

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

Chapter 4. Antibody detection methods for laboratory confirmation of measles, rubella, and CRS

Overview

The network laboratories in WHO Regions with both measles and rubella elimination targets should consider routinely testing all suspected cases of measles or rubella for both measles and rubella IgM. Depending on the existing epidemiology of rubella and the status of rubella control activities, laboratories in Regions without a rubella elimination target may perform rubella IgM testing to detect rubella infection among suspected measles cases that are detected outside of known measles outbreaks. Since testing all samples for both measles and rubella is often not a feasible option, initial testing of all samples for measles IgM, followed by testing the measles IgM-negative samples for rubella IgM provides an alternative strategy.

Detection of measles- or rubella- specific immunoglobulin M (IgM) in a single serum specimen is the standard method for the rapid laboratory confirmation of measles and rubella. However, oral fluid (OF) is an alternative sample for IgM detection that offers a minimally invasive collection method and may offer reduced cost for transportation. Serum (or OF) specimens tested from individuals with a rash illness due to causes other than measles and rubella may produce false positive IgM results in measles- or rubella-specific IgM EIAs, a problem that may be encountered regardless of the IgM assay used. This is especially true in settings with low prevalence of disease, since no test is 100% specific. Guidance for additional testing to address the lower predictive value of positive IgM results in elimination settings is discussed in chapter 8.

Although not used for routine diagnosis of measles and rubella infection, assays that measure virus-specific IgG by EIA may aid in case classification (section 4.5). The measurement of measles and rubella IgG for assessing population immunity in serosurveys is covered in chapter 9. Commercial kits that measure the avidity of rubella virus-specific IgG antibody are widely available and may be utilized as an additional test in certain situations (section 4.6).

In this chapter:

- 4.1 Selection and comparison of EIAs for IgM detection
- 4.2 Interpretation of IgM results for case classification of measles and rubella
 - 4.2.1 Timing of serum collection

- 4.2.2 Cross-reactions and interference: false positive IgM results
- 4.3 Interpretation of IgM results among suspected cases with recent vaccine history
- 4.4 Alternative specimens for IgM detection
- 4.5 IgG assays and interpretation for case classification
- 4.6 Determination of measles- or rubella-specific IgG avidity
- 4.7 Interpretation of IgG avidity results
- 4.8 Serologic confirmation of suspected CRS cases
- 4.9 New serological techniques and methodologies

4.1 Selection and comparison of EIAs for IgM detection

Suspected cases of measles or rubella are most frequently confirmed by detection of specific IgM antibody in serum specimens. The most common method of detection used is a commercial enzyme immunoassay (EIA), which has largely replaced the immunofluorescence assay (IFA), which is a much less sensitive and specific method than EIA.

Most commercially available IgM EIA kits for measles and rubella use either an indirect format or a capture format. The indirect format includes an absorbent in the diluent or a pre-treatment step to bind IgG antibodies to reduce non-specific reactions. The IgM assays based on the capture format do not require the removal of IgG antibodies and are generally considered to be more sensitive and specific than indirect EIAs. All assays have an equivocal range and the standard procedure is to repeat the test if an equivocal result is obtained. If the IgM result for the serum specimen after re-testing remains equivocal, a second sample may be required. Refer to 'Timing of the serum specimen' below.

There are many considerations to guide the selection of an EIA kit for measles and rubella IgM detection in addition to high sensitivity and specificity. The reliability of the manufacturer to produce a sufficient quantity of kits, the cost of kits and required reagents, and required purchase of necessary equipment are among the most important factors to consider. In addition, the type of specimen (serum or OF), the workload, and ease of use will also influence the choice of kits. The assays used for testing IgM in oral fluid (OF) must be evaluated for testing OF, as discussed in section 4.4 below.

Eleven different kits were used by the GMRLN to test the WHO proficiency panels for measles IgM in 2016. The Enzygnost® Anti-Measles Virus EIA kit, most recently produced by Siemens (Marburg, Germany,) has been utilized by many network laboratories for many years. However, as these kits are

no longer available in some markets, other commercial kits are being utilized, including the Serion kit (Institut Virion/Serion, Würzburg, Germany), and Euroimmun (Luebeck, Germany).

