SARS-CoV-2 Ag Saliva Rapid Test
Package Insert

REF VCD17-01-018	English

INTENDED USE AND PRINCIPLE

SARS-CoV-2 Ag Saliva Rapid Test is for the rapid, qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in human saliva[1]. The test is for *in vitro* diagnostic use only. For professional use only. It is intended for clinical laboratories and healthcare professional use only for point-of-care testing[3]. It provides only an initial screening test result. More specific alternative diagnosis methods (molecular diagnostic and / or CT) should be performed in order to obtain the confirmation of SARS-CoV-2 infection. Not for at-home testing.

SARS-CoV-2 Ag Saliva Rapid Test is based on immunochromatography technology. Each test device has one line of anti-SARS-CoV-2 monoclonal antibody on the detection line (T line) and one line of anti-mouse IgG polyclonal antibody on the quality control line (C line). When extracted specimen is added to the specimen well, it will react with the labeled antibody to form a complex, the mixture then migrates through the membrane by capillary action and interacts with the coated anti-SARS-CoV-2 monoclonal antibody on the detection line. If the specimen contains SARS-CoV-2 antigen, the detection line will appear purplish-red indicating the SARS-CoV-2 antigen is positive. Otherwise, the test result will be negative. The test device also contains a quality control line C which should appear purplish-red for all valid tests. If the quality control line C does not appear, the test result will be invalid even if the detection line appears.

COMPOSITION

Each test kit contains test devices, extraction solution (in the sealed tube), extraction tube tips, sterile swabs, tube stand and package insert.

Materials required but not provided: timer.

STORAGE AND HANDLING

- Store the test kit in a cool, dry place between 2-30°C. Keep away from light. Exposure to temperature and / or humidity outside the specified conditions may cause inaccurate results.
- Do not freeze. Use the test kit at temperatures between 15-30°C.
- Use the test kit between 10-90% humidity.
- Do not use the test kit beyond the expiration date (printed on the foil pouch and box).

Note: *All expiration dates are printed in Year-Month-Day format. 2022-06-18 indicates June 18, 2022.*

WARNINGS, PRECAUTIONS AND LIMITATIONS

- Results from SARS-CoV-2 antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status[2].
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic and / or CT should be considered to rule out infection in these individuals.
- Positive results may be due to present infection with SARS-coronavirus strains, see “cross-reactivity” for details. Follow-up testing with a molecular diagnostic and / or CT should be considered to confirm the testing result.
- False results may be due to sticky specimen, insufficient specimen volume or bubbles during applying.
- Do not use unverified UTM, which may lead to false positive or false negative results.
- For *in vitro* diagnostic use only. Not for at-home testing.
- Further molecular diagnostic and / or CT is recommended to identify the actual physical situation.
- Do not open the foil pouch of the test device exposing it to the ambient environment until the test device is ready for immediate use.
- Do not use any damaged test device or material. Do not reuse the test device.
- Handle extraction solution with caution, do not contact with eyes or skin. If spilled on eyes or skin, wash thoroughly with water.
- Do not use test kit beyond the expiration date.
- Specific training or guidance is recommended if operators are not experienced with specimen collection and handling procedures.
- Only use saliva as specimen. Follow the package insert to obtain accurate results.
- Wear protective gears such as laboratory coats, disposable gloves, and eye protection when specimens are collected and evaluated.
- All parts of kit are considered biohazardous and can potentially transmit infectious diseases from pathogens, even after you have performed cleaning and disinfection. Follow proper precautions and all local regulations when disposing of the used test kits.
- Wash hands thoroughly after handling.
- Since the outbreak of the pandemic, the SARS-CoV-2 variant with D614G mutations in the spike protein has replaced the original form in the most regions all over the world[5]. In December 2020, a novel strain of the virus, named ‘VUI-202012/01’, was identified in the England with a set of 17 mutations[6]. Another mutant strain 501Y.V2 of SARS-CoV-2, originally detected in South Africa, shares the same key mutation N501Y. The N501Y mutation locates the receptor-binding domain (RBD) of the spike protein that the virus uses to bind to the human ACE2 receptor, which might be associate with increased transmissibility[7]. The nucleocapsid phosphoprotein (N Protein), linking the viral envelope to the viral RNA, plays a central role in the packaging signal RNA recognition and subsequent RNA encapsidation[8]. Based on its vital role in transcription and replication of the virus, the N protein is suggested to be more sensitive for the early detection of infections[9]. SARS-CoV-2 Ag rapid tests produced by VivaChek employ the interaction with antigen sites in N protein. Till now, there is no clear evidence indicating that mutations found in Spike protein can affect the performance of N protein based antigen tests.

