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(54) Titre : TECHNIQUE RELATIVE A UNE EPREUVE D'IMMUNITE MENEES AVEC DES PARTICULES D'UN VEHICULE
INSOLUBLE ET REACTIF A CET EFFET
(54) Title: METHOD OF IMMUNITY EXAMINATION WITH INSOLUBLE CARRIER PARTICLE AND REAGENT
THEREFOR

(57) **Abrégé/Abstract:**

A reagent for immunity examination which contains insoluble carrier particles, can be stored over long, and gives highly accurate and reliable found values; a method of immunity examination with the reagent; and a method of stabilizing the reagent. The method of immunity examination comprises conducting an antigen-antibody reaction with the aid of insoluble carrier particles in an aqueous medium containing both a buffer having the ability to buffer in a neutral to alkaline region (provided that the buffer is not a carbonate-based one) and a carbonic acid compound releasing a hydrogen carbonate ion. The reagent for immunity examination comprises a buffer having the ability to buffer in a neutral to alkaline region (provided that the buffer is not a carbonate-based one), a carbonic acid compound releasing a hydrogen carbonate ion, and insoluble carrier particles. The method of stabilizing the reagent for immunity examination containing insoluble carrier particles comprises incorporating the insoluble carrier particles into an aqueous medium containing both a buffer having the ability to buffer in a neutral to alkaline region (provided that the buffer is not a carbonate-based one) and a carbonic acid compound releasing a hydrogen carbonate ion.



Abstract

The present invention provides a reagent for an immunoassay comprising insoluble carrier particles which can
5 give the values to be determined with high accuracy and reliability, and can be stored for a long time; an immunoassay using the reagent; and a method for keeping the reagent stable. The present invention provides an immunoassay which comprises carrying out an antigen-antibody reaction using insoluble
10 carrier particles in an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion; a reagent for an immunoassay comprising a buffer having its buffer capacity in
15 a neutral or alkaline region (except for a carbonate-based buffer), a carbonic acid compound releasing bicarbonate ion, and insoluble carrier particles; and a method for keeping the reagent for an immunoassay comprising insoluble carrier particles stable, which comprises allowing insoluble carrier
20 particles to co-exist in an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion.

DESCRIPTION**TITLE OF THE INVENTION**

METHOD OF IMMUNITY EXAMINATION WITH INSOLUBLE CARRIER PARTICLE
5 AND REAGENT THEREFOR

Technical Field

The present invention relates to an immunoassay using
insoluble carrier particles, a reagent for an immunoassay
10 comprising such insoluble carrier particles, and a method for
keeping a reagent for an immunoassay comprising such insoluble
carrier particles stable.

Background Art

15 In the field of clinical tests, insoluble carrier
particles are widely used for immunoassays, which comprise
determination of antigens or antibodies in the samples.
However, there is a problem that the reproducible values to be
determined are not obtained when using a reagent for an
20 immunoassay comprising insoluble carrier particles having been
stored for a long time. Thus it is impossible to diagram a
calibration curve with the same sensitivity, which results in
an error of measurement.

An object of the present invention is to provide a reagent
25 for an immunoassay comprising insoluble carrier particles that
can give the values to be determined with high accuracy and high
reliability and can be stored for a long time, an immunoassay
using the reagent, and a method for keeping the reagent stable.

30 **Disclosure of the Invention**

The present invention relates to an immunoassay, which comprises carrying out an antigen-antibody reaction using insoluble carrier particles in an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion (claim 1); the immunoassay according to claim 1, wherein the carbonic acid compound releasing a bicarbonate ion is an alkali metal compound (claim 2); the immunoassay according to claim 1 or 2, wherein the concentration of the bicarbonate ion is 0.05 to 500 mmol/L (claim 3); the immunoassay according to any of claims 1 to 3, wherein the concentration of the buffer having its buffer capacity in a neutral or alkaline region is 0.1 to 500 mmol/L (claim 4); the immunoassay according to any of claims 1 to 4, wherein the insoluble carrier particles are those which antibodies reactive with substance to be determined are bound to (claim 5); the immunoassay according to any of claims 1 to 4, wherein the insoluble carrier particles are those which substance to be determined is bound to (claim 6); the immunoassay according to any of claims 1 to 6, wherein the insoluble carrier particles are latex (claim 7); the immunoassay according to any of claims 1 to 7, wherein the immunoassay is a nephelometric immunoassay (claim 8); a reagent for an immunoassay comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), a carbonic acid compound releasing a bicarbonate ion, and insoluble carrier particles (claim 9); the reagent for an immunoassay according to claim 9, wherein the buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), the carbonic acid

