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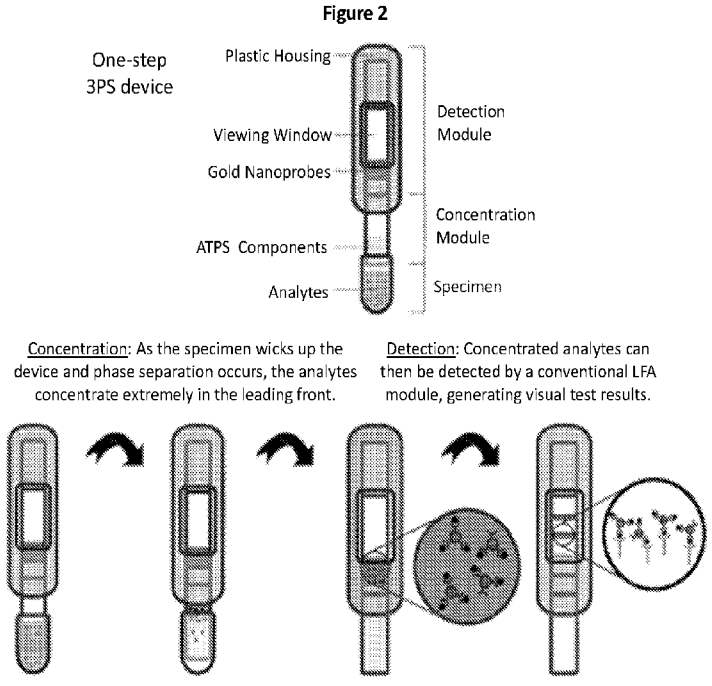
Figure 2



(57) Abstract: The present invention provides a method and device for concentration and detection of a target analyte in a solution using a pre-coated porous material embedded with ATPS components. In one embodiment, the water soluble nanoparticles include, but are not limited to, silicon dioxide, iron oxide, titanium dioxide, silver oxide and any combinations thereof. In one embodiment, the present invention provides a device comprising ATPS as a concentration module linked with a lateral flow immunoassay (LFA) component as a detection module. In one embodiment, as the sample wicks up the device and ATPS components undergo phase separation, the analytes are concentrated substantially in the leading front, the concentrated analytes are then detected by the LFA module generating visual test results. In one embodiment, the present method and device are used to detect Streptococcus mutans and thereby dental caries.

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[Continued on next page]

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METHOD AND DEVICE FOR ACCURATE DIAGNOSIS OF DENTAL DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/478,021, filed March 28, 2017. The entire contents and disclosures of the preceding application are incorporated by reference into this application.

[0002] Throughout this application, various publications are cited. The disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

FIELD OF THE INVENTION

[0003] The present invention relates to a method and device for improving detection accuracy and diagnostic performance. The method is related to components of ATPS (aqueous two-phase system) which are embedded entirely within a porous material, allowing spontaneous phase separation and therefore, purification and concentration of analytes with only a single-step required from the end user. The present invention further provides a diagnostic detection device for carrying out the method of the present invention.

BACKGROUND OF THE INVENTION

[0004] New biomarkers and medicines for many diseases have been discovered or invented in recent years. However, numerous patients still die of these diseases every year. One of the reasons accounting for the death is the failure of detecting the diseases at an early stage or the failure of detecting the diseases at all. Many of these diseases are symptomless at their early stages. By the time symptoms develop, the patients are already at late or end stages. There are not much health care professionals can do to cure the patients or to save their lives. If these diseases are diagnosed early, patients are more likely to be cured fully by applying the right procedures or using the suitable medicines. While examining the patients, experienced health care professionals may suspect that the patients have certain diseases. However, the concentration of the biomarkers in the patients at early stage is usually so insignificant and is not detectable. Health care professionals cannot prescribe the suitable medicines if the diagnostic technique cannot confirm the existence of the diseases. The predicament is that biomarkers of a disease exist in a patient but they are

unable to be detected. Medicines exist to cure the disease but they are not prescribed. All these issues are the result of not detecting the disease early.

[0005] It has been a huge challenge to detect the existence of an analyte which has an extremely minute concentration. The analyte can be a biomarker of a disease such as a cell free DNA (cfDNA), circulating tumor DNA (ctDNA) or a protein which may exist in a sample such as saliva, blood, urine and other bodily fluids of the patient. Many of the existing diagnostic or detection methods may falsely report that the analyte does not exist if the analyte concentration is too low. For instance, the gold standard of diagnostics such as Polymerase Chain Reaction (PCR) and Enzyme-Linked Immune Sorbent Assay (ELISA) may produce a false negative result if the targeted analyte has extremely low concentration.

[0006] Cell free DNA (cfDNA) extracted from liquid biopsies has become an attractive biomarker for several pathologic conditions. For example, cfDNA likely released from apoptotic or necrotic tumor cells can be used to monitor cancer progression and relapse throughout treatment with potentially greater sensitivity than current gold standard imaging methods like Computed Tomography (CT), Positron Emission Tomography (PET), and Magnetic Resonance Imaging (MRI). In comparison to a tumor biopsy that is taken from a region of the solid tumor, liquid biopsies are noninvasive, can be collected at multiple time points, and can yield cfDNA that better represents the diversity of the tumor, which is useful for identifying drug-resistant mutations.

[0007] Additionally, cfDNA of the fetus can indicate various abnormalities during pregnancy such as aneuploidies, which can be done earlier than current prenatal testing methods like chorionic villi sample and amniocentesis, without the risk of spontaneous abortion. Liquid biopsies of heart transplant patients can reveal donor cfDNA, which can be a warning sign that the patient will reject the transplant in the upcoming weeks or months after the transplant.

[0008] Despite discovery and invention of new biomarkers for many diseases in recent years, diagnostic tests based on these new biomarkers, such as cfDNA testing, have not been adopted in routine clinical procedures due to lack of sensitivity and specificity. Where biomarkers are present in low quantities, accurate detection hinges upon isolation methods that can concentrate the biomarkers from background. Depending on the isolation method, this challenge can be complicated with variance in fragment size and influences by test inhibitors.

[0009] There are purification products widely used in literature to concentrate cfDNA. However, these expensive product kits are limited by the maximum sample volume they can process, as well

as the amount of DNA that can be purified before clogging (in the case of blood samples). A method to concentrate the analyte by porous material embedded with ATPS has been disclosed in patent WO2017041030. Unfortunately, the fold of concentration was only about 10 to 60-fold for detecting *Streptococcus mutans*, which is the dominant bacterium that could lead to dental caries (cavities). The low concentration hardly afford an accurate and sensitive diagnosis of dental caries. An improved method and device to achieve more than 60-fold concentration is highly desired.

[0010] To overcome these limitations, the present invention provides an improved method and device to purify target analytes from samples and concentrate the purified target analytes for further analysis, the above can be easily and quickly done in a single step without the need of complex equipment. Using porous material embedded with ATPS, the present methods and devices can concurrently perform a number of tasks including cell lysis, isolating targeted biomolecules, removing non-targeted biomolecules and impurities, and concentrating targeted biomolecules. Concentrating biomolecules or removing inhibitors will improve detection accuracy and diagnostic performance.

[0011] Using porous material embedded with ATPS, the present method and device can purify target analytes from samples and concentrate them for up to 100x or more.

[0012] Overall, the methods and device described herein can improve the accuracy, sensitivity and efficiency of the detection and quantification of biomolecules and therefore are capable of improving the performance of various analytical or diagnostic technologies relying on the detection and/or quantification of biomolecules. Many life-threatening diseases are cured if the diseases are detected early.

SUMMARY OF THE INVENTION

[0013] The foregoing summary, as well as the following detailed description, are better understood when read in conjunction with the appended figures. The figures are intended to be illustrative and not limiting. The disclosure is not limited to the precise arrangements and examples shown herein.

[0014] The present invention relates to an improved method and device for improving detection accuracy and diagnostic performance. The present method and device is related to components of ATPS (aqueous two-phase system) which are embedded entirely within a porous material, allowing spontaneous phase separation and therefore, purification and concentration of analytes with only a single-step required from the end user.

[0015] In one embodiment, the present invention provides a method and device for concentration and detection of a target analyte in a solution using a pre-coated porous material embedded with ATPS components. The porous material is pre-coated with water soluble nanoparticles. The water soluble nanoparticles include, but are not limited to, silicon dioxide, iron oxide, titanium dioxide, silver oxide and any combinations thereof.

[0016] In one embodiment, the present invention provides a device comprising ATPS as a concentration module linked with a lateral flow immunoassay (LFA) component as a detection module. In one embodiment, the detection module is housed in a plastic housing with a viewing window and gold nanoprobes. As the sample wicks up the device and ATPS components undergo phase separation, the analytes are concentrated substantially in the leading front. Concentrated analytes are then detected by the LFA module generating visual test results.

BRIEF DESCRIPTION OF DRAWINGS

[0017] Figure 1 shows phase separation within different porous materials embedded with two-component ATPS. The pre-coated porous materials used include fiberglass paper (FG), cotton-based paper (C), single-layer matrix paper (SM) and polyolefin foam pad (PO) from 3 different vendors.

[0018] Figure 2 shows a schematic diagram of the present device where the analytes are concentrated in the leading front of the fluid flow and detected, generating visual test results.

[0019] Figure 3 shows one embodiment of the present invention in which biomolecules were concentrated in the leading front of the ATPS on the porous material up to various concentration factors.

[0020] Figure 4 shows concentration of biomolecules from different types of sample (urine and saliva) using the present pre-coated porous material embedded with ATPS components.

[0021] Figure 5 shows that the present device comprising a porous material embedded with two-component ATPS enhance the sensitivity of detecting *S. mutans*. The result demonstrates a 100-fold improvement in the limit of detection of *S. mutans* over detection using LFA module alone

[0022] Figure 6 shows various designs of the present device using porous material with embedded two-component ATPS as a concentration module.

[0023] Figure 7 illustrates one embodiment of the manufacturing process of a concentration module.

[0024] Figure 8 demonstrates the feasibility of changing the placement and ordering of ATPS components on the concentration module.

[0025] Figure 9 shows various designs of the present device. **Figure 9A** shows a closed (assembled) and an open configuration of a vertically oriented cassette housing design for the present device which contains sample in a separate well component. **Figure 9B** shows a closed (assembled) and an open configuration of a vertically oriented cassette housing design for the present device which contains sample in an integrated well component. **Figure 9C** shows a closed (assembled) and an open configuration of a horizontally oriented cassette housing design for the present device which allows sample to be added from above the test strip. **Figure 9D** shows a closed (assembled) and an open configuration of a horizontally oriented cassette housing design for the present device which allows sample to be added from below the test strip.

[0026] Figure 10 shows several CAD models of cassette housing designs for the present device.

DETAILED DESCRIPTION OF THE INVENTION

[0027] In the following description, several embodiments of the invention are described. For purposes of explanation, specific configurations and details are set forth in order to provide a thorough understanding of the embodiments. In addition, to the plural or singular forms of a word and to the extent that orientations of the embodiments are described as, “top”, “bottom”, “front”, “back”, “left”, “right” and the like, these wordings are to aid the reader in understanding the embodiments and are not meant to be limiting physically. It is apparent to a person skilled in the art that the present invention may be practiced without specific details. The invention will be better understood by reference to the examples which follow, but those skilled in the art will readily appreciate that the specific examples are for illustrative purposes only and should not limit the scope of the invention which is defined by the claims which follow thereafter. It is to be noted that the transitional term “comprising” or “including”, which is synonymous with “containing” or “characterized by”, is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[0028] The present invention relates to an improved method and device for improving detection accuracy and diagnostic performance. The present method and device is related to components of ATPS (aqueous two-phase system) which are embedded entirely within a porous material, allowing spontaneous phase separation and therefore, purification and concentration of analytes

with only a single-step required from the end user.

