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Extraction validation results of Sullivan Nicolaides

Compare ANDiS with KingFisher Flex (MagMax kit)

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Sample options

SARS-CoV-2 Coronavirus Materials

A variety of materials containing either entire genomic RNA of the SARS-CoV-2 virus, or synthetic sequences matching PCR target regions was obtained for evaluating RNA recovery.

1. Cell culture derived materials

Gamma irradiated inactive Vero cell line materials infected with SARS-CoV-2 was prepared in Liquid Amies in a ten fold dilution series from 10-1 through 10-9 of the neat material.

2. Commercial quantitated material

Seracare Accuplex SARS-CoV-2 synthetic RNA material was obtained from a commercial supplier. This control contains synthetic RNA sequences matching common SARS-CoV-2 PCR target regions, and is calibrated at 5000 copies/mL.

RNA virus

A data extract was performed on the SNP clinical database to identify patients positive for respiratory viruses for which the clinical sample was still held on site. Samples were recovered from refrigerated storage for use in the evaluation. To ensure sufficient material was available for cross comparisons 1mL of Liquid Amies was added to each patient sample, and the sample vigorously vortexed to resuspend materials. It was expected that this dilution may reduce Ct values to non-detectable in some late cycle samples.

Extraction Efficiency Test

Results of Seracare Accuplex Commercial positive control material SARS-CoV-2 for ANDiS system for extraction efficiency study

Assessment of the equivalency of the extraction methods was determined through a direct comparison of the performance of ANDiS Viral RNA Auto Extraction & Purification Kit to MagMAX Viral/Pathogen Nucleic Acid Isolation Kit with KingFisher Flex. Seracare Accuplex SARS-CoV-2 Commercial Control:5000 copies/mL and Vero Cell Line – Gamma Irradiated SARS-CoV-2 Infected Cells were used.

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- Seracare Accuplex SARS-CoV-2 Commercial Control: 5000 copies/mL
- According to the basis of qPCR, $160 * R_{MagMax} * 2^{Ct\_MagMax} = 40 * R_{ANDiS} * 2^{Ct\_ANDiS}$,

  so $R_{ANDiS} : R_{MagMax} = 160 * 2^{Ct\_MagMax}) / (40 * 2^{CtANDiS}) = 1.82$

	MagMax					ANDiS					DeltaCt	Relative Yield (R_{ANDiS}:R_{MagMax})
	Input	Elution	RT-qPCR	Ct	Concentration factor	Input	Elution	RT-qPCR	Ct	Concentration factor		
Volume	400 µL	50 µL	4 µL	31.04	8 fold	200 µL	100 µL	4 µL	32.18	2 fold	-1.14	182%
Copy Number	2000	2000 *R_{MagMax}	160 *R_{MagMax}			1000	1000*R_{ANDiS}	40*R_{ANDiS}				

R_{MagMax} : Recovery Rate of MagMax Kit with KingFisher
R_{ANDiS}: Recovery Rate of ANDiS System

Extraction Efficiency Test

Gamma irradiated inactive Vero cell line materials infected with SARS-CoV-2 was prepared in Liquid Amies in a ten fold dilution series from 10-1 through 10-9 of the neat material for extraction efficiency study. Same equation was used as last slide.

Dilution of Vero cell line materials infected with SARS-CoV-2	Ct_ MagMax		Ct_ANDiS		Ct(ANDiS) - Ct(MagMax)	Relative Yield （ANDiS / MagMax）
	Ct	Concentration factor	Ct	Concentration factor		
10^{-1}	14.19	8	16.08	2	-1.89	107.92%
10^{-2}	18.03	8	19.52	2	-1.49	142.41%
10^{-3}	21.26	8	22.89	2	-1.63	129.24%
10^{-4}	24.59	8	26.25	2	-1.66	126.58%
10-5	28.08	8	29.67	2	-1.59	132.87%
10-6	32.11	8	32.18	2	-0.07	381.06%
10-7	35.6	8	35.8	2	-0.2	348.22%
10-8	37.41	8	N	2		
10-9	N	8	N	2		

Clinical Test

A number liquid amies collected samples from patients confirmed to be infected with SARS-CoV-2 were extracted by MagMAX and the ANDiS system. Results are presented below.

SARS-CoV-2

		Test 2 (Kingfisher FLEX/MagMAX)	
		Positive	**Negative**
Test 1 (ANDiS method)	**Positive**	24	0
	Negative	0	1

Average Ct Difference (MagMAX – ANDiS): -1.05

Greatest Ct Difference: -2.68

Lowest Ct Difference: +0.48

The ANDiS sytem was found to recover SARS-CoV-2 RNA with 100% concordance to the Thermofisher MagMAX Virus/Pathogen kit on the Kingfisher instrument. Adjusted for differences in input and elution volume the ANDiS appears to offer equivalent utility.

