Research Use Only Manual

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. This test is designed for use with fluorescence microplate readers capable of fluorescence measurements and is intended for the qualitative detection of the spike protein antigen(s) from SARS-CoV-2 virus. The test uses 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 and high complexity tests.

Results are for the identification of SARS-CoV-2 Spike protein antigen. The antigen is generally detectable in upper 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 for Research

Overview and Test Principle

The **Facible Q-LAAD SARS-CoV-2** high-throughput test is an aptamer-based fluorescence test. Based on our revolutionary Quantum-Logic Aptamer Analyte Detection (Q-LAAD) platform, our **Q-LAAD SARS-CoV-2 test** is designed to detect the SARS-CoV-2 antigen in **anterior nares swabs collected in 0.9% saline solution** from patients who are suspected of having COVID-19 by their healthcare provider within the first 7 days of symptom onset, or for screening of individuals without symptoms or other reasons to suspect COVID-19 infection. Facible's Q-LAAD SARS-CoV-2 test was specifically engineered to work on standard laboratory equipment using readily available materials and fit within standard moderate and high-complexity laboratory workflows.

We have developed an aptamer with two distinct binding pockets that are made allosteric through a communication linker. This type of aptamer is called a Fluorogenic Logic-gated Aptamer (FLA). We developed this dual-logic aptamer has to allow for an easy and accurate detection of the binding of the target SARS-CoV-2 spike protein. The specific binding of SARS-CoV-2 spike protein triggers the folding of the second "logic" binding pocket to enable binding of a reporter molecule (or fluorophore). We have attached the biotin modified FLA to the plastic surface in the bottom of each well of standard 96-well microplates using biotin-Streptavidin chemistry. The biotin-streptavidin arrives to the customer already conjugated and ready to be used. No conjugations steps are needed by the technologist to perform this assay.

This test does not have a sample extraction or preparation steps that require specific instruments or reagents to incubate the sample for analyte detection, reducing test complexity. The sample is simply added directly to the wells containing buffer and incubated for 10 minutes at room temperature. After specimen incubation and removal of unbound sample, the detection reagent is added. The detection reagent contains a fluorogenic small molecule which is subsequently recognized and bound by the second logic pocket. The second binding of the fluorophore is conditional upon binding of the SARS-CoV-2 spike protein. Once the fluorophore is bound, a change in fluorescent signal is measured using a microplate reader at the endpoint of the 5-minute room temperature incubation.

Our high throughput Q-LAAD SARS-CoV-2 test combines the speed and relative simplicity of an antigen test with the sensitivity of a molecular test. This fluorescence-based assay utilizes a 96-well plate format that will fit into the normal workflow of any ***moderate complexity*** lab. The test is easy to setup, it is significantly faster for the technician to prepare, and requires only 5 minutes on the instrument. The BioTek plate reader with Gen5 software provides results without technician interpretation. With an optimized workflow, Facible's Q-LAAD SARS-CoV-2 assay can yield a throughput of 348 of tests per hour per instrument.

MATERIALS INCLUDED

Component	*Description*	*Quantity*
Q-LAAD CoV-2 Probe Plate	Functionalized microplate containing bound DNA aptamer for the detection of SARS-CoV-2	(5) Individual plates, foil packaged with desiccant
Q-LAAD CoV-2 Buffer	pH 7.5, Sterile, salt-based buffer.	(1) bottle of 250 mL total volume
Q-LAAD CoV-2 Detection Reagent	5 µM reporter molecule in solution of 50% ethanol.	(1) 2 mL bottle of 500 µL total volume
Microplate Seals	Acrylic, Adhesive, Clear seal to prevent microplate spillage	(1) Package containing 5 Microplate sealers
Package insert	Instructions for use	(1) paper pamphlet
Positive Quality Control solution	Recombinant SARS-CoV-2 spike-1 protein (0.85 µg/mL) in saline. Native Antigen Company, Catalog No. REC31806	(1) 2 mL vial
Negative Quality Control Solution	Heat-inactivated Vero cells at a concentration of 2.4x105 cells/mL in saline. Vero, ATCC, Catalog No. CCL-81	(1) 2 mL vial

