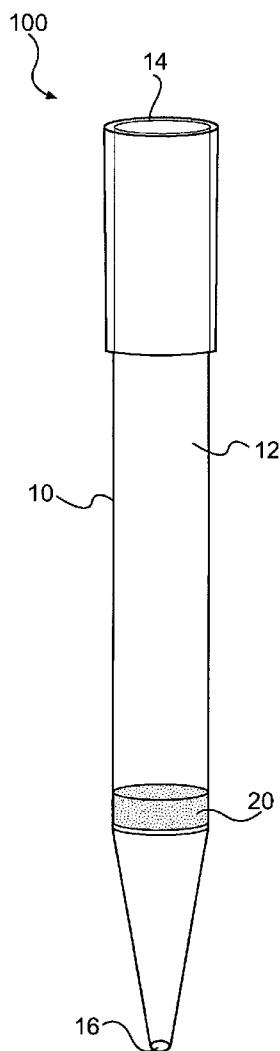




US 20090111193A1

(19) **United States**(12) **Patent Application Publication**
Cooney et al.(10) **Pub. No.: US 2009/0111193 A1**(43) **Pub. Date: Apr. 30, 2009**(54) **SAMPLE PREPARATION DEVICE****Publication Classification**(76) Inventors: **Christopher G. Cooney**, Severn,
MD (US); **Phil Belgrader**, Severna
Park, MD (US)Correspondence Address:
ANDREWS KURTH LLP
Intellectual Property Department
Suite 1100, 1350 I Street, N.W.
Washington, DC 20005 (US)(51) **Int. Cl.**
G01N 1/34 (2006.01)
B01L 11/00 (2006.01)
B01L 3/02 (2006.01)
C12M 1/33 (2006.01)
(52) **U.S. Cl. 436/178; 422/101; 422/100; 422/103;**
435/306.1(57) **ABSTRACT**

A sample preparation device is disclosed. The sample preparation device includes a housing defining a passage way between a first opening and a second opening; and a sample filter occupying a section of said passage way. The sample filter contains a monolith adsorbent that specifically binds to nucleic acids. Also disclosed are sample filters containing glass frit is coated with an capture agent that binds specifically to an analyte of interest, sample filters containing a hydrophilic matrix with impregnated chemicals that lyses cell membranes, a cartridge base and an integrated sample preparation cartridge.

(21) Appl. No.: **12/213,942**(22) Filed: **Jun. 26, 2008****Related U.S. Application Data**(63) Continuation-in-part of application No. 11/933,113,
filed on Oct. 31, 2007.

