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(54) Title: USE OF POLYMERIZED IMMUNOGLOBULIN TO REDUCE THE INCIDENCE OF FALSE-POSITIVE RESPONSES IN IMMUNOASSAYS

(57) Abstract

The present invention relates to a method to reduce false positive results in immunoassays. The method uses polymerized immunoglobulin to overcome binding which is commonly referred to as non-specific binding. The falsely elevated signal generated in the presence of human serum in assays for infectious disease markers, cancer related antigens and peptide hormones is reduced in assays performed in the presence of the polymerized immunoglobulin. The polymerized immunoglobulin can be prepared using different methods of polymerization.

1

TITLE

USE OF POLYMERIZED IMMUNOGLOBULIN TO REDUCE THE
INCIDENCE OF FALSE-POSITIVE RESPONSES IN IMMUNOASSAYS

TECHNICAL FIELD

This invention relates to a method for reducing
false positive responses in enzyme immunoassays, more
specifically to the method of using polymerized
immunoglobulin for removing false positive responses in
immunoassays.

BACKGROUND ART

Immunometric assays are widely in use for detection
of antigens having multiple epitopes. Typically, such
an assay involves the use of at least two antibodies, a
capture antibody being immobilized onto a solid support
and a detector antibody conjugated to a label. One of
the most commonly used labels is an enzyme while
colorometric, radioactive, fluorescent or
chemiluminescent and other labels are available.

An enzyme label offers high sensitivity, ready
availability and stability compared to a radioactive
label. However, the enzyme being a large protein
contributes to non-specific protein-protein interactions
which induce false positive responses, also refered to
as non-specific responses. By a false positive response
is meant a response that is not specifically associated
with the binding of the capture or the detector antibody
to the antigen of interest. Although the problem of
false positives is less frequent in immunoradiometric or
competitive radioimmunoassays, it manifests itself in
high sensitivity assays requiring large sample size and
long incubation step [Hunter et al, Lancet, Vol. ii,
1136, 1980]. Thus, it remains a universal problem to be
solved.

2

The false positive response can be observed in
assays for many different types of clinical parameters,
both quantitive and qualitative in nature, including,
for example, hormones of peptide nature, such as hCG,
5   LH, somatotropin, FSH, ACTH, LPH, prolactin, MSH, beta-
endorphinns, enkephalines, vasopressin, oxitocin, and
the like. In addition, false positive results can be
observed in determination of markers which are
indicators of malignancy, such as, carcinoembryonic
10   antigen (CEA), alpha-feto protein, prostatic specific
antigen, cancer antigens ca 19.9, ca 125, ca 15-3, beta-
2-microglobulin, ferritin, prostatic acid phosphatase,
and the like. Assays for specific microbial and viral
disease markers are also subject to false positive
15   results. Such markers are hepatitis B surface antigen,
hepatitis B core antigen, antibody to hepatitis B core
antigen, antibody to HIV, p17 antigen, p24 antigen, HTLV
1 and HTLV 2 antigen and specific peptide markers of
infection with HTLV 1 and HTLV 2, markers for hepatitis
20   A, C, D and E.
One form of non-specific interaction involves IgM
rheumatoid factor (RF). Methods for removal of RF
interference using non-specific aggregated IgG are well
known in the art. It is also well known that aggregated
25   IgG has enhanced reactivity with RF compared to
unmodified monomeric IgG [Oreskes et al, Immunology,
Vol. 51, 115-121, 1984, and Dissanayake et al,
Immunology, Vol. 32, 309-318, 1977]. In fact, Hallgren,
US 4,184,847 and US, 4,153,417 discloses a method for
30   detection of RF using labeled aggregated IgG.
Most of the methods for removing RF interference
involve a sample pretreatment process. Sakai et al, US
4,680,274, issued July 14, 1987 disclose the use of
ultrafine particles of less than 0.2 micrometers in size
35   having immobilized immunoglobulins thereon to absorb

