Comparison of TaqPath™ COVID-19 Combo Kit and BGI Real-Time Fluorescent RT-PCR Kit for detecting SARS-CoV-2 in nasal swabs

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INTRODUCTION

Since the onset of the COVID-19 pandemic, more than 200 RT-qPCR tests have received Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration for detection of SARS-CoV-2. The TaqPath™ COVID-19 Combo Kit targets three SARS-CoV-2 genes (ORF1ab, N, S), and uses bacteriophage MS2 as an exogenous processing control. The BGI Real-Time Fluorescent RT-PCR Kit targets a single SARS-CoV-2 gene (ORF1ab) and uses human beta-actin as an endogenous processing control. (Fig.1) In this study, we compared clinical performance of the two above-mentioned EUA-approved RT-PCR diagnostic tests in nasal swabs.

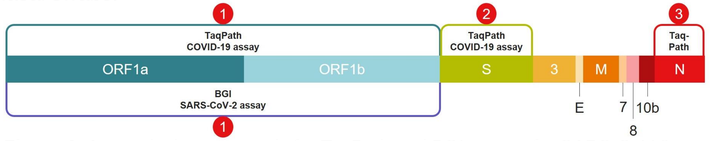

***Figure 1**. Assay design schematic for TaqPath and BGI assays for SARS-CoV-2*

METHODS

A retrospective study was conducted on residual nasal swab specimens collected as part of routine clinical testing at Quantigen BioSciences in August and September 2020. All specimens were transported in 2-4 ml viral transport medium and stored at 4° C until tested. 334 specimens were selected based upon TaqPath results. All samples were tested with the TaqPath protocol within 1-2 days following clinical collection, followed by testing with the BGI protocol within one week following initial testing. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated and discordant samples were evaluated by Sanger sequencing in a blinded fashion.

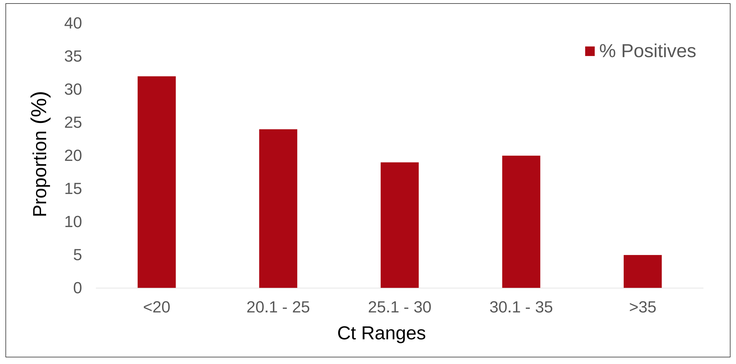

***Figure 2.** Distribution of Ct values for positive cohort based on initial TaqPath testing*

RESULTS

Of the 334 samples, 24 samples were excluded from the cohort due to negative b-actin results with the BGI assay. The final cohort included 310 samples, with COVID specific Ct values ranging between 12-36 with an average of 24.26. The distribution of Ct values of positive cohort is shown in Figure 2.

The PPA between the two assays was 85.23%, with 13 of 88 TaqPath positive samples testing negative with the BGI assay. The NPA was 99.55%, with 1 of 222 TaqPath negative samples testing positive with the BGI assay.

		TaqPath™ COVID-19 Combo Kit		
		Positive	Negative	Total
BGI SARS-CoV-2 Assay	Positive	75	1	76
	Negative	13	221	234
	Total	88	222	310
Positive Percentage Agreement		**85.23%**	76.06% to 91.89%	
Negative Percentage Agreement		**99.55%**	97.52% to 99.99%	

***Table 1.** Concordance between TaqPath™ COVID-19 Combo Kit and BGI SARS-CoV-2 Assay*

Sample ID	TaqPath Result	BGI Result	Sanger Sequencing
43	Positive ✓	Negative	Positive
46	Negative ✓	Positive	Negative
97	Positive ✓	Negative	Positive
99	Positive	Negative ✓	Negative
102	Positive	Negative ✓	Negative
196	Positive ✓	Negative	Positive
279	Positive ✓	Negative	Positive
282	Positive ✓	Negative	Positive
292	Positive ✓	Negative	Positive
306	Positive ✓	Negative	Positive
324	Positive ✓	Negative	Positive
325	Positive ✓	Negative	Positive
327	Positive ✓	Negative	Positive
340	Positive ✓	Negative	Positive

***Table 2.** Discordant sample resolution showing agreement of each of the assays with Sanger Sequencing*

RESULTS (Contd)

In 12 of 14 samples, Sanger sequencing results agreed with the TaqPath assay. In 2 out 14 discordant results, Sanger sequencing agreed with BGI assay. (Table 2). After arbitration testing by Sanger Sequencing, the PPA and NPA for TaqPath assay was 100% and 99.11% respectively and for the BGI assay was 87.21% and 99.55% respectively. (Table 3)

	TaqPath™ COVID-19 Combo Kit	BGI SARS-CoV-2 Assay
Positive Percent Agreement	**100%**	**87.21%**
Negative Percent Agreement	**99.11%**	**99.55%**

***Table 3.** Agreement of the 2 assays after blinded arbitration testing using Sanger Sequencing*

6 samples in TaqPath positive cohort showed amplification of 2 out the 3 targets (Orf1ab and N genes) and failed to show amplification of S-gene. However, all 6 samples showed high Ct values (Ct>30) on the 2 other targets. (Table 4) The high Ct values in these cases indicate low viral loads as a plausible reason for lack of S-gene amplification rather than presence of del69-70 mutation.

Sample ID	Orf1ab	N-gene	S-gene
99	35.50	34.15	ND
282	33.10	30.75	ND
289	34.37	30.92	ND
304	36.55	34.45	ND
329	36.76	35.95	ND
333	35.83	30.99	ND

***Table 4.** Ct values of the 6 cases that failed show amplification of S-gene*

CONCLUSIONS

Significant agreement differences, especially PPA, were observed between the multi-target TaqPath™ COVID-19 Combo Kit and single-target BGI SARS-CoV-2 assay. TaqPath test had excellent agreement after arbitration testing by Sanger sequencing (100% PPA, 99.11% NPA). Inadequate sample collection and/or poor extraction efficiency (as evidenced by weak b-actin signals) or storage degradation may account for some of the BGI-negative discordances.