STANDARD OPERATING PROCEDURE

Title: VIRAL HAEMAGGLUTINATION TEST AND HAEMAGGLUTINATION INHIBITION TEST FPR DETECTION OF NEW CASTLE DISEASE VIRUS(RD)

Reference: Alexander & Allan, 1974

1.0 INTRODUCTION

1.1 PURPOSE/SCOPE OF THIS SOP

This SOP outlines the method of inoculation of RDV in embryonated chicken eggs. Preferably eggs are collected from unvaccinated and disease free flock. . It encompasses virus and antibody assay techniques.

2.0 DETECTION OF RD VIRUS

Haemagglutinating activity (HA) activity detected in bacteriologically sterile fluids harvested from inoculated eggs may be due to the presence of any of the ten subtypes of APMV (including RDV) or 16 haemagglutinin subtypes of influenza A viruses, or. Non-sterile fluid could contain bacterial HA. RDV can be confirmed by the use of specific antiserum in a haemagglutination inhibition (HI) test. Usually chicken antiserum that has been prepared against one of the strains of RDV is used.

3.0 MATERIALS:

3.1	**Chemicals and reagents** Chemicals and reagents listed below are those currently used. Equivalent reagents, which are fit for purpose, may be used.	
3.2	**Consumables**	**Suppliers**
	Microtitre plate, Pasteur pipette, Lyophilizing vials	Tarson
	Equipments	**Suppliers & Model**
	BOD Incubator **Egg cadler** **Refrigerator** **Deep freezer** **Laminarair flow (Vertical)** **Centrifuge machine** **Lyophilizer**	

4.0 PROCEDURES:

4.1 Haemagglutination and haemagglutination inhibition tests

Chicken sera rarely give nonspecific positive reactions in the HI test and any pretreatment of the sera is unnecessary. Sera from species other than chickens may sometimes cause agglutination of chicken red blood cells (RBCs), so this property should first be determined and then removed by adsorption of the serum with chicken RBCs. This is done by adding 0.025 ml of packed chicken RBCs to each 0.5 ml of antisera, shaking gently and leaving for at least 30 minutes; the RBCs are then pelleted by centrifugation at 800 g for 2–5 minutes and the adsorbed sera are decanted. Variations in the procedures for HA and HI tests are practised in different laboratories. The following
recommended examples apply in the use of V-bottomed microwell plastic plates in which the final volume for both types of test is 0.075 ml. The reagents required for these tests are isotonic PBS (0.01 M), pH 7.0–7.2, and RBC taken from a minimum of three
unvaccinated birds free from antibodies to NDV. Cells are washed three times in PBS before use as a 1% (packed cell v/v) suspension. Positive and negative control antigens and antisera are run with each test, as appropriate.

4.1.1 Haemagglutination test

i) 0.025 ml of PBS is dispensed into each well of a plastic V-bottomed microtitre plate.
ii) 0.025 ml of the virus suspension (i.e. infective or inactivated allantoic fluid) is placed in the first well. For accurate determination of the HA content, this is done from a close range of an initial series of dilutions, i.e. 1/2, 1/4, 1/8, etc.
iii) Twofold dilutions of 0.025 ml volumes of the virus suspension are made across the plate.
iv) A further 0.025 ml of PBS is dispensed to each well.
v) 0.025 ml of 1% (v/v) chicken RBCs is dispensed to each well.
vi) The solution is mixed by tapping the plate gently. The RBCs are allowed to settle for about 30 minutes at room temperature, i.e. about 20°C, or for 60 minutes at 4°C if ambient temperatures are high, when control RBCs should be settled to a distinct button.
vii) HA is determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration should be read to the highest dilution giving complete HA (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the initial range of dilutions.

4.1.2 Haemagglutination inhibition test

i) 0.025 ml of PBS is dispensed into each well of a plastic V-bottomed microtitre plate.
ii) 0.025 ml of serum is placed into the first well of the plate.
iii) Twofold dilutions of 0.025 ml volumes of the serum are made across the plate.
iv) 4 HAU virus/antigen in 0.025 ml is added to each well and the plate is left for a minimum of 30 minutes at room temperature, i.e. about 20°C, or 60 minutes at 4°C.
v) 0.025 ml of 1% (v/v) chicken RBCs is added to each well and, after gentle mixing, the RBCs are allowed to settle for about 30 minutes at room temperature, i.e. about 20°C, or for about 60 minutes at 4°C if ambient temperatures are high, when control RBCs should be settled to a distinct button.
vi) The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (positive serum, virus/antigen and PBS controls) should be considered to show inhibition.

vii) The validity of results should be assessed against a negative control serum, which should not give a titre >1/4 and a positive control serum for which the titre should be within one dilution of the known titre.

The value of serology in diagnosis is clearly related to the expected immune status of the affected birds. HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 or more against 4 HAU of antigen. Back titration of antigen should be included in all tests to verify the number of HAU used. In vaccinated flocks that are being monitored serologically, it may be possible to identify anamnestic responses as the result of a challenge infection with field virus (Alexander & Allan, 1974), but great care should be exercised as variations may occur from other causes. For example, it has been demonstrated that APMV-3 virus infections of RD-virus-vaccinated turkeys will result in substantially increased titres to RDV (Alexander *et al.*, 1983).