Comparison of Three Enzyme-Linked Immunosorbent Assays for Detection of Immunoglobulin G Antibodies to Tetanus Toxoid with Reference Standards and the Impact on Clinical Practice

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Accurate determination of the concentrations of immunoglobulin G (IgG) antibody to tetanus toxoid is important in order to evaluate the immunogenicity of tetanus toxoid vaccines, determine immune competence in individual patients, and measure the prevalence of immunity in populations. The performance of three commercially available enzyme-linked immunosorbent assays (ELISAs) for IgG antibodies to tetanus toxoid were evaluated. Serially diluted NIBSC 76/589 and TE-3 human tetanus IgG immunoglobulin international reference standards were analyzed in quadruplicate using ELISAs manufactured by The Binding Site, Inc. (VaccZyme); Scimedx; and Euroimmun. In addition, IgG antibodies to tetanus toxoid were measured in 83 deidentified serum specimens using each manufacturer’s ELISA. Each ELISA provided linear results when evaluated with the reference preparations. The Binding Site ELISA provided results that closely corresponded to the reference preparations ($y = 1.09x − 0.08$), whereas the Scimedx ELISA gave results that were consistently lower ($y = 0.21x − 0.07$) and the Euroimmun ELISA gave results that were consistently higher ($y = 1.5x + 0.30$) than the reference preparation concentrations. Using the recommended cutoff for each ELISA (<0.10 IU/ml), the overall agreement of all of the ELISA methods was 78%. Three of eighty-three (3.6%) human serum samples demonstrated inadequate immunity with all three assays. The Binding Site ELISA yielded nonprotective antibody concentrations in only these 3 samples, whereas 19 samples (22.9%) according to the Scimedx ELISA and 6 samples (7.2%) according to the Euroimmun ELISA demonstrated nonprotective concentrations. The performance characteristics of ELISAs for tetanus immunoglobulin titers were manufacturer dependent, and the differences translated into important disparities in reported results.

Accurate determination of tetanus toxoid immunoglobulin G (IgG) concentrations is clinically important for evaluating the immunogenicity of tetanus toxoid vaccines (6); determining the immune competence to tetanus in individual patients (5, 8), as part of an evaluation of humoral immune function in general (2); and measuring the prevalence of immunity to tetanus in populations (1, 11).

The gold standard assay for the determination of specific IgG antibodies to tetanus toxoid is the in vivo toxin neutralization test, which is time-consuming, is relatively expensive, is subjective, and raises ethical issues regarding the use of live mammals. The use of accurate and automated in vitro assays is therefore desirable for ethical, clinical, and economic reasons. Moreover, highly reproducible, sensitive, and specific in vitro testing improves the efficiency of the clinical laboratory.

The accurate calibration of these in vitro assays to an internationally recognized reference material is essential for maintaining reproducible and accurate results. The World Health Organization First International Standard for human tetanus immunoglobulin, coded TE-3, was established in 1992, was developed from a pool of 10,628 human plasma donations from Germany, and was calibrated by an international collaborative group from 15 countries representing 15 laboratories.

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**MATERIALS AND METHODS**

Reference materials. NIBSC reagent 76/589 was supplied by NIBSC (Potters Bar, Hertfordshire, United Kingdom) in a lyophilized vial containing 9.2 IU. It was reconstituted in 9.2 ml of sterile distilled water to yield a working concentration of 1 IU/ml. Serial dilutions of NIBSC 76/589 were performed to yield final concentrations as shown in Table 1.

An ampoule of the first International Standard for human tetanus immunoglobulin, coded TE-3, was also obtained from the NIBSC. TE-3 was supplied lyophilized at 120 IU, reconstituted in 1 ml of sterile distilled water to yield a working concentration of 120 IU/ml. It was then further diluted to 10 IU/ml by adding 50 μl of the initially reconstituted solution to 550 μl of sterile distilled water. TE-3 was then rediluted to a starting concentration of 7 IU/ml by adding 350 μl of the previously diluted fluid to 150 μl of sterile distilled water. Serial dilutions of TE-3 were performed to yield final concentrations as shown in Table 1. One set of dilutions was made and tested with all three ELISAs.
calculated by dividing the final result with the expected result and multiplying by the coefficient of variation for the quadruplicate runs. The recovery percentage was reported in the present study. All assays were performed in quadruplicate at each dilution. Serum samples were assayed once in accordance with general laboratory procedures. Each run included high and low control samples (7.2%) according to Euroimmun demonstrated inadequate protective concentrations of IgG antibodies.

### RESULTS

Results of controls provided by all manufacturers’ ELISAs were within acceptable limits (Table 2). Intra-assay imprecision ranged from 1.22 to 16.68% for the Scimedx ELISA, from 0.00 to 9.11% for The Binding Site ELISA, and from 2.96 to 24.62% for the Euroimmun ELISA.

The Binding Site ELISA provided results that closely corresponded to the reference preparations (1.09x – 0.08), whereas the Scimedx ELISA gave results that were consistently lower (0.2x – 0.07), and the Euroimmun ELISA gave results that were consistently higher (1.5x + 0.30) than the reference preparations (Fig. 1). All assays appeared linear, with correlation coefficients of 0.99 (The Binding Site), 0.97 (Scimedx), and 0.98 (Euroimmun). Because the cutoff for adequate immunity is 0.1 IU/ml for each manufacturer’s assay, the results from each of the quadruplicate runs and imprecision at reference values near these clinically important thresholds and at the lower limits of detection are shown in Table 3.

