Interlaboratory comparison exercises (ILC) of SARS-CoV-2 molecular detection methods used by veterinary diagnostic laboratories

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Introduction

- SARS-CoV-2 continues to be detected in wild and domestic animals around the world
- Veterinary diagnostic laboratories use qRT-PCR for human and animal testing
- Ensuring that laboratories have reliable SARS-CoV-2 testing methods is a critical component of the pandemic response
- Three interlaboratory comparison exercises were conducted since 2020 (Round 1, Round 2, Round 3).

Methods

Participants used routine SARS-CoV-2 detection procedures

Most assays detected the *N* gene, specifically markers N1 and N2. Other gene markers include: ORF1ab, S, or E genes

Round 1
- Detected Wuhan SARS-CoV-2 RNA in Tris-EDTA (TE) buffer or molecular transport medium (MTM)

Round 2
- Evaluated sensitivity and specificity of the methods to detect the RNA of B.1 lineage, Alpha and Beta variants in MTM with and without confounding RNA

Round 3
- Evaluated sensitivity and specificity of the methods to detect Delta and Omicron variants in canine nasal matrix with and without confounding RNA

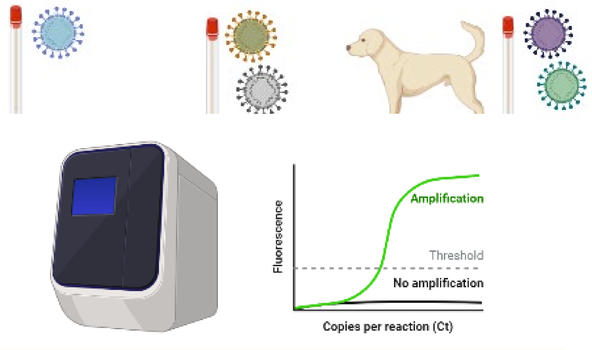

Figure 1. Methodology for SARS-CoV-2 detection by laboratories

Results

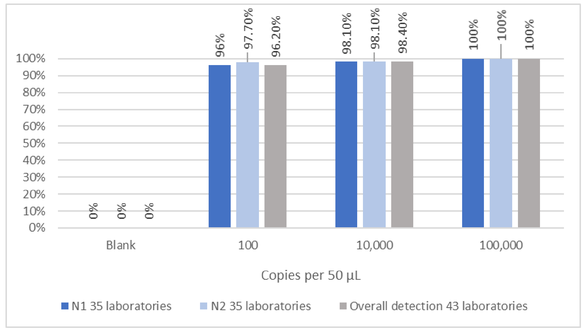

Figure 2. Rate of detection (ROD) of Wuhan for N1 and N2 markers as well as the overall detection from ILC Round 1

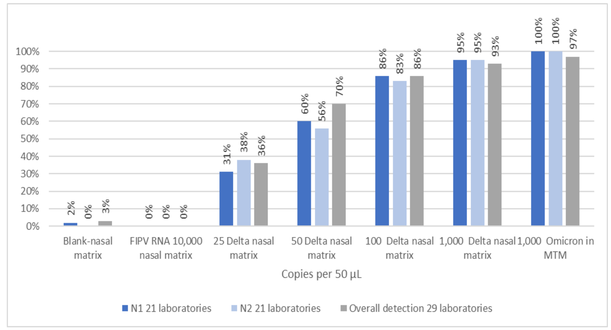

Figure 4. Rate of detection (ROD) N1 and N2 markers as well as the overall detection from ILC Round 3

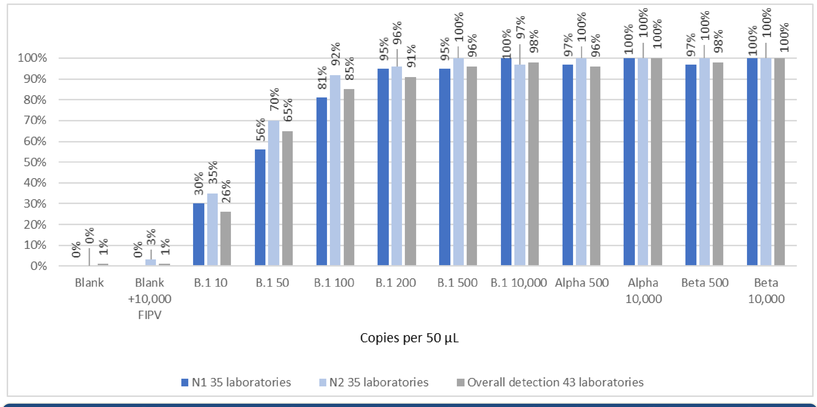

Figure 3. Rate of detection (ROD) for N1 and N2 markers as well as the overall detection from ILC Round 2

Round 1 publication link

Round 2 publication link

Round 3 publication link

Conclusions

Round 1
- 66% of laboratory methods had a Level of Detection (LOD) at ≤20 copies per PCR reaction
- Reproducibility standard deviation was higher for the RNA stored in MTM than those in TE buffer

Round 2
- 95% of laboratories detected the SARS-CoV2 RNA in MTM at ≥500 copies for all 3 variants
- For markers N1 and N2, 81% and 92% of the laboratories had LOD ≤20 copies in the assays, respectively
- Confounding RNA (FIPV) did not interfere with vast majority of assays (97%)
- Specificity of the evaluated methods was >99%

Round 3
- 93% detection for Delta virus and 97% for Omicron virus at 1,000 copies per test portion
- Specificity was 97% for blank samples and 100% for blank samples with confounding RNA (FIPV)
- Confounding RNA (FIPV) did not interfere with any of assays (100%)
- The canine nasal matrix did not significantly affect SARS-CoV-2 detection

Public Health Relevance

Veterinary Diagnostic Laboratories test both human and animal samples for SARS-CoV-2 and play a critical One Health role in assessing the impact of COVID-19.

Ensuring accurate and reliable results is critical during pandemic response.

Collaborative nature of the project ensures that numerous laboratories can assess and potentially improve their method capabilities for SARS-CoV-2 detection.

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