

APPARATUS FOR SEPARATING BIOLOGICAL SAMPLE AND SEPARATING METHOD OF THE SAME

FIELD OF THE INVENTION

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The present invention relates to an apparatus for separating a biological sample and a separating method of the same, specifically to a blood-separating apparatus for separating collected blood into blood cells and plasmas or serums, and the separating method of the collected blood.

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BACKGROUND OF THE INVENTION

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Generally in conventional laboratory examination, a person having a certain license or a specialized technician such as a doctor, a nurse, or a clinical laboratory technician collects a biological sample by means of for example blood sample collection, and then performs a predetermined test based on the collected biological sample.

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In manner of conventional biochemical examination however, it has been necessary for a subject to visit a hospital or a center for clinical examination where the licensed person or the specialized technician such as a doctor, a nurse, a clinical laboratory technician or the like is available, or conversely, such a licensed person or specialized technician may have to visit a place where the subject is located, in order to collect the biological sample. Thus, collecting biological samples has been very troublesome and caused an increase in the examination cost.

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Further, since raw blood changes quickly with time, fluctuation in accuracy of the examination has occurred. Accordingly, there has been a need to develop an examination apparatus capable of producing more accurate test result by

separating the blood immediately after the blood sample is collected.

In addition, although a method of collecting the biological sample by the subject by himself has been practiced in order to simplify the operation of collecting the biological sample, this method has been problematic in that it is
5 only effective in some examination items and not applicable to other examination items.

The present invention has been carried out in the light of the above-described conditions and it provides the apparatus for separating the biological sample and the separating method of the same, which are capable of simplifying
10 the collecting operation of the biological sample, improving the accuracy of the examination, and lowering the examination cost.

SUMMARY OF THE INVENTION

15 The present invention comprises a biological sample collecting container for receiving a collected biological sample, a filter for passing a predetermined component within the collected biological sample therethrough, a separated component container capable of being fitted into the biological sample collecting container, for receiving the predetermined
20 component that is passed through the filter, and a reverse-flow preventing member for preventing the predetermined component received in the separated component container from flowing backward into the biological sample collecting container through the filter, wherein once the biological sample is collected in the biological sample collecting container and the
25 separated component container is fitted into the biological sample collecting container, and after the predetermined component within the biological sample is passed through the filter, the reverse-flow preventing member closes a flow path between the separated component container

and the biological sample collecting container to prevent a reverse flow of the predetermined component, and the predetermined component of the biological sample is separately received in the separated component container.

5 Preferably, the present invention comprises a blood-collecting container for receiving collected blood, a filtration film for allowing plasmas or serums within the collected blood to pass through while blocking the passage of blood cells, a cylindrical body capable of being fitted into the blood-collecting container and of receiving the plasmas or serums that are passed through the
10 filtration film, and a sealing cap for preventing the plasmas or serums received in the cylindrical body from flowing backward into the blood-collecting container through the filtration film, wherein once the blood is collected in the blood-collecting container and the cylindrical body is fitted into the blood-collecting container, and after the plasmas or serums within the blood
15 are passed through the filtration film, then the sealing cap closes a flow path between the cylindrical body and the blood-collecting container to prevent a reverse flow of the plasmas or serums, and the blood cells and the plasmas or serums are separately received in the blood-collecting container and the cylindrical body respectively.

20 More preferably, a diluent solution is placed within the blood-collecting container.

 An diluent solution to be used for the present invention is not limited to special ones but may include, for example, a deionized water, a distilled water and a buffer solution, and preferably it may be the buffer solution.
25 A buffer to be used for the buffer solution may be any buffer so far as it has buffer capacity, and it may include, for example, Good buffer of pH 1 to 11, such as a lactic acid buffer, a citric acid buffer, an acetic acid buffer, a succinic acid buffer, a phthalic acid buffer, a phosphoric acid buffer, a

triethanolamine buffer, a diethanolamine buffer, a lysine buffer, a barbiturate buffer, tris (hydroxymethyl) aminomethane buffer, an imidazole buffer, a malic acid buffer, an oxalic acid buffer, a glycine buffer, a boric acid buffer, a carbonic acid buffer, a glycine buffer, 3-morpholino-propanoic acid (MOPS), 1,4-piperazinebis (ethanoic sulfonic acid) (PIPES), 2-[4-(2-hydroxyethyl)-1-piperadiny] ethanoic sulfonic acid (HEPES). A concentration of the buffer solution is not specifically limited to some values, but it may preferably within a range of 0.1 to 1000 mmol/L, more preferably 1 to 500 mmol/L.

Further, the buffer solution may, if desired, contain a surface active agent, an antiseptic agent or the like. The surface active agent may include, for example, a cationic surface active agent, an anionic surface active agent, an ampholytic surface active agent, or a nonionic surface active agent. The antiseptic agent may include, for example, a sodium azide, an antibiotic agent or the like.

When the whole blood is used as the biological sample, the diluent solution is preferably prepared to be an isotonic solution by using salts, sugars, buffer solution or the like which has no effect on a quantification of elements to be quantified in order to prevent a variation of the concentration of element in a blood serum possibly caused by an expansion or a contraction of a blood cell such as a red blood cell or the like.

As for the kind of salts, there is no specific limitation, but it may include alkali metal halide such as sodium chloride, potassium chloride or the like.

As for the kind of sugars, there is no specific limitation, but it may include sugar alcohol such as mannitol, sorbitol or the like. As for the kind of buffer solutions, those described above may be included therein.

