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Novel Spatial Multiplex Screening of Uropathogens Associated with Urinary Tract Microbiota Research using a Nanofluidic qPCR Platform

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ABSTRACT

Introduction: Accurate identification of uropathogens in a timely manner is important to correctly understand urinary tract infections (UTI's), which affects nearly 150 million people each year. The current standard approach for detecting the UTI pathogens is culture based. This method is time consuming, has low throughput, and can lack sensitivity and/or specificity. In addition, not all uropathogens grow equally well under standard culture conditions which can result in a failure to detect the species. To address these gaps, we have developed a unique workflow from sample preparation to target identification using the nanofluidic OpenArray™ platform for spatial multiplexing of target specific assays. In this study, we tested pre-determined blinded research samples and confirmed the subset of results with orthogonal Sanger sequences.

Methods: The in-house solution allows for the detection of 17 uropathogens including 16 bacterial and 1 fungal target. All assays have been verified with different sample types including synthetic plasmid control and ATCC gDNA samples for sensitivity and specificity testing. More than 120 pre-determined blinded samples from relevant sources were processed using the MagMAX DNA Multi-Sample Ultra Kit on the KingFisher Flex platform and screened by a nanofluidic qPCR platform using target specific TaqMan® pathogen detection assays(Table 1).

Results: More than one pathogens were detected simultaneously in most of the samples using our research assays and nanofluidic platform. We observed greater than 98% concordance with the result generated at different site using the different OpenArray™ build with most of the assays similar to our panel. Results were highly reproducible between two different geographical sites. To confirm the accuracy of the OpenArray™ plate results we further investigated the subset of samples with orthogonal testing using capillary electrophoresis DNA sequencing. We observed 100% concordance for the sample tested. This further testing demonstrated that our workflow for UTI related pathogens detection is accurate.

Conclusions: Based on these study results, we concluded that our application produced highly concordant results that are more sensitive and accurate. In summary, we have developed highly efficient, cost-effective research application for urinary tract microbiota pathogen profiling using high performance verified assay for each microorganism.

MATERIALS AND METHODS

Organism Type	Targets	Gram Positive/Negative
Yeast	Candida albicans	
Bacteria	Acinetobacter baumannii	Gram Negative
Bacteria	Citrobacter freundii	Gram Negative
Bacteria	Klebsiella aerogenes	Gram Negative
Bacteria	Enterobacter cloacae	Gram Negative
Bacteria	Enterococcus faecalis	Gram Positive
Bacteria	Enterococcus faecium	Gram Positive
Bacteria	Escherichia coli	Gram Negative
Bacteria	Klebsiella oxytoca	Gram Negative
Bacteria	Klebsiella pneumoniae	Gram Negative
Bacteria	Morganella morganii	Gram Negative
Bacteria	Proteus mirabilis	Gram Negative
Bacteria	Proteus vulgaris	Gram Negative
Bacteria	Providencia stuartii	Gram Negative
Bacteria	Pseudomonas aeruginosa	Gram Negative
Bacteria	Staphylococcus saprophyticus	Gram Positive
Bacteria	Streptococcus agalactiae	Gram Positive

Table 1: Urinary Tract Research Pathogen Detection Assay Collection : The panel consist of 17 species specific assays which are closely associated with urinary tract infection including 13 Gram negative bacteria, 3 Gram positives bacteria and 1 fungal target.

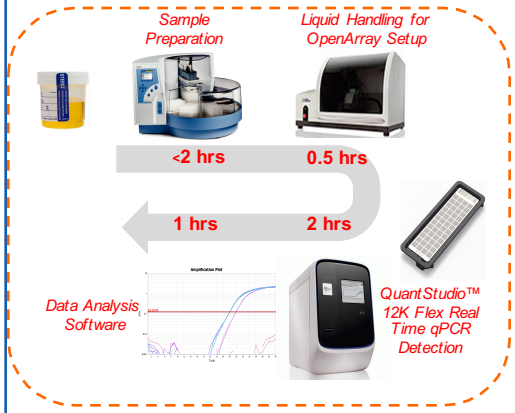

Figure 1: Complete Workflow Solution: Sample to answer in less than 5.5 hours. Each OpenArray™ plate consists of 48 subarrays each containing 56 TaqMan™ assays spotted according to customer's specifications. Up to 4 OpenArray™ plates can be included in a single QuantStudio™ 12K Flex run allowing for a throughput of 192 samples.

