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(54) Title: DEVICE AND METHODS FOR DETECTION OF AN ORGANISM

(57) Abstract: Disclosed are devices and methods for detecting a microbial organism that include a detection apparatus with a sample collection device. The sample collection device generally includes a collection surface and an elongate shaft, coupled to a proximal end of the collection surface and a substrate fluidly coupled to said collection surface, wherein said substrate comprises at least one detection agent vitrified thereto.

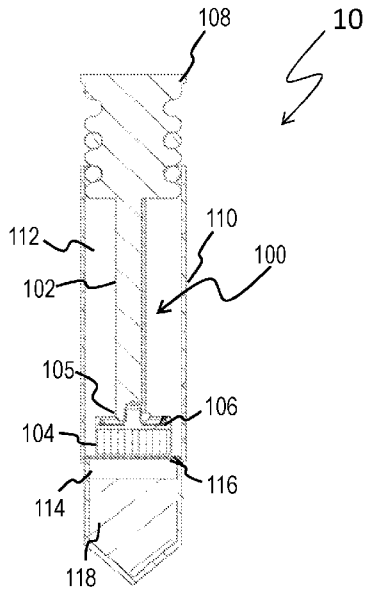

FIG. 1A

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DEVICE AND METHODS FOR DETECTION OF AN ORGANISM

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application depends from and claims priority to U.S. Provisional Application No. 63/582,974 filed September 15, 2023, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

[0002] The present disclosure generally concerns apparatuses and methods for detecting the presence of an agent such as a chemical or biological agent or organism.

BACKGROUND

[0003] Current methods of detecting microbial organisms are multi-step processes that typically require collecting a sample, preparing the sample for analysis, analyzing the sample, and interpreting the presence of a microbial organism in the sample. After sample collection, detection agents must be contacted to the sample to allow identification of the presence or absence of the sample by a reaction or binding of a detection agent to the target analyte. This may result in a colorimetric, fluorescent, or other readout allowing the investigator to identify the target analyte.

[0004] Detection agents, however, are commonly liable to degradation during storage and prior to use. Degradation of detection agents is prevented by the use of cold chain storage the increase the lifetime of the detection agent. Cold chain storage, however, is not always available, particularly in the field in areas where electrical service is irregular or absent or other considerations prevent the presence of infrastructure suitable to allow for such cold chain storage. Thus, when analyzing a surface or space for the presence or absence of an agent or organism, samples must be collected and shipped to a separate location for analysis. This increases the time to detection and introduces potential issues with the sample during transfer to an analysis location.

[0005] Accordingly, a need exists to develop devices and methods to allow for remote detection of agents or organisms and without the need for expensive and often unreliable infrastructure.

SUMMARY

[0006] Example embodiments disclosed herein are directed to devices and methods for rapidly analyzing a biological sample for the presence of an agent or organism. Embodiments of the present disclosure overcome obstacles in microbial detection by providing devices and methods for rapid microbial detection using a detection apparatus with vitrified detection agents built directly into the device such that the detection agents are immediately available for detection of the agent or organism and without the need for cold chain storage of such detection agents.

[0007] Provided herein are detection apparatuses that include a sample collection device having a collection surface and an elongate shaft, coupled to a proximal end of the collection surface. The detection apparatus also includes a substrate fluidly coupled to said collection surface, where said substrate includes at least one detection agent vitrified thereto.

[0008] Also provided are methods for detecting an organism using the detection device of the present disclosure. Optionally, a method includes collecting a sample to be tested for the organism on the collection surface, contacting the substrate with elution buffer, where the elution buffer rehydrates the vitrified detection agent, whereby the agent or organism, if present in the sample, reacts with or otherwise contacts the detection agent, and detecting the presence or absence of the agent or organism by the presence or absence of a detectable signal.

[0009] These and other features, aspects, and advantages will become better understood with reference to the following description and the appended claims.

[0010] Additional features and advantages of the embodiments described herein will be set forth in the detailed description that follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the embodiments described herein, including the detailed description that follows, the claims, as well as the appended drawings.

[0011] It is to be understood that both the foregoing general description and the following detailed description describe various embodiments and are intended to provide an overview or framework for understanding the nature and character of the claimed subject matter. The accompanying drawings are included to provide a further understanding of the various embodiments, and are incorporated into and constitute a part of this specification. The drawings illustrate the various embodiments described herein, and together with the description serve to explain the principles and operations of the claimed subject matter.

[0012] A need persists for a rapid, robust assay for the detection of one or more analytes, including but not limited to antibodies or pathogens, in a sample, on a surface, or in a space.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The drawings are not necessarily to scale; some features may be exaggerated or minimized to show details of particular components. Therefore, specific structural and functional details disclosed herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the present disclosure. Exemplary aspects will become more fully understood from the detailed description and the accompanying drawings, wherein:

[0014] FIG. 1A illustrates an exemplary device according to one or more embodiments described herein;

[0015] FIG. 1B illustrates the exemplary device of FIG. 1A, wherein the internal partition has been ruptured according to one or more embodiments described herein;

[0016] FIG. 1C illustrates an exemplary sample collection device according to one or more embodiments described herein;

[0017] FIG. 2A illustrates an exemplary configuration of a sample collection surface and substrate in a sample collection device according to one or more embodiments described herein;

[0018] FIG. 2B illustrates an exemplary configuration of a sample collection surface and substrate in a sample collection device according to one or more embodiments described herein;

[0019] FIG. 2C illustrates an exemplary configuration of a sample collection surface and substrate in a sample collection device according to one or more embodiments described herein;

[0020] FIG. 3 illustrates an exemplary device according to one or more embodiments described herein;

[0021] FIG. 4 graphically illustrates comparative results of organism detection according to one or more embodiments described herein.

DETAILED DESCRIPTION

[0022] Provided herein are devices and methods that allow for detection of one or more agents or organisms on a surface or in a space. The devices employ a collection surface whereby a substrate housing one or more detection agents therein is fluidly connected to the collection surface, optionally within the collection surface, adjacent thereto, or the collection surface itself. The collection surface is optionally on an end of an elongate shaft such that the device may be contacted to a surface or space for analysis of the presence or absence of an agent or organism. The presence of the detection agents in stable form that does not require cold chain storage to the collection surface allows for rapid and robust detection upon dissolving the one or more detection agents with a fluid. This allows for rapid, remote, and robust detection of the presence or absence of an agent or organism on a surface optionally without the need for separately housed detection agents.

[0023] The following terms or phrases used herein have the exemplary meanings listed below in connection with at least one aspect.

[0024] "Vitrification", as used herein, is a process of converting a material into an amorphous material. The amorphous solid may be free of any crystalline structure.

[0025] "Vitrification mixture" as used herein, means a heterogeneous mixture of biological material(s) and a vitrification medium containing vitrification agents and optionally other materials.

[0026] "Vitrification agent", as used herein, is a material that forms an amorphous structure, or that suppress the formation of crystals in other material(s), as the mixture of the vitrification agent and other material(s) cools or desiccates. The vitrification agent(s) may also provide osmotic protection or otherwise enable cell survival during dehydration. In some aspects, the vitrification agent(s) may be any water soluble solution that yields a suitable amorphous structure for storage of biological materials. In other aspects, the vitrification agent may be imbibed within a cell, tissue, or organ.

[0027] "Hydrophilic," as used herein, means attracting or associating preferentially with water molecules. Hydrophilic materials with a special affinity for water, maximize contact with water and have smaller contact angles with water relative to hydrophobic materials.

[0028] "Hydrophobic," as used herein, means lacking affinity for water. Materials that are hydrophobic naturally repel water, causing droplets to form, and have large contact angles with water.

[0029] "Biological sample," as used herein, refers to a sample that may contain an organism or portion of an organism of interest or any biologically derived agent. The biological sample may be any sample capable of flowing through the matrices described herein and potentially harboring the agent or organism of interest. In some embodiments, the biological sample comprises saliva, blood, serum, plasma, urine, bronchoalveolar lavage fluid, sputum, nasal fluid, skin secretions, or combinations thereof. In other embodiments, the biological sample is collected from a surface.

[0030] As used herein, the terms "surface" or "environmental surface" generally refer to any surface from which a sample can be collected. Exemplary, non-limiting examples of surfaces include walls, doors, floors, ceilings, drains, appliances, furniture, refrigeration systems, ducts, vents, toilet seats, handles, doorknobs, handrails, bedrails, countertops, tabletops, eating surfaces, working surfaces, equipment surfaces, clothing, etc.

