Comparison of Five Commercial Anti-Tetanus Toxoid Immunoglobulin G Enzyme-Linked Immunosorbent Assays†

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Five commercially available enzyme-linked immunosorbent assays for the measurement of anti-tetanus toxoid immunoglobulin G (IgG) antibodies were evaluated for performance. The data suggest that there are manufacturer-dependent differences in sensitivity and accuracy for the determination of tetanus toxoid IgG antibodies that could result in different diagnostic interpretations.

The measurement of anti-tetanus toxoid immunoglobulin G (IgG) levels is of value in (i) determining the rates of immunity within broad populations or the immune status of individuals who may be at risk of infection (15), (ii) assessing responses to vaccination and immunization schedule efficacy (2, 5, 15), and (iii) evaluating individuals for potential immunodeficiency disorders (1, 3).

The World Health Organization guidelines suggest that IgG antibody titers above 0.01 IU/ml afford minimal protection against infection and titers above 0.1 IU/ml provide substantial protection (2).

Significant differences in performance against international reference preparations and clinical interpretation of a number of patient samples have been found previously in commercial anti-tetanus toxoid enzyme-linked immunosorbent assays (ELISAs) (14). The present study confirms these findings, increases the number of commercial assays evaluated, and extends the comparison of their performance in terms of diagnostic interpretations.

Normal human serum samples were obtained from Research Sample Bank, Inc., Pompano Beach, FL, and Golden West Biologicals Inc., Temecula, CA, and stored at −20°C prior to testing. The reference sample NIBSC 76/589 (NIBSC, Hertfordshire, United Kingdom) was used to evaluate the calibration of the ELISA kits. It was chosen because it is correlated against the mouse in vivo neutralization test, the concentration was more appropriate for the measuring ranges of clinically relevant ELISA kits, and it has been used previously (14). For use, NIBSC 76/589 was reconstituted according to the manufacturer’s instructions (working concentration of 1 IU/ml), serially diluted with distilled water to a final concentration of 0.03 IU/ml, and further diluted immediately into the appropriate sample diluents.

Anti-tetanus toxoid IgG antibodies were measured according to the manufacturers’ instructions by using the following ELISA kits, with the measuring ranges in parentheses: Euroimmun, Lübeck, Germany (0.01 to 10 IU/ml); Scimed Corp., Denville, NJ (0.1 to 5 IU/ml); Serion-Verion, Würzburg, Germany (0.1 to 5 IU/ml); and Genzyme Virotech, Rüsselsheim, Germany (0.1 to 5 IU/ml). Results were generated in accordance with the manufacturer’s instructions. Assays were considered valid when quality control parameters were in the range specified in the manufacturer’s product insert. Intraassay precision was measured by using three serum samples (low, medium, and high levels) and assayed in seven-well repeats at the same time. For interassay precision, the same measurements were performed in triplicate over 3 consecutive days. Intra- and interassay precision was assessed by calculating the coefficient of variation. Calibration was assessed by calculating the “recovery” of NIBSC 76/589. To calculate results, values (IU/ml) for serially diluted NIBSC 76/589 were obtained from a calibration curve and compared to the expected values according to the equation (obtained NIBSC value/expected NIBSC value) × 100. The results are expressed as a percentage.

All P values were two tailed, and results were considered significant at a P < 0.05.

The intra- and interassay precision of each kit was calculated (Table 1). The measuring ranges of the Virotech, Serion, and Scimedx assays were only designed to measure antibody levels as low as 0.1 IU/ml. The precision data for Scimedx were limited because the high-level sample values were significantly lower (P < 0.0001) than those obtained in the other four assays. Precision ranged from 3% to 23%, with the lowest values achieved with the TBS (3.2% to 9.8%) and Virotech (3.6% to 10.8%) assays. Poor interassay precision (>20%) was evident with the high-level samples in the Scimedx and Serion ELISAs.

To assess calibration, the recovery of serially diluted NIBSC 76/589 reference material with known values read from the respective calibration material was assessed and expressed as a percentage of the target value (Table 2). The TBS assay (mean recovery, 99.8%), followed by the Serion assay (mean recovery, 90.8%), gave the most consistent and accurate recovery of the reference material across the range tested, with a coefficient of variation (CV) of <12%. Recoveries around the 0.1-IU/ml cutoff were assessed. Only the TBS and Virotech assays showed recoveries within 10% of the target value (103 to 107%, respectively). Only the TBS and Euroimmun assays are...
designed to detect titers below 0.1 IU/ml, and only the TBS assay had a recovery within 10% of the target value.