RESULTS

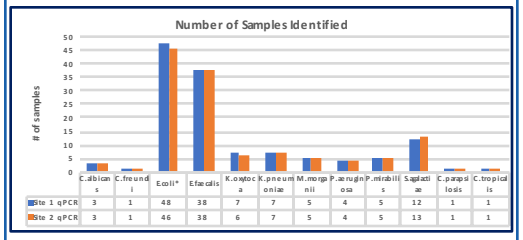

Figure 2: Number of Sample Identified: Each urine samples underwent nucleic acid extraction using MagMAX™ Kit and the extracted DNA samples were run using OpenArray™ plates at two different geographical sites. qPCR results were highly reproducible between two sites with >98% concordance

*E.coli assay was different in both OpenArray™ panel

Sample Name	C.albicans	C.freundii	E.coli	E.faecalis	K.oxytoca	K.pneumoniae	M.morganii	P.mirabilis	S.agalactiae
PUX17-000345			+						
PUX17-000346			+	+	+				
PUX17-000348				+		+			
PUX17-000453			+	+		+		+	
PUX17-000452						+			
PUX17-000442					+	+			
PUX17-000563								+	+
PUX17-000362			+				+		+
PUX17-000562		+	+						
PUX17-000349									
PUX17-000363			+						+
PUX17-000365			+						+
PUX17-000451			+	+				+	+
PUX17-000574			+	+		+	+	+	
PUX17-000577	+			+					
PUX17-000580			+	+	+				
PUX17-000764			+	+					+

Table 2: Multiplex Research Screening of Urinary Tract Microbiota on the OpenArray™ Platform. Subset of sample results for OpenArray™ are shown as presence(+) or as absence (blank cell) for respective uropathogens. The OpenArray results from 2 different geographical sites were identical for this subset of samples and pathogens detected in both sites were highlighted in green. Both sites were able to detect multiple pathogens including fungal and bacterial targets which were hard to culture

Research Samples	Assays for CE Sequencing	Confirmed by both site qPCR	Confirmed by Sanger Sequencing
PUX17-000566	S.agalactiae	YES	YES
PUX17-000574	K.pneumoniae	YES	YES
	P.mirabilis	YES	YES
	E.faecalis	YES	YES
PUX17-000577	E.faecium	YES	YES
	C.albicans	YES	YES
PUX17-000580	K.oxytoca	YES	YES
	K.pneumoniae	NO	NO
PUX17-000764	E.coli	YES	YES
	E.faecalis	YES	YES
	S.agalactiae	YES	YES
PUX17-000777	E.coli	YES	YES
	E.faecalis	YES	YES
PUX17-000375	S.aureus	YES	YES

Table 3: Orthogonal Testing of Samples by Sanger Sequencing to Confirm OpenArray™ Results: Sequencing was used to confirm results obtained using OpenArray™ qPCR for 7 different samples tested. These results indicate that OpenArray™ qPCR can be used to accurately identify the correct pathogens.

CONCLUSIONS

- All assays for urinary tract research pathogen detection were verified for sensitivity, specificity, and reproducibility using plasmids , target specific genomic DNA controls and repository cultured samples
- OpenArray™ data was verified as having a high degree of accuracy by qPCR and sequencing.
- OpenArray™ qPCR using urinary tract microbiota research assays are accurate in identifying multiple pathogens in a single sample
- Our results demonstrate that multiplex profiling on Nanofluidic TaqMan™ OpenArray™ platform along with MagMAX™ sample prep is a successful method for detection of pathogens associated with human urinary tract microbiota imbalance.

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TRADEMARKS

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