There was less concordance in the results obtained for rubella IgM among the 18 different kits used for the 2016 proficiency panel. However, there is a greater choice of *mu*-capture rubella IgM kits on the market, which are generally less likely to produce false positive IgM results. Although there is data available from proficiency panel testing that may provide some indication of the relative performance of the various kits, proficiency testing does not provide a true comparison of how the kits perform. The samples included in the panels are selected primarily to test the competence of the laboratory and not to compare kit function. A summary with an analysis of results obtained using different IgM kits for measles and rubella for proficiency testing is available in **Annex 4.1**. Additional information regarding kit selection will be made available through links on the GMRLN website upon completion of the evaluation of measles and rubella IgM kits.

4.2 Interpretation of IgM results for case classification of measles and rubella

With a few exceptions, most EIA kits for IgM testing used in the GMRLN perform well. With the quality assurance activities implemented throughout the network, confidence in the accuracy of laboratory classification provided by routine IgM testing is very high. However, there are limitations associated with IgM detection for case classification. As discussed below, ‘false negative’ IgM results can occur due to ‘early’ collection of specimens.

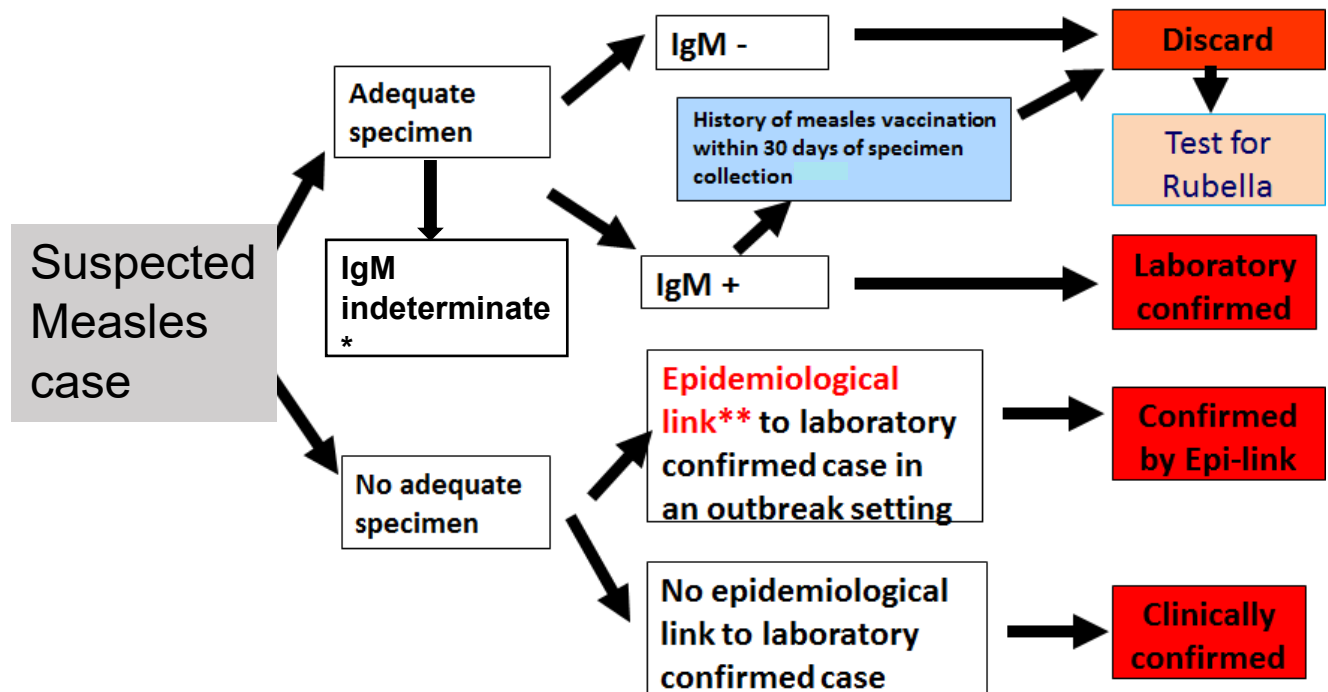
Note: A serum specimen is considered an ‘early’ specimen when it is collected ≤ 3 days from rash onset (suspect measles case) or ≤ 5 days after rash (suspect rubella case). The guidelines for the interpretation of results obtained by EIA for IgM detection includes a caveat, based on Helfand et al. [1], that a “false negative” may be obtained when serum is collected early. This refers to the possibility that the negative IgM result by EIA may not reflect the true disease status (a proportion of early serum specimens from true cases will have a negative IgM result). A negative IgM result under such circumstances is not technically a false-negative result, but represents a valid result as measured by the EIA since the patient has either not yet produced an IgM response or the level of virus-specific IgM is below the detection threshold for the EIA.