[0031] The devices and methods as provided herein may be used to detect the presence or absence of an agent or organism on a surface or within a space or the environment. Optionally, the organism is a microbial organism. In some embodiments, the microbial organism may be a bacteria or portion thereof. Optionally, the organism is a fungi or portion thereof. In other aspects, the organism is a virus or a portion thereof.

[0032] In some embodiments, the presence or absence of the microbial organism is determined by the presence or absence of a target analyte on a collection surface after collection. A target analyte or agent may be any chemical or biological material. Optionally, a target analyte may be a biomolecule. "Biomolecule" as used herein refers to proteins, polysaccharides, nucleic acids, and nucleotides such as ATP, GTP, NAD, and NADP, that indicate the presence of one or more organisms. An exemplary bacteria used and exemplified throughout this disclosure for exemplary purposes alone is *Escherichia coli* (*E. coli*). It is appreciated the devices and methods as provided herein are equally described with respect to any other bacteria, virus, fungus, or portion thereof. It is further appreciated that the devices and methods as provided herein are equally described as detecting one or more chemical agents, including but not limited to toxins, chemical warfare agents, industrial chemicals, or other. This disclosure provides devices and methods that can specifically detect the presence or absence of an agent or organism or other target analyte in a specific and robust system.

[0033] FIGS. 1A-1C depict an exemplary embodiment of a detection apparatus 10 in accordance with the present disclosure. It is noted that positional terms such as "upper," "lower," "proximal," distal," "top," and "bottom" are used herein to assist in describing an embodiment of the present disclosure but are not limiting. The detection apparatus 10 may be inverted or positioned differently than shown.

[0034] The detection apparatus 10 generally includes a sample collection device 100. The sample collection device 100 generally includes an elongate shaft 102 and a collection surface 104. coupled to the elongate shaft 102.

[0035] The elongate shaft 102 may be constructed from a variety of materials, such as plastic, metal, wood, or combinations thereof. In some embodiments, the elongate shaft 102 is flexible such that it can reach sampling sites that would otherwise be inaccessible. In other

embodiments, the elongate shaft 102 can be relatively rigid, so that force can be applied to the elongate shaft 102 to collect a sample.

[0036] The sample collection device 100 also includes the collection surface 104. Generally speaking, the collection surface 104 is adapted for receiving a biological sample. In embodiments, the collection surface 104 is formed from one or more liquid permeable materials, such as cotton, fibers, sponges, foams, non-woven fabrics, bamboo, hydrophilic or hydrophobic polymers, and the like. In embodiments, the collection surface 104 is sterile to prevent contamination of the sample or inaccurate results.

[0037] The collection surface 104 may be coupled to the elongate shaft 102. Optionally, a proximal end 105 of the collection surface 104 is coupled to the elongate shaft 102. The collection surface 104 may be coupled to the elongate shaft 102 using any suitable mechanism, including, but not limited to fasteners, adhesives, heat sealing, thermal bonding, and the like. In some embodiments, the collection surface 104 is coupled to the elongate shaft 102 by entanglement of the liquid permeable material on and/or around the elongate shaft 102. In some embodiments, the collection surface 104 is coupled to the elongate shaft 102 by one or more threaded fasteners.

[0038] In operation, the sample collection device 100 is used to contact a sampling site. The biological sample may be collected by contacting the collection surface 104 to the sampling site. For example, the collection surface 104 is gently rubbed or rotated over the sampling site, wherein the collection surface 104 will absorb or collect the biological sample. Exemplary sampling sites include skin, nails, mucous membranes, wounds, environmental surfaces, and the like.

[0039] The sample collection device 100 may include a substrate 106 fluidly coupled to the collection surface 104. The substrate 106 may be a membrane scaffold or any other structure operable to function within this disclosure. Optionally, the substrate is present in the form of a fibrous random or ordered mesh that defines channels or other continuous or discontinuous routes of access for a fluid or a target analyte. Optionally, a substrate is in the form of a non-woven fibrous mesh with high porosity (e.g. greater than 50% by volume). In some embodiments, the substrate is a foam material. Illustrative examples of suitable substrates can be found in International Patent Application Publication No. WO 2020/086812.

[0040] The substrate 106 may be formed from one or more matrix materials into or onto which one or more vitrified components may be associated at one or more locations. The matrix material may be formed into an underlying patterned ridged support or of a porous material such as a membrane may be made of a material that is not toxic and not reactive to the biomaterials or biological samples and does not react chemically or physically with the vitrification medium. The material can be of a suitable polymer, metal, ceramic, glass, or a combination thereof. In some aspects, a matrix material is formed from a material of polydimethylsiloxane (PDMS), polycarbonate, polyurethane, polyethersulphone (PES), polyester (e.g. polyethylene terephthalate), among others. Other illustrative examples of matrix materials include polymers, optionally including but not limited to, collagen, elastin, hyaluronic acid and derivatives, sodium alginate and derivatives, chitosan and derivatives, gelatin, starch, cellulose polymers (for example, nitrocellulose (e.g. Sartorius CN95), methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose, cellulose acetate phthalate, cellulose acetate succinate, hydroxypropylmethylcellulose phthalate), poly(diol citrate) (e.g., poly(octanediol citrate), etc.), casein, dextran and derivatives, poly(caprolactone), poly(hydroxyl acids), poly(L-lactide) poly(D,L lactide), poly(D,L-lactide-co-glycolide), poly(L-lactide-co-glycolide), copolymers of lactic acid and glycolic acid, copolymers of ε-caprolactone and lactide, copolymers of glycolide and ε-caprolactone, copolymers of lactide and 1,4-dioxane-2-one, polymers and copolymers that include one or more of the residue units of the monomers D-lactide, L-lactide, D,L-lactide, glycolide, ε-caprolactone, trimethylene carbonate, 1,4-dioxane-2-one or 1,5-dioxepan-2-one, poly(glycolide), poly(hydroxybutyrate), poly(alkylcarbonate) and poly(orthoesters), polyesters, poly(hydroxyvaleric acid), polydioxanone, poly(ethylene terephthalate), poly(malic acid), poly(tartronic acid), polyanhydrides, polyphosphazenes, poly(amino acids), and copolymers of the above polymers as well as blends and combinations of the above materials.

[0041] Polymers that may be used in a substrate herein optionally can be formed into a porous mesh, such as in the form of a filter, symmetric mesh, or other such porous sheet-like material. Illustratively, a polymer is formed into a fibrous network such as by methods that include electrospinning. In electrospinning, desired polymers are placed in a desired solvent (e.g. 2,2,2-trifluoroethanol (TFE) or hexafluoroisopropanol (HFIP)) and subjected to electrospinning processes so as to form a fiber of desired cross-sectional dimension and length

and arranged in a desired orientation (optionally random) so as to have a resulting pore size (average distance between strands) to allow materials, analytes, active agents, etc. to pass through or be retained within or by the polymer network. Optionally, the substrate includes 2 or more layers of polymer, optionally 3, 4, 5, 6, or more layers of polymer.

[0042] In some embodiments, the substrate comprises a pore size, the pore size optionally suitable for controlling sample flow or regulating the size of molecules that may pass through the system. In some aspects, the pore size within the matrix ranges from about 5 micrometers (μm) to about 500 μm, or any value or range therebetween. In some aspects, the pores may be of an average opening of from about 20 μm to about 0.1 μm, including about 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, and 0.2 μm. A capillary channel may have a length optionally defined by the thickness of a substrate that forms the channels or by one or a plurality of individual channels themselves. A capillary channel length is optionally about one millimeter or less, but is not to be interpreted as limited to such dimensions. Optionally, a capillary channel length is of about 0.1 microns to about 1000 microns, or any value or range therebetween. Optionally, a capillary channel length is of about 5 to about 100 microns, optionally of about 1 to about 200 microns, and/or optionally of about 1 to about 100 microns. A capillary channel length is optionally about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 microns. In some aspects, the length of the capillary channels varies throughout a plurality of capillary channels, optionally in a non-uniform variation. In some aspects, the pore size of the substrate is selected to permit the flow of a liquid and/or any reporters or target analytes of interest present in the biological sample. In multi-layer embodiments, different layers of the substrate component may comprise different pore sizes.

