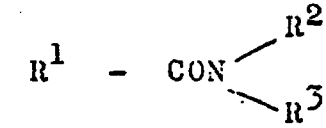
An improved Reagent for Latex Agglutination and a method employing the improved reagent.

2042170

The present invention relates generally to a reagent for latex agglutination and more particularly to an improvement in latex agglutination reactions.

In recent years there have been increasingly and frequently employed clinical diagnostic methods using immunological procedures, because they are specific and have a high sensitivity. Among them, a measurement utilizing the agglutination reaction of latex particles sensitized with an antigen or antibody has the advantages that the procedures are simple and that the test results are obtained in a very short time. However, this method using the prior art latex diagnostic agents often misleads the diagnosis, because these agents are prone to cause non-specific agglutination reactions in the measurement of immunologically active substances contained in urine or body fluids such as e.g. serum or plasma.

We have now unexpectedly found that the aforementioned non-specific agglutination can be prevented by conducting latex agglutination reactions in the presence of one or more members selected from di-lower alkyl sulfoxides and compounds of the formula:



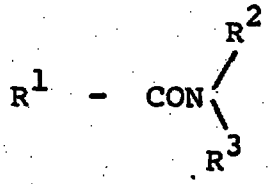
(I)

wherein R^1 is a hydrogen atom, or lower alkyl group or an amino group, which may be substituted with a lower alkyl group and R^2 and R^3 are each independently a hydrogen atom or a lower alkyl group.

The present invention is based on these findings.

The present invention provides a diagnostic agent which comprises:

(1) 2-52% (weight/volume) of one or more members selected from (a), compounds of the formula (I):



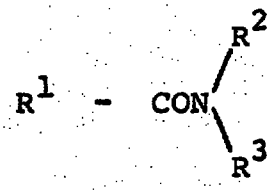
(I)

wherein R^1 is a hydrogen atom, a lower alkyl group or an amino group, which may be substituted with a lower alkyl group and R^2 and R^3 are each a hydrogen atom or a lower alkyl group, and (b) a di-lower alkyl sulfoxide and

(2) 0.4 to 1.4% (weight/volume) of latex particles sensitized with an antigen or antibody, in

(3) a buffer with a pH range of 7-9.

and a diluent for a test sample in a latex agglutination reaction immunoassay which comprises (1) 2-52% (weight/volume) of one or more members selected from (a) compounds of the formula:



(I)

wherein R^1 is a hydrogen atom, a lower alkyl group or an amino group, which may be substituted with a lower alkyl group and R^2 and R^3 are each a hydrogen atom or a lower alkyl group, and (b) a di-lower alkyl sulfoxide in

(2) a buffer with a pH range of from 6-9.

The invention also covers improved latex agglutination reactions effected with the use of said compounds. Other features and advantages of the invention will be made clear from the description and claims hereinafter.

In the formula (I), the lower alkyl group for R^1, R^2 and R^3 has preferably up to 2 carbon atoms, and methyl is especially advantageous. The amino group for R^1 may be substituted with one or two lower alkyl groups which preferably contain up to 2 carbon atoms, methyl being especially preferred. The lower alkyl group in the di-lower alkyl sulfoxide preferably contains up to 2 carbon atoms methyl being the most convenient.

Representative examples of the compound of the

formula (I) and the di-lower alkyl sulfoxide are urea, N-methylurea, N,N'-dimethylurea, N-methylformamide, N,N-dimethylformamide, N,N-diethylformamide, N-methylacetamide, N,N-dimethylacetamide, N,N-diethylacetamide, dimethyl sulfoxide and diethyl sulfoxide. These compounds may be used alone or in various combinations. The combinations of urea and N,N-dimethylformamide is especially advantageous.

In the present invention, the above-mentioned non-specific agglutination can be prevented by conducting a latex agglutination reaction in the presence of one or more of the compounds (I) and di-lower alkyl sulfoxides (hereinafter referred to as the instant compound), thereby reducing false-positive results in the latex agglutination reaction, and thus remarkably increasing the reliability of the test. The instant compound is preferably present in the latex agglutination reaction system (after being mixed with a test sample) at a concentration of from 1 to 13% (weight/volume) (hereinafter all the percentages are expressed as weight/volume percent), especially 1 to 7%.

