

Guidance for testing of
measles and **rubella**
in the laboratory network of
the Region of the Americas



Pan American
Health
Organization



World Health
Organization

REGIONAL OFFICE FOR THE Americas

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REGIONAL OFFICE FOR THE
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Washington, D.C.
2019

Also published in Spanish

Orientaciones sobre las pruebas de sarampión y de la rubéola realizadas en la
red de laboratorios de la Región de las Américas

ISBN: 978-92-75-31997-0

Guidance for testing of measles and rubella in the laboratory network of the Region of the Americas

ISBN: 978-92-75-11997-6

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Suggested citation. Pan American Health Organization. Guidance for testing of measles and rubella in the laboratory network of the Region of the Americas. Washington, D.C.: PAHO; 2019.

Cataloguing-in-Publication (CIP) data. CIP data are available at <http://iris.paho.org/xmlui/handle/123456789/34932>

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FOREWORD

The countries of the Region of the Americas established the goal of interrupting endemic measles transmission by the year 2000 and rubella transmission by 2010. Due to the successful implementation of measles and rubella vaccination strategies, the countries interrupted endemic transmission of measles in 2002 and rubella in 2009. After a process of verification, the Americas were declared free of endemic rubella and congenital rubella syndrome (CRS) in 2015, and free of measles on 2016.

To maintain the elimination of measles and rubella in the Region, it is essential for laboratory surveillance to follow the recommendations established within PAHO's Regional Measles and Rubella Laboratory Network (RMRLN) and to ensure that accurate and reliable results are provided by all national laboratories participating in the World Health Organization (WHO) Global Measles and Rubella Laboratory Network (GMRLN) and in RMRLN.

As a part of the provision of laboratory support and technical expertise by PAHO in the post-elimination phase (since elimination was declared), there has been continuous development and dissemination of technical guidance on testing strategies, correlation and interpretation of results, training, and technology transfers to enhance national laboratories' ability to provide results. This process will accurate case classification and optimize the capacity of the countries' surveillance systems to detect imported viruses and provide laboratory guidance for the study of transmission chains.

The purpose of this document is to provide information about the clinical specimens required and the assays available to support the national laboratories' surveillance of measles and rubella, and maintenance of the quality assurance established by WHO's GMRLN. It also provides guidance for the standardization of laboratory testing of measles and rubella in sporadic cases with initial IgM-positive results, for which testing algorithms have been developed; and for the study of transmission chains, with a view to providing the evidence required by the system and optimizing the use of resources. This document is essentially a tool to help improve measles and rubella laboratory investigation and case classification in the Region of the Americas, a critical requirement for maintaining the elimination of these two viruses in the Region.



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ACKNOWLEDGMENTS

This document has been prepared by the Pan American Health Organization (PAHO) based on the experience of the Member States during the process of elimination and verification of measles and rubella elimination in the Region of the Americas. PAHO would like to thank everyone who participated in the regional, expert, and face-to-face meetings that generated this content, including those who contributed to the technical reviews that led to the final preparation and edition of this guidance document. The following people made important contributions: William Bellini, Ana Maria Bispo, Rodrigo Fasce, Joseph Icenogle, Jennifer Rota, Paul Rota, Marilda M. Siqueira, and Gloria Rey-Benito. Finally, we thank Andrea Patricia Villalobos, consultant for the Family, Health Promotion, and Life Course Department, Family Immunization Unit (FPL/IM), for her thorough review and feedback.

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ABBREVIATIONS AND ACRONYMS

CDC	United States Centers for Disease Control and Prevention
CFT	Complement fixation test
CRS	Congenital rubella syndrome
DNA	Deoxyribose nucleic acid
ELISA	Enzyme-linked immunosorbent assay
GMRLN	Global Measles and Rubella Laboratory Network (WHO)
GSL	Global specialized laboratory
HHV-6	Human herpesvirus 6
HI	Hemagglutination inhibition (assay)
IFA	Immunofluorescence antibody (assay)
IgG	Immunoglobulin, G class (type of antibody)
IgM	Immunoglobulin, M class (type of antibody)
MMR	Triple-viral vaccine (measles, mumps, and rubella)
MR	Double-viral vaccine (measles and rubella)
NL	National laboratory
NPS	Nasopharyngeal swab
NS	Nasal swab
PAHO	Pan American Health Organization
PBS	Phosphate-buffered saline
PRNT	Plaque reduction neutralization test
PT	Proficiency testing
RLC	Regional Laboratory Coordinator (PAHO)
RMRLN	Regional Measles and Rubella Laboratory Network (PAHO)
RNA	Ribonucleic acid
RNase	Ribonuclease
RRL	Regional reference laboratory
RT-qPCR	Reverse transcription quantitative-polymerase chain reaction
TAG	Technical Advisory Group on Vaccine-preventable Diseases
TS	Throat swab
VIDRL	Victorian Infectious Diseases Reference Laboratory
VTM	Viral transport medium
WHO	World Health Organization



**Measles and Rubella
Laboratories Network
Region of the Americas, 2018**

- 21 national laboratories
- 2 regional reference laboratories
- 1 global specialized laboratory

110 subnational laboratories

- 21 in Argentina
- 27 in Brazil
- 26 in Canada
- 5 in Colombia
- 31 in Mexico

SUMMARY

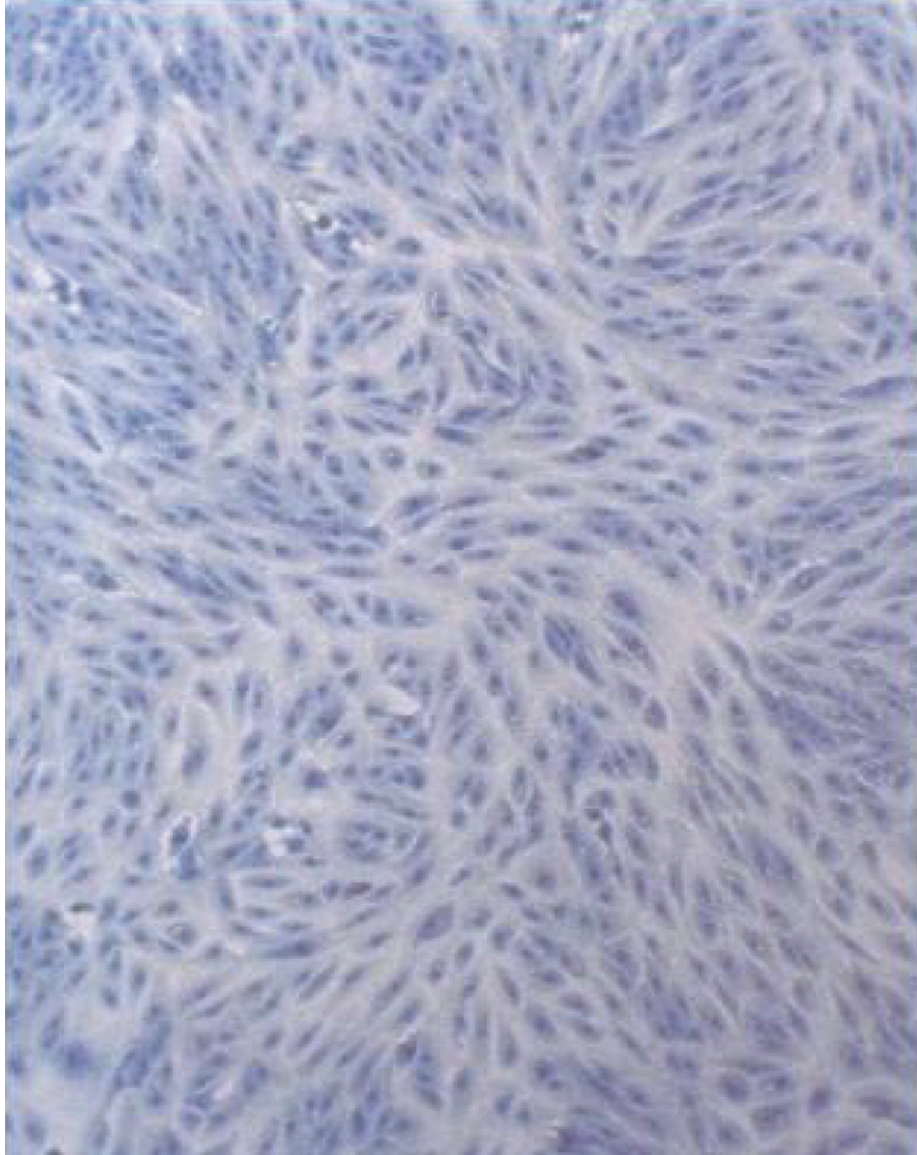
The purpose of this document is to guide and standardize sample testing in suspected cases of measles and rubella in the laboratory network of the Region of the Americas. It presents relevant data on elimination of measles and rubella in the Region, collection of the biological specimens necessary for laboratory tests, and the methods and assays available in the regional laboratory network. The document also describes the main activities related to quality assurance in the Global Measles and Rubella Laboratory Network, notification of detected cases, and reporting of results to the surveillance system.

This document will serve as a tool to increase health workers' capability for case analysis, improve the correlation of laboratory results with clinical and epidemiological data, and optimize the use of laboratory tests to benefit communities, the surveillance system, and decision-makers. For this purpose, it presents a routine algorithm and a complementary algorithm for testing of specimens from suspected cases with an initial IgM-positive or indeterminate result. One section discusses laboratory functions during an outbreak of measles or rubella, and another section focuses on sporadic imported cases and the study of transmission chains.

This document also includes model forms that should be attached when sending samples of sporadic cases with IgM-positive or indeterminate results, to provide basic information to facilitate the interpretation of these results for final case classification.

Many technical and scientific documents have been published and will continue to be published on laboratory tests to diagnose measles and rubella, confirm acute infection and reinfection, detect primary or secondary vaccine failure, and detect immunity, among other aspects. Some of these publications have been consulted and cited in this document with a view to facilitating the interpretation of test results, optimizing the use of laboratory assays, and properly classifying each case; however, readers interested in further study should consult the available up-to-date scientific literature on these subjects.

By reading this guidance and implementing it in routine surveillance, health workers will improve their competencies in laboratory investigation from suspected cases of measles and rubella in contexts of low incidence of disease. This is an essential component of keeping the countries of the Region free from these diseases.



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Monolayer of Vero cells colored with methylene blue and phenol.

1. Introduction

In 1994, the countries of the Region of Americas set the goal of interrupting the transmission of endemic measles by 2000 (1). To achieve the elimination goal, the Pan American Health Organization (PAHO) developed strategies that include vaccination activities intended to achieve high population immunity together with sensitive surveillance to detect suspected cases, along with laboratory confirmation of acute measles virus infection and virus isolation or detection of ribonucleic acid (RNA) to enable molecular identification of the source of the virus (2). With this purpose, PAHO, working with the Centers for Disease Control and Prevention (CDC, Atlanta), created the Regional Measles and Rubella Laboratory Network of (RMRLN).

At the 18th Meeting of the PAHO Technical Advisory Group (TAG) on Vaccine-preventable Diseases, held in San José (Costa Rica) in August 2009 (3), PAHO presented the Plan of Action for the Documentation and Verification of Measles, Rubella, and Congenital Rubella Syndrome Elimination in the Region of the Americas (4). An essential component of the plan is to maintain laboratory surveillance of measles and rubella and to generate evidence on the molecular epidemiology of these viruses. The genetic data obtained through research by this network have provided important evidence documenting the interruption of endemic transmission of measles and rubella, as well as maintenance of their elimination status in the Region.

The RMRLN joined the Global Measles and Rubella Laboratory Network (GMRLN), established by WHO in 2000. The RMRLN has introduced standardized diagnostic methods and reagents as well as comprehensive quality assurance, which includes the following: proficiency testing (with assessment of the quality of laboratory diagnosis through testing serum panels); confirmatory testing; in-house quality control; a laboratory accreditation process; monitoring of the timeliness of results; and weekly notification of performance indicators to the national measles-rubella surveillance system (hereinafter the “surveillance system”), as well as the participation of PAHO as coordinator of the regional surveillance system.

Virological surveillance conducted by the RMRLN is used to observe changes in viral genotypes and sequences over time in a particular country, and in the Region as a whole. The information is analyzed in conjunction with routine epidemiologic data to help document the interruption of transmission of endemic measles in the Region and to provide data on its molecular epidemiology—a vital tool for documenting the absence of an endemic genotype and/or importations of genotypes from other regions.

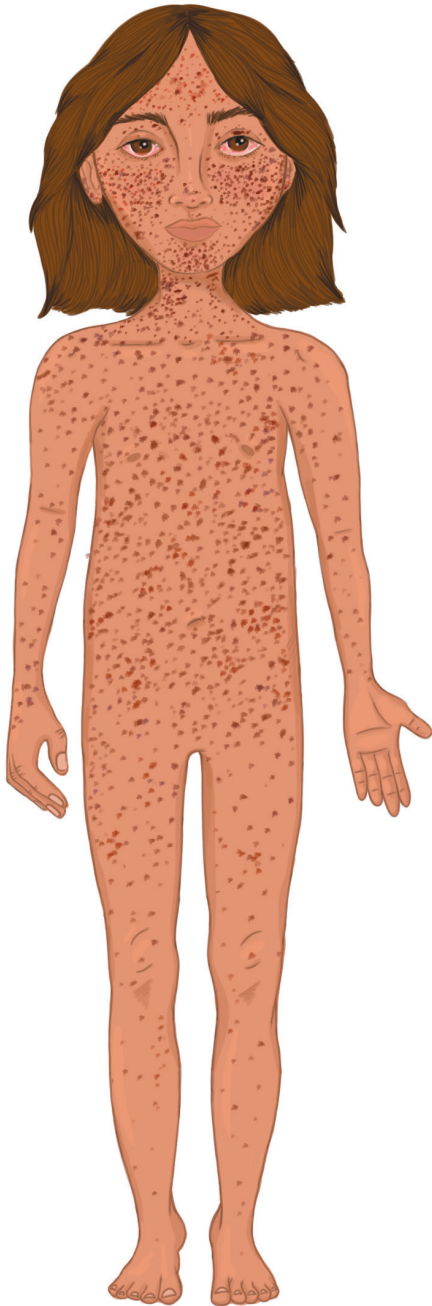
Elimination is defined as the absence of endemic measles or rubella transmission in a defined geographical area (for example, a country or a region) for ≥ 12 months in the presence of a well-performing surveillance system (5). In an elimination setting, a single laboratory-confirmed measles case is considered to be a confirmed measles outbreak (6, 7) and a rapid response is required.

The International Expert Committee declared the elimination of rubella and CRS in April 2015, and announced the elimination of measles on 27 September 2016, making the Americas the first region in the world to reach these goals. However,

as measles continues to circulate in several regions worldwide, countries in the Region continue to be at risk for importation of these viruses. This guidance for laboratory testing of specimens from sporadic cases of measles and rubella is designed to help protect the Region's achievements in eliminating rubella and measles and to help guarantee that good-quality, accurate results are provided by all regional and national laboratory participants.

Development and dissemination of this guidance was initially proposed by a group of experts during a meeting at PAHO headquarters in Washington, DC, on 27 August 2008. The content provided herein has been reviewed and updated based on PAHO member countries' experience with the process of verification of elimination of measles and rubella in the Region. The persons responsible for the surveillance system in the countries should endeavor to follow this guidance, while recognizing that in some situations there may not be enough available information for complete certainty about final classification of cases and that this document is a tool to maintain good laboratory research, facilitate final case classification, and support the sustainability of elimination in order to keep the Region free from measles and rubella.

SARA

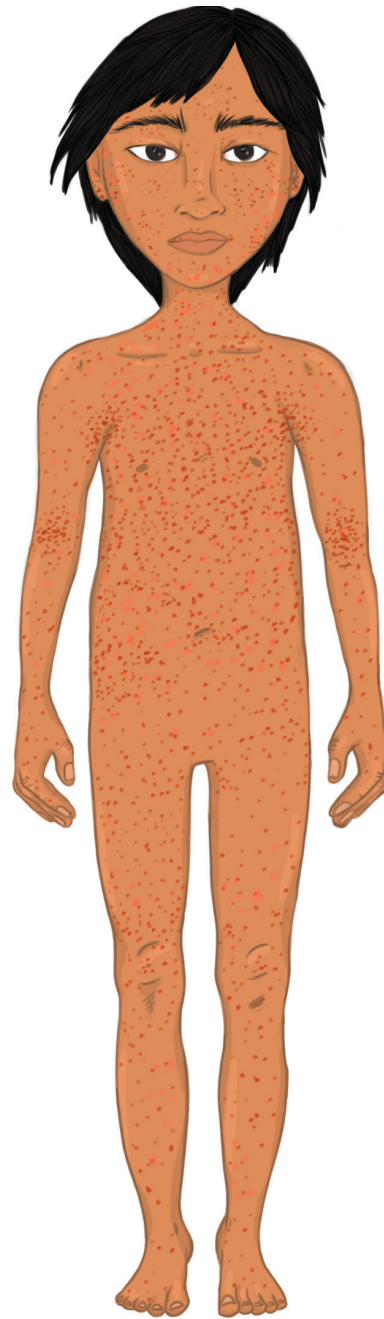


Sara, 3 days after measles rash onset

She presented high fever, conjunctivitis, cough, and coryza; two days later, a general maculopapular rash appeared behind the ears and on face, extending progressively to the neck, chest, back, upper limbs, and abdomen, and finally to the lower limbs.

