

INSTRUCTIONS FOR USE

For use under the Emergency Use Authorization (EUA) only the information in this document has not been evaluated by the FDA

For in vitro diagnostic use

Facible Q-LAAD SARS -CoV-2 Test

Intended Use:

Facible's Quantum-Logic Aptamer Analyte Detection (Q-LAAD) SARS-CoV-2 test is a high throughput fluorescence-based test designed for use with fluorescence microplate readers capable of fluorescence measurements intended for the qualitative detection of the ***spike protein*** antigen from SARS-CoV-2 in anterior nares swab specimens stored in saline solution from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform ***moderate complexity***.

Results are for the identification of SARS-CoV-2 Spike-1 protein antigen. Antigen is generally detectable in respiratory specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results should be treated as presumptive, and do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary, for patient management.

The Q-LAAD SARS-CoV-2 test is intended for use by trained clinical laboratory personnel specifically instructed and trained in vitro diagnostic procedures]. The Q-LAAD SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Component	Description
Q-LAAD SARS-CoV-2 probe plate	ready-to-use 96-well microplate containing covalently attached probe for the detection of SARS-CoV-2 spike-1 antigen
Q-LAAD SARS-CoV-2 test buffer	250 mL, 1x concentrated salt-based buffer for testing
Q-LAAD SARS-CoV-2 assay detection reagent	500 µL, concentrated solution containing reporter molecule in 50% ethanol solvent
Clinical Microplate seals	Transparent sheet coated in acrylate adhesive to minimize any possible contamination during the analyzing of the microplate after completion of the assay
Positive Control	Solution of recombinant spike-1 protein in Staphylococcus epidermidis

Materials Required but not Included:

- Multichannel pipettes for 10 and 200 microliter volumes
- Pipettes for 200 and 1000 microliter volumes
- Disposable, low retention, RNase/DNase free pipette tips with aerosol filters suitable for the above volumes
- 95% ethanol
- 2mL tubes
- 200 microliter tubes
- Negative control

Component Required but not supplied

- Fluorescence Microplate reader
 - Biotek Synergy Lx with filters or H1 Synergy model with monochromator
- Gen5 Software

Warnings and Precautions

Caution: Cut corners on plate should be facing user

Warning: If probe at bottom of well is damaged during pipetting, results for well are invalid

Warning: Do not remove clear plate cover during disposal process

Kit Storage and Stability

Store the microplates at 4 deg Celsius.

Positive Control material should be stored at frozen -20 Celsius

Store all other components at room temperature

Biotek Synergy Lx Microplate Reader Configuration

- See Biotek configuration instructions for step-by-step details
- Read: Fluorescence Endpoint, Full Plate
- Filter Set 1 (575/610)
- Excitation 575/10, Emission: 610/10
 - Mirror: Top 595 nm, Gain: 70
 - Light Source Tungsten
 - Standard Dynamic Range
 - Read Speed: Normal, Delay: 100 msec, Measurement/ Data Point :10
 - Read Height: 8.25 mm

Test Procedure

Sample Collection

Facible's Q-LAAD SARS-CoV-2test is designed to test anterior nares samples collected by trained professional and stored in 0.9% saline solution.

- **Supplies for collection**
 - 15 mL conical containing 2 mL Medline saline.
 - 6" plastic handle swabs
- **Instructions for sample collection**

- 1) Remove swab from packaging.
- 2) Insert the swab into the anterior nares (high into the nose) cavity and rotate the swab for 15 seconds. Repeat on other anterior nares.
- 3) Break the swab two inches from the non-cotton side.
- 4) Place the swab into the collection tube cotton side down. Store vertically.
- 5) Allow the swab to incubate in the collection buffer for a minimum of 20 minutes. Vortex sample with swab in the conical for 5 seconds.
- 6) Remove the swab from collection buffer using forceps in a sterile manner. If removing multiple swabs, sanitize forceps with 70% ethanol between each swab to prevent cross-contamination.