[0029] In one embodiment, the present invention provides a method and device for concentration and detection of a target analyte in a solution using a pre-coated porous material embedded with ATPS components. The porous material is pre-coated with water soluble nanoparticles. The water soluble nanoparticles include, but are not limited to, silicon dioxide, iron oxide, titanium dioxide, silver oxide and any combinations thereof.

[0030] In one embodiment, the present invention provides a device comprising ATPS as a concentration module linked with a lateral flow immunoassay (LFA) component as a detection module. In one embodiment, the detection module is housed in a plastic housing with a viewing window and gold nanoprobes. As the sample wicks up the device and ATPS components undergo phase separation, the analytes are concentrated extremely in the leading front. Concentrated analytes are then detected by the LFA module generating visual test results.

[0031] In one embodiment, the present invention provides a method that improves the performance of various detection and diagnostic techniques, for example, in terms of accuracy, sensitivity and efficiency.

[0032] In one embodiment, the present invention further provides a diagnostic or detection device for carrying out the methods described herein.

Type of Samples and Target Analytes

[0033] In one embodiment, the present method and device can separate a target analyte from non-target molecules (e.g., small molecules and macromolecules which are typically of natural origin and may interfere with the detection or quantification of target analyte) in a sample and thereby allows a more accurate detection and diagnosis.

[0034] In one embodiment, the present method and device are applicable to any types of sample. In one embodiment, samples include but are not limited to blood, plasma, serum, tissues, bacteria, viruses, RNA viruses, smear preparations, bacteria cultures, cell cultures (e.g. cell suspensions and adherent cells), urine, saliva, fecal matters, and bodily discharges (e.g. tears, sputum, nasopharyngeal mucus, vaginal discharge and penile discharge), polymerase chain reaction (PCR) mixtures and *in vitro* nucleic acid modification reaction mixtures.

[0035] In one embodiment, the present method and device are used to purify and concentrate a target analyte from blood. The present method is able to lyse the blood cells to release their

contents, separate the target analyte from non-target molecules including cell debris, and concentrate the target analyte simultaneously.

[0036] In one embodiment, the present method and device are used to purify and concentrate a target biomarker from saliva or urine. As shown in **Figure 4**, the present invention was applicable to urine and saliva. Despite additional organic and inorganic components present in other sample medium, the concentration phenomenon behaved similarly to PBS. Additionally, the porous materials can filter out any large molecules that would disrupt the LFA performance.

[0037] In one embodiment, the target analytes include but are not limited to proteins, nucleic acids, carbohydrates, lipids, bacteria, virus and nanoparticles and the like.

[0038] In one embodiment, the target analyte is a cell-free molecule including but is not limited to a cell-free DNA (cfDNA), a cell-free protein, an exosome and a cell-free molecule circulating in the body fluid of the subject. In one embodiment, the biomarker is a molecule attached to the surface of a cell, or included in a cell.

[0039] In one embodiment, the target analyte is a nucleic acid of various types (e.g. DNA including cDNA, RNA including mRNA and rRNA), forms (e.g. single-stranded, double-stranded, coiled, as a plasmid, non-coding or coding) and lengths (e.g. an oligonucleotide, a gene, a chromosome and genomic DNA), originated from the subject or an exogenous agent or both.

[0040] In one embodiment, the target analyte is a protein which is a peptide or a polypeptide, including an intact protein molecule, a degraded protein molecule and digested fragments of a protein molecule. In one embodiment, biomarkers include but are not limited to antigens, receptors and antibodies, originated from the subject or an exogenous agent or both.

[0041] In one embodiment, the target analyte is a small molecule such as a metabolite. In one embodiment, the metabolite is a disease-related metabolite which is indicative of the presence or extent of a disease or a health condition. In one embodiment, the metabolite is a drug-related metabolite such as a drug by-product of which the level changes in a subject body consuming the drug.

[0042] In one embodiment, the target analyte is originated from the subject himself or herself (e.g. molecules that are derived or released from any organs, tissues or cells of the subject), an exogenous source (e.g. a pathogen such as virus or bacteria associated with a particular disease), or a food or drug taken by the subject.

[0043] In one embodiment, the target analyte is a molecule produced by a tumor or cancer, or by the body of the subject in response to a tumor or cancer.

[0044] In one embodiment, the target analyte is not normally found in healthy subject. In one embodiment, the target biomarker is a molecule that is normally found in a healthy subject but the level of which is indicative of a particular disease or a health condition.

ATPS (Aqueous two phase system)

[0045] In one embodiment, there is provided a two-component ATPS (aqueous two phase system) embedded entirely within a porous material for purifying a target analyte from a sample and concentration of the target analyte.

[0046] In one embodiment, the ATPS components are embedded entirely within a porous material, allowing spontaneous phase separation and therefore, purification and concentration of analytes with only a single-step required from the end user.

[0047] In one embodiment, a two-component ATPS is embedded entirely within a porous material which is pre-coated with water soluble nanoparticles.

[0048] In one embodiment, when the ATPS is applied on a porous material (e.g. paper), the two phases of the ATPS separate from each other as the mixed phase solution flows through the porous materials. The resulting phase solutions are of different physicochemical properties and each phase travels through the porous matrix at different rates. Different molecules in a mixture would be distributed differentially between the two phase solutions due to their different properties, and it is possible to separate and concentrate target molecules using ATPS with minimal set up and human intervention. No power or equipment is necessary to bring about the phase separation, as the fluid flow relies purely on capillary action which is based on isothermal-dynamic principles.

[0049] The advantage of the invention is that high purity and concentration of the target analyte can be obtained in a simple way and compatible with downstream application analysis without further step of purification or concentration.

[0050] The methods and devices provided herein are robust, inexpensive, simple, easy to handle, safe, user friendly and fast. The present method and device is able to purify and concentrate the target analyte and thereby ensures the performance of the downstream applications using the purified and concentrated analyte will not be affected by impurities in the original sample. Since the present invention does not require any additional power source, complex instrumentation or

electronic hardware to operate, it provides a fast and affordable means for rapid analyte isolation and purification.

[0051] Because of the unique features described herein, the present invention can purify and concentrate the target analyte conveniently and rapidly without the use of external power source or complex instrumentation, and is applicable to samples containing the target analyte in a very low amount, or of a small volume. Furthermore, the present method is readily adaptable to automation including high throughput screening systems.

Design of ATPS-embedded porous material

[0052] In one embodiment, the present invention provides a porous material embedded with ATPS components. Various ATPS systems can be used in the present invention, including but are not limited to polymer-polymer (e.g.PEG-dextran), polymer-salt (e.g. PEG-salt), and micellar (e.g. Triton X-114). Porous material may be made of any suitable porous material which can absorb and transfer liquid. Suitable porous materials for this invention include but are not limited to fiberglass paper, cotton-based paper, other types of paper, polymer foams, cellulose foams, other types of foams, rayon fabric, cotton fabric, other types of fabric, wood, stones, and any other materials that can absorb and transfer liquid.

[0053] In one embodiment, the porous material is commercially available or manufactured in-house.

[0054] **Figure 1** illustrates the ability of several types of porous materials (fiberglass paper, cotton-based paper, single-layer matrix paper and polyolefin foam pad) from three vendors in inducing phase separation when impregnated with ATPS components. Target analytes were indicated by a purple color. As shown in Figure 1, fiberglass paper FG1 from vendor 1, FG1 and FG2 from vendor 2 and polyolefin foam pad from vendor 3 were the best at concentrating analyte with a strong leading purple front.

Porous material pre-coated with water soluble nanoparticles

[0055] In one embodiment, the present invention provides a method and device for concentration and detection of a target analyte in a solution using a pre-coated porous material embedded with ATPS components.

[0056] In one embodiment, the present invention provides a porous material that is pre-coated with

water soluble nanoparticles and embedded with ATPS components. The pre-coated porous material is capable of increasing the concentration of target analyte significantly by enhancing the adhesion property and facilitating the embedding of ATPS on the porous material.

[0057] In one embodiment, the present porous material is pre-coated with water soluble nanoparticles. The water soluble nanoparticles include, but are not limited to, silicon dioxide, iron oxide, titanium dioxide, silver oxide and any combinations thereof.

[0058] In one embodiment, the porous material comprises a surface and plurality of pores/capillary system. In one embodiment, the surface of the porous material is pre-coated with water soluble nonparties. In another embodiment, pores/capillary system of the porous material are pre-coated with water soluble nonparties. In yet another embodiment, both the surface and pores/capillary system of the porous material are pre-coated with water soluble nonparties. In one embodiment, both the surface and pores/capillary system of the porous material are pre-coated with silicon dioxide.

[0059] Water soluble nanoparticles (e.g. silicon dioxide) carry both positive and negative charges which play an important role to influence the interaction between the molecules in ATPS and the porous material. In this invention, it was found that pre-coating the porous material with water soluble nanoparticles significantly facilitates the efficient of embedding of ATPS on the porous material. With better embedding, ATPS is more stable on the porous material and ultimately improve the concentration performance. In one embodiment, concentration fold achieved by this invention can be up to 100 or more.

[0060] In one embodiment, porous material, for example, porous fiberglass paper, is coated with addition of water soluble nanoparticles, such as silicon dioxide, by the following preparation process: 5M concentration of silicon dioxide is dispersed in aqueous solution. Porous fiberglass paper is soaked in the 5M silicon dioxide aqueous solution followed by the vigorous stirring for an hour. The system is kept at the temperature of 80°C for 24 hours. Then the paper is taken out and washed twice with water, and finally dried under compressed air.

Adjustment of concentration factors

[0061] In one embodiment, the relative amounts of ATPS components in the porous material can be changed.

[0062] By changing the amounts of ATPS components embedded on the porous material and

thereby the volume ratio of the two phases, the target analyte can be preferentially concentrated in one phase. In one embodiment, the target analyte is retained in the leading front of the ATPS, which is then collected and optionally further analyzed using appropriate technologies. In one embodiment as illustrated by the examples provided herein, target analyte is purified at the top of the porous material embedded with ATPS components and concentrated for up to 50-100 folds as compared to a control stack having no ATPS components.

[0063] In one embodiment, the order of ATPS components on the porous material can be adjusted to achieve the desired phenomenon. In one embodiment, one or more components can be embedded onto the porous material.

[0064] To better quantify the phenomena associated with the present invention, an assay was developed to evaluate the correlation between the relative amounts of ATPS components embedded on the porous material and the fold of concentration achieved. With this, the concentration factor can be selected and fine-tuned by adjusting the relative amount of the ATPS components as needed.

[0065] In one embodiment, to integrate the ATPS components into the porous material, the ATPS components were solubilized in water (or appropriate buffer) and applied on the porous material in certain ratios. The porous materials were then placed in a lyophilizer to remove water, resulting in the ATPS components embedded directly on the porous material. Upon introduction of the sample to the porous materials, the ATPS components instantly undergo rehydration and thereby separate the molecules in the sample and concentrate the target analyte at the front of the fluid flow without any external power or equipment to provide a driving force.

