

Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome

Chapter 8. Laboratory testing in support of measles and rubella surveillance in elimination settings

Chapter 8 overview

The laboratory confirmation of suspected cases of measles and rubella becomes more challenging in settings where significant progress has been made toward elimination (near-elimination) or where the absence of endemic circulation of these diseases has been successfully demonstrated and verified (post-elimination). Because the strategy for achievement and maintenance of elimination requires a case-based surveillance programme, greater demands are placed on the laboratory to confirm suspected cases of measles or rubella or to discard a suspected case [1,2]. When a suspected case is detected, rapid laboratory confirmation and implementation of control measures are critical elements for minimizing additional exposures and onward transmission of virus. However, where there is a low prevalence (or absence) of measles and rubella, there is a greater likelihood that time and resources directed toward investigations of suspected cases may include a higher proportion of IgM positive cases that are not true cases.

As discussed in Section 8.1.1, the reliability of an IgM positive EIA result to accurately identify a true case of measles or rubella is significantly diminished due to the low prevalence of disease in elimination settings. However, the investigation for a clinically suspicious case should not be delayed pending the completion of additional testing or collection of additional specimens that may be planned to resolve lingering questions about the case.

This chapter provides guidelines for additional testing strategies including specialized testing that may provide helpful for the final classification of suspected cases of measles and rubella in an elimination setting. In most circumstances, routine testing for IgM will provide sufficient laboratory evidence to confirm a suspected case, as most cases will have an epidemiologic association with another confirmed case or other known risk factor for exposure to virus.

The epidemiologic context is extremely important in deciding which cases warrant further testing. To resolve the true disease status for a challenging case, it is essential that all information regarding the suspected case are shared between health care providers, epidemiologists, and laboratory staff. Countries in elimination settings should consider simultaneous testing for prevalent diseases that can mimic measles or rubella infections, particularly where diseases such as dengue and chikungunya are endemic. Network laboratories are encouraged to consult with the regional laboratory coordinator (RLC) to discuss the best approach when confronted with a difficult case, particularly those without a known source of infection.

In this chapter:

- 8.1 Challenges for accurate case classification in elimination settings
 - 8.1.1 Positive predictive value and low prevalence of disease
 - 8.1.2 Suspected cases among individuals with a rash following MR/MMR vaccination
 - 8.1.3 Measles and rubella cases in presumptively immune, vaccinated individuals
- 8.2 Utility and limitations for molecular testing in elimination settings
- 8.3 Difficult cases and situations that may require additional testing
- 8.4 Additional testing to aid case classification
 - 8.4.1 Guidelines for testing paired serum specimens for IgG titre measurements
 - 8.4.2 Criteria for discarding a suspected case of measles or rubella
- 8.5 Measles reinfections: characteristics and case confirmation

8.1 Challenges for accurate case classification in elimination settings

The laboratories in the GMRLN must follow established criteria to discard a suspected case that had a positive or equivocal IgM result when the clinical and epidemiologic data based on the case investigation indicates that the result may be a false positive or due to recent vaccination [3]. However, it is understood that some non-cases will be included as confirmed cases based on laboratory confirmation by IgM detection. Collection of a second serum specimen to test for a rise in IgG may be warranted in certain instances to support an IgM positive or equivocal result when prevalence of disease is low. An accurate vaccination history is critical since an individual

may have a rash due to a recent vaccination (section 8.1.2). Detection of virus-specific RNA by RT-PCR is a valuable diagnostic tool to support a positive or equivocal IgM result. In addition, collection of good quality virologic specimens can provide the means to distinguish vaccine strains from wild-type virus. However, as discussed in section 8.2, a negative RT-PCR result should not be relied upon as sole justification to discard a case.

8.1.1 Positive predictive value and low prevalence of disease

In low prevalence settings, there is a considerable decrease in the positive predictive value (PPV) for IgM positive results since the specificity of EIAs (and most serologic assays) is less than 100%. The PPV is the proportion of suspected cases with a positive laboratory result that are correctly identified as having the disease (true positive cases). While the relationship of PPV to disease prevalence is well-understood, the extent of the effect due to low prevalence is often not fully appreciated. In a study of IgM positive results in Ontario (2009-2014), the PPV for IgM positive cases that was calculated for measles was 17.4% and the PPV for rubella was 3.6% [4]. However, the authors of that study speculated that some of the IgM positive results in the study were obtained from testing healthy individuals because the IgM tests were requested inappropriately by the physician. Regardless, the study highlights the fact that an assay with <100% specificity will produce a PPV that is quite low when the disease is rare, even when only individuals who meet the case definition for measles or rubella are tested.

It is critical that the interpretation of laboratory results for the classification of suspected cases of measles and rubella is consistent throughout the GMRLN. The default interpretation for a suspected case with a positive or equivocal IgM result is to confirm the case. Communication between the laboratory and health personnel investigating the suspected case is essential to determine whether additional testing is appropriate and feasible. Information provided about local outbreaks or recent notifications of non-measles/non-rubella rash illnesses in the area can aid in decisions regarding whether other testing and/or collection of additional specimens may be justified. Section 8.4 provides guidelines for additional testing including a flowchart (Figure 8).

