Feasibility of Unbiased Metagenomic Next-Generation Sequencing (mNGS) for Urinary Tract Pathogen Detection from Symptomatic Patients

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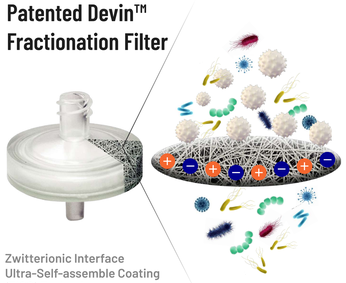
Background

- The etiology of Urinary Tract Infections (UTIs) is varied, but in approximately 90% of cases, enteric bacteria are implicated, especially *Escherichia coli*, which produces more than 70% of these infections. Other urinary tract pathogens are *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Pseudomonas* spp., *Enterococcus* spp., and *Staphylococcus saprophyticus*.
- Although urine culture is still the gold standard for the microbiological confirmation of UTIs, it takes 24 to 48 h to provide results and detects as little as 12% of clinically significant species that can cause infections[1]. There is an urgent clinical need for rapid screening methods.
- Metagenomic Next-Generation Sequencing (mNGS) has emerged as an enabling technological platform for the detection of microorganisms in clinical samples. The major challenge of mNGS is host-cell contamination. Micronbrane Medical's workflow incorporates a proprietary host depletion method that has been proven to improve the sensitivity of bacterial DNA detection by reducing sequencing real estate loss due to the sequencing of host DNA. The objective of this study is to investigate the applicability of this mNGS workflow to urine samples.

Patented Devin™ Fractionation Filter

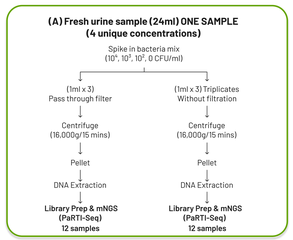


1. *J Clin Microbiol* 54(5). 1216-1222.

Methods

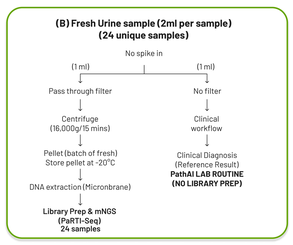
Part A: Does the host depletion filter work in urine samples spiked with bacteria?

Negative urine specimens were spiked with pathogens at known concentrations (10^4, 10^3, 10^2, 0 GE/ml). The ability of the Devin™ filtration device to deplete human neutrophils was tested, by testing equal fractions, one with cell depletion with Devin, and another as unfiltered control.



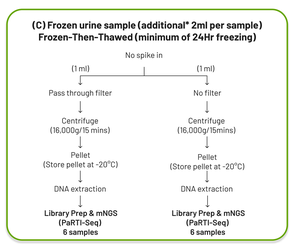
Part B: Are mNGS results concordant with lab routine testing (in-house PCR)?

Metagenomic NGS results were checked against routine clinical practice (PCR), which served as the reference standard to test clinical correlation.



Part C: Does the filter work for Frozen-Then-Thawed urine samples?

To test the efficacy of the workflow for archival frozen urine samples, remaining urine from a subset of clinical specimens were frozen for defined durations, with the experiment repeated using such samples after they are retrieved and thawed. All samples were sequenced by mNGS and interpreted using a customized software.

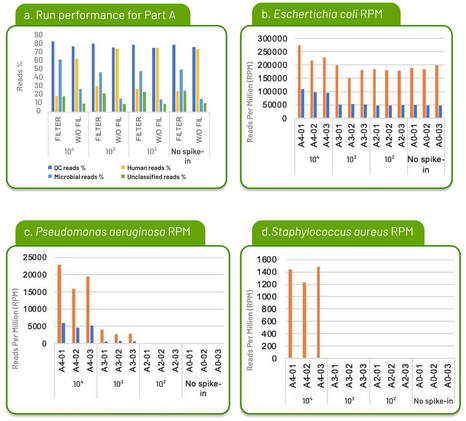


Results

Part A: Does the host-depletion filter work in urine samples spiked with bacteria?

- Host depletion filtration led to reduction in human reads (%) and increased microbial reads (%) **(Figure 1 a.)**
- *P. aeruginosa* could be detected down to 10^2 GE/ml. However, *S. aureus* could be detected at 10^4 GE/ml with filter but was undetected in unfiltered samples **(Figure 1 c.–d.)**
- 10^3 GE is approximately 10pg for both *P. aeruginosa* and *S. aureus*
- The use of host depletion filter significantly enhanced the RPM, increasing the confidence of detection of both *P. aeruginosa* and *S. aureus*
- High background of *E. coli* did not permit evaluation of detectability of *E. coli*

Figure 1



Part B: Are mNGS results concordant with lab routine testing (in-house PCR)?

