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Canadian Intellectual Property Office

CA 3078004 A1 2021/10/23

(21) 3 078 004

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(22) Date de dépôt/Filing Date: 2020/04/23

(41) Mise à la disp. pub./Open to Public Insp.: 2021/10/23

(51) Cl.Int./Int.Cl. *C12Q 1/70* (2006.01), *C12Q 1/68* (2018.01), *C12Q 1/6806* (2018.01), *C12Q 1/6844* (2018.01), *C12M 1/34* (2006.01)

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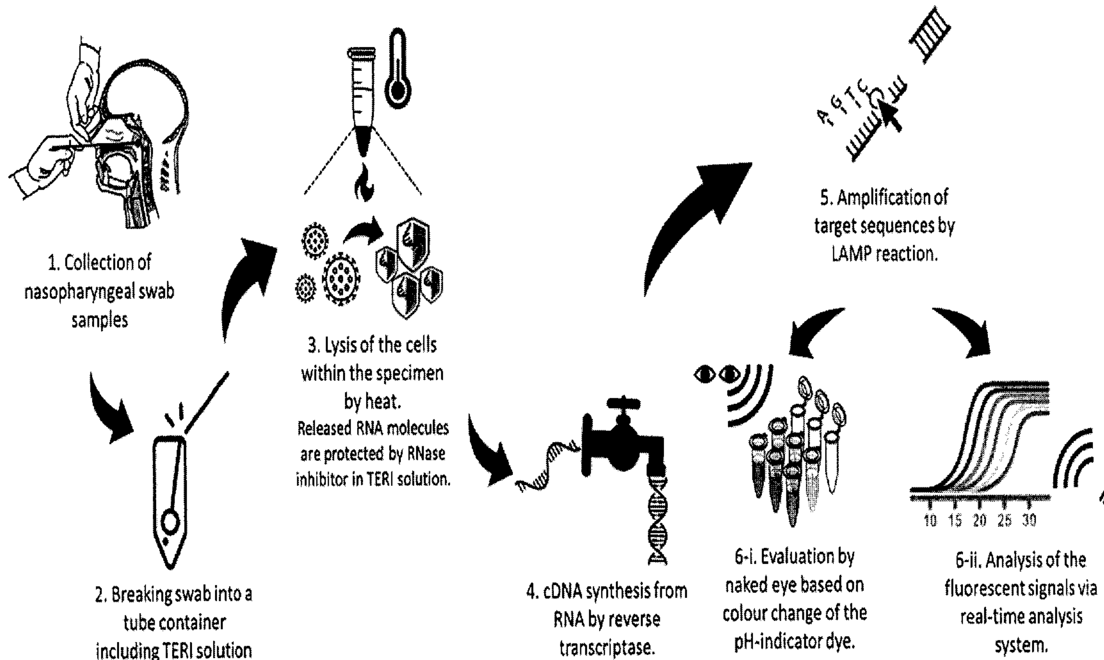
(54) Titre : PIPELINE DE DETECTION DE LA COVID-19
(54) Title: COVID-19 DETECTION PIPELINE



(57) Abrégé/Abstract:
Current laboratory procedures for collection of specimens from upper respiratory systems involve RNA isolation from the specimen, cDNA synthesis via reverse transcription, and a LAMP reaction. The present invention performs alternative ways of LAMP reactions in which every single key component in the traditional system was reorganized to achieve operational LAMP protocols. The present invention, skips the isolation step which decreases the overall cost and the test time by more than 20 minutes. This method can also be applicable for analysis of other viruses.

ABSTRACT

Current laboratory procedures for collection of specimens from upper respiratory systems involve RNA isolation from the specimen, cDNA synthesis via reverse transcription, and a

5 LAMP reaction. The present invention performs alternative ways of LAMP reactions in which every single key component in the traditional system was reorganized to achieve operational LAMP protocols. The present invention, skips the isolation step which decreases the overall cost and the test time by more than 20 minutes. This method can also be applicable for analysis of other viruses.

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COVID-19 DETECTION PIPELINE

TECHNICAL FIELD

There is provided a novel pipeline for detection of SARS-CoV-2 virus from clinical
5 specimens.

