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A Review of Enzyme Immunoassay for Detection of Antibody to *Brucella abortus* in Cattle

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**ABSTRACT**

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Enzyme immunoassay has gained wide acceptance for serological diagnosis of bovine brucellosis because of its ability to detect antibody of all isotypes unlike the conventional tests. The indirect enzyme immunoassay, however, presents several parameters that require careful analysis. These parameters include the choice of antigen and antiglobulin-enzyme conjugate reagents for use in the assay, dealing with the large amount of data the semi-automatic or automatic assay can generate and the inter- and intralaboratory standardization and quality control. This review considers the various methods described in the literature and, briefly, how some of the problems have been overcome or how they might be dealt with.

**INTRODUCTION**

Brucellosis is an acute or chronic infectious disease of man and animals. The incidence of human brucellosis in many developed countries is low: 12 and 114 cases were diagnosed in Canada and the U.S., respectively, in 1987 (Canada Diseases Weekly Report, 1988; Morbidity and Mortality Weekly Report, 1988). While infection with *Brucella* in animals has been eradicated in some countries, it is endemic in others, thereby creating serious human health problems and causing considerable economic loss.

Detection of antibody has been a useful tool in the bovine brucellosis eradication programs established by many countries. Because of the cost of these programs, most research into methods of antibody detection has taken place in this species. Research efforts have led to the development of primary binding assays such as the enzyme immunoassay (ELISA) for both antibody and antigen detection in serum and milk. Such work has generally been undertaken to overcome the limitations of conventional serological tests not detecting one
TABLE 1

Reactivity of the four major bovine immunoglobulin isotypes in serological tests (Nielsen et al., 1984a)

| Assay   | IgM  | IgG₁ | IgG₂ | IgA  |
|---------|------|------|------|------|
| SAT     | 20ᵇ  | 125  | 650  | 650  |
| BPAT    | 550  | 8500 | 7500 | 650  |
| Card    | 600  | 7500 | 7500 | 650  |
| Riv     | 650ᵈ | 1550 | 2750 | 650  |
| CFT     | 290  | 290  | 290  | 290  |

ᵃSAT = Standard tube agglutination test (neutral pH); BPAT = Buffered plate antigen test (acid pH); Card = Card test (buffered antigen); Riv = Rivanol agglutination test; CFT = Complement fixation test.
ᵇBased on the minimum weight of affinity purified antibody (ng) required to agglutinate 50% of a bacterial cell suspension.
ᶜNegative using 20 000 ng/test.
ᵈBased on the minimum weight of affinity purified antibody (ng) required to fix 50% of 3CH₅₀ units of guinea pig complement.

or more of the relevant antibody isotypes (Table 1). These limitations require the use of two or more tests to ensure effective (sensitive and specific) serological diagnosis. For example, the complement fixation test (CFT) and the tube agglutination test (SAT), both used as confirmatory tests, fail to detect IgG₂ and IgG₁, respectively (Table 1). These shortcomings can be eliminated by using an indirect ELISA, given careful selection of antigen, anti-immunoglobulin and enzyme-based detection system. In some laboratories the ELISA has been implemented and studies are underway in many other laboratories to determine if these technical improvements can be turned to practical advantage in terms of the cost and effectiveness of diagnostic testing for disease control or eradication programs.

In this review, antigens of *B. abortus*, literature pertaining to various ELISA test methods, antiglobulin reagent selection and application of computer technology to ELISA are described.

BRUCELLA ANTIGENS

A comprehensive review of the antigens of *Brucella abortus* is beyond the scope of this paper (for reviews see Olitsky, 1970; Jones and Berman, 1976; Berman, 1981; Raybould, 1982 and Corbel, 1985).

