Sensitive and Specific Detection of Severe Acute Respiratory Syndrome Coronavirus 2 by the Fast and Automated POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent

Abstract

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a huge impact on global public health and economics. Nucleic acid amplification testing is highly recommended for the detection of SARS-CoV-2. POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent, targeting *orf 1ab* region, is a fully automatic qualitative insulated isothermal polymerase chain reaction (iiPCR) assay working on the compact and user-friendly POCKIT™ Central Nucleic Analyzer. Clinical performance of POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent was compared to a real-time RT-PCR method routinely run in clinical laboratory in North Cumbria, United Kingdom, using 182 upper respiratory specimens collected from SARS-CoV-2-suspected patients. Interrater agreement calculated by 2x2 contingency analysis showed that negative percentage agreement between the two RT-PCR assays was 97.0% (CI 95%, 93.5 - 100%) and positive percentage agreement was 96.0% (CI 95%, 88.8 – 100%), indicating that POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent had performance equivalent to that of laboratory real-time RT-PCR. Providing great diagnostic sensitivity and specificity, POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent with the easily deployable POCKIT™ Central device can serve as an effective tool for point-of-need detection of SARS-CoV-2.

Challenges behind the testing of COVID-19 during the pandemic

The coronavirus disease of 2019 (COVID-19) has caused more than 696,000 deaths and more than 18.3 million confirmed cases worldwide (as of August 5, 2020) [1]. Its etiological agent is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense single-stranded RNA virus belonging to the *Betacoronavirus* genus and closely related to the SARS-CoV [2]. The pathogen is primarily spread via droplets, contact and fomites. Those infected may either be asymptomatic or symptomatic, and the disease can progress into pneumonia and even death in some cases [3]. Acute respiratory distress syndrome

caused by SARS-CoV-2 infection, respiratory/cardiac failure, and sepsis due to secondary bacterial infection are major known causes of mortality in COVID-19 patients [4, 5].

Nucleic acid amplification testing (NAAT) is the recommended method to aid in diagnosis of SARS-CoV-2 infection [6]. Recommended specimens for NAAT include nasopharyngeal (NP) swab, oropharyngeal (OP) swab, mid-turbinate swab, anterior nares swab, and nasopharyngeal wash/aspirate or nasal wash/aspirate [7]. Even though real-time RT-PCR is a well-accepted method, the processes from sample collection, RNA extraction, to amplification, detection and data interpretation remains challenging [8]. Poor clinical sensitivity could be resulted by poor RNA quality due to loss, degradation, or contamination of viral RNA during sample transportation and at the RNA extraction step which often requires additional equipment investment and trained personnel. Traditionally, real-time PCR amplify target sequence by several cycles of heating and cooling. Sophisticated thermal cycler is required for reaction temperature adjustment, which also makes the thermal cycler relatively expensive. In addition, processing and analysis of real-time RT-PCR results requires well-trained personnel. For COVID-19, there is an urgent need for an easy, rapid and reliable near-patient diagnostic assay to help implement effective control and preventive measures.

POCKIT™ Central makes COVID-19 testing easy and simple

Sophisticated NAAT protocols become easy and simple and NAAT can be deployed at any time and any places with the POCKIT™ Central platform. The bench-top POCKIT™ Central Nucleic Acid Analyzer automates magnetic bead-based nucleic acid extraction as well as fluorescence-based insulated isothermal PCR (iiPCR), signal detection, and data processing/interpretation to offer a simple sample-in-answer-out protocol for up to 8 samples within 85 minutes (Figure 1). Complicated operation procedures and human operation errors are minimized. Unlike conventional PCR, iiPCR is accomplished

Figure 1. Specifications of POCKIT™ Central Nucleic Acid Analyzer

- Throughput: 1-8 samples
- Detection channel: 2
- Extraction Included
- Run time: hands-on time less than 5 mins; total reaction time 85 mins
- Dimensions: 31(W) x 48 (D) x 40 (H) cm
- Weight: 21 kg

through the temperature gradient generated from the thermal convection with significantly shortened reaction time. Most remarkably, the device is relative affordable.

