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

VERFAHREN ZUR INTERFERENZUNTERDRÜCKUNG DURCH KOMPONENTEN AUSSERHALB EINES IMMUNOLOGISCHEN LATEX-AGGLUTINATIONSSYSTEMS

PROCÉDÉ POUR RÉDUIRE L'INTERFÉRENCE DUE À UN COMPOSANT EXTÉRIEUR AU SYSTÈME DE DOSAGE IMMUNOLOGIQUE UTILISANT L'AGGLUTINATION DE LATEX

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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.

[0009] EP 2 508 885 is a post-published document pursuant to Article 54(3) EPC and relates to a homogenous measurement method using insoluble carrier particles that suppresses the matrix effect originating from the sample and also suppresses differences in measurement accuracy among different models of automated analyzers, and to a respective measuring agent. Inclusion of a silicone-based defoaming agent in the reagent reduces the matrix effect originating from the sample and reduces variability of measurement accuracy among different automated analyzers having differing specifications.

[0010] JP H11 14628 relates to a measurement method for a substance with a specific connection capability such as protein or ligand, wherein the change in turbidity based on the reaction between protein and ligand is detected as a change in absorption light or scattered light while utilizing the specific connection capability between protein and ligand, independent of an antigen/antibody reaction. This allows quickly measuring protein or ligand, independent of an immunity reaction, with a high sensitivity and specificity with simple operation. Especially, hyaluronic acid can be measured accurately even without using a competition reaction.

[0011] EP 679 892 relates to sensitized carboxylate-modified latex microparticles for immunoassays having a certain diameter and parking area and immunoassay reagents that are both sensitive and specific and which require no sample pretreatment.

[0012] US 4,536,478 discloses a method for reducing interferences in latex immunoagglutination assays by adding a chemical additive comprised of at least one halogen substituted carboxylic acid in the reaction mixture comprising the latex particles and sample.

CITATION LIST

NON-PATENT LITERATURE

[0013]

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

[0014] 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

[0015] 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) X-100) having structures different from water-soluble silicones can also be reduced, leading to the completion of the present invention.

[0016] The present invention comprises the following.

(1) A method of suppressing the reduction in measurement value due to water soluble silicone and/or surfactant

contaminating the measurement system of a latex immunoagglutination assay for the measurement of an analyte in a biological sample,
characterized in that the method includes adding a polyether-modified silicone oil to a reagent solution containing latex particles supporting a substance having high affinity for the analyte;
incubating the reagent solution with the added polyether-modified silicone oil at a temperature from 30 to 65 degrees C; and
performing the latex immunoagglutination assay with the sample and the incubated reagent solution.
(2) A latex immunoagglutination assay method for the measurement of an analyte in a biological sample,
characterized in that the method includes adding a polyether-modified silicone oil to a reagent solution containing latex particles supporting a substance having high affinity for the analyte;
incubating the reagent solution with the added polyether-modified silicone oil at a temperature from 30 to 65 degrees C;
bringing the reagent solution with the added polyether-modified silicone oil into contact with the sample; and
performing the latex immunoagglutination reaction using said reagent solution.
(3) Use of a polyether-modified silicone oil for suppressing the reduction in measurement value due to water soluble silicone and/or surfactant contaminating the measurement system of a latex immunoagglutination assay for the measurement of an analyte in a biological sample,
wherein the use involves adding a polyether-modified silicone oil to a reagent solution containing latex particles supporting a substance having high affinity for the analyte
incubating the reagent solution with the added polyether-modified silicone oil at a temperature from 30 to 65 degrees C; and
performing the latex immunoagglutination assay with the sample and the incubated reagent solution.
(4) 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 and a polyether-modified silicone oil, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.002 % to 1 %.

