MicroGEM

Sal6830

MicroGEM Sal6830 SARS-CoV-2 Saliva Test Instructions for Use (IFU)

For use with MicroGEM Sal6830
Point of Care PCR System

MicroGEM Sal6830 SARS-CoV-2 Saliva Test (30 kits): SCF0030
MicroGEM Sal6830 Point of Care PCR System: SCFMA

IVD

℞ ONLY

MicroGEM

1 INTRODUCTION

1.1 Intended Use

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is a real-time RT-PCR assay intended for the qualitative detection of RNA from SARS-CoV-2 in saliva specimens from individuals 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 high, moderate, or waived complexity tests. The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in saliva specimens 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. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be confirmed with different authorized or cleared molecular tests in a CLIA-certified laboratory that meets requirements to perform high or moderate complexity tests. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is intended for use by health professionals or trained operators who are proficient in using the MicroGEM Sal6830 Point of Care PCR System. The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

1.2 Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. SARS-CoV-2 is an enveloped, single-stranded RNA virus of the ß genus. The virus can cause mild to severe respiratory illness and has spread globally.

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is a molecular qualitative RT-PCR test for use with the MicroGEM Sal6830 Point of Care PCR System for the detection of 2019 novel coronavirus (SARS-CoV-2) RNA in saliva.

Immediately after collection, the saliva sample is automatically processed by the instrument system. The test kit contains all the components needed to perform testing, including the primers and probes used for nucleic acid RT-PCR amplification and detection of SARS-CoV-2 virus, and internal controls to monitor the various assay steps.

1.3 Principle of the Procedure

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is based on fully automated sample preparation (nanoparticle mediated viral concentration, nucleic acid extraction and purification), followed by RT-PCR amplification and detection. The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is performed on the MicroGEM Sal6830 Point of Care PCR System, which automates and integrates sample preparation, nucleic acid extraction and amplification, and detection of the target sequences. The system consists of an instrument and preloaded software for running the test, interpreting, and viewing the results. The system requires the use of single-use disposable saliva collection cups and cartridges that hold the reagents.

Saliva is first pooled in the mouth and then allowed to flow into the collection cup. When the collection cup is sealed, capture beads concentrate the virus particles in preparation for the extraction step. The cup and tube assembly is inserted into the detection cartridge and then into the instrument where RNA is extracted by lysis using a thermophilic proteinase and elevated temperature. The lysate is pushed by thermally responsive polymers through a purification matrix and into the PCR chamber where a rapid RT-PCR is conducted using gene specific primers and probes designed to target SARS-CoV-2 E and N genes.

Each cartridge contains the following internal controls:

- Sample Process Control for monitoring sample addition, processing, and nucleic acid extraction
- Reverse Transcriptase (RT) Control for monitoring reverse transcription
- Negative and Blank controls for monitoring background

Test validity and SARS-CoV-2 results are interpreted automatically by the software based on the test and controls' signals.

2 REAGENTS AND MATERIALS

Each MicroGEM Sal6830 SARS-CoV-2 Saliva Test kit contains sufficient reagents to process one individual saliva test. A carton of MicroGEM test kits includes 10 individual tests and access to Quick Reference Instructions (QRI).

2.1 Kit Components

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test kit contains the following:

- Cap (Pouch A) - seals the saliva cup after saliva is deposited and releases the capture particles and diluent
- Saliva Cup (Pouch B) - contains capture beads, reagents for sample lysis and nucleic acid extraction
- Test Cartridge (Pouch C) - a microfluidic cartridge that contains the purification column, and primers, probes, enzymes, and other reagents required for amplification and detection of viral targets and controls
- Test ID Card – contains stickers with bar codes for traceability of samples and results
- Quick Reference Instructions (QRI)

2.2 Equipment and Software Required

MicroGEM Sal6830 Point of Care PCR System (Product Number SCFMA)
MicroGEM Sal6830 System Software (Core) Version 1.2.4971 or higher

2.3 Reagent Storage and Handling

Ensure all kit contents are stored between 15°C and 30°C, protected from direct sunlight and protected from water.

3 WARNINGS AND PRECAUTIONS

3.1 General

- For in vitro diagnostic use.
- For prescription use only.
- For use under Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved; but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid 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 in vitro diagnostics 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.
- Laboratories and patient care settings should report all SARS-CoV-2 results to the appropriate public health authorities.
- This product is for single use only; do not reuse the MicroGEM Sal6830 SARS-CoV-2 Saliva Test.
- Federal or other laws may restrict this device for sale by or on the order of a licensed practitioner.
- Closely follow the Instructions For Use (IFU), the Quick Reference Instructions (QRI) and the MicroGEM Sal6830 Point of Care PCR System User Guide to ensure the test is performed correctly. Any deviation from these instructions may affect optimal test performance.
- Choose a level and clean surface to place the MicroGEM Sal6830 Point of Care PCR System according to the installation conditions described in the MicroGEM Sal6830 Point of Care PCR System User Guide.
- All test steps are to be performed immediately one after the other and without delay. The saliva sample should be tested within 20 minutes from the time the sample was collected.
- The person being tested should not eat, drink, use mouthwash, smoke, or chew gum in the 30 minutes prior to providing the saliva sample. If the person has done any of these activities, wait until 30 minutes have passed before saliva collection to ensure a valid saliva sample is collected.
- Wash hands for a minimum of 20 seconds, or use hand sanitizer, before and after saliva collection. If wearing gloves, change them or wipe them with hand sanitizer between handling each saliva sample. Handle all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Follow universal precautions when handling samples, this kit, and its contents. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention, Clinical and Laboratory Standards Institute and World Health Organization.
- Do not eat, drink, or smoke in designated testing areas.
- Do not open the door of the system during the test. If the door is opened, the test will be canceled and re-testing with a new saliva sample and test kit will be required.

- Do not disassemble used saliva cups or cartridges. Dispose all used test components according to institution, local and country requirements.
- Thoroughly clean and disinfect all work surfaces with diluted household chlorine bleach as follows:
  - Mix 1 part bleach with 9 parts water (10% dilution).
  - Replace this diluted bleach every 24 hours.
  - Wearing gloves, wipe surfaces with bleach solution, followed by wiping the surface with 70% ethanol.
  - Do not use a bleach product if the sodium hypochlorite is not within 5.25% to 8.25% percent or is not specified.

3.2 Test Kit Components and System

- Always check the expiry date of consumables prior to running a test. Do not use a kit that is expired.
- Do not open the saliva cup pouch or the test cartridge pouch until ready to conduct the test.
- If spills occur on the collection cup while collecting the saliva sample, immediately wipe the outside of the collection cup with sanitizing wipes before proceeding to the next step.
- Do not use any kit components that have leaked, cracked, or are damaged.
- Do not use a saliva cup or test cartridge that has been dropped after removing it from the pouch.
- Do not place the sample ID sticker label around the tube. Only place it on the flat surface on top of the cap.
- Lock the cap after sample collection before proceeding with the test. Failure to lock the cap can lead to erroneous results.
- Once twisted and locked, the cap cannot be reopened.
- Each single-use saliva cup and test cartridge should only be used once.
- Clean barcode reader using 10% bleach on a damp, lint free cloth. Do not use any other type of cleaner on the barcode reader.
- Wipe the instrument, including exterior surfaces visible behind the door including the latch, with 70% isopropanol available in commercial wipes or on a damp, lint free cloth. Do not spray isopropanol directly onto the instrument as spray may cause damage.
- If spills occur on the instrument or surrounding area, wipe with 10% bleach on a damp, lint free cloth followed by 70% isopropanol available in commercial wipes.
- Do not clean the instrument with soap or other cleaning solutions other than as directed in these instructions.
- Complete instructions for instrument cleaning and maintenance are found in the MicroGEM Sal6830 Point of Care PCR System User Guide.

4 INSTRUCTIONS FOR ADMINISTERING THE TEST

4.1 Starting the System

Read the MicroGEM Sal6830 Point of Care PCR System User Guide for instructions on setting up the system.