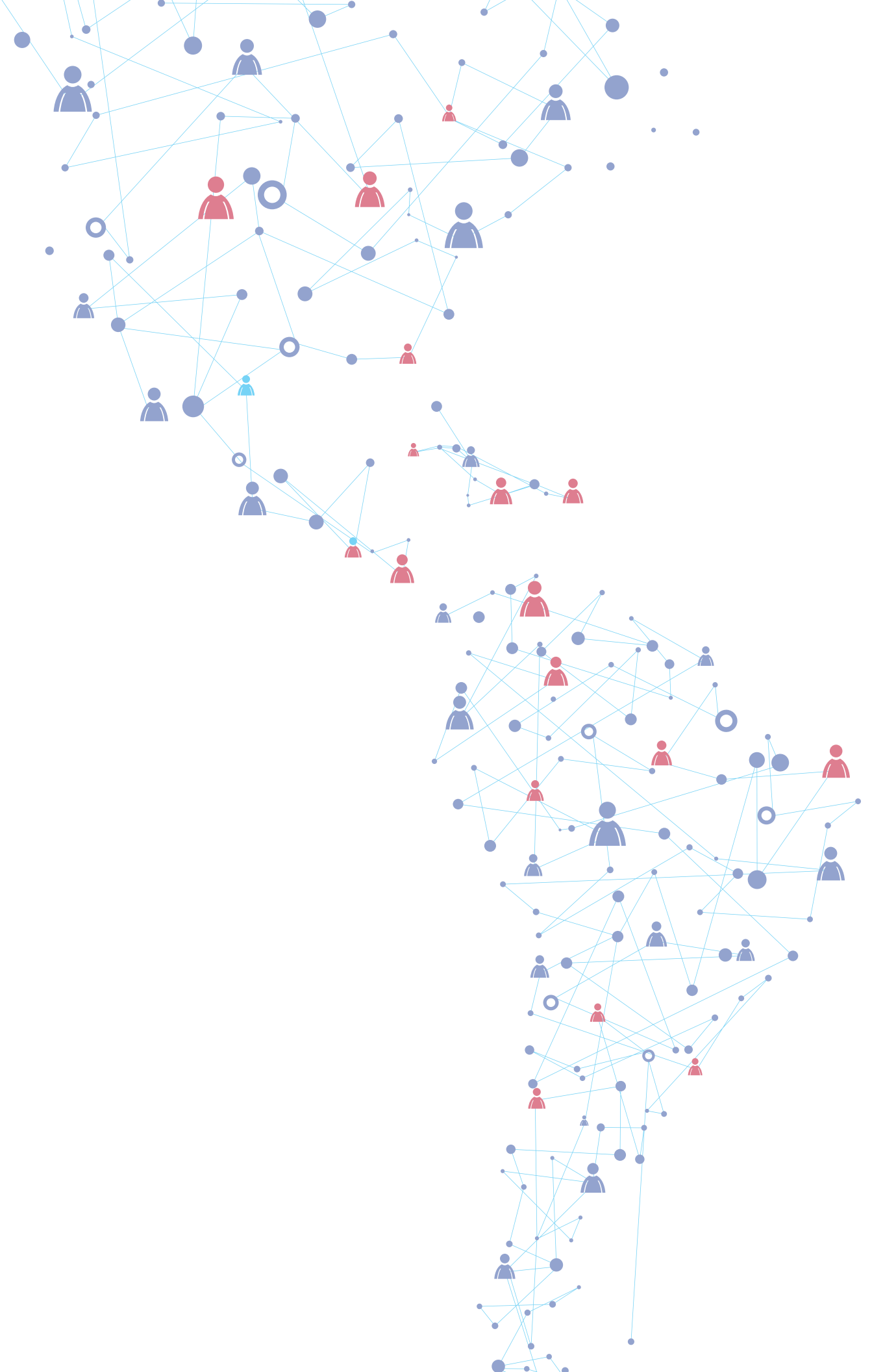
The rash changed from red to dark brown; it was desquamative and non-pruritic.

RUBEN



Ruben, 3 days after rubella rash onset

He presented fever, lymphadenopathies, and general macular rash behind the ears, extending rapidly to the entire body, mainly the trunk and near folds of skin. The rash was reddish with well-defined borders and was non-pruritic.



2. Collection and transportation of biological specimens

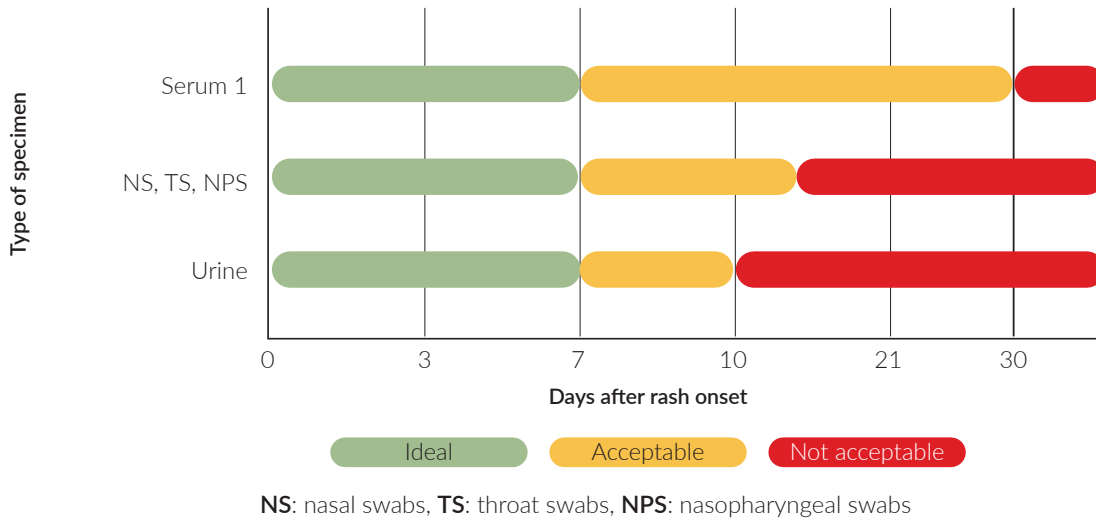
Considering the similarity of measles and rubella in terms of their clinical presentation, epidemiological investigation, and laboratory diagnosis, since 2003 the Region has fully integrated its surveillance of measles and rubella. And serum specimens from suspected cases are tested simultaneously to detect the IgM antibodies specific to each of these viruses (6)

A serum specimen and at least one specimen for viral isolation should be obtained from each suspected measles or rubella case at first contact with the patient. Because serologic results can sometimes be inconclusive, use of an adequate specimen for virus detection can improve case classification. A specimen collected within the first 3 days of rash onset has a higher probability of successfully detecting the virus. Virus detection also allows for genetic characterization of measles and rubella viruses associated with the infection.

Collecting respiratory samples by swab is the preferred method for viral detection; swabs can be pharyngeal, nasal, or nasopharyngeal (either a single sample or combined samples). Depending on the situation and the availability of devices, oral fluid or urine may also be collected. Collecting both respiratory and urine specimens increases the likelihood of viral detection from single cases occurring in connection with recent international travel and/or a high degree of suspicion. Once the specimens are collected, they must be shipped to the laboratory in adequate conditions as soon as possible.

In the collection of clinical specimens, some researchers refer to the date of rash onset as “day zero,” while others refer to it as “day one.” For purposes of standardization, in this document we recommend using “day zero” for the date of rash onset.

Figure 1. Type of recommended specimen, according to days after rash onset



2.1 Serum

For serologic testing, a blood specimen is collected by phlebotomy in a serum-gel separator tube. The blood is allowed to clot and then centrifuged to separate the serum. The serum is aseptically transferred to a sterile screw-cap vial. Serum should be kept refrigerated until tested or shipped on a wet ice pack. To avoid hemolysis, never freeze a tube containing whole blood specimen.

The serum specimen is used in serological testing for specific IgM and IgG antibodies. In measles and rubella surveillance, collection of the serum specimen is recommended at the time of first contact with the health institution and no more than 30 days after rash onset (8).

Routine surveillance of measles and rubella is based on identifying IgM in a single serum specimen, preferably collected during the acute phase of the disease (first 7 days after rash onset).

Some cases may require a second serum specimen, which should be collected during the convalescent

phase of the disease, preferably within 14 to 21 days (range of 10 to 30 days) after the first specimen collection.

2.2 Respiratory specimens

For virological testing, specimens can be collected from the respiratory tract by nasal swab (NS), pharyngeal swab (PS), or nasopharyngeal swab (NPS). It is important to obtain a sufficient number of epithelial cells (rubbing or swirling the swab on the epithelium) to detect the virus, using polyester, rayon, or nylon swabs. The swab should not be allowed to dry out and should be placed in a tube containing sterile viral transport medium (VTM) or phosphate-buffered saline (PBS). The specimen should be kept refrigerated (at 2–8 °C) until shipment and during transport. At the laboratory, the specimen can be frozen at –70 °C. The ideal time for collection of the respiratory specimen is within 7 days of rash onset, but these samples can be collected up to 14 days after rash onset (9).

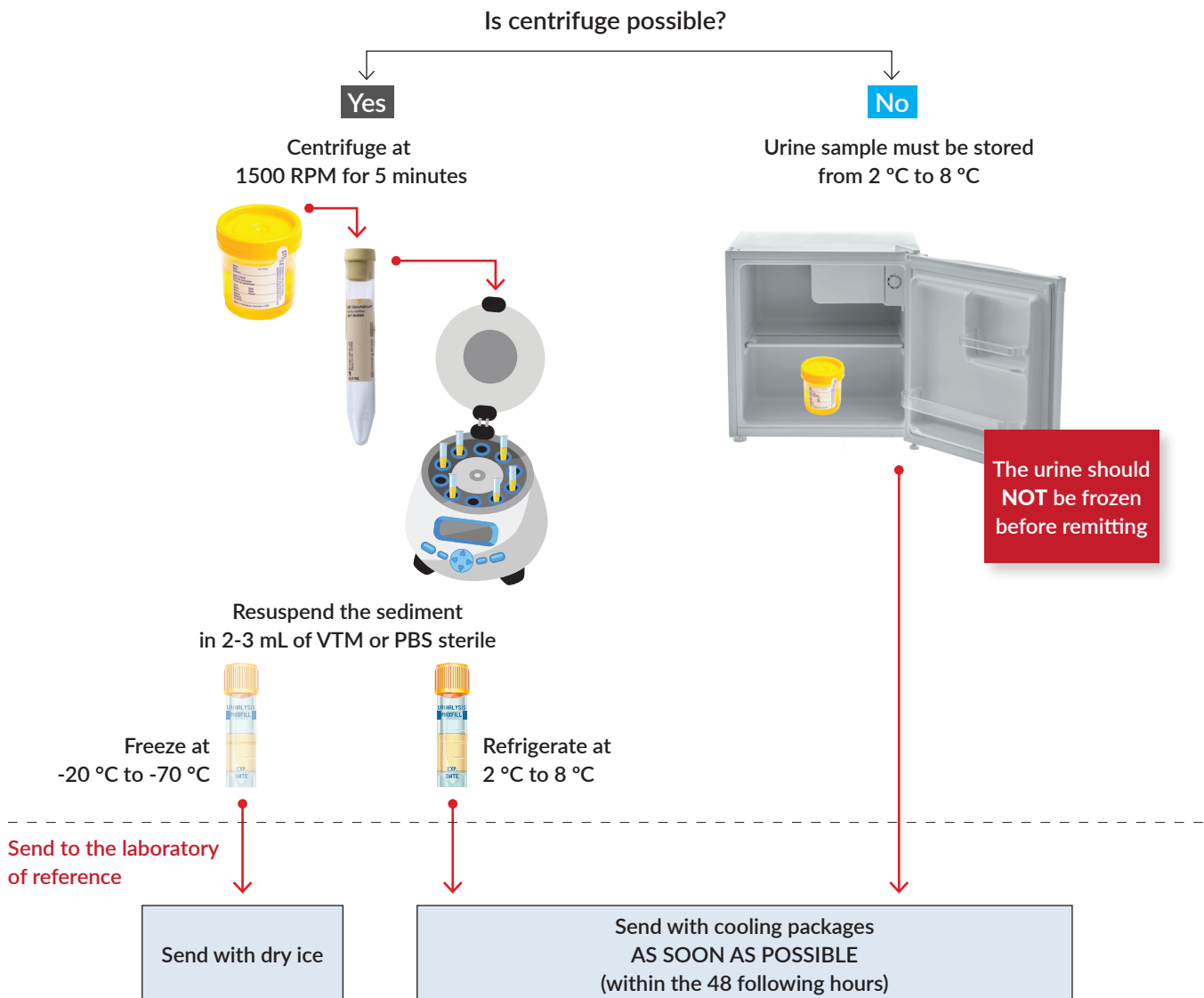
2.3 Urine

Urine specimens can be collected in an appropriate wide-mouth plastic container. The urine should be centrifuged, and the sediment resuspended in 2-3 mL of sterile VTM or PBS. The resuspended urine specimen should be frozen at -70°C . If a centrifuge is not available, the urine sample should be refrigerated (at $2-8^{\circ}\text{C}$) until it can be shipped on refrigerant packs ("cold packs") within 48 hours to a laboratory that is able to perform viral isolation or RNA detection using Reverse Transcription quantitative-Polymerase Chain Reaction (RT-qPCR). The recommended time for

collection of urine specimens is within 7 days of rash onset, but these can be collected up to 10 days after rash onset (Figure 2).

Additional information and protocols for collecting, shipping, processing, and storing these and other specimens are available in the WHO laboratory manual (9) and from the following documents at the WHO and CDC websites: https://www.who.int/ihr/elibrary/manual_diagn_lab_meas_rub_en.pdf and <https://www.cdc.gov/vaccines/pubs/surv-manual/chpt22-lab-support.pdf>.

Figure 2. Pretreatment and preservation of urine specimens for viral isolation or RNA detection



2.4 Packaging and safe transport of specimens

The safe transport of specimens should ensure compliance with applicable national and international regulations, and specifically with recommendations for triple packaging (Figure 3), documentation, and relevant biosafety measures.

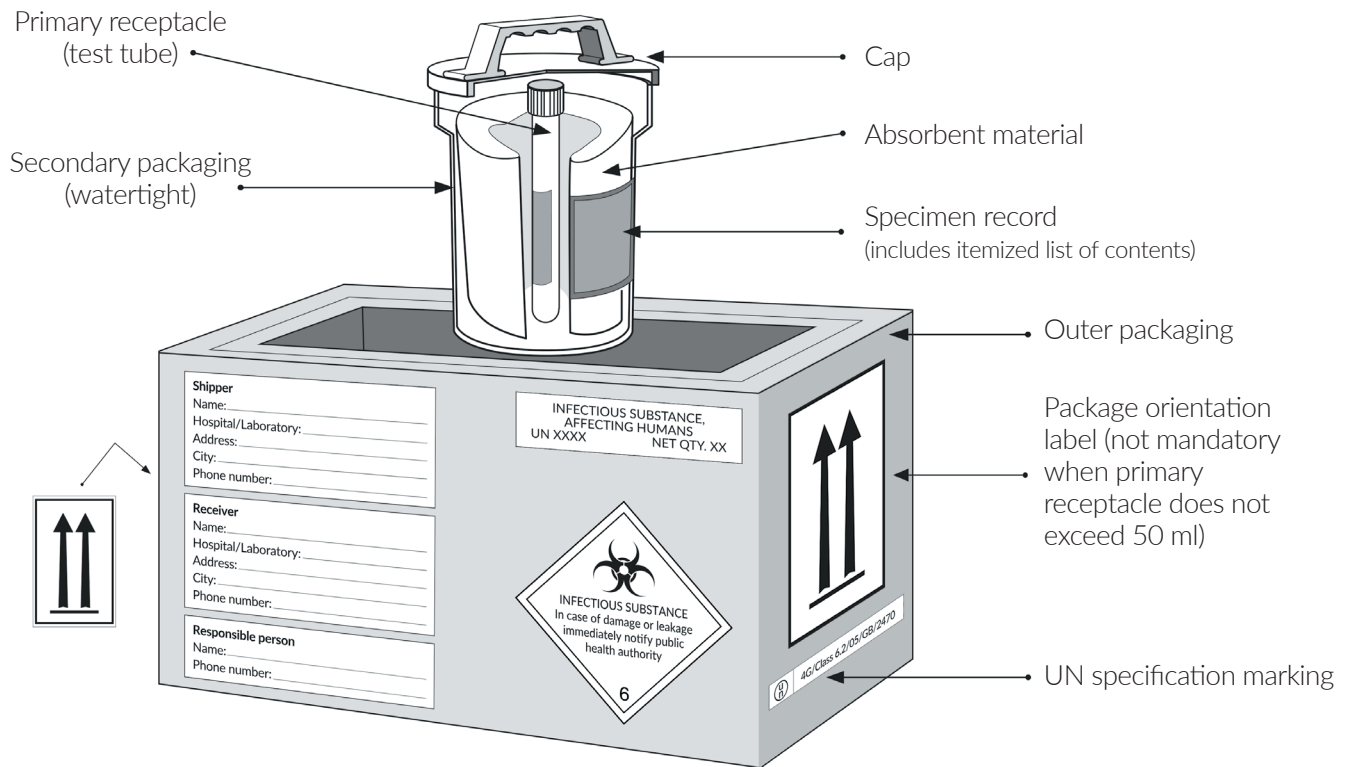
The transport of biological specimens should meet the recommendations of the WHO Guidance on Regulations for the Transport of Infectious Substances (28), with the required documentation attached. Users of the International Air Transport Association (IATA) Dangerous Goods Regulations (DGR) are

responsible for monitoring all amendments, updates, and corrections. Those responsible for international shipments should have valid shipper certification.

