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Report Title:

LIFE RIVER SARS-CoV-2 EXTRACTION AND RT-PCR: Evaluation of this system by the National Virus Reference Laboratory.

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BRIEF OVERVIEW OF THE SYSTEM:

The Life River 3600 extractors were received at the NVRL (N=17). Each system is capable of extracting 3 X 12 samples in 20 minutes. The extraction kit consists of a pre-loaded deep-well plate, which contains all the reagents for the extraction procedure. Each preloaded plate is capable of extracting 12 samples. 3 of the pre-loaded plates may be loaded on board the system at the one time. Each extraction kit contains 5 preloaded plates (i.e. 60 extractions). The Life River Real-time RT-PCR kits were also received. Each PCR kit can test up to 24 samples.

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METHOD OVERVIEW:

EXTRACTION:

i. 500 µL of carrier RNA buffer was added to the lyophilised carrier RNA.

ii. 390 µL of the prepared carried RNA was added to the 1.3 mL of proteinase K. This allows for enough Carrier RNA/ Proteinase mix for 65 extractions.

iii. **OPTIONAL:** Add 65 µL of Internal control (from the Life River RT-PCR kit) to the Carrier RNA/ Proteinase mix.

iv. The pre-loaded extraction plate was removed from its plastic packaging. A visual volume check for lysis buffer in well positions A1 – A12 was performed and recorded on the run template *(*WARNING: Some of the lysis buffer has leaked from the preloaded plates during transit)*

v. 26 µL of the Carrier RNA/ Proteinase mix was added to wells A1 – A12 of the pre-loaded extraction plate on the bench **OR** if using the internal control add 27 µL of the Carrier RNA/ Proteinase/Internal Control mix to the appropriate wells.

vi. The samples were ordered in lots of 12 and the barcode was scanned into an excel template, and printed. A sample check was performed and recorded on the template.

vii. 300 µL of sample was added to the pre-loaded well in positions A1 – A12 and mixed by pipetting.

a. 'Live' prepared respiratory specimens were added in the laminar flow hood using strict Biosafety level 2+ conditions. The sample racks and pre-loaded extraction plates were decontaminated by wiping the outside with Virkon, followed by incubation at room temperature for 10 minutes. The incubation time allowed for both lysis of the 'live' samples and decontamination.

b. 'Pre-Lysed' samples, prepared in either Biomerieux Nuclisens Lysis buffer or Roche MagNA Pure lysis buffer were added directly to the pre-loaded extraction plate on the bench.

viii. A second sample check was performed following sample addition and recorded on the assay template.

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ix. The pre-loaded extraction plates were loaded onto the machine. The machine ID and extraction plate position on the machine was noted down on the template.

x. The magnetic caps were loaded onto the system. They should be located directly above wells A1 – A12 of the pre-loaded extraction plate.

xi. RNA isolation 2 protocol was selected and the run was started. (*WARNING: *the machine is quite noisy – do not be alarmed!)*

xii. The extraction took approximately 20 minutes

xiii. The eluates from well positions E1 – E12 were removed and transferred in a 96-well plate. The position of the eluates in the 96-well plate was recorded on the assay template.

xiv. The pre-loaded plate and the magnetic caps were discarded in the yellow bin.

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RT-PCR:

i. The master mix for the life River RT-PCR was prepared. There is 19 µL of Supermix and 1 µL of enzyme per RT-PCR reaction. The limited kit volume meant that the master mix had to be added manually to the reaction plate. It was not possible to use the liquid handler to drop the RT-PCR master mix.

ii. 5 µL of extracts was added to 20 µL RT-PCR reaction master mix in the appropriate wells using either the liquid handler / multi-channel pipette..

iii. The RT-PCR was carried out using the ABI7500fast platform (Applied Biosystems) and set-up according to the product insert.

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RESULTS:

- Excellent result concordance of the Life River extraction with MagNA pure and Abbott M200 extraction when LIVE samples were processed. Pre-lysed samples did not perform adequately.

