ANNEX III

DIAGNOSTIC PROCEDURES FOR THE CONFIRMATION AND DIFFERENTIAL DIAGNOSIS OF NEWCASTLE DISEASE

CHAPTER 2

Virus isolation

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 to 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 present the fluids may be passed through a 450 nm membrane filter, further antibiotics added and inoculated into embryonated eggs as above.