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Short communication

## Real-time PCR-based SARS-CoV-2 detection in Canadian laboratories

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## ABSTRACT

With emergence of pandemic COVID-19, rapid and accurate diagnostic testing is essential. This study compared laboratory-developed tests (LDTs) used for the detection of SARS-CoV-2 in Canadian hospital and public health laboratories, and some commercially available real-time RT-PCR assays. Overall, analytical sensitivities were equivalent between LDTs and most commercially available methods.

## 1. Introduction

Coronaviruses are part of a large family of viruses that infect humans and animals, and are classified into 4 genera ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ). Human coronaviruses belong to  $\alpha$  and  $\beta$  genera. [1] Most human

coronaviruses (229E, NL63, OC43, and HKU1) are causes of the common cold, and serious outcomes are rare. Some human  $\beta$ -coronaviruses have been associated with high morbidity and mortality, such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) [1]. In

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December 2019, novel  $\beta$ -coronavirus now termed SARS-CoV-2 [2–5] emerged from China, and was associated with high morbidity and mortality in older adults or individuals with underlying medical conditions. [6–10]. Due to the severity and spread, the disease caused by SARS-CoV-2 (i.e. 2019 coronavirus disease, or COVID-19) was declared a pandemic on March 11, 2020 [6–12].

Laboratory testing for COVID-19 is an essential component of containment and mitigation strategies, as it allows the appropriate clinical management and public health interventions. [13–15] Nucleic acid amplification tests (NAAT) such as real-time reverse transcription polymerase chain reaction (rRT-PCR) are the methods of choice for SARS CoV-2 diagnostic testing. With the rapid availability of genome sequences [4,16], laboratory-developed tests (LDTs) for the detection of the SARS-CoV-2 were quickly developed. The first LDTs relied primarily on the detection of SARS-CoV-2 envelope (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid (N) genes [17,18], but more recent rRT-PCR method targets include open reading frame 1 a/b (Orf1a/b) and the gene encoding spike (S) protein. Recognizing the performance characteristics of PCR methods can vary with reagents, PCR, and instrumentation [19,20], this study compared the analytical sensitivity of LDTs used in hospital and provincial public health laboratories across Canada, as well as some commercially available NAATs that are available in Canada.

## 2. Methods

The lower limit of detection (LoD) of each method was determined by testing serial dilutions of RNA extracted from a cultured stock of SARS-CoV-2/Canada/VIDO-01/2020 [originally cultured by VIDO-Intervac at the University of Saskatchewan from a clinical specimen originating from Sunnybrook Health Sciences Centre, and propagated at the National Microbiology Laboratory (NML)]. To reduce inter-laboratory variability, specimen preparation, nucleic acid extraction and dilutions were carried out at the NML in Winnipeg (Manitoba, Canada). The viral stock was extracted using the QIAamp Viral RNA Mini kit (Qiagen Inc.), as recommended by the manufacturer. Ten-fold dilution of RNA was prepared in 10 mM Tris (Invitrogen, ThermoFisher) as a single batch. Five replicates of each dilution were shipped on dry ice to participating sites in sufficient volumes to ensure there would be only a single freeze thaw for each of the five independent experiments. Viral RNA was maintained at  $-70^{\circ}\text{C}$  until tested. The viral RNA used for the relative method comparison was quantified using a standard curve generated with *in vitro* transcribed RNA (Doc S1). All commercial assays were performed as recommended by the respective manufacturers and the various LDT were performed using reagents, instrumentation, and conditions summarized in Table 1. Results of each laboratory's tests were collated, and the LoD estimated using a Probit analysis ([https://biostats.shinyapps.io/LOD\\_probit/](https://biostats.shinyapps.io/LOD_probit/)) at a probability of 95%. The LoD of each method was expressed as  $\log_{10}$  copies/mL in the PCR reaction, with 95% confidence intervals (CI) (Table 1).

## 3. Results

Our data shows that there is a wide spectrum of instruments, reagents, PCR targets, and reaction conditions between the different diagnostic and commercial rRT-PCR methods for the identification of SARS-CoV-2 (Table 1). Despite this variability, the LoD of the various LDTs were between 3.4 and 4.5  $\log_{10}$  copies/mL. Most commercial methods were equivalent to LDTs, with only a few assays that showed reduced sensitivity (Table 1).

