Appendix IV

INDIRECT IMMUNOFLUORESCENT ANTIBODY (IFA) STAINING FOR VIRAL CULTURE CONFIRMATION

I. Introduction

The IFA technique is used to identify viral isolates in the cells obtained from shell vials and tube cultures. The indirect method consists of two steps. In the first step, primary antibodies are allowed to react with viral antigens in the cells. These specific complexes are detected in a second step using a species-specific antibody conjugated with a fluorochrome. Viruses which we currently identify by IFA staining include cytomegalovirus immediate early antigen (CMV-IE) and enteroviruses.

II. Reagents and Materials

Virus-specific antibody
FITC-conjugated antimouse antibody
Phosphate buffered saline (PBS)
Distilled water
Cold acetone ($4^{o}C$)
Mounting fluid
Sterile pipettes
Cytospin and accessories (for tube cultures)
Humidified chamber
Sterile freezer vial
Glass slides
Coverslips
Paper towels for blotting
Humidified chamber (for tube culture)

II. Procedure

1. Shell Vial

Follow outline in Appendix II to determine if staining should be done in the shell vial itself or if a cytospin needs to be prepared. If the staining is to be done in the shell vial itself, proceed to step i) below.

i. Discard cap. Remove maintenance medium from the shell vial using a clean sterile pipette.

c) Add 1 mL of cold acetone. Cover and fix for 10 minutes.

d) Decant acetone and blot on paper towel.

e) Gently rinse with PBS from squirt bottle, filling vial 3/4 full. Decant PBS.

f) Add 75 µl (2 drops from bottle) of appropriate antibody. Cover.

vi. Incubate at 36^{o}C for 30 minutes.

vii. Gently rinse with PBS from squirt bottle, filling vial 3/4 full. Decant PBS. Repeat.

viii. Add 75µl (2 drops from bottle) of appropriate FITC -conjugated antibodies, cover and repeat steps vi and vii.

x. Remove the coverslip and place cell side down onto a drop of mounting fluid on a glass slide.

xi. Read using fluorescence microscope with the FITC/Evans Blue filter and the 40x objective.

2. Tube Culture

i. Prepare cytospin preparation from cell culture tube as outlined in Appendix XX.

ii. Add 20 µl of appropriate antibodies onto the fixed cytospin slide.

iii. Incubate in a humidified chamber for 30 minutes at 36^{o}C.

iv. Wash each slide 3 times with fresh PBS for 2 minutes each in a coplin jar.

v. Wipe excess PBS from the slide without touching the cell spot.

vi. Add 20 µl of appropriate FITC-conjugated antibodies.

vii. Incubate in a humidified chamber at 36^{o}C for 30 minutes.

viii. Wash each slide 3 times with fresh PBS for 2 minutes each in a coplin jar.

ix. Wash with distilled water for 1 minute in a coplin jar.

x. Wipe excess water from the slide without touching the cell spot.

xi. Mount using coverslip and mounting fluid.

xii. Read with fluorescence microscope with the FITC/Evans Blue filter and the 40x objective.

Interpretation of Results

Positive: CMV-IE:
An even matte green fluorescence covering the entire nucleus which may include specks of brighter fluorescence.

Enteroviruses:
Distinct apple green fluorescence of the cytoplasm and/or nucleus of the infected cells.

Negative: Red cells with no apple-green fluorescence.

IV. Quality Control

Appropriate positive and negative control slides should be stained with each batch.

V. Reporting

See individual specimen protocols.

VI. Reference

Isenberg, H.D., 1992. Clinical Microbiology Procedures Handbook Vol. 2. ASM Press.