Analytical Performance of a Precision Metagenomics Approach for Pathogen Detection in Urinary Tract Infections

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Background
Laboratory testing still heavily relies on culture and often fails to provide an etiologic diagnosis, even in common infectious diseases. More than 1 million patients suffer from recurrent or difficult-to-treat urinary tract infections annually in the US alone. Challenges include large numbers of pathogens; low sensitivity, dependence on viable pathogens, and subjective nature of culture; and limited molecular testing alternatives. Target-enriched next-generation sequencing (Precision Metagenomics, PM) can detect, quantify, and genotypically characterize hundreds of pathogens and thousands of antimicrobial resistance markers. Given its broad range, PM can improve diagnostic yield and may also prove useful in ruling out infections. As high analytical sensitivity is required in both cases, we determined the limit of detection (LoD) for representative pathogens in a variety of contrived samples using a commercial PM test.

Methods
Urinary Pathogen ID/AMR Panel (UPIP) uses a targeted-enrichment approach, and can detect >190 pathogens, including 135 bacteria, 35 viruses, 14 fungi and 7 parasites, and >2,000 antimicrobial resistance (AMR) markers associated with resistance to 47 antimicrobials within 24 hours in a single assay. For analytical evaluation, quantified and commercially available DNA was used for testing and spiked into 10 ng of human genomic DNA background (Promega, #PAG3041). Serial dilutions of contrived samples were tested in triplicate, and directly used to generate sequencing libraries. Libraries were then enriched using a triplex reaction and sequenced using the NextSeq 550 System (Illumina), down to a depth of 1M reads/sample. Sequencing data was analyzed with the Explify Software Platform. The last dilution with positive results in all 3 replicates was defined as limit of detection.

Results
UPIP identified all expected pathogens. LOD for 1 DNA virus, 2 fungi, 1 Gram-positive and 1 Gram-negative bacteria, 1 mollicute, and 1 protozoal ranged between 1 and 100 copies/mL (Figure 1).

Conclusions
UPIP demonstrated high analytical sensitivity across representative bacterial, viral, and protozoal pathogens on par or superior to existing tests. With the ability to detect hundreds of pathogens in a wide range of sample types with a standardized and automatable workflow in <24h, PM provides a powerful solution for syndromic testing.

Keyword 1: Next-Generation Sequencing
Keyword 2: Precision Metagenomics
Keyword 3: Urinary Tract Infection

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PM Matches or Exceeds Reported Sensitivity

Pathogen	Pathogen Group	LOD (cp/mL)
Escherichia coli	Gram-negative	100
Staphylococcus aureus	Gram-positive	100
Chlamydia trachomatis	Mollicute	10
Aspergillus flavus	Fungi (mold)	100
Candida albicans	Fungi (yeast)	100
HSV-2	DNA virus	10
Trichomonas vaginalis	Protozoon	1

Conflicts of Interest:
Do you have any conflicts of interest to declare? – I have no potential conflict of interest to report.
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