

Method and unit for preparing sample for microbiological analysis of liquid

The present invention concerns the preparation of a sample for the microbiological analysis of a liquid.

5 It is known that there are liquids which may contain different types of microorganisms, among which the microbiological analysis must only concern one or several types.

These liquids exist for example at the end of a manufacturing chain for monoclonal antibodies. More particularly, such liquids containing eukaryotes,
10 which enable antibodies to be produced, may possibly be contaminated with bacteria and/or viruses.

The presence of eukaryote cells may hinder the microbiological analysis of bacteria and viruses either due to released toxins which may adversely affect the growth of microorganisms in the case of conventional detection on a gel
15 growth medium (detection of false negatives) or due to perturbed reading of results in the case of fast detection (detection of false positives) by luminescence, fluorescence, or amplification or hybridization or by any other analytical method for nucleic acids of those microorganisms.

In order to carry out a reliable analysis of this type of liquid, it is thus
20 appropriate to prepare a sample in which selection has been made from only microorganisms belonging to the types of microorganisms desired to be detected (i.e., bacteria and/or viruses in the example given above).

Methods are already known for preparing a sample for the analysis of a liquid in which the microorganisms to be detected are selected by exploiting the
25 differences in morphological characteristics (physical and/or chemical) from among the different types of microorganisms.

More particularly, a method of preparation is already known, the method comprising a step of selection consisting of adding into the liquid to be analyzed a reagent constituted in such a manner as to specifically lyse microorganisms
30 belonging to the types of microorganisms which are not desired to be kept in the sample and filtering the liquid by a membrane so as to collect on that membrane a sample containing solely the microorganisms to be detected, which have not reacted specifically due to lysate materials (those having been destroyed, lysed

and passed through the membrane). The sample so collected is then recovered to be analyzed with conventional or fast microbiological analysis techniques.

Another solution for forming a sample includes a step of separating microorganisms from the others based on the differences in mass by centrifuging
5 at low speed.

The invention concerns preparing a sample by a similar method, which at the same time has improved performance and is simpler and more convenient for implementation.

To that end, the invention provides a method of preparing a sample for
10 the microbiological analysis of a liquid which may contain microorganisms of several different types each having predetermined morphological characteristics; this method comprises a step of making a selection of microorganisms from said different types of microorganisms, wherein said step of making a selection comprises:

- 15 - a step of procuring a unit for preparing said sample, the unit comprising a generally tubular body within which a first membrane and a second membrane are fixed, said body having an opening for introduction of said liquid, a first compartment between said introduction opening and said first membrane, a second compartment between said first and second membranes and a third
20 compartment on the opposite side of said second membrane from said first membrane, said first membrane having a predetermined first pore diameter and said second membrane having a predetermined second pore diameter smaller than said predetermined first pore diameter of said first membrane;
- a step of passing a predetermined volume of said liquid from the first
25 compartment to the second compartment through said first membrane;
- a step of passing all the filtrate, having so reached the second compartment, from the second compartment to the third compartment through said second membrane in order to collect said sample on said second membrane, the sample containing microorganisms selected from said different types of
30 microorganisms and having the size smaller than the predetermined pore diameter of said first membrane and greater than the predetermined pore diameter of said second membrane; and
- a step of recovering said sample so collected on said second membrane.

In the method according to the invention, a sample containing microorganisms to be analyzed (bacteria and/or viruses for example) is collected on the second membrane after they were separated from the undesired microorganisms (for example eukaryote cells), which have too great a size to pass
5 through the first membrane.

Such a selection of microorganisms on the basis of their differences in size is particularly effective, often more so than in the aforementioned methods in the prior art.

This is because in the case of centrifuging, a portion of microorganisms
10 to be collected is captured by centrifugal force together with microorganisms to be eliminated; and in the case of chemical lysis, the lysing agents are never perfectly selective such that a small but non-zero proportion of microorganisms to be collected undergoes lysis and is thus not collected on the membrane, whereas in the method according to the invention (selection by size), such marginal effects
15 are negligible, or even non-existent.

According to preferred features, for reasons of simplicity and convenience for both production and use:

- the step of procuring a preparation unit comprises a step of selecting a preparation unit in which the body thereof comprises a first portion to which said
20 first member is fixed and a second portion to which said second member is fixed, said portions being detachable from each other, and the step of recovering said sample comprises a step of detaching said second portion of said body from said first portion of said body; and/or

- the step of procuring a preparation unit comprises a step of selecting
25 a preparation unit in which the body thereof has an auxiliary opening whose stopper can be removed, the opening communicating with said second compartment, and the step of recovering said sample comprises a step of removing said opening, a step of depositing an agent for lysing said microorganisms selected on said second membrane through said auxiliary
30 opening, and a step of passing the lysate so formed from the second compartment to the third compartment through said second membrane.

