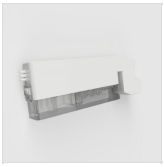

Sep 08, 2020

VPH_AUTOGENE-COVID_Diagnosis_Protocol_XPRIZE

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XPRIZE Rapid Covid Testing | Valetude Primus Healthcare, New Delhi

Shruti Ahuja
Valetude Primus Healthcare

ABSTRACT

Valetude Primus Healthcare (VPH) is a healthcare spinoff from IIT Delhi, India. VPH has developed an automated portable device, called AutoGene-COVID, to detect SARS COV 2 infection from a patient's nasopharyngeal, oropharyngeal, nasal swab, or saliva sample within 2 hours. The one-step rapid process includes RNA extraction and purification using magnetic capture (< 30 minutes) followed by the confirmation of COVID-19 infection using reverse transcription-polymerase chain reaction (1 hour). The automated system comprises of an ingeniously designed single-use cartridge that has pre-filled reagents for RNA extraction and RT-PCR. The qualitative RT-PCR test detects the presence of Nucleocapsid genes N1, N2, and N3 and RNAse P gene by SYBR Green I based fluorescence. The over-all test costs less than $13.29/sample with sensitivity and specificity of 100% and a limit of detection of 10 copies/µL. The automation of the test ensures a contained system and reduces the risk of infection to the user. It obviates the need for infrastructure and trained personnel for the operation of the device. The battery-operated system is a portable device and can also be deployed in resource-poor settings.

DOI

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PROTOCOL CITATION

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KEYWORDS

RNA extraction, magnetic capture, Reverse transcription-polymerase chain reaction, SYBR Green I fluorescence

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GUIDELINES

Store the cartridge at -20^{0}C.
The nylon swab, Viral transport medium vial, and micropipette are not provided with the kit.

MATERIALS

NAME	CATALOG #	VENDOR
Ethanol	100983	Merck Millipore
SuperScript™ III Platinum™ SYBR™ Green One-Step qRT-PCR Kit	11736051	Thermo Fisher
Proteinase K from Tritirachium album	P2308	Millipore Sigma
Guanidine thiocyanate	80272	Sisco Research Laboratories Pvt. Ltd.
Tris-HCl	89781	Sisco Research Laboratories Pvt. Ltd.
EDTA Dipotassium Salt extrapure	62196	Sisco Research Laboratories Pvt. Ltd.
Triton-X	64518	Sisco Research Laboratories Pvt. Ltd.
Isopropanol (IPA) for molecular biology 99.8%	38445	Sisco Research Laboratories Pvt. Ltd.
sicastar®-M plain 1.5 µm 50 mg/ml	39-00-153	Micromod Partikeltechnologie GmbH
Molecular grade water	ML024	Himedia
Primer (Forward and Reverse primers for N1 N2 N3 RdRp and RNase P gene)		
Single stranded RNA (ssRNA) fragments of SARS-CoV-2	EURM019-1EA	Sigma Aldrich

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Guanidine thiocyanate	80272	Sisco Research Laboratories Pvt. Ltd.
EDTA Dipotassium Salt extrapure	62196	Sisco Research Laboratories Pvt. Ltd.
Triton-X	64518	Sisco Research Laboratories Pvt. Ltd.
Proteinase-K	BP1700-500	Fisher Scientific
Tris-HCl	89781	Sisco Research Laboratories Pvt. Ltd.
Isopropanol (IPA) for molecular biology 99.8%	38445	Sisco Research Laboratories Pvt. Ltd.
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SuperScript™ III Platinum™ SYBR™ Green One-Step qRT-PCR Kit	11736051	Thermo Fisher

MATERIALS TEXT

Micropipette, nylon swab

SAFETY WARNINGS

Citation: Dr Vikas Pandey, Dr Saurabh Singh, Shruti Ahuja, Avishek Dev, Manu Kadyan, Mukesh Pandit, Amir Khan, Sambit Dam, Tanish Garg (09/08/2020). VPH_AUTOGENE-COVID_Diagnosis_Protocol_XPRIZE. https://dx.doi.org/10.17504/protocols.io.bkxjkxkn

While pipetting 200 µl of a sample from VTM tube to the reaction slot in the cartridge, wear PPE and avoid any spill or bubble formation.
After the results have been noted discard the cartridge as per WHO guidelines.