compound releasing a bicarbonate ion, and insoluble carrier particles are comprised in an aqueous medium (claim 10); the reagent for an immunoassay according to claim 9 or 10, wherein the carbonic acid compound releasing a bicarbonate ion is an alkali metal compound (claim 11); the reagent for an immunoassay according to claim 10 or 11, wherein the concentration of the bicarbonate ion is 0.05 to 500 mmol/L (claim 12); the reagent for an immunoassay according to any of claims 10 to 12, wherein the concentration of the buffer having its buffer capacity in a neutral or alkaline region is 0.1 to 500 mmol/L (claim 13); the reagent for an immunoassay according to any of claims 9 to 13, wherein the insoluble carrier particles are those which antibodies reactive with substance to be determined are bound to (claim 14); the reagent for an immunoassay according to any of claims 9 to 14, wherein the insoluble carrier particles are latex (claim 15); a method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable, wherein the insoluble carrier particles are comprised in an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion (claim 16); the method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to claim 16, wherein the carbonic acid compound releasing a bicarbonate ion is an alkali metal compound (claim 17); the method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to claim 16 or 17, wherein the concentration of the bicarbonate ion is 0.05 to 500 mmol/L (claim 18); the method for keeping a reagent for an immunoassay comprising insoluble particles

stable according to any of claims 16 to 18, wherein the concentration of the buffer having its buffer capacity in a neutral or alkaline region is 0.1 to 500 mmol/L (claim 19); the method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to any of claims 16 to 19, wherein the insoluble carrier particles are those which antibodies reactive with substance to be determined are bound to (claim 20); and the method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to any of claims 16 to 20, wherein the insoluble carrier particles are latex (claim 21).

Best Mode of Carrying Out the Invention

As a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) for the present invention, there is no specific limitation as long as the buffer has its buffer capacity at over pH 7, and the buffer having its buffer capacity at pH 7 to 12 is preferable, and the buffer having its buffer capacity at pH 8 to 11 is more preferable. Examples include an organic amine buffer, a Good's buffer, a buffer for biochemical use, and the like.

Examples of the above-mentioned buffer for biochemical use include imidazole buffer, sodium dihydrogenphosphate - disodium hydrogenphosphate buffer, citrate- disodium hydrogenphosphate buffer, hydrochloric acid -veronal sodium-sodium acetate buffer, potassium dihydrogenphosphate - disodium hydrogenphosphate buffer, potassium dihydrogenphosphate - borax buffer, potassium dihydrogenphosphate - sodium hydroxide buffer, hydrochloric acid-collidine buffer, hydrochloric acid - veronal sodium

buffer, hydrochloric acid - tris(hydroxymethyl)aminomethane
 buffer, hydrochloric acid - borax buffer, borate - sodium
 carbonate buffer, borate - borax buffer, hydrochloric acid -
 aminomethylpropanediol buffer, ammonium chloride- ammonia
 5 buffer, glycine - sodium hydroxide buffer, borate - sodium
 hydroxide buffer, hydrochloric acid - dimethylglycine sodium
 buffer, borax - sodium hydroxide buffer, borax-sodium carbonate
 buffer, glycine - sodium chloride - hydrochloric acid buffer,
 disodium hydrogen citrate - hydrochloric acid buffer, disodium
 10 hydrogen citrate - sodium hydroxide buffer, borax - sodium
 chloride buffer, veronal sodium - sodium acetate - hydrochloric
 acid buffer, borate - potassium chloride - sodium hydroxide
 buffer, Tris - maleate buffer, maleate buffer, veronal - acetate
 buffer, veronal buffer, Michaelis buffer, Clark-Lubs' buffer,
 15 Atkins-Pantin buffer, Paritish buffer, Kolthoff's buffer,
 MacIlvaine buffer, Hasting-Sendroi buffer, Britton-Robinson
 buffer, Sørensen buffer.

Examples of the above-mentioned organic amine buffer
 include diethanolamine buffer, 2-ethylaminoethanol buffer,
 20 2-amino-2-methyl-1-propanol buffer, N-methyl-D-glucamine and
 the like.

Examples of the above-mentioned Good's buffer include MES
 (2-morpholinoethanesulfonic acid) buffer, bis-tris [bis(2-
 hydroxyethyl)imino tris(hydroxymethyl)methane] buffer, ADA
 25 [N-(2-acetamido)iminodiacetic acid] buffer, PIPES
 [piperazine-N,N'-bis(2-ethanesulfonic acid)] buffer, ACES
 {2-[N-(2-acetamido)amino]ethanesulfonic acid} buffer, MOPSO
 (3-morpholino-2-hydroxypropanesulfonic acid) buffer, BES
 {2-[N,N-bis (2-hydroxyethyl)amino]ethanesulfonic acid}
 30 buffer, MOPS (3-morpholinopropanesulfonic acid) buffer, TES