8.1.2 Suspected cases among individuals with a rash following MR/MMR vaccination

The proportion of individuals (without exposure to wild-type measles) who develop rash 7-14 days following vaccination with a measles-containing vaccine (generally the rash is attributed to the measles component of the MR or MMR vaccination) is estimated to be 5%. However, heightened surveillance for rash illness can inflate that number. In an elimination setting, particularly when recent measles or rubella activity has raised the index of suspicion, specimens from individuals with a vaccination-associated rash may surge.

The cases that have a possible exposure to wild-type measles virus and a recent vaccination cannot be distinguished by IgM testing. Often, the determination of true case status for these suspected cases is critical for decisions on whether exposed infants receive immune globulin for post exposure prophylaxis. This situation may occur following outbreak control measures that include vaccination of susceptible contacts and as well as unimmunized individuals in the surrounding community. Consequently, the increased vigilance for measles will result in notifications of suspected measles cases among recently vaccinated children with rash, most with a mild rash that would not normally generate suspicion of a wild-type infection.

Identification of a vaccine strain by sequence analysis of clinical specimens from a recently vaccinated, suspected measles case demonstrates that the rash illness was not due to a wild-type infection, and the case can be discarded. If genotyping cannot be performed, the case may be discarded and classified as a vaccine-related rash provided that the suspected case meets all the necessary epidemiologic and clinical criteria for a vaccine-associated rash, including the absence of any possible exposure to wild-type measles (see chapter 4, section 4.3). A specialized real-time RT-PCR assay that targets nucleotide sequences common to measles vaccine strains (genotype A) may be available in some NLs and RRLs to provide more rapid confirmation of vaccine reactions [11]. This method is particularly helpful when amplification of the full sequence required for genotyping is unsuccessful (chapter 7).

8.1.3 Measles and rubella cases in presumptively immune, vaccinated individuals

In elimination settings, where vaccine coverage is high, many of the confirmed cases of measles/rubella will occur among individuals who have a past history of vaccination and are

presumptively immune. This situation can be confusing and may sometimes lead to delays in appropriate specimen collection and testing. For example, upon evaluation of a rash illness in a previously vaccinated patient, a healthcare provider may consider an alternative etiology such as Kawasaki disease rather than measles or rubella.

Many of the confirmed measles cases, and nearly all of the confirmed rubella cases that occur among vaccinated individuals will be due to a primary vaccine failure (PVF), in which the individual failed to seroconvert following vaccination. The clinical presentation and laboratory evaluation for a case with a PVF is no different from that for a primary measles infection in an unvaccinated individual. A confirmed measles or rubella case with a PVF can be demonstrated by measurement of low avidity, virus-specific IgG (for a discussion of avidity testing, see chapter 4).

While symptomatic reinfections with rubella are extremely rare, there are numerous reports of confirmed measles cases that were identified as having a measles reinfection by demonstration of the presence of high avidity measles IgG in acute serum [5-10]. The clinical presentation of a measles reinfection case varies, from a mild fever with transient rash to an illness that meets the clinical case definition. Regardless of the clinical presentation, the serologic confirmation of measles reinfection cases can be challenging, and RT-PCR offers the best method to confirm these cases. The characteristics of measles reinfection cases and the additional testing that may be necessary to identify these cases are presented in section 8.5.

8.2 Utility and limitations for molecular testing in elimination settings

Collection of clinical samples for molecular testing from all suspected cases is recommended not only for genetic characterization (chapter 7), but also case confirmation by detection of virus-specific RNA by RT-PCR (chapter 6). This is especially important if a positive IgM result is obtained from a sporadic case with an unknown source. Unless there is an epidemiologic link or a high index of suspicion (e.g., recent travel), a positive IgM result may be questioned due to the low PPV in elimination settings. When appropriate precautions are in place to reduce the risk of

cross-contamination, and adequate controls are included to detect cross-contamination, a positive RT-PCR result for virus-specific RNA confirms the case.

In addition to providing support for a positive IgM result, detection of virus-specific RNA by RT-PCR is particularly useful for case confirmation when a negative IgM result is obtained from serum collected in the first few days after rash onset. Among suspected cases that have **not** been recently vaccinated, a positive RT-PCR result can provide confirmation of acute infection even if the IgM result is negative. Occasionally, the reliability of a positive RT-PCR result may be questioned if a high Ct value (near the cut-off) is obtained and/or a repeat test produces an indeterminate or negative result (chapter 6). When RT-PCR results are considered inconclusive, an attempt may be made to re-extract the RNA and repeat the test if there is any clinical specimen remaining.

However, because negative RT-PCR results may be obtained due to poor sample quality, failure to detect virus-specific RNA should not be used to rule out a case. Timeliness of collection, while a primary determinant of the sample quality, is not the only variable that can compromise the ability to detect virus-specific RNA (see [chapter 6](#)). In addition, negative RT-PCR results may be obtained from measles reinfection cases, even when clinical specimens are promptly collected (see section 8.5). If RT-PCR results are inconclusive or adequate samples were not collected, the case must be classified based upon the results of IgM testing. Depending on the epidemiologic context, additional specimen collection and/or laboratory testing may be pursued to resolve a challenging case with conflicting or inconclusive results (sections 8.3, 8.4).