As indicated in Table 2, a variety of antigen preparations ranging from whole cells to purified components have been used in ELISA for antibody. In most assay procedures, antigen mixtures such as *Brucella abortus* soluble antigen (BASA) have been used, although smooth-lipopolysaccharide (sLPS) was probably the major antigenic component both in terms of antigenicity (im-
**TABLE 2**

Compendium of enzyme immunoassay techniques for detection of bovine antibody and antigens of to *Brucella abortus*

### Antibody detection

| Assay type | Sample | Antigen | Specificity | Sample ref. |
|------------|--------|---------|-------------|-------------|
| Indirect   | Serum  | Whole   | —           | Ruppanner et al., 1980a |
|            |        | cell    | G           | Cargill et al., 1985 |
|            |        | Sonicated | —         | Ruppanner et al., 1980b |
|            |        | γ glob. | G,M         | Oliver and Cooper, 1981 |
|            |        | Autoclaved | γ glob.   | Saunders et al., 1977; |
|            |        |         |             | Heck et al., 1979 |
|            |        |         | G           | Byrd et al., 1979; |
|            |        |         |             | Heck et al., 1980a,b |
|            |        |         | G,G2,M      | Berman et al., 1980 |
|            |        |         | G,G2,M,A    | Butler, 1981 |
|            |        | Protein | G           | Tabatabai and Deyoe, 1984 |
|            |        | LPS     | γ glob.     | Rylatt et al., 1985 |
|            |        |         | G           | Heck et al., 1984; |
|            |        |         |             | Nielsen et al., 1984a,b; |
|            |        |         |             | Wright et al., 1985; |
|            |        |         |             | Dohoo et al., 1986 |
|            |        |         | G1,G2       | Stemshorn et al., 1980 |
|            |        |         | G1,G2,M     | Lamb et al., 1979; |
|            |        |         |             | Nielsen et al., 1983a |
|            |        |         | G1,G2,M,A   | Nielsen et al., 1984a,b |
|            |        | Indirect | Milk       | Van Aert et al., 1984 |
|            |        | Whole   | γ glob.     | Thoen et al., 1979a, 1980 |
|            |        | cell    | G1,G1,A     | Thoen et al., 1983 |
|            |        | Sonicated | G,M         | Oliver and Cooper, 1981 |
|            |        | Autoclaved | γ glob.   | Thoen et al., 1979a, 1980 |
|            |        | Filtered | G           | Heck et al., 1980b |
|            |        | Poly B  | G,G2,A      | Boraker et al., 1981 |

### Antigen detection

| Assay type | Sample | Specificity | Sample ref. |
|------------|--------|-------------|-------------|
| Indirect   | Cultured vaginal secretion | A + M | Thoen et al., 1979b |
|            |        | S2308       | Chen et al., 1984 |
| Indirect sandwich | Blood leukocytes | LPS | Perera et al., 1983 |

*Not described.*
munodominance) and in its ability to adsorb to polystyrene. Another possibility for an antigen is the so-called poly B, a polysaccharide shown to distinguish strain 19 vaccinated animals from *B. abortus* field infected animals by gel diffusion. The chemistry and uses of sLPS and poly B will be discussed below.

Smooth-lipopolysaccharides (sLPS) are long chain molecules consisting of a region of several polysaccharide repeating units (the O-chain), a small chain of a few carbohydrates (the core) and lipids (Lipid A) which bind the complex to the cell wall (Luderitz et al., 1971). For *Brucella*, it is the sLPS-complex which is involved in almost all serological tests (Diaz et al., 1968). Some controversy exists as to whether the sLPS of *Brucella* is complexed to proteins (Perera et al., 1984) or can be isolated (Caroff et al., 1984a). Possibly both groups are correct or, depending on the preparation, the sLPS may be a population of molecules varying in the amount of covalently bound proteins. In either case, sLPS can be purified with only minor amounts of protein (Moreno et al., 1979) to yield a standardized antigen. The sLPS readily adsorbs to plastics (Nielsen et al., 1983b) and has been used in ELISA studies (Lamb et al., 1979; Stemshorn et al., 1980; Schurig et al., 1984; Bundle et al., 1984).