A false positive IgM result may be obtained when serum is tested for measles or rubella IgM from patients with rash illnesses due to other causes. A false positive IgM result, or nonspecific positive result, may also be due to components within the serum specimen. The possible causes for a nonspecific reaction in the IgM assay are discussed in section 4.2.2. In addition, there are guidelines that should be considered if a positive IgM result is obtained from a suspected case that has been recently immunized with the measles monovalent or the MR/MMR vaccine (section 4.3).

Laboratory test results obtained by IgM EIAs should be reported promptly with appropriate accompanying information as described in chapter 11. Additional serologic testing for virus-specific IgG may aid in case classification when the IgM result is equivocal or to provide additional results to resolve an IgM positive result that is doubtful or unexpected due to a non-classic clinical presentation and/or the epidemiologic investigation fails to identify a source of infection (section 4.6). If equivocal results cannot be resolved, the result is reported as equivocal. Additional testing is not indicated in settings where disease is endemic or during outbreaks and the cases are classified as confirmed. See Figure 4.1, Flowchart for case classification of measles in non-elimination settings. The detection of virus-specific RNA by RT-PCR can be helpful to support a doubtful positive or an equivocal IgM result. The application of RT-PCR for case classification is discussed in chapter 6.

Note: In areas that are close to elimination, or those that have achieved elimination, the flowchart for case confirmation and for discarding cases has been modified to include testing strategies that can provide enhanced laboratory support for accurate case classification. A flowchart (figure 8) for measles and rubella cases classification in near or post elimination settings is included in chapter 8.

Figure 4.1 Flowchart for case classification of measles in non-elimination settings



*All serum specimens with indeterminate measles IgM results should undergo a second test before being labeled “indeterminate” and being classified as “Compatible”.

**A suspected measles case that is linked (in place, person and time) to a lab confirmed case; i.e., living in the same or in an adjacent district with a lab confirmed case where there is a likelihood of transmission; onset of rash of the two cases are within 30 days of each other.

Figure based on WHO 2003 surveillance standards and African regional guidelines for measles and rubella surveillance-Draft version April 2015.

4.2.1 Timing of serum collection

An adequate serum specimen for surveillance purposes is one that is collected within 28 days from onset of rash. As discussed above, although most commercial IgM EIAs are highly sensitive, a negative IgM result may be obtained from a proportion of measles cases if serum was collected ≤ 3 days (≤ 5 days for rubella cases) after rash onset. Particularly in elimination settings, a second serum specimen is recommended if a negative IgM result is obtained from serum collected during the timeframe that is considered early for IgM detection by EIA. Despite the possibility that a false negative result may be obtained, serum should be collected at first contact with suspected case. At the same time, collection of a virologic specimen is recommended for molecular surveillance and for RT-

PCR testing, which can improve the ability to rapidly confirm cases. Refer to chapter 3 for best practices for collection of serum specimens.

4.2.2 Cross-reactions and interference: false positive results for IgM

Many pathogens and medical conditions can produce a rash and other symptoms that mimic measles or rubella infection. Many of these etiologic agents or conditions can also cause a non-specific or false-positive IgM result in measles or rubella IgM assays. Medical conditions in which high levels of rheumatoid factor (RF) are present can cause interference and generate false positive IgM results.

