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(54) METHOD FOR REDUCING INTERFERENCE BY COMPONENT OUTSIDE ASSAY SYSTEM

(57) An object of the present invention is to provide a method of reducing interference to a measurement system from water-soluble silicone and/or surfactant contaminating the measurement system in a latex agglutination immunoassay. The execution of a latex immunoagglutination reaction in the presence of a silicone compound can reduce the interference to the measurement system from a component derived from a micro blood-collection tube (water-soluble silicone) and/or surfactant mixed from outside of the measurement system.

EP 2 554 991 A1

Description

TECHNICAL FIELD

[0001] The present invention relates to i) a method of reducing interference to a measurement system from a component outside of the measurement system and water-soluble silicone and/or surfactant contaminating the measurement system in particular, characterized in that a latex immunoagglutination reaction is performed in the presence of a silicone compound in a latex agglutination immunoassay (latex immunoagglutination assay) using latex particles supporting a substance having a high affinity for the analyte or supporting the analyte, ii) a reagent used in the method of reducing the interference, and iii) the latex agglutination immunoassay with the interference reduced.

BACKGROUND ART

[0002] A latex immunoagglutination assay (latex turbidimetric immunoagglutination method) (hereinafter also referred to as an LTIA method) is frequently used in the field of clinical examination as a measurement (assay) method of an analyte (hereinafter also referred to as a target component) in a biological sample. The LTIA method is a measurement method using, for example, latex particles supporting an antibody to a target component (hereinafter also referred to as antibody-supporting latex particles) so as to detect a degree of agglutination (turbidity) of latex particles generated due to binding of an antigen, i.e., the target component, and the antibody-supporting latex particles, with an optical means (e.g., a turbidimetric method measuring transmitted light, a nephelometric method measuring scattering light) etc.

[0003] It is known that a surfactant-like substance interferes with immunological measurement systems including the LTIA method. The presence of a certain surfactant in an immunological measurement system may cause a problem such as inhibiting of an antigen-antibody reaction itself and dissociating of the antigen-antibody binding formed by the antigen-antibody reaction. The LTIA method is a homogeneous measurement method in which an antigen-antibody reaction is performed in one liquid phase, and results in an environment in which materials making up a measurement system such as the antibody-supporting latex particles are continually exposed to surfactant during measurement and, therefore, this may lead to the occurrence of interferences to the measurement system in a composite manner, such as causing a change in the structure of an analyte itself, the formation of a complex with an analyte, the nonspecific adsorption to the antibody-supporting latex particles, and the detachment of antibodies and blocking proteins supported by latex particles, due to the surfactant.

[0004] When blood is collected from a subject in the case where blood (whole blood, serum, or plasma) is used as a biological sample, preventive components (hereinafter also referred to as blood collection tube-processing agents) may have been applied to the inner wall and the cap of a blood collection tube so as to prevent blood (blood clot) from sticking to the inner wall of the blood collection tube and bubbling in the blood collection tube and the cap portion and to prevent insufficient coagulation for acquiring serum and insufficient separation of serum layer and blood cell layer. A certain kind of silicone compound is used by itself as a blood collection tube-processing agent and is used as a medium for applying other blood collection tube-processing agent than silicone compounds to the inner wall and the cap in some cases.

[0005] Some reports have been made for the interference to the LTIA method from a component applied to the inner wall of a blood collection tube. Non-Patent Literature 1 reports that a reduction in measurement value is observed when a measurement sample acquired with a commercially available micro blood collection tube is measured with the LTIA method. Non-Patent Literature 1 points out water-soluble silicone released from the inner wall of the blood collection tube as a causative substance and describes measurement results in which measurement samples with water-soluble silicone added were measured with a plurality of LTIA reagents. In Non-Patent Literature 1, consideration is also given to the interference when surfactants (Brij (registered trademark) 35, Tween (registered trademark) 20, Triton (registered trademark) X-100) were added to measurement samples and it is reported that the reduction in measurement value was observed as was the case with water-soluble silicone. Non-Patent Literature 2 reports that measurement samples acquired with a plurality of blood collection tubes were measured with a plurality of LTIA reagents and that the reduction in measurement value was observed when a measurement sample acquired with a micro blood collection tube was measured as was the case with Non-Patent Literature 1, and water-soluble silicone released from the inner wall of the blood collection tube is also pointed out as a causative substance in this case.

[0006] A micro blood collection tube is often used when blood is collected from newborns having a smaller body weight as compared to adults. Even if the micro blood collection tube is used, a predetermined amount of blood cannot easily be collected in some cases, resulting in collected blood less than the predetermined amount. In such a case, a concentration of the blood collection tube-processing agent is increased in the measurement sample and the interference to the measurement system is expected to be prominent.