3

non-specific factors from the sample. Vejtorp, J.
Virol. Methods, Vol. 1, 1-10, 1980 describes the use of
latex particles coated with aggregated human IgG as
means for removing RF from the sample in an ELISA based
5   rubella IgM antibody assay. Forghani et al, J. Clin.
Microbiol. Vol. 18, 652-657, 1983, also describe a
sample pretreatment method using polymerized human IgG.
Here, the sample is pretreated with glutaraldehyde-
aggregated human IgG followed by centrifugation to
10   remove the interferant. These references do not discuss
nor suggest the use of polymerized immunoglobulin, which
is of the same animal species of the capture and
detector antibodies, in sample or conjugate diluent.
          Briantais et al, J. Virol. Methods, Vol. 9, 15-26,
15   1984, describe the use of a radiolabeled (Fab')2
antibody to overcome RF interference in rubella and
hepatitis B core IgM assays. In their tests for human
IgM, they describe specific IgG binding non-specifically
with the immobilized IgG antibody. This type of
20   interference is said to be reduced only by the addition
to the dilution medium of non-specific human IgG or
fetal calf serum (FCS). They use unmodified IgG to
reduce the IgG-IgG interaction in an IgM capture assay.
The advantageous use of polymerized Immunoglobulin as in
25   assay milieu is not recognized.
          Gerlich et al, J. Med. Virol. Vol. 4, 227-238,
1979, describe the use of excess heat-aggregated human
IgG in a sample diluent to remove false positive
responses in a hepatitis B core IgM antibody assay.
30   Vejtorp, Acta Path. Microbiol. Scan., B, Vol. 89,
123-128, 1981, also describes the additon of heat-
aggregated human IgG to the serum diluent eliminating RF
interference in a rubella IgM assay. These are methods
for reducing RF interference applicable only in IgM
35   assays. The present invention is useful in immunoassays

4

in which the analyte of interest can be viral markers,
hormones or tumor markers, when both RF-positive and
RF-negative samples are tested.

    A mechanism for false positive responses in two
5   site immunoassays has recently been proposed by Levinson
et al, Clin. Immunol. Letter, Vol. 9, 101-116, 1988. It
involves endogeneous immunoglobulins (heterophile
antibodies) in a sample having broad specificities
toward animal antibodies. These heterophile antibodies
10 are capable of bridging capture antibodies with detector
antibodies in the absence of an antigen, producing a
non-specific reponse. Removal of this type of
interference requires either a sample pretreatment with
immunoglobulins from several species or addition of
15 these immunoglobulins to the assay milieu.

    The addition of non-specific immunoglobulins
derived from the same species as the antigen-specific
antibody is commonly used to block non-specific binding
caused by heterophile antibodies. For example, the
20 addition of a non-specific murine monoclonal antibody
that is of the same subclass as those of the specific
monoclonal antibodies is shown to reduce non-specific
interactions in a sandwich assay using murine monoclonal
antibodies.[Fukuyama et al, JP Application, 63-12,960,
25 published January 20, 1988]

    R. C. McCarthy et. al., Arch. Pathol. Lab. Med.,
Vol. 112, pp. 901-907, 1988, describe the nature of
"heterophile antibody" as primarily human IgM having
reactivity to mouse IgG. They were able to inhibit this
30 type of interference by adding an excess of unmodified
mouse IgG in the sample (final concentration of IgG:
1.3 mg/mL). They also report that the false positive
responses can be reduced, but not completely eliminated,
by using Fab' or (Fab')2 of IgG as the detector
35 antibody. The present invention employs polymerized IgG

5

with a defined range of molecular weight in the assay
media, which is much more effective in eliminating the
false positive responses.

These cited methods do not solve all of the false
5   positive problems. For example, it is often observed in
correlating enzyme-immunoassay (EIA) to radio-
immunoassay (RIA) that EIA values in certain samples are
always higher than the RIA values. Furthermore, due to
the complex nature of protein-protein interactions
10   involved, the incidence of false positives is highly
dependent on the assay configuration. For example, a
forward sandwich assay, wherein a sample is incubated
with the capture antibody on a solid support, washed to
remove all of the unwanted components, then incubated
15   with the detector antibody, is less prone to non-
specific interactions.

