MatMaCorp COVID-19 Test (SARS-CoV-2)

Qualitative assay for use on the MatMaCorp Solas 8 Instrument

For in-vitro diagnostic use

MatMaCorp COVID-19 Test kit	Part Number: ST-CV19-L
Solas 8 device	Part Number: SOL8

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1 Intended Use

MatMaCorp's COVID-19 assay performed on the Solas 8 Instrument is a molecular in vitro diagnostic test utilizing a combined RT-PCR and isothermal nucleic acid amplification technology intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs from individuals who are suspected of COVID-19 by their healthcare provider. Testing is for laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, to perform high complexity tests. Use of this test is limited to laboratories certified to perform high complexity testing, including testing at the point-of-care when the site is covered by the laboratory's CLIA certificate for high-complexity testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The MatMaCorp COVID-19 test is intended for use by medical professionals or trained operators who are proficient in performing tests using the Solas 8 Instrument. The test is not authorized by FDA and is being distributed in accordance with Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised).

2 Summary and Explanation of the Test

2.1 Test Principle

The MatMaCorp COVID-19 test is used for the qualitative detection of SARS-CoV-2 from nasopharyngeal swabs. The MatMaCorp COVID-19 kit contains all components required to carry out an assay for SARS-CoV-2 on the Solas 8 device. The MatMaCorp COVID-19 test utilizes a combined RT-PCR and isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral nucleic acids. The assay comprises two procedures: 1) sample preparation and 2) amplification and detection. Sample can be swab samples collected in universal/viral transport media (UTM/VTM).

2.1.1 Sample Preparation

Fifty (50) μl of each sample is treated with an equal volume of lysis buffer and incubated for 10 minutes in the big block of the Solas 8 device, following the on-screen instructions. After this, 5 μl of the lysed sample is used directly in the detection step.

2.1.2 Amplification and Detection Step

As prompted by the user interface on the device, the RNA template from the Sample Preparation step above is added to the designated well of an 8-well PCR strip tube. From here on, there are 3 steps involved in getting the final data and they are all done in the same tube:

STEP 1

The initial cDNA synthesis and PCR amplification (RT-PCR) occurs at this step. The Step 1 Master Mix contains all the reagents required for amplification of SARS-CoV-2, as well as an internal control. The master mix has primers designed to target SARS-CoV-2 RNA that amplifies a unique region of the RdRp segment. The Step 1 Master Mix is added to all the wells of the 8-well PCR strip tube that have samples as directed by the instructions on the device.

STEP 2

After the initial PCR reaction (Step 1), a method using padlock probes are used to confirm that the amplification is specific to the SARS-CoV-2 target sequence. For this, a circularizing oligonucleotide probe (also called padlock probe) specific to the amplified region of the RdRp segment is added in Step 2 along with a thermostable ligase. The thermostable ligase allows a cycling ligase reaction which happens during Step 2. This padlock probe is extremely specific that it will only ligate if the correct amplified segment from the SARS-CoV-2 virus is present.

STEP 3

The ligated probe is amplified using by an isothermal amplification method called rolling circle amplification (RCA). Step 3 reagents include Bst DNA polymerase and fluorescent labeled primers with quenchers that are used to identify each of the amplified RNA targets. There are two primers used in this step for each target. One is a forward primer (labeled) and the second is a reverse primer that is unlabeled. Real-time fluorescence is detected by the Solas 8 device.

The MatMaCorp Solas 8 can run standard PCR reactions or isothermal reactions and is also a 4-channel fluorescence detection device. It is operated by a graphical user interface on a touch screen. It incorporates multi-channel fluorescence detection with two, 8-well heating blocks - a large well heating block for DNA/RNA isolation and a small block that allows PCR and or isothermal assays to be run using 0.2 ml tubes or 0.2 ml 8-well strip PCR tubes

2.2 Internal Control

MatMaCorp COVID-19 test has built-in procedural controls. This is referred to on the system as Human Internal Positive Control (HIPC). The result of the HIPC is displayed on the screen and is automatically stored in the instrument with each test result. This can be reviewed later by selecting the Reports function on the instrument. If needed, all the raw data generated by the instrument can be reviewed later.

2.3 External Positive and Negative Controls

MatMaCorp COVID-19 kits contains a separate Positive Control reagent that is provided. The kits are formulated in 8 well PCR strips. The first well is hard-coded as a Negative Control and the 8th well is hard-coded as a positive Control. The Positive control is a synthetic DNA target that is identical to the RdRp segment of the SARS-CoV-2 genome. The negative control is used to monitor contamination introduced by the laboratory environment and the users in their routine use of the kit. The positive control is used to monitor the proper functioning of the reagents, particularly the reagents used in the detection of the SARS-CoV-2 RNA.

