichever sampling strategy is selected, it should be one that minimizes bias and must be well documented, implemented and monitored.

Decision process flow at sentinel hospital for patient recruitment and specimen collection (Figure 1)

- Check whether the patient meets the SARI or extended SARI case definition
  a) Confirm that child is less than 2 years age
  b) Ask mother or check case-sheet if child had acute onset cough and / or difficulty in breathing; if child is less than 6 months, also check case-sheet if child had temporary apnea or signs of sepsis
     i) If yes, enrol patient
        (1) Check for fever, other clinical data required to fill Specimen Submission Form
        (2) Collect specimen
        (3) Send specimen and RSV specimen submission form to laboratory
     ii) If child does not meet above criteria, proceed to the next eligible child

Decision process flow at laboratory for specimen testing and reporting of results

- Check eligibility for testing
  a) Check RSV specimen submission form to confirm age group of child and presence or absence of fever
  b) If fever marked as present on the specimen submission form, test specimen for both RSV and influenza
     i) Report RSV and influenza results to RSV database on FluMart
     ii) Report influenza results separately to FluNet on FluMart
  c) If no fever, test specimen for RSV only
     i) Report RSV results to RSV database on FluMart
  d) Do not report influenza results (even if available) to FluNet database for specimens from afebrile patients

- Surveillance specimens received by the laboratory more than the required weekly sample size, may be tested for RSV and / or influenza based on available resources
- Laboratory should monitor the number of specimens tested in each of the four age groups on a weekly basis and adjust to ensure that all age groups are tested as required

7.3 Seasonality

Seasonality can be assessed by different analytic approaches (weekly proportion of specimens testing positive[1], average epidemic curve, moving epidemic method, etc.). To statistically ascertain the onset of the season prospectively, using the 10% threshold for the weekly proportion of positive cases approach, would require about 300 specimens to be tested in a week. Given resources that limit testing of up to 8 specimens per week, the WHO RSV surveillance allows the start and end of a season to be defined only in retrospect using data accumulated over the year.

8 Laboratory testing [2]

8.1 Collection of clinical specimens

1. The optimal type of clinical specimens for the detection of RSV depends on the age of the patient.
2. For infants and young children, a nasal swab taken from the mid-turbinate of each nostril has been found to yield high recovery of respiratory viruses [3,4] [5](Appendix D).
3. Nasopharyngeal aspirates may also be collected particularly from young children.
4. Collection of specimens must be done using flocked nylon swabs. Cotton-tipped, calcium alginate or wooden stick swabs should not be used as they may contain substances that inactivate viruses and inhibit PCR testing.
5. In severe hospitalized cases, lower respiratory specimens may also be collected where indicated. These include tracheal aspirate and bronchoalveolar lavage.
6. Specimens should ideally be collected within three days of the onset of clinical symptoms.

For each specimen collected, a corresponding RSV data collection form must be duly completed. The forms must be sent in a separate envelope to the laboratory along with the specimen.

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[1] http://www.cdc.gov/rsv/research/us-surveillance.html

[2] WHO Manual for the laboratory diagnosis and virological surveillance of influenza.2011 http://apps.who.int/iris/bits.tream/10665/44518/1/9789241548090_eng.pdf, accessed 27 June 2016

[3] Pan-American Health Organization. Influenza and other Respiratory Viruses. Videos: Sample Collection. 2015. http://www.paho.org/hq/index.php?option=com_content&view=article&id=7918%3A2012-videos-sample-collection&catid=2407%3Ainfluenza-respiratory-viruses&Itemid=39737&lang=en, accessed 27 June 2016

[4] Centre for Disease Control and Prevention. Influenza Specimen Collection. https://www.cdc.gov/flu/pdf/freeresources/healthcare/flu-specimen-collection-guide.pdf, accessed 27 June 2016

[5] Nasal Mid-Turbinate (NMT) Specimen Collection Steps. https://www.cdc.gov/coronavirus/2019-ncov/downloads/lab/NMT-Specimen-Collection-Infographic.pdf

8.2 Transport and storage of clinical specimens

Storage of clinical specimens at the site of collection and transport to the laboratory should follow guidelines as for influenza. Specimens should be placed at 4 °C immediately after collection and promptly transported to the laboratory. If the specimens cannot be processed within 48 hours, they should be kept frozen at or below -70 °C. Repeated freezing and thawing must be avoided. For storage and transport, triple packaging should be used in keeping with international and national biosafety recommendations which must be adhered to [1,2]. Specimens should be aliquoted in 3-4 vials of approximately 0.5 ml each on arrival at the laboratory.

[1] Pan-American Health Organization. Influenza and other Respiratory Viruses. Videos: Specimen Packaging. 2015. https://www.paho.org/hq/index.php?option=com_content&view=article&id=7917:2012-videos-proper-use-ppe&Itemid=40295&lang=en accessed 14 June 2019

[2] World Health Organization. Guidance on regulations for the transport of infectious substances 2017–2018. 2017. https://www.who.int/ihr/publications/WHO-WHE-CPI-2017.8/en/ accessed 14 June 2019

8.3 Algorithm for RSV specimen collection and testing

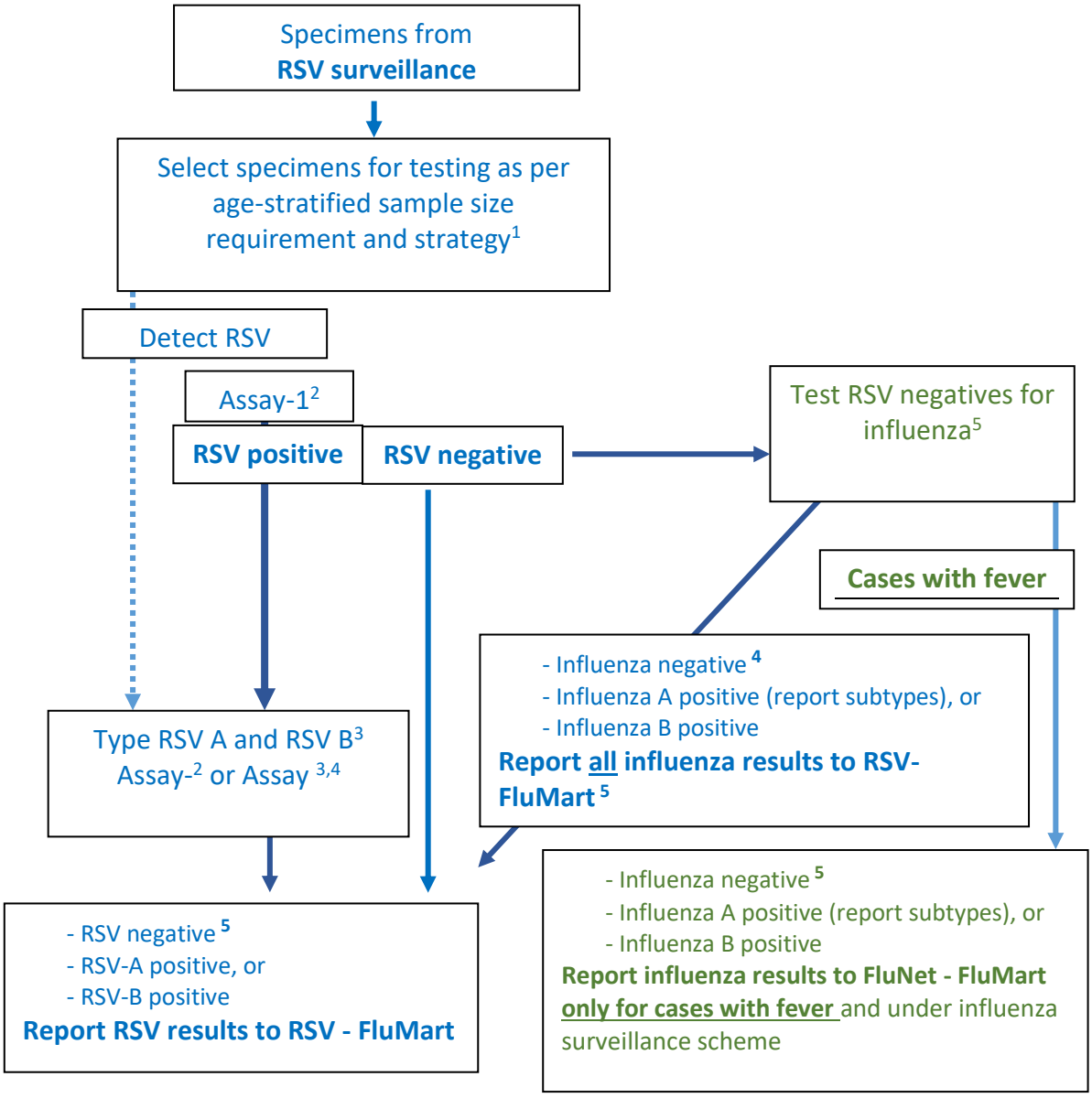

Figure 1: Algorithm for selection of specimens for testing and reporting

[1] Stratification of specimen selection. Section 8.4
[2] Assay 1: CDC Real-Time RT-PCR Assay for Respiratory Syncytial Virus (RSV)- ver.003.2016-Section 8.5
[3] Assay 2a: VIDRL Duplex Real-Time RT-qPCR for the Typing of RSV using the SensiFAST™ Probe Lo-ROX One-Step Kit (Bioline, London, England)
Assay 2b: VIDRL Duplex- Real-Time RT-qPCR for the Typing of RSV using the TaqMan® Fast Virus 1- Step Master Mix Kit (ABI, Life Technologies, Waltham, MA) Section 8.5
[4] Assay 3: CDC Respiratory Syncytial Virus Real-time RT-PCR Panel – (RSV_RUO-01)
[5] RSV negative specimens are recommended to be tested for influenza. (Section 8.5). Laboratories may test for SARS-CoV2 if RSV and influenza are negative.
..... ▶ Direct multiplex detection and typing using Assay 2 or 3- Section 8.5

Each laboratory will determine the algorithm best suited for laboratory testing of both RSV and influenza without compromising influenza testing. Some laboratories may opt to test in parallel for both influenza and RSV once sample selection of RSV has been completed.

