Office de la Propriété Intellectuelle du Canada
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Canadian Intellectual Property Office
An agency of Industry Canada

CA 2005204 C 2002/08/06

(11)(21) 2 005 204

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 1989/12/12

(41) Mise à la disp. pub./Open to Public Insp.: 1990/06/12

(45) Date de délivrance/Issue Date: 2002/08/06

(30) Priorité/Priority: 1988/12/12 (PJ 1917/88) AU

(51) Cl.Int.^{5}/Int.Cl.^{5} G01N 33/53

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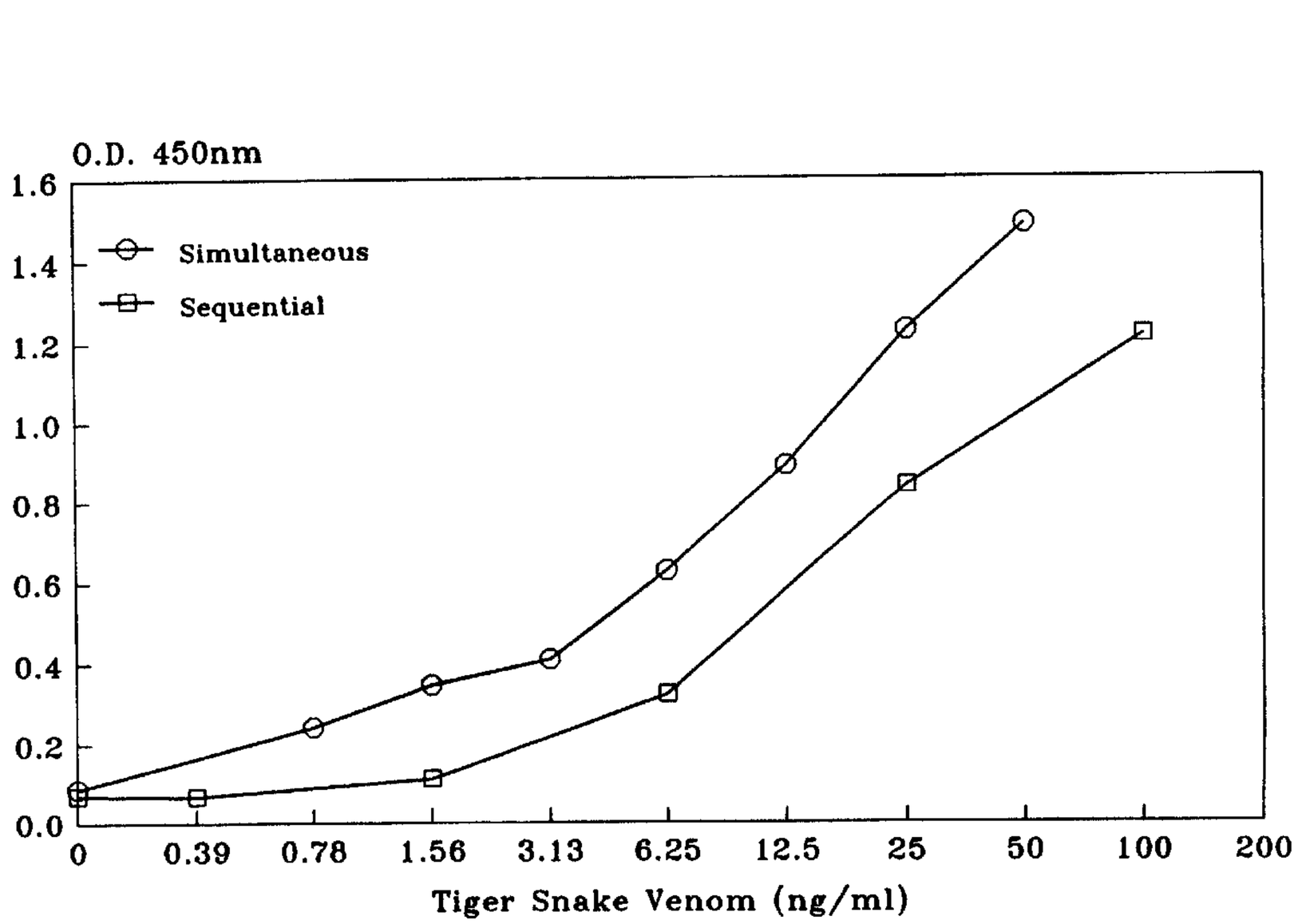
(54) Titre : IMMUNO-ESSAI EN PHASE SOLIDE AVEC CONJUGUE LYOPHILISE
(54) Title: SOLID PHASE IMMUNOASSAY WITH LYOPHILISED CONJUGATE



(57) Abrégé/Abstract:
Means for the detection of the presence in a sample of a first member of a specific binding pair, includes a solid substrate comprising the internal surface of a reaction vessel having the other member of the specific binding pair absorbed or coupled thereto, and having a conjugate in lyophilised form associated therewith, the conjugate comprising a binding member capable of detecting the presence of the first member bound to the other member of the specific binding pair on the solid substrate and having a label conjugated thereto.

ABSTRACT

Means for the detection of the presence in a sample of a first member of a specific binding pair, includes a solid substrate comprising the internal surface of a reaction vessel having the other member of the specific binding pair absorbed or coupled thereto, and having a conjugate in lyophilised form associated therewith, the conjugate comprising a binding member capable of detecting the presence of the first member bound to the other member of the specific binding pair on the solid substrate and having a label conjugated thereto.

