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EVALUATION OF PERFORMANCE OF NINE SEROLOGICAL ASSAYS FOR THE DETECTION OF SARS-COV-2 ANTIBODIES.

Virology

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ABSTRACT

Introduction: Currently, polymerase chain reaction (PCR) based viral RNA detection is the standard for COVID-19 diagnosis [2]. Though, RNA testing based on throat or nasopharyngeal swabs has shown a number of false-negative results. Antibody detection tests have been developed to detect specific antibodies, IgM and IgG, to SRAS-CoV-2 virus. The clinical relevance of these tests is still under evaluation and is highly related to their clinical performance. Our objective is to assess analytical performances of nine SARS-CoV-2 antibodies immunoassays.

Materiel and Method: We collected 80 blood samples from PCR-confirmed COVID-19 patients diagnosed in our Virology department (20 samples collected at day 10 after the onset of symptoms, 60 collected after day 14 following the onset of symptoms) and 20 blood samples from patients SARS-CoV-2 RT-PCR negative. All sera were tested with nine SARS-CoV-2 antibodies immunoassays ARCHITECT SARS-CoV-2 IgG® (Abbott), COVID-19 VIRCLIA® IgG MONOTEST (Vircell), COVID-19 VIRCLIA® IgM+IgA MONOTEST (Vircell), COVID-19 ELISA IgG® (Vircell), COVID-19 ELISA IgM+IgA® (Vircell), Elecsys® Anti-SARS-CoV-2 (Roche), FREND® COVID-19 IgG/IgM Duo (NanoEntek), COVID-PRESTO® (AAZ) and COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Kit® (Labnovation Technologies).

Results: Sensitivity of tests increases once the seroconversion to anti-SARS-CoV-2 IgG positive in most individuals occurs toward the end of week 2 post-infection. COVID-19 PRESTO had the best accuracy in our study showing 100% sensitivity after day 14 following the onset of symptoms. All of the tests had a specificity of 100%.

Conclusion: Serological tests are sensitive for the latest stages of COVID-19 infection. Recommendations on using SRAS-COV-2 antibody detection tests are continuously improving based on current knowledge of host antibody responses during infection. They are of great value in cases presenting COVID-19 symptoms with negative RT-PCR.

KEYWORDS

SARS-CoV-2, Antibody testing, Analytical performance

1. INTRODUCTION

At the end of 2019, pneumonia of unknown cause was first reported in Wuhan, On January 9th, 2020, the Chinese health authorities and the World Health Organization (WHO) officially announced the discovery of a novel coronavirus, first named 2019-nCoV, then officially termed SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2). The virus that caused this disease is designated severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2. On the 12th of March, WHO declared COVID-19 (CoronaVIrus Disease) as a world pandemic [1].

In order to provide appropriate treatment for patients and to limit further spread of the virus, the diagnosis of the SARS-CoV-2 must be accurate and well-timed. Currently, polymerase chain reaction (PCR) based viral RNA detection is the standard for COVID-19 diagnosis [2]. Though, RNA testing based on throat or nasopharyngeal swabs has shown a number of false-negative results.

In response to the growing COVID-19 pandemic, antibody detection tests have been developed to detect specific antibodies, IgM and IgG, to SRAS-CoV-2 virus in human blood, serum or plasma. Detection of the IgG and IgM antibody against SARS-CoV-2 can be an indicator of infection (whether the individual was symptomatic or not). Detection of antibodies could provide information on the virus infection time course. Also, detection of IgM and IgG can shed the light on SARS-CoV-2 epidemiology (seroprevalence, mortality rate…). The clinical relevance of these tests is still under evaluation and is highly related to their clinical performance.

Our objective is to assess analytical performances of the following seven SARS-CoV-2 antibodies immunoassays:

- ARCHITECT SARS-CoV-2 IgG® (Abbott)
- COVID-19 VIRCLIA® IgG MONOTEST (Vircell)
- COVID-19 VIRCLIA® IgM+IgA MONOTEST (Vircell)
- COVID-19 ELISA IgG® (Vircell)
- COVID-19 ELISA IgM+IgA® (Vircell)
- Elecsys® Anti-SARS-CoV-2 (Roche)
- FREND® COVID-19 IgG/IgM Duo (NanoEntek)

The laboratory of virology additionally assessed the performance of two points of care (POC) tests developed to detect specific antibodies, IgG and IgM, to SARS-CoV-2 virus.

