COVID-19 Antigen Rapid Test

ANALYTICAL VALIDATION

BIOLAN HEALTH

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1. INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a novel coronavirus that causes the disease COVID-19, a respiratory illness that can range from a common cold to more serious illnesses that can lead to death. It was first detected in December 2019 in the Chinese city of Wuhan and has since spread rapidly around the world[1]. According to current evidence, human-to-human transmission of the COVID-19 virus is primarily spread through respiratory droplets and contact routes[2]. Although the incubation period for COVID-19 ranges from 1 to 14 days, on average, it takes 5–6 days since someone is infected with the virus for symptoms to show[3]. Furthermore, SARS-CoV-2 viral load in the upper respiratory tract tends to peak within the first week after the onset of symptoms[4] so the patient tends to be more infectious at this stage of the disease[5]. Therefore, it is critical the early detection and isolation of these potentially infectious individuals to avoid a further spread of the virus.

To date, the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) remains the gold standard method for detecting SARS-CoV-2. Although its high sensitivity and specifity, the sample must be sent to a central lab to perform the analysis and requires waiting a few hours to get the result. In this context, the BIOLAN HEALTH COVID-19 Antigen Rapid Test is a lateral flow immunoassay that allows the rapid (15-20 min) and user-friendly detection of the nucleocapsid antigen of SARS-CoV-2, an antigen generally detectable in nasal swab specimens during the acute phase of infection. Therefore, the BIOLAN HEALTH COVID-19 Antigen Rapid Test can be a highly useful tool for the early detection of potentially infectious individuals and for preventing further spread of the COVID-19 with prompt identification and isolation of potentially infectious cases.

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[1] https://www.who.int/es/emergencies/diseases/novel-coronavirus-2019

[2] https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations

[3] https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19

[4] Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. The Lancet Microbe. 2021;2(1):e13-e22. doi:10.1016/S2666-5247(20)30172-5.

[5] Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT, Lin HH. Contact Tracing Assessment of COVID-19 Transmission Dynamics in Taiwan and Risk at Different Exposure Periods before and after Symptom Onset. JAMA Internal Medicine. 2020;180(9):1156-1163. doi:10.1001/jamainternmed.2020.

2. OBJECTIVE

To evaluate the performance of the BIOLAN HEALTH COVID-19 Antigen Rapid Test for detecting SARS-CoV-2 in clinical samples.

3. PROCEDURE AND RESULTS

3.1. Clinical performance

The clinical performance of the BIOLAN HEALTH COVID-19 Antigen Rapid Test was evaluated in a prospective study carried out at Hospital Universitario de Cruces in which a total of 430 volunteers were enrolled and tested. Among the volunteers, some were presenting symptoms compatible with a SARS-CoV-2 infection (within 7 days of onset) and others were asymptomatic with or without a known positive contact.

For all volunteers, one direct nasal swab was collected, handled and tested on site with the BIOLAN HEALTH COVID-19 Antigen Rapid Test by a qualified staff and according to the instructions described in the package insert of the tested device. In this study, the perfomance of the BIOLAN HEALTH COVID-19 Antigen Rapid Test was compared to the results obtained with a real-time Polymerase Chain Reaction (RT-PCR) assay for the detection of SARS-CoV-2 in nasofaringeal swab specimens. To this aim, in addition to the nasal swab, a nasopharyngeal swab was also collected by the same health professional. This swab was eluted and stored in a viral transport media (VTM) to be tested at the central laboratory of the Hospital Univesitario de Cruces by RT-PCR.

Clinical sensitivity, specificity and accuracy

Table 1. Clinical performance of the BIOLAN HEALTH COVID-19 Antigen Rapid Test compared to the RT-PCR.

BIOLAN HEALTH COVID-19 Antigen Rapid Test	Reference Method RT-PCR		
	Positive	Negative	Total
Positive	94	6	100
Negative	22	308	330
Total	116	314	430
PPA (sensitivity): 81,0% (95% CI: 73,90-88,16%)			
NPA (specificity): 98,1% (95% CI: 96,57-99,60 %)			
OPP (accuracy): 93,5% (95% CI: 91,15-95,82 %)			

Explanation of terms:

95% C.I.: 95% Confidence Interval

PPA: Positive Percent Agreement (sensitivity) = True Positives/True Positives + False Negatives. The percent of observed agreement on positive values between the tested product and the reference test.

NPA: Negative Percent Agreement (specificity) = True Negatives/True Negatives + False Positives. The percent of observed agreement on negative values between the tested product and the reference test.

OPP: Overall Percent Agreement (accuracy) = True Positives + True Negatives/ Total Samples. The percent of observed agreement on total values between the tested product and the reference test.

As with all antigen tests, the sensitivity in tested population increases with increasing the viral load of the person tested. In Table 2, the performance of the BIOLAN HEALTH COVID-19 Antigen Rapid Test results stratified by the RT-PCR Ct values (which is inversely related to the viral load) are presented. As it can be observed, the sensitivity of the BIOLAN HEALTH COVID-19 Antigen Rapid Test increases in samples with higher viral load, being **98,1%** for samples with a **RT-PCR Ct value ≤ 25.**

Table 2. Performance of the BIOLAN HEALTH COVID-19 Antigen Rapid Test result against the RT-PCR Ct value.

