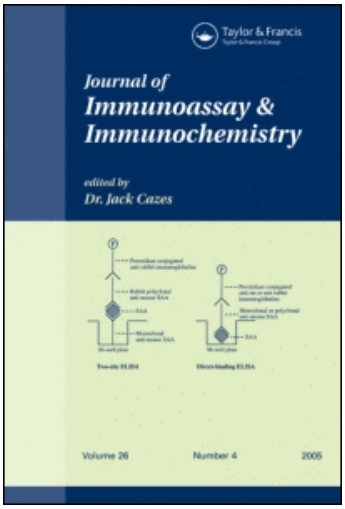


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# Procedure for Selecting Monoclonal Antibodies for use in a Ligand Displacement Assay of Serum Antibody Levels

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# PROCEDURE FOR SELECTING MONOCLONAL ANTIBODIES FOR USE IN A LIGAND DISPLACEMENT ASSAY OF SERUM ANTIBODY LEVELS

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ABSTRACT

Monoclonal antibodies for use in a ligand displacement assay were selected for specificity and affinity/avidity properties that result in their release and displacement in the presence of specific sample antibody but not in the presence of antibodies against other antigens. A screening process is described which involves measurement of displacement of antibody by an ELISA procedure using an enzyme labeled anti-immunoglobulin, providing a means of demonstrating usefulness of a candidate antibody in a ligand displacement format without necessitating the production of enzyme conjugates of each candidate antibody to be screened. The procedure was used to screen a set of eleven monoclonal antibodies (initially selected for anti-Trichinella spiralis specificity by conventional screening methods), and successfully discriminated between antibodies which were useful in the ligand displacement format and those which were not.

(KEY WORDS: enzyme immunoassay, ligand displacement, Trichinella spiralis, trichinosis, monoclonal antibody screening)

## Introduction

The term ligand displacement has been used to describe immunoassays and immunoassay analogues where a labeled substance (usually an antigen) which has been allowed to specifically bind to

a ligand (usually an antibody) attached to a solid phase support is displaced in the presence of an analyte (usually an antigen identical or similar to the labeled antigen used in the assay) which is present in a sample (e.g. a serum sample). Determination of the free, labeled antigen enables calculation of the original amount of displacing antigen (analyte) present in the sample. The assay is thus a modified competitive immunoassay in which all immunological reactants can be bound to the solid support and the assembly can be stored in dry form pending the addition of sample. The format and protocol of the ligand displacement assay minimizes separation and wash steps, and thus makes the assay a particularly suitable candidate for use in dry film format for manual or automated screening of samples. Ligand displacement assays for the detection of antigen have been reported which use either radiolabeled or enzyme labeled components. Ligand displacement radioimmunoassays have been used to measure thyroxin (1) and human chorionic somatomammotropin (2). Ligand displacement enzyme immunoassays and immunoassay analogues have been used to measure histamine (3, 4), serum theophylline (5), and serum or plasma phenobarbital and phenytoin (6). Displacement immunoassays in which labeled antibody is bound to antigen attached to a solid phase support have been less widely reported. Lotwick et al. (7) described a displacement immunoassay for measurement of antibodies specific for acetyl choline receptors and associated with myasthenia gravis.

Antibodies for use in a ligand displacement assay for serum antibody should have specificity and affinity properties that result in their release and displacement in the presence of specific sample antibody but not in the presence of antibodies against other antigens. The present report describes a simple screening procedure, named the indirect ligand displacement assay, which can be used to identify monoclonal antibodies with the desired properties for use in a normal (direct) ligand displacement assay for serum antibodies. From a panel of monoclonal antibodies against Trichinella spiralis antigens, such antibodies were identified and

used in a ligand displacement assay for quantitation of anti-Trichinella antibodies in serum from infected rabbits.

## MATERIALS AND METHODS

### Maintenance of T. spiralis

T. spiralis was maintained in Swiss mice by serial passage at six weeks intervals. The method of Despommier and Lacetti (8) was used to obtain infective muscle larvae ($L_1$) following digestion of eviscerated infected mouse carcasses in a solution of 0.7% pepsin (w/v) in 1% (v/v) HCl. The preparations of infective muscle larvae were either fed to mice (approximately 200-300 larvae per mouse) to initiate a new infection) or were used to make antigen preparations as described below.



### Antigen preparations

Crude T. spiralis antigen was obtained from a commercial source (Difco Laboratories, Detroit, Michigan), or was prepared from a saline extract of muscle larvae. To produce the latter preparation, approximately 30,000 larvae were suspended in 1 ml of phosphate buffered saline (PBS) (0.01M, pH 7.4) and homogenized by ten strokes in a tissue grinder with a mortar driven pestle. The homogenate was centrifuged at 49,000 x g for 1 hour at 4°C to remove particulate debris. The supernatant fluid, containing the extracted antigen, was collected and the protein content was determined by the method of Lowry et al. (9). Crude antigen preparations were stored at -20°C pending use.

Excretory-secretory antigen was produced by culturing infective muscle larvae in tissue culture medium using procedures described by Gamble et al. (10). Antigen released into the culture fluid was concentrated 100 x using an Amicon concentrating chamber with a YM-5 filter under high pressure. Concentrated preparations were dialyzed against PBS and stored at -20°C pending use. Protein content was determined by the Lowry method.

