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Detection of tetanus toxoid antibodies in human sera in New Zealand by ELISA

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SUMMARY

An enzyme-linked immunosorbent assay (ELISA) incorporating the sensitive biotin-streptavidin system was developed to detect IgG antibodies to tetanus toxoid in human serum. Serum samples obtained from 557 normal persons aged 1–65 years from different areas in New Zealand were tested. The proportion of those immune ranged from 60–93 % in males, and from 46–86 % in females. In the 1–9 years age group 85 % were immune. The indirect ELISA is suitable for serological surveys as it is simple to perform, economical and reproducible.

INTRODUCTION

The steady reduction in the incidence of communicable diseases in New Zealand was particularly noticeable in the 1940s, 1950s and 1960s (NZ Department of Health Annual Report, 1969). Although improvements in civic sanitation have played a significant role in the decline, the utilization of effective vaccines has probably been the major contributor. Since 1960 when the triple vaccine of diphtheria, tetanus and pertussis was introduced in this country, the number of notified cases of tetanus has fallen substantially.

This paper describes a simple-to-perform and reproducible ELISA test which can be used in sero-epidemiological surveys to define the immune status of different population groups to tetanus toxoid. The results of such a survey in New Zealand are presented. Although indirect ELISA tests for detecting tetanus antibodies have been described (Melville-Smith, Seagroatt & Watkins, 1983; Sedgwik *et al.* 1983), they have not incorporated the sensitive biotin-streptavidin system which we have used.

MATERIALS AND METHODS

Population studies

Serum samples were obtained from 557 normal persons aged 1–65 years who came from different parts of 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 (New Zealand Official Yearbook, 1981). The sera were collected between November 1978 and March 1980 and stored at −20 °C until tested.

ELISA tests

Flat-bottom, polystyrene plates (Nunc, Denmark) were coated with purified tetanus toxoid (2000 Lf/ml; Connaught Laboratories, Ontario, Canada) diluted 1/800 in 0·05 M sodium carbonate/bicarbonate buffer pH 9·6. Each well was coated with 100 μl of toxoid overnight at 4 °C. Plates were washed three times with phosphate-buffered saline (PBS) containing 0·1 % 'Tween' 20 (PBS/T20), dried, and stored in an air-tight container with desiccant at 4 °C. Sensitized plates could be kept for at least 4 weeks at 4 °C and used without further treatment. Sera diluted 1/800 in PBS/T20 containing 5 % foetal calf sera (PBS/T20/FCS) (Smith Biolab, Auckland, New Zealand) were added in 100 μl volumes, in duplicate, to the coated wells and incubated at 37 °C in a humid atmosphere for 1 h. The plates were then washed three times with PBS/T20 and 100 μl biotinylated anti-human IgG (Amersham, England), diluted 1/800 with PBS/T20/FCS was added to each well and incubated at 37 °C for 1 h. Plates were washed, 100 μl streptavidin-biotinylated peroxidase complex (Amersham) diluted 1/1400 with PBS/T20/FCS added to each well, and again incubated at 37 °C for 1 h. After washing the plates, 100 μl of the substrate, 1 % tetramethyl benzidine (TMB) (Miles, Illinois, USA) in 0·1 M sodium acetate buffer containing 0·07 % H_2O_2, pH 6·0, was added to each well. The reaction was allowed to proceed for 6 min at room temperature, after which it was stopped by the addition of 100 μl of 2·5 M sulphuric acid. As the reaction time was short, the timing was monitored using a stop watch. The absorbance was read at 450 nm on the Titertek Multiskan spectrophotometer (Flow, West Germany). The mean value of two wells was taken as the absorbance value for each serum sample. The results were standardized by correcting the mean values against a standard reference positive serum (> 0·01 i.u./ml; 1·10–1·30 absorbance units) in each assay, to compensate and minimize day-to-day variation.

