COMMISSION DECISION

of 2 June 1992

on testing of poultry for Newcastle Disease prior to movement, in application of Article 12 of Council Directive 90/539/EEC

(92/340/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 90/539/EEC of 15 October 1990, on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs [1], as last amended by Council Directive 91/496/EEC [2], and in particular Article 12 (1) thereof,

Whereas the methodologies, for the performance of serological tests for Newcastle Disease and the isolation of the Newcastle Disease virus, must include details of the sampling procedure, the procedure for carrying out the tests and the interpretation of the test results;

Whereas the measures provided for in this decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

The representative serological test to detect Newcastle Disease antibodies referred to in the third indent of Article 12 (1) (c) of Directive 90/539/EEC must comply with the requirements of Annex 1.

Article 2

The testing to isolate Newcastle disease virus referred to in the second indent of Article 12 (1) (d) of Directive 90/539/EEC, must comply with the requirements of Annex 2.

Article 3

This Decision is addressed to the Member States.

Done at Brussels, 2 June 1992.

For the Commission

Ray MAC SHARRY

Member of the Commission

[1] OJ No L 303, 31. 10. 1990, p. 6.
[2] OJ No L 268, 24. 9. 1991, p. 56.

ANNEX I

Serological testing to detect Newcastle Disease antibodies in poultry

1. *Sampling of blood*

Poultry subject to the conditions of this annex shall originate from flocks where blood samples have been obtained from at least 60 birds randomly chosen and examined by the Haemagglutination inhibition (HI) test in accordance with the procedure listed under 2.

2. *Procedure*

(a) Dispense 0,025 ml PBS into all wells of a plastic microtitre plate (with V-bottomed wells).

(b) Place 0,025 ml of serum into first well of plate.

(c) use microtitration diluter to make two-fold dilutions of serum across plate.

(d) Add 0,25 ml of diluted allantoic fluid containing four or eight HAU.

(e) Mix by tapping and place plate at 4° C for a minimum of 60 minutes or room temperature for a minimum of 30 minutes.

(f) Add 0,025 ml 1 % RBCs to all wells.

(g) Mix by gentle tapping and place at 4° C.

(h) Plates are read after 30-40 minutes when control RBCs are settled. This is done by tilting and observing the presence or absence of tear-shaped streaming at the same rate as control wells containing RBCs (0,025 ml) and PBS (0,05 ml) only.

(i) The HI titre is the highest dilution of antiserum causing complete inhibition of four or eight units of virus (An HA titration to confirm the presence of the required HAU should be included in each test).

(j) The validity of the results is dependent on obtaining a titre of less than 2^3 for four HAU or 2^2 for eight HAU with the negative control serum and a titre of within one dilution of the known titre of the positive control serum.

3. *Interpretation of tests*

The antigen used will affect the level at which a serum is considered positive : for four HAU a positive serum is any showing a titre of 2^4 or greater, for eight HAU a positive serum is any showing a titre of 2^3 or greater.

ANNEX II

Newcastle Disease virus isolation from slaughter poultry

Poultry subject to the conditions of this annex shall originate from flocks which have been tested for the presence of Newcastle Disease virus with negative results, no virus isolated, in accordance with the following procedure :

1. *Sampling*

   At least 60 samples comprising cloacal swabs or faeces shall be taken from each flock.

2. *Treatment of samples*

   Not more than five samples may be pooled. Swabs should be placed in sufficient antibiotic medium to ensure full immersion. Faeces samples should be homogenized (in an enclosed blender or using a pestle and mortar and sterile sand) in antibiotic medium and made to 10-20 % w/v suspensions in the medium. The suspensions should be left for about two hours at ambient temperature (or longer periods at 4 °C) and then clarified by centrifugation (e.g. 800 to 1000 × g for 10 minutes).

   High concentrations of antibiotics are required for faeces samples and a typical mixture is : 10 000 units/ml penicillin, 10 mg/ml streptomycin, 0,25 mg/ml gentamycin and 5 000 units/ml mycostatin in phosphate buffered saline. For control of Chlamydia organisms 50 mg/ml oxytetracycline may be added. It is imperative when making the medium that the pH is checked after the addition of the antibiotics and readjusted to pH 7,0-7,4.

3. *Virus isolation in embryonated fowls' eggs*

   The clarified supernatent fluid should be inoculated in 0,1-0,2 ml amounts into the allantoic cavity of each of a minimum of four embryonated, fowls' eggs which have been incubated for 8-10 days. Ideally, these eggs should be obtained from a specific pathogen free flock, but when this is impracticable it is acceptable to use eggs obtained from a flock shown to be free of antibodies to Newcastle Disease virus. The inoculated eggs are held at 37 °C and candled daily. Eggs with dead or dying embryos as they arise, and all remaining eggs six days after inoculation should be chilled to 4 °C and the allantoic-amniotic fluids tested for haemagglutination activity. If no haemagglutination is detected the above procedure is repeated using undiluted allantoic/amniotic fluid as inoculum.

   When haemagglutination is detected the presence of bacteria should be excluded by culture. If bacteria are presented the fluids may be passed through a 450mn membrane filter, further antibiotics added and inoculated into embryonated eggs as above.