3 Reagents and Materials

3.1 Components Included with the Test Kit

All components of the test are manufactured by MatMaCorp and they include enough reagents to do

- 100 sample preparations,
- 100 COVID-19 test reactions - Amplification and Detection including negative and positive controls.

All these components are packed in a kit box labeled MatMaCorp COVID-19 Test Kit. The contents of the kit box are:

Kit Component	Description	Quantity
Aluminum Pouch 1	Step 1 Master Mix	17 screw cap tubes
Aluminum Pouch 2	Step 2 Master Mix	17 screw cap tubes
Aluminum Pouch 3	Step 3 Master Mix	17 screw cap tubes
Plastic Bag 1	8 well PCR strip tubes	17 strips with attached lids
Plastic Bag 2	1.5 mL locking tubes for sample prep	100 tubes
Plastic Bag 3	Lysis Buffer	17 screw cap tubes
Aluminum Pouch 4	Positive Control pellet	17, 3/8" Medi-cup blister packs

3.2 Reagent Ingredients

Kit Component	Label on Tube	Reagent Ingredients
Step 1 Master Mix	S1 Mix	Tris buffer, K-acetate, Taq enzyme, forward and reverse transcriptase enzyme, dNTPs
Step 2 Master Mix	S2 Mix	Primer probe, dNTPs, forward and reverse primers, ligase enzyme, NAD+, Triton X100
Step 3 Master Mix	S3 Mix	Tris buffer, Mg-sulfate, K-Chloride, dNTPs, forward and reverse primers, Bst DNA polymerase enzyme
Lysis Buffer	Buffer CNL	Tris buffer, EDTA and Guanidine isothiocyanate
Positive Control	Positive Control	Tris buffer, artificial DNA

NOTE: All reagents are provided with overages.

3.3 Reagent storage and handling requirements

All reagents are stored at room temperature. Their expiration dates are provided on the kit box and the storage container that each reagent is packed in.

Components required but not included with the kit

- Sterile Water or DEPC treated Sterile Water
- Pipettes
- Tips for pipettes (filter/barrier tip)
- Nasopharyngeal swabs, collected in Copan UTM-RT or equivalent, stored according to CDC guidelines

3.4 Instrumentation Required

The Solas 8 instrument (part number: SOL8) is required. It comes with built-in software. For additional information, please refer to the Solas 8 User Guide that comes with the instrument.

4 Precautions and handling requirements

4.1 Warnings and Precautions

Good laboratory practices are essential to ensure that the test is performing as expected. As with any molecular test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- All patient samples for testing should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.1,2.
- Personnel proficient in handling infectious materials and the use of the Solas 8 device should perform this procedure.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- If spillage of sample occurs, follow appropriate site procedures or immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10).
- When using pipettes, the use of sterile disposable pipette tips with filters are recommended.
- Safety Data Sheets (SDS) are available on request from MatMaCorp customer support (+1) 402-387-7900. It is also available online at `http://matmacorp.com/product_matmacorp_covid_19_test_system.html`.
- Follow protocol steps and all provided recommendations to ensure that the test is performed correctly. Any deviation may affect test performance.
- To prevent false positive results that may occur, ensure that carryover of samples are adequately controlled during sample handling and processing.

4.2 Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practices. This will prevent carryover of samples or controls into patient samples.
- Before use, visually inspect each reagent packaging, to ensure that all packing is intact. If there is any evidence of breakage or damage, do not use that material for testing.
- The MatMaCorp Lysis buffer (CNL Buffer) contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- Do not allow CNL buffer which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

4.3 Good Laboratory Practice

- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents.
- Avoid contaminating gloves when handling samples and positive controls. If there is any question of having contaminated the gloves, immediately change gloves between handling samples and any test kit components.
- Wash hands thoroughly after after removing the gloves, particularly if samples were handled.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite (bleach) in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol. As stated above, do not allow CNL buffer which contains guanidine thiocyanate, to come in contact with sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- If spills occur on the Solas 8 instrument, follow the instructions in the User Guide to properly clean and decontaminate the surface of the instrument and the work surface.
- Do not eat, drink, or smoke in a laboratory.
- Use standard laboratory pipettes - mechanical or electronic but do not use any other pipetting method including mouth pipetting.

