

VETERINARY SERVICES MEMORANDUM DOC. NO. 352

TO: Veterinary Services Management Team
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

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SUBJECT: Conversion Formulas for S/P Ratio to Titer in Diagnostic Test Kit Inserts

I. PURPOSE

The purpose of this memorandum is to provide guidance to interested parties for relating the response obtained in an ELISA immunodiagnostic kit to a titer.

II. BACKGROUND

Antigen/antibody-based immunodiagnostic test kits for the diagnosis of animal disease are evaluated by the Center for Veterinary Biologics (CVB) as authorized by Title 9 Code of Federal Regulations, Part 114.9(f). Typically, immunodiagnostic kits are designed to differentiate positive from negative individuals by the response of their sera in the kit. In many kits, a continuous response is recorded; and the response is then dichotomized using a cut-off point such that values on one side are designated positive and those on the other are negative.

The sample-to-positive ratio (S/P) in an enzyme-linked immunosorbent assay (ELISA) is such a response. A predetermined single dilution of an unknown serum specimen is applied to the plate and the response measured, usually as optical density (OD). This is compared to the response of a reference serum, the positive control (PC). The final value that is calculated is the S/P, the ratio of the unknown's OD to the PC's OD, after correcting both for a blank or negative control.

The instructions for some commercial immunodiagnostic kits contain a formula giving a linear transformation of S/P or $\log(S/P)$ to serum titer, for example:

$$\log(\text{titer}) = a + b \log(S/P)$$

A serum titer, defined as the reciprocal of the greatest dilution in a dilution sequence that produces a response, is valuable when it reflects a meaningful immunological event, such as virus neutralization or hemagglutination. In the absence of such an event, an arbitrary response threshold may be set, below which the response is deemed negative (cut-off

point). Commercial diagnostic kit ELISA titers defined in that way refer only to the test itself and are not directly related to a meaningful immunological event.

The S/P is intended to be a yes or no response that designates a specimen as positive or negative by comparing it to a reference serum. By itself, S/P is not quantitative and not a titer.

Since a binary response is all that is necessary for diagnostic purposes, designing diagnostic assays for S/P is a convenience and only requires a single dilution rather than a dilution series. Usually the S/P-titer relationship is not linear and varies depending on the dilution that is selected for use in the assay. However, if the conditions are defined and one makes certain assumptions, the relationship between titer or $\log(\text{titer})$ and S/P may be approximately linear.

Under these conditions the S/P may be a crude guide to antibody titer and included in the product literature, provided the relationship is justified. This may be included in product literature under the following conditions.

III. ACTION

For new products seeking licensure and currently licensed products wishing to add a formula for conversion of the S/P to titer to the product insert, the formula must be supported by data from appropriate dilution sequences.

Existing products that already have a conversion formula in the product insert may continue to use the conversion formula.

The product insert of all products with a conversion formula should indicate the following:

- The S/P is intended as a diagnostic response to differentiate positive from negative serum specimens.
- The conversion formula may give a rough indication of the specimen's antibody titer. For accurate quantitative assessment, conduct a titration with a dilution sequence.
- The relationship between immunity and either S/P or antibody titer has not been established for this product.

This information may be included in the product literature but may not be used as the criteria for serial release or as validity criterion for establishing that a test run is valid. A consistent formula and positive control are essential in order to compare test results from one time period to another when using the same test kit. Therefore, the formula should not change, and criteria for accepting a new positive control must be established.