Harmonisation and increased comparability of SARS-CoV-2 serological assays by WHO IS

Project leader: Giada Mattiuzzo
First WHO International Standard for SARS-CoV-2 RNA

**Intended use:** calibration and harmonisation of NAT assay for the detection of SARS-CoV-2 RNA

- Acid/heat inactivated England isolate (20/146) with an assigned potency of $7.4 \log_{10} \text{IU/ampoule}$

Approximately 2500 ampoules available for distribution
Why we make it (use of the antibody Standard)

- Serological assays are needed to understand the real impact of COVID-19, as asymptomatic cases or those with mild symptoms are often undetected
- Evaluation and comparison of vaccine responses
- Evaluation and comparison of other therapeutics (mAbs, CP)
- Immunological surveillance

How we make it....

- Collaborative study
- Establishment by WHO Expert Committee on Biological Standardization
Source Material

Convalescent plasma was provided by:
• ISARIC4C through University of Liverpool, UK
• NHS Blood and Transplant, UK
• Oslo University Hospital, Norway

Convalescent sera was provided by:
• Papworth Hospital, Cambridge, UK

All donors gave written informed consent.
All material has been collected >28 days post diagnosis and solvent-detergent treated at NIBSC; blood virology report negative for usual blood borne viruses
Candidate International Standard and Reference Panel

**IS**- Pool of plasma from 11 donors from UK
0.25 mL plasma per ampoule, freeze-dried

**Reference Panel**- 0.25 mL plasma pools per ampoule, freeze-dried
High titer
Mid titer
Low anti-S, high anti-N
Low
Negative
Collaborative study samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>formulation/vol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-CP high (20/130)</td>
<td>20/130, Convalescent plasma from one patient, positive</td>
<td>liquid 0.1</td>
</tr>
<tr>
<td>B-CS high</td>
<td>Convalescent sera pool, positive</td>
<td>liquid 0.2</td>
</tr>
<tr>
<td>C-CS low</td>
<td>Convalescent sera pool, very weak positive</td>
<td>liquid 0.2</td>
</tr>
<tr>
<td>D-CP low</td>
<td>Convalescent plasma from one donor, weak positive</td>
<td>liquid 0.2</td>
</tr>
<tr>
<td>E-RP low S, high N</td>
<td>20/144, Reference Panel member, weak S, high N</td>
<td>f/d 0.25</td>
</tr>
<tr>
<td>F-RP high</td>
<td>20/150, Reference Panel member, high</td>
<td>f/d 0.25</td>
</tr>
<tr>
<td>G-IS</td>
<td>20/136, Candidate WHO IS</td>
<td>f/d 0.25</td>
</tr>
<tr>
<td>H-RP neg</td>
<td>20/142, Reference Panel member, negative</td>
<td>f/d 0.25</td>
</tr>
<tr>
<td>I-RP low</td>
<td>20/140, Reference Panel member, low</td>
<td>f/d 0.25</td>
</tr>
<tr>
<td>J-RP Mid</td>
<td>20/148, Reference Panel member, mid</td>
<td>f/d 0.25</td>
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</tbody>
</table>
Collaborative study participants

• 44 participants from 15 countries
• Vaccine developers, NCL/NRL, diagnostic labs, kit manufacturers, non-profit vaccine research organisation and academic laboratories
Methods: 125 data sets

**NEUTRALISATION ASSAY**
(27)
- LIVE SARS-CoV-2* (15)
  - PRNT/FRNT
  - CPE
  - MN
- PSEUDOTYPED VIRUS(12)
  - VSV(Luc)
  - HIV(Luc)

* 9 different isolates

**ELISA (78)**
- IgG:
  - In house (44)
  - Commercial kit * (18)
    - IgM (7), IgA (9)
  - RBD, S1, Spike, N, M, E and S2

* 13 different kits

**OTHERS (20)**
- Flow cytometry-based binding Ab assay
- Lateral flow immunoassay
- Fusion inhibitory assay
- ACE2 binding inhibitory assay
Neutralisation assays

A

B

NT50

100000

10000

1000

100

10

1

A-CP high (20/130) B-CS high C-CS low D-CP low E-RP low S, high N F-RP High G-IS H-RP Neg I-RP Low J-RP Mid

