VITASSAY

SARS-CoV-2+Flu A+B+RSV+Adeno Resp.

Rapid test for the simultaneous qualitative detection of nucleoprotein antigen of SARS-CoV-2, Influenza type A, Influenza type B, RSV and Adenovirus from nasopharyngeal and nasal swabs.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp. is a rapid, immunochromatographic assay for the simultaneous qualitative detection of nucleoprotein antigen of SARS-CoV-2, Influenza type A, Influenza type B, RSV and Adenovirus from nasopharyngeal swabs or nasal swabs samples from patients suspected of COVID-19 infection and/or Influenza A and/or Influenza B and/or Respiratory Syncytial Virus (RSV) and/or Adenovirus infection.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of SARS-CoV-2, and/or Influenza type A, influenza type B, and/or RSV and/or Adenovirus 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

Clinically, patients with SARS-Cov-2 infection tend to suffer from mile symptoms such as fever, dry cough, anosmia, fatigue, dyspnea, 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.

The clinical presentation of respiratory infections caused by different viral pathogens can be very similar, making etiological diagnosis difficult.

Influenza virus, respiratory syncytial virus (RSV) and adenovirus are of primary importance since infections produced by them range from mild respiratory illness to fatal pneumonia, and cause considerable morbidity and excess deaths in children, elderly people, and in immunocompromised individuals throughout the world.

Influenza A and B are two types of influenza viruses that cause epidemic human disease. Uncomplicated influenza illness is characterized by the abrupt onset of constitutional and respiratory signs and symptoms (e.g. fever, myalgia, headache, malaise, nonproductive cough, sore throat, and rhinitis). Among children, otitis, nausea, and vomiting are also commonly reported with influenza illness.

RSV is a frequent cause of flu-like symptoms. It can sometimes cause lower respiratory tract illness, which can be severe, and should be considered in the differential diagnosis in such cases.

Typically, adenovirus infections result in self-limiting respiratory, gastrointestinal or ocular infections, however, adenovirus can cause severe disseminated disease in immunocompromised patients.

PRINCIPLE

Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp. is a qualitative immunochromatographic assay for the simultaneous qualitative detection of nucleoprotein antigen of SARS-CoV-2, Influenza type A, Influenza type B, RSV and/or Adenovirus from nasopharyngeal swab or nasal swab samples from patients suspected of COVID-19 infection and/or Influenza A and/or Influenza B and/or Respiratory Syncytial Virus (RSV) and/or Adenovirus infection.

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

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Influenza type A.

Strip C: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Influenza type B.

Strip D: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against RSV.

Strip E: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Adenovirus.

During the process, the sample reacts with the antibodies against SARS-CoV-2 (strip A), Influenza A (strip B) and/or Influenza B (strip C) and/or RSV (strip D), and/or Adenovirus (strip E) forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is SARS-CoV-2 positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip A. If the sample is Influenza type A positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip B, if the sample is Influenza type B positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip C, if the sample is RSV positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip D and if the sample is Adenovirus positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip E. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears (for all the strips).

The presence of these green lines (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.**

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- 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 (especially samples from patients suspected of SARS-CoV infection) 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. These containers should be discarded in accordance with local or national laws or regulations.
- Sterile swabs provided in the kits should be only used for taking the nasopharyngeal or nasal 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+ Flu A+B+RSV+Adeno Resp.** Do not use any other commercial kit component.
- Use proper infection control practices when collecting and handling specimens. These practices should include, but are not limited to, personal protective equipment (PPE), such as laboratory coat, surgical or appropriate mask, or face shield, disposable gloves, and eye protection. Follow local or national regulations regarding collecting and handling specimens.
- 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).

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
▪ **10** Tests/kit **Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp.** ▪ 10 Vials with Reagent (sample diluent). ▪ 10 Swabs. ▪ Instructions for use.	▪ Specimen collection container (other personal protective equipment that will be considered necessary). ▪ Timer. ▪ Vortex (optional).

