



Laboratory Testing for Measles

For Public Health Staff

The California Department of Public Health (CDPH) Viral and Rickettsial Disease Laboratory (VRDL) encourages submission of specimens from suspected measles cases who meet the CDC clinical case definition.

CDC measles clinical case definition

An illness characterized by all the following:

- a generalized rash lasting ≥ 3 days;
- a temperature $\geq 101.0^{\circ}\text{F}$ ($\geq 38.3^{\circ}\text{C}$); and
- cough, coryza, or conjunctivitis.

Measles laboratory confirmation

Acute measles infection can be confirmed by:

- positive serologic test for serum measles IgM antibody; or
- significant rise in measles IgG antibody between acute and convalescent titers; or
- isolation of measles virus; or
- detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR)

Specimen collection

Collection of a serum specimen for serologic detection of measles antibody in conjunction with a respiratory sample (nasopharyngeal swab or aspirate or throat swab), and a urine specimen for molecular determination or virus isolation is helpful in the laboratory confirmation of a measles case.

IgM/IgG testing:

- Collect 7-10 ml of blood* in a red top or serum separator tube (SST).
- The optimal time for collection of acute blood is as soon as measles is suspected and up to 7 days after symptom onset.
- If initial IgM testing is negative and measles is strongly suspected, a convalescent serum sample should be collected 2-4 weeks after symptom onset.

*Capillary blood (finger or heel stick) can be used for serology if venous blood cannot be obtained (page 3).

IgM interpretation

- Detection of IgM in acute serum is the recommended test for rapid determination of acute measles infection.
- In unvaccinated persons, IgM antibody is generally detectable around the time of rash onset and can be detected for 1–2 months.
- If blood is drawn earlier than 72 hours after rash onset and the measles IgM antibody result is negative, draw another blood specimen and run for repeat testing.
- Other diseases, such as parvovirus infection, infectious mononucleosis, or rheumatologic disease can cause false positive measles IgM antibody results.
- If IgM results are positive, the original serum sample should be retained for confirmatory testing at a reference lab, if possible. This is particularly important if the patient does not have epidemiologic links suggesting measles.

IgG interpretation

- Obtain acute and convalescent serum specimens for measles-specific IgG antibody to confirm a measles diagnosis. This is especially important if the measles IgM antibody titer is drawn at the wrong time or if other infections are present.
- In unvaccinated persons, the IgG response starts at about 5–10 days after rash onset, but typically persists for a lifetime.
- In vaccinated persons, existing IgG antibody will begin to rise soon after exposure/infection and IgG may be quite elevated in the acute-phase blood sample, which may obviate the fourfold rise in IgG titer in the convalescent serum specimen.
- Paired acute and convalescent serum specimens that demonstrate a fourfold increase in IgG titer or a seroconversion from IgG negative to positive are considered positive diagnostic test results for measles.

Interpretations of a single positive measles IgG antibody result

- Current infection to which the individual is developing immunity; OR
- Immunity due to either a past infection or vaccination; OR
- Presence of maternal antibody (in infants < 15 months).

In recently vaccinated persons (6-45 days prior to rash onset), neither IgM nor IgG antibody responses can distinguish measles disease from a response to vaccination. If there is concern about measles disease, a viral specimen should be obtained to distinguish between vaccine virus and wild-type virus.

PCR and viral culture

- Preferred specimens are nasopharyngeal swabs or aspirates or throat swabs.
- Urine is also an acceptable specimen for measles isolation or detection by RT-PCR.
- Attempt to obtain specimens as soon as possible after rash onset up to 10 days after onset. Virus is more likely to be isolated in culture when the specimens are collected within 4 days of rash onset.

Specimen collection for PCR and isolation

Respiratory specimens: (all are acceptable)

- Nasopharyngeal aspirate: use a syringe attached to a small, plastic tube and 2-3 ml of viral transport media (VTM). After placing VTM in the nose, aspirate as much of the material as possible and rinse the tube with 2 ml of VTM.
- Nasopharyngeal swab: firmly rub the nasopharyngeal passage with a sterile Dacron swab to dislodge epithelial cells. Place swab in sterile VTM. Do not use special (e.g., anaerobic) media.
- Throat swab: vigorously swab tonsillar areas and posterior nasopharynx with a sterile Dacron swab.

Specimen storage and shipping (all specimen types):

Measles virus is sensitive to heat and desiccation and viability decreases when samples are not kept cold. Transport samples with cold packs as soon as possible following collection. If samples cannot be transported immediately, they can be held at 4°C for 72 hours before shipping. Otherwise, specimens should be frozen, preferably at -70°C and shipped on dry ice (except unprocessed urine – see above). Avoid repeat freeze-thaw cycles. For more information on laboratory testing for measles, please see:

<http://www.cdc.gov/measles/lab-tools/index.html>

Use a tongue blade to depress tongue to prevent contamination of swab with saliva. Place swab into VTM.