The shipper, receiver, and carrier should establish sufficient coordination, in advance, to ensure that materials are properly packaged, transported safely, and arrive at their destination on time and in good condition.

If the selected samples need to be sent to the CDC, the laboratory should request authorization from PAHO's Regional Laboratory Coordinator (RLC) before beginning the shipment process.

Figure 3. Triple-packaging scheme for infectious substances

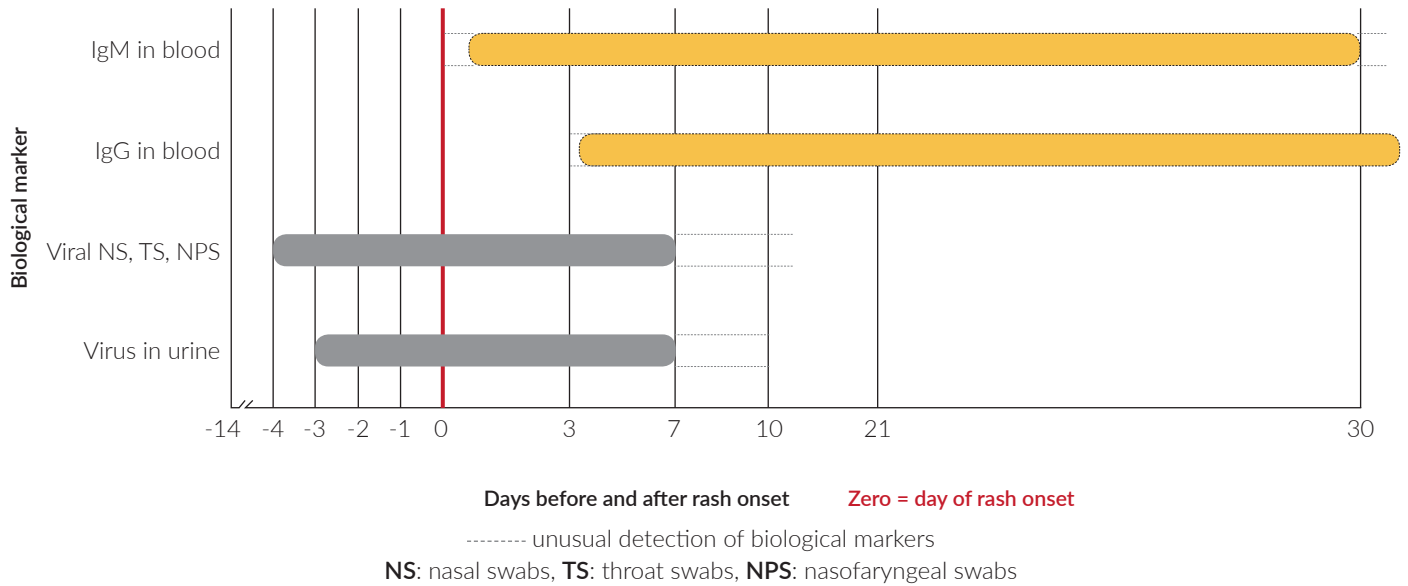


Source: Adapted from IATA Montreal, Canada. Example of triple packaging system for the packaging and labelling of Category A infectious substances.

3. Methods available in the regional measles and rubella laboratory network

This section describes the laboratory tests that are available for confirmation of acute measles and rubella infection (11). With the exception of ELISA testing for IgM and IgG, additional tests such as IgG-avidity may not be available in all national laboratories (NLs). Because few specimens require additional testing (and due to resource limitations), it may be more efficient for NLs to refer selected specimens to a regional reference laboratory (RRL) for additional testing. NLs should contact PAHO's Regional Laboratory Coordinator (RLC) and the PAHO/Immunization focal point to coordinate referral of the specimen to either the RRL or the Global Specialized Laboratory (GSL). Correct use of the assays requires clinical correlation with biological markers of measles or rubella virus infection (Figure 4).

Figure 4. Some biological markers of measles or rubella virus infection



3.1 Serological methods

All serum specimens will be routinely tested for IgM for both measles and rubella. Interpretation of the results should follow the validation criteria recommended by the manufacturer of the test. To facilitate monitoring and technical guidance in this document, “indeterminate” means any indeterminate or equivocal result.

Tests will be repeated immediately if they fail to meet any of the test validation criteria; only results of validated tests will be reported to the surveillance system. Every serum with an IgM-positive or indeterminate result should be reprocessed to ensure reproducibility of the result.

For susceptible persons exposed to the measles or rubella virus (wild-type or vaccine-strain), the test for IgM response is based on the time of rash onset. Antibodies can be detected within about one month for measles and about two months for rubella. IgG production starts after IgM production, about 5-10 days after rash onset, and persists throughout an exposed person’s lifetime (12).

Serological diagnosis of an acute infection can be based on detection of: 1) IgM-specific antibodies in

a single serum specimen; or 2) a significant increase in the titer of IgG-specific antibodies in two serum specimens from acute phase and convalescent phase, “i.e. paired sera”) (13).

3.1.1 Testing for IgM and IgG antibodies

There are various methods for determination of IgM and IgG antibodies, such as the enzyme-linked immunosorbent assay (ELISA), the hemagglutination inhibition (HI) assay, the immunofluorescence antibody (IFA) assay, the complement fixation test (CFT), and the plaque reduction neutralization test (PRNT). However, the ELISA test is recommended for the surveillance system given its accuracy and sensitivity, as well as its applicability in terms of ease in performing the test and obtaining fast, accurate, and reliable results.

All NLS use commercial ELISAs to detect IgM and IgG antibodies to measles and rubella, following PAHO/WHO’s evidence-based recommendations (14-16). These tests kits have shown good performance and high sensitivity and specificity. However, public health workers should be aware that false-positive and false-negative results can sometimes be obtained, and that additional testing

may be required. When a false-negative result is suspected in a highly suspicious case, a new serum specimen can be obtained 4-30 days after rash onset to test for IgM/IgG antibodies. Seroconversion of IgM or IgG will confirm the case.

3.1.2 IgG seroconversion

With virus infection in a naïve individual, virus-specific IgG antibodies can be detected a few days after IgM antibodies. IgG should be detectable 5-7 days after rash onset and peaks about 2-3 weeks after rash onset; antibodies will persist throughout an infected person's lifetime.

When infection occurs in a person previously vaccinated and suspected of being infected with measles or rubella, the acute serum will be IgM-negative and IgG-positive, in which case, collection of a second serum sample one or two weeks later is useful to: 1) determine the IgG response in paired sera (an acute specimen and a convalescent specimen); and 2) provide evidence of any significant increase in the titer of specific IgG antibodies.

Measles- and rubella-specific IgG can be detected using an ELISA; commercially produced kits are available. An increase in the titer of virus-specific IgG can be detected using a semi-quantitative ELISA or quantitative assays such as HI or PRNT (17). Both PRNT and HI are very time consuming and require training and additional resources, so ELISAs are the preferred method for use in the RMRLN. The method employed will vary depending on the type of ELISA kit used, and the interpretation of the results and use of quantitative algorithms must follow the manufacturer's instructions. Some authors have reported that elevated ELISA values do not directly correspond to increases in the titer antibodies, but others have asserted that there is a correlation between the value of an ELISA and the level of antibodies in a titer based on the PRNT (16). PAHO's RLC, the GSL, and the RRL can provide guidance on the use of ELISAs for measuring an IgG titer.

3.1.3 IgG avidity

"Avidity" refers to the strength of binding of IgG antibodies to the antigen. The initial response of

the immune system on first contact with an antigen is to produce IgM. Soon after, low-avidity IgG can be detected, and within a few months high-avidity IgG can be detected. IgG avidity depends on the maturation of IgG from weak- to strong-binding IgG antibodies to an antigen.

Avidity testing differentiates primary from secondary immune responses. This type of testing is particularly useful to demonstrate previous immunity in pregnant women, avoid incorrect interpretations of IgM, and help identify primary or secondary vaccine failure, as well as final classification of cases (18,19). IgG avidity testing can also be useful for documenting cases when the serum sample was collected more than 30 days after rash onset, if IgM is not detected but clinical and epidemiological evidence suggests a true case of measles or rubella. In these situations, a low avidity result can help confirm the case (20).

RRLs can perform avidity testing for measles and rubella and some commercial assays are available (21). Validation of the test is recommended before its use. Currently, the RMRLN sends its specimens to the GSL at the CDC for avidity testing. The IgG avidity tests for measles and rubella at the CDC require extensive validation and the use of well-characterized controls. Laboratories should report to the RLC to obtain authorization before initiating the process of shipping the selected specimens to the CDC for avidity testing.

3.2 Virological methods

Exposure to the virus (wild-type or vaccine-strain) cannot be detected through an IgM- or IgG-specific antibody response. For patients who were recently vaccinated or have a suspected false-negative IgM response, a NS, TS, NPS, or urine specimen for viral RNA detection and sequencing would be useful for accurate diagnosis of acute infection.

In an elimination setting, it is desirable to obtain information about circulating viral genotypes from at least 80% of transmission chains (5). This may be difficult with sporadic cases, but genotype information should be obtained from at least 80% of outbreaks (4). According to PAHO, in 2010-2015 the 80% target was achieved in the Region of the Americas for outbreaks with two or more cases (country reports to PAHO, data not published).

RMRLN participants that do not perform virus isolation or RT-qPCR should forward clinical specimens from confirmed cases to the designated RRL for virus detection and sequencing. Shipment of clinical specimens from confirmed cases must be completed within 15 days of detection, after the necessary permits and permissions have been obtained. Likewise, NLs that perform virus isolation but do not perform sequencing should forward the original specimen and virus isolates for genetic testing to the designated RRL within 15 days of detection, after all necessary permits and permissions have been obtained.

The RLC and the PAHO immunization focal point in the country should be notified, as appropriate, to expedite arrangements for clinical specimens and viral isolates to be forwarded to the RRL no less than one month after collection or isolation. The submitting NL must notify the RLC, RRL, and PAHO immunization focal point regarding any specimens to be forwarded to the RRL or a GSL for technical assistance, prior to shipment, using the forms presented in Annexes 1 and 2, or similar forms required by the reference laboratory.

If a case is confirmed by serological analysis, the specimens for virus detection (NS, TS, NPS, or urine sample) should be analyzed or sent to the RRL for genetic characterization by virus isolation or RNA detection.

3.2.1. Virus isolation and RT-qPCR

Isolation of measles or rubella viruses in a cell culture or direct detection of RNA in the clinical specimen provide useful evidence of recent infection when serology results are not conclusive.

Isolation of the virus in a Vero/hSLAM¹ cell culture or detection of viral RNA by RT-qPCR in a specimen can be useful to confirm recent infection. However, when a recent measles, mumps, and rubella (MMR) vaccination is reported close to rash onset, sequencing is required to differentiate the wild-type from the vaccine strain. It is important to note that a negative result by culture or RT-qPCR should not be used to rule out the case because detection of the virus is greatly affected by the quality, handling, and time of collection of the specimens (22).

NLs that are not performing viral isolation/detection should have a plan for referring specimens to one of the RRLs. NLs performing RT-qPCR should have a plan for shipping PCR products to an RRL for sequence analysis. The CDC can provide standard protocols for viral isolation and RT-qPCR. In addition, the CDC can provide primer/probe kits for RT-qPCR.

¹ Vero cells are cells for which a plasmid that codes the human gene of the molecule activator of the signaling of the lymphocyte (SLAM) have been introduced by transfection; the cells were developed by Dr. Yusuke Yanagi, of the University of Kyushu, Kukyoka (Japan). The use of these cells has been authorized by the WHO GMRLN; every published work using the VERO/hSLAM cell line should make reference to the original publication (Ono et al.; J. Virol. 2001; 75: 4399-4401)

3.2.2 Sequence analysis

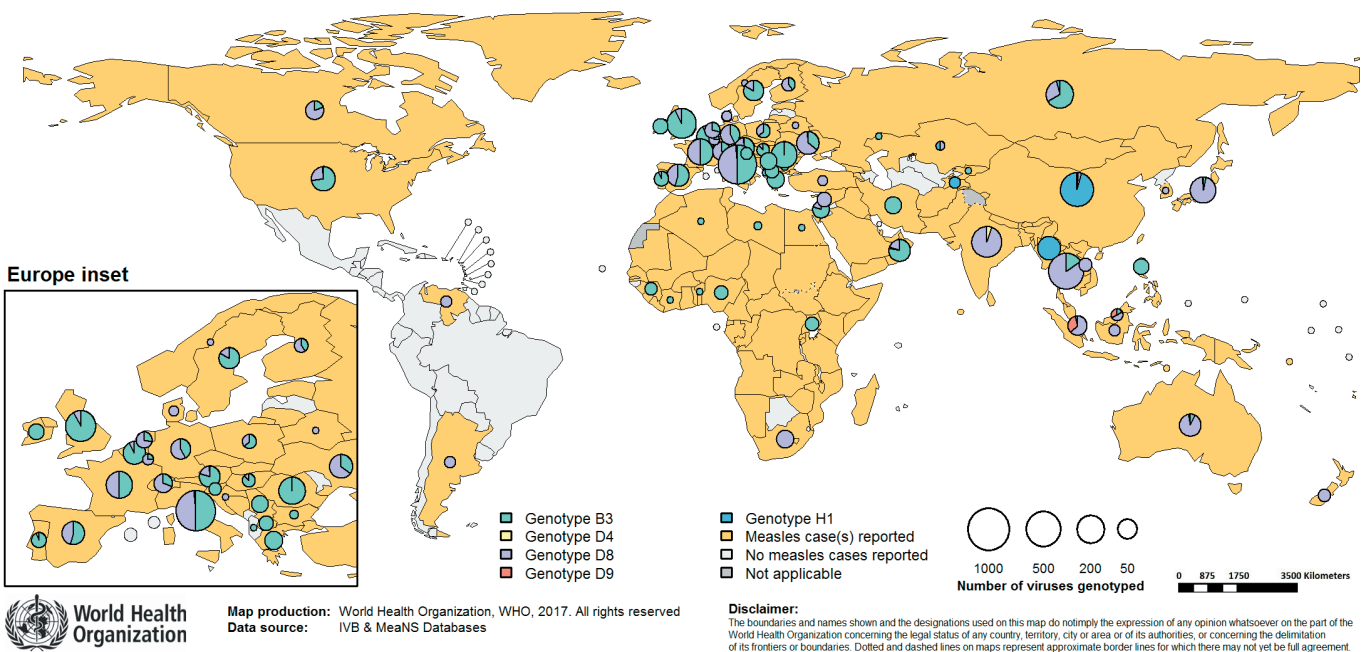
Determination of the genotype is the only way to differentiate the effects of a recent vaccination from a wild-type virus infection. Sequence analysis allows for 1) identification of the genotype in sporadic cases and 2) tracking of the transmission pathways. Molecular and epidemiologic data have proven useful for outbreak investigations in: 1) determining the source of the virus; and 2) providing evidence of the interruption of endemic measles and rubella transmission (23-25).

In accordance with the WHO recommendation for genotype determination, it is necessary to establish at least 450 nucleotides that code for the 150 amino acids of the carboxyl-terminal of the nucleoprotein (N) of the measles virus and 739 nucleotides in the E1-coding region of rubella. For the denomination of the measles and rubella sequences, it is recommended to follow standard international nomenclature for the respective viruses (26,27).