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent works on the POCKIT™ Central Nucleic Acid Analyzer. It allows automated qualitative detection of SARS-CoV-2 RNA from patients suspected of SARS-CoV-2 infection during the acute phase. An Internal Control is included in the reagent to monitor the performance of PCR. This assay is based on the well proven iiPCR technology [9-11]. The assay involves simple operation procedures with 2 ready-to-go cartridges. Users simply add sample to the Extraction Cartridges, place it and a Transfer Cartridge onto POCKIT™ Central, input info of sample and reagent, and start the run through a simple user interface. The device completes all processes automatically, and results are available on the monitor and ready to be retrieved in 85 minutes for up to 8 samples in one run (Figure 2). POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent was demonstrated to have clinical performance equivalent to that of a laboratory real-time RT-PCR assay and excellent analytical specificity in this report.

Clinical performance

A total of 182 retrospective NP swabs, OP swabs and NP/OP swab samples from SARS-CoV-2-suspected patients were analyzed by the POCKIT™ Central SARS-CoV-2 and cobas® 6800 SARS-CoV-2 assays in parallel. The clinical samples were collected from the diagnostic laboratories of North Cumbria Integrated Care National Health Service Trust from March to June, 2020 and were tested in a clinical laboratory in North Cumbria, United Kingdom (UK). The assays were performed by following manufacturer's instructions.

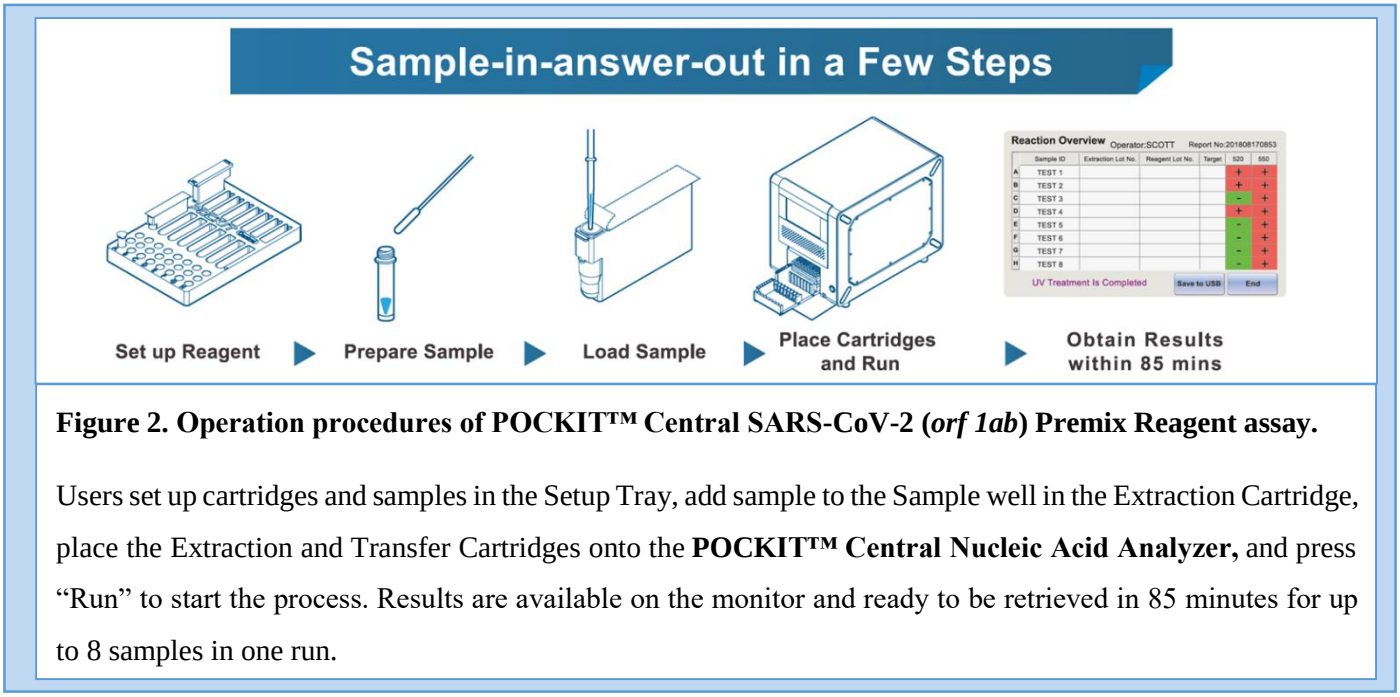

Figure 2. Operation procedures of POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent assay.

Users set up cartridges and samples in the Setup Tray, add sample to the Sample well in the Extraction Cartridge, place the Extraction and Transfer Cartridges onto the **POCKIT™ Central Nucleic Acid Analyzer,** and press "Run" to start the process. Results are available on the monitor and ready to be retrieved in 85 minutes for up to 8 samples in one run.