8.3 Difficult cases and situations that may require additional testing

Many suspected cases of measles or rubella that are tested in an elimination setting may not be highly suspicious for these diseases, and yet may be confirmed following detection of virus-specific IgM. For these doubtful cases, without a positive RT-PCR result to corroborate the IgM, there will be increased pressure on the laboratory to conduct additional testing that will provide the evidence needed to discard the case. A checklist is provided below that may be helpful to evaluate the likelihood that the positive or equivocal IgM result was nonspecific or to support a request for additional testing:

- Confirm that the IgM assay used for testing was a validated EIA. If not, the specimen should be referred to a WHO-accredited laboratory and re-tested with a validated EIA
- Confirm that the patient had not received an MR/MMR within 56 days since antibody from immunization could be detectable
- Re-test the serum using a different commercial IgM EIA kit (where available). If the IgM is negative, the case can be discarded
- If serum was collected within 3-4 days of rash onset, test for the presence of measles IgG antibody (or rubella IgG, if suspect rubella case). Detection of IgG is not consistent with a primary infection and could indicate a higher likelihood that the positive IgM result is nonspecific
- If IgM is positive for measles, test for IgM for rubella. If both are IgM positive or equivocal, this may suggest that the IgM result was due to a nonspecific reaction. (The likelihood of simultaneous infection with both viruses is very low)

Details regarding the case may come to light during the course of the investigation and laboratory testing that indicate an alternative etiology. The clinical presentation, patient medical history, and the findings of the epidemiologic investigation may indicate that the positive or equivocal IgM result was obtained due to a non-specific reaction:

- A source of infection for the measles/rubella case could not be identified after a thorough investigation
- Clinical, medical and epidemiologic aspects of the case suggest the rash was due to an alternative etiologic agent, medical condition, or drug reaction (e.g., treatment with antibiotics)
- Identification of a local outbreak of other febrile rash illnesses

In elimination settings, there may be an increase in the proportion of suspected cases that occur as single, sporadic cases in elimination settings. The total number of cases with an unknown source is an important component that is reviewed for the verification of elimination. Frequently, there is a missed opportunity to collect virologic specimens when a sporadic case occurs. This will increase the number of sporadic cases that are confirmed solely due to a positive or equivocal IgM result. If the source of infection is unknown and there is a low index of suspicion, additional laboratory testing may be pursued in an attempt to discard the case as a non-measles, non-rubella case.

For the verification of elimination or as a requirement for countries that have achieved elimination, no more than 20% of confirmed cases* should be categorised as having an unknown source [12,13]. The total number of cases with an unknown source of infection may include some cases for which the genotype was identified. The source may be classified as unknown if there is insufficient evidence to classify the case as an importation or an importation-associated case despite identification of the genotype (chapter 7).

*Note: The requirement for verification of elimination has some subtle differences from the indicator for adequate molecular surveillance. For molecular surveillance, the indicator requires that a genotype should be identified for $\geq 80\%$ of all chains of transmission. Refer to chapters 7 and 10 for additional information.

8.4 Additional testing to aid case classification

Specimens for serologic testing (serum or oral fluid) and clinical specimens for RT-PCR (oral fluid, nasopharyngeal (throat), urine) should be collected at the first contact with a suspected case of measles or rubella. Recommendations for collection of specimens for antibody testing and for molecular testing to provide high-quality surveillance for measles and rubella are presented in [chapter 3](#). The collection of both types of specimens is recommended for accurate case classification. The flowchart for testing in elimination settings (Figure 8) provides options for serologic and molecular testing that may be appropriate for laboratory confirmation of suspected cases of measles and rubella. Figure 8 (A) consists of the steps involved in RT-PCR testing. In Figure 8 (B) and (C), routine IgM testing as well as additional options for serologic testing are included if RT-PCR results are inconclusive.

Figure 8 (A-C). Flowchart for laboratory testing for suspected measles or rubella case in an elimination setting.