Connect the 24V power supply to the system. Plug the adapter into an appropriate electrical outlet. Once the power is connected, press the Power Button on the right side of the system to power up and start the system.

The Settings button on the screen also provides instructions for setting up the system.

It is recommended running positive and negative external controls before testing patient specimens when you first set up your MicroGEM Sal6830 Point of Care PCR System. Refer to Quality Controls.

4.2 Before You Begin

Use hand sanitizer or wash your hands thoroughly for 20 seconds before starting the test.

The person providing the saliva sample should not eat, drink, use mouthwash, smoke, or chew gum 30 minutes before collecting saliva to run the test.

Use the step-by-step instructions on the touch screen for real-time instructions as you do the test.

4.3 Run the Test

Start a new test (1).

Go to the touch screen and click “Start New Test” on the screen.

Open a new test kit. Remove the pouches, Test ID card, and QRI card from the kit.

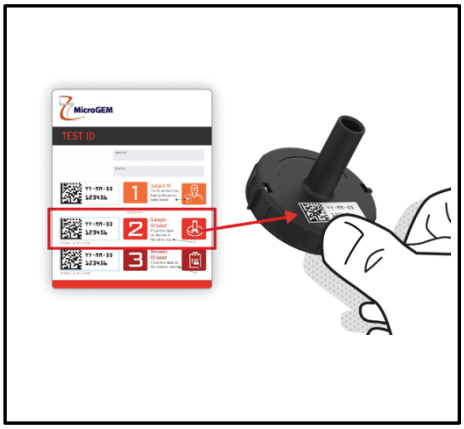
Open Pouch A, Pouch B, and Pouch C. Remove the cap and cup from Pouch A and B. **Leave the cartridge in Pouch C until ready to use.**

1

Remove the sample ID sticker label #2 from the card and place it on the flat surface (top) of the cap (2).

Remove the records ID sticker label #3 from the card and place it on the facility record.

Give the Test ID card, including label #1, to the patient being tested.

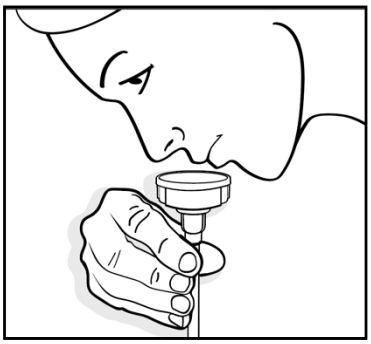


2

Hand the saliva cup and the cap to the patient being tested to self-collect the saliva sample (3). Ask the patient to follow these steps:

How to provide a saliva sample

- Pool saliva in your mouth.
- Press the cup against your lip.
- Tilt your head forward.
- Let your saliva flow into the cup.
- Think of a favorite food or make chewing motions to help your saliva flow.



3

Resources showing the process for collecting a good saliva sample can be found at www.microgembio.com/salivatips.

Instruct the patient providing the saliva sample to fill the cup to the dotted line as shown in the picture (4). When enough saliva has been collected the patient should loosely place the cap on the cup and hand it to you, the operator.

NOTE: The patient should NOT close and lock the cap.

Inspect to ensure the saliva is filled to the black line.
Bubbles should be above the dotted line as shown in the picture (4).
Make sure liquid saliva fills the space below the dotted line.

4

Check for any saliva spills outside the cup. If saliva has dripped outside the cup, wipe the outside of the cup with sanitizing wipes.

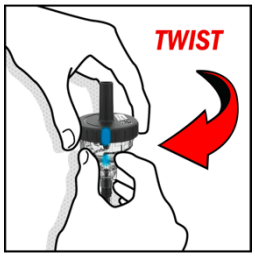
The saliva sample should be processed and run within 20 minutes from the time it was collected.

5

The test operator should align the tab on the sample cap with the tab on the collection cup, as highlighted in the picture (5).

Close the cap by pushing down and twisting the cap until it is fully closed and locked (6). Once locked, the cap cannot be opened.

Lock the cap before proceeding. Failure to lock the cap can lead to false results.



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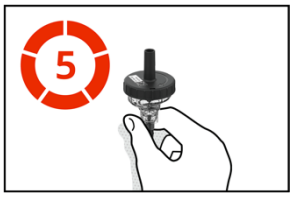


7

The test operator should mix the sample by keeping the cup upright and moving the cup assembly in a circular motion for 5 seconds (7).

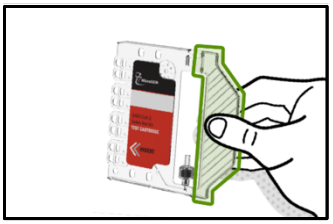
After mixing, the saliva sample should be orange indicating the cap was properly attached (8). If orange, proceed to the next step. If the sample IS NOT orange, use a new test kit with a fresh saliva sample and repeat the saliva collection process.

8

Scan the test ID number by holding the cap near the scanner above the touch screen until it beeps (9). After scanning the barcode, the screen will display the test ID number.

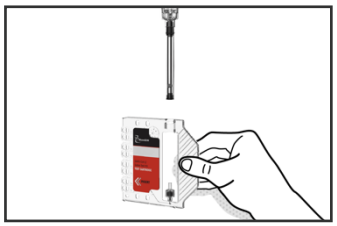
9

Remove the test cartridge from Pouch C by the handle as highlighted in the picture (10).



10

Hold the test cartridge as shown and slide the saliva cup into the test cartridge (11). **Do not twist the cup while sliding it into place.** The cup will click into place.



11

Open the door of the system. The latch should be pointing up.

Slide the assembled test cartridge and saliva cup in slot (12). Insert the side of the cartridge with the label into the slot. The cartridge will click into place.

Rotate the latch clockwise. Close the door of the system.

12

Select “Start Test.” The test takes less than 30 minutes and concludes with an audible ‘beep’ (13).

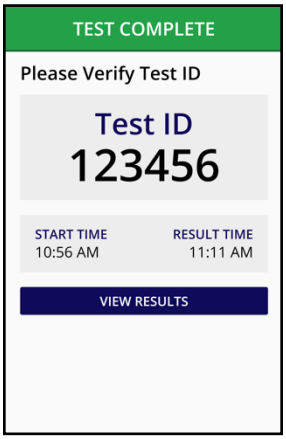
13

Follow the directions on the screen if any error messages occur. Information about error messages is also found in the MicroGEM Sal6830 Point of Care PCR System User Guide.

Wash your hands for a minimum of 20 seconds, or use hand sanitizer, after completing the saliva collection and test process. If you are wearing gloves, replace them or wipe them with hand sanitizer.

4.4 View Results

When the test run is complete, verify the Test ID on the results screen with the person's Test ID card and the ID label on the facility record (if used) to be sure they match (14).



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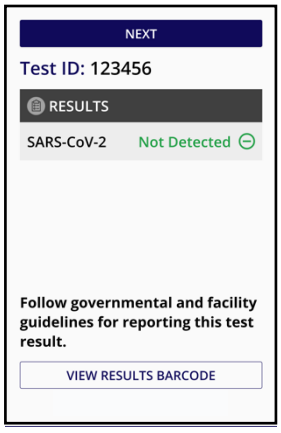
Click "View Results" (14)

Test validity and SARS-CoV-2 results are interpreted automatically by the MicroGEM Sal6830 Point of Care PCR System software and are shown on the View Results screen at the end of the run (15). See Table 1 for interpretation of results and follow-up actions.



