Chapter 3. Clinical specimens for the laboratory confirmation and molecular epidemiology of measles, rubella, and CRS

Overview
This chapter provides the best practices for collection of appropriate specimens for diagnostic and molecular investigations to support surveillance for measles and rubella. Protocols that include specific instructions for collection of adequate specimens for measles and rubella confirmation and molecular surveillance should be available in regional surveillance guidelines or field guides. These protocols should be updated and reviewed with input from both laboratory and epidemiological staff at appropriate intervals. However, when suspected cases occur, ongoing communication between the laboratory and field staff is essential to ensure that adequate specimens are provided to meet the requirements for case ascertainment and genetic characterization of outbreaks as every situation is different and alternative options may need to be adopted and implemented.

The full integration of surveillance for measles and rubella has been recommended for all Regions in the GMRLN. The investigation of suspected cases of measles or rubella should include adequate and appropriate sample collection for case classification and the genetic characterization of the virus. Ideally, samples for molecular testing from suspected cases of measles or rubella should be collected simultaneously with serologic specimens for antibody testing. Where feasible, the combination of IgM detection and RT-PCR to detect virus-specific RNA can enhance the ability to rapidly confirm suspected cases of measles or rubella.

3.1 Guidelines for the preparation and transport of clinical specimens
3.2 Safety procedures for incoming clinical specimens
3.3 Best practices for serum specimens for measles and rubella IgM detection
3.4 Alternative specimens for IgM antibody testing
3.5 Clinical specimens for molecular testing and virus isolation
3.6 Serologic and clinical specimens for confirmation of congenital rubella syndrome (CRS)
3.1 Guidelines for the preparation and transport of clinical specimens

Proper packaging and labelling of the clinical specimens or materials being shipped is vital to maintain the integrity of the specimens, prevent accidents, and to ensure that there are no delays due to failure to adhere to shipping regulations. All specimens collected for analysis must be properly labelled and should be accompanied with paperwork that includes the identification number that matches the label on the vial. In addition, all necessary details about each specimen should be provided to the receiving laboratory, including all relevant clinical and epidemiologic information regarding the suspected case. The onset and collection dates, as well as the date of the most recent vaccination are critical data to include for appropriate testing and interpretation of results. The documentation required and the patient information that should accompany specimens for measles and rubella testing is discussed in chapter 11. A standardised laboratory request form is recommended to ensure that all the pertinent information is provided. An example of an appropriate laboratory request form is provided in Annex 11.1.

Shipment of specimens within the country must meet all national requirements and laboratories should work closely with domestic carriers to ensure that the packaging and description of contents are appropriate. For international shipments of clinical specimens, it is essential that laboratories adhere to requirements for documentation, standardized packaging, and labelling for international shipments of clinical specimens. Successful shipment of materials within the country, region, or the global laboratory network requires advanced planning and communication between the sender, carrier, and the receiver. It is the responsibility of the sender to ensure that all requirements are met for shipment of materials sent to or from the laboratory. While much of this section pertains specifically to international shipments of clinical specimens, the complexity of the process- whether shipments are made within country or shipped internationally- requires an ongoing effort to keep informed of any updates or modifications made to shipping or packaging requirements.

The receiving laboratory should be contacted prior to shipment and informed of the quantity and type of specimens that will be sent. If the clinical specimens are being shipped internationally, the sender should inquire about any import permits required by the receiving laboratory’s national government. The receiving laboratory must obtain the current permit or other required paperwork and provide them (by pdf file, email, or fax) to the shipping laboratory. The sender should inquire whether the receiver
prefers a specific carrier or can provide helpful information regarding previous experiences or problems encountered with incoming shipments. The carrier should be familiar with handling infectious substances and diagnostic specimens. The carrier may require specific documents and may provide advice on packaging or specific instructions necessary to ensure safe arrival of the shipment. The sender and receiver should then make advance arrangements for a mutually convenient time for shipment to ensure that the appropriate staff are available to receive the shipment. It is recommended that weekend arrivals are avoided.

The packaging requirements for some types of laboratory materials are subject to international and national regulations. The updated International Air Transport Association (IATA) Dangerous Goods Regulations governing the transportation of biological specimens from 1 January 2017* are applicable. The document entitled, Guidance on Regulations for the Transport of Infectious Substances 2017-2018, covers all aspects of shipping including packaging and documentation [1]. The internet URL is provided in the bibliography at the end of this chapter, however the publication referred to here may be superseded on an annual basis.

