The Comparative Verification of the Kurabo QuickGene-810 Nucleic Acid Isolation System and QIAGEN EZ1 Advanced DSP Virus Kit for the RNA extraction of SARS-CoV2

April 2020

INTERNAL METHOD VERIFICATION

Test method evaluated: Kurabo QuickGene-810 Nucleic Acid Isolation System

1 Tests:

1.1 Intended purpose of test:

The Kurabo QuickGene-810 Nucleic Acid Isolation System is a semi-automated extraction platform that is intended for use as an additional RNA extraction method to support the increase in respiratory sample PCR testing during the 2020 SARS-CoV2 pandemic. Rapid and high-throughput molecular diagnostic testing is essential when considering individual patient care, healthcare facility preparedness, and public health surveillance and control.

1.2 Test method summary:

The Kurabo QuickGene-810 is a compact bench-top machine for the rapid isolation for DNA or RNA from various samples, including human tissue samples. Using the Kurabo RNA tissue kit SII, the QuickGene-810 processes 8 samples at a time taking 30 minutes from start (sample preparation) until finish (nucleic acid elution). The Quick Gene –mini 480 Nucleic Isolation system was also evaluated. This system uses the same kits and consumables and can perform 48 extractions that take approximately 60 minutes from sample preparation to RNA elution.

1.3 The Reference method summary:

The reference extraction platform used for comparison is the Qiagen EZ1 Advanced Extraction Platform with the EZ1 DSP Virus Kit, which effectively extracts both DNA and RNA. Both these Qiagen products have been previously validated for *in vitro* use by AusDiagnostics on their commercial SARS-CoV-2 real-time PCR assay (targeting ORF1a, ORF8 genes, Influenza A and B, and RSV).

2 Sample types

The sample types used for the purpose of this nucleic acid extraction comparison were predominantly respiratory swabs (nasal, throat, or nasopharyngeal) collected in UTM® medium. One expectorated sputum sample that had been treated with Sputasol was included. All sample types were deemed acceptable for use on the AusDiagnositics SARS-CoV2 assays as per the instructions for use (IFU).

3 Method

The AusDiagnostics SARS-CoV2 PCR assay had been previously verified at Concord Repatriation General Hospital's Microbiology Department in March 2020 using the EZ1 Advanced Extractor and DSP Viral kit (available if required). Positive and negative clinical samples obtained from Prince of Wales Hospital and Liverpool Hospital patients were tested as part of the original verification. These samples were extracted using RNA tissue kit SII using the Kurabo QuickGene-810 and Quick Gene – mini 480 Nucleic Acid Isolation System and subsequently run on the AusDiagnostics SARS-CoV2 PCR assay.

4 Results:

A total of 111 clinical specimens originally screened by the AusDiagnostics SARS-CoV-2 assay, and that had been extracted using the EZ1 Advanced extractor and DSP Virus Kit, were re-extracted from the original sample using the Kurabo QuickGene-810 and RNA Tissue Kit SII. Of these samples, there was a total of 27 positive and 84 negative sample extracts compared. All of the 111 Kurabo QuickGene-810 sample extracts were concordant with the results obtained from the EZ1 Advanced Extractor as displayed in Table 1. The average difference in the take-off value in positive samples differed between the Kurabo QuickGene-810 and the EZ1 Advanced Extractor which is displayed in Appendix 1. The difference was 3.04 cycles and 4.42 cycles for the SARS-CoV2a and SARS-CoV2b targets, respectively.

The performance characteristics can be seen in Table 2 and show that the sensitivity, specificity and the negative and positive predictive values were all determined as 100%.

Table 1. Kurabo QuickGene-810 results in comparison with the Qiagen EZ1 Advanced

Extraction Method		*Qiagen EZ1 Advanced*	
		Detected	**Not Detected**
Kurabo QuickGene-810	**Detected**	27	0
	Not Detected	0	84

Table 2. Performance characteristics of the Kurabo QuickGene-810 results in comparison with the Qiagen EZ1 Advanced

Sensitivity %	100
Specificity %	100
PPV %	100
NPV %	100

5 Limit of detection (LOD)

The LOD can be defined as the lowest concentration of an analyte that can be reliably detected by the assay. This is crucial when considering the overall acceptable level of confidence in an identified positive sample.

