FAECES/RECTAL

I. Introduction

Faecal specimens may be submitted for the detection of viruses from patients with gastroenteritis, aseptic meningitis, viral encephalitis, or exanthem type rashes. Most cases of viral gastroenteritis are caused by viruses which do not propagate in cell culture. Faecal specimens from such cases can be examined directly by antigen detection methods for rotavirus, Norwalk-like agents and adenovirus type 40 and 41 or by electron microscopy (EM). Aseptic meningitis may be due to enteroviruses and many can propagate in routine cell cultures. Herpes simplex virus is commonly implicated in cases of viral encephalitis and is not isolated from faecal specimens. Enteroviruses may cause exanthem type rashes and stool may be cultured for these viruses.

II. Collection and Transport

Stool is collected in a dry sterile container. Although rectal swab is less satisfactory than stool specimen, it will be processed if a stool sample cannot be obtained. Rectal swab should be placed in viral transport media. Specimens should be transported to the laboratory as soon as possible. If a delay in transport or processing is anticipated, keep the specimen at 4^{o}C.

III. Procedure

A. Processing of Specimens:

a. **Stool**

i. Stool specimens will be processed according to the clinical information provided. Specimens from patients with gastroenteritis or diarrhea will be referred to the Public Health Laboratory (PHL) for rotavirus, Norwalk-like agents or enteric adenoviruses.

ii. Specimens from patients with rashes, aseptic meningitis or other symptoms involving the central nervous system will be processed in-house for enteroviruses.

iii. Specimens received without any clinical information will be referred to PHL for Electron Microscopy.

Stool specimens being processed in-house should be processed as follows:

a) Transfer 1.5 to 2.0 g of stool to a clean, sterile 50 ml tube containing 4 to 6 sterile glass beads.

b) Add 15 ml of maintenance medium.

c) Vortex for 30 to 60 seconds.

d) Centrifuge at 3000 rpm (700 x g) for 15 minutes.

e) Transfer 2 ml supernatant to a sterile freezer vial. Add 4 drops gentamicin and 2 drops fungizone to a final concentration of 100 μg/ml and 10 μg/ml respectively.

f) Allow to stand at room temperature for 10 minutes.

ii) **Rectal swab**

a) Vortex the swab in transport medium for 30 seconds. Remove excess fluid from the swab and discard the swab.

b) Transfer maintenance medium to a conical bottom centrifuge tube and centrifuge at 3000 rpm (700 x g) for 15 minutes.

c) Transfer 2 ml supernate to a sterile freezer vial. Add 4 drop gentamicin and 2 drops fungizone to a final concentration of 100 mg and 10 mg respectively.

d) Allow to stand at room temperature for 10 minutes.

B. Direct Examination:

If electron microscopy is requested or symptoms of gastroenteritis reported, forward specimen to PHL. Otherwise, direct examination is not indicated.

C. Isolation and Identification:

Method	**Cell Lines[a]**	**Incubation at 36°C**	**Stain used/Read**
Shell Vial	MRC-5 (only if requested)	2 days	CMV-IE
Tube	CMK RD	14 days 7 days	3x Reads/week 3x Reads/week

[a]MRC-5 = Human diploid fibroblast cells; CMK = Cynomolgus Monkey Kidney cells; RD = Rhabdomyosarcoma cells

D. Interpretation and Processing of Cultures:

a) For shell vial procedure:

i) If CMV is requested, fix and stain after 2 days (or next working day) .

See Appendix II for detailed shell vial procedure.

b) Tube cultures should be examined a minimum of 3x per week for Cytopathic effect (CPE). Any culture demonstrating 2+ to 3+ CPE should be confirmed using appropriate monoclonal antibodies and 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.

i) **Contaminated Culture:** If the tube culture is visibly contaminated and uninterpretable, issue a report indicating contamination.

IV. Reporting Results

Tube Culture:

Negative Report:	"No virus isolated"
Positive* Report:	"_____ virus isolated."
Toxicity Report:	" Specimen toxic to cell culture."
Contaminated Report:	"Specimen is heavily contaminated with bacteria and/or fungus. Unable to perform Virology Tube Culture."

*** Telephone all positive results to ward/ordering physician.**
***** 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. Isenberg, HO. 1992. Clinical Microbiology Procedure Handbook, Vol. 2. American Society of Microbiology.