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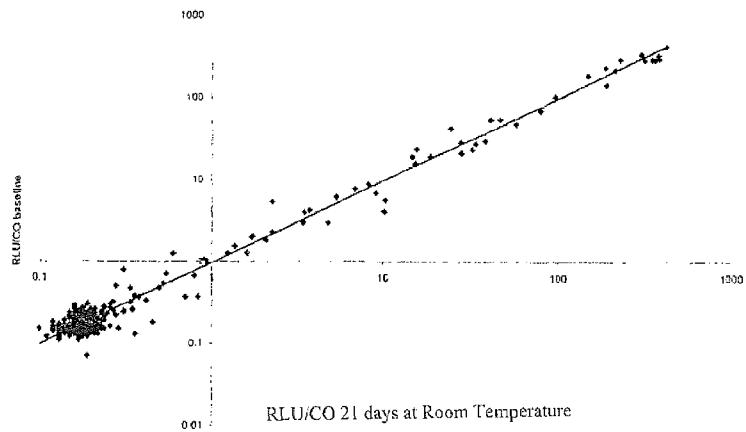
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[Continued on next page]

(54) Title: FAST RESULTS HYBRID CAPTURE ASSAY AND SYSTEM

Figure 3

Clinical Specimen Stability at Room Temperature



(57) Abstract: The present invention comprises a method that provides fast and reliable results for detecting the presence of a target nucleic acid molecule in a sample.



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FAST RESULTS HYBRID CAPTURE ASSAY AND SYSTEM

[0001] This application claims priority to both U.S. Provisional Patent Application No. 61,108,687, filed October 27, 2008, and U.S. Provisional Patent Application No. 5 61/174,848, filed May 1, 2009. The contents of all applications are herein incorporated by reference in their entirety.

FIELD

[0002] The present invention relates to methods, reagents, systems, and kits for 10 determining the presence of a nucleic acid in a sample.

BACKGROUND

[0003] The detection and characterization of specific nucleic acid sequences and sequence changes have been utilized to detect the presence of viral or bacterial nucleic acid 15 sequences indicative of an infection, the presence of variants or alleles of mammalian genes associated with disease and cancers, and the identification of the source of nucleic acids found in forensic samples, as well as in paternity determinations.

[0004] For example, the RNA or DNA for many microorganisms and viruses have 20 been isolated and sequenced. Nucleic acid probes have been examined for a large number of infections. Detectable nucleic acid sequences that hybridize to complementary RNA or DNA sequences in a test sample have been previously utilized. Detection of the probe indicates the presence of a particular nucleic acid sequence in the test sample for which the probe is specific. In addition to aiding scientific research, DNA or RNA probes can be used to detect 25 the presence of viruses and microorganisms such as bacteria, yeast and protozoa as well as genetic mutations linked to specific disorders in patient samples.

[0005] Nucleic acid hybridization probes have the advantages of high sensitivity and 30 specificity over other detection methods and do not require a viable organism. Hybridization probes can be labeled, for example with a radioactive substance that can be easily detected, or with biochemical markers such as, for example, biotin, that allows for their capture and detection. Nucleic acid molecules may also be captured by a first antibody that is specific to DNA hybrids, wherein the hybrids may comprise DNA-RNA hybrids, DNA-DNA hybrids or

RNA-RNA hybrids. The hybrids may subsequently be detected by a second, labeled, antibody that may be, for example, labeled with a biochemical marker such as alkaline phosphatase or any other marker capable of detection.

[0006] As nucleic acid sequence data for genes from humans and pathogenic organisms accumulates, the demand for fast, cost-effective, and easy-to-use tests increases. There is a need to provide novel and effective methods, compositions, and kits for determining target nucleic acids in a rapid, cost-effective, and reliable manner in geographical areas where access to medical care is not readily available. There is also a need to provide these assays in a rapid-screen format that can be used in developing countries. The methods and assays of the present invention meet these needs and may be used in manual, partially automated, automated, and non-automated systems.

[0007] Clinical analysis in developing countries and geographical areas where access to medical care is not readily available presents unique challenges. The invention described herein achieves an acceptable resolution that balances the importance of these challenges in such countries and areas. For instance, speed in obtaining results is particularly important in locations where women travel great distances to provide specimens for analysis. In such locations, it is advantageous that results are obtained within several hours or the same day while the patient is still present to avoid loss to follow-up associated with traveling from home to the test site.

[0008] Other factors facing developing countries are the cost of running the assay and the instrumentation needed to run the assay. Repeat pipettors and single pipettes are just two types of devices that are routinely used in developed countries but are potentially cost prohibitive in developing countries. Accordingly, there is a need for medical devices and products which employ cheaper and more readily accessible alternatives in developing countries.

SUMMARY

[0008a] According to a first aspect of the present invention there is provided a method for determining the presence of a target nucleic acid molecule in a sample, comprising:

- a) suspending a sample in a collection medium comprising an anionic detergent and a non-ionic detergent;
- b) denaturing a target nucleic acid molecule;

c) contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, thereby forming a double-stranded nucleic acid hybrid; and

5 d) capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded nucleic acid hybrid.

[0008b] According to a second aspect of the present invention there is provided a composition comprising:

(a) a biological sample suspended in a collection medium, wherein said collection medium comprises an anionic detergent and a non-ionic detergent;

10 (b) a denaturation reagent;

(c) at least one polynucleotide probe capable of binding to a target nucleic acid molecule;

(d) a support coated with a first antibody; and

(e) a second antibody labeled with a detectable marker.

15 **[0008c]** According to a third aspect of the present invention there is provided a composition comprising:

(a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, and about 10 mM to about 50 mM EDTA, and

20 (b) a polynucleotide probe; and

(c) a support coated with a first antibody.

[0008d] According to a fourth aspect of the present invention there is provided a kit, for the detection of a target nucleic acid molecule in a sample, comprising:

a) a collection medium comprising an anionic detergent and a non-ionic detergent;

25 b) a denaturation reagent;

c) a support coated with a first anti-hybrid antibody;

d) a detection reagent comprising a second anti-poly hybrid antibody, wherein the second antibody is detectably labeled;

e) a detergent-based wash buffer; and

30 f) a second detection reagent comprising a substrate for the label on the second antibody.

2b

[0009] One aspect relates to a method for determining the presence of a target nucleic acid molecule in a sample containing biological material. The biological material can include a cervical epithelial cell or nucleic acid from a cervical cell. Using the disclosed methods, the determination of whether a target nucleic acid molecule is present in a sample 5 can be obtained relatively rapidly, for example within a period of less than about two or three hours.

[0010] In an aspect, a method for determining the presence of a target nucleic acid molecule in a sample comprises:

- a) suspending the sample in a collection medium;
- b) releasing target nucleic acid molecules from the sample into the collection medium;
- 5 c) converting double-stranded target nucleic acid molecules to single-stranded target nucleic acid molecules;
- d) contacting one or more probes with the single-stranded target nucleic acid molecules under conditions that allow the probes and target single-stranded target nucleic acid molecules to hybridize forming double-stranded nucleic acid hybrids;
- 10 e) capturing the double-stranded nucleic acid hybrids;
- f) separating the double-stranded nucleic acid hybrids from un-bound single-stranded target nucleic acid molecules; and
- g) detecting the double-stranded nucleic acid hybrids, thereby indicating the presence of the target nucleic acid.

15 **[0011]** In one aspect, the method may be predominantly manual, requiring human input. Another aspect relates to the rapid detection of target nucleic acid molecules in a sample. The detection method may be automated, either fully automated, or partially automated – in other words requiring some human input.

20 **[0012]** Another aspect relates to the detection of target nucleic acid molecules in multiple samples at the same time or within a very short period of time, for example in a machine or a series of machines.

[0013] Yet another aspect relates to an instrument for running a method for the detection of a target nucleic acid molecule in a simple footprint. The instrument combines many, or all, of other individual instruments that perform the steps of the method.

25 **[0014]** Another aspect relates to a portable system for evaluating the detection of a target nucleic acid molecule in a sample.

[0015] Another aspect relates to a kit for the detection of a target nucleic acid molecule in a sample.

30 **[0016]** A further aspect relates to reagents within a collection medium into which a sample containing a target nucleic acid molecule are collected. The target nucleic acid molecule can be kept in the collection medium with minimal degradation of the target nucleic acid molecule over a time period of weeks or months. In an aspect, DNA-based target

sample material can be kept in the collection medium with minimal degradation of the target nucleic acid molecule over a time period of weeks or months. In an aspect the detergent-based collection medium allows for the rapid analysis and processing of a sample.

5

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 shows that the detergent-based collection medium holds magnetic beads in microtiter plate wells better than known collection or sample transport medium (STM) (non-detergent based medium).

[0018] FIG. 2 shows that samples having only 0.2 pg target nucleic acid (DNA) per ml of sample provide a readable signal using methods of the present invention.

[0019] FIG. 3 shows clinical specimen stability at room temperature for 21 days.

[0020] FIG. 4 shows clinical specimen stability at 33°C for 21 days.

[0021] FIG. 5 shows test results demonstrate an S/N ≥ 2.0 for a 0.2 pg/ml HPV 16 plasmid which is equivalent to 1000 copies of HPV 16 DNA.

15 [0022] FIG. 6 shows a system for detecting the presence of a target nucleic molecule acid in a sample including a heater configured for heating multiple samples; a luminometer; and a monitor.

[0023] FIG. 7 shows different reagents associated with the detection assay. The reagent vials are color coded for ease of use and can be included in a kit.

20 [0024] FIG. 8 shows a monitor used in conjunction with the system for detecting the presence of nucleic acid molecules.

[0025] FIG. 9 shows 11 day stability data for soft pellets suspended in a detergent-based collection medium and stored at room temperature.

25

DETAILED DESCRIPTION

[0026] The present disclosure covers methods, compositions, reagents, systems, and kits for rapidly determining the presence of a nucleic acid molecule in a sample. The methods, compositions, reagents, systems, and kits may be used for clinical diagnostic purposes, including but not limited to the detection and identification of pathogenic 30 organisms and the detection of a genetic predisposition to a particular disease.

[0027] In one aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample. The method comprises:

- a) suspending the sample in a collection medium comprising a detergent;
- b) denaturing the target nucleic acid molecule;
- 5 c) contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize, thereby forming a double-stranded nucleic acid hybrid;
- d) capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid, thereby forming a
- 10 double-stranded nucleic acid hybrid/solid support complex;
- e) separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid;
- f) conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded
- 15 nucleic acid hybrid/solid support antibody complex; wherein the second antibody is labeled with a detectable marker;
- g) washing the double-stranded nucleic acid hybrid/solid support antibody complex with a wash buffer comprising a detergent; and
- 20 h) detecting the label on the second antibody wherein the detecting indicates the presence of the target nucleic acid molecule.

[0028] In another aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, and capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid.

[0029] In an aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind,

capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid and separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid.

[0030] In an aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid, thereby forming a double-stranded nucleic acid hybrid/solid support complex; separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid; and conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded nucleic acid hybrid/solid support antibody complex.

[0031] In another aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample including suspending a sample in a collection medium including a detergent; denaturing a target nucleic acid molecule; contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded hybrid nucleic acid hybrid, thereby forming a double-stranded nucleic acid hybrid/solid support complex; and separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid; conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded nucleic acid hybrid/solid support antibody complex; wherein the second antibody is labeled with a detectable marker; and washing the double-stranded nucleic acid hybrid/solid support antibody complex with a wash buffer comprising a detergent.

[0032] In another aspect, the present disclosure provides a method for determining the presence of a target nucleic acid molecule in a sample, the method comprising:

- a) suspending the sample in a collection medium comprising a detergent;

- b) denaturing the target nucleic acid molecule in the sample;
- c) forming a double-stranded nucleic acid hybrid by contacting at least one polynucleotide probe with the target nucleic acid molecule;
- d) forming a double-stranded nucleic acid hybrid-support complex by capturing the double-stranded nucleic acid hybrid on a support, wherein the support comprises a first antibody;
- 5 e) forming a double-stranded nucleic acid hybrid-support-second antibody complex by contacting the double-stranded nucleic acid hybrid-support complex with a second antibody, wherein the second antibody is labeled with a detectable marker;
- f) washing the double-stranded nucleic acid hybrid-support-second antibody complex with a wash buffer; and
- 10 g) detecting the marker on the second antibody wherein the detecting indicates the presence of the target nucleic acid molecule.

15 [0033] In one aspect, the solid support comprises a modified paramagnetic bead that is coated or has attached thereto a first antibody immunospecific for double-stranded hybrid nucleic acids. A magnetic field is used to separate the double-stranded nucleic acid-magnetic bead-antibody complex from non-bound nucleic acid.

20 [0034] In an aspect, the method does not include a sample pre-treatment step. For example, the detergent-based collection medium allows for reduced sample preparation time which, in turn, can lead to accelerated detection of target nucleic acid molecules. The sample can be analyzed by methods, assays, or the apparatus of the disclosure in a direct-to-assay manner. In an example, purification steps are not performed on the sample prior to evaluation using assays of the disclosure. In an aspect, crude lysate is directly analyzed by the methods, assays, or the apparatus of the disclosure. In another aspect, the sample does 25 not undergo a target amplification step.