The present invention can be applied to any reaction to detect immunologically active substances contained in the urine or body fluids (e.g. serum or

plasma) of mammals, especially human, by using the agglutination with latex particles sensitized with an antigen or antibody corresponding to the substances. Representative examples of such immunologically active substances are serum proteins such as human immunoglobulin G, human immunoglobulin M, human immunoglobulin A, human albumin, human fibrinogen (fibrinogen, fibrin and their decomposed products), α-fetoprotein, C-reactive protein, β_2-microglobulin or myoglobin; hormones such as human chorionic gonadotropin (hereinafter referred to as HCG), human placental lactogen, insulin or steroids; or immunoglobulin fractions, especially specific antibodies belonging to immunoglobulin G or immunoglobulin M (e.g. an antiviral antibody or a rheumatoid factor).

In the present invention, the instant compound may be caused to be present in the latex agglutination reaction system by adding it to a diagnostic agent containing sensitized latex particles, or by incorporating it in a diluent for a test sample.

The latex particles which are sensitized with the antigen or antibody corresponding to the immunologically active substances may be of any latices which are employable for the latex agglutination test, and are exemplified by those of homopolymers and copolymers produced from styrene or its derivatives (e.g. methylstyrene, ethylstyrene or chlorostyrene), olefins (e.g. ethylene or propylene), acrylic acid or its esters (e.g. methyl acrylate or ethyl acrylate), methacrylic acid or its derivatives (e.g. ethyl methacrylate, acrylonitrile or acrylamide), dienes (e.g. butadiene, chloroprene or isoprene etc), vinyl chloride, vinylidene chloride or vinyl acetate. Among them, the latices of the homopolymer or copolymer latices comprised of styrene, chlorostyrene, acrylic acid, vinyltoluene or methyl methacrylate are advantageously used. These latices are preferably employed as those having a particle size of up to about 1 μ, particularly of about 0.01 to 1.0 μ, more particularly, about 0.1 to 0.6 μ.

The treatment to sensitize the afore-mentioned

latex particles with the antigen or antibody corresponding to the immunologically active substances can be effected by methods known in the art. The treatment conditions vary to some degree depending on the physiochemical properties of the sensitizing substance and latex particles, and, for example, where the latex particles are to be sensitized with an antibody, the sensitizing is conveniently effected as follows. Antiserum is subjected to salting out in a conventional manner to obtain the γ-globulin fraction, which is then dissolved in a 0.005 to 0.2 M buffer of a pH of 7 to 9 at a concentration of 0.01 to 1%. As the buffer, glycine buffer, phosphate buffer, borate buffer and Good's buffers such as a buffer of N-2-hydroxyethylpiperadine-N'-2-ethanesulfonic acid (hereinafter referred to as HEPES) and N-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (herein referred to as TES) are advantageously employed. A suspension of latex particles at a concentration of 0.1 to 10% is added to the solution obtained above, which is then incubated at room temperature for 1 to 6 hours or at 37°C to 60°C for 0.5 to 3 hours by allowing it to stand or with stirring to sensitize

the latex particles, and the mixture is then centrifuged. The thus obtained sensitized latex particles are suspended again in a buffer to which the instant compound has already been added to give the reagent for the diagnosis. As the buffer, the aforementioned glycine buffer, phosphate buffer, borate buffer or Good's buffers such as HEPES and TES buffers having a pH of 7 to 9 are preferably employed. The preferred concentrations of the instant compound and the sensitized latex particles in the diagnostic agent in accordance with the present invention can be chosen from the range of 2 to 52% and the range of 0.4 to 1.4%, respectively, depending on whether a test sample is diluted or not before the latex agglutination reaction. More particularly, where the diagnostic agent is to be mixed with a test sample diluted with a conventional diluent, the diagnostic agent preferably contains the instant compound at a concentration of four times the aforemention concentration range of 1 to 13% in the latex agglutination reaction system (preferably 1 to 7%), i.e. at a concentration of 4 to 52%, preferably 4 to 28%, and the sentisitzed latex particles at a concentration of 0.9 to 1.4%. In the case of a diagnostic agent which is to be directly mixed with a non-diluted

test sample, an agent is preferably employed which contains the instant compound at a concentration of twice the aforementioned concentration range (of 1 to 13%, more particularly 1 to 7%), i.e. at a concentration of 2 to 26%, preferably 2 to 14%, and the sensitized latex particles at a concentration of 0.4 to 0.7%.