5 Sample Collection, Transport and Storage

5.1 Sample Collection

Collect nasopharyngeal (NP) specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 1 mL or 3 mL of Copan Universal Transport Medium (UTM-RT) or equivalent.

5.2 Transport and Storage

Sample stability when using MatMaCorp COVID-19 test has not been established for suggested temperatures and time, but is based on viability data from testing similar viruses in the UTM-RT or UVT Systems as stated in, for example, on the Copan UTM-RT System Instructions For Use and shown below:

- After collection, the specimen should be stored at 2 to 25°C and processed within 48 hours.
- If delivery and processing exceed 48 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder.

6 Instruction For Use

6.1 Procedural notes

- Do not use reagents after their expiry dates.
- All consumables provided in the kit are for one-time use only.
- Refer to the COVID-19 Test User Guide for proper maintenance and use of of the instrument.

6.2 Running the MatMaCorp COVID-19 Test

Samples collected in Copan UTM-RT or equivalent can be used. Minimum required sample volume for the test is 50 μL:

- Use caution when transferring specimens from a primary collection tube to a sample prep tube (tubes with locking lid).
- Use pipettes with aerosol-barrier (filter tip) when handling samples.
- Use a new pipette tip for each sample.
- If samples are frozen, ensure that samples are completely thawed before transfer to a sample prep tube.

To transfer patient sample from a primary collection tube into a sample prep tube (with locking lid):

- Label sample prep tube with the same sample ID as the sample tube.
- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube.
- Transfer 0.050 mL (50 μL) into the appropriately labeled sample prep tube. Immediately close the lid.
- Place sample prep tube in to a tube rack.
- Close the primary sample tube cap and return to original location.

6.3 MatMaCorp COVID-19 Test Procedure

The detailed test procedure is provided in the MatMaCorp COVID-19 User Guide and the on-screen instructions on the Solas 8 for running the COVID-19 Test. The summary of the procedure is given below:

- Enter sample ID, sample type, and test for each sample. Up to 6 patient samples can be tested at a time.
- Perform RNA extraction by following instructions on the screen.
- Load prepared samples in test strip supplied with the kit. Add master mix as instructed.
- Follow Instructions on Screen.
- Review and export Results.

7 Results

The MatMaCorp Solas 8 instrument automatically detects SARS-CoV-2 and the internal control for each individually processed sample as well as the controls. The results for the patient samples and the controls are displayed as well as the validity of the results and controls.

7.1 Quality Control and Validity of Results

Each batch of 6 samples that are run together has one negative control and one positive control, making it a total of 8 reactions done together. In the Solas 8 report for each set of samples, check for the results, POSITIVE or ND (Not Detected), and the associated scores for the samples. The results from each batch of samples are valid if the negative and positive control are valid. Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

In addition to providing "POSITIVE" or "ND" (Not Detected) calls, the software also provides a numerical value or score. This numerical value is not the final call but an indication as to how close to the limit of detection the particular reaction is. For each call of "ND" or "POSITIVE", a score between 50 and 100 is given. A score above 70 indicates a satisfactory separation between the signal and the limit of detection so that the call can be accepted confidently. If there is ambiguity (score below 70) and a resolution is needed, it can be done by viewing the raw fluorescence signal data. Instructions for viewing and interpreting the raw data and for a detailed description of the scores, see the Solas 8 User Guide for running the COVID-19 test.

7.2 Interpretation of Results

Note that results provided by this test should only be interpreted along with clinical evaluation of the patient as well as available patient history. **Reports to healthcare providers should state that the manufacturer has validated the test used but it has not been independently reviewed by FDA**.

For each patient sample, the viral target will have a call of "POSITIVE" if the SARS-CoV-2 RNA was detected or "ND" (Not Detected) if the SARS-CoV-2 RNA was not present above the limit of

detection. Each sample (wells 2 to 7) should have a result of "Positive" for the human internal control (HIPC). In some cases, where the viral load is high, it is possible that the HIPC call may be "ND". In other cases, if the swab was not used properly during specimen collection, there may not be enough sample, and a "ND" call can be made for the internal control (HIPC). In such situations, if the positive and negative controls (wells 8 and 1) performed as expected, and the call made for the SARS-CoV-2 is POSITIVE, the result should be considered as a valid test.

Results and their corresponding interpretation for detecting SARS-CoV-2 using the MatMaCorp COVID-19 test kit can be summarized as shown below.