BACKGROUND

Currently, two detection methods are widely used for pathogen screening and disease
10 diagnosis, namely serological and molecular detection systems. SARS-CoV2 viral infections have lesser viral loads especially in the early stages of the disease which is very difficult to detect with conventional detection methods. There is an imminent need to develop highly sensitive, accurate, fast, and low-cost detection systems. The lack of sensitivity of the current gold standards (RtqPCR) in SARS-CoV2 infections requires special
15 attention to reduce false negative rates in overall viral monitoring.

Current solutions use the LAMP based detection system for COVID-19 screening. LAMP can detect few-copies (5·2 log10 copies per mL) which is very difficult to detect with RtqPCR based methods. There are other RT-LAMP based detection kits in the market;
20 however none of them provide a highly sensitive, accurate, fast, and low-cost detection system.

SUMMARY

25 Centers for Disease Control and Prevention (CDC) suggests collection of the specimens from upper respiratory system, ideally from nasopharynx using swab (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html). Current laboratory procedures involve RNA isolation from the specimen, cDNA synthesis via reverse transcription, and a LAMP reaction. Due to COVID-19 demand the inventors
30 were unable to obtain the required materials for Viral detection. This inadequacy in supplies led to the present invention performing alternative ways of LAMP reactions in which every

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single key component in the traditional system was reorganized to achieve operational LAMP protocols. The present invention, skips the isolation step which decreases the overall cost and the test time by more than 20 minutes. This method can also be applicable for analysis of other viruses.

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The present system is a LAMP-based detection kit and has been proven to be highly sensitive making it the right detection method for high-throughput population screening efforts. The present invention is in the field of molecular detection systems, where the proposed process has a better resolution in screening viral loads of patients. The present invention provides a

10 LAMP kit production process that is optimized at every stage of the process in order to become a competent COVID-19 diagnosis system.

The present system optimizes the procedure for enzymes that are not developed for LAMP reactions and creates a highly sensitive, fast, accurate and low-cost SARS-COV2 detection

15 kit. During the optimization, the system uses

*an AI based LAMP primer design algorithm

* a protocol to remove RNA-isolation step

* a milder reaction buffer composition that does not inhibit reverse transcriptase enzyme

20 * volume adjustment for providing adequate template for polymerization

* a customized highly sensitive real time monitoring system

* dynamic AI based algorithm that learns from tested samples the range of the signals coming from negative and positive samples to improve the accuracy detection system

25 In one aspect of the present invention there is provided a highly sensitive protocol that responds to a few copies of the viral genome. This femtomolar sensitivity requires careful sample treatment by the end-user. Although not necessary, it is recommended to carry out the protocol in BSL2 laboratories.

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The protocol gives reproducible results whenever proper sampling carried out. Although not necessary, it is recommended that both OP and NS sampling are performed for the same patient to increase the amount of viral RNA in the sampling tube.

5 In another aspect of the present invention, the protocol works best between a temperature of 60 °C to 72 °C with proper machinery that can keep the reaction temperature constant. It is preferred for the reaction temperature to not fluctuate over +-0.1 °C as the continuous polymerization should be assured on the template.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

The invention will be further understood from the following description with reference to the attached drawings.

Figure 1 illustrates a sample process for detecting the virus.

15

DETAILED DESCRIPTION

A preferred embodiment of the present invention will be set forth in detail with reference to the drawings, in which like reference numerals refer to like elements or method steps throughout.

20

Current laboratory procedures for a pipeline involve RNA isolation from the specimen, cDNA synthesis via reverse transcription, and a LAMP reaction. The present invention skips the isolation step. To skip the RNA isolation stage, there is provided a protocol wherein the specimen is concentrated and hence increases the chance of catching viral particles for 25 molecular testing. To this end, alternative collection protocols have been developed and each has shown high RNA yield efficiency. Some examples follow:

- Decreasing the container and solution volume; using 0.5 or 1.5 mL microcentrifuge tube as container with Tris-EDTA and RNase inhibitor mixture (TERI) in 0.1-0.3 mL volume, and/or
- 30 Decreasing high volume of specimen collected in 1-3 mL of TERI via lyophilisation, and/or

- Passing the specimen in TERI through HPLC quality filter system that sustains viral RNA material between 10kb-40 kb and eliminates other cellular debris.

In one example of the present invention, an AI algorithm can design optimum LAMP primers for evolving pathogens. The RNA isolation step is eliminated by use of a customized swab solution. A milder reaction buffer (pH7.2 -pH8 20mM Tris 2mM EDTA) is used for Reverse Transcriptase function.

