New immunogenicity strategies to meet the needs of a developing pandemic

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(Unwanted) Immunogenicity – the anti-drug antibody format

MSD Bridging assay (ECLIA) - commonly used in the assessment of unwanted immunogenicity

Why not an ELISA?

- Better sensitivity and analytical working range
- Homogenous solution phase incubation simplifies workflow
- Shorter assay times – higher throughput
- Not species specific
Early challenges in method development

• Early prototype assays used S1 and RBD fragments to investigate bridging assay potential
• Full length Spike protein was not available until June/July 2020
• Full length assays required additional development to reduce background
  > Buffer optimization
  > Different challenge ratios
  > Concentration in assay
• Positive controls not specific (only cross reactive from SARS)

The Virus Itself
Final assay – format and precision

- Block Streptavidin MSD Plate
- Dilute sample 1 in 20
- Diluted sample is incubated for 1 hour with master mix containing equal concentrations of biotinylated- and sulfo- tagged full length spike protein
- Reaction mix is added to blocked plate for 1 hour
- Plate is washed and read on sector imager
- All liquid handling performed on an Integra ViaFlo

<table>
<thead>
<tr>
<th></th>
<th>left</th>
<th>middle</th>
<th>right</th>
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<tbody>
<tr>
<td>NC</td>
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<td>145</td>
<td>135</td>
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<td>CP-PC</td>
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<td>1299</td>
<td>1223</td>
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<td>EPC</td>
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<td>5408</td>
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<tr>
<td>EPC</td>
<td>73628</td>
<td>71590</td>
<td>70321</td>
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</table>

Mean (n=6)  
%CV  
NC 148 5.4  
CP-PC 1270 4.0  
PC 5298 4.5  
EPC 71559 3.2
Sample types – serum and WB micro-sample

Neoteryx Mitra® VAMS and collection kits

**Benefits**
- At home sampling, no need for a clinic visit or venipuncture
- CE Marked, FDA Class 1 devices
- Devices can be shipped directly to us
- Barcoded, logged straight into our LIMS system for chain of custody and ease of reporting
Questions over approach

- Do we need a tiered approach? What about confirmatory analysis??
- What about how we normally validate an ADA assay? Selectivity etc..
- Analytical Sensitivity, PCs are not as good as real positive samples – can we justify not having the 100 ng/mL box ticked?
- Should we not be analyzing in duplicate?
- What regulations should we be working to?
The pathway to assay roll out*

- Method development
  - Serum
  - Mitra VAMS

- Analytical Validation
  - Serum
    - Cut point
    - Precision
    - Stability

- Clinical Verification
  - Serum
    - Verify CP with greater n
    - Cross-reactivities
    - Sensitivity/Specificity
    - Equivalence with other assays
  - Mitra VAMS
    - Verify CP
    - Concordance with serum

Given the purpose of the test, the threshold was set such that the specificity is as close to 100% as possible, while maximising the sensitivity. The point on the curve which is closest to the top left corner is at specificity 1 and sensitivity 0.9691, which is attained at thresholds between 385 and 1,400 RFU.

ROC Curve, the red dot represents a perfect test with 100% specificity and sensitivity

Bootstrapped values for the 99th percentile of the distribution of pre-pandemic samples
Assay Validation – Precision & Stability

• Precision – Intra/Inter-run and inter-analyst (3 analysts, 6 runs each of three plates)

<table>
<thead>
<tr>
<th>NC</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>115</td>
<td>973</td>
<td>4181</td>
</tr>
<tr>
<td>Std Dev.</td>
<td>16.6</td>
<td>169</td>
<td>741</td>
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<tr>
<td>CV%</td>
<td>14.5</td>
<td>17.4</td>
<td>17.7</td>
</tr>
<tr>
<td>n</td>
<td>156</td>
<td>156</td>
<td>156</td>
</tr>
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</table>

• PC1 and PC2 = Seracare Accurun controls. PC3 is high responding clinical sample
• All plate values are normalized to PC1, hence a positive sample is >1.

