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A rapid and accurate mNGS method for detecting RNA pathogens in BALF samples

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BACKGROUND

In the post-COVID-19 era, the demand for RNA virus detection has significantly increased, drawing renewed attention to metagenomic next-generation sequencing (mNGS) technologies. However, most workflow enabled by commercially available kits are still labor-intensive and difficult to standardize into a streamlined protocol for clinical applications. Moreover, the sample processing steps aiming to reduce host derived RNA such including human ribosomal RNA has been reported to yield varied efficiencies. The low viral load and small viral genome make the detection from ultralow biomass input extremely challenging. We aim to develop a mNGS workflow together with our patented Devin™ host depletion filter for a more streamlined, more sensitive and faster RNA pathogen detection.

METHODS

Inactivated SARS-CoV-2 and RSV-A viruses were spiked into simulated BALF samples. The RNA virus detection workflow integrates the Devin™ host depletion filter with a standard magnetic bead-based RNA purification step to effectively reduce host-derived RNA contamination through a simple 2-minute filtration process. The workflow also includes a high-efficiency reverse transcription module with an optimized conversion rate, and utilizes an ultralow biomass-compatible NGS library preparation system to construct libraries from clinical samples. Sequencing was performed using 150 bp single-end reads, generating 1 Gb of data on the NovaSeq 6000 platform.

RESULTS

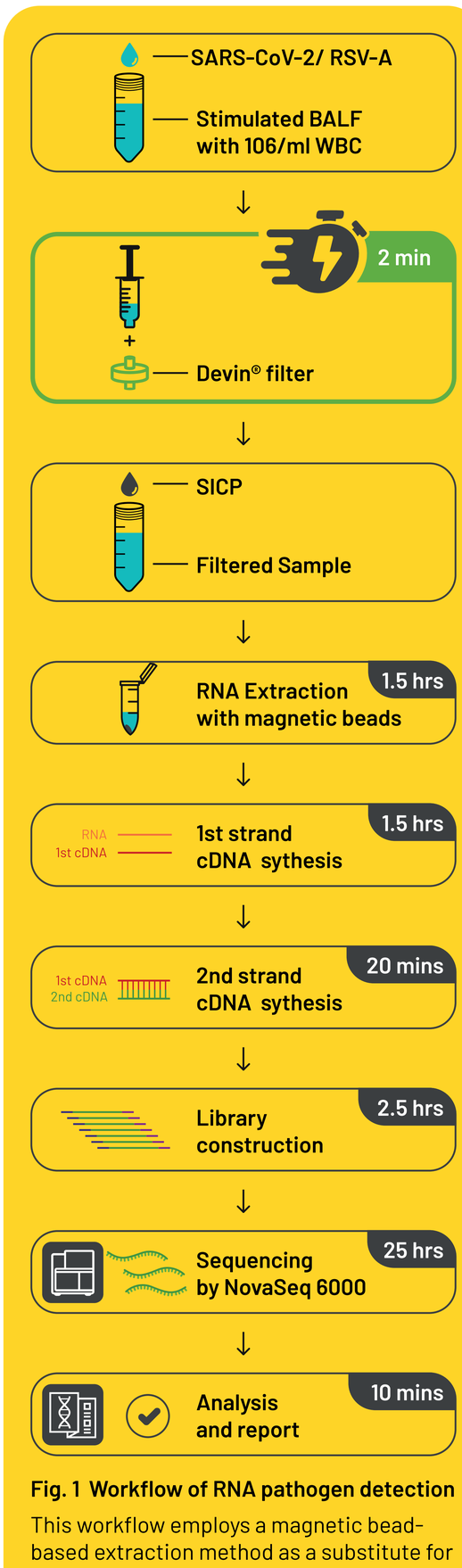

Fig. 1 Workflow of RNA pathogen detection

This workflow employs a magnetic bead-based extraction method as a substitute for the classical Trizol protocol. Additionally, it incorporates 2nd-strand cDNA synthesis to enhance transposase digestion efficiency. From sample preparation to library construction, the entire process can be completed within 6 hours.

Table 1. Comparison of different RNA extraction method

	Trizol	Magnetic Beads	Column
RNA con. (ng/uL)	480	110	30
23s/16s	1.4	1.3	0.0
RQN	10.0	9.1	6.7

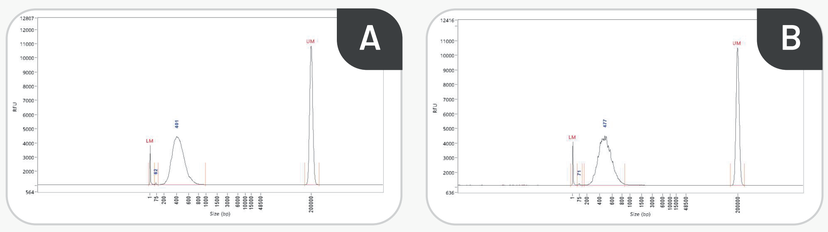

Table 2. Comparison of different cDNA reverse efficiency

	Our reagents	B brand
Input RNA (ng)	300	300
1st cDNA after RT (ng)	273	110
1st RT efficiency (%)	91%	36.7%
2nd cDNA after RT (ng)	411.9	214.5
2nd RT efficiency (%)	137.3%	71.5%

Fig. 2 Fragment analysis of 1st-strand & 2nd-strand cNDA

A shows the fragment size distribution of 1st-strand cDNA, while B displays that of 2nd-strand cDNA. The average fragment sizes were comparable between the two. However, the 2nd-strand cDNA exhibited less defined peaks, suggesting preferential amplification of certain fragments by the polymerase, potentially leading to overrepresentation of specific regions.

