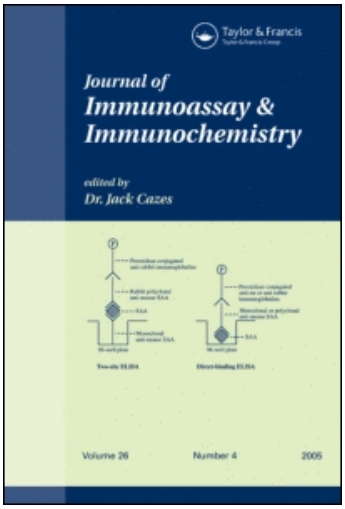

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Evaluation of Repeated Immunoassays (Mouse Intracerebral Potency Tests) of the Second International Standard of Pertussis Vaccine

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EVALUATION OF REPEATED IMMUNOASSAYS (MOUSE INTRACEREBRAL POTENCY TESTS) OF THE SECOND INTERNATIONAL STANDARD OF PERTUSSIS VACCINE

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ABSTRACT

During one year 27 potency assays of second International Standard of Pertussis Vaccine were performed, when the Indian Standard of pertussis vaccine was being standardized. In all the 27 assays, the 50 % immunizing dose (ImD_{50}) was calculated both by Wilson-Worcester method and by probit analysis. The ImD_{50} of the International Standard varied from 0.022 I.U. to 0.076 I.U. (Mean 0.041 I.U.) when it was calculated by Wilson-Worcester method and from 0.0226 I.U. to 0.0704 I.U. (Mean 0.0402 I.U.) when it was calculated by probit analysis. There were no statistically significant differences between ImD_{50} values calculated by Wilson-Worcester method and by probit analysis. The challenge dose of Bordetella pertussis in terms of LD_{50} units had no effect on the ImD_{50} values. The average slope (b) of the assays was 1.92 when calculated by probit analysis and the average homogeneity factor (χ) in these assays calculated by Wilson-Worcester method was 1.73.

(KEY WORDS: Second International Standard of Pertussis Vaccine; Immunoassays)

INTRODUCTION

The intracerebral mouse potency assay for pertussis vaccine developed by Kendrick et al[1] is the only available model that provides a laboratory measure of vaccine potency which, as in the

British Field Trials[2], has been correlated with the clinical efficacy. This type of assay with slight modification has also been recommended for acellular pertussis vaccine developed by Japanese workers[3]. For the potency assay of pertussis vaccine, a reference preparation is always included in each assay. Finney et al[4] analysed the data from various assays in an attempt to exclude the use of reference preparation in each assay as a large part of the expenditure of time, effort and materials in a series of assays is devoted to work with the reference preparation. Finney et al[4] found that the use of reference preparation in an assay can not be omitted by using the average of the ImD_{50} over a series of assays and found about 8 times variations in the ImD_{50} values. Cameron[5] also found similar observations. On the other hand, Maloney[6] found different results accummulated from the data of Bureau of Biologics.

During the standardization of Indian Standard of pertussis vaccine against the International Standard, a total of 27 assays of the latter standard were performed with consistent technique in our laboratory over a period of one year. In most of the developing countries, the ImD_{50} is calculated by Wilson-Worcester method[7] as described in the WHO manual[8] due to non-availability of computer facilities. In this report the variations observed among various assays of the Second International Standard of Pertussis Vaccine have been determined with ImD_{50} calculated by Wilson-Worcester method and by probit analysis.

MATERIALS AND METHODS

International Standard of Pertussis Vaccine

The Second International Standard of Pertussis Vaccine with a unitage of 46 I.U. per ampoule[9] was received from the Statens Serum Institute, Copenhagen, Denmark. The contents of each ampoule were reconstituted to 8 I.U. per ml in 5.75 ml of sterile normal saline. For each assay 1 ml of the reconstituted vaccine was used and one ampoule was sufficient for 5 assays over a period of about 2-3 months. During this period the reconstituted vaccine was stored at 4-8°C.