[0007] The surfactants considered in Non-Patent Literature 1 are used as nonspecific reaction-preventing agents and cleaning agents in immunological measurement methods including ELISA and are also frequently used in biochemical automated analyzers used in clinical assays as cleaning agents of: probes for dispensing or stirring measurement

samples and reagents; flow passages of reagents; and repeatedly used reaction tanks. Thus, attention must be given to the interference due to mixing of the surfactants into a measurement system in the case of LTIA agents which are necessarily used in the automated analyzers.

[0008] Despite such a situation, no report has been made of a method of avoiding the interference from these surfactants and a method of reducing the interference from the surfactants when a measurement sample acquired through a micro blood collection tube is measured with the LTIA method in particular.

CITATION LIST

NON-PATENT LITERATURE

[0009]

Non-Patent Literature 1: Japanese Journal of Medical Technology, Vol. 49, No. 10 (2000), pp.1399-1403.
Non-Patent Literature 2: Okayama Journal of Medical Technology, Vol. 40, No. 2 (2003), pp.6-10.

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0010] An objective of the present invention is to provide i) a method of reducing interference to a measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system in a latex agglutination immunoassay using latex particles supporting a substance having a high affinity for the analyte or supporting the analyte, ii) a reagent used in the method of reducing the interference, and iii) the latex agglutination immunoassay with the interference reduced.

SOLUTION TO PROBLEM

[0011] The present inventors have attempted verification from various viewpoints and have conducted extensive research for solving the problem in the LTIA method and have totally unexpectedly found that when a latex immunoagglutination reaction is performed in the presence of polyether-modified silicone oil, which is classified as the same silicone compound as water-soluble silicone considered as a cause of interference in Non-Patent Literatures 1 and 2, the interference to a measurement system from a component that is mixed from outside of the measurement system and derived from a micro blood collection tube can be reduced. The present inventors have also unexpectedly found that when a latex immunoagglutination reaction is performed in the presence of polyether-modified silicone oil, the interference from surfactants (Brij (registered trademark) 35, Tween (registered trademark) 20, Triton (registered trademark) Y-100) having structures different from water-soluble silicones can also be reduced, leading to the completion of the present invention.

[0012] The present invention comprises the following.

(1) A method of reducing interference to a measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system, wherein a latex immunoagglutination reaction is performed in the presence of a silicone compound in a latex agglutination immunoassay.
(2) The method of (1) above, wherein the silicone compound contains polyether-modified silicone oil.
(3) The method of (1) or (2) above, wherein the silicone compound is present by allowing a latex reagent solution to contain the silicone compound.
(4) The method of (1) to (3) above, wherein the process of allowing a latex reagent solution to contain the silicone compound is based on a blocking treatment.
(5) The method of (1) to (4) above, wherein the concentration of the silicone compound at the time of the latex immunoagglutination reaction is 0.0001 to 1 %.
(6) A method of reducing interference from water-soluble silicone and/or surfactant mixed in a latex agglutination immunoassay comprising the steps of:

bringing i) latex particles supporting a substance having a high affinity for the analyte and ii) a silicone compound into contact with a sample including the analyte derived from living body and the mixed water-soluble silicone and/or surfactant; and
measuring an agglutination reaction of the analyte and the latex particles.

(7) A latex agglutination immunoassay comprising the step of:

bringing i) latex particles supporting a substance having a high affinity for the analyte and ii) a silicone compound into contact with a sample including the analyte derived from living body.

(8) A kit for a latex agglutination immunoassay comprising:

a first reagent including a buffering agent; and
a second reagent including latex particles supporting a substance having a high affinity for the analyte, wherein at least one of the first reagent and the second reagent includes a silicone compound.

(9) A reagent for a latex agglutination immunoassay comprising:

i) a buffering agent; ii) a silicone compound; and iii) latex particles supporting a substance having a high affinity for the analyte.

ADVANTAGEOUS EFFECTS OF INVENTION

[0013] The present invention provides a method of reducing the interference to an LTIA measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system. The present invention enables accurate measurement even if the measurement is performed with the LTIA method using a measurement sample collected through a micro blood collection tube.