The present invention provides a method useful for
reducing non-specific interactions in immunoassays
wherein the capture antibody and detector antibody are
20   derived from the same or different animal species. It
is useful regardless whether the false positive sample
is RF-positive or RF-negative. The method can be used
either with a whole antibody or (Fab')2 fragment.

SUMMARY OF INVENTION

25   The present invention provides a method for
reducing false positive responses in an immunoassay for
detection of an antigen comprising:

(a) forming a heat or chemically polymerized
immunoglobulin that has no specific reactivity with said
30   antigen,

(b) treating a sample suspected of containing
said antigen with said polymerized immunoglobulin, and

(c) subjecting the treated sample to an
immunoassay employing antibodies of the same animal
35   species as the polymeric immunoglobulin.

6

EXAMPLE I: SYNTHESIS OF POLYMERIC
IMMUNOGLOBULIN (POLY IG)

The polymeric Ig can be generated by heating as
described in the references mentioned in Background Art.

5   Alternatively, it can be synthesized under defined
chemical conditions as follow. In all cases the
procedures are optimized with the goal of maintaining
the solubility of the Poly Ig in buffered aqueous
solution.

10   I.1 Reaction with S-Acetylmercaptosuccinic Anhydride
(SAMSA) and N,N'-1,4-Phenylenedimaleimide (PDM):

Sulfhydryl groups are introduced into a monoclonal
IgG by treatment with SAMSA at 50 molar excess in
phosphate buffer, pH 7.0. The sulfur protecting group

15   is cleaved with hydroxylamine and the protein fraction
is obtained by chromatography on Sephadex G-25, eluting
with phosphate buffer pH 6.5.

The thiolated IgG is treated with a 20 molar excess
of PDM and the reaction mixture is allowed to rock at

20   room temperature. The progress of the polymerization
reaction is monitored by HPLC analysis on a Zorbax(R)
Bio Series GF-250 column (9.4 X 250 mm). When the yield
of IgG polymer of optimal size reaches a maximum the
reaction is quenched with 2-mercaptoethylamine or

25   directly fractionated by preparative HPLC on a Zorbax(R)
Bio Series GF-250XL column (21.1 X 250 mm).

I.2 Reaction with Disuccinimidyl Suberate (DSS):

A purified monoclonal IgG (IgG1 subclass) at
approximately 5 mg/ml concentration, is reacted with DSS

30   at 12 to 25 fold molar excess to the antibody. The
mixture is stirred magnetically for 2 hours.

A sodium azide solution is added to the reaction
mixture in order to achieve a final sodium azide
concentration of 0.1%. After stirring for 0.5 hour, the

35   mixture is transferred to a dialysis bag and dialyzed

                                    7

against a buffer solution containing 0.1 M Tris, 0.15 M
Sodium chloride and 0.1% azide for 24 hours.
I.3 Reaction with Glutaraldehyde:
        Monoclonal IgG solution at a concentration of 4 to
5   10 mg/ml is reacted with a 0.005% to 0.03% solution of
glutaraldehyde at 20 to 24°C for 16 to 24 hours.
Preferred conditions are IgG at 5 mg/ml, reacting with
0.02% glutaraldehyde at 22°C for 20 hours.
        The solution is dialyzed against phosphate buffered
10 saline overnight.
I.4 Molecular Weight Distribution:
        The polymerized IgG preparations are analyzed by
molecular sieving chromatography. The solution of
polymer is fractionated in a glass column (approximately
15 2.5 cm in diameter and 85 cm in length) packed with a
gel such as Sephacryl S300 (Pharmacia Co.) and
equilibrated with 0.1 M phosphate buffered saline
solution, pH 7.4.
        In addition to monomeric IgG, four major polymeric
20 ractions are obtained. By comparing to standard
molecular weight markers, the median molecular weights
of the four fractions are estimated to be 1,500,000 to
2,000,000; 1,000,000; 700,000 and 400,000 Daltons.
These values translate to polymers of 10-15, 6-8, 4-6
25 and 3-4 IgG molecules respectively. All fractions are
soluble in aqueous buffer, such as phosphate buffered
saline.
        Poly Ig prepared by different procedures described
above consists of somewhat different proportions of the
30 four fractions. However, the effectiveness in reducing
false positivity can not be differentiated. The
preferred poly Ig preparation therefore consists of 3 to
15 IgG molecules.

