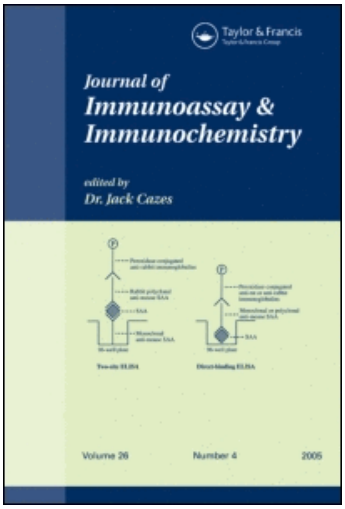

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

The Communication of ELISA Data from Laboratory to Clinician

D. de Savigny[a]; A. Voller[b]
[a] Department of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine, London, England [b] Zoological Society of London, Nuffield Laboratories of Comparative Medicine, London, England

To cite this Article de Savigny, D. and Voller, A.(1980) 'The Communication of ELISA Data from Laboratory to Clinician', Journal of Immunoassay and Immunochemistry, 1: 1, 105 — 128
To link to this Article: DOI: 10.1080/01971528008055779
URL: http://dx.doi.org/10.1080/01971528008055779

JOURNAL OF IMMUNOASSAY, 1(1), 105-128 (1980)

THE COMMUNICATION OF ELISA DATA FROM LABORATORY TO CLINICIAN

D. de Savigny
London School of Hygiene and Tropical Medicine, Department of Clinical Tropical Medicine, Gower Street, London WC1E 7HT, England.
and
A. Voller
Nuffield Laboratories of Comparative Medicine, Zoological Society of London, Regent's Park, London NW1 4RY, England.

ABSTRACT

The data generated by quantitative enzyme-immunoassay of antibody activity are unsuitable for direct communication from the laboratory to clinician. In this paper, the fundamental problems of reporting ELISA results are highlighted and a list of desirable characteristics is set out for such reports. The available methods for processing ELISA data are reviewed in relation to these ideal requirements and the deficiencies and advantages of each method are discussed. The performance of each method is demonstrated by the use of actual test data from a model ELISA system. No single method has been found which satisfies all criteria.

INTRODUCTION

There is a need for reconsideration of the means by which laboratory results of enzyme immunoassays (EIA) are communicated to the clinician. This situation has arisen following the rapid advances in EIA technology in recent years and pertains especially to the enzyme-linked immunosorbent assay (ELISA) for

measuring specific antibody activity. ELISA is currently emerging from the realm of the research tool to that of routine application in the diagnostic laboratory where it is gaining ready acceptance. The majority of published papers on ELISA have been of a highly technical nature and are intended to communicate information between research laboratories and among research workers. However, for routine diagnostic applications, the meaning of the assay result is of paramount importance, for no matter how sensitive and accurate the technique, the result has little value unless it is in a form intelligible to the user, the clinician. In this paper, the commonly used means of communicating laboratory data generated by ELISA are reviewed and illustrated using test data from a model ELISA system. A novel method is also proposed. Our purpose is not so much to offer solutions or to promote any one method but to identify the problems and examine the relative merits in existing methods as a guide to discussion and further work.

MATERIALS AND METHODS

The Model Assay

The Toxocara ELISA system (1) was selected in this work as the model by which to examine the various reporting methods. This assay is known to be highly sensitive, specific and reproducible in its ability to detect toxocaral specific antibody in human serum over a large concentration range. Briefly, antigen, in the form of Toxocara canis larval secretory product, is adsorbed to polyvinyl chloride microtiter plates at a

concentration of 10 ng per well and stored dry. Sera are routinely assayed at one dilution (10^{-3}) for two hours preceding an overnight incubation with alkaline phosphatase labelled anti-human IgG conjugate. Results are recorded as absorbance values (A_{405}) read photometrically following incubation with 4-nitrophenyl phosphate substrate. The timing of the substrate is critically controlled by the use of reference sera such that intra- and inter- assay variation ranges between 5 and 12 CV%.