Three positive samples had a serial 1:10 dilution performed and were extracted simultaneously using both the Kurabo QuickGene-810 and the Qiagen EZ1 DSP Virus Kit. The three selected samples consisted of a confirmed low positive, a mid-range positive, and a high positive concentration. These samples were classified by observing the original take-off values and concentrations given by the AusDiagnostics results analysis software when previously tested. The comparison of the LOD values in Table 3, 4 and 5 show that the Qiagen EZ1 Advanced has a greater LOD when compared to the Kurabo QuickGene-810, for all three samples as determined by the AusDiagnostics results analysis software. This differs at most by 1 dilution factor for both targets (SARS-CoV2a and SARS-CoV-2b) in all three samples and is marked in red. Due to the lack of volume in the positive samples, the LOD was unable to be repeated with the same sample therefore reproducibility was not assessed.

Table 3. Comparison of the Limit of Detection from the Kurabo QuickGene-810 and the Qiagen EZ1 Advanced from a High positive sample

		Neat	1:10	1:100	1:1000	1:10 000	1:100 000
High positive sample (MB-20-129171) Kurabo QuickGene-810	SARS-CoV-2a (ORF1a)	+	+	+	+	check*	-
	Concentration	33 376	3805	84	29	-	-
	Take-off	11.50	15.03	20.91	22.72	**25.16**	-
	SARS-CoV-2b (ORF8)	+	+	+	+	+	-
	Concentration	112 840	15 563	347	211	**7**	-
	Take-off	9.60	12.84	18.7	19.62	**24.75**	
High positive sample (MB-20-129171) EZ1 Advanced DSP Virus Kit	SARS-CoV-2a (ORF1a)	+	+	+	+	-	-
	Concentration	172 806	18 349	537	**69**	8	-
	Take-Off	8.65	12.25	17.36	**20.73**	24.09	
	SARS-CoV-2b (ORF8)	+	+	+	+	+	+
	Concentration	529 511	64 416	2928	392	39	**11**
	Take-off	6.90	10.30	14.71	18.03	21.54	**23.83**

Table 4. Comparison of the Limit of Detection from the Kurabo QuickGene-810 and the Qiagen EZ1 Advanced from a mid-range positive sample

		Neat	1:10	1:100	1:1000	1:10 000	1:100 000
Mid-range positive sample (MB-20-130404) Kurabo QuickGene-810	SARS-CoV-2a (ORF1a)	+	check*	-	-	-	-
	Concentration	15		-	-		-
	Take off	23.69	**25.31**				
	SARS-CoV-2b (ORF8)	+	+	-	-	-	-
	Concentration	65	**14**	-	-	-	-
	Take-off	21.39	**23.89**				
Mid-range positive sample (MB-20-130404) EZ1 Advanced DSP Virus Kit	SARS-CoV-2a (ORF1a)	+	+	+	-	-	-
	Concentration	166	11	**5**	-	-	-
	Take-off	19.57	23.83	**24.85**			
	SARS-CoV-2b (ORF8)	+	+	+	-	-	-
	Concentration	642	72	**7**	-	-	-
	Take-off	17.46	20.83	**24.52**			

Table 5. Comparison of the Limit of Detection from the Kurabo QuickGene-810 and the Qiagen EZ1 Advanced from a low positive sample