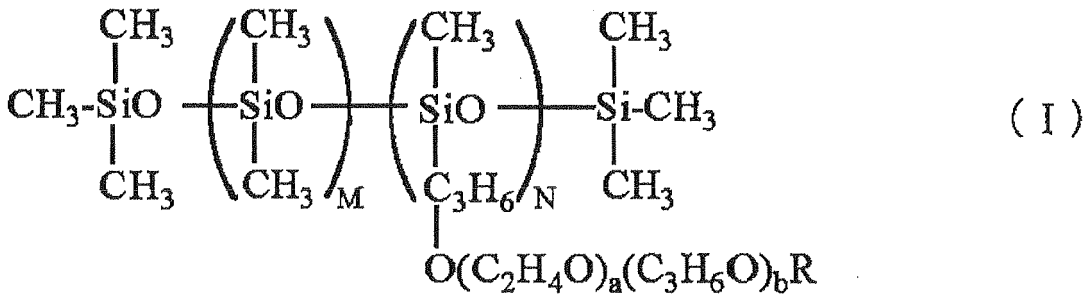
DESCRIPTION OF EMBODIMENTS

(Silicone Compound)

[0014] Polyether-modified silicone oil may preferably be used as a silicone compound of the present invention. Preferable polyether-modified silicone oils include a copolymer of alkyl (having one to three carbon atoms) siloxane and polyoxyalkylene (preferably having two to five carbon atoms in an alkylene group) and a copolymer of dimethylsiloxane and polyoxyalkylene is particularly preferable. Polyoxyalkylene refers to polyoxyethylene, polyoxypropylene, and a random or block polymer of polyoxyethylene and polyoxypropylene. Examples of such polyether-modified silicone oil include a compound expressed by the following general formula (I).

[0015]

[Chem 1]



(I)

(In this formula, M, N, a, and b are average degrees of polymerization and R is hydrogen or an alkyl group)

[0016] In this case, preferably, M and N are 10 to 10,000 and 1 to 1,000, respectively, and satisfy M>N and, more preferably, M and N are 10 to 1,000 and 1 to 50, respectively, and satisfy M>N. Preferably, a is 2 to 100 and b is 0 to 50. It is preferable that R be hydrogen or an alkyl group having one to four carbon atoms.

[0017] Specific examples of commercially available products containing the polyether-modified silicone oil used in the present invention include SILWET FZ-2166 manufactured by Nippon Unicar Company Limited, KF-618 manufactured by Shin-Etsu Chemical, SH3749, SH7090, SF8410, SH8700 manufactured by Dow Corning Toray Silicone Co., Ltd., and TSA775, TSF4440 manufactured by GE Toshiba Silicone or Momentive Performance Materials Japan LLC., and one of these products can solely be used or two or more products can be used as a mixture. These products may be mixtures with polyalkylsiloxane or silica as in the case of TSA775.

[0018] A preparation method of an LTIA reagent using a silicone compound of the present invention will hereinafter

be described by taking as an example the case of using antibody-supporting latex particles as latex particles supporting a substance having a high affinity for the analyte or supporting the analyte. Although the silicone compound of the present invention may be added to either of reagent solutions, which make up the LTIA reagent, containing or not containing the antibody-supporting latex particles, the silicone compound is preferably added to the reagent solution containing the antibody-supporting latex particles. If the silicone compound is added to a reagent solution containing the antibody-supporting latex particles, the silicone compound may be added to a reagent solution containing latex particles either before or after they support an antibody while the silicone compound is preferably added to the reagent solution containing latex particles after they support an antibody. The temperature at the time of the addition of the silicone compound of the present invention is selectable to be a suitable temperature from 1 to 65 degrees C, at which the solubility of the silicone compound of the present invention is expected to be increased, as long as the function (activity) of a supported antibody is not lost, for example.

[0019] After adding the silicone compound of the present invention to the reagent solution containing latex particles after supporting an antibody, incubation can additionally be performed at a suitable temperature between 1 to 65 degrees C for a suitable time. As a result, it can be expected that the same effect as the blocking effect is added to the antibody-supporting latex particles. If the incubation is performed, the incubation is preferably performed at 30 to 65 degrees C. If the temperature is less than 30 degrees C, the blocking effect may not sufficiently be added and, if the temperature exceeds 65 degrees C, the antibody etc., may be denatured as protein, resulting in the loss of antibody activity. The incubation at approximately 37 degrees C can be given as one example of preferred incubation temperature. When the incubation is performed, the time is not limited and can empirically be selected in accordance with temperature so as to acquire the expected blocking effect. In this description, such heating or incubation operations may be referred to as a blocking treatment.