RESULTS AND DISCUSSION

Comparative Problems of Reporting Antigen and Antibody Concentration by ELISA

Antigen Concentration

The end results of quantitative enzyme immunoassay of antigen or hapten concentration (determined by standard curve method) is self-evident because the result is expressed in absolute terms (e.g. nmol/ℓ). This is an ideal report for clinical purposes, especially when accompanied by the range of normal or pathologic values. The use of the standard curve method in antigen assay is made possible by certain fundamental features of such assays. Firstly, the concentration range of the substance assayed is known and rarely spans more than two $\log_{10}$ dilutions and often is less than one $\log_{10}$ dilution. Secondly, the primary ligand is a single antibody preparation, so the affinity constant remains the same for all tests. Hence, a single standard curve can be prepared and conditions of the test adjusted so that the required

concentration range falls largely within the linear phase of the curve. For greater accuracy this dose-response curve can be transformed to a linear approximation using a choice of mathematical transforms such as logit-log (2), spline-fit (3), third order polynomial (3), or four-parameter logistic curve fit (4). The latest generation of automated and semi-automated ELISA microplate readers have designed-in a capability to accept programmable calculators which are able to compute standard curve parameters and transform ELISA values into concentration units for print-out. The vast experience with standard curve-fitting gained from immunoradiometric (RIA) applications is largely applicable to EIA for quantitation of antigen and will not be further discussed here.

Antibody Concentration

In contrast to antigen quantitation, the measurement of antibody by ELISA is more difficult. The concentration range of specific antibody in pathological sera can span up to six $\log_{10}$ dilutions (Fig. 1). The antibody affinities of different sera, even from the same patient, cannot always be assumed to be identical, thus there are many possible dose-response curves, some of which are non-parallel. To complicate the problem, in tests for a specific antibody response of a particular immunoglobulin class, the dose-response curve can be altered by the presence or absence of responses in the other immunoglobulin classes (Fig. 2). For example, the removal of IgM from a reactive serum can diminish the prozone phenomenon and increase the slope

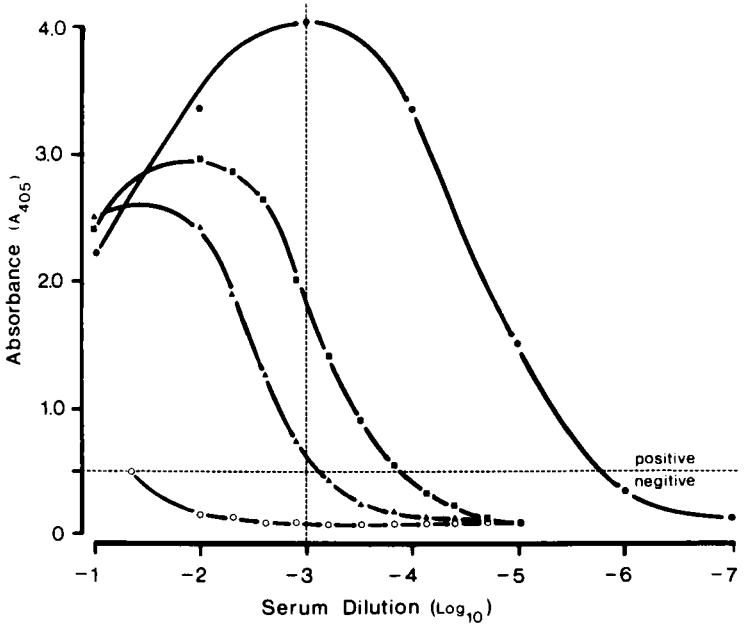

FIGURE 1. Indirect ELISA serum dose-response curves for human IgG anti-Toxocara antibody, illustrating the range of antibody activity that can occur. Intercepts with the vertical line indicate the absorbance values at working serum dilution (10^{-3}). Intercepts with the horizontal line indicate titre.

(●)	Strong Positive	: Titre = 1:800,000	A_{405}	= 4.0
(■)	Moderate Positive	: Titre = 1:8,000	A_{405}	= 1.80
(▲)	Weak Positive	: Titre = 1:1,100	A_{405}	= 0.55
(○)	Negative	: Titre = 1:30	A_{405}	= 0.09

of the specific IgG titration curve (Fig. 2). Furthermore since ELISA measures the combined effects of antibody concentration and antibody affinity (5) the standard curve method has rarely been used to produce results in absolute units (e.g. specific antibody concentration = 70 ng/IgG mℓ). A variety of alternative approaches has evolved to communicate an estimate of relative "antibody activity" rather than absolute concentration. Some are borrowed from traditional serology, while others are unique contributions, but none is entirely satisfactory.

