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Detection of diphtheria toxin antibodies in human sera in New Zealand by ELISA

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SUMMARY

An enzyme-linked immunosorbent assay (ELISA) was developed to detect IgG antibodies to diphtheria toxin in human serum. Serum samples obtained from 557 normal persons aged 1–65 years from different areas in New Zealand showed maximum antibody levels in the 1–9 years age group (95·1 %) and the least in the 60–65 years age group (38·1 %). The indirect ELISA is suitable for sero-epidemiological survey study as it is simple to perform, economical and precise.

INTRODUCTION

Primary vaccination against diphtheria in childhood started in New Zealand (Maclean, 1964) around the mid 1920s as was the case in Canada (Mathias & Schechter, 1985). In New Zealand, the vaccination programme was slow in being adopted due to a large extent to the apathy of parents (Maclean, 1964). By the end of 1928 only about 11500 school children had been immunized. In 1941, as a matter of Health Department's policy, immunization was offered to children below 7 years of age throughout New Zealand. Therefore, throughout the 1940s immunization was carried out with more encouraging results. By 1948 immunization against diphtheria was well-established throughout the country and thereafter the incidence declined to insignificance. At present vaccination is offered routinely to those aged 3, 5, and 18 months and again at school entry.

To investigate the immune status of a normal population in New Zealand, a useful method would be to detect the immunoglobulin G (IgG) antibody levels against diphtheria toxin. Therefore, in this paper a simple to perform and precise ELISA was developed and used to determine the immune status of a normal population in New Zealand.

MATERIALS AND METHODS

Normal population studied. Serum samples were obtained from 557 normal persons aged 1–65 years from different areas throughout New Zealand. The number studied from each age group was approximately proportional to the corresponding age distribution in the New Zealand population based on the 1976 Census. The sera were collected between November 1978 and March 1980 and stored at −20 °C until tested by ELISA.

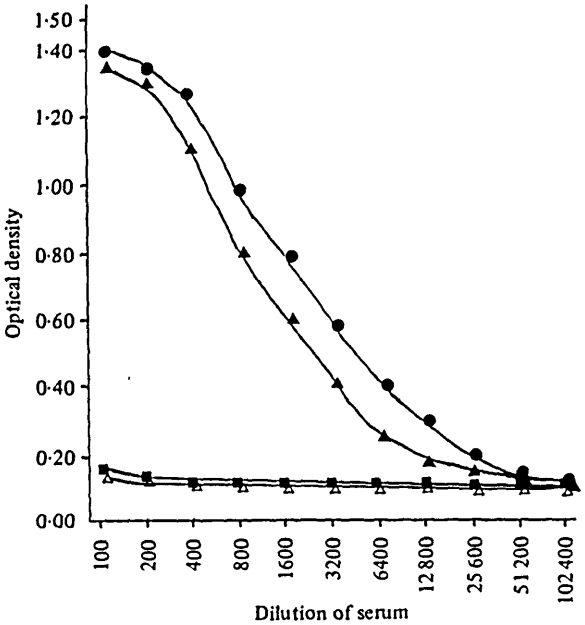

Fig. 1. Optical density readings against dilutions of diphtheria IgG positive standard sera (●, ▲) and negative sera (■, △), assayed by ELISA.

Standard diphtheria sera. Thirteen positive and seven negative standard diphtheria sera were kindly provided by Dr M. E. Camargo, University of São Paulo, Brazil. Sera were considered to be negative when the antitoxin titre was less than 0·01 U/ml, which is regarded as the minimal protective level.

ELISA. Polystyrene, flat-bottom plates (Nunc, Denmark) were coated with purified diphtheria toxoid (3050Lf/ml; Connaught Laboratories, Ontario, Canada) diluted 1/800 in sodium carbonate buffer (pH 9·6). Each well was coated with 100 μl toxoid and left overnight at 4 °C. After washing three times with phosphate-buffered saline (PBS) containing 0·001 % Tween 20 (PBS/T20), plates were dried and stored in an air-tight container with desiccant and stored at 4 °C. When required the plates were used without further treatment. Sensitized plates could be kept for at least 4 weeks at 4 °C. Sera were tested at a single dilution of 1/200. Test sera diluted in PBS/T20 containing 5% foetal calf sera (Smith Biolab, Auckland, New Zealand), (PBS/T20/FCS), were added in 100 μl volume in duplicate to the coated wells and incubated at 37 °C in a humid atmosphere for 1 h. Plates were then washed three times with PBS/T20 and 100 μl purified goat anti-human IgG peroxidase conjugate (Tago, California, USA; diluted 1/11000 with PBS/T20/FCS) was added to each well and incubated at 37 °C for 1 h. Plates were washed and the amount of bound conjugate in each well was measured with 100 μl of tetramethyl benzidine (TMB; Miles, Illinois, USA) substrate buffer (containing 1 % TMB and 0·07 % H_2O_2 in 0·1 M sodium acetate, pH 6·0). The plates were incubated at room temperature for 6 min. The reaction was stopped by the addition of 100 μl of 2·5 M sulphuric acid and absorbance was read at 450 nm on the Titertek Multiskan spectrophotometer (Flow, West Germany). The optical