[0020] The concentration of the silicone compound of the present invention may be prescribed, for example, as a concentration at the time of the latex immunoagglutination reaction. Preferable concentrations include 0.0001 % to 1 %, 0.0002 % to 1 %, 0.0004 % to 1 %, 0.0008 % to 1 %, 0.002 % to 1 %, 0.003 % to 1 %, 0.006 % to 1 %, 0.01 % to 1 %, 0.03 % to 1 %, 0.05 % to 1 %, 0.0001 % to 0.5 %, 0.0002 % to 0.5 %, 0.0004 % to 0.5 %, 0.0008 % to 0.5 %, 0.002 % to 0.5 %, 0.003 % to 0.5 %, 0.006 % to 0.5 %, 0.01 % to 0.5 %, 0.03 % to 0.5 %, 0.05 % to 0.5 %, 0.0001 % to 0.2 %, 0.0002 % to 0.2 %, 0.0004 % to 0.2 %, 0.0008 % to 0.2 %, 0.002 % to 0.2 %, 0.003 % to 0.2 %, 0.006 % to 0.2 %, 0.01 % to 0.2 %, 0.03 % to 0.2 %, 0.05 % to 0.2 %, 0.0001 % to 0.1 %, 0.0002 % to 0.1 %, 0.0004 % to 0.1 %, 0.0008 % to 0.1 %, 0.002 % to 0.1 %, 0.003 % to 0.1 %, 0.006 % to 0.1 %, 0.01 % to 0.1 %, 0.03 % to 0.1 %, and 0.05 % to 0.1 %. In general, a preferred concentration is 0.0001 % to 1 %, preferably 0.001 % to 0.5 %, and more preferably 0.01 % to 0.1 %. Although some commercially available silicone compound products are distributed as mixtures with other components (e.g., polyalkylsiloxane and silica), concentrations (dosage of individual products) for acquiring the effect of the present invention may empirically be confirmed by reference to a method described in the examples.

[0021] As described above, the silicone compound of the present invention may be selected from a group of silicone compounds (silicone products) having an ability to reduce the interference to a measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system in the LTIA method with consideration given to the availability of the measurement sensitivity, measurement range, and reproducibility desired for the measurement system or the stability of reagent, and thus, practically optimum type, concentration, and LTIA reagent preparation method may be utilized as needed. In this description, "reduce (reducing) the interference" means that the reduction in measurement value due to water-soluble silicone and/or surfactant is suppressed.

Although the details of water-soluble silicones used for micro blood collection tubes are not clear, commercially available water-soluble silicones include KS-538 (Shin-Etsu Silicone), KM-70 (Shin-Etsu Silicone), KM-72F (Shin-Etsu Silicone), TSA770 (Comentative), TSA732 (Comentative), TSA7341 (Comentative), AntifoamSI (Wako Pure Chemical Industries), SM5571 (Toray silicone), etc. Whether these water-soluble silicones actually cause the interference to the measurement system of the latex agglutination immunoassay can be examined by performing experiments as needed and if confirmed as an interfering component, the water-soluble silicone can be used as a material for screening a silicone compound of the present invention.

[0022] Although description above has been made by taking as an example the case of using the antibody-supporting latex particles as the latex particles supporting a substance having a high affinity for the analyte or supporting the analyte, the case of using an antigen as a supported substance must obviously be understood in the same way. From the viewpoint of a high-affinity-binding substance, the analyte is the high-affinity-binding substance. If a target component other than antigen or antibody binds to latex particles supporting a binding partner specific to the target component, and thus, agglutination of the latex particles is formed depending on the abundance of the target component, such reaction is also included in the latex immunoagglutination reaction according to the present invention.

(Latex Particles)

[0023] Although the latex particles in the present invention refer to polystyrene latex particles etc., when the latex

particles are included in the latex immunoagglutination reaction described above and when a method of supporting a bonding partner specific to the target component is based on a physical precess, such as hydrophobic bonding, the latex particles of the present invention include metal colloid, silica, carbon, etc. The size of the latex particles may be selected as needed from the range of 0.05 to 1 μm so as to acquire desired measurement sensitivity, measurement range, etc., in consideration of an optical measurement method used (e.g., a turbidimetric method measuring transmitted light, a nephelometric method measuring scattering light). An average particle diameter used in an optical measurement in automated analyzers is generally 0.1 to 0.4 μm and preferably 0.1 to 0.2 μm. An average particle diameter of the latex particles can be checked by a particle size analyzer, transmission electron microscope imaging, or other methods. The concentration of the latex particles in reagent solution can be selected as needed in accordance with the particle diameter of the latex particles used and the overall design of the measurement system from a range of 0.0001 mg/mL to 10 mg/mL, for example.

(Configuration etc., as LTIA reagent)

[0024] In addition to the main components for the reaction, the LTIA reagent (reagent solution) of the present invention may contain a component for buffering and adjusting the pH, ionic strength, osmotic pressure, etc., of the sample, such as acetic acid, citric acid, phosphoric acid, tris, glycine, boric acid, carbonic acid, and Good's buffer as well as sodium salts, potassium salts, and calcium salts thereof. The LTIA reagent may further contain a component for enhancing agglutination, such as macromolecules including polyethyleneglycol, polyvinylpyrrolidone, and phospholipid polymers. The LTIA reagent may also contain one or more of components for controlling agglutination, such as proteins, amino acids, saccharide, metal salts, surfactants, reducing agents, and chaotropic agents that are generally used for this purpose. Any components that tend to cause foaming may also be added to the assay reagents of the present invention.