<2-{N-[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid>
 buffer, HEPES [N-(2-hydroxyethyl)-N'-(2-
 sulfoethyl)piperazine] buffer, DIPSO {3-[N,N-bis(2-
 hydroxyethyl)amino]-2-hydroxypropanesulfonic acid} buffer,
 5 TAPSO <2-hydroxy-3-{{N-tris(hydroxymethyl)
 methyl]amino}propanesulfonic acid} buffer, POPSO
 [piperazine-N,N'-bis(2-hydroxy-3-propanesulfonic acid)]
 buffer, HEPPSO [N-(2-hydroxyethyl)-N'-(2-hydroxy-3-
 sulfopropyl)piperazine] buffer, EPPS [N-(2-hydroxyethyl)-
 10 N'-(3-sulfopropyl)piperazine] buffer, tricine {N-
 [tris(hydroxymethyl)methyl]glycine} buffer, bicine [N,N-
 bis(2-hydroxyethyl)glycine] buffer, TAPS {3-[N-
 tris(hydroxymethyl)methyl]aminopropanesulfonic acid} buffer,
 CHES [2-(N-cyclohexylamino)ethanesulfonic acid] buffer, CAPSO
 15 [3-(N-cyclohexylamino)-2-hydroxypropanesulfonic acid] buffer,
 CAPS [3-(N-cyclohexylamino)propanesulfonic acid] buffer, and
 the like.

The buffer having its buffer capacity in a neutral or
 alkaline region in the present invention is a buffer other than
 20 a carbonate-based buffer. The carbonate-based buffer mentioned
 here represents a buffer based on a carbonate, and examples of
 the carbonate-based buffer include sodium carbonate - sodium
 bicarbonate buffer, potassium carbonate - potassium
 bicarbonate buffer and the like.

25 Furthermore, there is no specific limitation for the
 concentration of the buffer having its buffer capacity in a
 neutral or alkaline region according to the present invention.
 A concentration of 0.1 mmol/L to 500 mmol/L is preferable, a
 concentration of 1 mmol/L to 100 mmol/L is more preferable, and
 30 a concentration of 5 mmol/L to 50 mmol/L is most preferable.

There is no specific limitation for the carbonic acid compound releasing a bicarbonate ion in the present invention, as long as the compound is a compound releasing a bicarbonate ion, and the carbonic acid releasing a bicarbonate-ion is exemplified by an alkali metal compound or an alkali earth metal compound and the like. Examples of the alkali metal compound and the alkali earth metal compound include sodium bicarbonate, potassium bicarbonate, magnesium bicarbonate, calcium bicarbonate, and the like. The alkali metal compounds such as sodium bicarbonate and potassium bicarbonate are preferably used.

Furthermore, there is no specific limitation for the concentration of the carbonic acid compound releasing a bicarbonate ion. A concentration of 0.05 to 500 mmol/L is preferable, a concentration of 0.1 mmol/L to 100 mmol/L is more preferable, and a concentration of 1 to 50 mmol/L is most preferable.

As an aqueous medium for the present invention, water, especially purified water is exemplified, and the aqueous medium may comprise, if necessary, enzymes, coenzymes, soluble salts such as sodium chloride, detergents such as Triton X-100 and Tween 20, stabilizing agents, antiseptics such as sodium azide, and the like.

The insoluble carrier particles used in the present invention is not specifically limited, and examples of the insoluble carrier particles include microparticles of organic polymers, microparticles of inorganic oxides, microparticles in which surface of the organic polymers or the inorganic oxides was treated with organic matters, and the like. There is no specific limitation for the materials of insoluble carrier

particles, and the preferred are the materials that enable the insoluble carrier particles to be suspended uniformly in the reaction solution.

Furthermore, there is no limitation for the particle
5 diameter of the insoluble carrier particles employable in the present invention, and the particles with the diameter of 0.4 to 0.8 μm are preferable. As more preferable insoluble carrier particles, latex such as polystyrene latex are exemplified. As a material of the latex, styrene latex such as polystyrene latex,
10 acrylic acid latex and the like are exemplified. Additionally, polystyrene latex, prepared by co-polymerization of a monomer of acrylic acid, a monomer having sulfonic acid and the like as a monomer component to give electric charges, can be used preferably.

15 There is no specific limitation for the concentration of insoluble carrier particles in the reaction solution or in the reagent, and the concentration of 0.005 to 2% by weight in the solution is preferable.

There is no specific limitation for substance to be
20 determined in an immunoassay according to the present invention, and examples of the substance include antigens such as ferritin and hemoglobin A1c (hereinafter referred to as HbA1c), antibodies to these antigens and the like.

In the present invention, "an antigen-antibody reaction
25 using insoluble carrier particles" refers to an antigen-antibody reaction using insoluble carrier particles as a solid phase. As insoluble carrier particles as a solid phase, those which substance to be determined or antibodies reactive with the substance to be determined are carried by (bound to) in
30 advance as well as those which neither substance to be

determined nor antibodies reactive with the substance to be determined are substantially carried by (bound to) are used. The immunoassay employable in the present invention is not specifically limited, as long as the immunoassay comprises an antigen-antibody reaction using insoluble carrier particles in an aqueous medium, and for example, a nephelometric immunoassay is mentioned.