An indicating material to be used for the present invention may be any

material so far as it is neither an element to be quantified in the biological sample nor an element contained in the biological sample, and preferably it may be the element which would have no effect on the element to be quantified in the biological sample. The indicating material may be, for
5 example, a pigment, a chromogen, a fluorescent material, a light emitting material or the like, and preferably a pigment or a chromogen. The pigment is preferable because the concentration thereof may be quantified directly by a colorimetric method.

The pigment may include, for example, an acid yellow 3, an acid yellow 23,
10 an acid yellow 25, an acid yellow 36, an acid orange 5, an acid orange 6, an acid orange 7, an acid orange 10, an acid orange 19, an acid orange 52, an acid green 16, an acid green 25, an acid violet 43, an acid blue 3, an acid blue 9 (a brilliant blue FCF), an acid blue 40, an acid blue 45, an acid blue 47, an acid blue 59, an acid blue 74, an acid blue 113, an acid blue
15 158, an acid red 1, an acid red 2, an acid red 14, an acid red 18, an acid red 27, an acid red 37, an acid red 51, an acid red 52, an acid red 87, an acid red 88, an acid red 92, an acid red 94, an acid red 95, an acid red 111, a food red 17, a food yellow 3, a basic yellow 1, a basic yellow 2, a basic yellow 11, a basic orange 1, a basic orange 22, a basic green 4 (a
20 malachite green), a basic violet 3, a basic violet 4, a basic violet 10, a basic blue 1, a basic blue 3, a basic blue 9, a basic blue 24, a basic red 1, a basic red 2, a basic red 5, a basic red 9, and a basic red 18.

As for a reductive coloring type chromogen, there may be included therein, for example, a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium
25 bromide (MTT), 2-(4-iodophenyl)-3-(nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1), 2-(4-iodophenyl)-3-(2,4-dinitrophenyl)-5-(2,4-disulfophenyl)-2H-tetra-zolium monosodium salt (WST-3) or the like.

As for an oxidative coloring type chromogen, there may be included therein, for example, a chromogen which is converted into a pigment by itself under a coexistence with an active material of peroxide such as hydrogen peroxide and peroxidase (hereafter, referred to as a leuco type chromogen), and another chromogen which generate a pigment through an oxidative coupling of two compounds (hereafter, referred to as a coupling type chromogen).

The leuco type chromogen may include, for example, 10-N-carboxymethyl carbamoyl - 3,7 -bis(dimethylamino)- 10H - phenothiazine (CCAP), 10 - N - methylcarbamoyl - 3,7 - bis(dimethylamino) - 10H -phenothiazine (MCDP), N-(carboxymethyl aminocarbonyl)-4,4'- bis (dimethylamino) diphenylamine sodium salt (DA-64), 4,4'- bis (dimethylamino) diphenylamine, bis[3-bis(4-chlorophenyl)methyl-4-dimethylaminophenyl]amine (BCMA) or the like.

The coupling type chromogen may include, for example, a combination of a coupler such as 4-aminoantipyrine (4-AA), 3-methyl- 2-benzothiazoline hydrazine or the like, and aniline such as N-ethyl- N-(3-methylphenyl)-N'succinyl ethylenediamine (EMSE), N-(3,5-dimethoxyphenyl)-N'succinyl ethylenediamine sodium salt (DOSE), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine, N-ethyl-N-sulfo-propyl-aniline, N-ethyl-N-sulfopropyl-3,5-dimethoxy-aniline, N-sulfopropyl-3,5-dimethoxy-aniline, N-ethyl-N-sulfopropyl-3,5-dimethyl-aniline, N-ethyl-N-sulfopropyl-m-toluidine, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-anisidine, N-ethyl-N-(2-hydroxy-3-sulfopropyl) aniline, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium salt 2 hydrate (TOOS), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (HSDA), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethylaniline, N-sulfopropyl-aniline, N-ethyl-N-sulfopropyl-anilinepropyl-

m-anisidine, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-4-fluoro-3,5-dimethoxy-aniline sodium salt (F-DAOS) or the like, or another combination of 4-AA and phenol or phenol such as 3-hydroxy-2,4,6-triiodo-acetic acid or the like.

- 5 The fluorescent material may include p-hydroxy phenyl-acetic acid, p-hydroxy phenyl-propionic acid, coumarin or the like.

The light emitting material may include a compound such as luminol, isoluminol, lucigenin, acridiniumester or the like.

- The biological sample to be used in the present invention may be any
10 sample including, for example, a whole blood, a blood plasma, a blood serum, a cerebrospinal fluid, a saliva, a urine, a sweat or the like, and it is preferable to use a whole blood, a blood plasma, or a blood serum therefor.

- Further, the biological sample may be taken from any organism, which is
15 not limited to a human being but may be an animal, a fish, a bird or the like.

- The animal may include a horse, a cattle, a pig, a wild bore, a sheep, a rabbit, a raccoon, a fox, a dog, a cat, a bear, a panda or the like, and the fish may include a conger, an ayu, a sardine, a char, an eel, a bonito, a sillaginoid, a salmon, a mackerel, a young yellowtail, a blowfish, a tuna or
20 the like, and the bird may include a chicken, a dove or the like.

- The sample for quantification used in the present invention is a solution composed of a specified volume of diluent solution containing a specified amount of indicating material and an unknown volume of biological sample. In this sample for quantification, said diluent solution containing a
25 specified amount of indicating material used to dissolve the unknown volume of biological sample thereinto will be hereafter referred to as a reference solution.

The sample for quantification may be prepared either by mixing an

unknown volume of biological sample collected without quantifying a volume thereof with a specified volume of diluent solution containing a specified amount of indicating material, or by adding a specified volume of diluent solution containing a specified amount of chromogen to a solution
5 made by mixing an unknown volume of biological sample and a specified volume of diluent solution. Accordingly, since there is no need to use a container for quantifying a volume of the biological sample, the sample for quantification can be easily prepared right on the spot of collection of the biological sample. Further, very small amount of biological sample may
10 be sufficient to prepare the sample for quantification.