- COVID-PRESTO® (AAZ)
- COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Kit® (Labnovation Technologies)

2. MATERIELAND METHODS:

Samples:

We collected 80 blood samples from PCR-confirmed COVID-19 patients diagnosed in the Laboratory of Virology, Center for Virology, Infectious and Tropical Diseases, Mohammed V Military Teaching

Hospital (20 samples collected at day 10 after the onset of symptoms, 60 collected after day 14 following the onset of symptoms) and 20 blood samples from patients SARS-CoV-2 RT-PCR negative. All samples have been centrifuged upon arrival at the laboratory, sera were separated, aliquoted and stored deep-frozen at -20°C until analysis.

Ethical approval:
All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration. All subjects have given their written informed consent.

Evaluation method:
When refrigerated, Sera were warmed to room temperature. Samples were tested on multiple commercially available (mostly automated) immunoassay platforms according to the manufacturer's protocol. Assays were performed on corresponding automated immunoassay platforms:

- ARCHITECT i2000SR® for ARCHITECT SARS-CoV-2 IgG® (Abbott)
- VIRCLIA® for COVID-19 VIRCLIA® IgG MONOTEST (Vircell) and COVID-19 VIRCLIA® IgM+IgA MONOTEST (Vircell)
- Cobas® e411 for Elecsys® Anti-SARS-CoV-2 (Roche)
- FREND™ System for the rapid test FREND® COVID-19 IgG/IgM Duo (NanoEntek)

Lateral Flow Test POC (COVID-PRESTO® (AAZ) and COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Kit® (Labnovation Technologies)) were performed according to the manufacturer's protocol.

Any equivocal test result has been excluded. To assess the sensitivity and specificity, we choose the RT-PCR method as gold standard.

Calculations of sensitivity, specificity, Predictive values Positive and Negative were performed using an online calculator. (https://www.medcalc.org/calc/diagnostic_test.php).

3. RESULTS:

Sensitivities obtained with the immunoassays are summarized in Table 1. Sensitivity was lower (<50%) when the test was performed on a sample obtained at day 10 following the symptoms with **ARCHITECT SARS-CoV-2 IgG®, COVID-19 VIRCLIA® IgM+IgA MONOTEST, VIRCELL COVID-19 ELISA IgM+IgA® and COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Kit® (Labnovation Technologies).**

Sensitivity of COVID-19 ELISA IgG®, FREND® COVID-19, Elecsys® Anti-SARS-CoV-2, COVID-19 VIRCLIA® IgG MONOTEST et COVID-PRESTO® was medium (between 60 and 85%) at day 10. All the tests had a specificity of 100% at >10 days after the onset of symptoms.

The sensitivity of IgG was >90% for: **ARCHITECT SARS-CoV-2 IgG® (** 98.44%) and **for VIRCELL COVID-19 ELISA IgG® (94.44%) .** Sensitivity for total Antibodies was ≥ 90% for **Elecsys® Anti-SARS-CoV-2.** Sensitivity for POC was greater than 90% for the two kits: **FREND® COVID-19 and COVID-PRESTO®. VIRCLIA® IgG MONOTEST** (Vircell) and **COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Kit®** (Labnovation Technologies) showed respectively a sensitivity of 87.5% and 33.33% after 14 days following the onset of symptoms.

Immunoassays aiming for **IgM or IgM+IgA (COVID-19 VIRCLIA® IgM+IgA MONOTEST, VIRCELL COVID-19 ELISA IgM+IgA®)** performed a low to medium sensitivity (from 25% to 60%) after 14 days following the apparition of symptoms. We excluded 3 equivocal results given by COVID-19 VIRCLIA® IgG MONOTEST (Vircell). **All of the tests had a specificity of 100% at >14 days of symptoms.**

Table 1: Sensitivity for the nine serological assays for the detection of SARS-COV-2 antibodies