The immunoassay in the present invention comprises the following steps: 1) the step of adding the above-mentioned insoluble carrier particles to an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion to give a suspension; 2) the step of reacting the suspension comprising the insoluble carrier particles with the sample comprising substance to be determined; 3) the step of measuring agglutination of insoluble carrier particles originated from the reaction.

In case insoluble carrier particles, which neither substance to be determined nor antibodies to the substance to be determined are carried by (bound to), are used as insoluble carrier particles, the step of reacting the suspension comprising the insoluble carrier particles with the sample comprising the substance to be determined is followed by the step of adding the reagent comprising antibodies reactive with the substance to be determined thereto.

There is no specific limitation for a reagent for an immunoassay according to the present invention as long as the reagent comprises a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), a carbonic acid compound releasing a bicarbonate ion,

and insoluble carrier particles. The buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), the carbonic acid compound releasing a bicarbonate ion, and insoluble carrier particles may be
5 comprised in an aqueous medium. As the buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), the carbonic acid compound releasing a bicarbonate ion, the insoluble carrier particles and the aqueous medium, those as mentioned above are exemplified,
10 respectively. The reagent according to the present invention may comprise, if necessary, enzymes, coenzymes, soluble salts such as sodium chloride, detergents such as Triton X-100 and Tween 20, stabilizing agents, antiseptics such as sodium azide, and the like.

15 A reagent for an immunoassay according to the present invention may be stored and used in the form of a kit. Examples of a form of a kit include a kit consisted of two reagents, a kit consisted of three reagents, and the like. Examples of a kit consisted of two reagents according to the present invention
20 include: a kit consisted of a first reagent and a second reagent, wherein the first reagent comprises in an aqueous medium a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), and a carbonic acid compound releasing a bicarbonate ion, and the second
25 reagent comprises in an aqueous medium insoluble carrier particles which the antibodies reactive with the substance to be determined are bound to, a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), and a carbonic acid compound releasing a bicarbonate
30 ion; a kit consisted of a first reagent and a second reagent,

wherein the first reagent comprises insoluble carrier particles which neither the substance to be determined nor the antibodies to the substance are not substantially carried by (bound to), a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), and a carbonic acid compound releasing a bicarbonate ion, and the second reagent comprises in an aqueous medium the antibodies reactive with the substance to be determined.

A method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to the present invention is not specifically limited, as long as the method comprises allowing insoluble carrier particles to present in an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion. As the buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), the carbonic acid compound releasing a bicarbonate ion, the insoluble carrier particles and the aqueous medium, those as mentioned above may be used, respectively. In the aqueous medium, the following may be comprised, if necessary: enzymes, coenzymes, soluble salts such as sodium chloride, detergents such as Triton X-100 and Tween 20, stabilizing agents, antiseptics such as sodium azide, and the like.

A condition for storage of a reagent for an immunoassay according to the present invention is not specifically limited, and for example, the reagent can be stored at 0 to 30.degree. C., preferably at 0 to 5.degree. C. The method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to the present invention is more

effective for keeping the reagent stable when the reagent is stored under an unsealed condition where deterioration of the reagent is considered to occur easily.

The present invention will be described in detail by the following examples, while the scope of the invention will not be limited to these examples.

Example 1

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1

10	bicine buffer (DOJINDO Laboratories, pH 8.4)	
		3.26 g/L
	Triton X-100 (Sigma)	0.1 g/L
	sodium chloride (Wako Pure Chemical Industries, Ltd.)	
		17.5 g/L
15	sodium azide (Kanto Chemical Co., Inc.)	0.01 g/L
	sodium bicarbonate (Kanto Chemical Co., Inc.)	
		0.2 g/L

Reagent R2 (a suspension of the latex carrying the antibodies thereon)

20	imidazole buffer (Nacalai Tesque, Inc., pH 8.4)	
		0.68 g/L
	Triton X-100 (Sigma)	0.15 g/L
	sodium bicarbonate (Kanto Chemical Co., Inc.)	
		0.2 g/L
25	the latex carrying the antibodies thereon	
	(prepared in reference example)	0.1% by weight

Example 2

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1

30	bicine buffer (DOJINDO Laboratories, pH 8.4)
----	--

		3.26 g/L
	Triton X-100 (Sigma)	0.1 g/L
	sodium chloride (Wako Pure Chemical Industries, Ltd.)	
		17.5 g/L
5	sodium azide (Kanto Chemical Co., Inc.)	0.01 g/L
	sodium bicarbonate (Kanto Chemical Co., Inc.)	
		0.4 g/L
	Reagent R2 (a suspension of the latex carrying the antibodies thereon)	
10	imidazole buffer (Nacalai Tesque, Inc., pH 8.4)	
		0.68 g/L
	Triton X-100 (Sigma)	0.15 g/L
	sodium bicarbonate (Kanto Chemical Co., Inc.)	
		0.4 g/L
15	the latex carrying the antibodies thereon	
	(prepared in reference example)	0.1% by weight

Example 3

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1

20	bicine buffer (DOJINDO Laboratories, pH 8.4)	
		3.26 g/L
	Triton X-100 (Sigma)	0.1 g/L
	sodium chloride (Wako Pure Chemical Industries, Ltd.)	
		17.5 g/L
25	sodium azide (Kanto Chemical Co., Inc.)	0.01 g/L
	sodium bicarbonate (Kanto Chemical Co., Inc.)	
		0.6 g/L

Reagent R2 (a suspension of the latex carrying the antibodies thereon)

30	imidazole buffer (Nacalai Tesque, Inc., pH 8.4)
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0.68 g/L

Triton X-100 (Sigma) 0.15 g/L

sodium bicarbonate (Kanto Chemical Co., Inc.)