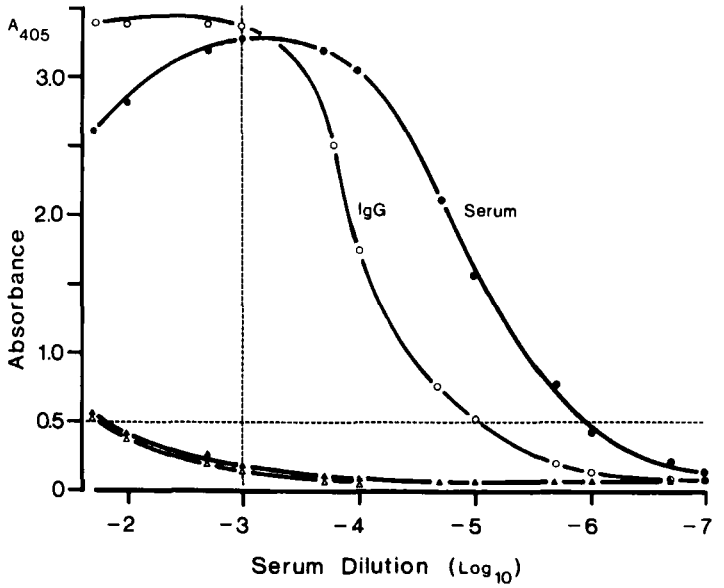

FIGURE 2. IgG anti-Toxocara antibody dose-response curves for positive (●) and negative (▲) sera and for the same sera (○ △) following the removal of IgM by fractionation on Bio-Gel A-5m. The absorbances at the serum working dilution (10^{-3}) remain unaltered, however the slope of the positive dose-response curve increases to produce a ten-fold drop in titre. The prozone phenomenon is also diminished.

Ideal Requirements of ELISA Serological Reports

No serological test can be both 100% specific and 100% sensitive, but it is still reasonable to list ideal characteristics desirable in the presentation of a test result to the clinician.

1. The ideal report must be easily understood by the clinician who will not necessarily be familiar with the laboratory details of ELISA techniques and data products.
2. The report should provide some qualitative information regarding the status of the patient; i.e. whether the specific

antibody activity is "Positive" or "Negative" or, more realistically, "Reactive" or "Non-reactive" or above or within the "normal" range.

3. The logical extension of the "positive" qualitative report is a quantitative report which will provide an index of the severity or recency of infection and will allow serial follow-up of the patient and comparison to other patients.

4. To be ideally quantitative, a scale is needed that is linearly proportional to antibody activity, numerical, continuous (i.e. step-less) and consistent in its unitage and significance.

5. It would be useful if the report is comparative such that it presents a measure of the patient's antibody activity relative to that found in a reference normal or pathologic group.

6. The method of processing the result must be reproducible. Although this is largely a function of technical aspects of the test system, it can be influenced by data-processing of the raw result.

7. Ideally the basis by which the test data are processed to a clinical report should not include assumptions (e.g. gaussian distribution of response values, or parallelism of dose-response curves).

8. Results generated by the ideal method should, in their final form, be adequate for retrospective and prospective sero-epidemiologic analyses.

9. To optimize the efficiency and economy of ELISA as a proper assay, the report should be based on tests at a single serum dilution.

Setting the Positive/Negative Discrimination Level

The discrimination between positive and negative is a crucial element in the design of all assays for specific antibody activity both qualitative and quantitative. Various methods for determining the "positive/negative cut-off" line have been used but the most common method is to set the minimum positive response value (e.g. Absorbance) at two (or three) times the mean absorbance value of the negative group. This method is used only when small numbers of reference normal sera (<10) are available and is not recommended because of inadequate data. When more reference sera (between 10 and 100) are available, authors tend to set the minimum positive absorbance at the mean negative value plus two (or three) standard deviations of the mean. This method is rarely valid because it assumes that ELISA values in the normal population are normally distributed when they, along with most other measures of serum constituents in normal populations, are distributed with a positive skew (1,6). Therefore Type 1 errors (false positives) occur at +2 and +3 S.D. Where absorbance values are not normally distributed, non-parametric methods should be used. The upper limit of normal can then be determined from a minimum of 120 sera from the reference negative group and set at the 97.5th or 100th percentile (7). Confidence intervals can be calculated although appropriate reference groups may be difficult or impossible to obtain. The use of variance ratios in setting the positive/negative level has also been proposed (8).