IMPROVED IMMUNOASSAY

This invention relates to an improved immunoassay for the detection of the presence in a sample of a member of a specific binding pair, such as an antigen-antibody binding pair.

Immunoassay kits, such as enzyme immunoassay kits, are widely used in diagnostic and research laboratories for detection and quantitation of, for example, antigens or antibodies, and usually include a labelled conjugate as a separate reagent of the kit. The present invention relates to a novel presentation of immunoassay kits whereby the conjugate is present in lyophilised form with the capture antibody or antigen. Most typically, the invention relates to a sandwich-type enzyme immunoassay, and as a simple example of kits using this immunoassay system, reference is made to detection of parvovirus in a canine faecal specimen. In their simplest form, available "ready-to-use" kits for this purpose would typically comprise the following separate reagents:

a. a tube or well to which is absorbed or coupled by chemical means a capture antibody preparation specific to canine parvovirus;

b. a diluent solution for resuspending the faecal specimen;

c. a conjugate solution diluted ready to use, where the conjugate is an enzyme-labelled antibody specific to canine parvovirus; and

d. an enzyme substrate solution.

Using these reagents, the assay may be performed either as a sequential or a simultaneous assay. In the former, incubation of the capture antibody with the faecal specimen and a subsequent brief wash precedes the conjugate incubation step. In the latter, these two reactions occur as a single incubation step. In this example, because of the multivalent nature of a whole virus particle, a simultaneous assay can be expected to be at least as sensitive as a sequential assay and significantly quicker and easier to perform and such expectation can be proven experimentally. Many similar examples are in the scientific literature.

It is an object of the present invention to further simplify the presentation and performance of such kits by removing the conjugate solution as a separate reagent and incorporating it in lyophilised form in association with the tube or well.

In accordance with a first aspect of the present invention, there is provided means for the detection of the presence in a sample of a first member of a specific binding pair, comprising:

a solid substrate having the other member of said specific binding pair absorbed or coupled and having a conjugate in lyophilised form associated therewith, said solid substrate comprising the internal surface of a reaction vessel, and said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having a label conjugated thereto.

The means defined above can be incorporated into an immunoassay kit. Accordingly, in a further aspect of the present invention there is provided an immunoassay kit for the detection of the presence in a sample of a first member of a specific binding pair, comprising: (a) a solid substrate having the other member of said specific binding pair absorbed or coupled thereto, and having a conjugate in lyophilised form associated therewith, said solid substrate comprising the internal surface of a reaction vessel, and said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having a label conjugated thereto; and (b) means for detecting said label.

More specifically, the present invention provides an immunoassay kit for the detection of the presence in a sample of a plurality of different analytes each of which comprises a first member of a specific binding pair, comprising: (a) a plurality of solid substrates each of which comprises an internal surface of a respective one of a plurality of wells in a micro titre strip or tray and each of said substrates having the other member of a said specific binding pair absorbed or coupled thereto, and a

conjugate in lyophilised form associated therewith in such a manner that upon addition of an aqueous solution of said sample, the lyophilised conjugate is reconstituted and freed into the aqueous solution, and each said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having an enzyme label conjugated thereto; and (b) means for detecting said label.

It will be appreciated that in the immunoassay kit broadly described above, the means for detecting the label indicates the presence of the conjugate bound to the solid substrate which, in turn, indicates the presence of the first member of said specific binding pair in said sample.

The present invention also extends to an immunoassay method for detection of the presence in a sample of a first member of a specific binding pair which comprises the steps of: (a) contacting said sample with a solid substrate having the other member of said specific binding pair absorbed or coupled thereto and having a conjugate in lyophilised form associated therewith, said solid substrate comprising the internal surface of a reaction vessel, and said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having a label conjugated thereto; and (b) detecting the binding of said conjugate to said solid substrate to indicate the presence of said first member of the specific binding pair in said sample.

More specifically the present invention provides an immunoassay method for detection of the presence in a sample of a plurality of different analytes each of which comprises a first member of a specific binding pair which comprises the steps of: (a) contacting said sample with a plurality of solid substrates each of which comprises an internal surface of a respective one of a plurality of wells in a microtitre strip or tray and each of said substrates having the other member of a said specific binding pair absorbed or coupled thereto, and a conjugate in lyophilised form associated therewith in such a manner that upon addition of an aqueous solution of said sample, the lyophilised conjugate is reconstituted and freed into the aqueous solution, and each said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having an enzyme label conjugated thereto; and (b) detecting the binding of said conjugates to said solid substrates to indicate the presence of one or more of said first members of the specific binding pairs in said sample.

Whilst the present invention has particular application in the detection of the presence of a member of an antigen-antibody binding pair or a hapten-antibody binding pair, the invention also has application in respect of other known ligand/ligand binding pairs, including ligand-receptor pairs where the ligand is a protein, steroid hormone, drug or other medicament, or the like.

Similarly, whilst specific reference is made herein to a sandwich-type enzyme immunoassay, it will be

appreciated that in its broadest aspect the invention is not restricted to the use of enzyme labels, and other types of labels which are well known in immunoassays, including

fluorochromes, radioisotopes and heavy metals, may also be used. Similarly, other well known types of assay formats may be used as well as the sandwich-type format, including the indirect and competitive immunoassay formats.

In one particularly preferred aspect of this invention, there is provided an immunoassay kit for the detection of a blood group antigen in a sample comprising:

a. a reaction vessel having an antibody binding to said blood group antigen absorbed or coupled to the internal surface thereof, and having a conjugate in lyophilised form associated therewith, said conjugate comprising the same or another antibody binding to said blood group antigen having a label conjugated thereto; and

b. means for detecting said label.

