MEASLES VIRUS ISOLATION

(Updated September 2002)

I. Background

The availability of a sensitive cell line (B95a) for isolation of measles virus from clinical specimens and the establishment of automated DNA sequencing techniques have allowed for rapid genetic characterization of a large number of wild-type strains of measles virus. This database of sequence information now makes it possible to use molecular epidemiological techniques to identify the source of wild-type viruses and to rapidly differentiate between wild-type and vaccine strains. As progress is made toward elimination of measles in the U.S., it will be critical to examine virus isolates from as many outbreaks and isolated cases as possible in order to identify the source of the virus.

Virus isolation and genetic characterization can take several weeks to complete. Therefore, laboratory diagnosis of measles should always be based on detection of measles-specific IgM in serum. The IgM-capture EIA test can be completed in one day, and is available from virtually all state health department laboratories and commercial sources. 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. However, urine or respiratory specimens should not be substituted for serum specimens for measles diagnosis.

The Measles Virus Section, CDC is currently testing the possibility of using saliva in addition to serum to diagnose measles using the IgM-capture EIA. During a measles outbreak, if you believe that you can collect saliva in addition to serum to help test this method, please contact the Measles Virus Section (contact information provided below), who will provide you with saliva collection kits. An aliquot of serum should be sent with the saliva.

Direct PCR from clinical specimens has improved the ability to provide genotyping information in many instances when virus culture was unsuccessful. However, isolation of virus is always preferable since it is the most reliable, direct evidence for measles virus infection. Isolated viruses can be used for virological analyses and further genetic characterization. **Therefore, isolation of virus is always attempted first.**

Although viral RNA in the clinical sample may survive short periods of time at ambient temperature, the RNA is subject to degradation. The best results using direct PCR are from samples that have been collected within 1-5 days of rash and have been kept cold or frozen at
-70° C.

The possibility for direct PCR detection and sequencing from specimens which do not produce virus in culture means that this technique can play an important role in surveillance of measles virus. This is especially true when a sporadic suspected measles case occurs and no other specimens are available. For this reason, laboratories which perform measles virus isolation should save an aliquot of the original clinical material that can be tested later by PCR at CDC or in a laboratory that has this capability.

II. Protocols for isolation of measles virus

Specimens for virus isolation should be obtained as soon as possible after the onset of rash. Always collect a urine specimen and, if possible, attempt to collect a respiratory specimen. Protocols are described below.

Respiratory Specimens

Materials:

- sterile swabs
- sterile saline
- 3 ml aliquots of viral transport medium (VTM: sterile PBS or suitable isotonic solution such as Hank's BSS, etc. containing antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin) and either 2 % fetal bovine serum or 0.5% gelatin in 15 ml polycarbonate or polystyrene centrifuge tubes
- 5 ml plastic syringes
- plastic aspirators or 30 ml syringe
- Styrofoam shipping containers

Instructions:

1. 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.
2. The preferred respiratory specimen is a nasal wash (nasopharyngeal aspirate) using a syringe attached to a small piece of plastic tubing and about 3 ml of sterile saline. After placing saline in the nose, aspirate as much of the material as possible and add to the centrifuge tube containing the VTM. Rinse the syringe and collection tubing into the VTM.
3. Alternatively, sterile swabs can be used to wipe the nose and throat. Place both swabs in a tube containing 2-3 ml of VTM. The virus is extremely cell-associated, so attempt to swab the throat and nasal passages to collect epithelial cells. If the specimen is to be shipped without freezing, the swab can be left in the tube of VTM.
4. Keep all specimens on wet ice or at 4° C and ship as soon as possible on cold packs (see address below).

   Note: 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

The best results for virus recovery are from urine samples collected within 5 days of rash onset, though urines collected up to 16 days after rash onset will be accepted. Virus has been isolated from the urine for up to one week after the onset of rash. In addition, we will accept urine collected from close contacts of measles cases (e.g., household contacts). First morning voided specimens are ideal, but any urine collection is adequate. 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 cool (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. Resuspend the sediment in 2 ml of VTM (above) or any cell culture medium (DMEM, EMEM, RPMI plus antibiotics) 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.

Virus isolation

An Epstein-Barr virus-transformed, B lymphoblastoid cell line, B95a, is the preferred cell line for primary isolation of measles virus (Kobune et al., 1990, J Virol. 64:700-705). Laboratorians should note that this cell line should be handled as an infectious cell line capable of yielding Epstein-Barr virus. Infected B95a cells show syncytium formation and giant-cell cytopathogenic effect (CPE) sometimes as early as 24 hours after inoculation. Isolation-attempt cultures should be followed for 7-8 days with subsequent passages 2-3 times before ruling out isolation of measles virus. B95-8 cells are available from the American Type Culture Collection (# CRL 1612). When cultured in Dulbecco's Modified Minimum Essential Medium (DMEM), these cells will adhere to the surface of the culture vessel and the adherent cells are referred to as B95a.

