Rapid Direct Nucleic Acid Amplification Test Without RNA Extraction for SARS-CoV-2 Using a Portable PCR Thermocycler

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Introduction

The coronavirus disease 2019 (Covid-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has impacted the world greatly.[1] As the number of cases increase worldwide, there is an imperative to develop rapid, simplified and cost-effective detection methods such that Covid-19 testing for diagnosis, screening and research could be widely used in countries and regions where laboratory capabilities are limiting.[2-4]

At present, confirmatory diagnosis is by reverse transcriptase polymerase chain reaction (RT-qPCR), typically taking several hours and requiring a molecular lab to perform.[5,6] **This paper explores and analytically validates a more efficient protocol using direct rapid extraction-free PCR (DIRECT-PCR) detection of SARS-CoV-2 without the need for nucleic acid purification.**

Aim

1. To develop a DIRECT-PCR protocol for SARS-CoV 2 detection and assess its analytical performance.
2. To develop a fast DIRECT-PCR protocol for use in a portable thermocycler and assess its analytical performance.

Methods

Sample collection and preparation

- ssRNA of amplicon sequence for SARS-CoV-2 nucleocapsid gene synthesised in vitro
- Collection of sputum and nasal exudate

RT- qPCR and DIRECT - PCR

DIRECT – PCR:

- Monoplex single-tube reaction mixtures
- PCR enhancer and inhibitor-resistant enzymes[7-9]

Optimisation for fast DIRECT - PCR

- Direct – PCR assays validated on portable thermocycler

Statistical Analysis

- Limit of detection (LoD) determined by plotting quantification cycle (Cq) against log10 copy number concentration.
- Amplification efficiency (E) = $1 + 10^{(-1/slope)}$

Results

Threshold of detection using RT-qPCR, DIRECT-PCR and Fast DIRECT-PCR

Amplification plots were used to determine LoD and mean Cq:

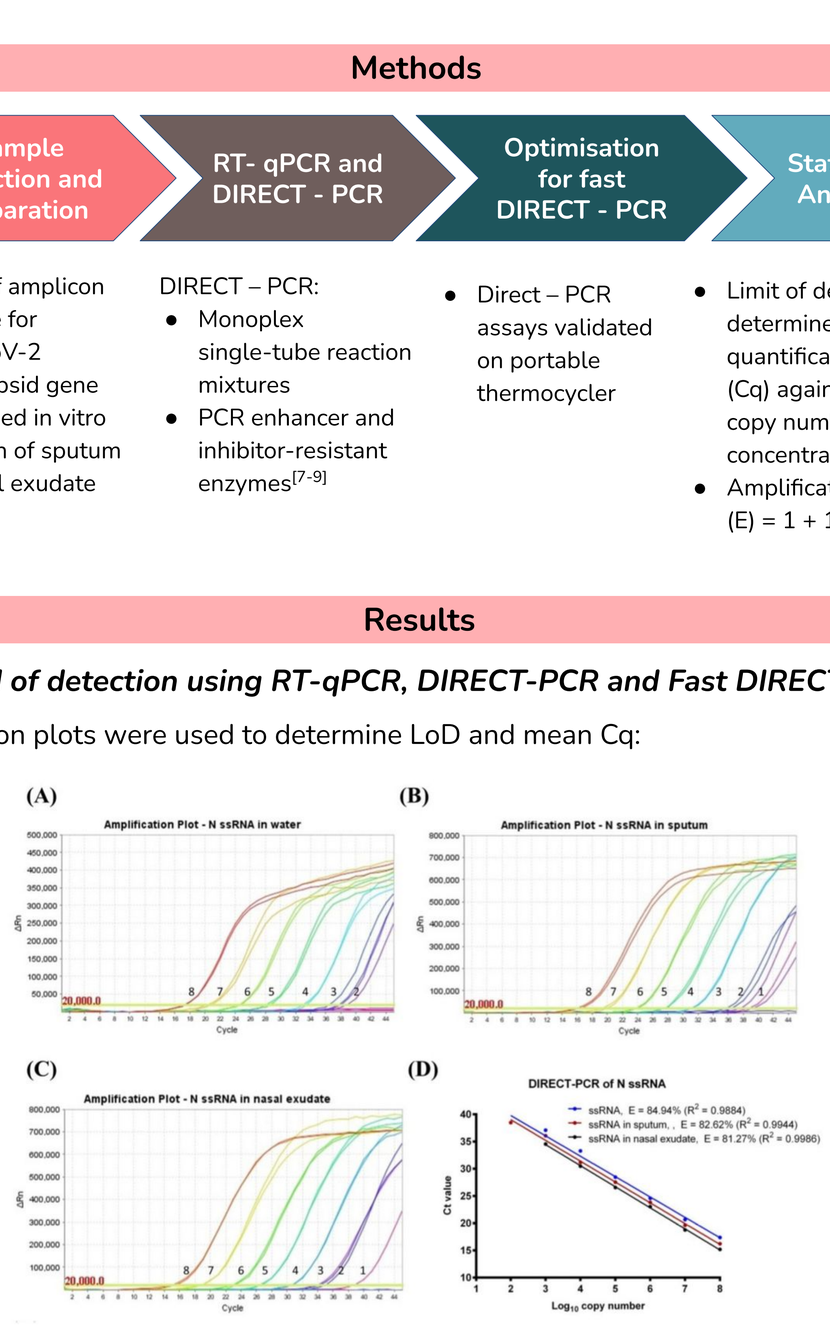

Fig. 1: DIRECT-PCR amplification plot *of SARS-CoV-2 N gene ssRNA in (A) water, (B) sputum, (C) nasal exudate conducted in same thermocycler run.*