[0043] The cross-sectional area of the capillary channel(s) may be of about 2000 μm^2 or less. Optionally a cross-sectional area is of about 0.01 μm^2 to about 2000 μm^2, optionally of about 100 μm^2 to about 2000 μm^2, or any value or range therebetween. Optionally, a cross-sectional area of the capillary channel(s) is of about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 μm^2 or less.

[0044] In some aspects, the substrate is of a hydrophilic material. In other aspects, the substrate may be of a hydrophobic material and further treated to be hydrophilic or more hydrophilic in nature, such as through exposure to a plasma.

[0045] In some embodiments, a substrate is or includes polycaprolactone (PCL), collagen, or combinations thereof. The primary characteristic of such water-stable polymers is that they are able to form networks or fibers such that one or more components may be vitrified thereon or therein. Thus, the substrate must have sufficient stability in an aqueous environment so as to serve as a suitable surface for vitrification of an aqueous vitrification medium containing one or more desired molecules such as detection agents.

[0046] Referring to FIGS. 2A-2C, exemplary embodiments of a sample collection device 100 are provided. In embodiments, such as shown in FIGS. 2A-2C, the substrate 106 is disposed on the sample collection device. As shown, the collection surface 104 is in fluidic contact with the substrate 106. Optionally, the substrate 106 is coupled to the elongate shaft 102. In some embodiments, the substrate 106 is disposed on a proximal surface of the collection surface 104. In some embodiments, the substrate 106 is disposed at least partially between the elongate shaft 102 and the collection surface 104. Optionally, the substrate 106 is a layer disposed underneath the collection surface 106. In some embodiments, the collection surface 104 partially or completely covers the substrate 106.

[0047] In some embodiments, such as shown in FIG. 2C, the substrate 106 and the collection surface 104 are coupled to the elongate shaft at discrete positions. For example, without being bound by theory, the substrate 106 and the collection surface 104 may be disposed on diametrically opposite points. In other embodiments, the substrate 106 and the collection surface 104 may be offset from one another by an angle, optionally 90 degrees.

[0048] The substrate 106 optionally contains or supports one or more vitrified components, such as one or more detection agents, buffering agents, or other desired material. Components may be vitrified to the substrate using any suitable method known in the art. For example, and not as a limitation, methods may include those detailed in U.S. Patent No. 10,433,540, U.S. Patent No. 10,568,318, International Patent Application No. PCT/US2022/035892, and

International Patent Application No PCT/US2021/060164, the contents of each of which are incorporated by reference in their entirety.

[0049] Briefly, a vitrification medium including one or more materials to be vitrified into or onto a substrate are combined and placed into or onto the matrix. A vitrification medium may include at least one vitrification agent. Illustrative examples of a vitrification agent include, but are not limited to, dimethylsulfoxide, glycerol, sugars (e.g. trehalose, etc.), polyalcohols, methylamines, betines, antifreeze proteins, synthetic anti-nucleating agents, polyvinyl alcohol, cyclohexanetriols, cyclohexanediols, inorganic salts, organic salts, ionic liquids, or combinations thereof. In aspects, 1, 2, 3, 4, or more vitrification agents are included in the vitrification medium.

[0050] The vitrification agent is included in the vitrification medium at a concentration that is dependent on the identity of the vitrification agent. In some aspects, the concentration of the vitrification agent below that which would be toxic to the material(s) being vitrified. As used herein, "toxic" means that functional or biological viability is not achieved upon subsequent sample use, or the material is not suitable for subsequent use or analyses. In various aspects, the concentration of the vitrification agent is greater than or equal to 500 micromolar (μM) and less than or equal to 6 molar (M), or any value or range therebetween. As one example, trehalose is included in various aspects in a concentration of greater than or equal to 1 millimolar (mM) and less than or equal to 6 M, optionally greater than or equal to 150 mM and less than or equal to 6 M. In some aspects, the total concentration of all vitrification agents when combined is greater than or equal to 1 mM and less than or equal to 6 M, optionally greater than or equal to 1 mM and less than or equal to 6 M.

[0051] It is contemplated that, in some aspects, the vitrification medium may further include other components, such as, by way of example and not limitation, water or other solvents, a buffering agent, one or more salts, RNase or DNAse inhibitors, or combinations thereof. A buffering agent is any agent with a pKa of 6 to 8.5 at 25 °C. Illustrative examples of buffering agents include choline, betaine, HEPES, TRIS, PIPES, MOPS, among others. In some aspects, the buffering agent is a buffering agent that contains large organic ions (greater than 120 kDa), such as choline, betaine, or HEPES. In embodiments including a buffering agent, the buffering

agent is provided at a concentration suitable to stabilize the pH of the vitrification medium to a desired level.

[0052] A vitrification medium may include one or more salts. Salts can include, by way of example and not limitation, magnesium salts, sodium salts, potassium salts, chloride salts, or combinations thereof. When included in the vitrification medium, the salts can be provided at a concentration of from greater than or equal to 1 mM to less than or equal to 500 mM.

[0053] A detection agent, reporter, buffering agent or any other desired material may be vitrified into or onto a particular device, optionally according to the teachings of U.S. Patent No. 10,433,540. Optionally, the material(s) to be vitrified may be combined in a vitrification solution including a sugar (e.g. trehalose) and that further includes a combination of divalent metal ions and a chelator. Illustrative examples of divalent metal ions includes salts of Ca, Mg, Co, Fe, Zn, Mn among others. Illustrative salts include chloride, sulfate, acetate, etc. Chelators include polyols, ethylenediaminetetraacetic acid (EDTA), egtazic acid (EGTA), among others. The molar ratio of divalent metal salt to the chelator in the vitrification solution may be 10:90 to 90:10, optionally 50:50.

[0054] The detection of the analyte may be performed by detection of the reporter in the detection apparatus 10 by any suitable method. Illustratively, detection includes chemiluminescence, a fluorescence reader matched to the detection agent, colorimetric assay, direct detection of label on the reporter molecule (e.g. gold or other), or other such assay systems.

[0055] Illustratively detection of an analyte is performed using an enzyme/substrate system (e.g. luciferase coupled with luciferin, horseradish peroxidase (HRP) or alkaline phosphatase (AP) coupled with a substrate such as 4-chloro-1-naphthol (4-CN), 3, 3′-diaminobenzidine (DAB), p-Nitrophenyl Phosphate (PNPP), CSPD and CDP-Star substrates, DynaLight Substrate with RapidGlow Enhancer, o-phenylenediamine dihydrochloride (OPD), 3,3',5,5'-tetramethylbenzidine (TMB) or its derivatives (e.g. TMBM, TMBMX), 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS), SuperSignal ELISA Pico Chemiluminescent Substrate, SuperSignal ELISA Femto Maximum Sensitivity Substrate, and the like, available from ThermoFisher Scientific (Waltham, MA) and other commercial

vendors. In some embodiments, the enzyme/substrate system is luciferase coupled with luciferin.

[0056] The substrate 106 and/or the collection surface 104 may include one or more vitrified assay reagents therein. Such vitrified assay reagents may include buffers, detergents, salts, or any other needed reagent. As one example, the collection surface may include vitrified therein a detergent and/or a buffer whereby rehydration of the collection surface allows the stored assay reagents to be functional to alter one or more characteristics of the sample applied (e.g. pH adjustment, lysing one or more components, etc.).

[0057] In some embodiments, the microbial detection apparatus includes one or more lysing reagents. Optionally, the lysing reagents may be vitrified to the collection surface or scaffold. Alternatively, the lysing reagents may be present as a liquid lysis buffer. Cell lysis reagents can be added to the moistening liquid to help permeabilize biological cells and facilitate the detection of an analyte associated with the cells. The detection of intracellular analytes (e.g., nucleic acids, proteins, oligopeptides, and small molecules such as ATP) and cell wall-associated or cell-membrane associated molecules (e.g., polysaccharides and proteins) can be facilitated by cell lysis reagents. Preferably, the cell lysis reagent does not substantially inhibit the activities (e.g., interfere with an enzyme activity or the ability of a protein to recognize and/or bind to another molecule) of proteins (e.g., enzymes, antibodies), if the protein is present in the sample or the test device. Cell lysis reagents and their effective concentrations are known in the art. Examples of cell lysis agents include detergents (e.g., TRITON X-100), biocides (e.g., chlorhexidine gluconate, benzalkonium chloride), enzymes (e.g., phospholipases, lysozyme, lysostaphin), and cytolytic peptides (e.g., phylloxin). In some embodiments, the lysing reagents include reducing agents that may assist in improving signal results in the assay system, maintain the reduced state of proteins, and prevent disruption of the lysis process. Illustrative reducing agents include, but are not limited to dithiothreitol (DTT), β-mercaptoethanol, glutathione, ascorbic acid, and the like.

