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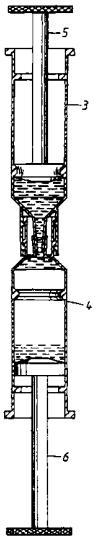
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(54) Title: METHOD AND DEVICE FOR THE HANDLING OF SAMPLES AND REAGENTS



(57) Abstract: Nucleic acids are extracted rapidly, safely and directly from a sample without pipetting steps by using predispensed, interconnectable vessels. These vessels are used separately or interconnected according to the mictrotitre standard format. The sample is mixed with lysis buffer and the nucleic acids bound to a matrix in a closed system, comprising at least two interconnectable volumes. By forcing the sample and buffer mixture back and forth from one volume to another, passing a narrow passage, thorough mixing is ensured.

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METHOD AND DEVICE FOR THE HANDLING OF SAMPLES AND REAGENTS

Field of the invention

The present invention concerns closed or so called dry handling of reagents and samples, and in particular the field of sample preparation, quantitative and qualitative extraction, purification and amplification of nucleic acids (DNA or RNA, including different species of nucleic acids) from organic samples, such as blood, serum, urine, cell suspensions, *in vitro* amplified samples and biopsy samples.

Background of the invention

There are a large number of different protocols for the isolation and purification of nucleic acids. Most of the methods aim at the isolation of highly purified samples, suitable for use in PCR amplifications.

One presently used protocol, utilising the Split Second™ DNA Preparation Kit (Boehringer Mannheim GmbH) comprises the following steps: a first buffer solution is dispensed in microcentrifuge tubes. The sample, e.g. human whole blood, is added to the tubes. The tubes are placed on a rocking platform for 10 minutes and then centrifuged at 2500 rpm for 5 minutes in a microfuge. The supernatant is then removed and discarded, and the pellet resuspended in a buffer solution. The resuspended pellet is then centrifuged at 2500 rpm for 3 minutes. Following this second centrifugation, the supernatant is removed and discarded. The pellet is resuspended in a second buffer and the mixture vortexed thoroughly. After incubation for 5 minutes in a 65°C water bath, the sample can be used directly for PCR.

Another protocol is the purification of DNA using a chaotropic agent and silica particles, as described by Dr. J. Kleiber (Preparation of DNA templates, Boehringer Mannheim GmbH, FRG). Silica suspension is added to lysis buffer in a microcentrifuge tube, and vortexed. EDTA-treated blood is then added to the lysis buffer containing the silica and the mixture vortexed. After a 10 minute incubation, during which the tube should be inverted regularly to prevent the silica from settling, the mixture is centrifuged. After removing the supernatant, the silica-nucleic acid pellet is subjected to a series of washes: first a wash buffer (guanidine thiocyanate, Tris/HCl), then ethanol (70 %) and finally acetone. Each wash step requires

vortexing of the mixture, centrifugation and removal of the supernatant. After the last wash, the silica-nucleic acid pellet is dried by heating it at 56°C for 10 min. Then, the pellet is resuspended in TE-buffer and the mixture incubated for 10 min at 56 °C. Finally, the mixture is centrifuged at 10.000 x g for 2 minutes. The supernatant will now contain the purified nucleic acid and it can be used for PCR.

It is easily recognised, that the multiple steps of the presently used protocols are labor intensive and require relentless concentration. Exact volumes have to be pipetted in a large number of microcentrifuge tubes and these tubes are subjected to different steps, such as centrifugation, vortexing and incubation.

The handling of samples taken from the human body, regardless if they are biopsy samples, blood or serum samples, or samples of other body fluids such as urine or saliva, cell suspensions and *in vitro* amplified samples, involves many practical complications and special considerations. Not only must the laboratory personnel be protected from disease causing agents, possibly contained in the samples, the samples themselves must be protected from contamination, either from other samples or from the personnel handling the samples.

The many steps involved in conventional nucleic acid purification increase the risk of transmission of nucleic acids from sample to sample. In determinations involving the extremely sensitive polymerase chain reaction (PCR) or other amplification systems, the slightest contamination can result in false-positive results.

The handling of human blood poses specific problems. As heparinised blood cannot be used (heparin inhibits the PCR) the samples tend to coagulate in the containers containing the samples, in pipette tips and microcentrifuge tubes. Further, blood has a tendency to adhere to surfaces and to dry, forming minute flakes, which easily become airborne. Finally, the psychological discomfort or stress, experienced when handling potentially contagious human blood samples, should not be neglected.

The present invention aims at improving, e.g. simplifying the purification of nucleic acids from organic samples in general and in particular the preparation of nucleic acid samples from human whole blood.

Notably, nucleic acids are present in the form of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA can in turn be subdivided into plasmide DNA and genomic

DNA. RNA is usually subdivided into messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). These groups are frequently referred to as "nucleic acid species". It would be desirable to make available an easy and reliable method for the qualitative extraction of nucleic acids, i.e. the differentiation between different nucleic acid species.

