Welcome to those who are currently logging in to the live Zoom Conference, as well as to those watching the live stream on our Facebook and YouTube channels. We are glad to have each and every one of you with us tonight. We will begin the presentation in just a few moments.
James F. Holmes, Jr., MD, MPH
University of California
Davis Health System
SAEM President

Moderator
Richard Eric Rothman, MD, PhD
Johns Hopkins University
School of Medicine

SAEM COVID-19 Planning Chair
Leana S. Wen, MD, MSc
Visiting Professor of Health Policy and Management
George Washington University
Milken Institute School of Public Health

Emergency Physicians and Public Health Providers: The New Pandemicists
5 LESSONS FROM THE FRONTLINES
#1: DO WHAT WE CAN, NOW
#2: EVALUATE, INNOVATE & IMPROVE
#3: DRAW ON OUR TRAINING
#4: FIND INTERVENTIONS WHEN WE CAN
#5: MAKE THE INVISIBLE, VISIBLE
THANK YOU
Special Session on COVID-19: Diagnostics

Diagnosis of SARS-CoV-2: An Update

will begin at
2 pm ET/1 pm CT/12 pm MT/11 am PT
Special Session on COVID-19: Diagnostics
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Diagnosis of SARS-CoV-2: An Update
Severe Acute Respiratory Syndrome Coronavirus 2

- SARS-CoV-2: agent responsible for current outbreak of COVID-19
- Genus Betacoronavirus; subgenus Sarbecovirus
- Zoonotic virus
- First reported in a patient on 12 Dec 2019 in Wuhan, China
- Other emergent beta coronaviruses: SARS-CoV (2002); MERS-CoV (2012)
- Coronaviruses that cause common cold: NL63, 229E, HKU1, OC43
Coronavirus Phylogenetics

Betacoronavirus, lineage B

Ref: Global Initiative for Sharing All Influenza Data; accessed 18 April, 2020

• Endemic “common cold” strains NL63, 229E, HKU1, OC43
  – Second most common cause of URI and rhinoviruses
For humans, this is not our first Corona rodeo

Many of these viruses have now become endemic, but were once probably pandemic

Source: Timothy Sheahan, UNC (reprinted from Wall Street Journal, March 11, 2020)
Two other Coronaviruses of pandemic potential have emerged in the past 18 years:

- **SARS-CoV**
  - Emerged 2002 in Guangdong Province, China
  - Zoonosis: bats → civets → humans
  - Pandemic <1 year
  - >8000 cases, 900 deaths (~11% CF rate)

- **MERS-CoV**
  - Emerged 2012 in Saudi Arabia
  - Zoonosis: ? → camels → humans
  - Ongoing regional epidemic
  - ~2500 cases, >850 deaths (~34% CF rate)
Laboratory Diagnosis of SARS-CoV-2

- Serology
- Antigen detection
- Nucleic acid amplification tests (NAATs)
- Viral culture
- Antibody neutralization assays
Opportunities & Challenges of Serological Testing

• Utility
  – Strongly suspected cases negative by PCR, especially late in disease course
  – Identification of convalescent plasma donors
  – Epidemiologic studies of disease prevalence
  – Vaccine effectiveness/ vaccine qualification
  – “COVID passport” in some countries (Germany, others)

• Challenges
  – False negatives, esp. early in disease course
  – False negatives or delayed seroconversion, mild or asymptomatic infection
  – False positives. X-react with RF, other CoV infections

Serological Diagnosis of SARS-CoV-2 Infection

• Most immunoassays are based on the major immunogenic proteins: Nucleocapsid and/or Spike proteins

• Methods
  – Lab-based ELISA, FIA (most accurate)
  – Lateral -flow assay (LFA) (less accurate)

• In general, antibodies reliably detected 10+ days post symptom onset (15-17 days or more, post exposure)

Serological Diagnosis of SARS-CoV-2 Infection

**Takeaway:** ELISA has high sensitivity 10-14 days post symptom onset


Ref: Wolfel R, et al. Nature https://doi.org/10.1038/s41586-020-2196-x (2020). Published online 1 April 2020
Antibody Response—Mild and Severe Disease

Takeaway: *Mild cases showed less robust antibody response, but all patients seroconverted between 13 and 21 days after onset of symptoms*
Receptor Binding Domain or Nucleicapsid are the most common targets of Next-Gen Immunoassays

SARS-CoV-2 Specific Domain

Wrapp et al, Science 2020 Feb 19
Spike/RBD Immunoassays show some cross-reactivity with SARS CoV, but not a practical limitation.


