

## Short Communication

### USE OF EGG YOLK IN SEROMONITORING AGAINST NEWCASTLE DISEASE

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Seromonitoring of layer flocks using egg yolk is economically feasible (Piela *et al.*, 1984). The chloroform extraction procedure enables egg yolks to be used for antibody estimation by haemagglutination inhibition (HI) test (Piela *et al.*, 1984). The present paper describes the use of Brij-35 (Polyoxy ethylene 23 lauryl ether) solution in egg yolks before use in the HI test for assessment of antibodies against Newcastle disease (ND).

Fertile eggs from layers immunised with ND vaccines were collected. The yolks were collected individually from 30 eggs and divided into 3 equal parts. One part was used for chloroform extraction another part was used for Brij-35 solution, a non-ionic detergent treatment and the third part was mixed with phosphate buffered saline (PBS). All the yolk samples after different treatments were subjected to HI test for assessment of antibodies against ND. Samples were also allowed to adsorb onto Whatman No. 1 filter paper and the eluate obtained from the filter papers on day 1 and day 7 were tested for antibodies against ND by HI test.

The chloroform extraction of yolk procedure described by Piela *et al.* (1984) was followed. One ml of yolk was mixed 1/1 with PBS and then mixed one half (v/v) with chloroform. After incubation for one hour at room temperature, centrifuged at 1,500 r.p.m. for 20 min, the clear supernatant was used for testing and this was considered to represent a yolk dilution of one half.

For non-ionic detergent (Brij-35) treatment of yolk, one ml of yolk was mixed 1/1 (v/v) with PBS and then mixed 1/2 (v/v) with 0.1 per cent Brij-35 in normal saline solution, after incubated for one hour at room temperature and centrifuged at 1,500 r.p.m. for 20 min. The supernatant was used for HI test and this was considered to represent a yolk dilution of one quarter.

Yolk dilution in PBS was prepared by mixing one ml of yolk with one ml PBS and incubated at room temperature for one hour. The tubes were centrifuged at 1,500 r.p.m. for 20 min. The supernatant was used for testing in HI test and this was considered to represent a yolk dilution of one half.

After treatment with chloroform, Brij-35 solution and PBS the supernatants were allowed to adsorb onto Whatman No. 1 filter paper and air dried. The filter papers were stored in polypropylene bags and kept at room temperature for one week. On day 1 and day 7 eluates were obtained from sample areas of filter papers stored at room temperature using Brij-35 solution and subjected to antibody assessment by HI test.

Elution of antibody from filter paper was done as described by Roy *et al.* (1992). Two discs of 5 mm diameter were punched from the sample area of each filter paper and placed in wells of a flat bottom microtitre plate. One hundred  $\mu$ l of Brij-35 solution was added to each well and incubated at room temperature for 2 hours for elution of antibody.