In many chemical and pharmaceutical applications, a reagent or pharmaceutical agent is stored and delivered to the user in lyophilised form and has to be reconstituted by mixing it with a suitable amount of an appropriate solvent. The solvent is often water or an aqueous solution, such as physiological saline solution. This technique is resorted to mainly in order to extend the stability and thus the storage life of the reagent or pharmaceutical agent. In some cases, a reaction between components, contained in the reagents, is made impossible or considerably retarded in a lyophilised state and initiated when the material is reconstituted.

When reconstituting a lyophilised substance, thorough mixing must be guaranteed. Concentration gradients, insufficient mixing and perhaps remaining solids can cause errors and risks in the further use of the reconstituted solution. The risk of contaminating the lyophilised substance must be taken into account, in particular when the reconstitution is done openly.

Examples of applications where contaminating material can totally disarrange the result include analytical and biochemical applications where genetic information from a sample is amplified. Important reactions of this type include the polymerase chain reaction (PCR), ligase chain reaction (LCR), "gapped-LCR-reaction", nucleic acid sequence-based amplification (NASBA), self-sustained replication (SSR), transcription mediated amplification (TMA), strand displacement amplification (SDA), target amplification, signal amplification, Hybrid Capture® reaction or a combination of any of the above. The present invention aims at minimizing or totally avoiding contamination in the preparation of samples for the above applications and others, where contamination causes risks for errors.

Another aim of the present invention is thus to make available a method and device for restitution of lyophilised substances, e.g. reagents for use in biochemical analyses or pharmaceutical agents, offering a higher degree of safety, reliability and user-friendliness than presently available systems.

Closest prior art

U.S. 5,330,916 discloses a method for the extraction of cellular components and a vessel suitable for use in this method. The so called double-ended extraction vessel has two compartments, separated by a filter, and a grinder – plunger movably positioned in one of the chambers. By moving the grinder – plunger, the sample is subjected to mechanical forces, breaking open the cells in the sample, releasing the cell content into an aqueous phase. Said aqueous phase passes the filter into the second compartment, the organic phase and cellular debris remaining in the first compartment.

U.S. 5,786,182 discloses a dual chamber disposable vessel for amplification reactions, wherein a first chamber contains an amplification reagent mix and a second chamber contains an amplification enzyme. These two chambers are connected by a fluid channel which can be opened or through which the sample can be forced to pass as the result of mechanical action, application of vacuum etc. The gist of the invention is to separate reagents with different resistance to heat, i.a. a heat labile enzymatic reagent and a heat stable amplification reagent, and to make it possible to bring them together at a chosen moment.

The vessel according to U.S. 5,786,182 is directed to amplification reactions and does not include the possibilities of interchanging the chambers constituting the dual chamber vessel, nor is it suitable for the extraction of nucleic acids, i.a. as it lacks means for a thorough mixing of the sample and reagent.

The method and vessel according to 5,330,916 constitutes the closest prior art as it concerns the extraction of nucleic acids. It however does not make it possible to customise the extraction procedure, exchange of buffers, sequential extraction etc in an easy and reliable manner.

Summary of the invention

The present invention solves the above problems by making available devices and methods according to the attached claims.

The present invention makes available a device for the extraction of nucleic acids, wherein said system comprises a first vessel (3) for containing the sample and at least one second vessel (4), containing at least one reagent (2), said vessels being detachably connected via a

narrow passage (7, 8), and means (5, 6) for forcing the sample from the first vessel into the second vessel and back.

The present invention also makes available a method for the extraction of nucleic acids from a sample, wherein said sample is provided in a first vessel, which is detachably connected to a second vessel, said second vessel containing a predispensed reagent, whereupon the sample is pumped into the second vessel and back, repeating this until sufficiently mixed, whereupon the vessel containing the mixture is attached to a third vessel, containing a predispensed reagent and the mixing repeated.

Further embodiments and their advantages of the invention will be evident or deducible from the description and examples, including the attached figures.

Short description of the drawing

The present invention will be disclosed in closer detail in the description and examples below, with reference to the accompanying drawing, in which

Figure 1 shows a cross section of two vessels, A and B, according to one embodiment of the invention;

Figure 2 shows the two vessels of fig. 1 interconnected according to the invention;

Figure 3 shows a vessel with a cap for separation of the matrix, carrying the nucleic acids; and

Figure 4 shows an assembly of fig. 3, placed in a centrifuge tube (A) and a microcentrifuge tube (B), respectively, for example for centrifugal emptying.

Description

The inventive method for purification, extraction and enzymatic treatment of nucleic acids according to the present invention makes possible the "dry" handling of a sample and necessary buffers, as pipetting steps become unnecessary.