Category B agents (viable measles and rubella virus cultures are included in this category) are considered to present a reduced risk because they are not easily transmissible and basic precautions and hygienic practices will serve to prevent exposure and infection in the event of an incident. Specimens or other materials that are not subject to dangerous goods requirements and regulations include patient diagnostic specimens if there is minimal likelihood that pathogens are present and if they are transported in packaging which will prevent any leakage and are correctly labelled. The exterior packaging must be marked as “Exempt human specimen”. For diagrams and more information, refer to Annex 3.1, Shipping clinical samples.

Clinical specimens collected for molecular characterization may be processed and shipped on commercially produced paper cards (FTA® Cards) that inactivate virus but preserve the nucleic acids. Shipment of the FTA cards does not require a cold chain and should be accepted as regular mail. The preparation, shipment and extraction protocols for dried blood spots and dried serum spots is provided in Annex 3.2. Information regarding the use of the FTA cards for molecular testing is discussed in chapter 5. Oral fluid samples and dried blood spots (DBS) for serology testing can usually be shipped
at ambient temperature, often by regular post shipment. Refer to specific sections in this chapter regarding these specimens.

Inclusion of adequate refrigerants is needed to ship clinical specimens for virus isolation or when virus cultures are being shipped for further analyses. Ice or ice packs that have been frozen at -20°C can maintain temperatures of +4-8°C in a well-insulated shipping container for up to 3 days. Ice should be placed in a leak-proof container outside the secondary receptacle and the outer packaging should also be leak-proof. In order to maintain cold-chain conditions for longer than 3 days, the use of dry ice is recommended if it can be readily obtained and the required packaging for dry ice shipments is available.

Dry ice must not be placed inside the primary or secondary receptacle because of the risk of explosions. A specially designed insulated packaging is used for dry ice shipments which permits the release of carbon dioxide gas as the dry ice undergoes sublimation. Dry ice is regulated as a hazardous material in air transport; ICAO/IATA Packing Instruction PI954 applies. The outermost packaging must carry the hazard label for dry ice and the appropriate marking.

The documentation required for shipping materials is determined by the nature of the materials being sent. In general, each shipment should be accompanied with the airway bill (if shipped by air), any required export/import documentation, and outer packaging should inform the receiving laboratory of the proper contents and proper storage conditions. For example:

**URGENT: DO NOT DELAY: Biological specimens – highly perishable – store at 4°C to 8°C**

Once the package has been sent, the receiver should be immediately notified of the following:

- estimated number of cartons and weight
- flight and arrival date/time
- airway bill number

A copy of the airway bill should be provided to the sender and the receiving laboratory should confirm that the airway bill was received. Upon receipt of the package and inspection of the contents,
the receiver should provide confirmation of delivery and inform the sender of the condition of the materials. This can be facilitated by the sender including a “fax back” form in the shipment. If the quantity or types of specimens received does not match the accompanying documentation or does not conform with the information that was provided prior to shipment, the receiver should immediately contact the sender and resolve the apparent discrepancy.

3.2 Safety procedures for incoming clinical samples

On arrival in the laboratory, shipping cartons or packages must be promptly unpacked in the designated area. Use of a Class II Biosafety Cabinet (BSC) is recommended to limit exposure of laboratory staff to potential pathogens. Personnel who receive and unpack specimens should be aware of the potential health hazards involved and should be trained to adopt standard precautions, particularly when dealing with broken or leaking containers. Primary specimen containers should be opened in a biological safety cabinet where possible. Disinfectants such as 70% ethanol are used to wipe down surfaces and a 1:10 solution of sodium hypochlorite (bleach) solution should be available in case of spills. All potentially contaminated materials should be placed inside discard containers that are lined with leak-proof bags.

All laboratory personnel should wear appropriate personal protective equipment (PPE) including latex or nitrile gloves when packages are opened, and the contents are removed for inspection and specimen accessioning. Blood products including serum specimens should always be considered as potentially infectious materials. Unpacking and recording of specimens should preferably be carried out by two persons: one records data while the other is gloved and is responsible for opening the package and checking for broken or damaged sample containers and evidence of leakage. Information that should be recorded when accessioning samples is provided in chapter 11.

Any potentially contaminated paperwork should be placed temporarily in the BSC while the information is manually recorded on a fresh sheet of paper. Contaminated documents should be handled in the same manner as infectious waste. Use of a BSC is recommended for transfer of specimens to another container, such as when preparing aliquots of clinical specimens. It is recommended that each laboratory develop specific standard operating procedures for opening packages and logging in specimens.
3.3 Best practices for serum specimens for measles and rubella IgM detection

The analysis of serum specimens for the presence of measles- or rubella-specific IgM antibodies is traditionally regarded as the gold standard for laboratory confirmation. The enzyme immunoassay (EIA) is the method recommended for the WHO measles and rubella laboratory network for the detection of virus-specific IgM antibodies in serum. The EIA kits that are commercially available vary in sensitivity and specificity, but many kits have been evaluated by the laboratories in the GMRLN. Additional guidelines for selection of EIAs are provided in chapter 4. The guidelines are based on analyses of the results obtained from network laboratories that have participated in the proficiency testing programme over many years. Several commercial measles and rubella IgM EIA kits (and in-house assays) have been utilized by network laboratories [2].