- Excellent result concordance of Life River RT-PCR with NVRL LDT / Altona / Abbott M2000 SARS-CoV-2 assays

- Please see the table and figures below for detailed review of the results.

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Table 1: LIFE RIVER EXTRACTION AND RT-PCR VALIDATION RESULTS.

Sample Name	Liferiver 'live' extraction NVRL LDT		Liferiver 'lysed' extraction		Liferiver 'live' extraction / Life River Rt-PCR				MagNA pure extraction / Altona PCR OR ABBOTT	Result interpretation LIFE RIVER EXTRACTION	Result interpretation LIFE RIVER RT-PCR
	E-gene Ct	Rnase P Ct	E-gene Ct	Rnase P Ct	E-gene Ct	IC Ct (IC not added	N-gene Ct	ORF1ab Ct			
20Z03027	15.7	ND	ND	ND	16.4	ND	18.0	17.9	18.3	Live Life River condordant	Live Life River condordant
20Z03046	17.5	38.8	ND	ND	17.9	ND	20.3	19.6	19.1	Live Life River condordant	Live Life River condordant
20Z03016	18.4	37.9	ND	ND	18.5	ND	20.4	19.8	17.2	Live Life River condordant	Live Life River condordant
20Z03107	19.1	ND	ND	ND	19.5	ND	21.6	21.1	18.8	Live Life River condordant	Live Life River condordant
20Z03105	19.1	26.0	ND	ND	19.3	ND	21.6	20.8	16.7	Live Life River condordant	Live Life River condordant
20Z03270	20.1	21.4	ND	ND	19.5	ND	22.2	20.8	CN: 9.7 (Abbott)	Live Life River condordant	Live Life River condordant
20Z03022	20.4	24.3	ND	ND	17.5	ND	18.9	18.9	19.7	Live Life River condordant	Live Life River condordant
20Z03062	22.3	38.3	ND	ND	27.5	ND	30.1	29.0	24.6	Live Life River condordant	Live Life River condordant
20Z03103	23.5	31.2	ND	ND	23.4	ND	25.8	25.4	22.7	Live Life River condordant	Live Life River condordant
20Z03032	26.8	26.2	ND	ND	27.1	ND	28.8	28.5	25.5	Live Life River condordant	Live Life River condordant
20Z03054	27.7	28.2	ND	ND	27.4	ND	30.0	28.7	26.8	Live Life River condordant	Live Life River condordant
20Z03262	27.8	29.8	ND	ND	22.8	ND	24.3	24.1	CN: 18.9 (Abbott)	Live Life River condordant	Live Life River condordant
20Z03010	29.7	30.7	ND	ND	30.1	ND	32.2	31.3	27.3	Live Life River condordant	Live Life River condordant
20Z03003	30.6	29.0	ND	ND	33.8	ND	38.1	35.2	30.6	Live Life River condordant	Live Life River condordant
20Z03252	33.0	32.8	ND	ND	34.7	ND	35.5	34.7	CN: 28.4	Live Life River condordant	Live Life River condordant