8.4 Laboratory techniques for the detection and typing of RSV

Four laboratories have been identified as RSV reference laboratories for Phase II. These include CDC Atlanta, PHE London, NICD Johannesburg and VIDRL Melbourne. The four reference laboratories will provide technical support to participating countries. These reference laboratories will also provide the rRT-PCR protocols for the detection of RSV (RSV detection protocol by CDC, USA) and typing (RSV A and RSV B multiplex protocol by VIDRL). WHO will supply the required reagents to participating countries for both the detection and typing of RSV along with extraction kits and enzymes.

Real-time, reverse transcription polymerase chain reaction (rRT-PCR) for RSV is the gold standard test to be used. Countries have the option of utilizing an established CDC USA and ViDRL rRT-PCR assays for the detection and typing of RSV. These assays can be used to detect RSV in clinical specimens. Alternatively, countries can use their existing rRT-PCR assay laboratory developed assay or commercial assay for the detection and typing of RSV provided they have validated their assay under the guidance of reference laboratories through external quality assurance program (Section 8.6).

Testing Strategies

The recommended test algorithm is detection first using the CDC RSV rRT-PCR assay followed by typing of RSV positives using the VIDRL or CDC multiplex real-time RT-PCR for the detection of RSV-A and RSV-B. Figure 2

i. Nucleic Acid Extraction
    1. Sample extractions must yield total nucleic acid for detection of both RNA and DNA viruses with sufficient volume to cover all viruses and controls tested. Participating WHO RSV laboratories will be provided with extraction reagents through the supplier QIAGEN (this does not reflect endorsement of any particular company by WHO). Alternate extraction methods may be used provided that the extraction performance is assessed through the detection of Human RNase P (RNP).

ii. Assay 1: CDC Real-Time RT-PCR Assay for Respiratory Syncytial Virus (RSV)- ver.003.2016
    1. Laboratories are required to test selected incoming specimens from sentinel hospitals for RSV according to age group stratification Section 8.4
    2. The CDC RSV detection assay contains Human RNase P (RNP) as an internal control. All clinical samples should be tested for Human RNAse P (RNP) gene to assess specimen quality and extraction performance.
    3. Accurate interpretation of rRT-PCR results requires consideration of all three controls (positive, negative and RNP control). Appendix E.A
    4. RNA from RSV positive specimens should be stored at -70 °C and typed (A/B) in batches (where applicable) according to laboratories operating protocol

iii. Assay 2a: VIDRL Duplex Real-Time RT-qPCR for the Typing of RSV using the SensiFAST™ Probe Lo-ROX One-Step Kit (Bioline, London, England) and

iv. Assay 2b: VIDRL Duplex- Real-Time RT-qPCR for the Typing of RSV using the TaqMan® Fast Virus 1- Step Master Mix Kit (ABI, Life Technologies, Waltham, MA)

Assay 2a and 2b:

1. All RSV positive specimens should be typed
2. The VIDRL assay uses multiplex real-time RT-PCR for both RSV-A and RSV-B detection in a single reaction. The RSV-A probe is labelled with FAM and the RSV-B probe is labelled with VIC. This assay does not contain Human RNase P (RNP) as an internal control hence the recommendation to perform Assay 1 prior to proceeding to Assay 2. Alternatively, laboratories may choose the algorithm of 8.5v below.
   or

v. Assay 3: CDC Respiratory Syncytial Virus Real-time RT-PCR Panel – (RSV_RUO-01)

1. All RSV positive specimens should be typed
2. The CDC Respiratory Syncytial Virus Real-Time RT-PCR Subtyping Panel is intended for the in vitro qualitative detection of human RSV and the identification of RSV A and B subtypes using real-time RT-PCR technology. This assay does not contain Human RNase P (RNP) as an internal control hence the recommendation to perform Assay 1 prior to proceeding to Assay 3.

vi. Use of alternate test algorithms or RSV detection and typing assays

1. The CDC and VIDRL assays and the above are recommended. Some laboratories however may choose alternate algorithms and/or use commercial or laboratory developed assays apart from the CDC or VIDRL assay. In such circumstances laboratories are requested to validated assays internally and externally with the technical support of the RSV Reference Laboratories prior to implementation. Additional RSV multiplex typing assays are under development by CDC & VIDRL and will be shared with laboratories once available.

vii. RSV negative specimens are recommended to be tested for influenza

1. All influenza results from specimens collected for RSV surveillance should be uploaded to the RSV database on FluMart
2. Influenza results from specimens collected from patients with fever should also be reported to FluNet/FluMart database under influenza surveillance.
3. Influenza results from specimens collected from patients without fever (Extended SARI) should not be reported to FluNet/ FluMart.

Refer to Appendix E for details of testing protocols from respective reference laboratories.

8.5 WHO RSV external quality assurance program

All national and reference laboratories are invited to participate in the WHO External Quality Assurance program for the molecular detection and typing of RSV prior to the implementation of laboratory testing. The EQA panel contains 12 unknown specimens of which RSV viruses are selected from recently circulating RSV virus's representative of different geographic regions. The purpose of the RSV EQA is to establish quality standards of all laboratories to detect and type RSV strains.

Laboratories participating in the RSV surveillance should use highly sensitive and specific rRT-PCR methods for detection and typing. Three molecular assays are provided to Phase 2 countries. Assay 1 is the CDC rRT-PCR assay for the detection of RSV contains an internal control and is recommended as the reference assay for detection. The VIDRL rRT-PCR multiplex assays (Assay 2a and 2b) and the CDC RSV rRT-PCR multiplex assay (Assay 3) are recommended for the typing of positive isolates. The WHO RSV EQA 2019 was dispatched to participating countries during the latter half of the 4th quarter of Year 2019. Countries were required to prepare import documents for the RSV EQA panel prior to dispatch. The required turnaround time for reporting of results is within one month of receipt of the RSV EQA panel. Laboratories that achieve suboptimal scores are asked to

review methodologies and implement corrective actions. Recommendations for corrective actions and technical support is provided by the RSV reference laboratories and WHO HQ RSV Technical Team.

8.6 RSV genetic sequencing

The objectives of RSV genomic characterization are to:

1. Characterize representative global viral diversity, particularly in LMICs
2. Better understand the global evolutionary phylogeographic dynamics of RSV
3. Demonstrate the feasibility of large-scale whole genome sequencing of RSV
4. Help to establish a baseline of RSV diversity prior to the introduction of RSV vaccines
5. Source suitable reference viruses for molecular testing and phylogenetic analyses
6. Identify RSV strains with potential resistance to monoclonal antibodies
7. Identify RSV strains with potential resistance to novel antiviral drugs

Retrospective sequencing of specimens from the pilot phase

Prior to sequencing specimens collected during Phase II, RSV positive specimens / isolates collected in the pilot phase will undergo genetic characterization by reference laboratories. The purpose of retrospective sequencing includes:

- Establish procedures for specimen labelling and shipping to RSV reference laboratories from participating countries
- Establish a standard approach to virus nomenclature for future data analysis and management purposes
- Investigate the compatibility of different amplicon-based sequencing methods at the reference laboratories for G gene, F gene or whole genome sequencing
- Establish standards, methods and bioinformatic pipelines for genome assembly and annotation
- Explore the establishment of a platform for reference laboratories to manage and share sequencing outputs with each other and participating countries
- Establish standards for analyses of genetic outputs:
    - Phylogenetic analyses
    - Interpretation of F protein variability
    - Relevance of genetic diversity for detection and typing

Countries from the WHO RSV surveillance pilot phase are invited to select RSV positive specimens collected in the 2016-17 and 2017-18 RSV seasons, and to share positive specimens/isolates with RSV reference laboratories for sequencing purposes. If countries can generate their own sequence data and would like to undertake this task, then these sequences will be added to the sequence data under this project, to make a more comprehensive overall analysis of the RSV genetic variation occurring globally. Countries implementing sequencing should follow the guidelines of the RSV reference laboratories. Outputs from analysis of sequence methodologies will be used to guide the finalization reference standards for RSV Sequencing.

Below is a guideline of selection of positive specimens to be shared with reference laboratories. Each reference laboratory has custom sample submission forms. Laboratories should contact RSV reference laboratories to obtain the electronic version of the respective submission form. An example of the submission form and instructions for VIDRL RSV reference laboratory is displayed in Appendix J.

- 15% of positive specimens/isolates from patients < 2 years
- 30% of positive specimens/isolates from patients 2-5 years of age
- All positive specimens/isolates collected from patients 5-65 years of age
- All specimens collected from patients > 65 years

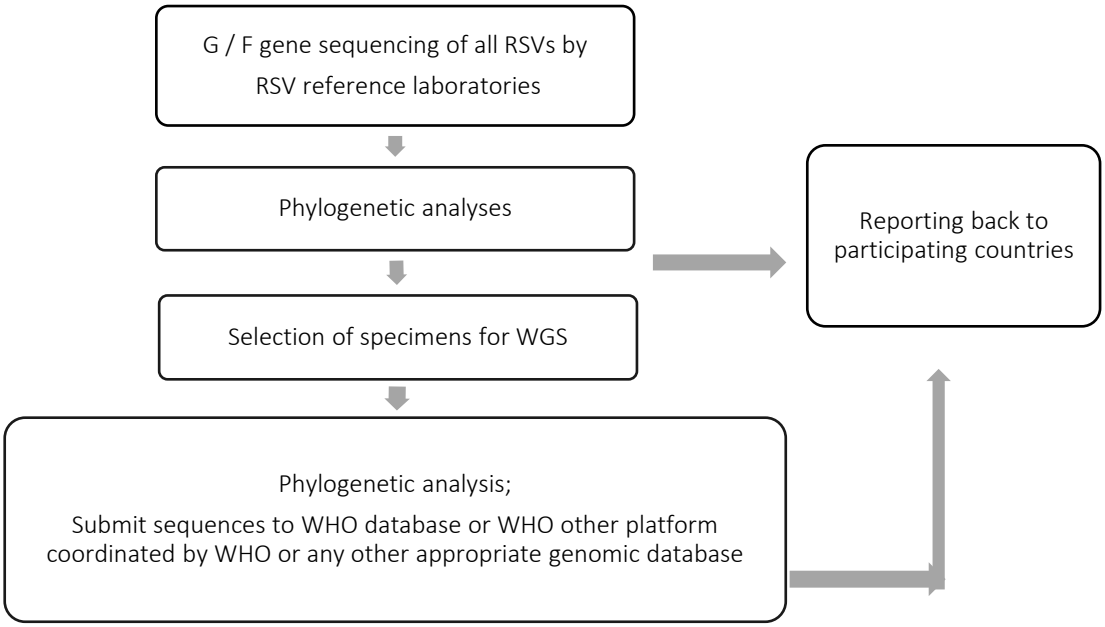

Figure 2: Schema for sequencing of RSVs detected in Phase 1

Sample shipments to reference laboratories (Phase II specimens)

Participating countries are requested to ship 20 RSV positive specimens, with representation of RSV types A and B viruses up to three times a year if possible (at start, middle and end of their RSV seasons, as determined by local data) to the reference laboratory. The criteria for selecting specimens for sequencing prospectively will be reviewed and revised in the second year. If countries can generate their own sequence data and would like to undertake this task, then these sequences will be added to the sequence data that will be generated by reference laboratories, to make a more comprehensive overall analysis of the RSV genetic variation occurring globally. Countries implementing sequencing should follow the guidelines of the RSV reference laboratories in accordance with agreed WHO RSV Sequencing Strategy.

