SERVEI ANDORRÀ D'ATENCIÓ SANITÀRIA

Lateral flow antigen test for diagnosis of SARS-CoV-2 infection

Designed and manufactured by: Linkcare Bioscreening Barcelona
Product: SARS-CoV-2 antigen test
Sample collection: Nasal swab
Comparison method: TMA, RT-PCR and RT-PCR (≤30 Ct)

Summary

The main challenge to control the COVID-19 pandemic has been the rapid detection of cases given the pre-symptomatic infectious period. The irruption of rapid lateral flow antigen tests (RAT) allows to implement a fast, economical and efficient safety layer against the control of people with high viral load levels of beta coronavirus SARS-CoV-2 in upper respiratory pathway regardless of symptomatology.

The comparative clinical analysis between *the Linkcare Bioscreening SARS-CoV-2 Antigen Rapid Test (TRA)* and the Transcription Mediated Amplification (TMA) diagnostic technique, reveal a high performance of the test, detecting 83% of cases with a positive predictive value of 97.

The sensitivity of the antigen test is increased to 90% when comparing with a quantitative method such as the RT-PCR (Reverse Trancription Polymerase Chain Reaction) test of GenXpert from Cepheid. This PCR has a sensitivity of 99.5% [1] to SARS-CoV-2, establishing a cut-off point at 39 amplification cycles (Cycle threshold or Ct) that allows establishing a detection limit of around 0.0200 to 0.0001 PFU/ml[2].. The sensitivity of the test is increased to 100% (CI: 86% - 100%) if ART is intended to detect people with a high viral load in the respiratory tract greater than or equal to thirty (Ct ≤ 30) and therefore high capacity. infective.

The anterior nasal sampling, a method authorized by the *American Food* and *Drug Administration* (FDA) for sample collection and PCR analysis with GenXpert[3], has been shown to be a simple and fast mechanism with greater acceptance than other techniques because it is non-invasive and efficient for SARS-CoV-2 detection. The nasal sampling opens up the possibility to perform self-testing sample collection, without the need of a healthcare professional for sample collection, which allows to increase the use and sensitivity of the test by repetition.

Background

Type 2 coronavirus that caused severe acute respiratory syndrome (SARS-CoV-2) was first identified in November 2019 in Wuhan, China. This disease-causing coronavirus whose global spread caused the Covid-19 pandemic.

The increasing use of SARS-CoV-2 detection tests is an important factor in controlling the pandemic through Test Trace Isolate (TTI) strategies. The availability of rapid antigen tests makes it possible to modify strategies by creating a regular test regimen that functions as a

filter against SARS-CoV-2, identifying people with both symptomatic and asymptomatic transmission capacity, and allowing immediate isolation and traceability actions.

Objective

Determine the level of sensitivity and specificity of the Linkcare Bioscreening SARS-CoV-2 Antigen Rapid Test, to assess the detection capacity of infectious people.

Place of study

The analytical study was carried out at the "Servei Andorrà d'Atenció Sanitària" (SAAS, Principality of Andorra's Public Health Care Service), c/ Dels Escalls s/n, AD700 Escaldes - Engordany, Andorra.

Materials

Swab.
The round-tipped swab used for the collection of nasal and oropharyngeal samples *was PS Stick with Polyester Tipped Applicator 6" 2.5mm Sterile* with reference GW-1237AP lot 160420.[4]

Means of transport and storage of samples.
The TMA and PCR samples transported to the clinical analysis laboratory of Hospital Nostra Senyora de Meritxell (HNSM), were stored in the Microtest M4RT transport medium from the company Remel and kept at room temperature (+ 18ºC ± 3) until their arrival. to the laboratory where they were kept at a refrigerated temperature of (+ 4ºC ± 2) until the analysis was carried out.[5]

Antigen rapid test.
The antigen rapid test used for this validation was the Linkcare Bioscreening SARS-CoV-2 Antigen Rapid Test lot:20201112. The kit contains buffer, extraction tube with dispensing tip, cassette and swab type C that has been replaced by the one mentioned above in order to perform an optimal nasal sample collection.[6]