Methods of Reporting Relative Antibody Activity by ELISA

A listing of the various methods used for reporting ELISA results, each evaluated in terms of the ideal requirements appears in Table 1 with further description below.

TABLE 1

COMMUNICATION OF QUANTITATIVE ELISA RESULTS FOR ANTIBODY ACTIVITY

	DESIRABLE FEATURES										
METHOD	Easily Understood	Highly Quantitative	Numerical Scale	Continuous Scale	Linear Relation with titre	Conversion to I.U.	Comparability	Reproducibility	No Assumptions	Seroepidemiology	Single Serum Dilution
Semi-Quantitative	●							●	●		●
Titration	●	●	●		●	●			●	●	
Absorbance			●	●				●	●	●	●
P/N Ratio	●		●	●			●	●	●		●
MONA	●		●	●			●	●			●
% Positive			●	●			●	●	●		●
Percentile			●	●			●	●	●	●	●
Effective Dose			●	●	●		●				
Standard Curve	●	●	●	●	●	●		●		●	●

Qualitative and Semi-Quantitative Reports

Qualitative reports (e.g. "Positve") and semi-quantitative reports (e.g. Positive +++) have the advantages that they allow visual reading of the test result and that the report is easily understood.

However, visual readings are subjective, rely heavily on the skill of the reader, and may be influenced by the relative frequency of positives and negatives. Even if results are read by photometry or fluorimetry, the qualitative and semi-quantitative reports fail to make full use of the quantitative capacity of ELISA as an assay and are of limited use in the seroepidemiologic comparison of patient groups or in following the time-course of infection.

Quantitative Reports

1. The Titration Method. In the titration method, antiserum under test is serially diluted to the point (titre) at which the specific antibody activity can no longer be detected, hence this method gives a truly quantitative measure of the relative antibody activity and is the standard by which other quantitative methods will be assessed (Table 2). Titres are commonly reported as serum dilutions and expressed as ratios (1:1024) or reciprocals (1024).

Advantages:

- Titres are highly quantitative measures of relative antibody activity.
- Visual readings can be used (see above).
- The concept is easily understood.

TABLE 2

MEANS of COMMUNICATING ESTIMATES of RELATIVE ANTIBODY ACTIVITY from ELISA DATA

RELATIVE SPECIFIC ANTIBODY ACTIVITY	ASSAY at a SINGLE SERUM DILUTION (10^{-3})							TITRATION	
	SEMI QUANTITATIVE	ABSORBANCE (A_{405} nm)	P/N	MONA	PERCENT OF POSITIVE STANDARD	PERCENTILE OF NORMAL POPULATION	UNITS FROM STANDARD CURVE	ED (Log)	TITRE 1 :
WEAK NEGATIVE	Negative	0.09	0.6	0.4	4.5%	0.02	35	-0.37	30
STANDARD NEGATIVE	Negative	0.15	1.0	1.0	7.5%	0.46	90	0	70
WEAK POSITIVE	Positive +	0.55	3.7	12	27.5%	0.98	1100	+1.19	1100
MODERATE POSITIVE	Positive ++	1.75	11.7	107	87.5%	1.00	7010	+1.90	5600
STRONG POSITIVE	Positive +++	3.80	25.3	463	190%	1.00	800,000	+3.89	550,000

Taken from actual ELISA data for human IgG anti-Toxocara antibody activity.

P/N = Positive:Negative Ratio MONA = Multiple of Normal Activity (n = 1.9). ED = Effective Dose

Disadvantages:

- Titres can be inaccurate and variable because ELISA dose-response curves flatten towards titre end-point (Fig. 1).
- A false sense of precision is implied (e.g. 1:1024 has a suggested accuracy of four significant figures).
- Serial dilutions are required, thus technical errors are compounded and reproducibility is only $\pm$ 50% (i.e. one two-fold dilution).
- Titration scales are not continuous, thus the patient is allocated to one of a limited range of discrete quantitative categories (usually less than 12 serial dilutions).
- Titrations are relatively work-intensive and costly of materials and reagents.
- The possibility of confusion exists for the clinician regarding the "significant titre" which varies greatly between assay systems.