Mice

Swiss albino mice of the LACA strain weighing 13-15 g of both sexes in equal numbers were used. A total of 20 randomised mice (10 male and 10 female) were used for each dilution.

Intracerebral potency assay

The active mouse protection test (potency assay) was performed in accordance with the recommendation of WHO[10]. Three dilutions of the standard pertussis vaccine in 5 fold steps were prepared in normal saline. The various dilutions contained 0.5 I.U., 0.1 I.U. and 0.02 I.U. per ml. Each dilution was injected intraperitoneally in a volume of 0.5 ml to each of 20 mice. Fourteen days after injection, each mouse was inoculated intrcerebrally with 0.03 ml of suspension of *Bordetella pertussis* 18323 (in 1% casamino acid saline) containing 100,000 organisms. Five fold dilutions of the challenge suspension were also inoculated in normal mice kept alongwith the immunized mice for the calculation of 50% lethal

dose (LD_{50}) of challenge suspension. On the 17th day of each assay 16 healthy mice (8 male and 8 female) were allowed to remain for each diluton and the extra mice were discarded. The deaths were recorded for the next 11 days after selection. The ImD_{50} in each assay was calculated initially by the Wilson-Worcester method[7] with the help of tables described in the WHO manual[8]. After that the ImD_{50} was also calculated by probit analysis for each assay using a programmed Seiko S-301 computer. This analysis was done using the computer facilities of the Takeda Chemical Industries Ltd., Hikari Plant, Japan when one of the authors was under training there.

Statistical analysis

The ImD_{50} values calculated by Wilson-Worcester method and by probit analysis were evaluated by analysis of variance using a programmed Sharp PC-1500 pocket computer. The ImD_{50} values and homogeneity factor (α) and slope (b) were evaluated also by mean, standard deviation (SD), standard error and 95% confidence intervals as recommended recently[11].

RESULTS

Table 1 shows the ImD_{50} values of various assays performed on the Second International Standard of Pertussis Vaccine. The ImD_{50} values varied from 0.022 I.U. to 0.076 I.U. (Mean 0.041 I.U.) showing 3.45 times variations among ImD_{50} values when these were calculated by Wilson-Worcester method. The ImD_{50} values calculated by probit analysis showed 3.12 fold variations with ImD_{50} values ranging from 0.0226 I.U. to 0.0704 I.U. (Mean 0.0402 I.U.). The

TABLE 1.

ImD_{50} values of the Second International Standard of pertussis vaccine.

Expt. No.	ImD_{50} (I.U. X 100) by Wilson-Worcester method	ImD_{50} (I.U. X 100) by Probit method	Challenge dose LD_{50} units
1	2.99(2.23-3.88)	3.23(2.00-5.23)	210
2	6.68(5.41-8.22)	6.28(4.36-9.03)	250
3	6.16(4.81-7.89)	5.57(3.68-8.44)	179
4	2.63(1.73-3.96)	2.63(1.22-5.65)	204
5	3.63(2.75-4.75)	4.46(2.77-7.13)	373
6	2.43(1.89-3.10)	2.57(1.63-4.70)	86
7	3.63(2.75-4.75)	3.71(2.29-5.99)	489
8	5.59(4.08-7.77)	5.03(2.69-9.39)	558
9	3.04(2.40-3.82)	3.69(2.44-5.59)	628
10	3.63(2.68-4.89)	3.57(2.04-6.25)	64
11	3.63(2.68-4.89)	3.87(2.22-6.77)	684
12	3.46(2.42-5.11)	3.41(1.70-6.84)	35
13	3.68(2.91-4.71)	3.53(2.34-5.34)	278
14	7.60(5.85-9.96)	7.04(4.39-11.3)	491
15	5.59(4.25-7.32)	4.79(2.96-7.74)	369
16	4.40(3.08-6.33)	4.57(2.20-9.49)	67
17	4.46(3.39-5.85)	4.23(2.61-6.88)	749
18	4.46(3.39-5.85)	4.23(2.61-6.88)	324
19	4.46(3.39-5.85)	4.23(2.61-6.88)	353
20	3.68(2.91-4.71)	3.53(2.34-5.34)	667
21	5.00(3.95-6.35)	4.74(2.74-7.85)	298
22	5.59(4.25-7.32)	4.79(2.96-7.74)	289
23	5.00(4.15-6.00)	5.00(3.62-6.91)	215
24	2.43(1.89-3.10)	2.57(1.63-4.70)	418
25	2.43(1.89-3.10)	2.57(1.63-4.70)	934
26	2.31(1.70-3.14)	2.37(1.32-4.22)	655
27	2.20(1.70-2.90)	2.26(1.42-4.01)	748
Mean	4.10	4.02	
S.D.	1.40	1.17	
Standard Error	0.27	0.23	
95% Conf. Intervals	3.55 to 4.65	3.56 to 4.48	