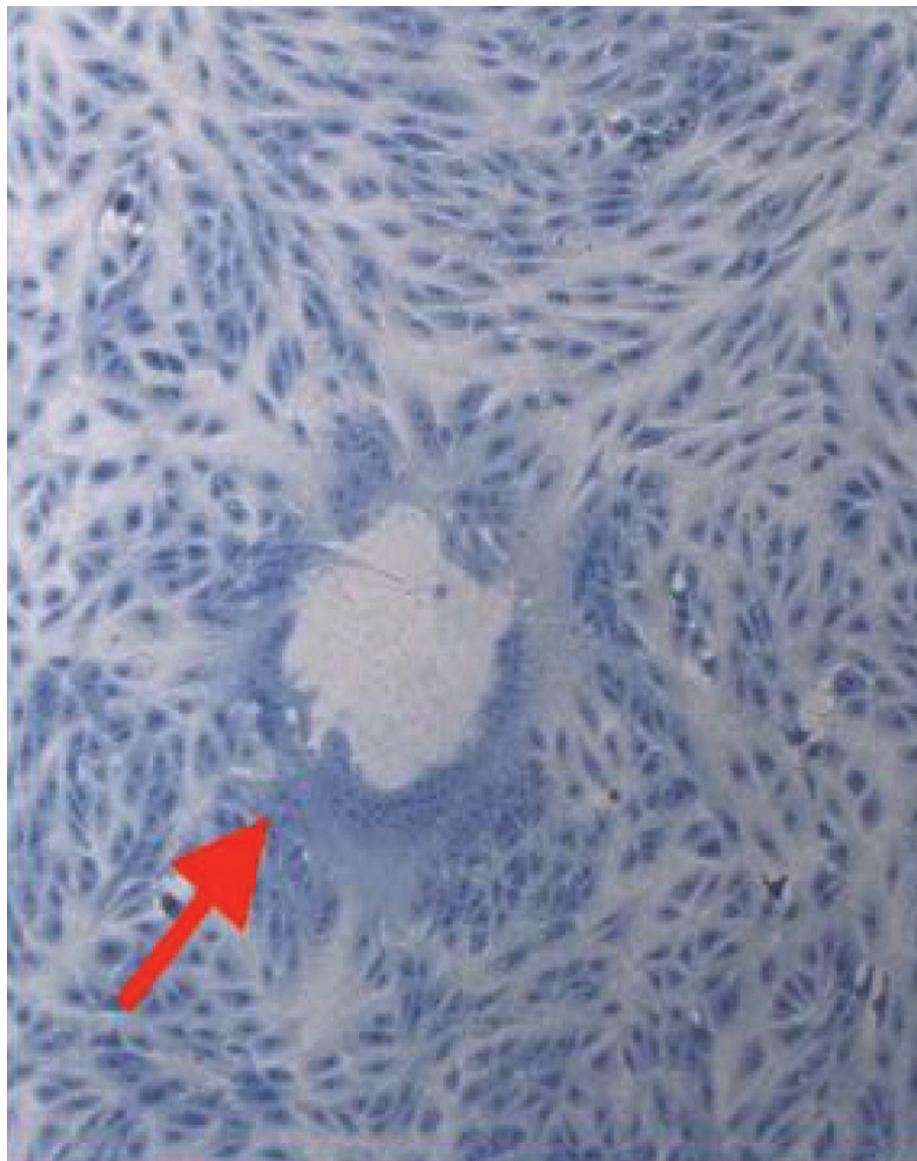
All RRLs and some NLs can perform a sequence analysis of measles and rubella viruses for genotyping. NLs are reminded that timely reporting of genotype information is critical for the success of the surveillance system. RRLs may accept viral isolates or PCR products for sequence analysis. NLs that are not performing sequence analysis should have a plan for referring specimens to one of the RRLs. NLs that perform sequencing must send the isolates and the PCR product to an RRL or the GSL for genotype confirmation within two months of receiving the specimens.

Information on the genetic sequence of the measles and rubella viruses must be reported to the WHO databases in order to document the global distribution of the viral genotypes (Figure 5). For timely reporting of the sequences to the system, the GMRLN has established a two-month period after receipt of specimens in the laboratory.

Figure 5. Distribution of measles genotypes in 2017



Source: MeaNS database. Genotypes reported from January to December 2017.



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Vero cells. Note the large multinucleate cells, which are a cytopathic effect characteristic of measles virus infection.

4. Confirmation of recent infection and active laboratory search

4.1 Criteria for laboratory confirmation of recent measles or rubella infection

In suspected measles or rubella cases, compliance with one of the following criteria confirms a recent infection:

- positive, virus-specific IgM;
- seroconversion or significant increase in IgG titers from paired sera (acute and convalescent);
- viral isolation or RNA detection by RT-qPCR;
- detection of a wild-type sequence; or
- direct epidemiologic linkage to a laboratory-confirmed case.

IgM testing continues to be the routine assay required in PAHO's surveillance system to confirm acute infection. While an IgM-positive result confirms a recent infection by the virus, in the context of elimination in the Region, it is desirable for each confirmed case to meet more than one of these criteria.

4.2 Active laboratory search

It is recommended that active laboratory searches be made for cases of measles and rubella in serum samples collected for surveillance of dengue or arboviral diseases, for the following reasons: 1) endemic areas and outbreaks of dengue and other arboviral diseases have been detected in different countries in the Region; 2) some probable cases of arboviral diseases can present fever and rash; 3) during the prodrome or initial stages of the disease it is difficult to clinically differentiate any infection by these viruses; and 4) some suspected measles or rubella cases may have been detected and reported as dengue or other arboviral diseases.

4.2.1 Active laboratory search in silent areas or municipalities

Active laboratory searches have been used in the process of verifying elimination and should be continued during the post-elimination phase to complement measles and rubella surveillance in “silent municipalities” that have not reported suspected cases. The objective is to obtain evidence of the absence of measles or rubella transmission in these epidemiologically silent areas. A reasonable number of serum samples should be analyzed to complete the wells of reaction-strips in the assays performed periodically in the RMRLN member laboratory.

The sera selected for IgM testing for measles and rubella should meet ALL the following criteria:

- a) case presented fever and rash;
- b) serum of a probable case of dengue or another arboviral disease;
- c) serum was negative for dengue or another arboviral disease;
- d) serum was obtained 30 days before IgM testing for measles and rubella; and
- e) case is from a “silent area” (no suspected measles or rubella case reported to the surveillance system).

Any positive or indeterminate result should be reported immediately, and all criteria defined in the surveillance system for the investigation of measles/rubella cases should be followed. The laboratory should maintain a registry of this activity and periodically revise the aggregate data with the epidemiologist responsible for the surveillance system.

4.2.2 Active laboratory search at the onset of an outbreak

An active laboratory search can be considered a useful tool in surveillance to document the presence of cases in areas where a measles case has been confirmed (index case) and there is no evidence of the source of infection or that the virus was introduced in the community.

It is necessary to analyze a reasonable number of samples obtained for laboratory surveillance of dengue, arboviruses, or other febrile rash diseases (depending on the country’s epidemiological situation and laboratory’s response capacity), meeting ALL the following criteria:

- a) case presented fever and rash;
- b) serum was from a probable case of dengue or another arboviral disease;
- c) serum was negative for dengue or another arboviral disease;
- d) samples were obtained within 30 days prior to the date of rash onset of the index case; and
- e) samples were obtained in the same municipality where the index case was confirmed.

The laboratory should keep a record of this activity and report the aggregate data to the surveillance system.

Together with epidemiological and vaccination criteria, the results of the active laboratory search offer data that help to verify, after an outbreak, that measles or rubella virus circulation has been interrupted.

4.2.3 Active laboratory search when closing an outbreak

Active laboratory searches have also been recommended for PAHO as one of the required criteria for closing an outbreak; the objective is to show that the transmission of the measles or rubella virus has ended.

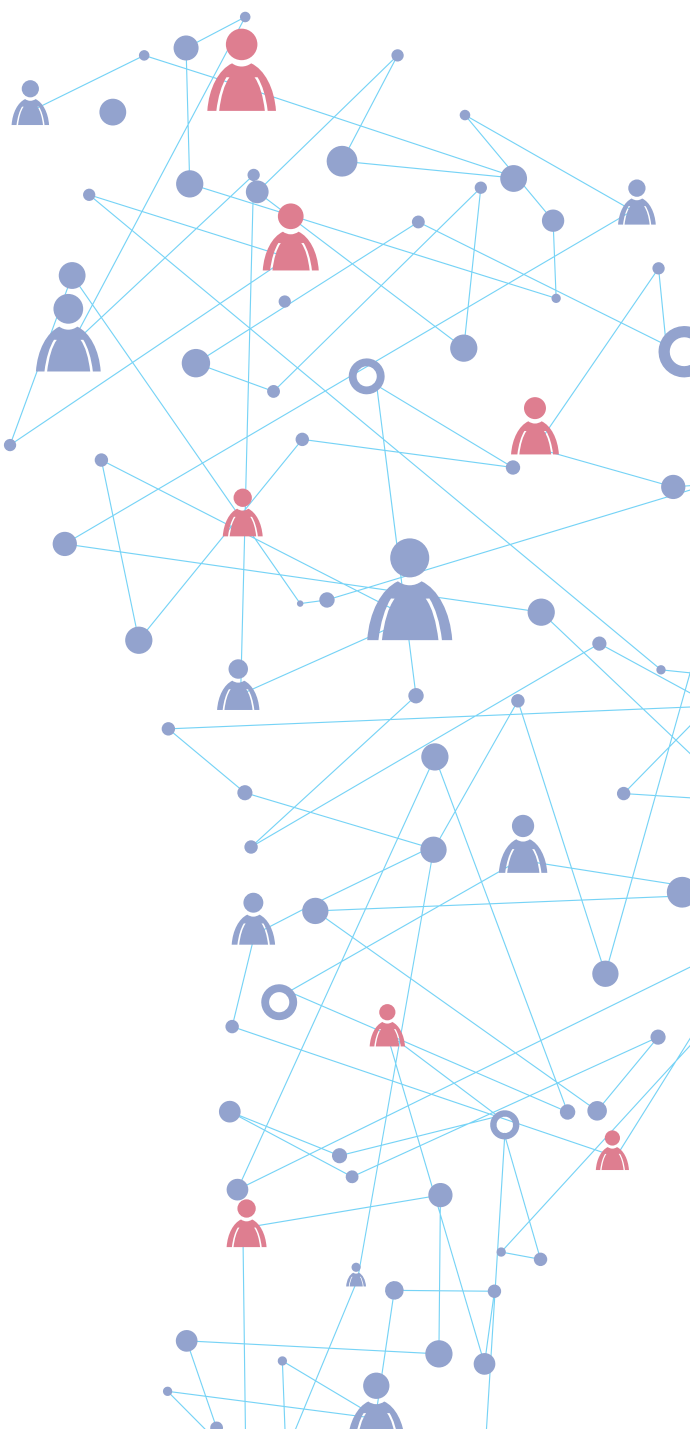
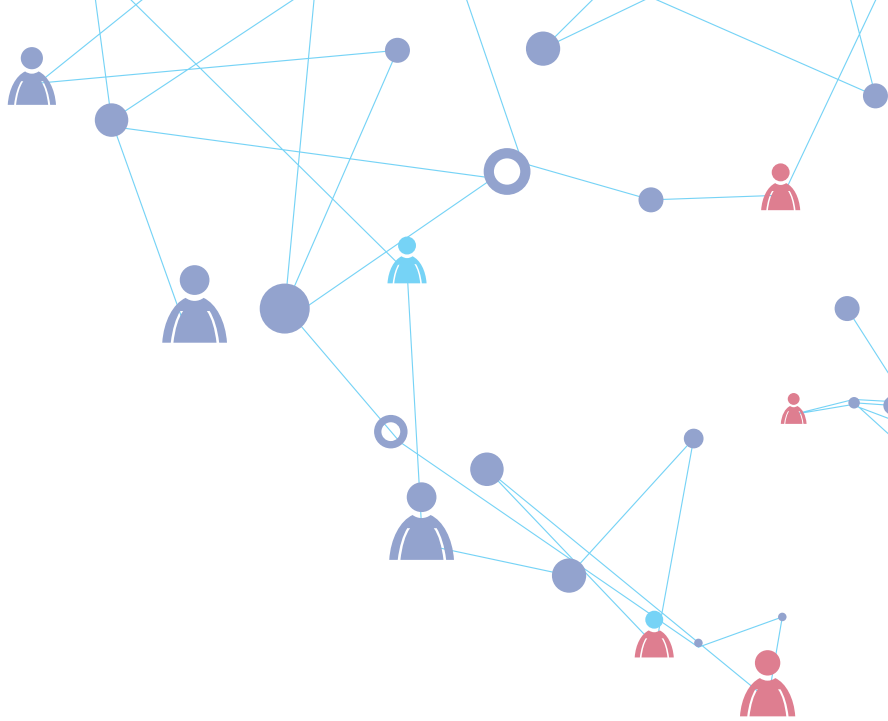
It is necessary to process a reasonable number of samples obtained to diagnose dengue or other arboviruses (depending on the country’s epidemiological situation). These samples should be processed for the detection of IgM against measles or rubella and must meet ALL of the following criteria:

- a) serum was obtained from a case with fever and rash;
- b) serum was from a probable case of dengue or another arboviral disease, from areas where confirmed cases of measles or rubella have been reported;

- c) serum tested negative for dengue or another arboviral disease; and
- d) serum was obtained within 12 weeks following the last confirmed measles or rubella case.

The laboratory should keep a record of this activity and report the aggregate data to the surveillance system.

Together with epidemiological and vaccination criteria, the results of the active laboratory search offer data that help to verify, after an outbreak, that measles or rubella virus circulation has been interrupted.



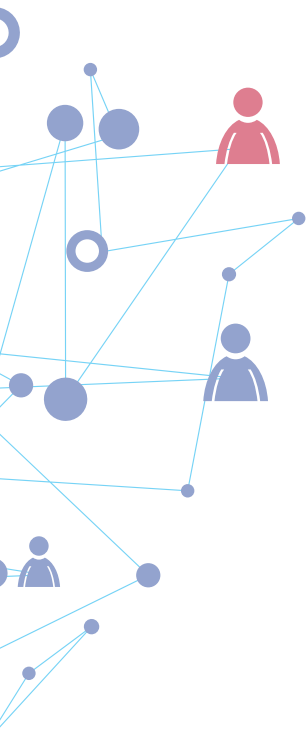
5. Laboratory testing of sporadic cases

5.1 Laboratory confirmation of sporadic cases

In an elimination setting, there will be some IgM-positive or indeterminate results for rubella or measles among samples received from cases of rash and fever. Depending on the epidemiological situation, the results may suggest false-positive IgM or be linked to a vaccine-related case (23,24). Indeterminate (or equivocal) results should be considered IgM-positive until more information and analysis are available.

To provide a high level of specificity in diagnoses and help confirm cases and outbreaks, the NLs should send serum specimens for case verification (complementary testing) to the RRL identified by the RLC. The form for shipping specimens from the NLs to the RRLs for laboratory confirmation of measles and rubella (see Annex 2) should be completed and enclosed in the shipment. The GSL or RRL will evaluate the need for other additional testing (IgG avidity, RT-qPCR, sequencing) according to the information available for each case, and the results will be reported to the RLC and the referral laboratory.

In the context of measles and rubella elimination, accurate case classification depends on careful analysis of all laboratory results and epidemiologic data. Therefore, it is recommended that cases be classified only after the laboratory team and epidemiologic team have reviewed all laboratory results and the clinical and epidemiologic data. Laboratory data will most likely be linked to demographic and epidemiologic data. Despite all this data, it may be difficult to resolve all cases based on the laboratory and epidemiologic results. For this reason, public health teams should strive to obtain complete information for IgM-positive cases so that these cases can be properly analyzed and classified.



To verify elimination of measles and rubella, PAHO recommends that adequate specimens for viral detection be collected and genotype information be available from at least 80% of outbreaks (4). WHO has specified that the proportion of laboratory-confirmed chains of transmission with samples collected and tested in an accredited laboratory should be at least 80% (5). During the post-elimination phase, laboratory testing can be challenging. Serologic and virologic testing will be needed to guarantee timely detection of an imported virus and a rapid response to implement activities to control the spread or transmission of the virus.

An adequate specimen for virus isolation and/or RT-qPCR can also improve the ability to classify cases, especially if specimens are obtained during the first few days of the disease, when serologic test results may be inconclusive (29). However, it is important to note that a negative result for viral culture or RT-qPCR does not rule out measles or rubella infection because the test is affected by the timing of specimen collection and specimen quality.

Some laboratories may opt to test for other viruses that cause rash illness, such as dengue and other arboviral diseases, parvovirus B19, human herpes virus 6 (HHV-6), and HHV-7 (roseola), among others, since some of these etiologic agents cause a nonspecific polyclonal reaction and more than one of the IgM tests may be positive; accordingly, the initial results should be confirmed with other laboratory tests. However, considering the cost, serologic testing for other viruses may not be feasible and each country will have to decide whether to assume the costs of differential diagnosis.

5.2 Sporadic cases where additional testing may be required

An IgM-positive result is sufficient to confirm a case of measles or rubella. However, in some situations other complementary tests may be necessary, as described in Annex 5. The following examples may facilitate decision-making about testing for certain cases.

SITUATION 1. IgM-positive result for measles OR rubella In an acute serum sample, more careful investigation is needed if the case had the following characteristics:

- a) not recently vaccinated (in the 8 weeks prior to rash onset) (6, 30, 31);
- b) no exposure to a confirmed case and no contact with international visitors; and
- c) no travel history 21 days before rash onset.

In these cases, **after reviewing the clinical and epidemiological variables, and in collaboration with the epidemiologist and the RLC**, it may be appropriate to attempt additional testing as described below (listed in preferred order of analysis):

- a) Any specimen obtained for viral detection should be tested using viral isolation and/or RT-qPCR. A positive result would confirm the case, while a negative result would be inconclusive.
- b) Specimens should be tested for IgM to dengue or other arboviruses, if appropriate, to rule out dengue or other arboviral diseases (e.g., in specimens from an arbovirus-endemic area).
- c) If a second serum specimen is obtained and the acute serum is IgG-negative, the second sample should be analyzed for IgG. Seroconversion will confirm the case. If the acute serum is IgG-positive, a validated assay can be used to document a significant increase in titer between the first and second samples (the two samples must be processed in the same assay).
- d) If only a single serum specimen is available, and it is IgG-positive, it should be tested for IgG avidity for measles or rubella. Low-avidity IgG can be used to confirm a case.