Where the instant compound is brought into a diluent for a test sample, any of the conventional buffers for diluting a test sample such as glycine buffer, borate buffer, phosphate buffer or Good's buffer, e.g. HEPES and TES buffers, can be employed as the fundamental component, and the instant compound may suitably be employed in a preferred pH range of 6 to 9 and at an electrolyte concentration of 0.05 to 0.3 M. A 0.1 M glycine buffer (pH 8.2) or a 0.1 M HEPES buffer (pH 7.5), for example, is especially advantageously employed. It is preferred that such buffer should contain the instant compound at a concentration of about four times the aforementioned concentration range (about 1 to 13%, particularly 1 to 7%).

If desired, the aforementioned reagents for the diagnosis may contain 0.01 to 0.2% of serum albumin, preferably bovine serum albumin, and 0.02 to 0.2% of a preservative such as ————————————

sodium azide.

With the use of the above-mentioned reagent, the latex agglutination reaction which comprises incubating a test sample and the sensitized latex particles to assess the occurrence of the agglutination of the latex particles is conducted in the presence of the instant compound. The reaction may be carried out by a *per se* known technique such as the slide method described in e.g., Am. J. Clin. Pathol. 58, 305-316 (1972) and the test-tube method described in, e.g., Am. J. Obstet. Gynecol. 131, No. 6, 701-702 (1978).

The test sample such as urine, serum or plasma may be pretreated using an appropriate filter or may be directly subjected to the reaction without filtration.

By the present invention, the non-specific agglutination in the latex agglutination reaction can be remarkably prevented, thus greatly improving the reliability of the test results.

The effects of the present invention will be further illustrated in the following Examples and Test Examples which are not intended to restrict the scope of the present invention.

Throughout the present specification as well

as in the claims, the abbreviations "mg", "g", "μℓ", "mℓ", "μ", "°C", "M", "N" and "r.p.m." respectively refer to "milligram(s), "gram(s), "microliter(s)", "milliliter(s)", "micron(s)", "degree(s)" centigrade", "molar concentration", "normality" and "revolution(s) per minutes", and percentages are weight/volume unless otherwise specified.

Example 1

Glycine	3.75	g
Urea	60	g
Sodium azide	0.5	g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.2 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 2

HEPES	11.9	g
N,N-Dimethylformamide	100	g
Sodium azide	0.5	g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.5 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a

diluent for a test sample.

Example 3

Glycine	7.5 g
N,N-Dimethylformamide	75 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjuted to 8.6 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 4

HEPES	5.95 g
Dimethyl sulfoxide	100 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.2 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 5

HEPES	11.9 g
Urea	50 g
N,N-Dimethylformamide	50 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ

of distilled water and the pH was adjusted to 7.5 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 6

Glycine	3.75 g
N,N-Diethylformamide	50 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.0 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 7

Glycine	7.5 g
N,N-Diethylacetamide	50 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.8 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 8

HEPES	11.9 g
Diethyl sulfoxide	30 g

Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.5 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 9

HEPES	11.9 g
N-Methylurea	75 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.2 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 10

Glycine	3.75 g
N-Methylformamide	100 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.6 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 11

Glycine	3.75 g
N-Methylacetamide	75 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.6 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 12

HEPES	11.9 g
Urea	60 g
N,N-Dimethylformamide	50 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.5 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a diluent for a test sample.

Example 13

(1) Anti-C-reactive protein serum obtained by immunizing rabbits was subjected to salting out using ammonium sulfate in a conventional manner to yield a γ-globulin fraction, which, after the dialysis, was dissolved in a 0.01 M phosphate buffer

of pH 8.2 to yield a 0.2% γ-globulin solution. To 10 mℓ of this solution was added an equal volume of a 2% polystyrene latex dispersion (particle size 0.2 μ; dispersed with a 0.1 M phosphate buffer of pH 8.2), and the latex particles were sensitized at 37°C for 2 hours with occasional shaking. Centrifugal separation at 12,000 r.p.m. for 15 minutes yielding latex particles sensitized with anti-C-reactive protein antibody as a precipitate.

(2)

Glycine	3.75 g
Urea	30 g
N,N-Dimethylacetamide	25 g
Bovine serum albumin	0.25 g
Sodium azide	0.25 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.2 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to obtain a solution, in which the sensitized latex particles prepared at (1) above were dispersed at a concentration of 0.6% to yield a diagnostic agent for measuring C-reactive protein which contained anti-C-reactive protein antibody sensitized latex. This agent

can be provided for the agglutination reaction without diluting a test sample.