- mNGS was able to detect all microorganisms that were detected by culture **(Table 1)**. PCR missed detection of *Serratia marcesans* in USB-11 which was detected by mNGS
- 8 out of 9 *E. coli* samples were successfully detected by mNGS. One case that was missed had borderline Ct-value of 30.42
- 4 out of 4 *K. pneumonia* samples were successfully detected by mNGS
- The predominant species may preclude the detection of others present at lower frequencies due to PCR amplification bias during library preparation
- mNGS was able to detect more organisms in samples that were negative by PCR **(Table 2)**

Table 1

Study ID	PCR Status	Ct	Microbial Culture Result	PathAI Standard Result	Taxonomy	Raw Reads (genome)	%	Reads Per Million
USB-01	POS	2.15	>100,000 cfu/ml Multiple organisms indicative of urogenital flora	*E. faecalis, 18.27*	*Enterococcus faecalis*	1,852	0.21	644
				E. coli, 15.94	*Escherichia coli*	338,417	38.48	117,685
				K. oxytoca, 25.84	Not detected	562	0.06	195
				K. pneumoniae, 21.08	*Klebsiella pneumoniae*	5,154	0.59	1,792
USB-04	POS	5.68	>100,000 cfu/ml ESCHERICHIA COLI	*E. coli, 15.51*	*Escherichia coli*	150,984	42.13	53,507
USB-06	POS	21.60	>100,000 cfu/ml ESCHERICHIA COLI	*C. albicans, 20.85*	Not detected	4	0.00	1
				E. coli, 22.48	*Escherichia coli*	694,339	42.23	180,687
				K. pneumoniae, 29.79	*Klebsiella michiganensis*	3,308	0.20	861
USB-07	POS	0.48	> 1000 cfu/ml < 10,000 cfu/ml Multiple organisms indicative of urogenital flora	*E. faecalis, 26.10*	Not detected	4	0.23	1
USB-10	POS	0.31	> 10,000 cfu/ml, <50,000 cfu/mL Multiple organisms indicative of urogenital flora	*E. faecalis, 26.99*	*Enterococcus faecalis*	109	1.54	45
				E. coli, 26.94	*Escherichia coli*	752	10.60	312
				U. urealyticum, 24.84	*Ureaplasma urealyticum*	862	12.15	357
USB-11	POS	2.41	>100,000 cfu/ml *Serratia marcescens* >100,000 cfu/ml *Pseudomonas aeruginosa*	*P. aeruginosa, 20.46*	*Pseudomonas aeruginosa*	8,363	1.87	2,634
				Not detected	*Serratia marcescens*	400,070	89.29	126,019
				E. faecalis, 30.73	Not detected	3	0.00	1
				E. coli, 30.42	Not detected	188	0.04	59
USB-12	POS	0.28	> 50,000 cfu/mL <100,000 cfu/mL *Klebsiella pneumoniae* ssp *pneumoniae*	*E. faecium, 28.69*	Not detected	323	0.01	97
				U. urealyticum, 30.75	Not detected	30	0.00	9
				K. pneumoniae, 30.52	*Klebsiella pneumoniae*	1,373,333	52.06	412,971
USB-14	POS	0.58	> 10,000 cfu/mL <50,000 cfu/mL ESCHERICHIA COLI	*E. coli, 22.50*	*Escherichia coli*	103,381	42.93	39,333
USB-15	POS	8.16	>100,000 cfu/ml *Klebsiella pneumoniae* ssp *pneumoniae*	*M. hominis, 23.39*	Not detected	NOT IN DATABASE		
				E. faecalis, 28.34	Not detected	212	0.00	39
				K. pneumoniae, 28.09	*Klebsiella pneumoniae*	4,073,430	86.82	741,651
USB-18	POS	1.41	> 50,000 cfu/mL <100,000 cfu/mL ESCHERICHIA COLI	*E. faecalis, 24.59*	Not detected	17	0.00	5
				E. coli, 24.55	*Escherichia coli*	242,275	40.55	68,031
				U. urealyticum, 26.93	Not detected	15	0.00	4
USB-19	POS	0.67	>100,000 cfu/ml Multiple organisms indicative of urogenital flora	*S. saprophyticus, 18.13*	*Staphylococcus saprophyticus*	171,053	76.35	52,933
				U. urealyticum, 26.39	Not detected	41	0.02	13
USB-21	POS	0.64	> 10,000 cfu/mL <50,000 cfu/mL ESCHERICHIA COLI > 1000 cfu/ml < 10,000 cfu/ml Enterococcus faecalis	*E. faecalis, 24.38*	Not detected	224	0.06	37
				E. coli, 17.60	*Escherichia coli*	143,113	41.19	23,896
USB-23	POS	2.76	> 10,000 cfu/mL <50,000 cfu/mL Multiple organisms indicative of urogenital flora	*E. faecalis, 27.01*	Not detected	2	0.00	0
				U. urealyticum, 28.76	Not detected	0	0.00	0
USB-25	POS	0.31	>100,000 cfu/ml Multiple organisms indicative of urogenital flora	*E. faecalis, 24.75*	*Enterococcus faecalis*	1,174,843	56.45	390,071
				E. coli, 23.62	*Escherichia coli*	364,204	17.50	120,923

