# Interlaboratory Comparison Exercise (ICE) of SARS-CoV-2 Molecular Detection Assays in Use by Veterinary Diagnostic Laboratories


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## Abstract


The recent mink-associated human COVID-19 cluster and the continued search for intermediate hosts and potential viral reservoirs make it clear that in outbreak response and prevention, diagnostic testing of animals is critically important. An important benefit of real-time RT-PCR assays to identify SARS-CoV-2 is that they can easily be adapted to different host species. Veterinary diagnostic laboratories (VDLs) in the United States have adapted assays from the Centers for Disease Control and Prevention or tests from other national reference laboratories to test animal samples, and many of these laboratories are also directly supporting public health efforts in various ways. The animal-adapted test methods, however, have only been evaluated using internal validation protocols. To help VDLs evaluate their SARS-CoV-2 test methods, an Interlaboratory Comparison Exercise (ICE) was designed and provided in collaboration with six organizations. Each of forty-two VDLs analyzed nineteen blind-coded RNA samples prepared in Tris-EDTA (TE) buffer and PrimeStoreTM transport medium. Results were analyzed according to the principles of International Organization for Standardization (ISO) 16140-2:2016. The performance of each laboratory method and results were qualitatively and quantitatively evaluated. Qualitative assessment suggests that 100% sensitivity was achieved for detecting the SARS-CoV-2 RNA in TE buffer, and the limit of detection (LOD) for a laboratory with median performance was close to the theoretical minimum of 3 copies per PCR reaction. This level of sensitivity is not expected in clinical samples since there would be more factors involved such as sample collection, transport and testing in the clinical matrix. Quantitative assessment implicated that reproducibility standard deviations for testing the RNA with the N1 marker were slightly lower than those with N2, and they were higher for the RNA in PrimeStoreTM medium than those in TE buffer. The experience of the analyst and the use of either a singleplex or multiplex PCR also affected the quantitative testing results. These findings demonstrate excellent results obtained by VDLs testing for SARS-CoV-2 in animals, and 22 of these laboratories have additionally be granted provisional Clinical Laboratory Improvement Amendments (CLIA) certification and are testing human samples.


## Introduction

COVID-19, caused by virus SARS-CoV-2, has rapidly spread throughout many countries including the United States since its discovery in December 2019. To better understand the scope and spread of the COVID-19 pandemic, it is very important to ensure that the nation's diagnostic laboratory have effective testing systems for human and animal specimens.

SARS-CoV-2 RNA has been detected from specimens of naturally or experimentally infected cats, dogs, ferrets, fruit bats, pigs, chickens, tigers, and rhesus monkeys. All these reports have used various equipment, reagents for RNA extraction and purification, and a variety of gene targets. The tests currently being used by US veterinary diagnostic laboratories have only been evaluated using their internal validation protocols. It is essential to design an ICE study to collect qualitative detect/undetected results from the laboratories, calculate performance scores by quantitative assessment, and allow a laboratory to evaluate its own method as well as compare its results with those of its peers.

## Objective

- To determine if participants can reliably detect SARS-CoV-2 RNA at various levels in buffer and virus transport medium;
- To comparatively evaluate results and methods from participants.

## Collaborations

- FDA- CVM-Vet-LIRN
- FDA/CFSAN/DFPST/PT
- QuoData Quality and Statistics GmbH, Germany
- IIT/IFSH (Illinois Institute of Technology/Institute for Food Safety and Health)
- USGS (United States Geological Survey)
- USDA NVSL/NAHLN
- Cornell University

## Materials & Methods

- **ICE Sample Preparation and Distribution:**
  - RNA was isolated from SARS-CoV2 virus (WA-1) propagated in Vero cells. Its copy number was determined by Standard Curves comparison with synthetic CoV RNA in a known quantity.
  - ICE Sample #1-6: RNA in TE buffer (negative, low, medium level)
  - ICE Sample #7-19: RNA in PrimeStore™ medium (negative, low, medium and high level)
  - Pre-shipment tests: by Moffett and USDA NVSL/NAHLN
  - Shipment condition: dry ice
- **Sample Analysis by provider laboratory:**

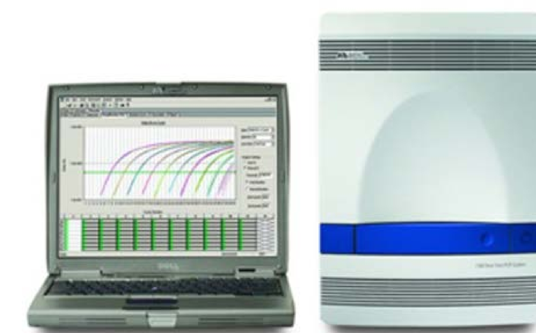
1). The RNA inoculated in Primestore samples was isolated using RNA extraction method routinely used in a VDL.