SITUATION 2. IgM-positive result for measles AND rubella, and the patient has no history of recent vaccination.

This situation (IgM-positive both for measles and rubella) may suggest a nonspecific reaction (i.e., another etiologic agent should be considered because simultaneous infections with both wild viruses have not been described, and sequential infections would be very rare in an elimination setting). In

collaboration with the investigating epidemiologist, other probable causes (such as parvovirus B19 or dengue or other arboviral diseases) should be ruled out, if possible. Additional testing, described below, should be attempted to confirm either measles or rubella infection.

- a) If specimens for viral detection were obtained, they should be tested using viral isolation and/or RT-qPCR. A positive result will confirm the case, while a negative result will be inconclusive.
- b) The specimen should be tested for IgM to dengue or other arboviral diseases, if appropriate, to rule out dengue or other arboviruses.
- c) If a second serum specimen is obtained and the acute serum was IgG-negative, the second sample should be tested for IgG. Seroconversion to measles or rubella will confirm the case.
- d) If only a single serum specimen is available, and it is IgG-positive, it should be tested for IgG avidity for measles and rubella. Low-avidity IgG can be used to confirm recent measles or rubella infection.

SITUATION 3. IgM-positive result for measles OR rubella and the case received vaccine in the 8 weeks before rash onset and has a recent history of exposure to measles or rubella. This is a common situation during outbreaks in which vaccination is part of the outbreak control strategy.

In this case, the serologic response cannot be used to differentiate between natural infection and a vaccine reaction. Genetic characterization of the viral isolate or PCR product is needed.

- a) If specimens for viral detection are obtained, an attempt should be made to grow the virus in cell culture (for specimens other than oral fluid specimens) and/or detect the virus using RT-qPCR. Confirmation of the presence of wild-type virus by sequence analysis will confirm the case, while detection of a vaccine sequence will rule it out.
- b) If only a single serum specimen is available, and the specimen was taken within 3 days of rash onset, an IgG-positive result can rule out a case of acute rubella (but this does not apply

for measles). An IgG-negative result requires virologic testing to confirm the case

SITUATION 4. IgM-negative result for measles OR rubella in an acute serum specimen obtained within 3 days of rash onset, with strong suspicion of measles or rubella due to recent travel or recent exposure, or because the case has no history of vaccination.

- a) If specimens for viral detection are obtained, they should be tested using viral isolation and/or RT-qPCR. A positive result will confirm the case, while a negative result will be inconclusive.
- b) If only a single serum specimen is available, it should be tested for the presence of measles or rubella IgG. An IgG-positive result can rule out the case if the date of rash onset is known and the specimen was taken within 3 days of rash onset (does not apply for measles). An IgG-negative result requires a second serum specimen and/or virologic testing to confirm the case.
- c) If a second serum specimen was taken after 3 days of rash onset, it should be tested for measles and rubella IgM, and a positive result would confirm the case. If the acute serum was IgG-negative, demonstration of IgG seroconversion would also confirm the case. IgM and IgG seroconversion would confirm the case with greater certainty.

SITUATION 5. An IgM-negative or indeterminate result for measles or rubella in an acute serum specimen obtained within 3 days of rash onset, with strong suspicion of measles or rubella due to recent travel, recent exposure to a confirmed case, and previous history of vaccination (>4 months before rash onset).

- a) The test for IgM should be repeated and IgG should be performed.
- b) If specimens for viral detection are obtained, they should be tested using viral isolation and/or RT-qPCR. A positive result would confirm the case, while negative results would be inconclusive.
- c) If only a single serum specimen is available, it should be tested for the presence of measles or rubella IgG. An IgG-positive result can rule out the case if the date of rash onset is known and the

specimen was taken within 3 days of rash onset (does not apply for measles). An IgG-negative result requires a second serum specimen and/or virologic testing to confirm the case.

d) A second serum specimen should be tested for measles or rubella IgM, obtained between 4 and

30 days after rash onset. Seroconversion from IgM-negative to IgM-positive will confirm the case more quickly.

e) Seroconversion of measles or rubella IgG can also confirm the case.

6. Algorithm for testing of specimens

To standardize the use of the assays available in the RMRLN, PAHO has developed two algorithms that should be followed by the laboratories to improve case study and optimize the use of resources. The first is a routine algorithm for testing of specimens from suspected measles and rubella cases (see Annex 6), including serologic and virologic testing. The second one, referred to as the “complementary algorithm” (see Annex 7), serves as a guide for testing of specimens when the result of previous tests has been IgM-positive or indeterminate (equivocal).

6.1 Routine algorithm for testing of specimens from a suspected measles and rubella case

Routine laboratory surveillance for measles and rubella is based on IgM detection by ELISA. All serum specimens from suspected measles and rubella cases must be tested simultaneously for both measles and rubella IgM antibodies. In an elimination setting, **any IgM-positive or indeterminate (equivocal) result should be retested**. If the **retested result** is indeterminate, the final result should be considered “indeterminate” and the guidance criteria in Annex 6 should be followed to document the action taken to attempt to classify the case.

All IgM-positive results should be reported immediately to the surveillance system and to the health institution that referred the sample. If specimens are available for viral detection, they should be tested using RT-qPCR and an attempt should be made to identify the genotype of positive specimens (for the recommended number of cases per outbreak, or per time interval, if the outbreak continues for several months).

For cases with IgM-positive results, where a false-positive IgM is suspected (i.e., the clinical or epidemiological evidence suggests the cases are not measles or rubella), the laboratory may consider performing differential diagnosis for other rash diseases such as arboviruses, parvovirus B19, or human herpes virus (HHV-6), among others. Each RMRLN member should consult with the epidemiologist and work together to determine the best course of action according to geographical spread, local circumstances, and available resources.

If the result is IgM-negative, it should be reported to both the surveillance system and the health facility that referred the specimen. If an IgM-false-negative result is suspected (because of the clinical presentations of the case and the epidemiological risk assessment), and the serum specimen was collected within 3 days of rash onset, the following steps should be taken: 1) The serum specimens should be tested for IgG; 2) if viral detection specimens are available, they should be tested for RT-qPCR; and 3) a second serum sample should be requested and tested using IgM/IgG, looking for evidence of seroconversion.

6.2 Complementary algorithm for serological testing of specimens with initial IgM-positive or indeterminate result

In a context of low incidence of the disease, the public health team will face the challenge of classifying an **IgM-positive** or **indeterminate** result when no confirmed cases have been reported for many months, or years, and/or there is no evidence of travel or contact with confirmed cases. All cases with an initial **IgM-positive or indeterminate** result should be subject to a detailed study.

If the first serum sample is consumed by IgM testing, a second serum specimen should be requested and tested for IgG antibodies. If the result of the second specimen is IgG-negative, the laboratory should report the result and the case should be discarded (32, 33). If the result is IgG-positive, the specimen

should be referred to the RRL or GSL for avidity testing. Low avidity confirms recent infection and high avidity suggests the exposure was >3 months the specimen was collected.

If the first serum specimen is available, IgG testing should be performed. An IgG-negative result indicates no immunity or previous exposure. An IgG-positive result will require careful interpretation to determine if it was produced by recent infection or if there is any evidence of previous immunity.² To clarify the IgG result for the first specimen, a second serum specimen should be requested and collected 14-21 days after collection of the first specimen.

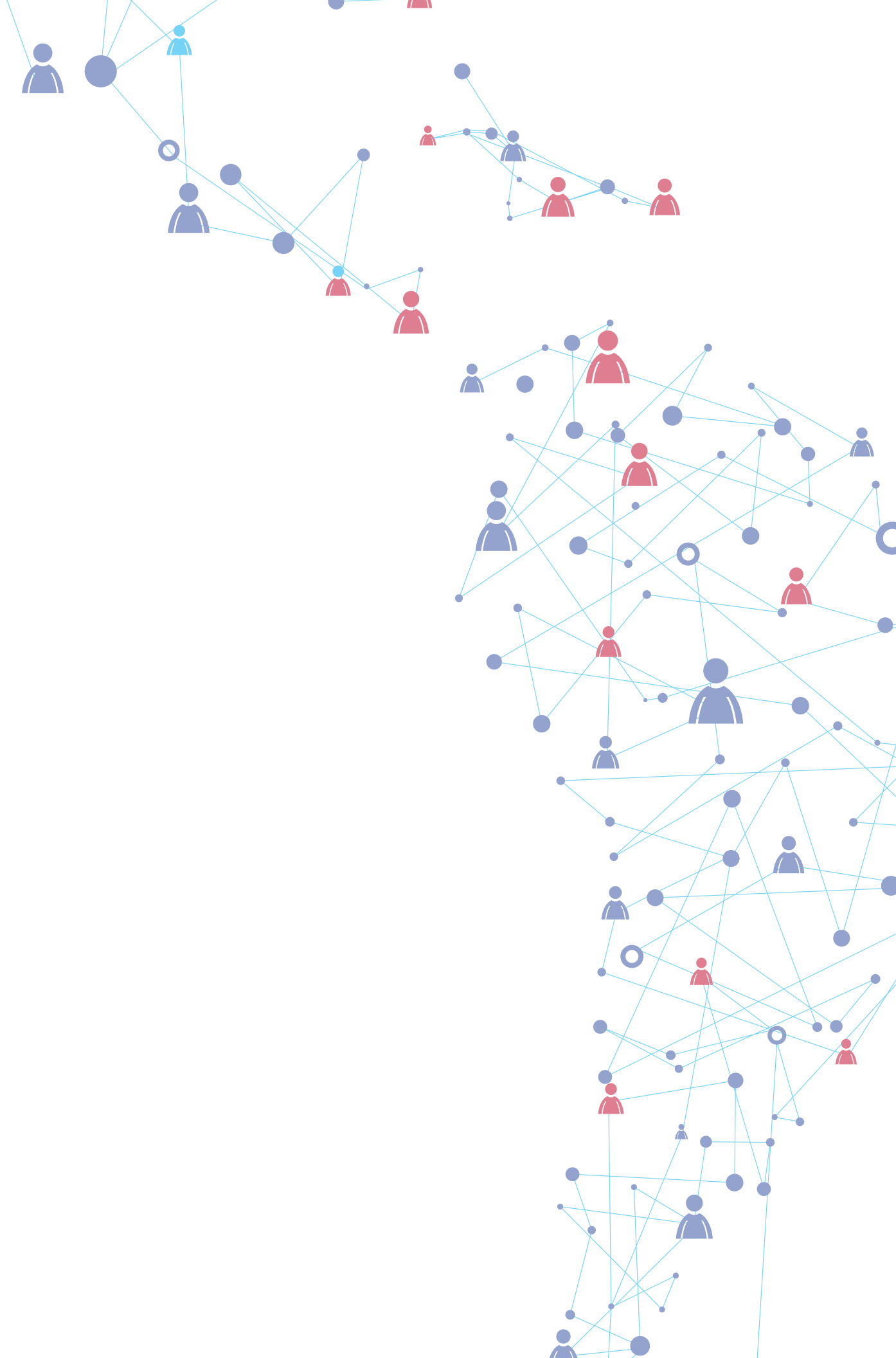
If the result of the first test was IgG-negative, and a second sample was obtained to test for IgG, the results should be interpreted as follows: 1) an IgG-positive result for the second specimen suggests seroconversion (from an IgG-negative to IgG-positive result) and recent contact with the virus; 2) if the second specimen has an IgG-negative result, there is no evidence of contact with the virus (either recent or past) and acute infection will be ruled out.

When the first specimen is IgG-positive, the second specimen should be tested in parallel with the first one (acute and convalescent paired sera) to determine the IgG titers. The results should be interpreted according to the following criteria: 1) if IgG titers remain stable, there is no evidence of recent contact with the virus; 2) if IgG titers increase significantly (following the interpretation criteria for the test), there is evidence of recent contact or infection with the virus; and 3) if IgG titers increase, but not significantly, the result is indeterminate (cannot confirm or reject the possibility of recent contact with the virus). It is advisable to check the specimen collection times to ensure the samples were collected during the acute and convalescent phases (with the first serum collected 7 or more days after rash onset, and the second sample collected 14–21 days after that). When sera samples are collected in a short period of time, even in a true case, the time allotted for the rise of IgG in the titer is not sufficient for a significant IgG increase.

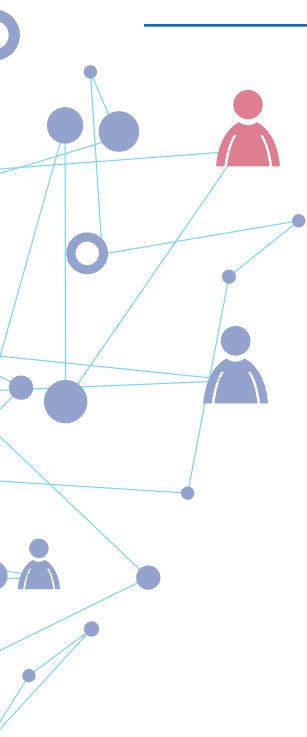
² These results must be correlated with the time of collection of the specimens.

In some cases, when it is not possible to obtain a second serum specimen and the result of the first specimen is IgG-positive, avidity testing must be performed. If the avidity testing reports IgG antibodies of low avidity due to low affinity, the case source of the specimen has had recent contact with the virus. The presence of IgG antibodies of high avidity is evidence that the case had a past immunological response to the virus, and recent infection can be ruled out. However, some cases of measles reinfection or secondary vaccine failure have high avidity and high titers of neutralizing antibodies (34-37).

For final classification of a case, public health teams (epidemiology, immunization, laboratory) should analyze the results of all the assays performed, and the clinical and epidemiological data available (contact with a previous confirmed case, contact with foreigners, recent visit to countries where the virus continues to circulate, signs and symptoms, etc.). Correct interpretation of the data and more accurate classification of cases can strengthen the implementation of appropriate control activities and help maintain the Region of the Americas free from measles and rubella.



7. Role of the measles and rubella laboratory during outbreaks



The laboratory has played an important role in the surveillance system for measles and rubella elimination by carrying out standardized tests, reporting results in a timely way, and complying with high-quality standards, which has allowed for the documentation and verification of the elimination of these diseases in the countries of the Region of the Americas.

Excellent laboratory performance must be maintained to guarantee early detection of imported cases and timely reporting of results to health authorities for adequate decision-making, minimizing the likelihood of virus dispersion and successfully controlling outbreaks of these diseases.

7.1 Dealing with an imported sporadic case

- It is not possible to know in advance whether a case is real, thus the importance of maintaining compliance with the timely results report (≤ 4 days).
- Immediately report the case to the sending health institution and the epidemiologist responsible for the surveillance system.
- Review sample availability for virologic and molecular testing. Perform viral detection and genotyping if samples are available.
- If you suspect a false-positive result, perform differential diagnoses according to the epidemiological situation of the case's area of precedence and the availability of reagents in the laboratory.
- Immediately request that health personnel (sending institution, epidemiology) obtain additional samples for complementary tests. It is recommended to specify the type of sample required and the appropriate time needed to collect them.
- Carry out additional laboratory tests, IgG seroconversion, IgG avidity, PCR, and sequencing, depending on the availability of samples and installed technical capacity.

- Notify epidemiology regarding the results of additional or complementary tests.
- Participate actively in the case study unit.
- Send samples with case information to the RRL or GSL for confirmatory testing and quality control.
- Review the availability of reagents and human resources for the laboratory to respond to an increase in reported cases (more samples, more tests).
- Notify laboratory management regarding available supplies and the need for additional resources.

7.2 Dealing with a transmission chain

- Compliance with surveillance indicators is of the greatest importance and efforts should be made to ensure these indicators are followed.
- All samples received from localities or municipalities with confirmed cases should be analyzed specifically for the virus identified in the transmission (5); it is not necessary to carry out differential diagnosis with the other virus that is the subject of integrated surveillance.
- Confirm the presence of the virus and document the viral genotype associated with the transmission chain.
- Virologic surveillance for a measles or rubella outbreak is recommended. To avoid saturation of the laboratory, optimize the use of resources and ensure laboratory support before, during, and after the outbreak. The following samples should be prioritized for RT-qPCR analysis:
 - The first 3 to 10 suspected cases directly related to the index case.
 - The first 3 to 10 suspected cases in a new locality or municipality.
 - The first 3 to 10 suspected cases that occur every two months in the same locality or municipality where cases have been confirmed.
- An active laboratory search should be considered a strategy to document the presence of cases, especially at the beginning of an outbreak in areas or time periods where surveillance has been weak. All criteria defined in section 4.2.1 (a, b, and c) of this document should be followed. Also, the specimen should be obtained within 30

days prior to the date of the index case of the outbreak, and in the same municipality. An active laboratory search should also be considered one of the components required to document the end of virus transmission in a community and as part of the evidence needed to close the outbreak. The criteria set out in section 4.2.3 of this document should be followed.

