CEREBRAL SPINAL FLUID

I. Introduction

Cerebral Spinal Fluid (CSF) will be routinely cultured for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV) and enteroviruses (coxsackie, echo and polio virus). PCR for these viruses will be performed if specifically requested. Other viruses that may be isolated from CSF include mumps virus and adenovirus. Requests for Rubella virus, JC virus, BK virus and arbovirus should be referred to the Public Health Laboratory (PHL).

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, the specimen should be kept at 4^{o}C until processed. If a delay of more than 72 hours is anticipated, the specimen should be frozen at –70^{o}C. Avoid repeated freeze-thaw cycles.

III. Procedure

A. Processing of Specimens:

Specimens should be set up as soon as possible after arriving in virology laboratory. After processing, an aliquot of up to 2 mL of the left-over specimen should be stored at -70°C in a cryovial.

a. If the specimen is requested for PCR and viral culture and
   i. The amount of specimen is <0.5 mL, perform PCR only.
   ii. The amount of specimen is between 0.5-1.0 mL, perform PCR and tube cultures.
   iii. The amount of specimen is >1.0 mL, perform PCR, tube culture and shell vial assay.

b. If specimen is requested for viral culture only and
   iv. The amount of specimen is <0.5mL, perform the culture only.
   v. If the amount of specimen is >0.5mL, perform tube culture and shell vial assay.

c. If PCR is requested, aliquot 0.2-1 mL first (freeze aliquot unless PCR can be performed immediately) before proceeding.

d. CSF specimens will be inoculated directly into shell vials and tube cultures without further processing.

B. Direct Examination:

Method	Virus(es)	Location
PCR	HSV / CMV / EBV/VZV	Research Lab
PCR	HHV6,7,8	Hospital for Sick Children
PCR	Adenovirus	Research Lab
RT-PCR*	Enteroviruses	Research Lab
RT-PCR*	West Nile virus	In-house

*RT-PCR = Reverse Transcription PCR using Qiagen Isolation Kit, RealArt reagents and Roche LightCycler. CMV= cytomegalovirus; EBV= Epstein-Barr virus; HSV= Herpes simplex virus; VZV= Varicella-zoster virus; HHV6,7,8= Human herpes virus types 6,7,8

Note: PCR and RT-PCR are performed only upon request and only for those viruses requested.

C. Isolation and Identification:

Method	**Cell Line[a]**	**Incubation at 36°C**	**Stain used/Read**
Shell Vial	MRC-5 MRC-5 (if requested) MRC-5 (if requested)	2 days 1 day 2 days	CMV-IE HSV-bivalent VZV
Tube	CMK HFF RD[b]	14 days 14 days 7 days	3 x Reads/week 3 x Reads/week 3 x Reads/week

[a]MRC-5 = Human Fibroblast cells; HEp 2 = Human Laryngeal Epidermoid Carcinoma Cells; CMK = Cynomolgus Monkey Kidney;
[b] RD = 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) Shell vial procedure:

i) For CMV, fix and stain 1 shell vial after 2 days (or next working day).
ii) If HSV is requested, fix and stain 1 shell vial after 1 day (or next working day).
iii) If VZV is requested, fix and stain 1 shell vial 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+ 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.

IV. Reporting Results

PCR: Negative Report: "Negative for _____virus. This is a research test"

Positive Report*: "POSITIVE for _____ virus. This is a research test."

Indeterminate Report: "Indeterminate by PCR. This is a research test"

Shell vial: Negative Report: "Negative for _____ virus."

Positive Report*: "POSITIVE for _____ virus."

Tube Culture: Negative Report: "No virus isolated," OR "See Shell Vial Assay."

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. References

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

2. Collier L, Balows A, Sussman M. Topley's and Wilson's Microbiology and Microbial Infections. Volume 1, Ninth Ed. 1998.