# Rapid and Safe Detection of SARS-CoV-2 and Influenza Virus RNA using Onsite qPCR Diagnostic Testing from Clinical Specimens Collected in Molecular Transport Medium

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**Running Title:**

Cobas Liat Testing from Clinical Specimens in PrimeStore MTM

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47
48 **Abstract:**
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51 **BACKGROUND**: The ability to rapidly detect SARS-CoV-2 and influenza virus infection is
52 vital for patient care due to overlap in clinical symptoms. Roche's cobas® Liat® SARS-CoV-2
53 & Influenza A/B Nucleic Acid Test used on the cobas® Liat® was granted approval under
54 FDA's Emergency Use Authorization (EUA) for nasopharyngeal (NP) and nasal swabs
55 collected in viral/universal transport medium (VTM/UTM). However, there is a critical need
56 for media that inactivates the virus, especially when specimens are collected in decentralized
57 settings. This study aimed to investigate the use of PrimeStore Molecular Transport
58 Medium® (PS-MTM®), designed to inactivate/kill and stabilize RNA/DNA for ambient
59 transport and pre-processing of collected samples. **METHODS:** A limit of detection (LOD)
60 using serially diluted SARS-CoV-2 RNA in PS-MTM® and routine UTM was established using
61 standard qPCR. Additionally, a clinical panel of NP and oral swabs collected in PS-MTM®
62 collected during the 2020 coronavirus disease 2019 (COVID-19) pandemic were evaluated
63 on the cobas® Liat® and compared to 'gold standard' qPCR on an ABI-7500 instrument.
64 **RESULTS:** SARS-CoV-2 RNA LOD using standard qPCR was equivalent on the cobas® Liat®
65 instrument. cobas® Liat® detection from oral/NP swabs in PS-MTM® media exhibited
66 equivalent positive percent agreement (100%) and negative percent agreement (96.4%).
67 **CONCLUSION:** PS-MTM® and the Roche cobas® Liat® are compatible and complimentary
68 devices for respiratory specimen collection and rapid disease detection, respectively. PS-
69 MTM® is equivalent to standard VTM/UTM with the added benefit of safe, non-infectious
70 sample processing for near-patient testing.

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## Impact Statement

This study was the first to evaluate PrimeStore Molecular Transport Medium®, an FDA-cleared collection device, with Roche's cobas® Liat®, a point-of-care, FDA-approved diagnostic system for detection of SARS-CoV-2 and influenza A/B RNA from clinical swabs. Specimens collected in PrimeStore MTM® combined with Liat® testing provides a safe and sensitive approach to standard VTM/UTM for rapid SARS-CoV-2 and influenza A/B virus detection.

## Introduction

The first documented cases of coronavirus disease 2019 (COVID-19) were reported in Wuhan City, China in December 2019[1,2]. Since then, COVID-19 has emerged as a global pandemic[3] with more than 150 million reported cases and 3.2 million deaths spanning most countries globally[3]. Severe accurate respiratory syndrome coronavirus virus-2 (SARS-CoV-2) and seasonal influenza virus infections present with similar clinical signs and symptoms. Therefore, it is important for individuals with evidence of a respiratory infection to be evaluated for both viruses, particularly during winter seasons[4,5].

The SARS-CoV-2 & Influenza A/B Nucleic Acid Test for use on Roche's cobas® Liat® System is a real-time RT-PCR assay approved on September 14, 2020 under the FDA's Emergency Use Authorization (EUA) for multiplex *in vitro* qualitative detection of SARS-CoV-2, influenza A and influenza B virus[6]. The assay runs on the Roche cobas® Liat®, an FDA-approved, fully automated, point-of-care (POC) system that employs onboard nucleic acid purification and

103 amplification (~20-minute run time) from nasopharyngeal (NP) or nasal swabs collected in
104 commercial viral or universal transport mediums (VTM and UTM)[6-8].