Example 14

(1) Anti-human fibrinogen serum obtained by immunizing rabbits was subjected to salting out using ammonium sulfate in a conventional manner to yield a γ-globulin fraction, which, after dialysis, was dissolved in a 0.01 M phosphate buffer of pH 8.0 to yield a 0.2% γ-globulin solution. To 10 mℓ of this solution was added an equal volume of a 2% polystyrene latex dispersion (particle size 0.25 μ ; dispersed with a 0.01 M phosphate buffer of pH 8.2), and the latex particles were sensitized at room temperature for 6 hours with occasional shaking. The ceentrifugal separation at 10,000 r.p.m. for 15 minutes yielded latex particles sensitized with anti-human fibrinogen antibody as the precipitate.

(2)

HEPES	5.95 g
Urea	25 g
N,N-Dimethylformamide	25 g
Bovine serum albumin	0.25 g
Sodium azide	0.25 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.5 by

adding 1N NaOH, after which distilled water was added to make the total volume up to 500 mℓ to yield a solution in which the sensitized latex particles prepared at (1) above were dispersed at a concentration of 0.6% to give a diagnostic agent for measuring fibrinogen, fibrin and their decomposition products. This agent can be provided for the agglutination reaction without diluting a test sample.

Example 15

Glycine	3.75 g
N,N-Dimethylformamide	10 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.5 by adding a 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to yield a solution in which the sensitized latex particles prepared in Example 13 (1) were dispersed at a concentration of 0.6% to give a diagnostic agent for measuring C-reactive protein. This agent can be provided for the agglutination reaction without diluting a test sample.

Example 16

(1) Human γ-globulin (Cohn's fraction II)

was dissolved in a 0.01 M phosphate buffer of pH 8.0 at a concentration of 0.3%. To 10 mℓ of the thus obtained solution was added an equal volume of a 2% polystyrene latex dispersion (particle size 0.15 μ; dispersed with a 0.01 M phosphate buffer of pH 8.0) and the latex particles were sensitized at 56°C for 2 hours with occasional shaking. Centrifugal separation at 15,000 r.p.m. for 30 minutes gave human γ-globulin sensitized latex particles for detecting the rheumatoid factor as the precipitate.

(2)

Glycine	3.75 g
N,N-Dimethylformamide	30 g
Bovine serum albumin	0.25 g
Sodium azide	0.25 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 8.0 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a solution, in which the sensitized latex particles prepared at (1) above were dispersed in a concentration of 0.6% to give a diagnostic agent for detecting the rheumatoid factor. This agent can be provided for the agglutination reaction without diluting a test sample.

Example 17

(1) Two mg of HCG (5000 IU/mg) were dissolved in 1 mℓ of saline, mixed with 1 mℓ of Freund's complete adjuvant and used to immunize rabbits at three-week intervals 4 times to obtain anti-HCG serum. The thus obtained antiserum was salted out using ammonium sulfate in a conventional manner to obtain γ-globulin fraction which was then, after the dialysis, dissolved in a 0.01 M phosphate buffer of pH 8.2 to yield a 0.2% γ-globulin solution. To 10 mℓ of the γ-globulin solution thus obtained was added an equal volume of a 2% polystyrene latex dispersion (particle size 0.3 μ; dispersed with a 0.01 M phosphate buffer of pH 8.2), and the latex particles were sensitized at 37°C for 2 hours with occasional shaking. The centrifugal separation at 9000 r.p.m. for 15 minutes gave latex particles sensitized with anti-HCG antibody as the precipitate.

(2)

Glycine	3.75 g
Urea	75 g
N,N-Dimethylacetamide	50 g
Bovine serum albumin	0.5 g
Sodium azide	0.5 g

The above ingredients were dissolved in 400 mℓ

of distilled water and the pH was adjusted to 8.2 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a solution, in which the anti-HCG antibody sensitized latex particles prepared at (1) above were dispersed in a concentration of 1% to give a diagnostic agent containing anti-HCG antibody sensitized latex for checking pregnancy. For use, this agent is mixed with a test sample diluted with a conventional diluent.

Example 18

HEPES	5.95 g
Urea	30 g
N,N,-Dimethylformamide	25 g
Bovine serum albumin	0.25 g
Sodium azide	0.25 g

The above ingredients were dissolved in 400 mℓ of distilled water and the pH was adjusted to 7.5 by adding 1N NaOH, after which distilled water was added to make the total volume 500 mℓ to give a solution, in which the anti-HCG antibody sensitized latex particles prepared in Example 17 (1) were dispersed at a concentration of 0.6% to give a diagnostic agent for checking pregnancy. This product can be provided for the agglutination reaction without diluting a test sample.