		Neat	1:10	1:100	1:1000	1:10 000	1:100 000
Low positive sample (MB-20-137199) Kurabo QuickGene-810	SARS-CoV-2a (ORF1a)	-		-	-	-	-
	Concentration	-	-	-	-	-	-
	Take-off	-	-	-	-	-	-
	SARS-CoV-2b (ORF8)	+	-	-	-	-	-
	Concentration	**13**	-	-	-	-	-
	Take-off	**23.59**	-	-	-	-	-
Low positive sample (MB-20-137199) EZ1 Advanced DSP Virus Kit	SARS-CoV-2a (ORF1a)	+	-	-	-	-	-
	Concentration	**16**	-	-	-	-	-
	Take-off	**23.28**	-	-	-	-	-
	SARS-CoV-2b (ORF8)	+	+	-	-	-	-
	Concentration	53	**14**	-	-	-	-
	Take-off	21.37	**23.18**	-	-	-	-

*NOTE: As per the AusDiagnostics High-Plex IFU, a check is defined as a case when the cycling acceleration is less than the pre-set parameters and therefore no concentration estimate is provided.[2] For this verification, a 'check' will be considered as positive as they are known positive samples.

The Take-off value is the point of maximum acceleration where the fluorescence emerges from the background.[2]

6 Discussion

All the samples tested were concordant between the Kurabo QuickGene-810 and the Qiagen EZ1 Advanced Extractor. Although results matched, there was a difference between the take-off values for both SARS-CoV2 targets for each sample suggesting that the EZ1 Advanced yields a higher concentration of nucleic acid extract. Therefore, it is recommended that in the case of a potential low positive or discrepancies between the two gene targets that re-extraction occur on the EZ1 Advanced Extractor.

For future investigation, as per the Kurabo manufacturer, use of Dithiothreitol (DTT) and carrier rRNA should be considered as a supplement for increasing the yield of purified nucleic acid product. The DTT acts as an agent that reduces the disulphide bonds of proteins, such as proteases, that degrade nucleic acid material.[3] Similarly, carrier RNA acts to increase the recovery of nucleic material through preventing the target material from being irretrievably bound.[4]

7 Conclusion

In conclusion, the Kurabo QuickGene-810 and QuickGene mini-480 using the RNA Tissue Kit SII are fit for purpose extraction platforms for RNA extraction from human respiratory samples. The platform performs comparatively with the Qiagen EZ1 Advanced Extractor and is suitable for use with the AusDiagnostics SARS-CoV2 PCR. However, the Quick Gene- mini 480 is more cumbersome and requires more hands on time for processing. Based on the high sensitivity and specificity results, this verification supports the introduction of the Kurabo QuickGene-810 into our laboratory to support COVID-19 testing.

The controls comply with stated criteria?	Yes
Limitations of the assay:	None
Acceptance of the validation/verification:	Verification - Yes
Comments including limitations of method:	

Date test introduced in laboratory: Pending Approval

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8th April 2020

Report reviewed by:

Lab Manager Signed: Date: 9/4/20

Approved by:

Snr Scientist Signed: Date: 9/4/20

Specialist Signed: Date: 9/4/20

References

1. Concord Hospital Microbiology. Verification of the AusDiagnositics SARS-CoV2 assay. March 2020.
2. AusDiagnostics High-Plex 24 System REF 91501. *Instructions for Use*. March 2019. pp 1-44.
3. He H, Li R, Chen Y, Pan P, Tong W, Dong X, et al. Integrated DNA and RNA extraction using magnetic beads from viral pathogens causing acute respiratory infections. *Scientific Reports*. 2017;7:45199.
4. Suliman BA. Comparison of five viral nucleic acid extraction kits for the efficient extraction of viral DNA and RNA from cell-free samples. *Trends Med*. 2019;19: DOI: 10.15761/TiM.1000202