Both DIRECT-PCR and Fast **DIRECT-PCR showed a much higher threshold of detection for the sputum and nasal exudate matrices**, compared to RT-qPCR.

No difference was observed for the water matrix.

Table 1: LoD and Mean Cq *of RT-qPCR, DIRECT-PCR and Fast DIRECT-PCR assays in various matrices.*

PCR	Template	Matrix	LoD (Cq Mean ± S.D)
RT-qPCR	ssRNA	Water	**120** (40.67 ± 0.29)
		Sputum	No amplification
		Nasal Exudate	No amplification
DIRECT-PCR	ssRNA	Water	**120** (38.48 ± 0.57)
		Sputum	**12** (38.79^)
		Nasal Exudate	**12** (38.72^)
Fast DIRECT-PCR	ssRNA	Water	**600** (39.42^)
		Sputum	**6** (39.28^)
		Nasal Exudate	**60** (39.34^)
	Plasmid	Water	**2** (39.75^)
		Sputum	**2** (38.93^)
		Nasal Exudate	**20** (38.02 ± 0.55)

Fast DIRECT-PCR using Portable vs Standard Benchtop Thermocycler
In nasal exudate, the threshold of detection for both thermocyclers was the same (LoD = 60). However, the portable had a lower threshold of detection in sputum (LoD = 600) compared to the standard benchtop thermocycler (LoD = 6).

Overall, **portable thermocyclers have a similar threshold of detection compared to benchtop thermocyclers.**

Discussion

DIRECT-PCR is a viable alternative to RT-qPCR.

DIRECT-PCR had the same threshold of detection in sputum and nasal exudates compared to that of RT-qPCR in water.

Benefits

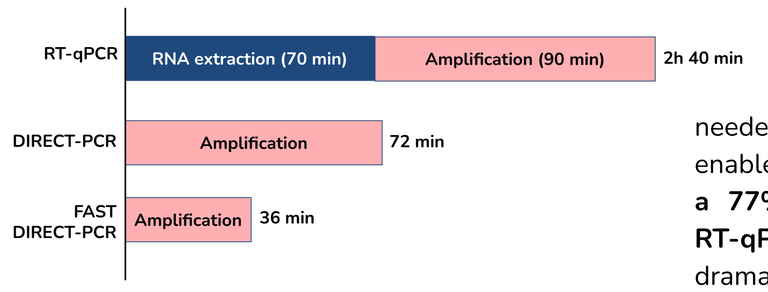

Fig. 2: Duration of RNA extraction and amplification *of RT-qPCR, DIRECT-PCR, FAST DIRECT-PCR.*

Shorter duration as RNA purification not needed (Fig 2). Fast DIRECT-PCR enabled amplification in **36 min – a 77% reduction from current RT-qPCR methods.** This could dramatically **shorten current turnaround time** (24-48h in Singapore).

Cost savings on nucleic acid extraction kits and other reagents.

Reduced carryover contamination, biosafety risk as Fast DIRECT-PCR is a single-tube homogeneous reaction.

Rapid point-of-care tests now possible with **portable thermocyclers**, negating transport time which can prolong result availability beyond 24h, **in resource-poor or rural areas**.[10,11]

Future endeavours

Other respiratory samples including endotracheal swabs and bronchoalveolar lavage fluid as effective clinical samples can be assessed.

Automation of DIRECT-PCR allows for a greater number of samples to be analysed and reduces human error. Results are also uploaded to national records with automation, reducing turnover time for samples.

Limitations

Portable thermocyclers are limited in speed. Amplification time takes 49 min instead of 36 min in a benchtop thermocycler.

Reduction in amplification efficiency (E) due to presence of PCR inhibitors that can interfere with polymerase activity, degradation of nucleic acids and efficient cell lysis.[12]

Greater sampling error may increase false negatives as only 1 µL of sample is used. Larger volume reactions may be necessary to reduce this risk.

Clinical validation for DIRECT-PCR will require approval by authorities (for example, HSA) in areas such as analytical sensitivity & specificity, precision, sample matrix validation and a usability test (for PoCT).[13]

Conclusion

This paper affirms that **DIRECT-PCR is a highly sensitive in detecting SARS-CoV-2 viral RNA from nasopharyngeal swabs and sputum samples.** The assay reduces hands-on time, time-to-results, and costs. Fast DIRECT-PCR can be achieved in a single-tube homogeneous reaction taking less than 1 hour and can be performed on a portable thermocycler.

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