## 4. Discussion

When COVID-19 first emerged in Canada, method validation in provincial public health and hospital laboratories was challenged by the

lack or limited availability of control material for SARS-CoV-2, and limited data to help guide the choice of SARS-CoV-2 real-time PCR methods. Given the novelty of the virus, initial Public Health Agency of Canada (PHAC) case definitions for COVID-19 required laboratory detection of SARS-CoV-2 by real-time RT-PCR with confirmation from either sequencing, or rRT-PCR using a second genetic target. As such, many LDTs were performed in sequential algorithm, or using multiplex rRT-PCRs that enabled simultaneous detection of at least two SARS-CoV-2 targets (Table 1). Most methods were LDTs derived from those developed by the Centers for disease Control and Prevention (CDC), or from published literature. [17,18] While LDTs for COVID-19 testing were quickly implemented in Canadian laboratories, different instruments, reagents, and genetic targets were used.

Despite these differences, this study showed that the analytical sensitivity of LDTs were consistent between laboratories, and few exceptions noted in commercial assays. Most LDTs had LoDs between 3.4 and 4.5  $\log_{10}$  copies/mL, which is fairly consistent with those reported by others. [17,20] Corman et al. [17] reported analytical sensitivities for E gene at RdRp targets at 3.9 [95% CI: 2.8–9.8] and 3.6 copies/reaction [95% CI: 2.7–11.2] copies/reaction, which correlates to 2.9 [95% CI: 2.7–3.3] and 2.9 [95% CI: 2.7–3.4]  $\log_{10}$  copies/mL, respectively. In another recent publication, Chan et al. [21] reported analytical sensitivity of 11.2 [95% CI: 7.2–52.6] copies/reaction for RdRp and 21.3 [95% CI: 11.6–177.0] copies/reaction for N gene. These values represent 3.4 [95% CI: 3.2–4.0]  $\log_{10}$  copies/mL and 3.6 [95% CI: 3.4–4.5]  $\log_{10}$  copies/mL, respectively, which is consistent with values from this study. Only a limited number of methods showed reduced sensitivity. For example, unlike the E gene target in the LightMix 2019-nCoV Real-time RT-PCR kit, the RdRp and N gene targets showed reduced sensitivity. This observation had previously been noted for N gene detection. [17]

It should be noted that the most recent case definition for COVID-19 requires detection of a single genetic target for laboratory detection of SARS-CoV-2. [22] While dual- or multi-target real-time RT-PCRs were initially developed to ensure specificity, sensitivity can in some cases be enhanced when the sum of all results from individuals targets in a multiplex PCR are considered. For example, the Allplex 2019-nCoV assay considers detection in any of its three SARS-CoV-2 targets as a positive result. Individually, each target showed LoD between 3.6 and 3.8  $\log_{10}$  copies/mL, whereas a LoD of 2.6  $\log_{10}$  copies/mL was observed with the sum of all results. As detection of SARS-CoV-2 can be variable at the LoD, relying on more than one target could increase the chances of identifying the virus in specimens with low viral loads. Low viral loads can occur in asymptomatic individuals, during the early or late stages of COVID-19 disease, or they could be attributed to improper specimen collection. [13,14] Such variables could greatly affect the performance of any laboratory method, and falls outside the scope of this study.

A noted limitation for this study includes the use of an RNA-based panel to assess the LoD of the various PCR methods. As such, evaluation of automated instrumentation with a paired nucleic acid extraction and nucleic acid amplification were excluded from the study. Using serial dilutions of cultured virus, future studies are underway to compare automated methods capable of SARS-CoV-2 detection.

As pandemic COVID-19 continues to spread, laboratory testing is essential, and understanding the limits of SARS-CoV-2 detection using rRT-PCR is fundamental. This study provided the first head-to-head comparison of LDTs and commercial real-time methods used for the detection of SARS-CoV-2 in Canada. These findings could help guide the choice of molecular methods in diagnostic laboratories looking at introducing COVID-19 testing, or, given the challenges in procuring reagents during the pandemic, these data may provide a list of suitable alternatives for laboratories already offering COVID-19 testing.

**Table 1**  
Limit of detection (LoD) at a 95% probability for various real-time RT-PCR methods for the detection of SARS-CoV-2.

