New IgM + IgG Antibody Test For The Novel Coronavirus (SARS-CoV-2): A Less Invasive And Safer Test Option

The Novel Coronavirus is a single-strand RNA coronavirus that first appeared in 2019, also known as Severe Acute Respiratory Syndrome Coronavirus 2, or SARS-CoV-2. The disease caused by this virus is called COVID-19 which stands for Corona Virus Disease first detected in 2019. This virus has caused a global pandemic, leading to hundreds of thousands to become infected around the world and causing thousands of deaths. Common symptoms include fever, fatigue, a dry cough, and shortness of breath. Some who are infected have relatively minimal symptoms, while others, particularly those most vulnerable (the elderly and those with pre-existing medical conditions) can become severely ill, often with pneumonia-like symptoms, sometimes with the infection leading to sepsis and death. Cases in the United States are on the rise and effective and accurate testing is vital to helping slow the spread of the virus.

Why is antibody testing for the SARS-CoV-2 virus more useful than RT-qPCR technology?

- A blood test is far less invasive and painful than the nasal swab RT-PCR test.
- With immunoassays, there is no risk for cross-contamination from nucleic acids that might contaminate the laboratory using RT-PCR technology.
- Combining both the IgM and IgG antibody tests helps determine whether someone is currently infected, if they’ve been infected in the past, and how long ago the patient was infected.

*The CDC strongly recommends that anyone with symptoms of COVID-19 have the RT-PCR test done to confirm whether or not they are infected.

Results and Clinical Significance

The Diazyme SARS-CoV-2 IgM/IgG tests are designed to complement RT-qPCR in the diagnosis of SARS-CoV-2 infections. Results are expressed in AU/mL (antibody arbitrary unit per milliliter). A negative result corresponds to a result strictly less than 1.0 AU/mL. A positive results corresponds to a result greater than or equal to 1.0 AU/mL. Samples having results near the 1.0 AU/mL cutoff (0.9-1.1 AU/mL) should have follow up testing and the patient should be assessed in conjunction with other tests (including RT-qPCR and computed tomography).
Many currently available tests for SARS-CoV-2 virus are a RT-PCR test (reverse transcription polymerase chain reaction) done via an invasive and often painful nasal swab. This method of sample collection also puts the staff collecting the sample at risk for exposure to the virus. The Great Plains Laboratory (GPL) has been conducting IgG and IgE antibody blood tests for more than 20 years, offering both serum and dried blood spot sample options. The SARS-CoV-2 Antibody Test from GPL is less invasive than the nasal swab test for the patient (currently available as a blood draw) and safer for the staff conducting the test, since they are less likely to be exposed to the virus during sample collection.

The SARS-CoV-2 Antibody Test from GPL, a fully automated, bead-based immunoassay, looks for both IgM and IgG antibodies to the virus. The presence of IgM antibodies indicates the patient recently contracted the virus, as IgM antibodies are the first to be produced in response to a virus. The presence of IgG antibodies indicates the patient has had the virus for a longer period of time, as IgG immunoglobulin starts to be produced as IgM antibodies begin to decrease. IgG may continue to be produced for years while IgM antibodies commonly disappear after some weeks.

RT-qPCR and IgM/IgG serological tests do not necessarily need to agree. A disagreement between the two tests, if any, can often be traced to the after-infection time points at which the tests were performed. Overall, while RT-qPCR testing may be appropriate for the detection of the SARS-CoV-2 virus during the acute phase, IgM/IgG is an appropriate test during the chronic phase. Since the exact time of infection is often unknown, combining RT-qPCR and IgM/IgG testing can improve the accuracy of the COVID-19 diagnosis.
**Performance Validation**

The performance of the Diazyme SARS-CoV-2 IgM/IgG CLIA kits has been validated in terms of precision, high-dose effect, matrix comparison, interference, cross-reaction, clinical sensitivity and clinical specificity. The following results were obtained for both assays:

- **Precision:** For positive samples, Repeatability CV ≤ 10%; Reproducibility CV < 15%.
- **No High-dose effect up-to 1000 AU/mL.**
- **The following types of specimens can be used with Diazyme DZ-Lite SARS-CoV-2 IgM/IgG CLIA Kit:**
  - Serum: standard sampling tubes, procoagulant inert separation tubes containing separating gel (SST).
- **There is no interference with assay with the following substances at the following concentrations:**
  - Bilirubin ≤ 40 mg/dL, Triglycerides ≤ 1000 mg/dL, Hemoglobin ≤ 2000 mg/dL, Rheumatoid Factor ≤ 1500 IU/mL, Anti-Mitochondrial ≤ 1:64 (titer), HAMA ≤ 30 ng/mL, Total IgG ≤ 1600 mg/dL, Total IgM ≤ 280 mg/dL, Interferon ≤ 1500 U/mL, Ribavirin ≤ 90 mg/dL, Oseltamivir ≤ 1.0 mg/dL, Levofloxacin ≤ 1.776 mg/dL, Azithromycin ≤ 1.201 mg/dL, Ceftriaxone sodium ≤ 81.03 mg/dL, Meropenem ≤ 80.15 mg/dL, Tobramycin ≤ 2.4 mg/dL, Diphenhydramine Hydrochloride ≤ 4.5 mg/dL, Oxymetazoline ≤ 2.5 mg/dL, Sodium chloride ≤ 45 mg/dL, Beclomethasone ≤ 2.5 mg/dL, Dexamethasone ≤ 18 mg/dL, Triamcinoloneacetonide ≤ 5.5 mg/dL, Budesonide ≤ 3.2 mg/dL, Mometasone ≤ 2.5 mg/dL, Fluticasone propionate ≤ 2.5 mg/dL.
- **No false positive results were observed when testing the following clinical interference samples:** Influenza virus type A antibody, Influenza virus type B antibody, parainfluenza virus antibody, respiratory syncytial virus antibody, adenovirus antibody, EBV NA IgG, EBV VCA IgM/IgG, Measles virus, CMV IgM/IgG, Varicella zoster virus antibodies, M. Pneumoniae IgM/IgG, chlamydiapneumoniae IgM/IgG, Candida albicans, ANA, HCoV-HKU1, HCoV-OC43, HCoV-NL63 and HCoV-229E.

**Clinical Sensitivity**

The clinical sensitivity of the Diazyme SARS-CoV-2 IgM CLIA assay was determined using confirmed novel coronavirus infected specimens from three hospitals in China (n=89). The clinical sensitivity for the SARS-CoV-2 IgM assay was found to be 78.7%. When used in combination with the Diazyme SARS-CoV-2 IgG CLIA assay, the clinical sensitivity increased to 89.9%.

The clinical sensitivity of the Diazyme SARS-CoV-2 IgG CLIA assay was determined using confirmed novel coronavirus infected specimens from two hospitals in China (n=91). The clinical sensitivity for the SARS-CoV-2 IgM assay was found to be 91.2%. When used in combination with the Diazyme SARS-CoV-2 IgG CLIA assay, the clinical sensitivity increased to 95.6%.

**Clinical Specificity**

The clinical specificity of the Diazyme SARS-CoV-2 IgM CLIA assay was determined in China (n=200) using non-novel coronavirus infected specimens (including normal samples and interference samples). The clinical specificity
for the SARS-CoV-2 IgM assay was found to be 97.5%. When used in combination with the SARS-CoV-2 IgG assay, the clinical specificity was found to be 96.5%.

The clinical specificity of the Diazyme SARS-CoV-2 IgM CLIA was determined in China (n=750) using non-novel coronavirus infected specimens (including normal samples and interference samples). The clinical specificity for the SARS-CoV-2 IgM assay was found to be 97.3%. When used in combination with the SARS-CoV-2 IgG assay, the clinical specificity was found to be 96.0%.

**Performance on the Field**
The Diazyme SARS-CoV-2 IgM/IgG CLIA kits are currently used worldwide (including China, Europe and the United States) to help diagnose SARS-CoV-2 infections.

**ITALY STUDY**
A recent article titled “Assessment of immune response to SARS-CoV-2 with fully-automated MAGLUMI 2019-nCoV IgG and IgM chemiluminescence immunoassays” by scientists of the university of Verona Italy compared the performance of the assay to that of RT-qpCR and a CE-marked Anti-SARS-CoV-2 IgA and IgG enzyme-linked immunosorbent assays (ELISAs; Euroimmun AG, Luebeck, Germany) 1. Overall concordance with the Anti-SARS-CoV-2 IgA and IgG enzyme-linked immunosorbent assay was found to be 90%, despite both assays testing for different classes of immunoglobulins (IgM versus IgA). Although the overall agreement with the results of RT-PCR on upper respiratory specimens was limited, the authors of the study stated that such findings are not surprising since results of the two methods depend on the time passed between the onset of symptoms and blood and swab collection.

**Reference**

**USA STUDY (BOSTON, MA)**
A recent study of the performance of the Diazyme SARS-CoV-2 IgM/IgG at a clinical laboratory in Boston, MA has yielded the following results:

Results obtained with 100 Random Subjects: Median IgM and IgG values were 0.21 AU/mL and 0.04 AU/mL, respectively, with 3.0% having IgG values > 1.0, and 1.0% having IgM values > 1.0 AU/mL, indicating some degree of SARS-CoV-2 population exposure.

