Current Avenues for COVID-19 Serology

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Abstract

Keywords
- rapid diagnostic tests
- COVID-19 serological tests
- convalescent plasma
- immune status
- antibody test

Development of rapid, reliable, and easy diagnostic tests with high-throughput is the need of the hour for laboratories combating the COVID-19 pandemic. While real-time polymerase chain reaction (RT-PCR) is the gold standard for diagnosing active infections, it is expensive and time-consuming. Serological diagnostic assays with a premise to aid rapid contact tracing, immune status determination, and identification of potential convalescent plasma donors hold great promise. Timely diagnosis, effective treatment, and future prevention are key to management of COVID-19.

Introduction

The Coronavirus disease 2019 (COVID-19) caused by a novel coronavirus (SARS-CoV-2, previously known as 2019-nCoV) is a new pandemic, spreading across countries and threatening the world.1 Viral epidemics pose a serious threat to public health. In the last 20 years, several such epidemics, such as severe acute respiratory syndrome coronavirus (SARS-CoV) between 2002 and 2003, H1N1 influenza in 2009, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, have led to great human loss. Coronaviruses are enveloped positive sense RNA viruses with spiked projections on their surface, giving them a crowned appearance; hence, the name “Coronavirus.”2

The current pandemic began with a cluster of pneumonia cases reported from Wuhan, China, on December 31, 2019, resulting in the identification of a novel Coronavirus SARS-CoV-2 and its associated disease “COVID-19,” an acronym of “Coronavirus disease 2019.” On March 11, 2020, the WHO declared it as a pandemic. Despite social distancing and large-scale lockdowns, it has led to 310,801 deaths worldwide till May 16, 2020,4 requiring additional epidemiological solutions5 6 to curtail its spread.

The microbiology laboratory is faced with multiple challenges related to reliable diagnosis and prognosis of COVID-19. Currently, the confirmation of active disease is approved by real-time polymerase chain reaction (RT-PCR) but this facility is expensive, time-consuming, and is only available in well-equipped laboratories with trained personnel. PCR results cannot predict past infection, immunity against the disease or prognosis. There is an urgent need for simpler, cheaper, and quicker point-of-care tests. Serological testing can enable testing of immune status, contact tracing and identification of potential convalescent plasma donors. Timely diagnosis, effective treatment, and future prevention are key to management of COVID-19.7

Basis for Serological Testing and Immunoassays in COVID-19

Serological testing for detection of antigen (Ag) and/or antibodies (Ab) is currently the focus of rapid diagnostic solutions for COVID-19. While RT-PCR for detection of true infection is clearly superior to serology, Ab testing may be a useful marker of immunity. IgM can be used to diagnose early infections, and IgG can be used as an indicator for current or past infections and postinfection immunity. These tests have a huge potential for establishing the epidemiology of COVID-198 12 as well as Table 1. The challenges faced by developers of immunoassays are: (1) False negative results during the serological window period before IgM becomes detectable; (2) Asymptomatic infections leading to seropositivity with discordant PCR negativity, without a way to confirm the same; (3) Unknown baseline levels of
Some currently available immunodiagnostics to detect viral proteins or antibodies against SARS-CoV-2

