SARS-CoV-2 Serology/Antibody testing

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Coronavirus Standards Working Group

https://coronavirus.jhu.edu/map.html
Adaptive immune response

Input (RNA)

Output

https://www.immunopaedia.org.za/immunology/advanced/5-mucosal-immunity-immunopathology/
[Reproduced with permission from Bellanti JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012]
Separation of Systemic and Mucosal Immune Monitoring

Types and Characteristics of Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Type</th>
<th>Characteristics</th>
</tr>
</thead>
</table>
| IgG      |      | • ~75% of systemic antibody in circulatory system.  
           |      | • Minor fraction of Ab in mucosal tissue. |
| IgM      |      | • Produced first upon antigen invasion. Increases transiently. |
| sIgA     |      | • 80+% of antibody in mucosal tissues.  
           |      | • Also expressed systemically at lower levels. |
| IgA      |      |                |

Main Location in the Body

- IgG
- IgM
- IgA

Figure modified from MBL
ELISA, Enzyme-Linked Immunosorbent Assay

Example Serial Dilution

User Inputs:
Total Mixing Volume = 150
Number of Dilutions = 11
Dilution Factor = 3

Opentrons example protocol
SARS-CoV-2 Structure and Proteome

Predicted ORFs in SARS-CoV-2 Genome (~29.8 kb)

- 5' UTR
- pp1ab
- pp1a
- nsp1  nsp6  nsp12
- nsp2  nsp7  nsp13
- nsp3  nsp8  nsp14
- nsp4  nsp9  nsp15
- nsp5  nsp10 nsp16

- 27 proteins
  - 4 structure proteins
  - 8 accessory proteins
  - 15 nonstructural proteins (nsp)

- Spike (S1 & S2)
- Nucleocapsid (N)
- Membrane (M)
- Envelope (E)
- ssRNA (+ sense, ~30kb in length)

Receptor binding domain (RBD)
Angiotensin converting enzyme 2 (ACE2)
Longitudinal profile of IgG, IgM, and IgA antibodies to SARS-CoV nucleocapsid protein in patients with pneumonia due to SARS-CoV.
SARS-CoV-2 Antibody Response

Figure 1

Time Courses of RNA and Antibody Tests

Additional info: Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients, Yu et al.
<table>
<thead>
<tr>
<th></th>
<th><strong>RNA</strong></th>
<th><strong>Antibody</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial</strong></td>
<td>Early, at symptom onset, variable False Negative rate</td>
<td>Mid to long term, day 7+, high early False Negative rate. Low but variable FN rate by day 15</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>Tapers off, reduced signal by day 10</td>
<td>IgM and IgA, moderate duration IgG, longer term ??</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>Ideal, lowest FP, FN</td>
<td>Ideal, lowest FP, FN</td>
</tr>
<tr>
<td><strong>Saliva</strong></td>
<td>Moderate to good, higher FN</td>
<td>Poor to Moderate, higher FN</td>
</tr>
<tr>
<td><strong>Nasal</strong></td>
<td>Moderate to good, higher FN</td>
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Angiotensin converting enzyme 2 (ACE2)
Receptor binding domain (RBD)
Developing Antibody/Serology Standards - Core

- Current targets (proven for SARS-CoV)
  - Spike protein (S protein)*
  - Spike receptor binding domain (RBD)*
  - Other portions of Spike: S1, S2
  - Nucleocapsid protein (N protein
    - Most sensitive, but noisier than Spike (SARS, Jiang et al)

- Current discordance, ELISA/IgG
  - Zhao et al showed 100% of patients with later-stage samples showed IgG+IgM seroconversion (Spike-RBD)
  - But Wu et al showed that ~30% of convalescent patients have very low/no titers of neutralizing IgG antibody (Spike RBD, S1, S2)

Amanat et al. A serological assay to detect SARS-CoV-2 seroconversion in humans
26 proteins + S1 + S2 + RBD

Codon optimization
Gene synthesis and cloned to pET32a/pGEX-4T-1
Protein expression and purification

Collect recombinant proteins from other sources

Protein microarray printing
Protein microarray quality control

Serum collection from recovered person

Profiling of SARS-CoV-2 specific serum IgG & IgM on the microarray

Jiang et al
Jiang et al.
Developing Antibody/Serology Standards - more...

- Epitope mapping
  - Proteome - proteins, low resolution
    - Cross-reactivity to other viruses/pre-existing patient antibodies
  - Proteome/epitopes - Hi resolution; peptide libraries/arrays
    - Convalescent protective antibody epitope mapping
    - Linear epitope testing/target validation (e.g. Spike-RBD epitopes not yet found in convalescent sera; possibly structural rather than linear epitopes)
    - Vaccine epitope selection
- And more...?
Summary

- RNA-based test is best to detect early infection
- Antibody based tests are essential as people recover
  - Immunity passports
- Technologies:
  - ELISA
  - Lateral flow assays (Cellex) including testing at home
  - Protein and peptide arrays
  - Immunome and PBMC transcriptomics + machine learning
- Open research questions
  - Best protein or peptide targets? S, S1, S2, N, portions?
    - Sensitivity and specificity
    - False positive and negative rates
  - Preexisting coronavirus antibody cross reactivity
- Aspirations: Standards for high quality antibody test of easily collected sample (saliva, nasal or oral swab, etc.)
Thanks and be safe!