Sample storage and transport

Samples should be tested as soon as possible. If testing immediately is not possible, store at 2-8° C for up to 48 hours before testing. If longer storage is expected, freeze each collection tube at -80° C.

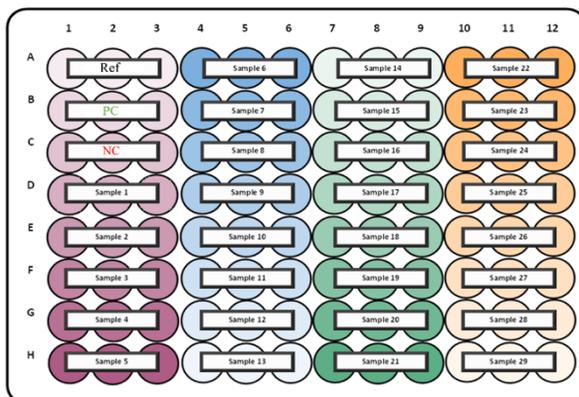
Sample Preparation

1. Allow samples to come to room temperature.
2. Prepare samples for testing by adding 35 microliters of each specimen or control to individual 200 microliter tubes.

Operator Instructions

1. Review microplate sample layout in figure 1 below. The Biotek plate reader software must be programmed to match the layout shown in **Figure 1** below.
2. Open the plate package and remove plate from package. Discard packaging.
3. Add 200 microliters of Q-LAAD sample buffer to each well of the dry microplate to prepare the plate.
4. Remove all buffer from each well using a multichannel pipette.
5. Use a multichannel pipette to add 100 microliters of Q-LAAD sample buffer to all wells.
6. Use a multichannel pipette to add 10 microliters of each sample to the appropriate wells from the prepared tubes and mix up and down 3x.
7. Incubate the plate for 10 minutes at room temperature.
8. Following the 10-minute incubation, use a multichannel pipette to remove the 110 microliters of solution from each well and discard in accordance with local state and federal regulations.
9. Add 100 microliters of Q-LAAD sample buffer to each well using a multichannel pipette.
10. Prepare the detection reagent.
11. Shake the 25x detection reagent tube by hand for 3 seconds. Do not vortex.
12. Prepare detection reagent by mixing 80 microliters of 25x detection reagent with 1920 microliters of 10% ethanol prepared from 95% ethanol in a tube and mix briefly. Do not centrifuge.
13. Add detection reagent to a reservoir boat compatible with the multichannel pipette and **use immediately**.
14. Using a multichannel pipette, dispense 5 microliters of detection reagent into each well and pipette up and down 3 times to mix. Avoid forming bubbles.
15. Seal with clear plate sealer and incubate for 5 minutes in the dark at room temperature.
Recommendation: incubate in the plate reader.
16. Place the microplate in the microplate reader, read the plate and collect the data and report.
17. Discard all hazardous waste materials in accordance with local state and federal regulations.

Figure 1. Microplate layout.



Well ID	Type
Ref	Reference Dye
PC	Pos Control
NC	Neg Control

Biotek programming instructions

Prepare Microplate reader

1. Designate a system Administrator.
2. Install Gen5 on the Administrator's computer
3. Change the Systems Administrator password
4. Determine the optimal way to store Gen5's protocol and experiment files
5. Install Gen5 for other users and connect a reader to each computer
 - a. Turn on reader
 - b. From the menu, select **System > Reader Configuration**
 - c. Click the **Add** button to define the Reader Settings
 - d. Use the drop-down list to select the **Reader Type**
 - i. Select **SynergyLX**
 - e. In the **Com Port** text field, enter the number of the communications port
 - f. Retain the default **Baud Rate**
 - g. Click **Test Comm**. Gen5 will attempt to communicate with the reader
 - h. After you receive a passing message, "The reader is communicating," click **OK** and then click **Close** at Reader Configuration