Solubilized ($S_3$) antigen was prepared from homogenized larvae by the differential centrifugation and detergent treatment method described by Despommier and Lacetti (8). The protein content of these solubilized antigen preparations was determined by the Lowry method and the preparations were stored at -20°C pending use.

Production of hybridomas

Balb/C mice were immunized by intraperitoneal injection of crude T. spiralis antigen (Difco) three times at intervals of 10 days. The first injection consisted of 100 μg of antigen in complete Freund's adjuvant, the second injection consisted of 100 μg of antigen in incomplete Freund's adjuvant, and the final injection consisted of 10 μg of antigen in PBS. Cell fusions and selection of hybridoma clones were performed following procedures described by Oi and Herzenberg (11) with slight modification. The myeloma cell line P3 x 63-Ag8.653 was used in the fusions. Lymphocytes from spleens of immunized mice were fused with equal numbers of myeloma cells using polyethyleneglycol (PEG 1000, Sigma, St. Louis, MO). Wells of microwell plates were seeded with $10^6$ cells from the fusion mixture suspended in RPMI 1640 medium supplemented with 15% fetal bovine serum (FBS) and conditioned by overnight exposure to myeloma cells in logarithmic growth (this conditioned medium was used in lieu of feeder cells). On the day following fusion, the conditioned medium was replaced with HAT medium (RPMI 1640 containing 10% FBS, $10^{-4}$ M hypoxanthine, $4 \times 10^{-7}$ aminopterin, and $2 \times 10^{-5}$ M thymidine). Cultures were supplied twice weekly with fresh HAT medium until growing clones were observed microscopically.

Developing hybridomas were initially screened for production of mouse immunoglobulin by enzyme-linked immunosorbent assay (ELISA). Rabbit anti-mouse immunoglobulin (reactive with IgG, IgM, and IgA) was allowed to bind overnight to 96-well polyvinyl microtest plates (Falcon), 0.5 μg/well in 50 μl coating buffer (0.1 M carbonate, pH 9.0, 4°C). Plates were washed with washing buffer (0.13 M NaCl, $1.4 \times 10^{-3}$ M $KH_2PO_4$, 0.01 M $Na_2HPO_4$, $2.7 \times 10^{-3}$ M KCl,

0.5% Tween-20, pH 6.3). Hybridoma supernatants were then added to wells (50 μl/well). After incubation for 1.5 hours at room temperature and washing, 50 μl of a 1:1000 dilution of rabbit anti-mouse immunoglobulin (IgA, IgG, IgM)-alkaline phosphatase conjugate (Sigma) in incubation buffer (0.01 M PBS, 0.05% Tween-20, pH 6.3) was added. After incubation for 1.5 hours at room temperature and washing, bound antibodies were detected by adding 50 μl of the substrate solution (p-nitrophenyl phosphate, 2 mg/ml) in diethanolamine buffer (9.7% diethanolamine, $10^{-3}$ M $MgCl_2$, pH 9.8). The chromogenic reaction was stopped after 30 minutes by the addition of 50 μl of 3 M NaOH and absorbance at 405 nm was measured.

All hybridoma clones that were positive for production of mouse immunoglobulin were next tested to determine whether the antibodies produced were specific for T. spiralis. An ELISA similar to that described above was used, except that wells were coated with 0.5 μg of crude T. spiralis antigen in 50 μl buffer. Negative controls consisted of supernatants from clones not producing mouse immunoglobulin and from hybridomas known to produce antibody not specific for T. spiralis.

Positive hybridomas were cloned and subcloned by the limiting dilution method and the subcloned hybridomas were tested by ELISA for production of antibodies binding to several T. spiralis antigen preparations, including crude antigen, excretory-secretory antigen, and solubilized ($S_3$) antigen. In these ELISA's, wells were initially coated with 0.5 μg of the desired antigen preparation in 50 μl coating buffer. The immunoglobulin isotypes of monoclonal antibodies produced by positive, subcloned hybridomas were determined by ELISA's using mouse isotype-specific enzyme-conjugated antibodies (Southern Biotechnology, Inc.).

Stocks of expanded positive subclones were stored in liquid nitrogen. Ascites tumors were induced in Pristane-primed mice by intraperitoneal injection of 5 x $10^6$ hybridoma cells per mouse. Both ascites fluid from these mice and hybridoma culture supernatants served as sources of monoclonal antibodies used for the remainder of the experiments described.

Polyclonal Antibody

A polyclonal antiserum against T. spiralis was produced in rabbits. An infection was established by feeding 1 x $10^4$ infective muscle larvae. After nine months 800 μg of T. spiralis crude antigen was injected subcutaneously along the neck and back, and a similar injection was given two weeks later. Two weeks after the final injection, blood was drawn from the animals and the serum was collected after clotting had occurred. The reactivity of this antiserum was measured using an ELISA procedure with T. spiralis crude antigen. The quantitated antiserum serum was stored in frozen aliquots pending use.