30 [0035] One aspect relates to a method of diagnosing cancer by utilizing methods, kits, assays, and the apparatus provided herein. In one aspect, cervical cancer is detected by identifying nucleic acid molecules associated with HPV and HPV variants. In another aspect, cervical intraepithelial neoplasia (CIN) can be screened for using methods, kits, assays, and the apparatus provided herein. The detected cancer can be subsequently treated after being diagnosis by the methods, kits, assays, and the apparatus provided herein. In an aspect, the diagnosed cancer is cervical cancer and variants thereof.

[0036] In one aspect, the disclosure provides a composition comprising a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 5 0.01% to about 0.10% sodium azide.

[0037] In an aspect, the disclosure provides for a composition comprising (a) a biological sample suspended in about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 10 0.10% sodium azide; and (b) at least one or more polynucleotide probes.

[0038] In an aspect, the disclosure provides for a composition comprising (a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 15 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide; (b) at least one or more polynucleotide probes; and (c) a first antibody.

[0039] In an aspect, the disclosure provides for a composition comprising (a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide; (b) a first antibody; and 25 (c) a second antibody.

[0040] In an aspect, the disclosure provides for a composition comprising (a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM 30 NaCl, and about 0.01% to about 0.10% sodium azide; (b) at least one or more polynucleotide probes; (c) a first antibody; and

(d) a second antibody.

[0041] In an aspect, the disclosure provides for a composition comprising

- (a) a biological sample suspended in a collection medium, wherein the collection medium comprises at least one detergent;
- 5 (b) a denaturation reagent;
- (c) at least one polynucleotide probe capable of binding to a target nucleic acid molecule;
- (d) a support coated with a first antibody; and
- (e) a second antibody labeled with a detectable marker.

10 [0042] In an aspect, any of the above compositions may be used with any of the collection mediums described herein.

[0043] In an aspect, the biological sample in the above compositions is a cervical cell sample or a human cervical cell sample. In another aspect, the nucleic acid molecules in the biological sample are denatured. The biological sample in the above compositions can

15 exhibit stability when stored in the collection medium for at least 21 days at 33°C. In an aspect, the second antibody is labeled with a detectable marker.

Biological Sample

[0044] Methods of the present invention may be used to detect the presence of a target nucleic acid molecule from samples, including, without limitation, a specimen or

20 culture (e.g., cellular, microbiological and viral cultures) including biological and environmental samples. Biological samples may be from an animal, including a human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Environmental samples include environmental material such as surface matter, soil, water

25 and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items.

[0045] Particularly preferred are biological samples including, but not limited to, cervical epithelial cells (e.g., a sample obtained from a cervical swab), adenoid cells, anal epithelial cells, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and

30 semen. The sample may comprise a double-stranded nucleic acid molecule or may comprise a single-stranded nucleic acid molecule. If a double-stranded nucleic acid molecule is

present, it may be prepared for hybridization analysis by a variety of methods known in the art, *e.g.*, using alkali, using proteinase K/SDS, chaotropic salts. The process of preparing a double-stranded nucleic acid molecule for hybridization analysis generally involves converting it into a single-stranded nucleic acid molecule. This process is generally known 5 as denaturation. However, it is also contemplated that a double-stranded nucleic acid molecule may be detected without denaturation, *e.g.*, through a triple-stranded construct.

10 [0046] The target nucleic acid molecule in a sample can be DNA or RNA or both DNA and RNA. The target nucleic acid molecule can be contained within a larger nucleic acid molecule. Detection of either the target nucleic acid molecule or the larger nucleic acid molecule containing the target nucleic acid molecule is contemplated by this disclosure.

15 [0047] The biological sample may comprise cervical cells, especially human cervical cells. The sample can be collected with any method or device known in the art, including a chemically inert collection device such as a DACRON tipped swab. Other acceptable collection devices may be used including, but not limited to, cotton swab, cervical brush, flocked swab (a swab shaped like a DACRON swab but made with nylon fibers enabling collection of more cells and easier release of cells), cervical broom, mini broom, lavage, or any collection device often used in Pap smear testing.

20 [0048] In an aspect, the methods include collecting a sample from a woman over 30 years of age. The method can also include collecting a sample from a woman over 30 years via a Pap smear or comparable test. The sample collected by the Pap smear or comparable test can be a cervical cell sample.

25 [0049] Once the sample is collected, it may be placed in a sample tube. The tube can be sealed to prevent contamination. The collection device (swab, brush, etc.) may further contain a mechanism by which it can be moved once it is inside the sample tube. In one aspect, the collection device contains an insert that can be moved using a magnet. In one aspect, this insert comprises a metal. In another aspect, this insert comprises a magnetic material. Magnetic material includes paramagnetic, ferromagnetic, and diamagnetic materials. One advantage of moving the collection device once it is inside the sample tube is to avoid the collection device from making contact with any sample extraction or sample 30 detection devices. Examples of a sample extraction device include pipettes, pipette tips, dropper bottles or other low tech extraction devices. Examples of sample detection devices include probes and probe tips.

[0050] The speed of this assay is also beneficial in screening samples from patients in remote living areas. Often patients will travel quite a distance to visit the doctor or clinic and will not likely return for some time thereafter. Thus, it is desirable to be able to test the patient and provide results while the patient waits at the clinic. In some circumstances, 5 tracking down the patient to provide test results and/or treat the patient after they have left the doctor's office may be difficult. Thus, the described assays provide results over a short time, for example, between about 2 hours to about 3 hours, between about 2 hours to about 4 hours, between about 3 hours to about 5 hours, between about 4 hours to about 8 hours, or between about 6 hours to about 12 hours. In another aspect, the described assays provide results in 10 less than about 2 hours, less than about 2.5 hours, less than about 3 hours, less than about 3.5 hours, less than about 4 hours, less than about 4.5 hours, less than about 5 hours, less than about 8 hours, less than about 12 hours, and less than about 24 hours. Such a short turnaround time allows the doctor to provide the patient with the results and/or treatment the same day the patient is at the clinic.

15 Sample Tube

[0051] Any type of sample tube may be used. Advantageously, the sample tube may be closed or sealed to minimize contamination. The closure may be permanent or removable. Examples of removable closures include snap caps, screw caps, rubber septa, foil, and film. The closure may contain one or more openings or perforations, which when pierced may be 20 re-sealable. One advantage of a closure that contains such openings or perforations is that the closure is not rendered ineffective when pierced by, for example, a sample extraction device or sample detection device. Once the sample extraction device or sample detection device is removed, the closure re-seals, thereby minimizing contamination.

Storage of the Biological Sample

25 [0052] Once the sample is in the sample tube, the sample may be stored by drying it with a substrate, or in a preservative medium, or both. Desiccation is accomplished by pressure drying or drying with chemicals. This removes most of the water and is suitable for long-term stability. Alternatively, the sample may be lyophilized (freeze-dried) with a substrate like trehalose to ensure stability of the sample.

30 [0053] Another possibility is that the sample may be stored by suspending in a preservative medium, known and apparent to one of skill in the art. The purpose of the

preservative medium is to protect biological components that can degrade. For instance, the sample cells, the probe mixture, the antibody: bead complex used in the capture step, and the secondary antibody used in the detection step are all susceptible to degradation. A preservative medium at the initial step of collection ideally provides sample stability and

5 integrity and can affect downstream steps in the process of nucleic acid capture and detection. In an aspect, the sample may be stored at room temperature, refrigerated, or frozen either prior to or after the addition of the preservative medium.

Collection Medium

[0054] In an aspect, the sample may be collected and stored in a collection medium.

10 The collection medium has several functions including as a preservative medium to preserve nucleic acids and inhibit nucleases to prevent degradation of nucleic acids prior to analysis. In one aspect, the collection medium contains at least one detergent. In another aspect, the collection medium contains at least two detergents, at least three detergents, or at least four detergents. In an aspect, each of the detergents is different. In another aspect, the detergent-

15 based collection medium comprises two different detergents, one which is able to control background signal and another detergent that improves magnetic bead behavior, for example, migration through a viscous sample. Because the detergent improves the bead behavior, the beads can be washed with a variety of less precise devices, such as a squirt bottle, and dropper bottle, without causing too much disruption of the beads. Such methodology may be

20 advantageous for use in developing countries where funds or access to technologically advanced equipment, such as pipetting devices, may not be available.

[0055] Further, the present invention provides a robust assay that can support using less precise reagent delivery devices. The reagents in this assay were designed to be robust and withstand variability in reagent volume delivery. For example, the neutralization step of

25 the assay utilizes a highly buffered pH neutral solution to bring the very basic pH of the reaction to the appropriate pH range for hybridization with very large volume tolerances.

[0056] The use of a detergent-based collection medium for use in the assay can include one or more detergents. In an aspect, heat is employed during the hybridization, capture, and detection steps of the assay. Even with detergent and the application of heat,

30 antibodies used in the assay remain functional.

[0057] FIG. 1 demonstrates that a detergent-based collection medium greatly improves the ability to prevent magnetic bead loss and migration during handling as compared to standard collection mediums. The detergent-based collection medium may comprise, consist essentially of, or consist of one, two, three, or four or more detergents.

5 Detergents are known in the art and may include, but are not limited to, cationic detergents such as but not limited to cetyl pyridinium bromide, cetyltrimethylammonium bromide (collectively known as cetrimonium compounds) and alkylbenzyldimethylammonium chlorides (collectively known as benzalkonium compounds), and alkyl-trimethyl-ammonium salts; anionic detergents such as, but not limited to, sodium dodecyl sulfate (SDS), and

10 Sarkosyl; and non-denaturing detergents such as NP-40; and other detergents. NP-40 is also known as Tergitol-type NP-40, which is nonyl phenoxylpolyethoxylethanol. NP-40 is not powerful enough to break the nuclear membrane, but can break the plasma membrane. As such, it can be used to obtain the cytoplasmic contents of a cellular culture.

[0058] Other detergents and combination of detergents may be used, and

15 advantageously their combination provides the ability to control background noise and improve magnetic bead behavior (when the solid support employed comprises magnetic beads). In certain aspects, one detergent is an anionic detergent and the second detergent is a nonanionic detergent. For example, in one aspect, the combination of non-ionic and anionic detergents helps to maintain low-background noise. In an aspect, a detergent-based

20 collection medium comprises an anionic detergent such as sodium deoxycholate, which controls background noise and NP-40, which improves magnetic bead behavior.

[0059] The combination of these two types of detergents provides synergistic benefits beyond a simple combination of adding two detergents together: control of background noise, better bead behavior, and increased assay speed. The presence of these detergents (in the

25 detergent-based collection medium) provides the ability to achieve faster assay results, but does not negatively impact the nucleic acid or capture antibody during downstream analytical steps.

[0060] In addition, the detergent-based collection medium improves removal of the specimen from the collection device as the sample is dissolved more easily. In addition, the

30 detergent-based collection medium improves the homogeneity of the sample compared with other collection media such as but not limited to PRESERV CYT (uses a 40% methanol

solution), STM (uses a chaotropic agent), and alcohol. The detergent-based collection medium also reduced sample viscosity after mixing (either manual or automated).

[0061] The concentration of NP-40 in the collection medium can range from about 0.5% to about 2.0%, from about 0.1% to about 1.0%, as well as any number within the recited ranges. In certain aspects, the NP-40 is present at a concentration from about 0.8% to about 1.5%; from about 0.9% to about 1.2% and in certain aspects is about 1.0%. In another aspect, the NP-40 is present at a concentration from about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, 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%, or about 2.0%. The concentration of sodium deoxycholate in the collection medium can range from about 0.10% to about 0.40%, from about 0.20% to about 0.30%, as well as any number within the recited ranges. In one aspect, the concentration of sodium deoxycholate is about 0.10%, about 0.15%, about 0.20%, about 0.25%, about 0.30%, about 0.35%, or about 0.40%.

[0062] The detergent-based collection medium may comprise, consist essentially of, or consist of a buffer, two detergents, a chelator and a preservative. The buffer may be Tris-HCl in a concentration of from about 25 mM to about 75 mM; from about 30 mM to about 60 mM; from about 40 mM to about 50 mM, and from about 45 mM to about 55 mM as well as any number within the recited ranges. The buffer may also be Tris-HCl in a concentration of about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, or about 75 mM.

[0063] Any preservative can be used and the choice can depend on factors such as desired functionality, minimization side-effects, cost, etc. Suitable preservatives include gentomycin, ProClin, dimersol, and sodium azide. The concentration of the preservatives in the collection medium depends on factors such as the type of preservative, its efficacy, its side-effects, etc. For example, for sodium azide, the concentration of sodium azide can range from about 0.01% to about 0.1%, from about 0.025% to about 0.075%, and from about 0.04% to about 0.06%, as well as any number within the recited ranges. The preservative, for example, sodium azide, can also be present at about 0.01%, about 0.02%, about 0.03%, about 0.04%, 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.10%.