0.6 g/L

5 the latex carrying the antibodies thereon

(prepared in reference example) 0.1% by weight

Comparative example 1

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1

10 bicine buffer (DOJINDO Laboratories, pH 8.4)

3.26 g/L

Triton X-100 (Sigma) 0.1 g/L

sodium chloride (Wako Pure Chemical Industries, Ltd.)

17.5 g/L

15 sodium azide (Kanto Chemical Co., Inc.) 0.01 g/L

Reagent R2 (a suspension of the latex carrying the antibodies thereon)

imidazole buffer (Nacalai Tesque, Inc., pH 8.4)

0.68 g/L

20 Triton X-100 (Sigma) 0.15 g/L

the latex carrying the antibodies thereon

(prepared in reference example) 0.1% by weight

Example 4

The following Reagent R1 and Reagent R2 were prepared.

25 Reagent R1 (a suspension of the latex)

bicine buffer (DOJINDO Laboratories, pH 7.8)

3.26 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

sodium bicarbonate (Kanto Chemical Co., Inc.)

30 0.1 g/L

the latex (particle diameter: 0.0775 μm , SEKISUI Chemical Co., Ltd.) 0.033% by weight

Reagent R2

bicine buffer (DOJINDO Laboratories, pH 7.0)
 5 3.26 g/L
 sodium chloride (Wako Pure Chemical Industries, Ltd.)
 15.0 g/L
 Tween 20 (Wako Pure Chemical Industries, Ltd.)
 2.0 g/L
 10 sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L
 anti-human HbA1c mouse monoclonal antibody
 0.025 g (in terms of IgG)/L
 anti-mouse IgG goat polyclonal antibody
 (Wako Pure Chemical Industries, Ltd.)
 15 0.04 g (in terms of IgG)/L

Example 5

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1 (a suspension of the latex)

bicine buffer (DOJINDO Laboratories, pH 7.8)
 20 3.26 g/L
 sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L
 sodium bicarbonate (Kanto Chemical Co., Inc.)
 0.2 g/L
 the latex (particle diameter: 0.0775 μm , SEKISUI Chemical
 25 Co., Ltd.) 0.033% by weight

Reagent R2

bicine buffer (DOJINDO Laboratories, pH 7.0)
 3.26 g/L
 sodium chloride (Wako Pure Chemical Industries, Ltd.)
 30 15.0 g/L

Tween 20 (Wako Pure Chemical Industries, Ltd.)

2.0 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

anti-human HbA1c mouse monoclonal antibody

5 0.025 g (in terms of IgG)/L

anti-mouse IgG goat polyclonal antibody

(Wako Pure Chemical Industries, Ltd.)

0.04 g (in terms of IgG)/L

Example 6

10 The following Reagent R1 and Reagent R2 were prepared.

Reagent R1 (a suspension of the latex)

bicine buffer (DOJINDO Laboratories, pH 8.4)

3.26 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

15 sodium bicarbonate (Kanto Chemical Co., Inc.)

0.2 g/L

the latex (particle diameter: 0.0775 μ m, SEKISUI Chemical Co., Ltd.)

0.033% by weight

Reagent R2

20 bicine buffer (DOJINDO Laboratories, pH 7.0)

3.26 g/L

sodium chloride (Wako Pure Chemical Industries, Ltd.)

15.0 g/L

Tween 20 (Wako Pure Chemical Industries, Ltd.)

25 2.0 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

anti-human HbA1c mouse monoclonal antibody

0.025 g(in terms of IgG)/L

anti-mouse IgG goat polyclonal antibody

30 (Wako Pure Chemical Industries, Ltd.)

0.04 g(in terms of IgG)/L

Example 7

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1 (a suspension of the latex)

5 bicine buffer (DOJINDO Laboratories, pH 8.4)

3.26 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

sodium bicarbonate (Kanto Chemical Co., Inc.)

0.3 g/L

10 the latex (particle diameter: 0.0775 μm , SEKISUI Chemical
Co., Ltd.) 0.033% by weight

Reagent R2

bicine buffer (DOJINDO Laboratories, pH 7.0)

3.26 q/L

15 sodium chloride (Wako Pure Chemical Industries, Ltd.)

15.0 g/L

Tween 20 (Wako Pure Chemical Industries, Ltd.)