8

EXAMPLE II: USE OF POLYMERIZED IMMUNOGLOBULIN
IN AN ENZYME IMMUNOASSAY FOR HUMAN
ANTIGEN (HBSAG ASSAY)

An enzyme immunoassay for human hepatitis B surface
5   antigen is used to demonstrate the efficacy of Poly Ig
in reducing the incidence of false-positive responses.
The assay steps are described as follows:
II.1 TWO-STEP FORWARD FORMAT:
1. Antigen Capture:
10   In a plastic test tube, serum sample (200 ul) is
first incubated with chromium dioxide particles
(100-200 ug), that are coated with anti-HBsAg monoclonal
antibodies (IgG1 subclass), and a sample diluent
(100 ul) containing 0.1 M sodium citrate, and 0.15 M
15   sodium chloride. The incubation is allowed to proceed.
for 30 minutes in a 37°C heating block.
2. Wash:
A wash solution containing 0.1 M Tris and 0.15 M
sodium chloride, pH 7.8 was added to the reaction
20   mixture. After mixing the slurry by vortexing, the test
tube is placed in a rack equipped with small cylindrical
magnets situated at both sides of the tube. The
chromium dioxide particles are attracted to the side
walls of the tube. The wash solution is then removed by
25   suction. The wash, particle separation and aspiration
steps are repeated two more times.
3. Enzyme Labelling:
A solution (50-250 ul) containing covalently
conjugate of anti-HBsAg monoclonal antibody (or its
30   (Fab')2 fragment, IgG1 subclass) and calf intestinal
alkaline phosphatase, 0.1 M Tris and 5% bovine serum
albumin, pH 7.8 is added to the washed particles. The
mixture is allowed to incubate at 37°C for 30 minutes.

9

4. Wash:

The same wash steps as described in Step (2) are
repeated.

5. Substrate Reaction and Measurement:

A substrate solution (240 ul) containing
4-methylumbelliferyl phosphate in diethyleneamine, pH
8.9, is added to the washed chromium dioxide particles.
The substrate reacts with alkaline phosphatase to
generate fluorescent 4-methylumbelliferone. The
reaction is allowed to proceed for precisely 5 minutes
at which time 0.5 M EDTA solution is added to stop the
reaction.

The particles are separated from the solution in
the magnetic rack and the clear supernatant is
transferred to a IMMUNLON-2(R) microtiter plate. The
fluorescent signals are measured on a Dynatech
MicroFLUOR(R) reader equipped with a 365 nm excitation
filter and a 450 nm emission filter.

Poly IgG, when used, is added either in the sample
diluent (Step 1) or in the conjugate reagent (Step 3) at
a concentration specified by each experiment.

II.2 SIMULTANEOUS FORMAT:

Alternately, the assay can be performed by the
simultaneous incubation of sample, sample diluent,
antibody-coated chromium dioxide particles and conjugate
reagent. This format necessarily omits the first wash
step (Step 2 of II.1). After the incubation, the assay
proceeds to Steps 4 and 5 as described above.

Again, Poly Ig, when used, is added either in the
sample diluent or in the conjugate reagent at a
concentration specified by each experiment.

10

EXAMPLE III: USE OF SAMSA/PDM POLYMERIZED
MONOCLONAL IGG (Poly IgG) IN HBSAG ASSAY
III.1 Efficacy on normal serum samples:

    Table 1 exhibits correlative decrease of the
5   fluorescent signals produced by sample No. 630 with the
    increasing concentration of Poly IgG added. This sample
    is rheumatoid factor (RF) negative. However, it
    produces a false-positive response with pepsin-
    fragmented anti-HBsAg/alkaline phosphatase conjugate
10 [(Fab')2-AP]. When other normal HBsAg-negative serum
    samples are tested with the inclusion of Poly IgG no
    significant effect is observed.

    The response of a HBsAg-containing positive control
    is not affected by poly IgG (see control values in
15 Tables 1). This is also apparent by the results shown
    in Table 3.

