

any analysis designed to determine the amounts or proportions of the target or signal. A detection is “qualitative” when it refers, relates to, or involves identification of a quality or kind of the target or signal in terms of relative abundance to another target or signal, which is not quantified.

The term “target” as used herein indicates an analyte of interest. The term “analyte” refers to a substance, compound or component whose presence or absence in a sample has to be detected. Analytes include but are not limited to biomolecules and in particular biomarkers. The term “biomolecule” as used herein indicates a substance compound or component associated to a biological environment including but not limited to sugars, amino acids, peptides proteins, nucleic acids, oligonucleotides, polynucleotides, polypeptides, organic molecules, haptens, epitopes, biological cells, parts of biological cells, vitamins, hormones and the like. The term “biomarker” indicates a biomolecule that is associated with a specific state of a biological environment including but not limited to a phase of cellular cycle, health and disease state. The presence, absence, reduction, upregulation of the biomarker is associated with and is indicative of a particular state. The term “biological environment” refers to any biological setting, including, for example, ecosystems, orders, families, genera, species, subspecies, organisms, tissues, cells, viruses, organelles, cellular substructures, prions, and samples of biological origin. Exemplary targets comprise molecular targets such as small molecules, proteins, nucleic acids, and also cells, tissues and organisms.

The term “nucleic acid” as used herein indicates a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, that comprise purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. Nucleic acids of the embodiments of the current disclosure include Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or DNA copies of RNA (complementary DNA or cDNA), which may be isolated from natural sources, recombinantly produced, or artificially synthesized. The nucleic acids may exist as single-stranded or double-stranded and any chemical modifications thereof, provided only that the modification does not interfere with amplification of selected nucleic acids. For example, the backbone of the nucleic acid can comprise sugars and phosphate groups or modified or substituted sugar or phosphate groups, and a nucleic acid may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs.

In several embodiments, the methods and apparatus herein described allow isothermal amplification of a target nucleic acid in a sample. For example, in some embodiments, the isothermal amplification of a target nucleic acid is performed by the Loop-mediated Isothermal Amplification (LAMP). In some embodiments, the isothermal amplification of a target nucleic acid is performed by Helicase-Dependent Isothermal Amplification (HDA). In other embodiments, the isothermal amplification of a target nucleic acid is performed by Recombinase Polymerase Amplification (RPA).

The term “isothermal amplification” as used herein indicates a method of DNA amplification using polymerase chain reaction that uses a single temperature incubation thereby obviating the need for a thermal cycler. By combining with a reverse transcription step, these amplification methods can also be used to isothermally amplify RNA.

The term “Loop-mediated Isothermal Amplification (LAMP)” as used herein indicates a isothermal nucleic acid amplification method. In LAMP, the target sequence is amplified at a constant temperature of 65° C. using either two or three sets of primers and a polymerase with high strand dis-

placement activity. More detailed information regarding LAMP can be found in Notomi T. et al., 2000, *Nucleic Acid Research*, Vol. 28, e63, herein incorporated by reference in its entirety.

The term “Helicase-Dependent Isothermal Amplification (HDA)” as used herein indicates another method of isothermal nucleic acid amplification wherein the use of a DNA helicase and single stranded DNA-binding proteins eliminates the need for a thermal cycler. In HDA, strands of double stranded DNA are first separated by a DNA helicase and coated by single stranded DNA-binding proteins. Two newly synthesized DNA products are then used as substrates by the DNA helicase, entering the next round of the reaction. Thus, a simultaneous chain reaction develops, resulting in exponential amplification of the selected target sequence. More detailed information regarding HDA can be found in Vincent et al., 2004, *EMBO reports*, Vol. 5, pp. 795-800, herein incorporated by reference in its entirety.

The term “Recombinase Polymerase Amplification (RPA)” as used herein indicates another isothermal nucleic acid amplification method wherein the use of a primer-recombinase complex and single-stranded DNA binding proteins eliminates the need for a thermal cycler. In RPA, the primer-recombinase complex attaches to the DNA template to be amplified and initiates the amplification process. Single-stranded DNA binding proteins attach to and stabilize the displaced strands of the template DNA during primer elongation by the polymerase. Two newly synthesized DNA products are then used as substrates of the primer-recombinase complex, entering the next round of the reaction. Thus, a simultaneous chain reaction develops, resulting in exponential amplification of the selected target sequence. More detailed information regarding RPA can be found in Hoff, 2006, *PLoS Biology*, Vol. 2, e222 herein incorporated by reference in its entirety.

FIG. 3A shows a schematic rendering of a disposable device according to several embodiments of a first structure. The device comprises a containment vessel (301), such as a sealable polypropylene tube, a sample collection element (302), such as a hollow polyester stick, comprising a lumen (310) and a swab tip (303), a nucleic acid binding element (304), such as a 4 mm disc of cellulose FTA card, positioned within the containment vessel, and a plurality of reagents (330) comprising reagents suitable for sample preparation such as nucleic acid purification and reagents suitable for nucleic acid amplification and detection. The sample collection element is configured for removable coupling to the containment vessel.

FIG. 3B shows a schematic rendering of an electronic heater (306). The heater comprises a control unit (320) and a heating unit (350) comprising a plurality of heating blocks (340) configured to house the containment vessel (301) as in FIG. 3A, and heat the nucleic acid binding element (304).

FIG. 5A shows a schematic rendering of a disposable device according to several embodiments of a second structure. The device comprises a containment vessel (501), such as a sealable polypropylene tube (501), a sample collection element (502) such as a hollow polyester stick, comprising a lumen (510) and a swab tip (503), a nucleic acid binding element (504) positioned within the containment vessel, a waste collection unit (507) coupled to the sample collection element, a plurality of reagent cartridges (515) coupled to the sample collection element and enveloping a plurality of reagents (530). The sample collection element is configured for removable coupling to the containment vessel. The plurality of reagent cartridges each comprises a capsule (516) and a plug (517). The plurality of reagents comprise reagents