

TML/MSH Microbiology Department Policy & Procedure Manual	Policy # MI/VIR/11/v02	Page 1 of 4
Section: Virology Manual	Subject Title: Sterile Fluids	
Issued by: LABORATORY MANAGER	Original Date: March 14, 2001	
Approved by: Laboratory Director	Revision Date: October 10, 2003	

STERILE FLUIDS

I. Introduction

Viruses which may be isolated from pleural, peritoneal and pericardial fluid are enteroviruses (Coxsackie Group A and B, poliovirus, and echoviruses). Viral isolation from joint fluid is exceptionally rare. Amniotic fluid will be set up for cytomegalovirus shell vial and tube culture. Specimens with requests for HSV or VZV also necessitate inoculation of shell vials. Refer to Appendix XV (Virus isolation and isolation and identification) to ensure the appropriate media are inoculated.

II. Collection and Transport

Specimens should be collected in a clean, sterile container and sent to the laboratory as soon as possible. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

III. Procedure

A. Processing of Specimens:

Specimens should be set up as soon as possible or stored at 4°C for up to 72 hours. All processed specimens should be stored at -70°C after inoculation. Bloody fluids should be spun at 2000 rpm (700xg) for 10 minutes and the supernate used for inoculation.

Refer to Appendix II for Shell Vial inoculation and staining.
Refer to Appendix III for Tube Culture inoculation.

B. Direct Examination:

Not done.

C. Isolation and Identification:

Specimens	Method	Cell Line^a	Incubation at 36°C	Stain used/Read
Amniotic fluid	Shell Vial	MRC-5	2 days	CMV-IE
		MRC-5 (if requested)	1 day	HSV1
		MRC-5 (if requested)	1 day	HSV2
		MRC-5 (if requested)	2 days	VZV
	Tube	CMK	14 days	3 x Reads/week
		HFF	14 days	3 x Reads/week
		RD ^b	7 days	3 x Reads/week
Pleural , Peritoneal , Pericardial, other fluids	Shell Vial	MRC-5 (if requested)	2 days	CMV-IE
		MRC-5 (if requested)	1 day	HSV1
		MRC-5 (if requested)	1 day	HSV2
		MRC-5 (if requested)	2 days	VZV
	Tube	CMK	14 days	3 x Reads/week
		HFF	14 days	3 x Reads/week
		RD ^b	7 days	3 x Reads/week

^aMRC-5 = Human Fibroblast cells; CMK = Cynomolgus Monkey Kidney; HFF= Human Foreskin Fibroblast cells

^bRD = Rhabdomyosarcoma cells are inoculated from May to November (and from December to April if enterovirus is specifically requested).

D. Interpretation and Processing of Cultures:

- a) For shell vial procedure:
- i) For amniotic fluid (or other fetal specimens), always set up CMV shell vial. Fix and stain after 2 days (or next working day).
 - ii) For other fluids, set up CMV, HSV1, HSV2 and VZV if requested. Fix and stain as indicated.

See Appendix II for detailed shell vial procedure.

TML/MSH Microbiology Department Policy & Procedure Manual	Policy # MI/VIR/11/v02	Page 3 of 4
Virology Manual		

- b) **Tube Cultures:**
Tube cultures should be examined a minimum of 3x per week for Cytopathic effect (CPE). Any culture demonstrating 2+ or more CPE should be confirmed using appropriate monoclonal antibodies immunofluorescent staining (Refer to Appendices IV and V). If positive, record in freezer program and freeze the cells and supernate (Refer to Appendix X and XII).
- c) Any culture demonstrating CPE for which a virus cannot be detected using monoclonal antibodies or other in-house methods and toxicity has been ruled out (see below) should be referred to the Public Health Laboratory (PHL) for electron microscopy and further work-up. Consult the charge/senior technologist or medical microbiologist.
- d) **Culture Toxicity:** If toxicity is suspected in a tube culture (rounding of cells, sloughing of cells, granular cytoplasm of cells or unusual CPE), the cells should be scraped and appropriate monoclonal antibody staining performed. Negative stain results indicate the need for a passage. Scrape cells and add 0.2 ml of these scraped cells to a fresh tube containing 2 ml of media (1:10 dilution) and proceed again with tube culture method. (Appendix III). If toxicity or CPE persists, refer to the charge/senior technologist for review.
- e) **Contaminated Culture:** If the tube culture is visibly contaminated and uninterpretable, replant the specimen.
Refer to Appendix II for Shell Vial staining interpretation.
Refer to Appendix III for Tube culture reading and interpretation.

IV. Reporting Results

Shell Vial: Negative Report: “Negative for _____ virus.”

 Positive Report*: “POSITIVE for _____ virus.”

Tube Culture: Negative Report: “No virus isolated”

 Positive Report*: “_____ virus isolated.”

Toxicity Report: "Virology Tube Culture: Specimen toxic to cell culture.

Contaminated Report: "Virology Tube Culture: Specimen is heavily contaminated with bacteria and/or fungus. Unable to perform Virology Tube Culture.

*** Telephone all positive results to ward/ordering physician.**

TML/MSH Microbiology Department Policy & Procedure Manual	Policy # MI/VIR/11/v02	Page 4 of 4
Virology Manual		

* When entering positive results in the Lab Information System (LIS), enter the virus name in the isolate window (under F7). See LIS Manual for entering results.

V. Reference

1. Gleaves, Curt A. et al. Cumitech 15A “Lab Diagnosis of Viral Infections”. American Society for Microbiology, August 1994.