Qiagen RNeasy kit

or

MagMAX-96 viral RNA isolation kit

2). The purified RNA was reverse transcribed to cDNA and subsequently amplified with specific primers (N1 and N2) and probe for the virus N gene, by following the CDC 2019-nCoV EUA Kit method. The RT-PCR was run at Applied Biosystems 7500 Fast Real-Time PCR Instrument with version 2.3 software.



## Results

### 1. Qualitative Assessment Results

Table 1. Rate of detection depending on the level for N1 and N2 markers as well as the "overall detection"

| Level | Number of PCR replicates | Rate of detection (ROD) across laboratories | | |
|---|---|---|---|---|
| | | N1<br>*Number of laboratories included in the calculations: 35* | N2<br>*Number of laboratories included in the calculations: 35* | Overall detection<br>*Number of laboratories included in the calculations: 42* |
| Blank | 2 | 0% | 0% | 0% |
| Low* | 5 | 96% | 98% | 96% |
| Medium* | 3 | 98% | 98% | 98% |
| High* | 3 | 100% | 100% | 100% |

* Low, medium and high levels were 100, 10,000 and 100,000 copies per 50 µl of PrimeStore™ respectively.

### 2. Quantitative Assessment Results

Table 2. Influence factors for adjusted Ct values for TE samples (A) and PrimeStore™ samples (B)

(A)

| Adjusted Ct values | | 30µl sample in Tris-EDTA buffer solution | | | | | |
|---|---|---|---|---|---|---|---|
| | | N1 | N2 | N1 | | N2 | |
| | | all samples | all samples | 100 copies/5 µl | 10,000 copies/5 µl | 100 copies/5 µl | 10,000 copies/5 µl |
| Instrument used | ABI 7500 | . | . | . | -0.40 | . | . |
| Analyst routinely | yes | . | . | . | -0.29 | . | . |
| Singleplex or multiplex | singleplex | 0.37 | 0.24 | . | 1.06 | . | . |
| Extraction kit | pathogen | . | . | . | -0.23 | . | . |
| Extraction kit | viral | . | . | . | . | . | . |

(B)

| Adjusted Ct values | | 150µl sample in PrimeStore™ medium | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N1 | N2 | N1 | | | N2 | | |
| | | all samples | all samples | 100 copies/50 µl | 10,000 copies/50 µl | 100,000 copies/50 µl | 100 copies/50 µl | 10,000 copies/50 µl | 100,000 copies/50 µl |
| Instrument used | ABI 7500 | -0.09 | -0.19 | . | -1.35 | -1.17 | . | -0.45 | -0.26 |
| Analyst routinely | yes | -0.20 | . | -0.36 | -0.66 | -0.32 | . | . | . |
| Singleplex or multiplex | singleplex | 0.69 | 0.55 | 0.32 | 1.97 | 1.47 | . | 0.61 | 0.05 |
| Extraction kit | pathogen | . | . | . | . | . | . | . | . |
| Extraction kit | viral | -0.12 | -0.02 | -0.07 | -0.50 | -0.28 | . | -0.01 | . |

The notation "." means that no effect of the factor in question was observed.

**Qualitative Result Summary:**

- Over 95% of the laboratories detected the virus RNA at all levels, with 100% of the laboratories detecting the RNA in the high concentration samples.
- Percent detection was slightly greater for RNA in TE samples than those for PrimeStore™ samples.

**Quantitative Result Summary:**

- The reproducibility standard deviation = ~ 2 Ct values, with N1 slightly lower than N2.
- The reproducibility standard deviation: PrimeStore™ medium samples > TE samples.
- The N1 Ct values: analysts who routinely performed the test < analysts who do not routinely perform the test.
- The N1 Ct values: "viral" labeled extraction kits < "pathogen" extraction kits.
- The Ct values for N1 and N2: singleplex PCR >multiplex PCR.
- Mean PCR amplification efficiency = 86%-98%, with N2 more variable than N1.

## Next Steps

In future ICEs, more technically challenging samples (samples with a low viral load and with confounding RNA) or in different matrices could be chosen to simulate more complex human and animal specimens.

## Conclusions

- The ICE facilitated validation efforts by providing standardized test materials and a statistical comparison of results among peer laboratories.
- The data show that for RNA in TE buffer, 100% of samples were detected.
- The data also show that where extraction was required, the PCR methods used provided reliable results.

## Mission Relevance

By conducting this ICE, the collaborating groups across government agencies, universities and private industry made a profound contribution to protect human and animal health by demonstrating the validity and performance of current SARS-CoV-2 RNA tests during the challenging times of the COVID-19 pandemic.