Panel 8A. Virologic specimen testing by RT-PCR

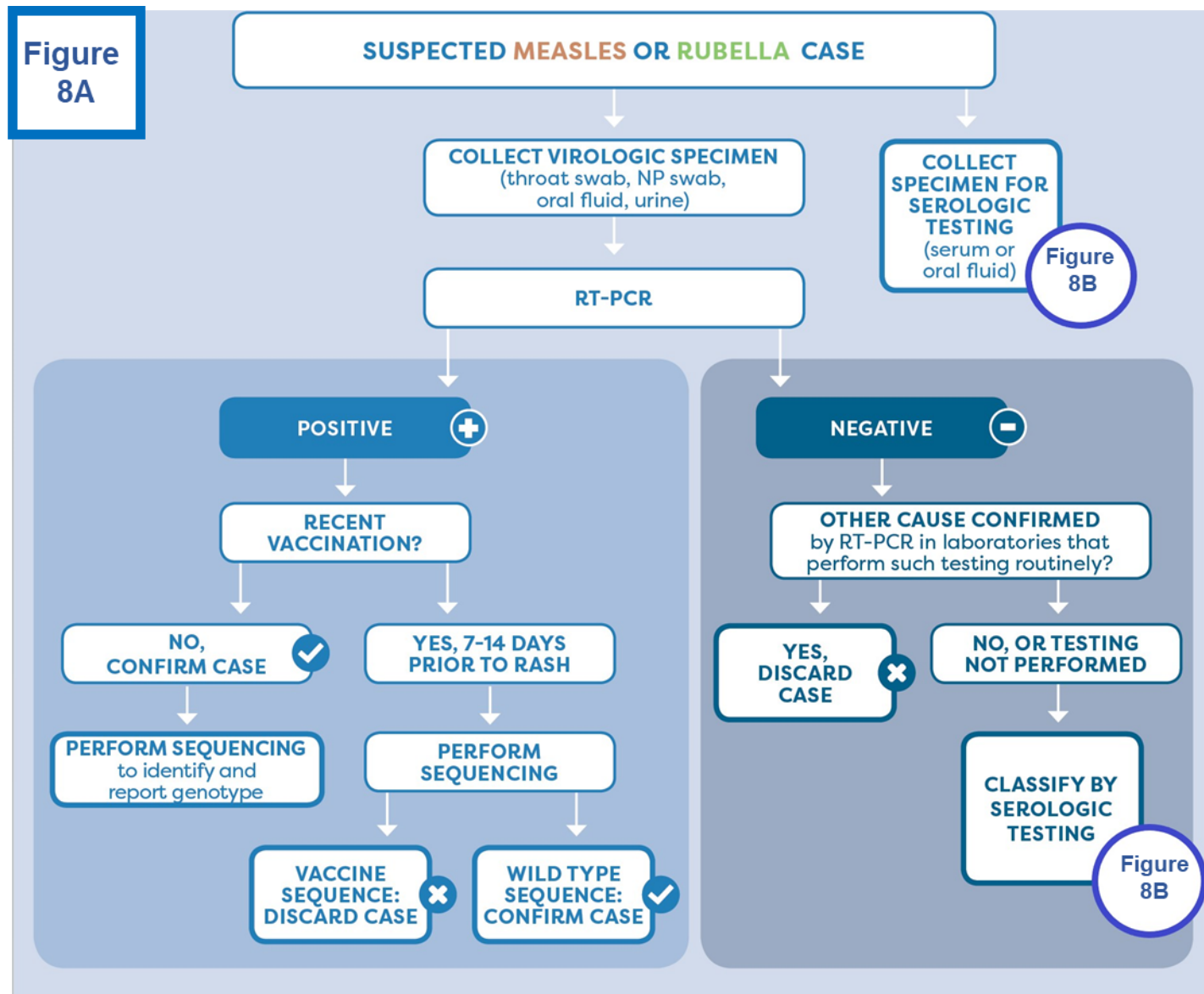
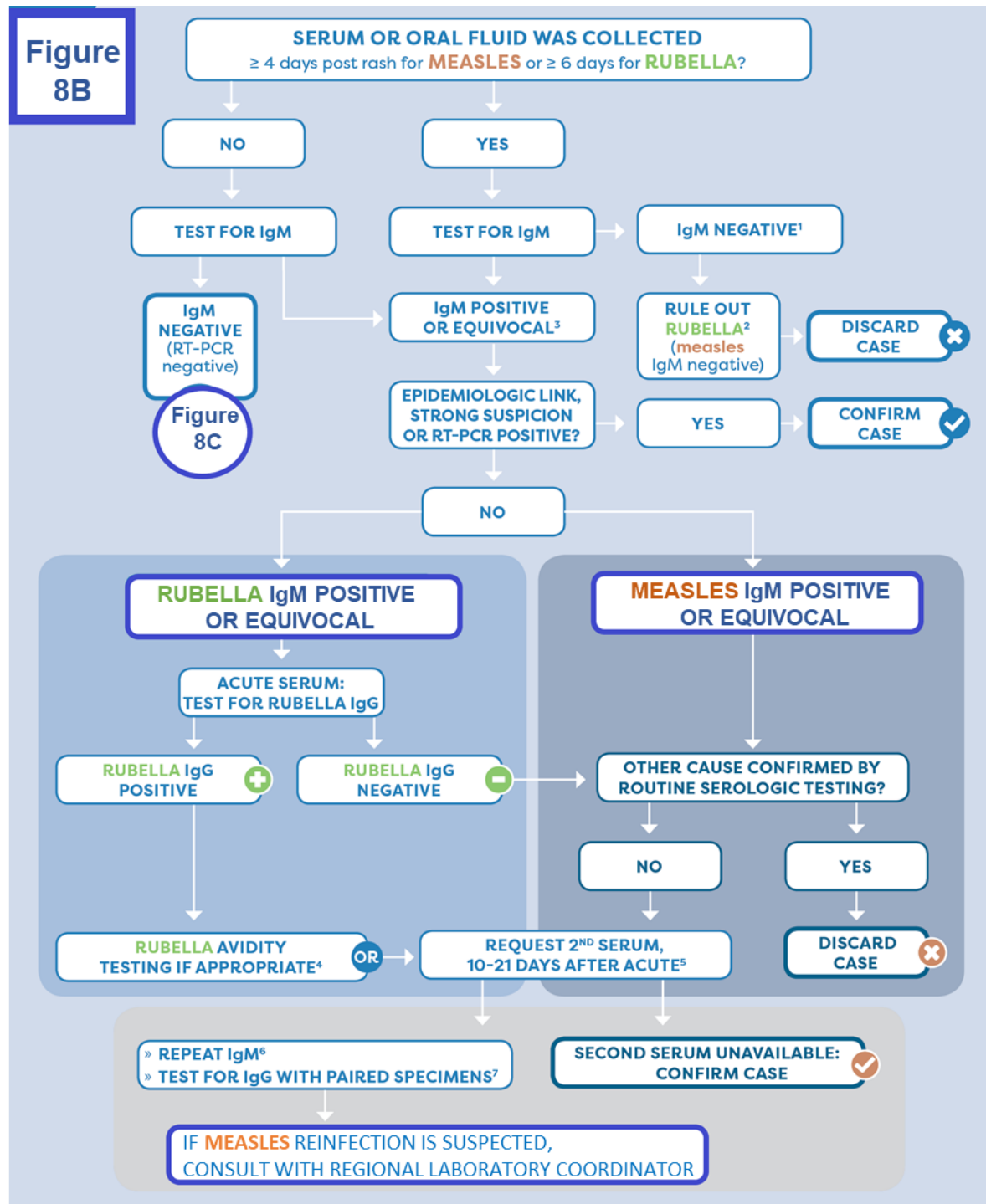