- All samples from suspected cases in municipalities or states where circulation of the measles or rubella virus has not been documented, and where there is no antecedent of a nexus or epidemiological link with a confirmed case, should continue to be analyzed following the established algorithm for integrated measles and rubella surveillance.

It is important to carry out a virologic follow-up of the outbreak, mainly to have evidence of new importation or introduction of a new genotype (Annexes 9 and 10). This will be very useful if transmission is eventually maintained for more than 12 months, which would be considered reestablishment of virus circulation meaning that elimination status has been lost.

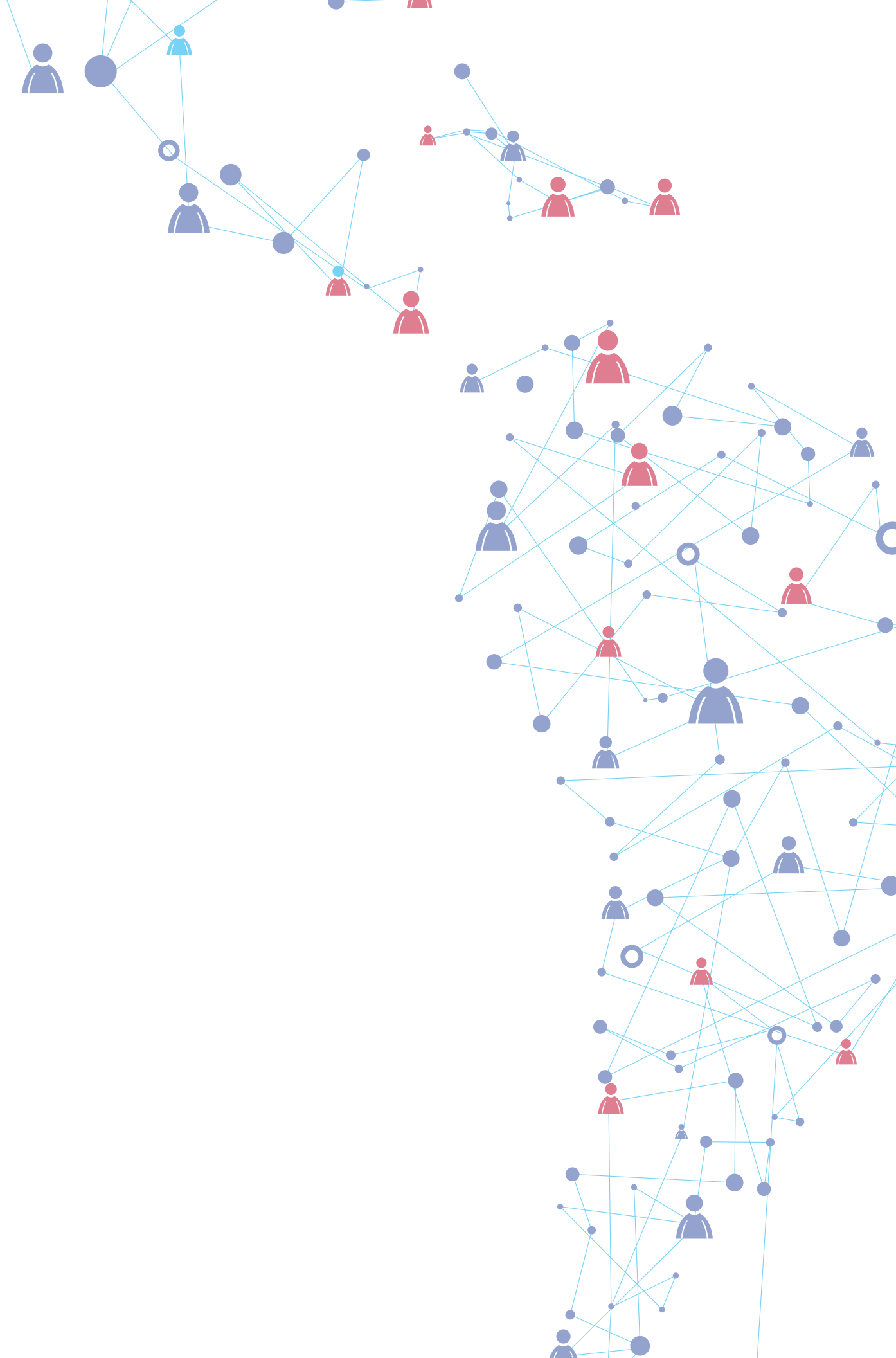
7.3 Additional recommendations for dealing with chains of transmission of measles or rubella

- Check the availability or existence of reagents and optimize the laboratory response according to the epidemiological situation and existing resources.
- Prioritize the analysis of samples from areas or localities where the circulation of the virus has not been previously confirmed, together with contact with positive cases or cases with a highly suggestive clinical picture.
- Maintain ongoing communication with personnel on the field; provide necessary recommendations to optimize the collection, handling and transportation of specimens.
- Be alert to possible cases of measles virus re-infection in order to evaluate the laboratory's capacity to perform the tests to analyze these cases properly.
- Maintain an up-to-date inventory of reagents and human resources to keep the laboratory's response capacity for several weeks or months.

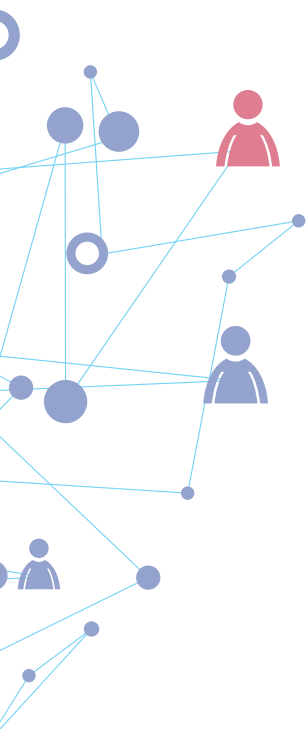
- Optimize the use of reagents, human resources, and specimens.
- Regularly notify those responsible for laboratory management of available stocks and the need for additional resources.
 - Request support from the reference laboratory and from PAHO, if necessary.
 - Maintain a database with an inventory of all the specimens received in the laboratory, the tests performed, the results reported, and corresponding dates.
 - Maintain good laboratory practices, ensuring the quality of the results reported to the surveillance system.

- Present an aggregated report of the activities carried out by the laboratory (see Annexes 8, 9, and 10) to the director of the laboratory and the epidemiologist responsible for outbreak response.
- Do not let yourself be pressured by others; maintain the technical criteria set out in RMRLN recommendations.

Epidemiological and laboratory information will allow the proper documentation of transmission chains, optimization of resources, and implementation of adequate control measures.



8. Quality assurance



Having the proper documentation to prove that elimination of measles and rubella has been achieved requires that the NLs produce the highest-quality laboratory surveillance data possible (38). This documentation includes reports from each country in the Region based on results from an accredited laboratory. Accreditation as a measles and rubella laboratory in the GMRLN is reviewed annually by PAHO/WHO (on-site or remotely), based on laboratory performance during the immediately preceding 12 months, and approved for the upcoming 12 months. The objective is to monitor the quality of the GMRLN and this is useful to identify possible problems and strengthen capacities, as needed.

The global accreditation review process assesses the following components:

- timely reporting of IgM results to the surveillance system;
- accuracy of IgM detection;
- implementation of internal quality control procedures;
- proficiency in IgM testing;
- proficiency in molecular testing;
- timely reporting of RNA detection and genotyping to the surveillance system and PAHO/WHO;
- timely forwarding of specimens for virus isolation and viral detection to the RRL;
- training and qualifications of laboratory staff;
- laboratory operating procedures and work practices:
 - space and rooms available
 - management and supervision
 - standardized operating procedures for management and inventory of specimens, including protocols for biosafety and containment of infectious material
 - equipment

- supplies
- communication with epidemiology and surveillance staff
- data management;
- score from on-site review of laboratory operating procedures and practices;
- capacity of the laboratory to perform virus isolation, RNA detection, sequencing, IgG avidity, and other tests for differential diagnosis, if resources are available.

In addition, the RMRLN assesses:

- Quarterly reporting of sporadic cases of measles or rubella with IgM-positive or indeterminate results to PAHO.
- Performance of subnational laboratory networks, where applicable.

Components related to quality assurance of routine testing, as well as result notification within the RMRLN, are covered in the sections below.

8.1 Participation in the global proficiency testing program for IgM tests

In 2000, the GMRLN launched a global proficiency testing program, using a panel of 20 serum samples from patients with a recent history of measles, rubella, or other rash illnesses.

Proficiency testing (PT) panels are provided to GMRLN members to assess their capacity to detect IgM against measles and rubella with ELISA testing. The PT panels are prepared by the Victorian Infectious Disease Laboratory (VIDRL) in Melbourne, Australia. VIDRL is a RRL for the Western Pacific Region and, on behalf of WHO's GMRLN, it is responsible for the production and feedback of PT panels (39). In the Region of Americas, the distribution of the panels to RMRLN members is coordinated by the CDC (as GSL) in conjunction with country program representatives, laboratory managers, and the RLC.

Testing and reporting of results should be timely, as described in the accreditation documents. Results are forwarded to PAHO, WHO headquarters in Geneva, and VIDRL. After submission of results, the

laboratory will receive a report from VIDRL within 10 days.

The RLC must 1) follow up on NL participation in the PT program for IgM and 2) develop improvement plans for any programs that show low performance, working collaboratively with staff from those laboratories to improve their capacity.

8.2 Indirect quality control of IgM assays

The provision of serum from routine testing at the NLs to the designated referral laboratory (RRL or GSL) for quality control (accuracy) is an important component of the quality assurance program of the GMRLN.

To ensure a high level of confidence in the quality of serologic testing carried out by the RMRLN to detect IgM (against measles and rubella), all NLs should send annual serum specimens to the RRL (identified by the RLC) to assess quality control. Specimens should be randomly selected from those processed in the previous 12 months (ensuring adequate volume) and organized consecutively by the IgM results. The following sample criteria should be followed:

- at least 10 IgM-negative measles specimens;
- up to 10 IgM-positive measles specimens;
- at least 10 IgM-negative rubella specimens;
- up to 10 IgM-positive rubella specimens;
- up to 10 IgM measles or rubella specimens with an indeterminate result.

The NL should complete the form for shipping the specimens to the RRL or the GSL for assessment of quality control (Annex 1) and include it in the shipment. The RRL will use the form to update the record of results.

The diagnostic tests carried out by the LN will be analyzed by the GSL or RRL and the results will be communicated to the NL that sent the specimens and to the RLC. In the case of discordant results, additional testing or consultation with the GSL or RRLs will be conducted through the RLC to address any perceived problems. The results of the quality

control assessment will be recorded on the checklist for annual PAHO/WHO accreditation and contribute to the accreditation score.

8.3. Participation in the global program for proficiency testing of molecular assays

Since 2015, WHO and CDC have implemented a PT program for molecular techniques. GMRLN members performing molecular tests participate in this program.

The PT panel consists of FTA® filter paper discs containing lysates of cells infected with wild-type measles or rubella viruses. All discs are non-infectious, and RNA should be extracted and tested in molecular assays that are used in the laboratory. Results should be reported using the report form provided with the PT panel.

The PT panel results submitted by the NLs and RRL are assessed by the GSL (CDC) based on the following criteria:

- ability to detect measles and rubella RNA by RT-qPCR;
- ability to generate the required amplicons for genotype analysis; and
- ability to perform sequencing and sequence analysis to correctly identified the viral genotype.

Based on these criteria, the molecular testing is evaluated by the GSL and communicated to the participating laboratory and the RLC. In some cases, the NLs are asked to retest one or more specimens and submit the correct result before the laboratory is considered proficient for molecular testing. These results should be included on the checklist for annual PAHO/WHO accreditation and will be taken into consideration in the accreditation score.

8.4. Quality control of molecular assays: RT-qPCR and sequencing

The high sensitivity of PCR methods can lead to false-

positive results caused by contamination with previous PCR products or amplicons.³ Cross-contamination between specimens is also possible (40).

Following the recommendations below can reduce the likelihood of contamination:

- maintain a unidirectional workflow;
- ensure there is no interchange of supplies or equipment between the sample preparation room and the reagent preparation room or between the amplification/detection and sample preparation rooms;
- use gloves throughout experiments to prevent contamination with ribonucleases (RNases) found on hands;
- change gloves after touching skin, door handles, and common surfaces;
- have a dedicated set of pipettes that are used only for RNA work;
- use filter tips and tubes that are tested and guaranteed to be RNase-free;
- use RNase-free chemicals and reagents;
- reduce RNase contamination by cleaning tube racks, micropipettors, and the work surface of the PCR hood with 70% ethanol and RNase Zap®⁴ wipes; and
- reduce deoxyribonucleic acid (DNA) contamination with exposure to ultraviolet light for 15 minutes (as per CDC protocols).

8.5 Documentation of sporadic IgM-positive cases

RLC will prepare summaries that include all the laboratory's performance data, including its accreditation status, results for the PT panels, and confirmatory testing. The summary will be distributed to all RMRLN members and to the individuals responsible for the measles and rubella programs in the countries. These data will be critical in the assessment of the quality of laboratory testing following the elimination of measles and rubella in the Region of Americas.

³ Segment of DNA that has undergone amplification and thus contains replicated genetic material.

⁴ <https://www.thermofisher.com/order/catalog/product/AM9780>

Documentation of sporadic IgM-positive cases, regardless of final classification (see section 5), should be provided quarterly to the RLC (see Annex 3). Sporadic IgM-positive cases are expected when disease prevalence is low (see Annex 4); these cases provide a way of measuring the capacity of the surveillance system (30). Recording these cases in a standard format will: 1) allow a consolidated evaluation of such cases as part of the review of overall laboratory surveillance; 2) facilitate case classification; 3) help to generate information that will be published in scientific papers; and 4) provide valuable evidence to generate guidelines.

8.6 Data reporting

Standardized reporting is essential for the success of the RMRLN. Adherence to the recommended protocols for data reporting is encouraged. Most of the laboratories report routine serologic results to PAHO via the Measles Elimination Surveillance System or the Integrated Surveillance Information System database. The following minimal information must be included in the results report: name of the

laboratory; type of specimen; laboratory register number; specimen identification number; last measles-rubella (MR) vaccine; date of rash onset; date of specimen collection; date of receipt; type of test; result by test; interpretation criteria by test; date of results reporting; and name of person in charge of the laboratory or person that performed the tests.

Timely reporting of sequencing data and viral genotyping information is a critical component of laboratory surveillance in an elimination and post-elimination setting. Laboratories that perform sequence analysis should notify the RLC as soon as possible after identification of a genotype. Due to complexities with sequence analysis, especially for rubella viruses, but also for measles viruses, genotype determinations (phylograms) should be shared with the RRLs or the GLS before submitting information to the databases. Viral genotype information should be transmitted to the WHO Global Measles and Rubella Sequences Databases (Measles Nucleotide Surveillance-MeaNS⁵ and Rubella Nucleotide Surveillance-RubeNS⁶).