Create New Protocol

6. Select **File>New Protocol**
7. Select **Protocol>Procedure**
8. Click the **Read** Button and change the **Detection Method** to **Fluorescence**
9. To set the filter Set, Use the drop-down list to select the filter for **Excitation** and **Emission**, enter **excitation- 575/10** and **emission- 610/10**
10. Enter **70** for the Sensitivity setting
11. Click **OK** twice to save and close the procedure

Plate Layout

12. Define plate layout in the usual way to reflect the arrangement of unknown samples, reference dye, positive and negative controls.
 - a. Select **Protocol>Plate Layout**
 - b. In the Well Settings box, select the Type of specimen, first **Reference**, then **Controls**, then **Samples**
 - c. Set the **Replicates** to 3
 - d. Assign the well IDs to their corresponding locations in the plate grid by clicking in the wells in the matrix
 - i. Use the Replicates options to speed up your work: Set the options and click and drag to fill multiple wells at once. Click a column or row header to fill it
 - ii. Select **OK** to save plate layout step
13. Select **Protocol>Data Reduction**
14. Click **Validation** in Data Reduction tab
 - a. In the **Label** section, label: **Cutoff Ranges**
 - b. For 1. **Data In** select 575/10,610/10
 - c. For 1. **Valid Formula** write the formula: PC<2047
 - d. For 2. **Data In** select 575/10,610/10

- e. For 2. **Valid Formula** write the formula: $NC > 2047$
 - f. For 3. **Data In** select 575/10,610/10
 - g. For 3. **Valid Formula** write the formula: $Ref > 1236$
 - h. For 4. **Data In** select 575/10,610/10
 - i. For 4. **Valid Formula** write the formula: $Ref < 5239$
 - j. Click **Ok** to save validation step
15. Click **Transformation**
- a. For the **Data In** tab, select 575/10,610/10
 - b. For the **New Data Set Name**, label it "Rel to Ref" and check the "show" option
 - c. In the **Current Formula** section, type the formula:
 $IF(Ref > 3400; x * (Ref / 3400); x * (Ref / Ref))$ and apply it to all sample wells. Note: Do not apply the formula to Ref, PC, NC wells
 - d. Click **OK** to save
16. Click **Transformation**
- a. **Data In:**
 - i. DS1: Current Plate Rel to Ref
 - ii. DS2: Current Plate 575/10,610/10
 - b. For the **New Data Set Name**, write "Mean Values" and check the "show" box
 - c. Select the first well, **A1**, and in the **Current formula** section write the formula:
 $Mean(Ref)$
 - d. Select the well, **B1**, and in the **Current formula** section write the formula: $Mean(PC)$
 - e. Select the well, **C1**, and in the **Current formula** section write the formula: $Mean(NC)$
 - f. Repeat previous steps for SPL1...SPL29 only adding a formula to first well
 - g. Click **OK** to save
17. Click **Cutoff**
- a. Data In: **Rel to ref**
 - b. Check the "Round values before evaluation" box
 - c. Significant digits: 5
 - d. Cutoff Formula 1: 2047
 - e. Symbol 1: **POS** (click the 3 dots to change color to green)
 - f. Symbol 2: **NEG** (click the 3 dots to change color to red)
 - g. Make sure both arrows between the Cutoff Formulas and Symbols are facing **Down**
 - h. Click **OK** to save
18. Click **Cutoff**
- a. Data In: **Mean Values**
 - b. Check the "Round values before evaluation" box
 - c. Significant digits: 5
 - d. Cutoff Formula 1: 2047
 - e. Symbol 1: **POS** (click the 3 dots to change color to green)
 - f. Symbol 2: **NEG** (click the 3 dots to change color to red)
 - g. Make sure both arrows between the Cutoff Formulas and Symbols are facing **Down**
 - h. Click **OK** to save
19. Click **OK** to save **Data Reduction** step

Build the Report

- 20. Open **Report Builder** from the menu tree
- 21. Click **New export to Excel**.
- 22. In the Properties section:

- a. Under scope, select: **Plate** (Most common. Results are grouped by plate)
- b. Under Content, select: **Automatic**
23. In the **Content section**:
 - c. Under **General Information**, Select:
 - i. System Description
 - ii. Procedure
 - iii. Plate Description
 - iv. Plate Layout Matrix
 - d. Under **Data**, Include:
 - i. Raw Data
 - ii. Calculated data
 - e. Under **General Format**, select: Matrix
 - f. Check the box: Regroup data in one matrix/table when possible
 - g. Under Kinetic/spectrum/scanning data format, select: Column-wise table
24. In the **Workflow Section**:
 - h. Check the “Auto-execute on completion of the procedure” box
 - i. Under **Export Mode**, Select: All plates in the same workbook
 - j. Select: As a new worksheet
25. In the **Format Section**:
 - k. Under **Text Format**, Select: Formatted text
 - l. Check the “Export row and column headers” box
26. Click **OK** to save
27. Click **New Report**.
28. In the **Properties** tab:
 - a. Scope: **Patient/Sample**
 - b. Content: **Automatic**
29. In the **Content** tab:
 - a. General Information: **Plate Description**
 - b. Data: **Raw data, Calculated data**
 - c. General Format: **Row-wise table**
 - i. Check the “**Regroup data in one matrix/table when possible**” box
 - d. Kinetic/spectrum/scanning data format: **Ignore**
30. In the Workflow tab: Select “**Auto-execute on completion of the procedure (reading the plate)**”
31. Click **OK** to save Report/Export Builder step

Run Experiment

32. Click the **Read** button
33. Select **File>Save** and give the experiment file a unique name
34. After reading plate, Open the Plate View and select **Matrix** tab
35. At the Data field, use the drop-down list to select the **Symbols Mean Values**
36. An excel document should pop up with a matrix containing; mean values and Positive/Negative calls for the plate

Limitations

- Viral transport media should not be used with Facible’s Q-LAAD SARS-CoV-2 test.
- Phosphate buffered Saline should not be used with Facible’s Q-LAAD SARS-CoV-2 test.

- The test is to be used for qualitative detection of SARS CoV-2 antigens from nasal swabs stored in 0.9% saline solution.
- A negative test result may occur if the concentration of antigen is below the limit of detection at the time of collection.
- A negative result may occur if the concentration of antigen decreases due to improper handling or transport of the sample.
- Failure to follow the test procedure may adversely affect test performance and /or invalidate the test result.
- Test results must be evaluated with other clinical data available to the physician.
- Positive results do not rule out co-infections with other pathogens.
- If the differentiation of specific SARS virus strains is required, additional testing is required.

Limitations

Performance data:

Clinical Blind Study		PCR	
		+	-
Q-LAAD	+	28	6
	-	2	24
		93%	80%

Q-LAAD demonstrated and overall agreement of 87% (PPV 93%, NPV 80%).

Ct Value	21	34	37	38
GE/mL*	6.03E+6	3.61E+02	3.82E+01	1.81E+01
Number of Correctly Identified Positives	19/20 (95%)	19/20 (95%)	19/20 (95%)	20/40 (50%)

Q-LAAD technology demonstrates 95% accuracy at a limit of detection of 38.2 virus/mL.*

*Genomic Equivalents per milliliter (GE/mL) are calculated from an inactivated virus standard curve.

Organism (ATCC cat #)	Stock (CFU/mL)	Final (CFU/mL)	Positive Results
Human coronavirus 229E (VR-740)	1.45E+07	1.00E+05	0/4
Human coronavirus OC43 (VR-1558)	3.10E+09	1.00E+05	0/4
Rhinovirus (VR-1645)	6.00E+06	1.00E+05	0/4
<i>Haemophilus influenzae</i> (51907)	4.50E+08	1.00E+06	0/4
<i>Streptococcus pneumoniae</i> (49619)	2.80E+09	1.00E+06	0/4
<i>Streptococcus pyogenes</i> (19615)	1.44E+08	1.00E+06	0/4