7.3 Patient Sample Results

For a valid assay, interpret the Patient Sample Results using the table below

SARS-CoV-2	HIPC	Interpretation of Results and Follow-Up Actions
POSITIVE	POSITIVE	Positive for COVID-19. Positive results do not rule out bacterial infection or coinfection with other viruses.
ND	POSITIVE	COVID 19 not detected. This result does not rule out co-infections with other pathogens. SARS-CoV-2 may be present below the level of detection (LOD).
POSITIVE	ND	Potentially positive for COVID-19 - repeat test.
ND	ND	Repeat testing of the sample using new test components. If repeated invalid results are obtained, results should be confirmed by another method prior to reporting the results.

In a valid test, samples are analyzed based on the detection of the viral target. If viral target is "ND" and the human internal control is "Positive", the sample is considered "Below LoD" and resampling may be required later, at the discretion of the physician. If all targets (viral and human internal control) are "ND", the sample is considered a "No Test" and should be repeated or re-sampled.

7.4 External Controls Result Interpretation and Troubleshooting

Instrument Calls for Well 1: Negative Control (NC)

SARS-CoV-2	HIPC	Issue
ND	ND	Expected result.
ND	POSITIVE	User contamination.
POSITIVE	ND	Contamination: clean and disinfect lab and equipment used - repeat testing of the sample using new test components.
POSITIVE	POSITIVE	User contamination - all results are invalid. Clean and disinfect lab and equipment used. Repeat testing of the sample using new test components.

Instrument Calls for Well 8: Positive Control (PC)

SARS-CoV-2	HIPC	Issue
POSITIVE	ND	Expected result.
POSITIVE	POSITIVE	User contamination.
ND	POSITIVE	Potential human sample contamination: repeat test.
ND	ND	External control has failed. Repeat test. If same result a second time - replace the MatMaCorp COVID 19 Kit.

8 Procedural Limitations

- The MatMaCorp COVID-19 Test has been evaluated only for use on the MatMaCorp Solas 8 instrument. None of the components in the kit will work with other instruments.
- How the sample was collected, stored and handled will influence the results that are generated from running this test.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swab samples collected in Universal viral transport media (Copan UTM-RT or equivalent) or Universal Viral Transport System (UVT) or equivalent media. Testing

of other sample types using the MatMaCorp COVID-19 test may result in inaccurate or no results.

- Different factors may influence the detection of SARS-CoV-2 RNA including sample collection methods, patient factors (e.g., presence or absence of symptoms), and/or stage of infection etc.
- As with any molecular test, mutations within the target region of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of the virus.
- A Human Internal Positive Control (HIPC) is included in each reaction of the MatMaCorp COVID-19 test to help identify the specimens containing substances that may interfere with nucleic acid isolation, amplification and detection. If interfering substances are present, false negative or invalid results may occur.
- Carefully following the recommendations provided in this Instructions For Use document along with good laboratory practices may help prevent contamination of samples.

9 Non-Clinical Performance evaluation - Key Performance Characteristics

9.1 Analytical Sensitivity

The goal of this study was to determine the lowest detectable concentration of SARS-CoV-2 at which 19 out of 20 replicates (95%) were positive. All the testing was performed on the MatMaCorp Solas 8 device. Limit of detection (LoD) was performed utilizing all the steps involved in using the MatMaCorp COVID-19 test from sample isolation to final detection. To determine the LoD, a gamma-irradiated SARS-Related Coronavirus 2, isolate (USA-WA1/2020 (NRC-52281 lot 7003364, provided by BEI Resources) was used. This had a titer of 2.8 x105 TCID50 per mL and 1.7 x 109 genome equivalents per mL according to the Certificate of Analysis provided with the sample. The genome equivalents were used to calculate the amount of viral RNA in each of the dilutions used in this study.
Clinical matrix used: Clinical samples collected in Universal Transport Media and determined to be negative using an Emergency Use Authorization (EUA) test authorized by the U.S. FDA were used to make dilutions of the virus. To get a representative negative sample, equal volumes from the 30 negative samples that were available were pooled to create the dilution matrix. As shown in the Table below, the following final viral amounts were used to determine the final LoD. The reactions were done as 20 independent reactions for each of the concentration levels shown below.

Concentration (genome equivalent/mL)	Total Results	Pos. Hit Rate (%)
6600	20/20	100
1600	19/20	95
800	20/20	100
400	17/20	85
0	0/20	0

Based on the above results, the limit of detection, or LOD, is determined to be 800 genome equivalents per mL (40 genome equivalents per 50 μl reaction).