In this aspect, there is also provided an immunoassay kit for the detection of a blood group antibody in a sample, comprising:

a. a reaction vessel having an antigen, hapten or ligand binding to said blood group antibody absorbed or coupled to the internal surface thereof, and having a conjugate in lyophilised form associated therewith, said conjugate comprising the same or another antigen, hapten or ligand binding to said blood group antibody or a second antibody binding to said blood group antibody having a label conjugated thereto; and

b. means for detecting said label.

This aspect of the invention also extends to corresponding immunoassay methods for the detection of blood group antigens and/or antibodies in a sample.

The essential aspect of the present invention resides in the use of the labelled conjugate in lyophilised form associated with the solid substrate. Depending on which member of the specific binding pair is absorbed or coupled to the solid substrate, the labelled conjugate may comprise an appropriate label attached to an antigen or hapten, or to an antibody, as the binding member capable of detecting the presence of the first member bound to the other member of the specific binding pair on the substrate.

Preferably, the solid substrate will usually comprise a tube or a well in an EIA or microtitre strip or tray or other appropriate container and the bound member of the specific binding pair is absorbed or coupled to the internal surface of the tube, well or other container. Where the surface of such a tube, well or similar container is the solid substrate, the lyophilised, labelled conjugate is formed therein and remains in close association with the container in which it was dried even though it is not chemically or physically bound in any way to the substrate. On addition of water or an aqueous solution, the bound member of the specific binding pair remains bound to the substrate but the lyophilised, labelled conjugate is reconstituted and passes into solution so that it is then able to react in the immunoassay in the same way as a separately-added conjugate solution.The incorporation of the conjugate in lyophilised form associated with the solid substrate offers a number of substantial advantages in the resulting kit, including:

1. Decreased production cost. The kit will contain one less reagent container, and as a result, one less bottle and label will be required, one dispensing run with subsequent sterility test will be saved and the overall kit size and weight can be reduced.

2. Increased shelf life. Lyophilisation is the best available procedure for retaining biological activity of reagents, particularly enzyme labelled antibody, upon long term storage. At present there are three available options for storage of enzyme labelled antibody.

   (i) Lyophilised in a separate container. This gives a good shelf life until first usage when the entire stock of conjugate has to be reconstituted. Stability is then that of diluted conjugate. Additionally, there are the costs of conjugate dispensing and lyophilisation and the need to supply reconstituting fluid to the user, and there is the added inconvenience of having to reconstitute conjugate before use. This is particularly inconvenient in a kit designed for field or irregular use.

   (ii) As a concentrate (usually 100X in 50% glycerol). This gives reasonably good shelf life but requires that conjugate be diluted for use before each test. Availability of accurate microdilutors is essential.

   (iii) Diluted ready for use. This presentation is least stable but most convenient to the user.

Since lyophilisation is a well-known technique, the preparation of the solid substrate having the lyophilised

conjugate associated therewith may be carried out using known techniques and apparatus. Where the solid substrate is in the form of separate tube or the well of a microtitre tray, the conjugate solution is simply added to the pre-treated tube or well and then lyophilised, followed by sealing of the tube or well to exclude moisture and air. Use of lyophilised conjugate in accordance with the present invention provides the shelf life of lyophilised conjugate, is cheaper to produce than any current alternative and is easier to use than any other option. Additionally, use of portion of the kit does not reduce the stability of the remainder. Thus, even in a simple assay kit the present invention confers the multiple advantages of decreased production cost and increased shelf life for manufacturer, and for the user, a quicker and simpler test format.

The advantages of this invention increase as the test complexity increases. Several examples are given below of tests which are designed to detect and distinguish between different analytes in a specimen. In these examples, in addition to the advantages cited above, the test can only be formatted in a simple and cost-effective way by utilising the improvement of the present invention.

A. Detection and identification of snake venom in a clinical specimen.

Currently in Australia, clinical snake envenomation usually results from the bite from 5 major species, viz. tiger snake, brown snake, death adder, king brown and taipan. Rapid identification of the species can permit treatment with monovalent rather than polyvalent antivenom with a concomitant vastly reduced injection of foreign horse protein. The current diagnostic kit used to identify snake

species uses affinity-absorbed antibodies to the above 5 snakes in five separate capillary tubes as the antigen capture phase and a carefully adjusted mixture of enzyme-labelled antibodies to each of the five venoms as label. The resulting test has been of considerable use but has sometimes given misleading and incorrect results with venoms from less common snakes. Extensive investigation of these false positive reactions has shown that if unabsorbed antibodies were used instead in the capture phase and followed by the homologous labelled antivenom (not a mixture) the correct antivenom for treatment would always be selected. However, to format such a kit would have required that five separate conjugate preparations be supplied, each to be added to its homologous incubation well. To perform such an assay under pressure in the field would have resulted in frequent errors. Utilising the present invention, such a test can be formatted so as to avoid these problems by lyophilising the homologous conjugate within the appropriate well. The test can then be performed under field conditions with minimal chance of error, or false diagnosis and in much less time than the current test.