Blood collection requires trained personnel, dedicated equipment for collection and processing, and a reliable reverse cold chain. Any staff involved with the collection of patients’ blood specimens should be fully trained in the process, the appropriate use and disposal of needles and syringes. Refer to WHO guidelines on drawing blood: best practices in phlebotomy [3]. The availability of a serum specimen from suspected cases provides a means to conduct testing using similar serologic assays for other causes of febrile rash illness. The specific testing performed for other rash illnesses varies among laboratories but may include diseases that are commonly encountered in a particular age group (e.g., young children and parvovirus B19) or diseases that are endemic in the area (e.g., dengue, zika virus).

Two topics are discussed below. The first, “3.3.1, Timing of blood collection for measles and rubella IgM detection”, covers the recommendations for optimal results for antibody detection and the circumstances under which the collection of a second serum specimen may be appropriate. The final subsection, “3.3.2, Collection, processing, storage and transport of serum specimens”, covers the technical and logistical issues for provision of quality serum specimens.

3.3.1 Timing of blood collection for measles and rubella IgM detection

Blood is collected at first contact with a suspected case of measles or rubella. In most instances, a single serum specimen will be sufficient to classify a suspected measles or rubella case based on the presence or absence of virus-specific IgM. For surveillance purposes, an adequate serum sample for measles or rubella is one that is obtained within 28 days after the onset of rash. However, when serum
is collected within the first few days following rash onset, a proportion of infected individuals may test negative for IgM due to low levels of virus-specific IgM that are undetectable by EIA (see discussion below). Refer to chapter 8 for guidance regarding laboratory confirmation including serologic testing that is specifically recommended for elimination settings.

Measles virus-specific IgM may not be detectable by EIA in serum that is collected in the first 72 hours (≤3 days) after rash onset in approximately 30% of measles cases [4]. In the study on which this guidance is based, day 1 was defined as ≤24 hours after onset of rash. Of 28 serum specimens collected on day 1, there were 20 (71%) that were IgM positive using an IgM capture EIA. By day 3 (48-72 hours after rash onset), the IgM positivity rate increased to 77% (20/26). For those patients whose second serum specimen was IgM positive, 100% of the serum specimens collected from day 4 through day 11 were IgM positive by the measles capture EIA.

Rubella-specific IgM may be undetectable by EIA in up to 50% of rubella cases from serum specimens that are collected on the day of rash onset. In >90% of rubella cases, serum that is collected at 5 days after rash onset will be IgM positive [5]. A discussion of the timing of blood collection for confirmation of CRS and CRI cases is provided in section 3.6.

During outbreaks that involve large numbers of cases, determination of epidemiologic links to laboratory-confirmed cases should be utilized as an adjunct for case confirmation in order to prioritize laboratory testing for suspected cases that may arise in new locations or in previously unaffected groups. Ongoing coordination between the laboratory and the field staff can reduce unnecessary blood collection and testing. However, periodic collection of clinical specimens for molecular testing during the course of ongoing outbreaks (extending beyond one month) is recommended to monitor the genotype over time (see chapter 7).

In the absence of an epidemiologic link to a laboratory-confirmed case, collection of an additional serum specimen may be required to classify the suspected case. However, in outbreak settings where measles is endemic, resources should not be used to collect follow-up serum specimens. The guidelines below are primarily intended for countries in near or post-elimination settings. More information for testing in these settings is available in chapter 8. However, regardless of the phase of
disease elimination, collection of a second serum specimen may be required under some circumstances to provide laboratory confirmation for suspected cases that may occur in locations outside of the initial outbreak area or when a suspected case has been identified in a previously unaffected group.

A **second or follow-up serum may be appropriate under the following circumstances:**

- Detection of virus-specific RNA by RT-PCR is either unavailable or the results were inconclusive
- The first serum specimen was collected ≤3 days after rash onset and is negative for measles IgM, or is negative in serum collected ≤5 days for rubella IgM by EIA
- Repeat testing of the initial serum specimen fails to resolve an equivocal result for IgM
- The IgM is positive but there is suspicion that the result is false positive, and the rash is due to another etiologic agent (see chapter 4, section 4.2.2)

Depending on the situation, it may be acceptable to delay blood collection so that the appropriate interval exists between the initial and second specimen to evaluate a diagnostically significant rise in IgG. A repeat IgM test is then performed with the follow-up serum. If necessary and feasible, IgG titres can be evaluated by a quantitative IgG EIA using paired serum specimens collected 10-21 days apart. The interval between the two serum specimens can be reduced if virus-specific IgG was not detected in the first serum specimen. When a seroconversion can confirm the case, the interval between serum collections can be shorter than 10 days but the second serum should not be collected until at least 9-10 days after rash onset when virus-specific IgG should be detectable. Refer to chapter 4 for additional information regarding testing strategies for case classification by IgM and IgG antibody testing.

