Appendix VII

HEMADSORPTION OF TUBE CULTURE MONOLAYERS (NOT ROUTINELY USED)

The hemadsorption (HAD) technique is used primarily to detect viruses that produce little or no cytopathic effect (CPE) in tube culture monolayers. Using guinea pig RBC, it is used to screen inoculated cell cultures for the presence of influenza, parainfluenza, mumps and Newcastle disease viruses.

I. Procedure

Reagents

guinea pig RBC in Alsever's solution
sterile phosphate buffered saline(PBS)

Materials

sterile pipettes
precision pipettes
inverted microscope
centrifuge
15 mL sterile centrifuge tube

Preparation of 10% stock guinea pig RBC suspension

The stock suspension should be prepared every Monday and stored at 4^{o}C and used within 7 days of preparation.

1. Transfer 5 mL of blood to 15 mL tube and add equal volume of PBS.

2. Centrifuge at 3000 rpm (700 x *g*) for 5 minutes at room temperature.

3. Discard the supernate and add 10 mL of PBS.

4. Centrifuge and wash the cells until the supernatant is clear. (approx. 2-3 washings).

5. Determine the packed-cell volume and add PBS equal to 9 times the packed-cell volume to yield a 10% suspension.

Preparation of 0.4% working guinea pig RBC suspension

The working suspension should be prepared from 10% suspension on the day of testing.

1. Add 0.4 mL of the 10% suspension to 9.6 mL of PBS.

HAD Test

The HAD test is performed on day 5 and day 10 for respiratory specimens with no evidence of CPE.

1. Select one RMK tube to be used for HAD. Transfer the medium in the tube to another sterile, labelled capped tube. Place medium at $4^{o}C$ pending HAD results.

2. Add 0.2 mL of the 0.4% RBC suspension to each culture tube, to be tested.

3. Incubate the tubes horizontally at $4^{o}C$ for 30 minutes. Make sure the RBC suspension is distributed over the monolayer.

4. Gently rotate or tap the tubes to resuspend nonadsorbed cells. Immediately examine the tubes with inverted microscope with the 40X objective. Do not handle the tubes in such a way that the monolayers will become warm.

Interpretation of Results

1. Positive HAD test should show RBCs firmly attached to the monolayer. Hemagglutinated cells (clumped RBCs) are also seen in the fluid overlaying the monolayer.

2. Negative HAD test should show no or minimal RBCs attached to the monolayers, with almost all cells floating above the monolayers.

II. Quality Control

Positive and negative controls should be set up for HAD test prior to the expected "flu" season.

Positive HAD Test

1. Place the tube in a 36^{o}C water bath for 15 minutes.

2. Wash the eluted monolayers twice with PBS.

3. Perform indirect immunofluorescence test for influenzae and parainfluenzae viruses. See Appendix III.

4. The culture medium harvested prior to the HAD test may be used for subpassage, storage or identification by hemagglutination inhibition.

Negative HAD Test

1. Discard tube that is HAD negative at 10 days after inoculation.

2. Tube inoculated after 5 days should be washed 3 times with 5 mL Hank's Balanced Salt Solution to remove all RBCs.

3. Refeed tubes with 2 mL of maintenance medium and reincubate the cultures for another 5 days.

4. Discard the culture fluid harvested from negative cultures unless subpassage is to be performed.

V. Reference

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