Facible

INSTRUCTIONS FOR USE

For use under the Emergency Use Authorization (EUA) only

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 from SARS-CoV-2. The test uses anterior nares swab specimens stored in saline solution from individuals who are suspected of COVID-19 by their healthcare provider. These specimens are to be collected within the first 7 days of symptom onset or for screening exposed individuals without symptoms. Results in asymptomatic individuals have not been fully evaluated. The results are generally expected to detect Spike protein if present in individuals without symptoms or other epidemiological reasons to suspect COVID-19 infection, when tested twice over two (or three) days with at least 24 hours (and no more than 36 hours) between tests. 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.*** This test is not for use in Point of Care (POC) settings, in low complexity labs, or for use in CLIA waived laboratory settings.

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 only for use under the Food and Drug Administration's Emergency Use Authorization.

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 medium-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.

FOR USE UNDER EMERGENCY USE AUTHORIZATION ONLY

The Q-LAAD SARS-CoV-2 test produced by Facible Biodiagnostics, LLC. offers this product with an FDA Emergency Use Authorization (EUA). This means that this product has not been FDA cleared or approved but has been authorized for emergency use by FDA under and EUA. This product has been authorized only for anterior nasal swab specimens as an aid in detection Spike protein antigen from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of medical devices for detection and/or diagnosis of COVID-19 under Section 564(b) (1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

WARNING

- Failure to follow the test procedure may adversely affect test performance and /or invalidate the test result.
- Use of appropriate PPE is required
- For *in vitro* diagnostic use only
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents
- Do not reuse the used 96-well Microplate, Fixed Volume Pipettes, Reagent Tubes, solutions, or Controls
- 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
- 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
- 50% EtOH dye 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 damaged/ faulty, or if any items are absent from the kit
- This Q-LAAD SARS-CoV-2 test kit has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA.
- 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 pathogen
- The emergency use of this Q-LAAD SARS-CoV-2 test kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

CAUTION

- 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

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	Solution containing recombinant Spike-1 protein with cryoprotectant.	(1) vial
Negative Quality Control Solution	Solution of suspended cell lysate with cryoprotectant	(1) vial

SPECIAL INSTRUMENT REQUIRED

The **Q-LAAD SARS-CoV-2** test is to be used with

- BioTek Microplate Reader
- BioTek Custom Filter Cube: Excitation wavelength 575±10nm; Dichroic mirror 595 nm, Emission wavelength 610±10nm
- BioTek Gen5 Software Features for Detection; microplate reading and data analysis

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
Ethanol	70% purity, ethanol solution, Molecular Biology grade, CAS#64-17-5 or similar	Fisher Scientific	BP201500
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 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

Summary of Microplate 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

Test Procedure

Sample Collection

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

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.

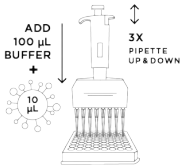
Sample Preparation

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.

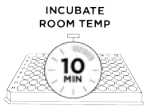
Operator Instructions

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.



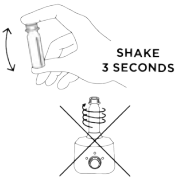
5. Use a multichannel pipette to add 100 microliters of Q-LAAD sample buffer to all wells.
6. Use a multichannel pipette to add 10 microliters of each sample to the appropriate wells from the prepared tubes and mix up and down 3x.



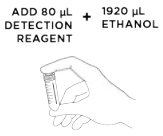
7. Incubate the plate for 10 minutes at room temperature.

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.

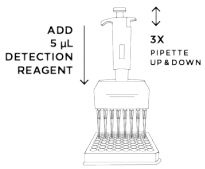


10. Prepare the detection reagent.
11. Shake the 25x detection reagent tube by hand for 3 seconds. Do not vortex.



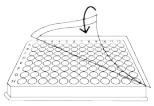
12. Prepare detection reagent by mixing 80 microliters of 25x detection reagent with 1920 microliters of 10% ethanol prepared from 95% ethanol in a tube and mix briefly. Do not centrifuge.

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

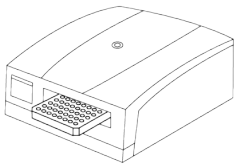


14. Using a multichannel pipette, dispense 5 microliters of detection reagent into each well and pipette up and down 3 times to mix. Avoid forming bubbles. Complete within 4 minutes.

