

EFFECT OF STORAGE AND REPEATED FREEZE-THAW OF SERA ON HAEMAGGLUTINATION ANTIBODY NEWCASTLE DISEASE (NDV) TITRES

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ABSTRACT

We investigated the effect of storage time and freeze-thaw on NDV antibody by HI method. 20 sera samples were divided into two groups, treatment A (storage) and treatment B (freeze-thaw). For treatment A, sera were aliquoted into 4 sets, stored at -20°C. Treatment B, 20 sera comprised one set, stored at -20°C, thawed at room temperature, tested and then cycle of freeze-thaw for 4 times. Each group tested after storage for 1w, 2w, 4w and 8w. The HI mean titre declined from 0 days to 8 weeks for storage time and freeze-thaw.

INTRODUCTION

The haemagglutination inhibition (HI) test is the most widely used for measurement of antibodies (Abs) against Newcastle disease virus (NDV). The basis of the test is the haemagglutination (HA) of glycoproteins on the surface of NDV antigen with agglutinate receptors on erythrocytes. The haemagglutination Inhibition (HI) test is the measure of interference of virus binding to receptors on the erythrocytes by serum antibodies to NDV. This study's objectives are to find out if a) storage of sera to 8 weeks after collection as well as b) repeated freeze-thaw of sera prior to testing has an effect on the HI test result.

MATERIALS AND METHODS

The collection of 20 sera samples was done by wing venipuncture. The blood was left to clot for 30 minutes at room temperature, then decanted into clean microcentrifuged tubes and labeled. After that, centrifuged for 5 minutes at 3000 rpm and inactivated at 56°C for 1 hour in water bath. The sera were divided into 2 treatments as follows—Group A (Storage) and Group B (Storage-Freeze-Thaw). Each sera were tested at different storage times i.e. Day-0, 1-week, 2-weeks, 4-weeks and 8-weeks). For Treatment A the sera were aliquoted into 4 sets of sera, stored at -20°C, then thawed to room temperature before testing at the specified storage times. For Group B, which comprised one set sera, the sample were stored at -20°C, then thawed to room temperature, tested and then cycle of storage-thawing and testing repeated at 1-week, 2-weeks, 4-weeks and 8-weeks.

The Haemagglutination inhibition test was conducted by using the reference method of 4 HA units of NDV antigen and 1 % chicken red blood cells as follows OIE method. (OIE *et al.*, 2012).

RESULTS AND DISCUSSION

Treatment A and treatment B showed declined in HI titre as compared from day-0 to 8-weeks. This study was to determine if a) storage time of up to 8 weeks and b) if repeated storage-freeze-thaw cycles for 8 weeks had an effect on the test result of the NDV HI titre. The present study showed that the storage time and freeze-thaw affected the HI titre. Generally, sera samples are claimed to be stable at -20C. But from the results in treatment A, the HI titre declined gradually from day-0 to 8-weeks. Proteins structural changes may occur when exposed to varying temperature. In the case of antibodies, they show structural instability via denaturation and aggregation. Normally, in an aqueous environment, protein fold spontaneously, so the hydrophobic side chains are encased by the polar chains with a surrounding bulk hydration layer. Freezing disrupts this bulk hydration layer, forming a crystal lattice of ice and rendering it inactive. Unfolding allows hydrophobic reaction to occur, thus denaturing the protein (Norman *et al.*, 2002). In conclusion, this study demonstrates that the NDV HI titre affected by the storage time and freeze-thaw cycles but still within in one dilution factor.

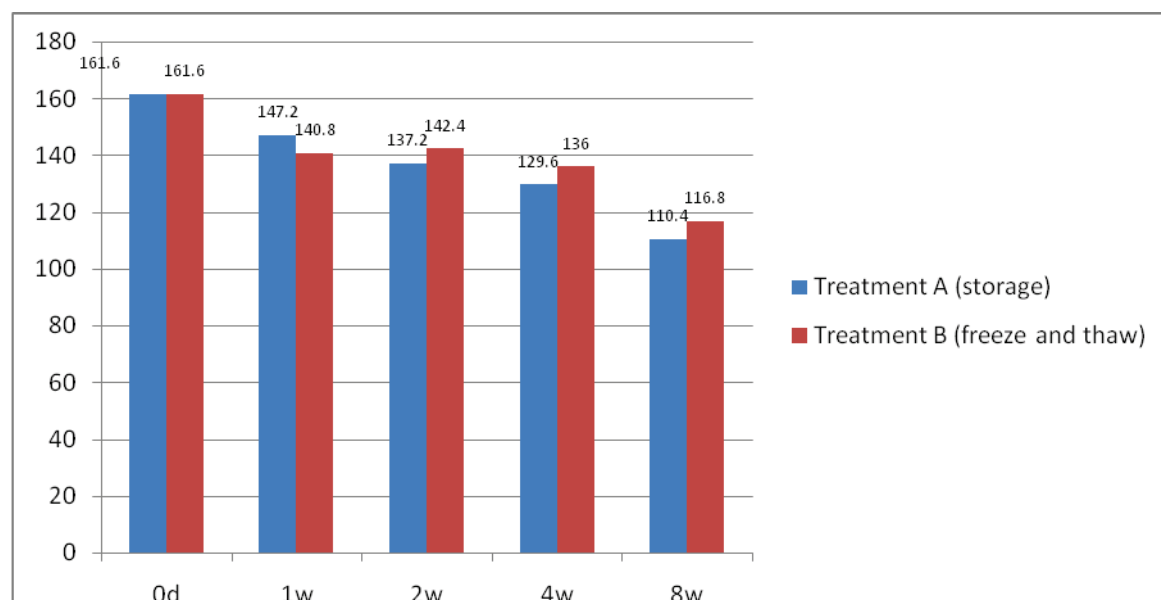


Chart 1: Mean titre of Treatment A and Treatment B

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