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. If the samples (only for nasopharyngeal samples) are preserved in validated transport media (VTM, UTM o Saline Buffer) could be preserved on it until 6 hours at room temperature or in the refrigerator (2-8ºC).

Samples must be brought to room temperature before testing.

Homogenize the samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

-Nasopharyngeal swab method:

1. Remove the sterile swab from its packing.
2. Use the sterile swab to collect the specimen from the nostril. Insert the swab into the nostril to the nasopharynx, rotating against the nasapharyngeal wall (to ensure swab contains cells as well as mucus). Remove the swab from the nostril carefully.
3. Consider repeating procedure using ther nostril only if the protocol of the professional taking the sample required do it.
4. Process the swab (sample) as soon as possible after collecting the specimen.

Follow the test procedure (samples).

-**Nasopharyngeal samples previously extracted by transport media:**

Even while using direct nasopharyngeal swab is the preferred protocol, the device can be also used with samples from nasopharyngela swabs previously diluted in transport media such as: VTM, UTM or Saline Buffer.

1. Use the minimun volume of transport media in order to avoid loss in sensitivity of the system.
2. Dilute te extract sample 1:1 in Reagent (sample diluent) provided.

Follow immediately with the point 3 of the test procedure (samples).

-Nasal swab method:

1. Remove the sterile swab from its packing.
2. Use the sterile swab to collect the specimen from the nostril. Recommended blow your nose once using a tissue before collection of the specimen.
   Insert the swab, rotating against the nasal wall (to ensure swab contains cells as well as mucus). Remove the swab from the nostril carefully and repeat the procedure with the other nostril.
3. Process the swab (sample) as soon as possible after collecting the specimen.

Follow the test procedure (samples).

PROCEDURE

Follow proper infection control practices. 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.

1. Open the cap of the vial for sample dilution with Reagent (figure 1).
2. Introduce the swab into the vial for sample dilution (figure 2) and mix the solution by rotating the swab forcefully against the side of the tube at least 15 seconds. Best results are obtained when the specimen is vigorously extracted in 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. Close the vial with sample and diluent. Shake the vial to assure a good sample dispersion, sake for 60 seconds (figure 2).
4. Remove **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp**. from its sealed bag just before using it (figure 3).
5. Take the vial for sample dilution containing the diluted sample (figure 4), place it inside the multiplex tube (figure 5). Screw the cap of the multiplex tube tighly (figure 6). The bottom of the vial for sample dilution will break and the diluent+sample solution reaches the sample zone of the strips (figure 7).

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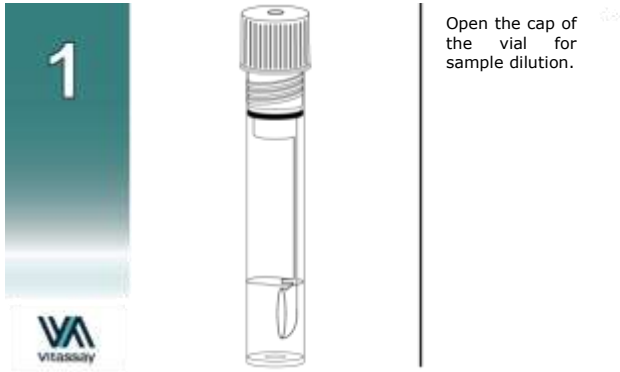
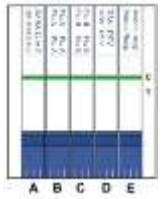
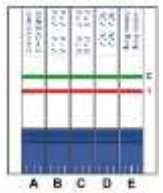
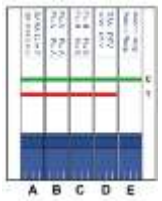
6. Read the results **at 10 minutes**. Do not read the test result later than 10 minutes.

If the test does not run due to solid particles (the sample is not homogenized), migration process can stop on one or more strips. In this case, tap the end of the multiplex tube on hard surface to allow migration to start again.