There are two limitations in the use of sLPS as antigen for the enzyme immunoassay and other serological tests. The first is that cattle vaccinated with attenuated *B. abortus* strain 19 have residual antibody specific for sLPS which cannot be distinguished from that resulting from field strain infection (World Health Organization, 1971). The second is that the sLPS of bacteria such as *Yersinia enterocolitica* 0:9 (Ahvonen et al., 1969; Granfors et al., 1981; Lindberg et al., 1982), *Vibrio cholerae* (Sandulache and Marx, 1978), *Escherichia coli* (Nielsen et al., 1980) and *Salmonella* Kauffmann–White Group N (Corbel, 1975) cross-react with *B. abortus*. For the latter, it has recently been determined that the O-chains of both *B. abortus* and *Y. enterocolitica* 0:9 are identical, consisting of a polymer of 4-formamido-4,6-dideoxy-D-mannopyranosyl units (Caroff et al., 1984a,b). *V. cholerae* has an N-acylated derivative of this sugar (Caroff et al., 1984b), *E. coli* 0157:H7 has a tetramer, with one of the carbohydrates being an N-acylated derivative of the above (Perry et al., 1986a), and the O-chain of *Salmonella landau* (Bundle et al., 1986), *S. urbana* and *S. godesberg* (Perry et al., 1986b) have the same derivative but as a pentamer of repeating sugars. It is likely, therefore, that the cross-reaction is due to the common N-acylated 4-amino-4,6-dideoxy-D-mannose in the O-chains of sLPS of the various species of bacteria. Using the cross-reaction between *B. abortus* and *Y. enterocolitica* 0:9 as an example, it may be possible to differentiate serological reactions by the use of specific antigens (Corbel and Cullen, 1970; Mittal and Tizard, 1979; Mittal et al., 1980). Because of its relative ease of preparation, its ability to passively attach to plastics and its immunodominance in the immune response of cattle, LPS is a widely used antigen in the ELISA for *B. abortus* antibody.
Another component of *Brucella* which has a potential use in ELISA is poly B (also called Component 1, second component or PB) a low molecular weight carbohydrate released from the rough strain *B. melitensis* B115 as well as other *Brucella* strains with trichloroacetic acid (Diaz et al., 1968, 1981).

Poly B has received considerable interest because *B. abortus* infected animals produce antibodies which precipitate this carbohydrate while vaccinated animals do not (Diaz et al., 1979; Jones et al., 1980). Its composition is unclear; it is chemically different from native hapten (NH) or acid hapten (AH) (Moreno et al., 1981; Perera et al., 1984); it is purified from rough rather than smooth strains (Jones et al., 1980), it is made within the cell of rough strains and it is immunologically distinct as determined by its inability to absorb out all antibodies to NH (Moreno et al., 1981). However, its similar cathodal mobility to sLPS (Diaz et al., 1968); partial immunological identity with NH (Fernandez-Lago et al., 1982) and partial identity with AH (Moreno et al., 1981) contrast the former differences. Although it does not bind antibodies when adsorbed to polystyrene tubes for an ELISA (Moreno et al., 1981) another interpretation might be that this carbohydrate does not adsorb to polystyrene (Diaz et al., 1984). Clearly more studies are warranted for poly B and its use as a discriminating antigen between vaccinated and infected cattle.

While the ideal of antigen(s) for ELISA diagnosis of *Brucella* infection is far from obvious at the present time, two features should be recalled at the time of decision. Firstly, it is very tedious to prepare a pure antigen totally free of sLPS contamination, and secondly, in addition to all the characteristics described above, the antigen must be one to which all field infected cattle produce a measurable immune response.

**ASSAY TECHNIQUES**

A compendium of ELISA techniques for the detection of bovine antibody to, and antigens of, *B. abortus* is presented in Table 2. All antibody assays described are ‘heterogeneous’ assays requiring immobilization of antigen, usually on a plastic surface (Voller, 1980). This method, suitable for most proteins and sLPS antigens, relies on the hydrophobic interaction between the antigen and the plastic for attachment. The choice of antigens used in the ELISA varies considerably, from whole cells to semi-purified cell components. Nearly all antigens contain sLPS which appears to be of major diagnostic importance in most *B. abortus* assays.

