VITASSAY

SARS-CoV-2

Rapid test for the qualitative detection of nucleoprotein antigen of SARS-CoV-2 from nasopharyngeal swabs.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay SARS-CoV-2 is a rapid, immunochromatographic, one step assay for the qualitative detection of nucleoprotein antigen of SARS-CoV-2 from nasopharyngeal swab samples from patients suspected of COVID-19 infection.

Simple and highly sensitivity immunoassay to make a presumptive diagnosis of SARS-CoV-2 respiratory infection.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) appeared in China the first time and subsequently has spread to over 200 countries of the world with thousands of health's workers infected

The SARS-Cov-2 an agent causing a disease called COVID-19, is a new species of coronaviruses. The world Health Organization (WHO) characterized COVID-19 as a pandemic on March 2020. International guidelines and general and local recommendations focus on the importance of hygiene measurements and rapid identification and isolation of COVID-19 positive patients for preventing infection spread.

Coronavirus belongs to the order of Nidovirales, identified by its envelope characteristics and positive-sense RNA as genetic material. The length of the coronavirus genome is about 26,4-31,7 kb. Coronavidae and Ronividae family are the largest RNA virus among other viruses. The SARS-CoV-2 genome is 29.9 kb.

SARS-CoV-2 infection can be affect individuals of any age, severe illness is uncommon in children (2). The disease currently causes an overwhelming hospitalization of infected patients. Clinically, patients with SARS-Cov-2 infection tend to suffer from mile symptoms such as fever, dry cough, anosmia, fatigue, dypspnea, headache, diarrhea, and sore throat followed by vascular and systemic complications such as leukocyte infiltration of the lungs, pneumonia, severe pneumonia, severe acute respiratory diseases syndrome (ARDS), sepsis and septic shock. Recent studies in COVID-19 patients commonly manifest olfactory and gustatory dysfunction even in the absence of rhinorrhea or nasal obstruction.

Truly asymptomatic infections were not frequent and did not appear to be a major driver of transmission. However, some recent papers propose transmission from pre-symptomatic or asymptomatic cases. Although the pre-symptomatic infectious period is not well defined, some preliminary data suggest that it might be around 2 days before the onset symptoms.

The current COVID-19 pandemic caused by SARS-CoV-2 virus demands the development of strategies not only to detect or inactivated the virus, but to treat it. COVID-19 is not only a critical threat for the population with risk factors, but also generates a dramatic economic impact in terms of morbidity and the overall interruption of economic activities. (4)

PRINCIPLE

Vitassay SARS-CoV-2 is a qualitative immunochromatographic assay for the detection of SARS-CoV-2 from nasopharyngeal swab samples from patients suspected of COVID-19 infection.

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against SARS-CoV-2.

During the process, the sample reacts with the antibodies against SARS-CoV-2, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears.

The presence of this green line (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Clean up spills thoroughly using an appropriate disinfectant.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device. Dangerous samples, handle with caution.
- Tests should be discarded in a proper biohazard container after testing.
- Sterile swabs provided in the kits should be only used for taking the nasopharyngeal sample collection. They cannot be reuse.
- Do not touch the head of the sterile swab provided when opening their primary packaging to avoid contamination.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay SARS-CoV-2**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

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- The presence of yellow lines in the result window (control line zone and test line zone), before using the test, is completely normal and does not imply failure of the test functionality.
- All positive results should be processed following local laws and regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/35.6-86ºF) on the sealed pouch.

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
25 tests/kit **Vitassay SARS-CoV-2.** ▪ 1 Reagent (sample diluent). ▪ 25 Swabs. ▪ 25 Disposable pipettes. ▪ 25 Testing tubes. ▪ Instructions for use. ▪ **Vitassay SARS-CoV-2 Positive Control swab**+ instructions for use.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer.

SPECIMEN COLLECTION

Samples should be collected in clean and dry containers.

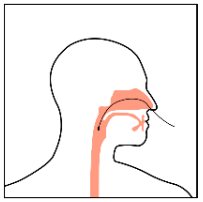
Samples should be process as soon as possible after collection. If this is not possible, the samples can be store in the refrigerator (2-8ºC/35.6-46.4ºF) for 8 hours prior testing.

Samples must be brought to room temperature before testing.