INTERPRETATION OF THE RESULTS

Strip A: SARS-CoV-2, Strip B: Influenza A, Strip C: Influenza B, Strip D: RSV and Strip E: Adenovirus Resp.

	**NEGATIVE** Only one green line in the control zone (C) in the four strips (A,B,C D and E)	There is no SARS-CoV-2, Influenza A, Influenza B, RSV and Adenovirus Resp.presence
	**POSITIVE** In addition to the green line (control line C), a red line appears in each strip, test line (T).	There is SARS-CoV-2, Influenza A, Influenza B, RSV and Adenovirus Resp.presence.
	**NEGATIVE** Strip E (Adenovirus Resp)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines	There is SARS-CoV-2, Influenza A, Influenza B and RSV presence.
	NEGATIVE Strip D (RSV)→ green line	There is SARS-CoV-2, Influenza A, Influenza B and

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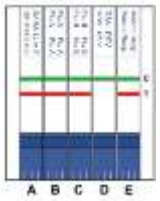
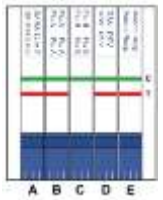
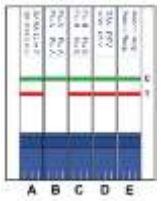
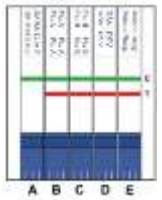
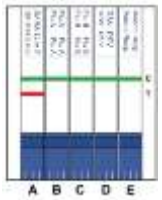
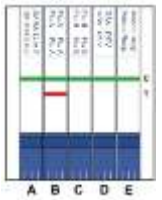
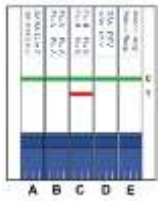
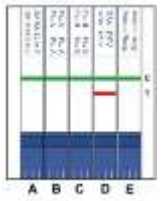
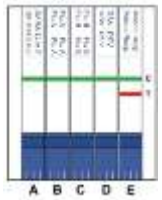
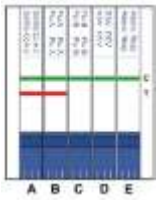
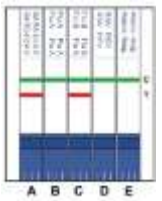
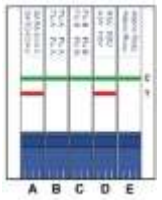
	**POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	Adenovirus Resp presence.
	**NEGATIVE** Strip C (Influenza B)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is SARS-COV-2, Influenza A, RSV and Adenovirus Resp. presence.
	**NEGATIVE** Strip B (Influenza A)→ green line. **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is SARS-COV-2, Influenza B RSV and Adenovirus Resp. presence
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is Influenza A, Influenza B, RSV and Adenovirus Resp presence.
	NEGATIVE	There is SARS-CoV-

	Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line Strip D (RSV)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines	2 presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip C (Influenza B)→ green line Strip D (RSV)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines	There is Influenza A presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line Strip D (RSV)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip C (Influenza B)→ green/red lines	There is Influenza B presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip D (RSV)→ green/red lines	There is RSV presence.

	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line Strip D (RSV)→ green line **POSITIVE** Strip E (Adenovirus Resp.)→ green/red lines	There is Adenovirus Resp presence.
	**NEGATIVE** Strip C (Influenza B)→ green line Strip D (RSV)→ green line Strip E (adenovirus Resp.)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines	There is SARS-CoV-2 and Influenza A presence.
	**NEGATIVE** Strip B (Influenza A)→ green line Strip D (RSV)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip C (Influenza B)→ green/red lines	There is SARS-CoV-2 and Influenza B presence
	**NEGATIVE** Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip A (SARS-COV-2)→ green/red lines Strip D (RSV)→ green/red lines	There is SARS-CoV-2 and RSV presence.