2. The Absorbance Method. Workers using ELISA for specific antibody activity commonly express results in absorbance values (e.g. A_{405} nm) when chromogenic substrates are used or in arbitrary fluorescence units when using fluorogenic substrates. However these values should be considered as raw laboratory intermediate data analogous to counts per minute in RIA, and should be further refined before communication to the clinician (9).

Advantages:

- Tests at a single serum dilution only are required.
- No data processing is necessary.
- A continuous numerical scale is used covering a broad range, thus patients can be categorized to one of at least 200 quantitative categories usually with a reproducibility of $\pm$ 10% (CV%).

Disadvantages

- Absorbance values are not linearly proportional to titres (Fig 3a) such that differences between sera with high antibody activities are minimized (i.e. a one log increase of titre is not reflected by a one log increase in absorbance.
- Absorbance values are not readily comprehensible to clinicians.

3. Ratio Methods. Ratio methods include both Positive:Negative (P/N) Ratio and the Percent Positive Method. By the former the specific antibody level of the patient is expressed as a ratio of absorbance units obtained from simultaneous tests on the patient and on the reference negative serum.

In the latter, the patient's result is expressed as a percentage of that simultaneously derived from the positive reference serum or pool. Both methods produce unitless results. P/N Ratios greater than 2 (or 3) are usually considered positive. No such constant feature applies to the Percent Positive Method.

Advantages:

- The ratio automatically compares the patient to the "normal" population (P/N) or positive reference in a quantitative fashion.

- The result is easily understood.
- The need for rigorous quality-control of substrate incubation time is minimized since ratios remain constant throughout substrate incubation (within limits).
- Requires tests at a single-serum dilution only.

Disadvantages:

- P/N: If the negative reference absorbance value is too low (e.g. A <0.10) ratios increase greatly and become difficult to reproduce.
- % Positive: If the positive reference absorbance value is too high the percent positive decreases and becomes difficult to reproduce.
- Neither method is linearly proportional to titre and provides no improvement over the absorbance method in this respect (Fig. 3c).

4. Multiple of Normal Activity (MONA). The Multiple of Normal Activity (MONA) proposed by Felgner (9) is a special case of the ratio method and represents a direct attempt to correct the deficiencies of reporting raw absorbance data from assays at a single serum dilution. To use MONA, the ELISA test system is optimized and the working dilution for test sera is determined. A titration of a standard reference serum is performed and the parabolic relationship of antibody activity and absorbance value is analyzed from the dose-response curve. The parabolic exponent constant (n) is derived from the absorbance values of the positive

reference standard at both the optimal working dilution (A_1) and at a higher dilution (A_2) according to equation [1]

$$[1] \qquad n = \frac{\text{Log Dilution Factor}}{\log A_1 - \log A_2}$$

where the Dilution Factor is the dilution of serum used for A_2 relative to that used for A_1. The parabolic exponent (n) remains constant for the test system and requires re-calculation only if test conditions change (e.g. new conjugate). For use, all subsequent test absorbance values are transformed to multiples of normal activity (MONA) by relating the test absorbance (A_x) to that of the "normal" reference pool (A_{Neg}) tested simultaneously according to either equations [2] or [3] .

$$[2] \qquad \text{MONA} = \text{antilog} \left(n \, (\log A_x - \log A_{Neg}) \right)$$

$$[3] \qquad \text{MONA} = \left(\frac{A_x}{A_{Neg}} \right)^n$$

Advantages:

- MONA compares the specific antibody activity of the patient to that of the "normal" population in a quantitative manner.
- The result is more linearly proportional to titre (Fig. 3d) than is the raw absorbance value or other ratio methods.
- The need for rigorous quality-control of substrate incubation time is minimized.

- Transformation of Absorbance to MONA can be computed automatically.
- Sensitivity is improved.
- Requires tests at a single serum dilution only.

Disadvantages:

- Calculation of (n) is greatly influenced by the choice of positive reference serum used and the portion of the dose-response curve in the calculation.
- MONA assumes dose-response curves of all test sera to be parallel.
- Not valid for high absorbance values (i.e. above the parabolic approximation).

5. Reference - Value/Percentile Method. In this method a large number (>120) of "normal" individuals from an appropriate reference group is tested for specific antibody by ELISA under standard conditions and a frequency distribution of these reference absorbance values is prepared (7). Percentiles of the reference population are determined from the cumulative percent of the population at each absorbance value and a standard curve of percentile vs absorbance value can be prepared. Absorbance values of test sera in subsequent tests can then be transformed to percentiles or P values from the standard curve. An example result would be expressed as "Anti-X antibody activity ELISA value = 0.52 (A_{405}) p = 0.98. Reference Group : Rural Nigerian Adults; n = 900". By comparing percentile markers, subtle seroepidemiologic differences between groups can be detected (1).

