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

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Chapter 4 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 test samples for rubella primarily because a proportion of suspected measles cases may be rubella cases. 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 sample 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 relative ease of transportation. For descriptions and best practices for the collection of serum and alternative samples (OF samples, and dried blood spots) for IgM antibody testing, refer to chapter 3.

Serum 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,
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).

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 for 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 sufficient kits, cost of kits and required reagents, necessary equipment, type of specimen, workload, and ease of use will influence the choice of kits. In 2016, 9 different kits were used for testing the WHO proficiency panels for measles IgM. The Siemens kit is utilized by the majority of network laboratories; however, the performance of other kits for measles IgM detection has been found to be satisfactory. The detection of rubella IgM was more variable among 18 kits used for the 2016 proficiency panel. Annex 4.1 provides an evaluation of commonly used kits based on observed
performance for completing the WHO proficiency panels for measles and rubella IgM in different years. The assays used for testing IgM in oral fluid (OF) must be evaluated for testing OF, as discussed in section 4.4 below.

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

As summarized in **Annex 4.1**, 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 within 3 days of rash (measles) or within 5 days of rash (rubella). Detection of IgM by EIA using serum specimens that are collected early include a caveat, described in many publications (including this one), consisting of the possibility that a “false negative” may be obtained. 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). The IgM result is not technically a falsely negative result, but reflects a result that is a ‘true negative’ when the level of virus-specific IgM is below the detection limit 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 be also be due to components within the serum specimen. The possible causes for a nonspecific result is 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 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. Some cases with equivocal results may be considered confirmed regardless of whether the clinical presentation is classical or in the absence of an epidemiologic link to a laboratory-confirmed case. However, there should be very few of these types of cases. See **Figure 4.1, Flowchart for case classification of measles in non-elimination settings.** The detection of virus-specific RNA by RT-PCR is often helpful to support a doubtful positive or an equivocal IgM result. The application of RT-PCR for case classification is discussed in chapter 6.

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**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. The flowchart for measles and rubella cases classification in near or post elimination settings is included in chapter 8 (figure 8.1).

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**Insert figure 4.1 here**

### 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 measles cases if serum was collected ≤3 days (<5 days for rubella cases) after rash onset, when levels of IgM may be below the threshold of detection of the assay. For this reason, a second serum specimen is required from highly suspicious cases if a negative or equivocal IgM result is obtained from serum collected during the early acute phase of illness. This requirement underscores the usefulness of collecting virologic specimens for RT-PCR testing to improve the ability to rapidly confirm cases. Refer to chapter 3 for best practices for the timing of 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 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 [1-4]. Enterovirus and adenovirus were also commonly identified. Among younger children with rash, HHV-6 was another frequent etiologic agent identified [1-4].

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 in individuals who were vaccinated between 8-56 days prior to rash onset cannot be determined by the detection of IgM. The virus-specific IgM detected by EIA does not distinguish between IgM elicited by a current wild-type infection and the IgM response following immunization with MR or MMR vaccine. While virus-specific IgM produced following vaccination in most individuals will be undetectable by 28-56 days post immunization or after natural disease, assays with higher sensitivity may extend this period.

A special situation applies to suspected measles/rubella cases that present with rash from 7-14 days after receipt of MR/MMR vaccine. While this timeframe is typical for a vaccine reaction, there may be recent measles activity or the possibility of exposure that raises the index of suspicion that the rash could be due to wild-type infection. Most rashes from vaccination are due to the measles component of the MR/MMR, and although it is rare, rubella can cause a vaccine-associated rash, extending the outer range from 14 days to 30 days after vaccination.
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 [2]. Therefore, a positive IgM result for measles combined with a negative result for rubella IgM in serum collected after a recent vaccination should not be interpreted as evidence of a measles wild-type infection. Because serologic techniques cannot distinguish between the immune response to natural infection and antibodies elicited by a recent immunization, collection of clinical specimens for molecular testing should be a top priority.

It is possible for a suspected case with a recent vaccination to be discarded without demonstration of a vaccine strain by molecular testing. Although a positive IgM result must be interpreted as a confirmed case and investigated appropriately, a retrospective re-classification as a vaccine-related rash is acceptable if epidemiologic and clinical criteria are met. These suspected cases would not include individuals who received vaccination as post-exposure prophylaxis (PEP), or were immunized as part of the outbreak response. If these conditions apply, in the absence of genotyping to confirm a vaccine strain, a positive IgM result should confirm the case. 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.

**Text box here:**

**Criteria for vaccine-associated rash**

The following 5 criteria must all be met in order for a positive IgM result from a suspected case to be considered as due to a measles/rubella vaccine-associated rash illness. These criteria are provided for situations where detection of a vaccine strain by genetic analysis of RNA collected from appropriate specimens was not available.

1. The patient had a rash illness, with or without fever, but did not have cough or other respiratory symptoms related to the rash
2. The rash began 7-14 days after vaccination with a measles/rubella-containing 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

The alternative approaches to serum collection for the determination of IgM that have been evaluated by specialized and reference laboratories in the GMRLN are the use of dried blood spots (DBS) from whole blood or collection of oral fluid (OF) [5]. Collection of these samples may be a viable option in areas where a cold chain and/or collection of venous blood is not feasible. 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. It is also important to ensure that there is sufficient total IgG in the sample for the result to be accurate [6,7]. Samples with insufficient IgG can give both false positive and false negative results. A capture EIA is used for IgM detection in OF. Details regarding modification of the protocol for the EIA is provided in Annex 4.2.

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 [5]. 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 [8]. 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 in IgM detection using DBS and serum sample pairs may vary in similar ways as was observed with 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 [9]. 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%. [9]. 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%. [10].

Eluted fluid from dried blood spots (DBS) can be tested using a commercially available IgM indirect EIA or a capture EIA. Methods for the elution of serum specimens from DBS have been published [11], 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 samples, usually collected 10-21 days apart, that are run together in the same test. 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 the virus-specific IgG in acute serum has high avidity 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 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 [12, 13]. 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. [14]. Refer to chapter 8 for more information regarding 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).

Insert Figure 4.2 here

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 still have detectable levels of rubella IgM in serum [15]. 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.
New serological techniques and methodologies
As improvements or alternative methodologies become available for measles and rubella diagnostics, updates should 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 (16,17). 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. Currently the assay is not available commercially.

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 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 [18-20]. 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


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