BEFORE STARTING

Before starting the experiment bring the cartridge to room temperature, if required the cartridge can be incubated for 5 minutes at 37^0C. Insert the test cartridge in the device and select 'RUN'. After the test cartridge run is finished after 30 minutes start the procedure detailed for the samples.

Sample Collection

1 Collect nasopharyngeal or oropharyngeal samples from patients at a healthcare facility using a flocked tapered nylon swab. For specimen collection of nasal swabs, follow the CDC Swab Collection Guidelines and swab manufacturers' recommendations. Tilt the patient's head back 70 degrees. While gently rotating the swab, insert swab less than one inch (about 2 cm) into the nostril (until resistance is met at turbinates). Rotate the swab several times against the nasal wall and repeat in other nostril using the same swab.

2 Place and soak the Patient Swab in the VTM (Viral transport medium) vial. Rotate and stir the swab up and down for **00:05:00** .

3 Scan the barcode on the cartridge and fill the patient's details in the device.

RNA extarction using magnetic capture

4 Transfer **200 µl** from the VTM tube in the reaction slot of the cartridge using a micropipette. Place the cartridge in the device. Select the slots with cartridges and click on the 'RUN' option.

The device can accommodate 10 samples at the same time.

5 In the cartridge, **200 µl** of lysis buffer is pumped in the reaction slot and incubated for **00:15:00** .
The composition of the lysis buffer is detailed below:
4 Molarity (M) Guanidine thiocyanate, **55 Milimolar (mM)** Tris-HCl, **25 Milimolar (mM)** EDTA, **3 % volume** Triton-X, and **0.1 mg/ml** Proteinase-K.

Guanidine thiocyanate
by Sisco Research Laboratories Pvt. Ltd.
Catalog #: 80272
CAS Number: 593-84-0

Citation: Dr Vikas Pandey, Dr Saurabh Singh, Shruti Ahuja, Avishek Dev, Manu Kadyan, Mukesh Pandit, Amir Khan, Sambit Dam, Tanish Garg (09/08/2020). VPH_AUTOGENE-COVID_Diagnosis_Protocol_XPRIZE. https://dx.doi.org/10.17504/protocols.io.bkxjkxkn

Tris-HCl
by Sisco Research Laboratories Pvt. Ltd.
Catalog #: 89781
CAS Number: 1185-53-1

EDTA Dipotassium Salt extrapure
by Sisco Research Laboratories Pvt. Ltd.
Catalog #: 62196
CAS Number: 25102-12-9

Triton-X
by Sisco Research Laboratories Pvt. Ltd.
Catalog #: 64518
CAS Number: 9002-93-1

Proteinase-K
by Fisher Scientific
Catalog #: BP1700-500

6 **265 µl** Binding buffer (Isopropanol) and **10 µl** of magnetic beads are pumped in the reaction slot and incubated for **00:10:00** .

Isopropanol (IPA) for molecular biology 99.8%
by Sisco Research Laboratories Pvt. Ltd.
Catalog #: 38445
CAS Number: 67-63-0

sicastar®-M plain 1.5 µm 50 mg/ml
by Micromod Partikeltechnologie GmbH
Catalog #: 39-00-153

Citation: Dr Vikas Pandey, Dr Saurabh Singh, Shruti Ahuja, Avishek Dev, Manu Kadyan, Mukesh Pandit, Amir Khan, Sambit Dam, Tanish Garg (09/08/2020). VPH_AUTOGENE-COVID_Diagnosis_Protocol_XPRIZE. https://dx.doi.org/10.17504/protocols.io.bkxjkxkn

7 An external magnetic field is applied to the reaction slot to separate the magnetic beads from the solution. The magnetic beads are washed twice and RNA bound to the beads are eluted in **50 µl** elution buffer.

Reagents:

Wash buffer 1: **500 µl** Nuclease free water

Wash Buffer 2: **750 µl** of **80 % volume** Ethanol

Elution Buffer: **10 Milimolar (mM)** Tris-HCl, **1 Milimolar (mM)** , **8.0**

Molecular grade water
by Himedia
Catalog #: ML024

Ethanol
by Merck Millipore
Catalog #: 100983

Reverse transcription PCR

8 **10 µl** eluted RNA is transferred to PCR Slots 1, 2, 3, and 4. The PCR Slots are pre-filled with primers and PCR master mix.