9.2 Inclusivity/Reactivity

In silico analysis concluded that the MatMaCorp COVID-19 test will detect all analyzed SARS-CoV-2 sequences in the NCBI database (GenBank) and the the Nextstrain database as of June 15, 2020.

Inclusivity was demonstrated by comparing the MatMaCorp SARS-CoV-2 assay primers and probes to an alignment of all SARS-CoV-2 sequences available in the databases that were used.

Of all the sequences that were identified, the probe sequence matched 100% coverage and 100% identity to all but two sequence accessions (99.91% inclusivity). Upon further investigation of the mismatched sequences, accessions MT419850 and MT419838 both contained an "N" (unknown nucleotide) in the subject sequence, likely due to incomplete sequencing of the isolates.

Of all the sequences searched, the assay primers also matched 100% coverage and 100% identity to all but a few with incomplete or variable sequencing. Accessions MT451798 and MT451786 both contained a "K" at base 9 of the Forward primer, for a total of 99.96% inclusivity. Accessions MT451660 and MT451663 both contained a "Y" at base 16 of the Reverse primer, while MT451617 contained a "W" at base 10, and MT536957 contained "N"s in the first two positions, for a total of 99.92% inclusivity.

For a deeper examination, the genomic epidemiology database as nextstrain.org was checked for additional mutations identified in a North America-focused subsampling of SARS-CoV-2 isolates. No significant events were identified between basepairs 15418-15456, covering the binding site of the padlock probe in ORF1b. However, a total of 8 sequences contained rare mutations within that region (1 at 15418, 2 at 15426, 2 at 15438, 1 at 15444, 1 at 15448, and 1 at 15450) out of 5310 genomes (99.85% inclusivity) at the time of the search. Upon further investigation of the mismatched sequences, no isolate contained more than one mutation within the region, and most importantly, no isolate contained a mutation at the critical position 15443, the clamp position of the 3' flanking sequence that allows for identification of single nucleotide polymorphisms (SNPs) in genotyping.

The Nextstrain search was repeated for the SARS-CoV-2 Forward and Reverse primers. No significant mutation events were identified between basepairs 15436-15455, covering the Forward primer, or 15526-15545, covering the Reverse primer. However, a total of 6 isolates contained rare mutations within the first region (2 at 15438 and 4 at 15444) and 6 more within the second region (1 at 15535 and 5 at 15540), out of the 3424 genomes that appeared in the database at the time of the revised search. Importantly, no isolate contained more than one mutation within the primer regions. This gives a total of 99.8% inclusivity for each the Forward and Reverse primers.

9.3 Cross-reactivity (Analytical Specificity)

The *in silico* analysis for possible cross reactions with all the organisms listed below was conducted by mapping primers in the and probes in the MatMaCorp COVID-19 test individually to sequences available in the NCBI (GenBank) database.

For any significant homology, >33% without regard to the gene or target (non-coding) sequence, the strain/isolate, GenBank accession #, and % homology are listed in the tables below. Based on the *in silico* analysis, none of the organisms are expected to cross-react with the test primers/probe.

