Vero/SLAM Cells for Isolation of Measles Virus

Introduction

The availability of a sensitive cell line for isolation of measles virus from clinical specimens and establishment of RT-PCR and 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 differentiate between wild type and vaccine strains.

The Vero/SLAM cell line has been recommended for use in the WHO laboratory network. These cells are Vero cells that have been transfected with a plasmid encoding the gene for the human SLAM molecule (Ono, et al., 2001). SLAM has been shown to be a receptor for both wild-type and laboratory-adapted strains of measles. Testing conducted to date, indicates that the sensitivity of Vero/SLAM cells for isolation of measles virus is equivalent to that of B95a cells. The advantage to the Vero/SLAM cells is that they are not persistently infected with virus, and therefore, are not considered as hazardous material like B95a cells. This provides a significant safety advantage for laboratorians and greatly facilitates international shipments. The disadvantage of the Vero/SLAM cells is that they must be cultured in medium containing geneticin to retain SLAM expression. This increases the cost of the tissue culture medium. Protocols for culture of Vero/SLAM cell are included at the end of this section.

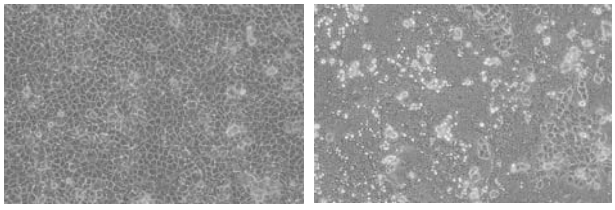

Figure (above) shows CPE from wild-type measles virus in Vero/SLAM cells (photo on right). Picture was taken 48 hours after inoculation. Uninfected Vero/SLAM cells are shown in the photo on the left.

Vero/SLAM Cells: Overview

Network laboratories should only accept Vero/SLAM cells from a WHO-approved source (Regional Reference Lab or Global Specialized Lab). Upon receipt, the cells should be passaged in medium containing 400ug/ml geneticin as described below. Laboratories should passage the cells 2 to 4 times in the presence of geneticin to prepare a sufficient number of cell culture vessels to prepare 20-50 vials for liquid N2 storage.

Note

Passage of Vero/SLAM cells for routine virus isolation: To prepare cells for virus isolation procedures, Vero/SLAM cells can be recovered from liquid N2 and passaged up to 15 times in medium without geneticin. These cells should be used for virus isolation attempts only and should be discarded after 15 passages. Even with addition of geneticin to the medium, the cells should not be passaged forward beyond about 20-30 passages, as with the standard Vero cell line. Cells that have been passaged without geneticin in the medium should never be used to prepare cell stocks for liquid N2 storage or shipped to another network laboratory for use in virus isolation.

Materials:

1.Dulbecco's Modified Eagle Medium (DMEM):
with 4,500 mg/L D-glucose (high glucose)
with L-glutamine
without sodium pyruvate
2. Antibiotics (100X)
10,000 units/ml penicillin G
10,000 ug/ml streptomycin sulfate
both in 0.85% saline
3. Trypsin-EDTA
0.05% Trypsin (porcine pancreas)
0.53 mM EDTA
in HBSS without Ca++ and Mg++

4. Fetal Bovine Serum (Defined)

5. Geneticin (G418), is available in solution (50mg/ml) or powder.

Procedure

1. Prepare DMEM by adding appropriate amount of penicillin/streptomycin solution and geneticin (G) to a final concentration of 400 ug/ml of DMEM. (If using the geneticin solution prepared as 50mg/ml, 1 ml is added to 125 ml of medium.) Use this medium (DMEM + G) for expanding culture for freezer stocks of Vero/SLAM cells.

2. When confluent, Vero/SLAM cells can be passaged by trypsinization as with any adherent cell line. We usually maintain cells in 75 cm^2 flasks, but volumes can be adjusted for larger or smaller vessels.

3. Wash cell monolayer 1X with 3-5 ml pre-warmed trypsin solution (or warm PBS) for about 30 sec to 1 minute. Discard wash medium. Add 5 ml pre-warmed trypsin solution and place flask in 37°C incubator. Observe flask every few minutes and to see if cells have begun to detach.

4. Resuspend cells in 5ml DMEM (see note regarding addition of geneticin above) plus 10% FBS and pipette up and down to break up clumps. Seed cells into flasks containing DMEM plus 10% FBS. Split ratios of up to 1:5 are acceptable. A 1:2 or 1:3 split usually will produce monolayers of sufficient density for virus isolation after 24 hours incubation.

5. For inoculations, virus adsorption and subsequent incubations should be in DMEM plus 2% FBS. For inoculation of a T-25 flask, (= virus passage #1), decant growth medium, add 5 ml of DMEM plus 2% FBS and 0.5- 1ml of specimen. Incubate at 37^o C for 1 hour and observe the cells under the microscope to ascertain if the sample was toxic to the cells (rounding of cells, cells floating).

6. Inoculated cells should be observed for CPE on a daily basis. Passage the infected Vero/SLAM cells by trypsinization every 4-5 days at a 1:2 or 1:3 split ratios. When CPE is visible, continue to feed the cells (replace the medium with fresh DMEM with 2% FBS, if necessary) until the CPE becomes extensive. It may be necessary to passage the cells 1-2 times to allow the infection to spread before cells become overgrown. When CPE is visible over at least 50-75% of the cell layer, the virus has reached a suitable titer for viral stock.

7. To prepare a viral stock, scrape the cells into the medium with a cell scraper. Mix and distribute to 4-6 vials and save remainder for IFA**. Always prepare viral stocks if isolation is successful.**

Preparation of stock (frozen) VERO/SLAM cells

It is important to prepare multiple frozen stock vials of Vero/SLAM cells as soon as they are available in the laboratory. Cells can be frozen using any standard cryopreservation technique. Commercial freezing media is available or the reagents described below should be adequate.

Cells should be removed from the flask by trypsinization (take care not to over-trypsinize). All of the cells from a 150 cm^2 flask should be placed in 10 ml DMEM plus 10% FBS and pelleted by centrifugation at 1500 rpm for 10 min at 4^oC. To the cell pellet add 5 ml DMEM containing 30% FBS and resuspend the cells by vortexing. Add an equal volume (5 ml) of DMEM and 15% DMSO (reagent grade). Pipette gently up and down briefly and dispense 1 ml into 10 plastic cryovials. The vials should be cooled slowly using a programmed cell freezer or a commercial product designed for gradual temperature reduction. Store vials in liquid nitrogen.

Use of trade names or commercial sources is for identification only and does not imply endorsement by CDC.