Figure 8 (A-C). Laboratory testing for suspected measles or rubella case in an elimination setting.

Panel 8B. Flowchart for serologic specimen testing ≥ 4 days post rash for measles or ≥ 6 days for rubella suspected cases (footnotes to 8B on following page).

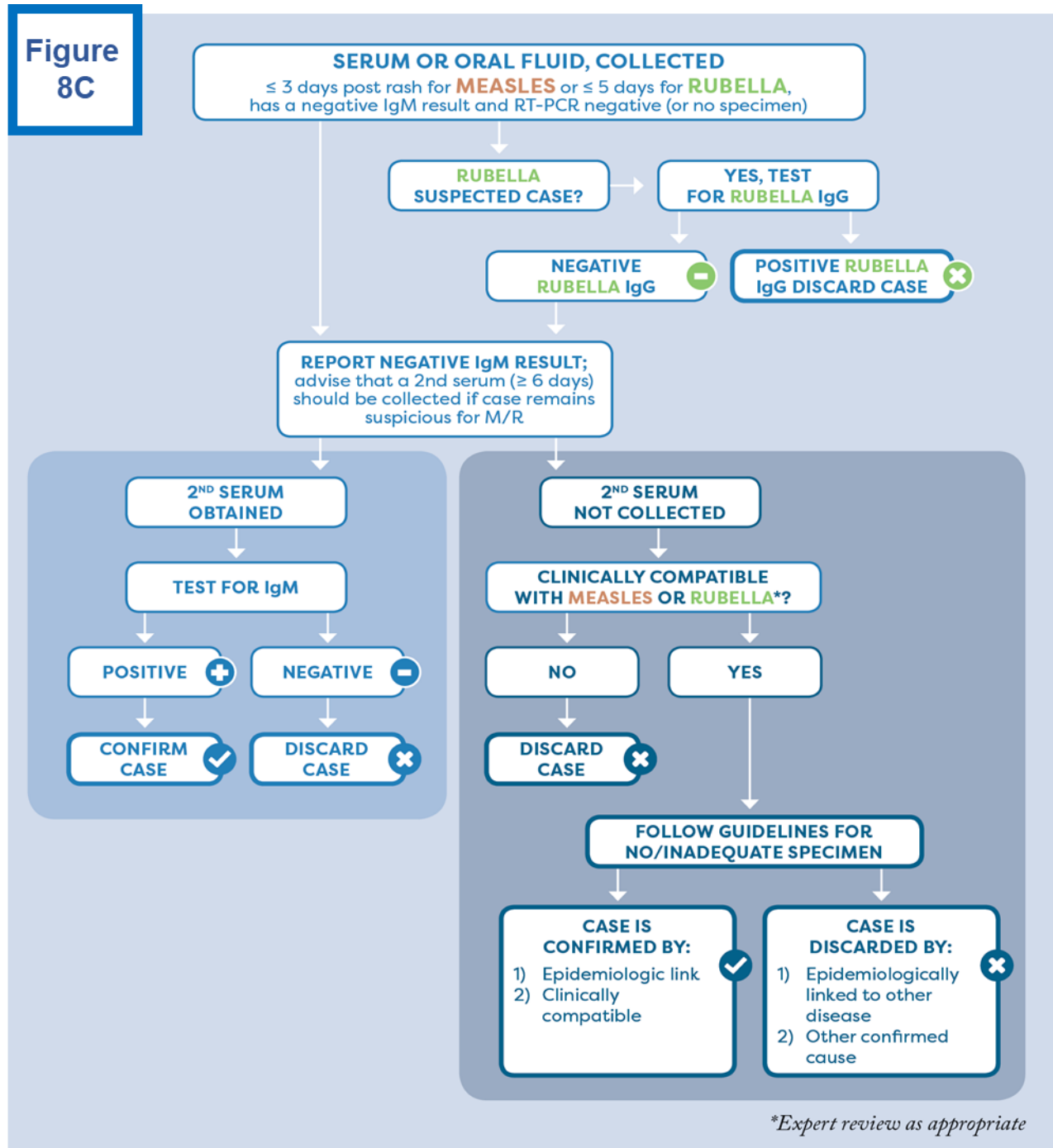


Footnotes for Figure 8, Panel 8B

1. A measles reinfection case can have a negative IgM result. If measles reinfection is suspected, consult with the regional laboratory coordinator. Reinfection cases can be confirmed by RT-PCR, a rise in IgG titer or by measuring high levels of measles neutralizing antibody levels ($\geq 40,000$ mIU/mL) by plaque reduction neutralization testing.
 2. Parallel, or reflex, testing should be performed according to the resources available and regional surveillance recommendations.
 3. An equivocal IgM result is obtained after repeat of test. The equivocal or positive IgM result was obtained using a validated assay in accredited laboratory.
 4. A positive IgG result and an equivocal IgM for rubella are inconsistent with primary rubella. If acute serum was IgM positive, rubella avidity testing or evaluation of IgG titers with paired specimens may be necessary to resolve the case. Low avidity is associated with recent primary rubella infection; high avidity is associated with past infection, vaccination, or reinfection.
 5. If the acute serum was IgG negative, the absence of seroconversion can be demonstrated with a second serum collected ≥ 10 days post rash.
 6. In most instances, a suspected case with an equivocal IgM result obtained from acute serum and a positive IgM from the second serum confirms the case. However, an evaluation of IgG titers may be deemed necessary to support the IgM result.
 7. Test for IgG if test is available (by semi-quantitative EIA) using appropriately timed paired specimens, tested together. Seroconversion or demonstration of a diagnostically significant rise confirms the case. Absence of seroconversion (both IgG negative) rules out the case.
- Note: failure to measure a diagnostically significant rise in titer must be interpreted with caution since the ideal timing for demonstration of a rise in titer can vary among individuals.
8. The rise in IgG titer from a measles reinfection case is rapid and remarkably high titers in acute serum are typical. Consultation with the regional laboratory coordinator is recommended to determine if additional testing is warranted and feasible.

Figure 8 (A-C). Laboratory testing for suspected measles or rubella case in an elimination setting.