In one example aspect of the present invention, there is provided a pipeline for COVID-19 detection, wherein for lysis of the cells and viruses a heat treatment at 90-99 °C for 5-15 minutes is applied. After cooling down to room temperature in 2-5 minutes, the tubes are centrifuged at 10-16,000 xg for 2-4 minutes to precipitate cellular debris. Supernatant is transferred into another sterile DNase/RNase-free microcentrifuge tube without disturbing the pellet. This step discards the possible inhibition of downstream methods resulted from cellular debris. If cDNA synthesis cannot be carried immediately, that supernatant can be stored at -20 °C or -80 °C. Before cDNA synthesis, RNA concentration is determined via Qubit fluorometer. If the concentration is high oligo(dT), random oligonucleotide are used in cDNA synthesis. If the concentration is low and cannot be detected by Qubit fluorometer, target-sequence specific primers are used to enrich targeted sequences. In synthesis, the amount of template is determined as 10% of the total volume of reaction mixture. Additionally, we add an in-house developed stabilizer complex composed of chemicals for enhancing reverse transcriptase's enzyme activity. After completion, synthesized cDNA is added as 10% of LAMP reaction volume. Those primers cause synthesis of cDNA's from all the RNA's within the sample. In this application, the LAMP step is carried on another mixture, and a portion of this synthesized cDNA is added as template. For LAMP assay, two strategies are adopted for detection of the amplification; (i) evaluation of turbidity or change in colour of pH-sensitive dyes by the naked eye or (ii) detection of the signals coming from double-stranded DNA-binding fluorescent dye via a real-time detection device. For the second option, there is a real-time detection system that is compatible with the fluorescent dye's excitation and emission wavelength probabilities.

In a further example embodiment, starting materials include pH7.2 -pH8 20mM Tris 2mM EDTA equivalents, 10mM Tris 2mM EDTA 0.040-0.065% NaCl pH7.2 -pH8 and 10mM Tris 2mM EDTA 5mM Acetic acid pH7.2 -pH8.

5 In a further example embodiment, the process involves the following steps:

1. Inactivation and Lysis of Viral Particles 90-99 °C for 5-15 minutes
2. Sample template volume: 2 to 10 ul of template for 25ul reaction volume
3. Reaction Temperature: 60-72 °C
4. Reaction Volume: 25 to 50ul
5. Optical Density Measurement: 430 to 730 nM measurement

10

After the sample process step 1 there are sufficient amounts of isolated of viral RNA. After the process step 2, the viral genome is converted to cDNA. At process step 3, continuous polymerization of Viral DNA and LAMP reaction occur. At process step 4, there is detection of the color change.

15

The same process can be applied to all of the pathogens from a wide range of industries, such as health, feed, and food industry.

In one aspect of the present invention there is provided higher sensitivity down to 5.6 viral

20 copy per ml and faster detection time 7-40 min. The system is easy to use with no need for a fully equipped laboratory. Custom designed LAMP primers are used and detection system.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples but should be given the broadest interpretation consistent with the description as

25 a whole. For example, the possible sizes are also constructible. Although it appears as a possible disadvantage, the operation cost will be logarithmic due to the required polymerization process.

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What is claimed is:

1. A method for detection of a virus, comprising the steps of:
   collecting a sample from a patient;
5 inserting the sample into a solution;
   heating the solution containing the sample to cause lysis of the cells;
   using reverse transcriptase to perform cDNA synthesis from RNA;
   using LAMP reaction to amplify target sequences; and
   evaluating whether the virus is present.
10
2. The method of claim 1 wherein the step of evaluating includes the step of using pH-indicator dye to determine colour change.
3. The method of claim 1 wherein the step of evaluating includes the step of analyzing a fluorescent signal using a real-time analysis system.

