Micronbrane Medical
Application note

PaRTI-Seq™ Workflow for Samples of Small Volume

Background

Endophthalmitis is the most challenging complication seen in ophthalmic practice. Culture of intraocular specimens like aqueous humour, vitreous humour and vitrectomy fluid, is still considered the gold standard in the diagnosis of endophthalimitis. However, the diagnostic yield of culture method is only 25-60% of the clinically diagnosed typical cases (Long C 2014)(Nicoara SD 2014).

Recent study showed that metagenomic next-generation sequencing (mNGS) has a higher positive rate of identifying pathogens in endophthalmitis than in culture (Zhu, et al. 2022). The positive rates of mNGS and culture were 88.89% (32/36) and 27.78% (10/36), respectively (Zhu, et al. 2022). *Staphylococcus epidermidis, Streptococcus pneumoniae,* and *Klebsiella pneumoniae* were the most pathogenic bacteria in traumatic, postoperative, and endogenous endophthalmitis, respectively.

Intraocular fluids for microbial testing in endophthalmitis cases are usually limited in sample amounts, which render the sample processing rather challenging. Devin™ Fractionation Filter utilizes Zwitterionic interface Ultra-Self-assemble Coating Technology (ZISC Technology) to specifically binds to human nucleated cells in the human biospecimen while the 15-20 μm membrane pores allow other components including microorganisms to pass through. The objective of this study is to test if the sample of small volume can be processed with PaRTI-Seq workflow with a modified protocol.

Material and Methods

A sample of vitreous humour (labelled VH; from infected eye) and an eye swab (labelled UI; from uninfected eye) were collected. The eye swab was intended as an "uninfected" control, representative of the microflora of the patient's eye surface, for comparison with the intraocular VH sample. The samples were dispatched immediately after collection and transported to Micronbrane's laboratory at temperature below 10°C.

The fluidic samples were processed with a modified protocol using Devin™ Fractionation Filter for samples of small volumes as illustrated in **Fig. 1**. First, the Devin™ filter was pre-wet with 500 μL of PBS. To the 50 μL VH sample received, 450 ul of PBS was added to make up to a volume of 500μL. The eye swab was immersed in 500 μL PBS. Both samples were then filtered through Devin™ filter using 3 mL syringe. Another 500 μL PBS wash was applied to filter to collect filtrations of both steps.

The final volume of the sample after filtration was about 1.0ml. The samples were then subject to centrifugation at 16,000g for 15 mins. DNA was purified from the microbial pellet and constructed into NGS library using Unison™ ultralow DNA NGS library kit for Illumina platforms*.

A non-template control (NTC) was prepared in parallel from the time of DNA extraction. Approximately 20 million NGS reads were obtained for both samples and analysed by in-house bioinformatic pipeline. The diagnostic performance was compared between mNGS and culture results from the same sample.

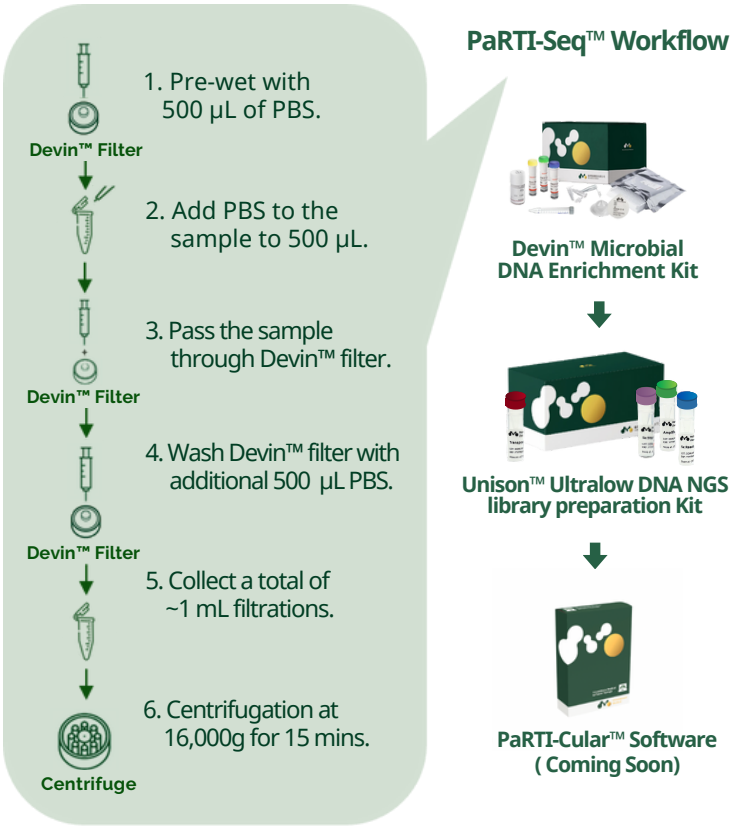

Fig. 1 PaRTI-Seq™ workflow with modified protocol for the liquid biopsy samples of small volume (< 500 μL) described in detail

*All the reagents and consumables required for DNA extraction and library construction for the PaRTI-Seq™ workflow are supplied by Micronbrane.

Result

NGS sequences of 100bp were used for the analysis in this application. The pipeline carries out human DNA subtraction and reports the number of microbial reads (Micro_Reads) mapped to the ~1400 microorganisms in the database. The pipeline also reports the percentage of Microbial reads (Micro_%) of each species among all the microorganisms reads.