Homogenize the samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

- **Nasopharyngeal swab:**
  1. Remove the swab from its packing.
  2. Use the sterile swab to collect the sample from the nostril. Insert the swab, rotating against the nasapharyngeal wall (to ensure swab contains cells as well as mucus).
  3. Repeat procedure using other nostril.
  4. Process the swab as soon as possible after collecting the specimen.



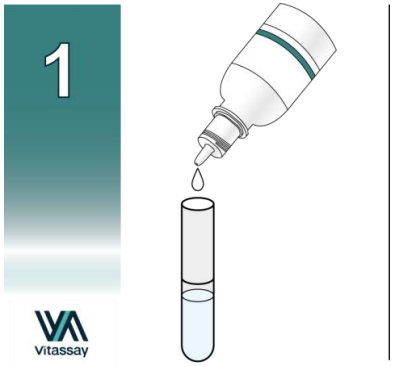
PROCEDURE

Allow the test, samples and reagents to reach room temperature (15-30ºC/59-86ºF) prior to testing.

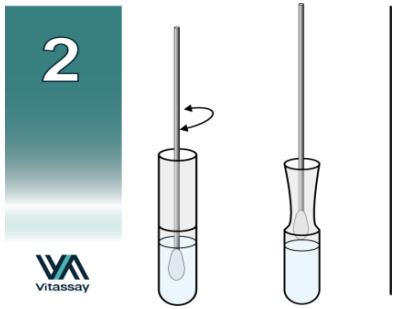
Do not open pouches until the performance of the assay.

Nasopharyngeal swab procedure:

1. Add 15 drops (figure 1) Reagent (SARS-CoV-2) and immediately put the swab into the tube.
2. Mix the solution by rotating the swab forcefully against the side of the tube at least 1 minute. Best results are obtained when the specimen is vigorously extracted from the solution (figure 2). Extract as much liquid as possible from the swab, squeezing the sides of the tube or rotating the swab against the side of the tube as the swab is withdrawn. Discard the swab.
3. Remove the **Vitassay SARS-CoV-2** from its sealed bag before testing. Dispense 3 drops from the testing tube into the circular window marked with letter S (figure 3).
4. Read the results at **10 minutes**. Do not read the test results later than 10 minutes.



Add 15 drops of Reagent.



Put the swab into the tube, rotating 1 minute and extract the liquid.

Dispense 3 drops in the circular window marked with the letter S.

If the test does not run due to the type of sample, stir the sample added in the sample window (S) with the pipette. If it does not work, dispense a drop of Reagent (sample diluent) until seeing the liquid running through the reaction zone.

INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone (**C**)	There is no SARS-CoV-2 presence.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears, (**test line T**)	There is SARS-CoV-2 presence.

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ANY OTHER RESULTS	Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation persist, discontinue using the test kit and contact your local distributor.

Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural control is included in **Vitassay SARS-CoV-2**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay SARS-CoV-2** must be carried out within 2 hours of opening the sealed bag.
- It should only be used with nasopharyngeal swabs. The use of other samples has not been established.
- The quality of **Vitassay SARS-CoV-2** depends on the quality of the sample; Proper nasapharyngeal specimens must be obtained.
- The intensity of test line may vary from very strong (high antigens concentration) to faint (antigens concentration is close to the detection limit).
- Positive results determine the presence of SARS-CoV-2 infection. A positive result should be followed up by a physician and must be based in the correlation of the results with further clinical observations.
- Positive results do not rule out co-infections with other pathogens.
- VTM, UTM and Saline Buffer are the transport media validated for use with **Vitassay SARS-CoV-2**. Following always proportion 1:1 (transport media and sample diluent provided with the device). When using transport media the sensitivity of the device can be reduce due to excessive dilution of sample. The preference is to use the sample immediately after taking it.
- Negative results should not be considered as conclusive; it is possible that the antigens concentration in nasopharyngeal samples is lower than the detection limit value. If symptoms or situation persist, it is recommended that all negative results undergo confirmatory testing using other method and/or virus identification by cell culture or PCR.

EXPECTED VALUES

The pandemic has caused until May 16, 2020, a total of cases and 302.059 deaths worldwide. Spain is the country of the European region that has been most affected by the infection, accounting for 230.183 cases and 33.998 deaths by May 16.

In general, most patients only develop mild (40%) or moderate (40%) disease, 15 % develop in the severe condition that requires oxygen support, and 5% have a critical disease with complications such as respiratory distress syndrome (ARDS), sepsis and septic shock, thromboembolism, and/or multiorgan failure, including acute kidney injury and cardiac injury.

