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(54) Method and device for detecting the presence of an analyte in a test sample

(57) A method and a device for determining the presence or the amount of an analyte in a test sample. The method comprises:

(a) introducing a test sample in a chamber (12),
(b) performing the following steps in that chamber (12):

(b.1) capturing nucleic acids contained in the test sample, said capturing being obtained by use of a binding surface (15) located within said chamber, said binding surface having a high binding affinity for capturing nucleic acids,
(b.2) amplifying a target nucleic acid sequence which is part of the captured nucleic acids, and
(b.3) detecting the presence of the amplified target nucleic acid sequence.

The device comprises the chamber (12). This chamber has a sealable inlet port (13) for introducing into chamber (12) a liquid containing the test sample and a sealable outlet port (14) allowing the exit of liquid from said chamber (12). Chamber (12) contains a binding surface which is adapted to capture nucleic acids contained in the test sample when said liquid containing said test sample flows through said chamber (12). Chamber (12) has at least one wall (16) which enables heating and cooling of the contents of said chamber (12), the temperature of said chamber (12) being thereby modifiable in order to carry out therein a process for amplifying a target nucleic acid sequence which is part of the captured nucleic acids. A part of chamber (12) has a zone (17) which allows examination of the chamber contents by optical means for detecting the presence of an amplified target nucleic acid sequence contained in said chamber (12).

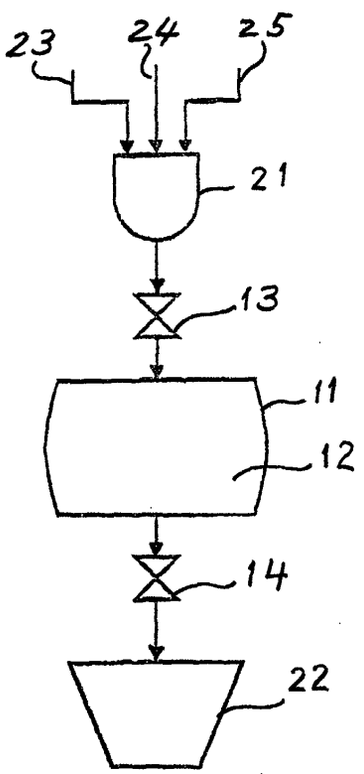


Fig. 1

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Description

FIELD OF THE INVENTION

[0001] The present invention concerns a method, an analytical device and a system for detecting the presence or the amount of an analyte in a test sample.

BACKGROUND OF THE INVENTION

[0002] According to a known method of the above mentioned kind the following analyzing steps:

(a) capturing a nucleic acid sample contained in a test sample,
(b) amplifying the nucleic acid sample to obtain an amplified nucleic acid sample, and
(c) detecting the presence of an analyte in said amplified nucleic acid sample

are carried out each in a separate chamber of an analytical device. This requires means for effecting the transfer of the material obtained by each step from one chamber to another, which increases the complexity and therefore the cost of the analytical device and consequently also the cost of analysis performed therewith. Moreover the transfer of the material can lead to a loss of the target material and therefore to a reduction in the overall recovery of the nucleic acid.

[0003] An aim of the present invention is to provide a method, an analytical device and a system which are suitable for carrying out all above mentioned steps fully automatically without any transfer of the nucleic acid between the analyzing steps, thus reducing the risk of sample contamination and loss of sample material. Furthermore the invention provides an analytical device with a simple structure, which therefore is particularly well suited for miniaturization and manufacturing at low costs.

SUMMARY OF THE INVENTION

[0004] A method according to the invention is defined by claim 1.

[0005] An analytical device according to the invention is defined by claim 6.

[0006] A system according to the invention is defined by claim 18.

[0007] Preferred embodiments of various aspects of the invention are defined by dependent claims.

[0008] The main advantages obtained with the invention are as follows:

- less fluidic interconnections and valves are needed, and this makes the device according to the invention more rigid and compact than prior art devices,
- practically all of the captured nucleic acid molecules are introduced into the amplification process,
- the risk of sample contamination is substantially reduced,
- the sample processing time is substantially reduced, since an elution step is not necessary and no transfer steps are involved.

[0009] In the following the term "analyte" refers to a sequence of nucleic acid, e. g. DNA or RNA, whose presence in a test sample is to be detected.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Preferred embodiments of a device, a system and a method according to the invention are described hereinafter as examples with reference to the accompanying figures.

Fig. 1 shows a schematic representation of an analytical device according to the invention;

Fig. 2 shows a first embodiment of an analytical cell which can be used as part of the device shown in Fig. 1;

Fig. 3 shows a second embodiment of an analytical cell which can be used as part of the device shown in Fig. 1;

Fig. 4 shows an electron-microscopic picture of a fleece used as a binding surface in the analytical cell of Figs. 2 and 3;

Fig. 5 shows schematically the flow behavior in an analytical cell of the type shown in Fig. 2;

Fig. 6 shows schematically the flow behavior in an analytical cell of the type shown in Fig. 3;

Fig. 7 shows a schematic side view of an analytical cell of the type shown in Figs. 2 or 3;

Fig. 8 shows the results obtained by the analytical device shown in Fig. 2.

Fig. 9 shows a top view of an analytical cell of the type shown in Fig. 3,

Fig. 10 shows a magnified view of a portion of the surface of the analytical cell shown in Fig. 9, and

Fig. 11 shows a scanning-electron-microscopic picture of a portion of the surface of the analytical cell shown in Fig. 9.

Fig. 12 shows an embodiment of an analytical system according to the invention.