Test Example 1

The anti-C-reactive protein antibody sensitized latex particles obtained in Example 13 (1) were dispersed in a 0.1 M glycine buffer (pH 8.2) containing 0.1% of bovine serum albumin and 0.1% of sodium azide to give a 1.2% dispersion. About 50 μℓ of human serum sample to be tested were dropped onto a cleaned glass plate. About 25 μℓ of each of the diluents described in Examples 1 to 5 were then added to the glass plate. The added controls were a 0.1 M glycine buffer with a pH of 8.2 and containing 0.1% of sodium azide (hereinafter referred to as Diluent G) and a 0.1 M HEPES buffer with a pH of 7.5 and containing 0.1% of sodium azide (hereinafter referred to as Diluent H). This was followed by the addition of 25 μℓ of the above sensitized latex suspension and by shaking for 3 minutes, after which the mixture was examined for the occurrence of the agglutination. Also, in a system which did not utilize a diluent for a test sample, about 50 μℓ of the diagnostic agent for measuring C-reactive protein obtained in Example 13 (2) were added to about 50 μℓ of a serum sample and shaken. After three minutes, the mixture was examined for the occurrence of the agglutination. The results are

shown in Table 1, as follows:

Table 1

Detection of C-Reactive Protein

Measuring System	Sample No											
	1	2	3	4	5	6	7	8	9	10	11	12
Diluent for Test Sample:												
Diluent G	+	+	+	+	+	+	+	−	±	+	−	−
Diluent H	+	±	+	+	+	+	+	−	±	±	−	−
Example 1	+	+	+	+	+	±	+	−	−	−	−	−
Example 2	+	+	+	+	+	+	+	−	−	−	−	−
Example 3	+	+	+	+	+	±	+	−	−	−	−	−
Example 4	+	+	+	+	+	±	+	−	−	−	−	−
Example 5	+	+	+	+	+	±	+	−	−	−	−	−
Example 13(2)	+	+	+	+	+	±	+	−	−	−	−	−
Capillary Method	+	+	+	+	±	±	+	−	−	−	−	−

+ : positive; ± : doubtful-positive; − : negative

(the same shall apply hereinafter)

As shown by the results of Table 1, when there was no urea, N,N-dimethylformamide, N,N-dimethylacetamide or dimethyl sulfoxide present in the diluent for a test sample or the latex dispersion, more fale-positive cases were observed as compared with the diagnostic results by the conventional capillary sedimentation method. On the other hand, in the systems using the diluents of Examples 1 to 5 and the diagnostic agent for measuring C-reactive protein obtained in Example 13

(2) respectively, the results showed good agreement with those obtained by the capillary sedimentation method.

Test Example 2

About 50 μl of a human serum sample were dropped onto a cleaned glass plate and about 50 μl of the anti-human fibrinogen antibody sensitized latex particle dispersion obtained in Example 14 (2) were added, after which the mixture was shaken for 2 minutes and then examined for the occurrence of the agglutination. Separately, as the control, the sensitized latex particles in the form of a precipitate obtained in Example 14 (1) were dispersed in a 0.05 M HEPES buffer of pH 7.5 containing 0.05% of bovine serum albumin and 0.05% of sodium azide at a concentration of 0.6% to obtain a suspension of anti-human fibrinogen antibody sensitized latex particles, and similarly tested for comparison. The results shown in Table 2 (just below) were obtained.

Table 2

Detection of Fibrin and Fibrinogen Decomposition Products

Diagnotic Agent Used	Sample No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Example 14(2)	-	+	+	-	-	-	+	-	±	+	-	+
Control	-	+	+	+	-	-	+	+	+	+	-	+

As shown in Table 2, there are two cases which fall in the category of different results (i.e. Samples Nos. 4 and 8). Since these samples were not absorbed even by treatment with anti-human fibrinogen antibody, it was judged that the results obtained using the control falsely showed positive.