The reference solution may be prepared either by mixing a specified amount of indicating material with a specified volume of diluent solution or by adding a specified volume of diluent solution containing a specified amount of chromogen to a specified volume of diluent solution.

15 The biological sample may be obtained by a normal method without using a specific one, that is, for example, the blood serum may be obtained from the whole blood by, after having left it for a while, applying a centrifugal processing thereto, and the blood plasma may be obtained by treating the whole blood with a separation processing such as a membrane separation.

20 In the present invention, a self-blood collection method where, for example, a test subject collects his/her blood in person by manipulating a blood collecting needle may be used preferably. In addition, since this operation may be performed without quantifying a volume thereof and accordingly no special technique is required for preparing the sample for
25 quantification, the test subject can prepare the sample for quantification by himself/herself. Further, the sample for quantification prepared by mixing the whole blood directly with a specified volume of diluent solution can be used as a sample for determining a concentration of the elements to be

quantified in the blood plasma or the blood serum from a value obtained by quantifying the elements to be quantified in the sample for quantification after, if necessary, having separated blood cell element by the separation processing such as a centrifugal separation and a membrane separation
5 and a dilution ratio determined based on a dilution ratio calculation method described below.

There is no special limitation on a manner for mixing the biological sample with the specified volume of diluent solution, but the sample obtained in the above-described method may be added directly or may be added
10 indirectly through a separation device installed in a container. The latter adding method may include, for example, a method where by using the blood separation instrument of the present invention, the blood plasma separated from the whole blood is added.

There is no special limitation on a range of the dilution ratio of the
15 indicating material in the sample for quantification, but it is preferably 2 to 10, more preferably 2 to 50, and most preferably 2 to 20.

It is to be noted that if the specified volume of diluent solution does not contain a specified amount of indicating material, after the biological sample having been mixed therewith, a specified volume of diluent solution
20 containing a specified amount of indicating material may be added thereto.

A process for quantifying the elements to be quantified in an unknown volume of biological sample comprises the steps of quantifying a concentration of the indicating material in a reference solution and a concentration of the indicating material in the sample for quantification to
25 determine the dilution ratio of the biological sample, and quantifying a concentration of the elements to be quantified in the sample for quantification.

The concentration (X) of the elements to be quantified in the biological

sample may be determined by an equation 1 as a function of the concentration (Y) of the elements to be quantified in the sample for quantification prepared by the above-described method and the dilution ratio (a) of the biological sample in the sample for quantification.

5 $X = a Y$ (eq. 1)

In the present invention, the dilution ratio can be determined as described below.

$$C2 = M1 / (V1+V2) \quad (\text{eq. 2})$$

where, C2 is the concentration of the indicating material in the sample for quantification, V1 is the volume of the diluent solution used for preparing
10 the sample for quantification, M1 is the amount of the indication material used therefor, and V2 is the volume of the biological sample used therefor (note: V2 is not measured).

On the other hand, the concentration C1 of the indicating material in the
15 diluent solution (= the reference solution) used for preparing the sample for quantification can be represented as:

$$C1 = M1 / V1 \quad (\text{eq. 3})$$

It is to be noted that in the method for preparing the sample for quantification from the biological sample, the reference solution is a
20 solution prepared without using the biological sample.

Since the dilution ratio (a) of the unknown volume of biological sample in the sample for quantification can be represented as:

$$a = (V1 + V2) / V2 \quad (\text{eq. 4})$$

the dilution ratio (a) may be rewritten by using C1 and C2 as shown in
25 equation 5.

$$\text{Dilution ratio (a)} = (V1 + V2) / V2 = C1 / (C1 - C2) \quad (\text{eq. 5})$$

Herein, the concentrations C1 and C2 of the indicating material can be determined by measuring an absorptivity when the indicating material is a

pigment or a chromogen, by measuring a light emitting intensity when the indicating material is a light emitting material, or by measuring a fluorescence intensity when the indicating material is a fluorescent material. When the indicating material is quantified by the absorptivity, since the concentration is in proportion to the absorptivity, they may be written as:

$$C2 / C1 = E2 / E1 \quad (\text{eq. 6})$$

where, C1 and E1 are the concentration and the absorptivity of the reference solution, and C2 and E2 are those of the sample for quantification, respectively. Accordingly, the dilution ratio can be determined also by an equation rewritten as:

$$\text{Dilution ratio (a)} = C1 / (C1 - C2) = E1 / (E1 - E2) \quad (\text{eq. 7})$$

As having been described above, the dilution ratio can be calculated based on the C1 and C2 values or the E1 and E2 values. It is to be noted that although the C1 or the E1 value may be set in advance to a known value, since they may be quantified by using the newly prepared solution, the amount of the indicating material in the reference solution may not be set in advance to a known value. That is, in the present invention, a volume of a solution to be mixed directly with the biological sample, and if this solution contains the indicating material, an amount or a concentration of the indicating material and a volume of the solution containing the indicating material and an amount or a concentration of the indicating material used for preparing the sample for quantification, each of them may not be known but arbitrary so far as it is constant.

The method for quantifying the indicating material may be any method so far as it can quantify the concentration of the indicating material. When the indicating material is a pigment, the absorptivity of the sample for quantification itself can be quantified. Further, in other cases, a specified

volume of sample is taken out of the sample for quantification and then the concentration thereof is quantified by a quantifying method for the indicating material to be quantified. Upon quantification, when the absorptivity is used therefor, the value of absorptivity can be used directly
5 without converting it to the concentration of the indicating material.