ADVANTAGEOUS EFFECTS OF INVENTION

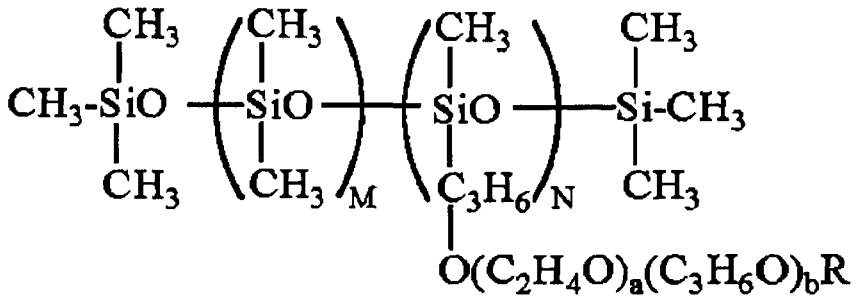
[0017] The present invention provides a method of reducing the interference to an LTIA measurement system from water-soluble silicone and/or surfactant mixed from outside of the measurement system. The present invention enables accurate measurement even if the measurement is performed with the LTIA method using a measurement sample collected through a micro blood collection tube.

DESCRIPTION OF EMBODIMENTS

(Silicone Compound)

[0018] Polyether-modified silicone oil is used as a silicone compound in 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).

[Chem 1]



(I)

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

[0019] 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.

[0020] 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.

[0021] A preparation method of an LTIA reagent using the silicone compound used in 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. The silicone compound is 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 is 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 used in the present invention is expected to be increased, as long as the function (activity) of a supported antibody is not lost, for example.

[0022] After adding the silicone compound used in the present invention to the reagent solution containing latex particles after supporting an antibody, incubation **is** additionally performed at a suitable temperature between **30** 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 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.

[0023] The concentration of the silicone compound used in 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.

[0024] As described above, the silicone compound used in 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.

[0025] 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 used in the present invention.

[0026] 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)

[0027] 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 used in 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)

[0028] In addition to the main components for the reaction, the LTIA reagent (reagent solution) 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 used in the present invention.

[0029] Although the type of the sample to be measured (assayed) with the LTIA reagent 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.

[0030] The LTIA reagent 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.

EXAMPLES

[0031] 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 used in the Present Invention (1)

[0032] Verified was the effect of the silicone compound used in the present invention in measuring a sample processed with a micro blood collection tube.

<Test Method>

(1) Conventional LTIA Reagent

[0033] SS Type Pure Auto (registered trademark) S, CRP Latex (manufactured by Sekisui Medical Co., Ltd.) was used.

(2) Test Reagent

(2-1) First Reagent

[0034] 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

[0035] 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

[0036] 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

[0037] 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

[0038] 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

[0039]

(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>

[0040] 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.

[0041] When the Control Reagent Solution not containing the silicone compound used in 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 %.

[0042] In contrast, when the Reagent Solutions of Examples 1a to 1c (containing eight types of the silicone compounds used in 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.

[0043]

[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**	**0.05mL**	**0.6mL**	**0.05mL**
None (Con.Re.So.)	**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

[0044] Consideration was given to a preparation method of the LTIA reagent using the silicone compound used in the present invention.

<Test Method>

(1) Conventional LTIA Reagent

[0045] The same conventional LTIA reagent as Example 1 was used.

(2) Test Reagent

(2-1) First Reagents

(i) Control Reagent Solution R1

[0046] Buffer Solution 1 of said conventional LTIA reagent was directly used.

(ii) Reagent Solution of Example 2a

[0047] 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

[0048] Latex Reagent Solution 2 of said conventional LTIA reagent was directly used.

(ii) Reagent Solution of Example 2b

[0049] 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

[0050] 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

[0051] 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

[0052] 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

[0053]

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

[0054] The conditions were the same as Example 1.

<Assay Result>

[0055] 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.

[0056] 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 (Comparison Example 2), the reduction in relative measurement value was confirmed.

[0057] 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 (Examples 2a to 2c), a slight or little variation in relative measurement value was recognized even if the 0.05-mL sample was measured.

[0058] In Examples 2a to 2c using the reagent solutions containing the silicone compound 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 was added to the reagents containing the antibody-supporting latex and heated at 37 degrees C for 24 hours.

[0059] 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.

[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 used in the Present Invention (2)

[0060] The effect of the silicone compound used in the present invention in measurement of a surfactant-added sample was verified.