SuperScript® III RT/Platinum® Taq Mix	0.5 µl
2X SYBR® Green Reaction Mix	12.5 µl
Forward Primer	0.125 µl
Reverse primer	0.125 µl
Nuclease free water	1.75 µl
Extracted RNA	10 µl
Total Volume	25 µl

PCR Slots 1,2,3 have primers targeting the N1, N2, and N3 genes respectively.
PCR Slot 4 has a primer targeting the RNAse P gene (Internal control).

The customized primer sequence is below:

Target Gene	**Sequence**

Citation: Dr Vikas Pandey, Dr Saurabh Singh, Shruti Ahuja, Avishek Dev, Manu Kadyan, Mukesh Pandit, Amir Khan, Sambit Dam, Tanish Garg (09/08/2020). VPH_AUTOGENE-COVID_Diagnosis_Protocol_XPRIZE. https://dx.doi.org/10.17504/protocols.io.bkxjkxkn

SARS CoV-2 nucleocapsid (N) gene; 2019-nCoV_N1- Forward Primer	GAC CCC AAA ATC AGC GAA AT
SARS CoV-2 nucleocapsid (N) gene; 2019-nCoV_N1- Reverse Primer	TCT GGT TAC TGC CAG TTG AAT CTG
SARS CoV-2 nucleocapsid (N) gene; 2019-nCoV_N2- Forward Primer	TTA CAA ACA TTG GCC GCA AA
SARS CoV-2 nucleocapsid (N) gene; 2019-nCoV_N1- Reverse Primer	GCG CGA CAT TCC GAA GAA
SARS CoV-2 nucleocapsid (N) gene; 2019-nCoV_N3- Forward Primer	GGGAGCCTTGAA TAC ACC AAA A
SARS CoV-2 nucleocapsid (N) gene; 2019-nCoV_N1- Reverse Primer	TGTAGCACG ATTGCAGCATTG
RdRp gene nCoV_IP2 Forward primer	ATGAGCTTAGTCCTGTTG
RdRp gene nCoV_IP2 Reverse primer	CTCCCTTTGTTGTGTTGT
(Internal Control) RNAse P Forward Primer	AGA TTT GGA CCT GCG AGC G
(Internal Control) RNAse P Reverse Primer	GAG CGG CTG TCT CCA CAA GT

Single stranded RNA (ssRNA) fragments of SARS-CoV-2
by Sigma Aldrich
Catalog #: EURM019-1EA

SuperScript™ III Platinum™ SYBR™ Green One-Step qRT-PCR Kit
by Thermo Fisher
Catalog #: 11736051

9 PCR setup for 40 cycles

cDNA synthesis	50°C	3 minutes
Initial denaturation	95°C	2 minutes`
Amplification (40 cycles)	95°C	15 seconds
	60°C	30 seconds

Citation: Dr Vikas Pandey, Dr Saurabh Singh, Shruti Ahuja, Avishek Dev, Manu Kadyan, Mukesh Pandit, Amir Khan, Sambit Dam, Tanish Garg (09/08/2020). VPH_AUTOGENE-COVID_Diagnosis_Protocol_XPRIZE. https://dx.doi.org/10.17504/protocols.io.bkxjkxkn

10 After completion of the PCR cycle, results are noted by determining the increase in the fluorescence intensity in the PCR slots.

The results are interpreted invalid if:
1) If the RNAse P (Internal control) shows no increase in fluorescence intensity (-).
2) If the positive control (P) in the test cartridge shows no increase in fluorescence intensity (-).
2) If the negative control (N) in the test cartridge shows an increase in fluorescence intensity (+).

1)The result is interpreted as positive if:
Any of the PCR slots 1, 2, and 3 exhibit an increase in fluorescence intensity (+).

2)The result is interpreted as negative if:
All the PCR slots 1, 2, and 3 show no increase in fluorescence intensity (-).

In case (1) and (2), the positive control and RNAse P (Internal control) show an increase in fluorescence intensity (+), and the negative control shows no increase in fluorescence intensity (-).

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