B. Detection of non-bacterial pathogens in human faeces.

Within Australia and indeed in most developed countries of the world, there are four major non-bacterial pathogens which cause diarrhoea in adults and children. These are the two protozoans Giardia and Cryptosporidia and the two viruses rotavirus and enteric adenovirus. The two protozoa can exist either as cysts or as free-growing trophozoites. These two forms have a varying array of surface antigens, few if any of which have been characterised. Specific identification can be achieved most reliably by use of a good polyclonal antiserum, production of which does not require detailed knowledge of antigenic

structure and which antigens are immunodominant. Conversely, viruses are antigenically far more simple and, in fact, specific identification of an enteric adenovirus from other closely related viruses is best achieved by use of a monoclonal antiserum specific for a single epitope. Thus, to identify these four enteric pathogens in a single diagnostic kit would necessitate four separate conjugate preparations or otherwise two separate preparations (one polyclonal, one monoclonal) each at double strength. This is because polyclonal antiserum from any species naturally will contain antibodies to the immunoglobulins of other species. Thus, for example, polyclonal rabbit serum can be expected to react with and precipitate mouse immunoglobulins etc. It is possible that such reactivity could be removed by affinity absorption permitting a single conjugate mix to be produced. However additional to the cost of this extra process the final conjugate
mixture would need to be at 4X strength. The present invention again overcomes all these problems because the homologous capture and label antibodies are presented in the incubation well ready for use.

C. Detection of meat species and meat contamination.

Irregularities in the meat industry has led, at various times, to meat from one species being substituted for that of another species. For religious, public health and other reasons, it is desirable to be able to detect such substitutions. For this purpose, a kit has been developed to identify bovine, buffalo, camel, donkey/horse, goat, kangaroo, pig and sheep. For reasons of economy, polyclonal antisera were raised in rabbits (for all species) and sheep (for all but anti-sheep where goats were used). In all cases, rabbits produced sera of lesser quality, and in some cases, following absorption to render them species specific,

their sensitivity was significantly less than the required 1%. However, although the sheep anti-goat was of high specificity and sensitivity, the goat anti-sheep was unsatisfactory and monoclonals to sheep were developed instead. Using these reagents, it was straightforward to produce a kit to examine large numbers of meat samples for the presence of any single species, but a preference was expressed in the market place for a test which would check for all eight species in a single kit. Once again, this was only possible by use of eight separate conjugates which had to be added to the eight appropriate wells after the primary incubation (otherwise the mouse anti-sheep would have precipitated the sheep-derived immunoglobulins). The present invention permits a single incubation assay for multi-species testing even though antisera could not be presented as single-mix conjugate.

D. Solid phase blood grouping.

Traditionally, determination of human blood groups has required forward and reverse matchings using suspensions of washed cells and plasma respectively. Presence of antigen or antibody is judged by eye as presence of agglutination. Various ways to automate these procedures and reduce subjectivity have been devised. However, all these attempts have been characterised by a number of shortcomings including the need to separate cells from plasma, expensive equipment and a continuing need to detect anti-red cell autoantibodies in a separate test. By use of the present invention, it is possible to separately detect antigens A, B, D, and H and antibodies to A, B, H and self red cells in a rapid, non-subjective, fully automatable test using existing equipment. In the absence of this new approach, the test would require two incubations with different conjugates to be added to different wells.

Further features of the present invention will be apparent from the following Example, which is included by way of exemplification and not limitation of the invention.

EXAMPLE 1

A kit for detection of canine parvovirus.

A. Preparation of plates with associated lyophilised conjugate.

Step 1. Purify monoclonal antibodies to canine parvovirus by protein A or other suitable procedure. Determine IgG concentration. This antibody will act as the capture phase for the assay.

Step 2. Use the above purified antibody to prepare enzyme conjugate according to any of a range of suitable known procedures.

Step 3. Dilute capture antibody to 10 µg/mL in 0.05M carbonate buffer, pH 9.5.

Step 4. Add 100 µL of diluted capture antibody to each well of a 96 well plate and incubate overnight at 4°C.

Step 5. Discard contents and add 100 µL post-coating buffer viz.:
0.010M phosphate, 0.145M NaCl, 1 mg/mL casein 50 mg/mL lactose pH 7.0. Incubate at 20°C for 1 hr.

Step 6. Discard contents and add 100 µL post-coat rinse buffer viz. 50 mg/mL glucosamine in distilled water.

Step 7. Immediately discard and add 5 µL of 40x conjugate in conjugate diluting buffer viz.: 0.010M phosphate, 0.145M NaCl, 50 mg/mL lactose, 10 mg/mL casein.

Step 8. Immediately transfer to the shelf of a freeze-drier and freeze to -50°C prior to application of vacuum.

Step 9. Freeze dry preferably with secondary drying over P_2O_5.

Step 10. Seal plates in laminated plastic pouches containing a sachet of silica gel.

B. **Evaluation of test plates.**

A number of batches of test plates with associated lyophilised conjugate prepared as described above have been evaluated in typical field-type assay in comparison with conventionally formatted (i.e. separate conjugate reagent) sequential and simultaneous assays. In all other respects, all reagents have been the same. Results are presented in Table 1 below:

TABLE Assay for presence of canine parvovirus in faeces from infected and uninfected dogs. Results are expressed as median, range and number of replicate tests. End point was the highest dilution of faecal preparation which gave a positive result under standard assay conditions (5 minutes at 20°C per incubation step).

	Assay Format		
Sample	Sequential	Simultaneous	Present Invention
Infected	512(256-1024)	1000(640-1280)	1000(640-1280)
No.of replicates	5	5	12
Uninfected	<2	<2	<2
No.of replicates	10	10	20

EXAMPLE 2

Snake Venom Detection Kit

(a) Preparation of Antisera.