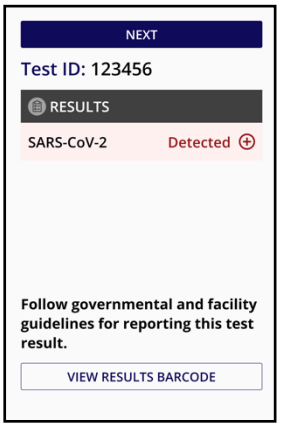


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Table 1. Interpretation of Results and Follow-up Actions

Test Result	Explanation	Next steps
SARS-CoV-2 Not Detected (presumptive negative)	Negative results should be treated as presumptive and if inconsistent with clinical signs and symptoms or necessary for patient management, should be confirmed with different authorized or cleared molecular test in a CLIA-certified laboratory that meets requirements to perform high or moderate complexity tests. Negative results do not preclude SARS- CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.	Follow the facility's guidelines for communicating results to the person being tested, recording the results and reporting the test result to relevant public health authorities. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
SARS-CoV-2 Detected (positive)	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. The agent detected may not be the definite cause of disease.	Follow the facility's guidelines for communicating the results to the person being tested, recording results and reporting the test result to relevant public health authorities. The person's healthcare provider will consider the test result with all other aspects of the person's history, such as symptoms and possible exposures, to decide how to care for the person.
Invalid Test	No reportable result due to failure of one or more of the internal controls included in the test or failure to pass acceptance criteria for amplification curves of targeted genes.	Repeat the test with a new kit and fresh saliva sample.
System Error	No reportable result due to power failure or instrumentation error.	Repeat the test with a new kit and fresh saliva sample. If the issue persists, contact customer support at techsupportdx@microgembio.com
Test Interrupted	No reportable result due to opening the door of the instrument while the test was running.	Repeat the test with a new kit and fresh saliva sample.

The instrument also produces a QR code (16) on the screen which may be scanned by an optional scanner available from Horiba Instruments (part number SMP) to export into their Lite DM version 3.0 middleware connectivity software. The results can also be exported to a USB device (17). Follow your facility procedures to report results.

Refer to the Export Results section in the MicroGEM Sal6830 Point of Care PCR System User Guide for additional information.

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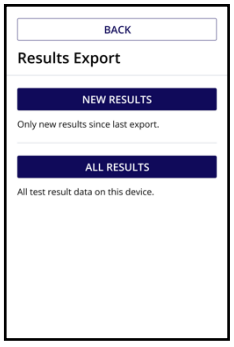

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4.5 Dispose test cartridge and prepare for next run

Open the door of the system and rotate the red-latch counterclockwise.
Remove the test cartridge and dispose according to the facility's guidelines for waste disposal.
Close the door of the system so it is ready for its next test.
Click "Done" on the screen.
It is recommended that the system be cleaned at the end of each day following the guidance below:

- Wipe the instrument, including exterior surfaces visible behind the door including the latch, with 70% isopropanol available in commercial wipes or on a damp, lint free cloth. Do not spray isopropanol directly onto the instrument as a spray may cause damage.
- If spills occur on the instrument or surrounding area, wipe with 10% bleach on a damp, lint free cloth followed by 70% isopropanol available in commercial wipes.
- Do not clean the instrument with soap or other cleaning solutions other than as directed in these instructions.
- Clean barcode reader using 10% bleach on a damp, lint free cloth. **Do not use any other type of cleaner on the barcode reader.**

5 QUALITY CONTROL

5.1 Internal Controls

Each MicroGEM Sal6830 SARS-CoV-2 Saliva Test cartridge includes internal controls to determine test validity: a sample process control, an RT control, and a negative control.

5.2 External Controls

External controls are not required to use this test kit.

In certain point-of-care settings, external controls may be tested, regularly or when new test kits are received, in order to train new operators or conform with local regulations, accrediting groups, or the lab's standard Quality Control procedures. MicroGEM recommends the use of commercially available positive and negative external run controls from Zeptometrix Inc, be run:

- Before running patient specimens after a system has been newly set up.
- Each time a new operator is performing the test (i.e., operator who has not performed the test recently).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by your institution's standard Quality Control (QC) procedures.

5.3 External Control Run Procedure

External controls* are run as if they were patient specimens. The only difference is that instead of collecting saliva from a patient, the contents of the external control vial are used. The user should open the vial, pour the entire contents of the vial into the saliva cup of a newly opened test kit, discard the empty vial per facility procedures, and run the external control per standard protocol in the QRI. It is recommended to run a positive control first and then a negative control. The positive control should give a positive result (i.e., SARS-CoV-2 detected) and the negative control should give a negative result (i.e., SARS-CoV-2 not detected).

*ZeptoMetrix NATtrol SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control (NATSARS (COV2)-ERC1) and the ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) Negative Control (NATSARS (COV2)-NEG1) positive and negative controls.

6 LIMITATIONS

- The performance of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test was evaluated using the procedures provided in this product insert only. Modifications to these procedures may alter the performance of the test.
- Negative results should be treated as presumptive and tested with an alternative FDA authorized or cleared molecular assay in a CLIA-certified laboratory that meets requirements to perform high or moderate complexity tests, if necessary for clinical management, including infection control.
- A false negative result may occur if saliva is improperly collected or handled. False negative results may also occur if the amount of virus present in the saliva is at a concentration below the limit of detection of the test. 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.
- As with any molecular test, viral mutations within the regions targeted by the test could affect primer and/or probe binding resulting in failure to amplify and/or detect the presence of virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- This test has been validated on saliva samples only. No other sample types have been validated for testing with this system.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The E gene targeted by the MicroGEM Sal6830 SARS-CoV-2 Saliva Test can detect, in addition to SARS-CoV-2, other coronavirus species within the Sarbecovirus subgenus.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.

7 CONDITIONS OF AUTHORIZATION FOR LABORATORIES

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

However, to assist authorized laboratories and Point of Care Settings using the MicroGEM Sal6830 SARS-CoV-2 Saliva Test (referred to in the Letter of Authorization as "Your Product"), the relevant Conditions of Authorization are listed below.

- Authorized laboratories using your product must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories using your product must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA Reporting@fda.hhs.gov) and (techsupportdx@microgembio.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- You, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[a]The letter of authorization refers to "authorized laboratories" as "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high, moderate or waived complexity tests. The MicroGEM Sal6830 SARS-CoV-2 Saliva Test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation."

8 PERFORMANCE CHARACTERISTICS

The following validation studies were conducted for the MicroGEM Sal6830 SARS-CoV-2 Saliva Test:

8.1 Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test was determined by testing serial half-log dilutions of pooled negative saliva specimens spiked with gamma irradiated SARS-CoV-2 virus (USA-WA1/2020; BEI Resources catalog number NR-52287, Lot number 70039068). A serial dilution was performed (Table 2) and the LoD was estimated to be 6,400 GE/ml by probit analysis and verified by testing an additional 20 replicates (Table 3). The LoD was generated at a system level, with results output of positive, negative, or invalid result as determined by the systems algorithm.

Table 2. Percent positivity of MicroGEM Sal6830 SARS-CoV-2 Saliva Test at different viral concentrations in clinical matrix.

SARS-CoV-2 (GE/ml)	MicroGEM Sal6830 SARS-CoV-2 Saliva Test Positive/Tested	MicroGEM Sal6830 SARS-CoV-2 Saliva Test % detected
498,561	5/5	100%
157,773	5/5	100%
49,928	5/5	100%
15,800	5/5	100%
5,000	5/5	100%
1,582	4/5	80%
501	0/3	0
159	1/4	25%
50	0/5	0
0	0/5	0

Table 3. Confirmation of LoD of MicroGEM Sal6830 SARS-CoV-2 Saliva Test.

SARS-CoV-2 (GE/ml)	Positive/Tested	% Positive (95% CI)
6,400	20/20	100% (83-100%)

8.2 Inclusivity (Analytical Reactivity)

The MicroGEM Sal6830 SARS-CoV-2 Saliva Test utilizes forward and reverse primers and probes targeting the E (envelope protein) and N (nucleocapsid protein) genes of the SARS-CoV-2 virus. *In silico* analysis was used to evaluate the extent of homology between each of the test primers and probe sequences included in the kit and sequences of SARS-CoV-2 isolates available in public databases. Analyses were performed using an in-house developed method and using ROSALIND DxM software.

ROSALIND DxM analysis. The N and E gene primer and probe sequences included in the MicroGEM Sal6830 SARS-CoV-2 Saliva Test were entered into the ROSALIND DxM system and compared against the 2,328,354 US GISAID sequences available on the ROSALIND DxM system as of February 16, 2022. This database included sequences up to the ROSALIND* DxM system's most recent update on February 10, 2022.