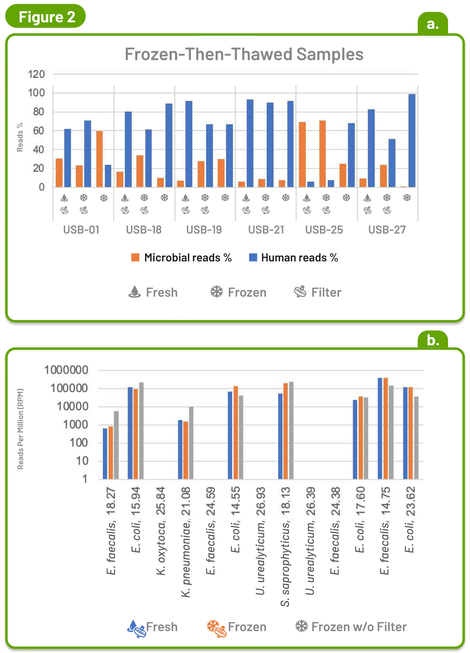
Table 2

Study ID	PCR Status	Microbial Culture	Taxonomy	Raw Reads (genome)	%	Reads Per Million	Genome Copies/mL	Possibility
USB-02	NEG	No growth at 2 days	*Bradyrhizobium sp. SK17*	22,876	50.84	113,308	188	high
			Sphingomonas sp. CL5.1	1,854	4.12	9,183	15	high
USB-03	NEG	No growth at 2 days	*Bradyrhizobium sp. SK17*	504	43.94	167	108	high
			Aspergillus versicolor	153	13.34	51	33	high
USB-05	NEG	> 1000 cfu/ml < 10,000 cfu/ml Coagulase negative Staphylococcus No further workup	*Bradyrhizobium sp. SK17*	12,904	40.94	115,310	104	high
			Aspergillus versicolor	1,406	4.46	12,564	11	high
USB-09	NEG	> 10,000 cfu/mL <50,000 cfu/mL Multiple organisms indicative of urogenital flora	*Lactobacillus jensenii*	880,916	46.36	381,183	56,460	high
			Lactobacillus gasseri	625,924	32.94	270,845	40,117	high
			Lactobacillus crispatus	308,340	16.23	133,423	19,762	high
USB-13	NEG	N/A	*Sneathia sanguinegens*	77,577	27.90	29,456	1,018	high
			Hoylesella buccalis	54,863	19.73	20,832	720	high
			Aerococcus christensenii	28,138	10.12	10,684	369	high
			Prevotella bivia	19,842	7.13	7,534	260	high
			Prevotella disiens	12,228	4.40	4,643	161	high
USB-17	NEG (Only tested for CT/NG, *T. vaginalis, M. genitalium*)	N/A	*Klebsiella pneumoniae*	2,258,970	73.63	527,737	729,359	high
			Raoultella ornithinolytica	164,706	5.37	38,478	53,179	high
			Klebsiella variicola	122,831	4.00	28,696	39,659	high
USB-20	NEG	N/A	*Enterococcus faecalis*	167,788	47.46	63,909	17,623	high
			Finegoldia magna	23,893	6.76	9,101	2,510	high
			Escherichia coli	18,779	5.31	7,153	1,972	high
USB-22	NEG	N/A	*Lactobacillus iners*	33,545	38.63	16,923	362	high
			Lactobacillus crispatus	16,537	19.05	8,343	178	high
			Aspergillus versicolor	4,869	5.61	2,456	53	high
USB-26	NEG	N/A	*Hoylesella buccalis*	202,720	50.76	60,407	27,657	high
			Prevotella bivia	40,776	10.21	12,151	5,563	high
			Prevotella disiens	37,187	9.31	11,081	5,073	high
			Prevotella corporis	27,202	6.81	8,106	3,711	high
USB-27	NEG	N/A	*Lactobacillus iners*	36,898	29.24	26,681	362	high
			Lactobacillus crispatus	17,725	14.05	12,817	174	high
			Aspergillus versicolor	17,387	13.78	12,573	171	high

Part C: Does the filter work for Frozen-Then-Thawed urine samples?

- Microbial reads % and human reads % were comparable between fresh with filter and frozen then thawed with filter samples
- Detection of pathogens in fresh with filter, frozen then thawed with filter, and frozen then thawed without filter samples, were 100% concordant
- The use of frozen then thawed samples did not adversely affect the efficacy of the host depletion filtration nor the calling of positive microbes in these samples

Figure 2



Conclusion

Part A: Does the host depletion filter work in urine samples spiked with bacteria?

Yes, the effect of host depletion and enrichment of relevant bacterial reads were readily observed in spiked-in experiments using known bacteria.

Part B: Are mNGS results concordant with lab routine testing (in-house PCR)?

A range of concordance was observed, with good concordance observed for *E. coli* and *K. pneumoniae*. This may be caused by difference in extraction efficiencies or analytical sensitivity between mNGS and PCR.

Part C: Does the filter work for Frozen-Then-Thawed urine samples?

Yes, the enrichment of microbial reads and detection of microbial species was not adversely affected by freezing and thawing of urine specimens.

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