### 3.3.2 Collection, processing, storage and transport of serum specimens

Collection of blood is performed by venepuncture using a sterile, plain collection tube (red-top tube) or serum separator tube without additives. Although a volume of 4-7 ml is typically collected from adults (1 ml will yield typically about 400 μl of serum), collection of 0.5 ml-1ml is acceptable from infants. Whole blood can be stored at (+)4–8°C (never freeze whole blood) for up to 24 hours or for 6 hours at (+)20-25°C before the serum is separated from the clotted blood through centrifugation. After
this time, whole blood must be transported to a facility equipped to separate the serum in order to avoid haemolysis.

Blood should be transported with adequate insulation on cold packs. After arrival at the laboratory, the blood tubes should be allowed to clot in an upright position, usually 30–60 minutes at room temperature. After the blood has clotted, the stoppered tube is centrifuged at 1000 × g for 10 minutes to separate the serum. The serum should be removed carefully without disturbing the red cell layer and transferred to a sterile, labelled vial. The vial should have a durable label attached with the patient’s name or identifier, date of collection and specimen type.

**Procedures for processing blood without a centrifuge:**

If a serum separator tube has been used, serum can be pipetted out aseptically after the red blood cells have separated (about 6 hours at +4°C). If the blood was collected in a plain tube, the blood may be refrigerated until there is complete retraction of the clot from the serum (no longer than 24 hours). At (+)20-25°C, the blood will clot in about 20 minutes. The serum should be carefully removed with a fine-bore pipette, to avoid extracting red cells, and transferred aseptically to a sterile, labelled vial.

Serum should be held at refrigeration temperature (4 to 8°C) until shipment, but ideally should not be stored at 4-8°C for longer than 7 days*.

*Note: The guidelines for storage of serum at refrigeration temperatures vary in regard to protecting the integrity of antibodies in serum. Some manufacturers of EIA kits specify that 2-3 days is the maximum time and others indicate serum maybe be stored at 4-8°C for up to 28 days. However, when a delay is anticipated in shipping or in testing, the serum specimens should be frozen at -20°C, or colder. A frost-free freezer, which has a periodic warming cycle to prevent ice build-up, should not be used.

The frozen serum specimens should be transported to a testing laboratory packed with frozen cold/ice packs, in a sufficiently insulated container (see details below). Since repeated freezing and thawing can have detrimental effects on the integrity of IgM antibodies, these cycles should be avoided, and aliquots of important serum specimens should be prepared prior to freezing. Generally, serum specimens should be shipped to the laboratory as soon as possible and shipment should not be delayed for the collection of additional specimens.
The requirements for shipping serum may vary according to local conditions and regulations, and depend on whether shipment of serum is within national borders or must be shipped internationally (see section 3.1, above). However, all serum specimens should be transported in properly labelled vials. The vials should be packaged with absorbent material and protected against crushing of the contents. The laboratory request form and any other paperwork for each specimen should be placed in a separate plastic bag. An insulated container with cold packs may be required. For transit times of several days, the insulated container should be lined with frozen cold packs with the samples placed in the centre of the container with more cold packs placed on top. The methods and details of the shipping (courier, date of shipping) should be arranged or communicated prior to shipment between the sender and the receiving laboratory.

As an alternative to shipping a refrigerated package, whole blood can be spotted on filter paper in place of serum if a cold chain or an economical method for shipment is not available (see next section on alternative samples for IgM testing). Efforts by the laboratory network to substitute dried serum spots using existing serum samples for confirmatory testing has not proven to have consistently acceptable performance for measurement of IgM. The procedure for preparation, shipment, and extraction of DBS samples is provided in Annex 3.2.

### 3.4 Alternative specimens for IgM antibody testing

While serum-based IgM detection is recommended by WHO for routine surveillance for measles and rubella, the use of dried blood spots (DBS) and oral fluid (OF) have proven to be acceptable alternative samples for antibody detection when logistical barriers exist for collection, proper processing and transport of serum specimens. However, external quality control programmes have not yet been established for DBS and OF. Guidelines for the use of DBS and OF are discussed in the specific sections (3.4.1, 3.4.2) that address each type of sample.