In the present invention, any one of a colorimetric method, a light emitting method and a fluorometric method may be used, and among them, the colorimetric method is most preferable.

The indicating material to be used in the colorimetric method may include,
10 for example, the above-described pigments and chromogens. The chromogens may include the reductive coloring type chromogen and the oxidative coloring type chromogen. The colorimetric method using the reductive coloring type chromogen may include a method in which the reductive coloring type chromogen is converted into the pigment by an
15 operation of an electron carrier of a reductive coenzyme such as NAD(P)H or the like, dihydrolipoamide dehydrogenase, 1-methoxy-5-methylphenaziummethylsulfate or the like and then the absorptivity of the generated pigment is measured by a spectrophotometer. The colorimetric method using the oxidative coloring type chromogen may include a
20 method in which the oxidative coloring type chromogen is converted into the pigment by an operation of active material of peroxide such as hydrogen peroxide, peroxidase or the like and then the absorptivity of the generated pigment is measured by the spectrophotometer. In the case where the chromogen is used, the method using the oxidative coloring type
25 chromogen is preferable.

When the chromogen is used as the indicating material, the chromogen is converted into the pigment by the method described below and then the absorptivity of the generated pigment is measured. In the case where the

reductive coloring type chromogen is used, the reductive coloring type chromogen is converted into the pigment by the operation of the electron carrier of the reductive coenzyme such as NAD(P)H or the like, dihydrolipoamide dehydrogenase, 1-methoxy-5-

- 5 methylphenaziummethylsulfate or the like and then the absorptivity of the generated pigment is measured. In the case where the oxidative coloring type chromogen is used, the oxidative coloring type chromogen is converted into the pigment by the operation of the active material of peroxide such as hydrogen peroxide, peroxidase or the like and then the
10 absorptivity of the generated pigment is measured.

The fluorometric method may include a method in which the fluorescence emitted from the above-described fluorescent material by the active material of peroxide such as hydrogen peroxide, peroxidase or the like is measured by a fluorophotometer.

- 15 The fluorometric method may further include another method in which the light (photon) emitted from the above-described fluorescent material by the active material of peroxide such as hydrogen peroxide, peroxidase or the like is measured by a luminometer.

It is to be noted that in the case where the coupling type chromogen is
20 used as the oxidative coloring type chromogen, either one of two compounds pertaining to the color development as the indicating material in the sample for quantification is contained and the other compound is preserved separately.

When the oxidative coloring type chromogen is used as the indicating
25 material, a mole-number of this oxidative coloring type chromogen should be controlled to be less than that of the hydrogen peroxide. When the coupling type chromogen is used as the indicating material, the mole-number of this chromogen should be controlled to be less than that of each

of the hydrogen peroxide and said the other compound.

The hydrogen peroxide to be used for converting the oxidative coloring type chromogen into the pigment may be hydrogen peroxide itself and it may be generated directly or indirectly from other materials by using
5 enzyme as well. A combination of the material and the enzyme for generating hydrogen peroxide directly or indirectly may include such ones as: cholesterol and cholesterol oxidase; uric acid and uricase; triglyceride and lipoprotein lipase & glycerol oxidase; free fatty acid and acyl-CoA synthetase & acyl-CoA oxidase; glucose and pyranose oxidase;
10 phospholipid and phospholipase D & choline oxidase; creatine and creatinase & sarcosine oxidase; creatinine and creatininase, creatinase & sarcosine oxidase; lactic acid and lactose oxidase; inorganic phosphorous and purine nucleotide phosphorylase & xanthine oxidase; 2,4-dimethoxybenzoyl-choline and cholinesterase & choline oxidase; allyllamine and
15 monoamine oxidase; and so on.

A reagent chemical for converting the oxidative coloring type chromogen as the indicating material in the sample for quantification into the pigment may be preserved as a single-reagent system or as a plural-reagent system. A preservation as the plural-reagent system is preferable and
20 that as two-reagent system is more preferable. In the case where hydrogen peroxide itself is used, the two-reagent system is preferable which avoids a coexistence of hydrogen peroxide and the active material of peroxide such as peroxidase. In the case where hydrogen peroxide is generated directly or indirectly from other materials by using enzyme, the
25 two-reagent system is preferable which avoids a coexistence of the enzyme to be reacted with the material directly and the material itself. Preferred embodiments of preserving manners of the reagent chemical for converting the oxidative coloring type chromogen into the pigment will be

described below. However, it is to be apprehended that the preserving manner is not limited to those embodiments below.

The chromogen in the sample for quantification: N-(2-hydroxy-3-sulfoethyl)-3,5-dimethoxyaniline sodium salt (HSDA)

- 5 A first reagent: A reagent prepared by subtracting HSDA from a first reagent of Determiner GL-E (reagent for measuring glucose: provided by Kyowa-medex Corp.) plus glucose.

A second reagent: A second reagent of Determiner GL-E

- There is no special limitation on the element to be quantified, but the
10 elements in the blood serum may be preferably included therein. Further, although the quantifying operation of the elements to be quantified is not limited to special manner but can be performed by a general method established as a quantifying method for the elements to be quantified, such a quantifying method is preferable that is not affected substantially by the
15 indicating material.

- The elements to be quantified and an example of the measuring method therefor will be described below with the latter indicated in parenthesis. Total protein (biuret method), GOT (JSCC method), GPT (JSCC method), L-lactate dehydro-genase (SSCC method), γ -GTP (JSCC method),
20 creatinine kinase (IFCC method), cholinesterase (p-HBC method), HDL cholesterol (enzyme method), LDL cholesterol (enzyme method), triglyceride (enzyme method), urea nitrogen (enzyme method), creatinine (enzyme method), uric acid (enzyme method), glucose (enzyme method), alkaline phosphatase (GSCC method), ammonium (enzyme method), sialic
25 acid (enzyme method), ceruloplasmin (colorimetric method), free cholesterol (enzyme method), free fatty acid (enzyme method), lactic acid (enzyme method), lipase (enzyme method), inorganic phosphorus (enzyme method), and monoamine oxidase (enzyme method).

