Targeted NGS Assay Kit in Unidentified Bacterial Urinary Tract Infections

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Abstract

Recurring and recalcitrant urinary tract infections remain an ongoing and unique healthcare challenge. Organisms often exhibit fastidious or unusual behaviors that contribute to the difficulty in a diagnosis. In the absence of a clear diagnosis, physicians frequently resort to an empiric therapeutic approach that may or may not yield a positive patient outcome. The first step in a successful treatment strategy is to detect and identify the nature of a putative infection. Urinary tract infections (UTIs) may arise from a variety of infectious etiologies including eubacteria, protozoa, and fungi. Microbial culture is widely considered the gold standard that enables isolation and identification of microbes; however, the time to results and the inability to detect and identify fastidious organisms remains problematic. While molecular detection methods (including quantitative and real time-PCR-based methods) have supplemented these deficits by reducing the turn-around-time and false-negative results, urinary tract infections still exhibit a 10-30% false-negative rate. In this study, we utilized NGS-based methods to detect and characterize unidentified microbes in 49 samples shown to be negative by PCR-based panels. Our results demonstrate that targeted PCR is insufficient to detect microbes in putative UTIs including microbes that would have been predicted to be detectable.

Sample Selection and Preparation

A total of 49 samples were collected from de-identified participants with diagnosed UTIs. Samples were residual samples that were previously tested by a quantitative PCR UTI screening panel (9 bacterial pathogens, 1 protozoal pathogen, 4 fungal pathogens, and 22 antibiotic resistance genes). All samples failed to yield a positive result even with qualitative features (turbidity, color, sediment, red and/or white-blood cells, and odor) suggesting the samples contained microbes. These samples were shipped on ice to the NGS laboratory where up to 8 mL of sample was processed via ultracentrifugation to generate a pellet of up to 200 µL in volume. With all supernatant removed, the resulting pellet was processed via the Qiagen DSP Blood Mini kit according to the protocol, except for substituting the urinary pellet for the intended blood sample. The resulting purified DNA was eluted into 30 µL AE buffer.

16S Microbial ID Kit

The 16S Microbial ID Kit (BioID Genomics) instructions were followed as described (Figure 1). A total of 1 µL DNA was loaded into each pair of wells in Amplification Plate 1 and 2. After the PCR amplification, two amplicon pools per sample were generated. An Illumina MiSeq was used with a Standard v2 Flowcell (Illumina) to sequence the subsequent amplicons. Data was automatically uploaded into BaseSpace and passed to the BioID Genomics analysis pipeline using the *BaseSpace BioID Linker* application. To ensure proper assay performance, Indices 1 and 2 were assigned to a urine extraction control that is spiked with a sequence-defined *Escherichia coli* in addition to an amplification control of sequence-defined *Gamella haemolysans* (both ATCC).

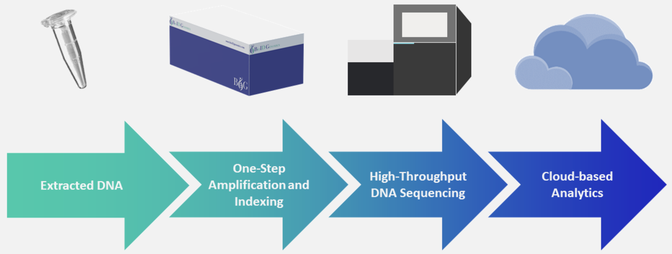

Figure 1 – The 16S Microbial ID Kit workflow according to the kit instructions.

Sequence Results

The submitted sequencing run generated a significant amount of high quality and indexable reads (Table 1) across both amplification plates as intended (Figure 2). The color-coded well pairs reflect the relative abundance of amplicons obtained for a specific DNA sample. The extraction and amplification controls were assigned to Index 1 and Index 2, respectively. The most abundant identified microbes and putative pathogens were tabulated (Table 2).

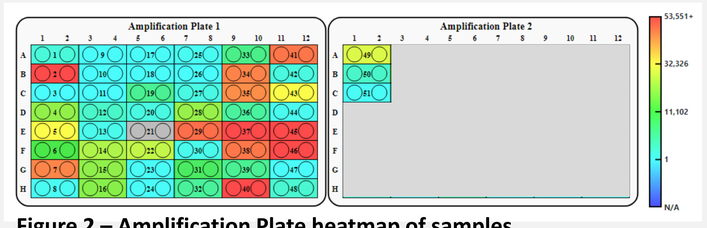

Figure 2 – Amplification Plate heatmap of samples.

Table 1 - Run Parameters

Run Parameters	Read Count	% Reads
Sequences PF	1154625	100.0%
Indexable	1013014	87.7%
Valid Results	988084	85.6%
Avg Reads / Index	18515	1.6%
Results / Index	18123	1.6%

Interestingly, bacteria were detected in all but 1 (2.0%) of the 49 putative UTI samples (Index 21). In total, 41 (85.4%) of the remaining 48 putative UTI samples exhibited microbial results consistent with a known pathogen that can cause UTIs, is associated with an infectious process, or may plausibly cause urinary tract infections. In 9 of the 41 cases (21.9%) where a putative pathogen was identified, the detected organism was included within the PCR target list. The remaining 32 cases (78.0%) revealed a putative pathogen that would not have been expected to be identified by the PCR assay. Of note, sample 36 exhibited a *Mycoplasma girendii* that has been associated with *Trichomonas vaginalis* infection; this is suggestive of an undiagnosed case of Trichomoniasis.