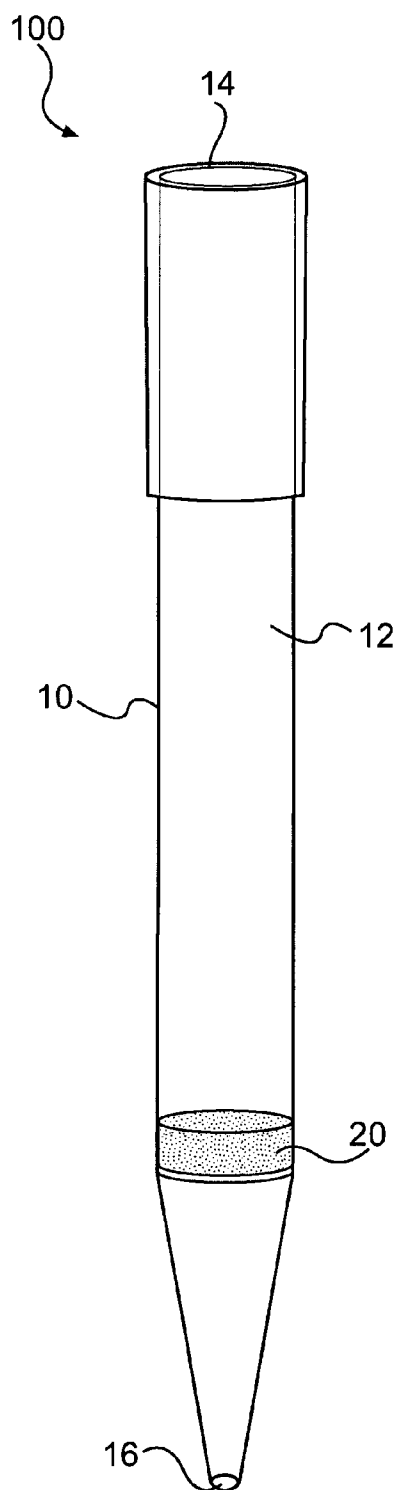


FIG. 1A

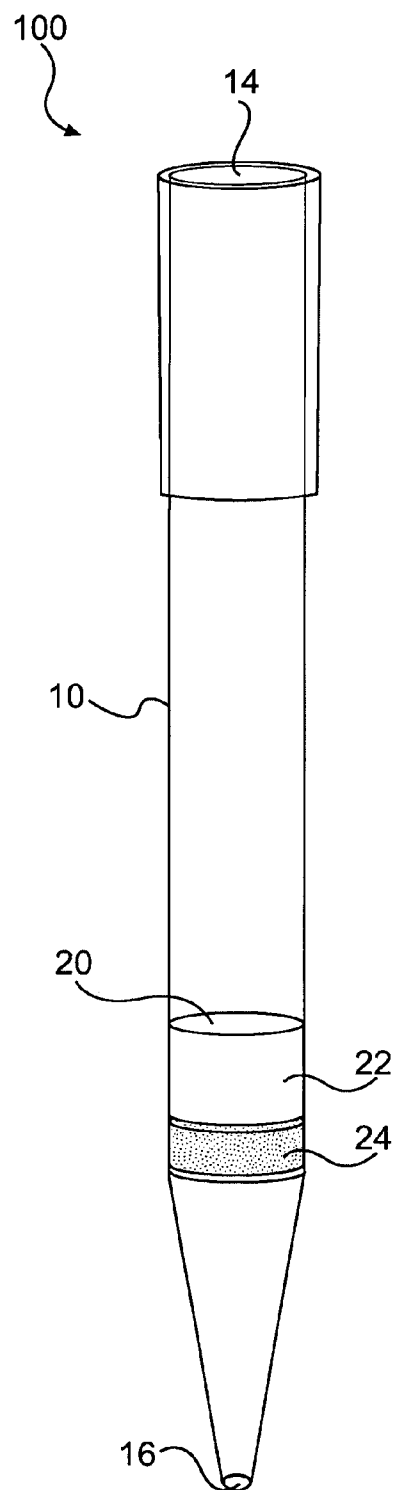


FIG. 1B

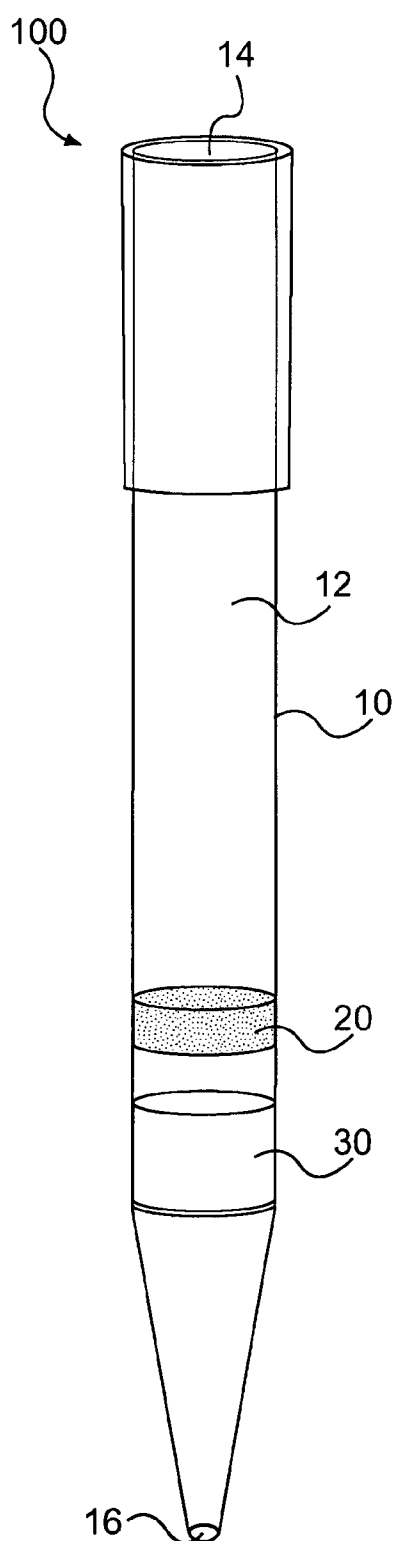


FIG. 1C

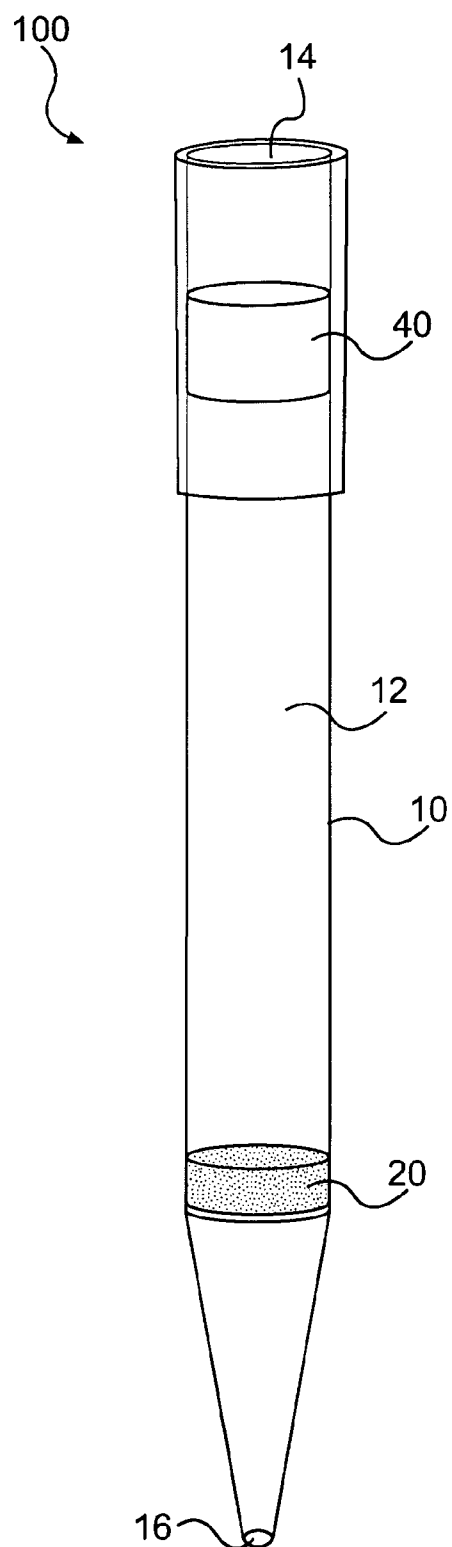


FIG. 1D

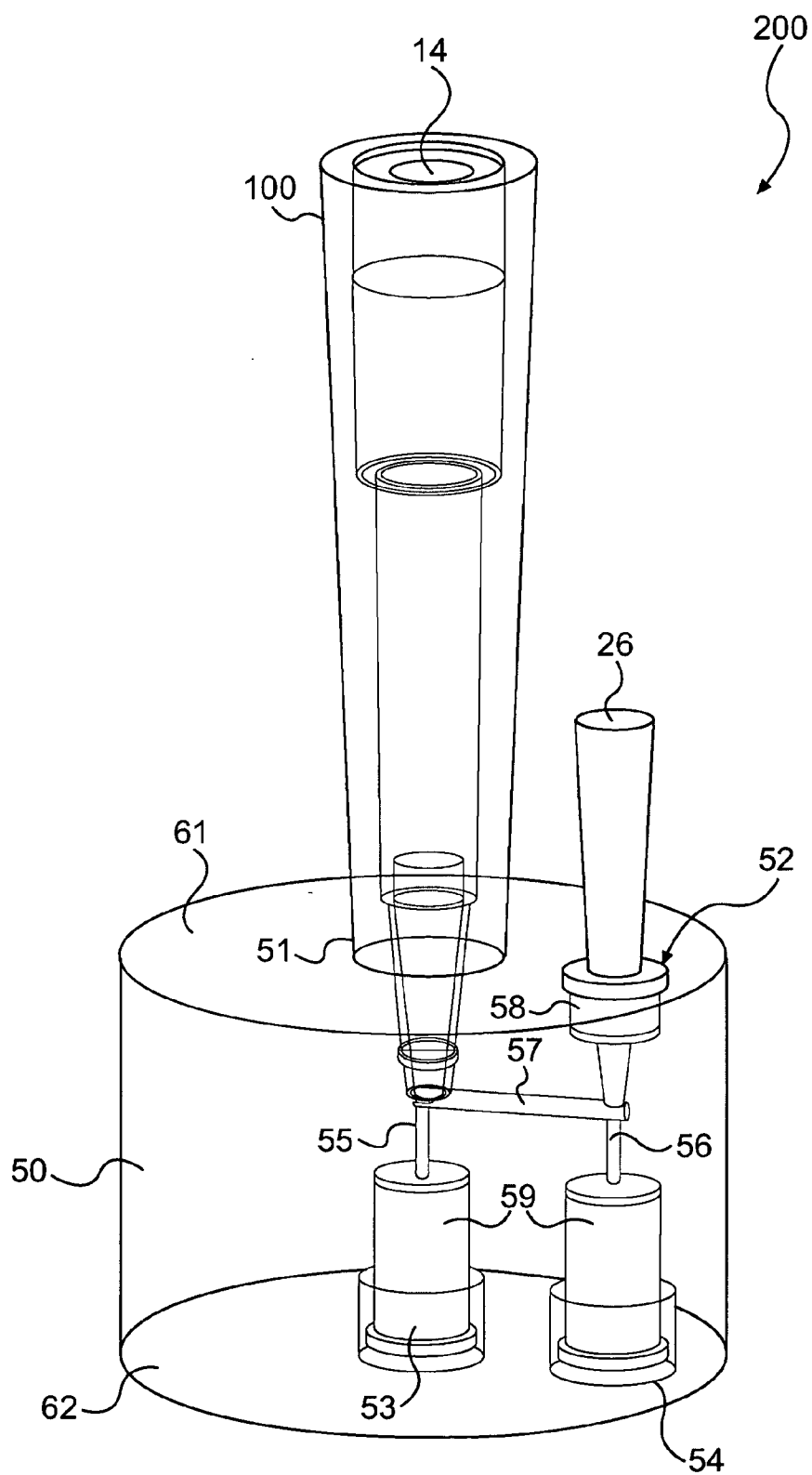


FIG. 2A

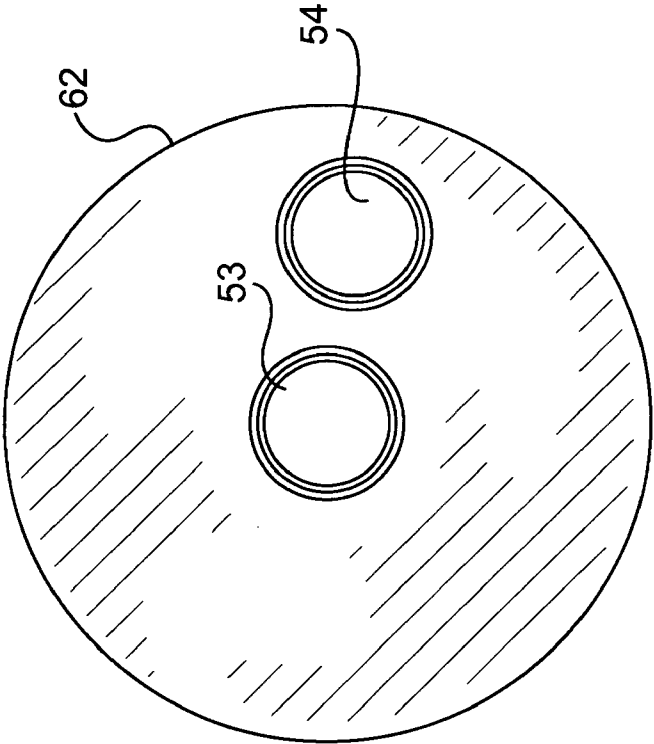


FIG. 2C

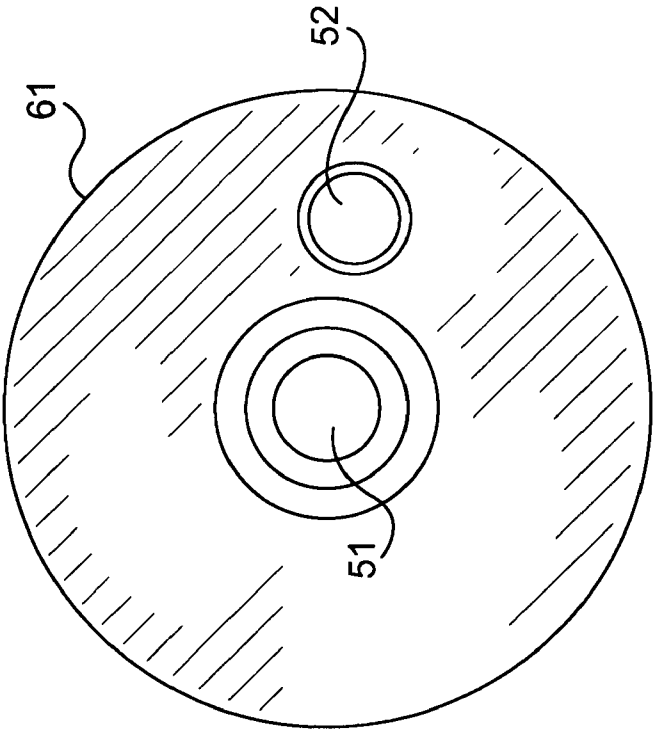


FIG. 2B

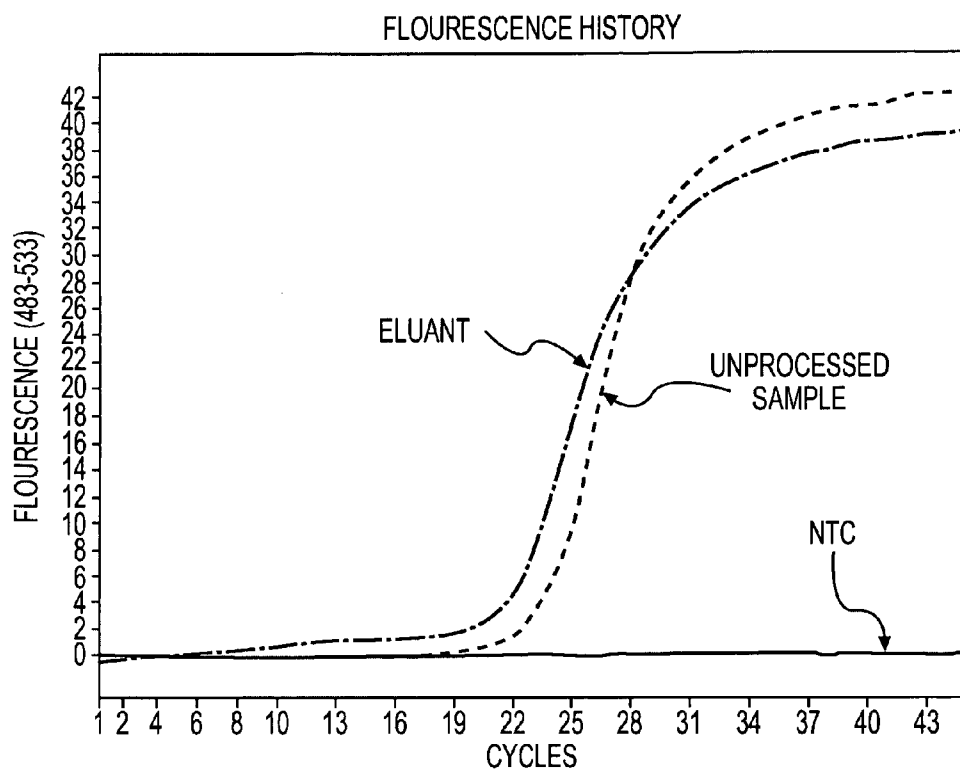


FIG. 3

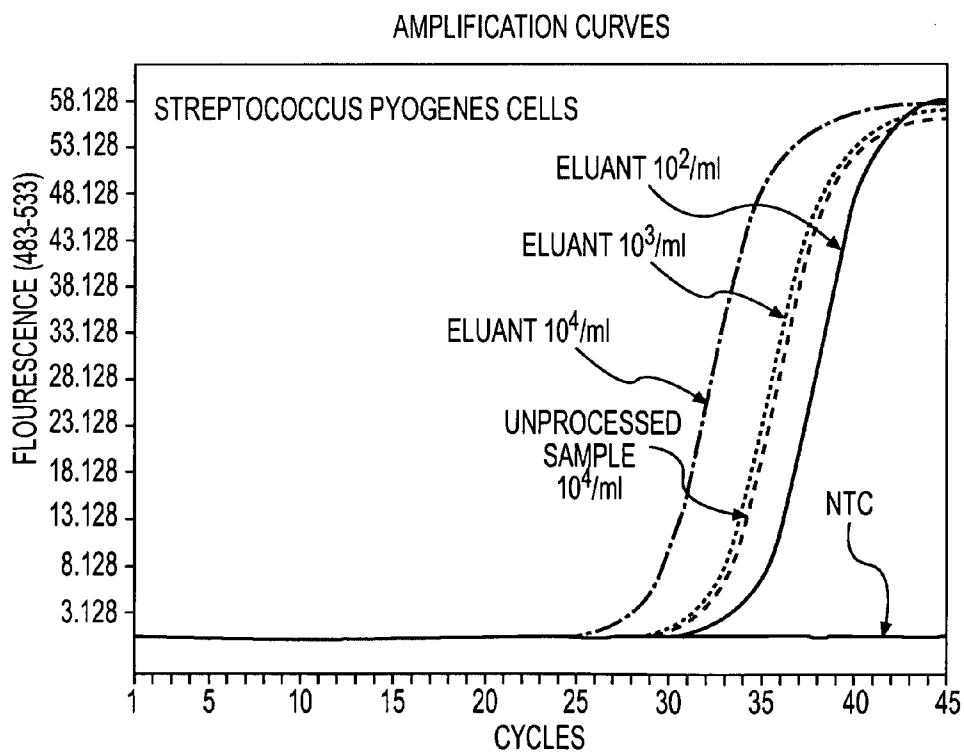


FIG. 4

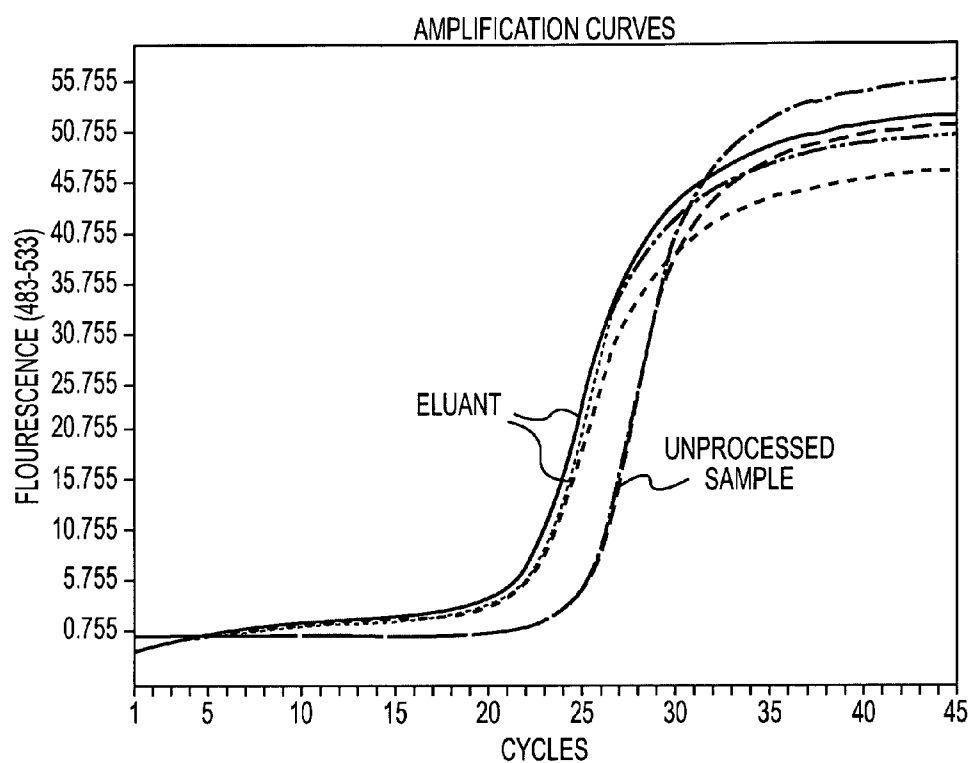


FIG. 5

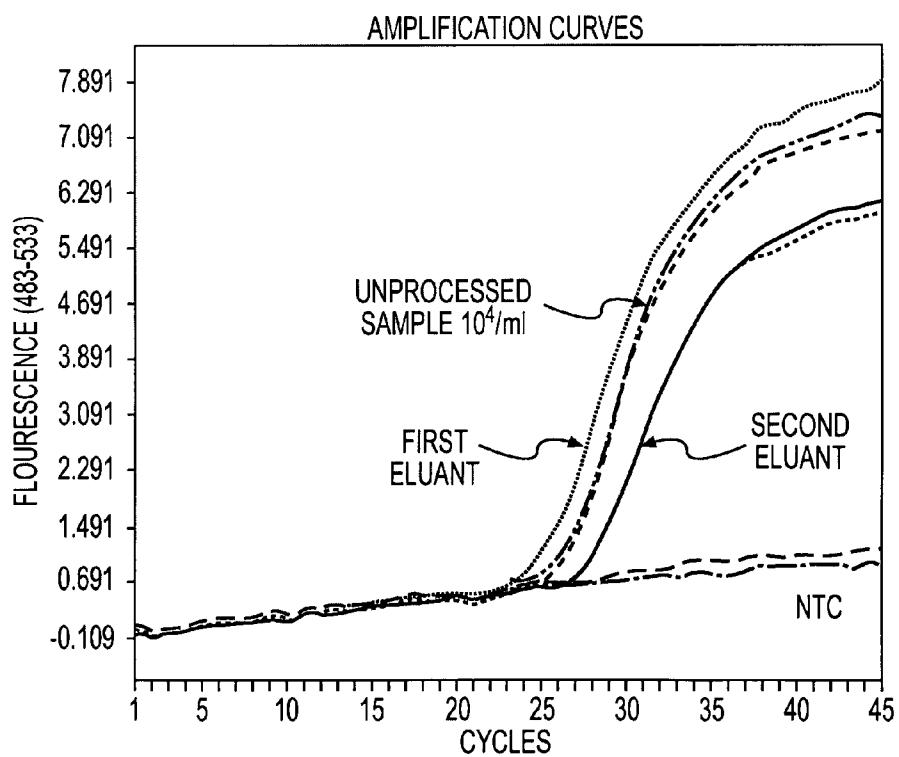


FIG. 6

WORK SEQUENCE			
SEQUENCES/STEPS	DEVICE NAME	FORMAT	PARAMETERS
<input type="checkbox"/> FRIT TIP SAMPLE TOGGLE			
<input checked="" type="checkbox"/> AIR BUBBLE			
FRIT TIP WAS 18 P	18-PORT	PORT #	10
MILLIGAT PUMP AS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
WAIT	SYSTEM	TIME(s)	3
MILLIGAT PUMP DIS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
MILLIGAT PUMP AS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
WAIT	SYSTEM	TIME(s)	3
MILLIGAT PUMP DIS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
MILLIGAT PUMP AS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
WAIT	SYSTEM	TIME(s)	3
MILLIGAT PUMP DIS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
MILLIGAT PUMP AS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
WAIT	SYSTEM	TIME(s)	3
MILLIGAT PUMP DIS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
MILLIGAT PUMP AS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1100,30,1
SELECT WASTE			
MILLIGAT PUMP DIS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	1000,30,1
FRIT TIP WAS 18 P	18-PORT	PORT #	10
MILLIGAT PUMP AS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	600,40,1
SELECT WASTE			
MILLIGAT PUMP DIS	MILLIGAT PU	VOLUME (μL), FLOW RATE(μL/s),HOLD ALL (0/1)	700,30,1
FLUSH TO WASTE			

