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Clinical performance of the Panbio assay for the detection of SARS-CoV-2 IgM and IgG in COVID-19 patients

Running title: Panbio LFA for SARS-CoV-2 IgM and IgG detection

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Abstract

Following the SARS-CoV-2 pandemic, numerous serological tests have been developed, including rapid diagnostic tests. This study aims at assessing the clinical performance of the Panbio IgG/IgM COVID-19 test (Abbott), a rapid lateral flow assay for the qualitative detection of IgG and IgM against SARS-CoV-2. One hundred and thirty-eight samples from 95 COVID-19 patients with a positive SARS-CoV-2 RT-PCR were analyzed to assess the clinical sensitivity. Seventy-six pre-COVID-19 samples were used to evaluate the clinical specificity. Two independent and blinded raters determined visually the presence or absence of the IgG, IgM and control lines for each test after 10 and 20 minutes. The sensitivity obtained with samples collected more than 14 days after the onset of symptoms was 95.2% for IgG. IgM were less frequently detected (highest sensitivity of 20.5%). The specificities obtained were 98.7% and 100% and for IgG and IgM respectively. In addition, the sensitivity of the assay was better when the reading was performed at 20 minutes than at 10 minutes, whereas the specificity was unchanged. The Panbio COVID-19 IgG/IgM rapid test

presents high sensitivities for IgG 14 days since symptom onset but a low sensitivity for IgM. The specificity was excellent for both IgG and IgM.

*these authors contributed equally.

Introduction

Rapid tests are designed for use where a preliminary screening test result is required and are especially useful in resource-limited countries or for broad screening campaign where access to blood sampling may be difficult or not obligatory. However, these tests have to be of high quality, user-friendly, quick and easy to perform and they have to require little or no additional equipment. In the context of COVID-19, all the above-mentioned criteria are of importance as serological tests may be useful for the diagnosis, for the characterization of the course of the disease, for identifying convalescent plasma donors directly on site, for lockdown exit programs, for epidemiological study and for the assessment of COVID-19 vaccine response [1]. Due to their widespread dissemination and the limited experience with these assays, it is crucial for laboratories to rigorously validate these methods before broad introduction into routine clinical practice. This study aims at evaluating the clinical performances of the Panbio COVID-19 IgM/IgG rapid test (Abbott, Chicago, United States) in a population of COVID-19 patients.

Materials and Methods

Sample Collection

This study was conducted from June 16, 2020, to June 24, 2020. Blood samples were collected from patients into serum-gel tubes (BD Vacutainer® 8.5 mL tubes, Becton Dickinson, New Jersey, USA) or lithium heparin plasma tubes (BD Vacutainer® 4.0 mL tubes) according to standardized operating procedure and manufacturer

recommendations. Samples were centrifuged for 10 minutes at 1,885 to 2,500 × g (ACU Modular® Pre Analytics, Roche Diagnostics®). A total of 214 samples were collected from April, 2019 to May 25, 2020, and stored in the laboratory biobank at -20°C. Pre-COVID-19 samples (n = 76) were all collected before March 2020, the start of the pandemic in Belgium. One hundred and thirty-eight samples from 95 COVID-19 patients were collected between March 21, 2020, and May 25, 2020. Frozen samples were thawed at room temperature. The study fulfilled the ethical principles of the Declaration of Helsinki.

Analytical Procedures

The Panbio IgG/IgM COVID-19 rapid test (Abbott) is a rapid lateral flow assay (LFA) for the qualitative detection of IgG and IgM directed against SARS-CoV-2 in human whole blood, serum or plasma specimens. The Panbio test was performed according to the manufacturer's instruction for use. Briefly, 10 µL of sample were applied into the specimen well and then two drops of buffer were applied. Raters determined visually the presence or absence of the IgG, IgM and control lines for each test 10 and 20 minutes after the addition of the buffer. As recommended by the manufacturer, even a slight colored striped was considered positive.

The reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2 determination in respiratory samples (nasopharyngeal swab samples) was performed on the LightCycler® 480 Instrument II (Roche Diagnostics®) using the LightMix® Modular SARS-CoV *E*-gene set.

Assessment of the Clinical Sensitivity

Samples (n=138) obtained from 95 patients with a confirmed RT-PCR SARS-CoV-2 diagnosis were assessed to determine the clinical sensitivity of the assay. Sensitivity

was defined as the proportion of correctly identified COVID-19 positive patients since symptom onset. Antibody kinetics was evaluated using all samples dividing in different categories based on the number of days after the symptom onset, as follows: 0-2 days (n=15); 3-5 days (n=6); 6-8 days (n=14); 9-11 days (n=9): 12-14 days (n=11): 15-17 days (n=13); 18-21 days (n=13); 22-25 days (n=15); 26-31 days (n=13); 32-40 days (n=12) and more than 40 days (n=17).