Levels of antibodies to tetanus toxoid in 94 serum samples were determined and quantified with the five ELISAs (data not shown). In the Euroimmun and Virotech assays, ~70% of the samples gave values of greater than 1.0 IU/ml, compared to values obtained in the TBS and Serion assays, where only ~55% of the samples had values above 1.0 IU/ml. In the Scimedx assay, only 15% of the samples had values of >1.0 IU/ml. The remaining samples all had values below 1.0 IU/ml but above the limit of detection (0.1 IU/ml). Generally, the Euroimmun and Virotech ELISAs gave higher antibody levels than the TBS and Serion assays.

Although it has been suggested that the antibody titer required for adequate protection is 0.1 IU/ml (2), several reports have suggested that quantitation of specific IgG levels between 0.01 and 0.1 IU/ml is also important, as these antibody levels may also provide protection. Wolters and Dehmel reported vaccinating themselves against tetanus toxin (16). The levels of specific IgG antibodies of one of the scientists rose from 0.004 to 0.08 IU/ml postvaccination, which is more than the accepted fourfold increase. Further, this response to vaccination offered protection against a self-administered dose of tetanus toxin corresponding to two to three times the estimated lethal human dose.

Sneath and colleagues suggested that 0.01 U of antibody is the critical level for protection against tetanus toxin in guinea pigs. Although 13% of these guinea pigs showed symptoms of tetanus, all of the guinea pigs with an antibody titer of at least 0.01 U survived (12). Scheibel (8) reported that guinea pigs with a level of 0.01 U/ml anti-tetanus toxoid antibody survived a challenge inoculation with 200 times the minimal lethal dose. It has also been shown that titers of anti-tetanus toxoid antibodies measured in vivo correlate well with the values achieved by using an in vitro ELISA even at titers of <0.01 IU/ml (6, 9, 10).

Both Ramsay and colleagues and Ullberg-Olsson and Eriks-son reported pre- and postvaccination titers of <0.1 IU/ml in

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**Table 1.** Calculated intra- and interassay precision of five commercial ELISAs

| Serum sample | Mean titer (IU/ml) | SD (CV %) |
|--------------|-------------------|-----------|
| Low          | 0.07              | 0.01 (10.89) |
| High         | 4.66              | 0.66 (14.11) |

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**Table 2.** Mean percent recovery from three repeat assays of the NIBSC 76/589 standard in five commercial ELISAs

| Target (IU/ml) or parameter | Recovery (%) |
|-----------------------------|--------------|
|                            | Euroimmun    | Scimedx      | Serion       | TBS           | Virotech      |
| 1.00                        | 97.86        | 118.40       | 106.36       | 101.18        | 124.52        |
| 0.60                        | 77.73        | 70.26        | 97.82        | 97.71         | 127.08        |
| 0.36                        | 55.89        | 41.75        | 85.45        | 92.77         | 106.69        |
| 0.22                        | 50.76        | 62.95        | 85.75        | 102.11        | 109.06        |
| 0.13                        | 41.96        | NA           | 78.68        | 107.06        | 103.74        |
| 0.08                        | 32.04        | NA           | NA           | 90.82         | NA            |
| 0.05                        | 40.32        | NA           | NA           | 112.98        | NA            |
| 0.03                        | 38.17        | NA           | NA           | 93.98         | NA            |
| Mean                        | 54.34        | 73.34        | 90.81        | 99.83         | 114.22        |
| CV                          | 41.53        | 44.15        | 12.22        | 7.59          | 9.44          |

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a To calculate results, values (IU/ml) for serially diluted NIBSC 76/589 were obtained from a calibration curve and compared to the expected value according to the equation (obtained NIBSC value/expected NIBSC value) × 100.

b NA, not applicable.
6 to 12% of the normal pediatric population (7, 13). It has been further suggested that 10% of immunized persons have anti-tetanus toxoid IgG antibody titers of ≤0.01 IU/ml and that the cutoff of 0.01 IU/ml represents true pure immunodominant antibodies that neutralize epitopes, whereas at 0.1 IU/ml there is the possible incorporation of levels of nonneutralizing antibodies (4, 11). The accurate measurement of low levels of anti-tetanus toxoid antibodies (<0.1 IU/ml), even in the presence of a fourfold increase in titer, may be critical in deciding whether or not to reimmunize and assessing potential deficiencies in humoral immunity.

In conclusion, we have performed a study that evaluated the measurement of anti-tetanus toxoid IgG levels by five different ELISAs. Significant manufacturer-dependent differences with regard to calibration and measuring ranges exist between different commercial kits which could result in differing clinical interpretations.

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