Updates on SARS-CoV-2 diagnostic methods

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Confirmatory/diagnostic test

Molecular test (RT-PCR)
- Detects: genetic material
  - High sensitivity
  - High specificity

Antigen test
- Detects: Proteins
  - Acceptable sensitivity (80-90%)
  - High specificity
Confirmatory/diagnostic test

Molecular test (RT-PCR)
- Detects: genetic material
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Antigen test
- Detects: Proteins
- Acceptable sensitivity (80-90%) - High specificity

Non-confirmatory/research test

Antibody detection (ELISA or RDT)
The assays...

Laboratory assay

Virologic assays
- Genetic material
- Proteins/Antigens

Serologic assay
- Antibodies

Confirmatory/diagnostic test

Non-confirmatory/ research test
The assays...

Laboratory assay

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Non-confirmatory/research test

Serologic assays
- Antibodies

Genetic material

Proteins/Antigens
PAHO is working to implement the first protocol made available by WHO, developed by the Charité Hospital, Berlin Germany. This protocol has been published and can be accessed on the following link:

**Molecular protocols: PCR**

At least 30 commercially available options have been validated.

La Charité *in-house* (gen E) is still the reference method.

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**Foundation for Innovative New Diagnostics**

### TABLE 1: Results for 21 manual (open) molecular tests included in the round 1 evaluation

<table>
<thead>
<tr>
<th>Company</th>
<th>Product name</th>
<th>Count number</th>
<th>Gene target</th>
<th>Verified LOD (copies/reaction)</th>
<th>Assay Ct (low cut-off, 10^5)</th>
<th>Clinical sensitivity (50% positive)</th>
<th>Clinical specificity (100% negative)</th>
<th>PCR platform</th>
<th>Reproducibility (10% of max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altona Diagnostics</td>
<td>RealTime SARS-CoV-2 RT-PCR Kit 1.0</td>
<td>221035/221035</td>
<td>E</td>
<td>1-10</td>
<td>35.45</td>
<td>99%</td>
<td>99%</td>
<td>Bioultiprint CFK6 deep well</td>
<td>0.03577</td>
</tr>
<tr>
<td>Alere Diagnosticia</td>
<td>AmpliPrep COV-19 Detection (nucleic acid amplification)</td>
<td>MF 0010</td>
<td>N</td>
<td>5-10</td>
<td>35.99</td>
<td>99%</td>
<td>99%</td>
<td>BioCycler CT6 deep well</td>
<td>0.03577</td>
</tr>
<tr>
<td>Biotest, Germany</td>
<td>Wnta SARS-CoV-2 RT-PCR Kit</td>
<td>WS-1241</td>
<td>N</td>
<td>5-10</td>
<td>36.50</td>
<td>99%</td>
<td>99%</td>
<td>BioCycler CT6 deep well</td>
<td>≤0.03057</td>
</tr>
<tr>
<td>Biogen Health, Ltd.</td>
<td>Real-Time Reverse Transcriptase RT-PCR for the Detection of SARS-CoV (E-gene)</td>
<td>MF 039010</td>
<td>N</td>
<td>1-10</td>
<td>32.43</td>
<td>99%</td>
<td>99%</td>
<td>BioCycler CT6 deep well</td>
<td>≤0.03057</td>
</tr>
<tr>
<td>Eurocorporation</td>
<td>AccuPower SARS-CoV-2 Real-Time RT-PCR Kit</td>
<td>E</td>
<td>1-10</td>
<td>35.18</td>
<td>99%</td>
<td>99%</td>
<td>99%</td>
<td>BioCycler CT6 deep well</td>
<td>≤0.03057</td>
</tr>
</tbody>
</table>

For questions relating to the evaluation of molecular tests, please contact our **Emerging Threats team**.

Visit the COVID-19 diagnostics resource centre.

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COVID-19 Laboratory Response in the Americas

• Currently, PCR molecular diagnosis is insufficient to respond, and labs are overwhelmed
• Implementing new methodologies and strategies to be more efficient should be a priority
Sample pooling
Sample pooling

RT-PCR

All samples are considered negatives
Sample pooling

All samples are run individually.

All samples are considered negative.

RT-PCR
Sample pooling

All samples are considered negative

All samples are run individually

Samples with low viral load might be missed (dilution = lower sensitivity)

Not efficient (reagents, TAT) is pool positivity is high
Sample pooling

Use of pooling should be **carefully evaluated**

Might be useful in areas with low incidence/positivity but not when high
COVID-19 Laboratory Response in the Americas

- Currently, PCR molecular diagnosis is insufficient to respond, and labs are overwhelmed
- Implementing new methodologies and strategies to be more efficient should be a priority
Decentralization of COVID-19 diagnosis: Implementation of Ag-based RDTs

• To implement the capacity for confirmation of cases at the first level of care and / or at the community level, outpatient services and remote areas.

