VivaDiag™ SARS-CoV-2 Ag Rapid Test

Evaluation Protocol

Clinical Evaluation

Ver.1.03

Date of Issue: Aug 26, 2020

Very Important Notices

When you perform the testing of VivaDiag™ SARS-CoV-2 Ag Rapid Test, please note:

- This test is only for professional use.
- The test result can't be used for diagnosis of COVID-19. If the result does not match the clinical evaluation, please do more testing.
- Please do not reuse the test device.
- The test device can only be used with nasal or throat swab specimen. If use other specimens, it may cause wrong results.
- Read the test result at 15 minutes. Don't read the result after 20 minutes If read at other time, it may cause wrong results.
- Please follow the package insert when testing.

Abstract

Since Dec. 2019, the novel coronavirus disease (SARS-CoV-2) quickly spread all over the world.

Due to the limitation of nucleic acid RT-PCR test which has become the standard method for diagnosis of COVID-19, an accurate and rapid test method to quickly identify large number of infected patients is urgently needed, to prevent virus transmission and also assure timely treatment of patients.

VivaChek™ have developed a rapid immunoassay test kit for qualitative determination of SARS-CoV-2 nucleocapsid protein in upper respiratory samples (nasal or throat swab specimen) from patients with signs and symptoms of infection that are suspected of COVID-19 in 15- 20 minutes.

The test devices contain:

1) Conjugate pad: murine anti-SARS-CoV-2 nucleocapsid protein monoclonal antibody and quality control antibody labeled with colloidal gold.
2) NC membrane: coated with one detection line (T line) and one quality control line (C

line). The T line coated with murine anti-SARS-CoV-2 nucleocapsid protein monoclonal antibody. The C line coated with quality control antibody.

Purpose

The purpose of this study is to provide healthcare professionals with a procedure for the clinical performance evaluation of VivaDiag™ SARS-CoV-2 Ag Rapid Test.

Applicable Materials & Equipment

- VivaDiag™ SARS-CoV-2 Ag Rapid Test contains all the substances and package insert.
- Laboratory Instrument (RT-PCR) and matching SARS-CoV-2 nucleic acid (RT-PCR) Test Device which is (FDA EUA) PCR test or a WHO EUL listed PCR test.
- Sterile swabs
- Clinical nasal or throat swab specimens
- Timer

Reference

WHO - Instructions and requirements for Emergency Use Listing (EUL) submission: In vitro diagnostics detecting SARS-CoV-2 nucleic acid and rapid diagnostics tests detecting SARS-CoV-2 antigens

Method

1) Training and Familiarization

All operators performing the evaluation should follow instructions in the VivaDiag™ SARS-CoV-2 Ag Rapid Test package insert and learn how to perform the following tasks:

- How to collect nasal or throat swab specimens using sterile swab (do not touch the tongue to avoid taking saliva)

- How to detect read the result

2) Patient and sample collection

Please take the sample from the patients who conform to the diagnostic criteria of suspected case of COVID-19 according to guideline of diagnosis and treatment of COVID-19 including typical epidemiological history and clinical characteristics.

VivaDiag™ SARS-CoV-2 Ag Rapid Test can use upper respiratory samples (nasal or throat swab specimens) from the patients.

Ideally, 100 prospective positive specimens should be tested (per specimen category, nasal or throat swab specimens). However, if a prospective study is not feasible, an acceptable alternative would be to test at least 100 retrospectively collected SARS-CoV-2 positive specimens from patients. At least 20% of specimens should have Ct values > 30 on the comparator PCR assay. Specimens should be taken at different time points (e.g. days 0-3 (40%); days 4-7 (40%); days >7 (20%)) post onset of symptoms to understand the dynamics of antigen shedding in respiratory specimens. In addition, a minimum of 30 paired, positive nasal swabs and 30 of oropharyngeal specimens (throat swabs) are required to be tested. The specific positive sample requirements are shown as below (Table 1).