[0064] In one aspect the detergent-based collection medium comprises, consists essentially of, or consists of 1.0% NP-40, 0.25% sodium deoxycholate, 50 mM Tris-HCl, 25

mM EDTA, 150 mM NaCl and 0.05% sodium azide. In another aspect the detergent-based collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide. In other aspects the detergent-based collection medium comprises, consists essentially of, or consists of about 0.8% to about 1.5% NP-40, about 0.20% to about 0.40% sodium deoxycholate, about 30 mM to about 60 mM Tris-HCl, about 20 mM to about 40 mM EDTA, about 100 mM to about 200 mM NaCl, and about 0.025% to about 0.075% sodium azide. In yet another aspect the detergent-based collection medium comprises, consists essentially of, or consists of about 0.9% to about 1.2% NP-40, about 0.20% to about 0.30% sodium deoxycholate, about 30 mM to about 60 mM Tris-HCl, about 20 mM to about 30 mM EDTA, about 100 mM to about 150 mM NaCl, and about 0.04% to about 0.06% sodium azide.

[0065] In an aspect, the collection medium comprises, consists essentially of, or consists of NP-40 and EDTA. In another aspect, the collection medium comprises, consists essentially of, or consists of NP-40, EDTA, and sodium azide. In one aspect, the collection medium comprises, consists essentially of, or consists of sodium deoxycholate, EDTA, and sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of about NP-40, sodium deoxycholate, EDTA, and sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of NP-40, sodium deoxycholate, Tris-HCl, EDTA, and sodium azide.

[0066] In another aspect, the collection medium comprises, consists essentially of, or consists of 0.5% to about 2.0% NP-40 and 10 mM to about 50 mM EDTA. In another aspect, the collection medium comprises, consists essentially of, or consists of 0.5% to about 2.0% NP-40, 10 mM to about 50 mM EDTA, and about 0.01 % to about 0.10 % sodium azide. In one aspect, the collection medium comprises, consists essentially of, or consists of about 0.10% to about 0.40% sodium deoxycholate, 10 mM to about 50 mM EDTA, and about 0.01% to about 0.10% sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, 10 mM to about 50 mM EDTA, and about 0.01% to about 0.10% sodium azide. In an aspect, the collection medium comprises, consists essentially of, or consists of about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium

deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, and about 0.01% to about 0.10% sodium azide.

[0067] In an aspect, the collection medium is a non-chaotropic medium. That is, for example, the collection medium does not include a chaotropic medium or chaotropic salts.

5 Without being limited, in an aspect, the collection medium does not include guanidine hydrochloride or urea. A potential advantage of using a non-chaotropic collection medium is better resuspension of a sample, more reproducible testing, and more uniform testing aliquots relative to a medium which includes a chaotropic medium or chaotropic salts.

[0068] An advantage of using a detergent-based collection medium is that it preserves 10 the stability of the sample. A sample stored in a detergent-based collection medium as disclosed is stable for at least 31 days, and, when held at temperatures from 15°C to 33°C is stable for at least 21 days. In an aspect, a sample is stable when frozen in a detergent-based collection medium at -20°C for at least six months. In another aspect, a cervical cell sample is stable for at least 31 days, for at least 21 days when held at temperatures from 15°C to 15 33°C, and for at least 6 months in a detergent-based collection medium at -20°C.

[0069] In an aspect, the detergent-based collection medium exhibits a sensitivity for 20 detecting cervical intraepithelial neoplasia or cancer of at least 80%, at least 90%, or at least 95% for a cut-off ratio of 0.5 relative light units. In another aspect, the detergent-based collection medium exhibits a specificity for detecting cervical intraepithelial neoplasia or cancer of at least 80%, at least 90%, or at least 95% for a cut-off ratio of 0.5 relative light units. In yet another aspect, the detergent-based collection medium exhibits a sensitivity for 25 detecting severe or moderate cervical intraepithelial neoplasia or cancer (CIN2+) of about 90% and a specificity of about 84% for a cut-off ratio of 0.5 relative light units. In an aspect, the detergent-based medium includes 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide.

[0070] A detergent-based collection medium also leads to improved assay 30 performance under rigorous hybridization and capture conditions (for example, at temperatures between 65° - 75°) relative to collection medium containing a denaturant.

[0071] The presence of one, two, three, four or more detergents can reduce sample viscosity, which aids in the removal of the liquid phase from the magnetic beads, as well as aids in the mixing of samples.

[0072] In one aspect, a sample such as blood or an exfoliated cervical cell specimen 5 can be collected and suspended in a detergent-based collection medium. The sample can be is collected with a chemically inert collection device such as a DACRON tipped swab. Any other suitable swab may be used such as nylon fiber swabs. The sample may be stored in a detergent-based collection medium, to prevent degradation of nucleic acids prior to analysis and to maintain stability of the sample.

10 [0073] Samples may be collected in other known collection mediums and then can be used in the methods described herein. Examples of other collection media include PRESERVCYT, SUREPATH, DCM (DIGENE Collection Medium), and STM (Sample/Specimen Transport Medium). Certain collection media are nucleic acid specific. For example DCM is not used when the target nucleic acid is RNA. Samples collected in 15 some of these media may require processing before the nucleic acids in the samples can be detected and analyzed. Various methods of processing samples (also known as preparing the samples) are known in the art. For example, cervical cell samples collected for cytological analysis in medium such as PRESERVCYT may be combined with a detergent-based lysis buffer followed by the addition of magnetic beads comprising nucleic acid binding surfaces.

20 In addition, other cell samples collected in other known commonly available collection mediums may be combined with a detergent-based lysis buffer followed by the addition of magnetic beads comprising nucleic acid binding surfaces.

Target Nucleic Acid Molecules

[0074] The target nucleic acid molecules include, without limitation, nucleic acid 25 molecules found in specimens or cultures (e.g., cellular, microbiological and viral cultures) including biological and environmental samples. The target nucleic acid molecules may be found in biological samples from an animal, including a human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Target nucleic acid molecules may be 30 found in environmental samples and include environmental material such as surface matter,

soil, water and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items.

[0075] The target nucleic acid molecules found in biological samples include, but not limited to cervical samples (e.g., a sample obtained from a cervical swab) or cervical cell samples, adenoid cells, anal epithelial cells, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum, urine and semen. The target nucleic acid molecules may be from other viral, bacteria, mycobacteria or plasmodia, for example cytomegalovirus (CMV), herpes, HIV, H1N1, chlamydia, gonorrhea, *Trichomonas vaginalis*, *Staphylococcus aureus*, tuberculosis, SARS-associated coronavirus or influenza. In an aspect the target nucleic acid molecules are at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 98%, at least 99%, or 100% identical to nucleic acid molecules associated with any one of cervical samples (e.g., a sample obtained from a cervical swab) or cervical cell samples, adenoid cells, anal epithelial cells, blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum, urine and semen, other viral, bacteria, mycobacteria or plasmodia, for example cytomegalovirus (CMV), herpes, HIV, H1N1, chlamydia, gonorrhea, *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis*, *Staphylococcus aureus*, tuberculosis, SARS-associated coronavirus or influenza.

[0076] In one aspect, the target nucleic acid molecules are human papillomavirus (HPV) and include genetic variants of HPV. A variant includes polymorphisms, mutants, derivatives, modified, altered, or the like forms of the target nucleic acid. In one aspect, the target nucleic acid is an HPV nucleic acid. In another aspect, the HPV nucleic acid is HPV DNA of a high risk HPV type. In another aspect, the HPV nucleic acid is HPV RNA of a high risk HPV type. In another aspect the target nucleic acids are any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 or any one of low risk HPV types 6, 11, 40, 43, 53, 61, 67, 69, 70, 71, 72, 81, and 83.

[0077] In another aspect, the target nucleic acid molecule is at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 98%, at least 99%, or 100% identical to nucleic acid molecules associated with any one of HPV, genetic variants of HPV, HPV DNA of a high risk HPV type, or HPV RNA of a high risk HPV type. In another aspect the target nucleic acids are at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 98%, at

least 99%, or 100% identical to nucleic acid molecules associated with any one of high risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 or any one of low risk HPV types 6, 11, 40, 43, 53, 61, 67, 69, 70, 71, 72, 81, and 83.

[0078] Using methods of the present inventions, the target nucleic acid molecule may 5 be present at concentrations less than about 1 pg per ml, less than about 0.75 pg per ml, less than 0.5 pg per ml, less than 0.25 pg per ml, and even as low as 0.2 pg per ml. As seen in FIG. 2 an excellent signal to noise ratio is obtained when HPV-16 DNA was used as the target nucleic acid molecule present at a concentration of 0.2 pg per ml.

[0079] As noted previously, the target nucleic acid molecule may be DNA or RNA. 10 When the target nucleic acid molecule is DNA, the probe is preferably RNA and when the target nucleic acid is RNA, the probe is preferably DNA. However, a DNA probe can be used with DNA target nucleic acid molecule and an RNA probe can be used with RNA target nucleic acid molecule. Also as indicated previously, the target nucleic acid molecule may determine the collection medium used.

15 Denaturation

[0080] After the sample is collected in a detergent-based collection medium as described above, the sample may be treated with a denaturation reagent to render the target nucleic acid molecule accessible to hybridization. In one aspect, the sample is denatured with an alkaline solution. Any alkali that can bring a solution pH to about pH 12, about pH 13, or 20 about pH 14 may be used. Additionally, any alkali that can bring a solution pH to a range of about pH 12 to about pH 13, from about pH 12 to about pH 14, and from about pH 13 to about pH 14 can be used. Suitable concentrations of alkali include from about 1.0 N to about 2.0 N, from about 1.25 N to about 1.75 N, and from about 1.25 N to about 1.5 N, and about 1.5 N as well as any number within the recited ranges. Without being limited, suitable alkali 25 include NaOH and KOH.

[0081] In one example, approximately one volume of the sample suspended in a detergent-based collection medium can be treated with about one-half volume of 1.75 N NaOH solution. For example, in certain aspects approximately a 50 μ l aliquot is removed from a sample suspended in a detergent-based collection medium and approximately 25 μ l of 30 1.75 N NaOH solution is added to the 50 μ l aliquot sample. At room temperature, the sample treated with the denaturation reagent can be mixed by hand mixing or mechanical shaking at

about 800 rpm, about 900 rpm, about 1000 rpm, between about 600 and about 1000 rpm, or between about 600 and 1200 rpm. The pH of the sample after addition of denaturation reagent can be about 14. In another aspect, the pH can be about pH 12 or pH 13. Such basic pH will both nick and denature a majority of the nucleic acid in the specimen. In addition,

5 alkaline treatment can disrupt interactions between peptides and nucleic acids to improve accessibility of the target nucleic acid and degrade protein.

[0082] Alkaline treatment of protein effectively homogenizes the specimen to ensure reproducibility of analysis results for a given sample. It can also reduce the viscosity of the sample to increase kinetics, homogenize the sample, and reduce background by destroying

10 any endogenous single stranded RNA nucleic acids, DNA-RNA hybrids or RNA-RNA hybrids in the sample. It also helps inactivate enzymes such as RNases and DNases that may be present in the sample. One skilled in that art would appreciate that if RNA is the target nucleic acid (as opposed to DNA), different reagents may be preferable including, but not limited to phenol extraction and TCA/acetone precipitation, and guanidinium thiocyanate-

15 phenol-chloroform extraction.

[0083] Other methods of denaturation may be employed such as utilizing a heating step, for example, heating the sample to about 95°C to separate the strands of nucleic acid. Enzymes such as helicase may be used as well.

[0084] In one aspect, 1.5 N to 2.0 N NaOH is added to the sample and heated. In

20 another aspect, 1.75 N NaOH is added to the sample and heated. The sample with denaturation reagent may be heated to about 60°C to about 80°C for about 30 minutes, to about 65°C to about 75°C for about 30 minutes, to about 67°C to about 70°C for about 30 minutes, or to about 70°C for about 30 minutes, or any number within the recited ranges. In another aspect, the sample with denaturation reagent is heated to about 60°C to about 80°C

25 for about 20 to about 40 minutes, or to about 65°C to about 75°C for about 20 to about 40 minutes, to about 67°C to about 70°C for about 20 to about 40 minutes, or to about 70°C for about 20 to about 40 minutes, or any number within the recited ranges. The goal of the described time and temperature conditions is to provide for maximal denaturation of the sample in a minimum amount of time, while leaving the target nucleic acid in a suitable

30 condition for carrying out the remaining steps of hybridization, capture, washing, and detection. Therefore, the sample may be heated in denaturation reagent for about 5 to about 120 minutes, about 10 to about 60 minutes, about 20 minutes to about 40 minutes, about 30

minutes, or any number within the recited ranges. It will be readily understood by one of ordinary skill in the art that longer periods of incubation at lower temperatures, or shorter periods of incubation at higher temperatures, may be balanced to provide a similar effect to the conditions described herein.

5 Hybridization and Binding of Probes

[0085] After the sample containing the nucleic acid is denatured, it is contacted with one or more polynucleotide probes under a condition sufficient for the one or more polynucleotide probes to hybridize to the target nucleic acid in the sample to form a double-stranded nucleic acid hybrid. The probe can be full length, truncated, or synthetic DNA or 10 full length, truncated, or synthetic RNA. If the target nucleic acid is DNA, then the probe may be RNA and if the target nucleic acid is RNA, then the probe may be DNA. Preferably, the one or more polynucleotide probes are diluted in a probe diluent that also can act as a neutralizing hybridization buffer (to neutralize the basic denaturation reagent).