This antigen is now well characterized (Caroff et al., 1984a,b), its preparation relatively simple (Nielsen et al., 1984b), it adsorbs very well to plastics (Nielsen et al., 1983b) and infected cattle (as well as vaccinated animals) appear to preferentially produce antibody to this antigen (i.e., it is immunodominant).
SAMPLE DILUTION

Antibody isotypes produced in response to *B. abortus* infection in cattle include IgM, IgG1, IgG2 and IgA (Rose and Amerault, 1964; Rose and Roepke, 1964; Rice et al., 1966; Rice and Boyes, 1971; Beh, 1973, 1974, 1975; Levieux, 1974a,b; Jones, 1977; Butler et al., 1981). In some cases in the very early antibody response IgM may be the main antibody class found in serum (Rose and Amerault, 1964; Rose and Roepke, 1964; Rice et al., 1964; Beh, 1974) while in other cases the IgG isotypes prevail (Rice et al., 1966; Beh, 1974). The former finding, however, has occurred very infrequently in our experience. All four isotypes of antibody are present in milk (Beh, 1973; Collin, 1976) although IgG1 and/or IgA predominate. These factors have a substantial impact on the choice of dilution of serum or milk to be used in the assay. Although IgM accounts for up to 5 mg protein per ml, the percentage of antibody of a given specificity is low. Therefore a high dilution may cause IgM antibody to be diluted to extinction. For example, at 5 mg per ml and containing 2% antibody to *B. abortus*, a 1:100 dilution would result in the application of 200 ng of IgM antibody in a 200-μl test sample. According to Table 1, 200 ng is detectable; however a 1:200 dilution would place only 100 ng in the test sample, possibly avoiding detection. Conversely, a low serum/milk dilution may result in false positive reactions due to non-specifically reacting IgM and perhaps negative reactions due to increased ‘background’ activity.

ANTIGLOBULIN REAGENTS

The specificity of the antiglobulin to be conjugated with enzyme and used as a detecting reagent in the indirect ELISA is also an important consideration. For a global estimate of antibody activity, a Coomb’s reagent with specificity for all heavy (H) and both light (L) chains could be used. However, this type of reagent would introduce a bias in favour of detection of IgM and therefore likely decrease assay specificity due to the non-specific nature of IgM reactivity. This bias could be reduced by using an anti-IgG (H and L chain) which would still allow for the detection of IgM based on anti-L chain reactivity. It has been our experience that this type of reagent works well when applied to cattle populations where vaccination with *B. abortus* strain 19 is not practised. In the testing of calfhood vaccinates (Fig. 1), we have found this type of reagent to be overly sensitive in the detection of low levels of residual vaccinal antibody, thus lowering or establishing separate diagnostic thresholds for non-vaccinated and vaccinated populations. The types of bias introduced by the use of reagents with H chain specificities could be further reduced by using an anti-L chain reagent (Fig. 1). We are currently using a monoclonal antibody specific for an epitope present on both lambda and kappa L chains. This re-
Fig. 1. Comparison of optical density values observed in the testing of sera (1/100) from 47 cattle vaccinated as calves with *B. abortus* S19; all cattle were greater than 18 months of age at time of testing. Values observed for the mouse monoclonal anti-bovine light chain (closed circles) were sorted by optical density for comparison with a rabbit anti-bovine IgG (H+L) (open circles); both antiglobulins were conjugated with HRPO.

agent has not compromised assay sensitivity but has considerably improved assay specificity, especially amongst vaccinated cattle.