[0066] As illustrated in **Figure 3** where two combinations of ATPS (micellar-based and polymer/salt-based ATPS) were tested, the concentration of a targeted analyte could be achieved up to 100X or more. Same amount of purple-colored analyte was loaded to porous materials embedded with varying amount of ATPS components as well as porous materials without ATPS components as a control. For the control, the analyte was homogenously distributed throughout the porous material with the light purple color. On the other hand, introduction of ATPS components into the porous materials allows for concentration of the analyte at the leading front of the fluid flow, as signified by a darker purple color. The data herein demonstrated that the concentration factors achieved were between 30X and 100X. More importantly, the data herein demonstrated that by adjusting the ratios of ATPS components embedded on the porous material,

the present invention can adjust the concentration factor as needed. Furthermore, the data indicated that multiple ATPS components (a polymer/salt system and micellar system) incorporated into porous material can achieve purification and concentration of analyte. Other polymer/salt, micellar, and polymer/polymer systems can be likewise implemented with further optimization.

[0067] In one embodiment, the present invention provides a device comprising a porous fiberglass paper that is impregnated with ATPS which is made of polymer to polymer-based PEG-dextran. When a sample containing a plurality of analytes is poured onto the device, the impregnated porous fiberglass paper preferentially causes the analyte-containing ATPS component to flow ahead of the other ATPS component. Therefore, the targeted analyte is concentrated in the analyte-containing ATPS component at the front of the fluid flow.

[0068] In one embodiment, the present invention provides a device comprising a porous fiberglass paper that is pretreated with ATPS which is made of polymer to salt based PEG-salt. When a sample containing a plurality of analytes is poured onto the device, the pretreated porous fiberglass paper preferentially causes the analyte-containing ATPS component to flow ahead of the other ATPS component. Therefore, the targeted analyte is concentrated in the analyte-containing ATPS component at the front of the fluid flow.

[0069] In one embodiment, the present invention provides a device comprising a porous fiberglass paper is impregnated with ATPS which is made of micellar based surfactant-containing solutions. When a sample containing a plurality of analytes is poured onto the device, the impregnated porous fiberglass paper preferentially causes the analyte-containing ATPS component to flow ahead of the other ATPS component. Therefore, the targeted analyte is concentrated in the analyte-containing ATPS component at the front of the fluid flow.

[0070] In one embodiment, sample subject to the present invention comprises a buffer solution includes, but are not limited to, phosphate-buffered saline (PBS), Tris-EDTA (TE) buffer. In this invention, prefer buffer is PBS buffer 9.57 Mm (8 mM Sodium chloride, 0.2 mM Potassium chloride, 1.15 mM Sodium monohydrogen phosphate, 0.2 mM Potassium dihydrogen phosphate, solution with pH 7.35-7.65). In one embodiment, the buffer is TE buffer containing 2% bovine serum albumin (BSA), 0.1% Tween-20, 0.1%PEG and 20 mM Tris, pH 7.5 respectively.

[0071] In one embodiment, multiple ATPS are incorporated into a porous material to achieve a control of concentration factors of the target analyte.

[0072] In one embodiment, a polymer/salt ATPS is implemented with the optimization in the ratios of the ATPS components to control the concentration factors.

[0073] In one embodiment, a polymer/polymer ATPS is implemented with the optimization in the ratios of the ATPS components to control the concentration factors.

[0074] In one embodiment, a micellar ATPS is implemented with the optimization in the ratios of the ATPS components to control the concentration factors.

[0075] In one embodiment, the ratios of the ATPS components are adjusted to demonstrate concentration factors between 10 folds and 100 folds.

[0076] In one embodiment, there are various ATPS systems including but not limited to polymer-polymer (e.g., PEG-dextran), polymer-salt (e.g. PEG-salt), and micellar (e.g. Triton X-114). The first and/or second component comprises a polymer. Polymer includes but is not limited to polyalkylene glycols, such as hydrophobically modified polyalkylene glycols, poly(oxyalkylene)polymers, poly(oxyalkylene)copolymers, such as hydrophobically modified poly(oxyalkylene)copolymers, polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl caprolactam, polyvinyl methylether, alkoxylated surfactants, alkoxylated starches, alkoxylated cellulose, alkyl hydroxyalkyl cellulose, silicone-modified polyethers, and poly N-isopropylacrylamide and copolymers thereof. In another embodiment, the first polymer comprises polyethylene glycol, polypropylene glycol, or dextran.

[0077] In one embodiment, the polymer concentration of the first component or second component is in the range of about 0.01% to about 90% by weight of the total weight of the aqueous solution (w/w). In various embodiments, the polymer solution is selected from a polymer solution that is about 0.01% w/w, about 0.05% w/w, about 0.1 % w/w, about 0.15% w/w, about 0.2% w/w, about 0.25% w/w, about 0.3% w/w, about 0.35% w/w, about 0.4% w/w, about 0.45% w/w, about 0.5% w/w, about 0.55% w/w, about 0.6% w/w, about 0.65% w/w, about 0.7% w/w, about 0.75% w/w, about 0.8% w/w, about 0.85% w/w, about 0.9%) w/w, about 0.95% w/w, or about 1% w/w. In some embodiments, the polymer solution is selected from polymer solution that is about 1% w/w, about 2% w/w, about 3% w/w, about 4% w/w, about 5% w/w, about 6% w/w, about 7% w/w, about 8% w/w, about 9% w/w, about 10% w/w, about 1 1% w/w, about 12% w/w, about 13% w/w, about 14% w/w, about 15% w/w, about 16% w/w, about 17% w/w, about 18% w/w, about 19% w/w, about 20% w/w, about 21% w/w, about 22% w/w, about 23% w/w, about 24% w/w, about 25% w/w, about 26% w/w, about 27% w/w, about 28% w/w, about 29% w/w, about 30% w/w,

about 31 % w/w, about 32% w/w, about 33% w/w, about 34% w/w, about 35% w/w, about 36% w/w, about 37% w/w, about 38% w/w, about 39% w/w, about 40% w/w, about 41% w/w, about 42% w/w, about 43% w/w, about 44% w/w, about 45% w/w, about 46% w/w, about 47% w/w, about 48% w/w, about 49% w/w, and about 50% w/w.

[0078] In one embodiment, the first and/or second component comprises a salt, the salt includes but is not limited to kosmotropic salts, chaotropic salts, inorganic salts containing cations such as straight or branched trimethyl ammonium, triethyl ammonium, tripropyl ammonium, tributyl ammonium, tetramethyl ammonium, tetraethyl ammonium, tetrapropyl ammonium and tetrabutyl ammonium, and anions such as phosphates, sulphate, nitrate, chloride and hydrogen carbonate. In another embodiment, the salt is selected from the group consisting of NaCl, Na3PO4, K3PO4, Na2SO4, potassium citrate, (NH4)2SO4, sodium citrate, sodium acetate and combinations thereof. Other salts, e.g. ammonium acetate, may also be used.

[0079] In one embodiment, the total salt concentration is in the range of 0.001 mM to 100mM. A skilled person in the art will understand that the amount of salt needed to form an aqueous two-phase system will be influenced by molecular weight, concentration and physical status of the polymer.

[0080] In various embodiments, the salt phase is selected from a salt solution that is about 0.001% to 90% w/w. In various embodiments, the salt solution is selected from a salt solution that is about 0.01% w/w, about 0.05% w/w, about 0.1 % w/w, about 0.15% w/w, about 0.2% w/w, about 0.25% w/w, about 0.3% w/w, about 0.35% w/w, about 0.4% w/w, about 0.45% w/w, about 0.5% w/w, about 0.55% w/w, about 0.6% w/w, about 0.65% w/w, about 0.7% w/w, about 0.75% w/w, about 0.8% w/w, about 0.85% w/w, about 0.9%) w/w, about 0.95% w/w, or about 1% w/w. In some embodiments, the salt solution is selected from polymer solution that is about 1% w/w, about 2% w/w, about 3% w/w, about 4% w/w, about 5% w/w, about 6% w/w, about 7% w/w, about 8% w/w, about 9% w/w, about 10% w/w, about 1 1% w/w, about 12% w/w, about 13% w/w, about 14% w/w, about 15% w/w, about 16% w/w, about 17% w/w, about 18% w/w, about 19% w/w, about 20% w/w, about 21% w/w, about 22% w/w, about 23% w/w, about 24% w/w, about 25% w/w, about 26% w/w, about 27% w/w, about 28% w/w, about 29% w/w, about 30% w/w, about 31 % w/w, about 32% w/w, about 33% w/w, about 34% w/w, about 35% w/w, about 36% w/w, about 37% w/w, about 38% w/w, about 39% w/w, about 40% w/w, about 41% w/w, about 42% w/w, about 43% w/w, about 44% w/w, about 45% w/w, about 46% w/w, about 47% w/w, about 48% w/w,

about 49% w/w, and about 50% w/w.

[0081] In one embodiment, the first component and/or the second component in the ATPS comprises a solvent that is immiscible with water. In some embodiments, the solvent comprises a non-polar organic solvent. In some embodiments, the solvent comprises an oil. In some embodiments, the solvent is selected from pentane, cyclopentane, benzene, 1,4- dioxane, diethyl ether, dichloromethane, chloroform, toluene and hexane.

[0082] In one embodiment, the first component and/or second component in the ATPS comprises a micellar solution. In some embodiments, the micellar solution comprises a nonionic surfactant. In some embodiments, the micellar solution comprises a detergent. In some embodiments, the micellar solution comprises Triton-X. In some embodiments, the micellar solution comprises a polymer similar to Triton-X, such as Igepal CA-630 and Nonidet P-40. In some embodiments, the micellar solution consists essentially of Triton-X.

[0083] In one embodiment, the first component in the ATPS comprises a micellar solution and the second component in the liquid phase comprises a polymer. In one embodiment, the second component in the liquid phase comprises a micellar solution and the first component in the liquid phase comprises a polymer. In one embodiment, the first component in the liquid phase comprises a micellar solution and the second component in the liquid phase comprises a salt. In one embodiment, the second component in the liquid phase comprises a micellar solution and the first component comprises a salt. In one embodiment, the micellar solution is a Triton-X solution. In one embodiments, the first component comprises a first polymer and the second component comprises a second polymer. In one embodiment, the first/second polymer is selected from polyethylene glycol and dextran. In one embodiments, the first component comprises a polymer and the second component comprises a salt. In one embodiment, the second component comprises a polymer and the first component comprises a salt. In some embodiments, the first component comprises polyethylene glycol and the second component comprises potassium phosphate. In some embodiments, the second component comprises polyethylene glycol and the first component comprises potassium phosphate. In one embodiment, the first component comprises a salt and the second component comprises a salt. In one embodiments, the first component comprises a kosmotropic salt and the second component comprises a chaotropic salt. In some embodiments, the second component comprises a kosmotropic salt and the first component comprises a chaotropic salt.

[0084] In one embodiments, ratios of the first component to the second component are in the range of 1:1 to 1:1000. In some embodiments, the ratio of the first component to the second component is selected from a ratio of about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, and about 1:10. In some embodiments the ratio of the first component to the second component is selected from a ratio of about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, and about 1: 100. In some embodiments the ratio of the first component to the second component is selected from a ratio of about 1:200, about 1:300, about 1:400, about 1:500, about 1:600, about 1:700, about 1:800, about 1:900, and about 1:1000.