⁵ <http://www.who-measles.org>

⁶ www.who-rubella.org

9. References

1. Pan American Health Organization. Elimination of measles in the Americas. XXIV Meeting of the Pan American Sanitary Conference, Washington, DC, 1995.
2. de Quadros CA, Olive JM, Hersh BS, Strassburg M, Henderson DA, Brandling-Bennett D, et al. Measles elimination in the Americas—evolving strategies. *JAMA* 1996;275:224-9.
3. Pan American Health Organization. XVIII Technical Advisory Group (TAG) Meeting on Vaccine-preventable Diseases, Costa Rica, 2009.
4. Pan American Health Organization. Plan of Action for the Documentation and Verification of Measles, Rubella, and Congenital Rubella Syndrome Elimination in the Region of the Americas. Washington, DC: PAHO; 2011.
5. World Health Organization. Framework for verifying elimination of measles and rubella. *WER* 2013;88(9):89-100.
6. Pan American Health Organization. Measles elimination field guide. 2nd ed. Washington, DC: PAHO; 2005. Available at: http://www1.paho.org/hq/dmdocuments/2010/FieldGuide_Measles_2nd_Ed_e.pdf.
7. Western Pacific Region. Measles Elimination Field Guide. 2013.
8. Venczel L, Rota J, Dietz V, Morris-Glasgow V, Siqueira M, Quiroz E, et al. The Measles Laboratory Network in the Region of the Americas. *J Infect Dis*. 2003;187(Suppl 1):S140-5.
9. Roush SW, Beall B, Cassidy P, Gentsch J, Icenogle J, Mayer L, et al. Chapter 22: Laboratory support for surveillance of vaccine-preventable diseases. In: Roush SW, Baldy LM, editors. *Manual for the surveillance of vaccine-preventable diseases*. 5th ed. Atlanta: Centers for Disease Control and Prevention; 2012.
10. World Health Organization. *Manual for the laboratory diagnosis of measles and rubella virus infection*. 2nd ed. Geneva: WHO; 2007.
11. Bispo de Filippis AM, Icenogle J, Matus CR, Andrus JK. Enhanced laboratory surveillance for the elimination of rubella and congenital rubella syndrome in the Americas. *J Infect Dis*. 2011;204(Suppl 2):S652-8.
12. McLean H, Redd S, Abernathy E, Icenogle J, Wallace G. Chapter 14: Rubella. In: Roush SW, Baldy LM, editors. *Manual for the surveillance of vaccine-preventable diseases*. 5th ed. Atlanta: Centers for Disease Control and Prevention; 2012.
13. Moss WJ, Griffin DE. Measles. *Lancet*. 2012;379(9811):153-64.
14. Abernathy E, Cabezas C, Sun H, Zheng Q, Chen MH, Castillo-Solorzano C, et al. Confirmation of rubella within 4 days of rash onset: comparison of rubella virus RNA detection in oral fluid with Immunoglobulin M detection in serum or oral fluid. *J Clin Microbiol*. 2009;47(1):182-8.
15. Ozanne G, d'Halewyn MA. Performance and reliability of the Enzygnost measles enzyme-linked immunosorbent assay for detection of measles virus-specific immunoglobulin M antibody during a large measles epidemic. *J Clin Microbiol*. 1992;30(3):564-9.
16. Tipples GA, Hamkar R, Mohktari-Azad T, Gray M, Parkyn G, Head C, et al. Assessment of immunoglobulin M enzyme immunoassays for diagnosis of measles. *J Clin Microbiol*. 2003;41(10):4790-2.
17. Cohen BJ, Dobals D, Andrews N. Comparison of plaque reduction neutralisation test (PRNT) and measles virus-specific IgG ELISA for assessing immunogenicity of measles vaccination. *Vaccine*. 2008;26(50):6392-7.
18. Pannuti CS, Morello RJ, Moraes JC, Curti SP, Afonso

- AM, Camargo MC, et al. Identification of primary and secondary measles vaccine failure by measurement of immunoglobulin G avidity in measles cases during the 1997 São Paulo epidemic. *Clin Diagn Lab Immunol.* 2004;11(1):119–22.
19. Paunio M, Hedman K, Davidkin I, Valle M, Heinonen OP, Leinikki P, et al. Secondary measles vaccine failures identified by measurement of IgG avidity: high occurrence among teenagers vaccinated at a young age. *Epidemiol Infect.* 2000;124(2):263–71.
 20. Mercader S, Garcia P, Bellini WJ. Measles virus IgG avidity assay for use in classification of measles vaccine failure in measles elimination settings. *Clin Vaccine Immunol.* 2012;19(11):1810–7.
 21. Mubareka S, Richards H, Gray M, Tipples GA. Evaluation of commercial rubella immunoglobulin G avidity assays. *J Clin Microbiol.* 2006;45(1):231–3.
 22. Kutty P, Rota J, Bellini W, Redd SB, Barskey A, Wallace G. Capítulo 7: Measles. In: Roush SW, Baldy LM, editors. *Manual for the surveillance of vaccine-preventable diseases.* 6th ed. Atlanta: Centers for Disease Control and Prevention; 2013.
 23. Centers for Disease Control and Prevention. Progress toward measles elimination—region of the Americas, 2002–2003. *MMWR Morb Mortal Wkly Rep.* 2004;53(14):304–6.
 24. Icenogle JP, Siqueira MM, Abernathy ES, Lemos XR, Fasce RA, Torres G, et al. Virological surveillance for wild-type rubella viruses in the Americas. *J Infect Dis.* 2011;204(Suppl 2):S647–51.
 25. Lazar M, Abernathy E, Chen M, Icenogle J, Janta D, Stanescu A, et al. Epidemiological and molecular investigation of a rubella outbreak, Romania, 2011 to 2012. *Euro Surveill.* 2016;21(38):pii=30345.
 26. World Health Organization. Measles virus nomenclature update: 2012. *WER.* 2012;87(9):73–81.
 27. World Health Organization. Rubella virus nomenclature. *WER.* 2013;88(32):337–48.
 28. World Health Organization. *Guidance on Regulations for the Transport of Infectious Substances.* Geneva: WHO; 2017. Available at: http://apps.who.int/iris/bitstream/handle/10665/149288/WHO_HSE_GCR_2015.2_eng.pdf?sequence=1
 29. Benamar T, Tajounte L, Alla A, Khebbba F, Ahmed H, Mulders MN, et al. Real-time PCR for measles virus detection on clinical specimens with negative IgM result in Morocco. *PLoS One.* 2016;11(1):e0147154.
 30. Dietz V, Rota J, Izurieta H, Carrasco P, Bellini W. The laboratory confirmation of suspected measles cases in settings of low measles transmission: conclusions from the experience in the Americas. *Bull World Health Organ.* 2004;82(11):852–7.
 31. Vicari AS, Dietz V, Bellini WJ, Icenogle J, Castillo-Solorzano C. Interpretation of measles and rubella serology. In: Andrus JK, de Quadros CA, eds. *Recent advances in immunization.* 2nd ed. Washington, DC: PAHO; 2006: 80–96.
 32. Pan American Health Organization. Measles case classification. II. Frequent dilemmas in the field. *EPI Newsl.* 2001;23(6):3–4.
 33. Rota JS, Hickman CJ, Sowers SB, Rota PA, Mercader S, Bellini WJ. Two case studies of modified measles in vaccinated physicians exposed to primary measles cases: high risk of infection but low risk of transmission. *J Infect Dis.* 2011;204 (Suppl 1).
 34. Hickman CJ, Hyde TB, Sowers SB, Mercader S, McGrew M, Williams NJ, et al. Laboratory characterization of measles virus infection in previously vaccinated and unvaccinated individuals. *J Infect Dis.* 2011;204(Suppl 1):S549–58.
 35. Rosen JB, Rota JS, Hickman CJ, Sowers SB, Mercader S, Rota PA, et al. Outbreak of measles among persons with prior evidence of immunity, New York City, 2011. *Clin Infect Dis.* 2014;58(9):1205–10.
 36. Sowers SB, Rota JS, Hickman CJ, Mercader S, Redd S, McNall RJ, et al. High concentrations of measles neutralizing antibodies and high-avidity measles IgG accurately identify measles reinfection cases. *Clin Vaccine Immunology.* 2016;23(8):707–16.
 37. Susan J. M. Hahné, Laura M. Nic Lochlainn, Nathalie D. van Burgel, Jeroen Kerkhof, et al. Measles outbreak among previously immunized healthcare workers, the Netherlands, 2014. *J Infect Dis.* 2016 214;(12):1980–86.
 38. Centers for Disease Control and Prevention. *Global Measles and Rubella Laboratory Network Support for Elimination Goals, 2010–2015.* *MMWR Morb Mortal Wkly Rep.* 2016;65(17):438–42.
 39. Featherstone DA, Rota PA, et al. Expansion of the Global Measles and Rubella Laboratory Network 2005–09. *JID* 2011;204(Supl 1):S491–98.
 40. United States Environmental Protection Agency. *Quality assurance/quality control guidance for laboratories performing PCR analyses on environmental samples.* Washington: EPA; 2004. Available at: <https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf>.

10. Annexes

Annex 1.A. Form for shipping specimens from the NL to the RRL or to the GSL for quality control of measles testing

Name of National Lab:

Country:

Date of shipment

Item	Lab No.	Patient name or initials	Age Y = years/ M = months	Sex M=male/ F=female	City	Date of last measles rubella vaccine DD/MM/YY	Date of rash onset DD/MM/YY	Date of sample collection		IgM-Measles 1 st serum sample		IgM-Measles 2 nd serum sample		IgG-Measles, 1 st and 2 nd sera samples	
								1 st serum DD/MM/YY	2 nd serum DD/MM/YY	Value OD*	Result	Value OD*	Result	Result (UI/mL) 1 st serum	Result (UI/mL) 2 nd serum
1	161	[Name or initials]	5 Y	M	San Carlos	15/01/12	15/05/16	18/05/16	14/06/16	0.212	Positive	0.095	No tested	No tested	No tested
2	198	[Name or initials]	14 M	F	San Juan	18/10/16	25/10/16	28/10/16	14/11/16	0.333	Positive	0.468	Positive	Positive (22 mUI/mL)	Positive (89 mUI/mL)
3															
4															
5															
6															
7															
8															
9															
10															

* OD = optical density.

Annex 1.B. Form for shipping specimens from the NL to the RRL or to the GSL for quality control of rubella testing

Name of National Lab: _____ Date of shipment _____

Country: _____

Item	Lab No.	Patient name or initials	Age Y = years/ M = months	Sex M=male/ F=female	City	Date of last Measles Rubella vaccine DD/MM/YY	Date of rash onset DD/MM/YY	Date of sample collection		IgM-rubella 1 st serum sample		IgM-rubella 2 nd serum sample		IgG-rubella, 1 st and 2 nd sera samples	
								1 st serum DD/MM/YY	2 nd serum DD/MM/YY	Value OD*	Result	Value OD*	Result	IgG-rubella, 1 st and 2 nd sera samples	IgG-rubella, 1 st and 2 nd sera samples
1	161	[Name or initials]	8 Y	M	San Carlos	15/01/12	15/05/16	18/05/16	14/06/16	0.212	Positive	0.095	Negative	No tested	No tested
2	198	[Name or initials]	14 M	F	San Juan	18/10/16	25/10/16	28/10/16	14/11/16	0.333	Positive	0.468	Positive	Positive (22 UI/mL)	Positive (89 UI/mL)
3															
4															
5															
6															
7															
8															
9															
10															

* OD = optical density.

Annex 2. Form for shipping specimens from the NL to the RRL or to the GSL for laboratory confirmation of measles and rubella

Name of National Lab: _____ Country: _____ Date of shipment _____

Item	Lab No.	Patient name or initials	Age Y= years/ M= months	Sex M= male/ F= female	City and State	Date of last measles rubella vaccine DD/MM/YY	Date of rash onset DD/MM/YY	Type of specimen (sera, urine, swab) 1st serum DD/MM/YY	Date of specimen collection		IgM results 1 st sample		IgM results 2 nd sample		IgG-measles*		IgG-rubella*		Other assays developed at the NL and results			Tests required to perform at RRL or GSL			
									2nd serum DD/MM/YY	Urine or swab DD/MM/YY	Measles IgM-result (OD value)	Rubella IgM-result (OD value)	Measles IgM-result (OD value)	Rubella IgM-result (OD value)	1 st sample Result (UI/ml)	2 nd sample Result (UI/ml)	1 st sample Result (UI/ml)	2 nd sample Result (UI/ml)	Rubella avidity	RT-PCR measles	PCR rubella		Not tested	Not tested	Not tested
1	161	[Name or initials]	8 Y	M	San Juan, PR		15/01/12	Sera, swab	15/01/12	31/01/12	15/01/12	Positive (0.459)	Indeterminate (0.158)	Positive (0.566)	Negative (0.028)	Positive (20 UI/ml)	Positive (87 UI/ml)	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	PCR measles	
2																									
3																									
4																									
5																									
6																									
7																									
8																									

*OD = optical density.

* Include the name of the kit and the interpretation criteria if the NLs use kits different from the standardized kits in the RMRLN.

Annex 3. Laboratory record of sporadic cases of measles or rubella with IgM-positive or indeterminate result

Name of patient: _____ Case ID: _____

Age of patient: _____ Residence (district/city): _____

Date of fever onset: _____ Date of rash onset: _____

Clinical diagnosis: Rubella Measles Not specified Other _____

Strong suspicion of measles or rubella: No Yes

History of vaccination? (MR or MMR)

Yes, vaccinated (respond to all the following questions that apply) Not vaccinated Unknown

1 dose Date: _____ Unknown

2 doses Date: _____ Unknown

Exposure to a confirmed case or contact with international visitors? No Yes

Travel history 21 days before rash onset? No Yes

Travel return date: _____

1. LABORATORY RESULTS

1.1 Serological methods

First serum sample				
Collection date:				
IgM	Positive	Negative	Indeterminate	OD / CO*
Measles				
Rubella				

Second serum sample				
Collection date:				
IgM	Positive	Negative	Indeterminate	OD / CO*
Measles				
Rubella				

IgG	Positive	Negative	Not tested	Value (IgG titer **)
Measles				
Rubella				

IgG	Positive	Negative	Not tested	Value (IgG titer **)
Measles				
Rubella				

IgG avidity	Low	High	Intermediate
Measles			
Rubella			

IgG avidity	Low	High	Intermediate
Measles			
Rubella			

* OD = Optical density / CO = Cut-off value.

** To determine IgG seroconversion, the first and second serum samples must be processed in the same assay.

1.2 Virological methods

Sample collected?	No	Yes	Date collected	Condition of sample
Throat swab				
Nasal swab				
Nasopharyngeal swab				
Urine				

Viral isolation: No Yes Result obtained: Negative Positive

RT-qPCR: No Yes Result obtained: Negative Positive

Genotype: No Yes Result obtained:

2. CASE ANALYSIS

Lab-confirmed: No Yes

Ruled out based on

Lab test: No Yes

Clinical data: No Yes

Epidemiological data: No Yes

Could not be ruled out based on available lab testing: No Yes

If any answer was "Yes," please provide details and add comments.

Name and title of people who participated in the revision:

Place and date of revision of the case:

Annex 4. Influence of the prevalence on the positive predictive value of the test

While a diagnostic test allows the determination of antibodies against the measles or rubella virus, it is important to remember that the test results depend on compliance with all validity criteria established by the manufacturer. Furthermore, results can depend of disease's prevalence in a certain population.

To better illustrate this situation using Bayes' theorem, we present a test to identify IgM antibodies against measles with a sensitivity of 97% and specificity of 99% in population of 500 people, in two different epidemiological scenarios: one scenario with a prevalence of 10% and another with a prevalence of 1%.

A summary of the results obtained in each scenario is presented in the 2x2 table below.

Test result	Sick individuals	Healthy individuals	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	N

a = sick individuals with positive result (true positive)

b = healthy individuals with positive result (false positive)

c = sick individuals with negative result (false negative)

d = healthy individuals with negative result (true negative)

a + b = total number of individuals with a positive result

c + d = total number of individuals with negative result

a + c = total number of sick individuals

b + d = total number of healthy individuals

N = total population

VPP = a/a+b

VPN= d/b+d

Scenario 1: Prevalence = 10%			
Test sensitivity: 97%			
Test specificity: 99%			
Test result	Sick individuals	Healthy individuals	Total tests
Positive	49	5	53
Negative	1	445	447
	50	450	500

PPV = $49/53 = 0.91$

NPV = $445/447 = 0.99$

Scenario 2: Prevalence = 1%			
Test sensitivity: 97%			
Test specificity: 99%			
Test result	Sick individuals	Healthy individuals	Total tests
Positive	5	5	10
Negative	0	490	490
	5	495	500

PPV = $5/10 = 0.50$

NPV = $490/490 = 1$

*Using Bayes' theorem, it is possible to calculate the positive predictive value (PPV) and negative predictive value (NPV) based on sensitivity and specificity at different prevalence rates.

Annex 5. Summary of situations of sporadic cases of measles or rubella in which additional testing may be necessary

Situation	Situation 1	Situation 2*	Situation 3**	Situation 4	Situation 5
Description of the sporadic cases	IgM-positive measles OR IgM-positive rubella	IgM-positive measles AND IgM-positive rubella	IgM-positive measles OR IgM-positive rubella	IgM-negative measles OR IgM-negative rubella	IgM-negative measles or indeterminate OR IgM-negative rubella or indeterminate
Strong suspicion of measles or rubella?	NO	NO	YES	YES	YES
History of vaccination in the 8 weeks prior to rash onset?	NO	NO	YES	NO	YES (>4 months)
Exposure to a confirmed case /contact with international visitors?	NO	NO	YES	YES	YES
Travel history 21 days prior to rash onset?	NO	NO	NO	YES	YES
Laboratory tests					
	Required	Required	Required	Required	Required
	If the acute serum sample is IgG-negative, it should be analyzed for the presence of IgG in a second sample. If only one serum sample is available, avidity testing is recommended.	If the acute serum sample is IgG-negative, it should be analyzed for the presence of IgG in a second sample. If only one serum sample is available, avidity testing is recommended.	If only one serum sample is available and the sample was collected within 3 days of rash onset: <ul style="list-style-type: none">an IgG-positive result can rule out a case of acute rubella. <i>This does not apply for measles.</i>an IgG-negative result requires virologic testing to confirm the case.	If only one serum sample is available: <ul style="list-style-type: none">an IgG-positive result for rubella can rule out the case if the sample was collected within the 3 days of rash onset. <i>This does not apply for measles.</i>an IgG-negative result will require a second serum sample for IgG testing. If a second serum sample is collected more than 3 days after rash onset, the case is confirmed if: <ul style="list-style-type: none">there is an IgM-positive resultthe acute serum is IgG-negative and the second serum sample is IgG-positive.	If only one serum sample is available for IgG testing for measles or rubella: <ul style="list-style-type: none">an IgG-positive result for rubella can rule out this disease if the sample was collected within 3 days of rash onset. <i>This does not apply for measles.</i>an IgG-negative result requires a second serum sample (collected between day 4 and day 30 after rash onset) to detect IgM against measles or rubellaIgM or IgG seroconversion against measles or rubella can confirm the case.
IgG test	Required	Required	Not Required	Required	Required
IgG seroconversion	IgG seroconversion will confirm the case if the first sample is negative and the second is positive, or if there is a significant increase in titers between coupled sera.	IgG seroconversion will confirm the case if the first sample is negative and the second is positive, or if there is a significant increase in titers between coupled sera.	Serology cannot be used to differentiate between natural infection and a vaccine immune response.	IgM or IgG seroconversion will confirm the case if the first sample is negative and the second is positive, or if there is a significant increase in IgG titers between coupled sera.	IgM or IgG seroconversion will confirm the case if the first sample is negative and the second is positive, or if there is a significant increase in IgG titers between coupled sera.