Monospecific reagents may be used where it is desirable to distinguish isotypic responses. In the case of IgM or to partially discriminate vaccinated from infected animals with an anti-IgG1, it may be preferable to use separate assays; however, this obviously also increases the cost. Antibody competition for limited antigenic determinants creates a problem when using monospecific antiglobulins in the indirect ELISA. It has been reported that IgM antibody could not be assessed because of such competition (Lamb et al., 1979). Competition between other isotypes has been reported with other antigens (Townsend et al., 1982). This problem can be overcome to a certain extent by increasing the number of available antigenic sites (however, steric hindrance or detachment may result) or by the use of an amplified ELISA (Butler, 1981). In addition, important consideration should also be given to whether antibody concentration or affinity is being measured (Butler et al., 1978). Perhaps a more appropriate technique for isotype detection is a class-capture ELISA in which anti-immunoglobulin is immobilized and used to capture immunoglobulin which is then reacted with antigen (as described in, for example, Vejtorp, 1981; Yolken and Leister, 1981; Payne et al., 1982; Gustafsson, 1984; Nielsen et al., 1985).

For diagnostic purposes, in laboratories receiving hundreds of thousands of
samples yearly, a test using a single polyvalent antoglobulin reagent such as anti-IgG (H and L) may be preferable due to ease of production and versatility. However, standardization of antoglobulin reagents for long-term diagnostic use will always remain a problem with polyclonal antisera prepared by conventional means. Each batch of antoglobulin will vary to some extent and enzyme conjugation, also susceptible to variations, may result in considerable discrepancies arising. At present, antglobulin reagents are not standard among laboratories (see Table 2) and direct interlaboratory comparison of ELISA data is more difficult than with conventional serological tests. Definitive criteria for performance of enzyme conjugated antoglobulin reagents with respect to analytical sensitivity and specificity may suffice to reduce batch variation and the lack of a universally available reagent. In our laboratory a monoclonal antibody is used to eliminate these difficulties, as the definable product of a cloned cell does not vary if handled properly. There are, unfortunately, two problems with monoclonal antibodies. Firstly, they are fairly expensive to produce, and secondly, the product of a single cloned cell line may be too specific to detect all molecules of a single antibody class, given that genetic variation within a species may occur, thereby requiring a blend of monoclonals. At present we are using a mouse monoclonal antibody to the bovine L-chain conjugated with HRPO as a diagnostic reagent. This reagent has proved stable as a HRPO conjugate for at least 20 months and no detectable differences were observed in conjugating some 25 batches of antibody. The cell line producing this antibody has been frozen and several batches have been thawed and used for preparation of ascites fluid without noticeable discrepancies in activity.

DATA EXPRESSION AND INTERPRETATION OF ANTIBODY ACTIVITY

The end result of any ELISA is the generation of a quantifiable product. The raw data generated from the measurement of a chromogenic product are expressed in optical density (OD) units. These data must then be expressed and interpreted in terms of antibody activity and diagnostic significance. There are several methods by which this can be done and the advantages and disadvantages of these various methods have been compared (De Savigny and Voller, 1980; Cremer et al., 1982; Malvano et al., 1982).

The indirect ELISA is semi-quantitative as a single dilution assay. The most simplistic form of data expression is the OD unit. Diagnostic thresholds have been established through application of statistical methods to OD values observed in the testing of antibody negative and positive reference populations (Heck et al., 1980a; Heck et al., 1982; Sutherland, 1984). In a more arbitrary fashion, threshold limits have been based on multiples of background activity (Tabatabai and Deyoe, 1984; Van Aert et al., 1984). Other thresholds have been determined with respect to percentage activity relative to a standard positive reference (Oliver and Cooper, 1981; Cargill et al., 1985). Thresholds
have also been defined in terms of the ratio of test sample activity relative to a standard negative reference (Saunders et al., 1977; Ruppanner et al., 1980a,b). In competitive assays, threshold optical density values have been determined relative to the ability of positive and negative reference populations to compete with a monoclonal antibody standard (Heck et al., 1984).