REFERENCE NUMERALS IN DRAWINGS

[0011]

11	analytical cell
11a	analytical cell
12	chamber
12a	chamber
13	sealable inlet port
14	sealable outlet port
15	body having a binding surface
15a	body having a binding surface
16	wall of chamber 12
17	zone of analytical cell which enables optical examination of contents of chamber 12
18	outer surface of wall 16 available for contact with a thermal instrument
21	container for pretreatment of test sample
22	waste container
23	inlet
24	inlet
25	inlet
26	air gap
33	inlet port
33a	inlet port
34	outlet port
34a	outlet port
41	fluorometer
42	source of excitation light
43	light detection module
44	excitation light beam
45	fluorescence light beam
51	heat transfer body of a thermal cycler

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

[0012] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

[0013] The present invention provides a method of detecting an amplified target nucleic acid sequence that is present in a sample. It is recognized by those skilled in the art that assays for a broad range of target nucleic acid sequences present in a sample may be performed in accordance with the present invention. Samples may include biological samples derived from agriculture sources, bacterial and viral sources, and from human or other animal sources, as well as other samples such as waste or drinking water, agricultural products, processed foodstuff, air, etc. Examples include blood, stool, sputum, mucus, serum, urine, saliva, teardrop, a biopsy sample, an histological tissue sample, a tissue culture product, an agricultural product, waste or drinking water, foodstuff, air, etc. The present invention is useful for the detection of nucleic acid sequences indicative of genetic defects or contagious diseases.

[0014] The following definitions will be helpful in understanding the specification and claims. The definitions provided herein should be borne in mind when these terms are used in the following examples and throughout the instant application.

[0015] As used in this invention, the term "amplification" refers to a "template-dependent process" that results in an increase in the concentration of a nucleic acid sequence relative to its initial concentration. A "template-dependent process" is defined as a process that involves the "template-dependent extension" of a "primer" molecule. A "primer" molecule refers to a sequence of nucleic acid that is complementary to a portion of the target or control sequence and may or may not be labeled with a hapten. A "template dependent extension" refers to nucleic acid synthesis of RNA or DNA wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the rules of complementary base pairing of the target nucleic acid and the primers.

EXAMPLE OF AN ANALYTICAL DEVICE ACCORDING TO THE INVENTION

[0016] An analytical device according to the invention for determining the presence of an analyte in a test sample is schematically represented in Fig. 1.

[0017] An essential component of this device is an analytical cell 11 described in detail hereinafter. Analytical cell 11 comprises a chamber 12 and has a sealable inlet port 13 and a sealable outlet port 14.

[0018] The analytical device comprises a pre-treatment container 21 adapted to be connected to chamber 12 of analytical cell 11 through inlet port 11, and a waste container 22 adapted to be connected to chamber 12 of analytical cell 11 through outlet port 14 of analytical cell 11.

[0019] Container 21 is adapted for mixing a test sample with reagents, and for preincubation for lysing microbiological particles in order to prepare the nucleic acids in the test sample to be transferred to chamber 12 of analytical cell 11 via inlet port 13. Container 21 is disconnectable from chamber 12 by sealing inlet port 13. Container 21 has several inlets: inlet 23 for receiving a liquid sample, inlet 24 for receiving a reagent, inlet 25 for receiving air. In operation container 21 is connected through these inlets to corresponding sources of liquid respectively air under pressure.

[0020] Waste container 22 is adapted for receiving through outlet port 14 waste generated by process steps carried out within chamber 12 of analytical cell 11. Container 22 is disconnectable from chamber 12 by sealing outlet port 14.

[0021] Fig. 2 shows a schematic representation of a first embodiment of analytical cell 11. Chamber 12 of cell 11 has an inlet port 33 and outlet port 34. In Fig. 2 these ports are open and allow flow of liquid through chamber 12.

[0022] Typical inner dimensions of chamber 12 shown

in Fig. 2 are a length L1 = 18.06 mm, a width W1 = 8.3 mm and a depth T1 = 1 mm, which yield a volume of 100 microliter.

[0023] Fig. 3 shows a schematic representation of a second embodiment of cell 11. Chamber 12a of cell 11 has an inlet port 33a and outlet port 34a. In Fig. 3 these ports are open and allow flow of liquid through chamber 12a.

[0024] Typical inner dimensions of chamber 12 shown in Fig. 3 are a length L2 = 7 mm, a width W2 = 3.5 mm and a depth T2 = 1 mm, which yield a volume of 24.5 microliter. This chamber 12 has wedge shaped inlet and outlet ports.

[0025] Fig. 5 shows schematically the flow behavior in an analytical cell of the type shown in Fig. 2. Fig. 6 shows schematically the flow behavior in an analytical cell of the type shown in Fig. 3. Fig. 7 shows a schematic side view of an analytical cell of the type shown in Figs. 2 or 3.

[0026] The upper part body of analytical cell 11 shown by Fig. 7 is preferably made of suitable plastic material e.g. of a transparent polycarbonate. Chamber 12 and inlet and outlet ports 33 respectively 34 are formed within this body. The bottom of cell 11 is an aluminum wall 16. Liquid flow through inlet 33 and through outlet 34 is represented by arrows. Wall 16 has an outer surface 18 which during the intended use of the cell is in thermal contact with heating or cooling means of a thermal instrument, e.g. a thermal cycler of the kind used to perform a polymerase chain reaction. As shown by Fig. 7, there is an air gap 26 within the body of analytical cell.

[0027] Also shown by Fig. 7 is that a part of chamber 12 has a zone 17 which allows examination of the chamber contents by optical means for detecting the presence of an amplified target nucleic acid sequence contained in chamber 12. In Fig. 7 arrow 19 represents an excitation light beam which irradiates body 15 located in chamber 12, and arrow 20 represents a fluorescent light beam emitted by reaction products in chamber 12.

[0028] Inlet port 33 and outlet port 34 allow flow of liquid through them. Inlet port 33 allows introduction of liquid containing a sample to be tested into chamber 12. Outlet port 34 of chamber 12 allows liquid to flow out of chamber 12.