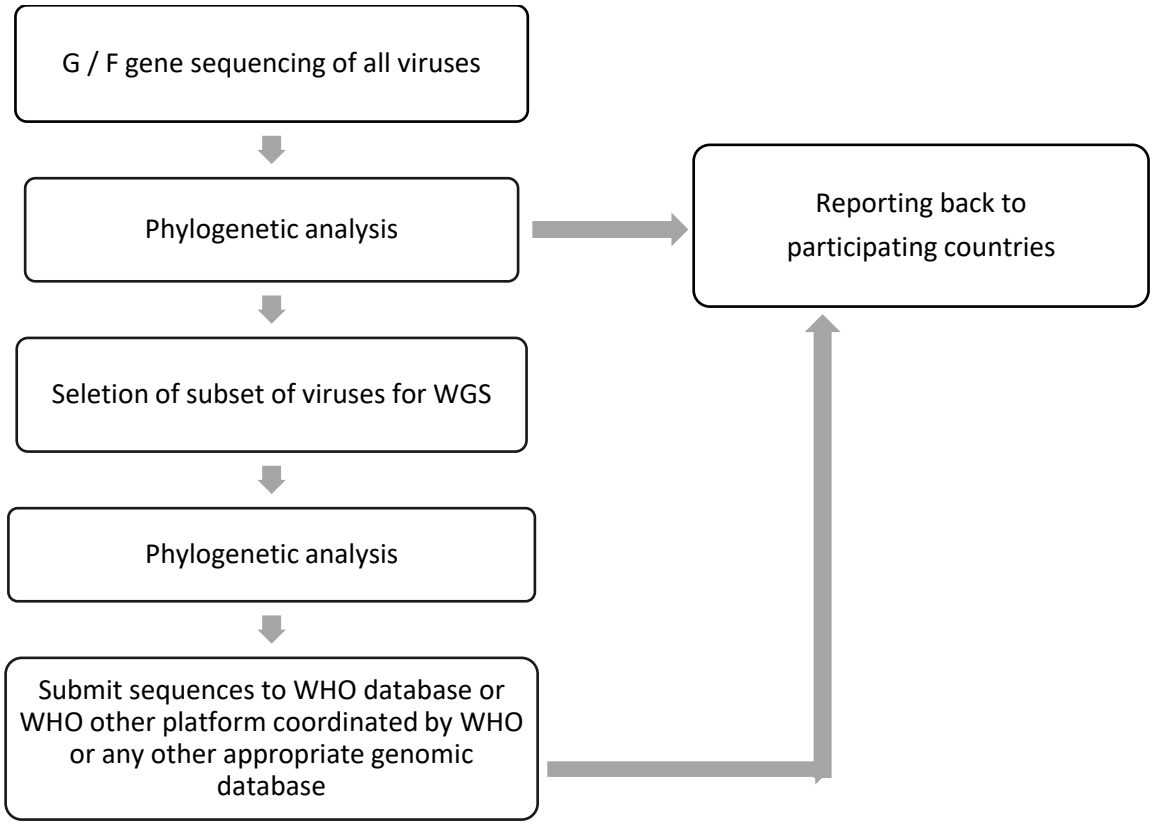

Figure 3: Schema for genetic sequencing of prospective RSVs from phase II

RSV nomenclature

The RSV reference laboratories along with RSV Experts and representatives from participating countries have proposed a basic nomenclature for designating RSV positive clinical samples and isolates. This interim nomenclature will be presented to the RSV community for discussions to reach a consensus on the nomenclature of RSV types and genetic strains. Proposals for nomenclature are under discussion and expected to be finalized in early 2021. .

RSV virus isolation is not a requirement for countries participating in Phase II of the WHO RSV surveillance project. RSV reference laboratories may culture selected isolates from participating countries to establish reference virus standards for genetics analyses and internal standards.

Genetic sequence data management

This component is under development and may change depending on the access and suitability of existing sequence databases such as GenBank, GISAID and others to host RSV sequences. As of 2020 a new RSV sequence database was developed and implemented by GISAID.

Participating laboratories will be able to access sequence data generated from respective specimens submitted. With approval from contributing laboratories, sequence data will also be shared between participating laboratories. Along the progress of the project and with approval from participating laboratories, representative sequences will be uploaded onto public databases. Countries will be informed of any changes to this proposal.

9. Data collection and reporting

The RSV specimen submission form (Appendix F) to be used is revised for the second phase and includes the optimal case-based, clinical, epidemiological and laboratory data that should be collected from sentinel hospitals and laboratories. The case-based clinical data will be assessed to determine the performance of the case definition for RSV infection in different age groups and to identify important clinical predictors for RSV infection. The epidemiological and laboratory data will be monitored for seasonality trends and disease burden.

Laboratory testing of surveillance specimens is not intended for diagnostic purposes or to aid patient care. It is not the intention of Phase II to provide a diagnostic service to submitting health care workers. Sentinel hospitals shall, however, receive laboratory results in keeping with surveillance guidance as established in the participating country.

To meet the objectives of this RSV Surveillance, case-based reporting of epidemiological, clinical information and laboratory results to WHO headquarters is required. Case-based data, as outlined in the RSV specimen submission form, will be submitted along with the specimen to the RSV designated national public health laboratory or National Influenza Centre (NIC) for testing for RSV. Countries are encouraged to adapt their specimen submission form without compromising the minimum information that is required.

Anonymized case-based data, along with the laboratory results, will be uploaded by the designated focal point at the FluMart portal. Countries are expected to upload data on a weekly basis. Queries in uploading and mapping the data should be communicated to the FluMart support team.

The RSV data will be used to generate real-time interactive outputs for age-stratified trends in RSV activity and seasonality. Additionally, the case-based data will be analysed separately to evaluate the most suitable case definition for RSV surveillance. Countries should take responsibility to assure confidentiality of case-based data and for quality control of data collection, management, storage and reporting (including electronic transfer) using locally-accepted procedures. Data access will be restricted to participating countries and WHO.

10. Burden of RSV associated hospitalization

The most common estimates for disease burden from an epidemiological perspective are population-based incidence rates. However, these require resource-intensive special studies implemented in a research mode and are unsuitable for sentinel hospital surveillance platforms. In the absence of resources, from a policy perspective, data on the total number of hospitalizations associated with RSV disease at each surveillance site is useful to indicate the hospital burden of disease at that surveillance site and estimate the proportional contribution of RSV-associated disease episodes to all-cause hospitalization and compare that with hospitalizations due to other diseases. A tiered approach is proposed based on resources and the specific objectives and priorities for the country (Appendix G). It is proposed that at the minimum, all countries collect burden-related data to estimate the age-stratified proportion of respiratory or pneumonia or all-cause hospitalizations due to RSV infection (Tier 1.1, 1.2 in Appendix G). A burden data collection tool will be standardized to collect burden-related data that may be comparable across sites (Appendix H). Countries with additional resources and interest may choose to opt for incidence and population-based burden estimates (Tiers 2 and 3 in Appendix G).

If the surveillance screens and tests all cases meeting the extended SARI case definition, then these should be reported by age group and by month. However, in many surveillance sites, the surveillance will not aim to recruit and test all cases, but only the recommended target number of cases. Not all cases meeting the case definition

will have been recruited and tested; hence, to estimate the total number of RSV-positive cases, this under-detection of RSV-associated disease episodes would need to be corrected for, as shown in the following example.

Example

A hospital-based RSV surveillance site admits 500 cases meeting the extended SARI case definition over a 1-year period in a specific age group. It tests 250 of these and finds that 100 of those tested are positive for RSV. The sampling proportion is therefore 250 / 500 or 0.5. The total number of RSV-positive cases can then be estimated to be about 200 (100 / 0.5) in this age group over the 1-year period.

The total number of RSV-positive cases in a specific age group in a hospital-based RSV surveillance site is thus:

$$\frac{\text{No. of cases identified using extended SARI definition that are RSV positive}}{\text{Proportion of cases identified using extended SARI definition that were tested for RSV}}$$

This equation is based on two assumptions: that the percentage of RSV-positive cases is similar in those who were tested and those who were not tested (during a particular period), and that there is no significant bias in the selection of patients for RSV testing. Because these assumptions are often not fully met, this estimation of the true number of RSV-positive cases can only be an approximation.

To carry out this calculation, the total number of cases meeting the extended SARI case definition in each group needs to be counted through a chart audit. This should be done:

- separately for each surveillance site; and
- separately for the main age groups (i.e. <3mo, 3–<6mo, 6-<12mo, and 12-<24mo).

Ideally, this should be done by calendar month and then aggregated to give an annual estimate. However, if this is not possible, then the calculation can be based on annual aggregate data.