Indirect Measure of Ligand Displacement

A procedure which will be referred to as the indirect ligand displacement assay (ILDA) was devised and used to screen a panel of monoclonal antibodies for the appropriate properties for use in a direct ligand displacement assay for serum antibody to T. spiralis antigen. The simplest ILDA protocol (hereafter referred to as the "basic ILDA") is proposed as a convenient mechanism for screening. The basic ILDA protocol is presented diagrammatically in Figure 1. Microtiter wells were coated with 0.5 μg of T. spiralis crude antigen. The monoclonal antibody preparation to be screened (typically the supernatant fluid from an actively growing hybridoma clone) was diluted 1/2 to 1/4 in 0.01 M PBS, pH 6.3, containing 0.05% Tween-20, and added to four replicate wells (50 μl per well). For the assay to be most efficient, all antigenic sites present in the wells should be saturated with antibody. Preliminary studies showed that supernatants from hybridomas surviving the first, conventional, screening, could typically be diluted at least 1/4 and still contain sufficient antibody to saturate all antigenic sites. Wells were incubated for two hours at room temperature and washed to remove unbound antibody. To two wells, 50 μl challenge antibody (rabbit polyclonal antiserum against T. spiralis crude antigen) in a dilution of 1/25 (in .01M PBS, pH 7.4) was added and allowed to

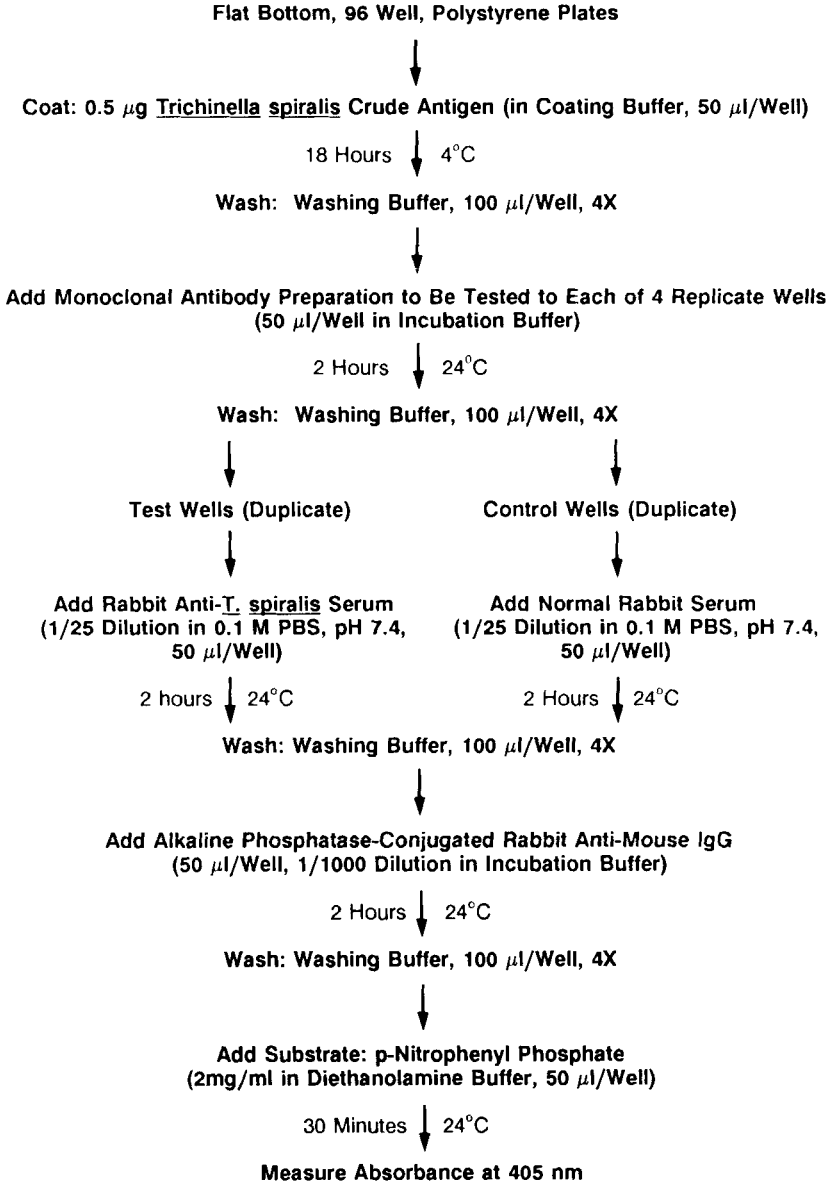


FIGURE 1. Flow diagram of the Basic ILDA protocol

displace bound monoclonal antibody during a two hour incubation. To the remaining two wells, 50 μl normal rabbit serum (diluted 1/25) was added and the wells were similarly incubated for two hours. The wells were washed and 50 μl of rabbit anti-mouse IgG-alkaline phosphatase conjugate (Sigma) was added to each well. After two hours incubation followed by washing, the chromogenic substrate p-nitrophenyl phosphate was added and the amount of enzyme-labeled antibody bound to the plate was determined by measuring absorbance at 405 nm.