[0058] Referring back to FIGS. 1A-1C, the sample collection device 100 may further include a plunger 108. In some embodiments, the elongate shaft 102 is sized and shaped to be inserted into or is formed into the shape of the plunger 108 so as to be integral therewith. Optionally, the plunger 108 serves as a grip or handle for collecting a sample with the sample

collection device 100, optionally without contaminating the collection surface. The elongate shaft 102 may be inserted into the plunger 108 after sample collection. In some embodiments, the plunger 108 forceably contacts a wall of the housing meaning that the plunger or a portion thereof or appendix thereof contacts a wall of a housing with a force that creates a pressure between the wall and the plunger or portion thereof, optionally forming a seal. Optionally, the plunger 108 forms a seal, optionally a seal that is sufficient to prevent fluid passing through or past the seal, with a housing 110 of the detection apparatus 10. Optionally, the plunger 108 includes one or more features (e.g., O. rings) to form the seal with the housing. In some embodiments, the plunger 108 is formed from a material that is relatively flexible and/or malleable such that the plunger 108 itself forms the seal with the housing 110, optionally when forceably contacting a wall of the housing.

[0059] As shown in FIGS. 1A-1B, the detection apparatus 10 may further include a housing 110 for analyzing the biological sample. The housing 110 is configured to receive the sample collection device 100. The housing 110 defines a first chamber 112 that is sized and shaped to receive the elongate shaft 102 of the sample collection device 100. Optionally, the housing 110 also defines a second chamber 114, fluidly coupled to the first chamber 112. Optionally, the first chamber 112 and second chamber 114 are divided by an internal partition 116. In some embodiments, the internal partition 116 is coupled to the housing 110. Optionally, the internal partition 116 is displaced for sample analysis.

[0060] The internal partition 116 may be formed from a rupturable material designed to break, tear, or rupture when force is applied to the internal partition 116. In some embodiments, the internal partition 116 is ruptured by the sample collection device 100 upon depressing the plunger. In other embodiments, the internal partition 116 is ruptured by an external force on the housing 110, such as pinching, twisting, bending, or snapping of a portion of the housing 110. Exemplary rupturable materials include, but are not limited to foils, polymer films, gelatin, glass, gels, waxes, metal films, and/or any pressure sensitive seal.

[0061] In embodiments, the detection apparatus 10 includes an elution liquid 118 disposed within the housing 110. An elution liquid is optionally any liquid suitable for dissolving the vitrified material in the substrate. Optionally, an elution liquid is water or aqueous material. Optionally, an elution liquid is or includes one or more organics solvents. Optionally, and

elution liquid includes one or more buffering agents. The elution buffer 118 may be present in the second chamber 114 as shown in FIG. 1A. Alternatively, and discussed in greater detail herein, the elution buffer 118 may be present in the first chamber 112, as shown in FIG. 3.

[0062] Illustrative examples of elution buffer agents include but are not limited to choline, betaine, HEPES, TRIS, PIPES, MOPS, among others. Optionally, the elution buffer can include, by way of example and not limitation, magnesium salts, sodium salts, potassium salts, chloride salts, or combinations thereof. When included in the elution buffer, the salts can be provided at a concentration of from greater than or equal to 1 millimolar (mM) to less than or equal to 500 mM. Optionally, the components of the elution buffer 118 may be provided at a concentration suitable to stabilize the pH of the elution buffer to a desired level. Optionally, elution buffer components may be vitrified to the substrate 106 in place of being included in the liquid fraction. Optionally, assay reagents that are not vitrified to the substrate 106 and/or collection surface 104 may be included in the elution buffer 118.

[0063] In some embodiments, the elution buffer 118 includes 1 part CCLR (25 mM TRIS, 2 mM DTT, 2 mM EDTA, 10% Glycerol, 1% Triton) and 5 parts buffer (20 mM TRIS-HCl, 0.4 M urea, 10% glycerol) in 4.5 mM $MgSO_4$, 25 mM EPPS. In other embodiments, the elution buffer 118 does not include TRIS, but instead includes EPPS in place of the TRIS. In yet other embodiments, the elution buffer 118 does not include EPPS, but instead includes TRIS in place of the EPPS. In some embodiments, the elution buffer has a pH of about 6.5 to about 8.0, including about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, or any range having endpoints by any two of the aforementioned values.

[0064] Referring again to FIGS. 1A-1B, in operation, the sample collection device 100 can be removed from the housing 110 to collect a biological sample from the surface of a sampling site. After the collection surface 104 contacts the surface, the sample collection device 100 is inserted into the housing. As shown in FIG. 1A, the sample collection device 100 may be inserted into the housing 110 at a first position. Optionally, a distal end 107 of the collection surface 104 is inserted into the housing 110, such as into the first chamber 112. Optionally, the distal end 107 is positioned adjacent to the internal partition 116. In the first position, the plunger 108 extends at least partially beyond a top edge of the housing 110.

[0065] As force is applied to the plunger 108, the sample collection device 100 may move to a second position within the housing 110, optionally by engaging a second ring or portion of a plunger, and/or by rupturing the internal partition 116, as shown in FIG. 1B. Optionally, the distal end 107 contacts and penetrates the internal partition 116. In the second position, the sample collection surface 104 and the substrate 106 are positioned in the second chamber 114. When the internal partition 116 is ruptured, the elution buffer 118 contacts the collection surface 104 and the substrate 106, facilitating detection of the analyte. Optionally, the plunger 108 is positioned entirely or substantially within the housing 110 in the second position.

[0066] Alternatively, and as shown in FIG. 3, the substrate 106 may be disposed in the second chamber 114. In some embodiments, the elution buffer 118 is disposed in the first chamber 112 to separate the elution buffer 118 from the substrate 106 until the internal partition 116 is ruptured. As force is applied to the plunger 108, the sample collection device 100 may move to a second position within the housing 110 by rupturing the internal partition 116. Optionally, the collection surface 104 may remain in the first chamber 112 while the elution buffer 118 that has interacted with the sample may move to the second chamber 114 to contact the substrate 106.

[0067] In embodiments, rupturing the internal partition 116 allows the elution buffer 118 to contact the collection surface 104 and/or the substrate 106. Upon contact with the elution buffer 118, the vitrified material is rehydrated and the components vitrified on the substrate 106 are released into the liquid and able to interact with the biological sample to facilitate the detection of the target analyte.

[0068] The device and methods herein may be used to detect the presence or absence of one or more agents or organisms. An agent is optionally a biological agent that includes, but is not limited to proteins (e.g. antibodies, blood proteins, intracellular proteins, membrane proteins, etc.), RNA, DNA, lipid, or other. Optionally, a material to be detected is an organism or portion thereof, optionally a bacteria, virus, fungi, or other organism. In some embodiments, the organism is a bacteria. As one non-limiting example, detection of bacteria (or other agent or organism) may be performed by collecting a sample on the collection surface that includes therein a vitrified detergent (e.g. Triton-X 100). The rehydration of the vitrified detergent

allows the detergent to lyse the bacterial cells in the biological sample thereby releasing intracellular analytes, such as ATP.

[0069] Optionally, detection of the analyte relies on an enzyme/substrate reaction. In embodiments, the enzyme/substrate reaction emits detectable light proportional to the amount of the target analyte in the biological sample. In embodiments, the enzyme and/or the substrate may be vitrified to the collection surface 104 and/or the substrate 106. In embodiments, the enzyme and the substrate are vitrified to the substrate 106. Optionally, the enzyme/substrate combination is luciferase/luciferin.

[0070] In embodiments, the emitted light is measured. Optionally, the emitted light is measured using a luminometer. The measured values may then be compared to a standard curve to quantify the amount of the analyte in the biological sample.

[0071] Various aspects of the present disclosure are illustrated by the following non-limiting examples. The examples are for illustrative purposes and are not a limitation on any practice of the present disclosure. It will be understood that variations and modifications can be made without departing from the spirit and scope of the disclosure.