High-titre antisera to snake venom is prepared by hyperimmunization of rabbits with increasing amounts of venom in Freund's adjuvant. The primary immunization course involves 10 weekly doses deep intramuscularly; booster doses are subcutaneous. Antisera are raised to each of the snake venoms of importance, viz: tiger (TSV), brown (BSV), king brown, taipan and death adder.

(b) Processing of antisera.

(i) Pure IgG is prepared by Protein A affinity purification according to established methods for absorption and elution of rabbit IgG. The material is checked for purity by poly-acrylamide gel electrophoresis (PAGE). A single band of MW 150 kD should be present.

(ii) Purified IgG is enzymically degraded to Fab'_2 by controlled reaction with solid-phase pepsin. The reaction is terminated by removal of the solid phase. The material is checked for purity by PAGE. A single band of MW 100kD should be present.

(iii) Purified IgG is chemically coupled to the enzyme horse radish peroxidase (HRP) by the periodate procedure of Nakane and Kawaoi (1974), J.Histochem. Cytochem. 22, 1084.

(c) Description of kit reagents.

An assay kit for field use comprises the following reagents:

(i) an 8 well strip

(ii) sample diluent buffer

(iii) enzyme substrate buffer (H_2O_2 solution stabilized) 2x strength

(iv) chromogen solution 2x strength.

(d) Preparation of 8 well strip.

The contents of the eight wells are summarized below:

Well 1 Coating antibody: Rabbit anti-TSV
Conjugate: Rabbit anti-TSV-HRP labelled

Well 2 Coating antibody: Rabbit anti-BSV
Conjugate: Rabbit anti-BSV-HRP labelled

Well 3 Coating antibody: Rabbit anti-Taipan
Conjugate: Rabbit anti-Taipan-HRP labelled

Well 4 Coating antibody: Rabbit anti-Death Adder
Conjugate: Rabbit anti-Death Adder-HRP labelled

Well 5 Coating antibody: Rabbit anti-King Brown
Conjugate: Rabbit anti-King Brown-HRP labelled

Well 6 Coating antibody: Normal rabbit IgG
Conjugate: Rabbit anti-TSV-HRP labelled

Well 7 Coating antibody: Normal rabbit IgG
Conjugate: Sheep anti-rabbit IgG-HRP labelled

Well 8 Blank - orientation well.

The 8 well strips are prepared according to the following steps:

1. Coat: 10µg/mL rabbit IgG in 0.05 M sodium bicarbonate buffer, pH 9.5. Add 100 µL/well and incubate overnight at 4°C then flick out.

2. Postcoat: 1 mg/mL casein, 5% lactose in PBS pH 7.2. Add 150 µL/well and incubate 1 hr at room temperature, then flick out.

3. Stabilization: 5% glucosamine in distilled water. Add 150 µl/well and incubate 5-10 minutes at room temperature. Flick out.

4. Conjugate: Add 5 µl conjugate at 12.5x optimal level (estimated when used wet, in a simultaneous assay - 50 µL sample, 50 µl conjugate). Conjugate concentrate diluted in the following diluent buffer - 1 µg/mL casein, 5% lactose in sorbitol buffer.

5. Lyophilization: Plates are immediately placed on shelves in a freeze dryer, and the shelf temperature dropped to -40°C. Lyophilization takes place under vacuum. Following lyophilization, 8 well strips are individually sealed to exclude moisture and air.

(e) Typical results for laboratory tests.

Results for 10 ng/mL venoms are shown in Table 2. It can be seen that the strongest signal is always given by the homologous venom; cross reacting signals being around one quarter.

(f) Examination of clinical specimens.

The kit has been designed for use with serum, urine and swab from a bite site. Although primarily intended for clinical use, it may also be used for various veterinary applications, especially for companion animals. The use of serum from whatever source can frequently lead to high, non-specific signals due to anti-species immunoglobulins (e.g. rheumatoid factor). Such non-specific reactivity can be effectively removed by use of one or more of:

(i) Fab'_2 rather than whole IgG as capture antibody.

(ii) Fab'_2 rather than whole IgG for conjugation to HRP.

(iii) Blocking IgG in the incubation buffer.

Such procedures have been used to completely remove non-specific reactivity from this test.

(g) Assay sensitivity.

Sensitivity was determined for the sequential and simultaneous assay systems. The results of a typical assay for tiger snake venom is shown in Figure 1. It can be seen that the simultaneous assay, which forms the basis for this assay format is actually more sensitive using a single 10 minute incubation than is the sequential assay with 10 minutes for each incubation stage. Sensitivity was less than 1 µg/mL (read as blue signal when controls were colourless).

(h) Kit Stability.

To determine kit stability, kits were stored at 37°C and 4°C and tested in parallel using three levels of venom. Stability was calculated as the OD for each venom level at 37°C as a percentage of that at 4°C

$$\text{i.e. \% drop} = \frac{\text{OD 4°C - OD 37°C}}{\text{OD 4°C}} \times 100$$

Tests were performed at doubling time intervals from week 1 and plotted to calculate a projected half life. The product half life at 37°C was estimated at 40 weeks.

TABLE 2 O.D. 450nm obtained for venoms at 10ng/ml

Identification of Well	Ti	Br	Tp	DA	KB
Ti	1.267	0.131	0.365	0.274	0.464
Br	0.101	0.528	0.074	0.092	0.078
Tp	0.037	0.042	0.957	0.088	0.065
DA	0.308	0.087	0.215	0.726	0.238
KB	0.156	0.125	0.143	0.141	1.340
-	0.227	0.075	0.164	0.083	0.070
+	1.160	1.525	1.076	1.154	1.114

(Ti = tiger; Br = brown; Tp = taipan; DA = death adder; KB = king brown.)

