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Protocol status: Working
 We have successfully deployed INSPECT for a real-time qPCR-based workflow for Covid19 testing from nasopharyngeal swab samples.

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INSPECT sample tracking system

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ABSTRACT

A specimen to data tracking tool for SEARCH SARS-CoV-2 tests. The application is used by SEARCH technicians to track samples as they proceed through each step within the RT-qPCR testing workflow. The app is currently hosted here:

http://inspect-covid.com/qpcr_records/

SAFETY WARNINGS

- INSPECT makes certain assumptions about plate format and automation at each step of the workflow :
 - 1) INSPECT expects 96-well plates for the Sample and RNA extraction steps and 384-well plates for the RT-qPCR reaction steps.
 - 2) Sample plating is not automated and performed manually. Technicians must follow the prompted order of sample plating to maintain data integrity within INSPECT
 - 3) RNA Extraction, RNA plate compression and RT-qPCR reaction plate preparation are automated using Kingfisher, EpMotion and Mosquito robots / machines. This permits INSPECT to transition well IDs from one plate to another in specific orders as followed by the machines.
 - 4) Only 1 decision review is permitted per plate.

Before Starting

- 1 Before starting ensure that the INSPECT system is publicly accessible and that you are registered on the INSPECT user list.

Sample Extraction and Plating

- 2 Freshly received samples can be registered into INSPECT by scanning the 2D sample tube barcode into the system. This is performed in conjunction to the sample extraction and plating step.
- 3 Enter the Lot # of the RNA Lysis buffer being used. The Lot # can also be scanned into the app. Enter the name of the assisting technician (if any).

The screenshot shows the INSPECT web application interface. At the top, there is a navigation bar with the INSPECT logo, 'Home', 'Search', and 'Dashboard' links, and a user profile 'ssathe'. The main content area is titled 'Perform Safety Checks' and contains a form with the following fields:

- Facemask**: ☒
- Hood**: ☒
- Gloves**: ☒
- Lysis Reagent Lot #***:
- Lysis Reagent Lot #**:
- Assisting Technician Name***:
- Name of Assisting Technician**:
- Start**:

At the bottom right of the form, there is a copyright notice: ©2020 Yeo Lab Design.

Submission form for recording the RNA lysis buffer lot #

- 4 Start sample plating by using the platemap guides provided by INSPECT.

4.1 Load control samples first. INSPECT assumes that the control samples are being loaded in well A1 and H1.

Input Sample Plate

Instructions

Load control sample into the indicated well below.

Control Wells: A1 & H1

Next Well End Plate

Plate Legend

- Sample
- Control
- Current Sample
- Loaded Samples

Scanned Barcodes

Well	Barcode
------	---------

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Initial sample plating window. INSPECT prompts user to load the control samples first into wells A1 and H1.

4.2 Scan sample barcode into the provided text area and load sample into the assigned well.

Input Sample Plate

Instructions

Scan sample barcode and load sample into the indicated well below.

Well: B1

Sample Barcode*

XYZ123 Next Well End Plate

Plate Legend

- Sample
- Control
- Current Sample
- Loaded Samples

Scanned Barcodes

Well	Barcode
------	---------

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Scan sample barcode and load sample into assigned well (B1, in this case)

Input Sample Plate

Instructions

Scan sample barcode and load sample into the indicated well below.

Well: **E1**

Sample Barcode*

Plate Legend

- Sample
- Control
- Current Sample
- Loaded Samples

Well	Barcode
D1	XYZ125
C1	XYZ124
B1	XYZ123

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Scan and load samples as guided by INSPECT. If you do not have 94 samples, you can end the sample plating step by clicking on "End Plate". INSPECT will proceed with the scanned barcodes only.

- 5
- Once all samples have been plated, end the sample plating step and proceed to the plate barcode scanning step. INSPECT requires the user to enter the Sample Extraction Plate (SEP) barcode. Additionally, if present, users can enter the Sample Storage Plate (SSP) and Sample Storage Bag (SSB) barcodes as well.

INSPECT

Home Search Dashboard

ssathe

Scan Plate Barcodes

3

Samples

Instructions

Scan the following plate barcodes.