SPECIMEN COLLECTION AND HANDLING

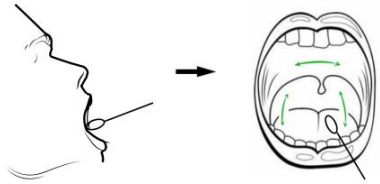
Please instruct the person who need to test to not place anything in the mouth for at least 10 minutes prior to collection including food, drink, gum, tobacco, and etc.

1) Specimen collection[4]

Step 1: Wash hands with soap and water or use hand sanitizer.

Step 2: Open the pack of sterile swabs and remove the sterile swab.

Step 3: It is important to obtain as much secretion as possible. Insert the sterile swab into mouth, then slowly wipe the upper jaw and the inside of the cheek, twist it more than 5 times and soak the saliva completely into the swab to ensure that both mucus and cells are collected.



Note:

- *False results can occur if the saliva is not collected properly.*
- *Do not use any damaged material.*

2) Specimen handling

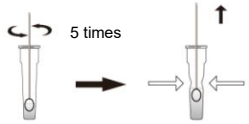
Freshly collected specimens should be tested as soon as possible. It is essential that correct specimen collection and preparation methods are followed.

TEST PROCEDURE

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

1. Open the extraction solution (in the sealed tube).

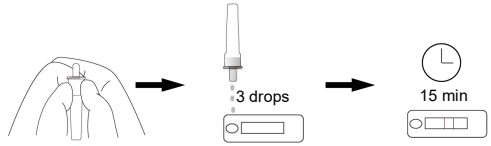
2. Collect specimen refer to **Specimen Collection.**
3. Insert the swab with collected specimen into the tube filled with extraction solution. Roll the swab 5 times while pressing the head against the bottom and side of the tube. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab. Try to release as much liquid as possible. Dispose the used swab as a biohazard waste.



Note:

- *Do not use the extraction solution if it leaks. Use a new extraction solution (in the sealed tube) and follow steps 1 to 3 to extract the specimen.*
- *Please mix specimen well with the extraction solution if the specimen is sticky.*

4. Put on the tube tip.
5. Take out a test device from sealed foil pouch and put it on a clean and level surface.
6. Apply 3 drops of the extracted specimen into the specimen well. Please avoid bubbles during applying and apply the extracted specimen within 1 hour.
7. Read the test result at 15 minutes. Don't read the result after 20 minutes.



Note:

- *Do not interchange or mix extraction solution from different lots.*
- *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.*

INTERPRETATION OF TEST RESULTS

1. Positive Result:

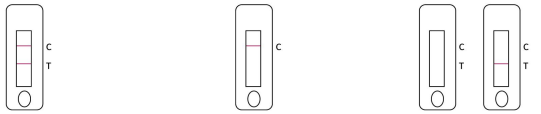
Both the quality control line C and the detection line T appear.

2. Negative Result:

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

3. 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 areas no matter the detection line (T) is colored or not.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is the internal procedural control. This procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

PERFORMANCE

1. Limit of Detection

The LOD for the SARS-CoV-2 Ag Saliva Rapid Test is established using dilutions of an inactivated virus culture. The starting material is supplied at a concentration of 1.51×10^6 $TCID_{50}$/mL. Studies are designed to estimate the LOD of the assay using saliva specimens, the starting material is spiked into a volume of pooled human saliva matrix obtained from healthy donors and confirmed negative for SARS-CoV-2 to obtain a series of different concentrations.