FIG. 7

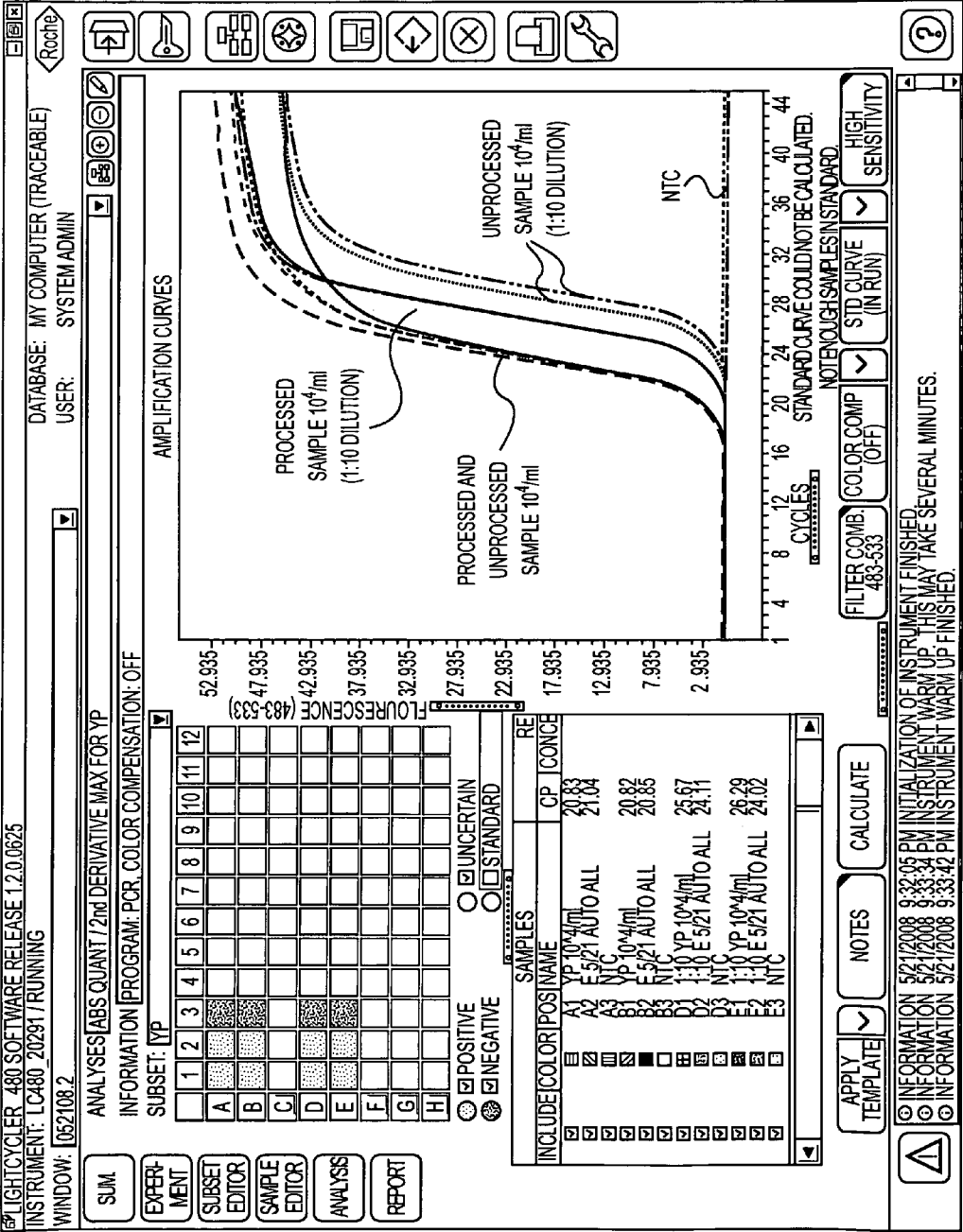


FIG. 8

SAMPLE PREPARATION DEVICE

CROSS-REFERENCE TO RELATED APPLICATION (S)

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 11/933,113 filed Oct. 31, 2007, entitled "APPARATUS, SYSTEM, AND METHOD FOR PURIFYING NUCLEIC ACIDS," which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[0002] The technical field is sample preparation devices in the biochemical art and, in particular, sample preparation devices using a porous monolith filter for sample filtration, separation and purification.

BACKGROUND

[0003] The presence of salts, detergents and other contaminants in a sample can be deleterious to the biochemical analysis of the sample. Many sample preparation devices have been developed in the biochemical art to remove solvent, solute and other molecules/materials from a liquid sample that contains the analyte of interest.

[0004] For example, U.S. Pat. Nos. 6,048,457 and 6,200,474 describes pipette tips with chromatography media in them for the purification of proteins and peptides. The chromatography media typically consists of functionalized glass beads with C4, C18, or a strong cationic resin such as polysulfone, polyethersulfone, polytetrafluoroethylene, cellulose acetate, polystyrene, polystyrene/acrylonitrile copolymer and PVDF. U.S. Pat. No. 6,537,502 also describes a sample purification pipette tip having a solid matrix coating on the interior surface of the pipette tip for sample separation and purification. The solid matrix is composed of a polymeric substance such as polytetrafluoroethylene, polysulfone, polyethersulfone, polystyrene, polystyrene/acrylonitrile copolymer, and polyvinylidene fluoride.

[0005] There still exists a need, however, for sample preparation devices that are easy to use and can be manufactured at low cost.

SUMMARY

[0006] A sample preparation device is disclosed. The sample preparation device includes a housing defining a passage way between a first opening and a second opening; and a sample filter occupying a section of said passage way. The sample filter contains a monolith adsorbent that specifically binds to nucleic acids. Also disclosed are sample filters containing a glass frit, coated with a capture agent that binds specifically to an analyte of interest, and sample filters containing a hydrophilic matrix with impregnated chemicals that lyse cell membranes.

[0007] Also disclosed is an integrated sample preparation cartridge. The integrated sample preparation cartridge includes a sample preparation device and a cartridge base. The sample preparation device includes a housing defining a passage way between a first opening and a second opening, and a sample filter occupying a section of the passage way, the sample filter includes an adsorbent that specifically binds to an analyte of interest. The cartridge base includes a first port configured to interface with the sample preparation device, a

second port configured to interface with a liquid delivery device, and a first channel connecting the first port to the second port.

[0008] Also disclosed is a sample purification system. The sample purification system includes a sample preparation device, a cartridge base and a liquid delivery device.

[0009] Also disclosed is a method for purifying an analyte from a sample. The method includes passing a sample through a sample preparation device comprising a housing defining a passage way between a first opening and a second opening, and a filter occupying a section of the passage way, wherein the sample filter comprising a material that specifically binds to said analyte and the sample passes through the sample filter while passing through the sample preparation device; and eluting analyte bound to the sample filter with an eluting solution, wherein the sample and the eluting solution enter and exit the housing through the same opening.

DESCRIPTION OF THE DRAWINGS

[0010] The detailed description will refer to the following drawings, wherein like numerals refer to like elements, and wherein:

[0011] FIGS. 1A-1D are schematics of various embodiments of a sample preparation device.

[0012] FIG. 2A-2C are schematics of the three-dimensional view (FIG. 2A), the top view (FIG. 2B) and the bottom view (FIG. 2C) of an embodiment of an integrated sample preparation device.

[0013] FIG. 3 shows the real-time PCR analysis of *Bacillus anthracis* nucleic acids purified from a blood sample. Controls include unprocessed *Bacillus anthracis* suspended in water at a concentration of 10^4 cfu/ml and NTC (no template control).

[0014] FIG. 4 shows the real-time PCR analysis of *Streptococcus pyogenes* nucleic acids purified from samples at various *Streptococcus pyogenes* concentrations. Controls are unprocessed sample of *Streptococcus pyogenes* suspended in water at a concentration of 10^4 cfu/ml and NTC (no template control).

[0015] FIG. 5 shows the real-time PCR analysis of *Bacillus anthracis* nucleic acids purified from a nasopharyngeal sample. Controls are unprocessed *Bacillus anthracis* suspended in water at a concentration of 10^4 cfu/ml FIG. 6 shows the real-time PCR analysis of Venezuela Equine Encephalitis virus nucleic acids purified from a blood sample. Controls are unprocessed Venezuela Equine Encephalitis virus suspended in water at a concentration of 10^4 pfu/ml and NTC (no template control). All samples are tested in duplicates.

[0016] FIG. 7 is a screen shot showing the Sample Toggle screen of the Flow Pro Fluidic Handling System FIG. 8 is a screen shot showing the real time PCR result screen of a Flow Pro Fluidic Handling System (Global FIA, Fox Island, Wash.). Controls are unprocessed *Yersinia pestis* suspended in water at a concentration of 10^4 cfu/ml and NTC (no template control).

DETAILED DESCRIPTION

[0017] This description is intended to be read in connection with the accompanying drawings, which are to be considered part of the entire written description of this invention. The drawing figures are not necessarily to scale and certain features of the invention may be shown exaggerated in scale or in somewhat schematic form in the interest of clarity and con-

ciseness. In the description, relative terms such as “front,” “back,” “up,” “down,” “top” and “bottom,” as well as derivatives thereof, should be construed to refer to the orientation as then described or as shown in the drawing figure under discussion. These relative terms are for convenience of description and normally are not intended to require a particular orientation. Terms concerning attachments, coupling and the like, such as “connected” and “attached,” refer to a relationship wherein structures are secured or attached to one another either directly or indirectly through intervening structures, as well as both movable or rigid attachments or relationships, unless expressly described otherwise.

[0018] In describing embodiments of the present invention, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. It is to be understood that each specific element includes all technical equivalents which operate in a similar manner to accomplish a similar purpose.

