

GUIDELINE ON THE USE OF DEEP THROAT SALIVA TO TEST FOR COVID-19 BY RT-qPCR

CONTENTS

Item		Page
1.0	Objective	1
2.0	Scope	1
3.0	Procedure	1
A	Things to make sure before the collection of the deep throat saliva sample	1
B	Methods of deep throat saliva collection	1
C	Packaging and transport of deep throat saliva specimen	2
D	Processing deep throat saliva specimen	2
4.0	Handling Precautions	3
5.0	References	4
6.0	Inquiries	4
7.0	Algorithm of testing using Deep Throat Saliva (DTS) for Real Time RT-PCR	5

**Guidelines on the use of Deep Throat Saliva as another sample to perform
Real Time RT-PCR for COVID-19**

1. OBJECTIVE

As a guideline to ensure the process of collection, handling and processing of deep throat saliva specimens received for virological tests are performed properly and systematically.

2. SCOPE

This work instruction shall be used for all deep throat saliva specimens collected for virology testing.

3. PROCEDURE

A Things to make sure before the collection of the deep throat saliva sample

- i. Patient or person under surveillance (PUS) must not eat or drink, smoke, chew tobacco/betel leaves, brush teeth or gargle with mouth freshener for at least 1 to 2 hours prior to the sample collection.
- ii. Let the patient or person under surveillance (PUS) sit comfortably, in a well ventilated space.

B Methods of deep throat saliva collection

- i. Instruct patient or PUS to drain mucus from the back of the nose and throat at least 3 times
- ii. Ask patient or PUS to forcefully breath in 3 times, with head tilt slightly up and cough out the deep throat saliva with mucus.
- iii. If patient or PUS find difficulty with earlier method, they can be asked to collect the saliva in mouth and bring at deep throat then gargle it for >30sec.

GUIDELINE ON THE USE OF DEEP THROAT SALIVA TO TEST FOR COVID-19 BY RT-qPCR

- iv. Ask patient or PUS to lift specimen collection cup close to his/her mouth and take a deep breath in and cough out or spit out the deep throat saliva into the collection cup.
- v. A minimum of 2 ml of deep throat saliva sample is required.

C. Packaging and transport of deep throat saliva specimen

- i. Sample collected in collection cup has to be labelled with patient's details and sealed.
- ii. Each sealed collection cups have to inserted in individual biohazard bag.
- iii. All samples sent or brought to lab has to be packed in triple packaging with ice.
- iv. Samples should reach the laboratory within the same day, on ice.

D. Processing deep throat saliva specimen.

- i. Unpacking
 - Move the sample packages near the biosafety cabinet, Disinfect the outside area of the sample package with a suitable disinfectant before unpacking.
 - Take out the samples and place them in biosafety cabinet. Disinfect outside area of the sample container with a suitable disinfectant.
 - Discard the sample package after taking out the samples.
- ii. As SARS-CoV-2 is a risk group 3 pathogen, the clinical sample must be heat treated to inactivate the virus. For this, please use either a heat block or an oven or a waterbath or any other heating equipment to set temperature to 65°C.
- iii. Carefully open the collection cup, and aliquot 200µL of deep throat saliva sample into microcentrifuge tube for inactivation and the remaining deep throat saliva sample in sample vial for storage in -80°C freezer.

GUIDELINE ON THE USE OF DEEP THROAT SALIVA TO TEST FOR COVID-19 BY RT-qPCR

- iv. In the event if the saliva has dried up in the container, then suitable volume of sterile saline or Viral Transport Media can be added to make up to the volume needed to proceed with RNA extraction.
- v. Confirm temperature of the heat blok or oven or waterbath or any other heating equipment reaches 65°C, before heat inactivating the samples for 60 minutes.
- vi. Once samples have been inactivated, proceed with RNA extraction with the existing kits used in the laboratory.
- vii. Upon completion of RNA extraction, proceed to use the extracts for Real Time RT-PCR for COVID-19, using existing protocols used in the laboratory.

4.0 HANDLING PRECAUTIONS

- i. Use adequate PPE (gowns, masks and gloves) when handling specimens for laboratory testing.
- ii. Disinfect surface of the specimen counter each time after sorting out the specimens. Use disinfectant at recommended in-use dilutions.