Panel 8C. Flowchart for serologic specimen testing ≤ 3 days post rash for measles or ≤ 5 days for rubella suspected cases, when result for IgM (and RT-PCR) is negative



Because not all SNLs or NLs have the capacity to perform all types of tests, especially those requiring specialized reagents, protocols should be in place for referral of samples when additional testing is deemed appropriate. The flowcharts in Figure 8 are intended to provide a comprehensive guide, however each country will need to tailor the approach to resolve challenging cases depending on the particular setting in which the suspected case occurs, the available resources, and the epidemiologic information. The appropriate NL or RRL should be consulted prior to referral of samples that have been identified for additional testing. Examples of additional and/or specialised tests that may aid in the final classification of suspected cases include:

- Kits or reagents for the detection of IgM or virus-specific RNA for alternative causes of rash illness
- Virus-specific IgG EIA kits
Quantitative/semi-quantitative IgG assays are required for evaluation of a diagnostic rise in titre between paired serum specimens.
- Rubella IgG avidity assays
- Measles IgG avidity assays
- Measurement of virus-specific neutralising antibody
- Enhanced sensitivity for detection of virus-specific RNA using real-time RT-PCR
- Detection of vaccine viruses using a real-time RT-PCR protocol that targets vaccine-specific nucleotide sequences
- Sequence analysis to rule out wild-type virus (confirm suspected vaccine-associated rash)

Many of the laboratories in the GMRLN have established protocols for differential testing to rule out alternative causes of rash illness that are typical in the country or region (e.g., dengue, chikungunya, zika). The decision to proceed with additional testing, particularly testing that would require referral to a RL or GSL, should be made in consultation with local epidemiologists and the RLC.

8.4.1 Guidelines for testing paired serum specimens for IgG titre measurements

For primary cases of measles or rubella, the follow-up serum specimen should be collected 10-21 days following collection of the initial (acute) serum specimen (which should be collected within 7 days after onset of rash). The serum specimens must be tested in parallel (i.e., tested together, in the same assay). The optical density values measured by the IgG EIA must fall into the acceptable range according to the kit parameters. Demonstration of a diagnostically significant rise (a 4-fold rise or as stipulated by the algorithm specific for the kit that converts OD values to titres) between paired specimens confirms the case.

