Measles Virus Isolation

(Updated 2009)

I. Background

Specimens for virus isolation should be taken at the same time that serum is obtained, since a delay in collection will reduce the chance of isolating the virus. While direct RT-PCR methodologies exist to detect and characterize measles viruses from clinical samples, successful virus isolation provides unlimited material for characterization of the virus and is not generally subject to the scrutiny given to RT-PCR results if contamination issues arise or results cannot be replicated.

The establishment of automated DNA sequencing techniques has allowed for rapid genetic characterization of a large number of wild-type strains of measles virus. Genetic analysis of the nucleotide sequence obtained from the virus can often identify the source of wild-type viruses and also may be important in distinguishing between wild-type strains and vaccine virus.

II. Samples for Virus Isolation (or RT-PCR Detection)

Throat (oropharyngeal) swabs (and/or nasopharyngeal [NP] swabs) are the preferred clinical samples for measles virus. Urine samples can be collected in addition to the throat swab. While throat swabs are generally more easily collected, processed and transported, urine samples provide an additional opportunity for successful isolation of virus and may prove superior to throat swabs if collection is delayed beyond about 5 days after onset of rash.

Respiratory specimens

Materials:

- Sterile swabs (Dacron or synthetic)
- Viral transport medium (VTM): Commercial sources of VTM are available. As an alternative, sterile phosphate buffered saline (PBS) or suitable isotonic solutions such as Hanks balanced salt solution or cell culture medium can be utilized. Addition of antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin) and a source of protein (either 2 % fetal bovine serum or 0.5% gelatin) is recommended to support viability of the virus.

Attempt to obtain the sample as soon as possible after onset of rash. Samples collected 5 days after rash onset have much lower chances for successful isolation of virus. Sterile swabs (Dacron or synthetic fiber) are used to absorb secretions and collect infected cells in the posterior nasal passages. The virus is extremely cell-associated, so the aim is to collect epithelial cells in the throat and nasal passages. Place both swabs in a tube containing 2–3 ml of VTM. If the specimen is to be shipped without freezing, the swab can be broken off and left in the tube of VTM.

Keep all specimens on wet ice or at 4° C and ship as soon as possible on cold packs. If immediate, cold shipment (within 48 hrs) cannot be arranged or is not convenient, nose and throat swabs should be removed from the VTM. Gently vortex or swirl the swab in the fluid and ream the swab against the side of the tube. These samples should be frozen and shipped at -70° C (dry ice).

Urine specimens

Although a respiratory sample (throat swab) is adequate as a viral sample, urine samples may serve as an additional source of virus. First morning voided specimens are ideal as these may contain greater numbers of sloughed cells, but urine collection at any time is adequate, more important is to collect as soon as measles is suspected. Collect 10–50 ml of urine in a urine specimen container. It is best to centrifuge the urine specimen as soon after collection as possible. After collection, keep the specimen cold (refrigerator or wet ice). If facilities are available, centrifuge the urine at 400 x *g* for 10 minutes at 4° C to pellet the sediment. Add 2 ml of VTM (above) or any cell culture medium (DMEM, EMEM, RPMI plus antibiotics) to resuspend the sediment and ship.

Preferably, specimens that have been centrifuged and resuspended should be frozen at -70° C and shipped on dry ice. If dry ice is not available, however, they can be stored at 4° C and shipped on cold packs. Avoid repeated freeze–thaw cycles. If centrifugation is not available, do not freeze the urine sample. The entire urine specimen should be stored at 4° C, and shipped to the lab on cold packs. Most urine collection cups are not leak-proof. Transfer the urine to sterile plastic centrifuge tubes.

III. Vero/SLAM Cell Line

The Vero/SLAM cell line is now recommended for routine isolation of measles. These cells are Vero cells which have been transfected with a plasmid encoding the gene for the human SLAM (signaling lymphocyte-activation molecule) protein (Ono et al., 2001). SLAM has been shown to be a receptor for both wild-type and laboratory-adapted strains of measles. The sensitivity of Vero/SLAM cells for isolation of measles virus is equivalent to that of B95a cells, and measles infection of Vero/SLAM cells produces the typical cytopathic effect that is readily observed, usually within 1–2 passages of the infected cells.

The advantage of the Vero/SLAM cells compared to B95a cells is that they are not persistently infected with Epstein-Barr virus, and therefore, are not considered as hazardous material. This provides a significant safety advantage for laboratory workers and greatly facilitates shipments. The disadvantage of the Vero/SLAM cells is that they must be cultured in medium containing the selective antibiotic G418 sulfate (Geneticin®) to retain SLAM expression. This does result in an increase in the cost of the tissue culture medium over that required for the B95a cell line. Contact the Measles Virus Laboratory for protocols and information on obtaining the Vero/SLAM cell line.

III. Shipping

Please attempt to notify CDC before virus isolates or clinical specimens are shipped. The CDC specimen submission form (Form No. 50.34; see Appendix 23) should include the mailing address of the submitting laboratory. If possible, supply a contact person and telephone number and e-mail address in case additional information is required.

Ship to:

Centers for Disease Control and Prevention
DASH Unit # 81—Att: Dr. William Bellini
1600 Clifton Rd.
Atlanta, GA 30333
Phone: 404-639-1156 or 404-639-3512
Fax: 404-639-4187 or 404-639-1516

IV. Reporting

Depending on the workload and the quality of the specimens, completion of the virus isolation and subsequent genetic analysis can take 1–3 weeks. The Measles Virus laboratory at CDC will send a report describing virus isolation and PCR results for all specimens received. Because PCR is more sensitive than virus isolation, all specimens from the U.S. will be screened using real-time RT-PCR to detect measles RNA. These results are generally available within 3 days to 1 week of receipt of sample, depending on current workload. Standard RT-PCR and genetic analysis may take an additional 1–2 weeks. Failure to detect virus by RT-PCR or by virus isolation should NOT be interpreted as evidence to rule out a clinically diagnosed measles case.