A sample, e.g. a volume of blood, serum, urine, saliva, a cell suspension, e.g. from a biopsy sample, or a sample amplified *in vitro*, is placed in a first space. This first space can be a space contained in a test tube, a Vacutainer®, a syringe or preferably a first space, contained

in a device according to the inven ion and described below. The sample is then brought in contact with a lysis buffer, which preferably also contains a matrix, capable of binding nucleic acids. A suitable lysis buffer is a salt solution, further containing a detergent, conventionally used agents, such as Tris (Boehringer Mannheim GmbH) and EDTA (Merck). A suitable matrix consists of glass or silica particles, diatoms, glass fibres, nylon fibres, cellulose slurry, para-magnetic beads, latex beads etc. The matrix can also be coated, e.g. coated with streptavidin or any other coating material for separating nucleic acid strands or a single nucleic acid strand exclusively.

This first buffer, preferably also comprising a solid matrix, is contained in a second space, having a narrow passage. When entering the second space, the sample forms a mixture with the lysis buffer and the matrix. This mixture is then forced to pass the above mentioned narrow passage and enters a space, which can be the first space, in case the sample was originally provided in a device according to the invention. When passing the narrow passage, or opening between the two spaces or vessels, the mixture is efficiently mixed. The mixture is then forced back into the second space and the procedure is repeated a number of times, guaranteeing a thorough mixing and lysis of the cells in the sample. After a sufficient number of passes, the mixture is practically homogenous. During this mixing, the nucleic acids bind to the matrix, contained in the lysis buffer. The mixing is terminated and the mixture contained in either the first or the second space.

The matrix with bound nucleic acids is then separated from the bulk of solution. In the case of using a particulate matrix, this separation can be achieved by closing the space containing the matrix with a membrane or with a separating cap, as described below, and centrifuging the device. The matrix remains on the membrane or in the separating cap.

When using a para-magnetic matrix, separation is achieved by applying a magnetic field around the vessel or in the movable element or plunger, used to force the contents of the vessels to pass from one vessel to another.

The mixture containing the lysis buffer and the matrix is then mixed with a rinse buffer, contained in a third space or vessel, connected to the second space or vessel. By forcing the mixture to pass back and forth between these two spaces, through a narrow passage, thorough mixing and rinsing is again ensured. The mixing is terminated and the mixture contained in either the second or third space, the other being discarded.

Finally, the purified nucleic acids can be removed from the matrix using an eluation buffer or in case of a para-magnetic matrix, by removing the magnetic field.

By using different matrixes, the extraction of different nucleic acid species can be achieved. This is done by choosing the material of the matrix, the structure of the matrix, its packing and other structural / physical properties. It can also be influenced by selecting appropriate chemical properties and/or chemical or biological pre-treatment, such as affinity favouring treatments, coating the matrix with antibodies, affibodies, streptavidin, biotin. Specific nucleic acid sequences can be extracted using complementary nucleic acid hybridised to the matrix. This way for example specific viral nucleic acids can be extracted as part of the preparation of the sample for a diagnostic test.

The extraction buffer or buffers can also be chosen or adapted to the extraction of specific nucleic acid species. According to one embodiment of the invention, a sample is passed back and forth between two vessels, one containing a matrix specifically adapted to or favouring the adsorption of one nucleic acid species, the other vessel containing a matrix specifically adapted to or favouring the adsorption of another nucleic acid species, and a buffer suitable for both matrixes. This way the extracted nucleic acid species are physically separated, which improves the kinetics of the reaction and leads to higher yields, both quantitatively and qualitatively.

According to an alternative embodiment of this method, the matrix can be added after lysis has been performed.

The device according to the present invention comprises at least two vessels having movable wall portions or elements for expelling the content of each vessel, and couplings that fit the corresponding part of at least the other vessel and preferably further vessels.

According to a preferred embodiment, one vessel contains a solid matrix permanently fixed to the vessel or physically hindered from leaving the vessel. This matrix can be a plug of a particulate or fibrous material, e.g. glass or silica particles, glass fibre, nylon fibre, cellulose or diatoms, optionally sintered or otherwise compressed to form a plug-like shape, unable to leave the vessel in the direction of the fluid stream when the vessel is emptied. The matrix can be specifically chosen, adapted or modified, with consideration to its physical / structural and/or chamical / biological properties, as described above.

Alternatively, when using para-magnetic particles, the movements of the matrix particles is regulated by applying a magnetic field to the vessel.

The matrix may also constitute a coating on the inside walls of one of the vessels, alternatively the walls of one of the vessels are made of glass, acrylic, polystyrene or other material known to bind reversibly e.g. to nucleic acids under specific conditions. In the latter case, some surface treatment, such as rugging or other enhancement of the active area of the walls, is preferred.

According to one embodiment of the invention, the possibilities of combining the vessels is restricted by the thread, shape or size of their nozzles or collars. A first vessel can for example be equipped with both an outside and an inside thread. A second vessel to be connected to said first vessel engages the inner thread. After the mixing involving the first vessel is terminated, the second vessel is discarded and the first vessel connected to a third vessel, engaging its outer thread.

Further, using colour codes, tactile marks and the like, the order of using the different vessels can be guided. By posing direct physical restrictions (different threads, different gauges of parts to be connected etc) on the coupling of the vessels, the possibility of errors is minimised.