    Table 1 also indicates that a minimum of 7 ug/test
    Poly IgG is needed to reduce the assay response to
    negative, but additional amounts do not induce much
20 further change. The preferred quantity of Poly IgG used
    in this type of heterogeneous enzyme immunoassay is 7 to
    15 ug/test (200 ul serum or plasma sample per test).

Table 1: Effect of the Quantity of Poly IgG on
HBSAG Assay Results##

Poly IgG added**	0 ug/test	7 ug/test	14 ug/test
Pos. Control(RFU)#	1882	1907	1885
Neg. Control(RFU)	45	42	49
Cut Off*	165	162	169
No. 630 (+/-)	264(+)	91(-)	61(-)

Footnotes:

A two-step forward format and (Fab')2-AP conjugate
are used in this experiment.

** Poly IgG is synthesized by SAMSA/DPM procedure.

RFU: Relative Fluorescent Unit

* Cut off = Neg. Control + 120, Values greater than Cut
off are assigned as positive.

Table 2 summarizes a study comparing no addition, addition of monomeric IgG and addition of poly IgG in the HBSAG assay. While a sample (#34) with normal response is not affected, false positive samples are reduced to negative much more effectively by Poly IgG than monomeric IgG.

12

Table 2: Comparison of Monomeric IgG to Poly IgG in
Reducing False Positive Responses in HBSAG Assay*

	Samples	No Additive	Monomeric IgG Added**	Poly IgG Added**
5				
	Pos. Control(RFU)	N/T	N/T	1504
	Neg. Control(RFU)	N/T	N/T	49
	Cut Off#	-	-	169
10				
	No. 34	98(-)	91(-)	89(-)
	No. 55	423(+)	189(+)	63(-)
	No. 57	1260(++)	214(+)	114(-)

15 Footnotes:

* A two-step forward format and intact IgG-alkaline
phosphatase conjugate are used in this study.

** Twenty (20) ug/test of either monomeric or polymeric
IgG is used. Poly IgG is synthesized by the SAMSA/DPM
20   Procedures.

\# Cut off = Neg. Control+120, Values greater than Cut
off are assigned as positive.

III.2 Efficacy of Poly IgG on rheumatoid factor positive
25 samples:

Rheumatoid factors are autoimmune antibodies which
are known to interfer with enzyme-linked immunoassays
and cause sporadic false-positive responses. Such
false-positive responses are not directly correlated to
30 the RF titers. The use of pepsin-fragmented IgG
[(Fab')2] to prepare the antibody-enzyme conjugate has
been reported to help reduce the incidence of false-
positive responses associated with RF-positivity.

Data presented in Table 3 indicate that certain
35 RF-positive samples still produce false-positive

13

results, even with the use of (Fab')2-enzyme conjugate.
These interferences are eliminated by the use of Poly
IgG.

Table 3: Effect of Poly IgG on Rheumatoid Factor
Positive Samples when (Fab')2-AP is Used as Enzyme
Conjugate

CONTROL VALUES:

Pos Control*	Neg Control	Cut-Off(Neg+120)
1189	92	212

		No PolyIgG		PolyIgG#	
Samples	RF Titer	RFU	+/-	RFU	+/-
129	N/T	76	-	101	-
137	10,240	143	-	90	-
142	10,240	111	-	73	-
147	10,240	94	-	99	-
173	10,240	101	-	78	-
176	10,240	71	-	113	-
177	10,240	767	+	76	-
187	10,240	108	-	139	-
385	10,240	333	+	115	-
190	10,240	97	-	90	-
279	10,240	87	-	78	-
327	>10,240	76	-	80	-
False positive		2/12		0/12	

Footnotes:

* A two-step forward format and a (Fab')2-AP conjugate
are used.

Poly IgG prepared by SAMSA/DPM Procedure, 15 ug/test
used.

14

EXAMPLE IV: EFFECT OF DSS-POLYMERIZED
IGG IN HBSAG ASSAY

Table 4 summarizes a study involving 80 negative
5 samples that are identified as likely to cause false
positive responses and 7 HBsAg-positive samples.