Assessment of the Clinical Specificity

Non-SARS-CoV-2 samples (n=76) collected prior to the COVID-19 pandemic (between April and June 2019) with potential cross-reactions (n=38) were also analyzed to assess the specificity. Samples included positive antinuclear antibodies (n=4), anti-thyroglobulin antibody (n=1), anti-*Treponema pallidum* antibodies (n=1), anti-TPO antibodies (n=3), direct coombs (n=1), hepatitis B Ag (n=3), IgA *Chlamydia pneumoniae* (n=1), IgG *Chlamydia trachomatis* (n=1), IgM *Borrelia burgdorferi* (n=1), IgM Cytomegalovirus (n=4), IgM *Mycoplasma pneumoniae* (n=1), IgM Parvovirus B19 (n=1), IgM *Toxoplasma gondii* (n=6), IgG polyclonal activation (n=1), IgM and IgG polyclonal activation (n=1), search for irregular agglutinins (n=5), rheumatoid factor (n=1), urinary infection with *Escherichia coli* (n=1), urinary infection with *Klebsiella oxytoca* (n=1), and samples from 38 healthy volunteers were included for the specificity calculation. Specificity was defined as the proportion of naïve patients classified as negative.

Evaluation of Reading Conditions

Two independent and blinded raters determined visually the presence or absence of the IgG, IgM and control lines for each test after 10 and 20 minutes. In case of discrepancies, a third blinded and independent rater checked the presence or absence

of the lines. Consensus results between all raters were used. The intra-rater (10 minutes *versus* 20 minutes) and the inter-rater (rater 1 *versus* rater 2) concordances were determined.

Statistical Analysis

Data analysis was performed using GraphPad Prism® software (version 8.2.1, California, USA) and MedCalc® software (version 14.8.1, Ostend, Belgium). Confidence intervals for sensitivity and specificity were "exact" Clopper-Pearson confidence intervals. The Cohen's kappa coefficient was used to assess the intra and inter-rater concordance.

Results

Clinical Performances

All the tests (n=214) were valid (i.e. the control line was visible). Kinetics of the sensitivity of the Panbio assay to detect IgG and IgM since the onset of the first symptoms is described in the **Figure 1**. After 14 days since symptom onset, the Panbio assay detected IgG in 95.2% (95% CI 88.1-98.7%). Before 14 days since first symptoms, sensitivities were not high enough to be reliably used in clinical practice (50.9%, 95% CI 37.1-64.7%).

Immunoglobulin M were less frequently detected by the Panbio assay, with sensitivities of 7.3% (95% CI 2.0-17.6%) and 20.5% (95% CI 12.4-30.8%) for samples the first 14 days and for those obtained more than 14 days since symptom onset respectively. The highest sensitivity for IgM obtained in a particular category based on the number of days after the symptom onset was 30.8% (95% CI 9.1-61.4%) (**Figure 1**).

Only one sample was positive for IgM and negative for IgG. This sample was collected 22 days after the first symptoms. The sensitivity of the Panbio assay to detect IgM and/or IgG within the first 14 days since symptom onset was unchanged compared to the sensitivity to detect IgG (50.9%; 95% CI 37.1-64.7%). After 14 days since symptom onset, the Panbio assay detected IgG and/or IgM in 96.4% (95% CI 89.8-99.3%) of samples.

Among the 76 samples collected before the COVID-19 pandemic, only one sample from a healthy volunteer gave a false positive result with IgG. Samples with potential cross-reaction gave no false positive result. The specificity was 98.7% (95% CI 92.9-100.0%) and 100% for IgG and IgM respectively.

Evaluation of Reading Conditions

The inter-rater variability was excellent when the tests were read at 10 minutes and 20 minutes for both IgG (Cohen's kappa coefficient at 10 minutes and 20 minutes were 0.972 and 0.991 respectively) and IgM (Cohen's kappa coefficient at 10 minutes and 20 minutes were 0.945 and 0.974). In addition, the sensitivity of the assay was better when the reading was performed at 20 minutes than at 10 minutes (**Table 1**), whereas the specificity was unchanged. Cohen's kappa coefficients for the different time of reading were lower for IgM than IgG, indicating that the time of reading influence more IgM results than IgG (**Table 1**). The positive lines (IgM and IgG) read at 10 minutes were always positive at 20 minutes.