COVID-19 *in silico* cross-reactivity results			Homology (0-100%)[1]		
Pathogen	**Strain**[2]	**GenBank Acc #**	**FP**[3]	**RP**[3]	**Probe**[4]
Human coronavirus 229E	HCoV-229E/BN1/GER/2015	KU291448.1	NSS[5]	60	38
Human coronavirus OC43	HCoV_OC43/Seattle/USA/SC831/2016	KY369905.1	75	55	33
Human coronavirus HKU1	SI17244	MH940245.1	85	55	43
Human coronavirus NL63	HCoV_NL63/Seattle/USA/SC0768/2019	MN306040.1	55	60	NSS
SARS-coronavirus	Urbani isolate icSARS-C7-MA	MK062184.1	95	75	92[6]
MERS-coronavirus	N/A	N/A	NSS	NSS	NSS
Adenovirus (e.g. C1 Ad. 71)	N/A	N/A	NSS	NSS	NSS
Human Metapneumovirus (hMPV)	N/A	N/A	NSS	NSS	NSS
Parainfluenza virus 1-4	N/A	N/A	NSS	NSS	NSS
Influenza A	A/Chicken/Hong Kong/G9/97 (H9N2)	AF156472.2	75	NSS	NSS
Influenza A	A/swine/South Dakota/A01678604/2018 (H1N2)	MK380012.1	NSS	65	NSS
Influenza B	B/clinical isolate SA8 Thailand/2002	AY880080.1	55	NSS	NSS
Influenza B	B/Temple/B17/2003	CY018203.1	NSS	50	NSS
Enterovirus (e.g. EV68)	C99 isolate YT31/SD/CHN/11	KJ857508.1	65	NSS	NSS
Enterovirus (e.g. EV68)	A71 strain EV71/Homo sapiens/VNM/228/2011	KJ686167.1	NSS	80	NSS
Respiratory syncytial virus	RSV Memphis-37	KM360090.1	NSS	60	NSS
Rhinovirus	strain 20693_1_HRV-A	MK989737.1	55	80	NSS
Chlamydia pneumonia	AR39	AE002161.1	55	55	NSS
Haemophilus influenzae	strain M15895	CP031249.1	60	60	NSS
Legionella pneumophila	strain D5945	CP017602.1	70	60	NSS
Mycobacterium tuberculosis	strain TCDC7	CP047163.1	65	65	NSS
Streptococus pneumonia	strain 180-15	LR129844.1	60	60	48
Streptococcus pyrogenes	strain emm197	CP035455.1	60	60	43
Bordetella pertussis	strain J494	CP032728.1	60	NSS	NSS

COVID-19 *in silico* cross-reactivity results			Homology (0-100%)[1]		
Pathogen	**Strain[2]**	**GenBank Acc #**	**FP[3]**	**RP[3]**	**Probe[4]**
Mycoplasma pneumoniae	19294	CP010539.1	55	65	NSS
Pneumocystis jirovecii (PJP)	RU7	XM_18374818	65	65	40
Influenza C	N/A	N/A	NSS	NSS	NSS
Parechovirus	6 isolate AFW	MG462718.1	65	NSS	NSS
Parechovirus	2 isolate 51Chzj674	KT879919.1	NSS	55	NSS
Candida albicans	strain TIMM 1768	CP032012.1	60	65	38
Corynebacterium diphtheriae	strain CHUV2995	LT990688.1	65	65	NSS
Legionella non-pneumophila	hackeliae	LN681225.1	NSS	NSS	69
Legionella non-pneumophila	spiritensis strain NCTC12082	LR134374.1	80	65	NSS
Bacillus anthracosis (Anthrax)	strain MCCC 1A02161	CP031642.1	60	60	41
Moraxella cararrhalis	strain MC8	CP010902.1	65	60	35
Neisseria elongate and miningitidis	animaloris strain NCTC12228	LR134440.1	60	60	48
Neisseria elongate and miningitidis	elongata strain M15910	CP031255.1	65	70	NSS
Pseudomonas aeruginosa	strain AR_0356	CP027169.1	60	60	NSS
Staphylococcus epidermis	strain SE95	CP024437.1	60	60	NSS
Streptococcus salivarius	JIM8777	FR873482.1	65	60	41
Leptospirosis	santarosai strain U164	CP028370.1	60	60	NSS
Chlamydia psittaci	RD1	FQ482149.1	65	55	41
Coxiella burneti (Q-Fever)	RSA 331	CP000890.1	55	60	38
Staphylococcus aureus	strain UP_1097	CP047803.1	70	65	NSS

[1]Homology = Coverage * Identity; no regard to gene or target
[2]Strain/accession with highest homology listed
[3]Primer parameters: Somewhat similar (blastn) Expect 1000, Word size 11, Match/Mismatch 1,-3
[4]Probe parameters: Somewhat similar (blastn) Expect 1000, Word size 15, Match/Mismatch 2,-3
[5]NSS = No Significant Similarity
[6]For SARS coronavirus, the probe has 3 mismatches in 14 basepairs of the 3' flanking sequence (79% homology)