It is thus possible, either by virtue of the body formed of two detachable portions, or by virtue of an aperture provided in that body, (for an operator) to have

access to the second membrane and more particularly to the surface of the second membrane on which the sample has been collected.

According to other preferred features, for the same reasons as those stated above:

- 5 - the steps of passing said liquid through said membranes are performed by placing said third compartment under reduced pressure;
- the steps of passing said liquid through said membranes are performed by placing said first compartment under increased pressure;
- the steps of passing said liquid through said membranes are performed by centrifuging said preparation unit;
- 10 - the step of procuring a preparation unit comprises a step of selecting a membrane of pore diameter greater than 1 μm as said first membrane;
- the step of procuring a preparation unit comprises a step of selecting a membrane of pore diameter smaller than 0.65 μm as said second membrane;
- 15 and/or
- the step of procuring a preparation unit comprises a step of selecting a membrane of pore diameter less than 0.1 μm as said second membrane;

According to a second aspect, the invention also concern a unit for preparing a sample suitable for the implementing the method as set forth above,

20 the unit characterized in that it comprises a generally tubular body within which a first membrane and a second membrane are fixed, said body having an opening for introduction of said liquid, a first compartment between said introduction opening and said first membrane, a second compartment between said first and second membranes and a third compartment on the opposite side of said second

25 membrane from said first membrane, said first membrane having a predetermined first pore diameter and said second membrane having a predetermined second pore diameter smaller than said predetermined first pore diameter of said first membrane, said unit being constituted in such a manner as to pass a predetermined volume of said liquid from the first compartment to the second

30 compartment through said first membrane and then to pass all the filtrate, having so reached the second compartment, from the second compartment to the third compartment through said second membrane in order to collect said sample on said second membrane; said body also comprising a means of recovering said sample so collected on said second membrane.

The preparation unit is thus designed to make it possible to pass the entirety of a predetermined volume through the two membranes (of course with the exception of portions retained by the membranes), and furthermore, (for an operator) to have access to a sample by virtue of a means of recovering the sample.

It will be noted that a simple filtering unit with several stages (for example a solution clarification unit) would not be able to serve as a preparation unit according to the invention and that since it is not constituted of membranes, such a filtering unit would not be constituted in such a manner as to pass all the filtrate arriving at an intermediate compartment through the second stage (a residual filtrate would always remain) and/or would lack a means of recovering a sample.

According to preferred features, for reasons of simplicity and convenience for both production and use, said first membrane is permeable to air when it is wet.

In this manner, when the liquid has been entirely emptied from the first compartment and the first membrane comes into contact with air on the surface facing that compartment, that membrane, by virtue of the sufficient size of its pores, remains permeable to air (no bubble point phenomenon) such that air may penetrate through that membrane into the second compartment to enable the liquid which it contains to be discharged.

According to other preferred features, for the same reasons as those stated above:

- the pore diameter of said first membrane is greater than 1 μm ;
- the pore diameter of said second membrane is smaller than 0.65 μm ;
- the pore diameter of said second membrane is smaller than 0.1 μm ;
- said body comprises a first portion to which said first membrane is fixed and a second portion to which said second membrane is fixed, and said recovering means is a means of separating said second portion from said first portion;
- said recovering means comprises an opening that may be formed in said body and communicating with said second compartment (i.e., the opening is not blocked);
- said body comprises a cup defining said third compartment; and/or

- said cup is detachable.

The features and advantages of the invention will become apparent from the following description, based on preferred but non-limiting examples, with reference to the accompanying drawings.

5 - Figure 1 is a cross-section view of a preparation unit according to the invention.

 - Figure 2 is a view of the unit similar to that in Figure 1, wherein two portions of a body fitted together have been separated.

10 - Figure 3 is a view similar to Figure 1 of a second embodiment of the preparation unit.

 - Figure 4 is a view similar to Figure 1 of a third embodiment of the preparation unit.

15 The preparation unit 1 illustrated in Figure 1 comprises a generally tubular body 2 within which a membrane 3, a membrane 4 and two porous supports 5 and 6 are fixed.