EXAMPLES

[0072] The following Examples are offered by way of illustration and are presented in a manner such that one skilled in the art should recognize are not meant to be limiting to the present disclosure as a whole or to the appended claims.

Example 1:

[0073] A device includes an elongate shaft under a collection surface. For detection of a biological agent, *E. coli* is grown in culture to confluence and serially diluted to about $0.1x10^6$ cells/ml. A sample of this cellular solution is coated onto a microscope slide and dried. These dried samples are used for the following studies demonstrating that the swab is functional to collect and detect cells as well as that vitrification of material into the a substrate or a collection surface is functional to provide materials for successful detection of cells.

[0074] In a first set of experiments, the use of a swab for the detection of *E. coli* dried onto a surface is demonstrated. A device in the form of a swab with a collection surface is contacted to the dried material on the surface of the microscope slide. This is then placed into a housing containing Biofix Buffer + 10% BSA, 0.875 µg luciferase (4.16 µg/mL), 139 µg luciferin (2.5 mM), 9 µg DTT (0.333 mM), 0.035 µg Triton-X100 (0.2%) and 2 mM EDTA. A sample of the eluted material is then subjected to analysis for the presence or absence of ATP by luminescence either following 20 minutes of incubation of the collection surface in the solution or within 1 minute of contact with the elution solution (Lysis Master Mix fast).

[0075] Comparative results are illustrated in FIG. 4. The swab is readily able to detect the presence of the *E. coli* nearly identical to control. Allowing lysis of the cells to occur for 20 minutes still allows excellent detection of the organism, but the 20-minute incubation time reduces the luminescence in the system likely due to degradation of the luminescent signal over the incubation time.

[0076] To demonstrate that detection agents may be vitrified onto substrates for use in detection, a CMV solution is made containing Biofix Buffer + 10% BSA, 0.875 µg luciferase (4.16 µg/mL), 139 µg luciferin (2.5 mM), 9 µg DTT (0.333 mM), 0.035 µg Triton-X100 (0.2%) and with or without 2 mM EDTA. The substrate is subjected to vitrification. This substrate is then placed in an elution buffer containing 4.5 mM Mg acetate, 25 mM TRIS, 2% glycerol at pH 7.2 and the vitrified materials are dissolved into the elution solution. A liquid sample is created with the identical amounts of materials in the vitrified material and used as a non-vitrified liquid control. A detection device as above is then dipped in either the liquid control or the vitrified/reconstituted CMV materials, swabbed to the dry slide surface then analyzed for the presence or absence of free ATP from the lysed cells. Results are illustrated in Table 1.

[0077] Table 1:

	Signal	Signal >LOD?	% of Liquid
Liquid 1e8 cells	40949.5	yes	
Liquid 1e5 cells	1121.167	yes	
CMV 1e8 cells	38161	yes	93%
CMV 1e5 cells	1446.833	yes	129%

[0078] Using both $1x10^8$ cells/ml and $1x10^5$ cells/ml the vitrified and reconstituted materials detected as much cell material as the non-vitrified/reconstituted control demonstrating that vitrification of the detection agents onto a substrate and subsequence reconstitution is successfully able to detect *E. coli* from a surface.

[0079] These studies were repeated seven times using either bacteria coated slides or mock coated slides. As illustrated in Table 2, both the liquid agents and the reconstituted vitrified agents were able to identify the presence or absence of bacterial with 100% accuracy.

Liquid				CMV			
Signal	S/N	Identification	ID Correct?	Signal	S/N	Identification	ID Correct?
601	1.1	negative	TRUE	1267	2.0	positive	TRUE
1613	2.9	positive	TRUE	1345	2.1	positive	TRUE
503	0.9	negative	TRUE	756	1.2	negative	TRUE
517	0.9	negative	TRUE	1126	1.8	positive	TRUE
1193	2.2	positive	TRUE	575	0.9	negative	TRUE
1217	2.2	positive	TRUE	1161	1.8	positive	TRUE
415	0.8	negative	TRUE	435	0.7	negative	TRUE

Table 2

[0080] Further studies were performed using substrates with or without CMV detection agents vitrified therein. A clean substrate and a substrate vitrified with the following were prepared as above. Vitrified substrate included Biofix Buffer + 10% BSA, 0.875 µg luciferase

(4.16 µg/mL), 139 µg luciferin (2.5 mM), 9 µg DTT (0.333 mM), 0.035 µg Triton-X100 (0.2%) and with or without 2 mM EDTA. The substrates were then contacted to a bacterial containing slide and placed in elution buffer in separate tubes with the control tube in which the clean substrate was included further including the same materials vitrified into the test substrate so that the test solutions were of identical composition. The materials were then analyzed for the presence or absence of ATP to detect bacteria. The presence of ATP was detected in both samples by luciferin luminescence.

[0081] These studies demonstrate that material such as buffers, detection agents, salts, lysing agents, etc. can be vitrified into a substrate, that substrate or collection surface contacted to a test surface, the vitrified material reconstituted and then successfully used to detect the presence of bacterial as an exemplary test agent.

Example 2:

[0082] A sample device is formed substantially as illustrated in FIG. 1A. Illustratively, to the substrate, 175 µL of a vitrification mixture containing Biofix Buffer + 10% BSA, 0.875 µg luciferase (4.16 µg/mL), 139 µg luciferin (2.5 mM), 9 µg DTT (0.333 mM), 0.035 µg Triton-X100 (0.2%) and with or without 2 mM EDTA is coated and vitrified according to the teachings of US Patent No. 10,433,540. This substrate is placed either under a collection surface, adjacent thereto, or otherwise in sufficient proximity to allow fluidic connection between the two within a housing.

Aspects Listing:

[0083] In a first aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus comprising: a sample collection device comprising: a collection surface; and an elongate shaft, coupled to a proximal end of the collection surface; a substrate fluidly coupled to said collection surface, wherein said substrate comprises at least one detection agent vitrified thereto.

[0084] In a second aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein the substrate is disposed on the sample collection device.

[0085] In a third aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein the substrate is disposed on the elongate shaft.

[0086] In a fourth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein the substrate is a layer disposed underneath the collection surface.

[0087] In a fifth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising a housing comprising a first chamber, said housing configured to accept said elongate shaft within the first chamber.

[0088] In a sixth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising a second chamber fluidly coupled to said first chamber.

[0089] In a seventh aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising an internal partition between said first chamber and said second chamber.

[0090] In an eighth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said internal partition comprises a rupturable material.

[0091] In a ninth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said substrate is connected to said elongate shaft proximal to said collection surface.

[0092] In a tenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said substrate is proximal to said second chamber relative to said rupture disk.

[0093] In an eleventh aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising a plunger connected to said elongate shaft distal from said collection surface.

[0094] In a twelfth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said plunger is configured to forceably associate with a wall of said housing.

[0095] In a thirteenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said plunger comprises one or more gaskets, wherein said one or more gaskets form a seal between the plunger and said housing.

[0096] In a fourteenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus comprising two or more gaskets.

[0097] In a fifteenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising a stop on or attached to said plunger, said stop distal from said collection surface.

[0098] In a sixteenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising an elution buffer.

[0099] In a seventeenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said elution buffer is housed in said first chamber.

[0100] In an eighteenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said elution buffer is housed in said second chamber.

[0101] In a nineteenth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said elution buffer is separated from said substrate by said internal partition.

[0102] In a twentieth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein said elution buffer is separated from said second chamber by said internal partition.

[0103] In a twenty-first aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus, wherein the detection agent is a colorimetric agent, fluorescent label, a luminescent label, a radioisotope label, or a combination thereof.

[0104] In a twenty-second aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein the detection agent is luciferase.

[0105] In a twenty-third aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein the detection agent is colorimetric.

[0106] In a twenty-fourth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus further comprising a lysis agent.

[0107] In a twenty-fifth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus, wherein the lysis agent is vitrified to the scaffold.

[0108] In a twenty-sixth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a detection apparatus wherein the lysis agent is in said elution buffer.

[0109] In a twenty-seventh aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism using a detection device the method comprising collecting a sample to be tested for the organism on the collection surface; contacting the substrate with elution buffer, wherein the elution buffer rehydrates the vitrified detection agent, whereby the organism, if present in the sample, reacts with the detection agent; and detecting the presence or absence of the organism by the presence or absence of a detectable signal.

[0110] In a twenty-eighth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism further comprising inserting the sample collection device into the sample chamber prior to the contacting.