[0025] Although the type of the sample to be measured (assayed) with the LTIA reagent of the present invention may be any one of a variety of biological samples, an analyte contained in the blood collected through a micro blood collection tube is preferred. The analyte (i.e. the substance of interest) can be protein, peptide, amino acid, lipid, carbohydrate, nucleic acid, or hapten, for example, or any other molecules that are quantifiable in theory. Examples of the analytes include CRP (C-reactive protein), Lp(a), MMP3 (matrix metalloproteinase 3), anti-CCP (cyclic citrullinated peptide) antibody, anti-phospholipid antibody, RPR, type IV collagen, PSA, BNP (brain natriuretic peptide), NT-proBNP, insulin, microalbumin, cystatin C, RF (rheumatoid factor), CA-RF, KL-6, PIVKA-II, FDP, D-dimer, SF (soluble fibrin), TAT (thrombin-antithrombin III complex), PIC, PAI, factor XIII, pepsinogen I/II, phenytoin, phenobarbital, carbamazepine, valproic acid, theophylline, and others.

[0026] The LTIA reagent of the present invention is made up of one or more reagent solutions, i.e., a plurality of reagent solutions, as described above. Examples of a plurality of reagent solutions include a reagent solution consisting of a buffer solution intended to adjust an analyte to a concentration preferred for measurement or to adjust an environment of the antigen-antibody reaction, a reagent solution containing antibody-supporting latex particles, etc. The silicone compound of the present invention may be contained in all of the constituent reagent solutions making up the reagent, or may be contained in any of selected constituent reagent solutions making up the assay reagent.

EXAMPLES

[0027] Although the present invention will hereinafter be described in detail by referring to the examples below, the present invention is not limited to the following examples.

[Example 1] Verification of Effect of Silicone Compound of the Present Invention (1)

[0028] Verified was the effect of the silicone compound of the present invention in measuring a sample processed with a micro blood collection tube.

<Test Method>

(1) Conventional LTIA Reagent

[0029] SS Type Pure Auto (registered trademark) S, CRP Latex (manufactured by Sekisui Medical Co., Ltd.) was used.

(2) Test Reagent

(2-1) First Reagent

[0030] Buffer Solution 1 (2-amino-2-hydroxymethyl-1,3-propanediol buffer solution (pH 8.5) 20 mmol/L) of said conventional LTIA reagent was directly used.

(2-2) Second Reagent

(i) Control Reagent Solution

[0031] Latex Reagent Solution 2 (antihuman C-reactive protein murine monoclonal antibody-sensitized latex 2.25 mg/ml) of said conventional LTIA reagent was directly used.

(ii) Reagent Solutions of Examples 1a to 1c

[0032] FZ-2166 (manufactured by Nippon Unicar Company Limited), KF-618 (manufactured by Shin-Etsu Silicone), SH3749, SH7090, SF8410, SH8700 (manufactured by Dow Coming Toray Co., Ltd.), and TSA775, TSF4440 (manufactured by GE Toshiba Silicone) were added as silicone compounds to the Control Reagent Solution at final concentrations of 0.01 %, 0.03 %, and 0.10 % and were used after heating at 37 degrees C for 24 hours.

(3) Preparation of Micro Blood Collection Tube-Processed Sample and Control Sample

[0033] Micro blood-collection tube: A predetermined amount, i.e., 0.6 mL, or 1/12 of the predetermined amount, i.e., 0.05 mL, of whole blood was dispensed to BD Microtainer Microguard tube (catalog number: 365985; with Lithium Heparin and plasma separator additive; manufactured by Becton, Dickinson and Company, Japan; fill volume: 0.4 to 0.6 mL) and was allowed to stand still for 30 minutes after inversion to prepare a micro blood collection tube-processed sample (hereinafter respectively referred to as a 0.6-mL sample and a 0.05-mL sample). A control sample was prepared by using Venoject II (code number: VP-HL050K; Lithium Heparin; manufactured by Terumo; fill volume: 5 mL).

(4) Assay Method

[0034] The four types of the second reagents (one Control Reagent Solution and three Reagent Solutions of Examples 1a to 1c) were combined with the first reagent (Buffer Solution 1 of the conventional LTIA reagent above) and used as test reagents, and the measurement samples (the control sample and the micro blood collection tube-processed samples (the 0.6-mL sample and the 0.05-mL sample)) were measured by using HITACHI 7170 Automated Analyzer (manufactured by Hitachi High-Technologies Corporation) with the following measurement parameters of (5).

