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Section: Virology Manual	Subject Title: Appendix XX Cytospin Preparation	
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Appendix XX

CYTOSPIN PREPARATION

I. Introduction

A cytospin preparation is a concentration of cells taken directly from specimens or from scraped cell cultures.

II. Reagents and Materials

- Virus-specific or pooled antibody
- Phosphate buffered saline (PBS)
- Cold acetone (4°C)
- Distilled water
- Mounting fluid
- Non-immune antibody
- vortex
- sterile pipettes
- cytospin and accessories
- humidified chamber
- coplin jars
- fluorescence microscope

III. Procedure

1. Shell Vial

- i. Remove all except 1 ml maintenance media from shell vial using a sterile pipette.
- ii. Scrape cells from top of coverslip using a sterile pipette. Break up cell clumps by pipetting the cells up and down several times.
- iii. Pipette 200 ul (4 drops) of scraped cells into funnel for each well.
- iv. Cytospin at 2000 rpm (700g) for 5 minutes.
- v. Remove slide and air dry.
- vi. Fix in cold acetone for 10 minutes in a coplin jar. Remove slide and air dry.

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- vii. Proceed to staining. Refer to Appendix IV for Indirect fluorescent antibody staining techniques or Appendix V for Direct fluorescent antibody staining techniques.

or

Refer to Appendices IV and V for immunofluorescent staining techniques for shell vials.

2. Tube culture

- i. Remove all except 1 ml maintenance media from the culture tube using a sterile pipette.
- ii. Scrape cells from side of tube using a sterile pipette. Break up cell clumps by pipetting the cells up and down several times.
- iii. Pipette 200 ul (4 drops) of scraped cells into funnel for each well.
- iv. Cytospin at 2000 rpm (700 x g) for 5 minutes.
- v. Remove slide and air dry.
- vi. Fix in cold acetone for 10 minutes in a coplin jar. Remove slide and air dry.
- vii. Proceed to staining. Refer to Appendix IV for Indirect fluorescent antibody stains or Appendix V for Direct fluorescent antibody stains.

or

Refer to Appendices IV and V for immunofluorescent staining techniques for shell vials.

3. Direct from specimen

IV. Reference

Thermo Shandon, cytopspin. Manufacturer's manual. Refer to Appendix VI for procedure.