BRONCHOSCOPY / BAL / SPUTUM / WASHINGS (SYMPTOMATIC)

I. Introduction

Viral pneumonia in immunocompromised patients is commonly due to cytomegalovirus (CMV) or respiratory syncytial virus (RSV). Other viruses that may be detected include influenza, parainfluenza, adenovirus, enterovirus and measles viruses. Requests for viruses other than the above may require the use of additional media. Refer to Appendix XV (Virus Isolation and Identification).

II. Collection and Transport

Sputum, bronchial washes and aspirates are to be submitted in a clean, sterile container. If a delay in transport or processing is anticipated, keep the specimen at 4^0C. Multiple bronchial washes and/or BAL samples received from the same patient at the same time should be pooled and processed as a single BAL sample.

III. Procedure

A. Processing of Specimens:

i) Bronchial washes and aspirates:

a) Approximately 2-3 mL of centrifuged sediment is to be received from the specimen receiving area (planting).
b) Prepare direct smears (if required) from the sediment. Very thick/mucoid specimens may require dilution of a portion of the sediment with sputolysin prior to direct smear preparation to avoid excessively thick smears. (See Appendix XXII)
c) Transfer 2 mL of sediment to a sterile freezer vial. Add 4 drops gentamicin and 2 drops of fungizone to a final concentration of 100μg/mL and 10 μg/mL respectively.
d) Allow to stand at room temperature for 10 minutes.

ii) Sputum:

a) Combine equal amounts of sputum and a 1:10 dilution of sputolysin and vortex gently.
b) Prepare direct smears, if required, from the above mixture.
c) Process as for Bronchoscopy specimens above steps c) and d)

B. Direct Examination:

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

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= 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 after 1 day (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, send out report indicating contamination.

IV. Reporting Results

Direct:	Negative Report:	"Negative for respiratory viruses."
	Positive Report*:	"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**.
***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.

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.