

person. For example, the heating can be performed by heating via an oxidative chemical reaction or a catalytic chemical reaction.

In some embodiments, detecting amplification of the target nucleic acid comprises detecting a color shift in a colorimetric dye or detecting fluorescence of a fluorescent dye. The dyes can be introduced concurrently with the nucleic acid amplification reagents or separately into fluid communication with the nucleic acid amplification reagents after the amplification, for example, from one of the reagent cartridges (see Example 3, FIG. 10). The colorimetric dye and the fluorescent dye can be the ones identifiable by a skilled person, including but not limited to hydroxynaphthol blue (HNB) and picogreen (see Example 2).

In other embodiments, detecting amplification of the target nucleic acid comprises detecting through a chromatography lateral flow dipstick (LFD).

In some embodiments, the method further comprises disposing the sample, the containment vessel, the sample collection element, nucleic acid binding element, the reagents and/or the heater after a single use.

According to another aspect of the current disclosure, a method for diagnosing a condition in an individual is described. The condition is associated to presence of a target nucleic acid in the individual, which is produced by certain pathogens. In some embodiments, the method comprises sealing a sample from the individual within a containment vessel, transferring the sample to a nucleic acid binding element position in the containment vessel, purifying nucleic acid from the sample in the containment vessel, amplifying the target nucleic acid in the containment vessel, detecting amplification of the target nucleic acid in the containment vessel, and diagnosing the condition. FIG. 4B shows a procedural flow chart illustrating the steps 4B.1-4B.5 according to these embodiments.

In some embodiments, the pathogen can be, for example, viruses, bacteria, fungi, and combinations thereof.

In some embodiments, the condition is an infectious disease. In particular, in some embodiments, the infectious condition can be, for example, foot and mouth disease, flu, swine flu, avian flu, MRSA, anthrax and combinations thereof.

Further details concerning the methods and apparatus can be identifiable by the person skilled in the art upon reading the present disclosure.

### EXAMPLES

The methods and apparatus for detecting a target nucleic acid in a sample are further illustrated in the following examples, which are provided by way of illustration and are not intended to be limiting.

A person skilled in the art will appreciate the applicability and the necessary modifications to adapt the features described in detail in the present section, to additional methods, apparatus, type of sample and pathogens according to embodiments of the present disclosure.

#### Example 1

##### Methods and Materials

The following materials and methods were used in performing the experiments illustrated in the examples herein described.

Primers, recombinant template and virus. MRSA primers were designed using LAVA-LAMP software and were purchased from Biosearch Technologies. MRSA primers are part

of a publication in progress (Tones et al. BMC Bioinform submitted for publication). *S. aureus* strain FPR3757/USA300 genomic DNA (BAA-1556D-5, American Type Culture Collection Manassas, Va.), used as positive MRSA template, was received as lyophilized powder and dissolved in 1×TE buffer (10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA, pH 8.0, sterile solution, (#T0226, Teknova, Inc., Hollister, Calif.) to an initial concentration of approximately 100 ng/μL. The exact DNA concentration was measured by the PicoGreen assay (P11496, Invitrogen Corp., Carlsbad, Calif.) on a Qubit fluorometer (Invitrogen Corp., Carlsbad, Calif.), and 1 ng/μL stock solutions were prepared and stored at -20° C. Our lowest level of detection, 0.05 pg, represents 17 copies of DNA.

The primers for pan-serotypic detection of FMDV and the performance of this assay (analytical sensitivity) have been previously published in Dukes et al., Arch. Virol., 2006, vol. 151, pp. 1093-1106, herein incorporated by reference in its entirety. Recombinant template was used for initial FMDV experiments. In order to produce recombinant template, a synthetic double-stranded DNA target sequence representing an ~200 nt portion of the 3D RNA polymerase gene was prepared by Genscript Corporation and inserted into the pUC57 and pGS-21a vectors. Approximately 4 μg of each were prepared. The pGS-21a vector allows for the preparation of single-stranded RNA template using T7 RNA polymerase. 100 μA recombinant RNA template was applied to prototype swabs at a 10<sup>-4</sup> dilution in DEPC water (Ambion).

For live virus testing, an epithelial homogenate (10% suspension in PBS) was prepared under BSL3 (UK SAPO4 containment—Specified Animal Pathogens Order) conditions from tongue tissue collected from cattle experimentally infected with O1-Manisa strain FMDV. The performance of the LAMP assay system was evaluated using prototype swabs that were dipped in 100 μl of the suspension at 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-5</sup> dilutions. Presence of FMDV in this material was verified by automated real-time RT-PCR targeting the 3-D region of the FMDV genome (Reid et al., J. Vet. Diagn. Invest., 2009, vol. 21, pp. 321-330).

LAMP and RT-LAMP. LAMP/RT-LAMP is a robust, isothermal nucleic acid amplification method. Master mix and enzymes were added to prototype tubes via the and heated to 63° C. for between 45 min and 1 h. Reactions included primer solutions prepared by combining 40 μL each of 100 μM FIP and 100 μM BIP, 5 μL each of 100 μM F3 and 100 μM B3, and 20 μL each of 100 μM LF and 100 μMLB, and 370 μl of TE buffer, resulting in 500 μL of combined primer solution. reaction volumes of 100 μL for MRSA assays comprised 25 μL of combined primer solution plus 70-μL base mix [1.4 mM each dNTPs (Roche Diagnostics, Basel, Switzerland), 0.8 M betaine (Sigma, St. Louis, Mo.), 4.1 mM MgSO<sub>4</sub> (New England Biolabs, Ipswich, Mass.), 1× Thermopol buffer (New England Biolabs), and 100 μM hydroxynaphthol blue (HNB) (Dojindo Laboratories) in DEPC water (Ambion)] and 5 μL BST polymerase (New England Biolabs).

FMDV Master Mix was slightly modified and included 4 μL BST polymerase, as well as 0.3 μL Thermoscript RT (Invitrogen, Carlsbad, Calif.) per 100-μL reaction volume.

HNB indicates target amplification to the unaided eye via a color shift that stems from changes in the concentration of Mg<sup>2+</sup> in solution: free Mg<sup>2+</sup> in the reaction solution binds to pyrophosphate that is generated as deoxynucleotide triphosphates are added to growing amplification product, forming magnesium pyrophosphate (Goto et al., Biotechniques, 2009, vol. 46, pp. 167-172). This is in contrast to fluorescent dyes,