Internal Control

HIPC *in silico* cross-reactivity results			Homology (0-100%)[1]		
Pathogen	**Strain[2]**	**GenBank Acc #**	**FP[3]**	**RP[3]**	**Probe[4]**
Human coronavirus 229E	N/A	N/A	NSS[5]	NSS	NSS
Human coronavirus OC43	N/A	N/A	NSS	NSS	NSS
Human coronavirus HKU1	N/A	N/A	NSS	NSS	NSS
Human coronavirus NL63	HCoV_NL63/Seattle/USA/ SC0768/2019	MN306040.1	NSS	48	NSS
SARS-coronavirus	BJ04	AY279354.2	NSS	48	NSS
MERS-coronavirus	P.khulii/Italy/206645-63/2011	MG596803.1	NSS	48	NSS
Adenovirus (e.g. C1 Ad. 71)	N/A	N/A	NSS	NSS	NSS
Human Metapneumovirus (hMPV)	Homo sapiens/PER/FPI01306/ 2011/A	KJ627415.1	52	47	NSS
Parainfluenza virus 1-4	HPIV4a/Seattle/USA/ SC0377/2019	MN306027.1	52	NSS	NSS
Influenza A	A/mallard/Alberta/152/ 2006(H1N3)	CY137666.1	57	NSS	NSS
Influenza B	B/Washington/48/2016	KX612329.1	52	NSS	NSS
Influenza B	B/New Jersey/12/2020	MT556940.1	NSS	47	NSS
Enterovirus (e.g. EV68)	90 isolate 10485a2	AY919469.1	57	NSS	NSS
Enterovirus (e.g. EV68)	E11 isolate AFP336-GD-CHN-2017	MN597924.1	NSS	57	NSS
Respiratory syncytial virus	N/A	N/A	NSS	NSS	NSS
Rhinovirus	A strain HRV-A76_p1194_sR843_2008	JX074055.1	67	NSS	NSS
Rhinovirus	C C9-NIID JPN-2015	LC428175.1	NSS	57	NSS
Chlamydia pneumonia	Wien3	LN847257.1	62	48	NSS
Haemophilus influenzae	strain M14951	CP031244.1	67	48	NSS
Legionella pneumophila	fraseri strain F-4198	CP021279.1	57	65	NSS
Mycobacterium tuberculosis	strain TCDC7	CP047163.1	62	NSS	NSS
Streptococus pneumonia	strain 6A-10	CP053210.1	57	61	NSS
Streptococcus pyrogenes	strain emm22.8	CP035438.1	62	52	35
Bordetella pertussis	strain J029	CP046995.1	62	52	NSS

HIPC *in silico* cross-reactivity results			Homology (0-100%)[1]		
Pathogen	**Strain[2]**	**GenBank Acc #**	**FP[3]**	**RP[3]**	**Probe[4]**
Mycoplasma pneumoniae	strain 16-734	CP039761.1	57	48	NSS
Pneumocystis jirovecii (PJP)	RU7	XM_018373345	52	52	NSS
Influenza C	N/A	N/A	NSS	NSS	NSS
Parechovirus	5 isolate G002-19/Vic/Jul/19	MN451649.1	NSS	52	NSS
Candida albicans	strain TIMM 1768	CP032015.1	62	52	NSS
Corynebacterium diphtheriae	strain CN2000	CP039522.1	71	48	NSS
Legionella non-pneumophila	TUM19329	AP022839.1	67	NSS	NSS
Legionella non-pneumophila	cherrii strain NCTC11976	LR134173.1	57	65	NSS
Bacillus anthracosis (Anthrax)	strain FDAARGOS_699	CP050973.1	57	52	NSS
Moraxella cararrhalis	strain MC8	CP010902.1	57	52	NSS
Neisseria elongate and miningitidis	strain AUSMDU00005726	LR134440.1	67	52	NSS
Neisseria elongate and miningitidis	elongata strain M15911	CP031252.1	67	52	NSS
Pseudomonas aeruginosa	strain IMP66	CP028959.1	67	61	NSS
Pseudomonas aeruginosa	strain PAC6	CP053705.1	67	NSS	35
Staphylococcus epidermis	strain SESURV_p2_0614	CP043788.1	62	57	NSS
Staphylococcus epidermis	strain NCTC4133	LR134242.1	57	52	35
Streptococcus salivarius	strain ICDC1	CP018186.1	57	48	35
Leptospirosis	mayottensis strain MDI222	CP030144.1	62	48	35
Leptospirosis	santarosai strain U160	CP027843.1	71	52	NSS
Leptospirosis	interrogans serovar Linhai str. 56609	CP006723.1	57	65	NSS
Chlamydia psittaci	strain AMK	CP047319.1	62	48	NSS
Coxiella burneti (Q-Fever)	strain RSA439	CP040059.1	52	48	NSS
Staphylococcus aureus	strain 14507	CP053356.1	NSS	52	57
Staphylococcus aureus	KUH140087	AP020315.1	NSS	65	NSS

