Challenges in COVID-19 Laboratory Testing
Presented by Dr. Carmen Charlton & Dr. Nathan Zelyas

Moderators
Sarah Forgie  MD, MEd, FRCPC
President
Association of Medical Microbiology and Infectious Disease (AMMI) Canada
Guillaume Poliquin, MD, PhD
A/Scientific Director General
National Microbiology Laboratory
Public Health Agency of Canada

All attendees will enter the meeting with their mic muted and will be unable to turn on their video.
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To ask a question:
- Click the Q&A button at the bottom of your screen
- Please type your question in the Q&A box (not in the chat box)
- At the end of the presentation questions will be answered live
Dr. Nathan Zelyas is a Medical Microbiologist at Alberta Precision Laboratories (APL) – Public Health and an Assistant Clinical Professor in the Department of Laboratory Medicine & Pathology at the University of Alberta. At APL – Public Health, his roles include Program Leader for respiratory virus testing and transplant virology. During the COVID-19 pandemic, Dr. Zelyas has supported the implementation of molecular diagnostic testing for SARS-CoV-2 infections in Alberta. He is also the Program Director of the Medical Microbiology residency training program at the University of Alberta.
Dr. Carmen Charlton is a Clinical Microbiologist at the Alberta Public Health Laboratory and an Assistant Professor in the Department of Laboratory Medicine & Pathology at the University of Alberta. She obtained her Ph.D. in biochemistry and biomedical sciences from McMaster University in 2010 and completed her clinical microbiology training at the University of California, Los Angeles (UCLA), in 2013. Dr. Charlton is the Virology Program Leader for HIV, Hepatitis, Prenatal and Immunity Screening, and COVID Serology at the Alberta Public Health Laboratory. She is Past-President of the Canadian Association for Clinical Microbiology and Infectious Disease (CACMID), served on the American Society for Microbiology’s (ASM) Practical Guidance for Clinical Microbiology Committee, and is a current member of the Canadian Public Health Laboratory Network (CPHLN) Serology Working Group, and the National COVID serology Testing Task Force.
Objectives

- Review molecular and serological testing for COVID-19

- Discuss common questions and controversies in COVID-19 laboratory testing
CHALLENGES IN COVID-19 DIAGNOSTICS

Nathan Zelyas MD MSc FRCPC D(ABMM)
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Public Health Laboratory (ProvLab)
Assistant Clinical Professor Univ of Alberta
DISCLOSURES

• I have no financial disclosures or conflicts of interest with the presented material in this presentation
SARS-COV-2 AND COVID-19

• A global pandemic that first gained attention Dec 31, 2019
MOLECULAR DIAGNOSTICS FOR COVID-19

• Nucleic acid detection of SARS-CoV-2 is the method of choice for diagnosing COVID-19

• Laboratory-developed tests (LDTs) had to be developed first

• Commercial kits eventually became available since they required Health Canada authorization for use
MOLECULAR TEST AVAILABILITY

Jan 2020
Feb 2020
Mar 2020
Apr 2020
May 2020
Jun 2020

Lab-developed tests

Health Canada-authorized tests

TaqPathTM
cobas
Xpert
Abbott
LYRA
Panther Fusion
NxTAG
1copy

DiaPlexQ
PerkinElmer
Allplex
Simplexa
BD
GeneFinder

BioFire
BGI
Qiastat-DX

Aries
Biomeeme
COVID-19 MOLECULAR TESTING IN CANADA

Tests performed in Canada

Tests in a day

Cumulative number of tests

Canada

Count of people tested for COVID-19

Note: Provincial/territorial (PT) data reported on their websites should be used if there are discrepancies. This can be due to lags, differing reporting cut-offs, or changes in lab testing criteria. For PTs that report the number of tests completed, a formula is used to estimate the number of individuals tested.
NUCLEIC ACID TEST CHARACTERISTICS

- Accuracy: percentage of “true” results measured against a standard
- Reproducibility: variability in repeated testing of the same specimen
- Limit of detection: copies or virus titre per specified volume
- Cross-reactivity: ability to discriminate against similar pathogens
- Clinical sensitivity: ability to accurately rule in COVID-19
- Clinical specificity: ability to accurately rule out COVID-19
FALSE-NEGATIVES IN COVID-19 TESTING