An evaluation and recommendations for the use of DBS and OF in network laboratories as diagnostic samples for measles and rubella was published in 2008 [6]. The figures based upon the evaluation are included below (Figures 3.1, 3.2).
Figure 3.1 Schematic of wild-type measles virus infection and sensitivity of alternative sampling methods


Figure 3.2 Schematic of wild-type rubella virus infection and sensitivity of alternative sampling methods

Some of the advantages these alternative specimens provide compared to serum collection are listed below.

- An equivalent combined cost for collection, extraction and testing
- The potential to greatly reduce transportation cost (a reverse cold chain generally is not required for DBS)
- DBS and OF can be used to detect both specific IgM and RNA* in the same sample
- OF may extend the window of opportunity for RNA detection post rash onset
- DBS samples are equivalent to serum for detecting IgG and offer versatility for use in sero-epidemiology studies

*RNA detection by real-time RT-PCR is more sensitive than conventional end-point RT-PCR. Although nested RT-PCR is often found to be more sensitive, it also imposes a greatly increased risk of cross-contamination and is not recommended to be used in a routine diagnostic setting. Refer to Chapter 6.

3.4.1 Dried blood spots on filter paper

As an alternative to serum, whole blood specimens that have been dried on filter paper (i.e., dried blood spots, DBS) have been used for a range of epidemiological studies. The use of DBS may be considered where a reliable cold chain is not available or logistical barriers for efficient transport of serum exist [6]. While routine blood collection (venipuncture) can be used to prepare DBS, in general, DBS samples are prepared using capillary blood, a less invasive blood collection method that may be perceived as more acceptable, particularly for young children and infants. Antibodies (IgM and IgG) are stable once the blood has dried on filter paper, making this method a good alternative option for collection of blood specimens in remote areas without a reliable cold chain. The added advantage is that DBS can also be utilized to detect both measles and rubella RNA when collected soon after disease onset (section 3.5).

To meet the surveillance requirement for an adequate sample of DBS, the blood volume collected must be sufficient to completely fill three of the four marked circles on a standardized filter paper card designed for blood collection (e.g., Whatman 903). However, efforts should be made to fill all four
marked circles (14-15 mm). This provides for a separate spot/extraction for measles testing, rubella testing, retesting, and an additional spot for referral if necessary. A drop of whole blood (50 μl) is necessary to fill each circle. As is true for serum specimens for antibody testing, the blood sample for DBS must be collected from suspected measles or rubella cases within 28 days of rash onset to meet the laboratory surveillance requirements.

Blood collection can be performed by finger- or heel-prick using a sterile, preferably using a single use, disposable lancet. The lancets currently available include a safety feature for activation and produce a consistent puncture depth (e.g., BD Microtainer 366594). Blood drops are then applied to marked circles on the filter paper card. For an example, see Figure 3.3, Example of an appropriate format for filter paper used for dried blood spots (DBS).

Blood specimens that have been spotted on filter paper must be allowed to air dry completely. The time for complete drying will vary according to humidity levels, but is usually 2-4 hours. Once dried, individual cards can be wrapped in wax paper, and placed in a sealable plastic bag with a desiccant pack. Ideally, the desiccant should have a colour indicator so the ability of the desiccant to absorb moisture can be monitored.

For elution of the serum, a standard hole punch is used to remove three 6mm discs from one of the filled filter paper circles. Each 6mm disc punched out from the DBS contains approximately 5μl serum. The remaining DBS are stored at -20°C if re-testing is necessary or for confirmatory testing.
Although properly prepared DBS samples are stable for a limited time at room temperature, ideally the DBS should be stored at 4°C until they can be shipped to the laboratory. Transport of DBS at ambient temperatures up to 42 °C is acceptable if the sample is delivered to the laboratory within 3 days [7]. Upon arrival to the laboratory the DBS should be held at 4°C until the serum can be extracted from the filter paper. The recommended protocols for the preparation, shipment and extraction of serum from DBS are provided in Annex 3.2.

### 3.4.2 Oral fluid samples

The use of oral fluid (OF) samples has been successfully implemented in the UK since the early 1990s and is used almost exclusively for measles, rubella and mumps laboratory-based surveillance. An OF specimen includes saliva and secretions from the parotid, submaxillary and sublingual glands. OF is a minimally invasive sample, is relatively easy to collect, and is more acceptable to both clinicians and patients compared to blood collection. Because OF is stable at moderate ambient temperatures, these samples may be easily and economically shipped if moderate ambient conditions exist with reliable local postal or delivery services. In the UK, the use of OF for laboratory testing for suspected cases of measles and rubella has resulted in a more comprehensive sampling of suspected cases.
Collection devices for OF are not commonly available in many regions and would need to be provided to health-care facilities by the surveillance programme. The Oracol™ device has been widely used in the GMRLN for OF collection (See Figure 3.4).

