1513
ID Week 2018

A New Method for Rapid Phenotypic AST Directly from Patient Samples

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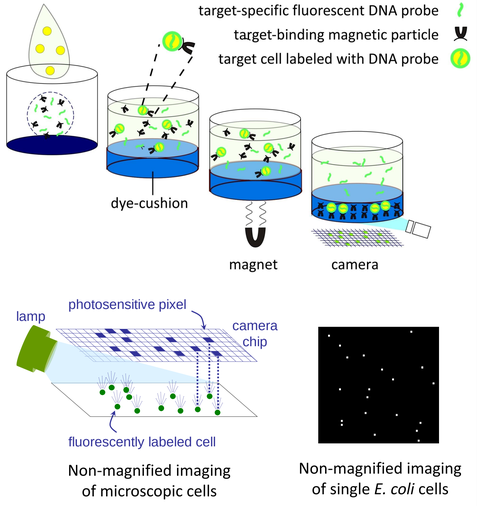
Introduction

Life-threatening syndromic hospital infections are becoming increasingly difficult to treat due to the high prevalence of antimicrobial-resistant pathogens. Since current culture-based antimicrobial susceptibility tests (ASTs) take days to identify an appropriate targeted therapy, broad-spectrum antimicrobials are initially prescribed. This empiric therapy may be medically sub-optimal or even ineffective leading to poor patient outcomes. The current approach often results in treatment of uninfected patients which accelerates the spread of antibiotic resistance. New rapid diagnostic methods that can determine the optimal narrow-spectrum antimicrobials at the onset of infection could significantly improve patient outcomes and attenuate the spread of resistance.

We present the MultiPath™ technology for detecting infections, identifying pathogens, and determining the appropriate targeted therapy in hours rather than days. Our results for detection of pathogens spiked into urine samples demonstrate the method's potential to detect syndromic infections and identify a broad range of bacterial pathogens directly from samples in 30 minutes. The MultiPath AST test delivers accurate results in just 4 hours while being robust to sample matrix and variable inoculum levels. The technology provides accurate AST results for multiple pathogens in polymicrobial infections and in non-sterile samples containing commensal microbes.

MultiPath ID Technology

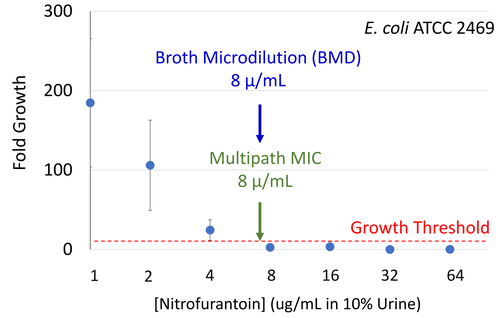


Robust to Sample Matrix

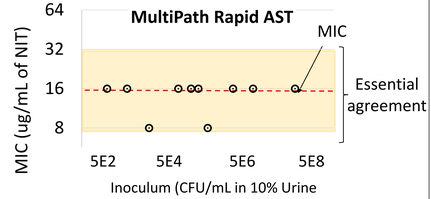
Target	Essential Agreement	Categorical Agreement
E. coli	100%	100%

n=56: 14 urine samples and 4 antibiotics

Accurate MIC Directly in Urine Samples



Robust to Variable Inoculum Levels



Target	Essential Agreement	Categorical Agreement
E. coli, Klebsiella spp., *P. aeruginosa*	100%	97%

N= 71: 4 antibiotics; Cell spikes ranging from 1E4 to 1E7 CFU/mL

Robust to Polymicrobial Infections

Compared MIC results from :

Individual pathogens (A or B) using BMD method **VS.** Pairs of UTI pathogens (A+B) in 10% urine using MultiPath method

Species tested individually and in combination: *E. coli, P. aeruginosa, K. pneumoniae, E. faecalis, & E. faecium*

Essential Agreement	Categorical Agreement
100%	100%

N= 48; 5 species; 1-2 isolates per species; 2-fold dilutions of Ciprofloxacin

Robust to Non-Sterile Samples

Off-target bacteria added:
Staphylococcus epidermidis
Micrococcus luteus
Corynebacterium minutissimum
Staphylococcus aureus
Acinetobacter baumannii
Citrobacter freundii
Klebsiella pneumoniae (OXA)