TMA.
TMA is a PCR-like technique that allows the detection of RNA of the virus with greater sensitivity, but is not quantifiable. For this analysis, the Hologic SARS-CoV-2 test was used to determine and classify all participants with SARS-CoV-2 infection and compare with the results of the antigen rapid test and PCR test.[7]

rt-PCR COVID.
PCRs were performed with Cepheid's GeneXpert point of care using the Xpert® SARS-CoV-2/Flu /RSV test that combines RSV, FLU-A/B and SARS-CoV-2 detection. This device allowed to assess the viral load level from the quantification of Ct, determining the positivity of the sample in a Ct below 39. PCR was used to analyze those discordant samples between the antigen test and TMA in which a false antigen test negative or false TMA positive was suspected.[8]

PCR respiratory panel.
Discordant samples suspected of a false antigen test positive or false TMA negative were analyzed using BIOFIRE's Respiratory Panel 2.1 plus[9] respiratory panel PCR, that allows detection of the following pathogens:

virus	virus	bacterium
adenovirus	Influenza A	Bordetella parapertussis
Coronavirus HKU1	Influenza A/H1	Bordetella pertussis
Coronavirus NL63	Influenza A/H3	Chlamydia pneumoniae
Coronavirus 229E	Influenza A/H1-2009	Mycoplasma pneumoniae
Coronavirus OC43	Influenza B	
SARS-CoV-2	Parainfluenza Virus 1	
Human Metapneumovirus	Parainfluenza Virus 2	
Human Rhinovirus/Enterovirus	Parainfluenza Virus 3	
Respiratory Syncytial Virus	Parainfluenza Virus 4	

Methods

This study evaluated the Linkcare Bioscreening rapid antigen test using clinical samples of nasal and oropharyngeal swabs. Those who attended the checking point enabled by the Andorran Health Care Service (SAAS) at the Andorra la Vella's "stop lab" (a point enabled by SAAS and Andorra's Ministry of Health for sample collection) were selected with suspicion of being infected, either by contact with a positive regardless of symptomatology, or by COVID-19 compatible symptomatology. Minors, people who did not have Andorran social security and or people who came to obtain COVID certificates for travel were excluded.

Samples were collected between February 16 and March 15. This period can be classified as low incidence, recording a daily average of 31.33 cases per 100,000 inhabitants and a daily incidence of positive results of 0.03% and a cumulative incidence at 14 days of 0.44%.

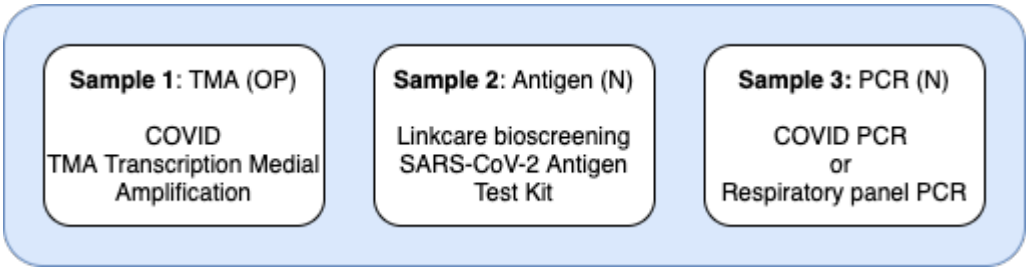

Figure 1: Samples and order of collection of each study participant

Each of the **147 participants** selected for the study got 3 samples collected with a round-tipped rayon fiber swab following the order of Figure 1 an oropharyngeal sample (OP) preserved in UTM for the TMA testing, a nasal sample (N) for rapid antigen testing immediately and a nasal sample Figure 2

