OCULAR SPECIMENS

I. Introduction

Viral infections of the eye (conjunctivitis, corneal ulcers, etc) are usually due to herpes simplex virus (HSV), varicella-zoster virus (VZV) and adenoviruses. Ulcerative lesions are usually due to HSV and VZV. For vitreous fluid, the two most common viruses isolated are cytomegalovirus (CMV) and varicella-zoster (VZV). Other viruses which may cause conjunctivitis, such as enteroviruses, will be looked for only if specifically requested.

II. Collection and Transport

Specimens should be collected using a clean, sterile swab and gently swabbing the conjunctiva or ulcerative lesion. Place the swab in viral transport medium and send to the laboratory as soon as possible. Vitreous fluid should be collected in a clean, sterile container. 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 immediately or stored at -70°C. Vortex patient sample in transport medium for 30 seconds. Remove excess fluid from the swab and discard the swab. Refer to Appendices II and III for Shell Vial and Tube culture inoculation, respectively.

B. Direct Examination:

If requested, prepare one double-well cytospin. Stain one well with HSV bivalent 1/2 antibody and the other with VZV monoclonal antibody.

Refer to Appendix V for immunofluorescent staining technique.

C. Isolation and Identification:

Specimen	**Method**	**Cell Line[a]**	**Incubation at 36°C**	**Stain[b] used/Read**
Conjunctiva, cornea	Shell Vial	MRC-5 MRC-5	1 day 2 days	HSV-bivalent VZV
	Tube	HEp2 CMK (if enterovirus is requested) RD (if enterovirus is requested)	10 days 14 days 7 days	3 x Reads/week 3 x Reads/week 3 x Reads/week
Vitreous fluid	Shell Vial	MRC-5 MRC-5 MRC-5	1 day 2 days 2 days	HSV-bivalent VZV CMV-IE
	Tube	HEp2 CMK (if enterovirus is requested) RD (if enterovirus is requested)	10 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 cells; RD = Rhabdomyosarcoma cells
[b]HSVbivalent= Monoclonal DFA stain for HSV1 and HSV2
[b]VZV= Monoclonal DFA stain for Varicella zoster virus
[b]CMV-IE= Monoclonal IFA stain for Cytomagalovirus Immediate Early antigen

D. Interpretation and Processing of Cultures:

a. For shell vial procedure:

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+ or more 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.

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

IV. Reporting Results

Direct:	Negative Report:	"Negative for _____ virus."
	Positive Report*:	"POSITIVE for _____virus."
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**.
* 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.