IRIS SARS CoV-2 IgG IgM Rapid Test

Technical Report and Clinical Data Analysis

1.0 Introduction

The IRIS SARS CoV-2 IgG IgM is a colloidal gold-based immunochromatographic strip assay produced by Alpha Pharma Industry (Bari, Italy). The test is rapid (10-14 min) and is based on a lateral flow immunoassay targeted to evaluate the presence or absence of anti-SARS-CoV-2-IgM and anti-SARS-CoV-2-IgG. The test is validated for different human specimens: capillary blood, venous whole blood, serum and/or plasma.

The IRIS SARS CoV-2 IgG IgM utilizes Anti-human IgG and anti IgM against the Receptor-Binding Domain of the COVID-19 spike protein recombinant antigen. The strip test consists by a nitrocellulose membrane incorporating mouse anti-human-IgM and IgG monoclonal antibody, plus anti-rabbit-IgG immobilized in different position (M and G lines) and in control line (C line), respectively.

The use of the test is extremely simple and rapid: the sample (10-15 ul) is deposited in sample-port and few second later 2 drops are added (the buffer is supplied together with the test in a dropper bottle). The blood macromolecules are moved forward by capillarity and "walk" along the nitrocellulose strip which has been absorbed by a mixture of recombinant antigen AuNP-COVID-19 and AuNP-rabbit-IgG. If anti-SARS CoV-2 IgM antibodies are present in the patient's blood, they bind to the viral antigen labelled with colloidal gold, then forming a sandwich with the anti-human IgM monoclonal antibody (present only on one line marked by the letter M); in this case the line will turn pink-red. Similarly, for IgG, in a second line in which monoclonal antibodies to human IgG are present.

If both lines are not dyeing, the test will be considered as negative; vice versa the development of a coloured band (even if not very intense) on the IgG and/or IgM line indicates the presence of anticovid antibody in the patient blood and the test will be considered positive. The device also contains a quality control line C, which must always colour and check that the kit is working properly.

2.0 Experimental data results

2.1.1 Preliminary test on sensitivity and specificity

Test are performed in the clinical setting of Catanzaro Lido Polyclinic (Dr Maurizio Cipolla, MD, Pathologist) in cooperation with the Catanzaro Hospital (Dept of Infectious Diseases) for the RT-PCR execution according with the Italian Government Guide Lines.

Sample size 15 adults of both sexes, aged 30-70 yr., with suspected diagnosis of Covid19 infection.

Operating Procedures: IRIS SARS CoV-2 IgG IgM test execution in the same day of pharyngeal swab for RT-PCR detection of Covid-19 according with WHO guideline and procedure advised by Regione Calabria and Italian Health Ministry. IRIS SARS CoV-2 IgG IgM test samples: capillary blood

Statistics: non parametric test (Cohen's K and exact McNemat test). Probability of Agreement, Concordance, and Cohen's K categorization according to DG Altman and R. Kwiecien[1]

The Results for IgG test: Cohen's k = 1.000; exact McNemar test: p > 0.999. Sensitivity 100,0% (95% Intervals of Confidence= 39.7-100.0). Specificity 100,0% (95%IC= 71,5-100.0).

Results for IgM test Cohen's k = 0.842; exact McNemar test: p > 0.99. Sensitivity 100,0% (95% Intervals of Confidence= 39.7-100.0). Specificity 90,1% (95%IC= 58.7-99.7).

2.1.2 Final test on sensitivity and specificity

The tests were performed as previously described (see 2.1.1). The overall sample (pooled data) consists of 161 adult subjects of both sexes

2.1.2.1 IgM results: Cohen's k = 0.963, McNemar exact test: p > 0.9999.

True disease status	Test result Neg.	Pos.	Total
Normal	77	1	78
Abnormal	2	81	83
Total	79	82	161

			[95% Confidence Interval]	
Prevalence	Pr(A)	52%	44%	59.5%
Sensitivity	**Pr(+|A)**	**97.6%**	91.6%	99.7%
Specificity	**Pr(-|N)**	**98.7%**	93.1%	100%
ROC area	(Sens. + Spec.)/2	.982	.961	1
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	51.1	10.5	249 (sf)
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	.0303	.0089	.103 (sf)
Odds ratio	LR(+)/LR(-)	1684	217	13057 (sf)
Positive predictive value	Pr(A|+)	98.8%	93.4%	100%
Negative predictive value	Pr(N|-)	97.5%	91.2%	99.7%

(sf) Likelihood ratios are estimated using the substitution formula; 0.5 is added to all cell frequencies before calculation

2.1.2.2 IgG results: Cohen's k = 0.963, McNemar exact test: p = 0.2500

True disease status	Test result Neg.	Pos.	Total
Normal	78	0	78
Abnormal	3	80	83
Total	81	80	161

			[95% Confidence Interval]	
Prevalence	Pr(A)	52%	44%	59.5%

[1] Categorization of values of Cohen's kappa: >0.20=Poor, 0.21–0.40=Fair, 0.41–0.60=Moderate, 0.61–0.80= Good, 0.81–1.0= Very good

Sensitivity	**Pr(+|A)**	**96.4%**	89.8%	99.2%	
Specificity	**Pr(-|N)**	**100%**	95.4%	100%	
ROC area	(Sens. + Spec.)/2	.982	.962	1	
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	151	9.55	2400	(sf)
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	.0419	.015	.117	(sf)
Odds ratio	LR(+)/LR(-)	3611	184	71054	(sf)
Positive predictive value	Pr(A|+)	100%	95.5%	100%	
Negative predictive value	Pr(N|-)	96.3%	89.6%	99.2%	

2.2.1 Clinical protocol (in- and out-patients)

Test are performed in the clinical setting of San Camillo Forlanini Hospital in Rome (Dr Gabriella Parisi) (Dept of Microbiology and Virology). The PCR test are performed according with Italian Istituto Superiore di Sanità (ISS) and Italian Health Ministry guideline and recommendations

Sample size 365 patients selected on the basis of the symptoms or candidate to surgery, from the Emergency, Surgery and Internal Medicine Departments. Patients of both sexes (no age-based exclusion criteria) with suspected diagnosis of Covid19 infection or candidate to the PCR before admission to surgery.