[0085] In one embodiment, the ratio of the second component to the first component is selected from a ratio of about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, and about 1:10. In some embodiments the ratio of the second component to the first component is selected from a ratio of about 1:20, about 1:30, about 1:40, about 1 :50, about 1:60, about 1:70, about 1:80, about 1:90, and about 1:100. In some embodiments the ratio of the second component to the first component is selected from a ratio of about 1:200, about 1:300, about 1:400, about 1:500, about 1:600, about 1:700, about 1:800, about 1:900, and about 1:1000.

Downstream processing of the isolated analyte

[0086] In various embodiments, the present invention can be used in combination with one or more processes or reagents for the purpose of washing and elution of the analytes retained in the porous matrix, or post-isolation treatment of the retained analytes.

[0087] In one embodiment, after contacting the analytes containing solution with the ATPS, a washing buffer is applied on the ATPS, once or for multiple times, to wash off non-target analytes or impurities from the porous matrix. Washing buffers may comprise solutions of varying ionic strength, pH values, or contain additives such as detergents. Examples of washing buffers include but are not limited to a solution of 20%-50% ethanol and 20%-50% isopropanol; a solution of about 0.1-4.0 M guanidinium hydrochloride, detergents and up to about 80% ethanol; or a solution of about 80% ethanol.

[0088] In one embodiment, target analytes enter the porous matrix of the ATPS and flow to one end of the porous matrix. In one embodiment, target analytes isolated by the present invention are eluted out of the porous matrix using appropriate elution butters or deionized water. In one

embodiment, isolated target analytes are not eluted but stored for future use in the porous matrix. For instance, after the isolation of analytes using the present invention, the porous material (e.g. a paper) containing the target analytes (e.g. a DNA) is dried and stored. In one embodiment, analytes retained on the porous matrix can be eluted for further analysis or treatment. The selection of the elution buffer may depend on the contemplated use of the purified biomarker. Examples of suitable elution buffers for nucleic acid type of analytes includes, but are not limited to, Tris-EDTA (TE) buffer, aqua bidest and PCR buffer. In one embodiment, the purified analyte on porous paper is eluted in a tube containing TE buffer (10 mM Tris.Cl, 1 mM EDTA solution with pH 7.5), and the purified analyte is recovered in a relatively small volume, e.g., less than 100 µl.

[0089] Analytes obtained by the present invention can be subject to a wide range of downstream applications such as detection or analysis of the analytes in forensic, diagnostic or therapeutic applications, and laboratory procedures such as sequencing, amplification, reverse transcription, labeling, digestion, blotting procedures and the like. It is expected that the present invention is able to improve the performance of downstream characterization or processing of the analytes.

[0090] In one embodiment, downstream applications of the analyte include, but are not limited to, any detection, analytical or diagnostic procedures involving the detection or quantification of the purified analyte. In one embodiment, detection, analytical or diagnostic procedures to be coupled with the present invention include but are not limited to any such procedure performed in commercial clinical laboratory, and laboratory procedures such as sequencing, amplification (e.g. PCR, RT-PCR, real-time PCR, and real-time RT-PCR), reverse transcription, labeling, digestion, blotting procedures, ELISA, RIA, immunoassays, enzymatic assays, GC/MS, proteomic-based approach, and the like.

[0091] In one embodiment, analyte obtained by the present invention can be analyzed by LFA.

Diagnostic detection Device

[0092] In one embodiment, the present invention provides a device comprising ATPS as a concentration module linked with a lateral flow immunoassay (LFA) component as a detection module. In one embodiment, the detection module is housed in a plastic housing with a viewing window and gold nanoprobes. As the sample wicks up the device and ATPS components undergo phase separation, the analytes are concentrated extremely in the leading front. Concentrated analytes are then detected by the LFA module generating visual test results.

[0093] In one embodiment, the present device is coupled with additional diagnostic assay such as a LFA. The seamless transition between concentration and detection of target analyte will allow a patient or end user to get a test result in one simple single step.

[0094] In one embodiment, the present device is a diagnostic device comprising a LFA detection module and a concentration module comprising ATPS components.

[0095] Lateral flow immunoassay (LFA) methods and devices have been previously described, e.g., Gordon and Pugh, U.S. Pat. No. 4,956,302; H. Buck, et al., WO 90/06511; T. Wang, U.S. Pat. No. 6,764,825; W. Brown, et al., U.S. Pat. No. 5,008,080; Kuo and Meritt, US 6,183,972, EP00987551A3. These assays involve the detection and determination of an analyte substance that is a member of a specific binding pair consisting of a ligand and a receptor. The ligand and the receptor are related in a way that the receptor specifically binds to the ligand, being capable of distinguishing a specific ligand or ligands from other sample constituents having similar characteristics. Immunological assays involving reactions between antibodies and antigens are one such example of a specific binding assay. Other examples include DNA and RNA hybridization reactions and binding reactions involving hormones and other biological receptors.

[0096] In one embodiment, ATPS components are embedded on a type of pre-coated porous material to create a concentration module for the purpose of integration with the lateral flow immunoassay (LFA).

[0097] **Figure 7** illustrates one embodiment of the manufacturing process of the present concentration module. Porous material (either paper or foam pad of different material types) was pre-coated and treated with different solutions containing ATPS solutions and dried in the lyophilizer. Pre-coated papers were then cut into strips (or other shape) such that there is a pointed tip for easy collection of the concentrated phase. Foam pad materials were cut first prior to treatments.

[0098] As seen in **Figure 7**, ATPS components are integrated into porous material. In accordance with one embodiment as shown in Figure 7, a polymer solution (PEG) in DI H_2O is added to the narrow end of each of the following porous materials including fiberglass paper, cotton-based paper, proprietary single-layer matrix paper and a polyolefin foam pad. The papers are of the same width but of variable length to account for differences in absorption. Then a Tris-buffered solution containing protein (e.g. bovine serum albumin), surfactant (Triton X-114) and Tween-20 and PEG polymer is added immediately adjacent to the first solution in certain ratios. The porous material

is then placed in a lyophilizer to remove solvent, resulting in embedding the ATPS components directly into the porous material.

[0099] In one embodiment, the papers are cut into 0.5 cm strip lengthwise. Pieces are then stacked together (e.g., 4 strips per stack) and cut at one end from the corner to form a pointed tip for easy collection of concentrated component. This stack is then placed in an indicator-containing buffer solution (e.g., colloidal gold) with salt with varying concentrations of salt (5%, 4.5% and 4% w/w of potassium phosphate in phosphate-buffered solution to achieve concentration factors of 30X, 50X and 100X, respectively. As salt concentration decreases, the concentration factor increases. There is a minimum amount of salt required. That depends on each individual polymer formulation.

[0100] **Figure 8** demonstrates the feasibility of changing the placement and ordering of ATPS components within the concentration module, showing that the pre-coated porous material can be impregnated with both polymer and salt either as separate zones (left) or as one continuous zone where the dehydrated polymer is overlaid on the dehydrated salt (right). The paper is treated in two steps. The first step involves drying salt and polymer in separate zones. This is similar to where different solutions are dried immediately proximal to each other. The second step involves drying the polymer zone in the lyophilizer first, then layering the salt solution directly on top of the dried polymer and dried once more in the lyophilizer for one hour. This approach results in the concentration of analyte. A running buffer containing analyte results in fluid flow and the rehydration of both ATPS components results in concentration of the purple indicator at the tip of the device. In this case, the running buffer does not require a predetermined amount of ATPS components, as any liquid sample including urine, saliva, etc. can be used, providing more flexibility for the end user, e.g., patient.

[0101] In one embodiment, a micellar version of the porous material stack identical to the polymer/salt version is made with the exception of the composition of the solution embedded on porous material and the composition of the running solution. To achieve concentration factors of 40X, 70X and 100X, varying amounts and concentrations of surfactant-containing solutions are embedded onto paper. Increasing surfactant concentration leads to a higher concentration factor. In one embodiment, phosphate-buffered saline containing indicator is used as the running buffer for all three concentration factors.

[0102] Considering various porous materials can be used for the concentration module, various designs are used to promote analyte concentration and transfer to the LFA sample pad and test strip. In one embodiment, all these designs can wick large amounts of volume and be optimized for a specific application.

[0103] This invention can be realized in several formats, including ones shown in **Figure 6**. ATPS concentration module is designed to promote analyte concentration and transfer to the LFA sample pad and test strip. The design can wick large amounts of sample volume. Additionally, various porous materials are designed differently for specific applications. In one embodiment, the present invention provides stack designs in which multiple porous materials were stacked side-by-side, and solution flowing vertically from the bottom of the stack to the top. The present stack designs are not limited to the detection of *S. mutans* or assays described herein. As described herein, one of the key feature of the present invention is the impregnation of porous material with ATPS components in any format such that any sample (urine, saliva, blood, buffer, etc.) will spontaneously phase separate and concentrate/remove the analyte(s) of interest.

Detection of *Streptococcus mutans*

[0104] As a demonstration of the utility of the present invention to benefit LFA limits of detection, a single-step diagnostic test is provided herein for detecting *Streptococcus mutans (S. mutans),* a bacteria related to the formation of dental caries. *Streptococcus mutans (S. mutans)* is the dominant bacterium that could lead to dental caries (cavities) [1, 2]. The concentration is therefore critical for diagnostic test. In this assay, a 100-fold improvement was achieved in the limit of detection of this bacteria from 10^6 CFU/ml to 10^4 CFU/mL as shown in **Figure 5**. This is done by using the micellar ATPS embedded directly into pre-coated porous material, and the concentration component was integrated with the LFA test strips. This single-step test can also be used with a polymer/salt ATPS in the same fashion.

[0105] **Figure 5** demonstrates a 100-fold improvement in the limit of detection of *S. mutans* over the detection using LFA alone. Left panel shows one embodiment of the 3PS device (comprising two-component ATPS for testing, a LFA detection module, a concentration module) used for testing. The LFA detection module is integrated with upstream of the concentration module. Right panel shows the test results using the LFA detection module without the concentration module (top) vs. that of the 3PS device (bottom) at the indicated concentrations of *S. mutans*. The presence

of a control line indicates a valid test, and the presence of a test line indicates a positive result. Test lines are indicated with a black arrowhead. "C" is the control line; "T" is the test line. In the LFA detection module alone, no test line is visible for the negative sample, confirming a valid control. At the *S. mutans* concentration of 10^6 CFU/ml, the test line is visible, confirming a positive result. However, no test line is visible at 10^4 CFU/ml. In the 3PS device, no test line is visible for the negative sample, confirming a valid control. At 10^6 CFU/ml, a test line is visible, and the test line intensity is stronger than that of LFA only at 10^6 CFU/ml, confirming that the concentration module successfully concentrated *S. mutans* and indicating that a concentration fold of about 100 in detecting *S. mutans* has been achieved. Furthermore, the present 3PS device is able to detect the bacteria at 10^4 CFU/ml, which could not be detected using LFA alone. Therefore, the present 3PS device is able to achieve a 100-fold improvement in the limit of detection of *S. mutans*.

[0106] In one embodiment, the diagnostic testing device of this invention can both concentrate and detect an analyte with only a single-step required from the end user.

[0107] In one embodiment, the present invention provides one-step diagnostic device comprising a concentration module of ATPS components and a detection module of LFA test. As seen in **Figure 2**, a diagnostic testing device is provided to concentrate and detect an analyte of interest with only a single-step required from the end user.

