TML/MSH Microbiology Department Policy & Procedure Manual	Policy # MI/VIR/16/09/v01	Page 1 of 2	
Section: Virology Manual	Subject Title: Appendix IX		
	Trypsinization & Maintenance of Monolayer		
	Cell Cultures		
Issued by: LABORATORY MANAGER	Original Date: March 14, 2001		
Approved by: Laboratory Director	Revision Date:		

## Appendix IX

## TRYPSINIZATION AND MAINTENANCE OF MONOLAYER CELL CULTURES

Monolayer cell cultures may be kept active and available for seeding of tubes, vials, dishes and plates. A constant supply of cell cultures can be maintained by routine subpassage of cell lines to new flasks. (Generally research use only.)

- 1. Discard medium from the cell culture flask (125 cm<sup>2</sup>). Rinse monolayer with 15 mL of Hank's Balanced Salt Solution and discard.
- 2. Add 5 mL of trypsin EDTA mixture (pre-warmed to 36°C) to flask.
- 3. Incubate the culture flask for 3 minutes (no more than 5 minutes) at 36°C. Observe after 3 minutes to see whether the cell sheet is breaking loose from the flask surface. Tap flask sharply against palm of hand to aid in loosening tissue.
- 4. When tissue has loosened completely, add 15 mL of pre-warmed growth medium into the flask.
- 5. Mix cells by drawing cells and fluid up and down in a pipette.
- 6. Adjust the volume to 90 mL with growth medium.
- 7. Aliquot 30 mL of suspended cell mixture into at least one new 125 cm<sup>2</sup> culture flask. Aliquot the remaining to shell vials at 1.5 mL each or tube culture (16 x 125 mm) at 2 mL each.
- 8. Incubate the culture flasks, shell vials or tube cultures at 36°C.
- 9. Observe daily for growth of cells (3 5 days) and for change in pH of medium. If the medium becomes acidic or basic, replace it with fresh growth medium.
- 10. Replace growth medium with maintenance medium when a confluent monolayer is obtained (usually after 2 3 days).

PROCEDURE MANUAL

TORONTO MEDICAL LABORATORIES / MOUNT SINAI HOSPITAL MICROBIOLOGY DEPARTMENT

TML/MSH Microbiology Department	Policy # MI/VIR/16/09/v01	Page 2 of 2
Policy & Procedure Manual		
Virology Manual		

11. Replace with fresh maintenance medium once a week.

**Note:** Freeze first passage of cell culture whenever a new shipment is received. (See Appendix VIII).