Brazilian Journal of Microbiology (2012): 429-431
ISSN 1517-8382

COMPARATIVE ANALYSIS OF THE INTRACEREBRAL MOUSE PROTECTION TEST AND SEROLOGICAL METHOD FOR POTENCY ASSAYS OF PERTUSSIS COMPONENT IN DTP VACCINE

Denise Cristina Souza Matos[1]*, Rugimar Marcovistz,[1] Andréa Marques Vieira da Silva, [1] Wagner Quintilio,[2] Ricardo Amaral Georgini[2]

[1]Laboratório de Tecnologia Imunológica, Bio-Manguinhos, Fundação Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil; [2]Instituto Butantan, São Paulo, SP, Brasil.

Submitted: April 07, 2009; Returned to authors for corrections: January 15, 2010; Approved: June 07, 2012.

ABSTRACT

The aim of this study was to compare the PSPT standardized in-house as an alternative to MPT for potency assays of pertussis component. Statistical analyses have showed similar pertussis potency values when PSPT was compared to MPT. Significant correlation between the potency results obtained by in vivo and in vitro assays was also been observed. Results by PSPT have demonstrated reproducibility and accuracy for potency pertussis control and this approach has been considered promising for use at least during the steps of production.

Key words: DTP vaccine, Pertussis whole cell, enzyme-linked immunosorbent assay, intracerebral mouse protection test.

In recent years serological models have been developed which can be used as a replacement for lethal challenge procedures in potency testing of Diphtheria and Tetanus Toxoid vaccines (3, 4). Russell and Buch (1959) have introduced the concept of three Rs: replacement, reduction and refinement as a starting-point for ethical behavior concerning experimental animals. They stated that researchers should continually examine their research for the possibilities of replacing the use of animals by microorganisms or non-biological material, reducing the number of animals used, or refining the experimental techniques to minimize animal stress or pain endured during an experiment (7). The importance of this approach lies especially in the possibility of combining the maintenance of scientific quality of research and the upholding of ethical principles (2). Improvements of reliability, reproducibility, and safety in the laboratory are also important considerations to replace traditional animal tests. The Mouse Protection Test (MPT) developed by Kendrick and collaborators (1947) is widely performed and still the only mandatory potency assay for the pertussis vaccine quality control (13, 15). Despite of this, it has a number of disadvantages, such as, time consuming, prone to high intra and inter-laboratory variations and poor reproducibility not reflecting natural disease and above all significant animal welfare concern. Nevertheless, it still remains the gold standard to evaluate the potency of the pertussis component from diphtheria-tetanus toxoid and whole cell pertussis vaccine lots (DTPw). In recent years, serological procedures have been

***Corresponding Author. Mailing address:** Laboratório de Tecnologia Imunológica, Bio-Manguinhos, Fiocruz, Av. Brasil, 4365, 21040-360 Rio de Janeiro, RJ, Brasil.; Fax.: 5521-22604727.; E-mail: dmatos@bio.fiocruz.br

developed to permit the replacement for lethal challenge tests, like the MPT, in the potency control of diphtheria and tetanus components of the DTP vaccine (1, 3, 4). In 1994, van der Ark and collaborators developed an enzyme-linked immunosorbent assay, the Pertussis Serology Potency Test (PSPT), as an unconventional method for pertussis potency control. These authors also demonstrated a weak intra and inter laboratory correlation when PSPT results were compared to those from MPT (8).

The PSPT was performed to determine the pertussis antibody concentration (IU/ml) in sera from mice immunized with 10 different lots of DTPw released previously by MPT. Groups of 20 mice were immunized with three dilutions (1:5, 1:25 and 1:125) of either pertussis reference vaccine or vaccine lots under test. Potencies of the pertussis component in DTPw vaccine lots obtained by PSPT were calculated by parallel line assay with log transformation of antibody concentrations using the software provided by Dr. Luís Rodriguez (Public Health Institute, Santiago, Chile) and the results express in IU/ml. The MPT method was performed according to the minimum requirements of production and control to DTP vaccine from the Brazilian Ministry of Health (7) based upon the requirements of WHO (11, 12, 13). Fisher's Exact Test (χ^2) has evaluated the repeatability precision of PSPT values and differences between PSPT and MPT results were assessed by the Wilcoxon signed rank test. The Spearman's rank correlation coefficient was employed to measure the association between PSPT and MPT tests.