The device according to the present invention is preferably made of a suitable thermoplastic. Examples of such materials include, but are not limited to, polypropylene (PP), polystyrene (PS), polyethylene (PE), high density polyethylene (HDPE), polycarbonate (PC), polyacetate (PA), poly-methylene-methacrylate (PMMA) and polyvinylidene-fluoride (PVDF). The choice of material is not only governed by chemical and thermal requirements, due to the reagents and buffers to be handled or the reactions to be performed in the device, but also economic considerations, such as material costs, production technology etc. One suitable method of production is injection moulding. Vacuum die-casting is another possible method of production. The inventive device is of course manufactured under conditions rendering it free from contaminants, which possibly influence the reaction or reactions it is intended to be used in.

The device is shown in Fig. 1 as two vessels 3 and 4. For the purpose of illustration, the vessels are shown as artefacts resembling conventional syringes, having a body, a movable plunger 5 and 6, an exit opening or nozzle 7 and 8. Around the exit openings, a collar 9 or 10 is arranged. As the collar on vessel 3 has inner threads 11, and the collar on vessel 4 has

corresponding outer threads 12, they can be securely connected to each other, ensuring a tight fit between the openings 7 and 8. The collars and threads can also be used for attaching a cover or cap to the vessels, protecting the integrity of their content, ensuring the sterility of the inner surfaces etc.

For illustrative purposes, the liquid contained in 3, in the volume indicated by dashed lines in the figure, can be held to be a sample, for example a whole blood sample. The liquid in 4, is then a lysis buffer, preferably also containing a matrix 2.

In order to regulate the movement of the plungers, the vessels here have a first annular edge 18 in the proximal end of the vessel and a second annular edge 19 in the distal end of the vessel. The first edge 18 will give a noticeable resistance and thus indicate to the user, that the plunger is near its lowest position. It is however possible to press the plunger past the first edge 18, for example when emptying the vessel. The second edge 19 will prevent the plunger from being withdrawn from the vessel, for example by mistake or as a result of too high pressure building up in the vessel.

In Fig. 2, two vessels 3 and 4 are shown when connected using the threaded collars described above. The connection opens a fluid path between the space of the two vessels, passing a narrow constriction or "neck" formed by the exit openings of the vessels. As the connection is tight, the two vessels define one volume. When the plunger 5 is depressed, the sample contained in vessel 3 is forced through the narrow passage into vessel 4, which contains the lysis buffer and matrix 2. As the connection is tight, the plunger 6 is forced back, by the pressure exerted by plunger 5. When most of the sample is emptied into vessel 4, the plunger 6 is depressed, forcing sample plus buffer into vessel 3. This way the contents are pumped back and forth between vessels 3 and 4, forming a mixture. When using non-heparinised blood – as necessary for PCR purposes - this mixing has shown to efficiently prevent the blood from coagulating. The mixing is terminated leaving the mixture in one of the vessels, either 3 or 4.

In an embodiment where vessel 4 contains a matrix unable to leave the vessel, e.g. a plug of glass fibres 2 or sintered silica particles etc, it is preferred that, after thorough mixing, the contents of 4 are emptied into 3, or another equivalent vessel, leaving only the matrix with bound nucleic acids. Vessel 3 is then discarded, together with its contents.

A third vessel (not shown) containing a rinse buffer is then attached to vessel 4 and the pumping procedure repeated. When the rinse is terminated, vessel 4 is emptied of all but the matrix with the bound nucleic acids and the third vessel, containing the used rinse buffer is discarded.

The nucleic acids can then be eluated from the matrix, for example by filling vessel 4 with an eluation buffer, preferably contained in a fourth vessel, according to the invention. Alternatively, a vessel containing an eluation buffer is connected to vessel 4 and the eluation performed by pumping the contents back and forth between these two vessels.

If the extracted nucleic acid is to be used as a template for an amplification process, e.g. PCR, a fourth vessel containing lyophilised reagents for the amplification can be connected to vessel 4. The content of vessel 4, including the eluated DNA is then forced into the fourth vessel and pumped back and forth, thus effectively solving and homogenising the reagents. In this embodiment, a complete reaction mixture including template for the amplification is prepared and dispensed directly to the reaction vessel used for PCR. This offers many benefits, as simplified handling and reduced risk of contamination etc.

The vessels can also be emptied by centrifugation. Fig. 3 shows an embodiment with a vessel 3 containing a mixture of buffer and matrix. At this stage, the nucleic acids are bound to the matrix and an eluation buffer added. A separation cap 13 having exit pores 14 is attached to the exit opening 7 of the vessel 3. The cap 13 can be secured to threads on the collar 9 (not shown). When centrifuged, the eluation buffer leaves the vessel through the exit pores 14, leaving the matrix in the separation cap. The eluated nucleic acids can then be used for amplification, e.g. PCR.