(5) Measurement Parameters of HITACHI 7170 Automated Analyzer

[0035]

(i) Liquid volumes: Measurement Sample, 3 μL; First Reagent, 150 μL; Second Reagent, 50 μL.
(ii) Analysis method: two-point end method (photometric point 19-34)
(iii) Measurement wavelength: 570 nm/ secondary-wavelength 800 nm
(iv) Calibration: spline
(v) Calibrator: SS Type Pure Auto (registered trademark) S, CRP Latex, Calibrator

<Assay Result>

[0036] The absorbance of the 0.6-mL sample and the 0.05-mL sample measured by using the four types of the second reagents (one Control Reagent Solution and three Reagent Solutions of Examples 1a to 1c) is divided by the absorbance of the control sample measured by using the Control Reagent Solution as the second reagent to obtain relative absorbance (%). The result is shown in Table 1.
When the Control Reagent Solution not containing the silicone compound of the present invention was used as the second reagent to measure the micro blood collection tube-processed sample (Comparison Example 1), the reduction in relative absorbance was confirmed. Particularly for the 0.05-mL sample (acquired by dispensing 0.05 mL, which is 1/12 of the predetermined amount), it was confirmed that the relative absorbance was considerably varied (reduced) to

75.6 %.

In contrast, when the Reagent Solutions of Examples 1a to 1c (containing eight types of the silicone compounds of the present invention each at three concentrations) were used as the second reagent to measure the micro blood collection tube-processed sample (Examples 1a to 1c), a slight or little variation in relative absorbance was recognized even if the 0.05-mL sample was measured.

[0037]

(Table 1]

	Comp. Example 1		Example 1a		Example 1b		Example 1c	
silicone Compound	0.00%		0.01%		0.03%		0.10%	
	Micro Blood Collection Tube-Processed Sample							
	0.6mL	0.05mL	0.6mL	0.05mL	0.6mL	0.5mL	0.6mL	0.05mL
None (Con.Re.Sol.)	92.7	75.6						
FZ-2166			98.9	101.8	98.8	99.7	99.9	101.1
KF-618			99.8	95.0	97.7	98.8	100.9	102.0
SH3749			98.4	96.8	100.8	98.3	100.1	100.2
SH7090			99.6	95.4	97.9	98.5	101.1	101.2
SH8410			97.2	93.9	100.8	97.8	99.8	99.3
SH8700			99.2	96.2	99.7	98.8	99.8	99.1
TSA775			100.3	97.6	99.5	99.0	99.0	100.2
TSF4440			97.6	95.5	99.7	98.4	98.9	98.9
Con.Re.Sol.= Control Reagent Solution								

[Example 2] Consideration of Preparation Method of LTIA Reagent Using Silicone Compound of the Present Invention

[0038] Consideration was given to a preparation method of the LTIA reagent using the silicone compound of the present invention.

<Test Method>

(1) Conventional LTIA Reagent

[0039] The same conventional LTIA reagent as Example 1 was used.

(2) Test Reagent

(2-1) First Reagents

(i) Control Reagent Solution R1

[0040] Buffer Solution 1 of said conventional LTIA reagent was directly used.

(ii) Reagent Solution of Example 2a

[0041] TSA775 was added to the Control Reagent Solution R1 at final concentrations of 0.01 % and 0.03 % and used after heating at 37 degrees C for 24 hours.

(2-2) Second Reagents

(i) Control Reagent Solution R2

[0042] Latex Reagent Solution 2 of said conventional LTIA reagent was directly used.

(ii) Reagent Solution of Example 2b

[0043] TSA775 was added to the Control Reagent Solution R2 at final concentrations of 0.01 % and 0.03 % and used after standing still at 10 degrees C or lower for 24 hours.

(iii) Reagent Solution of Example 2c

[0044] TSA775 was added to the Control Reagent Solution R2 at final concentrations of 0.01 % and 0.03 % and used after heating at 37 degrees C for 24 hours.

(3) Preparation of Micro Blood Collection Tube-Processed Sample and Control Sample

[0045] The 0.6-mL sample, the 0.05-mL sample, and the control sample were prepared as is the case with Example 1.

(4) Assay Method

[0046] The first reagents and the second reagents were used as test reagents in the following four types of combinations, and the measurement samples (the control sample and the micro blood collection tube-processed samples (the 0.6-mL sample and the 0.05-mL sample)) were measured by using HITACHI 7170 Automated Analyzer with the following measurement parameters of (5).

Comparison Example 2: Control Reagent Solution R1 and Control Reagent Solution R2

[0047]

Example 2a: Reagent Solution of Example 2a and Control Reagent Solution R2
Example 2b: Control Reagent Solution R1 and Reagent Solution of Example 2b
Example 2c: Control Reagent Solution R1 and Reagent Solution of Example 2c

(5) Measurement Parameters of HITACHI 7170 Automated Analyzer

[0048] The conditions were the same as Example 1.