- False negative COVID-19 molecular testing has been reported
- May result in inappropriate relaxation of measures to reduce transmission

**Table 1.** Age Distribution of COVID-19 Cases Identified Through RT-PCR Test for SARS-COV-2 on Upper Respiratory Specimens Collected Through Nasal Swabs, University Hospital of Udine, Italy, Between March 1, 2020, and April 12, 2020

<table>
<thead>
<tr>
<th>Age Group</th>
<th>COVID-19 Cases</th>
<th>Cases With at Least 2 Exams After the Positive Test, No. (%)</th>
<th>Cases with False-Negative Result of All With at Least 2 Additional Exams, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–14</td>
<td>11</td>
<td>7 (63.6)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>15–44</td>
<td>207</td>
<td>138 (66.7)</td>
<td>26 (13.8)</td>
</tr>
<tr>
<td>45–64</td>
<td>280</td>
<td>187 (66.8)</td>
<td>37 (13.8)</td>
</tr>
<tr>
<td>65–74</td>
<td>120</td>
<td>58 (48.3)</td>
<td>13 (22.4)</td>
</tr>
<tr>
<td>75–89</td>
<td>162</td>
<td>37 (22.8)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>≥90</td>
<td>80</td>
<td>6 (7.5)</td>
<td>2 (33.3)</td>
</tr>
</tbody>
</table>

Valent et al. 2020 Infec Control & Hosp Epi
WHAT FACTORS CONTRIBUTE TO FALSE-NEGATIVES?

• Timeline of illness and virus shedding
• Specimen issues
• Assay performance and design
• Large NY study looking at lab data of repeat COVID-19 PCRs
• 22,338 patients tested
• Among 2,413 initially negative patients, 18.6% became positive on repeat testing on subsequent days

Table 2: Distribution of repeat tests per day after a first day result of "Not Detected" or "Indeterminate" (A) or after a first day result of "Detected" (B). Percentages in parenthesis reflect the proportion of each result relative to the total tests per day.

<table>
<thead>
<tr>
<th>Day of testing</th>
<th>Not Detected</th>
<th>Indeterminate</th>
<th>Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1995 (97.4%)</td>
<td>54 (2.5%)</td>
<td>0 (0.0%)</td>
<td>2049</td>
</tr>
<tr>
<td>2</td>
<td>556 (80.1%)</td>
<td>39 (5.6%)</td>
<td>99 (14.3%)</td>
<td>694</td>
</tr>
<tr>
<td>3-6</td>
<td>601 (87.0%)</td>
<td>7 (1.0%)</td>
<td>83 (2.0%)</td>
<td>691</td>
</tr>
<tr>
<td>7-9</td>
<td>327 (89.6%)</td>
<td>6 (1.5%)</td>
<td>32 (8.8%)</td>
<td>365</td>
</tr>
<tr>
<td>10-15</td>
<td>469 (87.0%)</td>
<td>6 (1.1%)</td>
<td>64 (11.9%)</td>
<td>539</td>
</tr>
<tr>
<td>&gt; 16</td>
<td>565 (86.3%)</td>
<td>6 (0.9%)</td>
<td>84 (12.8%)</td>
<td>655</td>
</tr>
</tbody>
</table>

• Literature review and pooled analysis of studies providing molecular test performance data
• Included 7 studies to estimate false-negative rate by day since exposure and symptom onset
• FNR estimated at 38% on day of symptom onset, 20% on day 3, and 66% on day 16
• Limited by study heterogeneity
## SPECIMEN ISSUES AND COVID-19 POSITIVITY

- NP swab vs throat swab vs nasal swab vs lower tract sample
- Transport media and temperature
- Specimen quality