Test Example 3

About 50 μl of a human serum sample were dropped onto a cleaned glass plate and about 50 μl of the human γ-globulin sensitized latex particle dispersion obtained in Example 16 (2) were added, after which the mixture was shaken for 2 minutes and then examined for the occurrence of the agglutination. Separately, as the control, the sensitized latex particles in the form of a precipitate obtained in Example 16 (1) were dispersed in a 0.1 M glycine buffer of pH 8.0 containing 0.05% of bovine serum albumin and 0.05% of sodium azide at

a concentration of 0.6% to obtain a suspension of human γ-globulin sensitized latex particles and used for comparison. The results shown in Table 3 (just below) were obtained:

Table 3

Detection of Rheumatoid Factor

Diagnostic Agent Used	Sample No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Example 16(2)	+	+	+	−	−	±	+	−	−	+	−	+
Control	+	+	+	−	+	+	+	−	+	+	−	+

As shown in Table 3, there are two cases denoting different results (Samples Nos. 5 and 9). When these samples were tested by avoiding the influence of the serum components by the 20-fold dilution method using a conventional diluent, both cases showed the same results, negative. Thus, the use of the diagnostic agent of this invention makes it possible to measure the rheumatoid factor without diluting the serum sample.

Test Example 4

The anti-HCG antibody sensitized latex particles obtained in Example 17 (1) were dispersed in a 0.1 M glycine buffer of pH 8.2 at a concentration of 1%. As the urine to be tested, 10 urine samples

which showed a tendency to cause the non-specific agglutination were taken from non-pregnant women. As the diluent for a test sample, each of the diluents of Examples 1 to 5 was used, and, as the controls, the afore-mentioned Diluents G and H were used. Two drops of the urine sample were dropped onto a cleaned glass plate and one drop of each diluent was added and mixed, after which one drop of the afore-mentioned anti HCG antibody sensitized latex suspension was added thereto and shaken for 3 minutes. The results of the examination for the occurrence of the agglutination are shown in Table 4 appearing below.

As is clear from the table, when the diluent for a test sample did not contain urea, N,N-dimethylformamide, N,N-dimethylacetamide or dimethyl sulfoxide i.e., in both the cases of Diluents G and H, the proportions in which the test indicated pregnancy were 10 out of 10 including the doubtful-positive cases. This is due to the non-specific agglutination reaction, although the samples were taken from non-pregnant women.

On the other hand, where the diluents prepared in Examples 1 to 5 were used, all the cases showed negative reactions, thus indicating

no wrong diagnosis.

Next, using each of the diluents of Examples 1 to 5, the sensitivity of the latex agglutination reaction was tested on the standard solutions having varying levels of 0.5 to 5,000 IU/mℓ and urine samples at the initial stage of pregnancy each containing a low level of HCG (about 1 IU/mℓ). The results are shown in Table 5 below.

Test Example 5

According to the procedures described in Test Example 4 and using the diluents of Example 1,2 and 5 and, as the controls, Diluents G and H, respectively, the latex agglutination reaction for diagnosing pregnancy was conducted on urine samples of 200 cases of non-pregnant women.

As shown in Table 6 (below), there were 6 positive cases and 20 doubtful-positive cases with Diluent G and 7 positive cases and 15 doubtful-positive cases with Diluent H, whereas all the cases showed negative when the diluents of Examples 1, 2 and 5 were used.

Test Example 6

According to the procedures described in Test Example 4 (above) and, using each of the diluents of Examples 1, 2 and 5 as the diluents for test samples,

the latex agglutination reaction for diagnosing pregnancy was conducted on urine samples of 200 women definitely diagnosed as pregnant by clinical diagnoses. With each diluent, all the cases showed positive results just in agreement with the clinical diagnoses.

Test Example 7

According to the procedures described in Test Example 4 and using the diluents of Examples 1,2 and 5, respectively, as the diluents for test samples, the latex agglutination reaction was conducted on each urine sample of 50 non-pregnant women and 50 women positively diagnosed as pregnant. With each diluent, all the urine samples of the non-pregnant women showed negative and all the urine samples of the pregnant women showed positive.