Laboratory	Thermocycler	RT-PCR reagents	Reaction vol. (µL)	Template vol. (µL)	Positivity cutoff (Ct value)	SARS-CoV-2 Target	LoD (log copies/mL; 95% CI)
AB (ProvLab)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	10	35	RdRp	4.243 (3.472–5.013)
						E	3.776 (3.122–4.430)
BC (BCCDC)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	35	Any <sup>3</sup>	3.776 (3.122–4.430)
						RdRp	3.591 (3.245–3.937)
BC(BCCW)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	35	E	3.579 (3.180–3.979)
						Any <sup>3</sup>	4.243 (3.472–5.013)
BC (SPH)	LightCycler 480 (Roche Diagnostics)	LightMix 2019-nCoV Real-time RT-PCR kit (Tib Molbiol) with Lightcycler Multiplex RNA Master (Roche Diagnostics)	20	5	40	RdRp	4.729 (3.683–5.774)
						E	3.901 (3.048–4.755)
BC (VGH)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	35	Any <sup>3</sup>	3.901 (3.048–4.755)
						E	3.901 (3.048–4.755)
BC (VIHA)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	35	RdRp	4.516 (3.677–5.354)
						E	4.516 (3.677–5.354)
MB (Cadhams)	CFX96TM (Bio-Rad Laboratories, Ltd.)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	36.5	Any <sup>3</sup>	4.243 (3.472–5.013)
						RdRp	4.375 (3.413–5.337)
NB (CHU-Dumont)	LightCycler 480 II (Roche Diagnostics)	Lightcycler Multiplex RNA Master (Roche Diagnostics)	20	5	40	E	3.809 (2.886–4.732)
						N1	3.901 (3.048–4.755)
NL	LightCycler 480 II (Roche Diagnostics)	Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs)	20	5	33	N2	3.776 (3.122–4.430)
						N3	3.632 (2.670–4.594)
NS (NSHA)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	35	E + N	3.901 (3.048–4.755)
						E + N2	3.776 (3.122–4.430)
ON (PHOL)	Quantstudio 5 (ThermoFisher Scientific)	TaqPath 1-Step Multiplex Master Mix (Applied Biosystems)	10	5	38	E	3.575 (3.141–4.009)
						RdRp	3.901 (3.048–4.755)
PE (QEH)	BD Max (Becton Dickinson and Company)	RealStar SARS-CoV-2 RT-PCR kit (Altona Diagnostics)	20	12.5	ND <sup>1</sup>	Any <sup>3</sup>	3.901 (3.048–4.755)
						E	4.292 (3.658–4.925)
QC	QuantStudio3 (ThermoFisher Scientific)	TaqPath 1-Step Multiplex Master Mix (Applied Biosystems)	20	5	37	S	3.437 (2.829–4.045)
						Any <sup>3</sup>	3.901 (3.048–4.755)
SK (RRPL)	ABI 7500 Fast (Applied Biosystems)	TaqMan Fast Virus-1-Step Master Mix (Life Technologies)	20	5	36	E	4.558 (3.498–5.618)
						N	3.809 (2.886–4.732)
ON (St-Joseph's Healthcare)	CFX96TM (Bio-Rad Laboratories, Ltd.)	Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs)	20	5	45	E	3.776 (3.122–4.430)
						N	4.234 (3.101–5.367)
ON (St-Joseph's Healthcare)	Rotor-Gene Q (QIAGEN Inc.)	Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs)	20	5	45	E	3.752 (3.237–4.266)
						N	3.566 (3.090–4.420)
ON (St. Michael's Hospital)	Quantstudio 5 (ThermoFisher Scientific)	RealStar SARS-CoV-2 RT-PCR kit (Altona Diagnostics)	30	10	ND <sup>1</sup>	Any <sup>3</sup>	3.078 (2.539–3.617)
						UTR	3.809 (2.886–4.732)
ON (Mt-Sinai)	CFX96TM (Bio-Rad Laboratories, Ltd.)	Allplex 2019-nCoV Assay (Seegene Inc.)	25	8	40	UTR + E	2.638 (2.068–3.209)
						UTR	3.878 (2.685–5.071)
ON (Mt-Sinai)	CFX96TM (Bio-Rad Laboratories, Ltd.)	Allplex 2019-nCoV Assay (Seegene Inc.)	25	8	40	UTR + E	3.632 (2.670–4.594)
						Any <sup>3</sup>	3.878 (2.685–5.071)
ON (St. Michael's Hospital)	Quantstudio 5 (ThermoFisher Scientific)	RealStar SARS-CoV-2 RT-PCR kit (Altona Diagnostics)	30	10	ND <sup>1</sup>	E	3.375 (2.796–3.954)
						S	3.493 (2.951–4.034)
ON (Mt-Sinai)	CFX96TM (Bio-Rad Laboratories, Ltd.)	Allplex 2019-nCoV Assay (Seegene Inc.)	25	8	40	Any <sup>3</sup>	3.078 (2.539–3.617)
						E	3.632 (2.670–4.594)
ON (Mt-Sinai)	CFX96TM (Bio-Rad Laboratories, Ltd.)	Allplex 2019-nCoV Assay (Seegene Inc.)	25	8	40	RdRp	3.809 (2.886–4.732)
						N	3.632 (2.670–4.594)
ON (Mt-Sinai)	CFX96TM (Bio-Rad Laboratories, Ltd.)	Allplex 2019-nCoV Assay (Seegene Inc.)	25	8	40	Any <sup>3</sup>	2.638 (2.068–3.209)
						E	3.375 (2.796–3.954)