<Test Method>

(1) Conventional LTIA Reagent

[0061] The same conventional LTIA reagent as Example 1 was used.

(2) Test Reagent

(2-1) First Reagent

[0062] Buffer Solution 1 of the conventional LTIA reagent was directly used.

(2-2) Second Reagent

(i) Control Reagent Solution

[0063] Latex Reagent Solution 2 of the conventional LTIA reagent was directly used.

(ii) Reagent Solution of Example 3

[0064] 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

[0065] 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

[0066] 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

[0067] The conditions were the same as Example 1.

<Assay Result>

[0068] 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.

[0069] In Example 3 where the silicone compound used in 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.

[0070] 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.

[Table 3]

Surfactant	Additive Conc.	Comp. Ex. 3	Example 3
Triton-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

[0071] 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 suppressing the reduction in measurement value due to water soluble silicone and/or surfactant contaminating the measurement system of a latex immunoagglutination assay for the measurement of an analyte in a biological sample,
characterized in that the method includes adding a polyether-modified silicone oil to a reagent solution containing latex particles supporting a substance having high affinity for the analyte;
incubating the reagent solution with the added polyether-modified silicone oil at a temperature from 30 to 65 degrees C; and
performing the latex immunoagglutination assay with the sample and the incubated reagent solution.

2. The method of claim 1, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.0001 to 1 %.

3. A latex immunoagglutination assay method for the measurement of an analyte in a biological sample,
characterized in that the method includes adding a polyether-modified silicone oil to a reagent solution containing latex particles supporting a substance having high affinity for the analyte;
incubating the reagent solution with the added polyether-modified silicone oil at a temperature from 30 to 65 degrees C;
bringing the reagent solution with the added polyether-modified silicone oil into contact with the sample; and
performing the latex immunoagglutination reaction using said reagent solution.

4. Use of a polyether-modified silicone oil for suppressing the reduction in measurement value due to water soluble silicone and/or surfactant contaminating the measurement system of a latex immunoagglutination assay for the measurement of an analyte in a biological sample,
wherein the use involves adding a polyether-modified silicone oil to a reagent solution containing latex particles supporting a substance having high affinity for the analyte;
incubating the reagent solution with the added polyether-modified silicone oil at a temperature from 30 to 65 degrees C; and
performing the latex immunoagglutination assay with the sample and the incubated reagent solution.

5. The use of claim 4, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.0001 to 1 %.

6. 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 and a polyether-modified silicone oil, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.002 % to 1 %.

7. The kit for a latex agglutination immunoassay according to claim 6, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.003 % to 0.5 %.

8. The kit for a latex agglutination immunoassay according to claim 6, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.006 % to 0.2 %.

9. The kit for a latex agglutination immunoassay according to claim 6, wherein the concentration of the polyether-modified silicone oil at the time of the latex immunoagglutination reaction is 0.01 % to 0.1 %.

10. The kit for a latex agglutination immunoassay according to any one of claims 6 to 9, wherein the second reagent is obtainable by incubating the latex particle with the polyether-modified silicone oil at a temperature from 30 to 65 degrees C.

Patentansprüche

1. Verfahren zur Unterdrückung der Reduktion des Messwerts aufgrund von wasserlöslichem Silikon und/oder einem

Tensid, die das Messungssystem eines Latex-Immunoagglutinationsassay zur Messung eines Analyten in einer biologischen Probe kontaminieren, **dadurch gekennzeichnet, dass** das Verfahren die Zugabe eines Polyether-modifizierten Silikonöls zu einer Reagenzlösung, die Latexpartikel umfasst, die eine Substanz mit hoher Affinität zum Analyten tragen;
Inkubieren der Reagenzlösung mit dem zugegebenen Polyether-modifizierten Silikonöl bei einer Temperatur von 30 bis 65°C; und
Durchführen des Latex-Immunoagglutinationsassays mit der Probe und der inkubierten Reagenzlösung einschließt.

2. Verfahren gemäß Anspruch 1, worin die Konzentration des Polyether-modifizierten Silikonöls zum Zeitpunkt der Latex-Immunoagglutinationsreaktion 0,0001 bis 1 % ist.