Results obtained with Negative or Positive Hospitalized Patients Based on RT-qPCR Nasal Swabs: In 26 negative patients, median IgM and IgG values were 0.27 AU/mL and 0.05 AU/mL, respectively, and all patients had IgG values < 1.0AU/mL, and 25/26 (96%) had IgM values < 1.0 AU/mL. In 10 positive patients, median IgM and IgG values were 3.39 AU/mL and 44.98 IU/mL, respectively. All patients had IgG values > 1.0 AU/mL and 9/10 (90.0%) subjects had IgM values>1.0 AU/mL (range: 3.23 – > 100.00 AU/mL).

Results with RT-qPCR Negative or Positive Outpatients: In 5 negative subjects, median IgM and IgG values were 0.35 AU/mL and 0.06 AU/mL, with all values being < 1.0 AU/mL. In 5 positive subjects, median IgM and IgG values were 6.01AU/mL and 5.85 AU/mL, respectively, with all values being > 1.0 AU/mL.
USA STUDY (CALIFORNIA)
A recent study of the performance of the Diazyme SARS-CoV-2 IgM/IgG at a clinical laboratory in California has yielded the following results:

**Sensitivity**
30 of PCR confirmed COVID-19 patient samples were tested with Diazyme SARS-CoV-2 IgG/IgM CLIA kits and the results are shown below:

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Positive Results</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Number of Negative Results</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Sensitivity percentage</td>
<td>93.3%</td>
<td>80%</td>
</tr>
</tbody>
</table>

**Specificity (75 Healthy Patients)**

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Positive Results</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>Number of Negative Results</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sensitivity percentage</td>
<td>100%</td>
<td>98.7%</td>
</tr>
</tbody>
</table>

**Specificity (100 Patients Samples collected before COVID-19 outbreak, -20°C storage)**

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Positive Results</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Number of Negative Results</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity percentage</td>
<td>98%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Regulatory Considerations**
Diazyme’s Emergency Use Authorization (EUA) from the FDA for both assays is approved. Diazyme utilized the notification process as outlined in Section IV D of the policy listed on the FDA’s site dedicated to serological (antibody) testing: www.fda.gov/medical-devices/emergency-situations-medicaldevices/faqs-diagnostic-testing-sars-cov-2.


**Sample Report**

**SARS-CoV-2 Virus Antibody Test (Serum)**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Result (AU/mL)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM Antibodies</td>
<td>0.190</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>IgG Antibodies</td>
<td>0.590</td>
<td>Non-reactive</td>
</tr>
</tbody>
</table>

**Interpretation**
Since both IgG and IgM antibodies are negative, the results mean the following:
1) The person has not been exposed to or infected by the SARS-CoV-2 virus prior to blood draw.

**OR**
2) The person has been exposed to the SARS-CoV-2 virus prior to their blood draw but the exposure was such a short time before blood draw that the immune system has not had a chance to develop antibodies. In this case, performance of the PCR test for the nucleic acid of the virus on a nasal or throat swab might be necessary to determine if the person is infected.
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**WHY TEST FOR SARS-COV-2 ANTIBODIES IN BLOOD?**
Many currently available tests for SARS-CoV-2 (the virus that causes COVID-19) are a RT-PCR test (reverse transcription polymerase chain reaction) done via an invasive and often painful nasal swab. This method of sample collection also puts the staff collecting the sample at risk for exposure to the virus. The Great Plains Laboratory (GPL) has been conducting IgG and IgE antibody blood tests for more than 20 years. The SARS-CoV-2 Antibody Test from GPL is less invasive than the nasal swab test for the patient (currently available as a blood draw) and safer for the staff conducting the test, since they are less likely to be exposed to the virus during sample collection.

Antibody testing reveals both active infections (IgM antibodies) as well as previous exposure (IgG antibodies). Antibodies are more evenly distributed throughout the blood stream than RNA is and the antibodies are highly specific to this virus.

The SARS-CoV-2 Antibody Test from GPL, a fully automated, bead-based immunoassay, looks for both IgM and IgG antibodies to the virus. Studies of the specificity of the antibody tests for the SARS-CoV-2 virus used at GPL show there is no interference with other coronaviruses such as CoV-OC43, CoV-229E and CoV-NL63, and CoV-HKU1 viruses.

<table>
<thead>
<tr>
<th>Infection Stage</th>
<th>RT-qPCR</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage detection</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Active infection</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Late or recurrent stage</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Recovery state</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Recovered from a past infection</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

Learn more at [gplcovidtest.com](http://gplcovidtest.com) or contact us at (913) 341-8949 or sales@gpl4u.com to set up your account and order the test today.