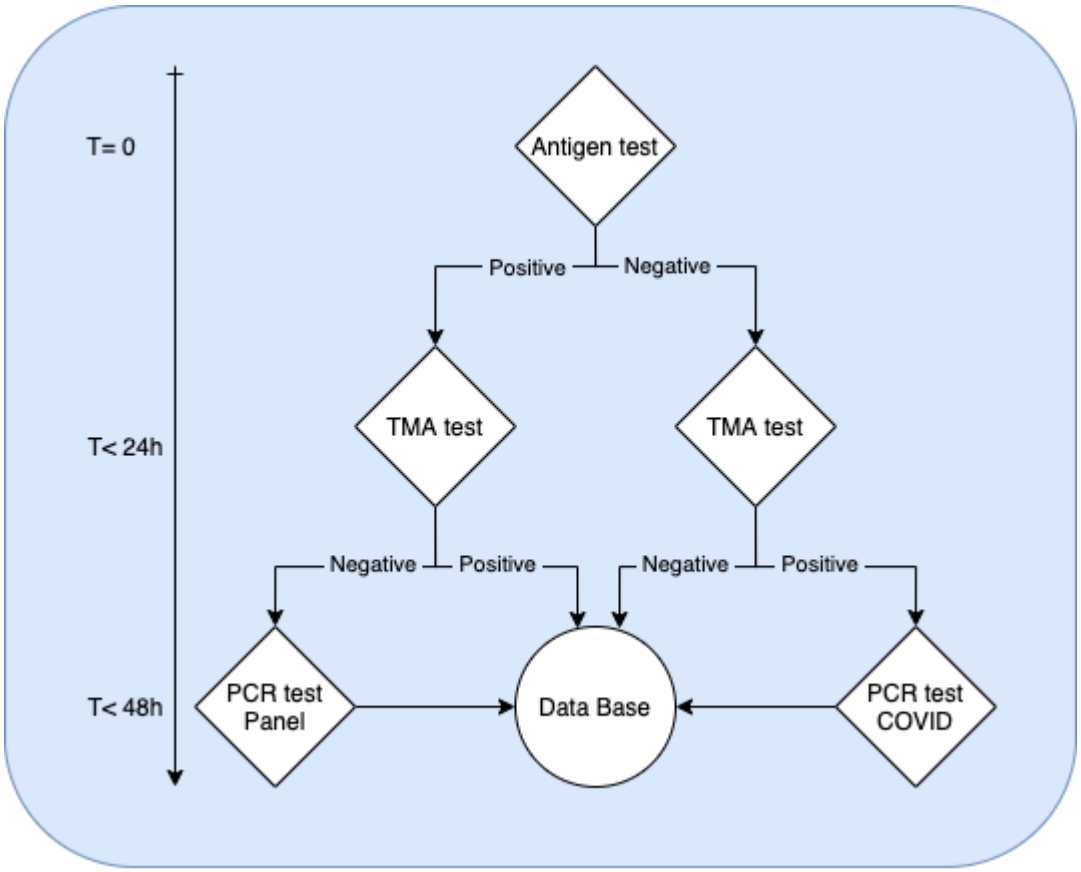

Figure 2: Decision tree for the PCR test confirmation of negative and positive discordant results

Samples obtained for the antigen rapid test were processed immediately following the directions and reading the results within 15 minutes. The results were recorded in primary data sheets linking the test result to the TMA analytical request number and the clinical history number of each participant to retrieve the result information.

Samples obtained for TMA were sent within 4 hours to the HNSM clinical analysis laboratory, where they were processed less than 24 hours after collection. The results of the TMA tests were incorporated into the unified medical history of the principality of Andorra, from where they could be recovered to be compared with the results obtained from the antigen rapid test.

PCR samples were stored at refrigerated temperature (+4ºC) in the HNSM clinical analysis laboratory until the TMA test results were compared with Linkcare Bioscreening antigen rapid test results. Following the preset decision tree, all necessary PCRs were requested and processed within 48 hours. COVID PCR was requested for those discordant results between the two tests in which a false negative result of the antigen test (negative antigen rapid test and positive TMA) is suspected. The result of COVID PCR allows us to confirm the false negative of the antigen test and determine the level of viral load detectable by the antigen rapid test or confirm the false positive of the TMA test.

In cases where a discrepancy was observed between the results of the TMA and the antigen rapid test suspecting a false positive of the rapid antigen test (negative TMA positive antigen test), the decision tree in Figure 2 was followed to perform a respiratory panel PCR. The results of the respiratory panel performed by PCR allows to confirm if the false positive result is produced by another known respiratory virus that may be interfering in the proper functioning of the rapid antigen test.

All the results obtained were stored in a local and private database, being analyzed with Rstudio using the epiR epidemiological analysis package to obtain the sensitivity and specificity values of the antigen test.

Results

A total of **147 people** participated in the study with the results raw data.

	antigen	PCR (Ct≤30)	PCR	Tma
Total positives	44	44	48	52
Total Negatives	103	103	99	95

From this data we have **obtained 10 discordant cases** (see table1.xls):

- 1 sample tested positive in antigen rapid test and negative in TMA. In this case, the corresponding check was performed processing the saved sample as a backup to which a biofire respiratory panel PCR was performed. This determination did not detect any respiratory viruses indicating that the false positive of the antigen test is not caused by known biological interference.
- 9 samples were negative in the antigen rapid test and positive for TMA. According to the established protocol, PCR samples were used as a backup to perform the analysis of Cepheid SARS-CoV-2/Flu/RSV PCRs with the following results:

5 positive samples per PCR	4 negative samples per PCR
muestra 1 Ct = 32,1 muestra 2 Ct = 30,5 muestra 3 Ct = 33,9 muestra 4 Ct = 34,7 muestra 5 Ct = 31,4	muestra 1 Ct > 39 muestra 2 Ct > 39 muestra 3 Ct > 39 muestra 4 Ct > 39

After the description of the positive cases, we proceed to analyze the sensitivity and specificity of the rapid antigen test by taking **the TMA as** Gold ***Standard.*** We observed a sensitivity value of 82.69% (CI: 69.67% - 91.77%) 98.95% specificity (IQ: 94.27% - 99.97%), with a positive predictive value (PPV) of 97.73% (CI: 87.98% - 99.94%) and a negative predictive value (NPV) of 91.26% (CI: 84.06% - 95.93%).

TMA Reference	Covid+	Covid-	total
Test +	43	1	44
Test -	9	94	103
total	52	95	147

Estimates with 95% confidence interval

TMA Reference	estimated	inferior	superior
Sensitivity	82,69	69,67	91,77
Specificity	98,95	94,27	99,97
Positive predictive value	97,73	87,98	99,94
Negative predictive value	91,26	84,06	95.93

In a second analysis we take **the PCR *as Gold Standard*** and considers six positive samples by TMA as negative, since the viral load of the sample is over 39 cycles of PCR amplification. By limiting the analysis viral load, we observe how sensitivity increases to 93.48% (CI: 82.10% - 98.63%) and specificity remains at 99.01% (CI: 94.61% - 99.97%).

RT-PCR Reference	Covid+	Covid-	total
Test +	43	1	44
Test -	5	98	103
total	48	99	147

Estimates with 95% confidence interval

RT-PCR Reference	estimated	inferior	superior
Sensitivity	89,59	77,34	96,53
Specificity	98,99	94,50	99,97
Positive predictive value	97,73	87,98	99,94
Negative predictive value	95,15	89,03	98.41

In the third analysis carried out we considered exclusively the samples **with viral load (Ct ≤33),** and classified all samples according to the result of the PCR determining as negative samples those with a Ct higher than the cut-off point.

RT-PCR Reference (Ct ≤33)	Covid+	Covid-	total
Test +	43	1	44
Test -	3	100	103
total	46	101	147

Estimates with 95% confidence interval

RT-PCR Reference (Ct ≤33)	estimated	inferior	superior
Sensitivity	93,48	82,10	98,63
Specificity	99,01	94,61	99,97
Positive predictive value	97,73	87,98	99,94
Negative predictive value	97,09	91,72	99,40

Finally, we carried out a fourth analysis considering exclusively samples with high viral load **(Ct ≤ 30).** We classified all samples based on the result of the PCR by determining as negative samples those with a Ct greater than the cut-off point. By limiting the viral load analysis we observe how sensitivity increases drastically to 100% (CI: 91.78% - 100%) and specificity remains at 99.04% (CI: 94.76% - 99.98%).

RT-PCR Reference (Ct ≤30)	Covid+	Covid-	total
Test +	43	1	44
Test -	0	103	103
total	43	104	147

RT-PCR Reference (Ct ≤30)	estimated	inferior	superior
Sensitivity	100,00	91,78	100,00
Specificity	99,04	94,76	99,98
Positive predictive value	97,73	87,98	99,94
Negative predictive value	100,00	96,48	100,00

Conclusions

We conclude that the *Linkcare Bioscreening SARS-CoV-2 Antigen Rapid Test* shows a very high positive predictive value of 97% and a similar negative predictive value of 91%. The observed predictive values tell us that any test result should be interpreted with a high degree of certainty.

Sensitivity and specificity data presented in the study reveal that the *Linkcare Bioscreening SARS-CoV-2 Antigen Rapid Test* obtains highly reliable results against RNA detection molecule tests and can be an effective and economical alternative if performed on a repetitive basis.

	TMA	PCR	Ct < 33	Ct < 30
Sensitivity	82,69%	89,59%	93,48%	100.00 %
Specificity	98,95%	98,99%	99,01%	99.04 %

The antigen rapid test analyzed has been shown to reliably detect people infected with a high viral load and therefore with high infective capacity. We can highlight the *Linkcare Bioscreening SARS-CoV-2 Antigen Rapid Test* as a fast and efficient layer of security that allows to detect people with the ability to infect and, therefore, a very useful tool to coordinate instant actions that allow to act before an infected person and contacts.

Bibliography

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