In theory, reduction of amount of mouse immunoglobulin bound to wells following challenge with immune rabbit serum compared to that present in wells following challenge with normal rabbit serum would indicate displacement by rabbit anti-T. spiralis antibodies. During development of the indirect ligand displacement assay, it was felt to be necessary to devise a more complete version of the assay to clearly demonstrate that the putative principle of the assay was indeed valid. This ILDA protocol (hereafter referred to as the extended ILDA) is presented diagramatically in Figure 2. A series of wells were coated with T. spiralis antigen followed by candidate antibody as described above. Challenge antibody was added in various dilutions to wells and the wells were incubated for two hours. Challenge antibody was either normal rabbit serum diluted 1/25 in PBS or immune rabbit serum initially diluted 1/25 in PBS with subsequent dilutions made using 1/25 normal rabbit serum as diluent. The supernatants containing displaced antibodies were transferred to other microwells designated as "displacement wells" 1, 2, and 3. Displacement wells 1 and 3 were pre-coated with T. spiralis crude antigen and displacement well 2 was pre-coated with anti-mouse IgG. The original microtiter wells (designated the "challenge wells") were washed and 50 μl rabbit anti-mouse IgG-alkaline phosphatase conjugate (Sigma) was added to each well, followed by incubation, washing, addition of substrate, and measurement of absorbance as described above. Displacement wells 1, 2, and 3 were processed in similar fashion, except that for displacement well 3, a goat anti-rabbit IgG-alkaline phosphatase

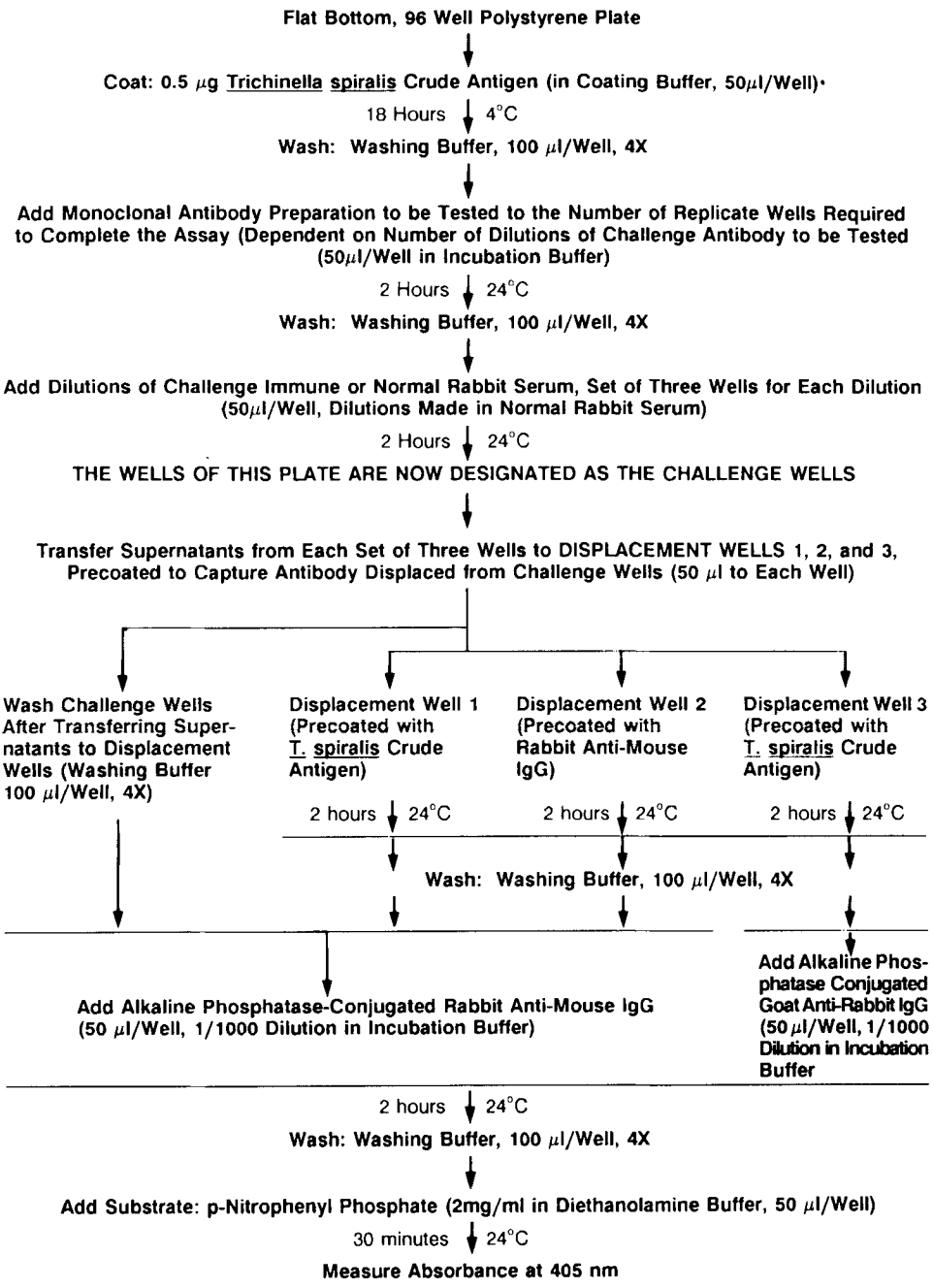


FIGURE 2. Flow diagram of the Extended ILDA protocol

conjugate was used. Displacement wells 1, 2, and 3 were designed respectively to detect mouse IgG against T. spiralis, total mouse IgG, and rabbit IgG present in the transferred supernatants from the challenge wells.

