Novel Human Cell Depletion Method Enables Rapid Pathogen Identification by Next Generation Sequencing

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Introduction

Human DNA depletion is the major bottleneck in rapid pathogen identification and timely treatment. Recently launched Devin® filter and Parti-Seq® platform enable not only fast depletion of host DNA but also rapid pathogen identification within 24 hours upon sample arrival. Objective of this study is to compare effectiveness of currently available depletion methods with Devin® and share results of using PaRTI-Seq® in clinical setting.

Contemporary Host DNA Depletion Methods comparison

Micronbrane utilises its own patent technology of Zwitterionic Interface Ultra-Self-assemble Coating (ZISC) in newly launched Devin® filter which can deplete 95% of human nucleated cells (Fig. 1) in the whole blood within 5 minutes. Devin® has also demonstrated high microbial passing efficiency (Fig. 2) including bacteria as well as viruses.

Fig. 1 Leukocytes reduction efficiency

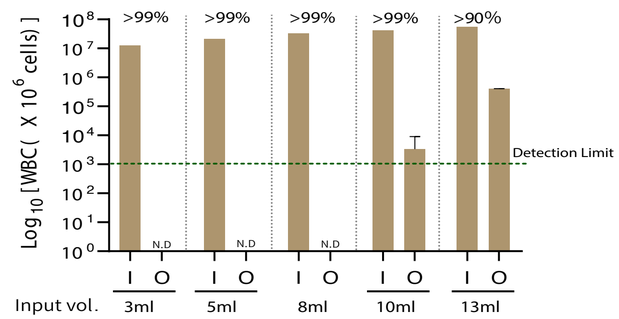


Fig. 2. Microbial passing efficiency

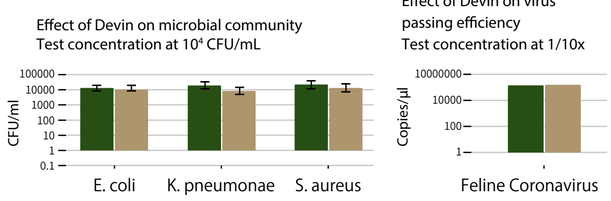
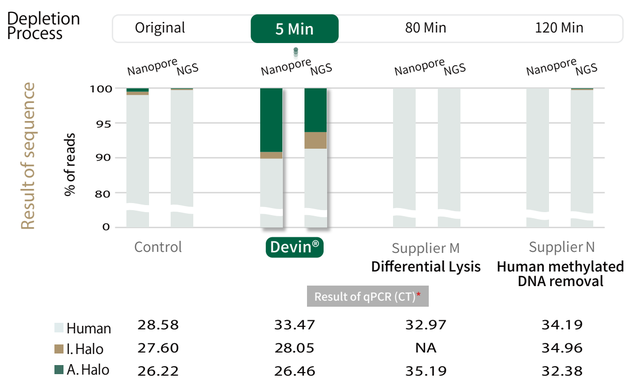
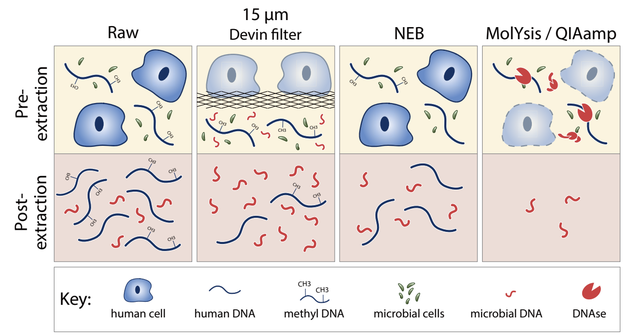


Fig. 4 Comparison of efficiency for different depletion methods



*Results of human and microbial identification using qPCR, NGS and Nanopore. Human blood samples (5mL) were added 10*4 Genome Copies/mL spike-in control from ZYMO Research and then processed using different depletion methods. Test results show that Devin® filter increases ratio of microbial DNA and decreases the ratio of human (host) DNA.

Fig 3. Contemporary depletion techniques



In this study, we compare different host DNA depletion techniques as illustrated in Fig.3 Pre-extraction methods use differentical lysis process to lyse human cells and digest human DNA by DNase first while keeping microorganisms intact. Commercial nuclease-based kits MolYsis® utilizes chemical/enzymatic DNA host depletion and decreases the average proportions of human-originated reads in saliva, sputum, respiratory samples within 40 mins to ~62.88%, ~ 87%, < 10%, respectively, at the same time this method could affect the microbial composition[1]. Similarly, QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany) eliminates host DNA with Benzonase with reported an average human reads in saliva and nasal samples of 29.17% and 57% in treated samples within 100 mins, respectively. The shortcoming of all pre-extraction methods is the removal of intracellular viral materials and the loss of significant part of metagenomic materials.

Post DNA extraction used in commercial kits like NEBNext reportedly removes 94-96% of human reads and increases from 8 to 43-fold reads from microbes compared to un-depleted samples however studies have shown significantly lower practical efficiency of such method.