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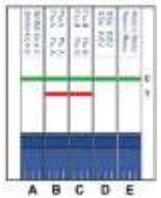
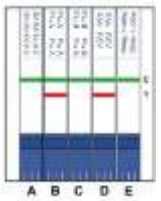
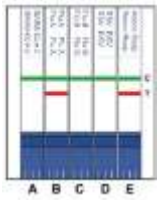
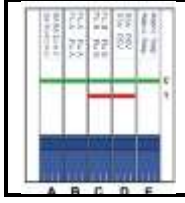
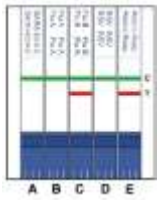
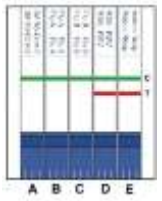
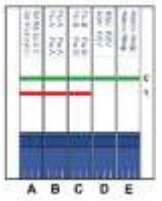
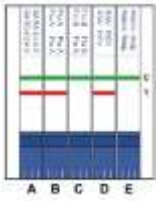
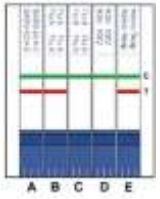
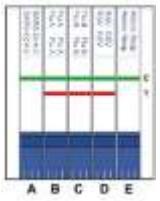
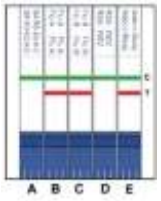
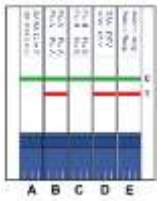
	**NEGATIVE** Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line Strip D (RSV)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is SARS-CoV-2 and adenovirus Resp presence
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip D (RSV)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines	There is Influenza A and B presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip C (Influenza B)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip D (RSV)→ green/red lines	There is Influenza A and RSV.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip C (Influenza B)→ green line Strip D (RSV)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is Influenza A and Adenovirus Resp.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE**	There is Influenza B and RSV presence.

	Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines	
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line Strip D (RSV)→ green line **POSITIVE** Strip C (Influenza B)→ l green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is Influenza B and Adenovirus Resp.presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line **POSITIVE** Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is RSV and Adenovirus Resp.presence.
	**NEGATIVE** Strip D (RSV)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines	There is SARS-CoV-2, Influenza A and Influenza B presence.
	**NEGATIVE** Strip C (Influenza B)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines Strip D (RSV)→ green/red lines	There is SARS-CoV-2, Influenza A and RSV.

	**NEGATIVE** Strip C (Influenza B)→ green line Strip D (RSV)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip B (Influenza A)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is SARS-CoV-2, Influenza A and Adenovirus Resp. presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip E (Adenovirus Resp.)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines	There is Influenza A, Influenza B and RSV presence.
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip D (RSV)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip C (Influenza B)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is Influenza A, Influenza B and Adenovrus Resp. presence
	**NEGATIVE** Strip A (SARS-CoV)→ green line Strip C (Influenza B)→ green line **POSITIVE** Strip B (Influenza A)→ green/red lines Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is Influenza A, RSV and Adenovirus Resp. presence
		There is Influenza B, RSV and

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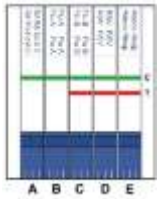
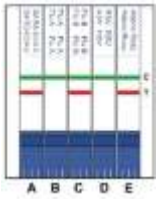
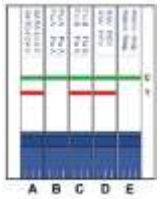
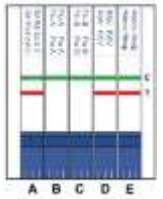
	**NEGATIVE** Strip A (SARS-CoV-2)→ green line Strip B (Influenza A)→ green line **POSITIVE** Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	adenovirus Resp. presence.
	**NEGATIVE** Strip B (Influenza A)→ green line Strip D (RSV)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip C (Influenza B)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is SARS-CoV-2, Influenza B and Andenovirus Resp. presence.
	**NEGATIVE** Strip B (Influenza A)→ green line Strip E (Adeno Resp.)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip C (Influenza B)→ green/red lines Strip D (RSV)→ green/red lines	There is SARS-CoV-2, Influenza B and RSV presence.
	**NEGATIVE** Strip B (Influenza A)→ green line Strip C (Influenza B)→ green line **POSITIVE** Strip A (SARS-CoV-2)→ green/red lines Strip D (RSV)→ green/red lines Strip E (Adenovirus Resp.)→ green/red lines	There is SARS-CoV-2, RSV and Adenovirus Resp presence.