105 For routine respiratory collection and testing, most laboratories collect swabs (NP, nasal,
106 or throat swabs) in VTM and UTM manufactured by Copan, Becton Dickinson and Thermo
107 Fisher. The various media recipes were designed and patented in the 1990's for the purpose
108 of maintaining microbial integrity until collected specimens can be cultured at reference
109 labs[9]. However, in the late 1990s, many laboratories began to transition from detection
110 solely by culture to molecular-based approaches including quantitative polymerase chain
111 reaction (qPCR). Many diagnostic labs, including those testing SARS-CoV-2, exclusively
112 employ qPCR due to increased sensitivity over culture[10]. However, some reagents in
113 VTM/UTM intended to maintain microbial viability, *i.e.,* gelatin and BSA (Daum *et al.,*
114 unpublished) may inhibit or reduce qPCR cycle threshold ($C_T$) values when co-extracted
115 during nucleic acid extraction amplification. Thus, most diagnostic testing manufacturers
116 list specific VTM/UTM products on the package insert to ensure that test performance is not
117 altered based on the media used for specimen collection.

118 PrimeStore Molecular Transport Medium® (PS-MTM®) is a microbial nucleic acid storage
119 and transport device cleared in 2018 by the U.S. Food and Drug Administration (US FDA)[11].
120 PS-MTM® is indicated for rapid killing/inactivation of viruses (including Influenza), and
121 bacteria (including *Mycobacterium tuberculosis*) or other respiratory pathogens, *i.e.,* SARS-
122 CoV-2 virus within a collected respiratory sample[11-13]. Importantly, RNA and DNA from
123 collected samples are subsequently stabilized and preserved to provide safer and more
124 efficient workflow for automated extraction, qPCR and sequencing[14-16].

125 PS-MTM® functions by disrupting and shearing lipid membranes, inactivating cellular
126 nucleases, and preserving released genetic material--RNA and DNA--at ambient temperature
127 or higher for extended periods. The nucleic acids from patient samples collected in PS-
128 MTM® do not require cold-chain and microbes are lysed (viruses in >5 minutes, bacteria in
129 >30 minutes) for safe shipping and transport using standard delivery[11-14]. Importantly, the
130 majority of collected samples do not require processing in BLS-II or III facilities. PS-MTM®
131 is compatible with most commercial extraction kits, *e.g.,* Roche's MagNA Pure, Qiagen kits[14-
132 17]. The collection medium is suited for respiratory specimens collected/tested at POC
133 diagnostic centers and has added utility for field collection in remote areas, triage centers,
134 and border crossings where cold-chain, transport, and dissemination of potentially
135 infectious pathogens are a concern[18-19]. During the 2020-21 COVID-19 pandemic, more than
136 60 million vials of PS-MTM® were distributed worldwide for collection and transport of
137 clinical respiratory samples.

138 This study evaluated the compatibility of Roche's cobas® Liat® with clinical respiratory
139 samples (NP and oral swabs) collected/transported in PS-MTM®. The main objectives were
140 to: *1)* compare limit of detection (LOD) at known concentrations of SARS-CoV-2 in PS-MTM®
141 or commercial UTM, and *2)* compare positive percent agreement and negative percent
142 agreement of clinical NP and oral swabs collected in PS-MTM® and analyzed on the cobas®
143 Liat® to standard qPCR using CDC's COVID-19 qPCR assay.

144

145 **Methodology**

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147 *Study Population and Clinical Samples*

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149 This evaluation was performed using a subset of positive and negative clinical specimens
150 (*N = 100*) selected from a prospective screening program initiated at Longhorn Vaccines and