2.0 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

20 anti-human HbA1c mouse monoclonal antibody

0.025 g (in terms of IgG)/L

anti-mouse IgG goat polyclonal antibody

(Wako Pure Chemical Industries, Ltd.)

0.04 g (in terms of IgG)/L

25 Comparative Example 2

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1 (a suspension of the latex)

bicine buffer (DOJINDO Laboratories, pH 7.8)

3.26 g/L

30 sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

the latex (particle diameter: 0.0775 μ m, SEKISUI Chemical Co., Ltd.) 0.033% by weight

Reagent R2

bicine buffer (DOJINDO Laboratories, pH 7.0)
 5 3.26 g/L
 sodium chloride (Wako Pure Chemical Industries, Ltd.)
 15.0 g/L
 Tween 20 (Wako Pure Chemical Industries, Ltd.)
 2.0 g/L
 10 sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L
 anti-human HbA1c mouse monoclonal antibody
 0.025 g (in terms of IgG)/L
 anti-mouse IgG goat polyclonal antibody
 (Wako Pure Chemical Industries, Ltd.)
 15 0.04 g (in terms of IgG)/L

Comparative Example 3

The following Reagent R1 and Reagent R2 were prepared.

Reagent R1 (a suspension of the latex)

bicine buffer (DOJINDO Laboratories, pH 8.4)
 20 3.26 g/L
 sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L
 the latex (particle diameter: 0.0775 μ m, SEKISUI Chemical Co., Ltd.) 0.033% by weight

Reagent R2

25 bicine buffer (DOJINDO Laboratories, pH 7.0)
 3.26 g/L
 sodium chloride (Wako Pure Chemical Industries, Ltd.)
 15.0 g/L
 Tween 20 (Wako Pure Chemical Industries, Ltd.)
 30 2.0 g/L

sodium azide (Kanto Chemical Co., Inc.) 0.1 g/L

anti-human HbA1c mouse monoclonal antibody

0.025 g (in terms of IgG)/L

anti-mouse IgG goat polyclonal antibody

5 (Wako Pure Chemical Industries, Ltd.)

0.04 g (in terms of IgG)/L

Reference Example (preparation of the latex carrying the antibody thereon)

Latex was diluted by adding 9 equivalents of 1/60 mol/L
 10 PBS solution (adjusted to pH 7.4 with 1 mol/L hydrochloric acid
 or sodium hydroxide solution) to one equivalent of 10%
 suspension of polystyrene latex (JSR) having the average
 particle diameter of 0.31 μ m to give the 1% latex solution. The
 anti-human ferritin antibody (polyclonal antibody, Kyowa Medex
 15 Co., LTD.) was diluted with a 1/60 mol/L PBS solution to give
 the antibody solution comprising the protein at a concentration
 of 50 μ g/mL, with which the antibody was carried by latex. With
 stirring the 1% latex suspension (600 μ L) with a magnetic
 stirrer at 25.degree. C. in an incubator, the antibody solution
 20 (1200 μ L) prepared above was quickly added to the suspension,
 and the mixture was stirred at 25.degree. C. for two hours. Then,
 blocking solution 1 (3 mL) prepared as described below was added
 thereto, and the mixture was further stirred at 25.degree. C.
 for two hours. The mixture was then centrifuged at 15,000 rpm
 25 at 4.degree. C. for one hour. Subsequently, the precipitate
 thus obtained was rinsed by adding blocking solution 1 (4 mL)
 and then by centrifuging the mixture under a similar condition.
 After rinsing three times, the precipitate obtained was used
 as the latex carrying the antibody thereon.

30

Blocking solution 1

BSA (bovine serum albumin)(6 g), Triton X-100 (Sigma)(0.15 g) were added to an aqueous solution of imidazole buffer (0.68 g). Then, the pH was adjusted to 7.4 by addition
5 of 1 mol/L hydrochloric acid or sodium hydroxide solution while measuring the pH level at 20.degree. C. The solution was made 1000 mL in total by addition of distilled water to give blocking solution 1.

Test Example 1

10 The concentration of ferritin in the serum samples was determined in the manner described below, by using each Reagent R1 and each Reagent R2 prepared in Examples 1 to 3 and Comparative Example 1. Each Reagent R1 and each Reagent R2 just after preparation were stored in bottles for autoanalyzer under a
15 sealed condition at 4.degree. C., and after three days, unsealed. Then, the concentration of ferritin was determined using each of the reagent just after unsealing (reagent just after unsealing), the reagent further stored under an unsealed condition at 4.degree. C. for 14 days after unsealing (reagent
20 stored for 14 days after unsealing), and the reagent further stored under an unsealed condition at 4.degree. C. for 28 days after unsealing (reagent stored for 28 days after unsealing).

Preparation of a serum sample

Human blood was collected with a blood tube (VENJECT Glass
25 Vacuum Tubes; TERUMO Corp.) and was left for two hours to give a supernatant fluid (serum), which was made a serum sample. The sample was frozen to store at -20.degree. C., and the frozen sample was melted just before use.