When the assay is conducted without Poly IgG, 24
samples exhibit falsely positive responses. With the
addition of DSS-polymerized Poly IgG, all samples are
10 statistically indistinguishable from the negative
control. The positive samples are not affected by the
Poly IgG.

Table 4: Effect of DSS-polymerized IgG on HBSAG Assay*
15 _____

	No Poly IgG	Poly IgG
	-----------	--------
Total Negative Samples Tested**	80	80
Total False Positive	24	0
Percent False Positive	30%	0%
False Positive Mean (St. Dev.)	1266(1305)	71(28)
Negative Control (St. Dev.)	65(6)	65(6)
Delta	1,201	6
(False Positive mean-Neg Control)		

25 ==

Total Positives Tested	7	7
Total True Positive (T/P)	7	7
T/P Mean (St. Dev.)	3162(976)	3115(1009)

_____

30 Footnotes:

* A two-step forward format and an intact IgG-Alkaline
Phosphatase conjugate are used in this study. Poly IgG
is used at 15 ug/test.

** These are samples identified as likely to cause false
35 positive responses.

EXAMPLE V: EFFECT OF GLUTARALDEHYDE-
POLYMERIZED IGG ON HBSAG ASSAY

Table 5 exhibits the results of using two Poly IgG preparations in the HBSAG assay with five identified false positive samples. These two lots were synthesized from IgG generated from two mouse monoclonal cell lines. Both preparations reduce the false positive responses to negative range.

This study indicates that glutaraldehyde-polymerized IgG performs as well as the SAMSA/DPM- and DSS- polymerized material in reducing the false positive responses. It also shows that Poly IgG prepared from two lots of IgG perform equally well.

16

Table 5: Effect of Poly IgG Synthesized by Glutaraldehyde Procedures on HBSAG Assay**

Control Values:	
Positive Control	1086
Negative Control	58
Cut-Off(=Neg Ctl+120)	178

SAMPLE ID*	No PolyG	Poly IgG #0619	Poly IgG #0620
9388	174	73	93
8523VS	1066	67	44
L155951	1180	82	76
E47458	4000	70	47
182999	74	46	63

* These are samples identified as likely to cause false
  positive responses in the HBSAG assay.

These two lots of Poly IgG are synthesized from two
  mouse monoclonal cell lines using the Glutaraldehyde
  Procedure. Poly IgG is used at 15 ug/test.

** A simultaneous format and intact IgG-Alkaline
  Phosphatase conjugate are used in this study.

EXAMPLE VI: EFFECT OF POLY IGG IN A HETEROGENEOUS IMMUNOASSAY FOR HUMAN CARCINOEMBRYONIC ANTIGEN (CEA)

The heterogeneous immunoassay for CEA is based on
the same principle as outlined in Example II. In the
assay, 50 ul of serum sample, 50 ul of anti-CEA-alkaline
phosphatase conjugate solution, and 25 ul of an anti-CEA
antibody coated chromium dioxide particle suspension are
incubated at 37°C for 30 minutes. The particles are
then washed and treated with the fluorescent substrate
solution in the same manner as described in Example II.

17

Fluorescent signals are then measured at an excitation
wavelength of 365 nm and an emission wavelength of
450 nm.

5   Many serum samples possess interfering material
which resulted in falsely elevated CEA results. In the
case of a quantitative determination such as measurement
of CEA, this false positive result is determined by
comparison of the result in a commercially available
assay with that obtained in the new assay. Since normal
10   humans will have from 5 to 8 ng/ml of CEA in their sera,
values which are greater than this are indicative of
disease. Discrepancies between two assays where the
value is above the normal range will be considered to be
the result of an interferring substance. The
15   interference is usually seen as a value higher in
analyte level that the reference, commercially available
assay. Inclusion of Poly IgG in the conjugate solution
at a concentration of 19 ug/test effectively eliminated
such false-positive responses. Table 6 exhibits a
20   summary of CEA tests which demonstrate such effect.