Figures in parenthesis show the 95% fiducial limits of ImD_{50} values.

TABLE 2
Analysis of variance between ImD_{50} values obtained by Wilson-Worcester method and by probit analysis.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F
Between calculation methods	0.00001	1	0.00001	1.4
Between experiments	0.00883	26	0.00034	45.7
Residual	0.00019	26	0.000007	
Total	0.00903	53		

LD_{50} values of the challenge dose used in each assay varied from 35 to 934. The challenge dose had no effect on the ImD_{50} values.

There were not much differences in mean, SD, standard error and 95% confidence limits of ImD_{50} values calculated by Wilson-Worcester method and by probit analysis. The results of analysis of variance also did not show statistically significant difference between ImD_{50} values calculated by Wilson-Worcester method and by probit analysis ($P>0.05$). These results are summarized in Table 2.

The slopes of various assays calculated by both the methods i.e. homogeneity factor (α) by Wilson-Worcester method and 'b' by probit analysis are shown in Table 3. The 'b' value was slightly higher than the 'α' value in most of the assays. The 'α' values ranged from 0.97 to 2.59 (Mean 1.73) while the 'b' values varied from 1.20 to 2.85 (Mean 1.92). The mean, SD, standard error and 95% confidence limits for 'α' and 'b' values were very similar.

DISCUSSION

The variability of the intracerebral potency assay of pertussis vaccine is well known[4,5,9,12]. Despite these variations and

TABLE 3
Homogeneity factor (α) and slope (b) of the fitted regression lines calculated by Wilson-Worcester method and probit analysis respectively.

Expt. No.	Homogeneity factor (α)	Slope (b)
1	1.80	1.92
2	2.59	2.53
3	1.97	2.22
4	0.97	1.20
5	1.69	1.95
6	2.05	2.05
7	1.69	1.91
8	1.22	1.47
9	2.20	2.22
10	1.43	1.70
11	1.43	1.65
12	1.07	1.32
13	2.02	2.23
14	1.73	1.95
15	1.63	1.91
16	1.06	1.26
17	1.63	1.92
18	1.63	1.92
19	1.63	1.92
20	2.02	2.23
21	1.93	2.11
22	1.63	1.91
23	2.59	2.85
24	2.05	2.05
25	2.05	2.05
26	1.43	1.59
27	1.82	1.93
Mean	1.73	1.92
S.D.	0.39	0.36
Standard Error	0.075	0.069
95% Confidence intervals	1.58 to 1.88	1.78 to 2.06

its doubtful usefulness in the control of acellular pertussis vaccine[13,14] and even in the whole cell pertussis vaccine[15], this is the only test which showed correlation with protection in children during MRC trials[2]. Moreover it is still the only mandatory potency test for acellular pertussis vaccine[3] and is

widely used in all countries for the control of pertussis vaccines in the absence of any reliable and practical alternative test[13].

