Nanofluidic PCR Assay Versus Urine Culture for Diagnosis of Urinary Tract Infection: A Comparison and Correlation of Cycle Threshold and Colony Forming Units

Arjuna Rao Vallabhaneni, Edward Ager, Pallavi Upadhyay, Adriana Rosato, Barbara Alexander and Vijay Singh
HealthTrackRx R&D, 1500 I35W, Denton, TX 76207

INTRODUCTION

Urinary tract infections (UTIs) are the leading cause of bacterial infections in the United States, contributing to nearly 10 million healthcare provider visits annually[1]. While the traditional urine is considered "gold standard" for UTI detections, the practical utilization of UTI cultures faces several methodological and operational challenges like longer turnaround times and difficulty isolating and diagnosing polymicrobial infections. This can either lead to missed detection or delayed results thus rendering providers to rely on empiric treatment options for effective patient outcomes[2]. In recent years, advanced molecular techniques such as multiplex PCR, have dramatically improved the accuracy of pathogen identification for UTIs[3]. PCR tests to detect UTIs can overcome several challenges urine culture presents, including (1) ability to detect anaerobic/anaerobic bacterial and fungal infections, (2) accurate identification of all species presents in a clinical sample, and (3) significantly shorter turn-around time. When adopting molecular testing for UTIs, one of the biggest challenges providers are faced with is understanding how the results obtained from PCR corresponds to that from traditional culture results. This study aims to compare Cycle Threshold (CT) values with Colony Forming Units (CFU) to assess the HealthTrackRx UTI multiplex panel result against traditional urine culture results. The correlation between CT and CFU is expected to assist clinicians in the interpretation of the UTI PCR report by estimating the microbial load in the urine sample.

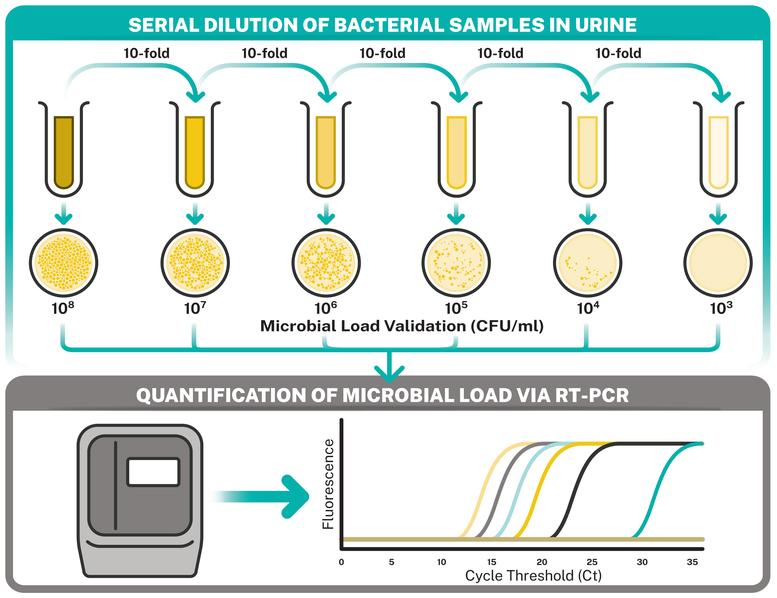

Figure 1: Schematic of Study Design: correlating Colony Forming Units (CFU/ml) and qRT-PCR CT Values

MATERIALS & METHODS

Five bacterial pathogens that are among the most reported UTI etiological agents detected by the HealthTrackRx UTI syndromic multiplex PCR panel were selected for inclusion in this study: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* BAA 977, and *Enterococcus faecalis* ATCC 51299.

Contrived urine samples with bacterial cell concentrations ranging from 10^8 to 10^0 were plated on agar plates in triplicates. Colony counts were performed in plates with countable colonies (30 to 300) and CFU/ml was estimated in the original sample and transformed into Log10 value.

The HealthTrackRX UTI-PCR test was used to determine the CT values of the various bacterial dilutions. The qRT-PCR test, employing the nanofluidic OpenArray™ technology, was performed on the QuantStudio™ 12K Flex Real-Time PCR platform *(ThermoFisher, California, USA)* as described by Singh et al. (2020).The average of three independent experiments are shown in Table 1.

RESULTS

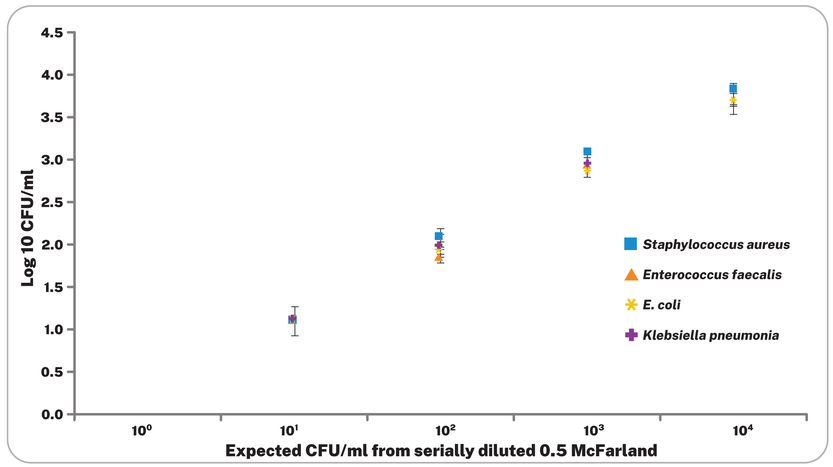

Figure 2: Observed CFU/ml values for the different bacterial strains spiked in synthetic urine.