Table 1 - Positive sample requirements (confirmed by RT-PCR)

Time Points	Nasal swabs	Throat swabs
Days 0-3	Preferably more than 40	At least 30 (from the same person)
Days 4-7	Preferably more than 40	
Days >7	Preferably more than 20	

A minimum of 400 COVID-19 negative specimens (confirmed by RT-PCR), collected from symptomatic individuals (preferably with respiratory symptoms) collected from the general population are required to be tested. If possible, select both nasal and throat swab

specimens to test by VivaDiag™ SARS-CoV-2 Ag Rapid Test. The specific negative sample requirements are shown as below (Table 2).

Table 2 - Negative sample requirements (confirmed by RT-PCR)

Nasal swabs	Throat swabs
At least 400	If possible, 400 case (from the same person)

The following basic information should accompany each specimen:

- The specimen type.
- The specimen collection date.
- Date of onset of symptoms (if present).
- Clinical diagnosis (if available).
- Severity of symptoms (if known).
- Tests used to identify COVID-19 patients.
- PCR test results (Ct values of SARS-CoV-2 targets and internal control).

Specimen collection

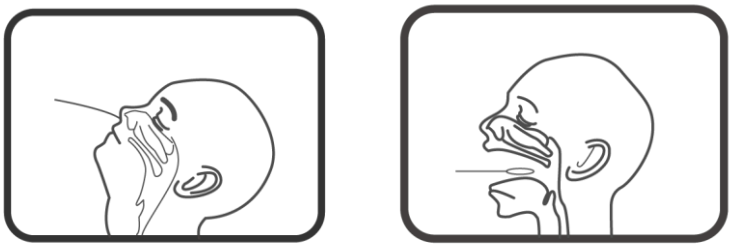
1) Specimen collection

- Nasal swab specimen

It is important to obtain as much secretion as possible. Insert the sterile swab into one nostril. The swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nostril. Roll the swab 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected. Repeat this process for the other nostril to ensure that an adequate specimen is collected from both nasal cavities (use the same swab).

- Throat swab specimen

It is important to obtain as much secretion as possible. Insert the sterile swab into throat that presents the most secretion from the red area of the throat wall and maxillary tonsils to collect throat swab specimen. Rub the bilateral throat tonsils and throat wall moderately to obtain the specimen. Please do not touch the tongue when remove the swab.



Nasal swab specimen　　Throat swab specimen

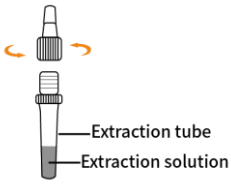
2) Specimen handling

Freshly collected specimens should be tested as soon as possible. It is essential that correct specimen collection and preparation methods are followed.

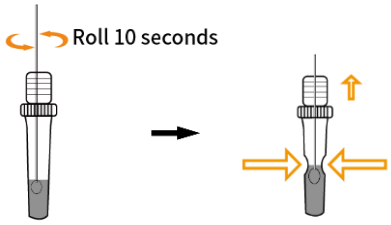
3) Testing Procedures

Allow the Test Devices and Extraction Solution to equilibrate to 15-30°C prior to testing.

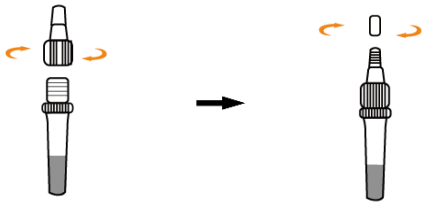
1. Take out a test device from sealed foil pouch and put it on a clean and level surface.
2. Gently unscrew the cap of an extraction tube (prefilled with 300 µL extraction solution), and place it on tube stand.



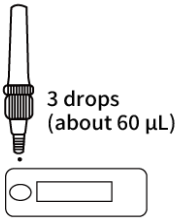
3. Collect specimen refer to **Specimen Collection**.
4. Insert sterile swab with collected specimen into extraction tube (prefilled with 300 µL extraction solution). Roll the swab at least 10 seconds while pressing the head against the bottom and side of the extraction tube. Roll the swab head against the inside of the extraction tube when remove it. Try to release as much liquid as possible. Dispose of the used swab in the biohazard waste.