Results from this analysis are shown in Table 4. All ROSALIND DxM reported mismatch incidents were filtered so as to only include incidents with a frequency in the total tested database of greater than 0.1%. All detected incidents had single mismatches and their effect decreasing their melting temperature was the main factor influencing the severity score. The table also shows the frequency of mismatches over time, indicating that the INC20210407-01327 incident is dominant, with the frequency of all other incidents approaching 0 cases over the 90 days prior to analysis.

Table 4. Incidents reported by ROSALIND DxM software with a frequency greater than 0.1% in full US database as of February 16, 2022.

ROSALIND Incident ID	Affected primer/probe	Mismatch frequency			
		Full US database	Previous 90 days	Previous 60 days	Previous 30 days
NC20210407-01327	N probe	10.992%	43.370%	77.644%	82.623%
NC20210407-01339	N probe	1.017%	0.163%	0.101%	0.012%
NC20210407-01347	N probe	0.102%	0.006%	0.005%	0.000%
NC20210407-01247	N F	0.268%	0.055%	0.034%	0.008%
NC20210407-01217	N F	0.140%	0.045%	0.044%	0.021%

In-house in silico analysis pipeline. The test primers and probes were aligned against a database of 5,000 randomly sampled SARS-CoV-2 genome sequences. This database was compiled on February 16, 2022, from the GISAID database. The sampled database was filtered so as to include only genome sequences marked "Complete", excluding "Low coverage", and isolated from human hosts. This sampled database included a total of 8,277,918 sequences. The primers/probes were also aligned against secondary, subset databases compiled for the emerging SARS-CoV-2 variants Alpha (B.1.1.7+Q.*), Beta (B.1.351+B.1.351.2+B.1.351.3), Gamma (P.1+P.1. *), Delta (B.1.617.2, AY.1, AY.2), and Omicron (B.1.1.529+BA.*). Subsets of 500 genome sequences for each of these variants were randomly sampled without replacement from the curated SARS-CoV-2 database, excluding all sequences uploaded prior to November 16, 2021.

If primers or probes contained mismatches to some sequences, a risk assessment was performed based on mismatch frequency within the database, number of mismatches in a single sequence, and mismatch proximity to the 3' end of the primers. Mismatch severity approximating the risk of failing detection of some sequences was stratified as high risk, mid risk, low risk or no risk.

Mismatch severity against general SARS-CoV-2 sequences

The great majority of the SARS-CoV-2 sequences analyzed showed either no or low predicted risk to the MicroGEM Sal6830 SARS-CoV-2 Saliva Test efficacy (Table 5, no risk and low risk). Here, the predicted frequency of all N gene primers and probes encountering no mismatches is 82.84%, and the predicted frequency of at least one N gene primer or probe encountering a low-risk mismatch is 16.02%. The predicted frequency of any N gene primer or probe encountering a sequence where mismatches present a high risk to N gene detection is only 0.62% (Table 5, high risk). Mismatches to the E gene are substantially fewer, with less than 1% of the gene sequences analyzed with mismatches and 0.06% of the gene sequences analyzed predicted with mismatches in positions that have a high risk of affecting E gene detection. Since only amplification and detection of the N gene or E gene is required to detect SARS-CoV-2 with the MicroGEM Sal6830 SARS-CoV-2 Saliva Test and the probability of SARS-CoV-2 variants with mutations affecting detection of both genes is low.

Table 5. Frequency of severity of primer / probe mismatches against general SARS-CoV-2 sequence database, as of February 16, 2022. Combined risk to test indicates the predicted frequency that the highest risk primer or probe in a reaction is within that severity band, and the whole reaction carries this risk.

Oligonucleotides in MicroGEM Sal6830 SARS-CoV-2 Saliva Test		Mismatch severity/risk to test			
		None	Low	Mid	High
N gene	Forward primer	95.80% (4790/5000)	3.76% (188/5000)	0.00% (0/5000)	0.44% (22/5000)
	Reverse primer	99.74% (4987/5000)	0.12% (6/5000)	0.00% (0/5000)	0.14% (7/5000)
	Probe	86.70% (4335/5000)	12.74% (637/5000)	0.52% (26/5000)	0.04% (2/5000)
	Combined	**82.84%**	**16.02%**	**0.52%**	**0.62%**
E gene	Forward primer	99.62% (4981/5000)	0.34% (17/5000)	0.00% (0/5000)	0.04% (2/5000)
	Reverse primer	99.94% (4997/5000)	0.06% (3/5000)	0.00% (0/5000)	0.00% (0/5000)
	Probe	99.92% (4996/5000)	0.06% (3/5000)	0.00% (0/5000)	0.02% (1/5000)
	Combined	**99.48%**	**0.46%**	**0.00%**	**0.06%**

Mismatch severity against emerging SARS-CoV-2 variants

The primer and probe sequence analysis against the five SARS-CoV-2 variant subset databases, indicated that the E gene primers and probe have 99.4% homology or higher with all sequences analyzed and only the N gene test showed mismatches against more than 2% of the tested variant databases (Table 6). Here, the N gene probe had single mismatches against 94.2% of Omicron sequences, and 5.2% of Beta sequences. Since amplification and detection of the N gene or E gene is required to detect SARS-CoV-2 with the MicroGEM Sal6830 SARS-CoV-2 Saliva Test and none of the oligos used for amplification and detection of the E gene have a mismatch frequency against either the Omicron or Beta variants greater than 0.4%, the probability of not detecting these variants is minimal. The single N gene probe mismatch identified as low risk in the total database (Table 5) is the same N gene probe mismatch observed in the Omicron Variant of Concern subset database.

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Table 6. Frequency of primer / probe mismatches against emerging SARS-CoV-2 variant databases, as of February 16, 2022.

Oligos in Sal6830 MicroGEM SARS-CoV-2 Saliva Test		Number of mismatches	Variant of concern				
			Alpha	Beta	Gamma	Delta	Omicron
N gene	Forward primer	0	98.8% (494/500)	98.6% (493/500)	99.0% (495/500)	98.6% (493/500)	100.0% (500/500)
		1	1.0% (5/500)	1.4% (7/500)	0.8% (4/500)	1.0% (5/500)	0.0% (0/500)
		2+	0.2% (1/500)	0.0% (0/500)	0.2% (1/500)	0.4% (2/500)	0.0% (0/500)
	Reverse primer	0	99.6% (498/500)	99.4% (497/500)	99.2% (496/500)	99.4% (497/500)	99.6% (498/500)
		1	0.4% (2/500)	0.4% (2/500)	0.8% (4/500)	0.6% (3/500)	0.4% (2/500)
		2+	0.0% (0/500)	0.2% (1/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)
	Probe	0	99.0% (495/500)	94.2% (471/500)	98.2% (491/500)	98.4% (492/500)	5.6% (28/500)
		1	0.8% (4/500)	5.2% (26/500)	1.0% (5/500)	1.4% (7/500)	94.2% (471/500)
		2+	0.2% (1/500)	0.6% (3/500)	0.8% (4/500)	0.2% (1/500)	0.2% (1/500)
E gene	Forward primer	0	99.4% (497/500)	100.0% (500/500)	99.6% (498/500)	100.0% (500/500)	99.6% (498/500)
		1	0.6% (3/500)	0.0% (0/500)	0.4% (2/500)	0.0% (0/500)	0.4% (2/500)
		2+	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)
	Reverse primer	0	99.6% (498/500)	100.0% (500/500)	100.0% 500/500)	99.6% (498/500)	100.0% (500/500)
		1	0.4% (2/500)	0.0% (0/500)	0.0% (0/500)	0.4% (2/500)	0.0% (0/500)
		2+	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)
	Probe	0	100.0% (500/500)	100.0% (500/500)	100.0% (500/500)	100.0% (500/500)	100.0% (500/500)
		1	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)
		2+	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)	0.0% (0/500)

8.3 Cross Reactivity and Microbial Interference (Analytical Specificity)

In-Silico Analysis

The potential for cross-reactivity of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test primers and probes with non-target organisms were determined by alignment of their sequences against a broad database of unintended target organism sequences described in Table 7, including microorganisms associated with other respiratory infections and common members of the human oral and nasal microbiome. Databases of influenza A (32,329 sequences), influenza B (10,151 sequences), influenza C (2 sequences), adenoviruses (4 sequences) and rhinoviruses (30 sequences) were compiled from all available sequences on the NCBI RefSeq database. A single genome sequence was provided for all other potential contaminating organisms, using a reference or representative genome sequence where available.