Target (CFU/mL)	Off-target Organism (CFU/mL)
1E5 *E. coli*	1E5, 1E6, 1E7

N= 72: 7 off-target species; 3 antibiotics

Essential Agreement	Categorical Agreement
98.6%	100%

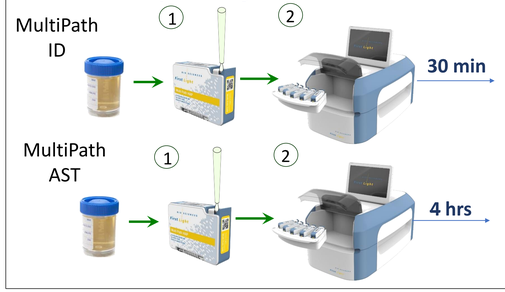
Challenging the technology: Does the presence of a carba-penemase–secreting *K. pneumoniae* (OXA) alter the MIC of an *E. coli* strain for imipenem?

Results: The MIC of the sensitive E. coli was identical to the BMD even in the presence of 5E7 CFU/mL of the carbapenemase secreting strain.

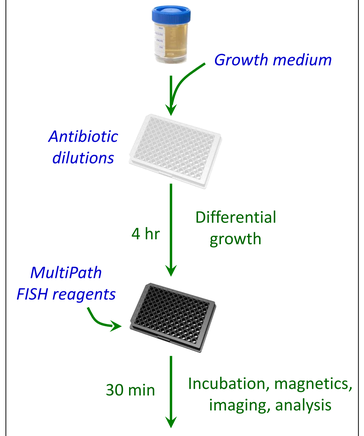
Technical Approach

- The MultiPath UTI ID/AST test uses FISH–based technology and non-magnified digital imaging to count cells labeled with target-specific DNA probes
- A novel dye-cushion eliminates sample preparation and wash steps
- All data was generated on microtiter plates except in the 'Proof of Concept: Automatic AST in 4.5 hours' section where we show data generated using the automated MultiPath platform (pictured below) that is currently under development

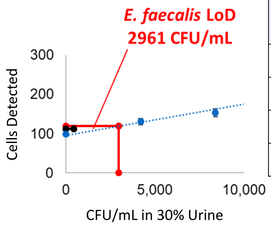
Product Concept Workflow



MultiPath AST Technology



Analytical Sensitivity



Limit of Detection (LoD) (CFU/mL in 30% Urine)	
E. coli	6E3 - 1E4
Enterococcus spp	2E3 - 5E3
Klebsiella spp.	6E3 - 1E4
P. aeruginosa	3E3 - 8E3

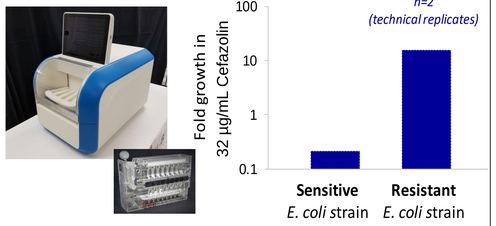
1 isolate/target group
4 urine samples/isolate

Inclusivity and Analytical Specificity

Target	Inclusivity (isolates detected[1])	Cross-reactivity[2]
E. coli	10/10	0/15
Klebsiella spp.	11/11	0/10
P. aeruginosa	10/10	0/10
Enterococcus spp.	6/6	0/10

[1]strains detected at 50K CFU/ml; [2]pairwise tests with relevant common urinary tract pathogens and commensals

Proof of Concept: Automated AST in 4.5hrs



The MultiPath UTI ID/AST test run in the MultiPath Cartridge and Analyzer with spiked samples in 30% urine

Summary

The results presented demonstrate the MultiPath technology's potential to:

- Detect infections and identify pathogens in 30 minutes; deliver MIC results in 4 hours
- Directly test samples with no sample preparation by the user
- Be robust to sample matrix effects and variable inoculum levels
- Deliver high analytical sensitivity, analytical specificity, and AST accuracy
- Provide AST results for non-sterile samples and polymicrobial infections
- Be processed by a fully automated, random-access, continuous-processing platform

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