Any other results	Invalid result either A, B, C, D or E, we recommend repeating the assay using the same sample with another test.

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

Positive results detailed in the above table should be followed up with additional confirmatory diagnostic procedures.

Single or dual simultaneous virus infections are more frequent than triple.

Invalid results: Total absence of any control coloured lines (green) indicates an invalid result, regardless of the appearance or not of the test lines (red**).** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. Review the procedure and repeat the assay with a new test. If the problem persists, discontinue using the kit and contact your local distributor.

QUALITY CONTROL

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

RECOMMENDATIONS

Recommendations World Health Organization: Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays- 11 September 202.

1. To optomize performance, testing should be conducted by trained operators in strict accoordance with the test procedure and within the first 5-7 days following the onset of symptoms.
2. Where possible, all positive samples giving positive results should be transported to laboratories with NAAT (Nucleid Acid Amplification Test) capability for confirmatory testing.
3. This test could be used to screen at risk individuals and rapidly isolate positive cases in NAAT- confirmed COVID-19 outbreaks.
4. To monitor trends in disease incidence in communities.
5. For early detection and isolation of positive cases in health facilities, where there is widespread community transmission.
6. A negative result cannot competely exclude an active COVID-19 infection, repeat testing or preferably confirmatory testing should be performed (NAAT) whenever possible, paticulary in symtomatic patients.
7. Even Vitassay SARS-CoV-2 test was not validated using samples form asymtomatic contacts of cases, asymptomatic cases have been demostrated to have viral loads similar to symtomatic cases, so Vitassay SARS-CoV-2 could detect as positive.

LIMITATIONS

- **Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp.** must be carried out within 2 hours of opening the sealed bag.
- The intensity of test line may vary depending on the concentration of antigens.
- The quality of **Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp.** depend on the quality of the sample; proper samples are from nasopharyngeal or nasal swabs.
- Positive results determine the presence of SARS-CoV-2, Influenza type A, Influenza type B, RSV and/or Adenovirus respiratory infection. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated 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.
- Only VTM, UTM and Saline Buffer has been validated with **Vitassay SARS-CoV-2 + Flu A + Flu B + RSV + Adeno Resp.** When using transport media, the sensitivity of the device can be reduced due to excessive dilution of sample. It is not recommended for nasal samples.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigen is lower than the test detection limit value. If symptoms or situation still persist, it is recommended that all negative results undergo confirmatory testing using other method and/or virus identification by cell culture, PCR or other technique.
- To obtain accurate results, do not use bloody nasopharyngeal or nasal samples.
- Very viscous nasopharyngeal or nasal samples may cause non-specific reaction in the test.

EXPECTED VALUES

In general, most patients with COVID-19 infection 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.

Respiratory infections caused by influenza virus type A, influenza virus type B, respiratory syncytial virus (RSV), parainfluenza virus are major causes of upper and lower respiratory tract diseases in infants and young children, causing croup, bronchiolitis, and pneumonia. Additionally, these viruses have all been identified as important causes of several lower respiratory tract diseases, with significant morbidity and mortality, in elderly and immunocompromised patients.

Sixty to ninety percent of the clinical syndrome of bronchiolitis is caused by respiratory syncytial virus (RSV) infection.

Adenoviruses are implicated in 4%-10% of cases of pneumonia, 2%-10% of cases of bronchiolitis, and 3%-9% of cases of croup.