[0111] In a twenty-ninth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism further comprising rupturing the internal partition so as to contact the substrate with the elution buffer.

[0112] In a thirtieth aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism wherein the sample and the substrate are contacted with the elution buffer simultaneously.

[0113] In a thirty-first aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism wherein the detectable signal comprises a fluorescent signal or a color change.

[0114] In a thirty-second aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism wherein the presence or absence of the organism is detected in about 10 minutes or less.

[0115] In a thirty-third aspect, alone or in combination with any other aspect provided herein, the present disclosure relates to a method for detecting an organism wherein the presence or absence of the organism is detected in about 5 minutes or less.

[0116] It is noted that the terms "substantially" and "about" may be utilized herein to represent the inherent degree of uncertainty that may be attributed to any quantitative comparison, value, measurement, or other representation. These terms are also utilized herein to represent the degree by which a quantitative representation may vary from a stated reference without resulting in a change in the basic function of the subject matter at issue. The term "substantially" is used herein also to represent the degree by which a quantitative representation may vary from a stated reference without resulting in a change in the basic function of the subject matter at issue. Thus, it is used to represent the inherent degree of uncertainty that may be attributed to any quantitative comparison, value, measurement, or other representation, referring to an arrangement of elements or features that, while in theory would be expected to exhibit exact correspondence or behavior, may in practice embody something less than exact.

[0117] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. The terminology used in the description herein is for describing particular embodiments only and is not intended to be limiting. As used in the specification and appended claims, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0118] It is noted that one or more of the following claims utilize the term "wherein" as a transitional phrase. For the purposes of defining the present technology, it is noted that this term is introduced in the claims as an open-ended transitional phrase that is used to introduce a recitation of a series of characteristics of the structure and should be interpreted in like manner as the more commonly used open-ended preamble term "comprising."

[0119] It should be understood that where a first component is described as "comprising" or "including" a second component, it is contemplated that, in some embodiments, the first component "consists" or "consists essentially of" the second component. Additionally, the

term "consisting essentially of" is used in this disclosure to refer to quantitative values that do not materially affect the basic and novel characteristic(s) of the disclosure.

[0120] It should be understood that any two quantitative values assigned to a property or measurement may constitute a range of that property or measurement, and all combinations of ranges formed from all stated quantitative values of a given property or measurement are contemplated in this disclosure.

[0121] While particular embodiments have been illustrated and described herein, it should be understood that various other changes and modifications may be made without departing from scope of the claimed subject matter. Moreover, although various aspects of the claimed subject matter have been described herein, such aspects need not be utilized in combination. It is therefore intended that the appended claims cover all such changes and modifications that are within the scope of the claimed subject matter.

[0122] Various modifications of the present invention, in addition to those shown and described herein, will be apparent to those skilled in the art of the above description. Such modifications are also intended to fall within the scope of the appended claims.

[0123] Patents, publications, and applications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents, publications, and applications are incorporated herein by reference to the same extent as if each individual patent, publication, or application was specifically and individually incorporated herein by reference.

[0124] The foregoing description is illustrative of particular embodiments, but is not meant to be a limitation upon the practice thereof.

CLAIMS

What is claimed is:

1. A detection apparatus comprising:
    a sample collection device comprising:
        a collection surface; and
        an elongate shaft, coupled to a proximal end of the collection surface; and
    a substrate fluidly coupled to said collection surface, wherein said substrate comprises at least one detection agent vitrified thereto.

2. The detection apparatus of claim 1, wherein the substrate is disposed on the sample collection device.

3. The detection apparatus of claim 1, wherein the substrate is disposed on the elongate shaft.

4. The detection apparatus of claim 1, wherein the substrate is a layer disposed underneath the collection surface.

5. The detection apparatus of claim 1, further comprising a housing comprising a first chamber, said housing configured to accept said elongate shaft within the first chamber.

6. The detection apparatus of claim 5, further comprising a second chamber fluidly coupled to said first chamber.

7. The detection apparatus of claim 6, further comprising an internal partition between said first chamber and said second chamber.

8. The detection apparatus of claim 7, wherein said internal partition comprises a rupturable material.

9. The detection apparatus of claim 1, wherein said substrate is connected to said elongate shaft proximal to said collection surface.

10. The detection device of claim 6, wherein said substrate is proximal to said second chamber relative to said rupture disk.

11. The detection device of any one of claims 1-10, further comprising a plunger connected to said elongate shaft distal from said collection surface.

12. The detection device of claim 11, wherein said plunger is configured to forceably associate with a wall of said housing.

13. The detection device of claim 11, wherein said plunger comprises one or more gaskets, wherein said one or more gaskets form a seal between the plunger and said housing.

14. The detection device of claim 13 comprising two or more gaskets.

15. The detection device of claim 13 further comprising a stop on or attached to said plunger, said stop distal from said collection surface.

16. The detection device of any one of claims 1-15 further comprising an elution buffer.

17. The detection device of claim 16, wherein said elution buffer is housed in said first chamber.

18. The detection device of claim 16, wherein said elution buffer is housed in said second chamber.

19. The detection device of claim 18, wherein said elution buffer is separated from said substrate by said internal partition.

20. The device of claim 17, wherein said elution buffer is separated from said second chamber by said internal partition.

21. The detection apparatus of any of claims 1-20, wherein the detection agent is a colorimetric agent, fluorescent label, a luminescent label, a radioisotope label, or a combination thereof.

22. The detection apparatus of claim 21, wherein the detection agent is luciferase.

23. The detection apparatus of claim 21, wherein the detection agent is colorimetric.

24. The detection apparatus of any of claims 1-23, further comprising a lysis agent.

25. The detection apparatus of claim 24, wherein the lysis agent is vitrified to the scaffold.

26. The detection apparatus of claim 24, wherein the lysis agent is in said elution buffer.

27. A method for detecting an organism using the detection device of any of claims 1-10, the method comprising:

collecting onto the collection surface a biological sample to be tested for the organism;

contacting the substrate with elution buffer, wherein the elution buffer rehydrates the vitrified detection agent, whereby the organism, if present in the sample, reacts with the detection agent; and

detecting the presence or absence of the organism by the presence or absence of a detectable signal.

28. The method of claim 27 further comprising inserting the sample collection device into the sample chamber prior to the contacting.

29. The method of claim 27 further comprising rupturing the internal partition so as to contact the substrate with the elution buffer.