SPECIAL INSTRUMENT REQUIRED

- BioTek Microplate Reader
- BioTek Custom Filter Cube: Excitation wavelength 575±10nm; Dichroic mirror 595 nm, Emission wavelength 610±10nm
- BioTek Gen5 Software

Materials Required but not Included:

Component	Description	Source	Catalog#
Polystyrene Pipette Basin	Argos Polystyrene, 55 mL capacity, Sterile, White, DNase-RNase free, Non-pyrogenic, 5 per pack, Used for Q-LAAD Buffer or similar	Fisher Scientific	03-391-536
Polypropylene Pipette Basin	Integra Polypropylene, 25 mL, 200 per pack, Used for Q-LAAD Detection Reagent or similar	Fisher Scientific	NC1529985
Saline Collection Vial	0.9% Saline solution, 3mL in 10mL Tube, 50/pack or similar	Fisher Scientific	NC1909168
Collection Swab	Flocked, Sterile, Nylon Fiber, individually wrapped or similar	Fisher Scientific	22-349-820
5 mL Polypropylene Snap cap tube	Argos Black 5 mL polypropylene snap cap tube or similar	Fisher Scientific	03-391-275
Ethanol	70% purity, ethanol solution, Molecular Biology grade, CAS#64-17-5 or similar	Fisher Scientific	BP82014
Pipette Tips, 0.1 – 10 µL	ep Dualfilter T.I.P.S.® LoRetention®, PCR clean and sterile, 0.1 – 10 µL S, 34 mm, dark gray, colorless tips, 960 tips (10 racks × 96 tips) or similar	Fisher Scientific	05-413-959
Pipette Tips, 2 – 200 µL	ep Dualfilter T.I.P.S.® LoRetention®, PCR clean and sterile, 2 – 200 µL, 55 mm, yellow, colorless tips, 960 tips (10 racks × 96 tips) or similar	Fisher Scientific	05-413-952
Pipette Tips, 50 – 1000 µL	ep Dualfilter T.I.P.S.® LoRetention®, PCR clean and sterile, 50 – 1,000 µL, 76 mm, blue, colorless tips, 960 tips (10 racks × 96 tips) or similar	Fisher Scientific	05-413-964
Multichannel Pipette, 0.5 – 10 µL	Gilson, PIPETMAN L Multichannel, P12x10L, 12 channel, 0.5-10 µL or similar	Fisher Scientific	FA10014G
Multichannel Pipette, 10 – 100 µL	Eppendorf Research® plus, 8-channel, variable, incl. epT.I.P.S.® Box, 10 – 100 µL, yellow or similar	Fisher Scientific	13-690-048
Multichannel Pipette, 20 – 300 µL	Gilson, PIPETMAN L Multichannel, P12x300L, 12 channel, 20-30 0µL or similar	Fisher Scientific	FA10016G

Kit Storage and Stability
Store the microplates at 2-8° C. for up to 6 months
Control materials should be stored at -20° C. up to 6 months
Store all other components at room temperature

BioTek Synergy Lx Microplate Reader Configuration

- 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

Troubleshooting
Refer to troubleshooting document

Q-LAAD SARS CoV-2 Test Procedure

Sample Collection

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

Suggested Supplies for Collection

- 15 mL conical containing 2 mL 0.9% Medline saline
- 6" plastic handle FLOQswabs

Instructions for sample collection

1) Remove swab from packaging.
2) Insert the swab into the anterior nares cavity and rotate the swab for 15 seconds. Repeat on other anterior nares.
3) Break the swab at the break line.
4) Place the swab into the collection tube swab-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 24 hours before testing. If longer storage is expected, freeze each collection tube at -20°-80° C after swab removal.

Sample Preparation

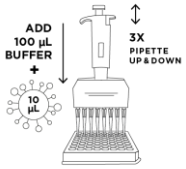
1. Allow samples to come to room temperature.
2. Prepare samples for testing by adding 35 microliters of each specimen or control to individual 200 microliter tubes.

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.

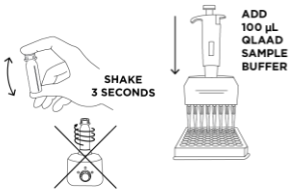


7. Incubate the plate for 10 minutes at room temperature.

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.