<table>
<thead>
<tr>
<th>Test name</th>
<th>Test type</th>
<th>Sample source</th>
<th>Ig/protein detected</th>
<th>Manufacturer</th>
</tr>
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<tbody>
<tr>
<td>m2000 SARS-CoV-2 assay</td>
<td>Chemiluminescent microparticle immunoassay</td>
<td>Serum/plasma/whole blood</td>
<td>IgG</td>
<td>Abbot</td>
</tr>
<tr>
<td>COVID-19 IgG/IgM LF</td>
<td>Lateral flow immunoassay</td>
<td>Serum/plasma/whole blood</td>
<td>IgG/IgM</td>
<td>Advagen Biotech</td>
</tr>
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<td>COVID-19 IgM/IgG rapid test</td>
<td>Lateral flow immunoassay</td>
<td>Serum/plasma/whole blood</td>
<td>IgG/IgM</td>
<td>BioMedomics Inc.</td>
</tr>
<tr>
<td>IgG antibody test kit for novel coronavirus 2019–nCoV</td>
<td>Magnetic particle-based chemiluminescence assay</td>
<td>Serum</td>
<td>IgG</td>
<td>Bioscience (Chongqing) Diagnostic Technology Co., Ltd.</td>
</tr>
<tr>
<td>One-Step COVID–2019 test</td>
<td>Lateral flow immunoassay</td>
<td>Serum/plasma/whole blood</td>
<td>IgG/IgM</td>
<td>Celer Biotechnologia</td>
</tr>
<tr>
<td>KT-1033 EDI Novel Coronavirus COVID-19 ELISA Kit</td>
<td>ELISA</td>
<td>Serum</td>
<td>IgG/IgM</td>
<td>Epitope Diagnostics</td>
</tr>
<tr>
<td>VITROS®-Immunodiagnostics Products Anti-SARS-CoV-2 total reagent pack</td>
<td>ELISA</td>
<td>Serum/blood</td>
<td>IgG/IgM</td>
<td>Ortho Clinical Diagnostics</td>
</tr>
<tr>
<td>Standard Q COVID-19 Ag</td>
<td>Chromatographic immunoassay</td>
<td>Nasopharyngeal swabs</td>
<td>Viral antigen</td>
<td>SD Biosensor</td>
</tr>
</tbody>
</table>

Abbreviation: ELISA, enzyme-linked immunosorbsent assay.

Rapid Antigen Detection Tests: While molecular methods are the current gold standard in diagnostic target detection, various rapid serological Ag detection tests using monoclonal Abs in different formats have been proposed. Such rapid tests employing colorimetry and chemiluminescence are available against SARS-CoV. A fluorescence-based lateral flow assay for the detection of SARS-CoV-2 nucleocapsid protein is currently under evaluation. Some rapid Ag detection tests, using monoclonal anti-CoV antibodies, have been recently approved by ICMR for India. (→ Table 2)

Enzyme-linked Immunosorbent Assays (ELISA): ELISA, based on Abs or Ags attached to a solid surface, can be used to detect Ags or Abs with high-specificity. It can use in-house protocols or commercial reagent kits, has high throughput–run on 96 or 324-well plates, can be conducted on different sample types, namely, plasma or serum, takes just 1 to 5 hours for results, and is highly sensitive. Being a laboratory-based test, it requires dedicated instruments (e.g., plate reader) and trained laboratory staff. All laboratory procedures must be performed in a facility using procedures equivalent to a BSL-2, be based on a risk assessment, and conducted by trained personnel. Initial processing of samples from patients suspected to have COVID-19 infection, as well as any aerosol-generating procedures such as vortexing and ELISA plate washing, must be done in a biological safety cabinet. The scientists at ICMR-NIV, Pune, have developed and validated the completely indigenous IgG ELISA test for antibody detection for SARS-CoV-2. On external validation, the IgG test kit produced by ICMR-NIV, Pune, has been found to have sensitivity and specificity of 98.7% and 100%, respectively. It is named...
Table 2  Rapid diagnostic kits approved by ICMR & MoHFW in India (as on June 6, 2020)