### Table 1. Stability of SARS-CoV-2 RNA detected by the Quest EUA rRT-PCR

<table>
<thead>
<tr>
<th>Media/sample type</th>
<th>Detector</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCM</td>
<td>N1</td>
<td>31.8 (0.2)</td>
<td>31.6 (0.4)</td>
<td>31.6 (0.3)</td>
<td>31.6 (0.4)</td>
<td>31.5 (0.8)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>31.3 (0.4)</td>
<td>30.8 (0.2)</td>
<td>30.8 (0.5)</td>
<td>30.8 (0.4)</td>
<td>30.7 (0.7)</td>
</tr>
<tr>
<td>UTM™-R</td>
<td>N1</td>
<td>31.8 (0.3)</td>
<td>31.6 (0.3)</td>
<td>31.3 (0.2)</td>
<td>31.9 (0.4)</td>
<td>33.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>31.2 (0.4)</td>
<td>30.8 (0.3)</td>
<td>31.0 (0.2)</td>
<td>31.1 (0.3)</td>
<td>32.2 (0.4)</td>
</tr>
<tr>
<td>ESwab™</td>
<td>N1</td>
<td>31.7 (0.4)</td>
<td>32.0 (0.4)</td>
<td>31.9 (0.5)</td>
<td>31.9 (0.4)</td>
<td>32.6 (1.0)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>31.3 (0.3)</td>
<td>31.1 (0.2)</td>
<td>30.9 (0.3)</td>
<td>31.0 (0.3)</td>
<td>31.6 (0.7)</td>
</tr>
<tr>
<td>M4</td>
<td>N1</td>
<td>31.8 (0.2)</td>
<td>32.1 (0.4)</td>
<td>31.5 (0.2)</td>
<td>32.4 (0.5)</td>
<td>32.6 (0.6)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>31.3 (0.4)</td>
<td>31.2 (0.4)</td>
<td>31.0 (0.1)</td>
<td>31.2 (0.2)</td>
<td>31.6 (0.4)</td>
</tr>
<tr>
<td>Saline</td>
<td>N1</td>
<td>25.2 (0.7)</td>
<td>29.9 (0.3)</td>
<td>30.3 (0.1)</td>
<td>30.7 (0.3)</td>
<td>31.1 (0.3)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>28.4 (0.6)</td>
<td>29.1 (0.2)</td>
<td>29.5 (0.1)</td>
<td>29.9 (0.5)</td>
<td>30.0 (0.2)</td>
</tr>
<tr>
<td>BAL</td>
<td>N1</td>
<td>31.8 (0.2)</td>
<td>31.5 (0.2)</td>
<td>32.2 (0.5)</td>
<td>31.4 (0.3)</td>
<td>32.4 (0.4)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>31.1 (0.2)</td>
<td>30.8 (0.2)</td>
<td>31.2 (0.4)</td>
<td>30.6 (0.3)</td>
<td>31.2 (0.4)</td>
</tr>
<tr>
<td>Sputum</td>
<td>N1</td>
<td>31.4 (0.5)</td>
<td>31.8 (0.6)</td>
<td>31.1 (0.4)</td>
<td>31.8 (0.4)</td>
<td>32.1 (0.3)</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>30.8 (0.6)</td>
<td>31.1 (0.7)</td>
<td>31.1 (0.5)</td>
<td>30.8 (0.3)</td>
<td>31.1 (0.3)</td>
</tr>
</tbody>
</table>

Rogers et al. 2020 JCM

### Inappropriate Nasopharyngeal Sampling for SARS-CoV-2 Detection Is a Relevant Cause of False-Negative Reports

2020 Otolaryngol Head Neck Surg

Antonio Piria, MD, Davide Rizzo, MD, PhD, Sergio Uzzau, MD, Giacomo De Ruvi, MD, Salvatore Rubino, MD, and Francesco Bussu, MD, PhD1,4

- Small series of 4 patients with septal deviation with false-negative COVID PCRs due to poor sampling
- Positive results obtained by ENT surgeons swabbing

- Suspected false-negative samples tended to have lower concentrations of human DNA

Kinloch et al. 2020 JID
ASSAY PERFORMANCE AND DESIGN

• Inappropriately designed primer/probe sequences

• Inadequate nucleic acid extraction

• Inadequate controls to rule out inhibition
FALSE-NEGATIVES HAPPEN

- Important to recognize the impact of pretest probability
- Important to mitigate risk of potential false-negatives
BUT WHAT ABOUT FALSE-POSITIVES?

• Generally more confusing than false-negative results
• Less discussed in the literature but can be impactful
• Causes: non-specific primer/probe design, contamination
• Suspect when there is an absence of exposure, inconsistent clinical findings, and negative testing before and soon after initial positive
WHAT ARE CT VALUES ANYWAYS?