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Table 1. *Immune status of a normal New Zealand population*

Age group (years)	No. studied	No. with immunity to diphtheria (percentage in parentheses)
1–9	41	39 (95·1)
10–19	113	88 (77·9)
20–29	108	57 (52·8)
30–39	98	61 (62·2)
40–49	100	48 (48·0)
50–59	76	37 (48·7)
60–65	21	8 (38·1)

density reading for each serum sample was taken as the mean value of two wells. Two positive and five negative standard sera (provided by Dr Camargo, Brazil) were used in each assay and the cut-off level for sera considered positive being taken as the mean absorbance value of the five negative controls plus 2 standard deviations. Results were standardized in each assay by correcting the test sera readings back to one standard reference positive serum to compensate for any day-to-day variation.

Inter- and intra-assay reproducibility of the ELISA was tested on 11 positive sera on two different days. Each day the sera were tested five times in one assay. The values obtained were used to establish the coefficient of variation (CV).

RESULTS

Figure 1 shows the optical density profile by ELISA of dilutions of two standard positive and two standard negative sera.

Table 1 shows the immune status of the various age groups in a normal New Zealand population studied. Maximum (95·1 %) antibody level for diphtheria was observed in the 1–9 years age group with a general gradual decrease in the level in older age groups. The least antibody level was found in the 60–65 years age group (38·1 %).

Inter-assay reproducibility showed a mean coefficient of variation (CV) of 5·6 %. Intra-assay reproducibility showed a mean CV of 6·9 %.

DISCUSSION

Studies have shown that after primary vaccination against diphtheria, the antitoxin titre fell over the first few years (Scheibel *et al.* 1966; Bojlen & Scheibel, 1955) and showed a continuing fall in the titre after 15 years (Scheibel *et al.* 1962), from which it was predicted that 25 years after primary vaccination 25 % of vaccinees would be unprotected. However, other studies (Gottlieb, Martin & McLaughlin, 1967; Trinca, 1975) which also looked at the fall of antibody levels suggest that the duration of immunity to diphtheria is long. In this present study of a normal New Zealand population, there was generally a gradual fall in antibody levels with increasing age. Even though this study showed that antibody levels fell with time in New Zealand, suggesting a corresponding fall in immunity, there

were only three notifiable cases of diphtheria in the country between 1978 and 1980 (The Public Health, 1983). Perhaps, in spite of the fact that diphtheria antibody levels wane with time, immunological memory does not, and that this is one reason why the immunization programme seemed to have controlled diphtheria well in New Zealand.

Mathias & Schechter (1985) suggest that routine boosters are not necessary once primary immunization is completed. However, in most European countries and the USA, primary vaccination and revaccination against diphtheria are generally recommended, and it has also been suggested that should diphtheria emerge in a community those who received their primary vaccination more than 2 years ago or were revaccinated more than 10 years ago ought to be revaccinated (Kjeldsen, Simonsen & Heron, 1985). In addition, they also advised those travelling to countries with endemic diphtheria to be revaccinated.

Using the indirect ELISA it was found that for the 1–9 years age group studied, 95·1 % had immunity to diphtheria based on the detection of IgG antibody to diphtheria toxin in human sera. Therefore, using antibody production and detection by ELISA in the 1–9 years age group is a good method of measuring the success of an immunization programme. Older age groups would not reflect clearly the success rate since antibody levels wane with time. As such, in the future in New Zealand, using the ELISA method described in this paper, and with a larger sample of children with better documented history of vaccination, more detailed information on the immune status of children in New Zealand could be ascertained. The ELISA described here is simple to perform, economical and precise for such a sero-epidemiological survey.

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