Table 7. In silico analysis of cross-reactivity

Target organism		Sequence type	GenBank genome accession	% Homology
Coronaviruses	**Severe acute respiratory syndrome coronavirus (SARS-CoV-1)**	Reference	GCA_000864885.1	**N gene** R: 92% Probe: 92% **E gene** F: 96% R: 100% Probe: 100%
	Middle East respiratory syndrome-related coronavirus (MERS-CoV)	Reference	GCA_000901155.1	< 80%
	Human coronavirus 229E (HCoV-229E)	Complete	GCA_000853505.1	< 80%
	Human coronavirus OC43 (HCoV-OC43)	Complete	GCA_003972325.1	< 80%
	Human coronavirus HKU1 (HCoV-HKU1)	Complete	GCA_000858765.1	< 80%
	Human coronavirus NL63 (HCoV-NL63)	Complete	GCA_000853865.1	< 80%
Influenza	Influenza A virus	Database	*NA*	< 80%
	Influenza B virus	Database	*NA*	< 80%
	Influenza C virus	Database	*NA*	< 80%
Other viruses	Adenoviruses	Database	*NA*	< 80%
	Rhinoviruses	Database	*NA*	< 80%
	Human alphaherpesvirus 1 (HHV-1) / Herpes simplex virus type 1 (HSV-1)	Reference	GCA_000859985.2	< 80%
	Human alphaherpesvirus 2 (HHV-2) / Herpes simplex virus type 2 (HSV-2)	Reference	GCA_000858385.2	< 80%
	Human alphaherpesvirus 3 (HHV-3) / Varicella-zoster virus (VZV)	Complete	GCA_000858285.1	< 80%
	Human betaherpesvirus 5 / Human cytomegalovirus (HCMV)	Complete	GCA_000845245.1	< 80%
	Human gammaherpesvirus 4 / Epstein-Barr virus (EBV)	Complete	GCA_002402265.1	< 80%
	Human metapneumovirus (HMPV)	Complete	GCA_002815375.1	< 80%
	Human parainfluenza virus 1 (HPIV-1)	Complete	GCA_000848705.1	< 80%
	Human parainfluenza virus 3 (HPIV-3)	Complete	GCA_000850205.1	< 80%

	Measles morbillivirus / Measles virus	Complete	GCA_000854845.1	< 80%
	Mumps orthorubulavirus (MuV) / Mumps virus	Complete	GCA_000856685.1	< 80%
	Parechovirus A	Complete	GCA_000861505.1	< 80%
	Human orthopneumovirus / Human respiratory syncytial virus (HRSV)	Complete	GCA_000856445.1	< 80%
Bacteria	*Actinomyces viscosus*	Reference	GCA_900637975.1	< 80%
	Bacillus anthracis	Reference	GCA_000008445.1	< 80%
	Bordetella pertussis (Pertussis / Whooping cough)	Reference	GCA_000306945.1	< 80%
	Chlamydia pneumoniae	Reference	GCA_000007205.1	< 80%
	Chlamydia psittaci	Reference	GCA_000204255.1	< 80%
	Corynebacterium diphtheriae (Diphtheria)	Reference	GCA_001457455.1	< 80%
	***Coxiella burnetii* (Q fever) ***	Reference	GCA_000007765.2	**N gene** R: 83%
	Eikenella corrodens	Reference	GCA_900187105.1	< 80%
	Eikenella exigua	Reference	GCA_008805035.1	< 80%
	Eikenella halliae	Reference	GCA_001648475.1	<80%
	Eikenella longinqua	Reference	GCA_001648355.1	<80%
	Escherichia coli	Reference	GCA_000005845.2	< 80%
	Haemophilus influenzae	Reference	GCA_000767075.1	< 80%
	Lactobacillus johnsonii	Reference	GCA_003316915.1	< 80%
	Legionella pneumophila (Legionnaires' disease)	Reference	GCA_001941585.1	< 80%
	Leptospira borgpetersenii	Representative	GCA_000013945.1	< 80%
	Leptospira interrogans (Leptospirosis)	Representative	GCA_000092565.1	< 80%
	***Moraxella catarrhalis* ***	Reference	GCA_000092265.1	**MS2 control** F: 80%
	Mycobacterium tuberculosis (Tuberculosis)	Reference	GCA_000195955.2	< 80%
	Mycoplasma pneumoniae	Reference	GCA_001272835.1	< 80%
	Neisseria elongata	Reference	GCA_003351545.1	< 80%
	***Neisseria meningitidis* (Meningococcus) ***	Representative	GCA_000008805.1	**N gene** F: 80%
	Nocardia asteroides (Nocardiosis)	Reference	GCA_900637185.1	< 80%
	Porphyromonas gingivalis	Reference	GCA_000010505.1	< 80%
	Prevotella oralis	Reference	GCA_000185145.2	< 80%
	Pseudomonas aeruginosa	Reference	GCA_000006765.1	< 80%
	Staphylococcus aureus	Reference	GCA_000013425.1	< 80%
	Staphylococcus epidermidis	Reference	GCA_006094375.1	< 80%
	Streptococcus mitis	Reference	GCA_000960005.1	< 80%
	Streptococcus anginosus	Reference	GCA_001412635.1	< 80%
	Streptococcus mutans	Reference	GCA_001558215.1	< 80%
	Streptococcus pneumoniae	Representative	GCA_000007045.1	< 80%
	Streptococcus pyogenes	Reference	GCA_001267845.1	< 80%
	Streptococcus salivarius	Representative	GCA_000785515.1	< 80%
Fungi	*Candida albicans*	Reference	GCA_000182965.3	< 80%
	Pneumocystis jirovecii	Reference	GCA_001477535.1	< 80%

The primers and probes included in the test showed less than 80% homology with the majority of the organisms evaluated, supporting high analytical specificity for the intended target organisms. The only instances when the primers and probes for SARS-CoV-2 or for the test internal controls show sequence homologies >80 % are as follows:

- N and E gene primers and probes have 92-100% homology with SARS-CoV
- N gene forward primer has 80% homology with *Neisseria meningitidis*
- N gene reverse primer has 83% homology with *Coxiella burnetii*

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- MS2 internal control forward primer has 80% homology with *Moraxella catarrhalis*

Since target detection (cross-reactivity) requires alignment of both forward and reverse primers, and corresponding probe, the only instance where these requirements are met is for the E gene primers and probe against SARS-CoV, which would lead to a false positive result if SARS-CoV is present in the specimen. However, in the wet testing described below, no such cross-reactivity was observed. The matches observed for the other microorganisms (*C. burnetii, M. catarrhalis, or N. meningitidis*) occur only in a primer, so they will not result in false positive result.

Laboratory testing

The analytical specificity of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test was also evaluated by testing the microorganisms described in Table 8 at the noted concentrations. Microorganisms were tested in the presence of SARS-CoV-2 gamma radiated virus at 3x LoD to assess microbial interference or in the absence of SARS-CoV-2 to assess cross-reactivity. Each sample was prepared in pooled negative saliva matrix and tested in triplicate. Table 8 shows the results obtained, which confirm the *in-silico* analysis indicating that the MicroGEM Sal6830 SARS-CoV-2 Saliva Test does not cross-react with any of the tested species and there is no evidence of microbial interference at the concentration tested.

