Red Blood Cell Preparation and Hemagglutination Assay

Purpose: This protocol describes the preparation of RBCs for storage and use in Hemagluttination assays, which are used to determine the La Sota B1 lentogenic Newcastle Disease virion hemaglutinin-neuraminidase titer relative to virion stock positive controls. The protocol was adapted from McGinnes et al. (2006) and "Detection of Hemagglutinating Viruses" (n.d.).

Materials:

- Whole Blood Cells
- 50mL conical tube
- Multichannel micropipette, micropipette, and tips
- PBS with 2mM Penicillin/Streptomycin
- SV3
- Alsever's Solution
- 96 round bottom well plate
- Microscope slide
- Virion stock
- Centrifuge

Procedure:

1. RBC preparation
    a. Obtain 25mL whole blood and add 25mL cold Alsever's Solution for anti-coagulation in a 50mL conical tube and keep on ice
    b. Centrifuge whole blood at 500 RCF for 10min
    c. Aspirate blood plasma, buffy layer, and top erythrocytes
    d. Wash 3 times by resuspending in PBS with 2mM Penicillin/Streptomycin to double the pellet volume, centrifuging at 500 RCF, and aspirating the supernatant
    e. Resuspend the pellet in PBS with 2mM Penicillin/Streptomycin or SV3 for a final concentration of 10% pellet volume per total volume, and store at 2-7˚C for 1 week or 42 days
2. HA Titer
    a. Thaw your virion stock and samples on ice
    b. Use a multichannel micropipette to add 50µL of cold PBS to each row on a 96 round bottom well plate for each sample in duplicates
    c. Add 50µL of each sample to column 1 of their respective duplicate rows
        i. Don't add anything to the negative control rows and add virion stocks to the positive control rows
    d. Use a multichannel micropipette to make a 2-fold serial dilution on every row except for the negative control
    e. Add 50µL of 1% RBC solution to each well and agitate the plate to mix
    f. Cover plate in sterile rap and incubate for 1hr at 2-7˚C

g. Observe HA titer of each sample from the inverse of the last serial dilution that indicates RBC agglutination
    i. Agglutinated RBCs will appear cloudy or be followed by cloudy wells in further dilutions as a result of the hook effect
    ii. Non-agglutinated RBCs will have a red button at the bottom of the well, where RBCs settled

3. HA Test
    a. Thaw your virion stock and samples on ice
    b. Add a drop of blood to a microscope slide for the positive control (Virion Stock), the negative control (PBS), and each sample
    c. Add a drop of undiluted samples and controls to their respective drops of blood
    d. Agitate the slide to mix for 1min
    e. Observe agglutination under the microscope
        i. Positive: chunky lattice structure forms
        ii. Negative: blood is closely packed and homogeneous

References

McGinnes, L.W., Pantua, H., Reitter, J., and Morrison, T.G. (2006). Newcastle Disease Virus: Propagation, Quantification, and Storage. In Current Protocols in Microbiology, R. Coico, A. McBride, J.M. Quarles, B. Stevenson, and R.K. Taylor, eds. (Hoboken, NJ, USA: John Wiley & Sons, Inc.), pp. 15F.2.1-15F.2.18.

"Detection of Hemagglutinating Viruses." Edited by Linda S. Snavely , *United States Department of Agriculture Center for Veterinary Biologics Testing Protocol*, United States Department of Agriculture Animal and Plant Health Inspection Service, 9 Jan. 2018, www.aphis.usda.gov/animal_health/vet_biologics/publications/VIRPRO0096.pdf.