2x2 contingency analysis of the test results showed that 48 were positive and 128 negative by both methods; while 4 were reference negative/index positive and 2 were reference positive/index negative (Table 1). Discrepant results were not resolved due to limited volumes of samples. Negative percent agreement (NPA) and Positive percent agreement (PPA) between POCKIT™ Central and the cobas® 6800 SARS-CoV-2 were 97.0% (CI 95%, 93.5–100%) and 96.0% (CI 95%, 88.8–100%), and the Cohen's kappa value was 0.92 (Table 1). The results indicated that the compact fully automated RT-PCR assay had performance equivalent to the laboratory real-time RT-PCR method for the detection of SARS-CoV-2 RNA.

Conclusions

SARS-CoV-2 virus has continued to spread around the world. There are critical needs for points of need NAAT tools to aid timely clinical diagnosis and public health surveillance of COVID-19. The fully automated sample-to-answer POCKIT™ Central Nucleic Acid Analyzer is a flexible benchtop NAAT tool to help reduce hands-on time and enhance test consistency. It involves minimal specimen handling and simple operation procedures, and can provide answers from sample in a short turnaround time (<120 minutes). Providing diagnostic performance comparable to laboratory real-time RT-PCR assay, POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent can serve as a sensitive and specific near-patient tool to aid quick diagnosis of SARS-CoV-2 infection for the in time medical response. This opens up a possibility of performing COVID-19 testing out of a clinical laboratory to the front lines, such as care homes, factories and airport quarantines, when in compliance with local laws and regulations.

Reference

1. CDC. Interim guidelines for collecting, handling, and testing clinical specimens from persons under investigation (PUIs) for coronavirus disease 2019 (COVID-19). 2020.

Table 1. 2x2 contingency analysis: Equivalent clinical performance between POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent and cobas® 6800 SARS-CoV-2 for the detection of SARS-CoV-2 RNA in upper respiratory specimens.

		cobas® 6800 SARS-CoV-2		
		Positive	Negative	Total
POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent	Positive	48	4	52
	Negative	2	128	130
	Total	50	132	182

	Value	95% Confidence Interval
Negative percent agreement (NPA)	97.0%	93.5% - 100%
Positive percent agreement (PPA)	96.0%	88.8% - 100%
Total agreement	96.7%	93.8% - 99.6%
Cohen's kappa values (κ)	0.92	

2. Chang HFG, Tsai YL, Tsai CF, Lin CK, Lee PY, Teng PH, et al. A thermally baffled device for highly stabilized convective PCR. *Biotechnology Journal.* 2012;7(5):662-6.
3. Feng W, Newbigging A, Le C, Pang B, Peng H, Cao Y, et al. Molecular diagnosis of COVID-19: Challenges and research needs. *Analytical chemistry*. 2020;92:10196-209.
4. Gorbalenya AE, Baker SC, Baric R, Groot RJd, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology*. 2020; 5:536–544
5. Pujadas E, Ibeh N, Hernandez MM, Waluszko A, Sidorenko T, Flores V, et al. Comparison of SARS-CoV-2 Detection from Nasopharyngeal Swab Samples by the Roche cobas® 6800 SARS-CoV-2 Test and a Laboratory-Developed Real-Time RT-PCR test. *Journal of Medical Virology.* 2020:1-4.
6. Tsai J-J, Liu W-L, Lin P-C, Huang B-Y, Tsai C-Y, Chou P-H, et al. An RT-PCR panel for rapid serotyping of dengue virus serotypes 1 to 4 in human serum and mosquito on a field-deployable PCR system. *PloS one.* 2019;14(3):e0214328.
7. Tsai Y-L, Wang H-TT, Chang H-FG, Tsai C-F, Lin C-K, Teng P-H, et al. Development of TaqMan probe-based insulated isothermal PCR (iiPCR) for sensitive and specific on-site pathogen detection. *PloS one.* 2012;7(9):e45278.
8. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *Jama*. 2020;323(11):1061-9.
9. WHO. Coronavirus disease (COVID-19): situation report, 198. World Health Organization, 2020.
10. WHO. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance, 19 March 2020. World Health Organization, 2020.
11. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *Jama*. 2020;323(13):1239-42.
12. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, *et al*. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *New England Journal of Medicine*. 2020;382(12):1177-9.

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