Urine:

- Urine from a first morning voided specimen during the first week after rash onset is best, although any urine specimen collected up to 10 days after rash onset will be accepted. Collect 50-100 ml urine in a sterile container, or centrifuge or sediment tube.
- Urine specimens should be shipped within 24 hours of collection. Otherwise, urine should be centrifuged at 2,500 X g for 15 minutes at 4°C to pellet the sediment. The sediment should then be resuspended in 2-3 ml of VTM and frozen, preferably at -70°C.
- If centrifugation is not available, do not freeze the urine specimen. The entire specimen should be stored at 4°C and shipped on wet ice.

RT-PCR/viral culture interpretation

- While detection of viral RNA by RT-PCR confirms measles infection, failure to detect measles virus RNA by RT-PCR in samples from a person who meets the clinical case definition for measles does not rule out measles. Successful detection of measles virus depends on the timing of collection and the quality of the viral sample.
- Virus may be isolated from a nasopharyngeal aspirate or swab, a throat swab, or urine collected at rash onset until up to 10 days after rash onset, but optimally within 4 days after rash onset.
- Persons vaccinated within 3 weeks of symptom onset may yield a positive RT-PCR result due to detection of vaccine virus. Genetic characterization of viral isolates or RT-PCR products is the only laboratory test that can differentiate between vaccine-associated cases and wild-type infection.

Measles Serology Results and Interpretation[†]

In early measles infection, one may expect to see any of the following four antibody profiles:

1. **IgM-/IgG-** No evidence of current infection. May be too early to detect antibody, therefore, follow-up sample needed for result comparison. In patients with otherwise healthy immune systems, the continued absence of both IgM and IgG antibodies to a specific viral agent is consistent with ongoing susceptibility to that agent and no evidence of prior infection or immunization.
2. **IgM+/IgG-** Possible primary infection or recent immunization.[‡] However, depending on the clinical history, a follow-up sample may be needed to rule out test non-specificity and/or a false positive IgM result. Failure to develop a subsequent detectable IgG antibody response is consistent with the latter two scenarios.
3. **IgM+/IgG+** Antibody response is consistent with recent infection and/or immunization.[‡]
4. **IgM-/IgG+** Presence of IgG antibodies in the absence of detectable IgM is consistent with past infection, prior vaccination, or ongoing infection (e.g., HIV).

[†]Adapted from Public Health Ontario materials:

http://www.publichealthontario.ca/en/eRepository/LAB_SD_044_Interpretation_viral_IgM_IgG_serology.pdf

[‡]MMR vaccination during the previous 6-45 days.

***Capillary Blood Specimen Collection** (may be collected from patients from whom it is difficult to collect venous blood)

1. Obtain supplies:
 - Two to three microcollection devices consisting of capillary tubes (heparinized tubes are acceptable) and a serum separator microtube; tubes are variable sizes and contain 50-200 µl each (consult local and/or state public health laboratories regarding acceptable collection devices)
 - Sterile safety lancet (fully automated devices recommended)
 - Biohazard container
 - Gloves
 - Alcohol wipes
 - Sterile gauze
 - Band-aid
2. Label each serum separator microtube with patient name (or other identifier), date of birth, and specimen collection date/time
 - If using capi-draw microcollection devices, make sure the capillary tube has not touched the serum separator gel; otherwise, capillary action may be compromised
3. Massage the puncture site to increase circulation and enrich blood flow
 - The heel is the recommended puncture site for infants 12 months of age or younger; the finger may be a suitable puncture site for children over 1 year of age
4. Clean the puncture site (heel or finger) well with alcohol; allow to dry.
5. Puncture the heel or the side of the pulp of the third or fourth finger with a sterile safety lancet.
6. Wipe away the first drop of blood with sterile gauze.
7. Touch the first capillary tube to subsequent free-flowing blood produced at the puncture site
 - Blood will fill the tube through capillary action
 - If blood flow is inadequate, gently massage the proximal portion and firmly press on the distal portion of the foot or finger (do not let blood run down the heel or finger); holding the microcollection device at a downward angle may improve collection results
8. Repeatedly touch additional capillary tubes to blood produced at the puncture site until 2-3 tubes are filled
 - Allow large blood droplets to form; avoid contact between the skin and capillary tube
 - A minimum of 100 µl of serum is required; however, it is recommended that 2-3 capillary tubes be filled even if the 100 µl volume requirement is met with the first tube
9. Express collected blood into the serum separator microtube by standing the microcollection device upright (capillary tube inserted in serum separator tube); after the capillary tube drains into the serum separator tube, lightly tap or shake the remaining blood out of the capillary tube.
10. Stop the bleeding with the gauze and cover the puncture site with a band-aid.
11. Remove the empty capillary tubes from the microcollection devices and discard the tubes and lancet in an appropriate biohazard container; cap the serum separator tube with the attached stopper.
12. Keep collected specimens at 4°C during transport (e.g., Styrofoam container with freezer packs).
13. Upon receipt at the laboratory, specimens must be microfuged before processing.