[0029] In preferred embodiments shown by Fig. 6, inlet port 33a is wedge shaped and has a decreasing cross-section in the sense of liquid flow.

[0030] In preferred embodiments shown by Fig. 6, and outlet port 34a is wedge shaped and has an increasing cross-section in the sense of liquid flow.

[0031] Chamber 12 of analytical cell 11 contains a binding surface which is adapted to capture nucleic acids contained in a test sample when the liquid containing the test sample flows through chamber 12.

[0032] In a preferred embodiment the binding surface is part of a body 15 which is manufactured separately from the other parts of the analytical cell 11 and which has a large surface-to-volume ratio. Such a body can e. g. consist of beads, membranes, gels or fibers forming a fleece. Fig. 4 shows an electron-microscopic picture of a glass fiber fleece which has a surface suitable as a binding surface in the analytical cells 11 and 11a shown by Figs. 2 and 3 respectively.

[0033] A preferred glass fiber fleece for making a suitable binding surface has e.g. the following composition:

Element	Atom %	Weight %
O	57.26	43.15
Na	8.41	9.11
Al	3.36	4.27
Si	27.14	35.91
K	1.84	3.40
Ca	0.88	1.66
Ti	1.11	2.50

[0034] SiO_x and Al_2O_3 are particularly important components of the fleece with regard to its capacity to bind nucleic acids.

[0035] A fleece consisting of pure SiO_2 shows an acceptable capacity to bind nucleic acids.

[0036] Fig. 12 shows another preferred embodiment of analytical cell 11. In this embodiment instead of positioning a body 15 having a nucleic acid binding surface within chamber 12, the central part of the inner surface of the upper wall chamber 12 is manufactured by a micromachining process which yields a microstructured cell which has a high surface-to-volume ratio and which is a binding surface for efficiently capturing nucleic acids. Figures 9 to 11 show such a surface. Figure 9 shows a top view of such a cell having a wedge shaped inlet and a wedge shaped outlet. Figure 10 shows a magnified view of the microstructured surface of the inner surface of a cell wall. Fig. 11 shows a scanning-electron-microscopic picture of columnar structures of such a surface which provide a high surface-to-volume ratio. Such a surface of analytical cell 11 is a particularly well adapted binding surface for efficiently capturing nucleic acids.

[0037] An homogeneous spatial distribution of liquid flow through chamber 12 is important in order to achieve a most efficient capture of nucleic acids by the binding surface located within chamber 12. For this purpose, the flow pattern represented in Fig. 6 is more advantageous than the flow pattern represented in Fig. 5. The shape of the inlet and outlet of chamber 12 shown in Fig. 5 yields a higher flow rate through the central part thereof where the flow path is shorter and the flow resistance lower than on paths aside of the central part. Therefore the flow rate is not as homogeneous as desirable. This disadvantage is overcome with the shape of the inlet and outlet of chamber 12 shown in Fig. 6 where the flow path length and the flow resistance is more uniformly distributed and ensures an homogeneous flow of liquid through chamber 12. In each of Figures 5 and 6 the

hatched area represents the functional binding area.

[0038] In a preferred embodiment the binding surface within chamber 12, either as part of the chamber walls or as part of a separate body, has chemical properties which are compatible with the chemical substances and reaction conditions necessary for performing a polymerase chain reaction within chamber 12.

[0039] In a preferred embodiment the binding surface within chamber 12 contains more than 30 weight percent silicon, and more than 35 weight percent oxygen.

[0040] In another preferred embodiment the binding surface within chamber 12 substantially consists of a metal oxide, e.g. of TiO_2, ZnO, ZrO_2, Al_2O_3 or mixtures thereof.

[0041] In another preferred embodiment the binding surface within chamber 12 substantially consists of a polymer, e.g. of polyamides, polystyrene, polypyrrole, cationic or anionic ion exchange resins.

[0042] As shown by Fig. 7, chamber 12 has at least one wall 16 which enables heating and cooling of the contents of chamber 12. The temperature of chamber 12 is thereby modifiable in order to carry out therein a process for amplifying a target nucleic acid sequence which is part of the nucleic acid captured by means of the binding surface.

EXAMPLE OF AN ANALYTICAL SYSTEM ACCORDING TO THE INVENTION

[0043] Fig. 12 shows an embodiment of an analytical system according to the invention. This system comprises an analytical device having the features described above and in addition the following components:

- means for causing a liquid flow through said chamber 12 of said analytical cell 11, e.g. pumping means and suitable conduits (not represented in Fig. 12),
- thermal instrument means, e.g. a thermal cycler, for heating and cooling the contents of chamber 12 of analytical cell 11 in order to carry out an nucleic acid amplification process within chamber 12, and
- optical means, e.g. a fluorometer 41, for examining the contents of said chamber 12 in order to measure the degree of amplification achieved by a nucleic acid amplifying process performed in said chamber 12 and thereby measuring the amount of an analyte present in the test sample.

[0044] A thermal cycler used as thermal instrument means has a heat transfer body 51 adapted to contact a surface 18 of wall 16 of chamber 12. This arrangement enables heating and cooling of the contents of said chamber 12 as required for carrying out a process for amplifying a target nucleic acid sequence within chamber 12.

[0045] Fluorometer 41 is adapted to irradiate the contents of chamber 12 with an excitation light beam 44 provided by a light source 42 and to measure fluorescence light 45 emitted by a tested sample within said chamber 12 by means of a light detection module43. This detection module monitors the fluorescence light energy integrated over the whole area of analytical cell which is accessible to optical examination.

EXAMPLE OF A METHOD ACCORDING TO THE INVENTION

[0046] At the outset a test sample to be analyzed for the presence of a predetermined nucleic acid sequence (analyte) is acquired. This nucleic acid sequence may originate e. g. from a virus, such as hepatitis B virus (HBV) which is present in a blood sample. Samples to be analyzed may contain nucleic acids from bacteria or particular cells.