**Figure 3.4** The Oracol™ device for collection of oral fluid

Oracol™ device (Malvern Medical Developments)

Oral fluid sampling devices which use a preservative for stabilising IgM (such as OraSure™) should not be used for collection of OF samples that would be tested by RT-PCR. The collection of an OF sample does not require professional medical personnel but does require instructions on the proper collection to obtain a good quality OF sample.

The technique for OF collection is intended to allow collection of gingival crevicular fluid at the gum and dentine interface which contains antibodies from the capillary beds along the gingival crevice. An adequate OF sample is one that is collected by gently rubbing along the base of the teeth and gums for at least 1 minute, which should allow the sponge to absorb about 0.5ml of crevicular fluid [7]. After collection, the sample is placed inside a microtube which is included. An OF sample for antibody testing is considered an adequate sample for surveillance if collected within 28 days of rash onset.
Note: There is evidence for the effectiveness of a new OF collection and extraction device (Oralight), although as of this writing the data is pending publication. The use of OF samples for virus-specific RNA detection is discussed in chapter 6.

A summary is provided in Table 3.1. Expected success rates for IgM or virus detection using serum, DBS and OF.

<table>
<thead>
<tr>
<th>Time of collection – Moment du recueil</th>
<th>Serum (%) – Sérum (%)</th>
<th>Dried blood sample (%) – Échantillon de sang séché (%)</th>
<th>Oral fluid (%) – Liquide buccal (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>IgM</td>
<td>Early (day 0-5) – Précoc. (jour 0-5)</td>
<td>60-70</td>
<td>60-70</td>
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<tr>
<td></td>
<td>Interactions (day 4-14) – Intermédiaire (jour 4-14)</td>
<td>90-100</td>
<td>90-100</td>
</tr>
<tr>
<td></td>
<td>Late (day 15-28) – Tardif (jour 15-28)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Early (day 0-5) – Précoc. (jour 0-5)</td>
<td>&lt;10</td>
<td>&lt;15</td>
</tr>
<tr>
<td></td>
<td>Interactions (day 4-14) – Intermédiaire (jour 4-14)</td>
<td>&lt;10</td>
<td>&lt; 25</td>
</tr>
<tr>
<td></td>
<td>Late (day 15-28) – Tardif (jour 15-28)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Measles – Rougeole                       | Early (day 0-3) – Précoc. (jour 0-3) | –50                                           | –50                               | –40                              |
|                                       | Intermediate (day 4-14) – Intermédiaire (jour 4-14) | 60-90                                         | 60-90                             | 50-90                            |
|                                       | Late (day 15-28) – Tardif (jour 15-28) | 100                                          | 100                               | 100                               |
|                                       | Early (day 0-3) – Précoc. (jour 0-3) | –20                                          | –20                               | Limited data – Données limitées  |
|                                       | Intermediate (day 4-14) – Intermédiaire (jour 4-14) | Limited data – Données limitées | Limited data – Données limitées | Limited data – Données limitées |
|                                       | Late (day 15-28) – Tardif (jour 15-28) | Limited data – Données limitées | Limited data – Données limitées | Limited data – Données limitées |

| Rubella – Rubéole                         | Early (day 0-3) – Précoc. (jour 0-3) | –50                                           | –50                               | –40                              |
|                                       | Intermediate (day 4-14) – Intermédiaire (jour 4-14) | 60-90                                         | 60-90                             | 50-90                            |
|                                       | Late (day 15-28) – Tardif (jour 15-28) | 100                                          | 100                               | 100                               |
|                                       | Early (day 0-3) – Précoc. (jour 0-3) | –20                                          | –20                               | Limited data – Données limitées  |
|                                       | Intermediate (day 4-14) – Intermédiaire (jour 4-14) | Limited data – Données limitées | Limited data – Données limitées | Limited data – Données limitées |
|                                       | Late (day 15-28) – Tardif (jour 15-28) | Limited data – Données limitées | Limited data – Données limitées | Limited data – Données limitées |

If the daily ambient temperature is below +22°C, OF samples may be shipped without refrigeration but ideally should be shipped to the laboratory within 24 hours. At higher temperatures, the OF samples should be kept at 4-8°C until the samples can be shipped to the laboratory on cold packs. The OF samples are not considered a biohazard and can be shipped without special documentation from the site of collection to the laboratory.

Oral fluids must be extracted from the swab as soon as possible after receipt in the laboratory. The processing required for the OF includes an extraction step (Annex 3.3, Processing oral fluid samples). External quality assessment programmes, such as those currently required for testing serum, have not yet been established for OF and assays must be validated for testing OF. However, one method of
assessing the quality of the OF for use in IgM assays is to verify that a minimum level of total IgG is present in the sample (protocol included Annex 3.3).