[0086] The probe diluent used for DNA or RNA probes will differ due to the different 15 requirements necessary for DNA versus RNA stability. For example, if the probes are RNA, it is preferable to neutralize the sample first and than add the probe or alternatively, add the RNA probe and neutralizing agent (probe diluent) to the sample at the same time as NaOH can destroy RNA. The probe diluent can be used to dissolve and dilute the probe and also help restore the sample to about a neutral pH, *e.g.*, about pH 6 to about pH 9, to provide a 20 more favorable environment for hybridization. Sufficient volume of probe diluent, preferably one-half volume of the sample, may be used to neutralize the base-treated sample.

[0087] In an aspect, the probe diluent comprises a buffer, polyacrylic acid, NaOH and sodium azide. The probe diluent may comprise acetic acid. In one aspect, the probe diluent comprises 2.2 M BES (N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 2.6% 25 polyacrylic acid (PAA), 0.7 N NaOH and 0.05% sodium azide. The probe diluent may contain from about 1.2 M to about 2.6 M BES, from about 1.5 M to about 2.5 M BES; from about 1.75 M to about 2.25 M BES; from about 2 M to 2.4 M BES, or about 2.2 M BES, as well as any number within the recited ranges. In one aspect the probe diluent may contain from about 2% to about 3.0% PAA or, as well as any number within the recited ranges. In 30 another aspect, the PAA concentration is from about 2.2% to about 2.7%. In yet another aspect, the PAA concentration is about 2.6%. In a further aspect the probe diluent may

contain from about 0.6 N to about 0.8 N NaOH, for example, about 0.7 N NaOH. The concentration of NaOH generally increases as the amount of BES increases.

[0088] For full length probes, a heated alkaline solution may be added to the sample, then probe diluent may be added to the sample at room temperature, and then the sample may 5 be reheated. Such a process can inhibit secondary structure from forming. Antibodies tend to irreversibly bind to structures with secondary structure. When using non-full length probes such as truncated or synthetic probes, heating the solutions or sample may not be necessary because secondary structures issues are not present. In an aspect, the sample is not heated when used with truncated or synthetic probes.

10 [0089] After treatment with the denaturation reagent, an aliquot of neutralization buffer, in an aspect the probe diluent described, in which the one or more probes are dissolved, can be added to the sample under appropriate conditions to allow hybridization or binding of the probe and the target nucleic acid to occur. The neutralization buffer may contain a single buffering salt. In an aspect, the neutralization buffer does not contain more 15 than a single buffering salt. The hybridization condition is sufficient to allow the one or more polynucleotide probes to anneal to a corresponding complementary nucleic acid sequence, if present, in the sample to form a double-stranded nucleic acid hybrid.

16 [0090] Hybridization conditions suitable for the particular probes and diluents described herein are employed. For example, the probes and sample nucleic acids can be 20 incubated for a hybridization time, preferably at least about 5 to about 30 minutes, about 5 to about 20 minutes, or from about 7 to about 15 minutes, or about 10 minutes, as well as any number within the recited ranges sufficient to allow the one or more polynucleotide probes to anneal to a corresponding complementary nucleic acid sequence. The hybridization condition can include a hybridization temperature of at least about 65°C, about 68.5°C, and 25 about 67°C to about 70°C, as well as any number within the recited ranges. For a given target nucleic acid and a given probe, one of ordinary skill in the art can readily determine desired hybridization conditions by routine experimentation. One of ordinary skill in the art will further appreciate that the time and temperature of hybridization must be optimized, one with respect to the other. Thus, higher hybridization temperatures may be carried out for 30 shorter periods of time and vice versa. Without being limited, stringent hybridization conditions may be controlled by increasing the temperature, increasing the ionic conditions to above 0.5M (for example, NaCl), or reducing the concentration of PAA. As a non-limiting

example, stringent hybridization conditions may include performing a hybridization reaction at elevated temperatures, such as of at least about 65°C, at least about 68.5°C, between about 67°C to about 70°C, and between about 69°C to about 70°C. Stringent hybridization conditions may also include elevated temperatures, such as of at least about 65°C, at least 5 about 68.5°C, and between about 67°C to about 70°C.

[0091] In a non-limiting aspect, the probe is capable of hybridizing or binding to nucleic acid molecules at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to nucleic acid molecules associated with HPV, genetic variants of HPV, HPV DNA of a high risk HPV type, or HPV RNA of a high risk HPV type, or any one of high risk HPV types 16, 18, 31, 10 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 or any one of low risk HPV types 6, 11, 40, 43, 53, 61, 67, 69, 70, 71, 72, 81, and 83. In another aspect, the probe is complementary to HPV, genetic variants of HPV, HPV DNA of a high risk HPV type, HPV RNA of a high risk HPV type, or any one of high risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 15 66, 68, and 82 or any one of low risk HPV types 6, 11, 40, 43, 53, 61, 67, 69, 70, 71, 72, 81, and 83.

[0092] In one aspect, the sample is suspended in detergent-based collection medium, the target nucleic acid is denatured with a denaturation reagent, and hybridized to nucleic acid probes suspended in a neutralizing buffer. In another aspect the neutralizing buffer is the 20 probe diluent of the present invention. The probe diluent can comprises 2.2 M BES (*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 2.6% polyacrylic acid, 0.7 N NaOH and 0.05% sodium azide.

Capture

[0093] After the probes are allowed to hybridize to the target nucleic acid molecule 25 and to form a double-stranded nucleic acid hybrid, the hybrid is captured by a molecule that is specific for the double-stranded nucleic acid hybrid. Molecules specific for the double stranded nucleic acid hybrids include, but are not limited to, monoclonal antibodies, polyclonal antibodies, proteins such as but not limited to RNase H, nucleic acids including but not limited to aptamers, or sequence specific nucleic acids. Aptamers are short stretches 30 of random sequences that are successively selected from a library of sequences by hybridizing to a target, amplifying the hybridized aptamers, and repeating the selection

process. In one aspect the molecule specific for the double stranded nucleic acid hybrid is captured by an antibody, known as an anti-hybrid antibody.

[0094] In one aspect, a first anti-hybrid antibody is immobilized onto a support using techniques that are standard in the art. Examples of suitable supports include covalent linkages or adsorption, for example, protein-protein interactions, protein-G beads, biotin-streptavidin interaction, EDAC to link to a carboxyl or tosyl group, etc., or hybridization directly onto the solid support using, for example, sequence specific nucleic acids in an affinity column.

[0095] Supports include but are not limited to beads, magnetic beads, which as indicated previously include paramagnetic, diamagnetic, ferromagnetic, ferromagnetic, and diamagnetic beads, columns, plates, filter paper, polydimethylsiloxane (PDMS), and dipsticks. Any support can be used as long as it allows extraction of the liquid phase and provides the ability to separate out bound and unbound antibodies. Magnetic beads are particularly useful in that they can be left in the solution and the liquid phase can be extracted or decanted, if a magnetic field is applied to immobilize the beads. Beads that are small and have a high surface area are preferable, such as beads about 1 μm in diameter. Other beads that employ charge switching or silica capture (as opposed to magnetic fields) may be used as well.

[0096] The hybrids are incubated with the anti-hybrid antibody attached to the support for a sufficient amount of time to allow capture of the double-stranded nucleic acid hybrids by the immobilized anti-hybrid antibodies. In an aspect, the support is a bead.

[0097] The anti-hybrid antibody may be monoclonal or polyclonal. In one aspect the antibody is monoclonal. In one aspect, the antibody is coupled to support by an 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDAC) linker. In one aspect, the support is a polystyrene bead. In an aspect, the support or bead coupled to the antibody is diluted in a bead dilution buffer. The bead dilution buffer is helpful in minimizing protein denaturation on the bead. One example of a bead dilution buffer comprises 6% casein, 100 mM Tris-HCl, 300 mM NaCl, and 0.05% sodium azide.

[0098] In an aspect, the beads coated with the anti-hybrid antibody are incubated with the sample at about 67°C to about 70°C for about 30 minutes. In another aspect, the beads and sample are incubated at about 68°C to about 69°C for about 30 minutes. In yet another aspect, the beads and sample are incubated at about 68.5°C for 30 minutes. The

incubation time can range from about 5 minutes to about 60 minutes, from about 15 minutes to about 45 minutes, from about 20 minutes to about 40 minutes, or any number within the recited ranges, and is generally inversely proportional to the temperature. It will be understood by those skilled in the art that the incubation time, temperature and/or shaking

5 conditions can be varied to achieve alternative capture kinetics as desired.

[0099] Following capture of the target nucleic acid/probe hybrid as described above, the captured hybrid may be separated from the rest of the sample by washing away of non-captured nucleic acids.

Conjugation

10 [00100] Another step in the method can involve providing a second antibody that is also specific for double stranded nucleic acids hybrids or alternatively is specific for the first antibody. The second antibody may be detectably labeled, either directly or indirectly, and may be a monoclonal or polyclonal antibody. In an aspect, the second antibody is monoclonal. In another aspect, the second antibody is directly labeled with a detectable

15 marker and is monoclonal. The second antibody is used to detect the presence of double-stranded nucleic acid hybrids. In one aspect, the second antibody has a label that must react with a substrate to provide a signal that can be detected. The second antibody may be dissolved in a suitable buffer. In one aspect the buffer comprises 100 mM TrisHCl, pH 7.4, 0.5 M NaCl, 0.1 mM ZnCl₂, 1.0 mM MgCl₂, 0.25% Tween 20, 0.2 mg/ml RNase A, 4%

20 hydroxypropyl-*b*-cyclodextrin (cyclodextrin), 30% bead dilution buffer as discussed previously, 0.05% goat IgG, 0.05% sodium azide.

[00101] In an aspect, the conjugation reaction takes place at room temperature. In an aspect, the conjugation reaction takes place at room temperature for between about 1 hour and 2 hours. In another aspect, the conjugation reaction takes place at room temperature for about 2 hours. In another aspect the conjugation reaction takes place at about 37°C, about 45°C, or about 50°C. In an aspect the conjugation reaction takes place at about 37°C, about 45°C, or about 50°C, between 35°C and about 40°C, between 40°C and about 50°C for between about 20 minutes and 40 minutes. In an aspect the conjugation reaction takes place at about 37°C, about 45°C, or about 50°C for between about 20 minutes and 40 minutes. In another aspect the conjugation reaction takes place at about 45°C for about 30 minutes.

[00102] It will be understood by those skilled in the art that any detectable label such as, but not limited to, an enzyme, radioactive molecule, fluorescent molecule, or metal particle such as gold particle can be used. In certain aspects, the detectable label is alkaline phosphatase. Methods of conjugating a label to an antibody are known. For example, an antibody can be reduced with dithiothreitol (DTT) to yield monovalent antibody fragments. The reduced antibody can then be directly conjugated to maleinated alkaline phosphatase by the methods of Ishikawa *et al.*, J. Immunoassay 4:209-237 (1983) and Means *et al.*, Chem. 1: 2-12 (1990), the contents of each of which are incorporated herein by reference in its entirety, and the resulting conjugate can be purified by HPLC. The conjugate may also be purified using any type of size-exclusion chromatography. One benefit of purification is that the conjugates of one protein to one antibody can be separated from those conjugates with other ratios of protein to antibody.

[00103] In another aspect, the double-stranded nucleic acid hybrids can be detected with a second anti-hybrid antibody that is not directly labeled. For example, the second antibody can be a mouse immunoglobulin that is detected by a labeled goat anti-mouse antibody.

Wash

[00104] Following conjugation with the second antibody, the sample is washed with a based wash buffer. The wash buffer may contain one or more detergents or may be free of a detergent. If the wash buffer contains a detergent, the detergent may be an ionic or a non-ionic detergent. One example of a non-ionic detergent is Triton-X. The detergent may be present in the wash buffer at a concentration of about 0.05% to about 1.5%, or from about 0.075% to about 1.0%, or from about 0.1% to about 0.75%, or about 0.5% or any number within the recited ranges. One example of a suitable wash buffer comprises 40 mM Tris, pH 8.2, 100 mM NaCl, 0.5% Triton-X 100 and 0.05% sodium azide.

[00105] The sample may be washed with the wash buffer from one to ten times, or from three to seven times, or from four to six times, or five times, or any number within the recited ranges. The sample may also be washed with a single wash buffer or with multiple wash buffers. Each wash may use the same wash buffer or a different wash buffer. For example, a detergent-containing wash buffer may be used for one wash while a detergent-free

wash buffer may be used for another wash. In an aspect, one of the wash buffers does not include Triton.

[00106] One benefit of the detergent-containing wash buffer is the positive effects on bead behavior when compared to detergent-free wash buffers. The detergent-containing 5 wash buffer allows for rapid, efficient, and resilient binding of the beads to the magnetic field. Binding of the beads to the magnetic field is strong enough that beads remain bound through physical inversion and decanting. While detergent-free wash buffers generally do not allow for physical inversion without bead loss, they may be used for other purposes. One example of the use of a detergent-free wash buffer is to remove or dilute a detergent in the 10 sample thereby reducing any likely detection problems.