[0019] One aspect of the present invention relates to a sample preparation device. In one embodiment, the sample preparation device includes a housing that defines a sample passage way between two openings, and a filter structure embedded in a section of the passage way. The filter structure includes a monolith adsorbent that specifically binds to nucleic acids.

[0020] The term “monolith adsorbent” or “monolithic adsorbent material,” as used in the embodiments described hereinafter, refers to a porous, three-dimensional adsorbent material having a continuous interconnected pore structure in a single piece. A monolith is prepared, for example, by casting, sintering or polymerizing precursors into a mold of a desired shape. The term “monolith adsorbent” or “monolithic adsorbent material” is meant to be distinguished from a collection of individual adsorbent particles packed into a bed formation or embedded into a porous matrix, in which the end product comprises individual adsorbent particles. The term “monolith adsorbent” or “monolithic adsorbent material” is also meant to be distinguished from a collection of adsorbent fibers or fibers coated with an adsorbent, such as filter papers or filter papers coated with an adsorbent.

[0021] The term “specifically bind to” or “specific binding,” as used in the embodiments described hereinafter, refers to the binding of the adsorbent to an analyte (e.g., nucleic acids) with a specificity that is sufficient to differentiate the analyte from other components or contaminants of a sample. In one embodiment, the dissociation constant of the adsorbent/ligand complex is less than about 1×10^{-6} M. A person of ordinary skill in the art understands that stringency of the binding and elution of the analyte to the adsorbent can be controlled by binding and elution buffer formulations. For example, elution stringencies for nucleic acids can be controlled by salt concentrations using KCl or NaCl. Nucleic acids, with their higher negative charge, are more resistant to elution than proteins. Temperature, pH, and mild detergent are other treatments that could be used for selective binding and elution. Thermal consistency of the binding and elution may be maintained with a heat block or a water bath. The manipulation of the binding buffer is preferable since the impact of the modified elution buffer on the downstream analyzer would need to be evaluated.

[0022] The term “nucleic acid,” as used in the embodiments described hereinafter, refers to individual nucleic acids and polymeric chains of nucleic acids, including DNA and RNA, whether naturally occurring or artificially synthesized (in-

cluding analogs thereof), or modifications thereof, especially those modifications known to occur in nature, having any length. Examples of nucleic acid lengths that are in accord with the present invention include, without limitation, lengths suitable for PCR products (e.g., about 50 to 700 base pairs (bp)) and human genomic DNA (e.g., on an order from about kilobase pairs (Kb) to gigabase pairs (Gb)). Thus, it will be appreciated that the term “nucleic acid” encompasses single nucleic acids as well as stretches of nucleotides, nucleosides, natural or artificial, and combinations thereof, in small fragments, e.g., expressed sequence tags or genetic fragments, as well as larger chains as exemplified by genomic material including individual genes and even whole chromosomes.

[0023] Referring now to FIG. 1A, an embodiment of the sample preparation device **100** includes a housing **10** and a sample filter **20**. The housing **10** defines a sample passage way **12** between a first opening **14** and a second opening **16**. The shape and size of the housing **10** are not particularly limited. The preferred housing configuration is substantially cylindrical so that the flow vectors during operation are substantially straight, thereby minimizing or avoiding dilutional washing that might occur with non-cylindrical configurations. In the embodiments shown in FIGS. 1A-1D, the housing **10** has a pipette tip geometry, i.e., the first opening **14** has a diameter that is greater than the diameter of said second opening **16**, and the first opening **14** is dimensioned to fit onto the tip of a pipettor. The sample filter **20** is placed in the close proximity of the second opening **16** so that samples are filtered immediately after being taken into the housing **10** through the second opening **16**. In one embodiment, the sample filter **20** is contiguous with the second opening **16**. In another embodiment, the sample filter **20** is separated from the second opening **16** by a distance of 1-20 mm. In another embodiment, the housing **10** has a column geometry.

[0024] In one embodiment, the housing **10** has a volume of about 0.1 μ l to about 10 ml. In another embodiment, the housing **10** has a volume of about 5 μ l to about 5 ml. Suitable materials for the housing **10** are not particularly limited, and include plastics (such as polyethylene, polypropylene, and polystyrene), glass and stainless steel.

[0025] The sample filter **20** can be made of any porous monolithic material that binds specifically to nucleic acids. The porosity of the porous monolithic material is application dependent. In general, the porous monolithic material should have a porosity that allows for a desired sample flow rate for a particular application.

[0026] In one embodiment, the sample filter **20** is made of a finely porous glass frit through which a liquid sample may pass. Porous glass frits, which are sintered glass that begins with crushing beads in a hot press to form a single monolithic structure, are excellent substrates for purifying nucleic acids. The uniform structure of the frit provides predictable liquid flow inside the frit and allows the eluent to have similar fluid dynamics as the sample flow. The predictable liquid flow also leads to a higher recovery during the elution process.

[0027] Exemplary glass frit pore sizes suitable for use with the present invention, including the various embodiments described herein, are between about 2 microns and about 220 microns. In one embodiment, the glass frit has a pore size between about 2 microns and about 100 microns. In another embodiment, the glass frit has a pore size between about 40 microns and about 75 microns. In another embodiment, the glass frit has a pore size between about 150 microns and about 200 microns. In yet another embodiment, the glass frit has a

pore size between about 2 microns and about 20 microns. For applications involving purification of microbial DNA, a glass frit size of between about 10 microns and about 15 microns is suitable.

[0028] In one embodiment, the glass frit has a thickness between about 1 mm and about 20 mm. In another embodiment, the glass frit has a thickness between about 2 mm and about 5 mm. In yet another embodiment, the glass frit has a thickness between about 2 mm and about 3 mm.

[0029] In another embodiment, the glass frit is chemically treated to functionalize its surface. For example, the glass frits may be derivatized with aminosilanes or treated with the ChargeSwitch® technology (Invitrogen, Carlsbad, Calif.) to create positive charges for better adsorption of the negatively charged nucleic acids.

[0030] While the glass frit is a good adsorbent for nucleic acids, a skilled artisan would recognize that the glass frit may also be used to absorb other types of molecules. For example, the glass frit may be coated with antibodies to extract other ligand of interest from the sample. In one embodiment, the glass frit is derivatized in polymethylmethacrylate (PMMA) and cyclo-olefin-copolymer (COC) with antibodies as capture moieties for microbes and toxin. The term “antibody”, as used herein, is used in the broadest possible sense and may include but is not limited to an antibody, a recombinant antibody, a genetically engineered antibody, a chimeric antibody, a monospecific antibody, a bispecific antibody, a multispecific antibody, a chimeric antibody, a heteroantibody, a monoclonal antibody, a polyclonal antibody, a camelized antibody, a deimmunized antibody, and an anti-idiotypic antibody. The term “antibody” may also include but is not limited to an antibody fragment such as at least a portion of an intact antibody, for instance, the antigen binding variable region. Examples of antibody fragments include Fv, Fab, Fab', F(ab'), F(ab')₂, Fv fragment, diabody, linear antibody, single-chain antibody molecule, multispecific antibody, and/or other antigen binding sequences of an antibody. In another embodiment, the glass frit is coated with lectins, which bind to carbohydrates found in bacteria coats and can be used to capture bacteria in a sample.

[0031] In another embodiment, the sample filter **20** is made of a porous glass monolith, a porous glass-ceramic, or porous monolithic polymers. Porous glass monolith may be produced using the sol-gel methods described in U.S. Pat. Nos. 4,810,674 and 4,765,818, which are hereby incorporated by reference. Porous glass-ceramic may be produced by controlled crystallization of a porous glass monolith.

[0032] Porous monolithic polymers are a new category of materials developed during the last decade. In contrast to polymers composed of very small beads, a monolith is a single, continuous piece of a polymer prepared using a simple molding process. In one embodiment, the housing **10** serves as the mold for the porous monolithic polymers. Briefly, a section of the passage way **12** of the housing **10** is filled with a liquid mixture of monomers and porogens. Next, a mask that is opaque to ultraviolet light is placed over the filled section. The mask has a small slit that exposes a small portion of the filled section. Finally, the monomers/porogens mixture in the filled section is irradiated with ultraviolet light through the tiny opening on the mask. The UV irradiation triggers a polymerization process that produces a solid but porous monolithic material in the filled section of the passage way **12**.

[0033] In yet another embodiment, the sample filter **20** is made of a hydrophilic matrix with impregnated chemicals that lyses cell membranes, denaturing proteins, and traps nucleic acids. In one embodiment, the hydrophilic matrix is FTA paper® (Whatman, Florham Park, N.J.). Biological samples are applied to the FTA® paper and cells contained in the sample are lysed on the paper. The paper is washed to remove any non-DNA material (the DNA remains entangled within the paper). The DNA is then eluted for subsequent analysis.

[0034] The sample filter **20** is shaped to fit tightly into the passage way **12** to prevent the sample from channeling or bypassing the sample filter **20** during operation. In one embodiment, the filter **20** is fitted into the passage way **12** through mechanical means such as crimping, press fitting, and heat shrinking the housing **10** or a portion thereof. In another embodiment, the filter **20** is attached to the interior of passage way **12** through an adhesive. In yet another embodiment, the side of the frit is tapered to the contour of the passage way **12**. In the embodiments shown in FIGS. 1A-1D, the housing **10** has the shape of a frustoconical pipette tip with the first opening **14** dimensioned to fit on the end of a liquid delivery system, such as a manual pipettor or an electronic pipetting device. Samples are taken up through the second opening **16**, passed through the sample filter **20** and then retained in the section of the housing **10** that is above the sample filter **20**. In one embodiment, the liquid delivery system is an electronic pipetting device, such as an electronic pipettor or a robotic pipetting station.

