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Section: Virology Manual	Subject Title: Broncho-Alveolar Lavage	
	(BAL) - CMV Surveillance (PMH)	
Issued by: LABORATORY MANAGER	Original Date: March 14, 2001	
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BRONCHO-ALVEOLAR LAVAGE (BAL) - CMV SURVEILLANCE

I. <u>Introduction</u>

Broncho-alveolar lavage specimens (BAL) for CMV surveillance collected on day 35 post-bone marrow transplant patients or routine screening of solid organ transplant patients are examined for CMV only.

II. Collection and Transport

BAL fluid should be collected into a clean, sterile container. If a delay in transport or processing is anticipated, keep the specimen at 4 °C.

III. Procedure

A. Processing of Specimen:

- a) Approximately 2-3 mL of centrifuged sediment should be received from the specimen receiving planting area.
- b) Transfer 2 mL of sediment to a sterile freezer vial containing 4 drops (0.2 mL) gentamicin (1 mg/mL) and 2 drops (0.1 mL) of fungizone (250 μ g/mL) to a final concentration of 100 μ g/mL and 10 μ g/mL respectively.
- c) Allow to stand at room temperature for 10 minutes
- d) Refer to Appendix II for Shell Vial inoculation.

B. Direct Examination:

Not done.

C. Isolation and Identification:

Method	Cell Lines ^a	Incubation at 36°C	Stain ^b used
Shell Vial	MRC-5	2 days	CMV-IE

^aMRC-5 = Human fibroblast cells

Refer to Appendix II for Shell Vial staining and interpretation.

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^bCMV-IE= Monoclonal antibody IFA stain for CMV immediate early antigen

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- D. Interpretation and Processing of Cultures:
 - a) At 48 hours observe shell vial coverslips using the inverted microscope:
 - i) If <50% or no CPE Perform DFA for CMV immediate early antigen (See Appendix V).
 - ii) If >50% CPE Prepare a cytospin slide of scraped cells and then perform DFA for CMV immediate early antigen.
 - iii) If positive, freeze shell vial supernate.

IV. Reporting Results

Shell Vial: Negative report: "Negative for Cytomegalovirus."

Positive report*: "POSITIVE for Cytomegalovirus."

V. Reference

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

^{*} 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.