• Real-time PCR measures fluorescence as the reaction proceeds

• As more PCR product is made during a positive reaction, more fluorescence is produced

• A cut-off of fluorescence is determined for each assay to define a positive result

• The cycle number needed to reach that cut-off is called the cycle threshold (CT) value

• The more starting nucleic acid is present, the faster the fluorescence reaches the cut-off and the lower the CT value

• The less starting nucleic acid is present, the more time it takes to reach the cut-off and the higher the CT value
CT VALUES AS THEY RELATE TO COVID-19

- CT values are a rough estimate of viral load
- It has been hypothesized that they may indicate infectivity
CT VALUES: CAUTION

- Not interchangeable between assays
- Not standardized viral loads
- Not homogeneous samples

- The inverse relationship between virus content and Ct value can be confusing
- Ct values are essentially raw data
ONGOING DIRECTIONS FOR MOLECULAR TESTING FOR COVID-19

- PCR assay characteristics in asymptomatic patients
- Multiplexing SARS-CoV-2 and other pathogens (especially influenza)
- Sample pooling
- Saliva and self-collections
- Determination of the true clinical sensitivity and specificity of PCR (serology may help in this regard)
COVID SEROLOGY

WHAT IS IT GOOD FOR?

Carmen Charlton PhD D(ABMM) FCCM
Clinical Microbiologist
Public Health Laboratory (ProvLab)
Assistant Professor Univ of Alberta
DISCLOSURES

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COVID-19 SEROLOGY

• Detection of antibodies specific to SARS-CoV-2 infection in blood, serum or plasma

• Limited role in acute COVID-19 diagnosis
  • Minimum of 7 to 14 days after symptom onset to develop a reliable and measurable Ab response

• Antibodies may not be detected at all (particularly in mild or asymptomatic cases)

• Unknown role in immunity or longevity of protection
COVID-19 SEROLOGY TIMELINE

Before symptom onset
- Detection unlikely

After symptom onset
- PCR - Likely positive
- PCR - Likely negative

Antibody detection

SARS-CoV-2 exposure

Week -2  |  Week -1  |  Week 1  |  Week 2  |  Week 3  |  Week 4  |  Week 5  |  Week 6
---------|----------|----------|----------|----------|----------|----------|----------
Nasopharyngeal swab PCR | Bronchoalveolar lavage/sputum PCR | IgM antibody
Virus isolation from respiratory tract | Stool PCR | IgG antibody

Wang W et al. 2020 JAMA
http://cnx.org/content/col11496/1.6/
### How well can antibodies be detected?

<table>
<thead>
<tr>
<th>Assay</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>Equ</td>
<td>Pos</td>
<td>Sens</td>
</tr>
<tr>
<td>0-14 days</td>
<td>6</td>
<td>0</td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>15-21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All time points</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioRad</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-14 days</td>
<td>9</td>
<td>0</td>
<td>12</td>
<td>57</td>
</tr>
<tr>
<td>15-21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All time points</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diasorin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-14 days</td>
<td>11</td>
<td>1</td>
<td>9</td>
<td>43</td>
</tr>
<tr>
<td>15-21 days</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&gt;21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All time points</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Euroimmun</td>
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<td></td>
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<td>0-14 days</td>
<td>7</td>
<td>0</td>
<td>14</td>
<td>67</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>&gt;21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All time points</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Roche</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-14 days</td>
<td>7</td>
<td>0</td>
<td>14</td>
<td>67</td>
</tr>
<tr>
<td>15-21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All time points</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Sensitivity and specificity values are provided for each assay and time point, indicating the percentage of samples correctly identified as positive or negative for antibodies. The CI values indicate the confidence intervals for these estimates. The table includes data from various assays (Abbott, Affinity, BioRad, Diasorin, Euroimmun, Roche) across different time points (0-14 days, 15-21 days, >21 days) and for both positive and negative samples collected pre-November 2019.

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**Charlton et al. 2020 Journal of Clinical Microbiology**
### WHAT ABOUT POINT OF CARE TESTING?