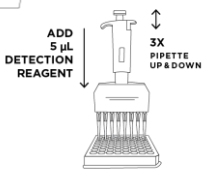
10. Prepare the detection reagent by following steps 11 and 12.
11. Shake the 25x detection reagent tube by hand for 3 seconds. Do not vortex.



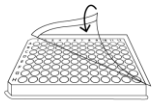
12. Prepare a stock of 10% ethanol from the 96% ethanol. Add 1920 microliters of the 10% ethanol to a 5 mL snap-cap tube. Then add 80 microliters of 25x detection reagent to the 5 mL tube containing 1920 microliters of 10% ethanol and mix briefly by hand for 3 seconds. Do not centrifuge or vortex.

13. Add detection reagent to a reservoir boat compatible with the multichannel pipette and **use immediately.**

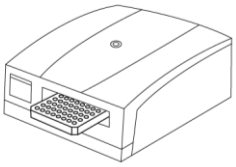
14. Using a multichannel pipette, dispense 5 microliters of detection reagent into each well and pipette up and down 3 times to mix. Avoid forming bubbles. **Complete within 4 minutes. Any delay in this step beyond 4 minutes may yield invalid results.**



15. Seal with clear plate sealer and incubate for 5 minutes in the dark at room temperature. **Recommendation: incubate in the plate reader.**

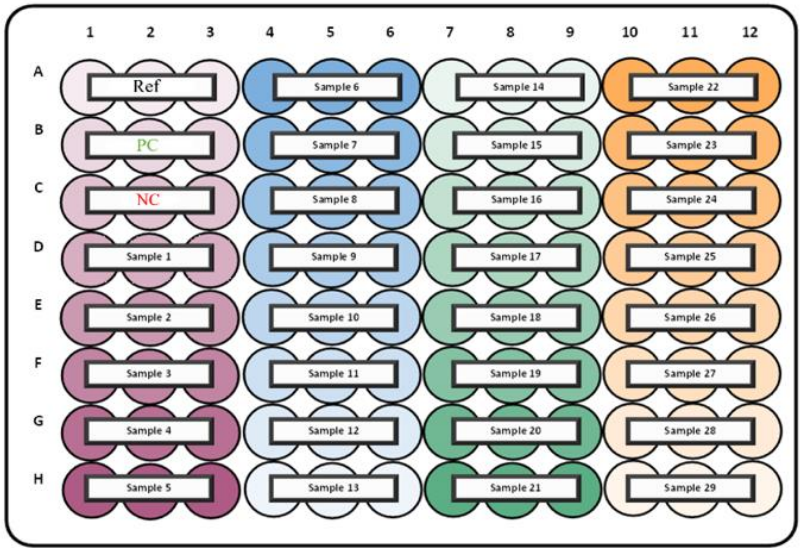


16. Place the microplate in the microplate reader, read the plate and collect the data and report.



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

Interpretation of Results

All test controls should be examined prior to interpretation of patient results by the Gen 5 software version number 3.10.06. If the controls are not valid, the patient results cannot be interpreted, and assay should be repeated.

Facible Q-LAAD SARS-CoV-2 Controls – Positive, Negative, and Others:

Level: Numerical Result in units*	Result	Test Result Interpretation
x ≥ **1995**	Negative	SARS-CoV-2 is not detected
x < ***1995***	Positive	SARS-CoV-2 is detected

Plate reader software will determine the appropriate call for every control result. The positive control with mean values less than 1,995 fluorescence units will be resulted as valid. If the positive control mean value is greater than 1,995 fluorescence units, the assay is invalid and should be repeated. The negative control with mean values greater than 1,995 fluorescence units will be resulted as valid. If the negative control mean value is less than 1,995 fluorescence units, the assay is invalid and should be repeated. ***At no time should the technician performing the assay interpret the control results.***

Table 1. Facible Q-LAAD SARS-CoV-2 Control Results Summary

Relative Fluorescence units (RFU)	Well Description	Control Result Interpretation (Software will interpret control and internal reference wells automatically)
x < 1995	Positive Control	Positive Control passed. Plate is valid.
x ≥ 1995	Positive Control	Positive Control failed. Plate is invalid. Retest.
x ≥ 1995	Negative Control	Negative Control passes. Plate is valid.

x < 1995	Negative Control	Negative control failed. Plate is invalid. Retest.
x < 350 or 10000 < x *	Average of any Control Result	Test failure: Too little/too much detection reagent. Retest.
< 100*	Individual well	Test failure: Too little/or no detection reagent. Retest.