Takeaway: S1 and RBD ELISAs specific for lineage B betacoronaviruses
Laboratory-based immunoassay shows high accuracy for detection of Nucleocapsid IgG responses, with >90% sensitivity post onset.

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<th>Minimum Specificity</th>
<th>Days From Onset</th>
<th>Threshold</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>pAUC</th>
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Rheumatoid Factor Interferes with SARS-CoV-2 IgM Tests

Takeaway: Rheumatoid Factor causes false positive IgM results, but avidity assay can differentiate between RF and SARS-CoV-2-specific IgM

UCSF Study- Rapid antibody tests are less sensitive


For rapid tests, IgM detection appears to offer a modest early detection advantage, and is associated with high false positive rates.
Interpretation of Coronavirus Serology

• IgM negative, IgG negative
  – No evidence for prior exposure to SARS-CoV-2 in months prior, but cannot exclude active infection
  – Negative IgM and IgG in the initial days after acute presentation
  – Some infected patients will have delayed seroconversion, probably depending on severity
  – PCR recommended for ruling out active infection

• IgM pos, IgG neg
  – Could indicate early infection
  – Expect higher rates of false positivity
  – PCR recommended for ruling out active infection
Rapid Antigen Tests

• Fast, relatively inexpensive
• Sensitivity? What we know from influenza:
  – LoD of Rapid influenza antigen tests ~1000-fold less than PCR
  – Diagnostic sensitivity compared to RT-PCR: 15-67%
• Recently authorized SARS CoV2 antigen test claims 85% clinical sensitivity (Sofia system, Quidel)
• Actual performance may vary depending on virus strain, some of which may be associated with higher average viral loads
Nucleic Acid Amplification Tests

- Single versus multiple genetic targets
- Broad-range targets
- Strain-specific targets
Nucleic Acid Amplification Tests

- Gold standard for diagnosis of acute COVID-19 cases
- Sensitive detection of SARS-CoV-2 RNA from URT
- LRT specimens remain RNA positive longer
- Viral cultures became negative after seroconversion
- Prolonged RNA positivity in stool, but virus never recovered by culture

Ref: Wolfel R, et al. Nature https://doi.org/10.1038/s41586-020-2196-x (2020). Published online 1 April 2020
Many EUAs of laboratory developed tests running custom RT-PCR protocols, often on high throughput systems

- Preferred approach of reference labs (+ drive-through testing)
- Batched collection, batched specimen shipping, batched testing generally results in 2-7 day TAT from time of collection

Several EUAs of conventional RT-PCR platforms that can be run at local hospital level (Roche, Abbott, Hologic, BD, Thermo-Fisher, Diasorin)

- Specimens arrive in lab individually or in batches, testing run in batches
- Shorter TAT (1-2 d) due to less transport time, depending on shift coverage for high complexity testing
- Not generally available in small to medium sized hospitals
- All generally perform at a high level (high sensitivity, high specificity)
### Sample-to-Answer NAATs

- Can be run on-demand (batch independent), potential for rapid triage, reduced exposure and PPE (high NPV needed for this)
- EUA products from Genmark, Cepheid, Abbott, compared to Hologic
- Tests varied widely in analytical and clinical performance

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<th>Molecular Assay</th>
<th>Reference Standard</th>
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<td>Positive</td>
<td>Negative</td>
<td>Kappa (κ)</td>
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<tr>
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<td>Negative</td>
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<td>0</td>
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<td></td>
<td>Negative</td>
<td>5</td>
<td>50</td>
<td></td>
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</table>

Zhen et al. [https://jcm.asm.org/content/jcm/early/2020/04/23/JCM.00783-20.full.pdf](https://jcm.asm.org/content/jcm/early/2020/04/23/JCM.00783-20.full.pdf)
On-demand NAATs vary in analytical sensitivity

<table>
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<td>N/A</td>
<td>10/10 (100%)</td>
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<td>7/10 (70%)</td>
<td>1/10 (10%)</td>
<td>1/4 (25%)</td>
<td>0/4 (0%)</td>
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Final LoD<sup>b</sup> copies/ml

100<sup>c</sup> 20,000 1,000

Clinical performance assessed on 483 upper and lower respiratory tract specimens previously analyzed by standard of care NAATs (mix of commercial platforms and LDTs)

- Positive percent agreement 99.5% (219/220)
- Negative percent agreement 95.8% (250/261), with all but three specimens resolving in favor of the Xpert after tie-breaker analysis
- 11/12 specimens called inconclusive by the NY Public health lab protocol were Xpert positive

Takeaway: Xpert performance was shown to be equivalent to reference methods
Antigen or PCR tests are only as good as the quality of the specimen.