Preparation of Antibody-Enzyme Conjugates

Monoclonal antibodies selected for conjugation with enzymes based on results of the ILDA screening were purified from ascites fluids either by precipitating in half-saturated ammonium sulfate followed by gel filtration, or by separation using a DEAE-Affi-Gel Blue (Bio-Rad) chromatography column. Specific antibody activity of purified preparations was measured by ELISA using T. spiralis crude antigen. Purified monoclonal antibody was conjugated to alkaline phosphatase by a one step glutaraldehyde procedure (12). Conjugates were dialyzed (14,000 M.W. cutoff) overnight at 4°C against 0.05 M Tris, pH 8.0, containing 1 mM $MgCl_2$. Following dialysis, 1.0% bovine serum albumin and 0.02% sodium azide was added and the dialysate was stored in the dark at 4°C. Specific binding activity of conjugates was measured by ELISA.



Direct Ligand Displacement Assays

Alkaline phosphatase labeled monoclonal antibody was used in direct ligand displacement assays of anti-T. spiralis antibody in immune rabbit sera. Labeled monoclonal antibody (50 µl/well in incubation buffer) was allowed to bind to microwells coated with 0.5 µg crude antigen or solubilized ($S_3$) antigen. Labeled preparations were diluted to contain a sufficient concentration of antibody in slight excess of that needed to saturate all antigenic determinant sites as determined be ELISA procedures. After washing, dilutions of rabbit immune and control sera in incubation buffer were added (50 µl/well) and plates were incubated for 1.5 hours at room temperature. The supernatants containing any displaced material were transferred to wells of an empty (uncoated) microwell plate and the amount of displaced antibody measured by adding 50 µl p-

nitrophenyl phosphate substrate per well and measuring absorbance at 405 nm after 30 minutes.

## RESULTS

Developing hybridoma clones were observed microscopically in 160 (20%) of 600 wells. Of these, 83 (52%) tested positive by ELISA for production of mouse immunoglobulins. The antibodies produced by these clones were tested for binding to T. spiralis crude antigen, and 15 (18%) were positive. These hybridomas were cloned and subcloned, then tested for production of antibodies binding to T. spiralis crude, solubilized ($S_3$) and excretory-secretory antigen. The isotypes of antibodies produced by these subclones were also determined.



Table 1 summarizes the characteristics of monoclonal antibodies produced by seven subclones, including four producing IgM and four producing $IgG_1$. Subclones 1P8D6E6 and 1P8D6F10 were obtained from the same original hybridoma (1P8D6). The differences in binding to excretory-secretory antigen suggest that these hybridomas represent two separate clones, not simply the result of isotype switching in vitro. The next step in screening these monoclonal antibodies for suitability for use in a ligand displacement format for quantitation of anti-T. spiralis antibodies in infected animal sera would typically require the production of conjugates of each candidate antibody with a suitable indicator enzyme (e.g. alkaline phosphatase), and evaluating each candidate conjugate in a standard (direct) ligand displacement assay. The production of such conjugates, especially where a large number of candidate antibodies must be screened, would be laborious and costly, hence the reason for exploring feasibility of screening with an indirect ligand displacement assay (which would avoid the need for producing a series of conjugates. Antibodies produced by each of the developmental hybridoma subclones listed in Table 1 were screened using the basic ILDA. The results of this screening are presented in Table 2. Based on reduction of the ELISA values of wells following challenge with immune serum, antibodies 2P5B7D7,

TABLE 1.

Binding of Developmental Monoclonal Antibodies to Microwell Plates Coated with Trichinella spiralis Antigen Preparations

| Hybridoma Subclone | Immunoglobulin Isotype | Antigen Preparation | | |
|---|---|---|---|---|
| | | Solubilized ($S_3$) | Excretory-Secretory | Crude |
| 1P8G3F4 | IgM | >1.999[1] | .352 | 1.708 |
| 1P7E4F2 | IgM | .555 | .032 | .252 |
| 1P8F8C4 | IgM | >1.999 | .321 | 1.452 |
| 1P8D6E6 | IgM | >1.999 | .219 | 1.253 |
| 1P8D6F10 | $IgG_1$ | >1.999 | 1.985 | 1.746 |
| 2P5B7D7 | $IgG_1$ | >1.999 | >1.999 | 1.598 |
| 2P5E7B7 | $IgG_1$ | .548 | .046 | 1.036 |
| 2P5F6C4 | $IgG_1$ | >1.999 | 1.878 | 1.738 |
| 1P7E3 | None | .029 | .005 | .008 |
| 3H5-1[2] | $IgG_1$ | .020 | .010 | .012 |
| IgM Blank[3] | - | .003 | .003 | .006 |
| IgG Blank[3] | - | .002 | .008 | .006 |

1. Figures represent absorbance at 405nm, average of two wells
2. American Type Culture Line HB 46. Produces antibody specific for Dengue Virus Type 2.
3. Blank wells were not pre-coated with immunoglobulin, but were incubated with antigen, alkaline-phosphatase-labeled anti-IgM or anti-IgG, and substrate.