Table 8. Evaluation of cross-reactivity and microbial interference

Organism Tested	Organism ID	In absence of SARS-CoV-2 (Cross-Reactivity)		SARS-CoV-2 Spiked (Microbial Interference)	
		Concentration Tested	Positive/ Tested	Concentration Tested*	Positive / Tested
Human coronavirus 229E	229E	$1.4x10^5$ $TCID_{50}$/mL	0/3	1.4 $x10^5$ $TCID_{50}$/mL	3/3
Adenovirus 1	AV1	$1.4x10^5$ $TCID_{50}$/mL	0/3	1.4 $x10^5$ $TCID_{50}$/mL	3/3
Bordatella pertussis	BP	$1.0x10^6$ CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Candida albicans	CA	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Corynebacterium sp.	CB	1.2 x 10^3 CFU/mL	0/3	1.1 x 10^3 CFU/mL	3/3
Chlamydia pneumoniae	CP	1.0 x 10^6 IFU/mL	0/3	1.0 x 10^6 IFU/mL	3/3
Escherichia coli	EC	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Enterovirus 68	EV68	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 $x10^5$ $TCID_{50}$/mL	3/3
Influenza A	FluA	1.4 x 10^5 CEID/mL	0/3	1.4 $x10^5$ CEID/mL	3/3
Influenza B	FluB	1.4 x 10^5 CEID/mL	0/3	1.4 $x10^5$ CEID/mL	3/3
Human Gammaherpesvirus 4	GHP	1.0 x 10^5 cp/mL	0/3	1.0 x 10^5 cp/mL	3/3
Human coxsackievirus	hCX	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x 10^5 $TCID_{50}$/mL	3/3
Haemophilus influenzae	HI	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Human metapneumovirus	hMPV	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 $x10^5$ $TCID_{50}$/mL	3/3
Human herpesvirus 1	HP1	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 $x10^5$ $TCID_{50}$/mL	3/3
Human herpesvirus 3	HP3	2.2 x 10^4 $TCID_{50}$/mL	0/3	9.4 x 10^3 $TCID_{50}$/mL	3/3

Organism Tested	Organism ID	In absence of SARS-CoV-2 (Cross-Reactivity)		SARS-CoV-2 Spiked (Microbial Interference)	
		Concentration Tested	Positive / Tested	Concentration Tested*	Positive / Tested
Human herpesvirus 5	HP5	1.9 x 10^4 $TCID_{50}$/mL	0/3	2.0 x 10^4 $TCID_{50}$/mL	3/3
Lactobacillus acidophilus	LA	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Legionella pneumonophila	LP	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Moraxella catarrhalis	MC	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Measles virus	MEA	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
MERS-coronavirus	MERS	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
Mycoplasma pneumoniae	MP	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Mycobacterium tuberculosis	MT	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Mumps rubulavirus	MUM	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
Human coronavirus NL63	NL63	9.4 x10^4 $TCID_{50}$/mL	0/3	1.5 x 10^4 $TCID_{50}$/mL	3/3
Neisseria meningitidis	NM	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Neisseria sp.	NS	3.0 x 10^2 CFU/mL	0/3	1.1 x 10^3 CFU/mL	3/3
Human coronavirus OC43	OC43	1.4 x 10^5 $TCID_{50}$/mL	0/3	9.5 x10^4 $TCID_{50}$/mL	3/3
Parainfluenza virus 1	P1	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
Parainfluenza virus 2	P2	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
Parainfluenza virus 3	P3	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
Parainfluenza virus 4	P4	1.4 x 10^5 $TCID_{50}$/mL	0/3	1.4 x10^5 $TCID_{50}$/mL	3/3
Pseudomonas aeruginosa	PA	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
P. jiroveci-S. cerevisiae	PJ	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Prevotella oralis	PO	1.2 x 10^3 CFU/mL	0/3	1.1 x 10^3 CFU/mL	3/3
Respiratory syncytial virus	RSV	8.0 x10^3 PFU/mL	0/3	1.0 x 10^5 PFU/mL	3/3
Rhinovirus 14	RV	1.0 x 10^5 PFU/mL	0/3	1.0 x 10^5 PFU/mL	3/3
Staphylococcus aureus	SA	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
SARS-coronavirus	SARS	6.5 x 10^3 PFU/mL	0/3	5.5 x 10^3 PFU/mL	3/3
Staphylococcus epidermidis	SE	1.0 x 10^6 CFU/mL	0/3	4.5 x 10^5 CFU/mL	3/3
Streptococcus pneumoniae	SPN	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Streptococcus pyogenes	SPY	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3
Streptococcus salivarius	SS	1.0 x 10^6 CFU/mL	0/3	1.0 x 10^6 CFU/mL	3/3

* All organisms tested were full organism.

8.4 Interfering Substances (Analytical Specificity)

A study was performed to assess substances with the potential to interfere with the performance of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test. Potential endogenous and exogenous interferents were tested at the concentration likely to be found in a saliva sample, as described in Table 9. Each interfering substance in pooled negative saliva was tested in triplicate in the presence or absence of Gramma Irradiated SARS-CoV-2, at 3X LoD to evaluate effects on test sensitivity or specificity, respectively. The results indicated that none of the substances evaluated affect test specificity at the concentrations tested.

Table 9. Test results with full dose of substances. Test results obtained using the specified concentrations of substances in the study protocol.

Interfering Substance		Concentration tested	MicroGEM Sal6830 SARS-CoV-2 Saliva Test results (Reactive/Tested)	
			In absence of SARS-CoV-2	SARS-CoV-2 spiked at 3xLoD
End-1	Whole Blood	5% v/v	0/3	3/3
End-2	Mucin, porcine gastric mucosa, (partially purified)	10 mg/mL	0/3	3/3
End-3	Human DNA	10 ng/µL	0/3	3/3
Exo-1	Nasal drops (Phenylephrine)	10% v/v	0/3	3/3
Exo-2	Nasal Corticosteroids (Budesonide)	10% v/v	0/3	3/3
Exo-3	Nasal Gel (Luffa operculata, sulfur)	10% v/v	0/3	3/3
Exo-4	Homeopathic allergy relief medicine (Histaminum hydrochloricum)	2 tablets in 4 mL	0/3	3/3
Exo-5	Throat lozenges, oral anesthetic, andanalgesic (Benzocaine, Menthol)	1 lozenge in 2 mL	0/3	3/3
Exo-6	FluMist© (Live intranasal influenza virus vaccine)	10% v/v	0/3	3/3
Exo-7	Anti-viral drugs (Zanamivir)	5 mg in 2 mL	0/3	3/3
Exo-8	Antibiotic, nasal ointment (Mupirocin)	10% v/v	0/3	3/3
Exo-9	Antibacterial, systemic (Tobramycin)	10% v/v	0/3	3/3
Exo-10	Toothpaste	10% v/v	0/3	3/3
Exo-11	Oral Rinse/Mouth wash	10% v/v	0/3	3/3
Exo-12	Chloraseptic/sore throat spray	10% v/v	0/3	3/3
Exo-13	Nicotine	0.03 mg/mL	0/3	3/3

8.5 Carryover Contamination

A study was conducted to evaluate the potential for carryover contamination when performing the MicroGEM Sal6830 SARS-CoV-2 Saliva Test. The evaluation consisted of alternate testing of high titer SARS-CoV-2 samples with negative samples. Samples consisted of negative saliva pool spiked with gamma irradiated SARS-CoV-2 at 1.0 x 10^6 GE/mL GE/ml. Twenty positive and twenty negative samples were tested on two instrument systems. The observed carryover rate was 0% with twenty of the twenty negative samples not detecting SARS-CoV-2.

8.6 Clinical Evaluation

The clinical performance of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test as a Point of Care (POC) test to determine the presence of SARS-CoV-2 in saliva specimens from individuals that are suspected of COVID-19 by their healthcare provider was evaluated in a prospective clinical study conducted at two sites, between January 2022 – February 2022. Testing was performed by six non-laboratory trained healthcare professionals who had no previous experience using the MicroGEM Sal6830 SARS-CoV-2 Saliva Test. The results of this study are shown in Table 10.