The potency values of the pertussis component as assayed by PSPT, represented by geometric means, were similar intra tests within the 95% confidence interval.

The results obtained by χ^2 have demonstrated a very good homogeneity intra tests per lot, and no significant difference ($P \leq 1.23$) has been observed (Table 1).

Table 1. PSPT repeatability precision

	Pertussis potency (IU/ml)									
Lots	1	2	3	4	5	6	7	8	9	10
GMT[a]	12.0	12.0	13.1	17.9	20.2	16.7	12.9	18.2	20.2	16.8
C.I.[b]	10.0 – 11.6	9.6 – 11.5	12.8 - 13.3	17.6 - 18.2	19.8 - 20.4	16.4 - 16.9	12.6 - 13.2	17.9 -18.4	19.8 -20.5	16.4 -17.1
C.V %[c]	10.76	10.54	2.30	2.17	1.88	2.12	3.01	1.74	2.17	2.63
					$P = 1.23$[d]					

[a] Geometric mean titer of seven independent experiments; [b] Confidence interval 95 %; [c] Coefficient of variation (%); [d] Fisher's exact test.

Figure 1A shows the pertussis potency titers of DTPw vaccine lots with values ranging from 12.0 to 20.2 IU/ml (mean 15.8 ± 3.5) by PSPT and from 12.5 to 17.1 (mean 14.6 ± 1.6) IU/ml by MPT. No significant difference (Wilcoxon test $P \leq 0.20$) between the pertussis potency results performed by both the PSPT and the MPT was seen. There was a significant correlation between PSPT and MPT methods (Spearman's r = 0.9, $P = 0.002$) (Fig. 1B).

The results presented herein showed that PSPT demonstrated significant homogeneity intra tests with small confidence interval. The pertussis potency values obtained by the PSPT were similar to those obtained by the MPT, and it was demonstrated a significant correlation coefficient between these results. It was also observed an excellent *in vivo/in vitro* ratio (0.90 ± 0.10). van der Ark and collaborators (1994 and 1996) had already reported a significant correlation coefficient between both the PSPT and the MPT for the pertussis potency of DTP vaccine produced in The Netherlands. They had also noticed a clear relationship between the induction of pertussis IgG antibody measured by PSPT and survival of mice in the case of MPT. This fact confirms the data obtained by van den Berg and collaborators (2001), showing that mice immunized

with whole-cell pertussis vaccine produced mainly IgG isotype antibodies against *B. pertussis* with a higher concentration of IgG1 than IgG2a specific antibodies. Therefore, considering the data presented, we can presume that the antibody response as measured by PSPT perfectly evaluates the relevant pertussis antibody response after DTPw immunization in mice for the vaccine quality control.

In conclusion, the present study suggest that the PSPT is a promising substitute for the MPT though further validation and additional studies should warrant replacement of the MPT by the pertussis serological potency test, at least during the control in process of the pertussis vaccine production. Moreover, PSPT is more reproducible and reduces the chance of re-testing compared to the MPT, allows a reduction in number of mice by at least 20%, and is less distressful to the animals.