If it is not possible to obtain data on the total number of cases meeting the extended SARI case definition, then in some settings it may be possible to obtain an approximation of the true number of cases identified using the extended SARI case definition by reviewing hospital discharge codes where these are available and of high quality. A review should be made of International Classification of Diseases (ICD) coding (in any of the first three diagnostic code positions for an episode) of admissions. The relevant ICD9 and ICD10 codes are given in the WHO publication, *A manual for estimating disease burden associated with seasonal influenza* [6]. These include:

- for ICD9:
    - codes 487, 488.01, 488.11 for SARI;
    - codes 771.81, 995.91, 995.92 for sepsis; and
- for ICD10:
    - codes J09.01, J09.11, J10.0, J11.0 for SARI; and
    - codes P36.0–36.9, R65.2, A40, A41 for sepsis.

Coding practices can vary between countries. Therefore, to better interpret the data, countries do an audit on how these ICD codes relate to the extended SARI case definition. An estimate could then be made of what proportion of those admitted with these ICD codes were recruited and tested for RSV in each surveillance site.

To be able to estimate the secondary outputs, countries should also collect monthly aggregated denominator data on the total number of all-cause or respiratory or pneumonia admissions for each sentinel hospital further aggregated by the specified age groups and by month.

Surveillance data on RSV cases do not provide population-based burden of disease estimates, because the denominator (or catchment) populations of the surveillance sites are not generally known. However, in settings in which population-based denominators are available, it may be possible to obtain these estimates using the methods described in the WHO publication, *A manual for estimating disease burden associated with seasonal influenza* [6].

11. RSV surveillance outputs

Epidemiological outputs

1. Seasonality – Number and percentage of cases identified using extended SARI case definition that are RSV positive by calendar week
2. RSV disease burden among hospitalized cases – Age-stratified proportion of all-cause or respiratory or pneumonia hospitalizations due to RSV infection
3. Age groups at risk – Age-stratified percent positivity for RSV

Additional epidemiological outputs can be reported if an estimate is made of the total number of cases identified using extended SARI case definition (by age group and month). These secondary outputs are:

4. estimated number of cases identified using extended SARI case definition that are RSV positive (by age group and month)
5. percentage of total hospital admissions that are due to RSV-positive disease (by the specified age group and by month)
6. relative number of cases of RSV-positive disease [1] compared to those for influenza and other locally defined priority conditions (by the specified age groups), and
7. proportion of RSV-positive cases identified using extended SARI case definition that would have been identified with the original SARI case definition (by age group)

Laboratory outputs

1. Participating national public health laboratories will build and improve capacity for RSV detection and typing by rRT-PCR
2. Standardization of RSV molecular testing through RSV EQA participation
3. Reporting of RSV results in a standardized format
4. Providing virologic data for use in assessing seasonality and burden and corresponding clinical related outputs of RSV in participating countries
5. Molecular characterization of selected RSV specimens

[1] RSV disease defined by cases identified using extended SARI case definition that are RSV-positive.

6.

12. Monitoring and evaluation

Monitoring

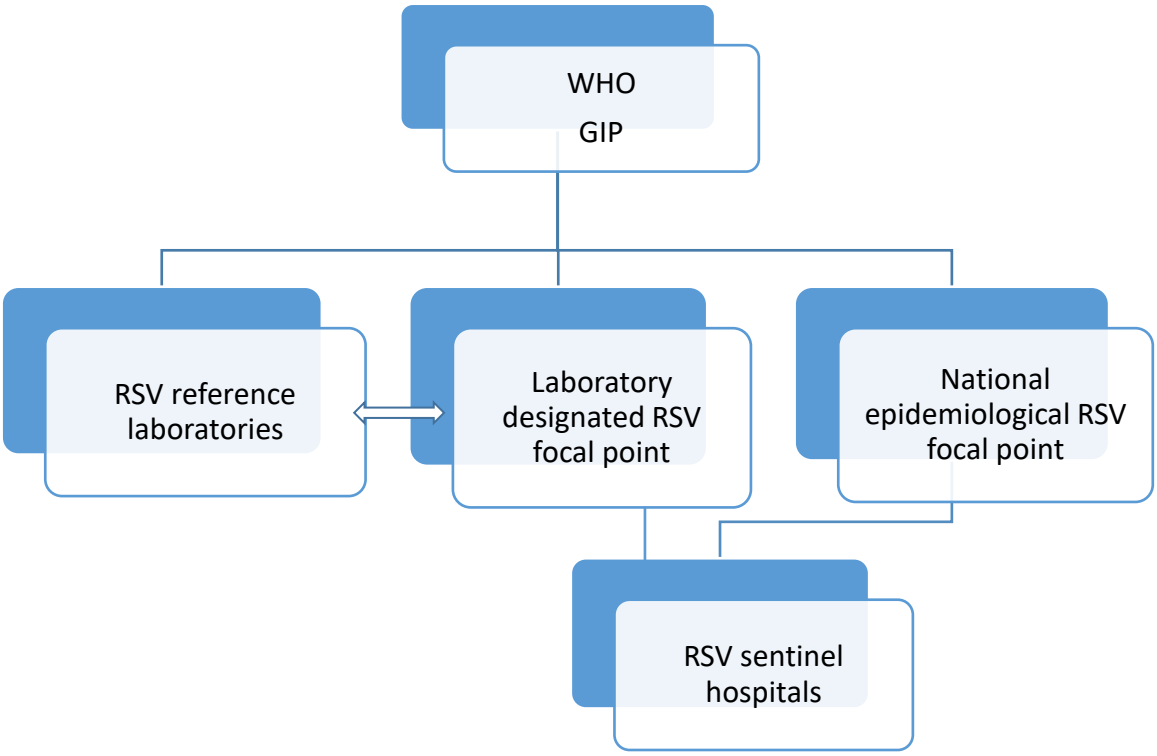

Figure 4: Organizational structure for the WHO RSV Surveillance

Continued evaluation, using designated indicators, will be used to ascertain the success of this surveillance. Monitoring will be conducted at all levels, including sentinel hospitals, national laboratories, national epidemiologists, reference laboratories and WHO headquarters.

A monthly report (Appendix I) will be generated that will monitor the parameters including:

- patient selection at the sentinel site
- quality of collected specimens including storage
- completeness, accuracy and reliability of specimen submission forms, and
- turnaround time of specimen transport to the laboratory, laboratory analysis, timely reporting of laboratory and case-based data to the GISRS platform, support provided by reference laboratories, and compilation and analysis of data at WHO headquarters

Compliance with regulations, including those on patient confidentiality, must be strictly maintained. Countries should monitor additional resources used such as reagents, staff training, data management and project logistics. Countries must ensure that the RSV surveillance does not have a negative effect on their existing influenza surveillance.

Monitoring at the sentinel site should include the following (to be performed by the national focal points assigned for virologic and epidemiological aspects):

- staff training
- adherence to criteria for RSV case definition
- adherence to strategy for selection of patients
- availability of appropriate specimen collection supplies
- completeness and accuracy of specimen submission forms
- storage and transport of specimens and submission forms
- interaction between sentinel site, laboratory and national focal point, and
- adequate documentation of surveillance-related activities

Monitoring at the RSV designated national public health laboratory should include:

- staff training
- availability of appropriate equipment and reagents
- performance in internal and external quality assurance
- adherence to standard operational procedures
- storage and laboratory facilities
- biosafety and biosecurity measures
- data entry and reporting to the GISRS platform
- reporting back to the sentinel hospitals
- interaction between sentinel site, national focal point and WHO headquarters
- adequate documentation of laboratory-related activities, and
- internal and external quality control

Monitoring at the RSV reference laboratories should include:

- performance in internal and external quality assurance
- communication with participating countries and WHO headquarters
- adherence to terms of reference (Appendix J), and
- adequate documentation of surveillance-related activities

Monitoring at the WHO GISRS level should include:

- availability of sufficient and qualified staff
- quality of laboratory and case-based data submitted (accuracy, completeness, timeliness and relevance)
- reliability and function of GISRS database
- feedback to RSV focal points;
- budget use, and
- effect on influenza surveillance

Evaluation

During and at the end of the 3-year period, the RSV surveillance will be evaluated as to whether the aims and objectives have been achieved. The surveillance will be evaluated by WHO, in collaboration with external experts and stakeholders.

Key aspects of the evaluation will include:

- establishment of baseline epidemiological data for RSV
- establishment of the feasibility of RSV surveillance built on the GISRS platform
- validity of case definitions for RSV
- defined seasonality of RSV in different geographical regions
- identification of at-risk groups for severe RSV infection. and
- effect of surveillance on improved RSV awareness at the national and international level

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5. Nyawanda, B.O., et al., *Evaluation of case definitions to detect respiratory syncytial virus infection in hospitalized children below 5 years in Rural Western Kenya, 2009-2013.* BMC Infect Dis, 2016. **16**: p. 218.
6. WHO, *A manual for estimating disease burden associated with seasonal influenza*. 2015, World Health Organization (WHO): Geneva.