Discussion

The detection of anti-SARS-CoV-2 antibodies represents an additional method for the diagnosis of COVID-19 which may significantly improve the sensitivity of pathogenic diagnosis for COVID-19 when combined with RT-PCR [7]. A wide range of

assays have been developed including ELISA, CLIA, ECLIA and rapid tests [8-13]. The main advantage of rapid diagnostic tests is that they do not require specific equipment and are easy to use. Furthermore, these tests are rapid, and they can be easily implemented in a low-resource laboratory.

The World Health Organisation (WHO) encourages laboratories to perform independent assay validation, in particular regarding the clinical utilization of rapid device [15]. Based on the conclusions of the study of the Frederick National Laboratory for Cancer Research (FNLCR), a Federally Funded Research and Development Center (FFRDC) sponsored by the National Cancer Institute (NCI), the FDA concluded that a list of 65 serological assays should not be distributed [16]. External validations of these tests are therefore paramount, and a plenty of data are arriving in the literature [8-13,17-21]. Given the leading position of Abbott for COVID-19 testing, independent external validation of their assays is mandatory to ensure the performance are in line with their claims.

In our evaluation, the sensitivity obtained for all samples collected more than 14 days after the onset of symptoms was 95.2% for IgG. The Panbio assay showed weak sensitivity for IgM (**Figure 1**). The specificities obtained were 98.7% and 100% and for IgG and IgM respectively. In the instructions for use, Abbott Diagnostics mentioned a sensitivity and a specificity of 95.8% and 94.0%, respectively [22]. In the manufacturer's study, 48 samples of PCR confirmed patients and 50 pre-COVID-19 samples were analyzed. Taken apart, IgG had a sensitivity and a specificity of 95.8% and 100% and IgM a sensitivity and a specificity of 56.3% and 94% [22]. Our results are in agreement with these claims and we even obtained a better specificity for IgM although the sensitivity was lower than claimed. However, in the information provided by the manufacturer, the details of the studied populations were lacking, i.e.

timing between symptom onset or since PCR positivity and the blood sampling as well as the characteristics of samples included for specificity calculation [22].

As observed on other assays and platforms, i.e. LFA, ELISA, CLIA, ECLIA [8,18,23,24], we found that sensitivities before 14 days since symptom onset were not sufficient to be reliably used in clinical practice. We therefore recommend obtaining a control or confirmatory sample after 14 days to increase the detection rate of possible past-COVID-19 infection.

Comparing the clinical performance of these rapid tests is hazardous. Indeed, the design of studies vary widely across studies, i.e. number of positive and negative samples, the definition of negative samples, number of days since symptoms or since PCR positivity, comparison to a neutralization test. Some studies included only a very limited number of patients [20], included control samples collected during the pandemic period [10,21], defined different categories since symptom onset (i.e. < or > 7 days [19], 0-6, 7-13, 14-25 days [18], or 5-9, 10-18 days [20]), or different categories since RT-PCR positivity [20]. Moreover, as with other rapid LFA [25], we showed that the result may depend on the reader and on the timing of reading (20 minutes better than 10 minutes). The utilization of an automated reader may be useful to decrease the inter-individual variation, especially when the colored stripe appears very thin.

Conclusions

The Panbio COVID-19 IgM/IgG rapid test presents high sensitivities for IgG 14 days since symptom onset but a very low sensitivity for IgM. The specificity was excellent for both IgG and IgM. Further investigations designed to evaluate the clinical performances of Panbio over a longer period of time is needed to further consider its use in seroprevalence studies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

Among the authors, JD is the chief executive officer and founder of Qualiblood sa, and reports personal fees from Diagnostica Stago, Roche, Roche Diagnostics, Daiichi-Sankyo, and Portola, outside the submitted work. The other authors have no conflict of interests to disclose.

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Author Contribution Statement

JD, JF and HH and were responsible for the conception and design of the study. JD, JF and HH were responsible for the acquisition, analysis and interpretation of data. JD, JF and HH were responsible for drafting the manuscript. CE, ME, JC, MT and JMD contributed to the final draft of the manuscript. All authors agree to be accountable for the content of the work.

