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Conceptual Design of an at-home COVID-19 detection device using a two-stage isothermal amplification (2SIA-method)

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ABSTRACT:
Since the emergence of the Covid-19 pandemic in 2020, there have emerged two main testing methods, PCR test (swab sample) and Antibody (serology test, using blood sample) testing, the former is more specific and sensitive (capable of testing even a single virus in a sample) and the latter is a faster means of testing.

PCR Testing	Antibody Testing
Accurate and sensitive	Results are obtained quick
Could result in false negatives	Highly inaccurate at the moment
Cannot be done at home	

As we can see that both these tests require sophisticated equipment, require medical professionals, laboratories (which are limited in number), and leave a lot of room for improvements in terms of time to obtain results and accuracy of the results.

This project aims to model a device that can provide easy (minimal training required for individuals), accurate, Covid-19 testing which can be done at the comfort of people's homes using a two-stage isothermal amplification (Covid-19 RAMP) and fluorescence detection.

I. INTRODUCTION:

Since the 2nd wave in India, we can see that there has been a severe strain on the medical infrastructure in India, the enormous surge of smails on campus is a testament to the severity of the situation.

During these times anyway to ease the burden on the medical industry will make a positive contribution. This project aims to use a two-stage isothermal amplification method to provide an at-home testing method, similar to the paper-based pregnancy test so that people need not have to go to hospitals for a preliminary test to check if they are Covid positive.

The 2SIA-method has 10 times better sensitivity than the LAMP assay method and the RT-PCR method.

The 2SIA-method has the potential to reduce the possibility of false positives while using minimal equipment and the need for trained professionals.

II. OBJECTIVE:

To design a device that can accurately, and conveniently (minimal technical requirements, minimal and relatively easy-to-use) detect the presence of the Coronavirus (SARS-CoV) using nasopharyngeal swabs which is easy to obtain.

III. MATERIALS AND METHODS:

Working Principle:

A nasopharyngeal swab is used to obtain the sample from the back of someone's nose (since the virus resides at the back of the nasal pathway) and the virus has RNA (Fig 1.) (Which is surrounded by a fatty membrane and spiked protein) as the genetic material, which is converted to cDNA using reverse transcription, then we use primers which will bind on to the virus DNA and starts a chemical reaction which makes copies of the DNA (billions of them) which makes the detection of the virus easy. My device consists of two isothermal amplification processes: Recombinase Polymerase Amplification (RPA) (at 38°C, which occurs in chamber 1, discussed later) and LAMP (at 63°C, which occurs in chamber 2, discussed later). To detect, we use an LCV dye that changes color from colorless in the absence of the cDNA to deep violet color in the presence of abundant dsDNA. This color change is visible to the naked eye and will indicate a positive result if it turns violet.

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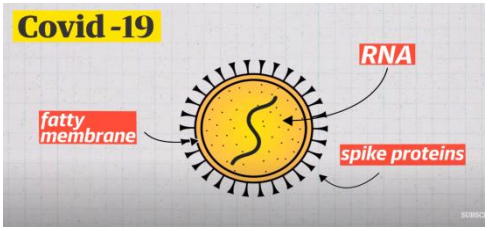

Fig. 1: A schematic representation of the Covid virus

Primers: we will use primerstargeting the open reading frame 1ab (ORF1ab) gene of the COVID-19 RNA because of its high homology among Covid-19 viruses. Apart from the primer, a LAMP reaction mixture is used, the LCV dye is mixed with the LAMP mixture.

The ratio of the Recombinase Polymerase Amplification mix to the LAMP mixture will be 1:9, but to avoid the need for centrifugation the device will contain 5 µL(in Chamber 1, discussed later) and LAMP volume of 70 µL(in Chamber 2, discussed later)

IV. DEVICE DESIGN AND FABRICATION:

Device components

- **Funnel**: This can be detached after loading the sample and closed with a rubber cap. This is to 'upload' the sample
- **Chamber 1**: this chamber will contain the RPA mix pre-loaded, here is where the Recombinase Polymerase Amplification (RPA) occurs at 38°C for 15-20 minutes
- **Removable Separator**: this is a flexible plastic-like material (such as polyethylene terephthalate (PET) or thermoplastic polyurethane (TPU)) which on removal will drop all the contents from chamber 1 to chamber 2, due to gravity.
- **Chamber 2**: this chamber will contain the LAMP mixture and LCV dye preloaded, and the mixture from chamber 1 will come here for the LAMP reaction, which will occur at 63°C for 40 minutes
- **Eye Piece:** This is for the patient to view if the mixture is violet or not in chamber 2, if the mixture is violet it indicates that the covid test is positive.