Table 4

Comparison of Diluents for Test Samples Tested on Urine Samples of Non-Pregnant Women

Diluent for Test Sample	Sample No.									
	1	2	3	4	5	6	7	8	9	10
Diluent G	±	±	±	+	±	+	±	±	+	+
Diluent H	+	±	±	+	±	+	+	±	+	+
Example 1	-	-	-	-	-	-	-	-	-	-
Example 2	-	-	-	-	-	-	-	-	-	-
Example 3	-	-	-	-	-	-	-	-	-	-
Example 4	-	-	-	-	-	-	-	-	-	-
Example 5	-	-	-	-	-	-	-	-	-	-

Table 5

Comparison of Diluent for Test Samples Tested on Standard HCG Solutions and Urine Samples at the Initial Stage of Pregnancy

Test Sample		Diluent for Test Sample						
		Dil. G	Dil. H	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5
Standard HCG (IU/mℓ)	0.0	−	−	−	−	−	−	−
	0.5	±	±	±	±	±	±	±
	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+
	2,000	+	+	+	+	+	+	+
	3,000	+	+	+	+	+	+	+
	5,000	+	+	+	+	+	+	+
Urine Samples at the Initial Stage of Pregnancy (ca. 1 IU/ml)	A	+	+	+	+	+	+	+
	B	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+
	D	+	+	+	+	+	+	+
	E	+	+	+	+	+	+	+

Table 6

Comparison of Diluent for Test Samples Tested on Urine Samples of 200 Non-pregnant Women

Diluent for Test Sample	Test Results (Numbers of Cases)		
	Positive	Doubtful-Positive	Negative
Diluent G	6	20	174
Diluent H	7	15	178
Example 1	0	0	200
Example 2	0	0	200
Example 5	0	0	200

Test Example 8

The anti-HCG antibody sensitized latex particles obtained in Example 17 (1) were dispersed in a 0.05 M borate buffer with a pH of 7.5 and containing 0.1% of sodium azide at a concentration of 1% to obtain a diagnostic agent for the latex agglutination reaction for checking pregnancy. Using the urine samples mentioned in Test Example 4 and the diluent for test samples of Examples 1 to 5, respectively, the latex agglutination was conducted by adding 1 drop (about 25 μℓ) of each diluent to 2 drops (about 50 μℓ) of each urine sample and mixing,

followed by the addition of 1 drop (about 25 μl) of the diagnostic agent obtained above to assess the effects of the diluents.

The results are shown in Table 7 (below) from which it is seen that, when the diluent for a test sample did not contain urea, N,N-dimethylformamide, N,N-dimethylacetamide or dimethyl sulfoxide, i.e., in the case of Diluents G and H, the proportions in which the tests indicated pregnancy were 10 out of 10 including doubtful-positive cases with both diluents due to the non-specific agglutination reaction although the samples were taken from non-pregnant women.

On the other hand, where the diluents prepared in Examples 1 to 5, respectively, were used, all the cases showed negative results, thus indicating no wrong diagnosis.

Next, using the diluents of Examples 1 to 5, respectively, the latex agglutination reaction was conducted on each urine sample of 200 non-pregnant women and 200 pregnant women. With each diluent, all the urine samples of the non-pregnant women showed negative and all the urine samples of the pregnant women showed positive.

Table 7

Comparison of Diluents for Test Samples Tested on Urine Samples of Non-Pregnant Women

Diluent for Test Sample	Urine No. 1	2	3	4	5	6	7	8	9	10
Diluent G	±	±	±	+	±	+	±	±	+	+
Diluent H	±	±	±	+	±	+	+	±	+	+
Example 1	-	-	-	-	-	-	-	-	-	-
Example 2	-	-	-	-	-	-	-	-	-	-
Example 3	-	-	-	-	-	-	-	-	-	-
Example 4	-	-	-	-	-	-	-	-	-	-
Example 5	-	-	-	-	-	-	-	-	-	-

Test Example 9

Two drops (about 50 μℓ) of a urine sample were dropped onto a cleaned glass plate and 1 drop (about 25 μℓ) of a 0.1 M glycine buffer of pH 8.2 containing 0.1% sodium azide was added and mixed. One drop (about 25 μℓ) of the diagnostic agent for checking pregnancy containing the anti-HCG antibody sensitized latex particles obtained in Example 17 (2) was added thereto and shaken for 3 minutes, after which the mixture was assessed for the occurrence of the agglutination.

When the latex agglutination reaction was conducted on each urine sample of 200 non-pregnant women and 200 women clinically diagnosed as pregnant, all the urine samples of the non-pregnant women showed negative and all the urine samples of the pregnant women showed positive.

Text Example 10

Two drops (about 50 μℓ) of a urine sample were dropped onto a cleaned glass plate and two drops (about 50 μℓ) of the diagnostic agent for checking pregnancy containing the anti-HCG antibody sensitized latex particles obtained in Example 18 were added thereto and shaken for 3 minutes, after which the mixture was assessed for the occurrence of the agglutination.

