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Section: Virology Manual	Subject Title: Throat / Nasopharyngeal / Nasal Swabs (Symptomatic)	
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THROAT/NASOPHARYNGEAL/NASAL SWABS (SYMPTOMATIC)

I. Introduction

These specimens are set up for routine isolation of respiratory viruses (Influenza A and B, Parainfluenza 1, 2, and 3, Respiratory Syncytial Virus, and Adenovirus). Direct antigen detection will be performed during the winter months (November to April, inclusive) and upon request only from May to October. Additional cell lines or shell vials may be required if specific viruses are requested. For other viruses requested, refer to Appendix XV (Isolation and Identification) to ensure that the appropriate media is inoculated.

II. Collection And Transport

Specimens should be obtained as early in the patient's illness as possible. Throat, nasopharyngeal or nasal swabs should be collected using a clean, sterile swab and placed into viral transport medium and transported to the laboratory as soon as possible. If a delay in transport or processing for up to 72 hours is anticipated, keep the specimen at 4°C.

III. Procedure

- A. Processing of Specimen:
Specimens can be kept refrigerated for up to 72 hours. Vortex patient sample in transport medium for 30 seconds. Remove excess fluid from the swab and discard the swab. Specimen in the transport medium can be transferred to a 2 mL cryovial. After preparation of slide (if needed) and inoculation of cultures, store cryovial at -70°C for 6 months. The original specimen container should be kept at 4°C for 1 week.

- B. Direct Examination:
Prepare one double-well cytospin slide for immunofluorescent staining using the SimulFluor influenza A/B monoclonal antibody for one well and SimulFluor respiratory virus screen monoclonal antibody for the second well. Refer to Appendix VI for SimulFluor Screen Protocol.

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NB: If a specific respiratory virus(es) is/are requested, prepare appropriate number of additional slides and stain using specific individual monoclonal antibodies.

Complete direct smear results the same day for specimens received in the virology section by 14:00 hours. For specimens received between 14:00 – 15:30 hours, processing and smear preparation should be completed, however, staining, reading and reporting results may be completed the next day (except on Fridays, consult charge/senior).

C. Isolation and Identification:

Specimens	Method	Cell Line ^a	Incubation at 36°C	Stain ^b used/Read
Specimens from infants in NICU	Shell Vial	MRC-5	2 days	CMV-IE
All patients	Tube	CMK CMK HEp2	5 days 10 days 10 days	RS/HSVbivalent RS/HSVbivalent 3 x Reads/week

^aMRC-5 = Human Fibroblast cells; CMK = Cynomolgus Monkey Kidney; HEp2= Human Laryngeal Epidermoid Carcinoma cells

^b CMV-IE = Monoclonal IFA stain for Cytomegalovirus Immediate Early antigen

^b RS= SimulFluor Respiratory virus Screen DFA staining

^b HSVbivalent= Monoclonal DFA stain for HSV1 and HSV2

D. Interpretation and Processing of Cultures:

a) For shell vial procedure:

- i) If HSV requested, fix and stain at 24 hours (or next working day).
- ii) If CMV requested, fix and stain at 48 hours (if >48 hours, 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).

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- 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

Direct:	Negative Report:	“Negative for respiratory viruses.”
	Positive* Report:	“POSITIVE for _____ virus.”
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.**

***Notify Infection Control of all positive respiratory virus results.**

*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.

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V. References

1. Gleaves, Curt A. et al. Cumitech 15A “Lab Diagnosis of Viral Infections”. American Society for Microbiology, August 1994.
2. Greenberg, S. et al. Cumitech 21 “Lab Diagnosis of Viral Respiratory Disease”. American Society for Microbiology, March 1986.