Using the recommended cutoff for each ELISA (<0.10 IU/ml), the overall concordance was 78%, with 62 samples demonstrating adequate anti-tetanus IgG immunoglobulin concentrations. Only 3 of 83 sera demonstrated insufficient immunity with all three assays (Table 3). The Binding Site ELISA yielded insufficient protection in only these 3 samples (3.6%), whereas totals of 19 samples (22.9%) according to Scimedx and 6 samples (7.2%) according to Euroimmun demonstrated inadequately protective concentrations of IgG antibodies.

### DISCUSSION

The performance characteristics of the three different ELISA kits were manufacturer dependent. The differences...
between the assays were manifested as discrepancies in performance against the reference standards and also as variability in the final laboratory results for a number of patient sera that were tested as part of the present study. The results from the Scimedx ELISA were consistently lower than the reference standards, and a relatively high percentage (23%, or 19 of 83) of the tested serum samples yielded nonprotective antibody titers. The Binding Site ELISA correlated most closely with the reference standards and produced the lowest percentage of nonprotective samples (4% [3 of 83]). The Euroimmun ELISA results were consistently higher than the reference standards, and yet nonprotective antibody titers were demonstrated in 7% of the samples (6 of 83). The explanation of this unexpected paradox is unclear but may be related to the relative avidity of the detected antibodies or the presence of an interfering substance in the samples. Other potential reasons for differences between assays may include the accuracy of the assay calibration and the optimization of the assay protocol on the automated ELISA processor.

The NIBSC distributes TE-3, the World Health Organization International Reference Standard that was derived from German human donor plasma and developed in 15 countries. The three ELISAs tested here were manufactured in unique locations (United States, United Kingdom, and Germany), and the patient samples were from the United States (9). Therefore, no geographical bias should affect the results. No other international reference standard for human tetanus immunoglobulin is currently available to our knowledge, nor are we aware of any equivalent human standard that was subject to such rigorous and stringent development criteria.

The World Health Organization has recommended 0.1 IU/ml as the protective level of specific tetanus toxoid IgG with ELISA testing, and the majority of manufacturers (including the three whose products were evaluated here) have adopted

| TABLE 3. Results of 21 samples showing nonprotective antibody levels with any assay and the corresponding values from all three assays* |
|-----------------|-----------------|-----------------|
| Scimedx (40 < 0.10 IU/ml) | Corresponding assay value (40 < 0.10 IU/ml) | Euroimmun |
| <0.10 | 0.07 | 0.05 |
| <0.10 | 2.90 | 3.64 |
| <0.10 | 0.15 | 0.13 |
| <0.10 | 0.89 | 1.29 |
| <0.10 | 0.28 | 0.25 |
| <0.10 | 0.19 | 0.26 |
| <0.10 | 0.14 | 0.08 |
| <0.10 | 0.19 | 0.06 |
| <0.10 | 0.10 | 0.07 |
| <0.10 | 0.11 | 0.12 |
| <0.10 | 0.08 | 0.07 |
| <0.10 | 0.06 | 0.05 |
| <0.10 | 0.31 | 0.80 |
| <0.10 | 0.32 | 1.17 |
| <0.10 | 1.06 | 4.18 |
| <0.10 | 0.25 | 0.84 |
| <0.10 | 0.54 | 2.19 |
| <0.10 | 0.44 | 1.88 |
| <0.10 | 0.62 | 3.63 |
| <0.10 | 2.06 | >10.0 |
| <0.10 | 2.23 | >10.0 |

*Values in boldface represent nonprotective levels. The remaining 62 samples demonstrated protective concentrations with all three ELISAs. The results of controls performed during the same run were as follows (IU/ml): high control (Scimedx), 1.30 (1.0 to 2.0); high control (The Binding Site), 1.61 (1.05 to 1.75); positive control (Euroimmun), 1.08 (1.058 to 1.966); low control (Scimedx), 0.28 (0.2 to 0.5); low control (The Binding Site), 0.26 (0.15 to 0.35); and negative control (Euroimmun), 0.01 (<0.09). Values in parentheses represent the acceptable range.
this recommendation (3). Accuracy of testing near the 0.1-IU/ml level is critical because lower concentrations are interpreted as nonprotective, and vaccination may be recommended.

Although testing for tetanus antitoxin levels is frequently used for academic purposes or to test the immunogenicity of vaccines that are in commercial development, specific recommendations have been issued for their appropriate uses in clinical practice. In the 2006 Recommendations of the Advisory Committee on Immunization Practices, there are several guidelines that describe when testing for serum tetanus antitoxin levels would be warranted. For individuals with a history of a prior Arthus reaction after tetanus vaccination, antitoxin levels can be obtained if the last vaccine was administered at least 10 years earlier (4, 7). Among adults or adolescents who probably received prior vaccination but for whom records are not available, serologic testing may also be considered to avoid unnecessary vaccination (4, 7). In the 2005 Practice Parameters for the Diagnosis and Management of Primary Immunodeficiency, it was stated that the determination of antibody levels to tetanus vaccination are often performed as one component of an evaluation of a specific immune response (2).

In the assessment of immune competence, pre- and postvaccination antibody levels may be compared, and a fourfold increase is generally considered desirable. Accuracy as well as reproducibility at the lower levels is therefore critical when immunodeficient patients are monitored because serial samples may be drawn over time to assess the efficacy of therapeutic immune globulin treatment and the potential development of immune competence.

The present study provides valuable comparative data for laboratories that are evaluating different manufacturers’ products for routine use in their own centers. Each product that was evaluated here performed well relative to internal precision, linearity, and internal controls. However, significant differences were shown relative to international reference preparations. Further work may be needed to confirm these findings, particularly if manufacturing processes or standards evolve or change over time.

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