Tetanus Antibody in Serum- A variety of assays for antibodies to tetanus and diphtheria toxoids have been introduced. The biological protection assay, introduced by the pioneers in the area, is still the most widely used assay. Other methods include passive hemagglutination assays, precipitation assays, radioimmunoassays, and enzymeimmunoassays. The Medical University of South Carolina began by developing a solid-phase immunoassay using TT immobilized in agarose beads (5). Because the antigenic portion of the toxin and toxoid are identical, TT can be used as the antigenic substrate for the immunoassay, and serum with known amounts of neutralizing antibodies expressed in Units can be used for calibration of the assay. The method used in NHANES III was developed later. The tetanus method used in NHANES III is a solid-phase ELISA in which purified TT is used to coat the microassay plates (5). After adequate washing and blocking of unreacted sites, calibration standards and unknowns are added to the TT-coated plates. The binding of antibody to immobilized TT is directly proportional to the antibody concentration. Serial dilutions of human hyper-tet, a commercial preparation of gamma globulin with known tetanus antibody concentration, are used to calibrate the assay. The amount of antibody is quantitated by adding an identical dilution of a peroxidase-conjugated polyvalent antiserum reactive with all classes of human antibodies. After washing the wells, a substrate is added which degrades peroxidase and acquires a dark green color. The intensity of the color is directly proportional to the amount of peroxidase-labeled anti-human immunoglobulin remaining bound to the plate and to the amount of tetanus antibody bound to the immobilized toxoid, and is quantitated in a colorimeter. A calibration curve is established by using the readings of the calibration standards, and concentrations of antibody in the unknowns are derived from this calibration curve. This EIA is relatively simple to perform and shows remarkable reproducibility with day-to-day run and within-run coefficients of variations of 11.2% and 7.3%, respectively. Because the method has adequate sensitivity to measure samples with low concentrations of antibody (0.032 U/ml), the lower limit of detection is 0.001 U/ml. Normal limits for antitetanus toxin antibodies have not been established. The levels of antitoxin antibodies depend upon a variety of factors, including genetic predisposition, immunization, and frequency of boosting. As a rule, the very young and very old have the lowest levels, and females have lower levels than males.

5. Virella G, Hyman B. Quantitation of anti-tetanus and anti-diphtheria antibodies by enzymoimmunoassay: Methodology and applications. J Clin Lab Anal 1991;5:43.

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