A New Method for Rapid Phenotypic AST Directly from Patient Samples

Lauren M. Priess, Annie Tran, Alejandra Garces, Grettel Crewe, Megan D. Warner, Ann Zuniga, Sadanand Gite, Michael P. Cappilli, Jayson Bowers, Don Straus
First Light Biosciences, 2 Omni Way, Chelmsford, MA 01824; P: (781)-271-0112 E: lauren@firstlightbio.com

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 antimiicrobials 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
- Target-specific fluorescent DNA probes
- Target-binding magnetic particle
- Target cell labeled with DNA probe

MultiPath AST Technology
- The MultiPath UTI/DiAST 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 plate wells 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

Technical Approach
- MultiPath UTI/DiAST
- MultiPath Rapid AST
- MultiPath Rapid AST

Product Concept Workflow
- MultiPath ID
  1. Rules out infection: Identities up to 64 targets
  2. AST results (Susceptible or Resistant) for up to 15 antibiotics
- MultiPath AST
  1. 30 min
  2. 4 hrs
  3. AST results
  4. Antibiotic susceptibility profile (MIC)

Analytical Sensitivity
- f. fuscus Loeb 2961 CFU/mL
  - Limit of Detection (LoD) (CFU/mL in 30% Urine)
    - E. coli
    - Entereococcus spp.
    - Klebsiella spp.
    - P. aeruginosa
  - MIC agreement
  - Cross-reactivity

Inclusivity and Analytical Specificity
- Target Isolates Included
  - E. coli
  - Klebsiella spp.
  - P. aeruginosa
  - Entereococcus spp.
- Cross-reactivity
  - Sensitive
  - Resistant

Robust to Variable Inoculum Levels
- MultiPath Rapid AST
  - MIC agreement

Robust to Non-Sterile Samples
- MultiPath Rapid AST
  - MIC agreement

Robust to Polymicrobial Infections
- MultiPath Rapid AST
- MIC agreement

Challengeing the technology:
- Does the presence of a carbapenemase-secretion 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 values

Proof of Concept: Automated AST in 4.5hrs
- The MultiPath UTI/DiAST test run in the MultiPath Cartridge and analyzed with spiked samples in 30% urine

Summary
- The technology 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

This project has been funded in whole or in part with Federal funds from the Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. HHSN26120200100022C and an NIH grant R01 AI117058

Lauren M. Priess, Annie Tran, Alejandra Garces, Grettel Crewe, Megan D. Warner, Ann Zuniga, Sadanand Gite, Michael P. Cappilli, Jayson Bowers, Don Straus
First Light Biosciences, 2 Omni Way, Chelmsford, MA 01824; P: (781)-271-0112 E: lauren@firstlightbio.com

Technical Approach
- MultiPath UTI/DiAST
- MultiPath Rapid AST
- MultiPath Rapid AST

Product Concept Workflow
- MultiPath ID
  1. Rules out infection: Identities up to 64 targets
  2. AST results (Susceptible or Resistant) for up to 15 antibiotics
- MultiPath AST
  1. 30 min
  2. 4 hrs
  3. AST results
  4. Antibiotic susceptibility profile (MIC)

Analytical Sensitivity
- f. fuscus Loeb 2961 CFU/mL
  - Limit of Detection (LoD) (CFU/mL in 30% Urine)
    - E. coli
    - Entereococcus spp.
    - Klebsiella spp.
    - P. aeruginosa
  - MIC agreement
  - Cross-reactivity

Inclusivity and Analytical Specificity
- Target Isolates Included
  - E. coli
  - Klebsiella spp.
  - P. aeruginosa
  - Entereococcus spp.
- Cross-reactivity
  - Sensitive
  - Resistant

Robust to Variable Inoculum Levels
- MultiPath Rapid AST
  - MIC agreement

Robust to Non-Sterile Samples
- MultiPath Rapid AST
  - MIC agreement

Robust to Polymicrobial Infections
- MultiPath Rapid AST
- MIC agreement

Challengeing the technology:
- Does the presence of a carbapenemase-secretion 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 values

Proof of Concept: Automated AST in 4.5hrs
- The MultiPath UTI/DiAST test run in the MultiPath Cartridge and analyzed with spiked samples in 30% urine

Summary
- The technology 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

This project has been funded in whole or in part with Federal funds from the Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. HHSN26120200100022C and an NIH grant R01 AI117058