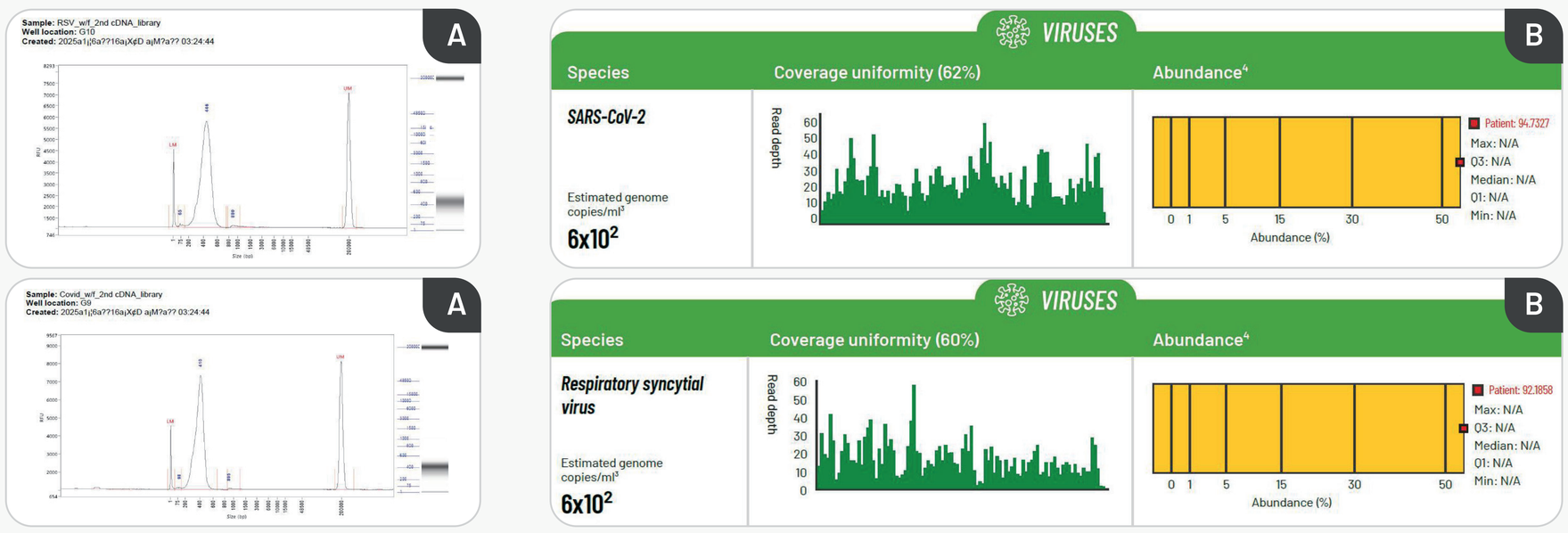

Fig. 3 SARS-CoV-2 and RSV spike-in test

A shows the fragment size distribution of cDNA libraries derived from each spiked in BALF samples, while B displays the corresponding sequencing report. Only 100 copies of inactivated SARS-CoV-2 particles were spiked into the simulated BALF sample. Despite the low input, the sequencing report demonstrated consistent calculated copy numbers and comprehensive genome coverage across the entire viral genome.

Table 3. Reads composition comparison between filtered and unfilted contrived BALF samples spiked with SARS-Cov-2 or RSV

Samples	Contrived BALF w/ Covid				Contrived BALF w/ RSV			
Per million QC reads		%	W/O filter	%		%	W/O filter	%
Allobacillus_halotolerans	842	0.08	3	0	248	0.02	60	0
Imtechella_halotolerans	1,357	0.14	1	0	2,600	0.26	298	0
Sample reads	997,801		999,996		997,152		999,642	
Covid reads	585,649	58.69	269,536	26.95	440,565	44.18	186,952	18.7
human reads	344,840	34.56	728,913	72.89	457,652	45.9	780,190	78.05
Plasmid reads	12,256	1.23	34	0	24,765	2.48	2,982	0.3
Microbial_reads	31,107	3.12	66	0.01	34,794	3.49	11,211	1.12
Unclassified reads	23,950	2.4	1,446	0.14	39,376	3.95	18,308	1.83

DISCUSSIONS

Magnetic bead-based extraction demonstrated superior performance compared to column-based methods in isolating nucleic acids from contrived ultra-low biomass specimens. Although 2nd-strand cDNA exhibited preferential amplification of certain fragments by the polymerase, it did not impact the overall sequencing results. The host cell depletion in the workflow by Devin filter alone, without any other depletion such as human rRNA depletion, did decrease the human reads % and increase viral reads in the sequencing output to enable higher sensitivity for detection and a more streamlined test.

CONCLUSIONS

In hospital diagnostic workflows, a rapid and convenient method for RNA virus detection is essential. This study presents a fast and efficient detection approach that utilizes mNGS technology with host depletion for sensitive RNA virus identification, offering hospital a novel diagnostic solution.

Acknowledgement

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