### 3.5 Clinical specimens for molecular testing and virus isolation

It is recommended that clinical specimens for the molecular surveillance of measles and rubella are collected in parallel with samples for antibody testing for case classification. Routine testing by RT-PCR in addition to antibody detection enhances the ability to rapidly confirm suspected cases of measles and rubella but will require additional resources.

An oropharyngeal (throat swab) is the recommended sample for both RNA detection and virus isolation for suspected cases of measles or rubella. Nasopharyngeal (NP) swabs will serve as good samples for both virus isolation and RNA detection but are more difficult to collect. NP aspirates and nasal swabs are variations that have been used successfully to detect measles virus.

**Note:** Some surveillance programmes include an option to use swabs to collect oral secretions (mouth swabs) for measles virus RNA detection as an alternative to a throat swab. Mouth swabs are often easier than throat swabs to obtain from young children. Saliva and crevicular secretions are collected by swabbing the inside cheek near the gum line.

If measles- and rubella-specific IgM is tested using OF rather than serum, the OF can also provide a sample for virus-specific RNA detection. However, OF is considered not suitable for virus isolation. For the purpose of RNA detection by molecular testing, the likelihood of obtaining a positive signal by RT-PCR is much improved when samples are collected closer to rash onset. As with other types of samples for molecular testing, the likelihood of obtaining a positive signal by RT-PCR is much improved when samples are collected closer to rash onset. However, it is also true that at any interval post rash onset, a negative RT-PCR result may be obtained from true cases of measles or rubella. Therefore, negative RT-PCR results alone are not sufficient to rule out a suspected case and serologic testing is generally required to corroborate negative RT-PCR results.

Countries that use OF as the primary specimen for antibody detection and virus-specific RNA detection should collect throat swabs to provide an opportunity to make virus isolates from
representative cases during an outbreak (see chapter 7). Serum specimens have been used in rare instances when better samples for genotyping are not available, and archival samples of serum have been used to aid in identification of circulating rubella strains prior to routine surveillance.

Urine specimens have been used successfully for both measles and rubella detection and virus isolation, but throat swabs are much more sensitive compared to urine samples, particularly for rubella. Throat/oropharyngeal swabs, NP swabs, and urine samples must be collected within 5 days of rash onset to be considered an adequate sample for virus isolation. While virus may be present through at least day 5 after rash onset, the success rate for virus isolation is much higher with specimens collected within 3 days from rash onset.

**Note:** Specimens for virus isolation and RNA detection from congenital rubella syndrome (CRS) and congenital rubella infection (CRI) cases are described in section 3.6.

Throat swabs and urine can be collected up to 14 days after rash onset for RNA detection by RT-PCR although RNA detection rates are much lower after 7 days post rash onset. Both measles and rubella virus-specific RNA can be detected in throat swab samples from a few days before onset of rash to several days afterwards. Prompt collection of samples at first contact with a suspected case of measles is the best approach to ensure a quality sample. Collection of both types of samples (throat swab and urine) may enhance the successful detection of RNA or the ability to isolate virus. The collection of both samples may be particularly useful and appropriate in elimination settings when a sporadic case is detected.

Both measles and rubella viruses are sensitive to heat, and infectivity decreases with time, even when samples are refrigerated for extended periods of time. While RNA detection by RT-PCR can often be successful despite disruptions in the maintenance of a cold chain, every effort should be made to transport and maintain all clinical specimens for RNA detection or virus isolation to freezers (-40 to -70°C) to best preserve RNA and the viability of the virus. Clinical samples transported at -20°C may preserve the integrity of the viral RNA for RT-PCR testing however, the viability of virus may be diminished or lost.
3.5.1 Collection, processing, and transport of upper respiratory specimens

The preferred sample for both measles and rubella virus detection is the throat (oropharyngeal) swab. Swabs should be collected using only synthetic fibre swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate viruses and/or inhibit PCR testing. The throat swab is collected by swabbing the posterior pharynx, avoiding the tongue. A tongue depressor may be used. Light pressure should be applied to collect the epithelial cells around the tonsil area. Similar swabs should be used for mouth swabs for measles RNA. For this sample, the swab is rubbed on the inside of the cheek area and the tongue.

The nasopharyngeal swab (NP) is often collected in a hospital or clinic setting, and provides a good specimen but may be less acceptable to the patient. A NP swab has a flexible shaft and the patient’s head should be tilted back. The swab is inserted into the nostril parallel to the palate and should contact the mucosal surface. The swab in held in place for a few seconds to absorb secretions. Both nostrils can be swabbed and the two swabs can be combined.