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

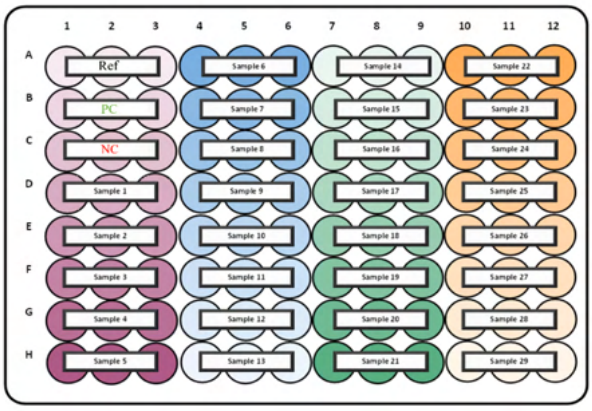


16. Place the microplate in the microplate reader, read the plate and collect the data and report.



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

Clinical Evaluation:

The clinical performance of the Q-LAAD SARS-CoV-2 test was established with a total of 70 anterior nasal swab samples collected from 2 different vendors (in the US) from symptomatic patients suspected of COVID-19. Nasal swabs were collected and eluted in saline buffer (0.9%) and stored until tested. Samples were prepared and tested with Q-LAAD SARS-CoV-2 test accordingly to the operator instructions. All subjects were confirmed as positive (≤7 days from onset of symptoms) or negative by a reference high sensitivity extracted EUA RT-qPCR method, used as comparator method for the study. All testing was conducted by operators blinded to the reference RT-PCR result, which was conducted on anterior nares swabs collected as part of the standard of care testing.

Positive samples by days onset symptoms				
Days	Not Detected	Detected	Total	% Correct
1-3	4	18	22	82%
4-5	0	6	6	100%
6-7	0	2	2	100%

		Comparator PCR +	Comparator PCR -
Q-LAAD	+	26	2
	-	4	38
		Sensitivity 87%	Specificity 95%

Limit of Detection (LoD) - Analytical Sensitivity:

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized **anterior nares clinical positive specimen. The claimed LOD is 36 genome equivalents / mL (N-gene Ct 34).**

LoD Range Finding		
Ct	Concentrations (GE/mL)	Agreement
31.0	3.40E+02	3/3
32.7	9.90E+01	3/3
34.3	2.88E+01	3/3
36.0	8.39E+00	1/3
37.6	2.44E+00	3/3
39.3	7.11E-01	1/3
40.9	2.07E-01	2/3

LoD Confirmation		
Viral Concentration (GE/mL)	3.61E+01	7.22E+00
Positive Results (n=20)	20/20	16/20

Cross-Reactivity and Interference by microorganisms

Conclusion: Cross-reactivity was observed for Mycoplasma pneumoniae at worst-case scenario concentration. To determine the concentration of Mycoplasma pneumonia that does not cross-react, a dilution study was performed. The results of the serial dilution showed that no cross-reactivity was observed at concentrations less than 10^5 CFU. No cross-reactivity was observed for all remaining microbes tested. No microbiological interference was observed with pooled microbes tested.

*No cross-reactivity was detected for SARS-CoV, however the *in silico* comparison between SARS-CoV-2 surface glycoprotein and SARS-CoV indicate a moderate level of homology and cross-reactivity may be likely.

**No cross reactivity was detected for MERS or Human Coronavirus NL63, and *in silico* comparison show low sequence homology to SARS-CoV-2, which suggest that cross-reactivity is unlikely.

Organism	**Strain**	**Source**	**Catalog number**	**Concentration tested (CFU/mL and PFU/mL)**	**Cross-reactivity**	**Interference**
Human coronavirus	229E	Isolate	ATCC Cat# VR-740	1.00E+05	0/3	3/3
Human coronavirus	OC43	Isolate	ATCC Cat# VR-1558	1.00E+05	0/3	3/3
Rhinovirus	B632	Isolate	ATCC Cat# VR-1645	1.00E+05	0/3	3/3
Haemophilus influenzae	Rd [KW20]	Isolate	ATCC Cat# 51907	1.00E+06	0/3	3/3
Streptococcus pneumoniae	262 [CIP 104340] (Klein) Chester	Isolate	ATCC Cat# 49619	1.00E+06	0/3	3/3
Streptococcus pyogenes	Bruno [CIP 104226]	Isolate	ATCC Cat# 19615	1.00E+06	0/3	3/3

Candida albicans	CBS 562 [572, CCRC 20512, CECT 1002, DBVPG 6133, IFO 1385, IGC 3436, JCM 1542, NCYC 597, NRRL Y-12983]	Isolate	ATCC Cat# 18804	1.00E+06	0/3	3/3
Pooled human nasal swab	N/A	N/A	N/A	N/A	0/3	3/3
Adenovirus 5	Adenoid 75	Isolate	ATCC Cat# VR-1516	1.00E+05	0/3	3/3
Parainfluenza virus 3	ATCC-2011-5	Isolate	ATCC Cat# VR-1782	1.00E+05	0/3	3/3
Parainfluenza virus 1	C35	Isolate	ATCC Cat# VR-94	1.00E+05	0/3	3/3
Influenza A (H1N1)	A/WS/33	Isolate	ATCC Cat# VR-1520	1.00E+05	0/3	3/3
Influenza B	B/Florida/78/2015	Isolate	ATCC Cat# VR-1931	1.00E+05	0/3	3/3
Enterovirus	H	Isolate	ATCC Cat# VR-1432	1.00E+05	0/3	3/3
Respiratory syncytial virus	Long	Isolate	ATCC Cat# VR-26	1.00E+05	0/3	3/3
Bordetella pertussis	18323 [NCTC 10739]	Isolate	ATCC Cat# 9797	1.00E+06	0/3	3/3
Mycoplasma pneumoniae	Eaton Agent [NCTC 10119]	Isolate	ATCC Cat# 15531	1.00E+06	1/3	3/3
Staphylococcus aureus	NCTC 8532 [IAM 12544, R. Hugh 2605]	Isolate	ATCC Cat# 12600	1.00E+06	0/3	3/3
Staphylococcus epidermidis	FDA strain PCI 1200	Isolate	ATCC Cat# CRM-12228	1.00E+06	0/3	3/3
**Human coronavirus	NL63 (heat-inactivated)	Isolate	ZeptoMetrix Cat# 0810228CFHI	1.70E+05 TCID 50/mL	0/3	3/3
Parainfluenza virus 2	Greer	Isolate	ATCC Cat# VR-92	1.00E+05	0/3	3/3