Subject participation in the study consisted of one visit. 120 participants were screened for inclusion or exclusion according to the study participation criteria, and informed consent and baseline survey information on subject demographics and COVID-19 status were collected. Then, a nasopharyngeal (NP) swab specimen for an EUA authorized highly-sensitive RT-PCR comparator test was collected by a health care professional according to the comparator test Instructions for Use. After that, a non-laboratory trained healthcare professional asked the subject to self-collect a saliva specimen, which was tested by the non-laboratory trained healthcare professional using the MicroGEM Sal6830 SARS-CoV-2 Saliva Test on the MicroGEM Sal6830 Point of Care PCR System. The positive percent agreement (PPA) was 87.2% and the negative percent agreement (NPA) was 97.2%. One sample that generated a negative result with the comparator test obtained an invalid result on the MicroGEM Sal6830 SARS-CoV-2 Saliva Test and was excluded from analysis. Specimen collection and testing followed only the instructions provided in the Quick Reference Instructions.

Table 10. Clinical Performance of the MicroGEM Sal6830 SARS-CoV-2 Saliva Test

Combine Sites		Comparator		
		Positive	Negative	Total
MicroGEM Sal6830 SARS-CoV-2 Saliva Test	Positive	41	2	43
	Negative	6	70	76
	Total	47	72	119

Positive Percent Agreement (PPA)	87.2%	CI = 74.8% - 94.0%
Negative Percent Agreement (NPA)	97.2%	CI = 90.4% - 99.2%

9 MICROGEM US CONTACT INFORMATION

MicroGEM
705D – Dale Avenue
Charlottesville, VA 22903
www.microgembio.com/covid-19

10 TECHNICAL SUPPORT

Technical support is available by email at techsupportdx@microgembio.com.

Please have the following information available:

- Product name
- Lot number
- Serial number of the instrument (located on the back of the instrument)
- Error message (if any)
- Software version (located on the instrument screen under settings)

11 TABLE OF SYMBOLS

SYMBOLS			
ANSI / AAMI / ISO 15223-1:2016		**OTHER**	
	Caution		USB 2.0 High Speed Interface Connector
	Biological risks		Hot Surface
	Consult instructions for use		DC Power
	Manufacturer		Power On-Off
SN	Serial Number		Federal Communications Commission
REF	Catalogue Number		Country of manufacture
	Do not re-use		Harmful to the environment
LOT	Batch code		For prescription use only
	Contains sufficient for <n> tests		High Voltage
CONTROL	Control		TUV certification
	Use-by date	PN	Part Number
	Temperature limit		Do not step
	Keep dry	UDI	Unique device identifier
	Do not use if package is damaged		Do not stack
	Keep away from sunlight		This way up
IVD	In Vitro Diagnostics		General symbol for recovery/recyclable
			Cap
			Saliva Cup
			Test Cartridge
			Test ID
			Sample ID Label
			Records ID Label
			Cut Here
			Handle With Care
			WEEE (Waste Electrical and Electronic Equipment)

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Sal6830

MicroGEM Sal6830
SARS-CoV-2 Saliva Test

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IVD Rx For Use Under an Emergency Use Authorization Only M0246 Rev5.4

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Sal6830

Quick Reference Instructions

MicroGEM Sal6830 SARS-CoV-2 Saliva Test

IVD

For Use Under an Emergency Use Authorization Only

M0247 Rev5.4

℞ ONLY

MicroGEM Sal6830 SARS-CoV-2 Saliva Test

Warnings and Precautions

- For in vitro diagnostic use.
- For prescription use only.
- For use under Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- 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 in vitro diagnostics 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.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The test kit should be used with the MicroGEM Sal6830 Point of Care PCR System.
- Do not open pouches until ready to use.
- Do not use a saliva cup or test cartridge that has been dropped after removing it from the pouch.
- The saliva sample should be processed and run within 20 minutes from the time it was collected.
- Do not place the sample ID sticker label around the tube. Only place it on the flat surface on top of the cap.
- Lock the cap after sample collection before proceeding with the test. Failure to lock the cap can lead to erroneous results.
- Do not use an expired kit.
- Do not use any cracked or broken kit parts.
- Dispose according to the facility's guidelines. Place kit contents and related materials in a waste bin according to the facility's guidelines.
- Ensure all kit contents are stored between 15°C and 30°C, protected from direct sunlight and protected from water.
- Read the Instructions for Use (IFUs) and the MicroGEM Sal6830 Point of Care PCR System User Guide for detailed instructions and product information.

Let's get started!

Before you begin:

Before collecting a saliva sample, first carefully read through the instructions below about how to collect a good saliva sample.

Confirm the patient did not eat, drink, use mouthwash, smoke, or chew gum in the last 30 minutes.

Read through these entire Quick Reference Instructions before starting a test. For help, contact MicroGEM technical support at techsupportdx@microgembio.com. Complete instructions can be found in the Instructions for Use (IFU) and the MicroGEM Sal6830 Point of Care PCR System User Guide.

Use hand sanitizer or wash your hands for 20 seconds before starting the test and after the sample has been collected.

Always check the expiry date of consumables prior to running a test. Do not use a kit that is expired.

Inside the kit:

- Cap (Pouch A) - seals the saliva cup after saliva is deposited and releases the capture particles and diluent
- Saliva Cup (Pouch B) - contains capture beads, reagents for sample lysis and nucleic acid extraction
- Test Cartridge (Pouch C) - a microfluidic cartridge that contains the purification column, and primers, probes, enzymes, and other reagents required for amplification and detection of viral targets and controls
- Test ID Card – contains stickers with bar codes for traceability of samples and results
- Quick Reference Instructions (QRI)

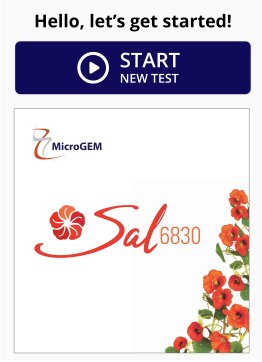
Use the step-by-step instructions on the touch screen for real-time instructions as you do the test.

Run the test

Go to the touch screen and start a new test.

Start a new test

- Click 'Start New Test' (1).
- Open a new test kit. Remove the pouches, Test ID card, and QRI card from the kit.
- Open Pouch A, Pouch B, and Pouch C. Remove the cap and cup from Pouch A and B. **Leave the cartridge in Pouch C until ready to use.**



1

Place ID sticker

- Place sticker #2 on cap and sticker #3 on facility record if required by your facility.
- The person being tested keeps the Test ID card with sticker #1.
- **Do not place the sample ID sticker label around the tube. Only place it on the flat surface on top of the cap (2).**

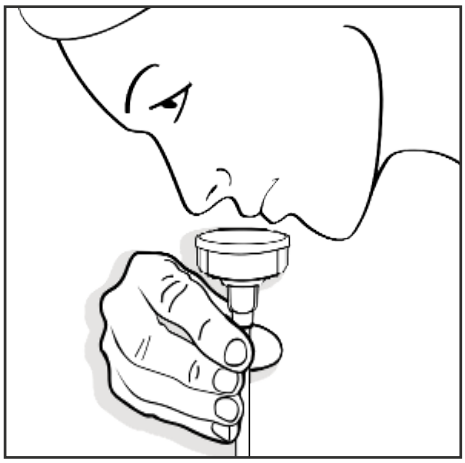
2

Collect saliva

- Hand the cup and cap to the patient being tested.
- Guide the patient through the following steps (3):

How to provide a saliva sample

> Pool saliva in your mouth.
> Press the cup against your lip.
> Tilt your head forward.
> Let your saliva flow into the cup
> Simply pool saliva and let it flow. Think of a favorite food or make chewing motions to help your saliva flow.