Fig. 4 shows two applications, one where a vessel with a separation cap 13 attached, is arranged in a centrifuge tube 17, and one where the vessel is arranged to empty its contents into a microcentrifuge tube 16. During centrifugation, the matrix remains in the vessel or is held back by the separation cap, while the eluation buffer and the nucleic acids pass the exit pores 14 and are transferred to the tube 16 or 17. The tubes 16 and 17 can then be subjected to further steps, e.g. as required by the PCR protocol.

The first vessel, which is the container of the sample, can also be the primary receptacle of the sample. It is conceived, that the first vessel is adapted for sample collection. According to one embodiment of the invention, the first vessel is adapted for holding a hypodermic needle. This

way a blood sample can be taken directly in the first vessel. According to another embodiment of the invention, the first vessel is adapted for taking a band from a gel or a colony from an agar plate. The vessel is then equipped with a nozzle for punching a band or a nozzle for lifting a colony from an agar plate. The pumping of the contents between vessels, as in the method described above, is very suitable for extracting nucleic acids from a gel or a clone grown on an agar plate.

According to a further embodiment, the inventive device and method can be adapted to a parallel format. In laboratories handling large numbers of samples, an embodiment having a manifold of vessels, e.g. 96, 384 or more vessels of microcentrifuge type or microtitre wells, can be assembled into the so called microtitre standard format or other parallel formats. Such an assembly of e.g. 8 x 12 vessels is preferrably injection moulded in one single piece. Corresponding plungers are also assembeld to form rows and columns in the same format, and also preferrably injection moulded in one single piece. The pumping described above can be performed manually. Preferrably, an automatic device is used. This can be a mechanic or pneumatic device, engaging the assembly of plungers. In this embodiment, the exit openings 7, 8 and the collars 9, 10 having threads, should be modified in a way permitting secure and tight connection without twisting. A twisting movement is difficult to perform when the vessels are assembled in a grid as in this embodiment. In other words, the vessels should be tightly engaged by aligning the vessels and pressing them together. This can be achieved using tight fitting connections, flanges, "snap-lock" mechanisms etc. A "push-and-lock" and "pull-and-release" mechanism can be devised by changing the shape of the collars 9 and 10.

Example

In the present example, a set of interconnectable syringes were used. Different matrixes were tested: silica particles, glass and nylon fibres. A lysis buffer consisting of a salt solution and detergent was used. The rinse buffer consisted of a salt solution and ethanol.

A sample of non-heparinised human whole blood was drawn into a first syringe, either directly from a patient, using a hypodermic needle, attached to the syringe, or from an intermediate container, a Vacutainer®, containing a patient sample.

A second syringe was provided, containing silica particles suspended in a lysis buffer. The syringe containing the blood sample was then connected to the second syringe, and the blood sample emptied into the lysis buffer. The mixed content of the two syringes was then pumped

back and forth between the two syringes. Each passage through the narrow waist of the interconnected syringes helped to mix the sample with the lysis buffer and ensured thorough mixing of the silica particles in the solution. This pumping was repeated for a few minutes, and the mixing terminated with the sample and lysis buffer being entirely in one of the syringes. The other syringe was detached and discarded.

Using a mixture of detergent and salt solution for the lysis of cells in a sample of non-heparinised blood, it was found that the coagulation of the blood was effectively prevented by the inventive treatment.

A third syringe, containing a rinse buffer was connected to the syringe containing the sample, silica and lysis buffer. The rinse buffer was then pumped back and forth between the third and second syringe. After the mixing was terminated, the combined solution was left in the second syringe.

When using a particulate matrix, moving freely in the contents of the vessel, the matrix was separated from the solution by attaching a separation cap on the tip of the syringe, placing the same in a centrifuge tube and subjecting it to centrifugation (as schematically shown in the attached fig. 4 of the drawings). The matrix remained in the separation cap whereas the solution was removed. A further rinse can be performed or the nucleic acid eluated from the pellet, present in the separation cap.

When the matrix was a glass fibre plug – or, as in a second example performed by the inventor, a length of nylon thread - no separation cap needs to be used. Instead, the matrix was rinsed and eluated *in situ*, by attaching different syringes containing the necessary buffers, to the syringe containing the matrix. Finally, the nucleic acids were eluated from the matrix. The purification result was confirmed by subjecting the eluated sample to gel electrophoresis. A distinct band showed that the purification of nucleic acids had been successful.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventor, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention as set forth in the claims appended hereto.

Claims

1. A device for the extraction of nucleic acids, **characterized** in that said system comprises a first vessel (3) for containing the sample and at least one second vessel (4), containing at least one reagent (2), said vessels being detachably connected via a narrow passage (7, 8), and means (5, 6) for forcing the sample from the first vessel into the second vessel and back.

2. Device according to claim 1, **characterized** in that said second vessel contains a pre-dispensed reagent.

3. Device according to claim 1, **characterized** in that said second vessel contains a lyophilised, pre-dispensed reagent.

4. Device according to claim 1, **characterized** in that said second vessel contains a matrix with nucleic acid binding properties.

5. Device according to claim 1, **characterized** in that said second vessel contains a lysis buffer and a matrix with nucleic acid binding properties.