15. Appendices

A. Contact information

WHO Global Influenza Programme

Email: gisrs-whohq@wo.int (attn: Wenqing Zhang, Siddhivinayak Hirve, Sandra Jackson)

WHO regional offices

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Facsimile: +47 241 39503
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Regional Office for the Eastern Mediterranean, Cairo, Egypt
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Facsimile: +202 2670 24 92 / 2670 24 94
Email: postmaster@emro.who.int (Attn: Abdinasir Abubakar)

Regional Office for the Western Pacific, Manila, Philippines
Telephone: +63 2 528 8001
Facsimile: +63 2 521 1036 / 526 0279
Email: postmaster@wpro.who.int (Attn: Masaya Kato)

Reference laboratories

Centers for Disease Control and Prevention (CDC), Atlanta
Aron Hall / Brett L. Whitaker
Respiratory Viruses Diagnostic Laboratory
Respiratory Viruses Branch
Division of Viral Diseases, NCIRD
CDC
Telephone: +1 404 432 6991
Email: esg3@cdc.gov/ ijr4@cdc.gov

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Maria Zambon/ Joanna Ellis
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National Infection Service
Public Health England (PHE)
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Facsimile: +27 011 386 6455
Email: annev@nicd.ac.za / JinalB@nicd.ac.za

Victorian Infectious Diseases Reference laboratory (VIDRL), Melbourne
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WHO Collaborating Center for Reference and Research on Influenza
Peter Doherty Institute for Infection and Immunity
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Email: Ian.Barr@influenzacentre.org / Yi-Mo.Deng@influenzacentre.org

B. List of participating countries

Pilot phase

- WHO African Region: Côte d'Ivoire, Mozambique, South Africa
- WHO Region of the Americas: Argentina, Brazil, Canada, Chile
- WHO Eastern Mediterranean Region: Egypt
- WHO European Region: Russian Federation, United Kingdom of Great Britain and Northern Ireland
- WHO South-East Asia Region: India, Thailand
- WHO Western Pacific Region: Australia, Mongolia

New countries expected to join in extension phase

- WHO African Region: Central African Republic, Madagascar, Senegal, Uganda
- WHO Eastern Mediterranean Region: Lebanon, Jordan, Morocco, Pakistan, Qatar
- WHO South-East Asia Region: Nepal
- WHO Western Pacific Region: Philippines

C. Selecting patients for testing

Below are some examples to illustrate sampling strategy for selection of patients for recruitment and collection of specimens. These examples can be variably adapted based on the local context, feasibility and resources.

Example 1: Patient selection and collection of specimens before admission

1. Based on the number of sentinel hospitals selected, decide the number of specimens to be collected per week from each sentinel hospital
2. For each sentinel hospital, decide the day/s of the week when patients would be screened, based on the average number of children aged <2 years admitted per week
3. Decide the timing and place (emergency room or outpatient clinic of the hospital) where patients will be screened, and specimens collected
4. Calculate the sampling interval as follows

$$sampling\ interval\ (n) = \frac{estimated\ respiratory\ admissions\ per\ day\ among\ patients\ aged < 2\ years}{expected\ number\ of\ patients\ aged < 2\ years\ to\ recruit\ per\ day}$$

5. Screen every n^{th} patient aged <2 years that gets admitted for respiratory illness
6. Collect specimen if patient fits the extended SARI case definition
7. Continue screening till the end of the outpatient clinic hours – do not stop screening if the expected number of recruited patients is met
8. If the expected number of recruited patients falls short of or exceeds the expected sample size, then adjust the sampling interval for the next round of screening and recruitment
9. At the end of the outpatient clinic hours, record the total number of recruited patients, the total number of eligible patients that were admitted and the number of patients who were selected who or were unsuccessful in obtaining a specimen for testing

Example 2: Patient selection and collection of specimens after admission

1. Based on the number of sentinel hospitals selected, decide the number of specimens to be collected per week from each sentinel hospital
2. For each sentinel hospital, decide the day/s of the week when patients would be screened, based on the average number of children aged <2 years admitted per week
3. Decide the timing and place (pediatric ward/s, pediatric intensive care unit of the sentinel hospital) where in-patients will be screened, and specimens collected
4. Review the hospital / ward admission register and list the total number of respiratory admissions among patients aged <2 years
5. Calculate the sampling interval as follows

$$sampling\ interval\ (n) = \frac{total\ respiratory\ admissions\ among\ patients\ aged < 2\ years}{expected\ number\ of\ patients\ aged < 2\ years\ to\ recruit\ per\ day}$$

6. Screen every n^{th} patient from the list of patients aged <2 years admitted for respiratory illness. Exclude if the patient was admitted more than 3 days prior to the day of screening
7. Collect specimen if patient fits the extended SARI case definition
8. If the expected number of recruited patients falls short of the expected sample size, then adjust the sampling interval for the next round of screening and recruitment
9. Record the total number of recruited patients and the total number of eligible patients that were admitted

Influenza Specimen Collection

	Nasopharyngeal Swab	Nasopharyngeal/Nasal Aspirate	Nasopharyngeal/Nasal Wash	Deep Nasal Swab	Combined Nasal & Throat Swab
Materials	• Sterile Dacron/nylon swab • Viral transport media tube *(should contain 1-3 ML of sterile viral transport medium)*	• Sterile suction catheter/suction apparatus • Viral transport media tube *(should contain 1-3 ML of sterile viral transport medium)*	• Sterile suction catheter/suction apparatus • Sterile normal saline	• Sterile polyester swab *(aluminum or plastic shaft preferred)* • Viral transport media tube *(should contain 1-3 ML of sterile viral transport medium)*	• 2 dry sterile polyester swabs *(aluminum or plastic shafts preferred)* • Viral transport media tube *(should contain 1-3 ML of sterile viral transport medium)*
Procedure	**1** Tilt patient's head back 70 degrees. **2** Insert swab into nostril. (Swab should reach depth equal to distance from nostrils to outer opening of the ear.) Leave swab in place for several seconds to absorb secretions. **3** Slowly remove swab while rotating it. (Swab both nostrils with same swab.) **4** Place tip of swab into sterile viral transport media tube and snap/cut off the applicator stick.	**1** Attach catheter to suction apparatus. **2** Tilt patient's head back 70 degrees. **3** Insert catheter into nostril. (Catheter should reach depth equal to distance from nostrils to outer opening of ear.) **4** Begin gentle suction. Remove catheter while rotating it gently. **5** Place specimen in sterile viral transport media tube. *Note: NP aspirate may not be possible to conduct in infants*	**1** Attach catheter to suction apparatus. **2** Tilt patient's head back 70 degrees. **3** Insert several drops of sterile normal saline into each nostril. **4** Insert catheter into nostril. (Catheter should reach depth equal to distance from nostrils to outer opening of ear.) **5** Begin gentle suction. Remove catheter while rotating it gently. **6** Place specimen in sterile viral transport media tube. *Note: NP aspirate may not be possible to conduct in infants*	**1** Tilt patient's head back 70 degrees. **2** While gently rotating the swab, insert swab less than one inch into nostril (until resistance is met at turbinates). **3** Rotate the swab several times against nasal wall and repeat in other nostril using the same swab. **4** Place tip of the swab into sterile sterile viral transport media tube and cut off the applicator stick.	**1** Tilt patient's head back 70 degrees. **2** While gently rotating the swab, insert swab less than one inch into nostril (until resistance is met at turbinates). **3** Rotate the swab several times against nasal wall and repeat in other nostril using the same swab. **4** Place tip of the swab into sterile viral transport media tube and cut off the applicator stick. **5** For throat swab, take a second dry polyester swab, insert into mouth, and swab the posterior pharynx and tonsillar areas. (Avoid the tongue.) **6** Place tip of swab into the same tube and cut off the applicator tip.

Packing:

- Label the specimen on viral transport media tube and ensure cap on tube is tightly sealed. (Do not use a pencil or pen for labeling, as they can rub off or smear. Instead, use a bar code or permanent marker).
- Fill out paperwork in accordance with state health department guidelines.
- Include a frozen cold pack with the specimen(s).
- Pack specimens in accordance with U.S. Department of Transportation regulations regarding shipment of biological substances, see **www.cdc.gov/flu/professionals/diagnosis/index.htm.**

Storing:

- Specimens should be placed into sterile viral transport media and immediately placed on refrigerant gel packs or at 4 degrees Celsius (refrigerator) for transport to the state public health laboratory.
- Keep specimens refrigerated (2-8 degrees Celsius, 26-46 degrees Fahrenheit) prior to shipping.

Shipping:

- Ship specimens for testing as soon as possible.
- If delivery will be delayed for more than 3-4 days, specimen should be frozen at -70 degrees Celsius (-94 degrees Fahrenheit).
- Ensure specimen will be received by the public health laboratory during normal business hours.

Considerations:

- A nasopharyngeal (NP) swab is the optimal upper respiratory tract specimen collection method for influenza testing. However, such specimens cannot be collected from infants and many older patients may not allow an NP specimen to be collected. Alternatively, a combined nasal and throat swab specimen or aspirate specimens can provide good influenza virus yield.
- Some influenza tests are approved only for use with certain kinds of respiratory tract specimens, so follow guidelines provided by test. Also, some tests (e.g., rapid influenza diagnostic tests) are only approved for certain kinds of respiratory tract specimens.
- For best results (i.e., highest influenza virus yield), collect respiratory tract specimens within four days of illness onset.
- Most sensitive and accurate tests for influenza virus detection are molecular or nucleic acid amplification tests (RT-PCR).
- Negative test results obtained from rapid influenza diagnostic tests (RIDTs) **that detect influenza viral antigens** do not exclude influenza virus infection in patients with signs and symptoms of influenza. A negative test result could be a false negative and should not preclude further diagnostic testing (such as RT-PCR) and starting empiric antiviral treatment.
- A surgical mask and gloves are recommended at a minimum for all procedures. For some patients and procedures, additional precautions may be indicated, see Standard Precautions at **www.cdc.gov/hicpac/2007IP/2007ip_part4.html#a4**.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CS246972

E. RSV rRT-PCR laboratory protocols, primers and probes

Assay 1: Real-Time RT-PCR Assay for Respiratory Syncytial Virus (RSV)- ver.003.2016-CDC)

Assay 1 protocol is available on request and with permission from GIP, WHO HQ through the US CDC, Atlanta, Georgia. Access to primers used in this protocol are published at the following link: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0015098

Assay 2a: VIDRL Duplex Real-Time RT-qPCR for the Typing of RSV using the SensiFAST™ Probe Lo-ROX One-Step Kit (Bioline, London, England)

The VIDRL multiplex protocol for Real-time RT-PCR for the detection of RSV-A and RSV-B was developed by and made available for sharing with WHO RSV surveillance participating countries by the WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty Institute for Infection and Immunity Melbourne, Australia

This method uses multiplex real-time RT-PCR for both RSV-A and RSV-B detection in a single reaction. The RSV-A probe is labelled with FAM, RSV-B probe is labelled with VIC. Primers are reactive to both RSV-A and RSV-B viruses. The following reaction set up and cycling condition are for SensiFAST kit only, any other real-time RT-PCR kit can be used, however, specific kit instructions (including cycling condition) should be followed.