The results of primary analysis of the samples and NTC are shown in **Table 1** below. The Micro_%(SPL): Micro_%(NTC) ratio of each species is computed for both samples. The top 10 species with the highest ratio is listed in **Table 1**. A threshold of 10 was arbitrarily set as the threshold for a valid positive call. As a result, the top positive call was *Klebsiella pneumoniae*, which was concordant with the identification of the same organism by microbial culture.

A plot of the Micro_%(SPL): Micro_%(NTC) ratio in the VH sample **(Fig. 2)** shows that a true positive is likely to have ratios of up to 1000 and can be easily identified from background noise. Interestingly, other *Klebsiella* spp such as *K. quasipneumoniae* and *K. varicola* were also called alongside *K. pneumoniae*. The alignment to these non-*K. pneumoniae Klebsiella* spp are probably due to sequences that are conserved across the *Klebsiella genus* and sequences were aligned using only 100 bp of read length.

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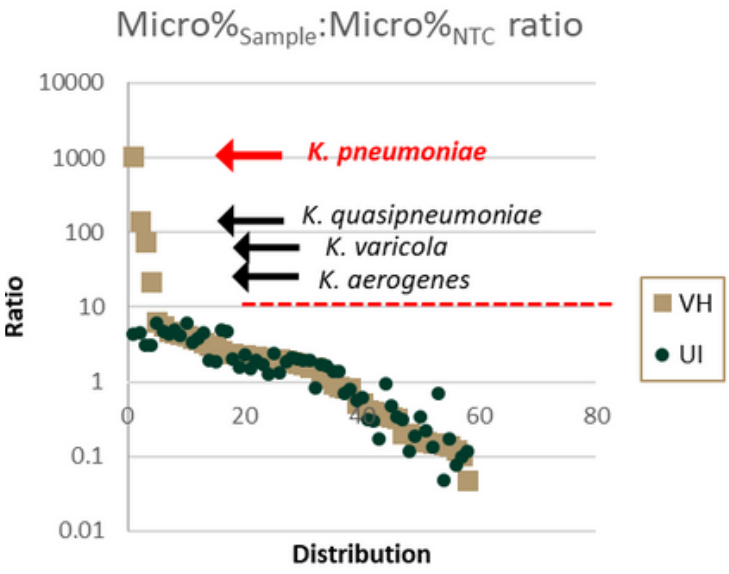

Fig 2. Plot of Micro%Sample:Micro%NTC ratio in VH and UI

However, as (Schlaberg 2017) pointed out, it is not possible to distinguish between partial coverage due to low bacterial genome copies and that due to conserved sequences. Another possibility is that these represent genuine species heterogeneity in a mixed infection that is easily missed by traditional culture.

Conclusion

The Endophthalmitis Vitrectomy Study (EVS) in 1995 showed that nearly 30% of vitreous culture specimens were culture negative (Group 1995). Subsequent studies have had more variable success, with culture-positive rates ranging from 38 to 64% from post trauma endophthalmitis for example (Long C 2014) (Nicoara SD 2014). The actual clinical performance of mNGS for eye infection is not known but is likely to have a higher diagnostic yield (ie. positive rates) as compared to traditional methods, like studies with other sample types have shown.

This study highlighted that small volume sample can be processed with PaRTI-Seq™ workflow with a modified protocol. In conclusion, we demonstrated Devin™ filter can be used to process biospecimen of small volume as low as 50 µL. The following DNA extraction and NGS library preparation reagents in PaRTI-Seq™ workflow were all compatible with low sample inputs such as vitreous humour samples. By extension, PaRTI-Seq™ is likely to be compatible with other scare samples such as CSF.

Table 1: Primary analysis results of VH and UI samples compared with NTC. Top 10 species with the highest Micro_%(SPL):Micro_%(NTC) ratio (largest to smallest) is listed. A threshold of 10 was arbitrarily set as the threshold for a valid positive call. Positive calls are highlighted in yellow

Organism	Sample VH			Sample UI			NTC	
	Micro_Reads	Micro_%	Micro_%(SPL): Micro_%(NTC) ratio	Micro_Reads	Micro_%	Micro_%(SPL): Micro_%(NTC) ratio	Micro_Reads	Micro_%
Klebsiella pneumoniae	2689	3.9795	1047.24	13	0.0168	4.42105	116	0.0038
Klebsiella quasipneumoniae	354	0.5239	141.595	13	0.0168	4.42105	112	0.0037
Klebsiella variicola	399	0.5905	74.7468	19	0.0246	3.11392	239	0.0079
Klebsiella aerogenes	127	0.1879	21.5977	21	0.0272	3.12644	264	0.0087
Citrobacter amalonaticus	34	0.0503	6.36709	37	0.0479	6.06329	240	0.0079
Malassezia globosa	31	0.0459	5.53012	31	0.0401	4.83133	253	0.0083
Burkholderia contaminans	10917	16.1561	4.70091	11311	14.629	4.25658	104592	3.4368
Mycobacteroides saopaulense	46	0.0681	4.57047	58	0.075	5.03356	453	0.0149
Mycobacteroides franklinii	368	0.5446	4.41687	396	0.5122	4.1541	3752	0.1233
Pseudomonas fulva	30	0.0444	4.18868	51	0.066	6.22642	322	0.0106

References

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