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Mirimus COVID-19 Pool Surveillance RT-PCR Testing

Prem Premsrirut[1], Ana Vasileva[1], Huiting Cheng[1]

[1]Mirimus, Inc.

1 Works for me dx.doi.org/10.17504/protocols.io.bkv3kw8n

XPRIZE Rapid Covid Testing

P Prem Premsrirut

ABSTRACT

Overview

At the start of the Pandemic in the US, New York was hit with more cases of COVID-19 than any single country in the world. Although case numbers are finally at an all-time low, the question of how to safely reopen the economy, school and businesses still remains unclear. Today, SARS-CoV-2 detection via RT-PCR methods remains the gold standard to detect active infection and recommend isolation of individuals in order to prevent further spread. Unfortunately, the cost and bottlenecks created from repeated massive testing efforts have become unsustainable. As a way to address this issue, we have devised a strategy of pooling samples and testing entire populations together as one unit. This cuts the cost of testing dramatically and still enables the detection of a susceptible group that would need to undergo individual testing.

To meet the COVID-19 testing demands, we created an organizational-based pooling strategy, whereby organizations can enroll in surveillance testing to monitor groups of people through pooled testing. By using organizational-based pooling, we avoid random pooling of samples. Instead, we pool groups of known contacts from a defined workplace, school or organization where people regularly interact. Therefore, a positive case would likely have an impact on the entire pooled population and the entire pooled population can be treated as an infected cohort until further individual testing, potentially, through a healthcare provider is recommended or offered.

Method of Pooled Testing

For pooled surveillance testing to be effective and widely adopted, sample collection methods must also be simplified and non-invasive in order to achieve high compliance and enrollment into a surveillance program. The method of testing must also be highly sensitive to accurately detect one positive case in a large pool of negative samples. For these reasons, we developed a simple saliva collection method with a validated pooled saliva specimens using RT-PCR and an acceptable limit of detection, which will be the most sensitive diagnostic test to date. We established automated methods to pool up to 24 saliva specimens and if a positive case is detected, the pools can be further broken down to pools of 2 specimens. The remaining 2 individuals within a pool will be referred to or, potentially, offered further clinical diagnostic testing by a healthcare provider.

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
L-1,4-Dithiothreitol	D-830	Gold Biotechnology
Eppendorf CryoStorage Vial	0030079485	Eppendorf
HBSS	14025-092	Gibco - Thermo Fischer
MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383	Thermo Fisher Scientific
Ethanol, Absolute, Molecular Biology Grade	BP2818500	Thermo Fisher Scientific
Deep Well Plate (96 well)	10045	Thermo Fisher
MicroAmp Optical 384-Well Reaction Plate with Barcode	4343814	Thermo Fisher Scientific
MicroAmp™ Clear Adhesive Film	4306311	Thermo Fisher
TaqPath™ COVID-19 Combo Kit	A47814	Thermo Fisher Scientific
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	A28523	Thermo Fisher
Water molecular-grade	BP24701	Fisher Scientific
Twist synthetic SARS-CoV-2 RNA control	Mt007544.1	Twist Bioscience

EQUIPMENT

NAME	CATALOG #	VENDOR
Integra Assist	4505	Integra
Integra Voyager 1250	4724	Integra
Hamilton LabElite DeCapper	193836	Hamilton
KingFisher	5400610	Thermo Fisher Scientific
QuantStudio 7 Pro	A45585	Thermo Fisher Scientific
Mantis	NA	Formulatrix
ViaFlow 96	6031	Integra

Sample Pooling 1h 31m 40s

1 Place 24 tubes 1m

Eppendorf CryoStorage Vial
by Eppendorf
Catalog #: 0030079485

on rack. Place pool tube in position F8. Place organization tube in position F1. Barcode scan the rack. Initiate automated decapping on the Hamilton.

Hamilton LabElite DeCapper
Robot
Hamilton 193836

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2 Initiate automated decapping. Move decapped rack to the Integra assist. 30s

Integra Assist
Liquid Handler
Integra **4505**

Integra Voyager 1250
Pipette
Integra **4724**
1250

3 Initiate the pooling automated program on the Integra Assist

3.1 1) Aspirate **50 µl** of **400 Milimolar (mM)** DTT 3m

L-1,4-Dithiothreitol
by Gold Biotechnology
Catalog #: D-830

2) Dispense **50 µl** of **400 Milimolar (mM)** DTT into each sample
3) Mix samples 5 times, using speed setting
4) Aspirate **200 µl** from each specimen
5) Dispense **200 µl** into the reservoir
6) Repeat for each row until all specimens are pooled
7) Transfer the pooled specimen to the pool tube
8) Place the pool tube in a new specimen rack