Detection

[00107] The label present on the second, or third, or more, antibody is detected to thus indicate the presence of the target nucleic acid molecule. Methods for detecting various labels are known in the art. For example, colorimetry, radioactive, surface plasmon 15 resonance, or chemiluminescence methods are described by *e.g.*, Coutlee *et al.*, *J. Clin. Microbiol.* 27:1002-1007 (1989), the contents of which are incorporated herein by reference in its entirety.

[00108] For example, a bound alkaline phosphatase conjugate can be detected by chemiluminescence with a reagent such as a LUMI-PHOS 530 reagent (Lumigen, Detroit, 20 MI) or DR2 (Applied Biosystems, Foster City, CA) using a detector such as an E/LUMINA luminometer (Source Scientific Systems, Inc., Garden Grove, CA), an OPTOCOMP I Luminometer (MGM Instruments, Hamden, CT), or the like, such as a Veritas Microplate Luminometer by Turner Biosystems. Multiple detection techniques can also be used in sequence or in parallel. For example, the conjugate may be detected by chemiluminescence 25 and fluorescence. In another aspect, the conjugate can be detected by chemiluminescence.

[00109] Detectors using different detection techniques for the conjugate may be reversible or irreversibly attached, for example in a modular fashion, to a machine that is capable of performing the method for determining the presence of a target nucleic acid molecule in a sample.

30 [00110] As described herein, detection of the label on the second antibody is indicative of the presence of one or more of the target nucleic acid molecules in the sample that are

complementary to the one or more probes. Following washing, the sample is suspended in a detection buffer that for example, contains the substrate for the label on the second antibody.

[00111] In one aspect, the sample is comprised of cervical cells. The method for determining the presence of a target nucleic acid molecule in a sample of cervical cells

5 comprises suspending the sample in a detergent-based collection medium and mixing by hand mixing. In another aspect the mixing is mechanical. An approximately 50 μ l aliquot of the sample is removed and mixed with about 25 μ l of a denaturation reagent. The sample is mixed by hand mixing or mechanical shaking at between about 600 to about 1200 rpm for about 30 to about 60 seconds and heated at about 70°C for about 30 minutes. High risk HPV

10 RNA probes are prepared in a diluent and diluted to about 375 ng/ml. About 40 μ l of diluted probe is added to the sample on a 70°C heating block. The samples are further incubated at approximately 68.5°C with shaking at about 1150 rpm for about 30 minutes. The supernatant can be removed by a dropper bottle or other low tech device. About 35 μ l of the detection reagent is added to the sample. The detection reagent contains a second antibody that is

15 labeled. The second antibody is specific for double-stranded nucleic acid hybrids. The sample containing the detection reagent is incubated at about 45°C for about 30 minutes, placed on a magnetic rack for about 30 seconds to 3 minutes and the supernatant is decanted. In another aspect the sample containing the detection reagent is incubated at room temperature. The sample is then washed with wash buffer about four or five times.

20 Anti-hybrid Antibodies

[00112] The double-stranded nucleic acid hybrids formed in accordance with the present invention can be captured and detected using antibodies that are specific to double-stranded nucleic acid hybrids. The antibody is specific to double-stranded hybrids, such as but not limited to RNA-DNA; DNA-DNA; RNA-RNA; and mimics thereof, where mimics refer to molecules that behave similarly to RNA-DNA, DNA-DNA, or RNA-RNA hybrids.

25 The anti-double-stranded nucleic acid hybrid antibody, *i.e.*, the anti-hybrid antibody that is utilized will depend on the type of double-stranded nucleic acid hybrid formed. In one aspect, the anti-hybrid antibody is immunospecific to RNA-DNA hybrids.

[00113] It will be understood by those skilled in the art that either polyclonal or

30 monoclonal anti-hybrid antibodies can be used and/or coupled to beads and/or immobilized on a support in the present assay as described below. Monoclonal antibody prepared using

standard techniques can be used in place of the polyclonal antibodies. Monoclonal antibodies may be produced by methods that are standard in the art. In an aspect, the antibodies used for capture and detection of the target nucleic acid are monoclonal antibodies. In an aspect, monoclonal antibodies support high stringency incubation temperatures during the capture 5 step. Without being limited, the high stringency incubation temperatures during the capture step may be between about 65° to about 75°C or between about 68° to about 75°C. The first and second antibodies may be the same for capture and detection (*i.e.*, produced by the same hybrid myeloma cell line) or may be different and produced by different hybrid myeloma cell lines. In one aspect, the first and second monoclonal antibodies used for capture and/or 10 detection are the same and are specific for RNA-DNA hybrids. Also included are immunofragments or derivatives of antibodies specific for double-stranded hybrids, where such fragments or derivatives contain binding regions of the antibody.

[00114] For example, a monoclonal anti-RNA-DNA hybrid antibody derived from myeloma cells fused to spleen cells that are immunized with an RNA-DNA hybrid can be 15 used. The hybrid-specific antibody can be purified by affinity purification against RNA-DNA hybrids immobilized on a solid support, for example as described in Kitawaga *et al.*, Mol. Immunology, 19:413 (1982); and U. S. Patent No. 4,732, 847, the contents of each of which are incorporated herein by reference in their entirety.

[00115] Other suitable methods of producing or isolating antibodies, including human 20 or artificial antibodies, can be used, including, for example, methods that select recombinant antibody (*e.g.*, single chain F_v or F_{ab}, or other fragments thereof) from a library, or which rely upon immunization of transgenic animals (*e.g.*, mice) capable of producing a repertoire of human antibodies (see, *e.g.*, Jakobovits *et al.*, Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits *et al.*, Nature, 362: 255 (1993); and U.S. Pat. No. 5,545,806 and U.S. Pat. No. 25 5,545, 807, the contents of each of which are incorporated herein by reference in their entirety).

[00116] In one aspect, the target nucleic acid to be detected is DNA (*e.g.*, HPV 30 genomic DNA or cDNA) or RNA (*e.g.*, mRNA, ribosomal RNA, nuclear RNA, transfer RNA, viral RNA, heterogeneous nuclear RNA), wherein the one or more polynucleotide probes are polyribonucleotides or polydeoxyribonucleotides, respectively. In a preferred aspect, the double-stranded nucleic acid hybrids are DNA-RNA hybrids formed by

hybridization of target DNA and probe RNA, and can be detected using an antibody that is immunospecific to RNA-DNA hybrids.

[00117] In an aspect of the present invention, a monoclonal anti-RNA-DNA hybrid antibody derived from a hybridoma cell line is used. Such hybridoma cell lines are described 5 in U.S. Pat. No. 4,865,980, U.S. Pat. No. 4,732,847, and U.S. Pat. No. 4,743,535, the contents of each of which are incorporated herein by reference in their entirety. Hybrid-specific monoclonal antibodies may be prepared using techniques that are standard in the art. The hybrid-specific monoclonal antibody may be used for both capturing and detecting the target nucleic acid.

[00118] While any vertebrate may be used for the preparation of polyclonal anti-RNA-DNA hybrid antibodies, goats or rabbits are preferred. Preferably, a goat or rabbit is immunized with a synthetic poly(A)-poly(dT) hybrid by injecting the hybrid into the animal in accordance with conventional injection procedures. Polyclonal antibodies may be 10 collected and purified from the blood of the animal with antibodies specific for the species of the immunized animal in accordance with well-known antibody isolation techniques. For the production of monoclonal antibodies, the spleen can be removed from the animal after a sufficient amount of time, and splenocytes can be fused with the appropriate myeloma cells 15 to produce hybridomas. Hybridomas can then be screened for the ability to secrete the anti-hybrid antibody. Selected hybridomas may then be used for injection into the peritoneal cavity of a second animal for production of ascites fluid, which may be extracted and used as 20 an enriched source of the desired monoclonal antibodies incorporated herein by reference.

Polynucleotide Probes

[00119] The polynucleotide probes are designed to hybridize or bind with the target nucleic acid molecules. In another aspect, the polynucleotide probes are designed to bind to 25 target nucleic acid molecules. In one aspect, the probes are capable of hybridizing or binding to HPV and HPV high risk variants. In an additional aspect, the polynucleotide probes are specific for HPV and HPV high risk variants. High risk (HR) nucleic acid probes can include probes for HPV high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 82. In other aspects the RNA or DNA probes are fragments. In an aspect, the probes are about 6 30 to about 8 kilobases in length, preferably about 7.5 kilobases, and may be produced using a plasmid template using a BLUESCRIPT vector. However, other plasmids, vectors and

methods are known in the art and could also be used to produce the RNA probes described herein.

5 [00120] The probes may vary in amount from about 7.5 ng to about 60 ng per HPV type per assay, or from about 20 ng to about 45 ng per HPV type per assay, or about 30 ng of probe for each HPV type per assay is used. Thus, in one aspect the HR probes consist of or consist essentially of one or more probes for HPV high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 or low risk HPV types 6, 11, 40, 43, 53, 61, 67, 69, 70, 71, 72, 81, and 83, wherein about 30 ng of each probe is used per assay for detection of the target nucleic acid molecule.

10 [00121] The RNA probes may be short synthetic RNA probes that specifically bind only to the target nucleic acid molecule. Examples are described in U.S. Pat. Appl. No. 12/426,076, filed on April 17, 2009, the contents of which are incorporated herein by reference in its entirety.

Cross-Reactivity

15 [00122] The present invention also provides for assay compositions, probes, and conditions wherein cross-reactivity between HPV HR probe sets and low risk HPV types is dramatically reduced when compared to the standard FDA approved HPV assay and probe set. In one aspect, the HPV HR probe set is selected from the group consisting of HPV high risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82 or low risk HPV types 20 6, 11, 40, 43, 53, 61, 67, 69, 70, 71, 72, 81, and 83. Using the present assay with these HR HPV probes, cross-reactivity between low risk HPV types and high risk HPV probes is reduced. *See, for example, U.S. Pat. Appl. No. 12/426,076.*

25 [00123] The present invention also provides a method for determining the presence of a target nucleic acid molecule, such as HPV, in a sample in about 2 hours or less, about 2.5 hours or less, about 3 hours or less, about 3.5 hours or less, about 4 hours or less, about 5 hours or less, about 6 hours or less, about 7 hours or less, about 8 hours or less, about 12 hours or less, about 24 hours or less, in other aspects, less than about 3.5 hours for at least 10 samples using the methods discussed above. One reason why the presence of HPV or other target nucleic acid molecules can be determined in short periods of time is because the 30 method does not amplify the target nucleic acid molecule prior to detection. Instead of target amplification, signal amplification may be used to accurately detect the presence of HPV or

other target nucleic acid molecules. In an aspect, the methods of the disclosure may include a signal amplification step. In an aspect, the methods of the disclosure do not include a target amplification step. In another aspect, the methods of the disclosure may include a signal amplification step and no target amplification step.

5 [00124] The present disclosure also provides methods and assays for detecting cancer, for example cervical cancer, by detecting the presence of a target nucleic acid molecule, such as HPV, in a sample in about 2 hours or less, about 2.5 hours or less, about 3 hours or less, about 3.5 hours or less, about 4 hours or less, about 5 hours or less, about 6 hours or less, about 7 hours or less, about 8 hours or less, about 12 hours or less, about 24 hours or less, in
10 other aspects, less than about 3.5 hours for at least 10 samples using the methods and assays discussed above.

[00125] It will be understood to those skilled in the art that the present invention can be carried out on a number of platforms including, but not limited to, tubes, dipsticks, microarrays, microplates, 384 well plates, other microtiter plates and microfluidic systems. It
15 will be understood to those skilled in the art that the present, as relevant to developing countries, can utilize low technology methods such as dropper bottles, rubber bulbs, Pasteur pipettes, or squirt bottles for steps involving movement of liquid. These devices deliver relatively precise volumes within the approximate ranges that are needed for the assay. In an aspect, the methods of the disclosure do not include automatic pipettors or other battery
20 powered or energy powered pipetting devices.

[00126] Another aspect of the present invention provides a collection medium into which samples containing the target nucleic acid are collected. The collection medium provides sample stability for several days, several weeks, or several months. For example, the collection medium may provide sample stability for at least 1 week, at least 2 weeks, at
25 least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, from about 1 week to about 4 weeks, from about 1 month to about 3 months, from about 3 to about 4 months, or from about 3 month to 6 months. In another aspect, the collection medium provides sample stability for at least 21 days at 33°C or at least 6 months at 20°C. In an aspect the above sample is a cervical cell
30 sample or a human cervical cell sample. Suitable collection media are described herein. In one aspect, the collection medium comprises, consists of, or consists essentially of NP-40, deoxycholate, Tris-HCl, EDTA, NaCl, and sodium azide. In other aspects, the collection

medium comprises, consists of, or consists essentially of 1.0% NP-40, 0.25% sodium deoxycholate, 50 mM Tris-HCl, 25 mM EDTA, 150 mM NaCl, and 0.05% sodium azide.