Sample Storage Plate Barcode*

Sample Extraction Plate Barcode*

Sample Storage Plate (SSP)

Scan or Enter Barcode of Sample Extraction Plate (SEP)

Sample Storage Bag*

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SSP, SEP and SSB barcode submission form

6

Expected result

The screenshot shows the INSPECT dashboard with a navigation bar (Home, Search, Dashboard) and a user profile (ssathe). A green notification bar at the top states "3 samples added successfully." The main content area features the INSPECT logo and the tagline "Instant Service Platform for Emergency COVID Tests".

Overall Testing Summary

Number of Samples	Number of Positives	Number of Negatives	Number of Undetermined
12105	1410 (11.65%)	9858 (81.44%)	567 (4.68%)

Sample Tracker

Step Name	Number of Samples in Step	Number of Plates Already Evaluated	Evaluated Plate IDs
Unprocessed Samples	0	-	-
Sample Extraction Plate (SEP)	3	1	SEP-0001
RNA Elution Plate (REP)	0	0	
RNA Working Plate (RWP)	0	0	
Running qPCR Plate (QRP)	0	0	
Recorded qPCR Plate (QRP)	0	-	-
Processed qPCR Plate (QRP)	0	-	-

INSPECT will display the number of samples successfully scanned. The live counter will also be updated to reflect the appropriate number of samples in the sample extraction steps.

6.1

The screenshot shows the INSPECT Search Results page. It includes a "Download All Results" link and a table with the following data:

Barcode	Sampling date	Ssp id	Ssp well	Sep id	Sep well	Sample bag id	Sampling plate csv	Rep id	Rep well	Rsp id	Rsp well	Rwp id	Rwp well	Qrp id	Qrp well	Ms2 ct value	N ct value	Orf1ab ct value	S ct value	D r
XYZ123	07/20/2020	SSP-0001	B1	SEP-0001	B1	SSB-0001	https://covidtest2.s3-us-west-2.amazonaws.com/SEP-0001_20-07-20.csv	-	B1	-	B1	-	-	-	-	-1.0	-1.0	-1.0	-1.0	U

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RNA Extraction

- INSPECT assumes that the RNA extraction from SEPs is an automated process, performed using Kingfisher or similar robotic machines. Thus, sample barcode scanning is not required. Users must enter the origin SEP and the destination RNA Extraction Plate (REP) barcodes. Only upon entering both will the samples be successfully linked between sample extraction and RNA extraction steps.

7.1 Enter Lot #s of the reagents being used in the RNA Extraction step

7.2 Enter the barcode of the SEP and the barcode of the new REP and proceed with extracting RNA from the samples.

The screenshot shows the INSPECT web application interface. At the top, there is a navigation bar with the logo and links for Home, Search, and Dashboard. A user profile icon labeled 'scathe' is in the top right corner. The main content area features a light blue background with a white form. A blue reminder box at the top states 'Reminder: Add MS2 PHAGE.' Below this, the form is divided into two columns. The left column contains fields for 'MS2 Phage Lot #*' (with value '2003001'), 'RNA Extraction Kit Lot #*' (with value 'XY2001'), and 'Carrier RNA Lot #*' (with value 'XY2003'). The right column contains fields for 'KingFisher ID*' (with value 'KF001') and 'Mag-bind Particles CNR Lot #*' (with value 'XY2002'). Each field has a small text prompt below it: 'Enter MS2 Control Lot #', 'Enter Kingfisher Number', 'Enter RNA extraction kit lot #', 'Enter Mag Bind particles CNR Lot #', and 'Enter Carrier RNA Lot #'. Below these fields, another blue reminder box states 'Reminder: RNA Elution Plate ID will be recorded as the RNA Storage Plate ID.' This is followed by two more fields: 'Sample Extraction Plate Barcode*' (with value 'SEP-0001') and 'RNA Elution Plate Barcode*' (with value 'REP-0001'). Small text prompts below these fields read 'Scan or Enter Barcode of Sample Extraction Plate (SEP)' and 'Scan or Enter Barcode of RNA Elution Plate (REP)'. At the bottom of the form is a blue button labeled 'Assign Plates'. The footer of the page includes the copyright notice '©2020 Yeo Lab Design'.

