LIAISON® SARS-CoV-2 SOLUTIONS

The combined testing solutions for the detection of antibodies to SARS-CoV-2
The comparison to PRNT90 was evaluated by testing 87 samples collected during the outbreak from subjects whose PRNT90 result was available: 69 were PRNT assay-negative, and 18 were PRNT assay-positive (Titer >1:40).

<table>
<thead>
<tr>
<th>LIAISON® SARS-CoV-2 S1/S2 IgG</th>
<th>PRNT titers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>Negative (&lt; 12.0 AU/mL)</td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>Equivocal (12.0 – 15.0 AU/mL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive (&gt; 15.0 AU/mL)</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>18</td>
</tr>
</tbody>
</table>

High positive concordance (94.4%) at LIAISON cut off for PRNT90 at 1:40 ratio, was observed between IgG assay and PNRT titers, indicating the presence of neutralizing antibodies.

Possible use for Plasma donors (3):

- 1:160 titer PRNT90 is a possible cut off to select donor serum for plasmapheresis in Italy and by CDC, i.e. hyperimmune serum donations
- At a LIAISON value of 80 AU/ml a positive agreement of 100% with a 1:160 PRNT90 Titer was observed.
- Our assay can be used as upstream pre-screening tool: LIAISON test first to identify donors >80AU/ml, then proceed with Quality Protocol for evaluating plasma donations (PRNT, screening for blood pathogens, etc)

FOCUS on the VALUE of neutralizing antibodies correlation

- Neutralizing antibodies are commonly considered to be protective (*) and DiaSorin assay positive agreement with PRNT 90 is 94.4%
- Although the role of neutralizing antibodies to SARS-CoV-2 is under investigation, information on their presence can be important from an epidemiological point of view
- The DiaSorin assay can be a valuable tool for plasma donation screening
- S1 and S2, the antigens that DiaSorin uses in his IgG test, are the most promising for vaccine (7)

(*) Reminder:
It should be noted that lack of scientific data at this time does not allow yet to determine if neutralizing IgG antibodies against SARS-CoV-2 provide long term immunity to the virus or if they protect patients against re-infection.

By blocking the virus entry to the cell, Neutralizing antibodies can block viral replication: this does not remove need for PCR testing to confirm patient is not infective

NOTE: Neutralizing antibodies are produced via a maturation process, can take longer to be produced than non-neutralizing Abs (6)

Potential use of Antibodies testing solutions:

- Shorten the diagnostic window (combined use with IgM + IgG)
- IgM testing could help to stratify patients to evaluate new vs previous infection
- IgG testing to identify potential donors of iperimmune plasma, thanks to the correlation with neutralizing antibodies
- Moreover, the Spike protein seems to be most promising target for the vaccines development
LIAISON® SARS-CoV-2

The value of DiaSorin assays
DiaSorin provides diagnostic laboratories with 2 antibodies detection kits to SARS-CoV-2:
• LIAISON® SARS-CoV-2 IgM
• LIAISON® SARS-CoV-2 S1/S2 IgG
The testing solutions are based on:
• 2 fully automated assays to be run on LIAISON® XL
• Two results from a single sample to determine both antibodies results
• The selection of Spike protein antigens for both assays, recognized to be the most likely target of future COVID-19 vaccines and for IgG neutralizing antibodies
• High sensitivity and specificity to ensure accurate results along the infection window

Timing of seroconversion is a key factor in establishing the appropriate time window to use serology tests. Seroconversion has been observed as early as within 5 days after symptom onset for IgM and within 5-7 days for IgG. Depending on the applied method, seroconversion is observed after a median of 10-13 days after symptom onset for IgM and 12-14 days for IgG.

The estimated variation over time in diagnostic tests for detection of SARS-CoV-2 Infection relative to symptom onset can be depicted for illustrative purposes as follows:

Furthermore, since viral RNA can be detected in patients after 20 or more days, a positive IgG result should not be interpreted as a sign that a patient has stopped being infective.

Both IgM and IgG assays use Spike protein on the solid phase.
IgM assay uses S1-RBD as antigen: more than 10 antigens were tested and S1-RBD provided the best combination of Specificity and Sensitivity.

IgG assay uses S1 and S2, subunits of the Spike protein.

Proteins are responsible for: binding (S1) & fusion (S2) of virus to cell.

The Spike Protein is the target for neutralizing antibodies and the most promising target for Vaccines. Internal viral protein, such as Nucleocapsid are not exposed to antibodies directly and unlikely to be neutralizing.

Combined detection of IgM and IgG can be used to assess the immune status of patients exposed to and infected by SARS-CoV-2. The use of IgG and IgM in combination can aid to shorten the diagnostic window improving the detection of seroconversion up to day 15 from PCR positivity.