IU/mL

100000

10000

1000

100

10

1

A-CP high (20/130) B-CS high C-CS low D-CP low E-RP low S, high N F-RP High G-IS H-RP Neg I-RP Low J-RP Mid
Neutralisation assays harmonisation

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<td>reported</td>
<td>249</td>
<td>179</td>
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<td>116</td>
<td>231</td>
<td>281</td>
<td>241</td>
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<td>161</td>
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<td>95</td>
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<td>250</td>
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<td>67</td>
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<td>115</td>
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<td>218</td>
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<td>-</td>
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<td>115</td>
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<tr>
<td>reported</td>
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<td>44%</td>
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<td>50%</td>
<td>46%</td>
<td>26%</td>
<td>22%</td>
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<td>70%</td>
<td>46%</td>
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<td>74%</td>
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<td>56%</td>
<td>65%</td>
<td>85%</td>
<td>-</td>
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<td>55%</td>
<td>81%</td>
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<tr>
<td>Relative to sample F</td>
<td>63%</td>
<td>70%</td>
<td>n/a</td>
<td>44%</td>
<td>58%</td>
<td>-</td>
<td>85%</td>
<td>n/a</td>
<td>50%</td>
<td>69%</td>
</tr>
<tr>
<td>UQ/LQ</td>
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<td></td>
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<tr>
<td>reported</td>
<td>4.039</td>
<td>3.759</td>
<td>n/a</td>
<td>4.118</td>
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<td>8.177</td>
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<td>Relative to sample G</td>
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<td>1.604</td>
<td>n/a</td>
<td>4.537</td>
<td>2.690</td>
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</table>
Binding antibodies-ELISA

Reported

Relative

IU/mL

In house

Commercial
Harmonisation ELISA titres

<table>
<thead>
<tr>
<th></th>
<th>A-20/130, CP high</th>
<th>B-CS high</th>
<th>C-CS low</th>
<th>D-CP low</th>
<th>E-RP low</th>
<th>F-RP high</th>
<th>G- IS</th>
<th>H-RP neg</th>
<th>I-RP low</th>
<th>J-RF mid</th>
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<tr>
<td>GM</td>
<td>reported</td>
<td>826</td>
<td>485</td>
<td>153</td>
<td>63</td>
<td>131</td>
<td>1217</td>
<td>1411</td>
<td>79</td>
<td>83</td>
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<tr>
<td></td>
<td>relative to G*</td>
<td>550</td>
<td>316</td>
<td>9</td>
<td>10</td>
<td>83</td>
<td>790</td>
<td>-</td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>%GCV</td>
<td>reported</td>
<td>1842</td>
<td>1437</td>
<td>307</td>
<td>604</td>
<td>1171</td>
<td>1557</td>
<td>1698</td>
<td>545</td>
<td>913</td>
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<td>relative to G</td>
<td>121</td>
<td>72</td>
<td>96</td>
<td>151</td>
<td>107</td>
<td>30</td>
<td>-</td>
<td>811</td>
<td>134</td>
</tr>
<tr>
<td>Lab GM: Med &lt;2</td>
<td>Reported</td>
<td>20%</td>
<td>24%</td>
<td>50%</td>
<td>52%</td>
<td>26%</td>
<td>26%</td>
<td>31%</td>
<td>25%</td>
<td>28%</td>
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<tr>
<td></td>
<td>relative to G</td>
<td>77%</td>
<td>79%</td>
<td>75%</td>
<td>81%</td>
<td>63%</td>
<td>98%</td>
<td>-</td>
<td>67%</td>
<td>69%</td>
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<tr>
<td>UQ/LQ</td>
<td>Reported</td>
<td>150.37</td>
<td>101.99</td>
<td>2.95</td>
<td>3.66</td>
<td>67</td>
<td>30.53</td>
<td>45.02</td>
<td>16.56</td>
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<td></td>
<td>relative to G</td>
<td>2.01</td>
<td>2.03</td>
<td>2.27</td>
<td>1.41</td>
<td>3.04</td>
<td>1.39</td>
<td>-</td>
<td>7.34</td>
<td>1.94</td>
</tr>
</tbody>
</table>