Adenoviruses are less frequent cause of lower respiratory tract infection in children than are respiratory syncytial virus and parainfluenza virus.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

Detection limit value (typical value) of **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip A: SARS-CoV-2) is $1.25x10^2$ PFU/mL ncoV-2019 D614G(S).

Detection limit value (typical value) of **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip B: Influenza A) is 12.5 ng/mL of Influenza A recombinant nucleoprotein.

Detection limit value (typical value) of **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip C: Influenza B) is 50.0 ng/mL of Influenza B recombinant nucleoprotein.

Detection limit value (typical value) of **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip D: RSV) is 20.0 ng/mL of RSV recombinant nucleoprotein.

Detection limit value (typical value) of **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip E: Adenovirus Resp.) 6.25 ng/mL Adenovirus Hexon recombinant protein.

Clinical sensitivity and specificity

Evaluations for SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (strip A: SARS-CoV-2)

An evaluation, with 556 nasopharyngeal samples from people suspected of infection by SARS-CoV-2 virus, was performed comparing the results obtained by **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip A) vs PCR tecnique.

Results were as follows:

		qPCR technique		
		Positive	Negative	Total
Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp (SARS-CoV-2)	Positive	93	1	94
	Negative	7	455	462
	Total	100	456	556

Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp vs qPCR technique		
	Mean value	95% (Confidence Interval)
Sensitivity (*)	93.0%	86.1-97.1%
Specificity	99.8%	98.8-100.0%
PPV	98.9%	94.2-100.0%
NPV	98.5%	96.9-99.4%

This multi-center evaluation (nasopharyngeal samples) with positive samples with Ct< 28 (*) showed the following results: sensitivity 95.1% (95% confidence interval: 88.0-98.7%) and specificity: 99.8% (95% confidence interval: 98.8-100.0%).

(*) 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.

Evaluations for SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (strip A_ SARS-CoV-2)

An evaluation, with 990 nasal samples from people suspected of infection by SARS-CoV-2 virus, was performed comparing the results obtained by Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp. (strip A) vs PCR technique (nasopharyngeal samples).

		Técnica qPCR		
		Positivo	Negativo	Total
Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (SARS-CoV-2 tira A)	Positivo	129	7	136
	Negativo	27	827	854
	Total	156	834	990

Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp. vs técnica qPCR		
	Valor Medio	95% intervalo de confianza
Sensibilidad (*)	82.7%	75.8-88.3%
Especificidad	99.2%	98.3-99.7%
VPP	94.9%	89.7-97.9%
VPN	96.8%	95.4-97.9%

This evaluation (nasal samples for the evaluation with the rapid test) with positive samples with Ct< 28 showed the following results: sensitivity 96.9% (95% confidence interval: 91.2-99.4%) and specificity: 99.2% (95% confidence interval: 98.3-99.7%).

Evaluations for SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (strip B and C: Influenza A and Infuenza B)

Respiratory samples were used in order to evaluate the results obtained by **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip B and C) with other immunochromatographic tests ((BinaxNOW® Influenza A&B (Alere).

Results were as follows:

		BinaxNOW® Influenza A&B		
		Positive	Negative	Total
Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp (Influenza A+B)	Positive	5	0	5
	Negative	0	6	6
	Total	5	6	11

Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp vs BinaxNOW® Influenza A&B			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Evaluations for SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (strip D_ RSV)

Respiratory samples were used in order to evaluate the results obtained by **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip D) with other immunochromatographic tests BinaxNOW® RSV (Alere).

Results were as follows:

		BinaxNOW® RSV		
		Positive	Negative	Total
Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp (RSV)	Positive	18	0	5
	Negative	1	10	11
	Total	19	10	29

Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp. vs BinaxNOW® RSV			
Sensitivity	Specificity	PPV	NPV
95%	>99%	>99%	91%

Evaluations for SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (strip E_ Adenovirus Resp.)

Respiratory samples were used in order to evaluate the results obtained by **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** (strip E) with other immunochromatographic tests, Adenovirus Respi, (CorisBioConcept) and a immunofluorescence test (PathoDx®Adenovirus, Remel).