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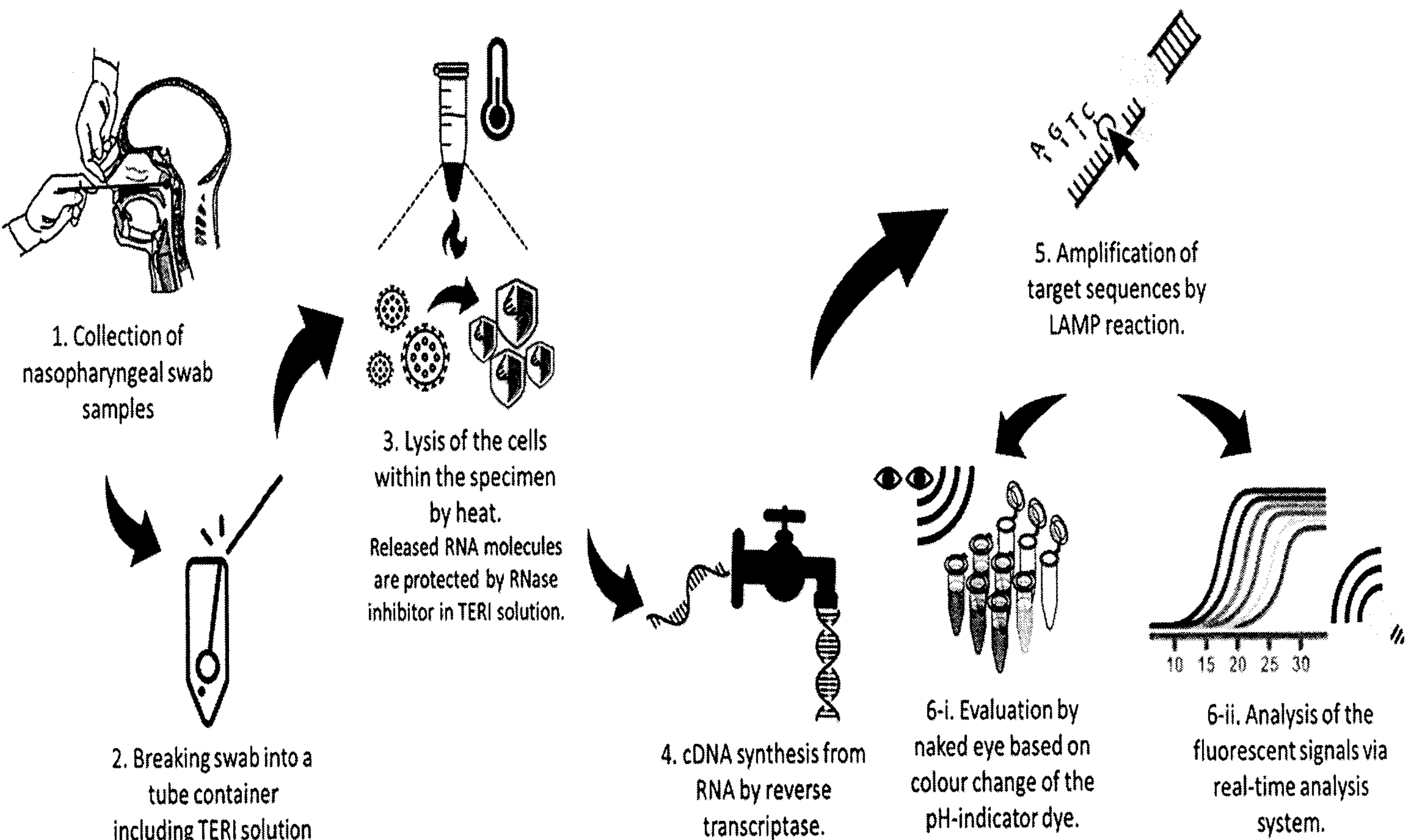
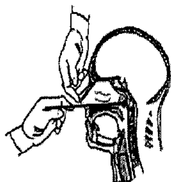
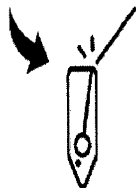


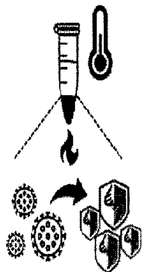
Figure 1



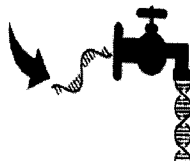
1. Collection of nasopharyngeal swab samples



2. Breaking swab into a tube container including TERI solution

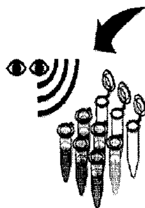


3. Lysis of the cells within the specimen by heat.
Released RNA molecules are protected by RNase inhibitor in TERI solution.

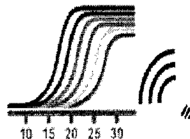


4. cDNA synthesis from RNA by reverse transcriptase.

5. Amplification of target sequences by LAMP reaction.



6-i. Evaluation by naked eye based on colour change of the pH-indicator dye.



6-ii. Analysis of the fluorescent signals via real-time analysis system.