Parainfluenza virus 4b	19503	Isolate	BEI Cat# 3238	1.00E+05	0/3	3/3
Influenza A H3N2	A/Hong Kong/8/68	Isolate	ATCC Cat# VR-1679	1.00E+05	0/3	3/3
**MERS-CoV	EMC/2012 (gamma-irradiated)	Gamma-irradiated	BEI Cat# NR-50549	1.00E+05	0/3	3/3
*SARS-CoV	Urbani (gamma-irradiated)	Gamma-irradiated	BEI Cat# NR-9323	1.00E+05	0/3	3/3
Human Metapneumovirus	TN/83-1211	Isolate	BEI Cat# NR-22227	1.00E+5	0/3	3/3
Chlamydiaceae, Chlamydia	AR-39	Isolate	ATCC Cat# 53592	1.00E+06	0/3	3/3
Legionella pneumophila	Concord 3 [NCTC 11985]	Isolate	ATCC Cat# 35096	1.00E+04	0/3	3/3

***In-silico* analysis**			
Compared to SARS-CoV-2 spike 1 amino acid sequence (QSJ03236.1)			
Organism	Method	Percent identity	Probability of cross reactivity
Pneumocystis jirovecii (taxid: 42068)	BLAST	No significant protein sequence homology	-
Mycobacterium tuberculosis (taxid: 1773)	BLAST	No significant protein sequence homology	-
Human Coronavirus HKU1(taxid: 168471)	BLAST	No significant protein sequence homology	-
MERS surface glycoprotein (ASJ26610.1)	BLAST	35.10%	Unlikely
Human Coronavirus NL63 spike protein (BBL54116.1)	BLAST	30.77%	Unlikely

Endogenous Interference Substances Studies

Conclusion: Studies were performed to demonstrate that these 19 potentially interfering substances do not cross-react or interfere with the detection of SARS-CoV-2 in the Facible Q-LAAD SARS-CoV-2 assay at the stated concentrations. Low viral-load positive samples were used at 1-3x LoD (GE 36/mL – GE 76/mL). No interfering substances were observed at the following concentrations.

Potential interfering substances	Active Ingredient	Concentration tested	Cross-reactivity results	Interference Results
Afrin	Oxymetazoline	5% v/v	0/3	3/3
Azithromycin	Azithromycin	250 ug/mL	0/3	3/3
Blood (Human)	Blood	0.005% v/v	0/3	3/3
Chroaspetic	Menthol	1.5 mg/mL	0/3	3/3
Clathromycin	Clathromycin	1 mg/mL	0/3	3/3
Flonase	Fluticasone Propionate	5% v/v	0/3	3/3
Homeopathic	Alkalol	1:10 dilution	0/3	3/3
Listerine	Menthol, Thymol, methyl salicylate	0.1% v/v	0/3	3/3
Mucin	Purified mucin protein	2.5 mg/mL	0/3	3/3
Mupirocin	Mupirocin	1 mg/mL	0/3	3/3
Nasal Drops	Phenylephrine hydrochloride	15% v/v	0/3	3/3
Nasal Spray	Cromolyn	15% v/v	0/3	3/3
Nelimed	Hyaluronic Acid	5% v/v	0/3	3/3
Phenol Throat Spray	Phenol	5% v/v	0/3	3/3
Saline Nasal Spray	Saline	5% v/v	0/3	3/3
Tamiflu	Oseltamivir	1 mg/mL	0/3	3/3
Tobramycin	Tobramycin	4 mg/mL	0/3	3/3
Zicam	Oxymetazoline hydrochloride	1% v/v	0/3	3/3
Zicam All clear	Hydroxpropryl methylcellulose, disodium phosphate	0.5% v/v	0/3	3/3

High-dose Hook Effect

Conclusion: A High-dose Hook Effect was not observed at the highest tested concentration of 1.13 x 10^8 genome copies/mL.

Quick Reference instructions:

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