An Update of COVID-19 Diagnostics

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As of July 7, 2020 11,645,109 Cases

538,780 Deaths

https://coronavirus.jhu.edu/map.html
Automated Molecular Testing Platforms
(March 12-present)

16 automated integrated diagnostic tests for SARS-CoV-2 testing, 5 modular cartridge based devices (4 of which are designed to be used at POC)

- **Cepheid Xpert Xpress**
  - **45 minutes**
  - Massive global penetration due to TB usage and concessional pricing

- **bioMerieux Biofire Filmarray**
  - **1 hour**
  - Has been used internationally for acute febrile illness and respiratory infection

- **Abbott ID NOW**
  - **13 minutes**
  - Sensitivity lower with VTM
  - Direct inoculation of swab
  - Popular in US for flu
  - Isothermal amplification RdRp

- **Mesa Biotech Accula**
  - **30 minutes**
  - No international usage
CRISPR-Based Tests: SHERLOCK and DETECTR
(Specific High-sensitivity Enzymatic Reporter unlocking/DNA Endonuclease-Targeted CRISPR Trans Reporter)

Cas 12a/13a RNA-guided DNAse – collateral ssDNA cleavage after target recognition
‘One Pot’ reactions that use thermostable CRISPR enzyme (AapCas12b), LAMP, LFA

Broughton JP Nature Biotech 38;p 870-4; Ding X et al., Guo et al., Lucia et al.
https://www.medrxiv.org/content/10.1101/2020.05.04.20091231v1.full.pdf
Antigen Assays: Sofia®2 SARS Antigen FIA

- Lateral flow immunofluorescent sandwich assay – 15 minutes
- Qualitative detection of SARS-CoV-2 nucleocapsid protein antigen in nasopharyngeal or nasal swabs directly or in VTM
- Does not distinguish between SARS-CoV and SARS-CoV-2
- Clinical sensitivity 80% (47/59) compared to EUA molecular device
- Clinical specificity 100% (84/84)
- No cross-reactivity with 79 specimens containing seasonal CoVs

Many more coming, though likely to have similar sensitivities, amenable to lateral flow RDT
Sample Types

- Nasal, nasopharyngeal, mid-turbinate: $10^6$-$10^9$ copies/swab
- Throat (oropharyngeal): $10^4$-$10^8$ copies/swab
- Sputum: $10^6$-$10^{11}$ copies/ml
- Stool: $10^4$-$10^8$ copies/g
- Blood: <5% positive
- Urine: not detectable

Weissleder R et al. Science Transl Med DOI: 10.1126/scitranslmed.abc1931
RT-PCR Performance Characteristics

• Similar sensitivity across automated, molecular platforms
• Variability occurs with sample type, timing during illness, quality of sample
  • Passive drool – similar range of cycle thresholds to NP swab, 2 positive salivary results on days when NP or Bronchoalveolar swab negative
  • Saliva – “coughed up by clearing the throat”

Azzi L. J Infect https://doi.org/10.1016/j.jinf.2020.04.005
To KKW Lancet Infect Dis https://doi.org/10.1016/S1473-3099(20)30196-1
Sample Type: Saliva superior to NP swab?

46 NP, 39 saliva (repeated spitting into a urine cup 1/3 full) – more sensitive, less variability

Wyllie A. [https://www.medrxiv.org/content/10.1101/2020.04.16.20067835v1](https://www.medrxiv.org/content/10.1101/2020.04.16.20067835v1)
Duration of RT-PCR positivity

Azamfirei R unpublished
RT-PCR Positive vs Culture Positive

How does RT-PCR positivity link with culture positivity? In turn, how does that link with transmissibility?

Wölfel R Nature 2020 doi: 10.1038/s41586-020-2196-x

Cultures negative when specimen obtained later OR with lower level or RNA

Details:
Manitoba, Canada
90 specimens
NP swabs and ETT specimens
E gene RT-PCR (single target)
Vero cell culture, CPE readout @ 4days

Repeated positive after symptomatic recovery: Korean CDC analysis of ‘re-positive’ cases

https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=1
Korean CDC: Findings from investigation and analysis of re-positive cases

**SUMMARY**

- Epidemiological investigation and contact investigation of 285 re-positive cases
- 60% tested as screening, others tested for symptom onset. Of the 284 cases for which symptoms were investigated, 126 (45%) were symptomatic.
- From the 285 re-positive cases, a total of 790 contacts were identified (351=family; 439=others). From the monitoring of contacts, as of now, no case has been found that was newly confirmed from exposure during re-positive period alone.

[https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=1](https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=1)
Declare the past, diagnose the present, foretell the future

-Hippocrates
Lessons from the 1918 Influenza Epidemic

- Study of weekly pneumonia and influenza mortality data for 43 US cities (1922, 22% of current US population)
- Nonpharmaceutical interventions
  - School Closure
  - Public Gathering Bans
  - Isolation and Quarantine

Lessons from the 1918 Influenza Epidemic

Reacted early
Sustained and rigid enforcement of isolation (hospital/facility) and quarantine procedures
Staggered business hour ordinance

Lessons from the 1918 Influenza Epidemic

2 peaks due to non-sustained response

Delayed school closure
Rescinded public gathering ban
Highest cumulative excess mortality

Lessons from the 1918 Influenza Epidemic

- Sustained implementation of multiple interventions → peak death rates ~50% lower than those that did not.
- Death rates climbed if interventions were lifted too early.
- “Second waves”: inverse correlation of height of first and second peak weekly mortality rates.
  - Cities with low first wave peaks at greater risk of large second wave
  - Low first wave peak cities experiences second wave sooner: 6-9 weeks after the first peak vs 10-14 weeks for cities with higher first peak mortality rates
- No city experienced a second wave with the main non-pharmaceutical battery in place.