Determination of the concentration of ferritin using
30 Reagent R1 and Reagent R2

A calibration curve was diagramed by using Reagent R1 and Reagent R2 just after unsealing, along with ferritin standard solutions with concentrations of 10.9, 21.9, 43.8, 87.5 and 175 ng/mL, respectively, which were prepared by using a ferritin standard (Scripps Laboratories Inc.).

Determination of the concentration of a serum sample was carried out as follows. Serum sample (10 iL) was added to Reagent R1 (140 iL) and the reaction was allowed to occur at 37.degree. C. for 6 minutes. Then, Reagent R2 (150 iL) was added thereto, and the reaction was allowed to occur at 37.degree. C. for 13 minutes. Then, changes in absorbance were measured by the 2 point-end method (photometric points: 21 and 39) with the main-wavelength of 750 nm and the sub-wavelength of 800 nm, wherein the measurement was carried out on Hitachi autoanalyzer 7170.

The results of the determination of the concentrations of ferritin using the reagents prepared in Examples 1 to 3 and Comparative Example 1 are shown in Table 1.

Table 1

days after unsealing of reagents (days)	Example 1	Example 2	Example 3	Comparative Example 1
0	61	62	62	62
14	66	62	60	74
28	69	62	57	83

As shown in Table 1, in the determination of ferritin concentration using the reagent of Comparative Example 1 consisting of Reagents R1 and R2, neither of which comprises no sodium bicarbonate, the values to be determined of ferritin

concentration changed significantly with the passage of days after unsealing of the reagent, while in the determination of ferritin concentration using the reagents of Examples 1 to 3 consisting of each Reagent R1 and each Reagent R2, both of which
5 comprise sodium bicarbonate, the values to be determined of ferritin concentration did not change so much even with the passage of days after unsealing of the reagent.

Test Example 2

The rate of HbA1c to hemoglobin in a sample (hereinafter
10 referred to as a concentration of HbA1c) was determined in the manner described below, by using each Reagent R1 and each Reagent R2, prepared respectively in Examples 4 to 5 and Comparative Example 2, and Examples 6 to 7 and Comparative Example 3. As Reagent R1 in the determination of the
15 concentration of HbA1c, the following reagents were used: the reagent just after unsealing of each Reagent 1, which was stored at 4.degree. C. for three days in bottles for autoanalyzer under a sealed condition just after preparation (reagent just after unsealing); the reagent stored at 4.degree. C. for 14 days under
20 an unsealed condition after unsealing of each Reagent R1, which was stored at 4.degree. C. for three days in bottles for autoanalyzer under a sealed condition just after preparation (reagent stored for 14 days after unsealing); and the reagent stored at 4.degree. C. for 28 days under an unsealed condition
25 after unsealing of each Reagent R1, which was stored at 4.degree. C. for three days in bottles for autoanalyzer under a sealed condition just after preparation (reagent stored for 28 days after unsealing). As Reagent R2 in the determination of the concentration of HbA1c, the following reagent was used: the
30 reagent just after unsealing of each Reagent R2, which was

stored at 4.degree. C. for three days in bottles for autoanalyzer under a sealed condition just after preparation.

Preparation of the samples

Human blood was collected with an EDTA blood tube (VENOJEC
5 Glass Vacuum Tubes; TERUMO Corp.). The blood was left for two hours to give the precipitated hemocyte layer. The hemocyte layer (10 μ L) was diluted with purified water (1 mL), and the mixture was frozen to store at -20.degree. C., and the frozen sample was melted just before use.

10 Determination of the concentration of HbA1c using Reagent R1 and Reagent R2

A calibration curve was diagramed using Reagent R1 and Reagent R2 just after unsealing, along with the standard samples with the values of HbA1c (=the concentration of HbA1c) of 0.0%,
15 4.2%, 7.7%, 11.3% and 14.8%, respectively, which were determined by an autoanalyzer of glycohemoglobin, HLC-723 GHbV (Tohso Corp.).

Determination of the concentration of HbA1c was carried out as follows. The sample (3.2 μ L) prepared as mentioned above
20 was added to Reagent R1 (240 μ L), and the reaction was allowed to occur at 37.degree. C. for 5 minutes. Then, Reagent R2 (80 μ L) was added thereto, and the reaction was allowed to occur at 37.degree. C. for 5 minutes. Then, changes in absorbance were measured by the 2 point-end method (photometric points:
25 16 and 34) with the main-wavelength of 450 nm and the sub-wavelength of 800 nm, wherein the measurement was carried out on Hitachi autoanalyser 7170.

The results of the determination of the concentration of HbA1c (%) by using the reagents prepared in Examples 4 to 5 and
30 Comparative Example 2 are shown in Table 2, and the results of

the determination of the concentration of HbA1c (%) by using the reagents prepared in Examples 6 to 7 and Comparative Example 3 are shown in Table 3.