[0047] A suitable test sample is necessary in order to perform a method according to the invention on such a sample. The preparation of a test sample is thus a preliminary step.

[0048] According to the invention this preliminary step comprises obtaining a test sample containing nucleic acids, e.g. by lysing a biological sample, and mixing it with a highly chaotropic salt in order to enable binding of the sample by a binding surface used in silica based extraction method. The mixture so formed is a test sample on which the method according to the invention is performed. The lysing and mixing steps just mentioned are preferably carried out in container 21 shown in Fig. 1.

[0049] According to the invention it is however also possible to introduce a non-lysed biological sample directly into chamber 12 of analytical cell 11 and to perform the above-mentioned lysing and mixing steps in chamber 12.

[0050] The following method description focuses on the essential steps of a method according to the invention as performed on a suitable test sample which is obtained by the above-mentioned lysing and mixing steps and which contains nucleic acids.

[0051] An example of a method for determining the presence or the amount of an analyte in a test sample is carried out by means of an analytical cell 11, an analytical device comprising such a cell, and an analytical system, which are described above with reference to Figures 1 to 12, and comprises the following essential steps:

(a) a test sample is introduced in chamber 12 of analytical cell 11,

(b) the following steps are performed in chamber 12:

(b.1) nucleic acids contained in the test sample are captured by a binding surface located within chamber 12,

(b.2) a target nucleic acid sequence which is part of the captured nucleic acids is amplified

by a suitable chemical reaction,
(b.3) the presence of the amplified target nucleic acid sequence is detected.

[0052] In a preferred embodiment, not only the presence of the amplified target nucleic acid sequence is detected, but the amount thereof and thereby the amount of a corresponding analyte is measured, e.g. by means of a fluorimetric measurement.

[0053] In a preferred embodiment nucleic acids are captured exclusively by the binding surface within analytical cell 11.

[0054] In a preferred embodiment the test sample is forced to flow through chamber 12 in order to effect capturing of nucleic acids contained in the test sample by the binding surface within analytical cell 11. In this embodiment the average flow rate is preferably substantially uniform over a substantial part of the binding surface.

[0055] After the nucleic acids capturing step in chamber 12 the binding surface is washed without elution by forced flow of a washing liquid through chamber 12. Waste liquid resulting from this washing step is collected in waste container 22.

[0056] After this washing step, a target nucleic acid sequence which is part of the captured nucleic acids is amplified within chamber 12 e.g. by means of a polymerase chain reaction. For this purpose, the necessary reagents for performing a polymerase chain reaction are introduced into chamber 12 by corresponding liquid flow through this chamber. After this step, chamber 12 is sealed at both ends and is subject to thermal cycling required for the polymerase chain reaction.

[0057] In a preferred embodiment the step of amplifying the target nucleic acid sequence is performed in the presence of a fluorescently labeled probe or a fluorescent agent that enables a quantitative measurement of the amplified product. In this embodiment the detection step is performed by measuring the fluorescence intensity of said fluorescently labeled probe or fluorescent agent.

[0058] Results obtained with the above described method are represented in Fig. 8, which shows a diagram showing the variation of the fluorescence intensity measured with the number of thermal cycles. These results are similar to those obtained with conventional instrument means having separate and independent chambers for performing each of the above mentioned essential method steps, i.e. the capturing, amplification and detection step.

[0059] In the above described example, the most commonly used polymerase chain reaction (PCR) has been mentioned has an example of a suitable reaction to perform the nucleic acid amplification step. However a variety of other amplification methods can be used within the scope of the invention, such methods are e.g.: self-sustained sequence replication (3SR) and strand-displacement amplification (SDA), ligase chain reaction(LCR), nucleic acid sequence-based amplification (NASB), QB replicase amplification (QBR), ligase activated transcription (LAT), repair chain reaction (RCR) and cycling probe reaction (CRC).

[0060] The general principles and conditions for amplification of NA using PCR are quite well known in the art; the details of which are provided in numerous references including U.S. Pat. No. 4,683,195, U.S. Pat. No. 4,683,202, U.S. Pat. No. 4,965,188, all to Mullis et al., all of which are specifically incorporated herein by reference.

[0061] In general terms, detection of amplified nucleic acid for clinical use relies largely on hybridization of the amplified product and a detection probe labeled with a fluorescent agent.

[0062] A preferred detection procedure used within the scope of the invention uses a so called TaqMan chemistry technique which is described in the following patent specifications: EP0512334 (B1), EP0640828 (B1), US6171785, US5994056, US5314809, EP0640828 (B1). The detection principle of this technique is based on a hybridization probe having a fluorescence label attached to one end and a fluorescence quencher on the other end of the DNA probe. During the amplification process the quencher and the fluorescence label become separated due to the exonuclease activity of the polymerase and the induced fluorescence increase relates quantitatively to the amount of specific products formed during the amplification. This technique allows highly specific real time monitoring of the amplified target.

[0063] A detailed example of processing of a sample with a method according to the invention comprises the following steps: 150 microliter of a primary probe (1e6 copies HBV) was mixed with 600 microliter of a lysing buffer (Guanidine isothiocyanate, GuSCN 4.2 M, pH 7.5) and lysed during 10 minutes. After that 750 microliter of Ethanol (EtOH) were added to the mixture and pumped through analytical cell 11 at a flow rate of 0.5 milliliter per second. Then the cell was flush out with air flow, rinsed with 300 microliter of a washing buffer (70% EtOH, pH 7.5) at a flow rate of 300 microliter per second, and flush out again with air. After this chamber 12 of analytical cell 11 was filled with a ready-to-use PCR reagent mix, the inlet and outlet ports of analytical cell 11 were sealed, and cell 11 was subject to a conventional PCR thermal cycling program for HBV.