Clinical Test

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Molecular Pathology Evaluation/Validation:

ANDiS 350 Automated Nucleic Acid Extraction System and AND RNA Auto Extraction and Purification Kit

The Ct difference between ANDiS and MagMax shows that RNA recovery rate of ANDiS is equal and even better than MagMax kits.

ID	Expected	MagMAX	ANDiS	Ct Diff	Therotical Ct Diff	Recovery Rate (ANDiS:MagMax)
38245	SARS-CoV-2	17.6	18.53	-0.93	-2	209.94%
38459	SARS-CoV-2	28.9	29.59	-0.69	-2	247.94%
38654	SARS-CoV-2	22	22.02	-0.02	-2	394.49%
38895	SARS-CoV-2	24.84	23.76	1.08	-2	845.61%
38911	SARS-CoV-2	27.6	27.12	0.48	-2	557.90%
38961	SARS-CoV-2	17.3	18.59	-1.29	-2	163.58%
38967	SARS-CoV-2	28.51	29.91	-1.4	-2	151.57%
39021	SARS-CoV-2	31	32.86	-1.86	-2	110.19%
39046	SARS-CoV-2	26	27.66	-1.66	-2	126.58%
39295	SARS-CoV-2	16	18.45	-2.45	-2	73.20%
39318	SARS-CoV-2	13.78	15.51	-1.73	-2	120.58%
39441	SARS-CoV-2	18	19.13	-1.13	-2	182.77%
39513	SARS-CoV-2	25.97	28.65	-2.68	-2	62.42%
39874	SARS-CoV-2	21.68	22.58	-0.9	-2	214.35%
39941	SARS-CoV-2	20.96	23.03	-2.07	-2	95.26%
40092	SARS-CoV-2	19.33	20.09	-0.76	-2	236.20%
40422	SARS-CoV-2	19.51	19.65	-0.14	-2	363.01%
40576	SARS-CoV-2	24.86	25.73	-0.87	-2	218.86%
40908	SARS-CoV-2	22.58	23.33	-0.75	-2	237.84%
40913	SARS-CoV-2	22.04	23.54	-1.5	-2	141.42%
40966	SARS-CoV-2	28.73	30.6	-1.87	-2	109.43%
40985	SARS-CoV-2	26.65	27.36	-0.71	-2	244.53%
41303	SARS-CoV-2	31.95	32.82	-0.87	-2	218.86%
41429	SARS-CoV-2	25.62	26.01	-0.39	-2	305.25%
				-1.04625	**Average Ct Diff**	**193.69%**
					Therotical Ct Diff	**100.00%**

The use of 200uL of sample materials eluted into 100uL of eluate introduced a ~2 Ct shift compared to the 400uL/50uL Kingfisher MagMAX comparator and this should be considered wen examining the data presented. The use of 50uL eluate volumes with the ANDiS instrument/kit resulted in a ~1Ct improvement in most samples and may be useful if chasing slightly improved sensitivity for low template patient samples.

Reduced Elution Volume Results

Reduced elution volume from 100 to 50 ul the Ct values of ANDiS are very close with MagMax Kit even the input volume is only half of MagMax kit.

Molecular Pathology Evaluation/Validation:

ANDiS 350 Automated Nucleic Acid Extraction System and ANDiS Viral RNA Auto Extraction and Purification Kit

		MagMAX	ANDiS 100uL	ANDiS 50uL	Ct Diff (MagMax-ANDiS 50µL)	Recovery (ANDiS:MagMax)
38245	SARS-CoV-2	17.6	18.53	16.92	0.68	320.43%
38459	SARS-CoV-2	28.9	29.59	29.24	-0.34	158.01%
38654	SARS-CoV-2	22	22.02	21.13	0.87	365.53%
38895	SARS-CoV-2	24.84	23.76	22.89	1.95	772.75%
38911	SARS-CoV-2	27.6	27.12	26.58	1.02	405.58%
38961	SARS-CoV-2	17.3	18.59	17.31	-0.01	198.62%
38967	SARS-CoV-2	28.51	29.91	29.19	-0.68	124.83%
39021	SARS-CoV-2	31	32.86	34.34	-3.34	19.75%

Reducing the elution volume from 100uL to 50uL brought in the Ct values by approximately 1 cycle (average 0.6) as expected. It appears the beads and RNA recovery works fine with the reduced eluate volume in columns 6 and 12 of the DWP's. This modification may be useful for slight improvements in detection of samples at very low template concentration. No bead carryover was observed in the wells of column 6 or 12 when utilising the lowered elution volumes.

Clinical Evaluation with other RNA Viruses

RNA Viruses

- **Virus tested:** Human Rhinovirus, Influenza A and B, Human Metapneumovirus, Parainfluenza, Respiratory Syncytial Virus
- Positive and Negative RNA Viruses were extracted with both ANDiS and MagMax method. Amplified with same detection kit.