5.0 REFERENCES

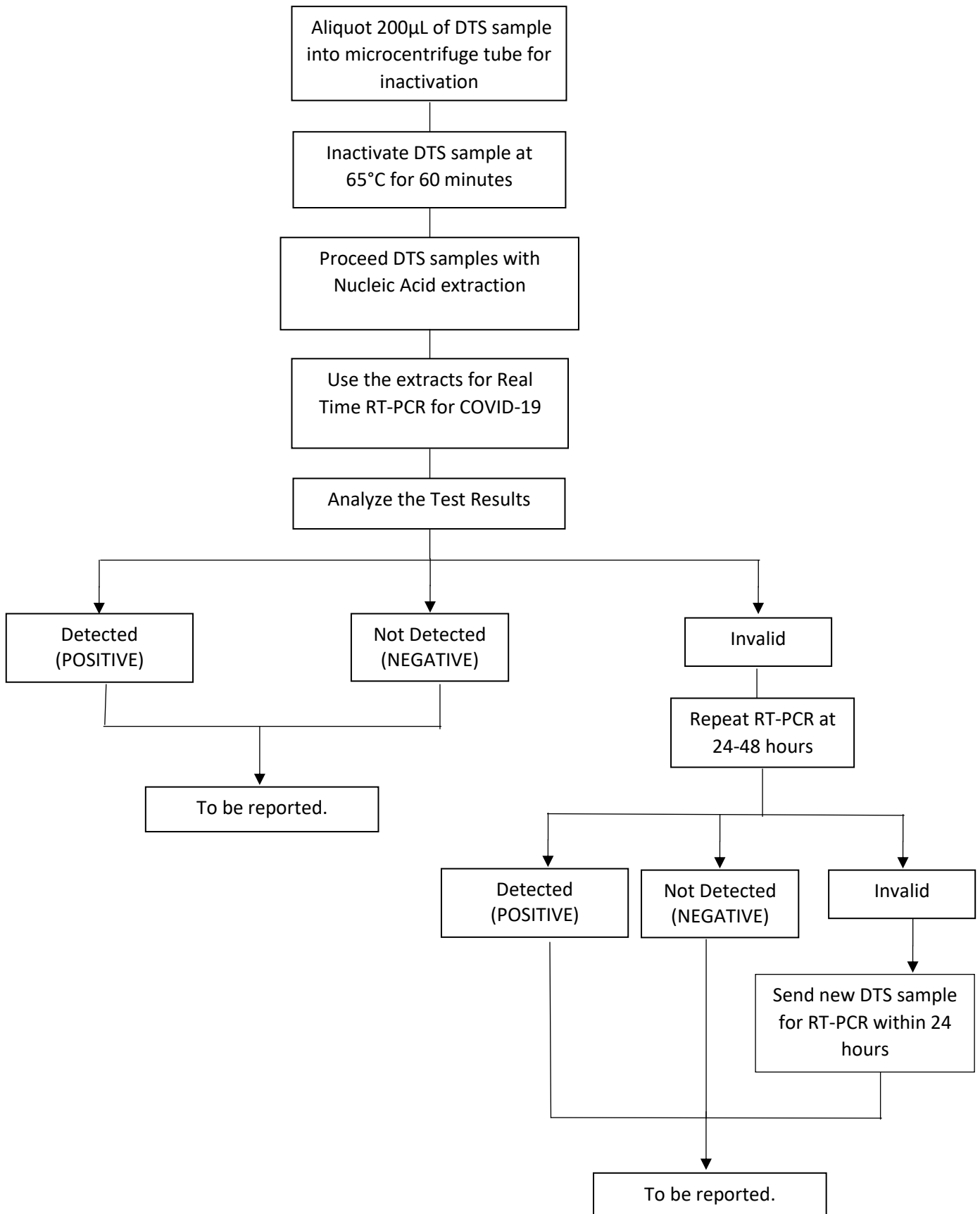
1. Mohan Rao, Fairuz A. Rashid, Fashihah S. A. H. Sabri, Nur Nadia Jamil, Rozainanee Zain, Rohaidah Hashim, Fairuz Amran, Huey Tean Kok, Md Anuar Abd Samad, and Norazah Ahmad 2020. Comparing Nasopharyngeal Swab and Early Morning Saliva for the identification of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clinical Infectious Diseases* 2020; ciaa1156:1–5.
2. Mohan Rao, Fairuz A. Rashid, Fashihah S. A. H. Sabri, Nur Nadia Jamil, Valentinus Seradja, Nurul A. Abdullah, Hanisah Ahmad, S.L. Aren, Shareh A.S. Ali, Mawaddah Ghazali, Anizan A. Mamat, Harishah Talib, Rohaidah Hashim, Rozainanee Zain, Ravindran Thayan, Fairuz Amran, Tahir Aris, and Norazah Ahmad 2021. COVID-19 screening test by using random oropharyngeal saliva. *Journal of Medical Virology*, DOI: 10.1002/jmv.26773.

6.0 INQUIRIES

Any inquiries about this document can be referred to:

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