When the latex agglutination reaction was conducted on each urine sample of 200 non-pregnant women and 200 women clinically diagnosed as pregnant, all the urine samples of the non-pregnant women showed negative and all the samples of the pregnant women showed positive.

Test Example 11

Two drops (about 50 μℓ) of a urine sample were dropped onto a cleaned glass plate and one drop (about 25 μℓ) of the diluent for a test sample obtained in Example

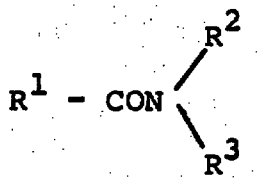
10 was added and mixed, after which one drop (about 25 μl) of a latex reagent obtained by dispersing the anti-HCG sensitized latex particles obtained in Example 17 (1) in a 0.1 M glycine buffer of pH 7.8 at a concentration of 1% and shaken for 3 minutes. The mixture was then examined for the occurrence of the agglutination.

When the latex agglutination reaction was conducted on each urine sample of 200 non-pregnant women and 200 women clinically diagnosed as pregnant, all the urine samples of the non-pregnant women showed negative and all the samples of the pregnant women showed positive.

CLAIMS

1. A diagnostic agent which comprises:

(1) 2-52% (weight/volume) of one or more members selected from (a), compounds of the formula (I):



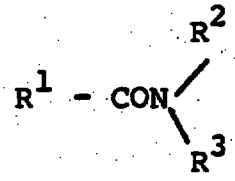
(I)

wherein R^1 is a hydrogen atom, a lower alkyl group or an amino group, which may be substituted with a lower alkyl group and R^2 and R^3 are each a hydrogen atom or a lower alkyl group, and (b) a di-lower alkyl sulfoxide and

(2) 0.4 to 1.4% (weight/volume) of latex particles sensitized with an antigen or antibody, in

(3) a buffer with a pH range of 7-9.

2. A diluent for a test sample in a latex agglutination reaction immunoassay which comprises (1) 2 - 52% (weight/volume) of one or more members selected from (a) compounds of the formula:



(I)

wherein R^1 is a hydrogen atom, a lower alkyl group or an amino group which may be substituted with a lower alkyl goup, and R^2 and R^3 are each a hydrogen atom or a lower alkyl group, and (b) a di-lower sulfoxide in (2) a buffer with a pH range of 6-9.

3. A diluent according to claim 2 which contains 4 to 52% (weight/volume), wherein the buffer has a pH range of from 6-9 and an electrolyte concentration of from 0.05 to 0.3 M

4. A diluent according to claim 2 and 3, which contains 4 to 52% of said member.

5. A diagnostic agent according to claim 1, which contains from 4% to 52% of said member.

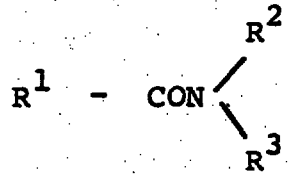
6. A diagnostic agent according to claim 4 which contains urea and N,N-dimethylformamide as said member.

7. A diagnostic agent according to claim 1, wherein the latex particles are composed of polystyrene.

8. A diagnostic agent according to any of claims 1 5, 6 and 7 wherein said member is N,N-dimethylformamide and the latex particles have been sensitized with an anti-C-reactive protein antibody.

9. In a method employing the latex agglutination reaction which comprises incubating a test sample and latex particles sensitized with an antigen or antibody to assess the occurrence of the agglutination of the latex particles, the improvements wherein the reaction is conducted in the presence of one or more members

selected from compounds of the formula:



(I)

wherein R^1 is a hydrogen atom, a lower alkyl group or an amino group which may be substituted with a lower alkyl group and R^2 and R^3 are each a hydrogen atom or a lower alkyl group and di-lower alkyl sulfoxide.

10. A method according to claim 9, wherein the concentration of said member is from 1% to 13% relative to the whole reaction system.

11. A method according to claim 10 wherein the concentration is from 1% to 7%.

12. A diagnostic agent for the latex agglutination reaction according to claim 1 substantially as herein described with reference to any of examples 13, 14, 15, 16 17 and 18.

13. A diluent for a test sample in a latex agglutination reaction immunoassay according to claim 2 substantially as herein described with reference to any of examples 1 to 12.

14. A method according to claim 9 substantially as herein described with reference to any of the specific examples.

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