Numerous investigations have been conducted to determine the etiologic agents responsible for rash illness from non-measles/non-rubella cases. Parvovirus B19 was identified as the cause of rash among a high proportion of cases of rash illness not due to measles or rubella [2-5]. Enterovirus and adenovirus were also commonly identified. Among younger children with rash, HHV-6 was another frequent etiologic agent identified [2-5].

Several of the GMRLN laboratories participate in national and international surveillance programmes for other infectious diseases including laboratory support for confirmation of many viral diseases associated with rash and fever, particularly those that are mosquito-borne diseases that occur as epidemics. Approximately one-third of primary dengue virus infections can present with rash and fever, and when a dengue epidemic occurs, the majority of rash and fever cases that are reported are caused by dengue. Chikungunya virus and zika virus are other mosquito-borne diseases of public health importance that can cause rash and fever. If IgM testing is conducted on these cases to rule out the possibility of measles or rubella, the test results may be positive when the rash and fever is due to one of these mosquito-borne diseases. The viremia from these agents can cause non-specific reactions or formation of immune complexes that can produce a false positive IgM result in measles or rubella IgM assays.

4.3 Interpretation of IgM results among suspected cases with recent vaccine history

The true case status of suspected cases of measles or rubella among individuals who were vaccinated between 8-56 days prior to rash onset cannot be determined by testing for the presence of IgM. The virus-specific IgM detected by EIA does not distinguish between IgM elicited by a current wild-type infection by measles or rubella and the IgM response due to recent immunization. While virus-specific IgM antibodies produced following vaccination in most individuals will decline to undetectable levels

at 28-56 days post immunization (or following natural disease), the use of assays with higher sensitivity may extend the period that IgM can be detected. Low levels of rubella IgM have been detected for months or years following infection or vaccination in some individuals [6].

A special situation applies to suspected measles or rubella cases that present with rash from 7-14 days after receipt of the measles monovalent or combination MR/MMR vaccine. While this timeframe is typical for a measles vaccine reaction, there may be contemporaneous measles activity or risk factors for exposure that raise the index of suspicion of a wild-type infection. While most rashes from vaccination are due to the measles component of MR or MMR vaccines, a vaccine-associated rash due to the rubella component of MR/MMR vaccine strain is possible, which extends the range that rash can develop following vaccination from 14 days to 30 days after receipt of rubella-containing vaccine.

The IgM response to the measles component of the vaccine typically precedes the IgM response to the rubella component by up to a week or more [3]. Therefore, in the first or second week after vaccination, a positive IgM result may be obtained for measles while rubella IgM is negative. These results should not be interpreted as evidence of a measles wild-type infection. Because serologic techniques cannot distinguish between an immune response to natural infection and antibodies elicited by a recent immunization, collection of clinical specimens for molecular testing are critical to determine the genotype.

If epidemiologic and clinical criteria for a vaccine-related rash are met, it is possible that a suspected case with a recent measles vaccination may be attributed to a vaccine reaction and discarded without identification of a vaccine strain by molecular testing. Since serology cannot discern a wild type infection from vaccination, the case must be investigated appropriately. However, further evaluation of the case may provide evidence for a vaccine-related rash. It is noted that a rash that occurs 7-14 days post-vaccination may be due to an infection with another rash-causing illness rather than a reaction to the recent vaccination. However, if the case meets the criteria for a vaccine-related rash, the rash is generally attributed to the vaccine unless laboratory confirmation of another cause of rash is identified.

Suspected cases that could potentially be discarded as having a rash due to vaccine would generally be limited to sporadic cases and would not include those individuals who received vaccination as post-

exposure prophylaxis (PEP), or were immunized as part of an outbreak response. In such instances, when genotyping is not available to confirm a vaccine strain, the case would be confirmed as measles by the positive IgM result. The rationale for confirmation of the case is that the patient was either epidemiologically linked to a confirmed case or was temporally associated with an outbreak. Although PEP is recommended as a control measure, the protective efficacy of post-exposure immunization has not been well documented. The guidelines for case classification of a suspected case with a positive IgM result and a recent MR/MMR vaccination are given in the box below.

Criteria for vaccine-associated rash

The following 5 criteria must all be met for a rash from a suspected case with a positive or equivocal IgM result to be attributed to a reaction from a recent vaccination. These criteria are provided for those situations where genotyping was unsuccessful or an appropriate virologic specimen was not collected.