In the present study, slightly more than a 3 fold variation was found in the ImD_{50} values in 27 assays performed in one year. The variation was not as high as that found by Finney et al[4]. The variations in the potency values of the Second International Standard of Pertussis Vaccine among various laboratories were about 3 fold[9]. The challenge dose had no effect on the ImD_{50} values (Table 1). For example in experiments No. 11 and 12 the variations among challenge doses were about 20 times whereas the ImD_{50} values for these 2 experiments were similar. The results reported by other workers also do not show any effect of challenge dose on potency values[9,12].

The ImD_{50} values calculated by 2 different statistical methods were similar. The simple Wilson-Worcester method for the calculation of ImD_{50} is quite satisfactory for developing countries, where the more complicated probit analysis method is difficult to follow because of lack of computer facilities. The slopes of the various assays were mostly between 1 and 2 as expected (Table 3). These results are similar to those reported earlier for the same preparation[9]. The slope (b) and the homogeneity factor (α) values calculated by probit analysis and Wilson-Worcester method respectively did not show much difference.

From the results, it is observed that the differences in the ImD_{50} values found in various assays are not as high as reported earlier and the average of various assays could act as a guide for

the further experiments in which the reference may be tested in small number of animals.

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REFERENCES

1. Kendrick,P.L., Eldering,G., Dixon,M.K. and Misner,J. Mouse protection tests in the study of pertussis vaccine. A comparative series using the intracerebral route for challenge. Am. J. Publ. Health 1947;37:803-810.
2. Medical Research Council. Vaccination against whooping cough-Final Report. Br. Med. J. 1959;1:994-1000.
3. Japanese Government Minimum Requirement of Biological Products. Precipitated-purified pertussis vaccine. Ministry of Health and Welfare 1982:129-135.
4. Finney,D.J., Holt,L.B. and Sheffield,F. Repeated estimations of an immunological response curve. J. Biol. Stand. 1975;3: 1-9.
5. Cameron,J. Pertussis vaccine: Control testing problems. In: Manclark,C.R. and Hill,J.C. Eds. International Symposium on Pertussis, November 1-3,1978 held at National Institutes of Health, Bethesda. Washington, D.C. US Government Printing Office. 1979:200-207.
6. Maloney,C.J. In: Discussion of Part 4. In: Manclark,C.R. and Hill,J.C. Eds. International Symposium on Pertussis, November 1-3,1978 held at National Institutes of Health, Bethesda. Washington, D.C. US Government Printing Office. 1979:240.
7. Worcester,J. and Wilson,E.B. A table determining LD_{50} or 50 percent end point. Proc. Nat. Acad. Sci. U.S.A. 1943;29:207-212.
8. WHO manual for the production and control of vaccines-Pertussis Vaccine 1977:BLG/UNDP/77.3 Rev. 1. (Unpublished working document)
9. Seagroatt,V. and Sheffield,F.W. A collaborative assay of the proposed Second Internation Standard for Pertussis Vaccine and of the proposed first British Standard for pertussis vaccine. J. Biol. Stand. 1981;9:351-365.
10. WHO Expert committee on Biological Standardization. Thirtieth Report. Requirements for pertussis vaccine. Tech. Rep. Series 1979;638:60-80.

11. Gardner,M.J. and Altman,D.G. Confidence intervals rather than P values: Estimation rather than hypothesis testing. Br. Med. J. 1986;292:746-750.
12. Murata,R., Perkins,F.T., Pittman,M., Scheibel,I. and Sladky,K. International collaborative studies on the pertussis vaccine potency assay. Part played by the challenge in the mouse protection test. Bull. W.H.O. 1971;44:673-687.
13. Robinson,A., Irons,L.I. and Ashworth,L.A.E. Pertussis vaccine: present status and future prospects. Vaccine 1985;3:11-22.
14. Developments in pertussis vaccine: Memorandum from a WHO meeting. Bull. W.H.O. 1985;63:241-248.
15. Preston,N.W. and Standbridge,T.N. Mouse or Man? Which are pertussis vaccine to protect? J. Hyg. 1976;76:249-256.