The throat and/or NP swabs are placed immediately into sterile tubes containing 2-3 ml of viral transport media (VTM) or PBS. It is important to prevent the swabs from drying out. (See Annex 3.4, Composition of viral transport media, antibiotics and reagents). A throat swab can be combined with an NP swab in one tube when both specimens are collected from the same patient. The throat and/or NP swabs may be refrigerated at 4-8°C for up to 48 hours and shipped on ice/frozen cold packs. If an interval of greater than 3-5 days will elapse prior to arrival at the receiving laboratory, it is best to preserve the sample at -70°C and transport on dry ice.

**Note:** Prior to freezing and the removal of the swab, the tubes should be vigorously mixed using a tube vortex for about 15 seconds to dissociate the cells from the swab material. The fluid should be drained from the swab by pressing the swab tip along the side of the tube before removal of the swab.

Efforts should be taken to avoid defrosting as a single defrosting step may render the specimen useless for virus isolation and may also affect the ability to extract intact RNA for RT-PCR. Upon arrival in the testing laboratory, the throat, nasal, or NP swab sample can be frozen at -70°C if the volume of the fluid is sufficient (2-3 ml) and the swab has been removed. If the swab has not been removed prior to
freezing, follow the steps provided (see Note, above) to maximize recovery of the sample. Cell culture medium (MEM or DMEM) is added to the sample to bring the total volume to 2-3 ml. The swab is discarded as potentially infectious material. If there is a large amount of debris in the fluid, the contents can be centrifuged at low speed and the supernatant removed to a labelled sample tube.

3.5.2 Collection, processing, and transport of urine samples

The first-voided urine of the day is preferred as it contains the highest concentration of cells sloughed off in the urinary tract, although in practice the sample is collected at first contact with the suspected case. The volume of urine collected should be at least 10 ml. A larger volume (up to 50 ml) will generally provide more infected cells. The urine is collected in a suitable sterile container that is leak-proof and should be stored at 4-8°C until the urine can be centrifuged. The original urine sample should not be frozen prior to centrifugation.

Whole urine samples may be shipped in sealed containers at +4°C, but centrifugation within 24 hours of collection is recommended. The urine is centrifuged at 500 x g (approximately 1500 rpm) for 5 to 10 minutes, preferably at 4°C and the supernatant is discarded. Sterile VTM, tissue culture medium or phosphate-buffered saline (PBS) is added to the urine sediment, to bring the final volume to 2 ml. If a pellet is not visible, remove all but the last 1-2ml at the bottom of the centrifuge tube and mix with an equal volume of sterile, isotonic fluid (VTM, PBS etc). The processed urine sample may be stored at +4°C and shipped within 48 hours to a laboratory that conducts measles virus isolation or RNA detection. Alternatively, the urine sample may be frozen at -70°C in viral transport medium and shipped on dry ice.

3.6 Serologic and clinical specimens for confirmation of congenital rubella syndrome (CRS)

Throat swab specimens are the preferred sample for CRS (or CRI) confirmation by RNA detection and for determination of the rubella genotype. However, other samples such as nasal swabs or aspirates, urine, and cerebrospinal fluid (CSF) specimens are acceptable for RNA detection to confirm CRS. Oral fluid samples have proven to be useful for rubella RNA detection from CRS cases [8]. Tissues from biopsy or autopsy may also be used for laboratory confirmation of CRS cases. In CRS
cases where cataracts are present, surgically removed cataracts can be submitted for molecular testing. Rubella virus RNA has been detected in cataracts up to three years after birth.

Serologic tests to confirm CRS include the demonstration of rubella IgM antibodies or the demonstration of increasing levels of IgG antibodies in the first year of life [5]. In CRS cases, IgM antibodies are often present shortly after birth, but re-testing should be performed since some infants do not develop antibody for a few weeks. The IgM may remain detectable for up to 1 year although IgM detection is most reliable between 3-6 months of age. For infants beyond 6 months of age, an evaluation of rubella-specific IgG is recommended to confirm CRS [8].

CRS cases can continue to shed virus for up to one year after birth and can be the source of virus for rubella infection in exposed persons, including susceptible pregnant women. Specimens for virus detection should be collected when a case is first suspected. Ideally, specimens should be collected in the first 3 months of age since about 50% of affected infants may not shed virus beyond 3 months of age. Infants with CRS should be monitored until they are at least 1 year of age. Alternatively, demonstration that virus shedding has ceased can be achieved by collection of two clinical specimens obtained one month apart and collected after the infant is older than 3 months of age [9]. Both specimens should have negative culture results.

**Bibliography for Chapter 3**