Hospitalization rates were estimated at 20.7%-31.4% in United States, and 23% in Iran. In our area this figure is 42% of reported cases. Then 60%-70% COVID-19 patients remain at home.
Transmission risk factors were age, presence of only two household members, and index case age. Age affectation in household contacts has little difference, suggesting that children may have an important role in the transmission.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

Detection limit value (typical value) of **Vitassay SARS-CoV-2** is 1.0 ng/mL of recombinant protein or $1{\cdot}10^3$ $TCID_{50}$/mL of 2019nCoV/USA-WA1/2020.

Clinical sensitivity and specificity

An evaluation, with 262 nasopharyngeal samples from people suspected of infection by SARS-CoV2 virus, was performed comparing the results obtained by **Vitassay SARS-CoV-2** vs PCR tecnique.

Results were as follows:

		qPCR technique		
		Positive	**Negative**	**Total**
Vitassay SARS-CoV-2	**Positive**	26	1	27
	Negative	2	233	235
	Total	28	234	262

Vitassay SARS-CoV-2 vs qPCR technique		
	Mean value	**95% (Confidence Interval)**
Sensitivity (*)	92.9%	76.5-99.1%
Specificity	99.6%	97.6-100.0%
PPV	96.3%	81.0-99.9%
NPV	99.1%	97.0-99.9%

(*) Taking into account the recommendations for *Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays (11 September 2020)* from WHO, the sensitivity of the test was calculated with nasopharyngeal samples with high viral load (high viral load is expected in early symptomatic phases of the illness (with the first 5-7 days of illness) in the range of Ag-RDT test detection.

The results showed that **Vitassay SARS-CoV-2** has a high sensitivity and specificity to detect SARS-CoV-2.

Hook effect

Vitassay SARS-CoV-2 does not show hook effect at the concentration of SARS-CoV-2 protein tested (202500.0 ng/mL).

Cross reactivity

No cross reactivity was detected against organisms that cause other infections:

Adenovirus	*Escherichia coli O157*	*Lactoferrin (human)*	*RSV*
Astrovirus	*Entamoeba histolytica*	*Legionella*	*Salmonella enteritidis/typhi/ typhimurium/ paratyphi*
Campylobacter jejuni	*Giardia (CWP1, a1-giardian)*	*Listeria monocytogenes*	*Shigella flexneri/boydii/ Sonnei/dysenteriae*
Calprotectin (Human)	*Helicobacter pylori*	*Norovirus GI/Norovirus GII*	*Streptococcus pyogenes*
C. difficile antigen GDH	*Haemoglobin (human/Pig/ Bovine)*	*Streptococcus pneumococcal*	*Transferrin (human)*
C. difficile Toxin *A/ C. difficile* Toxin B	*Influenza A/ Influenza B*	*Rotavirus*	*Yersinia O3/ Yersinia O9*
Coronavirus (strains 229E, NL63, OC43)			

Vitassay SARS-CoV-2 showed some cross reaction with SARS and very low with MERS.

Interference

It was performed an evaluation to determine the cross reactivity of Vitassay SARS-CoV-2. There is not cross reactivity with common respiratory pathogens, other organisms and substances that could cause infections:

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Metronidazole	*Loratadine*	*Loperamide hydrochloride (Fortasec)*	*Phenoxymethylpenicillin potassium*
Ampicillin	*Dexchloropheniramine (Polaramine)*	*Heparin (Hibor)*	*Ambroxol hydrochloride (Mucosan)*
Oseltamivir	*Ebastine (Ebastel)*	*Almagato (Almax)*	*Macrogol 3350 (Movicol)*
Amantadine	*Acetyl Salicylic (Adiro)*	*Fosfamycin (Monurol)*	*Lysine Carbocysteinate (Pectox)*
Ribavirin	*Ibuprofen (Espidifen)*	*Aceltylcysteine (Fluimucil)*	*Hydroxyzine dihydrochloride*
Codeine (Toseina)	*Paracetamol (Dolocatil)*	*Dexketoprofen trometamol (Enantyum)*	*Lorazepam*
Benzocaine (Angileptol)	*Metamizole (Nolotil)*	*Levofloxacin*	*Amoxicillin*
Cloperastine (Flutox)	*Prednisone*	*Ciprofloxacin*	*Mercaptopurine*
Carbocisteine (Iniston mucolítico)	*Omeprazole*	*Rifampicin (Rifaldin)*	

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

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