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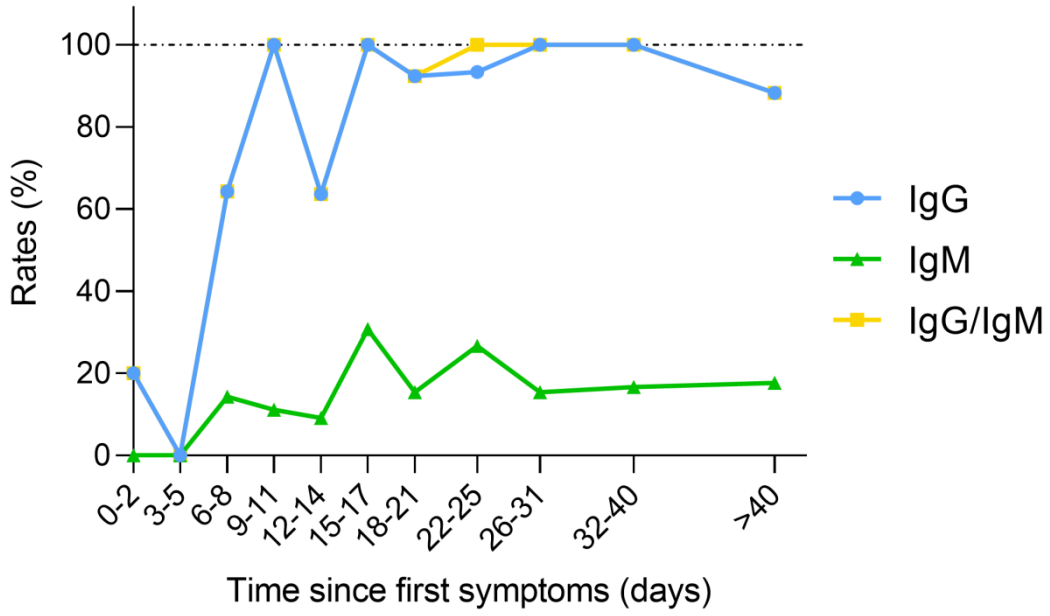
Figure legend

A



B

Days since first symptoms (number of samples)	0-2 (15)	3-5 (6)	6-8 (14)	9-11 (9)	12-14 (11)	15-17 (13)	18-21 (13)	22-25 (15)	26-31 (13)	32-40 (12)	>40 (17)
IgG positive (n)	3	0	9	9	7	13	12	14	13	12	15
IgG sensitivity (%)	20.0	0.0	64.3	100.0	63.6	100.0	92.3	93.3	100.0	100.0	88.2
IgG sensitivity 95% CI (%)	4.3 - 48.1	0.0 - 45.9	35.1 - 87.2	66.4 - 100.0	30.8 - 89.1	75.3 - 100.0	64.0 - 99.8	68.1 - 99.8	75.3 - 100.0	73.5 - 100.0	63.6 - 98.5
IgM positive (n)	0	0	2	1	1	4	2	4	2	2	3
IgM sensitivity (%)	0.0	0.0	14.3	11.1	9.1	30.8	15.4	26.7	15.4	16.7	17.7
IgM sensitivity 95% CI (%)	0.0 - 21.8	0.0 - 45.9	1.8 - 42.8	0.3 - 48.3	0.2 - 41.3	9.1 - 61.4	1.9 - 45.5	7.8 - 55.1	1.9 - 45.4	2.1 - 48.4	3.8 - 43.4
IgG/IgM positive (n)	3	0	9	9	7	13	12	15	13	12	15
IgG/IgM sensitivity (%)	20.0	0.0	64.3	100.0	63.6	100.0	92.3	100.0	100.0	100.0	88.2
IgG/IgM sensitivity 95% CI (%)	4.3 - 48.0	0.0 - 45.9	35.1 - 87.2	66.4 - 100.0	30.8 - 89.0	75.3 - 100.0	63.8 - 99.8	78.2 - 100.0	75.3 - 100.0	73.5 - 100.0	63.6 - 98.5

Figure 1: Kinetics of the sensitivity of the Panbio assay since the onset of symptoms.

A. Kinetics of the sensitivity of the Panbio assay since the onset of first symptoms to detects IgG (blue dots), IgM (green triangle), and IgG and/or IgM (yellow squares).

The result of each test was determined visually after 20 minutes by two independent and blinded operators. **B.** Sensitivities of the Panbio assay for IgG, IgM, and IgG and/or IgM since the onset of first symptoms.

Table 1: Evaluation of the impact of the rater and the time of reading on the IgG (A) and IgM (B) test results.

A.

	Number of samples read positive for IgG / total number of samples		κ coefficient between reading time
	Reading after 10 min	Reading after 20 min	
Rater 1	105/138 (76.1%)	106/138 (76.8%)	0.991
Rater 2	106/138 (76.8%)	107/138 (77.5%)	0.991
κ coefficient between raters	0.972	0.991	

B.

	Number of samples read positive for IgM / total number of samples		κ coefficient between reading time
	Reading after 10 min	Reading after 20 min	
Rater 1	21/138 (15.2%)	22/138 (15.9%)	0.922
Rater 2	19/138 (13.8%)	21/138 (15.2%)	0.945
κ coefficient between raters	0.945	0.974	