between the nucleic-acid and the adsorbing carrier caused by excessive drying. Consequently, long time and great effort must be paid.

[0033]

Thirdly, initial costs for preparing operation environment must be high, because they require: operation using the centrifugal machine and the micropipette; and an installation site and/or a working space for used equipment.

[0034]

Reference 1: Japanese registered patent No. 2680462

Reference 2: Japanese patent application Laid-open No. 10-72485

Reference 3: Japanese registered patent No. 3812696

Reference 4: Japanese patent application Laid-open No. 2014-30364

Reference 5: R Boom et al, J. Clin Microbiol, 28 (3), 495-503 (1990)

Reference 6: B Vogelstein and D Gillespie, PNAS, 76 (2), 615-619 (1979)

OBJECTS AND SUMMARY OF THE INVENTION

[0035]

In view of the above background, an object according to the present invention is to provide a method capable of realizing pretreatment process of genetic screening according to a POCT mode.

[0036]

A first aspect of the present invention provides: a pretreatment method of extracting nucleic-acid by means of a solid-phase extraction method prior to a nucleic-acid-amplifying process, the pretreatment method comprising: making a sample, extraction liquid for extracting nucleic-acid contained in the sample, silica particles, and a filtering material contact with each other; making the filtering material carry composite material of the nucleic-acid and the silica particles thereon; and then delivering the filtering material to a nucleic-acid-amplifying process by means of reaction solution for amplifying nucleic-acid, wherein particle diameters of the silica particles and concentration of the silica particles in the reaction solution for amplifying nucleic-acid are set up within a predetermined range, thereby allowing, in

advance of the nucleic-acid-amplifying process, to omit both a drying process and an elution process; the predetermined range is defined as follows such that based on 100% of nucleic-acid contained in the sample 5 through 38% of the nucleic-acid is recovered from the sample: the concentration of the silica particles is 0.0625 through 4 [μg/μl]; an average particle diameter of the silica particles is 0.01 through 100 [μm]; and a surface area calculated based on the average particle diameter is 1 x 10^4 through 1 x 10^8 [μm^2]; and the pretreatment method further comprises: a first extraction process of adding the sample to the extraction liquid to extract the nucleic-acid contained in the sample; a first absorption process of: making the silica particles contact with the extracted nucleic-acid to obtain the composite material of the nucleic-acid and the silica particles; and making the composite material contact with the filtering material; and a first washing process of: washing the composite material and the filtering material with purified water; and delivering the washed composite material and the washed filtering material under a wet condition to the nucleic-acid-amplifying process.

[0037]

A second aspect of the present invention provides: a pretreatment method of extracting nucleic-acid by means of a solid-phase extraction method prior to a nucleic-acid-amplifying process, the pretreatment method comprising: making a sample, extraction liquid for extracting nucleic-acid contained in the sample, silica particles, and a filtering material contact with each other; making the filtering material carry composite material of the nucleic-acid and the silica particles thereon; and then delivering the filtering material to a nucleic-acid-amplifying process by means of reaction solution for amplifying nucleic-acid, wherein: particle diameters of the silica particles and concentration of the silica particles in the reaction solution for amplifying nucleic-acid are set up within a predetermined range, thereby allowing, in advance of the nucleic-acid-amplifying process, to omit both a drying process and an elution process; the predetermined range is defined as follows such that based on 100 % of nucleic-acid contained in the sample 5 through 38 % of the nucleic-acid is recovered from the sample: the concentration of the silica particles is 0.0625 through 1 [μg/μl]; an average particle diameter of the silica particles is 0.01 through 10 [μm]; and a surface area calculated based on the average particle diameter is 1 x 10^5 through 5 x 10^7 [μm^2]; and the pretreatment method further comprises: a first extraction process of adding the sample to the extraction liquid to extract the nucleic-acid contained in the sample; a first absorption process of: making the silica particles contact with the extracted nucleic-acid to obtain the composite material of the nucleic-acid and the silica particles; and making the composite material contact with the filtering material; and a first washing process of: washing the composite material and the filtering material with purified water; and delivering the washed composite material and the

washed filtering material under a wet condition to the nucleic-acid-amplifying process. In one example, the predetermined range is defined as follows: the concentration of the silica particles is 0.0625 through 4 [μg/μl]; an average particle diameter of the silica particles is 0.01 through 100 [μm]; and a surface area calculated based on the average particle diameter is $1x10^4$ through $1x10^8$ [μm^2].

In one example, the predetermined range is defined as follows: the concentration of the silica particles is 0.0625 through 1 [μg/μl]; an average particle diameter of the silica particles is 0.01 through 10 [μm]; and a surface area calculated based on the average particle diameter is $1x10^5$ through $5x10^7$ [μm^2].

[0038]

In one example, the pretreatment method further comprises: a first extraction process of adding the sample to the extraction liquid to extract the nucleic-acid contained in the sample; a first absorption process of: making the silica particles contact with the extracted nucleic-acid to obtain the composite material of the nucleic-acid and the silica particles; and making the composite material contact with the filtering material; and a first washing process of: washing the composite material and the filtering material with purified water; and delivering the washed composite material and the washed filtering material to the nucleic-acid-amplifying process.

In one example, prior to the delivering the filtering material to the nucleic-acid-amplifying process, the composite material is separated from the filtering material, and then the separated composite material is delivered to the

nucleic-acid-amplifying process.

[0039]

In one example, the first absorption process and the first washing process are performed at the same time.

In one example, the first extraction process and the first absorption process are performed at the same time.

In one example, the first extraction process, the first absorption process, and the first washing process are performed at the same time.