In some instances, there may be an observed rise in titre but the increase in titre does not reach the threshold required by the kit parameters (or 4-fold) for confirmation of infection. The ability to demonstrate a significant rise in titre is dependent on the appropriate interval between collection of the serum specimens. Because of individual differences in immune responses, the ideal interval may vary to some extent. The uncertainty and logistical difficulty associated with collection of paired serum specimens requires a careful evaluation to justify ruling out a suspected case based on the absence of a significant rise in titre.

A rise in IgG antibody titre among individuals with a measles reinfection case is rapid and the results obtained from testing paired specimens can vary. Testing paired serum specimens with a gap of 2-3 weeks between blood collections may yield a diagnostically significant rise in titre. However, it is also possible that no rise is apparent, or a slight decrease in titre may be observed. This is due to the fact that the levels of antibody may peak much closer to rash onset (e.g., 3-10 days post rash onset) among measles reinfection cases [9].

If a reinfection is suspected, paired serum specimens can both be collected during the acute phase, with an interval of 3-5 days between collection of the specimens. Measles reinfection cases typically produce levels of IgG antibody that are much higher than what is generated following a primary response to measles. The conversion from the OD values obtained by EIA to titres is only valid within a specified range of OD values (per the specific manufacturer kit insert), which were developed and validated for the IgG assay using serum panels with much lower reactivity.

8.4.2 Criteria for discarding a suspected case of measles or rubella

To **discard** a case with a possible false positive IgM or equivocal result, one of the following test results or circumstances must be documented:

- Repeat testing for IgM on the same specimen using a validated method is IgM negative
- The first serologic specimen is negative for virus-specific IgG and a second specimen collected at least 10 days after rash onset, is also IgG negative when tested in the same assay performed together on the same day
- Repeat testing for IgM on the same specimen remains equivocal but virus-specific IgG is positive. This applies to rubella suspect cases as well as measles suspected cases unless a measles reinfection is suspected*
- The patient was vaccinated 7-14 days before rash onset (unless it was for post-exposure prophylaxis or outbreak control). See additional criteria for a vaccine-associated case provided in chapter 4
- Appropriately-timed, paired serum specimens do not show any change in IgG titre**
- The cause of rash was demonstrated to be due to an alternative etiologic agent by additional testing*** (e.g., chikungunya, dengue or parvovirus B19)

** Often, it is the epidemiological circumstances which lead to suspicion of measles, rather than the clinical presentation, since symptoms exhibited by an individual with a measles reinfection may be less severe, and the rash may not follow the usual progression.*

*** The rule-out of a suspected case based on the absence of a rise in IgG should be carefully evaluated in conjunction with the epidemiologic setting and the clinical presentation.*

**** Either RT-PCR or demonstration of a seroconversion is required to confirm a dengue infection. An IgM positive result for dengue may be obtained months after the acute dengue infection.*

8.5 Measles reinfections: characteristics and case confirmation

Because symptoms can be mild, measles reinfection cases are often only recognized during investigations of individuals who had contact with a confirmed measles case. A high intensity of exposure (force of infection) is thought to play an important role in the occurrence of measles

reinfection cases. Prolonged exposure to an acutely ill, confirmed case of measles in a medical setting has resulted in several reports of measles reinfections among health care workers [7,10,14,15].

Measles reinfection cases may have a history of one or two doses of MCV, and some cases have occurred in older individuals who claim to be unvaccinated [8]. The clinical presentation varies widely; a reinfection can be indistinguishable from a primary infection or may present with milder disease and modified symptoms. The initial site for the appearance of rash may be the trunk and arms rather than the face. The rash may not follow the usual progression or result in a full body rash. Similarly, the fever is generally less severe in an individual with a measles reinfection compared to a primary measles infection and cough, conjunctivitis and/or coryza, if present at all, are mild. If typical signs and symptoms of measles are present, they often resolve quickly.

Measles-specific IgG antibody in acute phase serum from measles reinfection cases is typically highly reactive ('high' positive) by EIA while IgM detection can vary. Depending on the sensitivity of the IgM assay, the IgM result may fall in the low positive or equivocal range. There may also be no detectable IgM in serum collected from measles reinfection cases. Therefore, detection of measles-specific RNA in appropriate clinical samples by RT-PCR is the best method to confirm a measles reinfection [16,17]. While it is not necessary to identify a reinfection case as such when there is a positive RT-PCR result, the occurrence of these cases can be documented by testing the avidity of the measles-specific IgG in acute serum [5]. Measurement of high avidity, virus-specific IgG is consistent with a prior immunologic response to measles from either vaccination or natural disease [5,8].

RT-PCR is the best method to confirm a measles reinfection; however, the window for RNA detection from measles reinfection cases may be shorter than for a primary case of measles [7,8,10]. Virologic specimens should be collected as soon as possible after rash onset to increase the likelihood of RNA detection. The paucity of reports that document secondary cases attributed to contact with measles reinfection cases may be due to the elevated and rapid production of

neutralizing antibody that quickly reduces the viral load in these patients. The absence of a cough (or a mild, unproductive cough) also reduces the likelihood of effective transmission of infectious virus.