A container equipped with a closable opening/closing device for adding the biological sample thereinto with a specified volume of above-described diluent solution precisely contained therein is used as the container for collecting and preserving the biological sample or for preparing the sample for quantification. Thus, the test subject can add a proper volume of biological sample such as his/her blood collected by himself/herself without any help of the specifically qualified person such as the doctor, the nurse and the clinical inspection engineer or the expert engineer, without any treatment, for example, as the whole blood and without quantifying the volume thereof one by one, to the container with the specified volume of diluent solution filled therein, and after having applied thereto a device for preventing a possible vaporization and leakage of the solution contained therein, send to the proper organization (inspection center or hospital) to ask said organization the quantification of the elements which he/she wants to be quantified.

Further, the present invention is characterized in that the blood-collecting container has the same outer diameter as a sample cup of a biochemical analyzer and has a screw part provided at an upper end part thereof, the filtration film is provided at a lower end part of the cylindrical body, the cylindrical body has a cap piston crowned thereon, the cap piston is provided with a grip part that can be threaded into the screw part and a stem part extending from the grip part into the cylindrical body, and the sealing cap is provided at a lower end of the stem part, and in that once the blood is collected in the blood-collecting container and the cylindrical body is fitted into the blood-collecting container followed by threading the grip part into the screw part, and after the plasmas or serums within the blood are passed through the filtration film, then the sealing cap closes the lower end part of the cylindrical body to prevent the reverse flow of the plasmas or

serums, and the plasmas or serums and the blood cells are separately received in the cylindrical body and the blood-collecting container respectively.

Furthermore, the present invention is characterized in that the
5 cylindrical body has an upper end part into which the cap piston can be fitted, and a main body part that can be separated from the upper end part and locked to the blood-collecting container, and in that once the grip part is threaded into the screw part of the blood-collecting container, the main
10 body part is locked to the blood-collecting container to restrain the movement of the main body part and to separate the upper end part from the main body part.

Further, the present invention is characterized in that the sealing cap is provided at a lower end of the stem part and at a lower end part of the cylindrical body such that the sealing cap can be detached from the lower
15 end of the stem part and fitted into the lower end part of the cylindrical body respectively, and once the mated condition between the grip part and the screw part of the blood-collecting container is released and the cap piston is pulled out, the sealing cap is broken off from the lower end of the stem part to maintain the sealed condition of the lower end part of the
20 cylindrical body.

The present invention comprises the steps of fitting a separated component container into a biological sample collecting container that has the biological sample collected, and receiving a predetermined component within the biological sample into the separated component container
25 through a filter, and after receiving the predetermined component into the separated component container, the steps of closing a flow path between the separated component container and the biological sample collecting container by a reverse-flow preventing member, preventing the reverse

flow of the predetermined component, and separately receiving the predetermined component into the separated component container.

Further, the present invention comprises the steps of fitting a cylindrical body into a blood-collecting container that has the blood collected, threading a grip part of a cap piston crowned on the cylindrical body into a screw part of the blood-collecting container, and receiving plasmas or serums within the blood into the cylindrical body through a filtration film, and after receiving the plasmas or serums into the cylindrical body, the steps of closing a flow path between the cylindrical body and the blood-collecting container by a sealing cap, preventing a reverse flow of the plasmas or serums, and separately receiving the plasmas or serums into the cylindrical body and blood cells into the blood-collecting container respectively.

According to the present invention described above, a simplified collecting operation of the biological sample is provided, which enables reduction of the examination cost. Also, since the biological sample can be completely separated, the temporal change of a specimen can be prevented to make the examination highly accurate.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 is a cross sectional view of a blood-separating apparatus according to an embodiment of the present invention;

Fig.2 is a side elevation view of a blood-collecting container according to the embodiment of the present invention;

Fig.3 is the cross sectional view of Fig.2 taken along the line A-A;

Fig.4 is the side elevation view of a cylindrical body according to the embodiment of the present invention;

Fig.5 is the cross sectional view of Fig.4 taken along the line B-B;

Fig.6 is the side elevation view of a cover according to the embodiment of the present invention;

Fig.7 is the cross sectional view of Fig.6 taken along the line C-C;

5 Fig.8 is the side elevation view of a cap piston according to the embodiment of the present invention;

Fig.9 is the cross sectional view of Fig.8 taken along the line D-D;

Fig.10 is the side elevation view of a sealing cap according to the present invention;

10 Fig.11 is the cross sectional view of Fig.10 taken along the line E-E;

Fig.12 is the cross sectional view illustrating the operation of the blood-separating apparatus according to the embodiment of the present invention; and

Fig.13 is the cross sectional view illustrating the operation of the blood-separating apparatus according to the embodiment of the present invention.

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DESCRIPTION OF THE PREFERRED EMBODIMENT

A biological sample separating apparatus and its separating method according to an embodiment of the present invention will now be described in
20 detail with reference to the drawings.

Figs.1-13 illustrate a case of using blood as a biological sample. A blood-separating apparatus 1 in this case comprises a blood-collecting container 2, a cylindrical body 3 capable of being fitted into the blood-collecting container 2, a cap piston 4 capable of being crowned on the cylinder body 3, and a sealing cap
25 5 provided at the lower end of the cap piston 4, wherein the upper end opening of the blood-collecting container 2 is sealed by a cap 6 via a packing 7 before use as shown in Fig.1.