False negative rates as high as 30% in case-defined COVID have been reported in China; Stanford false negative rate is reportedly less than 5%.

This is probably a function of swab collection technique and test sensitivity.

False negative swabs associate with lower human DNA content.

Combining sampling sites may reduce risk of false negatives (eg. OP+NP).

Suboptimal biological sampling as a probable cause of false-negative COVID-19 diagnostic Kinloch et al
https://www.medrxiv.org/content/10.1101/2020.05.05.20091728v1
Specimen Sources for NAAT

- **NPS, OPS, combined NPS/OPS, nasal, mid-turbinate, nasal wash/aspirate- which is best?**
  - In general, NP more sensitive than nasal swabs
  - In one study of a patient infected status standard of any site positive vs individual sites, NP 90%, OPS 87%, nasal swab 80% (Berenger et al, JCM)
  - Combined NP/OPS samples gave higher viral titers in GeneXpert Multicenter study

- **Sputum, tracheal aspirate, BAL all exhibit high titers**

- **Saliva?**
  - reduces biosafety risk during specimen collection, not swab dependent
  - can be self collected

Berenger et al https://www.medrxiv.org/content/10.1101/2020.05.05.20084889v1.full.pdf
Is saliva a “naturally pooled” specimen type?

Takeaway: saliva sampling may reduce the impact of sampling variability

Saliva testing: more human DNA on board

Takeaway: saliva sampling may be a convenient option for asymptomatic screening protocols

Symptomatic vs. Asymptomatic RNA detection

- UW Study: Testing of residents of LT skilled nursing facility.
- 76 residents tested
- 53 (69.7%) negative
- 23 (30.3%) positive
  - 10 (43.5%) symptomatic. 8 typical symptoms. (Cts 18.6-29.2). 2 atypical (malaise, nausea) (Cts 24.3-26.3)
  - 13 (56.5%) asymptomatic. Cts 21-9-31.0
    - 10 developed symptoms within 1 week later. Cts 15.3-37.9 (when presymptomatic)
- No significant differences between mean Ct values in 4 groups.
- Subsequent report in NEJM showed that asymptomatic cases were culture positive
Medical center employee screening

- 5,515 RT-PCRs performed as part of employee screening program
- 0.4% positivity rate overall (28/5,515)
- Median CT value 38.0
- Three individuals identified with high viral loads (CTs: 20.8, 21.6, 26.3)

First RNA positive blood donor identified

- Patient was asymptomatic in mid-March, but had been asymptomatic for at least 40 days prior to donation
- Plasma positive by RT-PCR for E and N genes
- IgG detected at the assay cutoff
- Donor will be called back at day 56 for evaluation
Can specimen pooling increase throughput?

- Stanford team looked for evidence of COVID in Santa Clara Valley prior to first reported cases.
- Tested 2,888 specimens negative for Flu/RSV in Jan/Feb 2020.
- 292 pools were screened, corresponding with 2740 nasopharyngeal samples and 148 bronchoalveolar lavage samples.
- 1 pool showed a positive E signal that was not reproducible with testing of the individual samples of that pool.
- Two pools of 10 specimens were positive, and deconvolution showed 2 positive samples showed detection of E and RdRp.
- Potential approach to asymptomatic screening?
Conclusions

• Serologic methods are rapidly evolving, but are not particularly helpful in acute patient management, since most of the result combinations need to be reflexed to molecular testing anyway.
• Laboratory-based viral serology is highly sensitive and specific, but not particularly fast, and rapid antibody tests are less sensitive and specific
• NAAT technologies are the gold standard for acute diagnosis, but vary in TAT and performance characteristics
• NAAT tests are only as good as the sample collected
• The diagnosis of COVID-19 is challenging enough outside of the respiratory virus season; we will need the right tools in place for syndromic management of febrile cases fall/winter