<Assay Result>

[0049] The measurement values (values acquired through concentration conversion by the calibrator) of the 0.6-mL sample and the 0.05-mL sample measured by using the four combinations of the first and second reagents (Comparison Example 2 and Examples 2a to 2c) are divided by the measurement value of the control sample measured by using the combination of the first and second reagents of Comparison Example 2 to obtain relative measurement values (%). The result is shown in Table 2.
When the micro blood collection tube-processed sample was measured with the combination of the Control Reagent Solution R1 and the Control Reagent Solution R2 containing no silicone compound of the present invention (Comparison Example 2), the reduction in relative measurement value was confirmed.
In contrast, when the micro blood collection tube-processed sample was measured by using the Reagent Solutions of Examples 2a to 2c containing the silicone compound of the present invention (Examples 2a to 2c), a slight or little variation in relative measurement value was recognized even if the 0.05-mL sample was measured.
In Examples 2a to 2c using the reagent solutions containing the silicone compound of the present invention, the average values of relative measurement values (four types) of the 0.6-mL sample and the 0.05-mL sample at two silicone compound concentrations were 96.0 % (Example 2a), 98.3 % (Example 2b), and 100.5 % (Example 2c). From the results above, the highest interference reducing effect was acquired among the three examined conditions when the silicone compound of the present invention was added to the reagents containing the antibody-supporting latex and heated at 37 degrees C for 24 hours.

It was considered that this may be attributed to the improvement in the solubility of the silicone compound in the reagent solutions or the addition of the same effect as the blocking effect due to the heating and incubation.

[0050]

[Table 2]

	Comp. Example 2	Example 2a		Example 2b		Example 2c	
First Reagent	Con.Re.Sol. R1	Example 2a		Con.Re.Sol. R1		Con.Re.Sol. R1	
Second Reagent	Con.Re.Sol. R2	Con.Re.Sol. R2		Example 2b		Example 2c	
Silicone Compound		Added to First Reagent		Added to Second Reagent and allowed to stand still at 10 degrees C or lower		Added to Second Reagent and heated at 37 degrees C for 24 hours	
		0.01%	0.03%	0.01%	0.03%	0.01%	0.03%
0.6mL	92.7	96.9	96.7	98.8	100.4	101.1	96.8
0.05mL	77.0	93.4	96.8	93.3	100.7	102.7	101.2
Con.Re.Sol. = Control Reagent Solution							

[Example 3] Verification of Effect of Silicone Compound of the Present Invention (2)

[0051] The effect of the silicone compound of the present invention in measurement of a surfactant-added sample was verified.

<Test Method>

(1) Conventional LTIA Reagent

[0052] The same conventional LTIA reagent as Example 1 was used.

(2) Test Reagent

(2-1) First Reagent

[0053] Buffer Solution 1 of the conventional LTIA reagent was directly used.

(2-2) Second Reagent

(i) Control Reagent Solution

[0054] Latex Reagent Solution 2 of the conventional LTIA reagent was directly used.

(ii) Reagent Solution of Example 3

[0055] TSA775 was added to the Control Reagent Solution at a final concentration of 0.01 % and used after heating at 37 degrees C for 20 hours.

(3) Preparation of Surfactant-Added Sample and Control Sample

[0056] Triton (registered trademark) X-100 (polyoxyethylene (10) octylphenylether), Tween (registered trademark) 20 (polyoxyethylene sorbitan monolaurate), and Brij (registered trademark) 35 (polyoxyethylene (23) lauryl ether) were added to serum acquired by dispensing whole blood into a glass test tube at additive concentrations (final concentrations) described in Table 3 to prepare the surfactant-added samples. Serum with saline was used as the control sample.

(4) Assay Method

[0057] The two types of the second reagents (the Control Reagent Solution and the Reagent Solution of Example 3) were combined with the first reagent (Buffer Solution 1 of the conventional LTIA reagent) and used as test reagents, and the measurement samples (the control sample and the surfactant-added samples) were measured by using HITACHI 7170 Automated Analyzer with the following measurement parameters of (5).

(5) Measurement Parameters of HITACHI 7170 Automated Analyzer

[0058] The conditions were the same as Example 1.

<Assay Result>

[0059] The measurement values (values acquired through concentration conversion by the calibrator) of the surfactant-added samples measured by using the two combinations of the first and second reagents (Comparison Example 3 and Example 3) are divided by the measurement value of the control sample measured by using the combination of the first and second reagents of Comparison Example 3 to obtain relative measurement values (%). The result is shown in Table 3. In Example 3 where the silicone compound of the present invention is contained, a fluctuation range of the relative measurement values was smaller as compared to Comparison Example 3. Particularly in the case of higher surfactant concentrations, the difference became significant.
It was also found out that the LTIA measurement system using the method of the present invention can avoid the interference of the surfactants described in Non-Patent Literature 1.
[0060]

[Table 3]

Surfactant	Additive Conc.	Comp. Ex. 3	Example 3
TritonX-100	0.10%	91.4	100.0
	1.00%	0.0	54.3
Tween20	0.10%	92.9	95.2
	0.50%	66.7	83.3
Brij35	0.10%	97.6	97.6
	1.00%	88.1	95.2
			(%)

INDUSTRIAL APPLICABILITY

[0061] The present invention provides a method of reducing interference to an LTIA measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system. The present invention enables accurate measurement even if a measurement sample collected through a micro blood-collection tube is used for performing measurement with the LTIA method.