151 Diagnostics addressing SARS-CoV-2 detection from employees and contractors including
152 household members. The subset of specimens were collected between June and October
153 (2020) as part of a voluntary quality control program to minimize potential contamination
154 of produced PS-MTM® reagent with SARS-CoV-2 RNA at production sites. Individuals or
155 household contacts of employees from Longhorn's kitting facilities in San Antonio, Lockhart,
156 and Boerne, Texas, USA where PrimeStore is manufactured and kitted were included in the
157 study. Clinical specimens analyzed were from nasopharyngeal (NP), nasal, and oral flocked
158 swabs (Puritan Medical Devices, Guilford, MA, USA) collected in cryotubes containing 1.5 mL
159 of PS-MTM® (Longhorn Vaccines and Diagnostics, San Antonio, Texas, USA) using standard
160 collection methodology. All specimens were originally tested for SARS-CoV-2 shortly after
161 collection and confirmed influenza-positive or negative using the approved CDC real-time
162 qPCR assay and PrimeMix™ Influenza A/B qPCR Blend (Longhorn Vaccines and Diagnostics,
163 San Antonio, Texas, USA). This study was deemed IRB Exempt in accordance FDA guidance
164 since patient screening was voluntary and did not impact patient care, and all samples were
165 deidentified and properly disposed after use[20].

166 *Laboratory Testing*
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168 For quantitative qRT-PCR testing (comparator test), a total of 0.2 mL PS-MTM® was added
169 to 0.2 mL of extraction buffer and 0.2 mL of 100% ethanol, mixed briefly, and subsequently
170 extracted using a PrimXtract Total Nucleic Acid Kit (Longhorn Vaccines and Diagnostics, San
171 Antonio, TX, USA) with a final elution of 50 µL according to manufacturer's
172 recommendations. For qRT-PCR detection of SARS-CoV-2 RNA, the CDC's primers are probes
173 targeting the N2 and RNASEP genes were used with an ABI-7500 instrument (ThermoFisher
174 Scientific, Waltham, MA, USA) according to thermocycling parameters described by CDC[21].

175 For influenza detection, Longhorn's PrimeMix™ Influenza A/B Universal Blend was utilized
176 according to thermocycling conditions previously reported[22]. For each thermocycling run,
177 duplicate positive and negative control reactions were included. Clinical samples testing
178 positive were recorded according the cycle threshold ($C_T$) value for viral targets and RNASEP
179 human genomic internal control. A lower $C_T$ value indicates a higher viral RNA
180 concentration, with a value >40 indicating no target amplification. PCR concentration ranges
181 were applied to divide samples into high ($C_T$ value <25), medium ($C_T$ 25–30), and low ($C_T$
182 30–40) viral RNA concentrations. All clinical specimens were stored at -80°C until use on
183 the Roche cobas® Liat® instrument.

184 Equivalence between UTM and PS-MTM® was evaluated by spiking purified SARS-CoV-2
185 RNA into Copan's UTM (Copan diagnostics, Brescia, Italy) and PS-MTM®. For Limit of
186 Detection (LOD) testing, quantified SARS-CoV-2 ribonucleic acid (RNA) from a U.S. isolate
187 collected in Texas, USA was diluted ten-fold, added to pooled negative NP swab matrix
188 (collected in UTM and MTM), and evaluated using: qPCR on an ABI-7500 (comparator test).
189 For this experiment, triplicate reactions of each 10-fold reduction (*e.g.,* 1,000 copies/µL to 1
190 copy/µL) were evaluated.

191 *Roche cobas® Liat® testing*
192
193 Before processing samples, Lot validation was performed on the cobas® Liat® system
194 which included running a *Positive* and *Negative* control sample. Clinical samples were
195 processing according instructions for use[6,7]. Briefly, a bulb pipette provided in each assay
196 kit was used to transfer ~0.2 mLs of clinical specimen inactivated in PS-MTM® into the
197 opening of the assay tube cartridge. The assay tube was closed, scanned for identification,
198 and inserted into the cobas® Liat® device. After a 20-minute run, a *Report* screen provides

qualitative results indicating detection of SARS-COV-2, influenza A, or influenza B from the patient sample.

Statistical analysis was performed using MEDCALC[23] for determination of positive percent agreement, negative percent agreement, confidence intervals (CI), and positive/negative predictive values (PPV/NPV).