*with an arbitrary value of 1000 IU/mL
Reference Panel neutralising antibodies
Reference Panel binding antibodies

RBD

S1
Reference Panel binding antibodies-II

Spike

titer (different units)

F- high
J-mid
E-low, high N
I- low
H- neg
Reference Panel binding antibodies-III

N

others

<table>
<thead>
<tr>
<th>Titer (different units)</th>
<th>24e</th>
<th>24b</th>
<th>34d</th>
<th>22c</th>
<th>22d</th>
<th>33c</th>
<th>4c</th>
<th>20c</th>
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<tbody>
<tr>
<td>S2</td>
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<td>E</td>
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<tr>
<td>Total lysate</td>
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<tr>
<td>Surrog neut</td>
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</tbody>
</table>
Other assays

Lateral flow immunoassay:
- IgG results provided same ranking of samples as ELISA;
- IgM detected in at least sample A, F, G, and J

Binding antibody detection by Flow cytometry analysis:
- IgG results provided same ranking of samples as ELISA;
- IgM detected in sample A, F and G against RBD, S1, Spike and N, and in sample E, I and J for Spike (antibody against S2 and E detected in all samples including the negative);
- IgA (flow cytometry only) detected in sample A, F, G for S1, S2, Spike and N, and in sample J for S2 and Spike, sample E for S2 only

Inhibition assays:
- Results provided same ranking of samples as neutralisation assays, with F>G;
- Cell fusion inhibition assay detected all positive plasma samples, but not the serum samples
- hACE2 binding inhibition assay only detected the high and mid titre samples
Summary

- Candidate IS sample G was evaluated in 125 methods including ELISA and neut assay.
- Candidate IS was scored as one of the top three highest titre samples in every assay.
- Expression of the titre as relative to the candidate IS reduced inter-laboratory variation in both neutralisation assay and IgG-based ELISA.
- The harmonization was more pronounced in the ELISA methods possibly because original results are reported in different units.
- The candidate IS was tested for IgA and IgM and found positive against RBD, S1, Spike and N.
- The candidate Reference Panel samples were ranked similarly in almost all the assays used with very few exceptions.

<table>
<thead>
<tr>
<th>Titer* (IU/mL)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
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</thead>
<tbody>
<tr>
<td>Neutralising Ab</td>
<td>1299</td>
<td>126</td>
<td>-</td>
<td>26</td>
<td>95</td>
<td>1473</td>
<td>1000</td>
<td>-</td>
<td>44</td>
<td>216</td>
</tr>
<tr>
<td>Binding antibody</td>
<td>550</td>
<td>316</td>
<td>9</td>
<td>10</td>
<td>83</td>
<td>790</td>
<td>1000</td>
<td>-</td>
<td>39</td>
<td>241</td>
</tr>
</tbody>
</table>
First WHO IS for SARS-CoV-2 immunoglobulin (20/136)

Assigned potency of 250 IU/ampoule for neutralising antibody activity

Approximately 3000 ampoules available for distribution

Recommended storage -20°C, and suitably stable for shipping at ambient temperature

Also, recommended as reference reagent for calibration of assays detecting binding antibody

1000 ELISA unit (EU)/mL for specific target

NIBSC Research reagent 20/130

1300 IU/mL neutralising antibody titre;
502 EU/mL anti-RBD IgG
588 EU/mL anti-S1 IgG
476 EU/mL anti-Spike IgG
747 EU/mL anti-N IgG
WHO Reference Panel (20/268)

Reference Panel will comprise:

- sample F, High (NIBSC code 20/150)
- sample J, Mid (NIBSC code 20/148)
- sample E, low S, high N (NIBSC code 20/144)
- sample I, low (NIBSC code 20/140)
- sample H, negative (NIBSC code 20/142).

- No unitage will be proposed for the Reference panel
- Approximately 2500 panels are available for distribution
  Recommended storage -20°C, and suitable stable for shipping at ambient temperature
**Acknowledgements**

**NIBSC –EV group**
- Emma Bentley
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**COLLABORATIVE STUDY PARTICIPANTS**