EXAMPLE 3

Solid phase blood grouping kit.

The kit described is a simple 8 well kit for forward and reverse grouping and direct Coombs.

(a) Preparation of reagents.

Antisera to selected blood group antigens are most conveniently sourced as monoclonal antibodies (MAbs) with defined specificity and affinity. However, polyclonal antisera can be used. The following antisera are required:

(i) MAb-anti A - must have specificity to all A sub-groups.

(ii) MAb-anti B

(iii) MAb-anti H

(iv) MAb-anti D

(v) anti-human immunoglobulin (rabbit, sheep or mouse).

(b) Processing of selected antisera.

(i) Pure immunoglobulin is prepared by ion exchange or affinity purification as appropriate according to established methods. The resulting material is checked for purity by PAGE. A single band should be present.

(ii) Purified immunoglobulin is chemically coupled to the enzyme horse radish peroxidase (HRP) by the periodate procedure of Nakane and Kawaoi (1974), J.Histochem. Cytochem, 22, 1084.

(c) Other reagents.

Synthetic glycoproteins (Biocarb Chemicals, Lund, Sweden) in which the oligosaccharide is specific to blood group antigens and is coupled via a spacer to a protein, e.g. casein.

(d) Description of kit reagents.

(i) a 96 well EIA plate containing 12x8 well strips

(ii) diluent buffer for whole blood

(iii) wash buffer 20x strength

(iv) enzyme substrate buffer 2x strength

(v) chromogen solution 2x strength

(vi) stop solution.

(e) Format of an 8 well strip.

Well 1 Coating antibody: MAb-anti-A
Conjugate: MAb-anti-H-HRP

Well 2 Coating antibody: MAb-anti-B
Conjugate: MAb-anti-H-HRP

Well 3 Coating antibody: MAb-anti-H
Conjugate: MAb-anti-H-HRP

Well 4 Coating antibody: MAb-anti-D
Conjugate: MAb-anti-H-HRP

Well 5 Coating antigen: A-trisaccharide-casein
Conjugate: A-trisaccharide-casein-HRP
Well 6 Coating antigen: B-trisaccharide-casein
Conjugate: B-trisaccharide-casein-HRP
Well 7 Coating antigen: 2'-fucosyllactosamine-casein
Conjugate: 2-fucosyllactosamine-casein-HRP
Well 8 Coating antibody: MAb-anti-H
Conjugate: antihuman immunoglobulin-HRP

In the above format, well 3 will act as a positive control (rare exception Bombay group) and well 7 as a negative control (rare exception Bombay group).

In one alternative configuration, the conjugate in wells 5, 6 and 7 will be, respectively MAb-anti-A-HRP, MAb-Anti-B-HRP and MAb-anti-H-HRP. The 8 well strips are prepared as described in Example 2. Monoclonal antibodies were coated at 1 µg/mL, synthetic glycoproteins at 5-10 µg/mL.

(f) Typical test results.

Whole blood is diluted 1 in 25 to 1 in 50 in diluent buffer then 100 µL added to each well of a strip, mixed then allowed to stand for 5 minutes. Wells are then washed thoroughly and substrate/chromogen added. In a typical set of results, readings as presented in Table 3 were obtained.

TABLE 3 Typical Test Results

		BLOOD GROUP						
		O Pos	A_1 Pos	A_2 Neg	A_2B Neg	B Pos	Neg	Coombs Pos. A_1 Pos
Well	1	.073	1.426	1.1281	1.200	0.084	0.057	1.523
	2	.102	0.083	0.077	1.277	1.520	0.064	0.071
	3	1.584	2.230	2.663	2.675	2.682	2.737	2.131
	4	1.176	2.286	0.127	2.371	1.706	0.053	1.982
	5	1.391	0.151	0.168	0.251	1.108	1.659	0.120
	6	0.976	1.112	1.380	0.126	0.098	1.504	1.365
	7	0.117	0.079	0.055	0.067	0.103	0.041	0.080
	8	0.051	0.062	0.041	0.040	0.055	0.053	1.720

* OD - 450 nm

EXAMPLE 4

Solid phase blood group antibody screen.

This antibody screen can be a 1 or 2 well test presented as a separate product or included as part of the 8 well test described in Example 3 above.

Two panels of group O red cells are prepared by careful selection. Between them, these panels must contain all of the following antigens: C, c, D, E, e, M, N, S, s, P_1, K, k, Fy^a, Fy^b, Jk^a, Jk^b, Le^a and Le^b.

As described previously, these cells would function as capture antigen either by direct attachment of cells to the walls of the 96 well plates or indirectly via MAb-anti-H capture. The conjugate-antihuman immunoglobulin-HRP, is lyophilized as described previously.

CLAIMS:

1. An immunoassay kit for the detection of the presence in a sample of a plurality of different analytes each of which comprises a first member of a specific binding pair, comprising:

a. a plurality of solid substrates each of which comprises an internal surface of a respective one of a plurality of wells in a microtitre strip or tray and each of said substrates having the other member of a said specific binding pair absorbed or coupled thereto, and a conjugate in lyophilised form associated therewith in such a manner that upon addition of an aqueous solution of said sample, the lyophilised conjugate is reconstituted and freed into the aqueous solution, and each said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having an enzyme label conjugated thereto; and

b. means for detecting said label.