BIOLAN HEALTH COVID-19 Antigen Rapid Test	Reference Method RT-PCR		
	Positive		
	Ct ≤ 25	Ct ≤ 30	Ct ≤ 35
Positive	53	81	94
Negative	1	11	19
Total	54	92	113
PPA (sensitivity) Ct ≤ 25: 98,1% (95% CI: 94,55-100%)			
PPA (sensitivity) Ct ≤30 : 88,0% (95% CI: 81,41-94,67%)			
PPA (sensitivity) Ct ≤ 35: 83,2% (95% CI: 76,29-90,08%)			

Our results (**a sensitivity of 98,1% for subjects with a Ct value ≤ 25 and a specificity of 98,1%**) show that the BIOLAN HEALTH COVID-19 Antigen Rapid Test, **fulfil the criteria defined by WHO[6] and the Health Security Committee of the EU Commision[7]; a sensitivity higher than 80% (and higher than 90% for subjects with a Ct value ≤ 25) and a specifity over 98%**, demonstrating that the test meets the requirements for its application in clinical practice.

3.2. Analytical performance

Limit of Detection (Analytical Sensitivity)

The limit of detection (LOD) of the BIOLAN HEALTH COVID-19 Antigen Rapid Test was determined by evaluating different concentrations of a heat-inactivated SARS-CoV-2 viral sample. To this aim, the starting material, a heat-inactivated SARS-CoV-2 virus sample at a concentration of 1 x $10^{4,95}$ $TCID_{50}$/mL, was spiked into a volume of pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2, to generate virus dilutions for testing.

Each virus dilution was then tested by adding 50 µL of the sample to the swab and by following to the test procedure described in the package insert.

An initial range finding study was performed testing devices in quintupled using a 10-fold dilution series. A concentration was chosen between the last dilution to

[6] Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: Interim guidance, 11 September 2020, WHO.

[7] https://ec.europa.eu/health/sites/default/files/preparedness_response/docs/covid-19_rat_common-list_en.pdf

give 5 positive results and the first to give 5 negative results. Using this concentration, the LOD was further refined. To verify the LOD, the last dilution demonstrating 100% positivity was then tested in an additional 20 replicates tested in the same way. The LOD was determined as the lowest virus concentration that was detected ≥ 95% of the time.

In this study, the BIOLAN HEALTH COVID-19 Antigen Rapid Test LOD (the concentration of virus at which at least 19/20 replicates produced positive results) was confirmed as $3,5 \times 10^{2,5}$ $TCID_{50}$/mL.

Starting Material Concentration	Estimated LOD	No. Positive/Total	% Positive
$1 \times 10^{4,95}$ $TCID_{50}$/mL	$3,5 \times 10^{2,5}$ $TCID_{50}$/mL	19/20	95%

Cross Reactivity (Analytical Specificity)

Cross reactivity of the BIOLAN HEALTH COVID-19 Antigen Rapid Test was evaluated by testing a panel of high prevalence respiratory pathogens that may be present in nasal cavity and that could potentially cross-react with the BIOLAN HEALTH COVID-19 Antigen Rapid Test. The final concentration of each organism is documented in the following table. No cross-reactivity was observed:

Potential Cross-Reactant	Concentration Tested	Final Test Concentration	Cross-Reactivity
Enterovirus Type 71	$4,17 \times 10^{5}$ $TCID_{50}$/mL	$3,79 \times 10^{4}$ $TCID_{50}$/mL	No
Adenovirus	$1,05 \times 10^{6}$ $TCID_{50}$/mL	$9,55 \times 10^{4}$ $TCID_{50}$/mL	No
Influenza B Virus	$4,68 \times 10^{4}$ $TCID_{50}$/mL	$5,51 \times 10^{3}$ $TCID_{50}$/mL	No
Influenza A H1N1 Virus	$4,17 \times 10^{5}$ $TCID_{50}$/mL	$4,91 \times 10^{4}$ $TCID_{50}$/mL	No
Parainfluenza Virus Type 1	$1,26 \times 10^{6}$ $TCID_{50}$/mL	$1,48 \times 10^{5}$ $TCID_{50}$/mL	No
HCoV-229E Nucleoprotein	0,25mg/mL	29,41µg/mL	No
HCoV-NL63 Nucleoprotein	0,25mg/mL	29,41µg/mL	No
HCoV-HKU1 Nucleoprotein	0,25mg/mL	29,41µg/mL	No
HCoV-OC43 Nucleoprotein	0,25mg/mL	29,41µg/mL	No

**Note: Human SARS-CoV and MERS-CoV were not tested due to lack of availability.*

Interfering substances (Analytical Specificity)

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated with the BIOLAN HEALTH COVID-19 Antigen Rapid Test at the concentrations listed below and were found not to affect test performance.

Potential Interfering substance	Concentration Tested	Final Test Concentration	Interference
Ibuprofen	175 mg/dL	25 mg/dL	No
Amoxicillin	35 mg/dL	5 mg/dL	No
Hemoglobin	700 mg/L	100 mg/L	No
Acetylsalicylic acid	70 mg/dL	10 mg/dL	No
Mucin	4% (v/v)	1% (v/v)	No
Biotin	1 mg/mL	100 ug/mL	No
Benzocaine	1 mg/mL	0,14 mg/mL	No
Tobramycin	140 ug/mL	20 ug/mL	No
Acetaminophen	21 mg/dL	3 mg/dL	No
Protein A from Staphylococcus aureus	175 ng/mL	25 ng/mL	No

High Dose Hook Effect

No high dose hook effect was observed up to the highest concentration of heat-inactivated SARS-CoV-2 stock available: 1 x $10^{4,95}$ $TCID_{50}$/mL.

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