Materials:

SensiFAST Probe Lo-Rox one-step RT-PCR kit (Bioline)

Primers:	RSVL1-F	AATACAGCCAAATCTAACCAACTTTACA	40 µM
	RSVL1-R	GCCAAGGAAGCATGCAATAAA	40 µM
Probes:	RSVL1-AP(FAM)	FAM-TGCTATTGTGCACTAAAG-MGBNFQ	10 µM
	RSVL1-BP(VIC)	VIC-CACTATTCCTTACTAAAGATGTC-MGBNFQ	10 µM

Methods:

1) Prepare master mix according to specific kit instruction in a clean reagent only room. The following table only applies to SensiFAST kit.

Reagent	Initial Conc.	Final Conc.	Vol/20µL 1x rxn
SensiFAST™ Probe One-Step Mix	2X	1X	10
RSV-L1-F	40µM	0.4µM	0.2
RSV-L1-R	40µM	0.4µM	0.2
RSV L1-ProbeA (FAM)	10µM	0.1µM	0.2
RSV L1-ProbeB (VIC)	10µM	0.1µM	0.2
Reverse Transcriptase			0.2
RiboSafe RNase Inhibitor	10U/µL		0.4

Nuclease-free Water	4.6
Total Mastermix Volume	**16**

2) Mix the master mix thoroughly by a quick and gentle vortex, pulse spin to collect master mix to the bottom of the tube.
3) Aliquot 16 µl of master mix to individual wells of the real-time PCR plate and seal the plate.
4) Take the sealed plate with master mix to a designated room to add 4 µl of RNA to each reaction well to a total volume of 20 µl per reaction. Add 4 µl water to the negative template control well. Add positive RSV-A and RSV-B controls to the positive control wells respectively.
5) Seal the plate and spin plate in a centrifuge to collect reaction at the bottom of all wells.
6) Put the sealed plate in a real-time PCR machine, for each reaction, both FAM and VIC should be selected, FAM for RSV-A, VIC for RSV-B, and set up the cycling condition as per kit instruction. The following cycling condition is only for the SensiFAST kit:

45°C	10 min	1 cycle
95°C	2 min	1 cycle
95°C	5 sec	
60°C	30 sec	40 cycles

7) At the end of the cycle, analyse the result as usual by setting up a proper threshold within the lower quarter of the linear phase of the amplification plot. Samples with Ct values of below or equal to 36 are considered to be positive, samples either with no amplification curve or with Ct values above 36 are considered to be negative.

Result interpretation:

A success run should produce a positive result for positive controls and negative result for the negative template control; otherwise the run should be repeated. Examples of possible results are listed below.

	RSV-A	RSV-B	Result
Sample 1	+	-	RSV-A
Sample 2	-	+	RSV-B
Sample 3	+	+	RSV-A and RSV-B co-infection
Sample 4	-	-	RSV negative

Assay 2b: VIDRL Duplex- Real-Time RT-qPCR for the Typing of RSV using the TaqMan® Fast Virus 1-Step Master Mix Kit (ABI, Life Technologies, Waltham, MA)

This method utilises a multiplex real-time RT-qPCR for the detection and typing of RSV-A and RSV-B in a single RT-qPCR reaction. The RSV-A specific probe is labelled with a FAM fluorophore while the RSVB specific probe is labelled with a VIC fluorophore. Primers are able to amplify both RSV-A and RSV-B viruses. The following master mix and thermocycling conditions are specific to the TaqMan® Fast Virus 1-Step Master Mix kit. Any other commercially available real-time RT-qPCR one-step kit can be used however specific kit instructions should be followed.

Materials:

TaqMan® Fast Virus 1-Step Master Mix Kit (ABI, Life Technologies, Waltham, MA)

Oligo	Sequence (5'-3')
RSV-L1-F	AATACAGCCAAATCTAACCAACTTTACA
RSV-L1-R	GCCAAGGAAGCATGCAATAAA
RSV L1-ProbeA (FAM)	6FAM-TGCTATTGTGCACTAAAG-MGBNFQ
RSV L1-ProbeB (VIC)	VIC-CACTATTCCTTACTAAAGATGTC-MGBNFQ

Methods:

1) Prepare master mix according to kit specific instructions in a clean, reagent only room. The following table only applies to the TaqMan® kit.

Reagent	Initial Conc.	Final Conc.	Vol/20µL rxn
TaqMan® Fast 1-Step Mix (4X)	4X	1X	5
RSV-L1-F	40µM	0.4µM	0.2
RSV-L1-R	40µM	0.4µM	0.2
RSV L1-ProbeA (FAM)	10µM	0.1µM	0.2
RSV L1-ProbeB (VIC)	10µM	0.1µM	0.2
Nuclease-free Water			10.2
Total Mastermix Volume			**16**

Add 4µL of template to 16µL of pre-aliquoted mastermix

2) Mix the master mix thoroughly by a quick and gentle vortex, pulse spin to collect master mix at the bottom of the tube.

3) Aliquot 16µL of master mix to individual wells of a real-time PCR plate and seal the plate.

4) Take the sealed plate with pre-aliquoted master mix to a designated room and add 4µL of extracted viral RNA to each well to give a total volume of 20µL per reaction. Add 4µL nuclease-free water to the non-template control well. Add positive RSV-A and RSV-B controls to the positive control wells.

5) Seal the real-time PCR plate containing the master mix and the RNA template and spin to collect the liquid at the bottom of all wells.

6) Place the sealed real-time PCR plate in a real-time PCR machine. For each reaction, both FAM and VIC dyes should be selected; FAM for RSV-A, VIC for RSV-B. Set-up thermocycling conditions as per kit specific instructions. The following thermocycling conditions are for the SensiFAST™ kit only:

Temperature (°C)	Time	Cycle(s)
50	5 mins	1
95	20 secs	1
95	3 secs	
60	30 secs	40

7) Upon completion of the assay, analyse results as per usual by setting up an appropriate threshold within the lower quarter of the linear phase of the amplification plot. Samples with Ct values of below or equal to 36 are considered to be positive, samples either with no amplification curve or with Ct values above 36 are considered to be negative.

Result Interpretation:

A successful run should produce positive amplification curves for positive controls and no amplification curves for the non-template control; otherwise the assay should be repeated. Examples of possible results are listed below:

Sample	RSV-A	RSV-B	Result
1	+	-	RSV-A detected
2	-	+	RSV-B detected
3	+	+	RSV-A and RSV-B co-infection detected
4	-	-	RSV not detected

Assay 3: CDC Respiratory Syncytial Virus Real-time RT-PCR Panel (RUO): RSV_RUO-01

The CDC Respiratory Syncytial Virus Real-Time RT-PCR Subtyping Panel is intended for the in vitro qualitative detection of human RSV and the identification of RSV A and B subtypes using real-time RT-PCR technology.

For details of this protocol please contact GIP, WHO HQ

F. RSV specimen & case submission form for sentinel sites

(RSV detection and typing request)

(to be completed for children <2 years)

Sentinel hospital ID: _____ Patient ID: _____ Laboratory ID: _____

Patient birth date: ☐☐/☐☐/20☐☐ *(dd/mm/20yy)*

Age (if birth date not known): _____months

Patient sex: ☐ Male ☐ Female Patient weight (kg): ☐☐.☐ kg

Clinical Information @ presentation

Patient location: Hospital ward ☐ Intensive care unit ☐

Date of onset of symptoms: ☐☐/☐☐/20☐☐ *(dd/mm/yyyy)*

Date of hospital admission: ☐☐/☐☐/20☐☐ *(dd/mm/yyyy)*

Date of specimen collection: ☐☐/☐☐/20☐☐ *(dd/mm/yyyy)*

Type of specimen: ☐ Nasal swab ☐ Nasopharyngeal / nasal aspirate

☐ Tracheal aspirate ☐ Broncho-alveolar lavage

Temperature: ☐☐.☐ ^{0}C

Respiratory rate: ☐☐☐ *breaths per minute*

SpO_2 *(when on room air)*: ☐☐☐ %

History of fever (last 10 days): ☐ Yes ☐ No

Lower chest wall indrawing[1]: ☐ Yes ☐ No

Wheeze: ☐ Yes (audible) ☐ Yes (auscultatory) ☐ No

Any IMCI danger sign[2]: ☐ Yes ☐ No

Apnea[3] (<6 mo age only): ☐ Yes ☐ No ☐ Not applicable

Respiratory support: ☐ Low flow O_2 ☐ High flow O_2 ☐ Positive Airway Pressure

[1] Lower chest wall indrawing (lower ribs goes IN when child breathes IN)

[2] Integrated Management of Childhood Illness (IMCI) danger signs include any of the following (i) Unable to drink or breastfeed, (ii) Vomits everything, (iii) Convulsions, and (iv) Difficulty to arouse

[3] Temporary cessation of breathing without any apparent cause as determined by physician

☐ Mechanical Ventilation ☐ None

Pre-existing illness

Premature (<37 weeks gestation): ☐ Yes ☐ No

Congenital heart disease: ☐ Yes ☐ No

Chronic lung disease[1]: ☐ Yes ☐ No

Immunocompromised: ☐ Yes ☐ No ☐ Don't know

Laboratory Results[2]

Date specimen received: ☐☐/☐☐/20☐☐ *(dd/mm/yyyy)*

RSV Results: ☐ Negative ☐ RSV-A ☐ RSV-B ☐ Positive (not typed)

CT value (if RSV positive): ☐☐.☐ cycles

Influenza Results: ☐Negative ☐A(H1N1)pdm ☐ A(H3N2) ☐ A (not subtyped) ☐ B

CT value (if flu positive): ☐☐.☐ cycles

[1] Chronic lung disease includes broncho-pulmonary dysplasia

[2] For quality control purpose, laboratories should maintain information on (1) CT values for Positive controls for RSV-A, RSV-B, and RSV, and (2) RNP result and RNP CT value for each specimen