TABLE 2

Results of Screening of Candidate Monoclonal Antibodies Using the Basic ILDA

| Monoclonal Antibody | ELISA Value of Wells Challenged With | |
|---|---|---|
| | Normal Rabbit Serum (1/25) | Immune Rabbit Serum (1/25) |
| 1P8G3F4 (IgM) | 1.458* | 1.376 |
| 1P7E4F2 (IgM) | .837 | .109 |
| 1P8F8C4 (IgM) | 1.200 | 1.118 |
| 1P8D6E6 (IgM) | 1.119 | 1.014 |
| 2P8D6F10 ($IgG_1$) | 1.440 | 1.322 |
| 2P5B7D7 ($IgG_1$) | 1.213 | .157 |
| 2P5E7B7 ($IgG_1$) | 1.078 | .612 |
| 2P5F6C4 ($IgG_1$) | 1.471 | .462 |

* Absorbance at 405 nm, average of two wells



2P5F6C4, and 1P7E4F2 were selected as candidates for further development for use in a direct LDA, as they showed the greatest indication of displacement by the challenge antibody. The remaining antibodies showed minimal to intermediate displacement, indicating that they were probably not as likely to be useful in a direct LDA.

Two antibodies were selected for use in an extended ILDA (as stated above, to demonstrate that the putative principle of the ILDA was valid). Antibody 2P5B7D7 was selected as a probably suitable (i.e. displaceable) candidate and 1P8D6F10 was selected as a probably non-suitable (i.e. non-displaceable) candidate. The results of the extended ILDA using these two antibodies are presented in Figure 3. The amount of monoclonal antibody remaining

in the challenge plate after challenge with rabbit polyclonal antibody is shown in Figure 3a. Antibody 2P5B7D7 was displaced to varying extent by dilutions of polyclonal antibody up to 1/400, whereas 1P8D6F10 showed little evidence of displacement over the range of dilutions of polyclonal antiserum used.

Based on these results it would be assumed that supernatants from the 2P5B7D7 plates should contain displaced antibody in amounts varying inversely to the dilution of polyclonal challenge antibody, and that supernatants from the 1P8D6F10 plates should contain a low, relatively constant level of displaced antibody (displaced by the washing procedure itself, or by non-specific displacement by normal rabbit serum). The results in displacement well 2 (Figure 3c), where antibody in the supernatant was captured by anti-mouse IgG bound to the wells, confirm this assumption, since the greatest amounts of displaced antibody 2P5B7D7 were found in supernatants from plates challenged with a 1/25 dilution of polyclonal antibody. In displacement well 1 (Figure 3b), where monoclonal antibody was captured by crude T. spiralis antigen bound to the wells, supernatants from wells challenged with the lowest dilution of rabbit polyclonal antiserum appeared to have the least displaced 2P5B7D7 antibody, with increasing amounts up to a polyclonal challenge dilution of 1/200, with a drop at higher dilutions. The level of 1P8D6F10 in supernatants is nearly the same or less than that of the baseline level found following challenge with normal rabbit serum, but like 2P5B7D7, it was apparently found in least amounts where the challenge plate was challenged with a 1/25 dilution of polyclonal antibody. When such results were first obtained it was reasoned that they could be explained on the basis of the presence of an excess of rabbit anti-T. spiralis antibodies (which would hence remain unbound) in the supernatants from the challenge plate. Since in displacement well 1 capture of displaced antibodies is a result of binding to T. spiralis antigen, the rabbit antibodies would compete with any displaced mouse antibodies for binding sites, reducing the apparent amount of mouse antibodies present. The results for displacement well 3 (Figure 3d), differing from