Accession	Run	SARS Cov2a	EZI Take Off	Kurabo Take Off	SARS Cov2b	EZI Take Off	Kurabo Take Off
MB-20-128934	92	POS	6	6	POS	4	5
MB-20-127132	92	POS	8	10	POS	6	8
MB-20-127944	92	POS	24	24	POS	21	22
MB-20-137199	92	POS	24	25	POS	23	23
MB-20-120037	95	POS	4	8	POS	3	7
MB-20-116934	95	POS	3	4	POS	3	4
MB-20-117791	95	POS	11	14	POS	9	13
MB-20-116927	95	POS	14	16	POS	12	14
MB-20-118855	95	POS	11	13	POS	9	12
MB-20-116959	95	POS	7	10	POS	5	9
MB-20-117791	95	POS	9	10	POS	7	8
MB-20-121084	95	POS	11	16	POS	9	14
MB-20-117066	95	POS	13	15	POS	11	15
MB-20-121442	95	POS	4	11	POS	3	11
MB-20-120754	95	POS	9	12	POS	7	10
MB-20-134741	95	POS	9	14	POS	7	13
MB-20-135163	95	POS	9	17	POS	7	17
MB-20-133924	95	LOW	19	24	LOW	17	24
MB-20-135156	95	POS	12	17	POS	10	16
MB-20-130404	97	POS	19	21	POS	17	19
MB-20-129171	97	POS	8	10	POS	6	9
MB-20-133690	97	POS	9	12	POS	7	11
MB-20-134672	97	POS	16	18	POS	14	16
MB-20-129927	97	POS	16	19	POS	12	16

							19
MB-20-134765	97	POS	19	21	POS	17	
MB-20-135345	97	POS	17	22	POS	15	21
MB-20-133503	97	POS	6	10	POS	5	9
MB-20-134910	92	NEG			NEG		
MB-20-134906	92	NEG			NEG		
MB-20-134924	92	NEG			NEG		
MB-20-134902	92	NEG			NEG		
MB-20-141205	96	NEG			NEG		
MB-20-134900	96	NEG			NEG		
MB-20-134901	96	NEG			NEG		
MB-20-134889	96	NEG			NEG		
MB-20-134893	96	NEG			NEG		
MB-20-134888	96	NEG			NEG		
MB-20-134897	96	NEG			NEG		
MB-20-134899	96	NEG			NEG		
MB-20-135535	96	NEG			NEG		
MB-20-135121	96	NEG			NEG		
MB-20-135116	96	NEG			NEG		
MB-20-135227	96	NEG			NEG		
MB-20-135237	96	NEG			NEG		
MB-20-135235	96	NEG			NEG		
MB-20-135243	96	NEG			NEG		
MB-20-135207	96	NEG			NEG		
MB-20-135412	96	NEG			NEG		
MB-20-135419	96	NEG			NEG		
MB-20-135415	96	NEG			NEG		
MB-20-135416	96	NEG			NEG		
MB-20-136561	96	NEG			NEG		

MB-20-136565	96	NEG	NEG
MB-20-136566	96	NEG	NEG
MB-20-135587	96	NEG	NEG
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MB-20-136900	96	NEG	NEG
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MB-20-136595	96	NEG	NEG
MB-20-136593	96	NEG	NEG
MB-20-136594	96	NEG	NEG
MB-20-139007	96	NEG	NEG
MB-20-139009	96	NEG	NEG
MB-20-139402	96	NEG	NEG
MB-20-139408	96	NEG	NEG
MB-20-138175	96	NEG	NEG

MB-20-138175-2	96	NEG	NEG
MB-20-138149	96	NEG	NEG
			NEG
MB-20-138150	96	NEG	
MB-20-138152	96	NEG	NEG
MB-20-138153	96	NEG	NEG
MB-20-138154	96	NEG	NEG
MB-20-138147	96	NEG	NEG
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MB-20-139796	96	NEG	NEG
MB-20-139866	96	NEG	NEG
MB-20-139797	96	NEG	NEG
MB-20-139801	96	NEG	NEG
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MB-20-139404	96	NEG	NEG
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MB-20-140877	96	NEG	NEG
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MB-20-134904	96	NEG	NEG
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MB-20-134794	96	NEG	NEG
MB-20-141325	96	NEG	NEG
			NEG
MB-20-141387	96	NEG	
MB-20-141505	96	NEG	NEG
MB-20-141202	96	NEG	NEG
MB-20-141203	96	NEG	NEG