ETT/AUGER SUCTION (INFANTS)

I. Introduction

Viral bronchitis, bronchiolitis and pneumonia are commonly caused by RSV or Parainfluenza in infants and young children. CMV pneumomitis may be seen in newborn/premature infants.

II. Collection and Transport

Endotracheal tube (ETT) aspirates or auger suctions are generally collected into a sterile container. If a delay in transport or processing is anticipated, keep the specimen at 4^0C.

III. Procedure

A. Processing of Specimens:

a) If sample is for virology only, flush approximately 2 mL of viral transport media through tubing and use for inoculation. If sample is to be split for other tests (i.e. C&S) use sterile saline.
b) For samples in saline add 0.2 ml (4 drops) of gentamicin (1 mg/mL) and 0.1 ml (2 drops) of fungizone (250 μg/mL) to a final concentration of 100 μg/ml and 10 μg/mL respectively.
c) Let stand at room temperature for 10 minutes before inoculating.
d) Refer to Appendix II and III for Shell Vial and Tube Culture inoculation, respectively.

B. Direct Examination:

Prepare one double-well cytospin slide for immunofluorescent staining. Stain one well with RSV/Para3 SimulFluor monoclonal antibody and one with SimulFluor Respiratory virus Screen monoclonal antibody. If a specific virus is requested, prepare appropriate number of additional slides and stain using specific individual monoclonal antibodies (Refer to Appendix VI Direct antigen Detection from specimens – SimulFluor Respiratory Screen Protocol Scheme 2)

Complete direct smear results the same day for specimens received in the virology section by 14:00 hours. For specimens received between 14:00 and 15:30

processing and smear preparation should be completed, however staining, reading and reporting results may be completed the next day (except Fridays).

C. Isolation and Identification:

Method	**Cell Line[a]**	**Incubation at 36°C**	**Stain[b] used/Read**
Shell Vial	MRC-5	2 days	CMV-IE
Tube	CMK CMK HEp2	5 days 10 days 10 days	RS/HSVbivalent RS/HSVbivalent 3 x Reads/week

[a]MRC-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:

If HSV requested, fix and stain at 24 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).

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.

1. **Reporting Results**

Direct:	Negative Report:	"Negative for respiratory virus"
	PositiveReport*:	"POSITIVE for _____virus."
Shell Vial:	Negative Report:	"Negative for Cytomegalovirus."
	Positive Report*:	"POSITIVE for Cytomegalovirus."
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.**
***Notifiy 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.

V. Reference

1. Greenberg, S. et al. Cumitech 21 "Lab Diagnosis of Viral Respiratory Disease". American Society for Microbiology, March 1986.