Validation of a Human Anti-Tetanus Toxoid IgG Immunoassay for Tetanus Hyperimmune Programs

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Background/Case Studies:

Plasma fractionators require quantitative testing for anti-tetanus antibodies in plasma samples collected from individual donors or plasma production pools as part of tetanus hyperimmune programs. This testing serves as a quality control test and helps estimate the antibody potency of the product. There are currently no anti-tetanus antibody assays for this quality control testing. The aim was to validate a quantitative Anti-Tetanus Toxoid IgG enzyme immunoassay kit using an automated enzyme-linked immunosorbent assay (ELISA) processor.

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Analytical sensitivity of the kit was performed by probit analysis on samples.

Study Design/Methods:

Specificity of the VaccZyme Anti-Tetanus Toxoid IgG Immunoassay Kit was determined by testing 20 replicates of sample diluent using the kit on an automated ELISA processor. Analytical sensitivity of the kit performed by probit analysis on samples containing various concentrations of anti-tetanus immunoglobulin (0, 0.375, 0.75, 1.5, and 6 IU/mL) using the 2nd International Standard for Anti-Tetanus Immunoglobulin Human; NIBSC code: 13/240. Each concentration was tested a minimum of 10 replicates for 3 days.The mean, standard deviation (SD), and %CV were calculated for each concentration of all samples in the probit analysis. Linearity of the immunoassay kit was performed by testing samples at various concentrations of anti-tetanus immunoglobulin (0, 8, 16, 24, 32, and 40 IU/mL) by using the 2nd International Standard for Anti-Tetanus Immunoglobulin Human; NIBSC code: 13/240. Each concentration was tested a minimum of 5 replicates. The mean, SD, %CV, and % recovery was calculated for each concentration in the linearity study and the limit of quantitation (LOQ) was also calculated.

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Results/Findings:

The specificity of the assay was acceptable, all 20 replicates of the sample diluent were nonreactive when screened. The probit analysis of the assay determined the 95% LOD to be 1.56 IU/mL. The qualitative accuracy of the assay passed since all anti-tetanus toxoid samples at 3 and 6 IU/mL were detected. The %CV for all anti-tetanus concentrations was ≤ 15% for both intermediate precision and precision within a run. Linear regression analysis demonstrated that r^2 was >0.95, % recovery was between 85 – 115%, and %CV was ≤ 15%. The linear range was determined to be 8 – 40 IU/mL and Limit of Quantitation (LOQ) of the assay was calculated to be 8 IU/mL.

Conclusion:

The data presented shows the successful validation of the Human Anti-Tetanus Toxoid IgG Immunoassay Kit for use on an automated ELISA processor.

Summary of findings:

- **Specificity:** All 20 replicates demonstrated acceptable nonreactive results.
- **Limit of Detection (LOD):** Probit analysis determined the LOD to be 1.56 IU/mL, indicating assay sensitivity detecting low concentrations of anti-tetanus antibodies.
- **Accuracy and Precision:** The qualitative accuracy of the assay was confirmed by detecting all anti-tetanus toxoid samples at concentrations of 3 and 6 IU/mL. The %CV values were ≤ 15% for both intermediate precision and precision within a run, showing the assay's reliability and reproducibility.
- **Linearity:** Linear regression analysis showed an r^2 value exceeding 0.95, indicating a strong linear relationship between assay result and the concentration of anti-tetanus immunoglobulin. The linear range was determined to be 8 - 40 IU/mL.
- **Limit of Quantitation (LOQ):** The calculated LOQ of the assay is 8 IU/mL, indicating the assay's ability to accurately quantify anti-tetanus antibodies at this low concentration.

The validation studies demonstrate excellent accuracy, precision, sensitivity, and linearity of the Human Anti-Tetanus Toxoid IgG Immunoassay Kit providing quality control and estimation of antibody potency in plasma products.

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