[0064] The composition of the PCR mix used is e.g. 50 mM Tricine pH 8.3; 100mM KOAc pH 7.5; 3.0 mM $Mn(OAc_2)$; Dimethyl sulfoxide (DMSO) 5%; Glycerin 5%; NTP's each 300 uM, Primer HBV1, HBV2 each 150 nM, HBV Probe 100 nM, Polymerase 40 U/reaction.

[0065] The conventional PCR thermal cycling program for HBV includes 120s at 95°C, followed by 60 cycles of 20s at 95°C followed by 40s at 59°C. The ramps for heating and cooling have a slope of 1.2°C/s.

[0066] Flow through chamber 12 is accompanied of a pressure loss which depends of the flow resistance pre-

sented by the cell and the viscosity of the liquid flowing through the chamber. With a chamber as shown in Figures 3, 5, and 9-11, a pressure loss of 100 millibar was measured for a liquid viscosity of 1 mPas, and an average pressure loss of 200 millibar was measured for a liquid viscosity of 4 mPas, with a flow rate of 0.5 milliliter per minute.

[0067] Although preferred embodiments of the invention have been described using specific terms, such description is for illustrative purposes only, and it is to be understood that changes and variations may be made without departing from the spirit or scope of the following claims.

Claims

1. A method for determining the presence or the amount of an analyte in a test sample, said method comprising

   (a) introducing a test sample in a chamber,
   (b) performing the following steps in said chamber:

   (b.1) capturing nucleic acids contained in the test sample, said capturing being obtained by use of a binding surface located within said chamber, said binding surface having a high binding affinity for capturing said nucleic acids,
   (b.2) amplifying a target nucleic acid sequence which is part of the captured nucleic acids, and
   (b.3) detecting the presence of the amplified target nucleic acid sequence.

2. The method according to claim 1, wherein said test sample is a non-lysed biological sample.

3. The method according to claim 1 or 2, wherein said capturing step includes forcing the test sample to flow through said chamber, the average flow rate being substantially uniform over a substantial part of said binding surface.

4. The method according to any of claims 1 to 3, wherein said amplifying step is performed by means of a polymerase chain reaction.

5. The method according to any of claims 1 to 4, wherein said amplifying step is performed in the presence of a fluorescently labeled probe or a fluorescent agent that enables a quantitative measurement of the amplified product, and wherein said detecting step is performed by measuring the fluorescence intensity of said fluorescently labeled probe or fluorescent agent.

6. An analytical device for determining the presence of an analyte in a test sample, said device including an analytical cell (11) comprising:

   a chamber (12) having a sealable inlet port (13) for introducing into said chamber (12) a liquid containing said test sample and a sealable outlet port (14) allowing the exit of liquid from said chamber (12), said inlet port (13) and said outlet port (14) allowing a flow of liquid through said chamber (12) when they are open,
   said chamber (12) containing a binding surface which is adapted to capture nucleic acids contained in the test sample when said liquid containing said test sample flows through said chamber (12),
   said chamber (12) having at least one wall (16) which enables heating and cooling of the contents of said chamber (12), the temperature of said chamber (12) being thereby modifiable in order to carry out therein a process for amplifying a target nucleic acid sequence which is part of the captured nucleic acids,
   part of said chamber (12) having a zone (17) which allows examination of the chamber contents by optical means for detecting the presence of an amplified target nucleic acid sequence contained in said chamber (12).

7. An analytical device according to claim 6, wherein said binding surface is part of a body (15) which is manufactured separately from the other parts of the analytical cell (11), said body having a large surface-to-volume ratio.

8. An analytical device according to claim 6, wherein said body (15) consists of beads, membranes, gels or fibers forming a fleece.

9. An analytical device according to claim 6, wherein said chamber (12) is manufactured by a micromachining process yielding a microstructured cell having a high surface-to-volume ratio and the surface being adapted for efficiently capturing nucleic acids.

10. An analytical device according to any of claims 6 to 9, wherein the chemical properties of said binding surface are compatible with the chemical substances and reaction conditions necessary for performing a polymerase chain reaction within chamber 12.

11. An analytical device according to any of claims 6 to 10, wherein said binding surface contains more than 30 weight percent silicon, and more than 35 weight percent oxygen.

12. An analytical device according to any of claims 6 to 10, wherein said binding surface consists substan-

tially of a metal oxide or of a mixture of metal oxides.

13. An analytical device according to any of claims 6 to 10, wherein said binding surface consists substantially of a polymer.

14. An analytical device according to any of claims 6 to 13, wherein said inlet port (33, 33a) is wedge shaped and has a decreasing cross-section in the sense of liquid flow.

15. An analytical device according to any of claims 6 to 13, wherein said outlet port (34, 34a) is wedge shaped and has an increasing cross-section in the sense of liquid flow.

16. An analytical device according to any of claims 6 to 15, which further comprises a first container (21) connectable to said chamber via said sealable inlet port (13), said first container (21) being adapted for mixing said test sample with reagents and for preincubation for lysing microbiological particles in order to prepare the nucleic acids in the test sample to be transferred to said chamber (12) via said inlet port (13), said first container (21) being disconnectable from said chamber (12) by sealing said inlet port (13).

17. An analytical device according to any of claims 6 to 15, which further comprises a second container (22) connectable to said chamber (12) via said sealable outlet port (14), said second container (22) being adapted for receiving waste generated by process steps carried out within said chamber (12), said second container (22) being disconnectable from said chamber (12) by sealing said outlet port (14).

18. A system for detecting the presence of an analyte in a test sample comprising:

(a) an analytical device according to any of claims 6 to 17,
(b) means for causing a liquid flow through said chamber (12) of said analytical cell (11),
(c) thermal instrument means adapted to contact a surface of said wall (16) which enables heating and cooling of the contents of said chamber (12) and comprising means for modifying the temperature of said chamber (12) in order to carry out therein a process for amplifying a target nucleic acid sequence, and
(d) optical means which are adapted for examining the contents of said chamber (12) in order to measure the degree of amplification achieved by a nucleic acid amplifying process performed in said chamber (12).