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									(Abbott)		
20Z03004	34.0	28.0	ND	ND	ND	ND	ND	ND	34.5	Live Life River condordant	Wk positive not detected by Life River PCR
20Z03005	ND	31.0	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z03006	ND	29.4	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z03018	ND	ND	ND	ND	ND	ND	ND	ND	ND	Live Life River inhibitory	Live Life River condordant
20Z03251	ND	28.6	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z03255	ND	27.6	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z03257	ND	29.2	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z03261	ND	28.7	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z03269	ND	30.2	ND	ND	ND	ND	ND	ND	ND	Live Life River condordant	Live Life River condordant
20Z01251	ND	25.8			ND	ND	ND	ND	ND (Abbott)	Live Life River condordant	Live Life River condordant
20Z01252	ND	25.3			ND	ND	ND	ND	ND (Abbott)	Live Life River condordant	Live Life River condordant
20Z01253	ND	26.2			ND	ND	ND	ND	ND (Abbott)	Live Life River condordant	Live Life River condordant
20Z01255	ND	32.2			ND	ND	ND	ND	ND (Abbott)	Live Life River condordant	Live Life River condordant
20Z01276	24.7	26.1			25.1	ND	26.6	26.4	23.9	Live Life River condordant	Live Life River condordant
20Z01281	34.6	25.2			34.5	ND	35.5	36.8	32.0	Live Life River condordant	Live Life River condordant
20Z01275	36.6	25.9			35.5	ND	ND	36.2	CN: 23.84 (Abbott)	Live Life River condordant	Live Life River condordant
20Z01299	17.7	28.8			17.9	ND	20.0	20.0	CN: 8.09 (Abbott)	Live Life River condordant	Live Life River condordant
20Z01301	18.6	35.6			19.6	ND	21.4	20.7	CN: 9.22 (Abbott)	Live Life River condordant	Live Life River condordant
20Z01302	23.6	28.4			24.0	ND	26.0	25.4	CN: 14.23 (Abbott)	Live Life River condordant	Live Life River condordant
20Z01304	32.6	30.1			32.7	ND	34.3	33.6	CN: 22.11 (Abbott)	Live Life River condordant	Live Life River condordant
20Z01309	21.1	32.9			21.5	ND	23.4	22.6	CN: 10.74	Live Life River condordant	Live Life River condordant

							(Abbott)		
20Z02709			ND	ND	ND	37.5	ND	N/A	Live Life River condordant
20Z02716			35.1	ND	38.4	38.2	34.5	N/A	Live Life River condordant
20Z02720			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02796			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02834			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02835			23.2	ND	26.4	25.2	22.5	N/A	Live Life River condordant
20Z02836			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02846			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02849			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02871			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02912			ND	ND	ND	ND	ND	N/A	Live Life River condordant
20Z02936			19.7	ND	23.2	21.9	19.3	N/A	Live Life River condordant

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Figure 1:

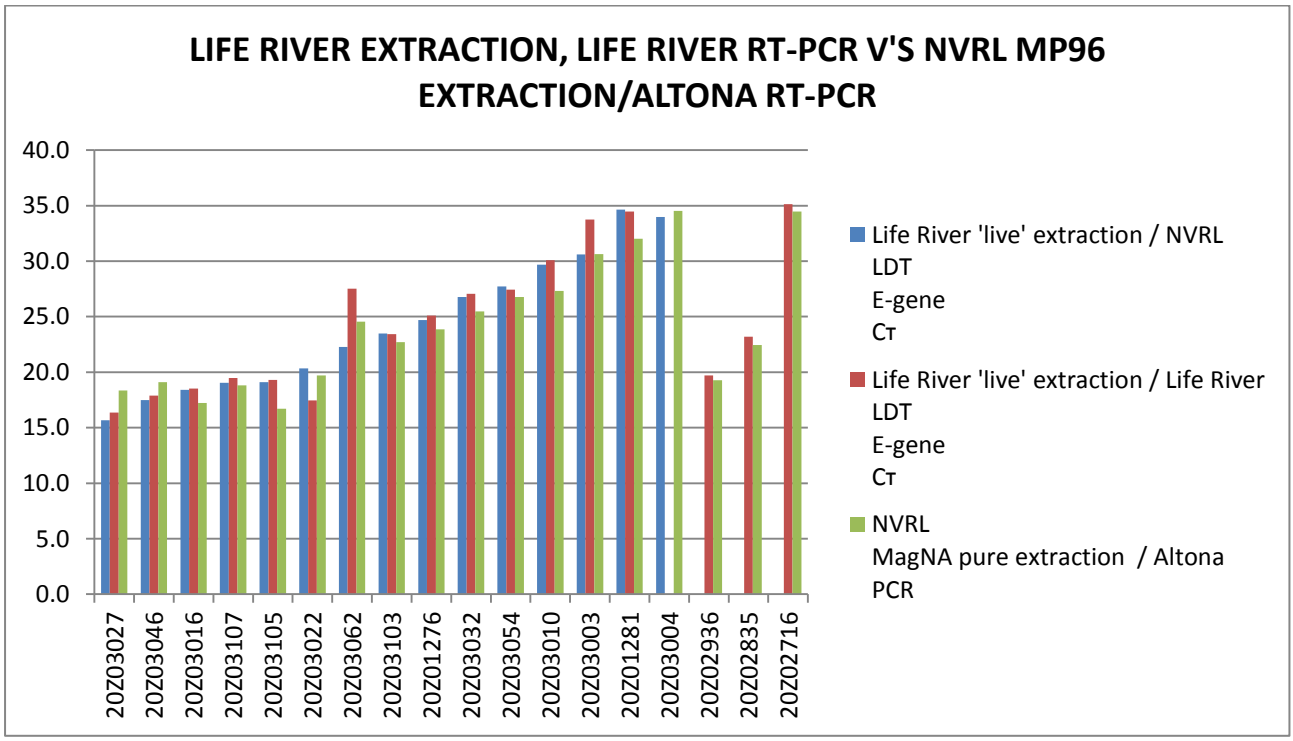
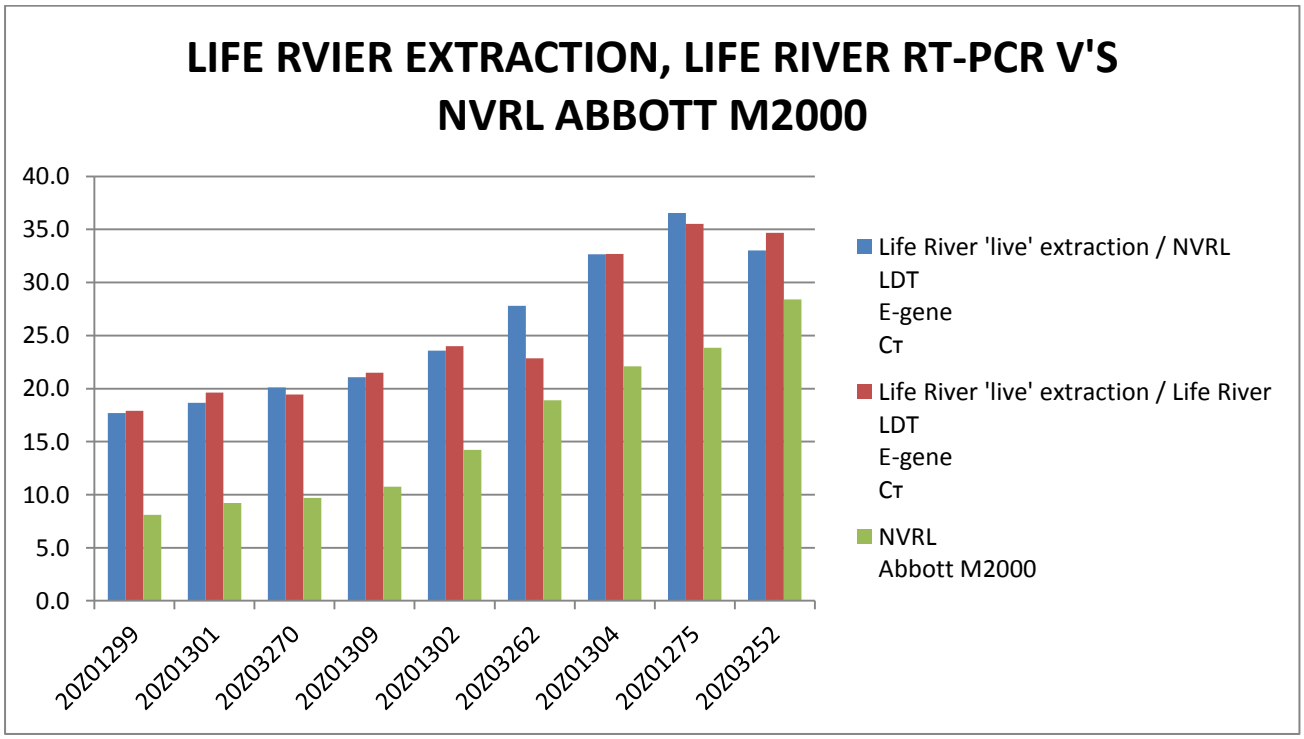