Results were as follows:

		PathoDx®Adenovirus		
		Positive	Negative	Total
Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (Adenovirus)	Positive	20	0	20
	Negative	0	5	5
	Total	20	5	25

		Adenovirus Respi		
		Positive	Negative	Total
Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp. (Adenovirus)	Positive	20	0	20
	Negative	0	5	5
	Total	20	5	25

Vitassay SARS-CoV-2+Flu A+B+RSV+Adeno Resp. vs PathoDx®Adenovirus Test and Adenovirus Respi Test			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

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The results showed that **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** has a high sensitivity and specificity to detect SARS-CoV-2, Influenza type A, Influenza type B, RSV and Adenovirus.

Hook effect

Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp. does not show hook effect at:

-The concentration of SARS-CoV-2 protein tested (202500.0 ng/mL).

-The concentration of Influenza A protein tested (200000.0 ng/mL).

-The concentration of Influenza B protein tested (200000.0 ng/mL).

-The concentration of RSV protein tested (395000.0 ng/mL).

-The concentration of Adenovirus Resp. protein tested (100000.0 ng/mL).

Cross reactivity

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

Strip A: SARS-CoV-2

Adenovirus	*Cryptosporidium*	*RSV*	*Salmonella enteritidis/typhi/ typhimurium/ paratyphi*
Astrovirus	*Escherichia coli O:157*	*Legionella pneumophila*	*Parainfluenza virus*
Bocavirus	*Enterovirus*	*Metapneumo virus human (hMPV)*	*Haemophilus influenzae*
Bordetella pertussis	*Mycobacterium tuberculosis/*	*Chlamydia pneumoniae*	*Mycoplasma tuberculosis*
Campylobacter jejuni	*Entamoeba histolytica*	*Listeria monocytogenes*	*Shigella flexneri/boydii/ Sonnei/dysenteriae*
C. difficile antigen GDH	*Giardia lamblia*	*Norovirus GI/Norovirus GII*	*Streptococcus pneumococcal/ pyogenes/ pneumoniae*
C. difficile Toxin *A/ C. difficile* Toxin B	*Helicobacter pylori*	*Staphylococcus aureus/epidermis*	*Yersinia O3/ Yersinia O9*
Coronavirus (strain 229E, NL63, OC43, HKU1)	*Pneumocystis jirovecii*	*Rotavirus*	*Influenza A/ Influenza B*
Pig/ bovine haemoglobin	*Pooled human nasal wash-representative of normal respiratory microbial flora*	*Rhinovirus*	

Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp. (SARS-CoV-2, strip A) showed some cross reaction with SARS and with MERS.

Strip B: Influenza A

Adenovirus	*SARS-CoV-2 (SARS-CoV-2)*	*Influenza B*	*RSV*
Astrovirus	*Coronavirus (strain 229, NL63, OC43)*	*Legionella pneumophila*	*Salmonella enteritidis/typhi/ typhimurium/ paratyphi*
Enterovirus	*Haemophilus Influenzae*	*Listeria monocytogenes*	*Shigella flexneri/boydii/ Sonnei/dysenteriae*
Campylobacter jejuni	*Escherichia coli 0:157/0:026/ 0:111*	*MERS*	*Streptococcus pneumococcal/pyogenes*
Norovirus GI/Norovirus GII	*Entamoebqa histolytica*	*Yersinia O3/ Yersinia O9*	*Pig/ bovine haemoglobin*
C. difficile antigen GDH	*Giardia lamblia*	*Rotavirus*	*Helicobacter pylori*
C. difficile Toxin *A/ C. difficile* Toxin B	*SARS-CoV-1 (SARS)*	*Cryptosporidium*	