G. Tiered approach to estimate RSV associated hospitalization burden

Tier	Burden estimate	Data source / Variables required (cumulative by month)	Adjustment / correction factor	Preconditions to avoid bias	Caveats / Limitations
Tier 0.1	None	a) Log of RSV positive b) Log of patients tested	None	None	- Can use ex-SARI or SARI - weekly aggregation - all-year round surveillance
Tier 1.1	Prop. of resp. OR pneumonia hosp. admissions due to RSV *(specify age-band (0-1y, 0-2y)*	a) Log of RSV positive b) Log of patients tested c) Log of patients screened (for enrolment) d) Log of admissions by resp. or pneumonia diagnosis (for non-enrolment)	- adjust for non-enrolment - adjust for weekends or days of non-enrolment	- Systematic sampling of enrolment days in week - systematic sampling of patients	- adjustment factor for non-enrolment estimated during season may overestimate burden during off-season period - assumes that % positivity of RSV to be same in those enrolled and those not enrolled - relationship between no. of ex-SARI cases and no. of resp. or pneumonia cases may vary by season - burden estimate biased if sampling strategy is non-random

Tier	Burden estimate	Data source / Variables required (cumulative by month)	Adjustment / correction factor	Preconditions to avoid bias	Caveats / Limitations
Tier 1.2	Prop. of <u>all-cause</u> hosp. admissions due to RSV *(specify age-band (0-1y, 0-2y)*	a) Log of RSV positive b) Log of patients tested c) Log of patients screened (for enrolment) a) Log of admissions (all-cause) diagnosis (for non-enrolment)	- adjust for non-enrolment - adjust for weekends or days of non-enrolment	- Systematic sampling of week days - systematic sampling of patients	- adjustment factor for non-enrolment estimated during season may overestimate burden during off-season period - assumes that % positivity of RSV to be same in those enrolled and those not enrolled - burden estimate biased if sampling strategy is non-random
Tier 2.1	RSV hosp. rate per 100,000 pop. *(specify age-band (0-1y, 0-2y)*	a) Log of RSV positive b) Log of patients tested c) Log of patients screened (for enrolment) d) Log of admissions by resp. or all-cause diagnosis (for non-enrolment) e) Catchment pop. f) Log of patients with resp. OR all-cause illness from catchment pop. that are admitted in non-sentinel hospitals	- adjust for non-enrolment - adjust for weekends or days of non-enrolment - adjust for patients with resp. illness from catchment pop. that seek care from other hospitals (HUS data)	- Systematic sampling of week days - systematic sampling of patients - secondary level hospital with defined catchment pop. - Health admissions survey OR health utilization survey	- based on WHO influenza disease burden estimation method - adjustment factor derived during season may overestimate burden during off-season period - burden estimate biased if sampling strategy is non-random - HUS / HAS data required

Tier	Burden estimate	Data source / Variables required (cumulative by month)	Adjustment / correction factor	Preconditions to avoid bias	Caveats / Limitations
Tier 2.2	Prop. of all-cause ICU admissions due to RSV *(specify age-band (0-1y, 0-2y)*	a) Log of ICU patients that are RSV positive b) Log of ICU patients tested c) Log of ICU patients screened (for enrolment) d) Log of ICU patients for all-cause diagnosis (for non-enrolment)	- adjust for non-enrolment - adjust for weekends or days of non-enrolment	- Systematic sampling of week days - systematic sampling of patients	- adjustment factor for non-enrolment estimated during season may overestimate burden during off-season period - assumes that % positivity of RSV to be same in those tested and those not tested - assumes no significant bias in selection of patients for testing - burden estimate biased if sampling strategy is non-random
Tier 2.3	Case fatality ratio *(specify age-band (0-1y, 0-2y)*	a) Log of RSV positive b) Log of patients tested c) Log of patients screened (for enrolment) d) Log of patients by resp. or all-cause diagnosis (for non-enrolment) e) Log of RSV deaths	- adjust for non-enrolment - adjust for weekends or days of non-enrolment	- Systematic sampling of week days - systematic sampling of patients	- adjustment factor derived during season may overestimate burden during off-season period - assumes that % positivity of RSV to be same in those tested and those not tested - assumes no significant bias in selection of patients for testing - burden estimate biased if sampling strategy is non-random - Need to follow up RSV positive cases till discharge to determine death

Tier	Burden estimate	Data source / Variables required (cumulative by month)	Adjustment / correction factor	Preconditions to avoid bias	Caveats / Limitations
Tier 2.4	Prop. of resp. OR pneumonia OR all-cause hosp. deaths due to RSV *(specify age-band (0-1y, 0-2y)*	a) Log of RSV positive b) Log of patients tested c) Log of patients screened (for enrolment) d) Log of patients by resp. OR pneumonia OR all-cause diagnosis (for non-enrolment) e) Log of RSV hosp. deaths f) Log of resp. OR pneumonia OR all-cause hosp. deaths	- adjust for non-enrolment - adjust for weekends or days of non-enrolment	- Systematic sampling of week days - systematic sampling of patients	- adjustment factor derived during season may overestimate burden during off-season period - assumes that % positivity of RSV to be same in those tested and those not tested - relationship between no. of ex-SARI cases and no. of resp. or pneumonia cases may vary by season - burden estimate biased if sampling strategy is non-random - Need to follow up RSV positive cases till discharge to determine death

Tier	Burden estimate	Data source / Variables required (cumulative by month)	Adjustment / correction factor	Preconditions to avoid bias	Caveats / Limitations
Tier 3.1	National estimate of RSV hosp. rate per 100,000 pop. *(specify age-band (0-1y, 0-2y)*	a) Log of RSV positive b) Log of patients tested c) Log of patients screened (for enrolment) d) Log of admissions by diagnosis (for non-enrolment) Census data: a) Mid-year pop. by specified age bands, by administrative division serving hosp. b) Adjusted for pop. increase c) Adjusted for the years of surveillance DHS data: a) to adjust admin division estimates to pop. estimates b) pneumonia OR influenza rates OR prevalence of risk factors (HIV, malnutrition, crowding, prematurity etc.) by region HUS data (if available): a) to adjust for non-medically attended resp. illness	- adjust for non-enrolment - adjust for weekends or days of non-enrolment - adjust for referrals from outside catchment population - adjust for non-medically attended illness (optional)	- Systematic sampling of week days - systematic sampling of patients - secondary level hospital with defined catchment pop.	- based on method described by Murray 2015, Theo 2017 - Census data required - DHS data on prevalence of certain morbidities required - HUS data optionally required if adjustment for non-medically attended illness - adjustment factor derived during season may overestimate burden during off-season period

H. Burden data collection tool

This sheet will provide the denominator for RSV as a % of total hospital admissions. Line listing of all new patients less than 2 years admitted to all paediatric medical wards of the hospital. To be completed on each weekday. Admissions on weekends may be completed the following Monday. Source for data usually a ward admission book.

Total Hospital Admission Log (tier 1.1 and 1.2)

Hospital name: _____ Ward no: _____

Date of Admission *(dd/mm/yyyy)*	Patient admission no.	Date of birth *(dd/mm/yyyy)*	Age in W or M or Y (if DOB not known)	Gender *(Male / Female)*	Diagnosis *(*use codes below)*	Screened *(Yes** / No)*

** **1**=LRTI / SARI, **2**=TB, **3**=Respiratory disease (other than SARI or TB), **4**=Diarrhoea, **5**=Diabetes Mellitus, **6**=Malignant disease/Cancer, **7**=Cerebrovascular disease/stroke, **8**=Cardiovascular disease, **9**=HIV, **10**=Pregnancy, **11**=Neurological disease, **12**=Other (specify), **13**=Poisoning, **14**=Malnutrition, **15**=Epilepsy/seizure **16**=Surgical Conditions*

*** If Screened=Yes, then include patient in the screening log*

This sheet will provide the denominator for RSV as a % of total hospital respiratory admissions. Line listing of all new patients less than 2 years admitted for respiratory illness to all paediatric medical wards of the hospital. To be completed on each weekday. Admissions on weekends may be completed the following Monday. Source for data usually a ward admission book.

Total Respiratory Admission Log (tier 1.1 and 1.2)

Hospital name: _____ Ward no: _____

Date of Admission *(dd/mm/yyyy)*	Patient admission no.	Date of birth *(dd/mm/yyyy)*	Age in W or M or Y (if DOB not known)	Gender *(Male / Female)*	Diagnosis *(*use codes below)*	Screened *(Yes** / No)*

* ***1****=URTI,* ***2****=LRTI (pneumonia, bronchopneumonia, bronchiolitis, TB, pleural effusion),* ***3****=Asthma*

*** If Screened=Yes, then include patient in the screening log*

This log provides the screened to enrolled ratio. Used to adjust for cases not enrolled. All patients who are screened should be included in this log.

Screening log (tier 1.1)

Hospital name: _____ Ward no: _____

Screening							Enrolment				
Patient admission no.	Date of admission *(dd/mm/yyyy)*	Date of screening *(dd/mm/yyyy)*	Date of birth *(dd/mm/yyyy)*	Age in W or M or Y (if DOB not known)	Case Definition Met*	Enrolled *(Yes / No)*	Reason not enrolled**	Flu or RSV	Date of enrolment	patient surveillance ID	Swab taken *(Yes / No)*

* ***1****=SARI,* ***2****=Extended SARI,* ***3****=Apnea,* ***4****=Sepsis,* ***5****=Does not meet case definition,* ***6****=Sampling complete for day/week*

** ***1****=too sick,* ***2****=parent refused,* ***3****=discharged,* ***4****=refused sample*

I. Monthly monitoring report

Country:

Month / year of monitoring report **May-19**

Name / code of hospital	Hosp 1	Hosp 2	Hosp 3	Hosp 4	Hosp 5	Total for May 2019	Cumulative total for 2019	Prop. over / under recruited (cumulative)
Sentinel site-related								
No. of specimens collected (0-<3mo)								
No. of specimens collected (3-<6mo)								
No. of specimens collected (6-<12mo)								
No. of specimens collected (12-<24mo)								
Total no. of specimens collected (0-<24mo)								
Prop. of enrolled patients (<2y) with fever								
List of clinical variables with >10% missing data								
List of clinical variables with >1% data error								
No. of specimens collected >3 days after onset of symptoms								
No. of specimens transported at 40C to laboratory after >48 hours of collection								

