

1. PURPOSE

The Veterans Health Administration is evaluating the safety and functionality of a variety of commercially available 3D printed nasal swabs for their use in acquiring samples to test for the SARS-CoV-2 virus. This test method aims to quantitatively measure a nasal swab's ability to release its inoculum in transport media. This test is adapted from the elution test standard using a bacteria sample described in *CLSI M40-A2 Quality Control of Microbiological Transport Systems; Approved Standard-Second Edition, June 2014*. The results from the test provide a quantitative value that is not intended to be an absolute indicator of a performant swab with respect to the SARS-CoV-2 virus. For example, this testing uses a solution of staphylococcus aureus bacteria instead of a viral sample for quantification of specimen release. In addition, in the clinical environment, swabs will come in contact with a much more viscous fluid sample. However, the results from this test are intended to be a comparative measure between standard of care nasal swabs and a 3D printed nasal swab. When combined with other laboratory testing, this test can help to evaluate how performant a 3D printed nasal swab will be in the clinical environment relative to the current standard of care. In order to fully assess clinical performance, data obtained from this test is intended to be followed up with a formal clinical trial during less critical times to further document swab performance.

2. MATERIALS / EQUIPMENT

- 2.1 20 Nasal Swabs (e.g., nasopharyngeal, mid-turbinate)
- 2.2 Test tubes (5 mL size)
- 2.3 Prepared staphylococcus aureus solution - ATCC, #29213 (1.5×10^7 CFU/mL)
- 2.4 Agar plates (e.g. Blood agar – 5% sheep's blood)
- 2.5 Pipettes
- 2.6 0.85% saline solution
- 2.7 Spectrophotometer

3. PROCEDURE

- 1) For each swab, prepare 7 test tubes. Place 1mL of 0.85% saline solution in test tubes #2-7. A full test tube rack will have 140 test tubes per swab type.
- 2) Prepare 1.5×10^7 CFU/mL inoculum solution and verify using a 0.5 McFarland Standard (Figure 1) by testing with a spectrophotometer at a wavelength of 625 nm. Acceptable spectrophotometer results are in the range of 0.05-0.07.

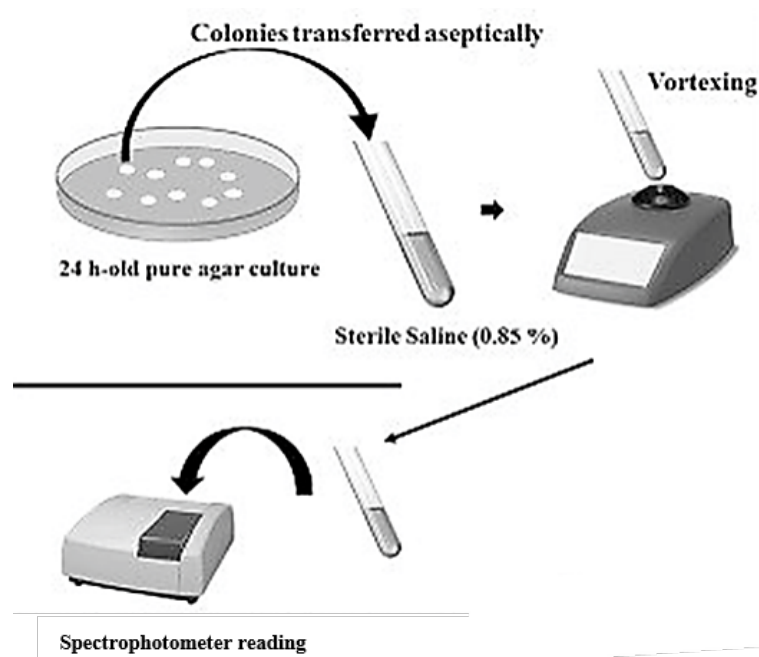


Figure 1: Preparation and verification of a 0.5 McFarland Standard.

- 3) Transfer 1mL of inoculum solution to test tube #1.
- 4) Place a swab in test tube #1 and swirl in solution for ~10 seconds.
- 5) Transfer swab to test tube #2 and vortex the swab and tube for ~10 seconds.
- 6) Begin serial dilutions of inoculum solution. With swab still in tube #2, pipette 100 μ L of inoculum solution from test tube #2 to test tube #3.
- 7) Then pipette 100 μ L of inoculum solution from test tube #3 to test tube #4, proceeding down the line until you've reached the last test tube.
- 8) Pipette tip should be exchanged when moving to the next swab's serial dilution set.
- 9) Using the lowest concentration dilution in test tube #7, transfer 100 μ L each to duplicate agar plates and spread across the plate. There should be a total of 40 plates per swab type.
- 10) Incubate agar plates at 35°C \pm 2°C under appropriate atmospheric conditions and for a minimum of 18 hours.
- 11) After incubation, count and average CFUs. The final colony count is expressed as an average of the CFU of the 40 plates.

4. RESULTS

Average CFU's and standard deviations for each swab will be reported for the incubated conditions.

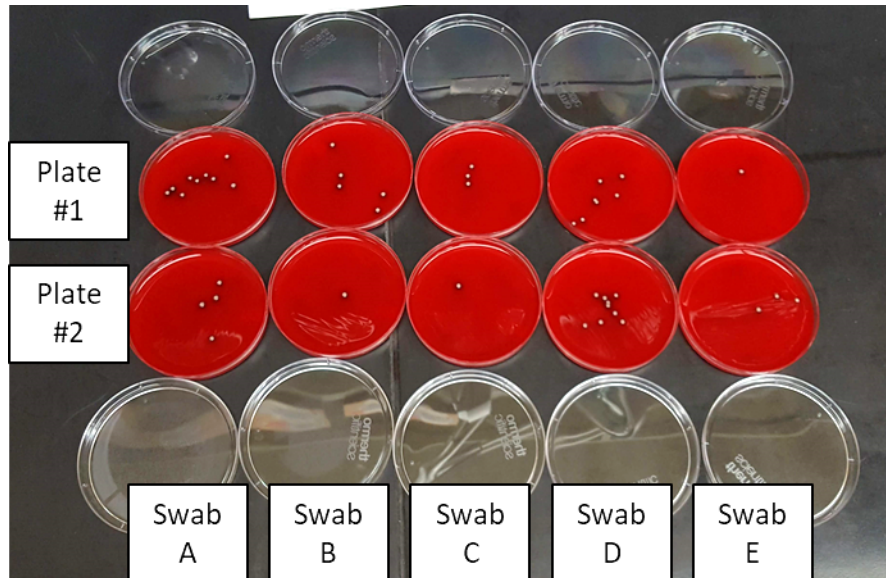


Figure 2: Example duplicate agar plates for 6 swabs.

Revision History

Revision	CHANGE DESCRIPTION	AUTHOR	APPROVAL	DATE
1.0	Start revision management	Arrianna Willis	pending	7.10.2020
1.1	Correction to item 2.3	Arrianna Willis	Pending	10.7.2020