Furthermore, the range and complexity of the microbial populations vary widely; however, a few trends were obtained. Microbial populations with very high read counts with valid results typically result in a single bacterial pathogen. Curiously, samples with lower or moderate reads often contain a mixed population of bacterial species that have been associated with UTIs, are considered to have pathogenic potential, or are considered opportunistic pathogens.

Table 2 - Top Result Per Sample

Index	Top Result	Sequences With Valid Results	Putative Pathogen
1	*Escherichia coli - Control*	2116	*NA*
2	*Gemella haemolysans - Control*	102371	*NA*
3	*Pedobacter jejuensis*	44	*None Detected*
4	*Pseudomonas aeruginosa*	13269	*Pseudomonas aeruginosa*
5	*Porphyromonas uenonis*	32284	*Porphyromonas uenonis*
6	*Gardnerella vaginalis*	11018	*Gardnerella vaginalis*
7	*Gardnerella vaginalis*	35755	*Gardnerella vaginalis*
8	*Ralstonia solanacearum*	626	*Acinetobacter junii*
9	*Aquihabitans daechungensis*	231	*None Detected*
10	*Exilispira thermophila*	55	*None Detected*
11	*Cutibacterium acnes*	57	*None Detected*
12	*Finegoldia magna*	3094	*Finegoldia magna - Aerococcus urinae*
13	*Actinotignum schaalii*	1034	*Actinotignum schaalii*
14	*Actinotignum schaalii*	18483	*Actinotignum schaalii*
15	*Lactobacillus jensenii*	12753	*Lactobacillus jensenii*
16	*Rodentibacter heylii*	11224	*Haemophilus influenzae*
17	*Rothia dentocariosa*	287	*None Detected*
18	*Cutibacterium acnes*	217	*Anaerococcus provencensis*
19	*Atopobium vaginae*	6523	*Atopobium vaginae*
20	*Corynebacterium pyruviciproducens*	1629	*Aerococcus urinae*
21	*None Detected*	0	*NA*
22	*Staphylococcus epidermidis*	21964	*Staphylococcus aureus*
23	*Veillonella atypica*	383	*Veillonella atypica*
24	*Porphyromonas uenonis*	270	*Prevotella buccalis*
25	*Streptococcus mitis*	672	*Streptococcus mitis - Winkia neuii*
26	*Corynebacterium durum*	82	*None Detected*
27	*Corynebacterium tuberculostearicum*	1581	*None Detected*
28	*Lactobacillus gasseri*	14191	*Lactobacillus gasseri*
29	*Klebsiella pneumoniae*	42501	*Klebsiella pneumoniae*
30	*Cutibacterium acnes*	1025	*Lactobacillus iners*
31	*Lactobacillus crispatus*	9541	*Lactobacillus crispatus*
32	*Porphyromonas somerae*	6026	*Porphyromonas somerae*
33	*Prevotella colorans*	7141	*Enterobacter asburiae*
34	*Gardnerella vaginalis*	36327	*Gardnerella vaginalis*
35	*Escherichia coli*	32803	*Escherichia coli*
36	*Mycoplasma girerdii*	5779	*Mycoplasma girerdii (T. vaginalis Associated)*
37	*Staphylococcus epidermidis*	104955	*Staphylococcus aureus*
38	*Lactobacillus iners*	39758	*Lactobacillus iners*
39	*Prevotella disiens*	6552	*Escherichia coli*
40	*Gardnerella vaginalis*	61587	*Gardnerella vaginalis*
41	*Klebsiella pneumoniae*	40162	*Klebsiella pneumoniae*
42	*Enterococcus faecium*	1856	*Enterococcus faecium*
43	*Lactobacillus iners*	32075	*Lactobacillus iners*
44	*Ralstonia solanacearum*	799	*Staphylococcus aureus*
45	*Fusobacterium nucleatum*	72282	*Actinotignum schaalii*
46	*Escherichia coli*	79692	*Escherichia coli*
47	*Veillonella atypica*	240	*Veillonella atypica*
48	*Lactobacillus iners*	2660	*Lactobacillus iners*
49	*Lactobacillus crispatus*	26608	*Lactobacillus crispatus*
50	*Streptococcus oralis*	3166	*Streptococcus pneumoniae*
51	*Schumannella luteola*	391	*Actinomyces oris*

Conclusions

Taken together, these results suggest that approximately 80% of putative UTI cases will be detectable in an initial PCR-screen with an additional 17% detectable using a follow-up NGS-based method. This would result in a total assay performance of 97%. Additionally, NGS-based methods assembled using a robust and reproducible kit would enable up-front detection by NGS, further eliminating the need for PCR-based methods. Precise identification of the putative pathogens from samples types that are plagued by false-negative results represents a significant improvement when compared to microbial culture, the current gold standard.

To learn more about the 16S Microbial ID Kit visit: www.bioidgenomics.com