We have developed a method of monitoring substrate conversion (Nielsen et al., 1984b; Wright et al., 1985) which permits the direct comparison of optical density values from plate-to-plate and day-to-day. Using this method, a large normal reference population was tested and OD values were arrayed in a frequency distribution. The mean of the 100th percentile was then used as a base for evaluation of the diagnostic performance of the assay (Dohoo et al., 1986). The OD threshold was extended upwards in arbitrary increments until the desired diagnostic specificity and sensitivity were obtained. A flexible threshold is important as the ultimate usefulness of an assay will depend on its diagnostic performance given a particular prevalence of disease.

If laboratory results are to be reported in a qualitative manner (i.e., positive, negative or 'questionable'), then the method of laboratory expression and interpretation is technically simple. However, if quantitative results are to be issued, then there is an obvious need to standardize a method for data expression. This is one of the principle shortcomings of the indirect ELISA technique. No single method of data expression has yet been adopted in brucellosis ELISA serology; however, some laboratories have addressed this problem (Stemshorn et al., 1980) and we are currently engaged in the development of a quantitative method of data expression.

GENERAL DATA ACQUISITION AND MANIPULATION

Because the reading, recording and manipulation of a large number of sample OD values can be a laborious task, automated reading equipment has been developed and linked to computers to expedite the process.

The result has been the development of ELISA protocols based on a variety of reaction vessels for which automated and semi-automated handling and reading equipment is available. This includes systems based on 96-well microtiter plates (Saunders et al., 1979; Ritchie et al., 1981; Richardson et al., 1983; Slezak et al., 1983; Stemshorn et al., 1983; Caulfield and Shaffer, 1984; Nielsen et al., 1984b), plastic cuvettes (Canellas and Karu, 1981; Barlough et al., 1983) and plastic tubes (Saunders et al., 1979; Seawright et al., 1981).

Accompanying the increased sample handling capacity of these systems is the problem of manipulating large amounts of raw data. This can be especially time-consuming if such operations as replicate mean determinations, sigmoidal curve fitting, linear regression analysis and quality control calculations are performed. In some cases the task of data manipulation has been handled through the use of custom computer programs which have been designed to
accept data directly from a plate reader (Ritchie et al., 1981; Richardson et al., 1983; Slezak et al., 1983; Caulfield and Shaffer, 1984) or a cuvette reader (Barragh et al., 1983) or from a magnetic tape storage device on which plate reader output has previously been reported off-line (Saunders et al., 1979; Ritchie et al., 1981; Richardson et al., 1983; Slezak et al., 183; Stemshorn et al., 1983; Nielsen et al., 1984b), or from manual keyboard entry (Gaines Das and Tyde, 1980; Raab et al., 1980; Fey, 1981; Morita et al., 1982).

The problem of data manipulation has also been addressed by manufacturers who offer plate readers with limited built-in ROM based data acquisition and handling routines which allow 'blanking' on specified wells, matrix printouts, flagging of OD readings within specified ranges, etc. Most plate readers feature a built-in serial RS-232-C port (some also have IEEE 488) to allow transmission of OD values to a computer either directly or over telephone lines through a modem. Some newer plate readers also use the serial port to receive computer commands which control plate movement, reading, 'blanking' and filter selection. In addition, some manufacturers offer general ELISA software to support their plate readers when interfaced to the more popular brands of North American microcomputers such as IBM and Apple. There is also a limited amount of ELISA software available from independent software companies. This commercial software typically accepts and files OD data directly from the plate reader, allows the user to specify the well placement of standards, test samples and replicates, and calculates the concentration of unknowns based on linear regression of a standard curve or as some percentage of the concentration of a known positive. Although this software usually offers more data-handling flexibility than the built-in plate reader routines, it is often too general to meet the specific needs of a particular ELISA protocol, especially if that protocol requires the calculation of reaction rates based on multiple time point readings or the calculation of a target time as is required by our ELISA for antibody to Brucella.