[00127] Another aspect is a detergent-containing wash buffer comprising, consisting of, or consisting essentially of 40 mM Tris pH 8.2, 100 mM NaCl, 0.1% to 0.5% Triton X-5 100, and 0.05% sodium azide. Yet another aspect is a detergent-free wash buffer comprising, consisting of, or consisting essentially of 40 mM Tris pH 8.2, 100 mM NaCl, and 0.05% sodium azide.

Sample Conversion for Recovery, Detection, and Analysis of Nucleic Acid Molecules

10 [00128] An aspect relates to adding a collection medium to a sample which has been previously prepared for diagnostic analysis. In one aspect, the sample to which the collection medium is added has been previously prepared using a liquid based cytology (LBC) assay. LBC media can contain tissue fixatives such as alcohol and formalin which serve to stabilize the sample, inhibit bacterial growth, preserve cell morphology and diagnostic clusters, and

15 assure the preparation of a tissue monolayer cytology slides. However, many compositions used to preserve biological samples, such as SUREPATH, contain alcohol or formalin which can be detrimental to analyzing nucleic acid molecules. In an aspect, the cytology slides contain cervical cell samples or any other biological sample capable of being evaluated. In an aspect, the SUREPATH media is used to prepare LBC sample.

20 [00129] In addition to cytology preparation, LBC samples can be used for detection of disorders, such as common sexually transmitted pathogens, including Human Papillomavirus (HPV), *Neisseria gonorrhoeae* (GC), and *Chlamydia trachomatis* (CT), among others. As a supplement to its application as a screening tool, LBC samples can be used to monitor patients' viral clearance after treatment for a particular disease, informing further follow-up and treatment regimens. In an aspect, HPV testing of LBC samples can be used to monitor patients' viral clearance after treatment for a cervical-based disease.

25 [00130] In an aspect, a biological sample is collected and preserved in a media, such as the SUREPATH media. The preserved media containing the biological sample is stored until further processing is required. The preserved media containing the biological sample can be removed and suspended in water thereby forming a "soft pellet." A portion of the soft pellet 30 may be removed and analyzed on a slide. In an aspect, the sample is prepared using a LCB assay. Instead of adding more preservation media, such as SUREPATH, to the remaining

soft pellet suspension, the detergent-based collection media described herein may be added to the remaining biological sample. This is advantageous in that a sample dispersed in the detergent-based collection media described herein can be directly analyzed in a nucleic acid molecule detection assay. Additionally, the biological sample suspended in the detergent-based collection medium is stable for at least 11 days at room temperature (Fig. 6).

5 [00131] Any of the disclosed detergent-based collection media are capable of being added to the soft pellet. In another aspect, a detergent and chelator media may be used to resolubilize the pellet. In a non-limiting aspect, a collection media including about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 10 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide can be used to solubilize the soft pellet. After the addition of the detergent-based collection media, the soft pellet sample may be analyzed in conjunction with any of the methods or assays described herein.

15 15 Kit

[00132] Also provided is a kit for the detection of a target nucleic acid molecule in a sample, the kit comprising, consisting of or, or consisting essentially of:

- 20 a) a collection medium;
- b) a denaturation reagent;
- c) at least one polynucleotide probe;
- d) a bead coated with a first anti-hybrid antibody;
- e) a detection reagent comprising a second anti-poly hybrid antibody, wherein the second antibody is detectably labeled;
- f) a wash buffer; and

25 g) a second detection reagent comprising a substrate for the label on the second antibody.

[00133] The collection medium, denaturation reagent, bead, first and second antibodies, polynucleotide probes, detection reagents, and wash buffers have been previously described.

30 [00134] The kit may also include instructions for describing procedures associated with the disclosed methods and assays. The kit may also include a means for transcribing patient information. In an aspect, the means includes paper, a computer, or a device capable

of transmitting patient information. The kit can include all the necessary components to complete the methods at the same location where the patient sample is taken.

[00135] In an aspect, the kit may include color coded reagents associated with the detection assay. The reagent vials are color coded for ease of use and can be included in a 5 kit. The reagent bottles may also be identified by symbols, letters, or other known identifiers.

[00136] As the individual components of the kit come together in an easy to use platform, one advantage of the kit described herein is that it provides for immediate testing of samples. This allows for rapid determination of patient results.

[00137] In an aspect, methods of the disclosure can include the collection and 10 processing of patient samples in the field. In one aspect, after the samples are collected, some of the method steps are conducted at the same location where the patient samples are collected. In another aspect, all of the method steps can be conducted at the same location where the samples are collected. The location may be a village, clinic, laboratory, or communal area where individuals receive medical checkups and evaluations. The location 15 may be permanent or temporary. In an aspect, the nucleic acid molecule is detected at a location, such as a laboratory or clinic, which is different from where the samples are taken. In an aspect, the kit is designed for use in a developing country or geographical areas where access to medical care is not readily available.

20 System

[00138] In an aspect, the sample may be analyzed using a portable system for detecting 25 the presence of a target nucleic molecule acid in a sample. The portable system may include a heater configured for heating multiple samples; a luminometer configured to detect the chemiluminescence of a target nucleic acid molecule on a support coated with a first antibody and conjugated to a second antibody labeled with a detectable marker; and a monitor 30 configured to report chemiluminescence data (Fig.7). In an aspect, the heater is a combination heater/shaker. In an aspect, the system is configured to simultaneously analyze 90 samples at a time together with 6 controls (96 samples altogether). In an aspect, both the luminometer and the heater are configured to simultaneously analyze 90 samples at a time together with 6 controls (96 samples altogether). The system is also capable of reporting the 35 results of at least 500 samples at a time. The portable system and associated reagents are set forth in Figs. 6, 7, and 8.

[00139] Without being limited, the individual components of the portable system can be easily transported by an individual, such as a doctor or laboratory technician, and weighs less than 10 pounds, less than 20 pounds, less than 30 pounds, or less than 40 pounds. In another aspect, the entire portable system for detecting target nucleic acid molecules weighs 5 less than 20 pounds, less than 30 pounds, less than 40 pounds, or less than 50 pounds, or less than 100 pounds and is designed for easy transportation.

[00140] The portable system and assay can be designed for use in the field or location where samples are collected and require a small footprint of bench-top work space (for example, about 25x50cm or less). In an aspect, the portable system is configured to work 10 without electricity, mains, or running water. The portable system may also run entirely on batteries. The portable system can be designed for use with the described kit, assays, with the methods described herein. Together, the kit and portable system provide an efficient and rapid way to analyze samples and detect nucleic acid samples in developing countries or other areas which may lack sophisticated laboratory equipment or facilities for analyzing 15 laboratory samples.

[00141] It is noted that when ranges are described herein, any number within that range is contemplated in the inventions described herein. The entire contents of all patents, patent applications, scientific publications, etc. listed are incorporated in their entirety by reference. The examples below are not meant to limit the scope of the invention.

20

EXAMPLES

Example 1: Assay Using Cervical Samples and HPV probes

[00142] A total of 324 physician collected cervical samples were collected in a detergent based collection medium and tested for the presence of high-risk HPV.

25 [00143] A 1 ml sample was vortexed to homogenize the sample and a 50 μ l aliquot was removed and combined with 25 μ l of denaturation reagent (1.75 N NaOH) in the assay microplate. This was shaken to mix and incubated at 70°C for 30 minutes to create single stranded DNA. To this, 40 μ l of a neutralization buffer (probe diluent - 2.2M BES, 2.6% PAA, 0.7 N NaOH and 0.05% sodium azide) containing RNA probes for 16 HPV types was 30 added to create a neutral pH and incubated at 68.5°C for 10 minutes.

[00144] Following this, 10 μ l of antibody conjugated paramagnetic beads (approximately 1 μ m carboxylated SERADYN beads from Thermo Fisher) were added to the

reaction and incubated for an additional 30 minutes at 68.5°C. The RNA probes and DNA target molecules that were complementary to each other bind and create RNA-DNA hybrids. The hybrids then captured by a RNA-DNA hybrid specific antibody coated on the paramagnetic SERADYN beads.

5 [00145] Following incubation, the paramagnetic beads are separated from the liquid phase/supernatant by exposure to a magnetic field. The supernatant waste is removed by decanting and 35 µl of detection reagent 1 (secondary antibody conjugated enzyme comprising a monoclonal anti-RNA-DNA hybrid antibody conjugated to alkaline phosphatase) is added and incubated at 45°C for 30 minutes. The secondary antibody binds
10 the RNA-DNA hybrid-antibody-conjugated paramagnetic bead complex. Non-bound secondary antibody is washed away using a detergent based wash buffer (40 mM Tris, pH 8.2, 100 mM NaCl, 0.1% Triton-X 100 and 0.05% sodium azide).

[00146] A substrate (dioxetane-based substrate from ABI, called DCP Star, with Emerald II enhancer) is added to the washed beads and wells that contain high-risk HPV
15 DNA create light that is detectable by a luminometer and measured in RLU (relative light units). An assay positive standard containing 1pg/ml of HPV DNA is used to establish the positive cutoff. All sample RLU values are divided by the RLU value for the positive standard creating a RLU/CO (RLU to cutoff value). Results are reported in RLU/CO and anything greater than or equal to 1.0 is considered positive.

20

Example 2: Stability Testing

[00147] Following initial testing, samples were stored at room temperature and 33°C to observe the stability of the samples. Testing was conducted as far as 21 days post collection. FIGS. 3 and 4 demonstrate that the RLU/CO value for each sample does not change with
25 time up to 21 days. A 2x2 analysis comparing baseline results to the results after 21 days of storage and scatter plot analysis demonstrated the linearity of the RLU/CO values with time. Based on these data, it is possible to conclude that samples collected and stored at either room temperature or 33°C for as long as 21 days provide comparable RLU/CO values as tested at baseline. Using linear mixed model comparison of RLU/CO values against the
30 temperature of storage the P values are 0.8803 for room temperature and 0.9517 for samples stored at 33°C indicating that values are equal.

Example 3: Limit of Detection Studies

[00148] The limit of detection (LOD) of HPV 16 was measured by serial dilution of HPV-16 plasmid target in the collection medium. The test results demonstrate a S/N ≥ 2.0 for a 0.2 pg/ml HPV 16 plasmid which is equivalent to 1000 copies of HPV 16 DNA. See FIG.

5 5. In addition, three-week stability studies were performed on clinical specimens at elevated temperatures to mimic the conditions in an area subject to relatively elevated temperatures, such as Rwanda. All biologically labile kit components (RNA probe, capture antibody, detection antibody-enzyme conjugate, and substrate) were stabilized as part of the production process and monitored for stability over 18 months at 37°C.

10

Example 4: SUREPATH Pellet Conversion and Recovery of Nucleic Acids

In this example, the typical workflow for SUREPATH pellet conversion and nucleic acid recovery is described. Workflow for SUREPATH media includes initial collection of the primary sample, which can be cytobrush employed for collection of cervical epithelium

15 15. cells at a transition zone or sample collection point.. An average of 2×10^8 cervical cells are collected per brush, which can be preserved in 10 mL of SUREPATH media in a collection vial. The vial can be sealed and cells incubated in the fixative media at room temperature or 4°C until further processing is performed. The cell suspension can then be subjected to an automated density gradient purification scheme, and the resulting cellular pellet, with an

20 20. average total cell number of 1.6×10^8 cells, can be suspended in a final volume of 1 mL water. This water pellet can be referred to as a "soft pellet" or "undiluted soft pellet".

200 μ L of the soft pellet can be used in an automated slide preparation protocol for cytology, leaving on average 1.3×10^8 total cells in an 800 μ L volume. After removal of the 200 aliquot for slide preparation, 1-2 mL of fresh SUREPATH media can be added to the

25 25. remaining 800 μ L soft pellet to stabilize and preserve the soft pellet in case the slide must be remade. In most cases, this remaining 2-3 mL sample is destroyed after cytology results are reported.

The detergent-based medium described herein can be added to the remaining 800 μ L soft pellet. Any of the detergent-based collection medium described herein can be added to

30 30. the remaining 800 μ L soft pellet. In an aspect, the media may contain 1.0% NP-40, 0.25% sodium deoxycholate, 50 mM Tris-HCl, 25 mM EDTA, 150 mM NaCl and 0.05% sodium azide. After the addition of the detergent-based collection media, the soft pellet sample can

be analyzed in conjunction with any of the methods or assays described herein.

5 **[00148a]** Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

10 **[00148b]** The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

The claims defining the invention are as follows:

1. A method for determining the presence of a target nucleic acid molecule in a sample, comprising:
 - a) suspending a sample in a collection medium comprising an anionic detergent and a non-ionic detergent;
 - b) denaturing a target nucleic acid molecule;
 - c) contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize or bind, thereby forming a double-stranded nucleic acid hybrid; and
 - d) capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded nucleic acid hybrid.
2. The method of claim 1, comprising:
 - a) suspending the sample in a collection medium comprising an anionic detergent and a non-ionic detergent;
 - b) denaturing the target nucleic acid molecule;
 - c) contacting one or more polynucleotide probes with the target nucleic acid molecule under conditions that allow the probes and the target nucleic acid molecule to hybridize, thereby forming a double-stranded nucleic acid hybrid;
 - d) capturing the double-stranded nucleic acid hybrid on a solid support coated with a first antibody specific for the double-stranded nucleic acid hybrid, thereby forming a double-stranded nucleic acid hybrid/solid support complex;
 - e) separating the double-stranded nucleic acid hybrid/solid support complex from unbound nucleic acid;
 - f) conjugating the complex with a second antibody that is specific for either the double-stranded nucleic acid hybrid or specific for the first antibody to form a double-stranded nucleic acid hybrid/solid support antibody complex; wherein the second antibody is labeled with a detectable marker;
 - g) washing the double-stranded nucleic acid hybrid/solid support antibody complex with a wash buffer comprising a detergent; and
 - h) detecting the label on the second antibody wherein the detecting indicates the presence of the target nucleic acid molecule.