(continued on next page)

**Table 1 (continued)**

Laboratory	Thermocycler	RT-PCR reagents	Reaction vol. (µL)	Template vol. (µL)	Positivity cutoff (Ct value)	SARS-CoV-2 Target	LoD (log copies/ml; 95% CI)
-	ABI 7500 Fast (Applied Biosystems)	Taqpath RT-PCR COVID-19 Kit with TaqPath 1-Step Multiplex Master Mix (Applied Biosystems)	20	5	ND <sup>1</sup>	Orfla/b N	4.243 (3.472–5.013) 4.434 (4.002–4.866)
-	ABI 7500 Fast (Applied Biosystems)	RIDA GENE SARS-CoV-2 (r-Biopharm AG)	20	5	ND <sup>1</sup>	Any <sup>3</sup>	3.776 (3.122–4.430)
-	ABI 7500 Fast (Applied Biosystems)	Lyra SARS-CoV-2 assay (Quidel Corp.)	15	5	30 <sup>2</sup>	E Orfla/b	3.901 (3.048–4.755) 4.712 (3.837–5.587)
-	ABI 7500 Fast (Applied Biosystems)	DiaPlexQ Novel Coronavirus (2019-nCoV) Detection Kit (SolGent Co., Ltd)	20	5	40	N	3.901 (3.048–4.755)
-	ABI 7500 Fast (Applied Biosystems) in Standard mode	New Coronavirus Nucleic Acid Detection Kit (Perkin-Elmer)	20	40	42	Orfla Any <sup>3</sup>	3.809 (2.886–4.732) 3.809 (2.886–4.732)
-	LightCycler 2.0 (Roche Diagnostics)	LightMix 2019-nCoV Real-time RT-PCR kit (Tib. Molbiol) with Lightcycler Multiplex RNA Master (Roche Diagnostics)	20	10	N/A	Orflab Any <sup>3</sup>	4.516 (3.677–5.354) 3.776 (3.122–4.430)
-					36	E	3.632 (2.670–4.594)
-					39	RdRp	5.591 (4.769–6.412)
-					37	N	5.523 (4.636–6.410)
-					N/A	Any <sup>3</sup>	3.632 (2.670–4.594)

<sup>1</sup> Not defined (ND).

<sup>2</sup> Amplification using Lyra SARS-CoV-2 assay (Quidel Corp.) on the ABI 7500 Fast includes 10 “blind” cycles where fluorescence is not capture, and therefore, the cutoff for positivity is set after the 30 cycles where fluorescence was captured.

<sup>3</sup> For multiplexed assays, LoD were assessed for individual targets; however, the LoD was also considered for the sum of results where any target detected would be considered a positive results. Note: Indeterminate results were characterized as positive for the Probit analysis. An indeterminate result on an rRT-PCR assay is defined as a late amplification signal, below the predetermined Ct value for positivity.

**Declaration of Competing Interest**

The authors have no conflicts to declare

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