[0035] In one embodiment, the sample filter **20** includes at least two sections, a first section **22** that binds specifically to nucleic acids and a second section **24** that specifically binds to another analyte of interest, such as proteins (FIG. 1B). In another embodiment, the housing **10** contains a pre-filter **30** placed between the second opening **16** and the sample filter **20** (FIG. 1C). The pre-filter **30** has a pore size that is larger than the pore size of the sample filter **20** and does not bind specifically to nucleic acids. In yet another embodiment, the housing contains an aerosol filter **40** in the proximity of the first opening **14** to prevent contamination from the pumping device (FIG. 1D).

[0036] In another embodiment, the housing **10** further contains a plurality of mechanical lysing beads, such as glass beads, in the space between the sample filter **20** and the aerosol filter **40**. The mechanical lysing beads are used to disrupt the cells and release the nucleic acid by vortexing the entire sample preparation device **100**. In this embodiment, the second opening **16** may be covered with a cap during vortexing to prevent the liquid from escaping from the second opening **16**.

[0037] Another aspect of the present invention relates to an integrated sample preparation cartridge. Referring now to FIGS. 2A-2C, an embodiment **200** of the integrated sample preparation cartridge includes a base **50** and the sample preparation device **100**. The base **50** contains a first sample port **51** and a second sample port **52** on the top surface **61**, a third sample port **53** and a fourth sample port **54** at the bottom surface **62**, a first channel **55** connecting the first sample port **51** to the third sample port **53**, a second channel **56** connecting the second sample port **52** to the fourth sample port **54**, and a third channel **57** connecting the first channel **55** and the second channel **56**.

[0038] The first sample port **51** is configured to receive the sample preparation device **100**, so that the sample preparation

device **100**, whether in a column configuration or pipette tip configuration, can be easily inserted into the first sample port **51** and form a liquid-tight seal with the base **50**.

[0039] Once attached to the first sample port **51**, the sample preparation device **100** maintains a vertical position. A sample may be loaded onto the sample preparation device **100** from either the first opening **14** (i.e., going down the sample passage way **12**) or the second opening **16** (i.e., going up the sample passage way **12**). Alternatively, the sample preparation device **100** may be attached to the first sample port **51** with a pre-loaded sample.

[0040] The second sample port **52** can also be used to introduce a liquid into the integrated sample preparation cartridge **200** or to take out a liquid from the integrated sample preparation cartridge **200**. The second sample port **52** is configured to receive the tip **26** of a liquid delivering device, such as a pipettor or a robotic pipetting station. In one embodiment, the second sample port **52** is a self-sealing inlet containing a seal **58** that can be punctured by a pipette tip and seals after the removal of the tip. Such a self-sealing entry port for a pipette allows easy introduction of the sample without the risk of opening caps, which are often a cause of contamination. In one embodiment, the seal **58** is a Multisip™ split septum plug from Abgene (Epsom, UK). In another embodiment, the seal **58** is a port valve, such as the Duckbill valves and dome valves from Minivalve International (Yellow Springs, Ohio). In another embodiment, the first sample port **51** also contains a self-sealing device, such as a dome valve or a septum, that is receptive to the sample preparation device **100**.

[0041] In another embodiment, either the first sample port **51** or the second sample port **52** or both ports can be sealed with a screw cap or a press fit cap to allow the introduction and removal of samples. The ports can also be sealed with a tape seal to prevent leaking during the automation process.

[0042] The first channel **55**, the second channel **56** and the third channel **57** connect the first sample port **51** to the second sample port **52** so that the nucleic acid purification process can be completed within the integrated sample preparation cartridge **200**. The third sample port **53** and the fourth sample port **54** may be connected to waste bottles to collect the flow-through from the sample preparation device **100**.

[0043] The integrated sample preparation cartridge **200** can be configured to be compatible with fluidic control systems, such as the Flow Pro Fluidic Handling System (Global FIA, Fox Island, Wash.). In one embodiment, the first sample outlet **53** and the second sample outlet **54** are fitted with Luer-activated valves **59**. The Luer-activated valves **59** are normally closed valves that may be opened only upon insertion of a Luer-type fitting. The Luer-activated valves **59** allow easy insertion into the fluidic control system and prevent leaking of sample from the sample preparation cartridge **200** after the sample preparation cartridge **200** is removed from the fluidic control system. In one embodiment, the integrated sample preparation cartridge **200** is designed to be plugged into a fluidic control system without the need for tightening screws or adjusting bolts.

[0044] A person of ordinary skill in the art understands that the general layout of the integrated sample preparation cartridge **200** allows for other sample introduction and elution withdrawal strategies. In one embodiment, the sample preparation cartridge **200** is connected to a fluidic control system. The sample preparation device **100** is inserted into the first sample port **51**. A sample is introduced into the integrated sample preparation cartridge **200** through the second sample

port **52**, which is sealed off after the introduction of the sample. A chaotrope, such as guanidine, is introduced into the integrated sample preparation cartridge **200** through the fourth sample port **54** by the fluidic control system and mixed with the sample within the integrated sample preparation cartridge **200**. The sample/chaotrope mixture is then pushed into the sample preparation device **100** from the second opening **16** of the sample preparation device **100**, passing the filter **20** and entering the section of the housing **10** that is above the sample filter **20**. The sample/chaotrope mixture is then withdrawn from the integrated sample preparation cartridge **200** through the fourth sample port **54** and discarded as waste. A washing buffer is introduced into the integrated sample preparation cartridge **200** through the third sample port **53** by the fluidic control system. Similar to the movement of the sample/chaotrope mixture, the washing buffer is forced into and then withdrawn from the sample preparation device **100**, passing the filter **20** twice during the process. The washing step may be repeated several times. Finally, an eluting buffer is introduced into the sample preparation device **100** through the second opening **16**, eluting the bound nucleic acids into the section of the housing **10** that is above the sample filter **20**, from where the eluant is removed for further analysis.

[0045] In another embodiment, the sample is introduced through the first opening **14** of the sample preparation device **100** which is attached to the first sample port **51**, and the eluant is removed from the second sample port **52**. In another embodiment, the sample is introduced through the first opening **14** of sample preparation device **100**, which is attached to the first sample port **51**, and the eluant is removed from the first opening **14** of sample preparation device **100**. In another embodiment, the sample is introduced onto the sample preparation device **100**, which is attached to the first sample port **51**, through the second sample port **52**, and the eluant is removed from the second sample port **52**. In another embodiment, the sample is pre-loaded into the sample preparation device **100** before the sample preparation device **100** is inserted into the first sample port **51** of the integrated sample preparation cartridge **200**. After the washing and eluting steps, the eluant is removed from the second sample port **52**. In yet another embodiment, the sample is pre-loaded into the sample preparation device **100** before the sample preparation device **100** is inserted into the first sample port **51** of the integrated sample preparation cartridge **200**. After the washing step, the analyte bound on the sample filter **20** are eluted into the sample preparation device **100**, which is then removed from the first sample port **51** with the purified analyte in the elution buffer within the space between the sample filter **20** and the aerosol filter **40**.

[0046] The integrated sample preparation cartridge **200** is easy to use. First, this device does not require centrifugation and thus eliminates the complexity associated with transferring samples from tubes to spin columns as well as simplifies the instrumentation required. Additionally a self-sealing entry port for a pipettor allows easy introduction of the sample without the risk of opening caps, which are often a cause of contamination. Additionally, the Luer-activated valves make cartridge insertion and removal simple and easy without the risk of losing sample due to leakage after the process is complete.

[0047] In addition, the vertical orientation of the sample preparation device **100** forces bubbles to rise to the top of the device from the sample filter **20**, which improves fluidic control and enhances analyte binding and elution. Addition-

ally, the small pores of the sample filter **20** reduces large air boluses into small bubbles which migrate to the top of the liquid column inside the sample passage way **12**, creating a vibrant mixing effect of the chaotrophe with the sample. It should be noted that the pipette tip configuration of the sample preparation device **100** allows bidirectional flow of the sample/washing/elution liquids through the sample filter **20**, while most sample preparation approaches rely on flow in only one direction through the filter. The bidirectional flow feature not only allows the sample liquid to be taken into the sample preparation device **100** and eluted out of the sample preparation device **100** from the same opening (e.g., the second opening **16**), but also permits a user to pipette a sample up and down for a number of cycles, thus providing the capability to process sample volumes larger than that of the sample preparation device **100**.

[0048] In one embodiment, the channels **55**, **56** and **57** are designed to have the shortest possible length to reduce unwanted biomolecular (nucleic acid) adsorption to the interior surfaces of the integrated sample preparation cartridge **200**. The channels may also be surface coated to reduce unwanted biomolecule adsorption. In one embodiment, the channels **55**, **56** and **57** have diameters in the range of 0.1-5 mm to reduce the surface-to-volume ratios and therefore reduce unwanted nucleic acid adsorption.

[0049] After the removal of the eluant, the integrated sample preparation cartridge **200** is removed from the Flow Control Station and discarded.

[0050] The base **50** of the integrated sample preparation cartridge **200** can be made of any material that is resistant to the chemicals commonly used in the sample solubilization/washing/eluting process. In one embodiment, the base **50** is made of a transparent material. Examples of the base **50** materials include, but are not limited to, polycarbonate and polypropylene.

[0051] The fluidic control system can be any fluidic control system that is capable of providing the desired flow rate in the integrated sample preparation cartridge **200**. In one embodiment, the fluidic control system is the Flow Pro Fluidic Handling System by Global FIA (Fox Island, Wash.).