1. The patient had a rash illness, with or without fever, but did not have cough or other respiratory symptoms
2. The rash developed 7-14 days after vaccination with a monovalent measles or MR/MMR vaccine
3. The blood specimen, which was positive for measles or rubella IgM, was collected 8-56 days after vaccination
4. A thorough field investigation did not identify any secondary cases of measles/rubella
5. Field and laboratory investigations failed to identify other causes for the rash illness

If all criteria are met, the case should be recorded as a vaccination-related rash case and discarded.

4.4 Alternative specimens for IgM detection

Two types of samples have been evaluated by specialized and reference laboratories in the GMRLN for detection of rubella- or measles-specific IgM as an alternative to testing serum. Dried blood spots (DBS) can be prepared from capillary or venous blood and may provide a viable option in areas where a cold chain and/or collection of venous blood is not feasible. Collection of oral fluid (OF) is minimally invasive and may not require refrigeration under certain conditions [7]. However, these alternative types of samples require that proper techniques for collection and processing are followed. The best practices for the collection, optimal timing, and preparation of DBS and OF are presented in chapter 3.

The use of oral fluid (OF) specimens for IgM antibody testing requires that the EIA kit has been validated for OF, since the OF sample is typically analysed without dilution. A sufficient amount of total IgG should be present in the sample as an indication that the specimen would potentially contain measurable IgM [8, 9]. A capture EIA is used for IgM detection in OF. Details regarding modification of the protocol for the EIA for testing OF is provided in [Annex 4.1](#).

In an evaluation of these alternative specimens, the sensitivity and specificity for measles- and rubella-specific IgM were comparable to measurement of IgM using serum specimens [7]. However, the early collection of the specimens is reflected in the concordance of IgM results obtained from both OF and serum. In a study of suspected rubella cases, detection of IgM from serum was somewhat higher in the first 4 days after onset of rash than the corresponding results for OF samples [10]. However, if the equivocal results for IgM from OF were considered as positive results, the concordance was much higher. The concordance of results for IgM detection from OF and serum improved considerably when specimens were collected ≥ 4 days after rash onset. As previously noted, the use of RT-PCR for RNA detection confirmed more rubella cases than IgM detection from serum among specimens that were collected within 2 days of rash onset.

The concordance observed for IgM detection using paired DBS and serum specimens may vary in similar ways as that noted for OF and serum. A study was conducted in Uganda to compare results for measles IgM detection using DBS and serum collected from hospitalised children with measles [11]. The concordance observed between IgM results from DBS (eluate) and serum when the specimens were collected on day 0-6 after rash onset was 95.6% (253 sample pairs). However, of 260 paired samples that were collected from week 2 to 5 after rash onset, the concordance was 100%. [11]. In a study of rubella IgM detection comparing DBS and serum, the concordance of results improved when equivocal results for IgM from DBS were considered as positives. In addition, the collection of samples at days 4-28 increased concordance from 76% to 88%. [12].

Eluted fluid from dried blood spots (DBS) can be tested using a commercially available IgM indirect EIA or a capture EIA which has been validated for the purpose of testing DBS. Methods for the elution of serum specimens from DBS have been published [13], and recommended protocols are provided in [Annex 3.2](#).

4.5 IgG assays and interpretation for case classification

Measurement of measles- or rubella-specific IgG can be useful as an additional serologic method for case classification. The most widely used assays for detection of IgG antibodies to measles and rubella are indirect EIAs, although capture IgG assays may also be available for measles testing. IgG testing may be utilized when an equivocal result is obtained for IgM or if a positive IgM result is questioned due to clinical or epidemiologic information that is inconsistent with a case of measles or rubella.

A seroconversion is demonstrated with paired serum specimens tested together in the same assay in which the acute phase sample has a negative result for IgG and the second or convalescent specimen has a positive IgG result. Demonstration of a diagnostically significant rise in IgG titre (by a validated, quantitative, indirect IgG EIA) confirms a positive IgM result. This method relies on the availability of two specimens, usually collected 10-21 days apart, that are tested together in the same assay. The specific criteria for documenting an increase in titre depend on the parameters for the particular commercial assay and may require multiple serial dilutions of the serum specimens. The EIA optical density (OD) values are not titres, and increases in OD values do not directly correspond to titre increases.