6. Device according to any one of claim 4 or 5, **characterized** in that said matrix is a particulate matrix moving freely in the vessel.

7. Device according to any one of claim 4 or 5, **characterized** in that said matrix consists of primary particles or fibres, forming a secondary shape which is prevented from leaving the vessel.

8. Device according to claim 6, **characterized** in that said matrix is chosen among glass or silica particles, diatoms, glass fibres, nylon fibres, cellulose slurry, para-magnetic beads and latex beads.

9. Device according to claim 4, **characterized** in that said matrix is given a specific affinity favouring treatment.

10. Device according to claim 4, **characterized** in that said matrix is prevented by a magnetic field from leaving the vessel.

11. Device according to claim 4, **characterized** in that the matrix is a plug of glass fibres.

12. Device according to claim 9, **characterized** in that the matrix is coated with streptavidin.

13. Device according to claim 9, **characterized** in that the matrix carries hybridises nucleic acids.

14. Device according to claim 1, **characterized** in that the first vessel (3) has means (9, 11) for engaging corresponding means (10, 12) on a second (4) vessel or further vessels.

15. Device according to claim 14, **characterized** in that said means (9, 11, 10, 12), by their size and/or function, regulate the sequence of attachment of the second and further vessels.

16. Device according to claim 1, **characterized** in that the vessels (3, 4) and further vessels attachable to these, have physical properties such as tactile marks, colour codes and the like, guiding their sequential use.

17. Device according to claim 4, **characterized** in that it comprises a detachable separation cap (13) which retains the solid matrix.

18. A device (13) for separation of a nucleic acid binding matrix from a liquid, **characterized** in that said device has at least one exit pore (14) having an opening, situated above the lowest inner surface of the device.

19. Device according to claim 18, **characterized** in that it attaches securely to the end (9 or 10) of a device (3 or 4) according to claim 1 and fits into a centrifuge tube.

20. Device according to any one of claim 1 – 19, **characterized** in that the vessels are connected to form a grid, corresponding to the microtitre standard format.

21. Method for nucleic acid extraction from an organic sample, **characterized** in that a device according to any one of claims 1 – 20 is used.

22. Method for nucleic acid extraction from an *in vitro* amplified sample, **characterized** in that a device according to any one of claims 1 – 20 is used.

23. Method according to claim 21, **characterized** in that said sample is one of blood, serum, urine, saliva, a cell suspension and a biopsy sample.

24. Method according to claim 21, **characterized** in that said sample is human whole blood.

25. Method for reconstitution of a lyophilised reagent, **characterized** in that a device according to claim 1 is used.

26. Method for the reconstitution of a reagent for any one of the following reactions: polymerase chain reaction (PCR), ligase chain reaction (LCR), "gapped-LCR-reaction", nucleic acid sequence-based amplification (NASBA), self-sustained replication (SSR), transcription mediated amplification (TMA), strand displacement amplification (SDA), Hybrid Capture® reaction, target amplification, signal amplification, or a combination thereof, **characterized** in that a device according to claim 1 is used.

27. A method for the extraction of nucleic acids from a sample, **characterized** in that said sample is provided in a first vessel, which is detachably connected to a second vessel, said second vessel containing a predispensed reagent, whereupon the sample is pumped into the second vessel and back, repeating this until sufficiently mixed, whereupon the vessel containing the mixture is attached to a third vessel, containing a predispensed reagent and the mixing repeated.

28. Method according to claim 26, **characterized** in that the second vessel contains a lysis buffer and a matrix, capable of binding nucleic acids.

29. Method according to claim 27, **characterized** in that the third vessel contains a rinse buffer.

30. Method according to any one of claims 27 - 29, **characterized** in that the purified sample is removed from a vessel (3, 4) by centrifugation.

31. Method according to any one of claims 27 - 29, **characterized** in that the matrix is separated from the liquid it is suspended in by centrifugation using a device according to claim 18.

32. Method according to any one of claims 27 - 29, **characterized** in that the matrix is separated from the liquid it is suspended in by use of a magnetic field.

33. Method according to any one of claims 27 - 29, **characterized** in that the sample is one of the following: a sample of blood, serum, urine, saliva, a cell suspension, an *in vitro* amplified sample or a biopsy sample.

34. Method according to any one of claims 27 - 29, **characterized** in that the sample is a human whole blood sample.

35. Method for the extraction of specific nucleic acid species, **characterized** in that a device according to claim 1 is used, said device containing a matrix treated in a manner favouring the attachment of said nucleic acid species to said matrix.

36. Method for the extraction of specific nucleic acid sequences, **characterized** in that a device according to claim 1 is used, said device containing a matrix having a complementary nucleic acid sequence hybridised thereon.