Claims

1. A method of reducing interference to a measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system, wherein a latex immunoagglutination reaction is performed in the presence of a silicone compound in a latex agglutination immunoassay.

2. The method of claim 1, wherein the silicone compound contains polyether-modified silicone oil.

3. The method of claim 1 or 2, wherein the silicone compound is present by allowing a latex reagent solution to contain the silicone compound.

4. The method of claims 1 to 3, wherein the step of allowing a latex reagent solution to contain the silicone compound is based on a blocking treatment.

5. The method of claims 1 to 4, wherein a concentration of the silicone compound at the time of the latex immunoagglutination reaction is 0.0001 to 1 %.

6. A method of reducing interference from water-soluble silicone and/or surfactant mixed in a latex agglutination immunoassay comprising the steps of:

bringing i) latex particles supporting a substance having a high affinity for an analyte and ii) a silicone compound into contact with a sample including the analyte derived from living body and the mixed water-soluble silicone and/or surfactant; and
measuring an agglutination reaction of the analyte and the latex particles.

7. A latex agglutination immunoassay comprising the step of:

bringing i) latex particles supporting a substance having a high affinity for an analyte and ii) a silicone compound into contact with a sample including the analyte derived from living body.

8. A kit for a latex agglutination immunoassay comprising:

a first reagent including a buffering agent; and
a second reagent including latex particles supporting a substance having a high affinity for an analyte, wherein at least one of the first reagent and the second reagent includes a silicone compound.

9. A reagent for a latex agglutination immunoassay comprising:

i) a buffering agent; ii) a silicone compound; and iii) latex particles supporting a substance having a high affinity for an analyte.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
G01N33/543(2006.01)i, *G01N33/531*(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N33/543, G01N33/531

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2011
Kokai Jitsuyo Shinan Koho 1971-2011 Toroku Jitsuyo Shinan Koho 1994-2011

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
JSTPlus/JMEDPlus/JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 11-014628 A (Mitsubishi Chemical Corp.), 22 January 1999 (22.01.1999), entire text (Family: none)	1-9
A	JP 07-301632 A (Mitsubishi Chemical Corp.), 14 November 1995 (14.11.1995), entire text & US 5486479 A & EP 679892 A1 & DE 69527877 D & DE 69527877 T & CN 1122913 A	1-9
A	JP 09-068529 A (Sanyo Chemical Industries, Ltd.), 11 March 1997 (11.03.1997), entire text (Family: none)	1-9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"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
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"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

Date of the actual completion of the international search	Date of mailing of the international search report
22 April, 2011 (22.04.11)	10 May, 2011 (10.05.11)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 09-107994 A (Toa Denpa Kogyo Kabushiki Kaisha), 28 April 1997 (28.04.1997), entire text (Family: none)	1-9
A	JP 07-198721 A (Kurabo Industries Ltd.), 01 August 1995 (01.08.1995), entire text (Family: none)	1-9
A	Shinji UJIIE, Nobuhiro SUZUKI, Tokuji SUZUKI, Tsutomu URAYAMA, "Interference of Agent for Coating Blood Collection Tubes with Latex Immunoaggulutination Reaction", The Japanese Journal of Medical Technology, 2000, vol.49, no.10, pages 1399 to 1403	1-9
A	Keiko UCHIYAMA, Kazuko EGUCHI, Takayuki NAKAO, Tomoko SENOO, Norio KOIDE, "Latex Gyoshuho o Mochiita Myoglobin Sokutei ni Okeru Saiketsukan no Eikyo", Okayama Eisei Kensa, 2003, vol.40, no.2, pages 6 to 10	1-9

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REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description

- *Japanese Journal of Medical Technology,* 2000, vol. 49 (10), 1399-1403 **[0009]**
- *Okayama Journal of Medical Technology,* 2003, vol. 40 (2), 6-10 **[0009]**

专利名称(译)	用于减少分析外部分析系统的干扰的方法		
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摘要(译)

本发明的一个目的是提供一种减少对乳胶凝集免疫测定中污染测量系统的水溶性硅氧烷和/或表面活性剂对测量系统的干扰的方法。在硅氧烷化合物存在下进行乳胶免疫凝集反应可以减少来自微量采血管（水溶性硅氧烷）和/或从测量系统外部混合的表面活性剂的组分对测量系统的干扰。