[0108] The present device requires only a single step from the end-user who would apply the specimens onto the analyte collection tip. The ATPS components are attached to analyte collection tip, forming the concentration module. In one embodiment, the detection module further comprises a plastic housing. The plastic housing contains a viewing window to show the testing result. In one embodiment, the LFA testing module and gold nanoprobes are contained inside the plastic housing. A simple direct junction is formed to connect the concentration module to the detection module. Tape is tightly wound around the intersection between the two modules to hold this junction together and promote flow. In one embodiment, said device can be slightly longer and accommodate more sample volume. After the end-user applies the specimen at the analyte collection tip, the specimen wicks up the device and rehydrate the ATPS components. Component separation occurs. The analytes concentrate substantially in the leading front of the flow when flowing into the plastic housing. Concentrated analytes will be conjugated with the gold nanoprobes and detected by the embedded conventional LFA detection module. Visual test results

identical to the LFA test will be observed at the viewing window of the plastic housing. By using the present device, the end-user will be able to observe an accurate test result with a single-step.

[0109] In one embodiment, the 3PS device of this invention can be enclosed in cassette housing.

[0110] As seen in **Figure 10**, the cassette housing in which the 3PS device is enclosed in, has many important functions:

1) It protects the fragile 3PS device components from damage, avoiding the potential for waste or invalid tests.
2) It provides an intuitive user interface to encourage the correct usage of the 3PS device;
3) The pressure points provided at dedicated locations can improve the overall flow and conjugate release during the test;
4) The orientation of the 3PS device test ensures that an end user can run a test in the correct manner;
5) It protects the user from exposure to any of the chemicals dehydrated on the various pads; and
6) The sample well that holds the desired sample releases users from constant interaction.

[0111] In one embodiment, the 3PS device cassette housing may take a design that is similar to that of a typical LFA test cassette housing. However, because the 3PS device is capable of processing larger volume for an improved concentration fold, its housing dimensions and sample well are larger than typical LFA test cassettes to accommodate the extra size and fluid. In one embodiment, integration of the concentration module with LFA creates a new device (3PS) which can handle a seamless transition between concentration and detection, allowing for the end-user to achieve results with a single step. In one embodiment, the concentration module can be attached to the LFA in a variety of ways, including with tape, encased in a housing as shown in Figure 9. In one embodiment, the concentration module can be of various sizes to accommodate different volumes of sample. Due to the nature of the 3PS device, large volumes (for example, 5 mL or more) are possible. In one embodiment, the format of this concentration module can be varied, including the formats shown herein. In one embodiment, the present device is a platform device that can be applied to many different applications other than *S. mutans* detection, including but not limited to detection of chlamydia, malaria, Zika virus, etc.

[0112] In one embodiment, the present invention provides various designs, including two vertically oriented and two horizontally oriented designs. In one design, the design is oriented vertically and is composed of a housing to protect the strip and a separate sample well, which the housing can interlock with to initiate flow (Figure 9A). In another design, it is oriented vertically and is composed of a single integrated housing, where the sample well and housing are inseparable (Figure 9B). In another design, the device is oriented horizontally and has a large sample well which is held above the strip (Figure 9C). In another design, the device is also oriented horizontally and has a large sample well which is below the strip, into which the strip is cantilevered (Figure 9D).

[0113] The major differences between the designs in **Figure 9** are:

1) in Figure 9A, the potential exposure of the actual test strip to the environment;
2) in Figure 9B, the requirement for additional sealant such as a rubber O-ring;
3) In Figure 9C, sample is added on top, and gravity forces the liquid down the well into the strip.
4) In Figure 9D, sample is added to a sample well which is actually under the strip, and the strip is cantilevered into the well to allow for capillary-mediated flow.

[0114] In one embodiment, the present invention provides a device for the concentration and detection of a target analyte in a sample solution, the device comprises:

(a) A sample well for holding a sample solution;

(b) A concentration module connected to said sample well, said module comprising a porous material coated with water soluble nanoparticles and embedded with ATPS (Aqueous Two Phase System) components which form two phase solutions when an aqueous solution travels through said porous material so that one phase solution travels faster than the other, wherein when a sample solution is introduced into the sample well, the porous material will wick said sample solution, wherein the target analyte in the sample solution partitions into the faster moving phase solution and becomes concentrated at the front of the faster moving phase solution; and

(c) A detection module connected to said concentration module for detecting the target analyte, said detection module comprising a lateral flow assay and a conjugate pad, wherein the porous material of the concentration module is conjugated with said conjugate pad, wherein the concentrated target analyte flows from said porous material

to said conjugate pad and is subsequently analyzed by said lateral flow assay, and wherein a display of color indicates the presence of the target analyte.

[0115] In one embodiment of the device described herein, the nanoparticles are selected from the group consisting of silicon dioxide, iron oxide, titanium dioxide, silver oxide, and any combinations thereof.

[0116] In one embodiment of the device described herein, the porous material is selected from the group consisting of fiber-glass paper, cotton-based paper, single-layer matrix paper and polyolefin foam pad.

[0117] In one embodiment of the device described herein, the ATPS components are selected from polymers, salts and surfactants.

[0118] In one embodiment of the device described herein, the polymers are selected from the group consisting of polyalkylene glycols, poly(oxyalkylene)polymers, poly(oxyalkylene)copolymers, polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl caprolactam, polyvinyl methylether, alkoxylated surfactants, alkoxylated starches, alkoxylated cellulose, alkyl hydroxyalkyl cellulose, silicone-modified polyethers, poly N-isopropylacrylamide, polyethylene glycol, polypropylene glycol and dextran.

[0119] In one embodiment of the device described herein, the salts are selected from the group consisting of kosmotropic salts, chaotropic salts, inorganic salts having a cation of trimethyl ammonium, triethyl ammonium, tripropyl ammonium, tributyl ammonium, tetramethyl ammonium, tetraethyl ammonium, tetrapropyl ammonium or tetrabutyl ammonium, and an anion of phosphate, sulphate, nitrate, chloride or hydrogen carbonate, NaCl, Na_3PO4, K_3PO_4, Na_2SO_4, potassium citrate, $(NH_4)_2SO_4$, sodium citrate, sodium acetate, ammonium acetate, and any combinations thereof.

[0120] In one embodiment of the device described herein, the surfactants are selected from the group consisting of nonionic surfactants, detergents, Triton-X, Igepal CA-630 and Nonidet P-40.

[0121] In one embodiment of the device described herein, the sample solution contains one or more of blood, plasma, serum, urine, saliva, fecal matters and bodily discharges.

[0122] In one embodiment of the device described herein, the analyte is a DNA, cell-free DNA, circulating tumor DNA, protein, nucleic acid, carbohydrate, lipid, bacterium, virus or nanoparticle.

[0123] In one embodiment of the device described herein, the ATPS components are completely separate or partly overlapping on said porous material.

[0124] In one embodiment of the device described herein, the analyte is concentrated by up to 100-fold.

[0125] In one embodiment of the device described herein, the porous material is in the form of multiple paper strips with one pointed end.

[0126] In one embodiment of the device described herein, the sample solution is a phosphate buffer or a Tris-EDTA buffer with an indicator.

[0127] In one embodiment of the device described herein, the concentration module and the detection module are separable and each in a housing, wherein the device optionally comprises a ring of rubber sealing the connection between the housings.

[0128] In one embodiment of the device described herein, the concentration module and the detection module are inseparable.

[0129] Throughout this application, it is to be noted that the transitional term "comprising", which is synonymous with "including", "containing" or "characterized by", is inclusive or open-ended, and does not exclude additional, un-recited elements or method steps.

[0130] This invention will be better understood by reference to the examples which follow. However, one skilled in the art will readily appreciate that the examples provided are merely for illustrative purposes and are not meant to limit the scope of the invention which is defined by the claims following thereafter.

EXAMPLES

Example 1- Embedding ATPS on with pre-coated porous material

[0131] Different porous materials, including fiberglass paper, cotton-based paper, a proprietary single-layer matrix paper, and a polyolefin foam pad, (of width 2.2 cm and variable length) were soaked separately into the 5M silicon dioxide aqueous solution followed by the vigorous stirring for an hour. The solution was kept at the temperature of 80°C for 24 hours. Then the porous materials were taken out and washed twice with water, and finally dried under compressed air.

[0132] The formulated ATPS components, 20% (w/w) PEG 4600, 2% (w/w) SDS, 16% (w/w) Triton-X114 and 18.5% (w/w) Na_2SO_4, were dehydrated onto those pre-coated porous paper prepared above. PEG solution (in DI H_20) was added to the narrow end of each porous material. 50 uL of a Tris-buffered solution containing 2% bovine serum albumin (BSA), 0.1% Tween-20, and 0.1%PEG, 20 mM Tris pH 7.5 respectively) was added immediately adjacent to the first

solution. A typical process is shown in Figure 7 according to one embodiment of the invention.

[0133] Those pre-coated porous materials with embedded ATPS were then dried in a lyophilizer for 1 hour and cut into 4 cm x 0.5 cm strips). Pieces were then stacked (four strips per stack) and cut at one end from the corner to a spot 0.5 cm from the end (forming a triangle, see Figure 7). This was then placed in an indicator-containing (colloidal gold) buffer solution with salt (in this case, potassium phosphate) in PBS (overall pH 7.4), resulting in capillary action-mediated flow.

Example 2 - Concentration of DNA in Saliva

[0134] A pre-coated porous fiberglass paper embedded with ATPS components prepared in Example 1, a blank paper (without ATPS or pre-coating) and a control paper (paper with ATPS but without pre-coating) were dipped in saliva mixture, which was a PBS buffer solution at pH 7.4 and 200 ng/mL of DNA ladder. The papers were wicked for approximately 2 minutes until the solution reached the top.

[0135] Solution was allowed to flow up the strip and the leading front was sampled and analyzed by UV-Vis spectrophotometry. This measurement was compared to a similar experiment using a blank paper (concentration factor of 1x). Note that the indicator used was colloidal gold. The concentration fold was summarized in Table 1.

TABLE 1. Concentration fold of DNA using the present invention

Test	ATPS embedded on pre-coated paper	Control Paper (ATPS embedded on paper without pre-coating)	Blank paper (without ATPS and pre-coating)
Yield of DNA (%)	98%	50%	7%
Fold Change in Concentration of DNA	100-fold	50-fold	1-fold

Example 3 - Detection using 3PS device

[0136] For the LFA detection module:

Nitrocellulose membrane was striped with: 1) anti-*S. mutans* antibody at a concentration of 1 mg/mL, and 2) Protein (Bovine Serum Albumin, BSA) at a concentration of 0.2 mg/mL. Colloidal

gold nanoparticles were conjugated to anti-*S. mutans* antibody as directed by manufacturer instructions. This conjugate was then dried onto conjugate pad material at using a lyophilizer. The sample pad comprised a sample pad material impregnated with a Tris buffer comprising 2% BAS protein, 16% surfactant (Triton X-114), 0.1% PEG polymer, and 20Mm Tris buffer salts. The absorbent pad consisted of untreated paper. To run the LFA test without the concentration module, various materials were assembled on an adhesive backing, the assembly was dipped into solutions containing S. *mutans* at various concentrations, and the results were analyzed by direct observation.