In order to successfully demonstrate a seroconversion or a diagnostically significant rise in titre, the first (acute) sample should be collected soon after rash onset (ideally within 7 days). If the first specimen is IgG negative, the second serum can be collected at any time on or after 10 days post rash. True cases of measles or rubella should have detectable IgG in serum collected ≥ 10 days after rash onset. The absence of detectable virus-specific IgG in both serum specimens is acceptable evidence to rule out/discard a suspected case.

4.6 Determination of measles- or rubella-specific IgG avidity

Antibody avidity refers to the net strength of the interaction of a multivalent immunoglobulin molecule with antigen. Depending upon the strength of this binding, the complex formed may or may not be easily dissociated. Avidity assays measure the antigen-binding avidity of IgG antibodies by incorporating a mild protein denaturant to distinguish low avidity antibodies produced at an early stage of a primary infection from those with high avidity.

Low avidity IgG is usually present in a current infection, but the avidity can remain low for up to 3 months after rash onset. Because avidity testing requires that detectable virus-specific IgG is present, avidity testing may require a second serum if the initial serum was collected within a few days after rash onset. Demonstration of low avidity IgG provides the additional laboratory evidence to support a positive IgM result from a suspected measles or rubella case that may be considered as a potentially nonspecific ('false positive') result. High avidity IgG is consistent with an infection in the past or a previous vaccination (>3 months prior to blood collection). A confirmed measles case with a history of vaccination and high avidity IgG is consistent with a measles reinfection case (see section 4.7).

Modifications of commercial EIA IgG assays are utilized for avidity assays that are developed in-house but these avidity assays are difficult to establish and require a range of avidity controls that must be validated. The controls must be included in each run so having a substantial volume of well-characterized validity controls is essential. There are commercial kits for determination of rubella avidity, but they may have limited distribution.

4.7 Interpretation of IgG avidity results

After primary antigenic challenge (from natural disease or immunization), IgG avidity is low for about 6-8 weeks. An acute phase serum sample with low avidity IgG can provide confirmation of a suspected case of measles or rubella and support a positive IgM result. However, the possibility of a recent immunization with MR or MMR should be ruled out, since avidity testing does not discriminate between low avidity from wild-type infection or response from a recent vaccination.

The demonstration of high avidity virus-specific IgG antibody in acute serum indicates that a suspected rubella case does not have a primary infection. The most common use for avidity testing is to rule out a primary rubella infection. An avidity test is frequently requested when a positive or equivocal rubella IgM result is obtained following inappropriate testing for rubella IgM from an asymptomatic pregnant woman. High avidity IgG antibody from such cases would be inconsistent with a current or recent rubella infection.

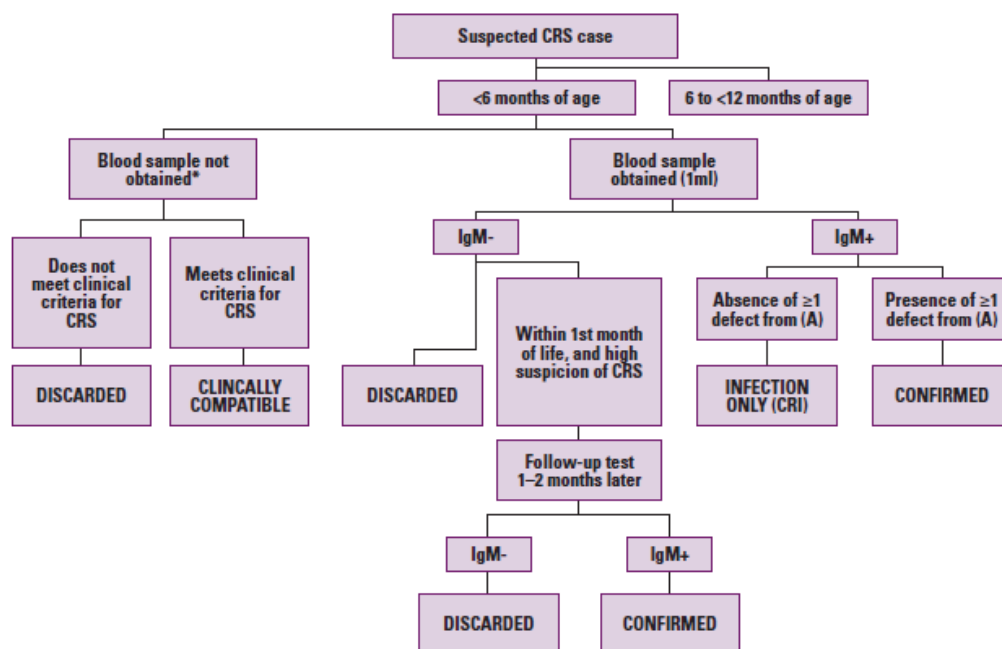
The presence of virus-specific, high avidity IgG indicates a mature immune response from past infection or vaccination. Although reinfections occur, symptomatic reinfections with rubella are extremely rare [14, 15]. Measles reinfections can be symptomatic although the presentation is usually