High levels of neutralizing antibody (IgG) may be present in serum specimens collected as soon as the first day of rash from measles reinfection cases. Because of the rapid rise in IgG, a rise in titre may not be apparent when testing conventionally-timed paired serum specimens, as discussed in Section 8.4. However, exceptionally high titres may be observed in the first serum specimen (or both specimens). A threshold concentration of measles neutralizing antibody of $\geq 40,000$ mIU/mL in a single serum specimen, as measured by the plaque reduction neutralization (PRN) assay [18], was demonstrated to identify and confirm a measles reinfection with 100% specificity [9]. Remarkably high levels of neutralising antibody titres measured by PRN from outbreaks involving previously vaccinated individuals with confirmed measles and high avidity IgG have also been reported [7-10]. The concentration of measles neutralizing antibody among some confirmed reinfection cases was $>200,000$ mIU/mL from serum specimens collected as early as 4 days after rash onset [8,9].

In a study of reinfection cases, remarkably high levels of neutralizing antibody were measured from most reinfection cases, but eight individuals (14%) produced much lower levels of antibody [9]. The eight confirmed reinfection cases had titres measured at 402 -29,367 mIU/ml in serum specimens that were collected at comparable intervals post rash onset as those collected from the reinfection cases with neutralising antibody titres $\geq 40,000$ mIU/ml.

Because the PRN assay is rarely performed outside of a GSL, measurement of high avidity measles IgG antibody and detection of measles-specific RNA by RT-PCR is the best method to identify a reinfection case. It may be possible that end-point IgG antibody titres determined by serial dilutions of serum tested by EIA could be correlated with the level of measles neutralizing antibody titres measured by PRN that provide confirmation for a measles reinfection case. However, demonstration of a threshold end-point dilution level for IgG measured in an EIA that identifies a measles reinfection case has not been described.

Bibliography to Chapter 8

1. Mulders MN et al. Global Measles and Rubella Laboratory Network support for elimination goals, 2010-2015. *MMWR Morbidity and Mortality Weekly Report*, 2016, 65:438-442.
2. Riddell MA et al. Laboratory testing and confirmation of suspected measles infection crucial in countries that have eliminated measles. *Annals of Emergency Medicine*, 2009, 54:639-640.
3. Dietz V et al. The laboratory confirmation of suspected measles cases in settings of low measles transmission: conclusions from the experience in the Americas. *Bulletin of the World Health Organization*, 2004, 82:852-857.
4. Bolotin S et al. The utility of measles and rubella IgM serology in an elimination setting, Ontario, Canada, 2009-2014. *PLOS One*, 2017, Aug 29;12(8):e0181172.
5. Mercader et al. Measles virus IgG avidity assay for use in classification of measles vaccine failure in measles elimination settings. *Clinical and Vaccine Immunology*, 2012, ;19(11):1810-1816.
6. Santibanez et al. Measles virus spread initiated at international mass gatherings in Europe, 2011. *EuroSurveillance*, 2014, 19(35):pii=20891.
7. Rota JS et al. Two case studies of modified measles in vaccinated physicians exposed to primary measles cases: high risk of infection but low risk of transmission. *Journal of Infectious Disease*, 2011, 204; Suppl 1:S559-563.
8. Rosen JB et al. Outbreak of measles among persons with prior evidence of immunity, New York City, 2011. *Clinical Infectious Disease*, 2014, 58:1205-1209.
9. Sowers SB et al. High concentrations of measles neutralizing antibodies and high-avidity measles IgG accurately identify measles reinfection cases. *Clinical Vaccine Immunology*, 2016, 23:707-716.
10. Hahné SJM et al. Measles outbreak among previously immunized health care workers, the Netherlands, 2014. *Journal of Infectious Diseases*, 2016, 214 (15 december):1980-1986.
11. Roy F et al. Rapid identification of measles virus vaccine genotype by real-time PCR. *Journal of Clinical Microbiology*, 2017, 55 (3): 735-743.
12. WHO. Monitoring progress towards measles elimination. *Weekly Epidemiological Record*, 2010, 49 (85):490-495.
13. Durrheim DN et al. Measles - The epidemiology of elimination. *Vaccine*, 2014, 32:6880-6883.
14. Ammari LK et al. Secondary measles vaccine failure in healthcare workers exposed to infected patients. *Infection Control and Hospital Epidemiology*, 1993, 14(2):81-86.

15. Vainio K et al. Measles virus genotyping an important tool in measles outbreak investigation in Norway, 2011. *EuroSurveillance*, 2012, 17(50):pii=20340.
16. de Oliveira SA et al. Atypical measles in a patient twice vaccinated against measles: transmission from as unvaccinated household contact. *Vaccine*, 2001, 19:1093-1096.
17. Oliveira SA et al. Use of RT-PCR on oral fluid samples to assist the identification of measles cases during an outbreak. *Epidemiology and Infection*, 2003, 130:101-106.
18. Cohen BJ et al. Plaque reduction neutralization test for measles antibodies: Description of a standardised laboratory method for use in immunogenicity studies of aerosol vaccination. *Vaccine*, 2007, 26(1):59-66.