3. Latex-Immunoagglutinationsverfahren zur Messung eines Analyten in einer biologischen Probe, **dadurch gekennzeichnet, dass** das Verfahren die Zugabe eines Polyether-modifizierten Silikonöls zu einer Reagenzlösung, die Latexpartikel enthält, die eine Substanz mit hoher Affinität zum Analyten tragen;
Inkubieren der Reagenzlösung mit dem zugegebenen Polyether-modifizierten Silikonöl bei einer Temperatur von 30 bis 65°C;
in Kontaktbringen der Reagenzlösung mit dem zugegebenen Polyether-modifizierten Silikonöl mit der Probe; und
Durchführen der Latex-Immunoagglutinationsreaktion unter Einsatz der Reagenzlösung einschließt.

4. Verwendung eines Polyether-modifizierten Silikonöls zum Unterdrücken der Reduktion des Messwerts aufgrund von wasserlöslichem Silikon und/oder Tensid, die das Messungssystem eines Latex-Immunoagglutinationsassay zur Messung eines Analyten in einer biologischen Probe kontaminieren,
worin die Verwendung die Zugabe eines Polyether-modifizierten Silikonöls zu einer Reagenzlösung einschließt, die Latexpartikel enthält, die eine Substanz mit hoher Affinität zum Analyten tragen;
Inkubieren der Reagenzlösung mit dem zugegebenen Polyether-modifizierten Silikonöl bei einer Temperatur von 30 bis 65°C; und
Durchführen des Latex-Immunoagglutinationsassay mit der Probe und der inkubierten Reagenzlösung.

5. Verwendung gemäß Anspruch 4, worin die Konzentration des Polyether-modifizierten Silikonöls zum Zeitpunkt der Latex-Immunoagglutinationsreaktion 0,0001 bis 1 % ist.

6. Kit für ein Latex-Agglutinations-Immunoassay, umfassend:

ein erstes Reagens, das ein Puffermittel einschließt; und
ein zweites Reagens, das Latexpartikel einschließt, die eine Substanz mit hoher Affinität zu einem Analyten tragen, und ein Polyether-modifiziertes Silikonöl, worin die Konzentration des Polyether-modifizierten Silikonöls zum Zeitpunkt der Latex-Immunoagglutinationsreaktion 0,002 bis 1 % ist.

7. Kit für ein Latex-Agglutinations-Immunoassay gemäß Anspruch 6, worin die Konzentration des Polyether-modifizierten Silikonöls zum Zeitpunkt der Latex-Immunoagglutinationsreaktion 0,003 bis 0,5 % ist.

8. Kit für ein Latex-Agglutinations-Immunoassay gemäß Anspruch 6, worin die Konzentration des Polyether-modifizierten Silikonöls zum Zeitpunkt der Latex-Immunoagglutinationsreaktion 0,006 bis 0,2 % ist.

9. Kit für ein Latex-Agglutinations-Immunoassay gemäß Anspruch 6, worin die Konzentration des Polyether-modifizierten Silikonöls zum Zeitpunkt der Latex-Immunoagglutinationsreaktion 0,01 bis 0,1 % ist.

10. Kit für ein Latex-Agglutinations-Immunoassay gemäß einem der Ansprüche 6 bis 9, worin das zweite Reagens durch Inkubieren der Latexpartikel mit dem Polyether-modifizierten Silikonöl bei einer Temperatur von 30 bis 65°C erhältlich ist.

Revendications

1. Procédé de suppression de la réduction d'une valeur de mesure en raison d'une silicone soluble dans l'eau et/ou d'un tensioactif contaminant le système de mesure d'un test d'immunoagglutination au latex pour la mesure d'un analyte dans un échantillon biologique,
caractérisé en ce que le procédé comporte le fait d'ajouter une huile de silicone modifiée par polyéther à une

solution de réactif contenant des particules de latex supportant une substance ayant une grande affinité pour l'analyte ;
d'incuber la solution de réactif avec l'huile de silicone modifiée par polyéther ajoutée à une température allant de 30 à 65 degrés C ; et
d'effectuer le test d'immunoagglutination au latex avec l'échantillon et la solution de réactif incubée.