mild, and these cases are detected most often among healthcare workers exposed to an acutely ill measles case. Reinfection cases may also be detected in the context of an outbreak or in household settings involving a confirmed measles case. [16]. Inadequate vaccine potency may be implicated in outbreaks where a large proportion of cases have evidence of previous vaccination and many cases are identified as measles reinfection cases. Refer to chapter 8 for more information regarding the confirmation of measles reinfections.

4.8 Serologic confirmation of suspected CRS cases

Suspected CRS cases can be laboratory confirmed in new-borns and young infants by detection of rubella-specific IgM (See Figure 4.2).

Fig 4.2 Algorithm for testing blood specimen from infants <6 months of age for CRS



Group (A) defects: cataract(s), congenital glaucoma, congenital heart disease, hearing impairment, pigmentary retinopathy. Figure from *Introducing Rubella Vaccine into National Programmes, A Step-by-Step Guide*, WHO, 2015

A suspected CRS case should be tested as close to birth as possible and again at 1 month of age if the initial IgM test is negative. At 6 months of age, only about 50% of cases will continue to have detectable levels of rubella IgM in serum [17]. Among infants that are clinically suspicious for CRS but were not identified within the timeframe to measure rubella IgM after birth (1-3 months), serial testing of serum after 9 months of age (when maternal antibody has waned) for IgG can confirm CRS. Maternal antibody normally diminishes at a rate of a 2-fold decline per month. It is necessary to demonstrate sustained levels of rubella IgG from the infant in order to confirm CRS.

4.9 New serological techniques and methodologies

As validated improvements or alternative methodologies become available for measles and rubella diagnostics, updates will be posted to the GMRLN website. Types of assays or techniques that show promise for expanding throughput or have specific utility in the field are described below.

Point of Contact Test (PoCT) for Measles IgM

Rapid (<30 minutes) tests based on Lateral flow technology are widely used for HIV and hepatitis diagnosis and screening. Recently an assay for detecting measles IgM has been described which has good sensitivity and specificity [18, 19]. These assays are designed to be read visually either in a clinic or by surveillance teams and as such give subjective results. Before they are implemented for measles diagnosis it is essential that training is provided and QA systems are established to monitor accuracy of the results produced. At the time of this writing, the assay is not yet commercially available.

Novel platforms for seroprevalence studies

The GMRLN has been investigating the use of platforms other than EIA for seroprevalence and vaccination studies. These novel platforms allow high throughput testing for multiple antigens at reduced overall running costs. In particular, multiplex bead assays (MBA) have the ability to simultaneously detect IgG antibodies to measles and rubella in very small volumes of serum [20-22]. MBA assays are being developed for use in GMRLN laboratories to improve the uniformity of serologic testing for seroprevalence studies. Research assays using microfluidic systems have been described for measles, but had not reached the implementation stage for measles diagnostic services at the time of the manual preparation.