19. A system according to claim 18, wherein said optical means is a fluorometer (41) which is adapted to measure fluorescence light (45) emitted by a tested sample within said chamber (12).

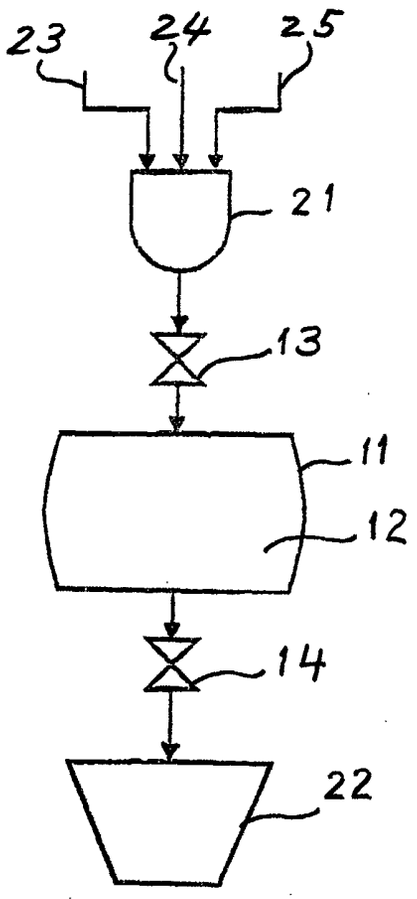


Fig. 1

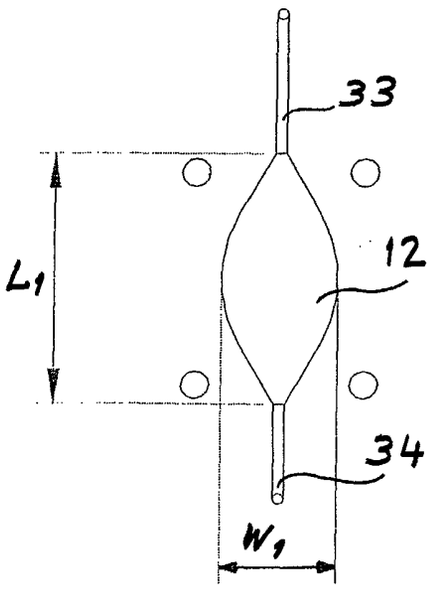


Fig. 2

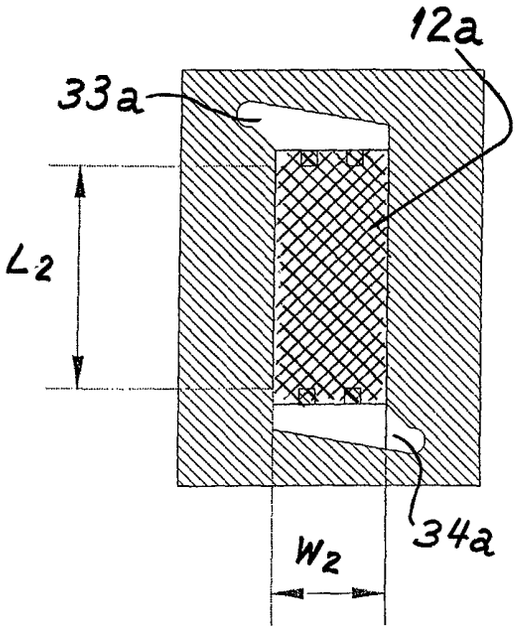


Fig. 3

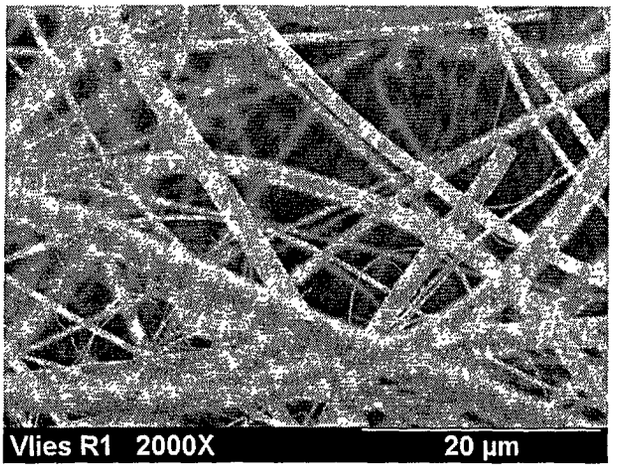


Fig. 4