4 Repeat pooling for each cohort 1h 30m

5 Rack all pool tubes and scan again. This scan will autopopulate the qPCR template for the run. 30s

RNA extraction 1h 1m 25s

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6 Set up RNA extraction using the reagents provided in the

MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit
by Thermo Fisher Scientific
Catalog #: A48383

7 Label 4 Deep Well Plates and generate 20s
1) Samples
2) Wash Buffer
3) [M]**80 % (v/v)** Ethanol
4) Elution

Deep Well Plate (96 well)
by Thermo Fisher
Catalog #: 10045

8 Create the sample plate 1 by combing the following from the 10m

MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit
by Thermo Fisher Scientific
Catalog #: A48383

For each pool specimen (N), use the following recipe
1) **265 µl** of Binding Solution [**265 µl** X (N+1) X 1.1]
2) **10 µl** of MVPII Binding Beads [**10 µl** X (N+1) X 1.1]. Be sure to vortex well before using.
3) **5 µl** of Proteinase K [**5 µl** X (N+1) X 1.1]
4) **5 µl** of MS2 Phage [**5 µl** X (N+1) X 1.1] from the

TaqPath™ COVID-19 Combo Kit
by Thermo Fisher Scientific
Catalog #: A47814

Vortext and then add **285 µl** of the mixture to each sample well using the multichannel pipettes.

9 Create the Wash Buffer plate 2. 2m
Add **500 µl** of Wash Buffer to each well.

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10 Create the [M]**80 % (v/v)** ethanol plate 3. 2m

Add **1000 µl** of [M]**80 % (v/v)**

Ethanol, Absolute, Molecular Biology Grade
by Thermo Fisher Scientific
Catalog #: BP2818500

to each well.

11 Create the Elution plate 4. 2m

Add **50 µl** of elution buffer to each well.

12 Scan the pool tube for QC. Initiate automated decapping. 2m

13 Aspirate and mix each sample 5 times. Aspirate and dispense **200 µl** from each pool tube into Sample Plate 1. 20m

14 Add **200 µl** of 15s

HBSS
by Gibco - Thermo Fischer
Catalog #: 14025-092

to the negative control well.

15 Load the King Fisher 28m

KingFisher
RNA Extractor
Thermo Scientific **5400610**

with each plate and initiate the RNA extraction. Use the standard program: MVP_2Wash_200_Flex

RT-PCR setup 12m

16 PERFORM THE NEXT STEPS IN THE CLEAN ROOM. Set-up the RT-PCR plate using the 2m

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Mantis
Liquid Handler
Formulatrix NA

. Select program and determine the volume required.

16.1 Generate TaqPath COVID-19 Master Mix according to the recipe per pool sample plus 1 positive control: 5m

1) **7.5 µl** of

TaqPath™ 1-Step Multiplex Master Mix (No ROX)
by Thermo Fisher
Catalog #: A28523

2) **1.5 µl** of primers from the

TaqPath™ COVID-19 Combo Kit
by Thermo Fisher Scientific
Catalog #: A47814

3) **6 µl** of

Water molecular-grade
by Fisher Scientific
Catalog #: BP24701

The following formula can also be use:
50% Taqpath 1-step master mix
10% Taqpath combo kit primers
40% H20

16.2 Run the program on the Mantis, to aliquot **5 µl** of Master Mix to each well. Plate in triplicates for each pool sample into 5m

MicroAmp Optical 384-Well Reaction Plate with Barcode
by Thermo Fisher Scientific
Catalog #: 4343814

. Include an additional reaction for the positive control.

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17 Transfer the purified RNA from the KingFisher Elution plate to the qPCR using the

1m

ViaFlow 96
Liquid Handler
Integra 6031

Add in the positive control

Twist synthetic SARS-CoV-2 RNA control
by Twist Bioscience
Catalog #: Mt007544.1

18 Seal the plate with

1m

MicroAmp™ Clear Adhesive Film
by Thermo Fisher
Catalog #: 4306311

19 Vortex for 30 sec. Centrifuge the plate for 10 sec.

2m

20 Place the plate in the

1h 20m

QuantStudio 7 Pro
Real Time Thermocycler
Thermo Fisher A45585

Use the program:

00:02:00 **25 °C** 1 cycle

00:10:00 **53 °C** 1 cycle

00:02:00 **95 °C** 1 cycle

45 cycles of:

00:00:03 **95 °C**

00:00:30 **60 °C**

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