Strip C: Influenza B

Adenovirus	*SARS-CoV-2 (SARS-CoV-2)*	*Influenza A*	*RSV*
Astrovirus	*Coronavirus (strain 229, NL63, OC43)*	*Legionella pneumophila*	*Salmonella enteritidis/typhi/ typhimurium/ paratyphi*
Enterovirus	*Haemophilus Influenzae*	*Listeria monocytogenes*	*Shigella flexneri/boydii/ Sonnei/dysenteriae*
Campylobacter jejuni	*Escherichia coli 0:157/0:026/ 0:111*	*MERS*	*Streptococcus pneumococcal/ pyogenes*
Norovirus GI/Norovirus GII	*Entamoebqa histolytica*	*Yersinia O3/ Yersinia O9*	*Pig/ bovine haemoglobin*
C. difficile antigen GDH	*Giardia lamblia*	*Rotavirus*	*Helicobacter pylori*
C. difficile Toxin *A/ C. difficile* Toxin B	*SARS-CoV-1 (SARS)*	*Cryptosporidium*	

Strip D: RSV

Adenovirus	*SARS-CoV-2 (SARS-CoV-2)*	*Influenza A/ Influenza B*	*Yersinia O3/ Yersinia O9*
Astrovirus	*Coronavirus (strain 229, NL63, OC43)*	*Legionella pneumophila*	*Salmonella enteritidis/typhi/ typhimurium/ paratyphi*
Enterovirus	*Haemophilus Influenzae*	*Listeria monocytogenes*	*Shigella flexneri/boydii/ Sonnei/dysenteriae*
Campylobacter jejuni	*Escherichia coli 0:157/0:026/ 0:111*	*MERS*	*Streptococcus pneumococcal/pyogenes*
C. difficile antigen GDH	*Entamoebqa histolytica*	*Cryptosporidium*	*Norovirus GI/Norovirus GII*
C. difficile Toxin *A/ C. difficile* Toxin B	*Giardia lamblia*	*SARS-CoV-1 (SARS)*	*Rotavirus*
Helicobacter pylori	*Pig/ bovine haemoglobin*		

Strip E: Adenovirus Resp.

Astrovirus	*Coronavirus (strain 229, NL63, OC43)*	*Influenza A/ Influenza B*	*Salmonella enteritidis/typhi/ typhimurium/ paratyphi*
Enterovirus	*Haemophilus Influenzae*	*Legionella pneumophila*	*Shigella flexneri/boydii/ Sonnei/dysenteriae*
Campylobacter jejuni	*Escherichia coli 0:157/0:026/ 0:111*	*Listeria monocytogenes*	*Streptococcus pneumococcal/pyogenes*
C. difficile antigen GDH	*Entamoebqa histolytica*	*MERS*	*Norovirus GI/Norovirus GII*
C. difficile Toxin *A/ C. difficile* Toxin B	*Giardia lamblia*	*Yersinia O3/ Yersinia O9*	*Rotavirus*
SARS-CoV-1 (SARS)	*Helicobacter pylori*	*Cryptosporidium*	*Pig/ bovine haemoglobin*
SARS-CoV-2 (SARS-CoV-2)			

Interference

An evaluation was performed to determine the possible interferences of **Vitassay SARS-CoV-2+ Flu A+B+RSV+Adeno Resp.** no interferences against the substances tested were detected:

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)*	*Acetylcysteine (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)*	*Biotine*
Naso GEL	*CVS Nasal Spray (Cromolyn)*	*Afrin (Oxymetazoli*	*CVS Nasal Drops*

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		ne)	*(Phenylephrine)*
Sore Throat Phenol spray	*Tobramycin*	*Mupirocin*	*Fluticasone Propionate*
ZICAM	*Homeopathic*	*HAMAs (Human anti-mouse antibodies*	*Chloraseptic (Menthol/Benzo caine)*
Human haemoglobin	*Human transferrin*	*Human calprotectin*	*Human Lactoferrinn*
Mucine	*Human blood*		

SARS-CoV-2 strip:

STREP A	*ADENO*	*RSV*	*Influenza A*
Influenza B			

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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		Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number
CE	CE marking		Do not re-sterilize
	Sterilized using ethylene oxide		Only one use
	Do not use if package is damaged		

Vitassay


Vitassay