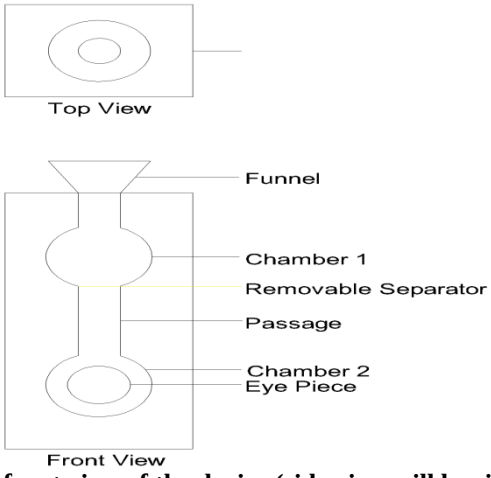

Fig. 2: A top view and front view of the device (side view will be similar to front view)

Device Design:

The device consists of 3 parts i) the silicon substrate, ii) either a polymer (such as PDMS or PMMA) or SiO_2 iii) a plastic covering with an eye-piece

Parts (i) and (ii) form the bulk of the device and it consists of two symmetric halves bound together to form the desired shape. The shape of one-half of the bulk is shown in Fig. 3. The plastic covering can be slid over or placed on top of the bulk.

In the passage between chamber 1 and chamber 2, there is a removable separator that can be removed to allow the contents of chamber 1 to drop down to the contents of chamber 2.

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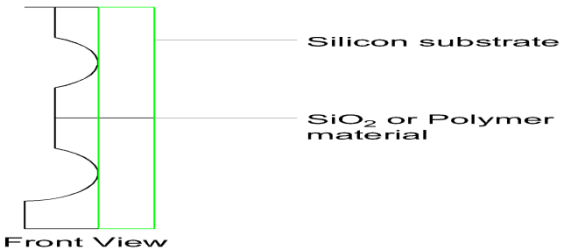


Fig. 3: Front view of one half of the bulk of the device

Device Fabrication:

The device fabrication must be carried out in a cleanroom environment.

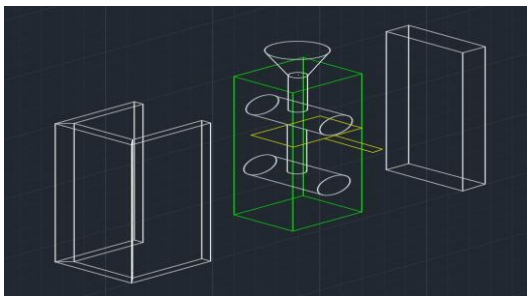
First, the substrate is cleaned by the RCA method to get rid of any contaminants, then heated at 120° for 5 mins.

If a SiO_2 is used, then it is deposited by sputtering or thermal deposition in a glass furnace, then it is cleaned once again either using IPA+DI water or by Piranha cleaning, then it is heated again 120° for 5 mins, then it is coated with HDMS for 5 mins, then positive PR coating is done by spin coating, followed by soft bake (100° for 1 min) to remove water molecules and firm up the PR, then the desired shape is obtained via UV lithography (masking process, since positive PR, the part exposed to the UV light will remove), followed by the development process, then hard bake (120° for 5 mins), then using RIE (reactive ion etching) the SiO_2 is etched to our desired shape, PR is removed using acetone.

If we use a polymer instead, the polymer will be deposited onto the substrate using the spin coating method, then a stamp/mold/master of the desired shape (50-100 bars pressure, 750-1450 psi) is used to thermal press the shape onto the polymer, and oxygen plasma etching can be used to remove any residue.

Two such symmetrical parts are fabricated and attached together to form the bulk of the device.

Fig. 4: A 3D model of the device (from left to right: the plastic slid-on cover, the bulk, silicon substrate)



Working:

Sample Collection

The device works on minimal sample processing, the patient collects a nasal sample with a swab, and elutes the swab in water, and heats the water above 65°C, this is enough to lyse the virus and suppress the inhibitors.

How-to-use

After collecting the sample, it is then poured into the device using the detachable funnel provided, the sample will reach chamber 1 which contains a pre-loaded RPA mixture, this is incubated at 38°C for 15-20 minutes, then the removable separator should be removed to allow the contents from chamber 1 to fall into chamber 2 (which contains the LAMP mix as well the LCV dye), the user must then shake the device to ensure thorough mixing of the contents, this should then be incubated at 63°C for 40 minutes. The incubation can be carried out with simple instrumentation (such as thermal water baths, or ovens that are readily available in the market) as no thermal cycling is needed.

Due to the presence of the LCV dye (changes color from colorless in the absence of cDNA to deep violet color in the presence of abundant cDNA), the

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color change is visible to the naked eye and does not require any instrumentation.
If the color is violet then the test is positive for the Covid virus.
All the reactants in-built in the device can be stored in dry form, ensuring easy and long-term storage.

V. EXPECTED RESULTS:

In terms of the testing, if the sample contains the covid virus, the color when viewed through the eye-piece at the end of the two stages will be violet. (as shown in Fig. 5)

The 2SIA method provides 100 times more sensitivity compared to the LAMP assay method when minimally prepared samples are used (as used by this device).

Fig. 5: Shows the color visible through the eye-piece when the test is negative and positive respectively

VI. CONCLUSION:

During these times of high stress on the hospitals and medical professionals, the 2SIA device provides an at-home testing method with high sensitivity, fast results, minimal training, minimal equipment requirements.
Although it requires minimal sample testing and is simpler than existing methods, it can perform better than them, This added sensitivity is important since it has been reported that a significant number of COVID-19 patients test negative with the COVID-19 PCR test.

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[6]. Apart from these websites like Google, YouTube, and other websites were used to better understand the topics involved in this project.