The cells grow lightly attached to the culture surface when grown in DMEM supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 0.25 µg/ml amphotericin (fungizone), and fetal bovine serum. Cell growth is sustained by adding 5-10% fetal bovine serum (FBS). FBS is used at a 2% concentration for cell maintenance during viral isolation. Grow cells in a moist CO_2 incubator at 37° C. Cell stocks can be frozen using standard cryoprotection medium.

The B95a cells can be passaged by briefly treating the cell monolayers with 0.05% trypsin-EDTA to release cells from tissue culture surface. Be careful not to over trypsinize. Neutralize trypsin by adding DMEM containing 10% FBS. Usually the cells from a single monolayer culture can be split 1:3. One will notice that more cells tend to become "floaters", growing in clumps suspended in the medium as the cell density increases. These cells are viable and can be passaged by gently pipetting to break up the clumps then replating to a lower cell density.

Cells can be transported at room temperature in a T-75 or T-25 tissue culture flask with additional medium added to help keep cells attached. After shipment, look at the cell sheet. If many cells are free-floating, a light spin of the medium will recover cells which can be added back to the flask (or to another flask for passage). Add 30-35 mls of the medium back to the flask for maintenance.

<u>Inoculation of specimens for measles isolation</u>

Please remember to save some of the original clinical specimen. This material can be used for a second isolation attempt if problems occur with the first as well as provide a specimen for PCR analysis. We do not routinely filter specimens before inoculation. However, if a culture becomes heavily contaminated, the specimen can

be diluted in DMEM and passed though a 0.45uM nitrocellulose filter and used to inoculate fresh B95a cells.

1. Prepare T-25 flasks for inoculation. Cells should be at 75-85% confluency when the specimen is inoculated.

2. For inoculation, decant medium, add 1-1.5 ml DMEM with 2X antibiotics and 0.2 to 1.0 ml of clinical specimen depending on concentration (original volume of urine). Usually 0.2ml is sufficient for respiratory samples.

3. Incubate at 37 C. for 1 hour. Specimens for virus isolation are sometimes toxic to the cell culture. Check for toxic effects after 15 minutes. Addition of extra medium can usually dilute out the toxic effect of the inoculum.

4. After the 1 hour incubation, add medium to sufficient volume (10-12 for a T-25 flask) with DMEM containing 2% FBS and 2X antibiotics.

5. Passage cells by splitting @ 1:2 after about 2 days. Check for CPE daily.

6. Attempt at least three passages before discarding the cell culture. Do not discard remaining original clinical specimens as they may still be used for PCR analysis.

7. If CPE is visible, continue to feed the cells until the CPE becomes extensive. It may be necessary to passage the cells one time to allow the CPE to progress. When CPE is maximal, pellet cells, resuspend the pellet in 1 ml of DMEM and freeze at -70° C.

Infected cells can be pelleted, resuspended in a small volume of DMEM and frozen at -70° C. before shipping on dry ice.

III. Shipping

Viral isolates (not clinical specimens) arriving from overseas will require CDC and USDA Import Permits. Please call numbers below to obtain a permit.

If Federal Express is available, contact the CDC to arrange pre-paid transport. If not, CDC will be able to reimburse for shipping costs.

Ship to:

Dr. William Bellini
Measles Virus Section, REVB, C-22
DASH Group #81
Centers for Disease Control and Prevention
1600 Clifton Rd.
Atlanta, GA 30333 USA

Phone: 404-639-3512 or 404-639-4183
Fax: 404-639-4187
E-mail: wjb2@cdc.gov

Please FAX CDC with any questions or to arrange shipping.

IV. Reporting

Please attempt to notify CDC before specimens are shipped. The CDC form 50.34 should include the mailing address of the submitting laboratory. If possible, supply a contact person and telephone number in case additional information is required. Depending on the workload and the quality of the specimens, virus isolation and subsequent genetic analysis can take several weeks. CDC will send a report describing virus isolation and PCR results for all specimens received from within the U.S. However, detection of measles infection is based on serology results.

Because virus isolation and sequence analysis are labor intensive, isolation will not be attempted from all specimens. For example, if many specimens from a single outbreak are submitted, virus isolation attempts will be discontinued after several isolates have been made from the outbreak. Our goal is to obtain at least one isolate from each chain of transmission in the U.S. Because PCR is more sensitive than virus isolation, all specimens from the U.S. will be screened using PCR to detect measles RNA. If no virus isolate is obtained from a chain of transmission, sequence data may be obtained from PCR products directly amplified from clinical material.