Figure 2:



CONCLUSIONS

- The Life River system is considered suitable for use in both the extraction and RT-PCR processes required for SARS-CoV-2 testing

OBSERVATION / LIMITATIONS / CONSIDERATIONS

- This is a low throughput manual system. There is no barcoding or sample traceability. I have attached a sample run template as a guide when running this assay (Appendix I)

- Unlysed samples must be added to the preloaded extraction plate. Laboratories must have a Class II laminar flow hood and SARS-CoV-2 sample preparation capabilities to use this system. This ensures the safety of staff when processing a 'live' respiratory sample. Technical support is available from China. They confirmed for us that the pre-loaded plate does contain lysis buffer.

SIGNED: Dr ALLISON WATERS **DATE:** 15/04/2020

APPLENDIX 1 – LIFE RIVER EXTRACTION SAMPLE TEMPLATE

LF-MVIR-*** LIFE RIVER EXTRACTION TEMPLATE

RUN DATE		BEFORE SAMPLE ADDITION		AFTER SAMPLE ADDITION	
PERSONNEL		Sample check		Sample check	
PIPETTE ID		Plate volume check		Plate volume check	
MACHINE ID:					

LIFE RIVER PRE-LOADED PLATE	LIFE RIVER PRE-LOADED WELL POSITION	NVRL ID	OUTPUT PLATE WELL POSITION		PRIMARAY SAMPLE HANDLER LIQUID HANDLER FILE INFORMATION		
			ROW	COLUMN	General:Pos	neral:Sample Nar	NCEG
A	A1	20M12345	A	1	A1	20M12345	X
	A2	20M12346	A	2	A2	20M12346	X
	A3	20M12347	A	3	A3	20M12347	X
	A4	20M12348	A	4	A4	20M12348	X
	A5	20M12349	A	5	A5	20M12349	X
	A6	20M12350	A	6	A6	20M12350	X
	A7	20M12351	A	7	A7	20M12351	X
	A8	20M12352	A	8	A8	20M12352	X
	A9	20M12353	A	9	A9	20M12353	X
	A10	20M12354	A	10	A10	20M12354	X
	A11	20M12355	A	11	A11	20M12355	X
	A12	20M12356	A	12	A12	20M12356	X
B	A1	20M12357	B	1	B1	20M12357	X
	A2	20M12358	B	2	B2	20M12358	X
	A3	20M12359	B	3	B3	20M12359	X
	A4	20M12360	B	4	B4	20M12360	X
	A5	20M12361	B	5	B5	20M12361	X
	A6	20M12362	B	6	B6	20M12362	X
	A7	20M12363	B	7	B7	20M12363	X
	A8	20M12364	B	8	B8	20M12364	X
	A9	20M12365	B	9	B9	20M12365	X
	A10	20M12366	B	10	B10	20M12366	X
	A11	20M12367	B	11	B11	20M12367	X
	A12	20M12368	B	12	B12	20M12368	X
C	A1	20M12369	C	1	C1	20M12369	X
	A2	20M12370	C	2	C2	20M12370	X
	A3	20M12371	C	3	C3	20M12371	X
	A4	20M12372	C	4	C4	20M12372	X
	A5	20M12373	C	5	C5	20M12373	X
	A6	20M12374	C	6	C6	20M12374	X
	A7	20M12375	C	7	C7	20M12375	X
	A8	20M12376	C	8	C8	20M12376	X
	A9	20M12377	C	9	C9	20M12377	X
	A10	20M12378	C	10	C10	20M12378	X
	A11	20M12379	C	11	C11	20M12379	X
	A12	20M12380	C	12	C12	20M12380	X