Submission Form to assign REP to an existing SEP

7.3

The screenshot shows the INSPECT dashboard with a sidebar on the left containing buttons for 'Upload PlateMap', 'Sample Plating', 'Update Existing Records', and 'Advanced Search'. The main content area features the INSPECT logo and an 'Overall Testing Summary' table.

Number of Samples	Number of Positives	Number of Negatives	Number of Undetermined
12105	1410 (11.65%)	9858 (81.44%)	567 (4.68%)

Below the summary is a 'Sample Tracker' table:

Step Name	Number of Samples in Step	Number of Plates Already Evaluated	Evaluated Plate IDs
Unprocessed Samples	0	-	-
Sample Extraction Plate (SEP)	0	0	SEP-0001
RNA Elution Plate (REP)	3	1	REP-0001
RNA Working Plate (RWP)	0	0	-
Running qPCR Plate (QRP)	0	0	-
Recorded qPCR Plate (QRP)	0	-	-
Processed qPCR Plate (QRP)	0	-	-

Provided with valid barcodes, INSPECT will link the given REP with the corresponding SEP entered in the form

- 7.4** INSPECT assumes that the RT-qPCR test is performed on a 384-well plate format. For this purpose, 4x 96-well plates are compressed into a single 384-well plate. Thus, each 384-well RNA Working Plate (RWP) must be linked with 4x 96-well REPs, and the well ID for each sample must be transformed into a 384-well plate format. This process is automated by INSPECT and can be achieved by simply scanning the new RWP and the associated REPs

The screenshot shows the INSPECT form for linking plates. It includes fields for 'EpMotion ID*', 'First 96-Well Plate in Array Position B2*', 'Second 96-Well Plate in Array Position B3', 'Third 96-Well Plate in Array Position B4', 'Fourth 96-Well Plate in Array Position B5', and 'RNA Working Plate Barcode*'. A blue 'Assign Plates' button is at the bottom.

7.5

Expected result

The screenshot displays the INSPECT dashboard with a navigation bar at the top containing 'Home', 'Search', and 'Dashboard' links. A notification banner at the top left states 'RNA working plate added successfully'. The main content area features a sidebar on the left with buttons for 'Upload PlateMap', 'Sample Plating', 'Update Existing Records', and 'Advanced Search'. The central panel is titled 'Overall Testing Summary' and includes a table with the following data:

Number of Samples	Number of Positives	Number of Negatives	Number of Undetermined
12105	1410 (11.65%)	9858 (81.44%)	567 (4.68%)

Below this is the 'Sample Tracker' table:

Step Name	Number of Samples in Step	Number of Plates Already Evaluated	Evaluated Plate IDs
Unprocessed Samples	0	-	-
Sample Extraction Plate (SEP)	0	0	SEP-0001
RNA Elution Plate (REP)	0	0	REP-0001
RNA Working Plate (RWP)	3	1	RWP-0001
Running qPCR Plate (QRP)	0	0	-
Recorded qPCR Plate (QRP)	0	-	-
Processed qPCR Plate (QRP)	0	-	-

The bottom section of the screenshot shows 'Search Results' with a 'Download All Results' link and a table of results for sample XYZ123.

Barcode	Sampling date	Ssp id	Ssp well	Sep id	Sep well	Sample bag id	Sampling plate csv	Rep id	Rep well	Rsp id	Rsp well	Rwp id	Rwp well	Qrp id	Qrp well	Ms2 ct value	N ct value	Orf ct value
XYZ123	07/20/2020	SSP-0001	B1	SEP-0001	B1	SSB-0001	https://cowitest2.s3-us-west-2.amazonaws.com/SEP-0001_20-07-20.csv	REP-0001	B1	REP-0001	C1	RWP-0001	C1	-	C1	-1.0	-1.0	-1.0

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Viral Gene Expression Test

- 8 Similar to previous steps, each qPCR Reaction Plate (QRP) must be linked to an existing RWP. Since the RWP and the QRP have the same platemap format, the well ID for each sample is carried over from the previous assignment.

8.1 Enter a valid RWP barcode and the new QRP barcode

RNA Working Plate Barcode*

qRT-PCR Plate Barcode*

RWP-0001 QRP-0001

Scan or Enter Barcode of RNA Working Plate (RWP) Scan or Enter Barcode of qRT-PCR Reaction Plate (QRP)

Assign Plates

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8.2

Expected result

qRT-PCR plate added successfully.