 The membranes 3 and 4 are of polyvinylidene fluoride (PVDF), the membrane 3 having pore diameter equal to 5 μm and the membrane 4 having a pore diameter equal to 0.4 μm .

20 The body 2 is formed of a portion 9 and a portion 10, wherein one nested in the other. The portion 9 has an opening 7 for introducing liquid while the portion 10 has an opening 8 for the outlet of that liquid.

25 The portion 9 has a first cylindrical part 15 defining the opening 7 on one side and on the opposite side joining to a second part 16 which is also cylindrical and has a diameter smaller than that of the part 15, the parts 15 and 16 being connected to each other by a transverse annular wall 17.

 Adjacent to the transverse wall 17, the porous support 5 is fixed and the membrane 3 sealed the membrane 3 contacting the porous support 5 through the face 12 from the same side as the opening 7 (Figure 1).

30 The portion 10 comprises a first cylindrical part 20 having a diameter substantially equal to the diameter of the cylindrical part 15, a second cylindrical part 21 having a diameter substantially equal to the diameter of the cylindrical part 16, and a third cylindrical part 22 having a diameter smaller than the diameter of part 21, the third cylindrical part 22 defining the opening 8.

The parts 20 and 21 are connected to each other by a transverse annular wall 23 whereas the parts 21 and 22 are connected to each other by a transverse annular wall 24.

5 Adjacent to the annular wall 23, the porous support 6 is fixed and the membrane 4 sealed, the membrane 4 contacting the porous support 6 through the face 14, and the face 13 of that membrane facing the porous support 5 (Figure 1).

The portion 9 with membrane 3 defines a compartment 26 for introducing liquid to be filtered, the portions 9 and 10 in the nested state defining an intermediate compartment 27 together with membranes 3 and 4 whereas the
10 portion 10 with membrane 4 defines a compartment 28 for discharging the liquid.

In the state where the portion 9 is nested in the portion 10 of the body 2, the outward surface of the cylindrical part 16 faces the inner surface of the cylindrical part 20 while they come into contact with each other by elastic deformation so as to maintain portions 9 and 10 to be fastened together.

15 A description will now be given to different steps of preparing a sample from a liquid to be analyzed, which may contain eukaryote cells and bacteria whose presence is desired to be detected among cells.

At a first step, the operator connects the cylindrical part 22 to a vacuum source (a vacuum pump for example), places compartment 28 under reduced
20 pressure and delivers a predetermined volume (for example 10 ml) of liquid into compartment 26.

Placing compartment 28 under reduced pressure leads to placing the compartment 27 under reduced pressure so that a predetermined volume of liquid can be suctioned and passed through the membrane 3 and the support 5 to
25 occupy the compartment 27.

The pores of membrane 3 are dimensioned such that only the microorganisms of the greatest size, which are eukaryote cells here, are retained by that membrane, whereas the other microorganisms, which are bacteria here, pass through it and the porous support 5 to arrive at a filtrate occupying the
30 compartment 27.

Furthermore, the large pore size of the membrane 3 mean that even when that membrane is wet, it remains permeable to air (absence of bubble point phenomenon) such that air can penetrate into compartment 27 through the membrane 3.

As this membrane allows air to pass, a filtrate contained in the compartment 27 flows through the membrane 4 and the support 6 to occupy the compartment 28 and then leave the body 2 through the outlet opening 8.

The pores of the membrane 4 are dimensioned so as to retain bacteria
5 which are desired to be detected.

Once these operations have been carried out, the operator disconnects the vacuum source from the cylindrical part 22.

Next, the operator separates the portions 9 from the portion 10 by sliding the portion 9 with respect to the portion 10 such that they disengage from
10 each other as illustrated in Figure 2.

The membrane 4 and in particular the face 13 of that membrane on which a sample has been collected is thus rendered accessible.

It is then possible to take out that membrane, bring it into contact with a gel growth medium and then place that assembly in an incubator to incubate
15 bacteria.

Another solution includes a step of treating that membrane (after having or not having taken it out of the portion 10) by depositing thereon from the face 13 side a reagent capable of revealing the presence of the ATP of bacteria by luminescence; or another possibility is to use a reagent capable of making it
20 possible to identify bacteria by fluorescence.

Still another solution includes a step of analyzing the DNA of bacteria collected on the membrane 4 by a conventional method (a method of PCR type, for example).

It is also possible, if it is desired to recover a sample independently of
25 the membrane, to deposit on that membrane an agent for lysing bacteria.