Host depletion using Devin® filter was compared with two methods mentioned above using whole blood samples from normal individuals spiked with 10^4 Genome Copies/mL of spike-in control (D6320, Zymo Research, Irvine, CA,USA) I. Extracted DNA using different host depletion methods were tested using NGS, Nanopore sequencing as well as qPCR to demonstrate the enrich level of two bacteria A. Halo (Gram+) and I. Halo (Gram-) of the spike-in control. The method with Devin® filter not only takes less than 5 minutes, significantly faster than the other two methods, but also shows much better enrichment results as shown in Fig. 4.

Host DNA Depletion of Devin® and Pathogen Identification Results in Clinical Samples Tested by PaRTI-Seq®

To measure the effectiveness of Devin® filter in the application of pathogen identification, we used NGS-based Pathogen Real Time Identification by Sequencing (PaRTI-Seq®) that Micronbrane developed for rapid pathogen identification within less than 24 hours. Patients admitted to Taipei Veteran General Hospital with suspected infection symptoms were recruited for this IRB-approved study. 7-8 mL of blood were drawn along with the blood draw for the routine blood culture. Half of the whole blood (3-4 mL) was filtered with Devin® blood fractionation filter. All the blood samples were then spiked with Spike-in control I at 10^6 Genome Copies/mL whole blood before subjected to PaRTI-Seq® test.

Briefly, Micronbrane PaRTI-Seq® workflow, as shown in Fig.5, includes microbial enrichment DNA extraction followed by optimized NGS library preparation before sequencing on Illumina platforms at single-read 150bp. The preliminary sequencing results and pathogen detection results of four patients were summarized in Table.1 as below. Pathogens significantly above the level in healthy individuals (internal database of Micronbrane) were identified (wF/healthy) and reported for the blood samples processed with PaRTI-Seq®. Results were consistent for the two samples(C and D) with positive blood culture results. In sample C, PaRTI-Seq® reported more pathogens with higher estimated genome copies in addition to the positive pathogen identified by the blood culture tests. PaRTI-Seq® also identified pathogens in the two samples (A and B) with negative blood culture results. Compared with blood samples without Devin® filter process (woF), pathogens reads in samples with total reads normalized to 5 millions reads, were significantly higher in the filtered samples (wF), increasement ranging from 10-1,000 fold. In addition, the genome copies of detected pathogens can be estimated using the concentration of Spike-in control I. The preliminary results suggested that the detection sensitivity of PaRTI-Seq® can be as low as 10^2 genome copies/mL.

Table 1. Clinical results of pathogen identified by PaRTI-Seq® with Devin® filter in comparison with Blood Culture test

Sample	Pathogen Detected	Reads in 5M	% in Sample	wF/Healthy	woF Reads in 5M	wF/woF	Estimated GC	Blood culture
A	Klebsiella oxytoca	257	0.0051	4.2	34	7.5	$1*10^2$	
	Streptococcus suis	899	0.0180	9.7	118	7.6	$5*10^2$	
B	Stenotrophomonas maltophilia	2828	0.0566	3.2	73	38.9	$2*10^3$	
C	Corynebacterium tuberculostearicum	28389	0.5678	1976.1	43	661.9	$2*10^4$	
	Corynebacterium amycolatum	19270	0.3854	2577.2	26	752.8	$1*10^4$	
	Corynebacterium kefirresidentii	4984	0.0997	270.9	9	555.2	$3*10^3$	
	Corynebacterium aurimucosum	1406	0.0281	113.6	2	650.8	$9*10^2$	
	Corynebacterium minutissimum	390	0.0078	71.3	0	2344.2	$2*10^2$	
	Corynebacterium singulare	271	0.0054	49.3	0	1630.4	$1*10^2$	
	Finegoldia magna	1293	0.0259	236.7	1	1296.8	$8*10^2$	
	Proteus mirabilis	1288	0.0258	106.9	1	968.6	$8*10^2$	+
	Staphylococcus hominis	983	0.0197	180.0	1	1183.2	$6*10^2$	
	Staphylococcus haemolyticus	764	0.0153	63.4	0	2298.1	$5*10^2$	
D	Enterococcus faecalis	4317	0.0863	130.4	447	9.7	$4*10^4$	+

Fig. 5 Workflow of PaRTI-Seq®
(Pathogen Real Time Identification by Sequencing)

Conclusions

Devin® blood fractionation filter is a novel device and method can be applied to efficiently deplete human cellular DNA background and enrich microorganisms in the whole blood samplesin less than 5 minutes. PaRTI-Seq® test, a metagenomic sequencing workflow built upon Devin® filter as illustrated in Fig. 5, can identify potential pathogens from blood samples within 24 hours with a sensitivity of 10^2 genome copies/mL.

References

1. Clarisse A. Marotz, Jon G. Sanders, Improving saliva shotgun metagenomics by chemical host DNA depletion; 2018 February 27.

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