Test 2 (Kingfisher FLEX/MagMAX)

Test 1 (ANDiS method)	Positive	Negative
Positive	47	1 hMPV
Negative	2 1 FluB, 1RSV	9

Overall Analytical sensitivity for Test 1 = TP/(TP+T1 FN) x 100% = 47/(47 +2) x 100% = 96%

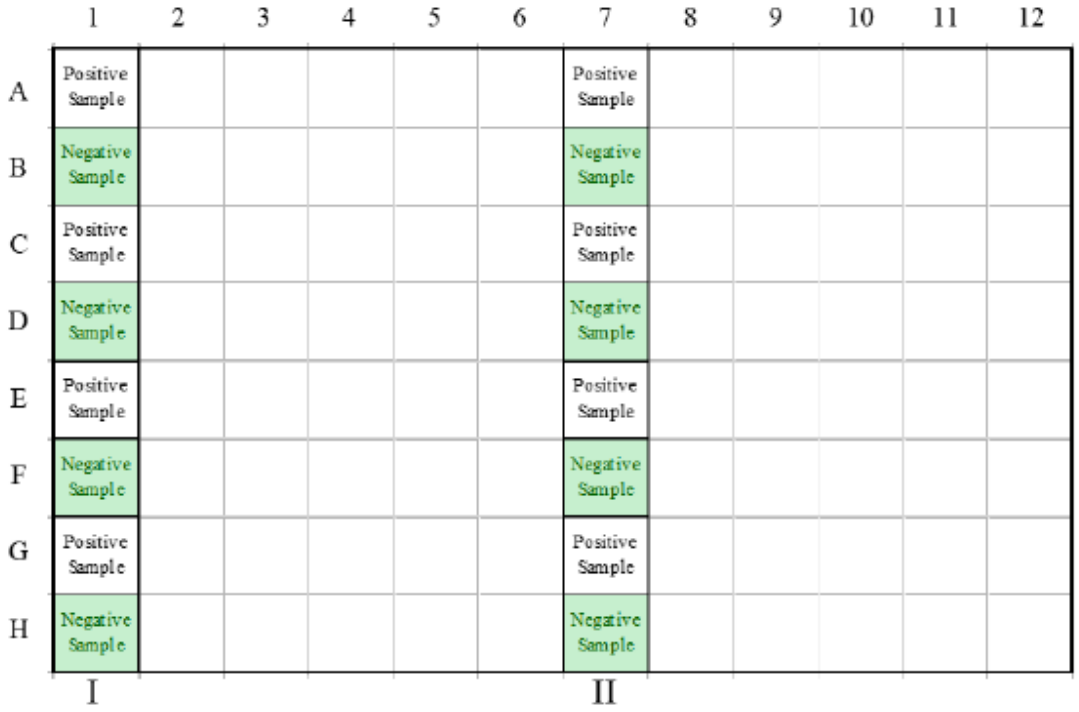
Overall Analytical specificity for Test 1 = TN/(TN + T1 FP) x 100% = 9/(9+0) x 100% = 100%

Equal or superior performance over industry standard

Cross – contamination Study :

- Positive and Negative sample were added next to each other for amplification

- **No cross-contamination** observed.



Column 1		Column 7	
RPIC		RPIC	
expected	as tested	expected	as tested
NEG	neg	NEG	neg
POS	23.4	POS	23.17
NEG	neg	NEG	neg
POS	24.03	POS	23.56
NEG	neg	NEG	neg
POS	23.92	POS	23.97
NEG	neg	NEG	neg
POS	23.6	POS	23.51

Overall conclusion

V) Overall conclusions

The performance of the ANDiS extractor and the ANDiS Viral RNA Auto Extraction and Purification kit was found to be in agreement with the manufacturers data and is fit for use for RNA respiratory viruses (96%), and also when adding in Adenovirus DNA and *Bordetella pertussis* DNA (92%). Further work on additional prospective patient samples is required to validate the kit for use with *Mycoplasma pneumoniae* DNA extraction.

The use of the kit for SARS-CoV-2 RNA extraction showed concordance rates of 100% and the instrument is fit for use in recovering Coronavirus RNA. No cross contamination was observed with the system.

The use of 200uL of sample materials eluted into 100uL of eluate introduced a ~2 Ct shift compared to the 400uL/50uL Kingfisher MagMAX comparator and this should be considered wen examining the data presented. The use of 50uL eluate volumes with the ANDiS instrument/kit resulted in a ~1Ct improvement in most samples and may be useful if chasing slightly improved sensitivity for low template patient samples.

The results of this study verify the manufacturers claims and the instrument and extraction chemistry is fit for use in a Sonic laboratory for the intended use of producing RNA for downstream assessment with the ANDiS SARS-CoV-2 and Influenza A/B RT-PCR detection kit

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