By again connecting the unit to a vacuum source, the lysate containing the biological materials of bacteria passes through the membrane 4 and then the compartment 28 to be collected in a container disposed beyond the opening 8. The liquid recovered in this container may then be analyzed by a fast
30 microbiological detection method (by luminescence for example).

The combined use of the unit according to the invention with a fast detection method for microorganisms makes it possible to verify in short periods of time and on a large scale all kinds of liquid samples that may contain several types of microorganisms.

Another embodiment of the preparation unit is shown in Figure 3.

Generally, for similar members, the same reference numerals added with 100 will be used.

A preparation unit 101 is configured in such a manner as to be placed in
 5 a centrifuge so as to make liquid pass through the two membranes without having to place a preparation unit under reduced pressure.

In this embodiment, the necessity of the annular part 24 and the cylindrical part 22 have been eliminated whereas a cylindrical part 121 is extended with respect to the part 21 in the preceding embodiment such that it is possible to
 10 fit it into a cup 130 belonging to a body 102 to take out a second filtrate when liquid comes out of an opening 108 or to recover a lysate in that cup after the second filtrate was emptied (by depositing a lysing agent on that membrane) when it is desired to recover a sample independently of the membrane 4.

Thus the cup is configured in such a manner as to be separated from
 15 portion 110 (by sliding along cylindrical part 121) either to empty the second filtrate or to obtain access to the lysate in order to perform a fast microbiological detection on that lysate by adding into the cup a reagent capable of revealing the presence of ATP by luminescence, for example.

Another embodiment is shown in Figure 4.

20 In this embodiment, a preparation unit 201 has a structure similar to that of the preparation unit 101 except for an opening 232 blocked in a fluid-tight manner by a removable stopper 233, which is provided laterally in a cylindrical part 220 of a portion 210 of a body 202.

Once a sample has been prepared and the stopper 233 removed, this
 25 opening makes it possible, if needed, (for an operator) to obtain access to the compartment 227 and in particular to the face 13 of the membrane 3 to deposit a lysing agent there, for example, without having to separate a portion 209 from the portion 210 of the body 202, and then the biological materials of microorganisms may be recovered in a cup 230.

30 In one variation, the pore diameter of the membrane 4 is smaller than $0.1\ \mu\text{m}$ so as to collect bacteria and/or viruses on the membrane.

In still another variation, liquid is passed through the membranes 3 and 4 by placing an admission chamber 26 under increased pressure using a piston engaged in the cylindrical part 15 by keeping a certain volume of air between the

piston and the liquid, for example. When it is actuated, the piston causes the displacement of the volume of liquid as well as the volume of air compressed between the piston and the liquid with the result that the compartment 26 is placed under increased pressure. The liquid therefore penetrates into the compartment 27
5 and is then flushed by air pushed by the piston, and then air in turn penetrates into the compartment 27 through the membrane 3 which remains permeable to air.

In a still further variation, microorganisms to be detected are not bacteria or viruses but yeasts (of sufficiently small size not to be retained by the first membrane) or molds, for example.

10 In yet another variation, the liquid to be analyzed does not contain eukaryotes but other types of microorganisms (which should be separated from microorganisms to be detected) such as yeasts and filamentous fungi.

In again another variation, the two portions of the body of the preparation unit snap-fit together, and the two portions can be separated by
15 breaking a snap-fitting foot portion.

In still another variation, the two portions form a single piece and are connected by a frangible belt to be broken in order to separate them from each other.

In yet another variation, the membrane collecting a sample is not sealed
20 to the body of a preparation unit but is pressed against an 'O' ring seal and/or the membrane is formed of Polyethersulfone (PES), polycarbonate, or polyester or cellulose ester as another possibility.

The present invention is not limited to the embodiment described and represented above but covers any variant embodiment.

CLAIMS

1. A method of preparing a sample for the microbiological analysis
5 of a liquid which may contain microorganisms of several different types each having predetermined morphological characteristics, the method comprising a step of making a selection of the microorganisms from among said different types of microorganisms, said step of making a selection comprising:
- a step of procuring a preparation unit (201) for preparing said
10 sample, the unit (201) comprising a generally tubular body (202) within which a first membrane (3) and a second membrane (4) are fixed, said body (202) having an opening (207) for introduction of said liquid, a first compartment (226) between said introduction opening (207) and said first membrane (3), a second compartment (227) between said first membrane (3) and second membrane (4)
15 and a third compartment (228) on the opposite side of said second membrane (4) from said first membrane (3), said first membrane (3) having a predetermined first pore diameter and said second membrane (4) having a predetermined second pore diameter smaller than said predetermined first pore diameter of said first membrane (3);
 - 20 - a step of passing a predetermined volume of said liquid from the first compartment (226) to the second compartment (227) through said first membrane (3);
 - a step of passing all the filtrate, having so reached the second compartment (227), from the second compartment (227) to the third compartment
25 (228) through said second membrane (4) in order to collect said sample on said second membrane (4), the sample containing microorganisms selected from said different types of microorganisms and having the size smaller than the predetermined pore diameter of said first membrane (3) and greater than the predetermined pore diameter of said second membrane (4); and
 - 30 - a step of recovering said sample so collected on said second membrane (4),
- the method characterized in that the step of procuring a preparation unit comprises a step of selecting a preparation unit (201) in which the body (202)