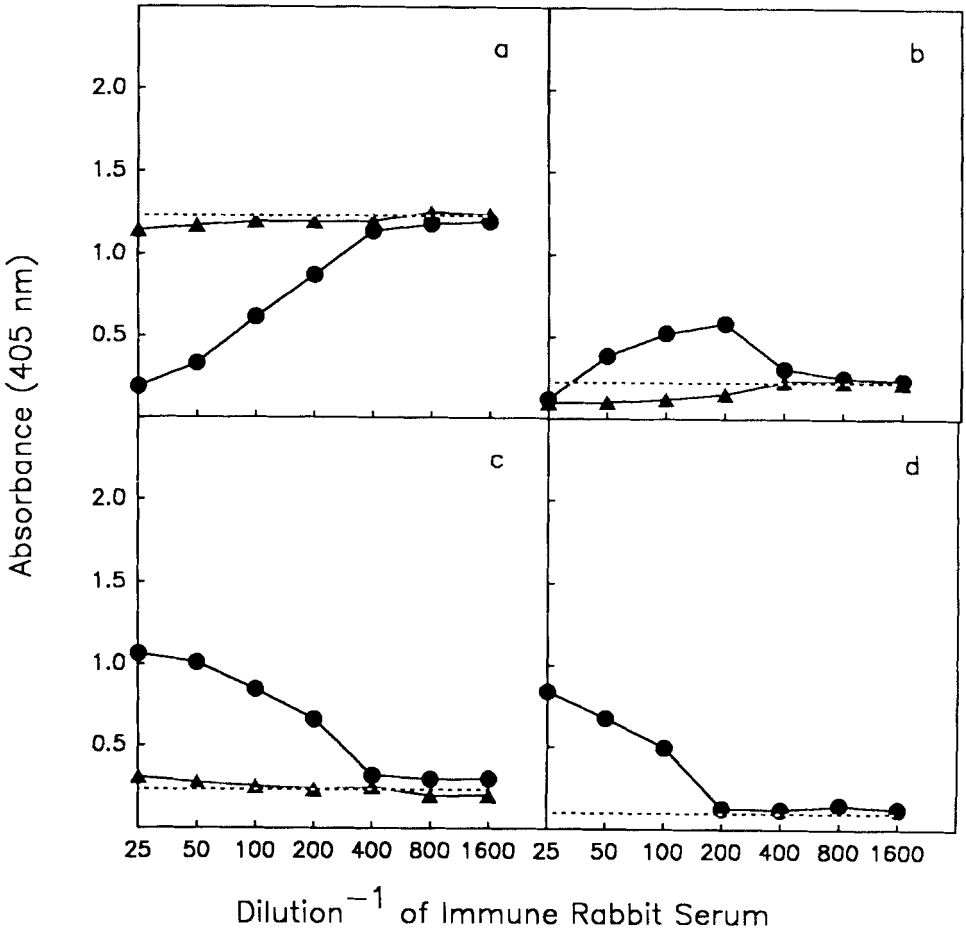


FIGURE 3. Comparison of extended indirect ligand displacement assays using monoclonal antibodies 2P5B7D7 and 1P8D6F10. a. Challenge well; b. Displacement well 1; c. Displacement well 2; d. Displacement well 3. Closed circles depict assay using 2P5B7D7; triangles depict results using 1P8D6F10; broken line depicts negative control with a 1/25 dilution of normal rabbit serum as challenge antibody. Each point represents average of at least two wells.

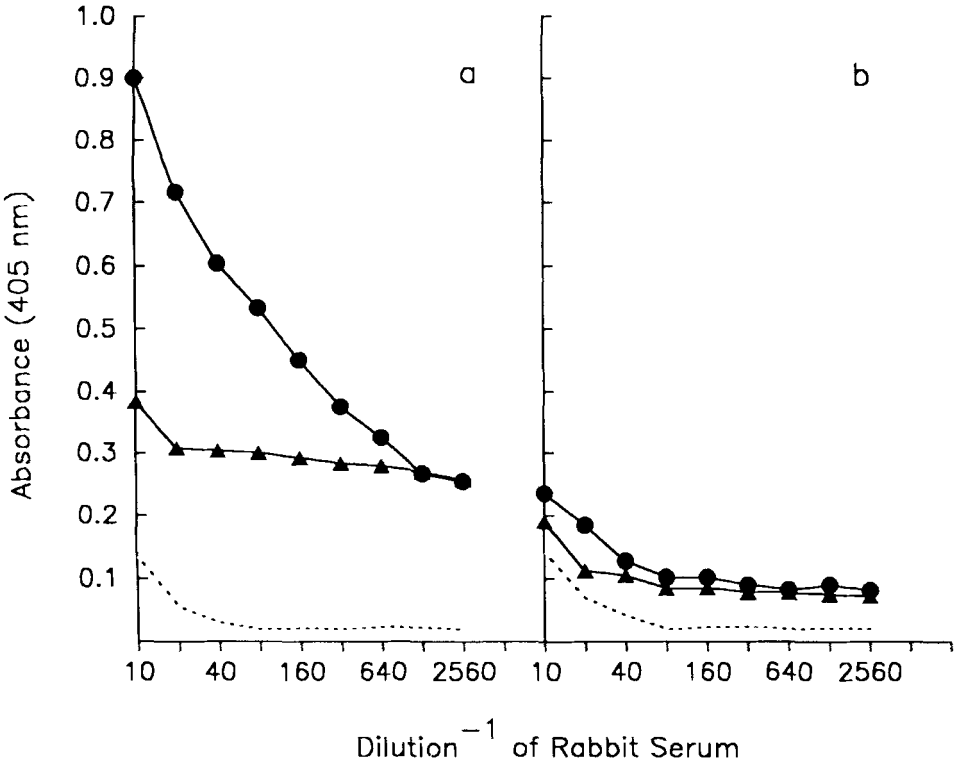


FIGURE 4. Direct ligand displacement assay of anti-T. spiralis antibodies in serum of infected rabbit. Comparison of assays using (a) alkaline phosphatase labeled monoclonal antibodies 2P5B7D7 and (b) 1P8D6F10. All wells were initially coated with T. spiralis solubilized (S3) antigen. Closed circles depict displacement by infected rabbit serum; triangles depict displacement by normal rabbit serum; broken line depicts alkaline phosphatase activity naturally present in rabbit serum.

displacement well 1 only in that antibody captured by T. spiralis antigen was detected using an anti-rabbit IgG-alkaline phosphatase conjugate, confirmed that considerable rabbit anti-T. spiralis antibody was indeed present in supernatants from challenge plates challenged with dilutions of immune rabbit serum up to 1/100.