The sensitivity was determined by investigating 268 samples collected over the course of time from 233 European patients. Infection with SARS-CoV-2 was confirmed by RT-PCR test at the time of the diagnosis.

The LIAISON® SARS-CoV-2 IgM and S1/S2 IgG test were performed on samples collected at the time of admission and the LIAISON® SARS-CoV-2 thereafter up to 30 days. The group included patients hospitalized with moderate symptoms, patients admitted at ICU with severe symptoms and patients not hospitalized without or with mild symptoms.

The following table describes combined diagnostic sensitivity in three groups, i.e. the early samples (≤ 7 days after diagnosis), the samples between 8 and 14 days after diagnosis, and the later samples (15-30 days after diagnosis).

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Number of LIAISON® IgG and/or IgM Positive results</th>
<th>Sensitivity (Wilson 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 7 days</td>
<td>73 / 105</td>
<td>69.5% (60.2% - 77.5%)</td>
</tr>
<tr>
<td>8-14 days</td>
<td>43 / 47</td>
<td>91.5% (80.1% - 96.6%)</td>
</tr>
<tr>
<td>15-30 days</td>
<td>114 / 116</td>
<td>98.3% (93.9% - 99.5%)</td>
</tr>
</tbody>
</table>

A total of 500 presumed SARS-CoV-2 negative samples were tested with LIAISON® SARS-CoV-2 S1/S2 IgG and LIAISON® SARS-CoV-2 IgM assays resulting in 99.2% combined clinical specificity (496 / 500, 95% CI: 98.0% – 99.7%).
The Value of detecting Neutralizing Antibodies:

- Neutralizing Antibodies are defined as an antibody that defends a cell from a pathogen or infectious particle by neutralizing any effect it has biologically.
- The presence of NAb's is commonly considered as a sign of protection against a pathogen, even if it should be noted that lack of scientific data at this time does not allow yet to determine if neutralizing IgG antibodies against SARS-CoV-2 provide long term immunity to the virus or if they protect patients against re-infection.

The LIAISON® IgG assay correlates with Plaque Reduction Neutralization Test (PRNT90)

A PRNT measures how effective are patient antibodies to stop the virus from infecting cells, that is the neutralizing activity.

The science behind PRNT90:

The plaque-reduction neutralization test determines relative concentrations of virus-specific (SARS-CoV-2) neutralizing antibodies in a serum sample by measuring the titer at which a serum sample produces 90% or reduction in plaques vs a no serum control.

The process has to be performed in a laboratory Biosafety Level 3.

Plaques are generally counted manually and the results, in combination with the dilution factor used to prepare the plate, are used to calculate the number of plaque forming units per sample unit volume. This result represents the number of infective particles within the sample and is based on the assumption that each plaque formed is representative of one infective virus particle.

The concentration of serum to reduce the number of plaques by 90% compared to the serum free virus gives the measure of how much antibody is present or how effective it is. This measurement is denoted as the PRNT90 value.
Ordering information & Specs - Kit

<table>
<thead>
<tr>
<th>KIT FORMAT</th>
<th>LIAISON® SARS-CoV-2 S1/S2 IgG 311450</th>
<th>LIAISON® SARS-CoV-2 IgM 311470</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME TO FIRST RESULT</td>
<td>35 min</td>
<td></td>
</tr>
<tr>
<td>THROUGHPUT</td>
<td>Up to 170 tests/hour</td>
<td>Up to 84 tests/hour</td>
</tr>
<tr>
<td>OPEN/ON BOARD KIT STABILITY and CALIBRATION</td>
<td>Up to 4 weeks</td>
<td>Up to 1 week</td>
</tr>
<tr>
<td>No Biotin potential inference observed</td>
<td></td>
<td>Up to 3500 ng/mL</td>
</tr>
</tbody>
</table>

Ordering information & Specs - Controls

<table>
<thead>
<tr>
<th>LIAISON® Control</th>
<th>SARS-CoV-2 S1/S2 IgG 311451</th>
<th>SARS-CoV-2 IgM 311471</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT FORMAT</td>
<td>2 levels x 2 vials (up to 40 runs)</td>
<td></td>
</tr>
<tr>
<td>Open/on board controls stability</td>
<td>Up to 4 weeks</td>
<td></td>
</tr>
</tbody>
</table>

References:
7. Shang, W., Yang, Y., Rao, Y. et al. The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. npj Vaccines 5, 18 (2020). https://doi.org/10.1038/s41541-020-0170-0

Please visit: [www.diasorin.com/covid19CE](http://www.diasorin.com/covid19CE) for more information and updates

**LIAISON®** is a registered trademark of DiaSorin

**Available only on LIAISON®**

Product availability subject to required regulatory approval

**DiaSorin S.p.A**

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