<i>Candida albicans</i> (18804)	3.96E+08	1.00E+06	0/4
Pooled human nasal wash	n/a	n/a	0/4
Adenovirus 5 (VR-1516)	5.80E+11	1.00E+05	0/4
Parainfluenza virus 3 (VR-1782)	3.50E+06	1.00E+05	0/4
Parainfluenza virus 1 (VR-94)	2.50E+06	1.00E+05	0/4
Influenza A (VR-1520)	1.60E+07	1.00E+05	0/4
Influenza B (VR-1931)	8.00E+06	1.00E+05	0/4
Enterovirus (VR-1432)	3.20E+06	1.00E+05	0/4
Respiratory syncytial virus (VR-26)	2.00E+06	1.00E+05	0/4
<i>Bordetella pertussis</i> (9797)	4.40E+09	1.00E+06	0/4
<i>Mycoplasma pneumoniae</i> (15531)	1.20E+08	1.00E+06	2/4
<i>Chlamydia pneumoniae</i> (53592)	n/a	1.00E+06	0/4
<i>Staphylococcus aureus</i> (12600)	1.20E+09	1.00E+06	0/4
<i>Staphylococcus epidermidis</i> (CRM-12228)	2.30E+09	1.00E+06	0/4

Q-LAAD exhibits slight non-specificity with *M. pneumoniae* at high doses concentrations (10^6 CFU)

Mycoplasma pneumoniae (15531) Dose Response			
CFU	Avg Signal	N	Result
1x10 ²	3346	3	Negative
1x10 ³	2935	3	Negative
1x10 ⁴	3659	3	Negative
1x10 ⁵	2285	3	Negative
1x10 ⁶	1765	3	Positive

Potential Interfering Pathogen	EUA Recommended Concentration	Pool	Interference
<i>Mycoplasma pneumoniae</i> (15531)	1.00E+06	1	None
<i>Streptococcus pneumoniae</i> (49619)	1.00E+06	1	None
<i>Haemophilus influenzae</i> (51907)	1.00E+06	1	None
Adenovirus 5 (VR-1516)	1.00E+05	1	None
<i>Bordetella pertussis</i> (9797)	1.00E+06	1	None
<i>Streptococcus pyogenes</i> (19615)	1.00E+06	2	None
Rhinovirus (VR-1645)	1.00E+05	2	None
Human coronavirus OC43 (VR-1558)	1.00E+05	2	None
<i>Candida albicans</i> (18804)	1.00E+06	2	None
<i>Staphylococcus aureus</i> (12600)	1.00E+06	2	None
Human coronavirus 229E (VR-740)	1.00E+05	3	None
Respiratory syncytial virus (VR-26)	1.00E+05	3	None

Parainfluenza virus 1 (VR-94)	1.00E+05	3	None
<i>Staphylococcus epidermidis</i> (CRM-12228)	1.00E+06	3	None
Parainfluenza virus 3 (VR-1782)	1.00E+05	4	None
Influenza A (VR-1520)	1.00E+05	4	None
Influenza B (VR-1931)	1.00E+05	4	None
Enterovirus (VR-1432)	1.00E+05	4	None

Pool	Expected Result	Actual Result
1	Positive	Positive
2	Positive	Positive
3	Positive	Positive
4	Positive	Positive

No microbial interferences observed.

Substance	Concentration	Results
Whole Blood	4%	No Interference
Mucin	0.50%	No Interference
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	No Interference
Naso GEL (NeilMed)	5% v/v	No Interference
CVS Nasal Drops (Phenylephrine)	15% v/v	No Interference
Afrin (Oxymetazoline)	15% v/v	No Interference
CVS Nasal Spray (Cromolyn)	15% v/v	No Interference
Zicam	5% v/v	No Interference
Homeopathic (Alkalol)	1:10 dilution	No Interference
Sore Throat Phenol Spray	15% v/v	No Interference
Tobramycin	4 µg/mL	No Interference
Mupirocin	10 mg/mL	No Interference
Fluticasone Propionate	5% v/v	No Interference

No endogenous interferences observed.

Quick Reference instructions:

See Facible Q-LAAD SARS-CoV-2 Quick Start Guide/Package Insert