*This "Invalid" result indicates insufficient volume of detection reagent, thereby minimizing the chances of false negatives due to insufficient reagent volume.

Examination and Interpretation of Patient Specimen Results:
Plate reader software will determine the appropriate call for every patient specimen result. Samples with mean values less than 1,995 fluorescence units will be resulted as "Positive". Samples with mean values equal to or greater than 1,995 fluorescence units will be resulted as "Negative". Sample ID along with the Positive/Negative determination will be exported for use in Laboratory Information Systems. ***At no time should the technician performing the assay interpret the patient sample results.***

Quick Reference instructions:
See Facible Q-LAAD SARS-CoV-2 Quick Start Guide/Package Insert.

WARNING

- Failure to follow the test procedure may adversely affect test performance and /or invalidate the test result.
- For *Research Use Only*
- Do not reuse the used 96-well Microplate, Fixed Volume Pipettes, Reagent Tubes, solutions, or Controls.
- Store test plates at 2-8°C. Do not use any 96-well Microplates that have been stored outside this temperature range.
- The user should never open the foil pouch of the 96-well Microplate exposing it to the ambient environment until it is ready for immediate use.

- Do not use 96-well Microplates if the desiccant packet is missing from the pouch.
- Do not use 96-well Microplates if the pouch has been punctured or previously opened.
- The reagent solution contains a salt solution (saline). If the solution contacts the skin or eye, flush with water. If irritation persists, seek medical advice.
- Keep out of reach of children.
- Detection reagent contains ethanol and is flammable. Keep away from heat, sparks, and open flames.
- A negative test result may occur if the concentration of antigen is below the limit of detection at the time of collection.
- Do not use if the kit or any items included within it are leaking, punctured, any items are absent from the kit or if the seal has been broken on the packaging.
- This Q-LAAD SARS CoV-2 test kit has been authorized only for maintenance of anterior nares swab specimens as an aid in detection of the ***Spike-1 protein*** antigen from SARS-CoV-2, not for any other viruses or pathogens.
- Strict adherence to the Facible Q-LAAD SARS-CoV-2 test instructions is necessary to obtain accurate results.
- Do not pool the contents of different vials of the same reagent (even if the reagents are from the same lot). Reagents are lot-specific and cannot be shared between kits.

CAUTION

- Patient specimens may be infectious. Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- Use universal laboratory precautions. Do not drink, eat, smoke, or other activities that may lead to both sample and infectious agent contamination. Use of appropriate PPE is required.
- Dispose of used 96-well microplates in a biohazard waste container. Proper handling and disposal shall be established in accordance with local regulations.
- Limit aerosolization of samples or reagents that may lead to contamination and transmission of possibly infectious substances.
- The BioTek plate reader should be cleaned and decontaminated on a routine basis. See the BioTek user manual for cleaning and decontamination procedures.
- Avoid cross contamination between wells.
- Avoid scaping probe during pipetting.
- Do not use variations of transport media other than 0.9% saline solution.
- To avoid plate orientation error, make sure cut corners on plate are facing user.

- Do not remove clear plate cover during disposal process to avoid splash remnants.

FOR Research Use Onl;y

The Q-LAAD SARS CoV-2 test produced by Facible Biodiagnostics, LLC. offers this product for Research Use Only. This product has been verified only for anterior nasal swab specimens as an aid in detection ***Spike-1 protein*** antigen from SARS-CoV-2, not for any other viruses or pathogens.

Symbols

The following symbols are used in labeling for Facible Biodiagnostics products

Symbol	Explanation
	Manufacturer

	Date of manufacture
	Consult instructions for use
	Do not re-use
	*In vitro* diagnostic medical device
	Batch code
	Catalog number
	Do not use if the kit or any items included within it, are damaged/ faulty, or if any items are absent from the kit
	US Only: Federal law restricts this device to sale by or on the order of a physician
	Temperature limit
	Use-by date
	Flammable hazard
QTY	Quantity