SARS-CoV-2 Titer	1.51×10^6 $TCID_{50}$/mL							
Dilution	1/10	1/100	1/1000	1/2500	1/5000	1/10000	1/20000	1/40000
Concentration in Dilution tested ($TCID_{50}$/mL)	1.51×10^5	1.51×10^4	1.51×10^3	6.04×10^2	3.02×10^2	1.51×10^2	75.5	37.8
Detection rates of 5 replicates	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	60% (3/5)
Detection rates of 20 replicates near cut-off	N/A	N/A	N/A	N/A	100% (20/20)	100% (20/20)	95% (19/20)	75% (15/20)

Lowest Concentration with Uniform Positivity per Analyte	75.5 $TCID_{50}$/mL
Limit of detection (LoD) per inactivated Virus Culture	75.5 $TCID_{50}$/mL

2. Clinical Sensitivity/Clinical Specificity

A total of 566 specimens are tested using the SARS-CoV-2 Ag Saliva Rapid Test. These specimens are obtained by saliva from symptomatic patients. The performance of the SARS-CoV-2 Ag Saliva Rapid Test is compared to a commercialized molecular assay.

Table Summary of sensitivity/specificity of the Ag Saliva Rapid Test compared to PCR.

SARS-CoV-2 Ag Saliva Rapid Test	PCR		
	Positive	Negative	Total
Positive	125	4	129
Negative	10	427	437
Total	135	431	566
Sensitivity	92.59% (125/135, 95%CI, 86.90%~95.93%)		
Specificity	99.07% (427/431, 95%CI, 97.64%~99.64%)		
Accuracy	97.53% (552/566, 95%CI, 95.89%~98.52%)		

The SARS-CoV-2 Ag Saliva Rapid Test shows a clinical sensitivity of 92.59%.

The SARS-CoV-2 Ag Saliva Rapid Test shows a clinical specificity of 99.07%.

The SARS-CoV-2 Ag Saliva Rapid Test shows a clinical accuracy of 97.53%.

Note: *Results from SARS-CoV-2 antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status. Follow-up testing with a molecular diagnostic and / or CT should be considered to confirm the testing result.*

CROSS-REACTIVITY AND INTERFERENCE

1. Cross-Reactivity: there is no cross-reaction with potential cross-reactive substances except SARS-coronavirus.

1) Cross-reaction with SARS-coronavirus

Virus	Strain	Concentration
SARS-coronavirus	Urbani	1 x $I0^6$ PFU/mL

2) No cross-reaction with potential cross-reactive substances

Virus/Bacteria/Parasite	Strain	Concentration Range
Influenza A	H1N1	1×10^4 ~1×10^6 $TCID_{50}$/mL
	H3N2	
	H5N1	
	H7N9	
Influenza B	N/A	
Adenovirus	Type1	
	Type2	
	Type3	
	Type5	
	Type7	
	Type55	
Respiratory syncytial virus	Type A	
	Type B	
Coronavirus	229E	
	0C43	
	NL63	
	HKU1	1×10^3 ng/mL
MERS-Coronavirus	Florida/USA-2_Saudi Arabia.2014	4×10^4 $TCID_{50}$/mL
Parainfluenza	Type1	1×10^5 $TCID_{50}$/mL
	Type 2	
	Type 3	
	Type 4	
Rhinovirus A16	N/A	
Legionella pneumophila	Bloomington-2	1×10^5 cells/mL
	82A3105	
Mycobacterium tuberculosis	K	
	Erdman	
	HN878	
	CDC1551	
	H37Rv	
Streptococcus pneumonia	475298 [Maryland(D1)6B-17]	
	178[Poland23F-16]	
	262[CIP 104340]	
	Slovakia14-10 [29055]	
Streptococcus pyrogens	Typing stain T1	1×10^5 cells/mL
Mycoplasma pneumoniae	Mutant22	
	FH strain of Eaton Agent	
	M129-B7	
Chlamydia pneumonia	AR-39	1×10^6 IFU/mL
Human Metapneumovirus	A1 (IA10-s003)	1×10^5 ~1×10^6 $TCID_{50}$/mL
Enterovirus	Type 68	
Haemophilus influenza	Type b; Eagan	1×10^6 ~1×10^9 CFU/mL
Candida albicans	CMCC(F)98001	
Bordetella pertussis	A639	
Staphylococcus aureus	NCTC 8325	
Staphylococcus epidermidis	MRSE; RP62A	
Pneumocystis jirovecii	W303-Pji	
Pooled human nasal wash	N/A	14% v/v