ASSAYS	SENSITIVITY (%)	
	AT DAY 10	>14 DAYS
ARCHITECT SARS-CoV-2 IgG® (Abbott)	38.24 (22.17 – 56.44)	98.44 (91.60 - 99.96)
COVID-19 VIRCLIA® IgG MONOTEST (Vircell)	82.35 (65.47 - 93.24)	87.50 (76.85 - 94.45)
COVID-19 VIRCLIA® IgM+IgA MONOTEST (Vircell)	41.18 (24.65 - 59.30)	37.5 (25.70 - 50.49)
COVID-19 ELISA IgG® (Vircell)	68.18 (45.13 - 86.14)	94.44 (72.71 - 99.86)
COVID-19 ELISA IgM+IgA® (Vircell)	40.12 (24.65 - 59.30)	60.03 (34.65 - 69.30)
Elecsys® Anti-SARS-CoV-2 (Roche)	62.50 (24.49 - 91.48)	98.1 (91.60 - 99.96)
FREND® COVID-19 IgG/IgM Duo (NanoEntek)	63.64 (25.49 - 92.48)	98.44 (91.47 - 99.96)
COVID-PRESTO® IgG+IgM (AAZ)	81 (3.05 - 36.34)	100 (81.47 - 100)
COVID-19 (SARS-CoV-2) IgM/IgG Antibody Test Kit® ((Labnovation Technologies)	10 (1.17 - 30.38)	33.33 (13.34 - 59)

4. DISCUSSION:

RT-PCR is the gold standard in the acute of very early phase of infection with SARS-COV-2. The role of antibody testing in the diagnosis of acute viral respiratory infections is usually restricted to epidemiological studies as seroconversion may take up to two weeks to develop in all patients. With the outbreak of COVID 19, the urge to find performant analytical tests, rapid and cost-effective had put detection of SARS-CoV-2 specific antibodies in use in clinical settings. Serology testing can be advantageous with a faster turnaround time, high throughput, automated, used for large sample sizes and with less workload. However; to be used in such settings, the sensitivity/specificity of these assays have to be acceptable according to some criteria. Criteria of validations of serological SRAS COV 2 tests were in issued in some countries [3].

This prospective observational study aimed to assess analytical performance of 9 tests designed to detect SARS-COV-2 antibodies IgA, IgG and IgM including two POCT tests. Sera used were from adult patients with PCR-confirmed diagnoses for SARS-CoV-2 (n=80) and from patients with RTPCR SRAS COV2 negative (n=20) collected at day 10 and after day 14 following the onset of symptoms.

Sensitivity of assays varied according to the moment of testing. We found lower sensitivity on the samples issued at day 10 after the onset of symptoms (10% to 70.28%) by contrast to higher rates after day 14 following the onset of symptoms (33.33% à 100%). In others studies, sensitivity of tests increases once the seroconversion to anti-SARS-CoV-2 IgG positive in most individuals occurs toward the end of week 2 post-infection [4], and our data continue to underscore the importance of not relying solely on such testing to diagnose infection in acutely symptomatic patients [5]. The SARS-CoV-2 IgM/IgG Antibody Test Kit® (Labnovation Technologies), showed the worst diagnosis accuracy (10% at day 10 et 33.33% after day 14 following the onset of symptoms).

POCT usually show the lower accuracy as reported before [6]. However the results indicate POCT COVID-19 PRESTO performed better than FREND® COVID-19 IgG/IgM Duo that also aims to detect both IgG and IgM. COVID-19 PRESTO had the best accuracy in our study showing 100% sensitivity after day 14 following the onset of symptoms. COVID-19 PRESTO was found to have high performance especially with IgG (sensitivity= 86.5%) [7] giving rapid results (10-15 minutes).

COVID-19 ELISA IgM+IgA®(Vircell) and COVID-19 VIRCLIA® IgM+IgA MONOTEST are both Vircell techniques use recombinant spike (protein S) and nucleocapsid (protein N) antigens. The manufacturer reports an overall sensitivity for IgM+A, an overall sensitivity of 66% and a specificity of 99%. We found high specificity but a medium sensitivity of 77.08% for ELISA and 62.05% for CLIA. The sensitivity of chemiluminescence assays was lower than ELISAs in line with previously published data [7,8]. Elecsys® Anti-SARS-CoV-2 showed a medium sensitivity at day 10 and performed better at day 14. This test does not discriminate IgA, IgM or IgG antibodies.

Regarding specificity, cross-reactivity of antibodies of endemic Coronavirus infected individuals or of individuals with other active infectious diseases (e.g. EBV or CMV) are known phenomenon [9,10]. We found excellent specificity with all the tests assessed.

CONCLUSION:

Serological tests are sensitive for the latest stages of COVID-19 infection. Recommendations on using SRAS-COV-2 antibody detection tests are continuously improving based on current knowledge of host antibody responses during infection [3,11]. They are of great value in cases presenting COVID-19 symptoms with negative RT-PCR.

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