<table>
<thead>
<tr>
<th>Assay</th>
<th>0-14 days</th>
<th>15-21 days</th>
<th>&gt;21 days</th>
<th>All time points</th>
<th>Negative Samples (serum collected pre Nov 2019)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg</td>
<td>Equ</td>
<td>Pos</td>
<td>Sens</td>
<td>CI</td>
</tr>
<tr>
<td>BTNX</td>
<td>IgM</td>
<td>7</td>
<td>5</td>
<td>40</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>8</td>
<td>1</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Overall IgM/IgG</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>65</td>
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<tr>
<td>Biolistics</td>
<td>IgM</td>
<td>14</td>
<td>2</td>
<td>4</td>
<td>20</td>
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<tr>
<td></td>
<td>IgG</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>65</td>
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<tr>
<td></td>
<td>Overall IgM/IgG</td>
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<td>1</td>
<td>15</td>
<td>65</td>
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<tr>
<td>Deep Blue</td>
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<td>11</td>
<td>55</td>
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<tr>
<td></td>
<td>IgG</td>
<td>11</td>
<td>3</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Overall IgM/IgG</td>
<td>5</td>
<td>4</td>
<td>11</td>
<td>55</td>
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<tr>
<td>Genrui</td>
<td>IgM</td>
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<td>0</td>
<td>16</td>
<td>70</td>
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<tr>
<td></td>
<td>IgG</td>
<td>10</td>
<td>0</td>
<td>16</td>
<td>50</td>
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<tr>
<td></td>
<td>Overall IgM/IgG</td>
<td>6</td>
<td>0</td>
<td>16</td>
<td>70</td>
</tr>
<tr>
<td>Getein BioTech</td>
<td>IgM</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>11</td>
<td>0</td>
<td>8</td>
<td>42</td>
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<tr>
<td></td>
<td>Overall IgM/IgG</td>
<td>11</td>
<td>0</td>
<td>8</td>
<td>42</td>
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<tr>
<td>Innovita</td>
<td>IgM</td>
<td>9</td>
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<td>15</td>
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<td></td>
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<td>4</td>
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</tr>
<tr>
<td></td>
<td>Overall IgM/IgG</td>
<td>8</td>
<td>7</td>
<td>25</td>
<td>60</td>
</tr>
</tbody>
</table>

Charlton et al. 2020 Journal of Clinical Microbiology
CROSS-REACTION WITH OTHER RESPIRATORY VIRUSES

Main concern is other common coronaviruses
- Antibody prevalence in the population
- Homology to S and NC proteins

Cross reaction has been identified against:
- CoV-229E
- CoV-NL63
- CoV-OC43
- PIV-4
- hMPV
- Rhinovirus/enterovirus

https://www.scientificanimations.com/wiki-images/
WHAT IS NEUTRALIZING ANTIBODY?

• Not all antibodies made during infection can prevent infection
• Antibodies that prevent infection by the SARS-CoV-2 virus are called neutralizing antibodies
• Neutralization tests are considered the gold-standard for antibody detection
  • High sensitivity and specificity
  • Limited throughput
  • Highly specialized
  • Need containment facilities

Photo credit: http://www.virus-vs-antibody.com/science
WHAT IS NEUTRALIZING ANTIBODY?

• Not all antibodies made during infection can prevent infection

• Antibodies that prevent infection by the SARS-CoV-2 virus are called neutralizing antibodies

• Neutralization tests are considered the gold standard for antibody detection
  • High sensitivity and specificity
  • Limited throughput
  • Highly specialized
  • Need containment facilities
PERCENT POSITIVITY OF COVID-19 IN CANADA

Figure 1. Provincial epidemic curve of incident cases by report date, British Columbia (BC), January 15 to June 30, 2020 (N=2,916)
FREQUENTLY ASKED QUESTIONS

What does a positive serology mean?

Does a positive mean I am immune?

Can I order serology to diagnose acute COVID-19 infection?

My patient was positive, can serology be used to track infection?

Can I use serology for retrospective diagnosis?

My patient had a negative COVID-19 PCR test, but still has symptoms. Can I use serology to diagnose infection?
WHEN CAN SEROLOGY BE USEFUL?

- MIS-C
- Sero-prevalence Studies
- Others?
- COVID-toes
  - Unusual neurologic or thrombo-embolic events
THANK YOU!  Questions?
Thank You For Joining Us!

Following the webinar we ask that you please complete the survey about today's presentation.

Please be sure to register for the next CUPA-tea Webinar on August 6th.

- Topic: **COVID-19 Epidemiology: the knowns and unknowns**
- Speaker: **Dr. Dominik Mertz**

More information and registration visit the AMMI Canada website.