30. The method of claim 27, wherein the sample and the substrate are contacted with the elution buffer simultaneously.

31. The method of claim 27, wherein the detectable signal comprises a fluorescent signal or a color change.

32. The method of claim 27, wherein the presence or absence of the organism is detected in about 10 minutes or less.

33. The method of claim 27, wherein the presence or absence of the organism is detected in about 5 minutes or less.

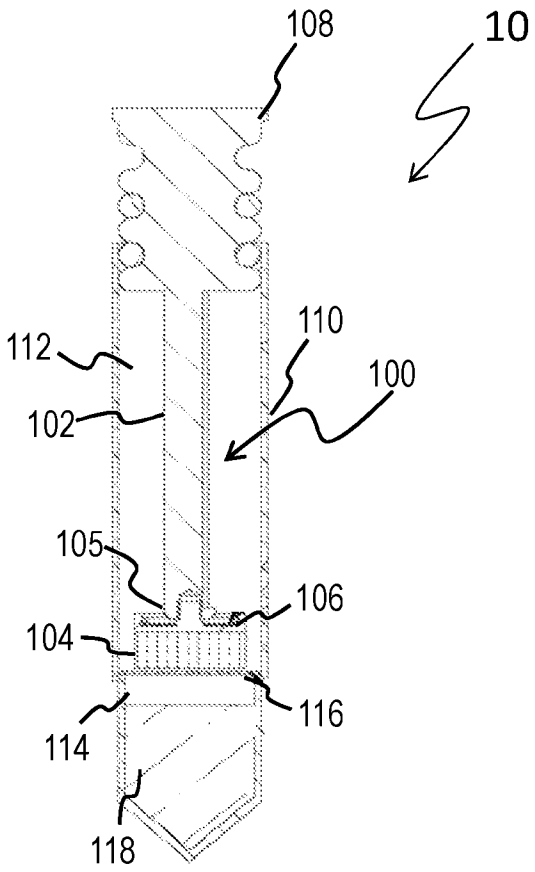

FIG. 1A

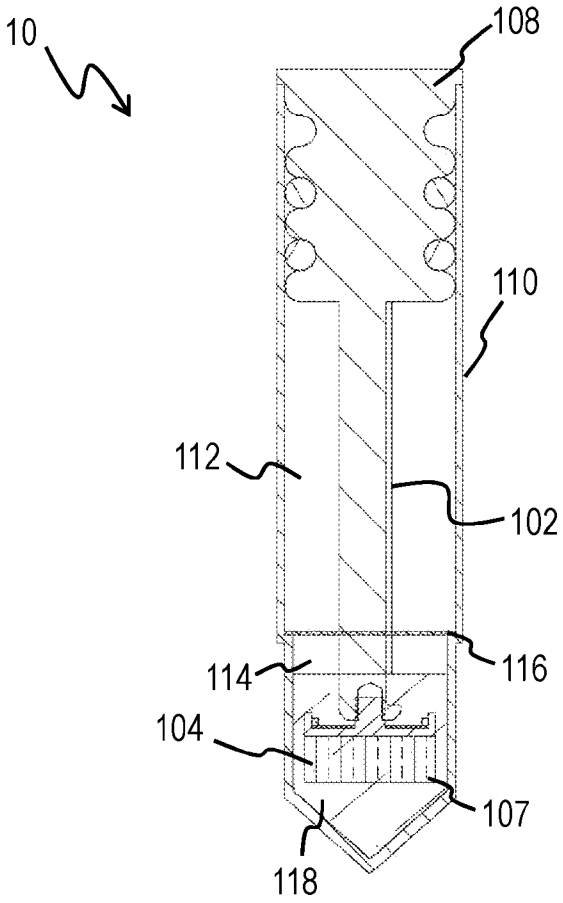

FIG. 1B

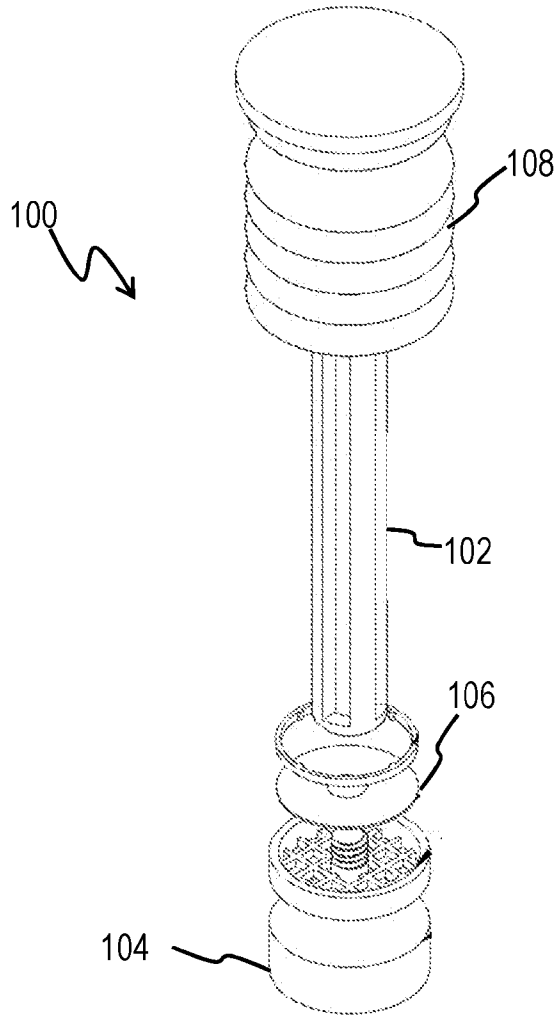

FIG. 1C

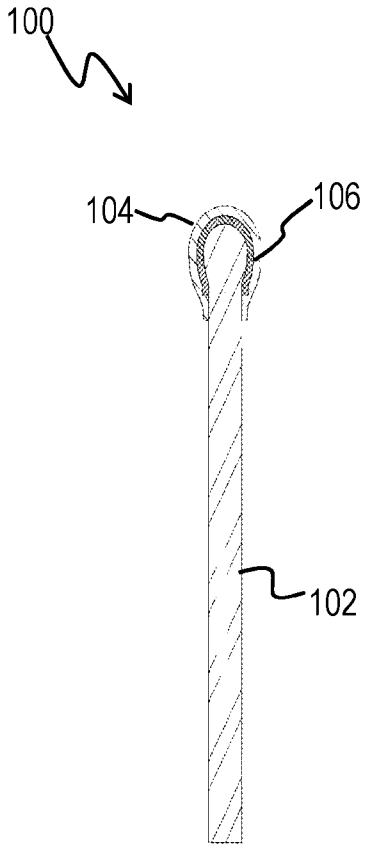

FIG. 2A

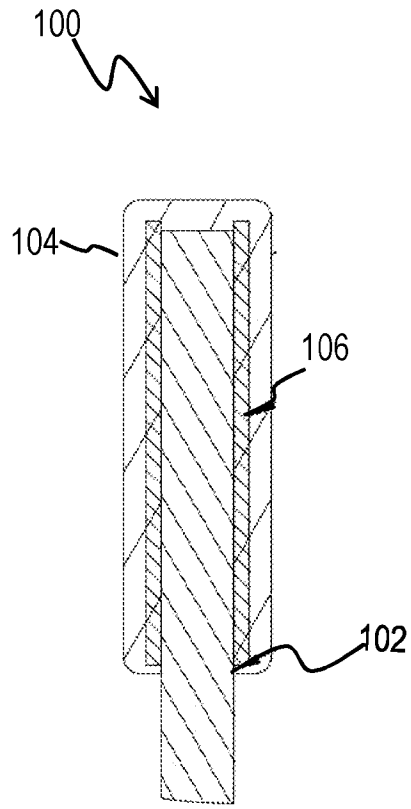

FIG. 2B

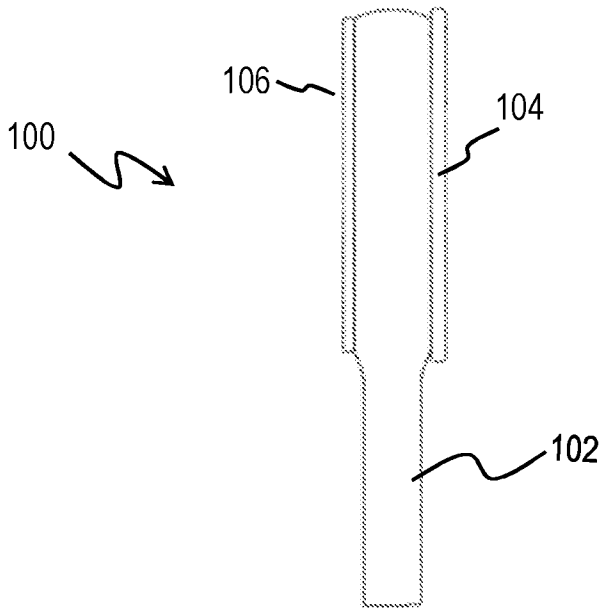

FIG. 2C

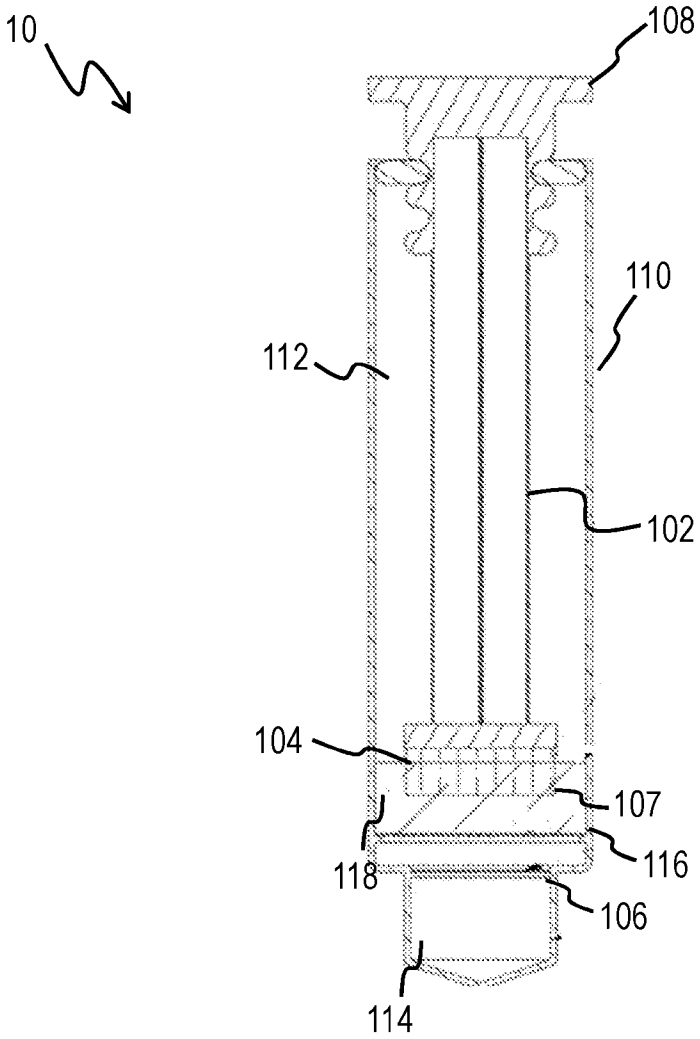

FIG. 3

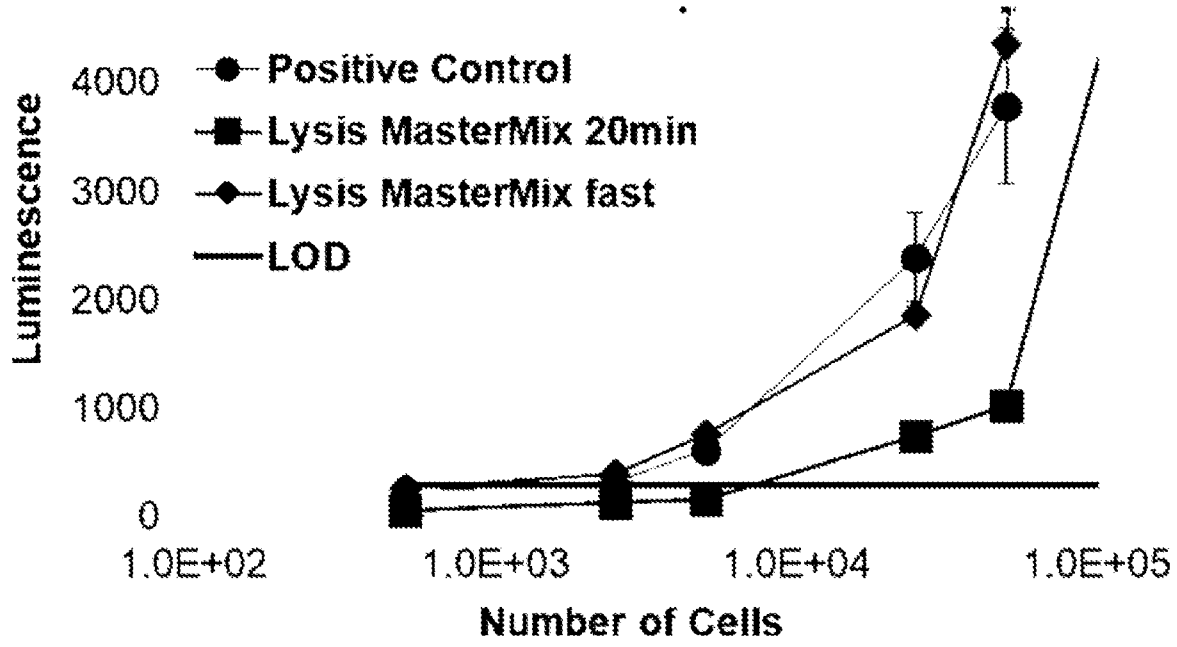
Positive Control
Lysis MasterMix 20min
Lysis MasterMix fast
LOD
Luminescence
4000
3000
2000
1000
0
1.0E+02
1.0E+03
1.0E+04
1.0E+05
Number of Cells

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/046881

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***G01N 1/02*** (2024.01); ***C12Q 1/00*** (2024.01); ***C12M 1/00*** (2024.01); *G01N 1/34* (2024.01)

CPC: ***G01N 1/02; C12Q 1/00; C12M 1/00;*** *G01N 1/34*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2023/0012231 A1 (DIAG-NOSE MEDICAL PTY LTD) 12 January 2023 (12.01.2023) para [0047]; [0052]; [0056]; [0058]; [0123]; [0124]; [0127]; [0129]; [0130]; [0137]; [0138]; [0192]	1-15
Y	WO 2023/278815 A1 (UPKARA, INC.) 05 January 2023 (05.01.2023) para [00102]; [00103]	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2024 (30.10.2024)	**15 January 2025 (15.01.2025)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**KARI RODRIQUEZ**
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/046881

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **16-26**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I, claims 1-15, directed to a detection apparatus comprising a sample collection device and a substrate.

Group II, claims 27-33, directed to a method for detecting an organism using a detection device.

The inventions listed as Groups I-II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of a detection apparatus comprising a sample collection device and a substrate, that is not required by Group II.

Group II has the special technical feature of a method for detecting an organism comprising: collecting onto a collection surface a biological sample to be tested for the organism, contacting a substrate with elution buffer, wherein the elution buffer rehydrates the vitrified detection agent, whereby the organism, if present in the sample, reacts with the detection agent, and detecting the presence or absence of the organism by the presence or absence of a detectable signal, that is not required by Group I.

Common Technical Features:

Groups I-II share the common technical features of (use of) a detection apparatus comprising:

a sample collection device comprising:

--a collection surface; and

--an elongate shaft, coupled to a proximal end of the collection surface; and

a substrate fluidly coupled to said collection surface, wherein said substrate comprises at least one detection agent vitrified thereto.