In each assay a blank, conjugate control, two positive and five negative (< 0·01 i.u./ml) sera were used. The cut-off level for sera considered positive was taken as the mean absorbance value of the five negative controls plus two standard deviations. Tetanus antitoxin standards (0·1, 0·01, and 0·002 i.u./ml), used to calibrate the negative and positive controls, were obtained from Labsystems, Helsinki, Finland. An antitoxin level of $\geqslant$ 0·01 i.u./ml was used as the criterion for protective immunity (Melville-Smith, Seagroatt & Watkins, 1983).

Twelve positive sera were tested on two different days to determine the inter- and intra-assay reproducibility of the ELISA. On each day the sera were tested five times in one assay. The values obtained were used to establish the coefficient of variation (CV).

RESULTS

The immune status of a normal New Zealand population is presented by age group in Table 1. In males the proportion of those immune ranged from 60 to 93 %, and in females from 46 to 85 %. In the 1–9 years age group 85 % were immune.

The falling number of notified tetanus cases in New Zealand is shown in Table 2.

Inter-assay reproducibility showed a mean coefficient of variation (CV) of 6·9 %. Intra-assay reproducibility showed a mean CV of 3·7 %.

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

	Male			Female			Total immune	
Age group (years)	No. studied	No. immune	%	No. studied	No. immune	%	No.	%
1–9	28	24	85·7	13	11	84·6	35	85·4
10–19	49	44	89·8	64	55	85·9	99	87·6
20–29	45	42	93·3	63	48	76·2	90	83·3
30–39	70	50	71·4	28	13	46·4	63	64·3
40–49	72	49	68·1	28	13	46·4	62	62·0
50–59	54	40	74·1	22	14	63·6	54	71·1
60–65	15	9	60·0	6	3	50·0	12	57·1
Total	333	258	77·5	224	157	70·1	415	74·5

Table 2. *Notified tetanus cases in New Zealand by age from 1951–84* *

	Age group (years)								
Years	< 1	1–4	5–9	10–14	15–24	25–44	45–64	≧ 65	Total
1951–55	6	25	35	22	10	15	19	12	144
1956–60	7	17	24	21	14	16	20	13	132
1961–65	2	4	10	15	8	9	16	12	76
1966–70	1	3	4	4	5	8	23	19	67
1971–75	—	1	—	3	1	10	17	9	41
1976–80	—	1	2	—	3	2	7	12	27
1981–84	—	—	—	—	2	4	5	10	21

* Compiled from New Zealand Department of Health *Annual Reports*, 1951–84.

DISCUSSION

Various serological methods have been used to measure antibody to tetanus toxin. These include the toxin neutralization test in guinea-pigs (Barile Hardegree & Pittman, 1970), radioimmunoassay (Habermann, Howath & Schaeg, 1977) and latex agglutination (Booth & Nuttal, 1978). However, these methods have either required the use of animals over several days, expensive equipment, reagents with short shelf-lives, larger quantities of reagents, or long incubation times. The ELISA test described in this paper provides economy of both time (3 h incubation time) and reagents which are used at high dilutions. As the human sera are tested at a dilution of 1/800, non-specific activity is greatly reduced and only very small amounts of sera are required. The incorporation of the biotin-streptavidin system has increased the sensitivity of the test and inter-assay and intra-assay tests confirm the reproducibility of the method.

The New Zealand Health Act 1956 and its subsequent amendments provide for the notification of at least 27 infectious diseases including tetanus (NZ Department of Health Annual Report, 1969). The near twofold reduction in the number of tetanus cases after 1960 in the 1961–5 quinquennium coincided with the intro-

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duction of triple vaccine in 1960 which is available free to general practitioners from the Department of Health. The decline has continued since then.

While neonatal tetanus is rarely seen in New Zealand, which may reflect the high proportion of females of child-bearing age with protective levels of specific antibody, countries in the less-developed world have a significant but usually unknown incidence of the disease. For example, in surveys carried out in three rural areas in Indonesia, there was a disturbingly high level (20–25 %) of infant mortality rate due to neonatal tetanus (Weekly Epidemiological Record, 1986). An easy to perform and sensitive ELISA test of the type described in this paper would be useful to screen pregnant women in these countries for antibodies to tetanus toxoid as part of an assessment of the need for a preventative vaccination programme. This ELISA method is clearly suitable for sero-epidemiological work in general in the control of this disease.

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