INSPECT
Instant Service Platform for
Emergency COVID Tests

Overall Testing Summary

Number of Samples	Number of Positives	Number of Negatives	Number of Undetermined
12105	1410 (11.65%)	9858 (81.44%)	567 (4.68%)

Sample Tracker

Step Name	Number of Samples in Step	Number of Plates Already Evaluated	Evaluated Plate IDs
Unprocessed Samples	0	-	-
Sample Extraction Plate (SEP)	0	0	SEP-0001
RNA Elution Plate (REP)	0	0	REP-0001
RNA Working Plate (RWP)	0	0	RWP-0001
Running qPCR Plate (QRP)	3	1	QRP-0001
Recorded qPCR Plate (QRP)	0	-	-
Processed qPCR Plate (QRP)	0	-	-

8.3

After the qPCR reaction has completed, the technician is required to upload the qPCR results file. This can be done using the results submission page. The filename for the results file must be the same as the QRP barcode.

Upload qPCR Results

Qpcr results file*

Browse... QRP-0001.xlsx

Upload

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- 8.4** Once the results are successfully uploaded, INSPECT will make decision calls on each sample (during file upload). These decisions have to be reviewed by a qualified technician. On the Review Results page, enter the barcode of the QRP for which you would like to review the results.

Barcode	Fake name	Sep id	Rep id	Rwp id	Qrp id	Qrp well	Ms2 ct value	N ct value	Orf1ab ct value	S ct value	Decision tree results	Final results
XYZ124	None	SEP-0001	REP-0001	RWP-0001	QRP-0001	E1	30.787	-1.0	-1.0	-1.0	Negative	Negative
XYZ125	None	SEP-0001	REP-0001	RWP-0001	QRP-0001	G1	30.84	-1.0	-1.0	-1.0	Negative	Negative
XYZ123	None	SEP-0001	REP-0001	RWP-0001	QRP-0001	C1	31.155	-1.0	-1.0	-1.0	Negative	Negative

Default results are the same as the decisions made by INSPECT. To change the decisions, use the dropdown menu to select 1 of 4 options : Negative, Positive, Invalid and Inconclusive.

Result Reporting

- 9** Once the results for a QRP have been reviewed, the RT-qPCR testing workflow is complete. Users can search for samples and corresponding results through the SEARCH tab.

Expected result

Dashboard Issue

Search Results

[Download All Results](#)

Barcode	Sampling date	Sep id	Sep well	Sep id	Sep well	Sample bag id	Sampling plate csv	Rep id	Rep well	Rep id	Rep well	Rwp id	Rwp well	Qrp id	Qrp well	Msc ct value	N ct value	Orf1ab ct value	S ct value	Decision tree results	Final results	Qpcr results file	Sample release
XY2124	07/20/2020	SSP-0001	C1	SEP-0001	C1	SSB-0001	https://covidtest2.s3-us-west-2.amazonaws.com/SEP-0001_20-07-20.csv	REP-0001	C1	REP-0001	E1	RWP-0001	E1	QRP-0001	E1	30.787	-1.0	-1.0	-1.0	Negative	Negative	https://covidtest2.s3-us-west-2.amazonaws.com/GRP-0001.xlsx	✖
XY2125	07/20/2020	SSP-0001	D1	SEP-0001	D1	SSB-0001	https://covidtest2.s3-us-west-2.amazonaws.com/SEP-0001_20-07-20.csv	REP-0001	D1	REP-0001	G1	RWP-0001	G1	QRP-0001	G1	30.84	-1.0	-1.0	-1.0	Negative	Negative	https://covidtest2.s3-us-west-2.amazonaws.com/GRP-0001.xlsx	✖
XY2123	07/20/2020	SSP-0001	B1	SEP-0001	B1	SSB-0001	https://covidtest2.s3-us-west-2.amazonaws.com/SEP-0001_20-07-20.csv	REP-0001	B1	REP-0001	C1	RWP-0001	C1	QRP-0001	C1	31.155	-1.0	-1.0	-1.0	Negative	Negative	https://covidtest2.s3-us-west-2.amazonaws.com/GRP-0001.xlsx	✖

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Users can download the results table from the Download link at top-left of the results table