Table 6: Effect of Poly IgG on False-Positive
Samples in CEA Assay

Sample ID	[CEA] No Poly IgG	[CEA] With Poly IgG	Tandem(r) [CEA]*
102	8.8 ng/mL	0.9 ng/mL	4.9 ng/mL
109	8.0	0.3	1.0
7721	10.1	2.7	4.2
E47458	48.9	10.7	8.6
L215797	65.8	2.5	3.0

* Manufactured and sold by Hybritech Inc. San Diego, CA.

18

EXAMPLE VII: EFFECT OF POLY IGG IN A HETEROGENEOUS
IMMUNOASSAY FOR HUMAN THYROID STIMULATING HORMONE (TSH)

The immunoassay for human TSH is based on the same
principle described in Example II. It is performed by a
5   simultaneous incubation of 50 ul of serum sample, 50 ul
of anti-TSH-alkaline phosphatase conjugate solution and
25 ul of anti-TSH coated chromium dioxide particles.
The washing, substrate reaction and fluorescent
measurement are performed in the same manner as
10   described in Example II.

Table 7 shows the effect of including Poly IgG in
the TSH test, when false positive samples are involved.
While true TSH concentrations are correctly measured in
samples 8523, L(10) and L(50), those exhibiting falsely
15   high results are corrected by the use of Poly IgG.

Table 7: Effect of Poly IgG on False-Positive
Samples in TSH assay

Sample ID	[TSH] No PolyIgG	[TSH] With PolyIgG#
L215797	2.92 mIU/L	0.85 mIU/L
8523	1.70	1.69
393ms	6.13	2.66
L155951	2.77	1.64
L(10)*	9.99	10.02
L(50)*	49.50	50.02

* Calibrators which have been spiked with purified TSH
30   to concentrations of 10 mIU/L and 50 mIU/L,
respectively.

Poly IgG used at 16 ug/test.

19

CLAIMS:

1. A method for reducing false positive responses
in an immunoassay for detection of an antigen in a
sample, said method comprising not in any order, the
5   steps of:

         forming a polymerized immunoglobulin from an
immunoglobulin that has no specific reactivity with said
antigen;

         contacting said sample suspected of containing
10   said antigen with said polymerized immunoglobulin; and
         subjecting the sample to an immunoassay
employing antibodies derived from the same animal
species as that of the polymeric immunoglobulin.

2. The method of Claim 1 wherein the
15   immunoglobuln is IgG.

3. The method of Claim 2 wherein the polymerized
IgG comprises about 3 to 15 IgG molecules.

4. The method of Claim 3 wherein the polymerized
IgG is present in an amount sufficient to provide about
20   7 to 20 μg per immunoassay.

5. The method of Claim 1 wherein the
immunoglobulin in IgM.

6. The method of Claim 2 wherein said sample is
pretreated with the polymerized IgG.

25   7. The method of Claim 1 wherein the polymerized
immunoglobulin is a component of the reagents used in
the assay.

8. The method of Claim 1 wherein the antigen to
be detected is selected from the group consisting of
30   hCG, LH, TSH, FSH, alpha-feto protein, prostatic
specific antigen, carcinoembrionic antigen (CEA), cancer
antigens ca 19.9, ca 125 and ca 15-3, human hepatitis B
surface antigen, hepatitis B core antigen and hepatitis
C, D and E.

9.   The method of Claim 1 wherein the antigen to be detected is a virus or viral marker.

10.   The method of Claim 1 wherein the antigen to be detected is a macromolecular hormone.

11.   The method of Claim 1 wherein the antigen to be detected is a molecular component of human malignant tumor cells.

12.   The method of Claim 8 wherein the antigen to be detected is human hepatitis B surface antigen.

13.   The method of Claim 8 wherein the antigen to be detected is human thyroid stimulating hormone (TSH).

14.   The method of Claim 8 wherein the antigen to be detected is carcinoembryonic antigen (CEA).

15.   A method of performing an enzyme immunoassay employing two antibodies of the same animal species for capture and detection of an antigen in a sample to be assayed, said method comprising the steps of:

a)   forming a polymerized immunoglobulin of the same species as those in the assay, said polymerized immunoglobulin having no reactivity to the antigen to be detected;

b)   contacting said sample with a solution containing the polymerized immunoglobulin of step a) while performing said assay;

c)   performing the assay to determine the presence or absence of antigen in said sample.

