# Immunological studies on Tetanus Toxoid

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## Abstract

Tetanus toxoid is one of the most successful vaccines used in immunization programme almost all over the world. Neonatal tetanus can be prevented by immunizing women of childbearing age with tetanus toxoid, either during pregnancy or outside of pregnancy. Tetanus vaccine is used either in mono or in combination with other antigens i.e. Diphtheria, Pertussis (whole cell or acellular), Hepatitis B, Haemophilus influenzae B, Inactivated polio vaccine etc. Tetanus toxoid is produced batch-wise using complex media, often containing poorly defined components. Therefore, batch related quality control to guarantee safety and potency is a statutory requirement.

In the new concept, quality control is seen as an instrument to monitor consistency of the critical steps in the production process and testing of vaccines. Monitoring consistency places emphasis on in-vitro methods, since in-vivo tests are less appropriates (expensive, time consuming and inaccurate) for this purpose. Immunochemical techniques may include the use of polyclonal antibodies for direct ELISA or monoclonal antibodies in capture ELISA and immunoblotting to indicate local differences in antigenicity.

There is no uniformity in the potency test of tetanus toxoid. Potency assays in animals may be seen as a way of estimating relative antigen contents parallel to the in- vitro estimations; e.g. by the flocculation tests or the Mancini test. In animal tests, however, it is the ability to provoke production of antibodies (immunogenicity) that is utilized and not just the ability to react with antibodies (antigenicity). This distinction might be carried even further. In challenge tests, the ability to create protection against toxin challenge is the reaction used (protective immunogenicity). In antibody production assays the ability to provoke production of antibodies reacting in a certain antibody detection system is used. In the past, the potency of tetanus toxoid was being expressed in Lf - units. United States Pharmacopoeia prescribed antibody induction method. British Pharmacopoeia, other European countries and World Health Organization recommended active challenge method for assaying the potency of tetanus component. However, Indian Pharmacopoeia prescribed both the methods viz. antibody titration method and active challenge method.

For the potency estimation of tetanus toxoid component in mono-valent or combination vaccines, the challenge test has been in use for many years. Despite the use of large number of animals (> 100 mice or guinea pigs) to test one batch of tetanus toxoid, this test has not been shown to correlate with immunogenicity in humans. However, toxin-neutralizing antibodies induced by the vaccine are generally accepted as correlates of protection. The three 'R's concept for the replacement, reduction and refinement of the use of laboratory animal testing is now widely accepted as not only need for ethical but also for scientific reasons.

The capture ELISA using monoclonal antibodies (characterized by determination of epitope specificity and sub unit specificity) was standardized and designed for assaying the antigenicity of tetanus toxoid bulk and tetanus component in DTP formulations. Statistically, there was a good correlation (Pearson correlation coefficient r = 0.888471) between potencies obtained by capture ELISA and active challenge method in DTP vaccine. There was no significant difference in the potencies of tetanus (bulk) obtained by active challenge method and capture ELISA method (t = 0.7621; tabulated t0.05 = 1.812). The results were also found to be correlating statistically (Pearson's correlation coefficient r = 0.77691).

Efforts have been made to correlate the results of potencies between antibody induction and active challenge methods so that the lethal challenge procedure can be replaced by a serological method, which requires only 7 guinea pigs for immunization and 12 – 15 mice for titration per batch. Based on statistical evaluation good correlation (r = 0.5760) was observed between antibody induction method and active challenge method. The ratio IU : AU was found to be 9.75 : 1.0 and 9.58 : 1.0 for reference preparation and vaccine preparation respectively. On the basis of these results, it is postulated that if ratio of 10 : 1 is considered between IU & AU the minimum requirement of potency for tetanus vaccine to give protection in human subject is 20 IU/dose in terms of challenge method. Finally, it is concluded that antibody induction method is a simple, economical and more reliable method which helps to practice three 'R's concept. In view of the statistically proven factor of relation, in this study, it is also possible to assign tetanus vaccine unitage of potency in terms of IU/ml even if antibody induction method is performed. Antibody induction method gives a true picture to determine the protection afforded by vaccine against tetanus disease.

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