3. The method of claim 1 or 2, wherein the solid support comprises magnetic beads.
4. The method of claim 3 wherein the magnetic beads comprise a modified paramagnetic bead that is coated or has attached thereto the first antibody immunospecific for double-stranded nucleic acid hybrid, and wherein a magnetic field is used to separate the double-stranded nucleic acid hybrid-magnetic bead-antibody complex from non-bound nucleic acid.
5. The method of any one of claims 1 to 4, wherein in the collection medium the anionic detergent is sodium deoxycholate and the non-ionic detergent is NP-40.
6. A composition comprising:
 - (a) a biological sample suspended in a collection medium, wherein said collection medium comprises an anionic detergent and a non-ionic detergent;
 - (b) a denaturation reagent;
 - (c) at least one polynucleotide probe capable of binding to a target nucleic acid molecule;
 - (d) a support coated with a first antibody; and
 - (e) a second antibody labeled with a detectable marker.
7. The composition of claim 6, wherein said collection medium comprises NP-40, sodium deoxycholate, and EDTA.
8. The composition of claim 6 or 7, wherein said collection medium comprises about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, and about 10 mM to about 50 mM EDTA.
9. The composition of any one of claims 6 to 8, wherein said at least one polynucleotide probe is selected from the group consisting of probes for HPV high risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82.
10. A composition comprising:

(a) a biological sample suspended in a collection medium comprising about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, and about 10 mM to about 50 mM EDTA, and

(b) a polynucleotide probe; and

(c) a support coated with a first antibody.

11. The composition of claim 10, further comprising

(d) a second antibody, wherein said second antibody is labeled with a detectable marker.

12. The composition of claim 10 or 11, wherein said collection medium further comprises about 0.01% to about 0.10% sodium azide.

13. The composition of any one of claims 6 to 12, wherein said biological sample is a cervical cell sample.

14. The composition of any one of claims 6 to 13, wherein said biological sample is stable when stored in said collection medium for at least 21 days at 33°C.

15. The composition of any one of claims 6 to 14, wherein the support coated with the first antibody comprises magnetic beads.

16. A kit for the detection of a target nucleic acid molecule in a sample, comprising:

a) a collection medium comprising an anionic detergent and a non-ionic detergent;

b) a denaturation reagent;

c) a support coated with a first anti-hybrid antibody;

d) a detection reagent comprising a second anti-poly hybrid antibody, wherein the second antibody is detectably labeled;

e) a detergent-based wash buffer; and

f) a second detection reagent comprising a substrate for the label on the second antibody.

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17. The kit of claim 16, wherein said collection medium has one of the following characteristics:

- (a) it comprises NP-40, Deoxycholate, and EDTA;
- (b) it comprises NP-40, Deoxycholate, and EDTA and further comprises sodium azide;
- (c) said detergent-based wash buffer comprises about 0.5% to about 2.0% NP-40, about 0.10% to about 0.40% sodium deoxycholate, about 25 mM to about 75 mM Tris-HCl, about 10 mM to about 50 mM EDTA, about 50 mM to about 200 mM NaCl, and about 0.01% to about 0.10% sodium azide;
- (d) said detergent-based wash buffer comprises about 40 mM Tris pH 8.2, about 100 mM NaCl, about 0.1% to about 0.5% Triton x-100, and about 0.05% sodium azide;
- (e) it comprises a diluent comprising BES, polyacrylic acid, NaOH and sodium azide;
- (f) the denaturation reagent is 1.75 NaOH.

18. The kit of claim 16 or 17, further comprising at least one polynucleotide probe capable of binding to human papillomavirus (HPV) and genetic variants thereof.

19. The kit of claim 18, wherein said at least one polynucleotide probe is selected from the group consisting of probes for HPV high risk types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82.