Advantages:

- The upper limit of normal can be established with known confidence intervals.
- Results can be expressed relating the patient's level of specific antibody to that found in a carefully selected and defined reference group.
- Useful for seroepidemiologic comparisons.

Disadvantages:

- Requires initial testing of large numbers of reference group subjects.
- Selection of the appropriate reference group can be difficult.
- If the reference group is "normal", only negative patients are quantified while most positive values are reported as >100th percentile or P >1.0 thus no quantitative information is available on those patients for whom it is most needed.
- Rigorous quality control is required.
- Results are not linearly proportional to titre (Fig. 3.b).

6. Effective Dose Method. The Effective Dose Method proposed by Leinikki et al. (10) is a special case of the titration method whereby improvement results from (a) determining the titre from the linear rather than flattening phase of the dose-response curve and (b) expressing the titre as the dilution difference (log distance) between the dose-response curves of the test and reference samples rather than as the dilution of the test serum itself. An example report would be:

Acute Phase Serum : ELISA ED = - 0.96

Convalescent Phase Serum : ELISA ED = + 1.23

Advantages:

- Reproducibility is improved.
- Seroconversion can be detected in serial samples taken less than three days apart (i.e. a change of 0.2 ED).
- Results are linearly proportional to titre (Fig. 3 e).

Disadvantages:

- The method assumes that dose-response curves are parallel to each other and to the standard curve although the authors report that acute and convalescent sera did not show significant changes in parallelism.
- At least three ten-fold dilutions, all near or within the linear part of the curve are required for each test.
- ED values (in Brigg's logs) are difficult to understand on their own.
- ED values fail to convey an impression of relative antibody activity and make it difficult to compare different patients.

Leinikki et al. (11) have recently reported a modification whereby the reference serum is prepared such that it contains a known concentration of IgG antibody purified by antigen-specific immunoadsorption. The test result can then be expressed in terms of absolute concentration (e.g. µg antigen-specific IgG per ml) using the effective dose method. The necessary calibrations can be computed automatically by a programmable calculator linked to the photometer. The method requires testing at more than one

serum dilution but results show a linear correlation with titre.

7. Units from Standard Curve. Experience with the Toxocara ELISA (1) has led to the following attempt to solve reporting problems.

A standard curve can be prepared from computor assisted or manual analysis of routine serum titrations obtained under standard test conditions. Sera (at least 40) representing the complete range of specific antibody activities from weak negative to strong positive are titrated in two-fold dilutions to their respective end-points. The dose-response curves of each serum are plotted on log paper and the titres taken at the serum dilution where the curve intersects with the positive negative cut-off line (Fig. 1). A scatter plot is then prepared by plotting for each serum the absorbance value at the routine test working dilution versus its respective titre end-point. A sigmoidal plot will result with non-uniform variance (scatter) occurring at the higher and lower absorbance values (Fig. 4).

The usual curve-fitting techniques and curvilinear regression (2,3,4) with weighting to correct for the variance (4) can be applied to produce a standard curve or equation which is used to transform ELISA values obtained at the working dilution to titre, international units or other units. This step can be assisted by a programmable calculator linked to the photometer. With careful use of reference standard sera to maintain inter-assay quality control the same standard curve parameters can be