As best shown in Fig.2 and Fig.3, the blood-collecting container 2 is made

of transparent material and of a cylindrical shape, wherein the upper end part of the blood-collecting container 2 has a screw part 8 on the outer surface thereof and a locking part 9 protrudingly formed on the inner surface thereof. Also, at the lower end part of the blood-collecting container 2 is formed a bottom 10 of the shape of an inverted cone and around the bottom 10 is formed a leg 11 of the shape of a cylinder. The leg 11 has the same outer diameter as a sample cup to be used in blood analysis and examination, and preferably has slits 12 formed vertically in the lower end of the leg at opposing positions respectively. Further, a predetermined amount of diluent solution 13, for example 500 mm³, is placed beforehand within the blood-collecting container 2 as shown in Fig.1.

As best shown in Fig.4 and Fig.5, the cylinder body 3 is made of the transparent material and of the cylindrical shape, wherein a diameter-extended part 14 is formed at the upper end part thereof. The diameter-extended part 14 is connected to a main body part 16 through a thin walled part 15, and stopper parts 17 are protrudingly formed vertically in the middle portion of the main body part 16. Also, a diameter-contracted part 18 is formed at the lower end part of the cylinder body 3, and the inner surface of the diameter-contracted part 18 is formed a protruded locking part 19. Further, the diameter-contracted part 18 has an outer flange 20 formed at the lower part thereof, and a lower end opening of the outer flange 20 is covered with a filtration film 21, which is adapted to allow plasmas or serums within the blood to pass through it, but block blood cells.

A cover 22, which is made of silicon rubber, is attached to the outer periphery of the diameter-contracted part 18 (see Fig.1). As best shown in Fig.6 and Fig.7, the cover 22 has a protruded part 23 formed on the outer periphery of the upper end part thereof and the lower end part 24 thereof has a gradually increasing diameter toward the end, so that the outer diameters of the protruded part 23 and the lower end part 24 are slightly larger than the outer diameter of the main body part 16. Further, the cover 22 has a peripheral groove 25 formed

in the inner surface thereof, and the outer flange 20 is fitted into the peripheral groove 25 so as to prevent the cover 22 from slipping off from the diameter-contracted part 18.

5 The cap piston 4, as best shown in Fig.8 and Fig.9, is comprised of a generally cylindrical grip part 26, and a stem part 27 extending downward concentrically with the grip part 26. The grip part 26 has a cylindrical space 28 formed in the inner upper end part thereof, into which the diameter-extended part 14 of the cylindrical body 3 can be fitted, and threads are formed in the part below the space so that the screw part 8 can be threaded into that part. The
10 stem part 27 has a lower end 29 formed into a pin shape, to which the sealing cap 5 is detachably attached (see Fig.1). The sealing cap 5 is made of the silicon rubber and has a generally cylindrical form with a lower part formed into an outer flange, thus creating a step part 31 on the outer periphery of the lower part of the sealing cap, as best shown in Fig.10 and Fig.11. Further, in the sealing cap 5
15 formed a groove 32 into which the lower end 29 of the stem part 27 can be detachably fitted.

The method of separating the blood according to the embodiment of the present invention will be now described with reference to Fig.1 and Figs.12-13.

20 After removing the cap 6 and the packing 7 from the blood-collecting container 2, the subject stings a blood-collecting needle on his finger or the like to collect a small amount, for example 1-2 drops of blood into the blood-collecting container 2. The collected blood is gradually separated into blood cells and plasmas or serums within the diluent solution 13, and the blood cells begin to clot and deposit in the diluent solution 13 while the plasmas or serums begin
25 to float within the diluent solution 13. In this condition, the cylindrical body 3 with the cap piston 4 crowned thereon is fitted into the blood-collecting container 2 while the screw part 8 is threaded into the grip part 26 as shown in Fig.12. At first, the cylindrical body 3 rotates with the grip part 26, but once the locking part 9 is

locked on the stopper part 17, the rotation of the cylindrical body 3 is restrained and the thin walled part 15 is twisted to rupture, resulting in separation of the cylindrical body 3 into the main body part 16 and the diameter-extended part 14. Then, when the grip part 26 is further rotated, the upper end part 33 of the main
5 body part 16 intrudes the space 28 into the inside of the diameter-extended part 14 and becomes to be pressed downward by the inner surface of a top 34 of the grip part 26, thus lowering the cylindrical body 3 further. As the cylindrical body 3 is lowered, the plasmas or serums suspended within the diluent solution 13 pass through the filtration film 21 to move into the cylindrical body 3, while the blood
10 cells cannot pass through the filtration film 21 and remain within the blood-collecting container 2. At this point of time, since the outer diameters of the protruded part 23 and the lower end part 24 of the cover 22 are greater than the outer diameter of the main body part 16 of the cylindrical body 3, the cylindrical body 3 is lowered with the inner surface of the blood-collecting container 2 being
15 closely contacted therewith. Accordingly, during the process of fitting the cylindrical body 3 into the blood-collecting container 2, there is no possibility of leaking the blood or the diluent solution 13 from the blood-collecting container 2 to the outside thereof through a gap between the blood-collecting container 2 and the cylindrical body 3. Then, when the grip part 26 is threaded into the screw
20 part 8 to its lowest part, the sealing cap 5 is mated with the diameter-contracted part 18 to seal the flow path between the blood-collecting container 2 and the cylindrical body 3, thereby maintaining assuredly the separated condition between the blood cells and the plasmas or serums.