thereof has an auxiliary opening (232) whose stopper can be removed, the auxiliary opening communicating with said second compartment (227), and in that
the step of recovering said sample comprises a step of removing said opening (232), a step of depositing an agent for lysing said microorganisms
5 selected on said second membrane (4) through said auxiliary opening (232) and a step of passing the lysate so formed from the second compartment (227) to the third compartment (228) through said second membrane (4).

2. A method according to claim 1, characterized in that the step of procuring a preparation unit comprises a step of selecting a preparation unit (201)
10 in which the body (202) comprises a first portion (209) to which said first membrane (3) is fixed and a second portion (210) to which said second membrane (4) is fixed, said portions being detachable from each other.

3. A method according to claim 1 or 2, characterized in that the steps of passing said liquid through said membranes (3, 4) are performed by
15 placing said third compartment (28) under reduced pressure.

4. A method according to claim 1 or 2, characterized in that the steps of passing said liquid through said membranes (3, 4) are performed by placing said first compartment (26) under increased pressure.

5. A method according to claim 1 or 2, characterized in that the
20 steps of passing said liquid through said membranes (3, 4) are performed by centrifuging said preparation unit (201).

6. A method according to any one of claims 1 to 5, characterized in that the step of procuring a preparation unit (201) comprises a step of selecting as said first membrane (3) a membrane of pore diameter greater than 1 μm .

25 7. A method according to any one of claims 1 to 6, characterized in that the step of procuring a preparation unit comprises a step of selecting as said second membrane (4) a membrane of pore diameter smaller than 0.65 μm .

8. A method according to claim 7, characterized in that the step of procuring a preparation unit comprises a step of selecting as said second
30 membrane a membrane of pore diameter smaller than 0.1 μm .

9. A unit for preparing a sample suitable for implementing the method according to any one of claims 1 to 8, the unit comprising a generally tubular body (202) within which a first membrane (3) and a second membrane (4)

are fixed, said body (202) having an opening (207) for introducing said liquid, a first compartment (226) between said introduction opening (207) and said first membrane (3), a second compartment (227) between said first membrane (3) and second membrane (4) and a third compartment (228) on the opposite side of said second membrane (4) from said first membrane (3), said first membrane (3) having a predetermined first pore diameter and said second membrane (4) having a predetermined second pore diameter smaller than said predetermined first pore diameter of said first membrane (3); said unit being constituted in such a manner as to pass a predetermined volume of said liquid from the first compartment to the second compartment through said first membrane (3) and then to pass all the filtrate, having so reached the second compartment (227), from the second compartment to the third compartment through said second membrane (4) in order to collect said sample on said second membrane (4); and said body further comprising a recovering means (232) for recovering said sample so collected on said second membrane (4), wherein said recovery means comprises an opening (232) whose stopper can be removed, the opening being formed in said body (202) and communicating with said second compartment (227).

10. A unit according to claim 9, characterized in that said first membrane (3) is permeable to air when it is wet.

20 11. A unit according to claim 10, characterized in that the pore diameter of said first membrane (3) is greater than 1 μm .

12. A unit according to any one of claims 9 to 11, characterized in that the pore diameter of said second membrane (4) is smaller than 0.65 μm .

25 13. A unit according to claim 12, characterized in that the pore diameter of said second membrane (4) is smaller than 0.1 μm .

14. A unit according to any one of claims 9 to 13, characterized in that said body (202) comprises a first portion (209) to which said first membrane (3) is fixed and a second portion (210) to which said second membrane (4) is fixed.

30 15. A unit according to any one of claims 9 to 14, characterized in that said body (202) comprises a cup (230), the cup defining said third compartment (228).

16. A unit according to claim 15, characterized in that said cup (230) is detachable.