[0040]

In one example, the pretreatment method further comprises: a second extraction process of adding the sample to the extraction liquid to extract the nucleic-acid contained in the sample; a second absorption process of: making the extracted nucleic-acid contact with the filtering material carrying the silica particles thereon to obtain the composite material of the nucleic-acid and the silica particles; and making the filtering material carry the obtained composite material thereon; and a second washing process of: washing the carried composite material and the filtering material with purified water; and delivering the washed composite material and the washed filtering material to the nucleic-acid-amplifying process.

In one example, prior to the delivering the filtering material to the nucleic-acid-amplifying process, the composite material are separated from the filtering material, and then the separated composite material is delivered to the nucleic-acid-amplifying process.

In one example, the second absorption process and the second washing process are performed at the same time.

[Effect of the Invention]

[0041]

With the methods according to the present invention, the following effects can be obtained.

Firstly, the extraction process of nucleic-acid in genetic screening can be simple, easy, safe, rapid, and less expensive, and capable of realizing the process of according to the POCT mode.

[0042]

Secondly, the combination of safe, simple, and easy operation enables to remarkably shorten operation time comparing with the conventional methods, and further the extraction of nucleic-acid can be performed at a low cost.

This is because the purified water is used as the wash fluid. Accordingly, it is unnecessary to prepare a plurality of kinds of organic solvent. It is also unnecessary to use the plurality of kinds of organic solvent while distinguishing thereof from each other. Furthermore, the organic solvent itself is unnecessary, and the operation is easy and safe.

[0043]

Thirdly, since the organic solvent itself is unnecessary, both drying operation upon the solid-phase carrier and the elution process of nucleic-acid can be omitted.

In short, immediately after the washing process, the target can be delivered to the nucleic-acid amplification reaction.

In addition, since the time of transfer of the solution can be remarkably reduced, environmental risks, such as splashes of nucleic-acid and/or cross contamination can be also reduced.

What is claimed is:

1. A pretreatment method of extracting nucleic-acid by means of a solid-phase extraction method prior to a nucleic-acid-amplifying process, the pretreatment method comprising:

making a sample, extraction liquid for extracting nucleic-acid contained in the sample, silica particles, and a filtering material contact with each other;

making the filtering material carry composite material of the nucleic-acid and the silica particles thereon; and

then delivering the filtering material to a nucleic-acid-amplifying process by means of reaction solution for amplifying nucleic-acid,

wherein:

particle diameters of the silica particles and concentration of the silica particles in the reaction solution for amplifying nucleic-acid are set up within a predetermined range, thereby allowing, in advance of the nucleic-acid-amplifying process, to omit both a drying process and an elution process;

the predetermined range is defined as follows such that based on 100 % of nucleic-acid contained in the sample 5 through 38 % of the nucleic-acid is recovered from the sample:

the concentration of the silica particles is 0.0625 through 4 [μg/μl];

an average particle diameter of the silica particles is 0.01 through 100 [μm]; and

a surface area calculated based on the average particle diameter is 1×10^4 through 1×10^8 [μm^2]; and

the pretreatment method further comprises:

a first extraction process of adding the sample to the extraction liquid to extract the nucleic-acid contained in the sample;

a first absorption process of: making the silica particles contact with the extracted nucleic-acid to obtain the composite material of the nucleic-acid and the silica particles; and making the composite material contact with the filtering material; and

a first washing process of: washing the composite material and the filtering material with purified water; and delivering the washed composite material and the washed filtering material under a wet condition to the nucleic-acid-amplifying process.

2. A pretreatment method of extracting nucleic-acid by means of a solid-phase extraction method prior to a nucleic-acid-amplifying process, the pretreatment method comprising:

making a sample, extraction liquid for extracting nucleic-acid contained in the sample, silica particles, and a filtering material contact with each other;

making the filtering material carry composite material of the nucleic-acid and the silica particles thereon; and

then delivering the filtering material to a nucleic-acid-amplifying process by means of reaction solution for amplifying nucleic-acid,

wherein: particle diameters of the silica particles and concentration of the silica particles in the reaction solution for amplifying nucleic-acid are set up within a predetermined range, thereby allowing, in advance of the nucleic-acid-amplifying process, to omit both a drying process and an elution process;

the predetermined range is defined as follows such that based on 100 % of nucleic-acid contained in the sample 5 through 38 % of the nucleic-acid is recovered from the sample:

the concentration of the silica particles is 0.0625 through 1 [μg/μl];

an average particle diameter of the silica particles is 0.01 through 10 [μm]; and

a surface area calculated based on the average particle diameter is 1 x 10^5 through 5 x 10^7 [μm^2]; and

the pretreatment method further comprises:

a first extraction process of adding the sample to the extraction liquid to extract the nucleic-acid contained in the sample;

a first absorption process of: making the silica particles contact with the extracted nucleic-acid to obtain the composite material of the nucleic-acid and the silica particles; and making the composite material contact with the filtering material; and

a first washing process of: washing the composite material and the filtering material with purified water; and delivering the washed composite material and the washed filtering material under a wet condition to the nucleic-acid-amplifying process.

3. The pretreatment method as defined in claim 1 or 2, wherein, prior to the delivering the filtering material to the nucleic-acid-amplifying process, the composite material is separated from the filtering material, and then the separated composite material is delivered to the nucleic-acid-amplifying process.

4. The pretreatment method as defined in claim 1 or 2, wherein the first absorption process and the first washing process are performed at the same time.

5. The pretreatment method as defined in claim 1 or 2, wherein the first extraction process and the first absorption process are performed at the same time.

6. The pretreatment method as defined in claim 1 or 2, wherein the first extraction process, the first absorption process, and the first washing process are performed at the same time.