16.   The method of Claim 15 wherein the immunoglobulin is IgG.

17.   The method of Claim 16 wherein said sample is pretreated with the polymerized IgG.

18.   The method of Claim 15 wherein the polymerized immunoglobulin is a component of the reagents used in the assay.

19. The method of Claim 15 wherein the detector antibody to enzyme conjugate is comprised of a pepsin-hydrolysis produced antibody fragment.

20. The method of Claim 15 wherein the detector antibody to enzyme conjugate is comprised of a papain produced antibody fragment.

21. The method of Claim 19 wherein the fragment is (Fab')2, F(ab)2 or Fab.

22. The method of Claim 15 wherein the antigen to be detected is a virus or viral marker.

23. The method of Claim 15 wherein the antigen to be detected is a macromolecular hormone.

24. The method of Claim 1 wherein the antigen to be detected is selected from the group consisting of hCG, LH, TSH, FSH, alpha-feto protein, prostatic specific antigen, carcinoembryonic antigen (CEA), cancer antigens ca 19.9, ca 125 and ca 15-3, human hepatitis B surface antigen, hepatitis B core antigen and hepatitis C, D and E.

25. The method of Claim 24 wherein the antigen to be detected is human hepatitis B surface antigen.

26. The method of Claim 24 wherein the antigen to be detected is human thyroid stimulation hormone (TSH).

27. The method of Claim 24 wherein the antigen to be detected is carcinoembryonic antigen (CEA).

28. The method of Claim 15 wherein the antigen to be detected is a molecular component of human malignant tumor cells.

29. A method for forming polymeric immunoglobulin comprising treating immunoglobulin with a heterobifunctional or a homobifunctional chemical linking reagent whereby the resulting polymeric molecules exist in soluble form; and wherein the polymeric molecules are comprised of 3 to 15 monomeric IgG molecules.

22

30. The method of Claim 18 wherein the heterobifunctional reagents is S-acetylmercaptosuccinic anhydride (SAMSA).

31. The method of Claim 18 wherein the
5 homobifunctional agent is disuccinimidyl suberate (DSS) or glutaraldehyde or their derivatives.

32. The method of Claim 7 wherein the amount of polymeric immunoglobulin in the assay is in the range of about 2 μg to 25 μg per test.
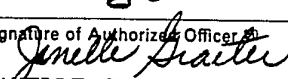
10 33. A diagnostic test kit for conducting an immunoassay for the detection of an antigen and for reducing false positive responses in said immunoassay, said test kit comprising:

a selected quantity of polymerized IgG
15 comprising about 3 to 15 IgG molecules in an amount sufficient to provide a minimum of about 7 μg per test, said polymerized IgG having no specific reactivity with the antigen to be detected; and

a labelled antibody to the antigen to be
20 detected.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05943

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S.CL.: 435/4,5,7.1,7.9,7.94; 436/512,536,547,825,826; 530/387,389,402
IPC(5): C07K 3/06; A61K 35/14; G01N 33/53,33/563,33/536

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴	
Classification System	Classification Symbols
U.S.CL.	435/4,5,7.1,7.9,7.94; 436/512,536,547,825,826; 530/387,389,402

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

APS, CAS, BIOSIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X P,Y	US, A, 4,914,040 (LENZ et al.) 03 April 1990, see column 3, line 1-column 2, line 43, column 5, lines 1-32, column 6, lines 17-30 and 55-62 and claims 1-9, 19-20, 23-27, 34-35, and 37.	1-2,4-5, 7-16,18-29, and 31-33 3,6,17 and 30
Y	US, A, 4,220,565 (KATZ) 02 September 1980, see column 2, lines 57-66, column 7, lines 7-42 and claim 4.	30 and 31

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²
03 January 1991	20 FEB 1991
International Searching Authority ¹	Signature of Authorized Officer
ISA/US	JANELLE GRAETER

Form PCT/ISA/210 (second sheet) (May 1986)