[0137] For the 3PS device:

To create the concentration module, 300 pL of a surfactant (Triton X-114) solution was added to FG1 pre-coated paper (dimensions 4 cm x 2.2 cm). This piece was subsequently dried in a lyophilizer and cut into 0.5 cm strips lengthwise. These four resulting strips were then stacked to form the concentration module.

[0138] To attach the concentration module to the LFA detection module described previously, a direct junction is formed between the concentration module and the sample pad of the detection module. To hold this junction together and promote flow, tape was tightly wound around the intersection between the two modules. Although this new device is slightly longer and requires more volume, running the test is the same.As shown in **Figure 5**, *S. mutans* was successfully concentrated by using the 3PS device and a concentration fold of 100 is achieved. The presence of a control line indicated a valid test, and the presence of a test line indicated a positive result. Test lines were indicated with a black arrowhead. “C” is the control line; “T” is the test line. In test using the LFA detection module alone, no test line was visible for the negative sample, confirming a valid control. At the *S. mutans* concentration of 10^6 CFU/ml, the test line was visible, confirming a positive result. However, no test line is visible at 10^4 CFU/ml. In the present 3PS device, no test line was visible for the negative sample, confirming a valid control. At 10^6 CFU/ml, a test line was visible, and the test line intensity was stronger than that of LFA only at 10^6 CFU/ml, confirming that the concentration module successfully concentrated *S. mutans* and indicating that a concentration fold of about 100 in detecting *S. mutans* has been achieved. Overall, the result indicated that the present 3PS device was able to detect the bacteria at 10^4 CFU/ml, which was not detectable by LFA alone. The present 3PS device achieved a 100-fold improvement in the limit of detection of *S. mutans*.

References:

1. Aas, J.A., Paster, B.J., Stokes, L.N., Olsen, I. and Dewhirst, F.E., 2005. Defining the normal bacterial flora of the oral cavity. Journal of clinical microbiology, 43(11), pp.5721-5732.
2. Corby, P.M., Lyons-Weiler, J., Bretz, W.A., Hart, T.C., Aas, J.A., Boumenna, T., Goss, J., Corby, A.L., Junior, H.M., Weyant, R.J. and Paster, B.J., 2005. Microbial risk indicators of early childhood caries. Journal of clinical microbiology, 43(11), pp.5753-5759.

What is claimed is:

1. A device for the concentration and detection of a target analyte in a sample solution, comprising:
   (a) A sample well for holding a sample solution;
   (b) A concentration module connected to said sample well, said module comprising a porous material coated with water soluble nanoparticles and embedded with ATPS (Aqueous Two Phase System) components which form two phase solutions when an aqueous solution travels through said porous material so that one phase solution travels faster than the other, wherein when a sample solution is introduced into the sample well, the porous material will wick said sample solution, wherein the target analyte in the sample solution partitions into the faster moving phase solution and becomes concentrated at the front of the faster moving phase solution; and
   (c) A detection module connected to said concentration module for detecting the target analyte, said detection module comprising a lateral flow assay and a conjugate pad, wherein the porous material of the concentration module is conjugated with said conjugate pad, wherein the concentrated target analyte flows from said porous material to said conjugate pad and is subsequently analyzed by said lateral flow assay, and wherein a display of color indicates the presence of the target analyte.

2. The device of claim 1, wherein said nanoparticles are selected from the group consisting of silicon dioxide, iron oxide, titanium dioxide, silver oxide, and any combinations thereof.

3. The device of claim 1, wherein the porous material is selected from the group consisting of fiber-glass paper, cotton-based paper, single-layer matrix paper and polyolefin foam pad.

4. The device of claim 1, wherein said ATPS components are selected from polymers, salts and surfactants.

5. The device of claim 4, wherein said polymers are selected from the group consisting of polyalkylene glycols, poly(oxyalkylene)polymers, poly(oxyalkylene)copolymers, polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl caprolactam, polyvinyl methylether, alkoxylated

surfactants, alkoxylated starches, alkoxylated cellulose, alkyl hydroxyalkyl cellulose, silicone-modified polyethers, poly N-isopropylacrylamide, polyethylene glycol, polypropylene glycol and dextran.

6. The device of claim 4, wherein said salts are selected from the group consisting of kosmotropic salts, chaotropic salts, inorganic salts having a cation of trimethyl ammonium, triethyl ammonium, tripropyl ammonium, tributyl ammonium, tetramethyl ammonium, tetraethyl ammonium, tetrapropyl ammonium or tetrabutyl ammonium, and an anion of phosphate, sulphate, nitrate, chloride or hydrogen carbonate, NaCl, Na_3PO4, K_3PO_4, Na_2SO_4, potassium citrate, $(NH_4)_2SO_4$, sodium citrate, sodium acetate, ammonium acetate, and any combinations thereof.

7. The device of claim 4, wherein said surfactants are selected from the group consisting of nonionic surfactants, detergents, Triton-X, Igepal CA-630 and Nonidet P-40.

8. The device of claim 1, wherein the sample solution contains one or more of blood, plasma, serum, urine, saliva, fecal matters and bodily discharges.

9. The device of claim 1, wherein the analyte is a DNA, cell-free DNA, circulating tumor DNA, protein, nucleic acid, carbohydrate, lipid, bacterium, virus or nanoparticle.

10. The device of claim 1, wherein said ATPS components are completely separate or partly overlapping on said porous material.

11. The device of claim 1, wherein said analyte is concentrated by up to 100-fold.

12. The device of claim 1, wherein the porous material is in the form of multiple paper strips with one pointed end.

13. The device of claim 1, wherein the sample solution is a phosphate buffer or a Tris-EDTA buffer with an indicator.

14. The device of claim 1, wherein the concentration module and the detection module are separable and each in a housing, wherein the device optionally comprises a ring of rubber sealing the connection between the housings.

15. The device of claim 13 or 14, wherein the concentration module and the detection module are inseparable.

Figure 1

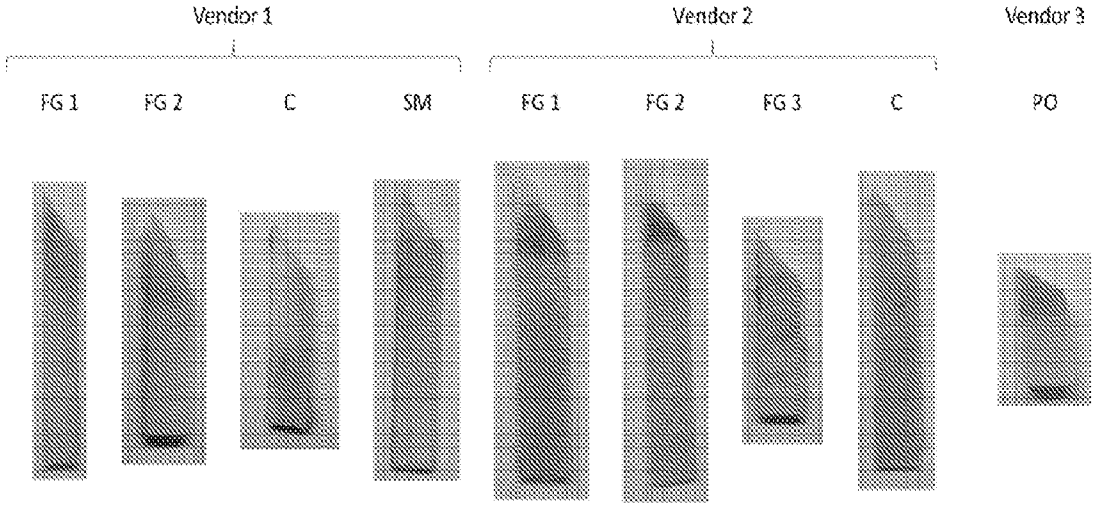
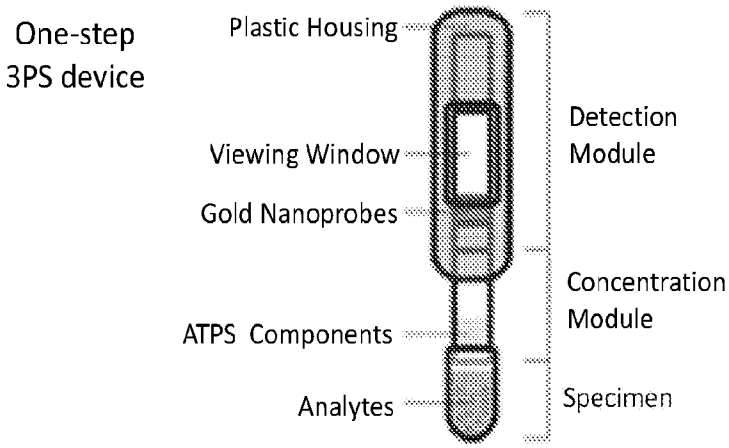


Figure 2



Concentration: As the specimen wicks up the device and phase separation occurs, the analytes concentrate extremely in the leading front.

Detection: Concentrated analytes can then be detected by a conventional LFA module, generating visual test results.