EXAMPLES

Example 1

Purification of Nucleic Acids from Blood Sample Containing *Bacillus anthracis* Using 2.0 ml Rainin Filtered Pipette Tip and 3 mm Glass Frit

[0052] In this experiment, nucleic acids were purified from a blood sample containing *Bacillus anthracis* using a 2.0 ml Rainin filtered pipette tip and a 3 mm glass frit (ROBU Glasfilter-Geraete GmbH, Germany) with the following protocol:

- [0053] 1. Label one 15 ml conical tube as: Flow Through, and four 1.5 ml centrifuge tubes as: Ethanol 1, Ethanol 2, Ethanol 3, and Eluant.
- [0054] 2. Mix 360 μ l of blood with 40 μ l of *Bacillus anthracis* (10^5 colony forming unit (cfu)/ml in water) in the Flow Through tube (final concentration 10^4 cfu/ml).
- [0055] 3. Add 1120 μ l of Qiagen AL Lysis Buffer to the mixture.
- [0056] 4. Add 80 μ l of Proteinase K (20 mg/ml).
- [0057] 5. Add 400 μ l of lysozyme, vortex and spin down.
- [0058] 6. Incubate the sample mixture at 55° C. for 1 hour.

- [0059] 7. Add 2000 μ l of 96-100% ethanol to the Flow Through Tube. Vortex and pulse spin down the mixture.
 - [0060] 8. Aliquot 100 μ l of elution buffer (10 mM Tris, pH 8.0) into the Eluant tube. Place the tube on the heat block set at 70° C. (Elution buffer must be heated at 70° C. for at least 5 minutes. Keep the buffer on the heat block until step 13.)
 - [0061] 9. Aliquot 1 ml of 70% Ethanol into each of the three Ethanol tubes.
 - [0062] 10. Pipette sample mixture into the Flow Through tube using frit tip (medium porosity) with a Rainin Electronic Pipettor. Pipette for 5 cycles (cycle=aspirate+dispense).
 - [0063] 11. Wash the bound nucleic acids by pipetting the 70% EtOH in Ethanol 1 tube for 10 cycles using a Rainin electronic Pipettor. Repeat the wash with the 70% EtOH in Ethanol 2 tube and Ethanol 3 tube (three washes total).
 - [0064] 12. Purge the EtOH from the frit tip by pipetting air for 20 cycles. Wipe the outside of the tip and tap the tip gently if a noticeable amount of ethanol is left.
 - [0065] 13. Elute the nucleic acids on the frit by pipetting the 70° C. elution buffer of step 8 for 10 cycles. The buffer may start to bubble but continue pipetting and spin down the microcentrifuge tube once complete. Make sure all the buffer has been purged from the tip.
 - [0066] 14. Collect the eluant and discard the frit tip
 - [0067] 15. Quantitating the eluted nucleic acids with real time PCR.
- [0068] As shown in FIG. 3, nucleic acids from *Bacillus anthracis* are detected in the eluant.

Example 2

Purification of Nucleic Acids from *Streptococcus pyogenes* Using 2.0 ml Rainin Filtered Pipette Tip and 2 mm Glass Frit

[0069] In this experiment, nucleic acids were purified from *Streptococcus pyogenes* suspensions of various concentrations using a 2.0 ml Rainin filtered pipette tip and a 2 mm glass frit (ROBU Glasfilter-Geraete GmbH, Germany) with the following protocol:

- [0070] 1. Label three 1.5 ml centrifuge tubes as: Sample+Guanidine, Ethanol, and Eluant.
- [0071] 2. Lyse by vortexing with glass beads.
- [0072] 3. Mix 500 μ l of *Streptococcus pyogenes* sample with 500 μ l of 6M guanidine, pH 6.5, by vortexing. An aliquot of unprocessed (i.e., the pre-guanidine) *Streptococcus pyogenes* were also saved as a control for real-time PCR analysis in step 10.
- [0073] 4. Aliquot 100 μ l elution buffer (1 mM NaOH) into the eluant tube. Pipette the sample/guanidine mixture with a frit tip (medium porosity) and a Rainin Electronic Pipettor. Pipette for 5 cycles (cycle=aspirate+dispense).
- [0074] 5. Pipette 1 ml 70% EtOH to wash bound nucleic acids using the Rainin electronic Pipettor for 5 cycles.
- [0075] 6. Pass air through the frit tip to purge EtOH using the electronic pipettor. Repeat pipetting for 20 cycles to remove traces of EtOH. Tap the frit tip gently if a noticeable amount of ethanol is left. Remove the EtOH on the outside of the tip with Kimwipe.

- [0076] 7. Elute the nucleic acids from the frit with the 70° C. elution buffer from step 4 by pipetting for 10 cycles. Make sure all the elution buffer has been purged from the tip.
- [0077] 8. Collect the eluant and discard the frit tip
- [0078] 9. Quantitating the eluted nucleic acids with real time PCR.
- [0079] As shown in FIG. 4, *Streptococcus pyogenes* nucleic acids are detected in samples prepared by the frit tip.

Example 3

Purification of Nucleic Acids from Nasopharyngeal Sample Containing *Bacillus anthracis* Using 2.0 ml Rainin Filtered Pipette Tip and 2 mm Glass Frit

[0080] In this experiment, nucleic acids were purified from nasopharyngeal sample containing *Bacillus anthracis* using a 2.0 ml Rainin filtered pipette tip and a 2 mm frit (ROBU Glasfilter-Geraete GmbH, Germany) with the following protocol:

- [0081] 1. Label three 1.5 ml centrifuge tubes as: Sample+Guanidine, Ethanol, and Eluant.
- [0082] 2. Prepare nasopharyngeal sample by mixing 450 µl of nasopharyngeal with 50 µl of *Bacillus anthracis* (10⁵ colony forming unit (cfu)/ml in water) in the Sample+Guanidine tube (final concentration 10⁴ cfu/ml). Save an aliquot of the nasopharyngeal sample as control in the real-time PCR analysis of Step 11.
- [0083] 3. Add 500 µl of 6M guanidine, pH 6.5, into the Sample+Guanidine tube and vortex.
- [0084] 4. Aliquot 100 µl of elution buffer (10 mM Tris, pH 8.0) into the Eluant tube. Place the tube on the heat block set at 70° C. (elution buffer must heat at 70° C. for at least 5 minutes). Keep the tube on the heat block until step 9.
- [0085] 5. Pipette the sample/guanidine mixture using a frit tip (medium porosity) with a Rainin Electronic Pipettor. Pipette for 5 cycles (cycle=aspirate+dispense).
- [0086] 6. Pipette 1 ml 70% EtOH to wash bound nucleic acids using the Rainin electronic Pipettor.
- [0087] 7. Pass air through the frit tip to purge EtOH using the electronic pipettor. Repeat pipetting for 20 cycles to remove traces of EtOH. Tap the frit tip gently if a noticeable amount of ethanol is left. Remove the EtOH on the outside of the tip with Kimwipe.
- [0088] 8. Elute the nucleic acids from the frit with the 70° C. elution buffer from step 4 by pipetting for 10 cycles. Make sure all the elution buffer has been purged from the tip.
- [0089] 9. Collect the eluant and discard the frit tip
- [0090] 10. Quantitating the eluted nucleic acids with real time PCR.
- [0091] As shown in FIG. 5, nucleic acids from *Bacillus anthracis* are detected in the eluant.

Example 4

Purification of Nucleic Acids from Blood Sample Containing Venezuela Equine Encephalitis Virus Using 1.2 ml Gilson Filtered Pipette Tip and 5 mm Glass Frit

[0092] In this experiment, nucleic acids were purified from blood sample containing Venezuela Equine Encephalitis

virus using a 2.0 ml Rainin filtered pipette tip and a 5 mm glass frit (ROBU Glasfilter-Geraete GmbH, Germany) with the following protocol:

- [0093] 1. Label six 1.5 ml centrifuge tubes as: Flow Through, Ethanol 1, Ethanol 2, Ethanol 3, Eluant 1 and Eluant 2.
- [0094] 2. Mix 90 µl of blood with 10 µl of Venezuela Equine Encephalitis virus (10⁵ plaque forming unit (pfu)/ml in water) in the Flow Through tube (final concentration 10⁴ pfu/ml).
- [0095] 3. Add 280 µl of Qiagen AL Lysis Buffer to the mixture.
- [0096] 4. Add 40 µl of Proteinase K (20 mg/ml).
- [0097] 5. Add 100 µl of lysozyme, vortex and spin down.
- [0098] 6. Incubate the sample mixture at 55° C. for 1 hour.
- [0099] 7. Add 500 µl of 96-100% ethanol to the Flow Through Tube. Vortex and pulse spin down the mixture.
- [0100] 8. Aliquot 100 µl of elution buffer (10 mM Tris, pH 8.0) into the Eluant tubes. Place the tubes on the heat block set at 70° C. (Elution buffer must be heated at 70° C. for at least 5 minutes. Keep the tubes on the heat block until step 13.)
- [0101] 9. Aliquot 1 ml of 70% Ethanol into each of the three Ethanol tubes.
- [0102] 10. Pipette sample mixture into the Flow Through tube using frit tip (medium porosity) with a Gilson Electronic Pipettor. Pipette for 5 cycles (cycle=aspirate+dispense). Wash the bound nucleic acids by pipetting the 70% EtOH in Ethanol 1 tube for 10 cycles using the electronic Pipettor. Repeat the wash with the 70% EtOH in Ethanol 2 tube and Ethanol 3 tube (three washes total). Purge the EtOH from the frit tip by pipetting air for 20 cycles. Wipe the outside of the tip and tap the tip gently if a noticeable amount of ethanol is left.
- [0103] 11. Elute the nucleic acids on the frit by pipetting the 70° C. elution buffer of step 8 for 10 cycles. Remove the elution buffer from the heat block once the cycles are completed. Make sure all the buffer has been purged from the tip.
- [0104] 12. Collect the eluant in Eluant 1 tube.
- [0105] 13. Repeat the step 13 with the same frit tip, collect the eluant in Eluant 2 tube, and discard the frit tip
- [0106] 14. Quantitating the eluted nucleic acids with real time PCR.
- [0107] As shown in FIG. 6, nucleic acids from Venezuela Equine Encephalitis virus are detected in both the first and second eluant. The first eluant, however, contains nucleic acids of Venezuela Equine Encephalitis at a much higher concentration.