CFU/ml	*Klebsiella pneumoniae*		*Psuedomonas aeruginosa*		*Escherichia coli*		*Staphylococcus aureus*		*Enterococcus faecalis*	
	CT	SC	CT	SD	CT	SD	CT	SD	CT	SD
10^3	28.64	0.9	28.24	1.2	27.20	1.7	29.53	0.8	29.16	1.0
10^4	25.74	1.5	25.70	1.0	25.47	2.7	28.13	1.4	27.52	1.9
10^5	21.74	1.2	22.26	0.9	22.25	2.7	25.80	2.5	26.22	2.5
10^6	18.53	1.2	18.41	1.1	19.09	2.8	23.35	2.2	22.98	1.9
10^7	14.82	1.0	15.16	1.4	14.01	1.9	19.90	2.4	19.33	1.1
10^8	12.43	2.0	12.10	1.3	10.46	1.1	16.73	2.4	16.90	1.4

Table 1: CT values for synthetic urine samples spiked with bacteria from 10^3 CFU/ml to 10^8 CFU/ml.

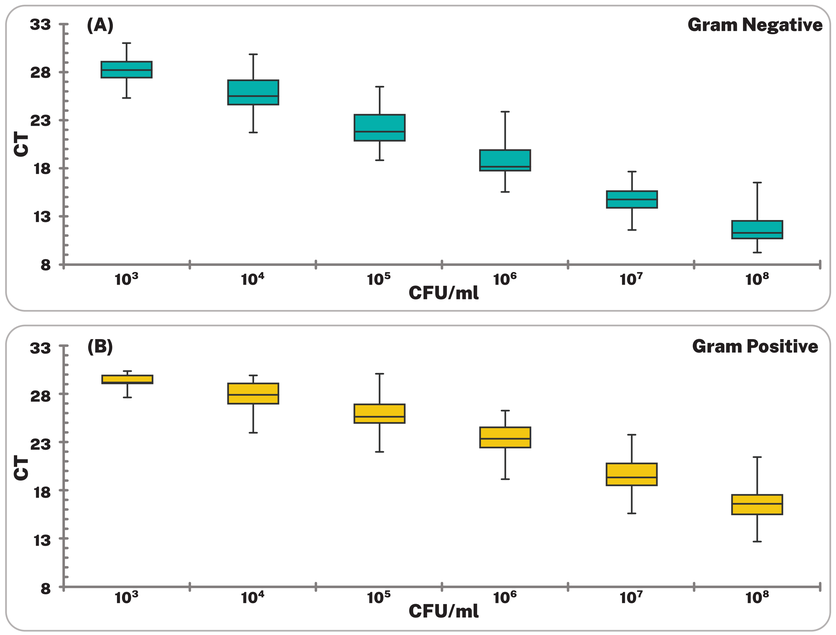

Figure 3: Correlation of CFU/ml and CT values of serially diluted Gram negative (A) and positive (B) bacterial strains

- The purpose of this study was to compare the CT values obtained by the HealthtrackRx UTI syndromic PCR test to CFU/ml values obtained and reported for traditional urine culture.
- The CFU/ml from urine culture spiked with various dilutions were as expected based on the optical density *(Figure 2)*. For urine samples spiked with ~10^3 CFU/ml, the following colony counts were obtained: *E. faecalis* 1223 (±30) CFU/ml, *S. aureus* 930 (±117) CFU/ml, *E. coli* 730 (±80) CFU/ml, *K. pneumoniae* 896 (±118) CFU/ml and *P. aeruginosa* 670 (±40) CFU/ml.
- At bacterial cell densities of 10^4 and 10^5 CFU/ml, the following CT Values were obtained: *E. faecalis* 27.5 (±1.9) and 26.2 (±2.4), *S. aureus* 28.1 (±1.3) and 25.7 (±2.4) *E. coli* 25.4 (±2.7) and 22.2 (±2.7), *K. pneumoniae* 25.7 (±1.4) and 21.7 (±1.4) *P. aeruginosa* 25.7 (±1.0) and 22.2 (±0.9) *(Table 1)*.
- On average, Gram positive bacteria had distinctively different CT values to that of Gram negative bacteria that were tested. *(Table 1 and Figure 3)*. In case of Gram negative bacteria at 10^5 CFU/ml the average CT values was 21.8 (±1.7), whereas for Gram positive bacteria it was 26.5 (±2.4). A similar pattern was observed at 10^4 CFU/ml as well.
- Based on this study we can correlate CT values obtained from HTRX UTI PCR to that of urine culture. At the clinically relevant value of 10^5 CFU/ml, an average CT value of 22.08 (±0.29) was determined for Gram negative bacteria. The corresponding CT value for Gram positive bacteria was determined as 26.01 (±0.29).

REFERENCES

1. Urinary Tract Infections. (2017). National Kidney Foundation. *https://www.kidney.org/atoz/content/uti.*
2. Wojno, K.J., et al. Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients. Urology. 2020; 136: 119–126
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CONCLUSIONS

This study establishes a direct correlation between the HealthTrackRx multiplex UTI PCR panel results and traditional urine culture results, thereby enabling quantitative interpretation of molecular results thus assisting providers in making treatment decisions based on PCR results. Due to inherent advantages of syndromic PCR panels, such as shorter turnaround times and superior sensitivity and specificity, as well as providing antimicrobial resistance information, these findings further enhance the appeal of a PCR-based syndromic UTI panel to providers for rapid and reliable results and improved patient outcomes.