2. A kit according to claim 1, wherein said wells are sealed to exclude moisture and air.

3. A kit according to claim 1 wherein said enzyme is horseradish peroxidase.

4. A kit according to any one of claims 1 to 3, for detection of a plurality of different antigens in a sample, wherein each of said solid substrates has an antibody specific for a said antigen absorbed or coupled thereto, and each said conjugate comprises the same or another antibody

specific for said antigen having an enzyme label conjugated thereto.

5. A kit according to claim 4, wherein said antigens are bacterial, viral or protozoal antigens.

6. A kit according to claim 5, wherein said antigens include canine parvovirus.

7. A kit according to claim 4, wherein said antigens are snake venoms.

8. A kit according to claim 4, wherein said antigens are blood group antigens.

9. A kit according to any one of claims 1 to 3 for detection of a plurality of different antibodies in a sample, wherein each of said solid substrates has an antigen, hapten or other ligand specific for a said antibody absorbed or coupled thereto, and each said conjugate comprises the same or another antigen, hapten or ligand specific for said antibody, or a second antibody specific for said first-mentioned antibody or competing with said first-mentioned antibody for binding to said first-mentioned antigen, hapten or ligand, having an enzyme label conjugated thereto.

10. A kit according to claim 9, wherein said antibodies are blood group antibodies.

11. A kit according to claim 8, which comprises a plurality of wells, each of said wells having an antibody specific for a different blood group antigen absorbed or

coupled to an internal surface thereof, and each said lyophilised conjugate comprising the same or another antibody specific for said blood group antigen having an enzyme label conjugated thereto.

12. A kit according to claim 11, wherein said plurality of wells comprises wells having antibodies selected from the group consisting of anti-A, anti-B, anti-H and anti-D antibodies absorbed or coupled thereto.

13. A kit according to claim 10, which comprises a plurality of wells, each of said wells having an antigen, hapten or ligand specific for a different blood group antibody absorbed or coupled to an internal surface thereof, and each said lyophilised conjugate comprising the same or another antigen, hapten or ligand specific for said blood group antibody or a second antibody specific for said blood group antibody or competing with said blood group antibody for binding to said first-mentioned antigen, hapten or ligand, having an enzyme label conjugated thereto.

14. A kit according to claim 13, wherein said plurality of wells includes one or more wells having synthetic glycoproteins absorbed or coupled thereto, each of said synthetic glycoproteins comprising an oligosaccharide binding to a blood group antibody coupled to a protein.

15. An immunoassay method for detection of the presence in a sample of a plurality of different analytes each of which comprises a first member of a specific binding pair which comprises the steps of:

a. contacting said sample with a plurality of solid substrates each of which comprises an internal surface of a

respective one of a plurality of wells in a microtitre strip or tray and each of said substrates having the other member of said specific binding pair absorbed or coupled thereto, and a conjugate in lyophilised form associated therewith in such a manner that upon addition of an aqueous solution of said sample, the lyophilised conjugate is reconstituted and freed into the aqueous solution, and each said conjugate comprising a binding member capable of detecting the presence of said first member bound to the other member of said specific binding pair on said substrate and having an enzyme label conjugated thereto; and

b. detecting the binding of said conjugates to said solid substrates to indicate the presence of one or more of said first members of the specific binding pairs in said sample.

16. A method according to claim 15, wherein said enzyme is horseradish peroxidase.

17. A method according to claim 15 or 16, for detection of a plurality of different antigens in a sample, wherein each of said solid substrates has an antibody specific for a said antigen absorbed or coupled thereto, and each said conjugate comprises the same or another antibody specific for said antigen having an enzyme label conjugated thereto.

18. A method according to claim 17, wherein said antigens are bacterial, viral or protozoal antigens.

19. A method according to claim 18, wherein said antigens include canine parvovirus.

20. A method according to claim 17, wherein said antigens are snake venoms.

21. A method according to claim 17, wherein said antigens are blood group antigens.

22. A method according to claim 16 or 17, for detection of a plurality of different antibodies in a sample, wherein each of said solid substrates has an antigen, hapten or other ligand specific for a said antibody absorbed or coupled thereto, and each said conjugate comprises the same or another antigen, hapten or ligand specific for said antibody, or a second antibody specific for said first-mentioned antibody or competing with said first-mentioned antibody for binding to said first-mentioned antigen, hapten or ligand, having an enzyme label conjugated thereto.

23. A method according to claim 22, wherein said antibodies are blood group antibodies.

24. A method according to claim 21, which comprises contacting said sample with a plurality of wells, each of said wells having an antibody specific for a different blood group antigen absorbed or couple to an internal surface thereof, each said lyophilised conjugate comprising the same or another antibody specific for said blood group antigen having an enzyme label conjugated thereto and detecting binding of said conjugates to said well surfaces to indicate the presence of one or more of said blood group antigens in said sample.

25. A method according to claim 24, wherein said plurality of wells comprises wells having antibodies selected from the group consisting of anti-A, anti-B, anti-H and anti-D antibodies absorbed or coupled thereto.

26. A method according to claim 23, which comprises contacting said sample with plurality of wells each of said wells having an antigen, hapten or ligand specific for a different blood group antibody absorbed or coupled to an internal surface thereof each said lyophilised conjugate comprising the same or another antigen, hapten or ligand specific for said blood group antibody, or a second antibody specific for said blood group antibody or competing with said blood group antibody for binding to said first-mentioned antigen, hapten or ligand, having an enzyme label conjugated thereto and detecting binding of said conjugates to said well surfaces to indicate the presence of one or more of said blood group antibodies in said sample.