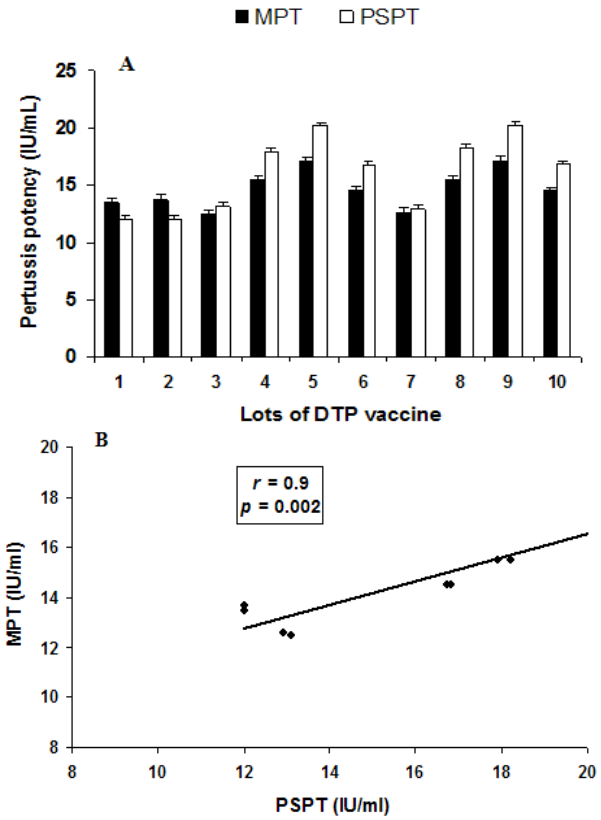

Figure 1. A) The figure shows the pertussis potency mean and ± 3 SD independent experiments of ten DTPw vaccine lots by MPT and PSPT tests. B) Statistical analysis showed a significant correlation coefficient between PSPT and MPT tests.

REFERENCES

1. Hendriksen, C.F.; Woltjes, J.; Akkermans, A.M.; van der Gun, J.W.; Marsman, F.R.; Verschure, M.H.; Veldman, K.A.; van Zutphen, L.F. (1994). Interlaboratory validation of in vitro serological assay systems to assess the potency of tetanus toxoid in vaccines for veterinary use. *Biological* 22, 257-68.
2. Hendriksen, C.F.M. (1996). A short history of the use of animals in vaccine development and quality control. *Dev Biol Stand* 86, 3-10.
3. Marcovistz, R.; Matos, D.C.S.; Cabello, P.H.; Georgini, R.A.; Sakauchi, D.; Leite, L.L. (2002). Potency control of diphtheria component in adsorbed vaccines by in vitro neutralization tests. *Biologicals* 30, 105-112.
4. Matos, D.C.S.; Marcovistz, R.; Cabello, P.H.; Georgini, R.A.; Sakauchi, D.; Leite, L.L. (2002). Immunogenicity test of tetanus component in adsorbed vaccines by Toxin Binding inhibition test. *Mem Inst Oswaldo Cruz* 97, 909-913.
5. Russel, W.M.S.; Burch, R.L. (1959). The principles of humane experimental technique. *Methuen & Co LTD*, London, Methuen.
6. Russell, W.M. (1995). The development of the three Rs concept. *Altern Lab Anim* 23, 298-304.
7. Secretaria de Vigilância Sanitária. (1998). Portaria no. 551. Normas de produção e controle da vacina tríplice (DTP). centro de documentação do Ministério da Saúde, Brasília, Brazil.
8. van der Ark, A.; van Straaten-van de Kappelle, I.; Akkermans, A.; Hendriksen, C.; van de Donk, H. (1994). Development of pertussis serological potency test. Serological assessment of antibody response induced by whole cell vaccine as an alternative to mouse protection in an intracerebral challenge model. *Biologicals* 22, 233-242.
9. van der Ark, A.; van Straaten-van de Kappelle, I.; Hendriksen, C.F.M. (1996). Pertussis Serological Potency Test as an alternative to the intracerebral Mouse Protection Test. "Replacement, reduction and replacement of animal experiments in development and control of biological products" In: F Brown, K Cussler and C Hendriksen, (eds). *Dev Biol Stand, Basel,* Karger, p. 271–281.
10. van den Berg, B.M.; David, S.; Beekhuizen, H.; Mooi, F.R.; van Furth, R. (2001). Protection and humoral immune responses against *Bordetella pertussis* infection in mice immunized with acellular or cellular pertussis immunogens. *Vaccine* 19, 1018-1128.
11. WHO-World Health Organization. (1990). Requirements for diphtheria, tetanus, pertussis and combined vaccines. International Requirements for Biological Substances.World Health Organization, Technical Report Series 800, 87-149.
12. WHO-World Health Organization BLG/95.1.1995. Manual of laboratory methods for potency testing of vaccine used in the WHO Expanded Program on Immunization: introduction to potency control of bacterial vaccines, WHO, Geneve.
13. WHO-World Health Organization TRS 941. 2007. WHO Expert Committee on Biological Standardization, Fifty-sixty report WHO, Geneve.