3

Resources for collecting good saliva can be found at microgembio.com/salivatips

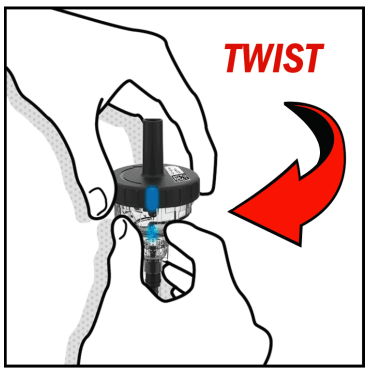
- Instruct the patient to fill the cup to the dotted line as shown in the picture (4). When enough saliva has been collected, the patient should loosely place the cap on the cup and hand it to you, the operator.
  NOTE: The patient should NOT close and lock the cap.
- **Inspect to ensure the saliva is filled to the dotted line. Bubbles should be above the dotted line as shown in the picture (4). Make sure liquid saliva fills the space below the dotted line.**
- Make sure that saliva has not dripped outside the cup. If saliva is observed, wipe the outside of the cup with sanitizing wipes.
- **The saliva sample should be processed and run within 20 minutes from the time it was collected.**

4

Cap the saliva cup

- The test operator should align the tab on the sample cap with the tab on the collection cup, as highlighted in the picture (5).

5

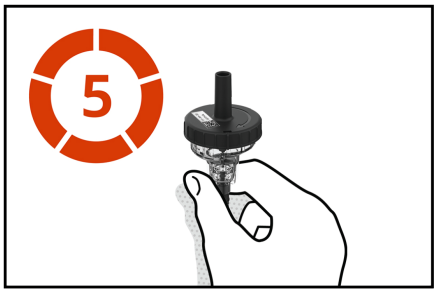


6

- Press down and twist the cap until it is fully closed and locks (6). Once locked, the cap cannot be reopened.
- **Lock the cap before proceeding. Failure to lock the cap can lead to false results.**

Mix the saliva

- Keep the cup upright. Move your entire hand in a circular motion for 5 seconds (7).
- After mixing, the saliva sample should be orange (8) indicating the cap was properly attached. If orange, proceed to the next step. If the sample IS NOT orange, use a new test kit with a fresh saliva sample and repeat the saliva collection process.



7

8

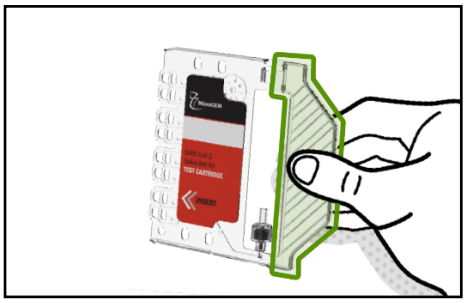
Scan the test ID

- Hold cap near scanner until it beeps (9). The scanner is located above the screen. After scanning, the screen will show the test ID number.

9

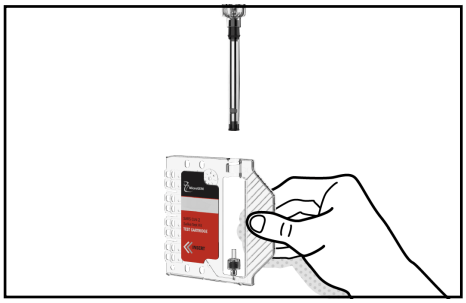
Prepare the test cartridge

- Remove the test cartridge from Pouch C by the handle as highlighted in the picture (10).



10

- Hold the test cartridge as shown and slide the saliva cup into the cartridge (11). **Do not twist the cup while sliding it into place.** The cup will click into place.



11

Insert the cartridge and start the test

- Open the door and slide the assembled test cartridge and saliva cup into the slot (12). Insert the side of the cartridge with the label into the slot. The cartridge will click into place.
- Rotate latch clockwise to lock the cartridge in place. Close the door and push start.

12

Wash hands for a minimum of 20 seconds or use hand sanitizer after completing the saliva collection and test process. If you are wearing gloves, replace them or wipe them with hand sanitizer.

View results

When the test run is complete, verify the test ID on the results screen with the person's test ID card and the ID label on the facility record (if used) to be sure they match. Click "view results". The results screen will show if SARS-CoV-2 has been detected (Table 1). The instrument also produces a QR code on the screen which may be scanned by an optional scanner available from Horiba Instruments (part number SMP) to export into their Lite DM ver 3.0 middleware connectivity software. The results can also be exported to a USB device. Follow your facility procedures to report results.

Table 1: Test results

Test Result	Explanation	Next steps
SARS-CoV-2 Not Detected (presumptive negative)	Negative results should be treated as presumptive and if inconsistent with clinical signs and symptoms or necessary for patient management, should be confirmed with different authorized or cleared molecular test in a CLIA-certified laboratory that meets requirements to perform high or moderate complexity tests. Negative results do not preclude SARS- CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.	Follow the facility's guidelines for communicating results to the person being tested, recording the results and reporting the test result to relevant public health authorities. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
SARS-CoV-2 Detected (positive)	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. The agent detected may not be the definite cause of disease.	Follow the facility's guidelines for communicating the results to the person being tested, recording results and reporting the test result to relevant public health authorities. The person's healthcare provider will consider the test result with all other aspects of the person's history, such as symptoms and possible exposures, to decide how to care for the person.
Invalid Test	No reportable result due to failure of one or more of the internal controls included in the test or failure to pass acceptance criteria for amplification curves of targeted genes.	Repeat the test with a new kit and fresh saliva sample.
System Error	No reportable result due to power failure or instrumentation error.	Repeat the test with a new kit and fresh saliva sample. If the issue persists, contact customer support at techsupportdx@microgembio.com
Test Interrupted	No reportable result due to opening the door of the instrument while the test was running.	Repeat the test with a new kit and fresh saliva sample.

Dispose test cartridge and prepare for next test

Open the door of the system and rotate the red-latch counterclockwise.

Remove the test cartridge and dispose according to the facility's guidelines for waste disposal.

Close the door of the system so it is ready for its next test.

Click "Done" on the screen.

It is recommended that the system be cleaned at the end of each day following the guidance below:

- Wipe the instrument, including exterior surfaces visible behind the door including the latch, with 70% isopropanol available in commercial wipes or on a damp, lint free cloth. Do not spray isopropanol directly onto the instrument as spray may cause damage.
- If spills occur on the instrument or surrounding area, wipe with 10% bleach on a damp, lint free cloth followed by 70% isopropanol available in commercial wipes.
- Do not clean the instrument with soap or other cleaning solutions other than as directed in these instructions.
- Clean barcode reader using 10% bleach on a damp, lint free cloth. **Do not use any other type of cleaner on the barcode scanner.**

External Controls

External controls* are run as if they were patient specimens. The only difference is that instead of collecting saliva from a patient, the contents of the external control vial are used. The user should open the vial, pour the entire contents of the vial into the saliva cup of a newly opened test kit, discard the empty vial per facility procedures, and run the external control per standard protocol in the QRI. It is recommended to run a positive control first and then a negative control. The positive control should give a positive results (i.e., SARS-CoV-2 detected) and the negative controls should give a negative results (i.e., SARS-CoV-2 not detected).

* ZeptoMetrix NATtrol SARS-Related Coronavirus 2 (SARS-CoV-2) External Run Control(NATSARS (COV2)-ERC1) and the ZeptoMetrix SARS-Related Coronavirus 2 (SARS-CoV-2) Negative Control (NATSARS (COV2)-NEG1) positive and negative controls.

Complete instructions can be found in the Instructions for Use and User Guide at:
www.microgembio.com/covid-19/resources

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www.microgembio.com/covid-19
techsupportdx@microgembio.com

PIC Card

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MicroGEM Sal6830 SARS-CoV-2 Saliva Test

This kit does not include all of the labeling (e.g. paper copy).
Scan here to obtain the latest version of the Instructions for Use, or visit:
www.microgembio.com/covid-19/resources
or
https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2.

For support, or to obtain a free paper copy of the labeling and Instructions for Use, please visit: www.microgembio.com/covid-19 or email us at: techsupportdx@microgembio.com.

This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

This product has been authorized only for the detection of nucleic acid 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 in vitro diagnostics 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.

Laboratories within the United States and its territories are required to report all results to the appropriate public health laboratories.

IVD

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For Emergency Use Authorization Only

PN M0233-RevA

MicroGEM

SAL6830 Label Set

System Quick Start Card