However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is previously made obvious over US 2013/0237780 A1 to Medtronic Ardian Luxembourg S.A.R.L. (hereinafter ‘Medtronic’), in view of WO 2023/278815 A1 to Upkara, Inc., (05 January 2023), (hereinafter 'Upkara').

Medtronic teaches a detection apparatus comprising:

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

a sample collection device (abstract "sampling assembly") comprising:

a collection surface (para [0055] - “The system 100 can further include an analyzer 120 (e.g., a biosensor) configured to receive and analyze the biological sample collected by the neuromodulation and sampling assembly 102”); and

an elongate shaft, coupled to a proximal end of the collection surface (para [0057] - “the analyzer 120 can be incorporated into the handle 112 of the treatment device 110 and can be configured to receive a collected biological sample via the elongated shaft 116");

and a substrate fluidly coupled to said collection surface (para [0057] - “The analyzer 120 can include one or more detection agents (e.g., a substrate for a target biomarker or an enzyme or catalytic antibody for which the target biomarker is a substrate) and/or capture agents (e.g., an agent that specifically binds to a target biomarker and/or binds to an enzymatic product or by-product of the target biomarker)”; para [0107] - “One or more exterior and/or interior surfaces of the test element 1900 can be coated and/or impregnated with a detection agent and/or capture agent”; para [0058] - “Upon receipt of the sample by the analyzer 120, detection and/or capture agents within the analyzer 120 can interact with target biomarkers of the collected sample”; para [0059] - “the analyzer 120 can be configured to receive a biological sample directly from the treatment device 110 (e.g., via a fluid conduit (not shown) (e.g., polymer tubing) within or separate from the connector 130)”), but does not explicitly teach wherein said substrate comprises at least one detection agent vitrified thereto.

Upkara teaches the use of vitrified detection agents deposited on the surface of a detection device (para [00102] - “A bioactive agent was deposited onto the substrate of sample prototype devices (labeled "scaffold device") and onto controls (labeled PES membrane) and samples of each were vitrified under vacuum with heat for 30 minutes”; para [00103] - “As shown, an anti-lgG detection antibody conjugated to H RP was stored at -20°C, 4°C or 55°C in either it's commercial product (liquid) state or vitrified in the prototype device for 5 days. The eluted protein was quantified and normalized using BCA. The antibody was then used as a detection antibody in an ELISA targeting IgG”). Since Medtronic teaches the deposition of detection reagents (para [0107]), it would have been obvious to one of ordinary skill in the art to apply the vitrified detection agent taught by Upkara in order to deposit a stable detection agent onto the substrate in the device taught by Medtronic.

As the technical features were known in the art, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I-II lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

INTERNATIONAL SEARCH REPORT

International application No.
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Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-15**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.