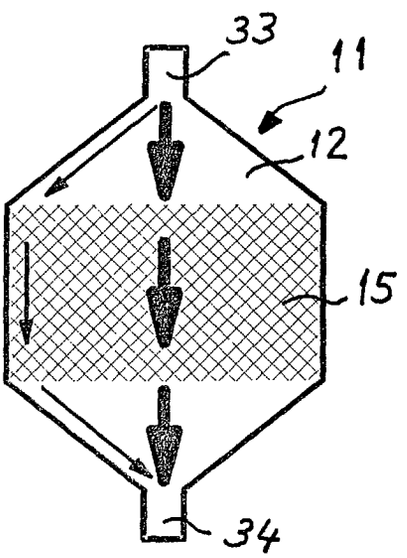


Fig. 5

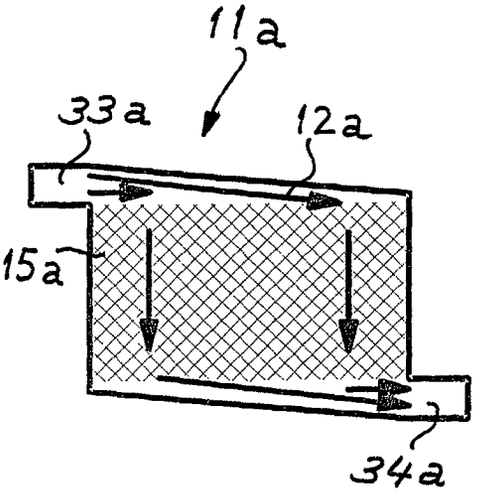


Fig. 6

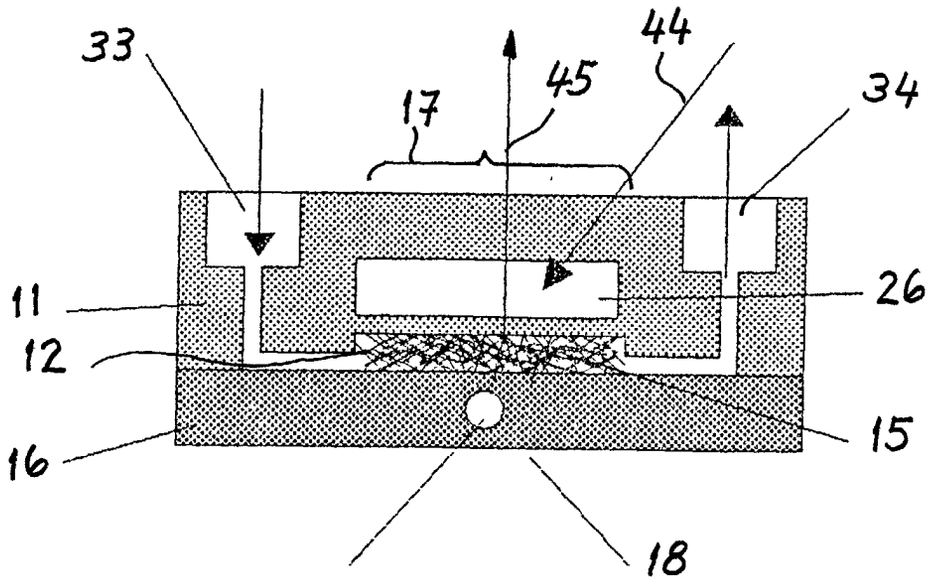

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Fig. 7

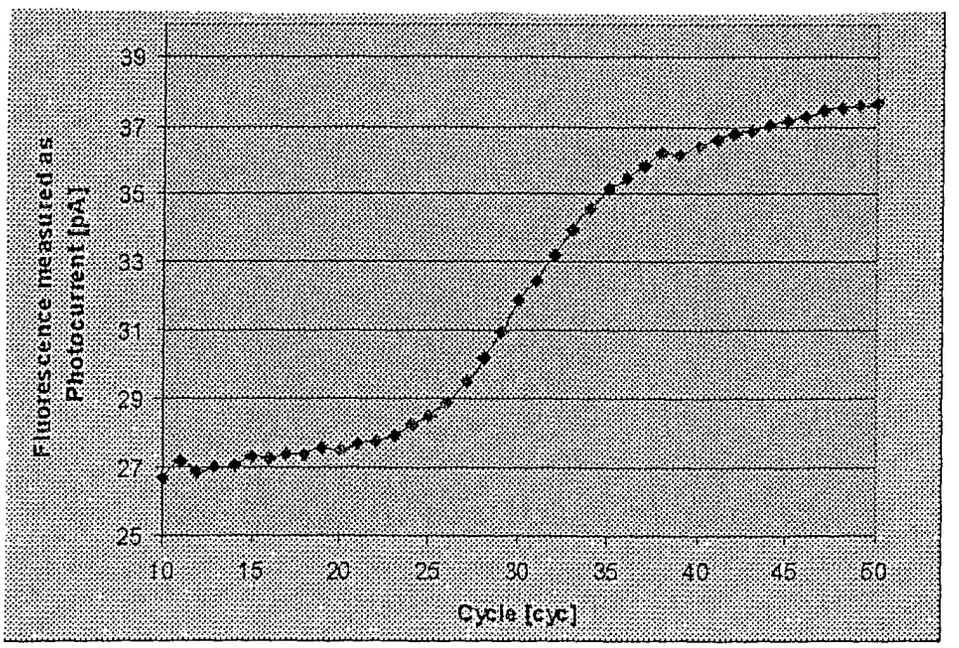

Fluorescence measured as Photocurrent [pA]
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45
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Cycle [cyc]

