ANNEX III

DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION AND DIFFERENTIAL DIAGNOSIS OF NEWCASTLE DISEASE

CHAPTER 5

Haemagglutination (HA) test

Reagents

1. Isotonic saline buffered with phosphate (PBS) (0,05 M) to pH 7,0-7,4.

2. Red blood cells (RBC) taken and pooled from a minimum of three specific pathogen free chickens (if not available blood may be taken from birds regularly monitored and shown to be free of NDV antibodies) into an equal volume of Alsever's solution. Cells should be washed three times in PBS before use. For the test a 1 % suspension (packed cell v/v) in PBS is recommended.

3. NDV strain Ulster 2C is recommended for use as standard antigen.

Procedure

(a) Dispense 0,025 ml PBS into each well of a plastic microtitre plate (V-bottomed wells should be used).

(b) Place 0,025 ml of virus suspension (i.e. allantoic fluid) in the first well.

(c) Use a microtitration diluter to make two-fold dilutions (1:2 to 1:4096) of virus across the plate.

(d) Dispense a further 0,025 ml of PBS to each well.

(e) Add 0,025 ml of 1 % red blood cells to each well.

(f) Mix by tapping gently and place at 4 °C.

(g) Plates are read 30 — 40 minutes later when controls are settled. Reading is done by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. Wells with no HA should flow at the same rate as the control cells with no virus.

(h) The HA titre is the highest dilution that causes agglutination of the RBCs. That dilution may be regarded as containing one HA unit (HAU). A more accurate method for determining the HA titre is to do HA tests on virus from a close range of initial dilutions i.e. 1:3, 1:4, 1:5, 1:6 etc. This is recommended for the accurate preparation of antigen for haemagglutination inhibition tests (see Chapter 6).