2. Interference Substances: there is no interference for potential interfering substances listed below.

Potential Interfering Substance		Concentration
Anti-viral drugs	Zanamivir (Influenza)	5 mg/mL
	Oseltamivir (Influenza)	10 mg/mL
	Artemether-lumefantrine (Malaria)	50 μM
	Dorxoycline hyclate (Malaria)	70 μM
	Quinine (Malaria)	150 μM
	Lamivudine (Retroviral medication)	1 mg/mL
	Ribavirin (HCV)	1 mg/mL
	Daclatasvir (HCV)	1 mg/mL
Respiratory Specimens	Mucin: bovine submaxillary gland, type I-S	100 μg/mL
	Blood (human), EDTA anticoagulated	5% (v/v)
	Biotin	100 μg/mL
Nasal sprays or drops	Neo-Synephrine (Phenylephrine)	10% (v/v)
	Afrin Nasal Spray (Oxymetazoline)	10% (v/v)
	Saline Nasal Spray	10% (v/v)
Homeopathic allergy relief medicine	Homeopathic Zicam Allergy Relief Nasal Gel	5% (v/v)
	Sodium Cromoglycate	20 mg/mL
	Olopatadine Hydrochloride	10 mg/mL
Anti-inflammatory	Acetaminophen	199 μM
medication	Acetylsalicylic acid	3.62 mM
	Ibuprofen	2.425 mM
Antibiotic	Mupirocin	10 mg/mL
	Tobramycin	5 μg/mL
	Erythromycin	81.6 μM
	Ciprofloxacin	30.2 μM

3. High-dose Hook Effect: cultured SARS-CoV-2 virus is spiked into specimen. No hook-effect was observed at 1.51 x 10^6 $TCID_{50}$/mL of cultured SARS-CoV-2 virus.

REFERENCES

1. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2 [J]. Nature Microbiology, 5, 536-544 (2020).
2. Perlman, S. Netland, J. Coronaviruses post-SARS: update on replication and pathogenesis. Nature Reviews Microbiology 7, 439-450, doi: 10.1038/nrmicro2147 (2009).
3. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020; 172 (9): 577-582. doi: 10.7326/M20-0504.
4. McCarron, MM, et al. Detection of Phencyclidine Usage by Radioimmunoassay of Saliva. J Anal Tox.1984 Sep-Oct.; 8 (5), pp 197-201.
5. B. Korber et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell, vol. 182, no. 4, pp. 812-827.e19, Aug. 2020, doi: 10.1016/j.cell.2020.06.043.
6. Investigation of novel SARS-CoV-2 variant: Variant of Concern 202012/01, GOV.UK.
7. New SARS-CoV-2 variant, GOV.UK.
8. D. C. Dinesh et al. Structural basis of RNA recognition by the SARS-CoV-2 nucleocapsid phosphoprotein. PLoS Pathog, vol. 16, no. 12, p. e1009100, Dec. 2020, doi: 10.1371/journal.ppat.1009100.
9. J. Mariën et al. Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay. Journal of Virological Methods, vol. 288, p. 114025, Feb. 2021, doi: 10.1016/j.jviromet.2020.114025.

INDEX OF SYMBOLS

	Consult instructions for use		Use by	Σ	Contains sufficient for <n> tests
IVD	For *in vitro* diagnostic use only	LOT	Lot number	REF	Catalog number
2°C-30°C	Storage temperature limitations		Manufacturer		Do not reuse
EC REP	Authorized Representative				

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CE
IVD

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