DATA ACQUISITION AND MANIPULATION FOR THE EIA FOR ANTIBODY TO BRUCELLA ABORTUS

A simple computer program in support of the indirect ELISA for detection of antibody to Brucella has been described (Nielsen et al., 1984b). This protocol calls for the placement of sample replicates in diagonally opposed quadrants of the microtiter plate with the target reference and quality control sera replicated in all four quadrants. It also requires that the mean OD of the target reference replicates be calculated after 4 min of substrate development and that this figure be used to calculate a target time at which the mean OD of the target reference replicates will reach 1.0 units.

The program is written in BASICA 2.0 and although it is intended to run on an IBM-PC operating under DOS 2.0 or higher, the version presented could
easily be modified for use on other brands of micro- or mini-computers which allow access to the internal clock, support serial communication and are not operating under time-sharing. The required plate reader is a computer-controllable FLOW LABORATORIES Titertek Multiskan MCC which is interfaced through the RS-232-C serial port to transmit data to and receive commands from the computer. All data are in ASCII (American Standard Code for Information Interchange) format. The program controls reader operations such as filter selection, blanking, plate movement and reading. It calculates a target time from a 4-min plate reading and automatically reads the plate again at that target time. Raw OD measurements for each well are then stored on a disk file and the mean, standard deviation (SD) and percent coefficient of variation (%CV) for each replicate sample pair are printed out along with the status (negative or positive) of each sample. The status is dependent on an OD threshold of 0.300 specified within the program. Although the version of the program described does not store the reduced data (i.e., mean, SD, %CV and status) on a disk, this feature is highly desirable if development of a database or further statistical analysis or quality control is required. However, the format to be used when storing these data is largely dependent on the software which in turn will access them. For example, some commercial statistical packages will accept only numeric data which would require omission of the status field. Many types of software, including some spreadsheet, database and graphics packages, will accept data which has been stored in a variety of formats including DIF (Beil, 1983), mailmerge, text-editor and fixed-length record. The format of choice would be one which takes up the least amount of disk space but can still be accessed by available software for further manipulation. (For further information contact Walter Kelly of this Institute.)

DISCUSSION

In the preceding brief review, consideration was given to the factors influencing the development a diagnostic ELISA with particular emphasis on assay performance and interpretation of results. While for the moment this test is very functional, application of research could probably further improve the performance of the test. Presently, a lipopolysaccharide antigen is used; however, at least two other options are being actively researched at the moment. Firstly, the use of the chemically defined O-chain (1,2 linked 4,6 dideoxy-4-formamide-α-D-mannopyranose) which can be synthesized and therefore eliminates any variability encountered with extraction of bacterial antigens. The second alternative, the use of polysaccharide, 'poly-B' as an antigen. An enticing glimpse of its potential has been described in the use of a gel diffusion test to differentiate sera from vaccinated and infected cattle. Until very recently, poly-B was relatively uncharacterized and difficult to purify; however, analysis and purification methods have advanced considerably (Perry et al.,
Research along these lines should lead to data that will eventually change the present ELISA methodology. With the advent of highly refined antigens, the ELISA test procedure itself may be revised to a much more manageable ‘homogeneous assay’ in which an epitope of the antigen is labelled with an enzyme, the function of which may be modulated by the binding of antibody to that epitope; in other words, a single-step assay that may be performed very quickly almost anywhere. Alternately, the application of the above-mentioned antigens to an indirect or competitive type of assay and the use of monoclonal antibody conjugated with enzyme may provide highly specific and sensitive assays which could now truly be standardized. Finally, further developments in cell culture technology may, in the future, allow production of stable monoclonal bovine cell lines that produce antibody of desired specificities. Thus a monoclonal bovine anti-Brucella antibody would greatly enhance the ultimate standardization of an indirect ELISA and could be used in a competitive ELISA (labelled with an enzyme) as well.

Utilization of micro-processors and robotics will in the very near future allow semi- or complete automation of ELISA procedures and with appropriate programming, once the test sample has been placed in the system, there would be no other human contact until after final data evaluation, regardless of the species being tested.

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