Background

- The plaque reduction neutralization test (PRNT) is the ‘gold standard’ for detecting neutralizing antibodies but requires a biosafety level 3 facility.
- We previously developed a SARS-CoV-2 pseudotyped MLV neutralization assay (MLV pseudotype) as a more accessible alternative.
- Several commercial IgG assays (Siemens, Abbott, Euroimmun) have been developed in order to measure SARS-CoV-2 binding antibodies but do not measure the neutralization antibodies which is critical for sterilizing immunity.
- Studies are ongoing to determine how long humoral immunity is maintained after SARS-CoV-2 seroconversion.

Objective

1. To compare SARS-CoV-2 antibody detection assays.
2. To ascertain the length of time SARS-CoV-2 neutralizing antibodies are maintained in convalescing individuals.

Methods

A) Isolate Serum
1.25 1.00 1.48 1.160
Serial Dilute Serum

Add virus to Serum

Add Serum + Virus to HeLa293
ACE2* cells

Incubate at 37°C for 1h

Incubate at 37°C for 48h

Neutralization (Remaining or No Infection)

Cell Lysis and Luciferase Assay

No Neutralization

(Failure)

B) Human Serum

SARS-CoV-2 spike pseudotyped MLV
(MLV pseudotype)

Neutralization test (PRNT)

Siemens SARS-CoV-2 IgG
Assay (Siemens)

Results

Table 1. Degree of Concordance for SARS-CoV-2 antibody detection assays.

<table>
<thead>
<tr>
<th>Test</th>
<th>PRNT</th>
<th>MLV Pseudotype</th>
<th>Siemens</th>
<th>Abbott</th>
<th>Euroimmun</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNT</td>
<td>X</td>
<td>95.87%</td>
<td>97.01%</td>
<td>94.64%</td>
<td>99.47%</td>
<td>94.29%</td>
</tr>
<tr>
<td>MLV Pseudotype</td>
<td>X</td>
<td>93.33%</td>
<td>88.57%</td>
<td>95.02%</td>
<td>90.24%</td>
<td>91.20%</td>
</tr>
<tr>
<td>Siemens</td>
<td>X</td>
<td>97.30%</td>
<td>97.5%</td>
<td>91.18%</td>
<td>91.00%</td>
<td>92.50%</td>
</tr>
<tr>
<td>Abbott</td>
<td>X</td>
<td>93.33%</td>
<td>94.87%</td>
<td>96.18%</td>
<td>96.18%</td>
<td>96.18%</td>
</tr>
<tr>
<td>Euroimmun</td>
<td>X</td>
<td>95.54%</td>
<td>95.54%</td>
<td>95.54%</td>
<td>95.54%</td>
<td>95.54%</td>
</tr>
</tbody>
</table>

Table 1. The degree of concordance (%) was calculated for positive and negative samples between assays. Ranges for each assay were as follows: PRNT (NT50 > 24); MLV pseudotype (NT50 > 24), Siemens (anti-RBD binding index > 1); Abbott (positive ≥ 1.4; negative < 1.4), and Euroimmun (positive ≥ 1.1; indeterminant ≥ 0.8 to < 1.1; negative ≤ 0.8) when possible.

Discussion

- The SARS-CoV-2 MLV pseudotype neutralization assay and the Siemens SARS-CoV-2 IgG Assay detect the presence of SARS-CoV-2 antibodies with a high degree of concordance to the PRNT assay.
- Neutralizing antibody titers (NT50) generated by the SARS-CoV-2 MLV pseudotype do not correlate with the PRNT assay.
- The Siemens SARS-CoV-2 IgG RBD binding antibodies values weakly correlate with the PRNT assay NT50 titers.
- Vaccinated donors C022 and C023 who previously received a positive COVID-19 PCR result appeared to develop high SARS-CoV-2 neutralizing antibodies within 10 days after receiving vaccine dose 1.
- Donor C119-DP who tested positive for COVID-19 twice (DP) prior to vaccination had a lower NT50 at day 32 post-vaccination compared to donors C022 and C023.
- The majority of donors (4/6) tracked immediately post-vaccination who self-reported no history of COVID-19 developed detectable neutralizing antibodies only after receiving the second vaccine.

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References