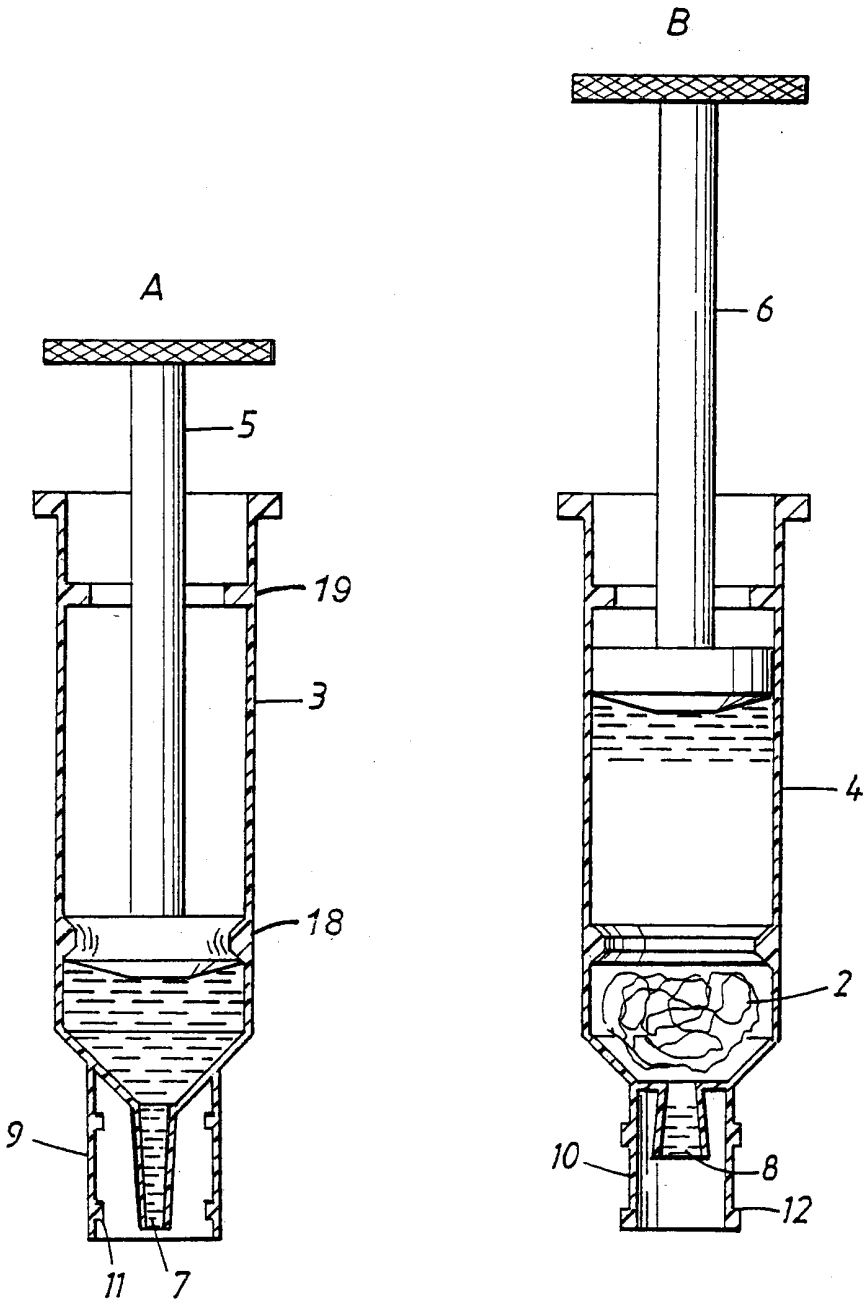


Fig. 1

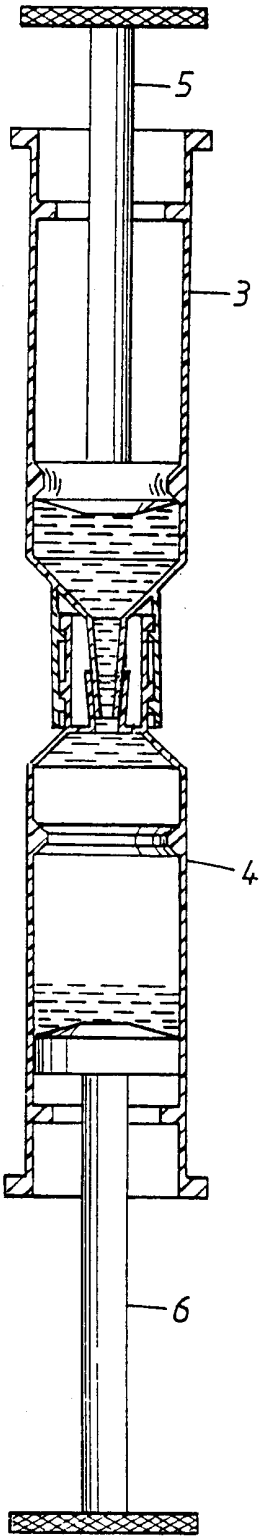


Fig. 2