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Kit detail</th>
<th>Lot no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COVID-19 IgM IgG rapid test: BioMedicoms (CE-IVD)</td>
<td>20200226</td>
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<tr>
<td>2</td>
<td>New Coronavirus (COVID-19) IgG/IgM rapid test: Vortx Bio Ltd, India</td>
<td>PCCV2003015</td>
</tr>
<tr>
<td>3</td>
<td>COVID-19 IgM/IgG antibody detection card test: VANGUARD Diagnostics, India</td>
<td>RCOVID200301T</td>
</tr>
<tr>
<td>4</td>
<td>MakeSure COVID-19 rapid test: HLL Lifecare Limited, India</td>
<td>CVCT030420</td>
</tr>
<tr>
<td>5</td>
<td>YHLO iFlash-SARS-CoV-2 IgM and IgG detection kit (additional equipment required): CPC Diagnostics</td>
<td>20200206</td>
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<td>6</td>
<td>ACCUCARE IgM/IgG lateral flow assay kit: LABORATORY-CARE Diagnostics (India Pvt. Ltd)</td>
<td>CVC 200401</td>
</tr>
<tr>
<td>7</td>
<td>Abchek COVID-19 IgM/IgG antibody rapid test: NuLifeCare</td>
<td>NUI/ COV-19/R&amp;D/001</td>
</tr>
<tr>
<td>8</td>
<td>One Step Coronavirus (COVID-19) IgM/IgG antibody test: Alpine Biomedicals</td>
<td>A10420 A20420</td>
</tr>
<tr>
<td>9</td>
<td>COVID 19 IgM/IgG rapid test kit: Medsource Ozone Biomedicals (ver 2.0)</td>
<td>COV-002</td>
</tr>
<tr>
<td>10</td>
<td>Immuno Quick rapid test for detection of novel Coronavirus (COVID-19) IgM/IgG antibodies: Immuno Science India Pvt. Ltd</td>
<td>E142001</td>
</tr>
<tr>
<td>11</td>
<td>Standard Q Covid-19 IgM/IgG Duo test–One Step rapid antibody test: SD Biosensors</td>
<td>E054002 E054004</td>
</tr>
<tr>
<td>12</td>
<td>COVID-19 IgG/IgM rapid test kit Rafael Diagnostic: BMT Diagnostics</td>
<td>COV20030059 COV20030059–1</td>
</tr>
<tr>
<td>13</td>
<td>One Step COVID-19 IgM/IgG antibody: SIDAK Life Care Pvt. Ltd.</td>
<td>COVID195004A COVID195004B COVID195004C</td>
</tr>
</tbody>
</table>

Abbreviation: ICMR, Indian Council for Medical Research.

“COVID KAVACH ELISA,” which is currently in the process of commercial production and distribution.\(^{22}\)

**Luminescent Immunoassay:** Luminescent immunoassays comprise chemiluminescence and fluorescence technology to improve specificity. Cai et al have developed a peptide-based magnetic chemiluminescence enzyme immunoassay for diagnosis of COVID-19, and Diazyme Laboratories, Inc. (San Diego, California) announced the availability of two new fully automated serological tests for SARS-CoV-2 that are run on the fully automated Diazyme DZ-lite 3000 Plus chemiluminescence analyzer.\(^{23,24}\)

**Biosensor Test:** This technology uses broad-range PCR primers that target conserved regions of bacterial genomes, such as ribosomal sequences and essential protein-coding genes, and rapidly identifies a variety of pathogens without prior knowledge of the pathogen’s nucleic acid sequence. The specific interaction of biomolecules can be converted into optical, electrical, enzymatic and other outcomes like surface plasmon resonance (SPR). An SPR-based biosensor had been developed for the diagnosis of SARS with a turnaround time of 10 minutes.\(^{25}\) Recently, PathSensors Inc. announced a CANARY biosensor to detect the novel SARS coronavirus. This platform utilizes a cell-based immnosensor that couples capture of the virus with signal amplification to provide a result in 3 to 5 minutes.\(^{15}\)

**Neutralization Assay:** Neutralization assays are highly specific for detecting Abs that inhibit viral infection and cytopathic effects of viral replication. For this assay, patient’s sample of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, they will bind and inactivate the virus. Their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures. The time required for results is typically 3 to 5 days but recent advances have reduced this to hours.\(^{30,32}\) This type of testing requires Biosafety Level 3 (BSL3) cell culture facilities which is only available in reference laboratories. Despite these limitations, determination of neutralizing antibodies is important in the short-term for the therapeutic application of convalescent plasma and in the long-term for vaccine development.

**Conflict of Interest**
None declared.

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