9.4 Microbial Interference

Based on the *in silico* analysis, none of the organisms are expected to interfere with the test primers/probe. Only SARS coronavirus, which is known to be genetically similar to SARS-CoV-2 has a high homology with one of the probe sequence. Using the broad parameters for the BLAST search, only three non-coronavirus organisms were found to have 80% homology with one of the test primers. However, none of Enterovirus A71 strain EV71 (KJ686167.1), rhinovirus strain 20693_1_HRV-A (MK989737.1), or Legionella spiritensis strain NCTC12082 (LR134374.1) had any significant similarity to the probe sequence. The more stringent default BLAST parameters do not identify these isolates as sequence matches. In fact, the short primer sequences are more likely to be randomly found in the human genome than these relatively small microbial genomes, yet we observe no effects when detecting SARS-CoV-2 RNA within a human sample matrix.

As explained in the previous section, the SARS coronavirus, which is known to be genetically similar to SARS-CoV-2 has a high homology with the probe sequence. We have accounted for this similarity in the design of the probe.

Endogenous Interference Substances Studies

So far, 30 known negative samples, 30 known positive samples and pools from 30 other negative samples (all confirmed by a different platform and test authorized by the FDA under the Emergency Use Authorization) have been tested using the MatMaCorp COVID-19 test. To date, all 60 samples tested individually have worked fine where both the virus and the internal HIPC test worked as expected showing no inhibition. Similarly, pooled negative samples worked as expected with the internal HIPC and when spiked with virus, both HIPC and the virus sample also worked. This shows that endogenous interference is not a major concern with the MatMaCorp Cocid-19 test.

9.5 Clinical Evaluation

The clinical evaluations were done with natural clinical positive samples, contrived clinical positive samples, natural clinical negative sample and with contrived clinical negative samples.

Natural Clinical Negative Samples used to generate Contrived Positive and Negative Samples

Clinical samples that were determined to be negative by a different test authorized by the FDA under the Emergency Use Authorization (EUA) were used to make dilutions of an inactivated virus. To get a representative negative sample, equal volumes from the 30 negative samples were pooled and mixed well. This was the dilution matrix used to create positive contrived samples.

Virus sample used

A gamma-irradiated SARS-Related Coronavirus 2, isolate USA-WA1/2020 (NRC-52281 lot 7003364, provided by BEI Resources). This had a titer of 2.8 x10^5 TCID50 per mL and 1.7 x 10^9 genome equivalents per mL according to the Certificate of Analysis provided with the sample.

Contrived Positive Clinical Samples

Using the clinical negative samples and the virus sample as described above, two sets of 24 samples each were made by spiking the inactivated virus into the negative samples. The first set were 2.5X LoD and the second set were 5.0X LoD.

Natural Clinical Positive Samples

A set of samples determined to be positive by a different test authorized by the FDA under the Emergency Use Authorization (EUA) were used in this study.

Natural Clinical Negative Samples

A set of samples, determined to be negative by different test authorized by the FDA under the Emergency Use Authorization (EUA) were used in this study

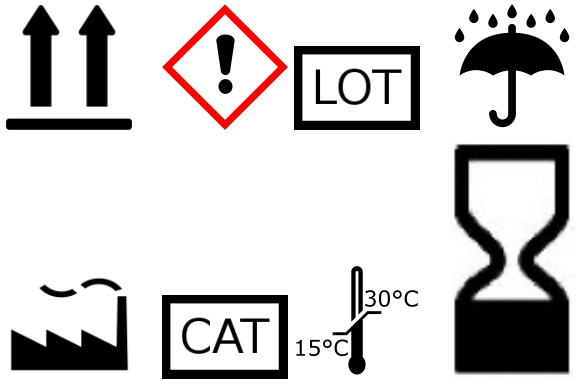
Clinical Study Results

Sample	Result	Pos. Hit Rate (%)	2-sided 95% CI
Dilution matrix (pooled negative)	0/20	0	-
2.5 x LOD	23/24	95.8	79.8 - 99.3
5 x LOD	24/24	100	86.2 - 100
Clinical Positive	29/30	96.7	83.3 - 99.4
Clinical Negative	0/30	0	-

As shown in the table above, 95.8% of low positives were called positive and 100% of all high positives were called positive and all negative samples were negative. The data in red was done with contrived samples. The pooled negatives were repeated 20 times. The data in black is the clinical samples provided by a regional hospital. Thirty negatives and 30 positives were checked individually.

10 Symbols

The following symbols are used in labeling of MatMaCorp COVID-19 test kits:



The Solas 8 instrument fulfills the following requirement:

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