5. Screw back the cover, and then unscrew the cap at the top of the cover.



6. Apply 3 drops (about 60 µL) of extracted specimen onto the specimen well.



Note:

- *For in vitro diagnostic use.*
- *Handle extraction solution with caution, do not contact with eyes or skin. If spilled on eyes or skin, wash thoroughly with water.*
- *Please follow local regulations to handle the used materials.*

4) Interpretation of Test Results

- Negative result

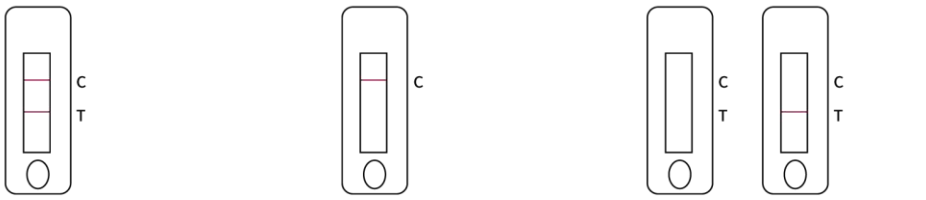
  Only the quality control line C appears, with no other line appearing on the detection line.

- Positive result

  Both the quality control line C and the detection line T appear.

- Invalid result

  Quality control line C fails to appear indicating the test is invalid, no matter if the detection line appears or not. Collect a new specimen and perform another test with a new test device.



Positive: Both detection line (T) and quality control line (C) appear purplish-red in the detection area.

Negative: Only the quality control line (C) appears in the detection area.

Invalid: No purplish-red quality control line (C) appears in the detection area no matter the detection (T) line is colored or not.

Data Analysis

Compare with Reference RT-PCR Results (Nasal or Throat swabs)

The sensitivity and the specificity between the reference RT-PCR results and VivaDiag™ results for SARS-CoV-2 should be calculated separately using a 2 x 2 table as follows:

VivaDiag™ Results	Reference RT-PCR Results		
	Pos.	Neg.	Total
Pos.	A	B	A+B
Neg.	C	D	C+D
Total	A+C	B+D	A+B+C+D

Sensitivity (PPA) = A/ (A+C);

Specificity (NPA) = D/ (D+B);

Overall Percent Agreement (OPA) = (A+D)/(A+B+C+D);

Consistency of swab samples from the nose and throat of the same person (Nasal swabs compare with throat swabs)

Calculate the number (M) of same result of swab samples from the nose and throat which tested by VivaDiag™ SARS-CoV-2 Ag Rapid Test. And the number of all subjects is as T.

Consistency of nasal swab sample and throat swab sample = M/ T.

Form 1: Data & Results

Product: VivaDiag™ SARS-CoV-2 Antigen Rapid Test Device								
Lot Number:								
Sample	RT-PCR Results		VivaDiagTM Results Pos. or Neg. (+ or -)		Collection Date	Date of onset of symptoms	Clinical diagnosis	Severity of symptoms
	Pos. or Neg. (+ or -)	Ct value	Nasal Swabs	Throat swabs				
1								
2								
3								
4								
5								
6								
7								
8								
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…								

Hospital/ Institution name:

Date:

Operator:

Tel:

Form2: Sensitivity, Specificity and Overall Percent Agreement

VivaDiag™ Results	Reference RT-PCR Results		
	Pos.	Neg.	Total
Pos.			
Neg.			
Total			
Sensitivity (PPA)			
Specificity (NPA)			
Overall Percent Agreement (OPA)			

Form3: Consistency of swab samples from the nose and throat of the same person (Nasal or nasopharyngeal swabs compare with oropharyngeal swabs)

Sample	VivaDiag™ Results Pos. or Neg. (+ or -)		whether or not the same?
	Nasal Swabs	Throat swabs	
1			
2			
3			
4			
5			
6			
7			
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13			
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Consistency of nasal swab sample and nasopharyngeal swab sample			