Collection of Specimens for Detection of Measles Virus RNA by PCR

1. Background

Measles virus can be assigned to one of 8 different clades and further subtyped in to 22 genotypes using PCR and sequence analysis of measles virus (MV) RNA. Measles genotyping is of real practical use in among other things distinguishing importation from ongoing local measles transmission in addition to distinguishing vaccine from wild type virus. The more we do, the more complete a picture we can build up, both in Australia & internationally.

The use of reverse transcriptase-polymerase chain reaction (RT-PCR) to detect measles virus RNA in various clinical specimens, including combined nose and throat swabs (NTS), nasopharyngeal aspirates (NPA), urine, CSF and blood has been described by several groups.

In 1999 the Victorian Infectious Diseases Reference Laboratory sought to determine the most appropriate specimen type and optimal sampling time post the onset of rash for the recovery of measles virus RNA *(Riddell et al J Clin Microbiol 2001 39(1): 375-376.)*

2. Specimen collection protocol

Identify patient with clinical symptoms of measles infection. (ie morbilliform rash, cough and fever at rash onset) within a few days (<5 days) after rash onset.

Virus is more likely to be present at the time of rash onset or within the first week after rash onset. Patients who have had rash onset within the last week should be sampled preferentially.

The specimen of choice is a nose swab and a throat swab from each patient combined together in a bottle of viral transport medium (VTM). Viral culture supernatant, positive for MV growth, can also be sent for molecular analysis.

Please follow the instructions below to optimise chance of obtaining MV RNA from the patient.

(i) Nose Swab

1. Insert a **STERILE DRY SWAB** into the nasal cavity of the patient and wipe the swab along the sides of the nasal passage. Place cotton tip into vial of viral transport medium, keep as sterile as possible.

2. Place the swab with the cotton tip end into the small bijou bottle (or leakproof screw top tube with 3-5 mls volume) of VTM. KEEP TIP AND MEDIUM AS STERILE AS POSSIBLE.
3. Break off (or cut off) the shaft of the swab at the top of the bottle so that the tip remains in the VTM and the lid can be screwed tightly shut.
4. Please ensure the lid is on properly to prevent leakage.
5. Label the VTM bottle with the patients ID, age, swab site (nose, throat or nose & throat) number of days post rash onset (if known). Swab tips (break or cut off shaft with scissors) from nose and throat of the SAME PATIENT can be combined into the same bijou bottle

(ii) Throat Swab

1. Insert a dry **STERILE DRY SWAB** into the mouth and wipe along the **back of the throat.** It is important to take the sample from the back of the throat and NOT the sides of the mouth or cheek cavity as the virus is more likely to be found in the cells at the back of the throat.
2. Follow steps 2-5 above.

(iii) Additional requirements for Specimen Collection

1. Sterile dry swabs must be sealed and sterile before use. Recommend use of a fresh sterile swab for each site to be sampled.
2. Viral transport medium: (sterile, usually consisting of Hanks balanced salt solution and antibiotics to reduce bacterial contamination growth.) These should be available from the in- country reference laboratory who are doing viral culture for poliovirus. If they do not have VTM, cell culture medium such as MEM would suffice. Sterile medium should be asceptically dispensed into sterile leakproof bottles or tubes of 3-5 ml volume.
3. Dry swabs or swabs placed into gel type transport media **ARE NOT SUITABLE** for recovery of viral RNA and are not recommended.

3. Specimen Transport

(i) Place the specimen at 4ºC (in the fridge) DO NOT FREEZE.

(ii) Samples should be transported to VIDRL, 10 Wreckyn Street, North Melbourne, VIC 3051, Australia ph (613) 9342 2600, fax (613) 9342 2666 on "wet ice"- DO NOT FREEZE as this may harm the MV-RNA and reduce the chances of a positive test result (please see attached shipping instructions).

(iii) Please contact the Measles Reference Laboratory at VIDRL (doris.chibo@mh.org.au or mike.catton@mh.org.au) when ready to ship specimens.