

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 characterize the compatibility of 3D printed nasal swabs (nasopharyngeal or mid-turbinate) with standard laboratory PCR tests for SARS-CoV-2. This test will assess two variables: 1) a 3D printed nasal swab's ability to absorb and elute enough viral material to obtain a positive rtPCR test and 2) whether the material properties of 3D printed nasal swabs interfere with the positive diagnostic accuracy of a standard laboratory PCR test for SARS-CoV-2. It is important to note that conditions used in this test (i.e. time between introduction of swab to transport tube and transfer of viral transport media to rtPCR) have been determined based on local clinical nasal swab test procedures. These variables should be altered to incorporate the "worst case scenario" encountered at your facility. In addition, this test method is meant to simulate a nasal swab procedure in a laboratory environment. It does not encompass all of the variables contributing to a clinical nasal swab procedure. Therefore, 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 Ten (10) standard of care (control) swabs
- 2.2 Ten (10) test swabs (e.g., 3D printed swab – n=10 for each type of swab)
- 2.3 Twenty (20) 5 mL test tubes
- 2.4 Twenty (20) transport tubes containing 1 mL of viral transport media
- 2.5 Additional viral transport media
- 2.6 Positive control viral sample (e.g.) AccuPlex™ SARS-CoV-2 Reference Material Kit (5000 copies/mL)
- 2.7 Pipettes and 200µL and 1000 µL pipet tips
- 2.8 rtPCR Test Kit
- 2.9 rtPCR Testing Machine

3. PROCEDURE

- 1) Perform testing for a total of n=10 control and n=10 test swabs. Run control and test swabs together on the same day, using the same type of positive control sample, to minimize differences in variables.
- 2) Label 10 test tubes and transport tubes with "test swab" and 10 test tubes and transport tubes with "control".
- 3) In each test tube, pipette 1 mL of positive control viral sample and 1 mL of viral transport media. Vortex to thoroughly mix. Final viral concentration in test tube must be at least 5000 copies/ mL.
- 4) One at a time, place a swab in its corresponding test tube and swirl the swab for ~10 seconds, to maximize swab uptake of viral transport media. When removing the swab from the test tube, tap gently to free any loose droplets of

viral sample.

- 5) Transfer swab from test tube to its corresponding transport tube (containing 1 mL of viral transport media). If needed, break off the handle of the swab at the break point so that the swab tip fits within the transport tube. Cap the transport tube with the swab tip still in the tube, as would be done during clinical use.
- 6) Place the transport tubes (with swab still in place) in a laboratory refrigerator (4°C) and store for 24 hours, which simulates worst case storage conditions for swab specimens at our facility (modify to match worst-case storage conditions at your facility if different).
- 7) Once ready for rtPCR, dispose of the remaining swab from each tube and follow rtPCR test kit manufacturer's Instructions for Use for preparation of samples.
- 8) Program rtPCR testing machine for PCR test of viral specimens and run according to the manufacturer's Instructions for Use.
- 9) Record whether the "test" and the "control" samples resulted in a positive or negative rtPCR test result.

		Control Result is Positive (+) (Reference Standard)
Test Swab Result		Condition Present (+)
	Positive (+)	True Positive (TP)
	Negative (-)	False Negative (FN)
	Total	TP + FN

Table 1: Possible rtPCR Test Results from Controlled Viral Sample.

- 10) Once results from all 20 swabs have been recorded, determine percent positive and negative tests for each swab type.

At this point, if positive results are obtained for all 10 test swabs: the swab has passed the test and is considered unlikely to interfere with the rtPCR test. It additionally suggests that the swab is adequate to absorb and elute virus at a concentration of at least 5000 copies/ mL during the process of sample testing. (This test does not assess the ability of the swab to absorb sample in a real-world clinical scenario from a patient's nares/midturbinates/nasopharyngeal region).

At this point, if false negative results are obtained for any of the test swabs: the possibility that the material used to create the test swab (e.g., 3D printed material) could interfere with the rtPCR test should be considered (other reasons for negative results include ineffective absorption/elution, amongst others). If possible, obtain the internal sample control results from any negative swab tests. Laboratory technicians should be able to verify whether the internal

control result is valid or invalid. Any invalid results indicate that the sample is interfering with the PCR tests. If you are unable to obtain internal sample control results to determine there is the possibility of swab material interference, continue to step 11 with 10 new control and test swabs.

****PART 2: Testing for swab material interference with rt PCR****

- 11) Prepare 20 new transport tubes with viral transport media, 10 tubes labeled “test swab” and 10 tubes labeled “control”.
- 12) Spike each transport tube with enough controlled viral sample to obtain a minimum concentration of 2500 viral copies/ mL by pipetting the positive control viral sample directly into the viral transport tube. Vortex for 10 seconds to mix.
- 13) Place a test swab into the transport tube spiked with viral transport media labeled as “test swab”. Swirl the swab in the tube for 10 seconds and tap before fully removing from the tube to release excess droplets. Break off swab at breakpoint. (Note: in contrast to steps 1-10, the test swab is not being used to transfer the virus. The test swab is simply being placed into the transport tube already spiked with virus.)
- 14) Place a control swab in the second tube labeled “control”. Swirl the swab in the tube for 10 seconds, rolling the swab along the side of the tube. Break off swab at breakpoint. (Note: in contrast to steps 1-10, the control swab is not being used to transfer the virus. The test swab is simply being placed into the transport tube already spiked with virus..
- 15) Store both samples next to one another in the same refrigerator (at 4°C) for 24 hours to allow sufficient time for interaction between the nasal swab and spiked viral transport media.
- 16) Once ready for rtPCR, dispose of the remaining swab from each tube and follow rtPCR test kit manufacturer’s Instructions for Use for preparation of samples.
- 17) Program rtPCR testing machine for PCR test of viral specimens and run according to the manufacturer’s Instructions for Use.
- 18) Record whether the “test” and the “control” samples resulted in a positive or negative rtPCR test result.

4. RESULTS

Positive agreement of the test swab with the control should be reported in both fraction (TP/TP+FN) and percentage ($[(TP/TP+FN)*100]$) format. 100% agreement is strongly suggestive that the test swab is compatible with the rtPCR test.

Less than 100% agreement of the test swab with the control swab raises the possibility that the test swab material could interfere with the rtPCR test (although there are many other explanations as well). Part 2 of this protocol is designed to isolate the variables and

question to: does the presence of a test swab interfere with the expected rtPCR result when running a positive viral control? Anything less than 100% agreement with the control swabs for this Part 2 of the protocol is highly suggestive that the material that the test swab is composed of interferes with the rtPCR test.

At all times, control swab tests should result in a positive result. If negative results occur for control swabs, the entire test should be invalidated and a search for the underlying cause should be carried out (e.g., expired or contaminated test reagents, rtPCR machine malfunction, inactivated positive viral control).

Revision History

Revision	CHANGE DESCRIPTION	AUTHOR	APPROVAL	DATE
1.0	Start revision management	Arrianna Willis	pending	7.10.2020
1.1	Revised Materials/Equipment and Procedure based on preliminary laboratory testing results.	Arrianna Willis	Pending	10.7.2020