Fig. 8

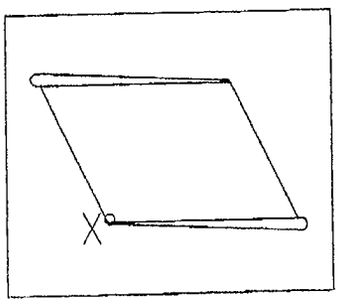


Fig. 9

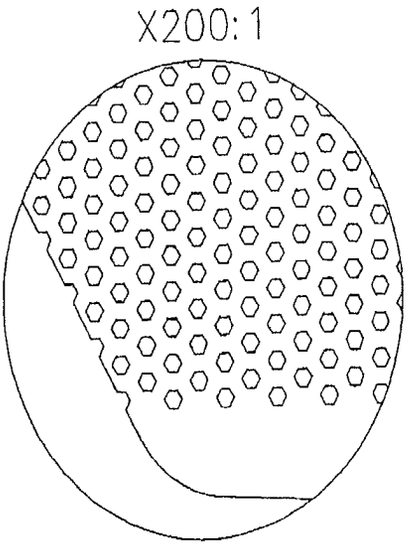


Fig. 10

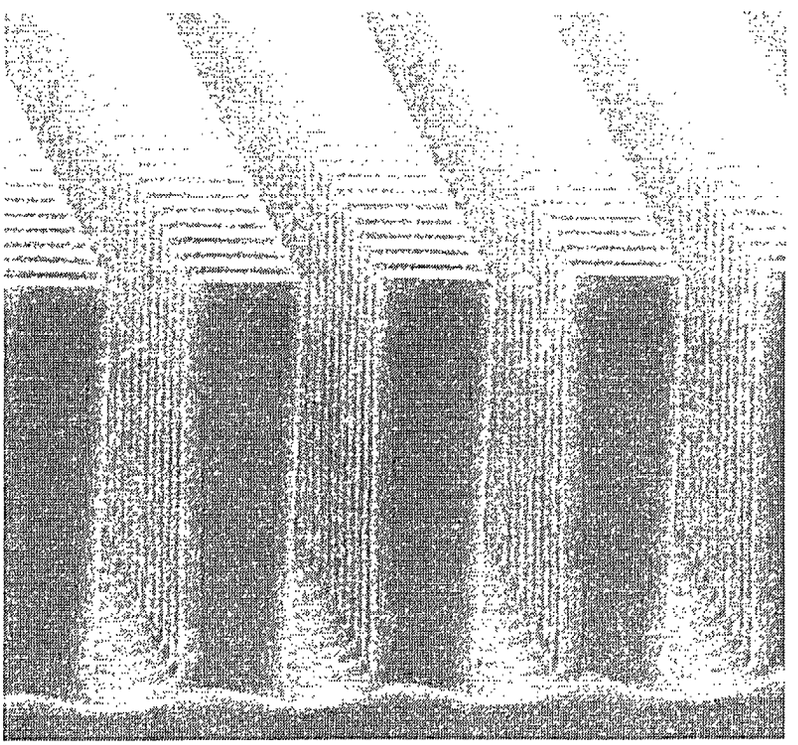


Fig. 11

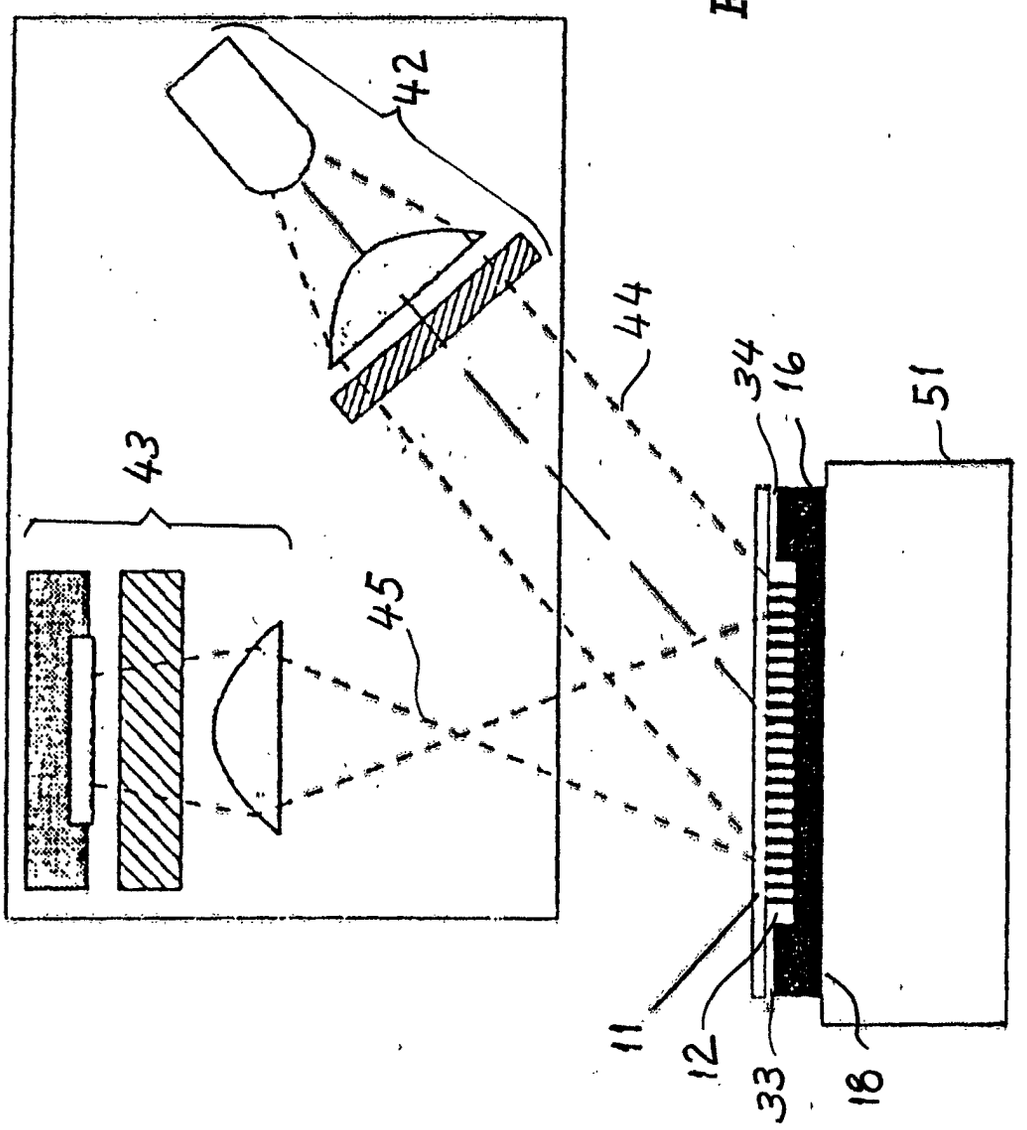


Fig. 12

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EUROPEAN SEARCH REPORT

Application Number
EP 02 07 7317

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	29 November 2002	Semino, D

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