Name / code of hospital	Hosp 1	Hosp 2	Hosp 3	Hosp 4	Hosp 5	Total for May 2019	Cumulative total for 2019	Prop. over / under recruited (cumulative)
Laboratory-related								
No. of RSV positives								
No. of specimens where RSV could not be typed								
No. of times in the month specimens received from sentinel hospital *(0=not recd: 1=once; 2=fortnightly; 3=weekly)*								
List of lab-related variables with >10% missing data								
List of lab-related variables with >1% data error								
Data and reporting								
No. of times clinical / laboratory data uploaded to FluMart *(0=no upload; 1=once; 2=twice; 3=thrice; 4=weekly)*								
Burden-related data								
Total no. of all-cause hospital admissions (<2y)								
Total no. of respiratory hospital admissions (<2y)								
No. of patients eligible for testing (<2y)								
Remarks (if any)								

J. Specimen submission forms for sequencing

(Please contact GIP WHO HQ or RSV Reference Laboratory for corresponding CDC, PHE and NICD forms and contact information)

RSV SPECIMEN SUBMISSION FORM EXAMPLE

WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA, MELBOURNE, AUSTRALIA

(Please request the electronic version of form when submitting positive RSV specimens for shipment)

Laboratory Information

Reporting Laboratory Information			
Name		**State**	
Institute/Facility Name		**Postcode**	
Address 1		**Country**	
Address 2		**Phone Number**	
City		**E-mail Address**	
Tracking Number		**Date of shipment (DD-MM-YYYY)**	
Comments			
Please save the file and email as an attachment to:	**whoflu@influenzacentre.org**		

WHOCC Melbourne Shipping Address
ATTN: Jayde Simpson WHO Collaborating Centre for Reference and Research on Influenza The Peter Doherty Institute for Infection and Immunity 792 Elizabeth Street Melbourne, VIC, 3000 Australia Telephone Number: + 61 3 9342 9300 E-mail: whoflu@influenzacentre.org

VIDRL specimen submission form for sequencing

	Sample Information					Patient/Host Information				Specimen Collection Location		Laboratory Results			
No	Sample ID	Sample Date DD/MM/YYYY	Specimen Source	Sample Type	Passage History (if culture isolate)	DOB DD/MM/YYYY	Sex	Patient Setting	Health Status	Country	City	Ct Value	RSV Subtype	Designation	Comments
1															
2															
3															
4															
5															
6															
7															
8															
9															
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17															
18															
19															
20															
21															

Instructions for shipping to VIDRL

1) **Sample ID:** This should be filled in with your laboratory's unique identifier or sample number. It needs to match the unique identifier or sample number on the label of the specimen submitted. No patient names please. Example: 30000234W.

2) **Sample Date:** Enter the date the specimen was collected from patient. Please express as DD/MM/YYYY.

3) **Specimen Source:** If submitting an original clinical specimen, please indicate the type of specimen. For example, "NPS" for nasopharyngeal swab; "NS" for nasal swab; "TS" for throat swab; "NPA" for nasal aspirate; "NW" for nasal wash; "NPS/TS" for nasopharyngeal swab plus throat swab in the same tube; "SPU" for sputum; "BAL" for bronchial wash; "TA" for tracheal aspirate; Unknown.

4) **Sample Type:** Indicate whether sending an original clinical specimen, viral isolate, both original clinical specimen and viral isolate, tissue or RNA sample.

5) **Passage History**: While virus cultures are not requested for surveillence there are unusual circumstances in which culture supernatents may be submitted (e.g., diagnostic). If submitting a culture isolate, passage history should be the laboratory history of the specimen as submitted, for example, HEp-2(2) means that the specimen has been passed two times in human epithelial type 2 cells including the initial inoculation. If the specimen was inoculated in another lab before being submitted to your lab, please include all passages. For example, HEp-2(1)HEp-2(2) means that it was passaged once in one lab and twice in another lab.

6) **DOB:** Date of birth of the patient. Please express as DD/MM/YYYY.

7) **Sex:** Select from the drop down options; F for Female, M for Male or Unknown.

8) **Patient Setting:** In what setting was the patient seen? As a hospital in-patient, hospital out-patient or in a general community/GP clinic? Select from drop down.

9) **Health Status:** Please note any unusual aspects of the patient's current health status. For example, immunocompromised, ICU, deceased, pregnant.

10) **Country**: Refers to the country where sample was collected (not the country of patient's residence or the locale of the lab).

11) **City:** Refers to the city where sample was collected (not the patient's residence or locale of the lab).

12) **Postcode:** Refers to where sample was collected (not postcode of the patient's residence or locale of the lab).

13) **Ct Value**: Please specify the Ct value of the RSV RT-qPCR assay, if known.

14) **RSV Subtype:** Select from drop down menu; A, B or Undetermined.

15) **Comments:** Include any additional information such as previous test results, important patient information, etc. Indicate type of medication used and start date if patient was on medication at the time of sample collection.

Submission form example

RSV SPECIMEN SUBMISSION FORM
WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA, MELBOURNE, AUSTRALIA

	Sample Information					Patient/Host Information				Specimen Collection Location		Laboratory Results			
No	Sample ID	Sample Date DD/MM/YYYY	Specimen Source	Sample Type	Passage History (if culture isolate)	DOB DD/MM/YYYY	Sex	Patient Setting	Health Status	Country	City	Ct Value	RSV Subtype	Designation	Comments
1	20-32453	2/1/2020	NPS	Specimen		1/1/2019	F	Hospitalised Inpatient	Deceased	Australia	Sydney	28.4	A	RSV/Australia.Vic/V001/2020	Example
2	20-32454	4/12/2020	NPS	Isolate		2/7/2020	M	Hospitalised Inpatient	Immunocompromised	Australia	Canberra	19.0	B	RSV/Australia.Vic/V002/2020	Example
3	20-32455	5/22/2020	TS	Specimen		9/22/2019	F	Hospitalised Inpatient	ICU	Australia	Brisbane	22.3	B	RSV/Australia.Vic/V003/2020	Example
4	20-32456	6/25/2020	NPA	Specimen		8/14/2019	F	Hospitalised Outpatient	Pregnant	Australia	Perth	19.8	B	RSV/Australia.Vic/V004/2020	Example
5	20-32457	1/30/2020	NW	RNA		10/29/2018	F	Surveillance GP	Unknown	Australia	Darwin	29.1	A	RSV/Australia.Vic/V005/2020	Example
6															

K. RSV Reference Laboratories – Terms of Reference

Background

Respiratory syncytial virus (RSV) is an important viral respiratory pathogen, causing acute and sometimes fatal lower respiratory tract infections in infants, young children and the elderly. With rapid progress in the development of RSV vaccines, it is expected that a vaccine will be available in the near future. In light of the significant public health impact of this virus, there is a critical need to develop and standardize RSV surveillance, and to provide evidence-based support for vaccination policies at the national, regional and global levels. Such evidence should include the documentation of RSV epidemiology, seasonality and virology, and identification of high-risk groups.

Two WHO consultations were held with both RSV and influenza scientists and public health experts in March 2015 and February 2016. After these consultations, a consensus was reached to establish global RSV surveillance based on the existing influenza surveillance platform, the WHO Global Influenza Surveillance and Response System (GISRS). It was agreed that an integrated virological and epidemiological RSV surveillance system should be launched in representative countries from all six WHO regions. Laboratories in these countries are referred to as RSV laboratories. It was also agreed that selected laboratories with technical expertise, capacity and experience on RSV be designated to provide technical guidance on the virological component of RSV surveillance. These specialized laboratories, referred to as "RSV Reference Laboratories" will function in the WHO global RSV surveillance according to WHO terms of reference for RSV Reference Laboratories. Additional Reference Laboratories may be designated as the WHO global RSV surveillance expands.

General conditions

RSV Reference Laboratories:

- work under the coordination of the WHO GIP;
- fulfil the terms of reference using financial support provided only by governmental or other non-commercial sources;
- assume full responsibility for complying with their respective national biosecurity and biosafety regulations on the understanding that such regulations and rules shall, at a minimum, meet the relevant and current WHO standards, and
- appropriately acknowledge, in presentations and publications, the contributions of collaborators, including RSV laboratories and countries participating in the WHO global RSV surveillance.

General activities

RSV Reference Laboratories:

- serve as a technical resource to WHO and national RSV laboratories as time and resources permit;
- guide RSV sequencing standardization activities and act as reference laboratories to support sequencing of specimens from participating countries
- guide the development and use of RSV genomic database platform
- guide the updating of RSV nomenclature for the RSV project and within the international community

- monitor national RSV laboratories in quality assessments of their assays for detection, typing and sequencing;
- prepare and distribute RSV diagnostic reagents and external quality assessment panels in coordination with WHO and as agreed with WHO, as time and resources permit;
- analyse the performance of national RSV laboratories on external quality assurance (EQA) panels and submit timely feedback and reports to national RSV laboratories and WHO;
- provide training and support to national RSV laboratories on laboratory techniques, as time and resources permit; and
- maintain and strengthen active communication and collaboration with national RSV laboratories and WHO to ensure that up-to-date information is exchanged.

L. Reports of WHO meetings

1. WHO Informal Consultation on Surveillance of RSV on the Global Influenza Surveillance and Response System (GISRS) Platform, 25–27 March 2015. http://www.who.int/influenza/resources/publications/report_rsv_meeting/en/
2. WHO Expert Working Group Meeting on RSV Surveillance Based on the GISRS Platform 2–3 February 2016. http://apps.who.int/iris/bitstream/10665/252625/1/WHO-OHE-PED-GIP-2016.7-eng.pdf
3. WHO Technical Meeting on Piloting RSV Surveillance Based on the Global Influenza Surveillance and Response System, 28–30 June 2016. http://apps.who.int/iris/bitstream/10665/252617/1/WHO-OHE-PED-GIP-2016.6-eng.pdf
4. WHO meeting of Mid-term Review of the RSV Surveillance Pilot Based on the Global Influenza Surveillance and Response System, 18-20 December 2017 https://apps.who.int/iris/bitstream/handle/10665/311960/WHO-WHE-IHM-2019.2-eng.pdf?ua=1
5. WHO meeting of Final Review of the RSV Surveillance Pilot Based on the Global Influenza Surveillance and Response System, 23-25 October 2018