27. A method according to claim 26, wherein said plurality of wells includes one or more wells having synthetic glycoproteins absorbed or coupled thereto, each of said synthetic glycoproteins comprising an oligosaccharide binding to a blood group antibody coupled to a protein.

28. A kit according to claim 1, wherein each said member of said specific binding pair absorbed or coupled to a solid substrate is stabilised with glucosamine prior to lyophilisation of said conjugate.

29. A kit according to claim 11, wherein each said antibody absorbed or coupled to the internal surface of a

well is stabilised with glucosamine prior to lyophilisation of said conjugate.

30. A kit according to claim 13, wherein each said antigen, hapten or ligand absorbed or coupled to the internal surface of a well is stabilised with glucosamine prior to lyophilisation of said conjugate.

31. A method according to claim 15, wherein each said member of said specific binding pair absorbed or coupled to a solid substrate is stabilised with glucosamine prior to lyophilisation of said conjugate.

32. A method according to claim 24, wherein each said antibody absorbed or coupled to the internal surface of a well is stabilised with glucosamine prior to lyophilisation of said conjugate.

33. A method according to claim 26, wherein each said antigen, hapten or ligand absorbed or coupled to the internal surface of a well is stabilised with glucosamine prior to lyophilisation of said conjugate.

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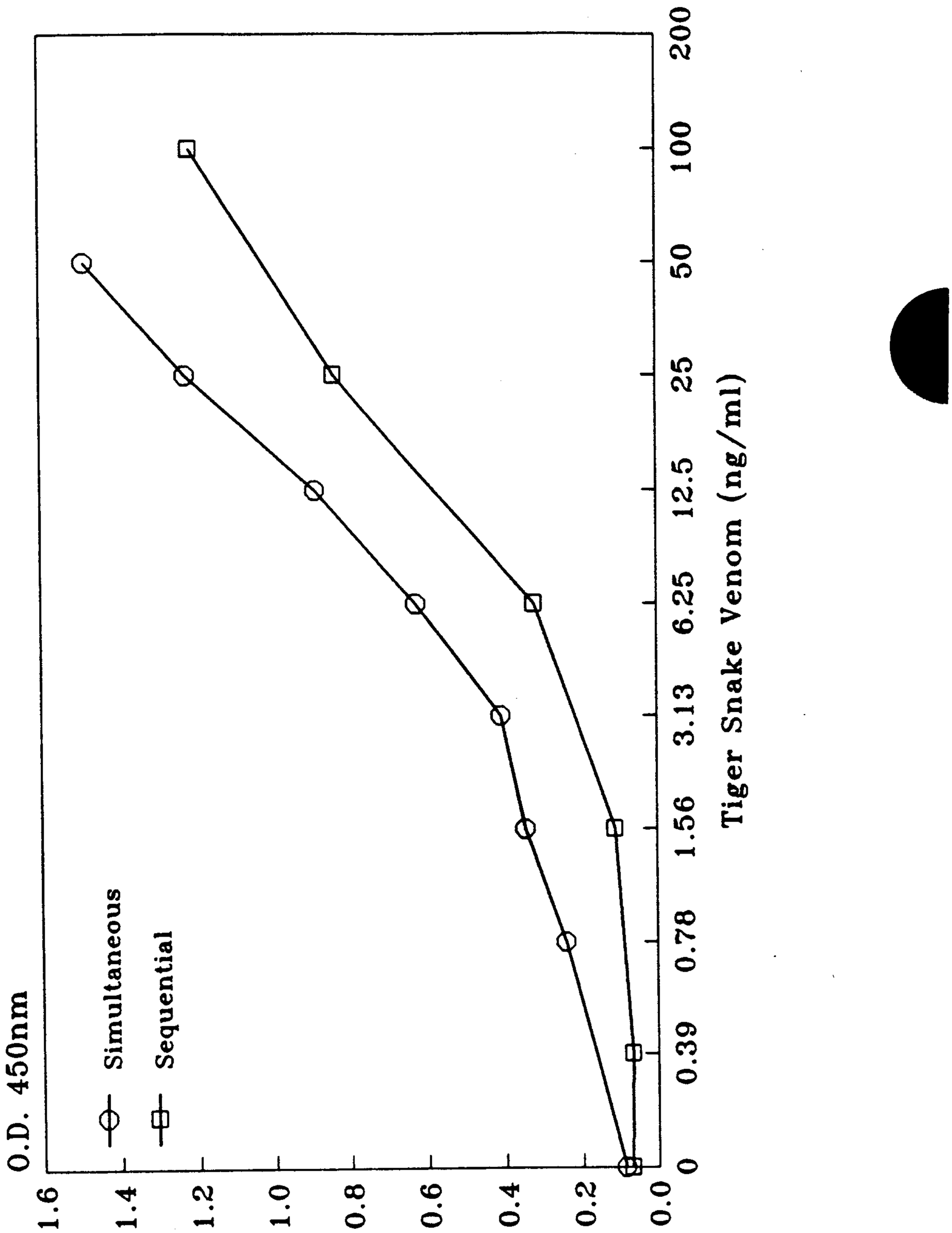
Figure 1. SENSITIVITY OF SEQUENTIAL V SIMULTANEOUS EIA FOR DETECTION OF TIGER SNAKE VENOM



SENSITIVITY OF SEQUENTIAL V SIMULTANEOUS EIA FOR DETECTION OF TIGER SNAKE VENOM