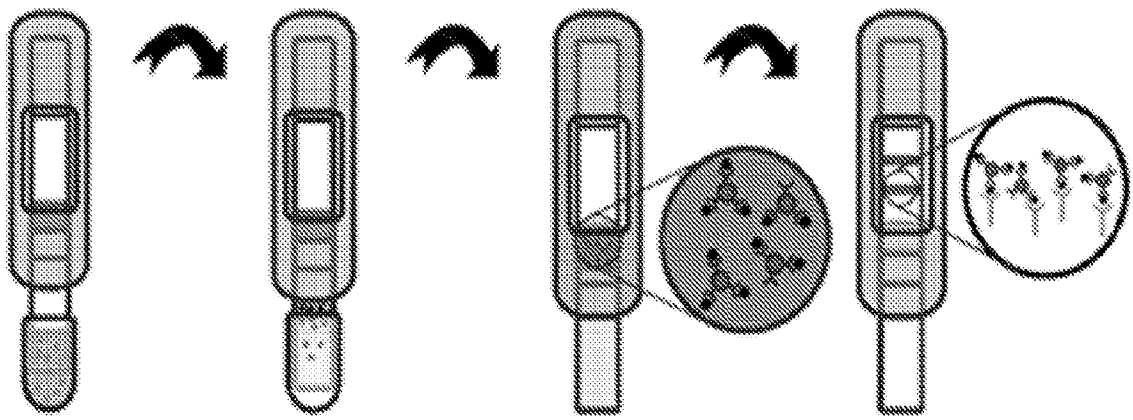


Figure 3

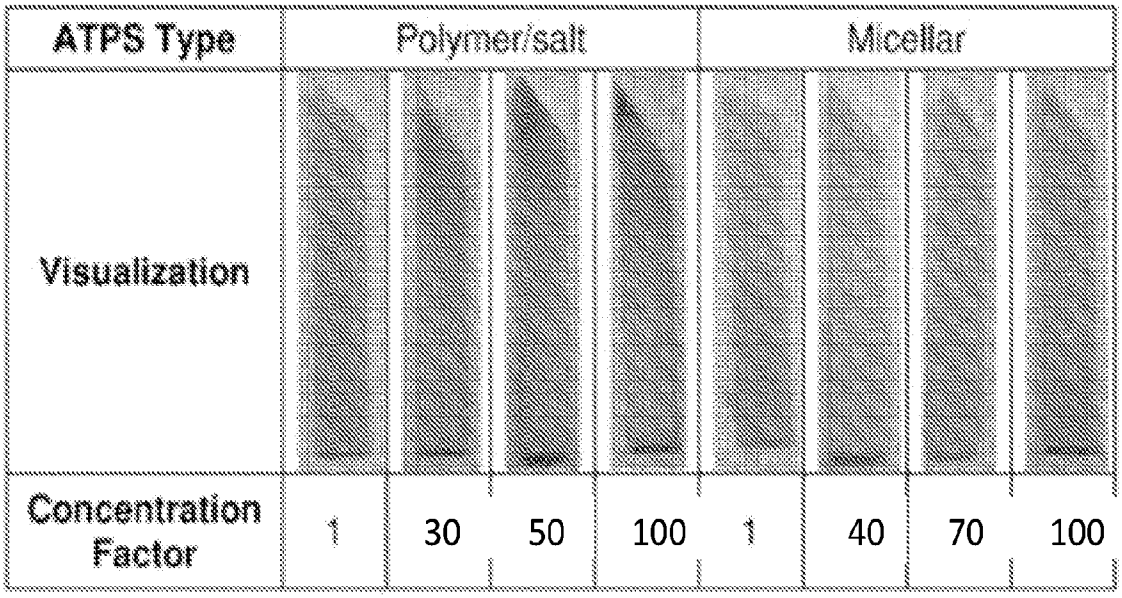


Figure 4

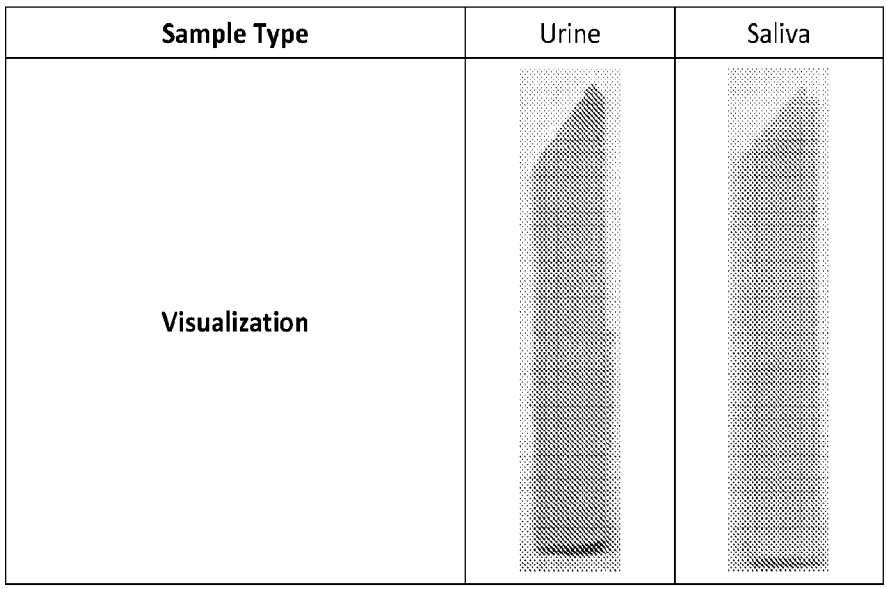


Figure 5

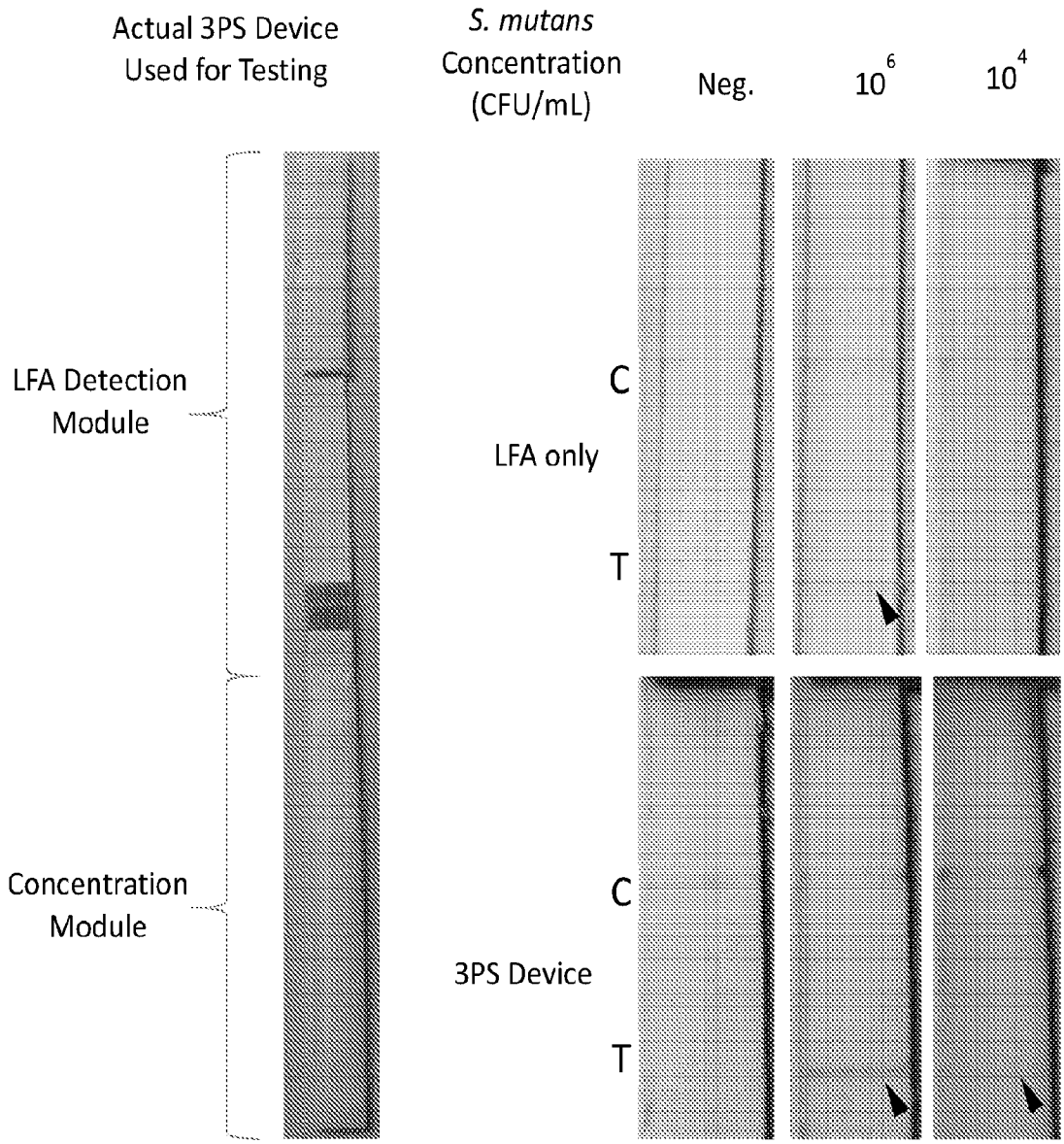

Actual 3PS Device Used for Testing
S. mutans Concentration (CFU/mL)
Neg.
10^6
10^4
LFA Detection Module
Concentration Module
C
LFA only
T
C
3PS Device
T

Figure 6

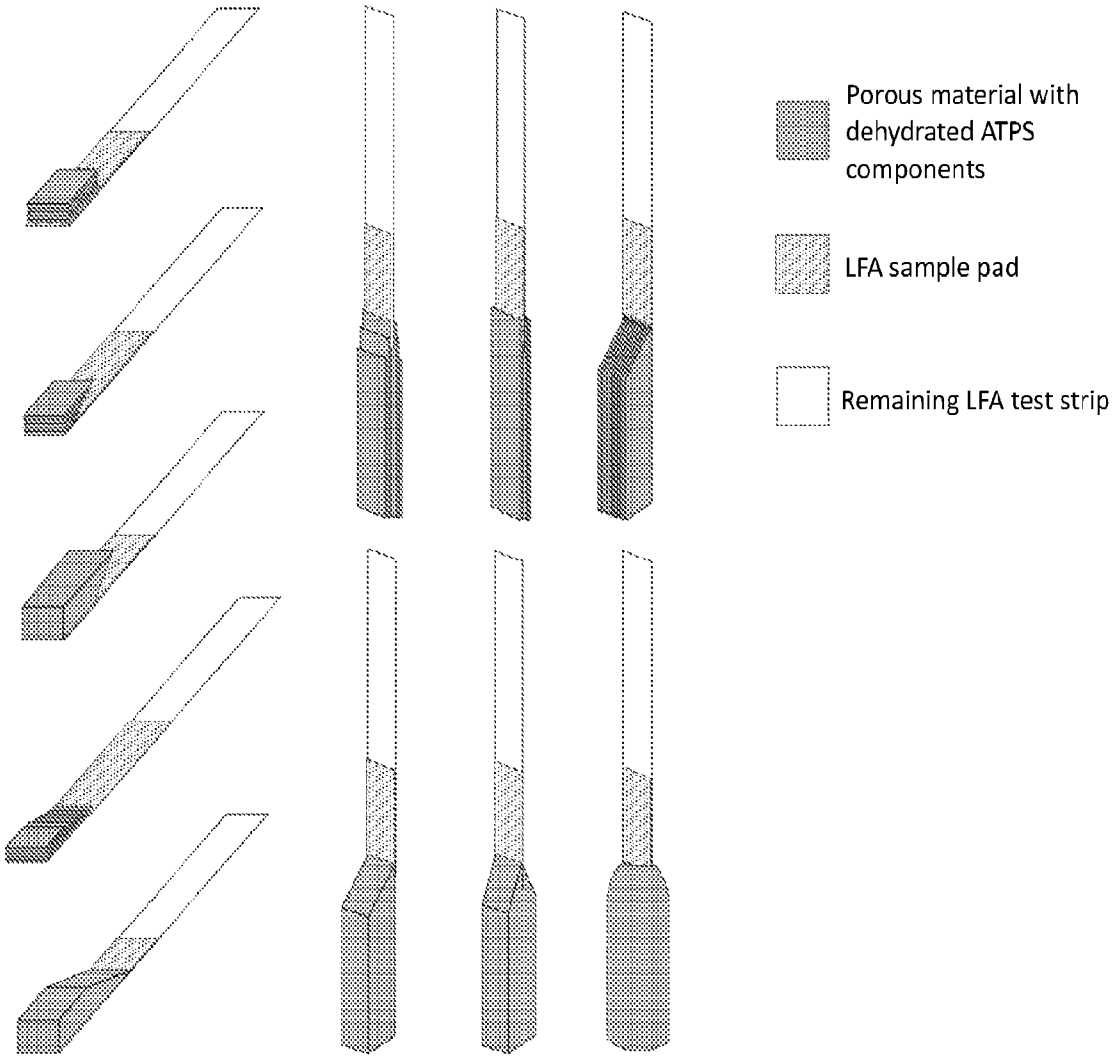

Porous material with dehydrated ATPS components
LFA sample pad
Remaining LFA test strip