Operating Procedures: IRIS SARS CoV-2 IgG IgM test execution within 36-48 hrs before or after pharyngeal swab for RT-PCR detection of Covid-19 according with WHO guideline and procedure advised by ISS and Italian Health Ministry. IRIS SARS CoV-2 IgG IgM test samples: venous whole blood or plasma after REB separation by centrifugation.

Results:

On 365 patients, 353 showed the same results both with PCR and IRIS SARS CoV-2 test. The concordance rate resulted of 97%. In 12 patients with discordant results, 7 resulted negative to the RT-PCR and positive for IgM only (n=6), for IgM and IgG (n=1<) while the RT-PCR resulted negative (see footnote 1, previous page). *After a second oropharyngeal swab* for RT-PCR second control performed on discordant patients, 5 patients tested positive to RT-PCR, thus the used test gave a 98.1% (358/365) concordance (e.g., accuracy), with an expected CI95% from 96.1% to 99.2%

2.2.2 Healthcare personnel survey

Test are performed on the Doctors and Nurses of the San Camillo Forlanini Hospital in Rome (Dr Gabriella Parisi, Dept of Microbiology and Virology). The PCR test are performed as previously described.

Sample size 180 doctors and nurses selected among the San Camillo-Forlanini Hospital healthcare personnel (adults, both sexes) on the basis of risk exposition to Covid-19 infection.

Operating Procedures: IRIS SARS CoV-2 IgG IgM test execution together with the pharyngeal swab for RT-PCR detection of Covid-19, according with WHO guideline and procedure advised by ISS and Italian Health Ministry. IRIS SARS CoV-2 IgG IgM test samples: venous whole blood or plasma and/or capillary blood.

Results: concordant results were documented in 176/180 hospital employees, the accuracy was 97.8% (176/180), with an expected CI95% from 94.4% to 99.4%. Discordant results (n=4): 1 false negative results -for both IgM and IgG rapid test- with positive oropharyngeal swab; 2 false

negative of RT-PCR; one was classified as "doubtful IgM positivity" (due to the difficulty of reading the IgM band): this patient had a positive PCR test and certainly negative IgG band and should be included among the possible false negatives of the rapid test.

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The test carried on the whole San Camillo sample (patients plus health workers) gave a **97.8% accuracy, with an expected CI95% ranging from 96.2% to 98.9%**.

2.3 IRIS SARS CoV2 test in the setting of the occupational medicine and industry worker protection

Tests were carried out on the employees of a company (Sorical SPA, General manager Pia Chiarella) working in the field of drinking water and water supplies of almost all the Calabrian municipalities. The aim is checking all employees of the industry (continuous monitoring of Covid-19 infection by rapid test and quarantine any COVID-19 positive employee).

Operating Procedures: all employees were invited to take the IRIS SARS CoV-2 IgG IgM rapid test during working hours, inside the factory. The test was repeated every 15 days. Employees were also given a computerized Covid card (with information on their health, co-morbidity and any symptoms present) and the data were sent to the competent doctor. The RC-PCR test was performed only if the rapid test was positive.

Sample size: 172 healthy employees (aged 20-70, males and females) were tested. At present two controls were performed (total 344 IRIS SARS CoV-2 tests).

Results: only 1 case resulted positive to the IgM test (IgG negative, first RT-PCR test negative): the employee was released from the work (quarantine) waiting the second RT-PCR test in accordance with the Italian legal provisions and with the guidelines. No new cases of seroconversion were detected during the first phase of the follow up.

3.0 Conclusions

The IRIS SARS-CoV-2 IgG / IgM rapid test has a very high sensitivity and specificity; has been successfully used in different settings, for monitoring and diagnosing patients (belonging to General Medicine, Emergency Dept or Internal Medicine) and for the periodic survey of industrial and healthcare personnel.

We consider this test valid and reliable in several sectors (food industry, hospitals and medical centres, other strategic industries) both for the high sensitivity and specificity (and therefore accuracy) and for the extreme ease of use and reading. In fact, over 900 tests carried out during the study and validation phase in Italy, no cases of difficulty / impossibility of execution were reported, even in non-specialist environments; in only one case was a doubt reported on reading the coloured band.

In our opinion, a test with such high sensitivity and specificity is also indicated for epidemiological purposes on large sections of the population (general population and/or high-risk groups). From a clinical point of view, it is indicated for the diagnosis of Covid19 infection and / or the differential diagnosis with respect to other diseases, according to good clinical practices, which require the doctor to consider all the diagnostic elements (clinic, instrumental, laboratory, included the RT-PCR). Furthermore, the use of the IRIS SARS CoV-2 test can reveal patients with false negative RT-PCR, as suggested by the WHO guidelines and the literature. It should be noted that the execution

of the rapid serological test, while not replacing the use of swabs to identify viral RNA, is not operator-dependent and therefore very suitable for the setting of primary care[2].

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