## Results

*Limit of Detection (LOD)*

Using the CDC's N2 assay, a qPCR LOD was performed to establish equivalency using quantified concentrations of SARS-CoV-2 RNA in PS-MTM® and UTM-RT (Figure 1; panel A). In this experiment, 3 of 3 replicates containing SARS-CoV-2 RNA were detected from samples containing 12,400 copies/mL down to 10 copies/mL, albeit $C_T$ values from PS-MTM were lower, *i.e.,* more targets detected at all dilution points (Figure 1: *c.f.*, panel A and B). For RNA dilution at the determined LOD, *i.e.* 1 copy/mL, 10 additional replicates in PS-MTM® (7 of 10 detected; average $C_T$ = 39.4; S.D. = 0.7, and 95% CI = 39 - 39.8) and Copan UTM-RT (6 of 10 detected; average $C_T$ = 39.7; S.D. = 0.4, and 95% CI = 39.5 - 39.7) were performed (*data not shown*).

As shown in Figure 1 (panel B), CDC's N2 qPCR $C_T$ values compared to Liat® detection (*e.g.,* positive or negative) from 10-fold reductions of SARS-CoV-2 RNA in PS-MTM® and UTM-RT (in triplicate) are shown. The Liat® detected 1 of 3 replicate reactions from PS-MTM® and UTM-RT medium at 1 copy/mL. For these dilutions, the mean $C_T$ value according to qPCR using the CDC's N2 assay was 39.2 and 39.6, respectively. The average $C_T$ value at each 10-

225 fold SARS-CoV-2 RNA dilution was lower (*i.e.,* more targets detected) for PS-MTM® samples;
226 however, no significant difference was noted in triplicate samples analyzed.

227 *Analysis of NP and oral swabs in PrimeStore*
228
229 Of 100 clinical specimens (37 NP and 63 oral flocked swabs), there were 47 positives and
230 53 negatives detected by the Roche cobas® Liat®. Of 53 total positives, there were 3 (6%)
231 influenza A (2 H3N2 and 1 H1N1 subtype), 2 (4%) influenza B, and 48 (90%) Sars-CoV-2
232 virus positive specimens detected. None of the evaluated samples were co-infections.
233 Positive percent agreement, defined as the percentage of specimens testing positive among
234 47 true-positive samples, was 100% (C.I. = 92.4-100%) compared to qPCR. The negative
235 percent agreement, defined as the percentage of specimens testing negative among 53 true-
236 negative samples, was 96.4% (C.I. = 86.0-99.5%). There were two false-positive samples
237 detected; however, both were from previously infected individuals. Table 1 summarizes the
238 positive and negative percent agreement, positive predictive value (PPV), and negative
239 predictive value (NPV) in comparison to qPCR testing.

240 The Roche cobas® Liat® exhibited no reduction in sensitivity from low copy clinical
241 samples with cycle threshold ($C_T$) values >30 according to qPCR testing. Of 47 positives, 23
242 (49%) had a $C_T$ value >30 ($C_T$ range: 30 - 38.2) according to qPCR, and all were readily
243 detected by the Liat® system.

244
245 **Discussion**
246
247 PS-MTM® is the first molecular transport device granted FDA 510(k) clearance (March
248 2018) for complete microbial inactivation and stabilization of nucleic acids and is covered
249 by several U.S. and international formulation and method patents. Several other inactivating
250 collection media are commercially available *e.g.,* Zymo Research Corporation's DNA/RNA