Figure 7

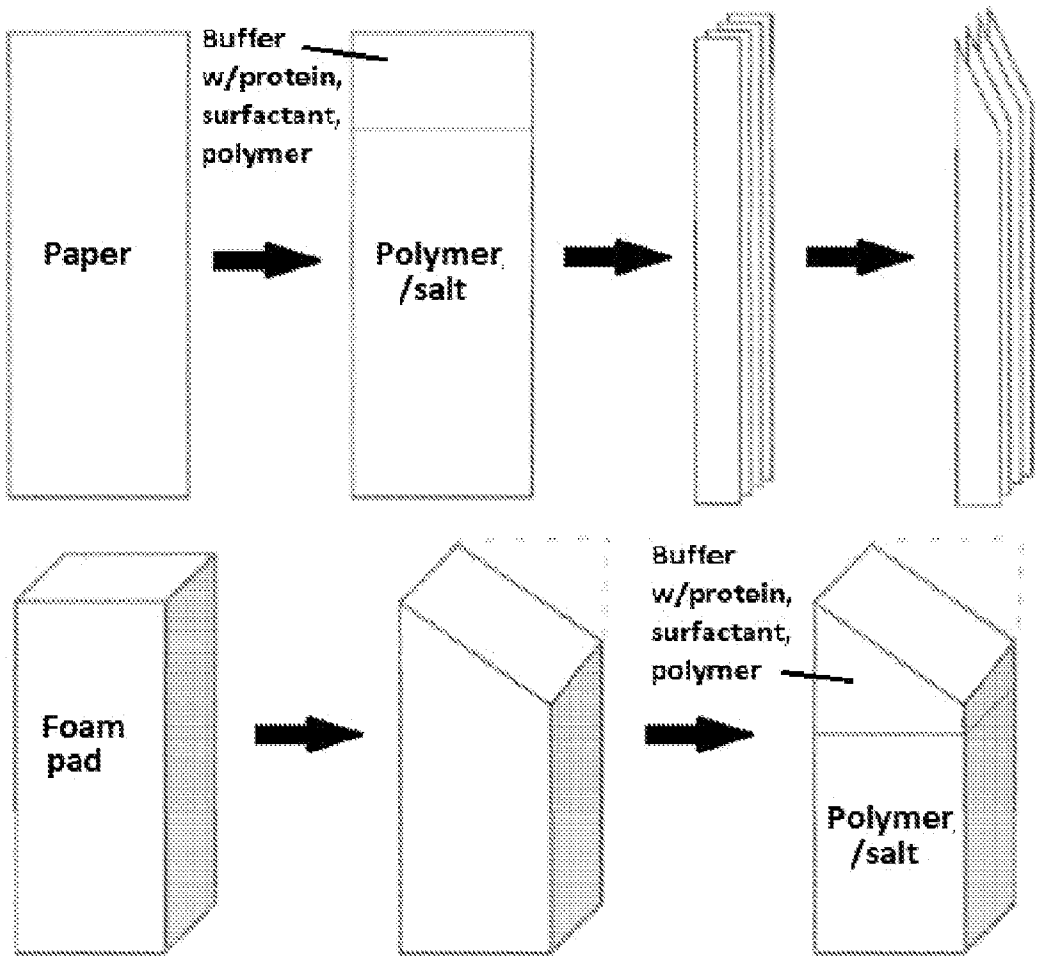

Buffer w/protein, surfactant, polymer
Paper
Polymer /salt
Foam pad
Buffer w/protein, surfactant, polymer
Polymer /salt

Figure 8

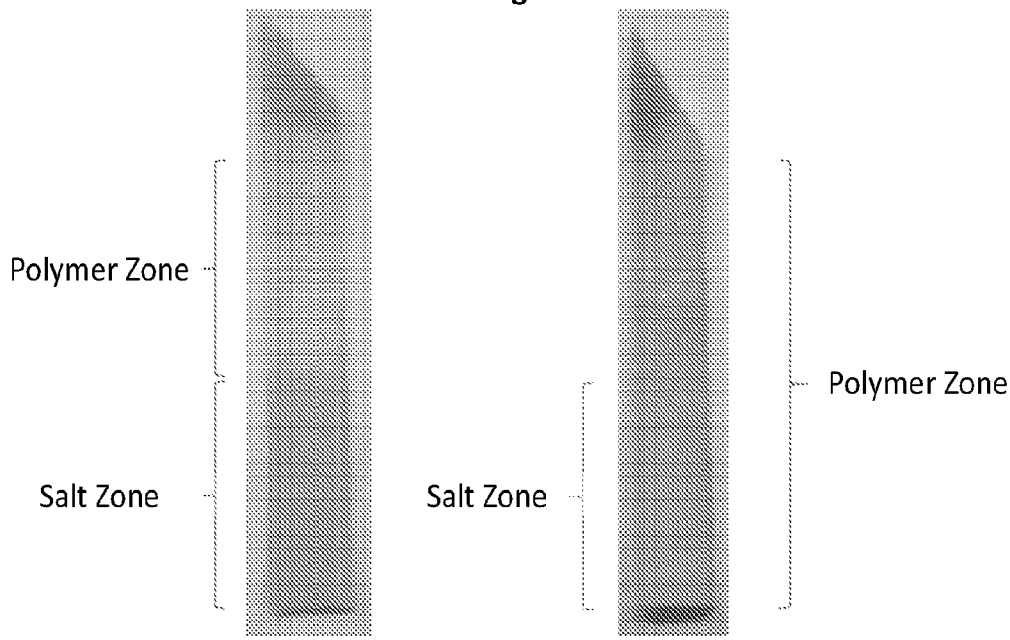


Figure 9

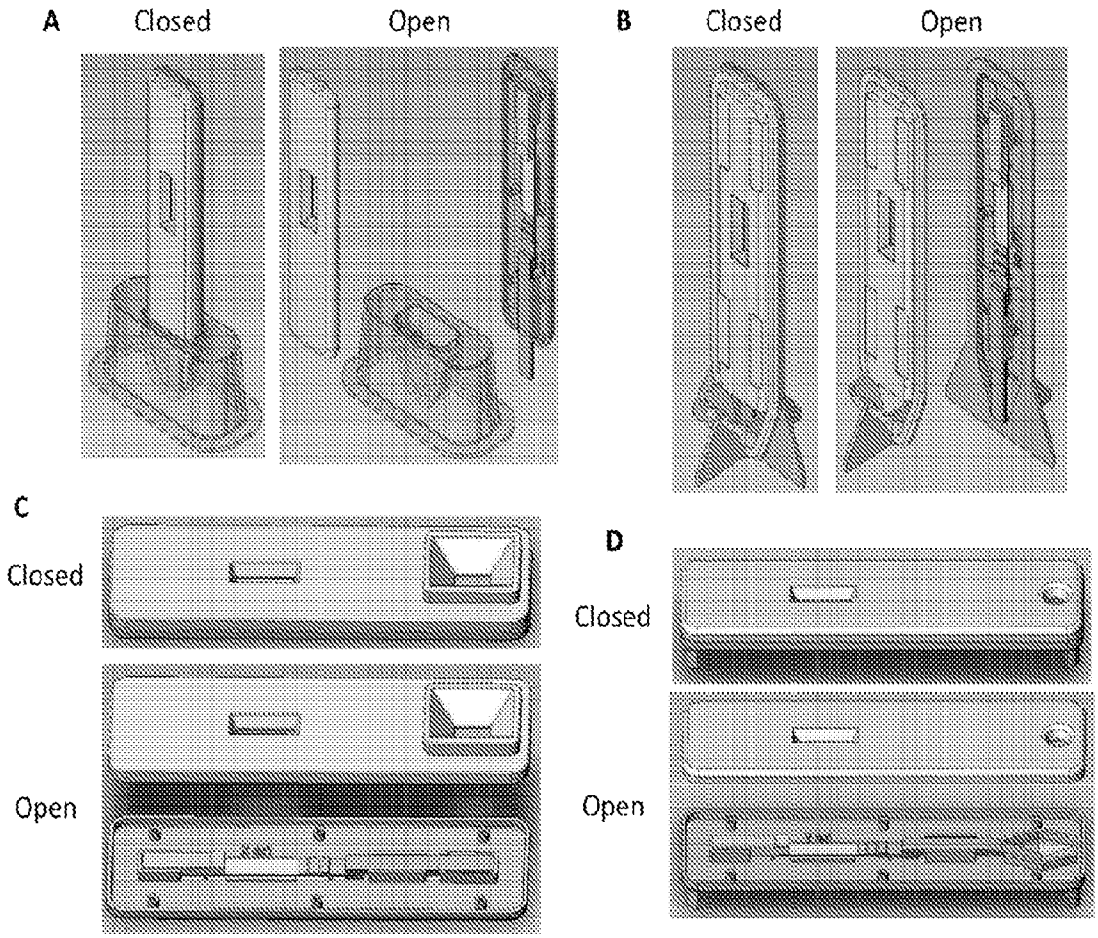
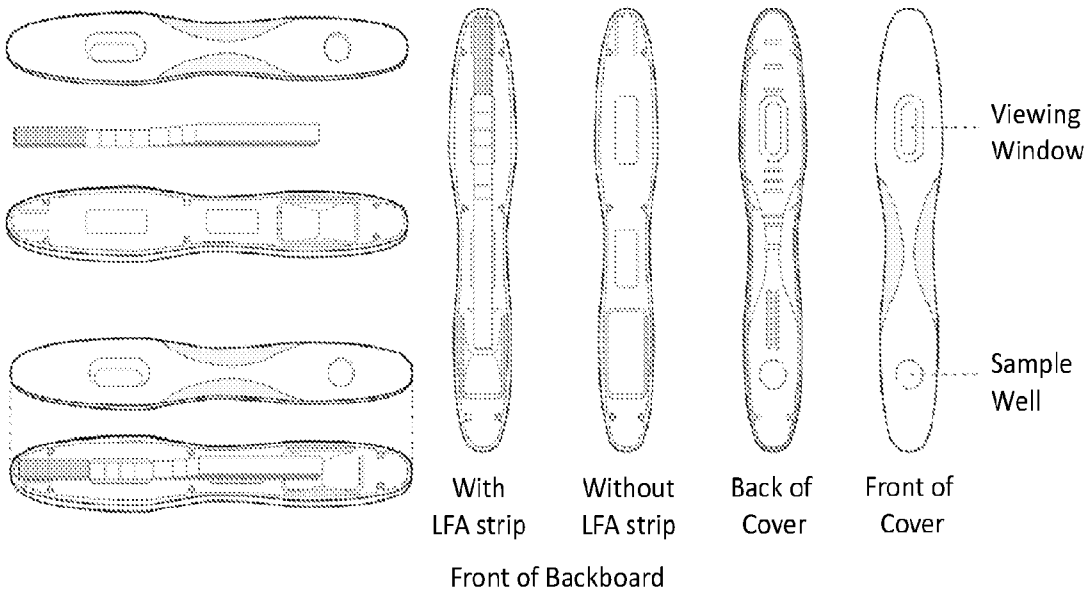


Figure 10


Viewing Window
Sample Well
With LFA strip
Without LFA strip
Back of Cover
Front of Cover
Front of Backboard

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/024779

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53; G01N 33/569; G01N 33/543 (2018.01)
CPC - G01N 33/5302; G01N 33/53; G01N 33/532; G01N 33/536; G01N 33/54346 (2018.05)

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

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.
A	US 2015/0253320 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 10 September 2015 (10.09.2015) entire document	1-15
A	WO 2017/041030 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 09 March 2017 (09.03.2017) entire document	1-15
A	WO 2011/159537 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 22 December 2011 (22.12.2011) entire document	1-15
P, A	WO 2018/039139 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 01 March 2018 (01.03.2018) entire document	1-15
P, A	WO 2017/214315 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 14 December 2017 (14.12.2017) entire document	1-15

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

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"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
24 July 2018	07 AUG 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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