• Stability
  o Serum
    • Benchtop 24 hrs, 3 x Freeze/Thaw, Long term frozen at -80°
  o Mitra sample
    • Dried tip stability at RT and 35°C for 7 days (covers postage period)
    • Eluate - Benchtop 24 hrs, 3 x Freeze/Thaw, Long term frozen at -80°
The assessment of sensitivity was performed on a cohort of COVID-19 patient samples where infection by SARS-CoV-2 had been confirmed by a PCR test 21 days prior to the sample being taken. In this case the assay demonstrated 98% Sensitivity.

<table>
<thead>
<tr>
<th>Days post PCR confirmation</th>
<th>N</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>% Positive</th>
<th>Sensitivity % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>19</td>
<td>19</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>44</td>
<td>44</td>
<td>1*</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>31-50</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>From 21 days</td>
<td>45</td>
<td>44</td>
<td>1*</td>
<td>98.0</td>
<td>88.4 – 99.6</td>
</tr>
</tbody>
</table>

* Sample confirmed as Ab negative by both Roche Elecsys and Siemens assays
Have we got the cut point in the right place?

Individual Screening Data from Clinical Verification

Proposed cut point window based on validation data

All samples were collected in 2018-2019
Serum clinical verification

- Assessment of serum samples for specificity used 377 pre-pandemic samples including the following disease state or interference assessments

  **Confounder samples**
  - 39 Coronavirus HKU Ab+
  - 39 Coronavirus OC43 Ab+
  - 40 Coronavirus 229E Ab+
  - 38 Coronavirus NL63 Ab+
  - 4 Parainfluenza Ab+
  - 4 Influenza A Ab+
  - 4 Influenza B Ab+
  - 4 Respiratory Syncytial Virus Ab+
  - 2 Rheumatoid Factor
  - 2 HIV+
  - 4 Enterovirus Ab+
  - 31 EBV Nuclear Antigen positive
  - 24 CMV Ab+
  - 16 HBs Ab+
  - 2 Immune thrombocytopenia (ITP)

  **Interference samples**
  - 2x hyperlipidaemia patient samples
  - 2x hyperlipidaemia (spiked to 4mg/mL)
  - 2x hyperbilirubinaemia (spiked to 30 µg/mL)
  - 2x haemolysed (3% equivalent to >250 mg/dL of free haemoglobin)
  - 2x Biotin (spiked to 1200 ng/mL)

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Reactive</th>
<th>Non-Reactive</th>
<th>Specificity (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative samples: Pre-COVID era</td>
<td>301</td>
<td>0</td>
<td>301</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Interference samples</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Confounder samples</td>
<td>66</td>
<td>0</td>
<td>66</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>377</td>
<td>0</td>
<td>377</td>
<td>100</td>
<td>98.7 – 100%</td>
</tr>
</tbody>
</table>
Additional Mitra Eluate clinical verification

Comparison between serum values and surrogate mitra samples (comprising of red blood cells from a healthy donor combined with serum from pre-pandemic or confirmed COVID-19 patients).

Paired venous draw serum and capillary “finger prick” Mitra samples, from volunteers at LGC, were assessed for concordance.
This is the Covid Home screen, the workflow is as follows:

- Create new clients/sites as required
- Upload Mitra tip kit barcodes
- Linking a shipment with a client.
- Sample batching and QC checking
- After analysis - run reviewed, accepted or rejected.
- Reporting

This is the plate review screen after data import. On the right hand side we have the plate level data.

Top table shows the cut point control data.

Middle table is the Positive and Negative QC data.

At the bottom we find the unknown sample results.

**All data is fake data created for testing so it may be inconsistent**
What's next...

The MHRA are very clear that an assay such as this would be classed as a diagnostic medical device.

As such, it requires a CE mark (done) and performance under an ISO15189 or ISO17025 quality system (pending inspection).
An end-to-end solution (it's not all about the assay...)

Neoteryx direct shipment to homes
1) Barcoded blood collection kit including pre-addressed shipping bag (for home testing)
2) Or distribution of kits via a hub

Barcode linked to named individual via app or other?

20µL dried blood collected onto Mitra tips

Royal Mail

Home test samples mailed directly to LGC using pre-paid packaging (ambient temperature)

Mitra samples are unpacked, logged into LIMS using barcode & placed in 96-well rack

Plates are read and data uploaded to LIMS

Data is processed and reported to individuals
Thank you for listening

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