251 Shield and Copan's eNAT. However, PS-MTM® is unique because FDA clearance includes
252 microbial inactivation and molecular analysis of RNA (influenza virus) and DNA
253 (*Mycobacterium tuberculosis*) based-microbes or other suspected respiratory pathogens
254 collected from clinical oral/NP swabs. Additionally, PS-MTM® is not restricted to any one
255 extraction device and is compatible with many bead-based and spin-column nucleic acid
256 extraction kits and platforms.
257
258 PS-MTM® may provide added value for sample collection at drive through self-testing
259 sites or for at-home testing. Moreover, collection in PS-MTM® enables reflex testing for
260 respiratory panels on the BioMérieux BioFire and GenMark Diagnostic ePlex Systems. In
261 these contexts, inactivating and subsequently stabilizing collected RNA/DNA at ambient
262 temperature for extended periods is critical. Furthermore, samples collected in PS-MTM®
263 are preserved in cryovials that can be biobanked for subsequent use to detect other
264 pathogens or for genomic sequencing analysis.
265
266 This study demonstrated equivalency of serial dilutions of SARS-CoV-2 RNA collected in
267 PS-MTM® compared to UTM-RT (Figure 1). Compared to CDC's qPCR test, positive and
268 negative percent agreement were 100 and 96.4 %, respectively for clinical NP and oral swabs
269 in PS-MTM® (Table 1). There were two discordant clinical samples (false-positives) that
270 were negative by standard CDC qPCR but positive by Roche Liat® testing. False-positivity on
271 the Roche Liat® platform has been reported previously[24-25]. However, both discrepant false-
272 positive tests in this study were from previously positive COVID-19 patients and may reflect
273 detection of low level viral RNA not detected initially by CDC's qPCR test. Unfortunately,

274 there is limited data correlating $C_T$ value with transmission events versus residual nucleic
275 acid from a previous infection. This is an area that will need to be explored further but is
276 out of the scope of this study. In this retrospective clinical study, the Liat® test-failure rate
277 was 2% (two tests). Both were from clinical samples testing negative by CDC's qPCR test
278 and both were determined as negative upon re-testing.
279
280 The Liat® is a multiplex system capable of simultaneous detection of SARS-CoV-2, and
281 influenza A and B RNA from patient samples. Furthermore, it can be used at point of care
282 without BSL-II/III containment on an open bench due to a closed system design for nucleic
283 acid extraction and amplification. This is important during winter months for detection of
284 COVID-19 and influenza infection or co-infections[26]. One beneficial feature of the Liat® is
285 that results are reported qualitatively, *i.e.,* as positive or negative for SARS-CoV-2, Influenza
286 A, or Influenza B to provide easily evaluated results for use in doctors' offices or emergency
287 departments where patient care decisions can be made immediately. The Roche Liat® is
288 often utilized at urgent care clinics for point-of-care testing by physicians, nurses, or office
289 staff where inactivated and stabilized clinical specimens in PS-MTM® are preferred prior to
290 uncapping and liquid transfer into test cartridges. PS-MTM® is compatible for use with the
291 Liat® System but laboratories must default the test to high complexity prior to use.
292
293 In this study, clinical NP and oral swabs collected in PS-MTM® and tested on the Liat®
294 demonstrated concordant positive percent agreement and negative percent agreement
295 compared to standard qPCR. Importantly, this study was the first to employ the use of FDA-
296 Cleared PS-MTM® on the cobas® Liat®. PS-MTM®, in addition to standard UMT/VTM

collection, compliments the Liat® device but offers added benefit since respiratory samples are inactivated at POC and can pipetted directly into the instrument without fear of accidental or infectious release of potentially dangerous agents. In addition, the RNA in PS-MTM® is preserved for additional molecular analysis, including sequencing. This is critically important for safe diagnosis, rapid treatment, and infection control, particularly in midst of the current COVID-19 pandemic.

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**Table 1**. Detection of SARS-CoV-2 viral RNA from clinical swabs (*N = 100*) collected in PrimeStore MTM® using the Roche Liat® compared to results by standard qPCR.

| **Roche cobas® Liat®** | | |
|---|---|---|
| Statistic | Value (%) | 95% Confidence Interval |
| Positive % Agreement | 100 | 92.5 - 100 |
| Negative % Agreement | 96.4 | 87.5 - 99.6 |
| PPV | 95.9 | 85.8 - 98.9 |
| NPV | 100 | |