Figure 1

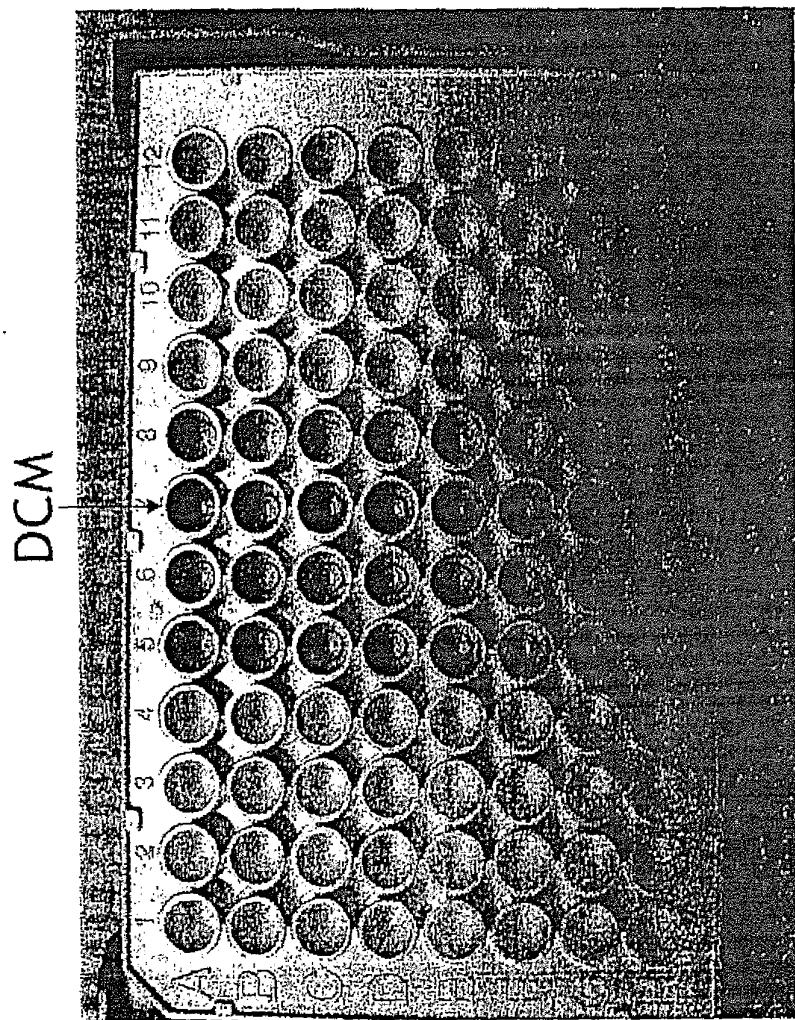


Figure 2

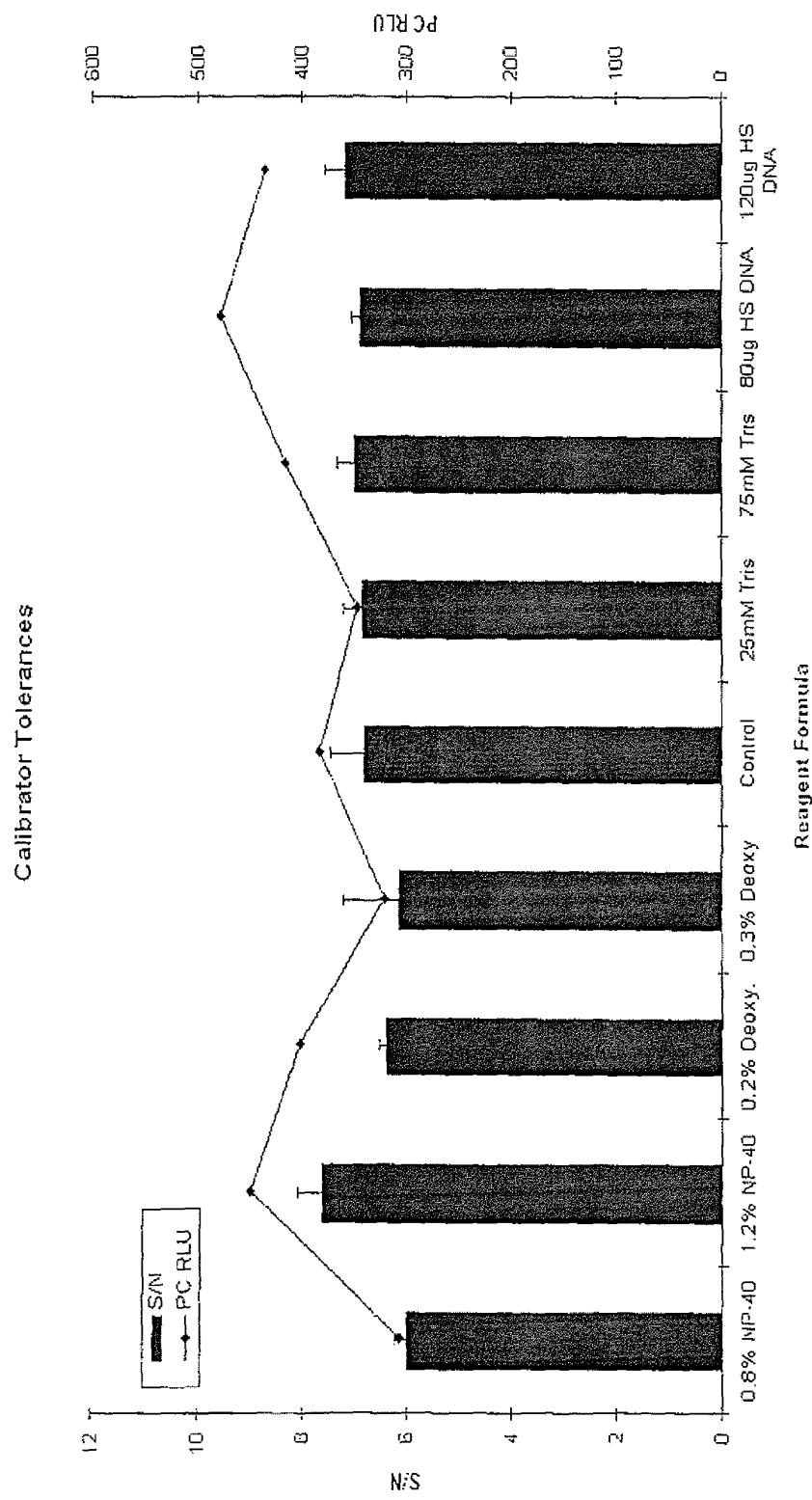


Figure 3

Clinical Specimen Stability at Room Temperature

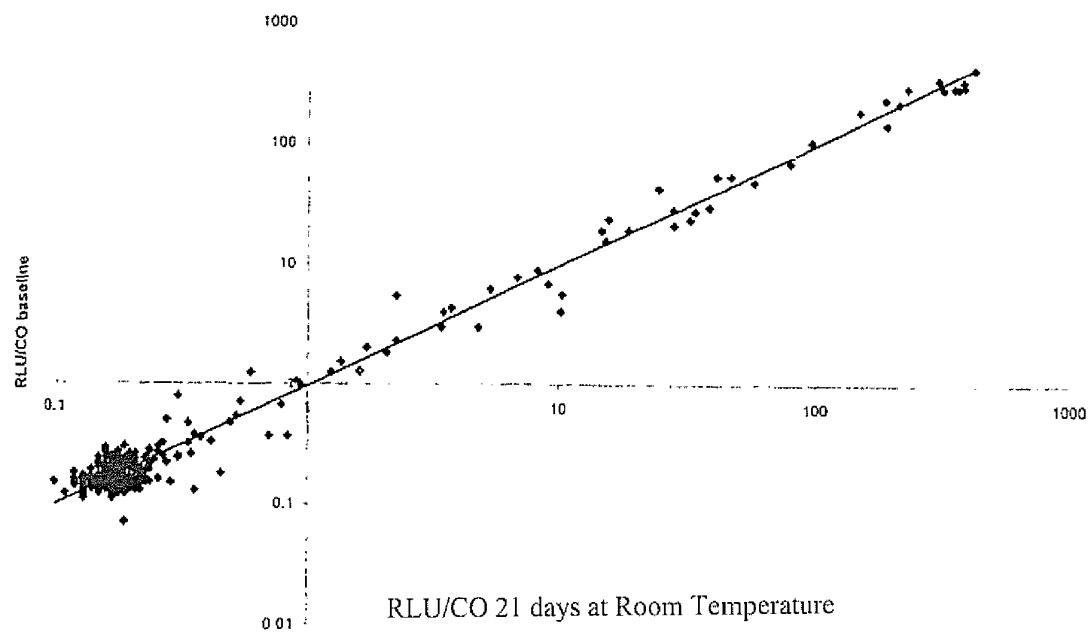
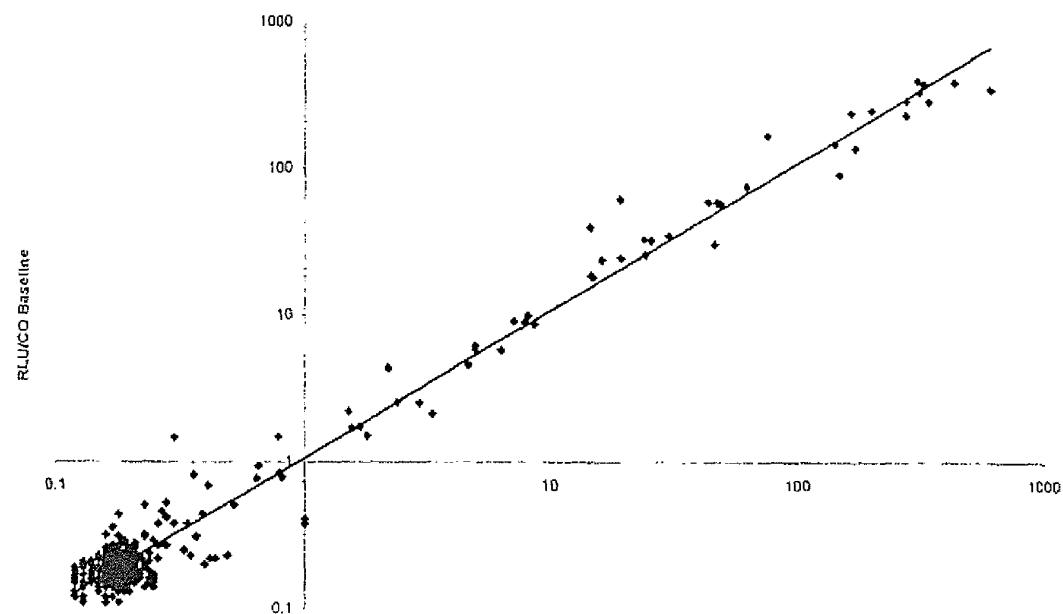


Figure 4

Clinical Specimen Stability at 33 degrees Celcius



RLU/CO 21 days at 33 degrees Celsius

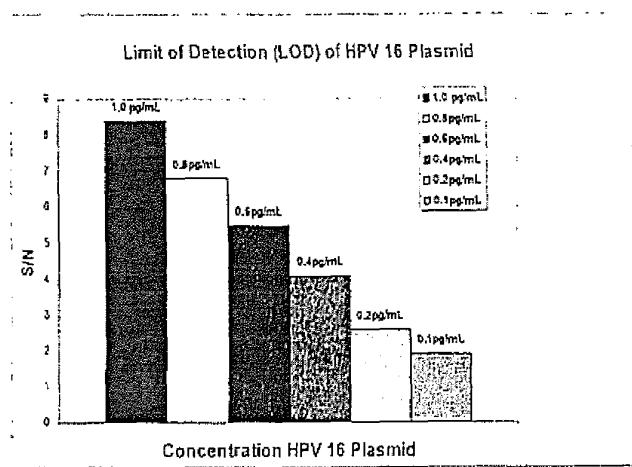
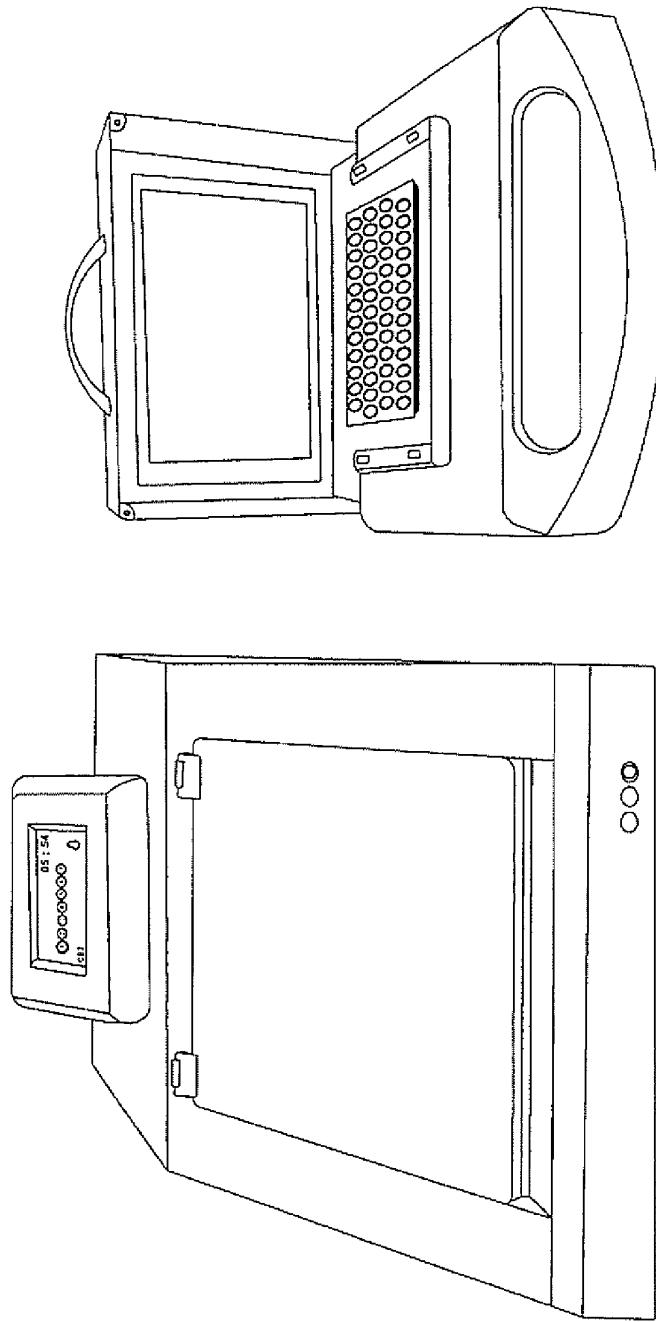


Figure 5: Test results demonstrate a S/N> 2.0 for a 0.2 pg/ml HPV 16 plasmid which is equivalent to 1000 copies of HPV 16 DNA

Figure 6



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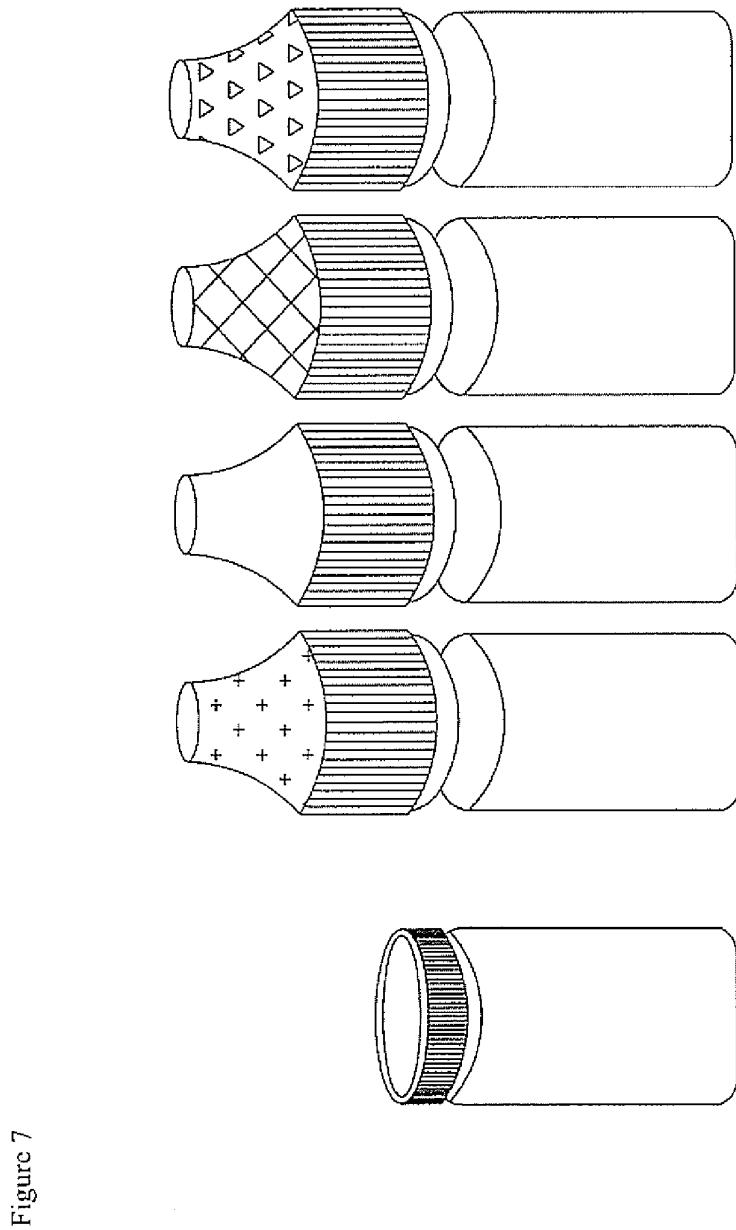
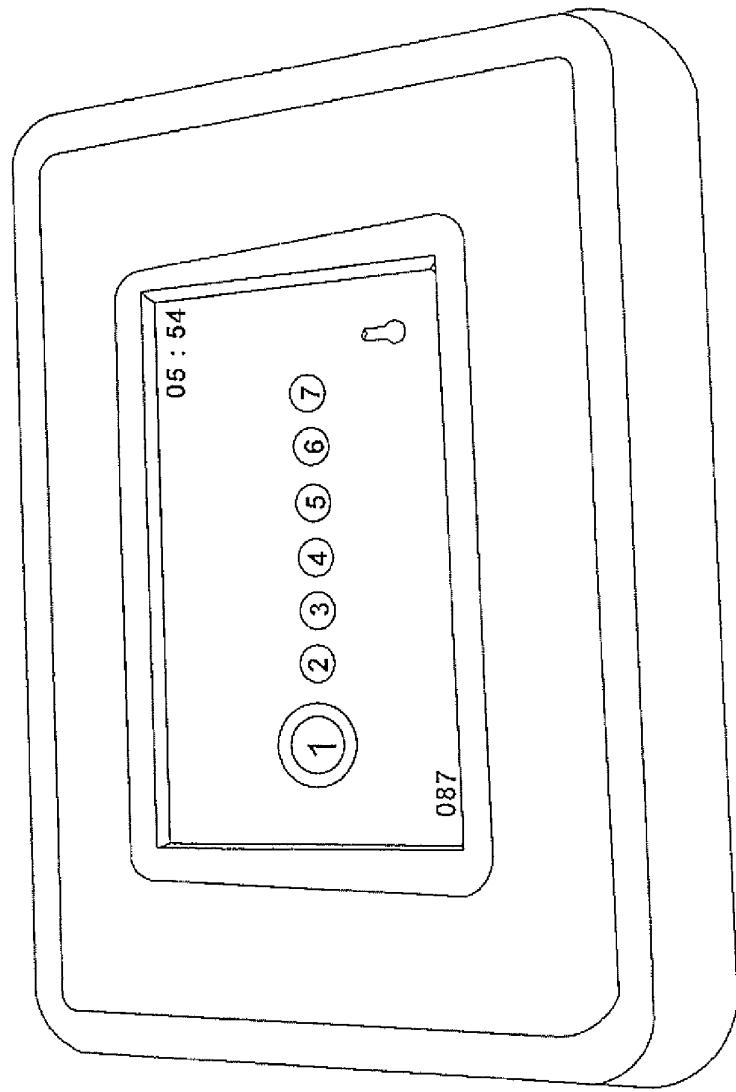


Figure 8



11 Day Stability and Reproducibility of Converted Soft Pellets
Stored At Room Temperature

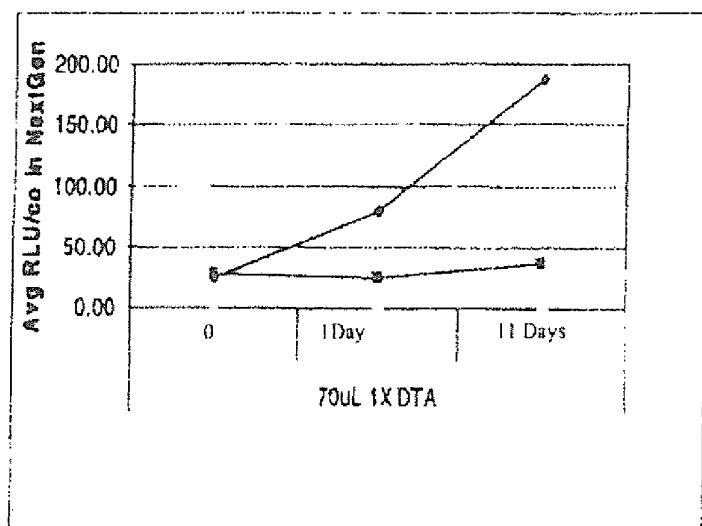


Figure 9