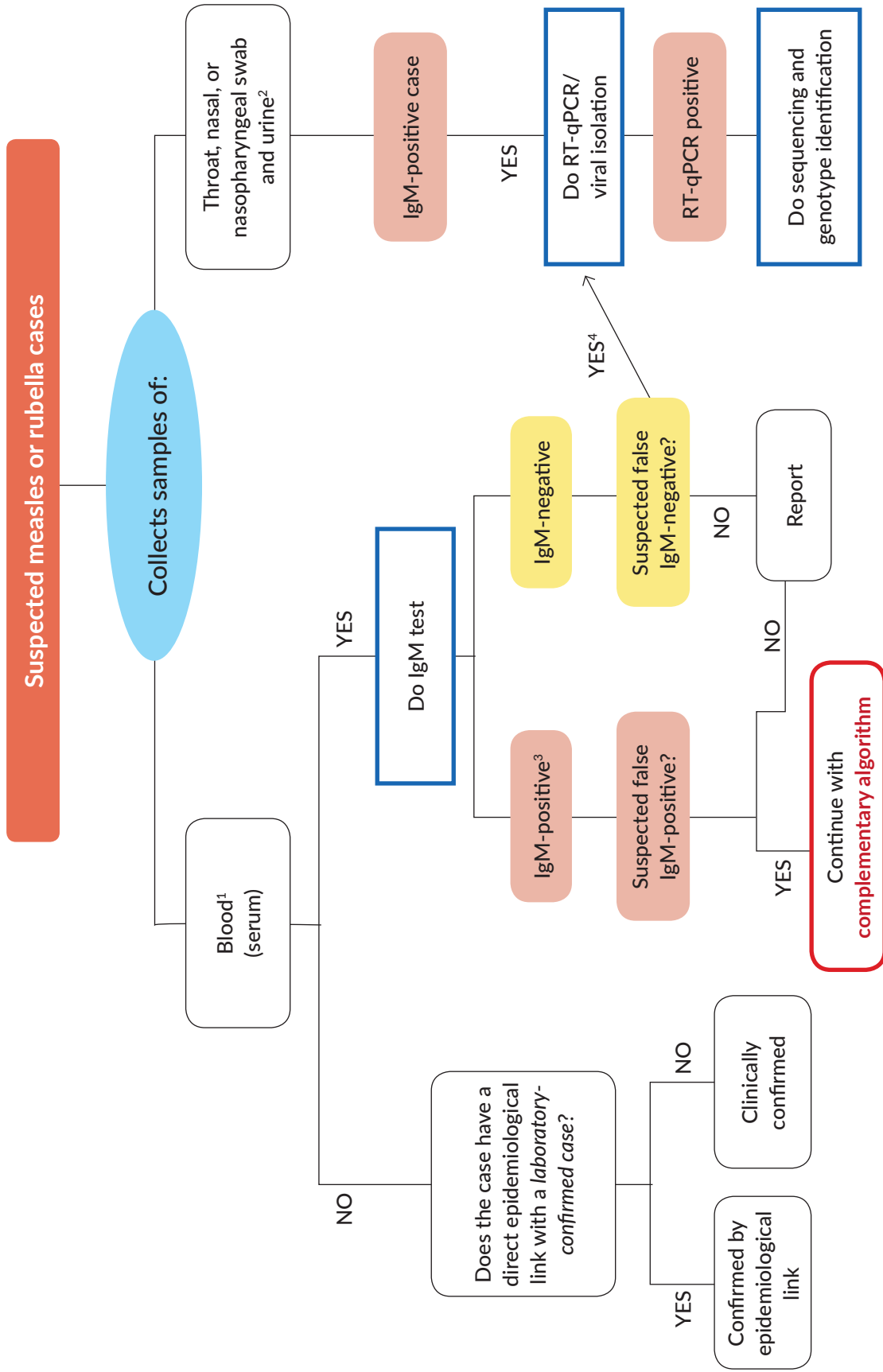
	Recommended	Recommended	Not Required	Not Required	Optional
IgG avidity	When only one serum specimen is available, and it is IgG-positive <ul style="list-style-type: none"> • Low-avidity IgG can be used to confirm a recent infection. 	When only one serum specimen is available, and it is IgG-positive <ul style="list-style-type: none"> • Low-avidity IgG can be used to confirm a recent infection 	Serology cannot be used to differentiate between natural infection and a vaccine immune response.		
Viral isolation or RT-qPCR	<ul style="list-style-type: none"> • A positive result will confirm the case • A negative result will not be conclusive 	<ul style="list-style-type: none"> • A positive result will confirm the case • A negative result will not be conclusive 	<ul style="list-style-type: none"> • An attempt should be made to isolate the virus in a cell culture (except for the samples of oral fluid) • Viral detection by RT-qPCR • A positive result requires genotype identification 	<ul style="list-style-type: none"> • A positive result will confirm the case • A negative result will not be conclusive 	<ul style="list-style-type: none"> • A positive result will confirm the case, while a negative result will not be conclusive
Sequence analysis	<ul style="list-style-type: none"> • A positive result will confirm the case • A negative result will not be conclusive 	<ul style="list-style-type: none"> • A positive result will confirm the case • A negative result will not be conclusive 	<ul style="list-style-type: none"> • Detection of a wild-type virus will confirm the case • Detection of a sequence of a vaccine strain rules out the case 	<ul style="list-style-type: none"> • A positive result will confirm the case • A negative result will not be conclusive 	<ul style="list-style-type: none"> • A positive result will confirm the case, while a negative result will not be conclusive

* This situation suggests a nonspecific reaction, i.e., the possibility that another causative agent should be considered because simultaneous infections have not been described by both wild-type viruses and sequential infections would be very uncommon in an elimination setting. In collaboration with the research epidemiologist, other probable causes should be ruled out, such as parvovirus B19, dengue, or other arboviruses, if possible. Additional tests should be carried out, as described above, in order to confirm measles or rubella infection.

** This is a common situation during outbreaks in which vaccination is part of the outbreak control strategy. In this case, serological response cannot be used to differentiate natural infection from a vaccine reaction, and genetic characterization of the isolated virus or a PCR result is necessary.

NOTE: Consideration should be given to the desirability of testing for other viruses that produce rash illnesses, such as dengue and other arbovirus, parvovirus B19, human herpes virus 6 (HHV-6), or HHV-7 (roseola).

Annex 6. Routine algorithm for testing of specimens from a suspected measles and rubella case



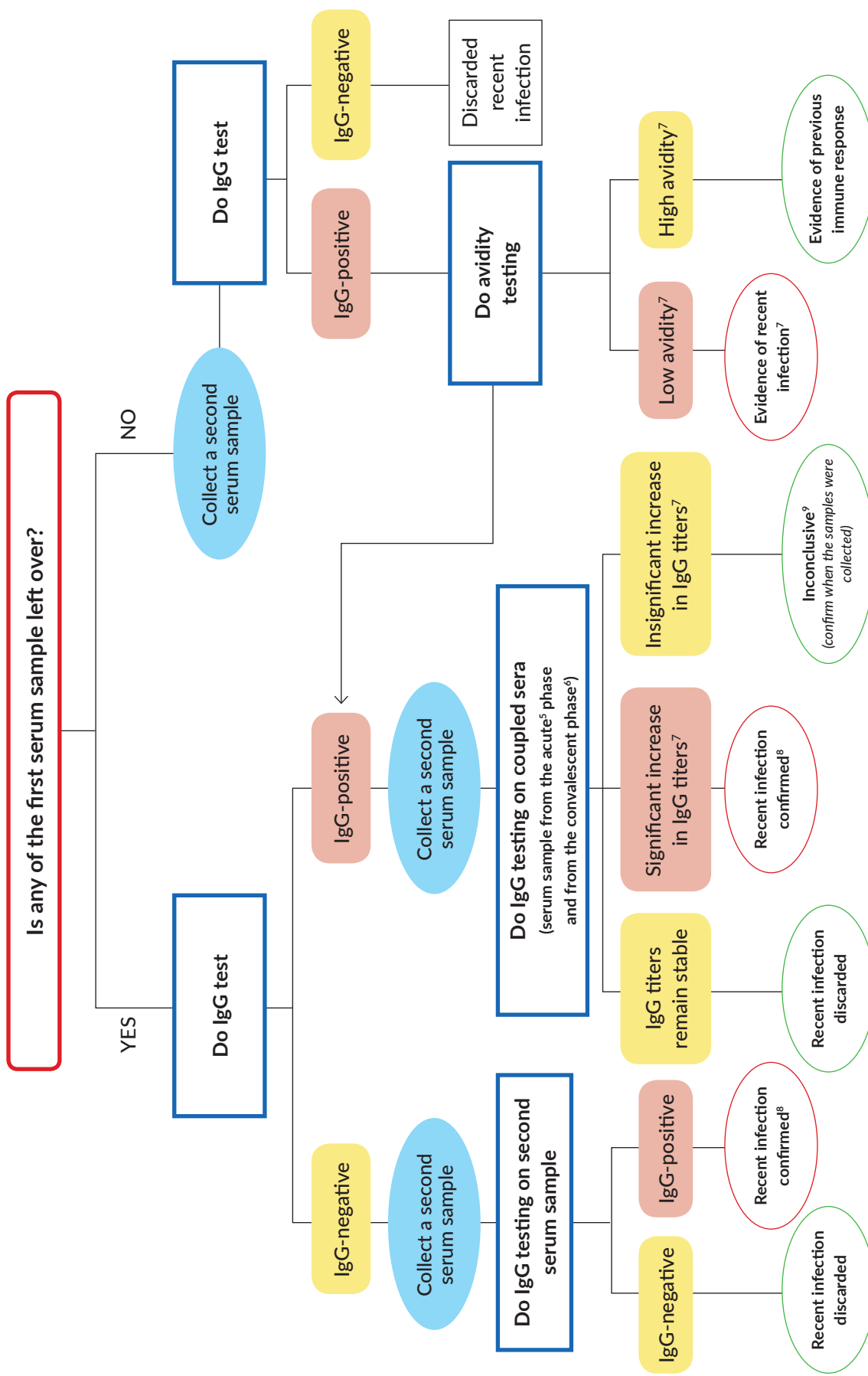
¹ Collect an adequate serum sample no more than 30 days after rash onset.

² Collect a respiratory specimen within 3 days of rash onset and no later than 10 days.

³ In an elimination setting, all IgM-indetermined results should be regarded as IgM-positive. Virological testing is recommended.

⁴ Analyze IgG in the first serum sample and request collection of a second sample for additional testing. Virological testing is recommended.

Annex 7. Complementary algorithm for serological testing of specimens with initial IgM-positive or indeterminate result



⁵ Collect an adequate serum sample within 7 days of rash onset.

⁶ Collect an adequate serum sample in the convalescent phase, 14–21 days after the sample from the acute phase.

⁷ Follow the criteria for test interpretation.

⁸ Evidence indicates a recent infection (either wild type or vaccine strain).

⁹ Correlate with IgG avidity results.

Annex 8. Total number of serum samples received, processed, and IgM-positive, by origin and date of collection *

Epi Week	EW1			EW2			EW3			EW4			EW5			EW6			EW7			EW8			EW9			EW10			EW11			EW12			
	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+	Rec	Test	IgM+							
Origin																																					
State A																																					
Municipality A1																																					
Municipality A2																																					
Municipality A3																																					
Municipality A4																																					
Municipality A5																																					
Total State A																																					
State B																																					
Municipality B1																																					
Municipality B2																																					
Municipality B3																																					
Municipality B4																																					
Municipality B5																																					
Total State B																																					
State C																																					
Municipality C1																																					
Municipality C2																																					
Municipality C3																																					
Municipality C4																																					
Municipality C5																																					
Total State C																																					
Total A + B + C																																					

Rec = Received.

Test = Tested Elisa-IgM.

IgM+ = IgM-positive.

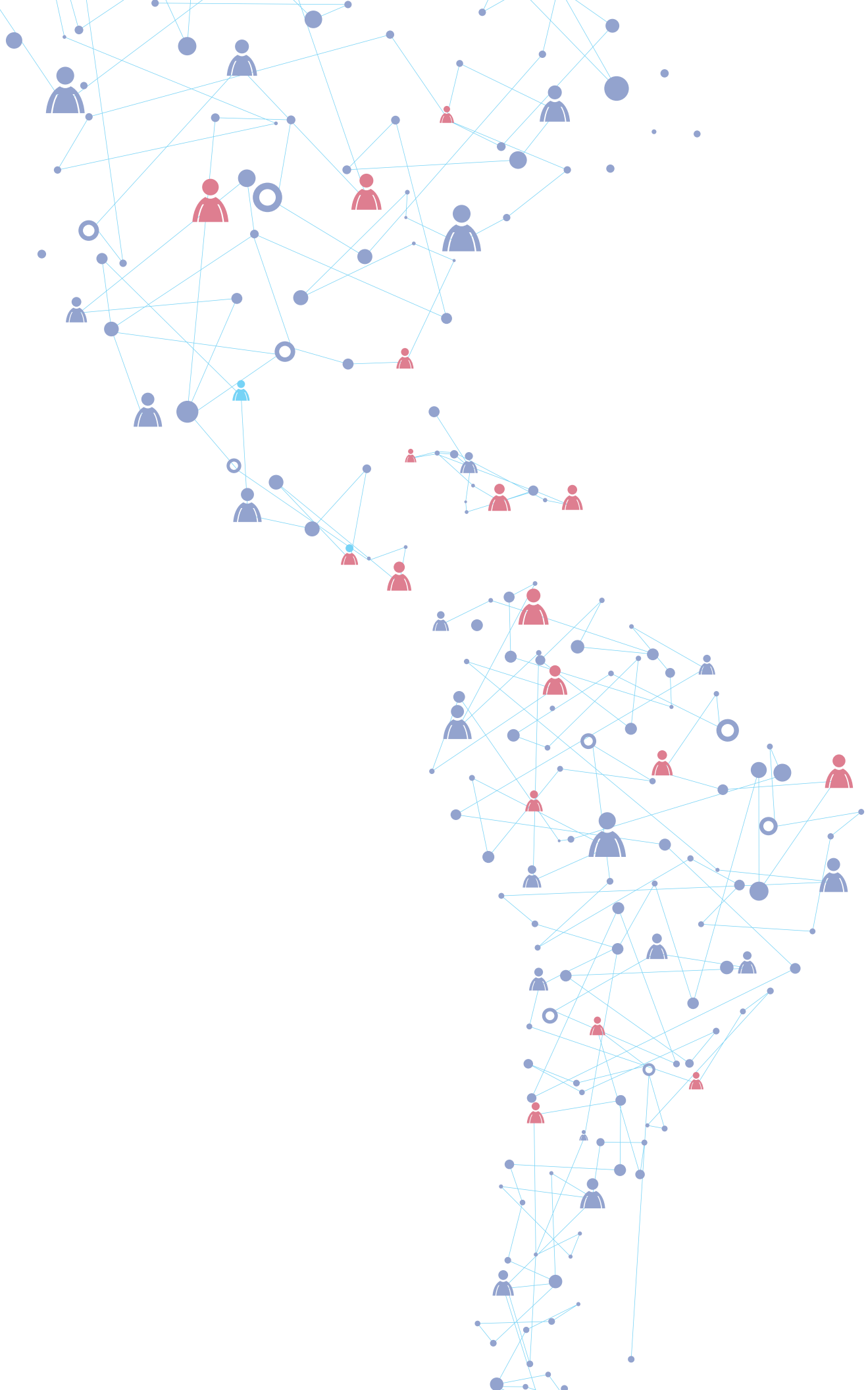
NOTE: The epidemiological week should correspond to the week of rash onset for the case.

Annex 10. Genotypes identified, and number of sequences detected, by origin and sample collection date*

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
State A												
Municipality A1	B3 (5)**		B3 (3)		B3 (8)							
Municipality A2												
Municipality A3												
Municipality A4												
Municipality A5												
Total State A	B3 (5)		B3 (3)		B3 (8)							
State B												
Municipality B1		B3 (5)		B3 (3)		B3 (8)						
Municipality B2												
Municipality B3												
Municipality B4												
Municipality B5												
Total State B		B3 (5)		B3 (3)		B3 (8)						
State C												
Municipality C1						B3 (5)		B3 (3)		B3 (8)		
Municipality C2												
Municipality C3												
Municipality C4												
Municipality C5												
Total State C												
Total A + B + C	B3 (5)	B3 (5)	B3 (3)	B3 (3)	B3 (8)	B3 (13)		B3 (3)		B3 (8)		

* = The month should correspond to the month of rash onset for the case.

** B3 (5) = B3 genotype, (5) five detected sequences.



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525 Twenty-third Street, N.W.
Washington, D.C. 20037

ISBN: 978-92-75-11997-6