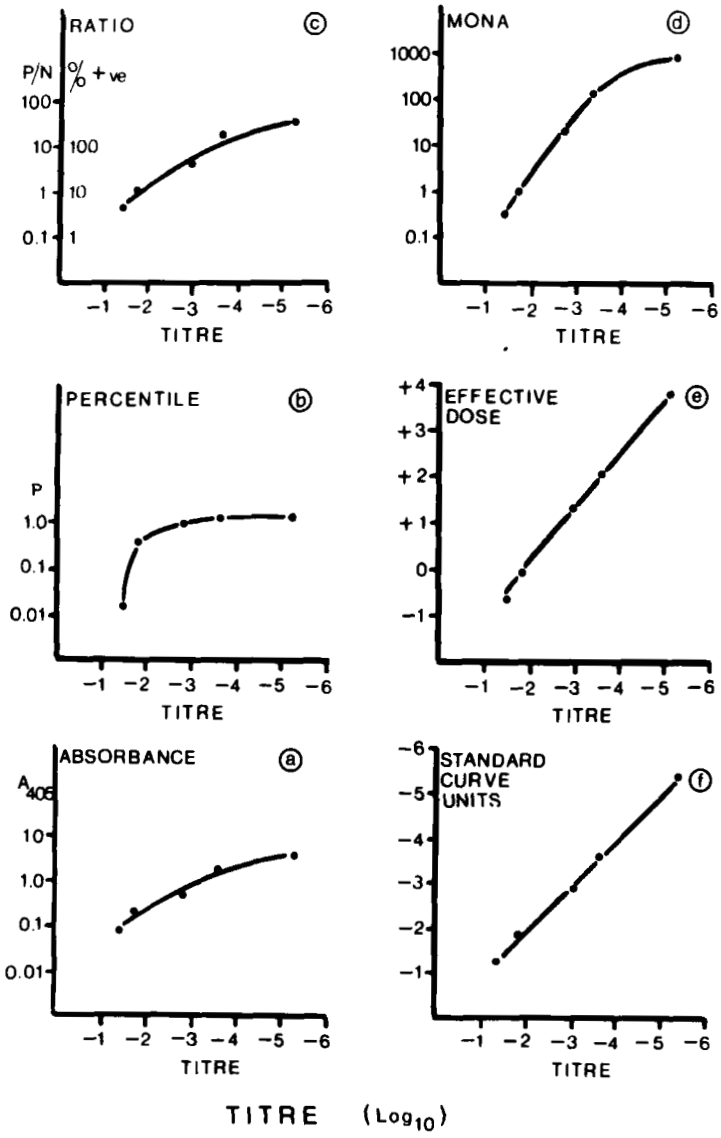

FIGURE 3. ELISA data obtained from tests on five patients with anti-Toxocara titres ranging from weak negative (1:30) to strong positive (1:300,000) and treated in accordance with each of the methods discussed in the text. The data products of each method have been plotted versus titre to illustrate the linear relationship of the results with titre. Correlation (r) and slope (a)

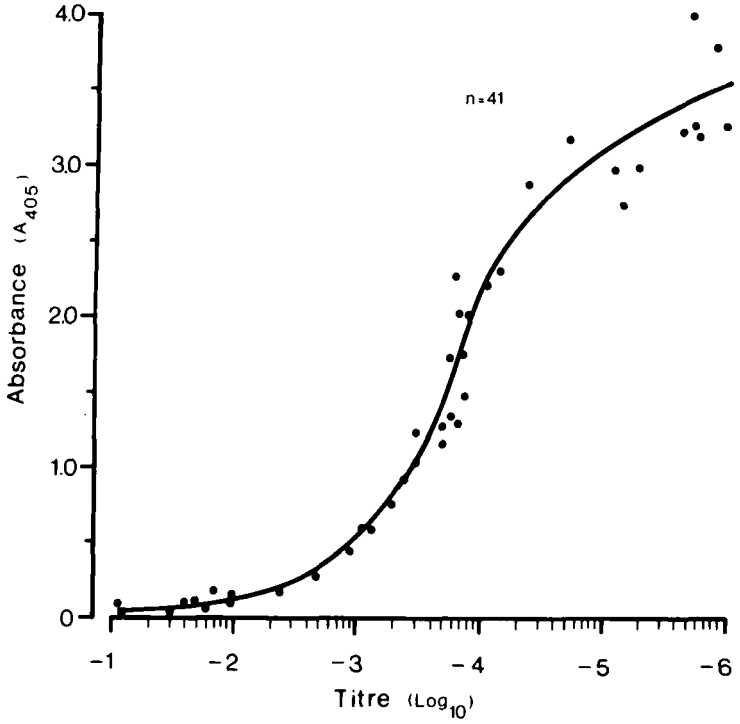

FIGURE 4. Absorbance values recorded at a serum working dilution of 10^{-3} are plotted versus their respective titre end-points for 41 sera with IgG anti-Toxocara antibody titres ranging from 1:12 to 1:1,000,000.

This scatter plot forms the basis of a standard curve relating ELISA value at a single serum dilution (10^{-3}) to serum titre. The accuracy of the curve can be improved by linear transformation by a variety of methods.