Example 5

Automatic Sample Preparation Using Fluidic Control System and the Integrated Sample Preparation System

[0108] A prototype of the integrated sample preparation device shown in FIG. 2A was connected to a Flow Pro Fluidic Handling System (Global FIA, Fox Island, Wash.). Nucleic acids were purified from *Yersinia pestis* suspension with the following protocol:

- [0109] 1. Label two 1.5 ml centrifuge tubes as: Sample and Eluant.

- [0110] 2. Aliquot 150 μ l of 1 mM NaOH into a tube designated "Elution Buffer" which is located on the Global FIA system.
- [0111] 3. Aliquot 500 μ l 70% EtOH into a Wash tube which is located on the Global FIA system.
- [0112] 4. Mix the 500 μ l of *Yersinia pestis* suspension (10^4 cfu/ml in water) with 500 μ l of 6M guanidine, pH 6.5, in the "sample" tube (step 1) and vortex. Save some un-mixed sample for analysis later.
- [0113] 5. Pipette the sample/guanidine mixture into frit tip (medium porosity) with a Rainin Electronic Pipettor.
- [0114] 6. Place the frit tip (with sample inside) onto the Sample Prep Cartridge located on the Global FIA device.
- [0115] 7. Perform the "Frit Tip Sample Toggle" sequence (FIG. 7) using the FloLV Software. 8. Perform the "Frit Tip EtOH Wash" sequence using the FloLV Software.
- [0116] 9. Perform the "Frit Tip EtOH Dry" sequence using the FloLV Software.
- [0117] 10. Perform the "Frit Tip Elution" sequence using the FloLV Software.
- [0118] 11. Once the sequence is completed, remove the frit tip from the Global FIA system, attach the frit tip to a Rainin Electronic Pipettor and dispense the eluant into the 1.5 ml centrifuge tube labeled "Eluant".
- [0119] 11. Discard the frit tip
- [0120] 12. Quantitating the eluted nucleic acids with real time PCR.
- [0121] As shown in FIG. 8, nucleic acids from *Yersinia pestis* are detected in the eluant.
- [0122] The terms and descriptions used herein are set forth by way of illustration only and are not meant as limitations. Those skilled in the art will recognize that many variations are possible within the spirit and scope of the invention as defined in the following claims, and their equivalents, in which all terms are to be understood in their broadest possible sense unless otherwise indicated.
1. A sample preparation device comprising:
 - a housing defining a passage way between a first opening and a second opening; and
 - a sample filter occupying a section of said passage way, said sample filter comprising a monolith adsorbent that specifically binds to nucleic acids.
 2. The sample preparation device of claim 1, wherein said sample filter is chemically treated to create positive charges.
 3. The sample preparation device of claim 1, wherein said monolith adsorbent is selected from the group consisting of glass frit, porous glass monolith, and porous monolithic polymers.
 4. The sample preparation device of claim 3, wherein said monolith adsorbent comprises a glass frit.
 5. The sample preparation device of claim 3, wherein said monolith adsorbent comprises porous glass monolith.
 6. The sample preparation device of claim 3, wherein said monolith adsorbent comprises porous monolithic polymers.
 7. The sample preparation device of claim 1, wherein said sample filter comprises a first section that binds specifically to nucleic acids and a second section that binds specifically to an analyte of interest.
 8. The sample preparation device of claim 7, wherein said analyte of interest is a protein.
 9. The sample preparation device of claim 1, wherein said first opening has a diameter that is greater than the diameter of

said second opening, and wherein said sample filter is placed in the close proximity of said second opening.

10. The sample preparation device of claim 9, wherein the side of said frit is tapered to the contour of said passage way.

11. The sample preparation device of claim 1, wherein said housing further comprises a second sample filter placed in the close proximity of said first sample filter.

12. The sample preparation device of claim 1, wherein said housing further comprises an aerosol filter placed in the proximity of said first opening.

13. The sample preparation device of claim 1, wherein said housing is in the configuration of a pipette tip.

14. An integrated sample preparation cartridge, comprising:

a sample preparation device comprising:

a housing defining a passage way between a first opening and a second opening; and

a sample filter occupying a section of the passage way, said sample filter comprising an adsorbent that specifically binds to an analyte of interest; and

a cartridge base comprising:

a first port configured to interface with the sample preparation device;

a second port configured to interface with a liquid delivery device; and

a first channel connecting said first port to said second port.

15. The integrated sample preparation cartridge of claim 14, wherein said cartridge base is made of a material selected from the group consisting of polycarbonate, polystyrene and polypropylene.

16. The integrated sample preparation cartridge of claim 14, wherein said liquid delivery device is a flow control station.

17. The integrated sample preparation cartridge of claim 14, wherein said second port comprises a Luer-activated valve.

18. The integrated sample preparation cartridge of claim 14, wherein said cartridge base further comprising:

a third port; and

a second channel connecting said third port to said first port.

19. The integrated sample preparation cartridge of claim 18, wherein said third port is a self-sealing port.

20. The integrated sample preparation cartridge of claim 18, wherein said third port comprises a septum or a one-way valve.

21. A cartridge base, comprising:

a base body having a top side and a bottom side;

a first port, formed on said top side of the base body and configured to interface with a sample preparation device;

a second port, formed on said bottom side of the base body and configured to interface with a liquid delivery device; and

a first channel connecting said first port to said second port.

22. The cartridge base of claim 21, further comprising one or more additional ports; and

one or more additional channels connecting said one or more additional ports to said first port.

23. A sample preparation device comprising:

a housing defining a passage way between a first opening and a second opening; and

- a glass frit occupying a section of the passage way, said glass frit is coated with an capture agent that binds specifically to an analyte of interest.
- 24.** The sample preparation device of claim **23**, wherein said capture agent is an antibody.
- 25.** The sample preparation device of claim **23**, wherein said capture agent is a lectin.
- 26.** A sample preparation device comprising:
a housing defining a passage way between a first opening and a second opening; and
a filter occupying a section of the passage way, said filter comprises a hydrophilic matrix with impregnated chemicals that lyses cell membranes.
- 27.** The sample preparation device of claim **26**, wherein said hydrophilic matrix is FTA paper®.
- 28.** A method for purifying an analyte from a liquid sample, comprising:
placing said liquid sample in a container;
withdrawing at least a portion of said liquid sample into a sample preparation device comprising:
a housing defining a passage way between a first opening and a second opening; and
a filter occupying a section of said passage way, said sample filter comprising a material that specifically binds to said analyte,
wherein said portion of liquid sample is drawn into said housing via the first opening and passing through said filter, and wherein said analyte binds to said filter while passing through said filter;
expelling said portion of liquid sample from said sample preparation device via the first opening, wherein said portion of liquid sample passes through said filter a second time while exiting said sample preparation device; and
eluting said analyte from said filter by withdrawing an eluting buffer into said sample preparation device via the first opening and expelling said eluting buffer from said sample preparation device via the first opening, wherein said eluting buffer passes through said filter while entering and exiting said sample preparation device.
- 29.** The method of claim **28**, further comprising:
washing said filter by withdrawing a washing buffer into said sample preparation device via the first opening and expelling said washing buffer from said sample preparation device via the first opening, wherein said washing buffer passes through said filter while entering and exiting said sample preparation device.
- 30.** The method of claim **29** wherein said washing step is repeated two or more times.
- 31.** The method of claim **28**, wherein said sample withdrawing and sample expelling steps are repeated until all said liquid sample passes through said filter at least once.
- 32.** The method of claim **28**, wherein said analyte is a nucleic acid and said filter comprises a glass frit.
- 33.** The method of claim **28**, wherein said sample preparation device has the configuration of a pipette tip.
- 34.** The method of claim **28**, wherein said withdrawing, expelling and eluting steps are controlled by an electronic pipettor.
- 35.** The method of claim **28**, wherein said withdrawing, expelling and eluting steps are controlled by an robotic pipetting station.
- 36.** A method for purifying an analyte from a sample, comprising:
passing said sample through a sample preparation device comprising:
a housing defining a passage way between a first opening and a second opening; and
a filter occupying a section of said passage way, wherein said sample filter comprising a material that specifically binds to said analyte and said sample passes through said sample filter while passing through said sample preparation device; and
eluting analyte bound to said sample filter with an eluting solution,
wherein said sample and said eluting solution enter and exit said housing through the same opening.
- 37.** The method of claim **36**, wherein said sample preparation device is attached to a cartridge base comprising a first port configured to receive said sample preparation device and a second port configured to receive a liquid delivery device.
- 38.** The method of claim **36**, further comprising:
washing said filter with a washing solution, wherein said washing solution enter and exit said housing through the same opening.
- 39.** A sample purification system, comprising:
a sample preparation device comprising:
a housing defining a passage way between a first opening and a second opening; and
a sample filter occupying a section of said passage way, said sample filter comprising a monolith adsorbent that specifically binds to an analyte of interest;
a cartridge base comprising:
a first port configured to receive said sample preparation device;
a second port configured to receive a liquid delivery device;
a channel connecting said first sample port with said second sample port; and
a liquid delivery device, connected to said cartridge base, that controls fluid flow in said sample preparation device and said cartridge base.
- 40.** The sample purification system of claim **39**, wherein said liquid delivery device delivers liquids into and removes liquids from said sample preparation device through the same opening on said housing.

* * * * *