Key Summary of Published Article

SARS-CoV-2 PCR Testing
Pooling Specimens

How does SARS-CoV-2 PCR testing perform using pooled specimens compared to single specimens?

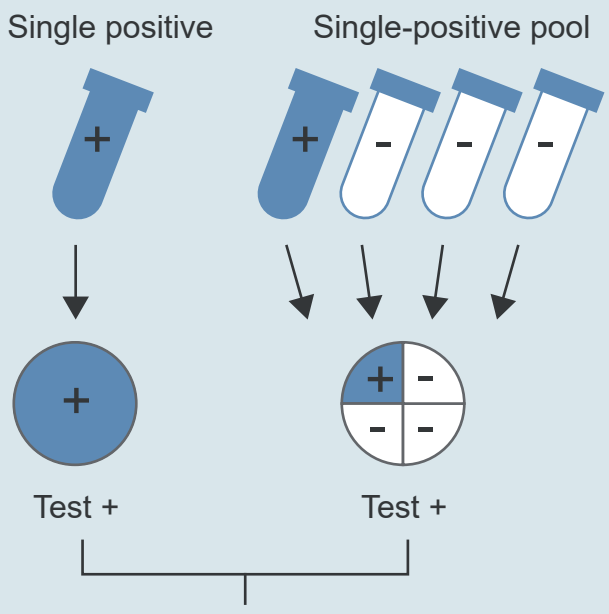
Background

Timely SARS-CoV-2 PCR testing is critical for managing the COVID-19 pandemic, but high daily testing volumes have put unprecedented demands on laboratories and supply chains. Pooling specimens could help reduce laboratory resources needed, but whether pooling affects performance of SARS-CoV-2 PCR testing was unknown.

Methods and Results

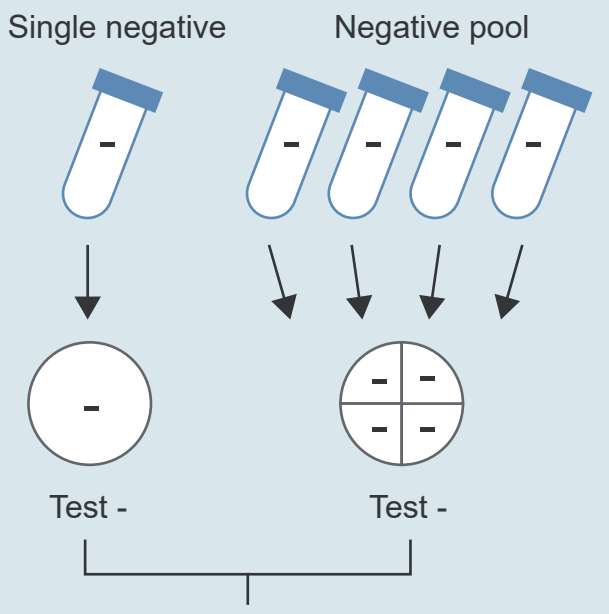
Study Design: Single vs Pooled Specimens

Sensitivity
(positive percent agreement)



100% Agreement

Specificity
(negative percent agreement)



>99% Agreement

Excellent test performance is maintained when 4 specimens are pooled, increasing SARS-CoV-2 testing capacity.

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Key Summary of Published Article

SARS-CoV-2 PCR Testing
Pooling Specimens

Article Title: Pooling of Upper Respiratory Specimens Using a SARS-CoV-2 Real Time RT-PCR Assay Authorized for Emergency Use in Low Prevalence Populations for High Throughput Testing

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Background

- High volumes of daily testing for SARS-CoV-2, which have regularly surpassed 1,000,000 in the United States,[1] combined with shortages of instrumentation and supplies, have put unprecedented demands on the laboratory and supply chains.
- By combining ("pooling") single specimens and testing them together, diagnostic laboratories can conserve resources while increasing laboratory throughput. This approach is used to screen for other viruses, such as HIV and hepatitis viruses,[2,3] and has been assessed for SARS-CoV-2 testing. However, each pooling strategy must be validated to ensure good performance.
- **Objective:** The investigators of this study report the performance of a real-time reverse-transcription PCR (RT-PCR) test authorized by the US Food and Drug Administration (FDA) for emergency use for pooled testing of upper respiratory specimens.

Methods

- Specimens were previously collected and tested for SARS-CoV-2 at Quest Diagnostics between May 2020 and July 2020.
- Results of single positive specimens were compared to those of pools with the same positive and 3 negative specimens.
  - Specimens positive for SARS-CoV-2 were selected from 3 groups representing different prevalence ranges (each group included ≥2 geographic locations): 0% to 3%, >3% to 6%, and >6% to 10%.
- Pools composed of 4 single negative specimens were also tested.
- Potential bias of the assay was assessed by comparing cycle threshold (Ct) data for single and pooled testing via regression analysis.
- Based on analysis of assay performance, previously reported results were examined to determine how many positive results may be missed by pooling.

Results

- Sensitivity (positive percent agreement) for pooled sample testing was 100% for all 3 prevalence groups.
- Specificity (negative percent agreement [NPA]) for pooled sample testing was 100% for 2 prevalence groups (>3% to 6%, >6% to 10%) and 99% for the other group (0% to 3%). Overall NPA across all prevalence groups was 99.6%.
- Regression analysis indicated pooling does not bias results (*r*: 0.96-0.99; slope = 1).
- In silico analysis of 44,217 previously reported positive results indicated only 1 would potentially have been missed by pooling (false-negative rate = 0.002%).

Conclusions

- The findings of this study demonstrate that excellent test performance is maintained when 4 upper respiratory specimens are pooled, increasing SARS-CoV-2 testing capacity.

References

1. *The Atlantic*. The COVID Tracking Project. Updated August 11, 2020. Accessed October 13, 2020. https://covidtracking.com/data/us-daily
2. Emmanuel JC, Bassett MT, Smith HJ, et al. *J Clin Pathol*. 1988;41(5):582-585. doi:10.1136/jcp.41.5.582
3. Mine H, Emura H, Miyamoto M, et al. *J Virol Methods*. 2003;112(1):145-151. doi:10.1016/S0166-0934(03)00215-5