The blood-separating apparatus 1 is then transported with keeping this
25 condition to an examination site where the grip part 26 is detached by rotating it. At this moment, the step part 31 of the sealing cap 5 is locked on the protruded locking part 19 and the sealing cap 5 is separated from the lower end 29 of the stem part 27 to remain within the diameter-contracted part 18, so that only the

grip part 26 and the stem part 27 are removed thereby creating no possibility of mixing the blood cells with the plasmas or serums. After that, the cylindrical body 3 is taken out from the blood-collecting container 2, and the blood cells in the blood-collecting container 2 and the plasmas or serums in the cylindrical body 3 respectively are analyzed using analytical instruments to conduct a predetermined examination. At this moment, since the blood-collecting container 2 has the same outer diameter as that of the sample cup, the blood-collecting container 2 can be placed directly in the analytical instrument without transferring the blood cells from the blood-collecting container into the sample cup, which provides for improvement in the operating efficiency and minimization of the amount of the blood to collect.

As such, the present invention can separate the blood into the blood cells and the plasmas or serums in situ immediately after the blood has been collected and transport it to the examination site with keeping the separated condition unchanged, which therefore prevents hemolysis, clotting and the like of the blood during transportation. Accordingly, the blood will be well preserved and improvement of the examination accuracy can be provided. In addition, since the blood separation does not use a centrifugal separator, only a minute amount of the collected blood may be required, and yet all the same examination items to those necessary for the usual examination can be conducted.

It is noted that, although in the above embodiment the cap piston 4 is adapted to be screwed into the blood collection container 2, the connection method is not limited to the use of threaded component and may include other methods such as forming a taper, as long as they provide a detachable connection capable of keeping its air-tightness.

Further, although in the above embodiment the implementation of the invention for the case of self blood-collection has been described where the subject collects his blood by himself, it will be obvious that the present invention

can be implemented for a general blood collection where a licensed person such as a doctor collects the blood by using an injection syringe.

Furthermore, although in the above embodiment the case of using the blood as a biological sample has been described, the present invention can be
5 implemented for the biological samples other than the blood, such as urine, feces, pleural effusion, ascites, saliva, and the like.

According to the present invention as described above, a simplified collecting operation of the biological samples is provided, which enables reduction of the examination cost. Furthermore, since the biological sample can
10 be completely separated, various superior effects may be demonstrated, including for example that the temporal change of a specimen can be prevented to make the examination highly accurate.

WHAT IS CLAIMED IS:

1. An apparatus for separating a biological sample, comprising:

5 a biological sample collecting means for receiving a collected biological sample;

a filtering means for passing a predetermined component within said collected biological sample therethrough;

10 a separated component containing means capable of being fitted into said biological sample collecting means, for receiving said predetermined component that is passed through said filtering means; and

a reverse-flow preventing means for preventing said predetermined component received in said separated component containing means from flowing backward into said biological sample collecting means through said filtering means;

15 wherein once the biological sample is collected in said biological sample collecting means and said separated component containing means is fitted into said biological sample collecting means, and after the predetermined component within said biological sample is passed through said filtering means, then said reverse-flow preventing means closes a
20 flow path between said separated component containing means and said biological sample collecting means to prevent a reverse flow of said predetermined component, and the predetermined component of said biological sample is separately received in said separated component containing means.

25

2. An apparatus for separating a biological sample, comprising:

a blood-collecting container for receiving collected blood;

a filtration film for allowing plasmas or serums within the collected blood

to pass through while blocking the passage of blood cells;

a cylindrical body capable of being fitted into said blood-collecting container and of receiving the plasmas or serums that are passed through said filtration film; and

5 a sealing cap for preventing the plasmas or serums received in the cylindrical body from flowing backward into said blood-collecting container through said filtration film;

wherein once the blood is collected in said blood-collecting container and said cylindrical body is fitted into said blood-collecting container, and
10 after the plasmas or serums within said blood are passed through said filtration film, then said sealing cap closes a flow path between said cylindrical body and said blood-collecting container to prevent a reverse flow of the plasmas or serums, and the blood cells and the plasmas or serums are separately received in said blood-collecting container and said
15 cylindrical body respectively.

3. The apparatus for separating a biological sample according to claim 2, wherein a diluent solution is placed within said blood-collecting container.

20 4. The apparatus for separating a biological sample according to claim 2, wherein said blood-collecting container has the same outer diameter as a sample cup and has a screw part provided at an upper end part of said container, said filtration film is provided at a lower end part of said cylindrical body, said cylindrical body has a cap piston crowned thereon, said cap piston is provided
25 with a grip part that can be threaded into said screw part and a stem part extending from said grip part into said cylindrical body, and said sealing cap is provided at a lower end of said stem part; and

wherein once the blood is collected in said blood-collecting container

and said cylindrical body is fitted into said blood-collecting container followed by threading said grip part into said screw part, and after the plasmas or serums within said blood are passed through said filtration film, then said sealing cap closes the lower end part of said cylindrical body to
5 prevent the reverse flow of said plasmas or serums, and the plasmas or serums and the blood cells are separately received in said cylindrical body and said blood-collecting container respectively.

5. The apparatus for separating a biological sample according to claim 4,
10 wherein said cylindrical body has an upper end part into which said cap piston can be fitted, and a main body part that can be separated from said upper end part and locked to said blood-collecting container; and

wherein once said grip part is threaded into the screw part of said blood-collecting container, said main body part is locked to said blood-
15 collecting container to restrain the movement of said main body part and to separate said upper end part from said main body part.

6. The apparatus for separating for a biological sample according to claim 5, wherein said sealing cap is provided at a lower end of said stem
20 part and at a lower end part of said cylindrical body such that said sealing cap can be detached from said lower end of said stem part and fitted into said lower end part of said cylindrical body respectively, and once the mated condition between said grip part and the screw part of said blood-collecting container is released and said cap piston is pulled out, said
25 sealing cap is broken off from the lower end of said stem part to maintain the sealed condition of the lower end part of said cylindrical body.