Michael Melia and Natasha Chida COVID Rounds Johns Hopkins CCGHE
US COVID-19 Cases
(as of July 5, 2020)

Serologic Immune Responses in Convalescent Patients

• Klein et al. 126 convalescent plasma potential donors ≥ 28 days after RT-PCR confirmed infection, IgG titers predominated (S1, full length S, S-RBD)
  • 80% with neutralizing titers
  • S-RBD IgG AUC had highest correlation with neutralizing titer AUC (0.79)
  • Male sex, older age, hospitalization with COVID-19 associated with increase antibody responses

Klein S et al. https://www.medrxiv.org/content/10.1101/2020.06.26.20139063v1
Superior doctors prevent the disease
Mediocre doctors treat the disease before evident
Inferior doctors treat the full-blown disease

-Huang Dee Nai-Chang 2600 BC
SARS-CoV-2 Superpower: Asymptomatic Presymptomatic Infection

Epidemiologic evidence

- Incubation periods for presymptomatic primary patients with distinct exposures ranged from 3-11 days
- Presymptomatic primary patients with travel history to an area with active transmission, the time from last exposure to symptom ≥2 - >9 days

Virologic Evidence

- Mean RT-PCR CT value 24.2 presymptomatic, 27.3 for asymptomatic. Viral culture positive 64% presymptomatic, none in asymptomatic (Arons et al.)
- 2 of 116 asymptomatic passengers from Wuhan to Germany were RT-PCR positive and Caco-2 cell positive

Arons M et al. NEJM DOI: 10.1056/NEJMoa2008457
Furukawa NW https://wwwnc.cdc.gov/eid/article/26/7/20-1595_article#r23
Modeling Transmission

- Total $R_0=2.0$
- Presymptomatic $R_0=0.9$
- Symptomatic $R_0=0.8$
- Environmental $R_0=0.1$

Implications

- Lower case-fatality rate
- Community interventions to slow transmission – social distance, face masks
- Increase capacity for widespread testing and contact tracing for asymptomatics

Ferretti L Science. 2020;eabb6936
Non-Pharmaceutical Interventions

<table>
<thead>
<tr>
<th>Studies and participants</th>
<th>Relative effect (95% CI)</th>
<th>Anticipated absolute effect (95% CI), eg, chance of viral infection or transmission</th>
<th>Difference (95% CI)</th>
<th>Certainty</th>
<th>What happens (standardised GRADE terminology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical distance ≥1 m vs &lt;1 m</td>
<td>Nine adjusted studies (n=7782); 29 unadjusted studies (n=10736)</td>
<td>aOR 0.18 (0.09 to 0.38); unadjusted RR 0.30 (95% CI 0.20 to 0.44)</td>
<td>Shorter distance, 12.8%; Further distance, 2.6% (1.3 to 5.3)</td>
<td>-10.2% (-11.5 to -7.5)</td>
<td>Moderate†</td>
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<tr>
<td>Face mask vs no face mask</td>
<td>Ten adjusted studies (n=2647); 29 unadjusted studies (n=10170)</td>
<td>aOR 0.15 (0.07 to 0.34); unadjusted RR 0.34 (95% CI 0.26 to 0.45)</td>
<td>No face mask, 17.4%; Face mask, 3.1% (1.5 to 6.7)</td>
<td>-14.3% (-15.9 to -10.7)</td>
<td>Low‡</td>
</tr>
<tr>
<td>Eye protection (faceshield, goggles) vs no eye protection</td>
<td>13 unadjusted studies (n=3713)</td>
<td>Unadjusted RR 0.34 (0.22 to 0.52)</td>
<td>No eye protection, 16.0%; Eye protection, 5.5% (3.6 to 8.5)</td>
<td>-10.6% (-12.5 to -7.7)</td>
<td>Low</td>
</tr>
</tbody>
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Chu DK Lancet 2020 [https://doi.org/10.1016/S0140-6736(20)31142-9](https://doi.org/10.1016/S0140-6736(20)31142-9)
Surveillance Testing Modeling: Impact of the sensitivity of the test used is minimal

https://www.medrxiv.org/content/10.1101/2020.06.22.20136309v2.full.pdf
Summary

• COVID-19 pandemic → over 11 million cases worldwide with evidence of sustained transmission particularly in the US
• Molecular (RT-PCR) and antigen tests directly detect the virus
• Relationship between RT-PCR positivity, culturable virus, and transmission need more data
• Only 80% of patients develop neutralizing immunity and the duration of protection is unknown
• Asymptomatic and presymptomatic patients account for a significant proportion of transmitted infection → diagnosing these people is key
• Social distancing, masking, and non-pharmaceutical interventions should be adopted