2. Procédé de la revendication 1, dans lequel la concentration de l'huile de silicone modifiée par polyéther au moment de la réaction d'immunoagglutination au latex est de 0,0001 à 1%.

3. Procédé de test d'immunoagglutination au latex pour la mesure d'un analyte dans un échantillon biologique, **caractérisé en ce que** le procédé comporte le fait d'ajouter une huile de silicone modifiée par polyéther à une solution de réactif contenant des particules de latex supportant une substance ayant une grande affinité pour l'analyte ;
d'incuber la solution de réactif avec l'huile de silicone modifiée par polyéther ajoutée à une température allant de 30 à 65 degrés C ;
d'amener la solution de réactif avec l'huile de silicone modifiée par polyéther ajoutée en contact avec l'échantillon ; et
d'effectuer la réaction d'immunoagglutination au latex en utilisant ladite solution de réactif.

4. Utilisation d'une huile de silicone modifiée par polyéther pour supprimer la réduction d'une valeur de mesure en raison d'une silicone soluble dans l'eau et/ou d'un tensioactif contaminant le système de mesure d'un test d'immunoagglutination au latex pour la mesure d'un analyte dans un échantillon biologique,
dans laquelle l'utilisation implique le fait d'ajouter une huile de silicone modifiée par polyéther à une solution de réactif contenant des particules de latex supportant une substance ayant une grande affinité pour l'analyte ;
d'incuber la solution de réactif avec l'huile de silicone modifiée par polyéther ajoutée à une température allant de 30 à 65 degrés C ; et
d'effectuer le test d'immunoagglutination au latex avec l'échantillon et la solution de réactif incubée.

5. Utilisation de la revendication 4, dans laquelle la concentration de l'huile de silicone modifiée par polyéther au moment de la réaction d'immunoagglutination au latex est de 0,0001 à 1%.

6. Kit pour un test immunologique d'agglutination au latex comprenant :

un premier réactif comportant un agent tampon ; et
un deuxième réactif comportant des particules de latex supportant une substance ayant une grande affinité pour un analyte et une huile de silicone modifiée par polyéther, où la concentration de l'huile de silicone modifiée par polyéther au moment de la réaction d'immunoagglutination au latex est de 0,002% à 1%,

7. Kit pour un test immunologique d'agglutination au latex selon la revendication 6, dans lequel la concentration de l'huile de silicone modifiée par polyéther au moment de la réaction d'immunoagglutination au latex est de 0,003% à 0,5%.

8. Kit pour un test immunologique d'agglutination au latex selon la revendication 6, dans lequel la concentration de l'huile de silicone modifiée par polyéther au moment de la réaction d'immunoagglutination au latex est de 0,006% à 0,2%.

9. Kit pour un test immunologique d'agglutination au latex selon la revendication 6, dans lequel la concentration de l'huile de silicone modifiée par polyéther au moment de la réaction d'immunoagglutination au latex est de 0,01% à 0,1%,

10. Kit pour un test immunologique d'agglutination au latex selon l'une quelconque des revendications 6 à 9, dans lequel le deuxième réactif peut être obtenu par l'incubation de la particule de latex avec l'huile de silicone modifiée par polyéther à une température allant de 30 à 65 degrés C.

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	减少胶乳免疫凝集测定系统外组分干扰的方法		
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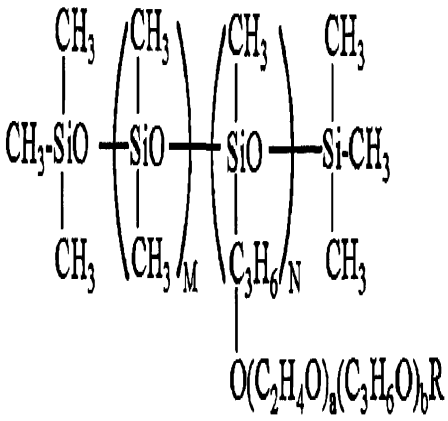
外部链接	Espacenet		

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。

[Chem 1]



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