Bibliography to Chapter 4

1. Helfand RF et al. Diagnosis of measles with an IgM capture EIA: the optimal timing of specimen collection after rash onset. *Journal of Infectious Diseases*, 1997, 175(1): p. 195-9.
2. Davidkin I et al.. Etiology of measles- and rubella-like illnesses in measles, mumps, and rubella-vaccinated children. *Journal of Infectious Diseases*, 1998, 178:1567-1570.
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. Yermalovich et al. Etiology of maculopapular rash in measles and rubella suspected patients from Belarus. *PLoS One*, 2014, 10:e111541.
5. Woods CR. False-positive results for immunoglobulin M serologic results: Explanations and examples. *Journal of the Pediatric Infectious Disease Society*, 2013, 2:87-90.
6. Vauloup-Fellous C, Grangeot-Keros L. Humoral immune response after primary rubella virus infection and after vaccination. *Clinical Vaccine Immunology*, 2007;14:644–647.
7. WHO. Measles and rubella laboratory network: 2007 meeting on use of alternative sampling techniques for surveillance. *Weekly Epidemiological Record*, 2008, 83(25): 229-232.
8. Maple PAC. Application of oral fluid assays in support of mumps, rubella and varicella control programs. *Vaccines (Basel)*, 2015, 3(4):988-1003.
9. Hutse et al. Oral fluid for the serological and molecular diagnosis of measles. *International Journal of Infectious Diseases*, 2010, 14:e991-e997.
10. Abernathy E 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. *Journal of Clinical Microbiology*, 2009, 47: 182-188.
11. Uzicanin A et al. Dried blood spots on filter paper as an alternative specimen for measles diagnostics: detection of measles immunoglobulin M antibody by a commercial enzyme immunoassay. *Journal of Infectious Diseases*, 2011, 204:(Suppl 1): S564-S569.
12. Helfand RF et al. Dried blood spots versus sera for detection of rubella virus-specific immunoglobulin M (IgM) and IgG in samples collected during a rubella outbreak in Peru. *Clinical and Vaccine Immunology*, 2007, 14:1522-1525.
13. Mercader S et al. Comparison of available methods to elute serum from dried blood spot samples for measles serology. *Journal of Virologic Methods*, 2006, 137:140-149.
14. Morgan-Capner P et al. Clinically apparent rubella reinfection with a detectable rubella specific IgM response. *British Medical Journal (Clin Res Ed)*, 1983, 286:1616.

15. Best JM et al. Fetal infection after maternal reinfection with rubella: criteria for defining reinfection. *British Medical Journal*, 1989, 299:773-775.
16. Rosen JB et al. Outbreak of measles among persons with prior evidence of immunity, New York City, 2011. *Clinical Infectious Diseases*, 2014, 58:1205-1210.
17. Bellini WJ, Icenogle JP. Measles and rubella viruses, in *Manual of Clinical Microbiology*, Murray PR, Baron EJ, Landry ML, Jorgensen JH, Pfaller MA, editors. 9th edition, 2007, ASM Press.
18. Shonhai A et al. Investigation of a measles outbreak in Zimbabwe, 2010: potential of a point of care test to replace laboratory confirmation of suspected cases. *Epidemiology and Infection*, 2015, 143(16):3442-50.
19. Warrener L et al. A point-of care test for measles diagnosis: detection of measles-specific IgM antibodies and viral nucleic acid. *Bulletin of the World Health Organization*, 2011, 89:675-82.
20. van den Berg JP et al. Lower transplacental antibody transport for measles, mumps, rubella and varicella zoster in very preterm infants. *PLoS One*. 2014; 9(4): e94714. Published online 2014 Apr 11. doi: 10.1371/journal.pone.0094714
21. Smits GP et al. Development of a bead-based multiplex immunoassay for simultaneous quantitative detection of IgG serum antibodies against measles, mumps, rubella, and varicella-zoster virus. *Clinical and Vaccine Immunology*, 2012, Mar;19(3):396-400.
22. Smits G et al. Seroprevalence of mumps in The Netherlands: dynamics over a decade with high vaccination coverage and recent outbreaks. *PLoS One*. 2013; 8(3): e58234. Published online 2013 Mar 8. doi: 10.1371/journal.pone.0058234