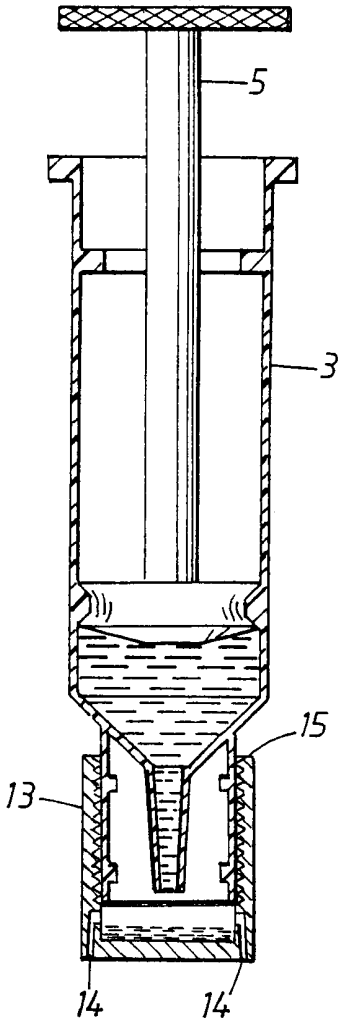


Fig. 3

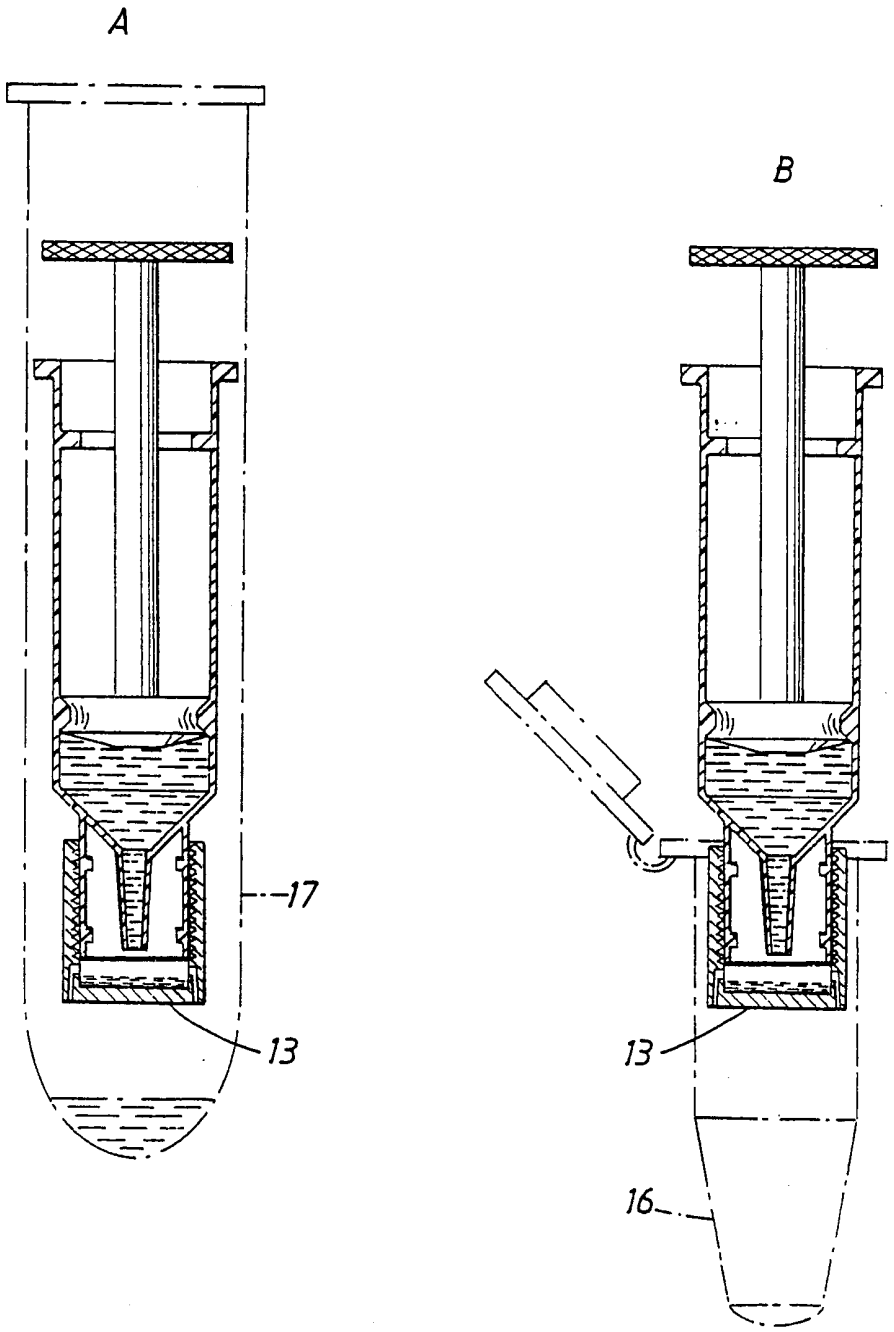


Fig. 4