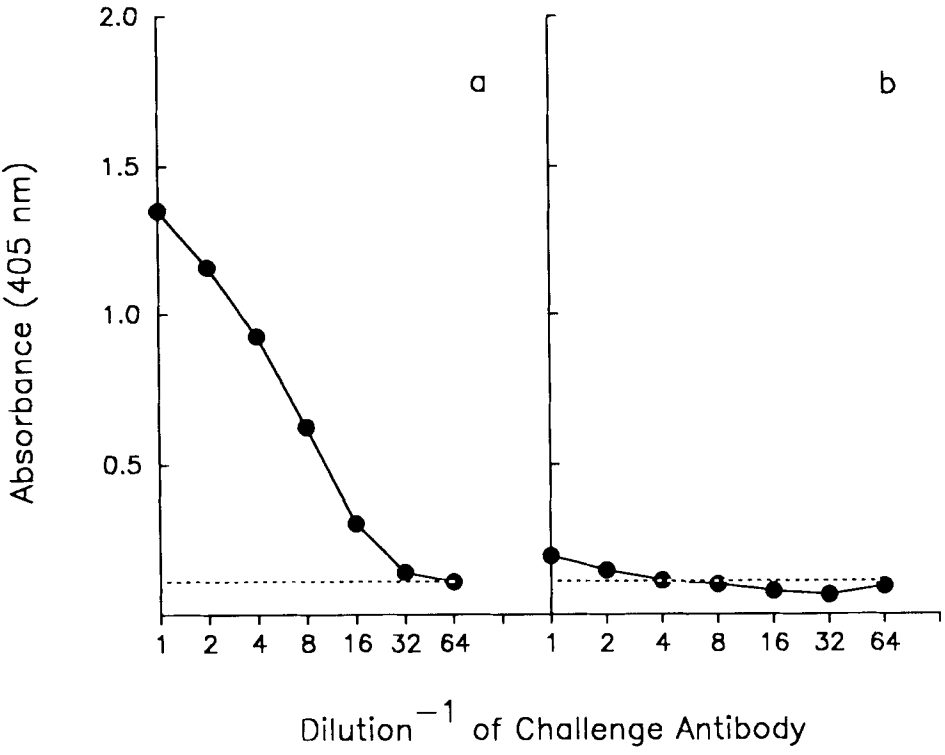


FIGURE 5. Reciprocal displacement by monoclonal antibodies 2P5B7D7 and 1P8D6F10 in direct ligand displacement assay. Wells were coated with T. spiralis solubilized (S3) antigen. a. displacement of alkaline phosphatase labeled 2P5B7D7 by 1P8D6F10; b. displacement of alkaline phosphatase labeled 1P8D6F10 by 2P5B7D7. Initial concentration of both monoclonal challenge antibodies was adjusted to 50 μg/ml (undiluted). Broken line indicates antibody displaced after challenge with 50 μg/ml normal mouse IgG.

The results of the extended ILDA validate the putative principle of the ILDA, and further indicate that the results of a simple basic ILDA provide a reliable predictor of usefulness of a monoclonal antibody for a displacement assay. Based on the results of both the basic and extended ILDA's, antibody 2P5B7D7 seemed to be an excellent candidate for use in a direct LDA, and antibody

1P8D6F10 clearly seemed to be unsuitable. As a crucial step in the demonstration that the ILDA is an effective screening tool, alkaline phosphatase conjugates of these two monoclonal antibodies were made, and direct LDA's were carried out using serum from a T. spiralis infected rabbit as the challenge antibody. The results of these assays (Figure 4) indicate that 2P5B7D7 does function as predicted, showing an essentially linear displacement dose response over a range of dilutions of rabbit antiserum from $10^{-1}$ to $10^{-3}$, and that 1P8D6F10 is displaced only slightly by the lowest dilutions of rabbit antiserum, hence is indeed unsuitable for use in the LDA format.

Direct LDA's were next carried out using solubilized ($S_3$) antigen-coated plates, where alkaline phosphatase labeled 2P5B7D7 was challenged with various dilutions of 1P8D6F10, or labeled 1P8D6F10 was challenged with dilutions of 2P5B7D7. As a control, some wells were challenged with normal mouse IgG. As would be predicted, 2P5B7D7 was displaceable by 1P8D6F10, but 1P8D6F10 was not displaceable by 2P5B7D7 (Figure 5).



## DISCUSSION

These studies indicate that the screening procedure herein referred to as the ILDA can be used to select, from a panel of monoclonal antibodies, those antibodies with properties making them potentially useful in a standard, direct LDA for antibodies against a specific antigen. Although the antigen-antibody system used in these studies was T. spiralis/ anti-T. spiralis, the technique should be applicable to many systems. In these studies the initial screening of hybridomas employed only conventional antigen capture ELISA methods to assemble a panel of monoclonal antibodies which were used to demonstrate the applicability of the ILDA. In actual practice, the basic ILDA procedure which was described could be used as the initial screening test for developing hybridomas. Supernatants from wells would be divided into two aliquots to be added to

wells coated with the appropriate antigen. After binding and washing, normal serum would be added to one well and immune serum to the other to attempt displacement of any antibody present. Antibody remaining bound in both wells would then be detected using an appropriately labeled anti-mouse immunoglobulin preparation. Such a screening procedure would be only slightly more complex than procedures normally employed. The screening well challenged with normal serum should give results almost identical to those obtained by screening by a standard ELISA. There would ordinarily be no need to employ the more extensive assay herein referred to as the extended ILDA. The extended ILDA was used in these studies only to demonstrate in detail various functional aspects and principles of the ILDA. Suitability of a monoclonal antibody for use in an LDA is presumably dependent at least in part on the affinity/avidity of the molecule, but determination of affinity/avidity for a large panel of candidate monoclonal antibodies would require extensive effort. The basic ILDA provides a simple but powerful screening tool, simultaneously screening for production of antibodies having both the desired specificity and the desired affinity/avidity properties for use in the LDA.



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