• To increase the access to diagnostic tests, in order to contribute to the interruption of community transmission through isolation of confirmed cases.

• To increase the daily number of samples processed and reduce the turnaround of the results.

• To increase the number of samples processed at the first level and reduce the number of samples sent to the NPHL (and then, the backlog).
During the first days after the onset of symptoms (approximately 1 to 5 days), viral proteins (antigens) can be detected by different assays (ELISA, immunofluorescence, or rapid tests).

In general, the detection of antigens presents good specificity (depending on the assay), therefore its detection can be used as confirmatory.

However, a negative result (at any stage of infection) not necessarily rule out a case.
The assays...

**COLLECTION OF SPECIMEN**

**Nasopharyngeal swab**
1. Insert a sterile swab into the nostril of the patient, swab over the surface of the posterior nasopharynx. Withdraw the sterile swab from the nasal cavity.
2. Insert the swab into an extraction buffer tube. While squeezing the buffer tube, stir the swab more than 5 times.
3. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab.

**Specimens in transport media**
1. Using a micropipette, collect the 350μl of specimen from the collection cup or VTM. Mix the specimen with an extraction buffer.
2. Press the nozzle cap tightly onto the tube.

**ANALYSIS OF SPECIMEN**
1. Apply 3 drops of extracted specimen to the specimen well of the test device.
2. Read the test result in 15-30 minutes.

![Image of swabbing procedure](https://asm.org/ASM/media/Article-Images/2020/April/Antigen-Testing.png?ext=.png)

![Diagram of assay components](https://asm.org/ASM/media/Article-Images/2020/April/Antigen-Testing.png?ext=.png)
General recommendation
To diagnose SARS-CoV-2 infection in symptomatic cases in settings where molecular testing (e.g., rRT-PCR) is limited or unavailable, or where is available with prolonged turnaround times
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To diagnose SARS-CoV-2 infection in symptomatic cases in settings where molecular testing (e.g., rRT-PCR) is limited or unavailable, or where is available with prolonged turnaround times.

The following scenarios might be considered:

• Primary and secondary care in remote areas with no or very limited access to molecular testing.
• Primary and secondary care in areas with access to molecular testing but turnaround times longer than 72 hours.
• Triage of symptomatic patients.
• Symptomatic health care workers when molecular testing is not timely available.
Implementation guidelines

Implementation of COVID-19 rapid antigen detection test - Pilot

01 October 2020

This document provides practical considerations for the implementation of COVID-19 rapid antigen detection test (Ag-RDTs) in the Region of the Americas. General considerations for the use of Ag-RDTs in the diagnosis of SARS-CoV-2 infection have been published (1, 2). Scientific and technical evidence on SARS-CoV-2 infection detection is evolving rapidly; this document will be updated as necessary.

1. User cases

In general, SARS-CoV-2 Ag-RDTs that meet the performance requirements can be used to diagnose SARS-CoV-2 infection in settings where molecular testing (e.g., RT-PCR) is limited or unavailable, or where is available with prolonged turnaround times (1).

The following user cases might be considered:

i. Primary and secondary care in remote areas with no or very limited access to molecular testing.
ii. Primary and secondary care in areas with access to molecular testing but turnaround times longer than 72 hours.
iii. Triage of symptomatic patients.
iv. Symptomatic health care workers when molecular testing is not timely available.
Challenges

• There is a very strong laboratory (NICs) network at PAHO Region; it was advantageous for COVID-19 response
  • Sampling
  • Molecular capacity/interpretation
  • Quality assurance and biosafety

• Nevertheless, the labs went overloaded and the capacity became insufficient.
  • Centralized response
  • Backlog and increased turnaround
  • Lack of material (global shortage)
  • Other pathogens surveillance was impacted

• Decentralization processes should be a priority (virological tests)
  • Strengthen National networks
  • Ag-RDTs
Challenges

• Still too many things to learn...
  • Immune response
  • Co / Re-infections
  • Vaccines
  • Genetic characterization and viral evolution

• Testing strategies should be clear; decisions should be coordinated with the laboratories
  • Type of test (virologic vs serologic)
  • When and how
  • Interpretation...
Thank you!!

PAHO
Laboratory Response Team