PPV= positive
predictive value
NPV= negative predictive value

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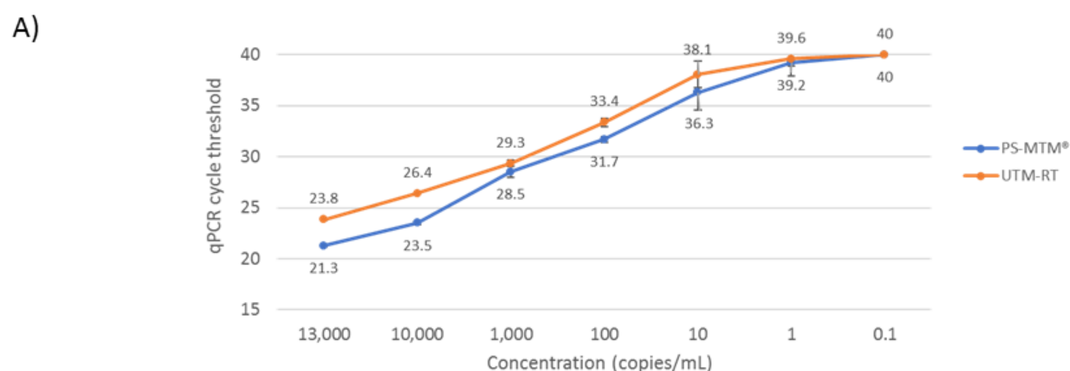
**Figure 1.** Limit of detection for SARS-CoV-2 viral RNA in PS-MTM® and Copan's UTM-RT using: A) CDC's N2 qPCR test on an ABI-7500 System, and B) Roche's Cobas® Liat®. Average triplicate reactions for each dilution with standard deviation bars are shown. Only qPCR $C_T$ values for the N2 CDC test are shown since the average $C_T$ value between N1 and N2 were within 1.1 $C_T$ at each dilution. *For dilution at LOD, *i.e.* 1 copy/mL, 10 additional replicates in PS-MTM® (7 of 10 detected, average $C_T$ = 39.4; S.D. = 0.7, and 95% CI = 39 - 39.8) and Copan UTM-RT (6 of 10 detected, average $C_T$ = 39.7; S.D. = 0.4, and 95% CI = 39.5 - 39.7) were performed (*data not shown*).




B)

| | | | PS-MTM® | | | | | | | UTM-RT | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Roche Cobas Liat | | | CDC-N2 Assay/ABI-7500 ($C_T$ *value*) | | | | Roche Cobas Liat | | | CDC-N2 Assay/ABI-7500 ($C_T$ *value*) | | | |
| Genomic copies (*copies/mL*) | Genomic copies (*copies/µL*) | Dilution (*PS-MTM® or UTM-RT*) | R1 | R2 | R3 | R1 | R2 | R3 | AVG $C_T$ | R1 | R2 | R3 | R1 | R2 | R3 | AVG $C_T$ |
| 13,000 | 13 | 3 to 1 | Pos | Pos | Pos | 21.2 | 21.2 | 21.4 | 21.3 | Pos | Pos | Pos | 23.9 | 23.9 | 23.7 | 23.8 |
| 10,000 | 10 | 10^-1 | Pos | Pos | Pos | 23.4 | 23.7 | 23.4 | 23.5 | Pos | Pos | Pos | 26.4 | 26.4 | 26.4 | 26.4 |
| 1,000 | 1 | 10^-2 | Pos | Pos | Pos | 28.2 | 29.2 | 28.2 | 28.5 | Pos | Pos | Pos | 29.7 | 29.2 | 29.1 | 29.3 |
| 100 | 0.1 | 10^-3 | Pos | Pos | Pos | 31.4 | 32 | 31.7 | 31.7 | Pos | Pos | Pos | 33.5 | 32.9 | 33.7 | 33.4 |
| 10 | 0.01 | 10^-4 | Pos | Pos | Pos | 34.3 | 37 | 37.6 | 36.3 | Pos | Pos | Pos | 37.8 | 39.5 | 36.9 | 38.1 |
| 1* | 0.001 | 10^-5 | Neg | Pos | Pos | 40 | 37.7 | 40 | 39.2 | Pos | Pos | Neg | 38.8 | 40 | 40 | 39.6 |
| 0.1 | 0.0001 | 10^-6 | Neg | Neg | Neg | 40 | 40 | 40 | 40 | Neg | Neg | Neg | 40 | 40 | 40 | 40 |