Figure 3 Legend Continued

of the calculated linear regression lines are as follows: (note r = 1.0 and a= 1.0 indicates perfect linearly proportional relation to titre).

Absorbance	r = 0.97	a = 0.39
P/N	r = 0.97	a = 0.39
% Pos	r = 0.97	a = 0.39
MONA	r = 0.97	a = 0.74
Percentile	r = 0.66	a = 0.29
E.D.	r = 0.99	a = 0.99
Standard Curve	r = 0.99	a = 1.02

Note: Only E.D. and Standard Curve Units have a satisfactory relation to titre and that MONA improves the linear relationship of P/N ratio.

used daily. If quality control has a record of high standard, this standard curve can be prepared retrospectively using titration data obtained from past assays thus increasing the data base and the validity of the curve.

Advantages:

- Absorbance values are transformed to highly quantitative, easily understood units on a continuous scale.
- Sera are tested at a single serum dilution.
- Results are linearly proportional to titre (Fig. 3f).

Disadvantages:

- Parallel dose-response curves are assumed.
- Rigorous quality-control is required.
- Not valid for high absorbance values.

SUMMARY

The transition of ELISA from use in research applications to routine diagnostic applications necessitates the re-thinking of reporting methods to produce a form of ELISA test result intelligible to clinicians. The quantitation of specific antibody activity by ELISA poses special problems due to the large range of activities being measured, the multiplicity of affinities, the possibility of non-parallel dose-response curves, and to the competitive effects of specific responses in different immunological classes.

In relation to a list of features characterising the ideal serological report (Table 1) no single method was found to satisfy all requirements. The Standard Curve method proposed in this

paper came closest to meeting the requirements however it has not been used in other test systems and cannot yet be recommended. Further efforts will be needed to devise a means of transforming raw ELISA data (absorbance values or fluorescence units) from assays at a single serum dilution to units on a continuous scale, linearly proportional to titre; the object being to provide the clinician with a reliable estimate of the relative antibody activity specific for the antigen in question.

ACKNOWLEDGEMENTS

This paper evolved from work done with financial support from the Ontario Ministry of Health and is based on a presentation prepared for the EEC Workshop on Immunoenzymatic Techniques, Pisa, April 23-27, 1979.

REFERENCES

(1) de Savigny DH, Voller A, Woodruff AW. Toxocariasis: Diagnosis by Enzyme Immunoassay. J. Clin Path 1979; 32:284-88.

(2) Ashton WD. The logit transformation with Special Reference to its Uses in Bioassay. Hainer Publishing Co. New York, 1972.

(3) Marschner I, Erhart F, Scriba PC. In: Radioimmunoassay and Related Procedures in Medicine. Vol. I. I.A.E.A. Vienna, 1974:111-22.

(4) Rodbard D, Hutt DM. In: Radioimmunoassay and Related Procedures in Medicine. Vol. I. I.A.E.A. Vienna, 1974: 165-92.

(5) Butler JE, Feldbush TL, McGivern PL, Stewart N. The enzyme-linked immunosorbent assay (ELISA): A measure of antibody concentration or affinity? Immunochemistry 1978; 15:131-36.

(6) Mainland D. Remarks on clinical "norms". Clin. Chem. 1971; 17:267-74.

(7) Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. Clin. Chem. 1971; 17:275-84.

(8) McLaren M, Draper CC, Robert JM, Minter-Goedblood E, Lighthart GS, et al. Studies on the enzyme-linked immunosorbent assay (ELISA) test for Schistosoma mansoni infections. Annals Trop. Med. Parasit. 1978; 72:243-53.

(9) Felgner P. Stepless antibody determination with the stick-ELISA technique. Results expressed as multiple of normal activity (MONA). Zbl. Bakt. Hyg. I. Abt. Orig. A. 1978; 242:100-05.

(10) Leinikki PO, Passila S. Evaluation of some of the parameters of the enzyme-linked immunosorbent assay. J. Infect. Dis. 1977; 136 (Suppl.): S294-99.

(11) Leinikki PO, Shekarchi I, Dorsett P, Sever JL. Enzyme-linked immunospecific assay determination of specific rubella antibody levels in micrograms of immunoglobulin G per milliliter of serum in clinical samples. J. Clin. Microbiol. 1978; 8:419-23.