5 Table 2

Days after unsealing of Reagent R1 (days)	Example 4	Example 5	Comparative Example 2
0	6.0	6.1	6.0
14	6.0	6.0	6.4
28	6.1	5.8	6.8

Table 3

Days after unsealing of Reagent R1 (days)	Example 6	Example 7	Comparative Example 3
0	6.0	6.1	6.1
14	6.4	6.1	6.8
28	6.5	6.1	7.5

As shown in Tables 2 and 3, in the determination of the concentration of HbA1c using the reagents prepared in Comparative Example 2 and 3, which comprise Reagent R1 comprising no sodium bicarbonate, the values to be determined of the concentration of HbA1c changed significantly with the passage of days after unsealing of the reagents, while in the determination of the concentration HbA1c using the reagents prepared in Examples 4 to 7, which comprise Reagent R1 comprising sodium bicarbonate, the values to be determined of the concentration of HbA1c did not change so much even with the passage of days after unsealing of the reagents.

20

Industrial Applicability

According to the present invention, the non-specific agglutination of the insoluble carrier particles is suppressed, which makes specific determination possible, and an immunoassay with high accuracy and reliability can be provided. The present
5 invention further provides a reagent for an immunoassay, in which non-specific agglutination is suppressed even after a long storage. The present invention furthermore provides a method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable even after a storage for a
10 long time.

Claims

1. An immunoassay, which comprises carrying out an antigen-antibody reaction using insoluble carrier particles in an aqueous medium comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer) and a carbonic acid compound releasing a bicarbonate ion.
2. The immunoassay according to claim 1, wherein the carbonic acid compound releasing a bicarbonate ion is an alkali metal compound.
3. The immunoassay according to claim 1 or 2, wherein the concentration of the bicarbonate ion is 0.05 to 500 mmol/L.
4. The immunoassay according to any of claims 1 to 3, wherein the concentration of the buffer having its buffer capacity in a neutral or alkaline region is 0.1 to 500 mmol/L.
5. The immunoassay according to any of claims 1 to 4, wherein the insoluble carrier particles are those which antibodies reactive with substance to be determined are bound to.
6. The immunoassay according to any of claims 1 to 4, wherein the insoluble carrier particles are those which substance to be determined is bound to.
7. The immunoassay according to any of claims 1 to 6, wherein the insoluble carrier particles are latex.

8. The immunoassay according to any of claims 1 to 7, wherein the immunoassay is a nephelometric immunoassay.

5 9. A reagent for an immunoassay comprising a buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), a carbonic acid compound releasing a bicarbonate ion and insoluble carrier particles.

10 10. The reagent for an immunoassay according to claim 9, wherein the buffer having its buffer capacity in a neutral or alkaline region (except for a carbonate-based buffer), the carbonic acid compound releasing a bicarbonate ion and the insoluble carrier particles are comprised in an aqueous medium.

15

11. The reagent for an immunoassay according to claim 9 or 10, wherein the carbonic acid compound releasing a bicarbonate ion is an alkali metal compound.

20 12. The reagent for an immunoassay according to claim 10 or 11, wherein the concentration of the bicarbonate ion is 0.05 to 500 mmol/L.

25 13. The reagent for an immunoassay according to any of claims 10 to 12, wherein the concentration of the buffer having its buffer capacity in a neutral or alkaline region is 0.1 to 500 mmol/L.

30 14. The reagent for an immunoassay according to any of claims 9 to 13, wherein the insoluble carrier particles are those which

antibodies reactive with substance to be determined are bound to.

15. The reagent for an immunoassay according to any of claims
5 9 to 14, wherein the insoluble carrier particles are latex.

16. A method for keeping a reagent for an immunoassay comprising
insoluble carrier particles stable, wherein the insoluble
carrier particles are comprised in an aqueous medium comprising
10 a buffer having its buffer capacity in a neutral or alkaline
region (except for a carbonate-based buffer) and a carbonic acid
compound releasing a bicarbonate ion.

17. The method for keeping a reagent for an immunoassay
15 comprising insoluble carrier particles stable according to
claim 16, wherein the carbonic acid compound releasing a
bicarbonate ion is an alkali metal compound.

18. The method for keeping a reagent for an immunoassay
20 comprising insoluble carrier particles stable according to
claim 16 or 17, wherein the concentration of the bicarbonate
ion is 0.05 to 500 mmol/L.

19. The method for keeping a reagent for an immunoassay
25 comprising insoluble carrier particles stable according to any
of claims 16 to 18, wherein the concentration of the buffer
having its buffer capacity in a neutral or alkaline region is
0.1 to 500 mmol/L.

30 20. The method for keeping a reagent for an immunoassay

comprising insoluble carrier particles stable according to any of claims 16 to 19, wherein the insoluble carrier particles are those which antibodies reactive with substance to be determined are bound to.

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21. The method for keeping a reagent for an immunoassay comprising insoluble carrier particles stable according to any of claims 16 to 20, wherein the insoluble carrier particles are latex.

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