7. A method of separating a biological sample, comprising the steps of:

fitting a separated component containing means into a biological sample collecting means that has the biological sample collected, and receiving a predetermined component within said biological sample into said separated component containing means through a filtering means;

5 and

after receiving said predetermined component into said separated component containing means, closing a flow path between said separated component containing means and said biological sample collecting means by a reverse-flow preventing means, preventing the reverse flow of said predetermined component, and separately receiving the predetermined component into said separated component containing means.

10

8. A method of separating a biological sample, comprising the steps of:

fitting a cylindrical body into a blood-collecting container that has the blood collected, threading a grip part of a cap piston crowned on said cylindrical body into a screw part of said blood-collecting container, and receiving plasmas or serums within said blood into said cylindrical body through a filtration film; and

15

after receiving said plasmas or serums into said cylindrical body, closing a flow path between said cylindrical body and said blood-collecting container by a sealing cap, preventing a reverse flow of said plasmas or serums, and separately receiving the plasmas or serums into said cylindrical body and blood cells into said blood-collecting container respectively.

20

9. A method for preparing a sample for quantification from a biological sample, which is to be used for quantifying elements to be quantified in said biological sample, said method characterized in comprising a step of mixing an unknown volume of biological sample collected without quantifying a volume thereof with a specified volume of diluent solution.

25

10. A method in accordance with claim 9, in which said specified volume of diluent solution contains a specified amount of indicating material.

5 11. A method in accordance with claim 9, characterized in further comprising a step of adding a specified volume of diluent solution containing a specified amount of indicating material.

12. A method in accordance with claim 9, in which said indicating material
10 is a pigment or a chromogen.

13. A method in accordance with claim 12, in which said chromogen is an oxidative coloring type chromogen.

15 14. A method in accordance with claim 9, in which said biological sample is either one of a whole blood, a blood plasma or a blood serum.

15. A method in accordance with claim 9, in which said diluent solution is a buffer solution.

20

16. A method in accordance with claim 9, in which said elements to be quantified are elements in the blood serum.

17. A method for quantifying elements to be quantified in a biological
25 sample, said method characterized in using a sample for quantification prepared by a preparing method comprising a step of mixing an unknown volume of biological sample collected without quantifying a volume thereof with a specified volume of diluent solution containing a specified amount of

indicating material.

18. A method in accordance with claim 17, in which said unknown volume
of biological sample collected without quantifying a volume thereof is
5 mixed with a specified volume of diluent solution.

19. A method in accordance with claim 17, said method characterized in
further comprising the steps of:

determining a dilution ratio of said biological sample in said sample for
10 quantification; and
quantifying a concentration of elements to be quantified in said sample for
quantification.

20. A method in accordance with claim 17, in which said indicating material
15 is a pigment or a chromogen.

21. A method in accordance with claim 20, in which said chromogen is an
oxidative coloring type chromogen.

20 22. A method in accordance with claim 17, in which said biological sample
is either one of a whole blood, a blood plasma or a blood serum.

23. A method in accordance with claim 17, in which said diluent solution is
a buffer solution.

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24. A method in accordance with claim 17, in which said elements to be
quantified are elements in the blood serum.

25. A container for preserving biological sample used for preserving an unknown volume of biological sample containing elements to be quantified, which has been collected without quantifying a volume thereof, until it will be quantified, said container being filled with a specified volume of diluent solution, and said container having a closable opening/closing means for adding said biological sample.

26. A container for preparing a sample for quantification used for preparing a sample for quantification from an unknown volume of biological sample containing elements to be quantified, which has been collected without quantifying a volume thereof, said container being filled with a specified volume of diluent solution, and said container having a closable opening/closing means for adding said biological sample.

27. A container in accordance with claim 25, in which said specified volume of diluent solution is a solution containing a specified amount of indicating material.

28. A container in accordance with claim 27, in which said indicating material is a pigment or a chromogen.

29. A container in accordance with claim 28, in which said chromogen is an oxidative coloring type chromogen.

30. A container for collecting a biological sample in accordance with either of claim 25, in which said biological sample is either one of a whole blood, a blood plasma or a blood serum.

31. A container for collecting a biological sample in accordance with either of claim 25, in which said elements to be quantified are elements in the blood serum.

5 32. A method for quantifying elements to be quantified in a biological sample, said method comprising the steps of:

1) preparing a sample for quantification composed of an unknown volume of biological sample containing elements to be quantified, which has been collected without quantifying a volume thereof, and a specified volume of
10 diluent solution containing a specified amount of indicating material;

2) determining a dilution ratio (a) of said biological sample from a concentration (C1) of the indicating material in said specified volume of diluent solution containing said specified amount of indicating material and a concentration (C2) of the indicating material in said sample for
15 quantification;

3) determining a concentration (Y) of the elements to be quantified in said sample for quantification; and

4) determining the elements to be quantified in the biological sample based on the dilution ratio (a) of the biological sample determined in the step 2) and said concentration (Y) of the elements to be quantified in the sample
20 for quantification determined in the step 3).

33. A method in accordance with claim 32, in which said diluent solution is a buffer solution.

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34. A method in accordance with claim 32, in which said indicating material is a pigment or a chromogen.

35. A method in accordance with claim 34, in which said chromogen is an oxidative coloring type chromogen.

36. A method in accordance with claim 34, in which an absorptivity (E1) of
5 said specified volume of diluent solution containing said specified amount of indicating material and an absorptivity (E2) of said sample for quantification are used in substitution for C1 and C2 respectively.