



# Analytical Validation of a Rapid Molecular Test for Semi-Quantitative Detection of Bacterial Pathogens and Antibiotic Resistance Genes in Urine

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**Background:** Phenotypic antibiotic susceptibility testing is the standard guide for antibiotic therapy, but it is slow and often leads to management with empiric therapy using broad-spectrum antibiotics. Rapid molecular tests detect pathogen and resistance genes but have not demonstrated the ability to comprehensively predict phenotypic resistance for Gram-negative infections.

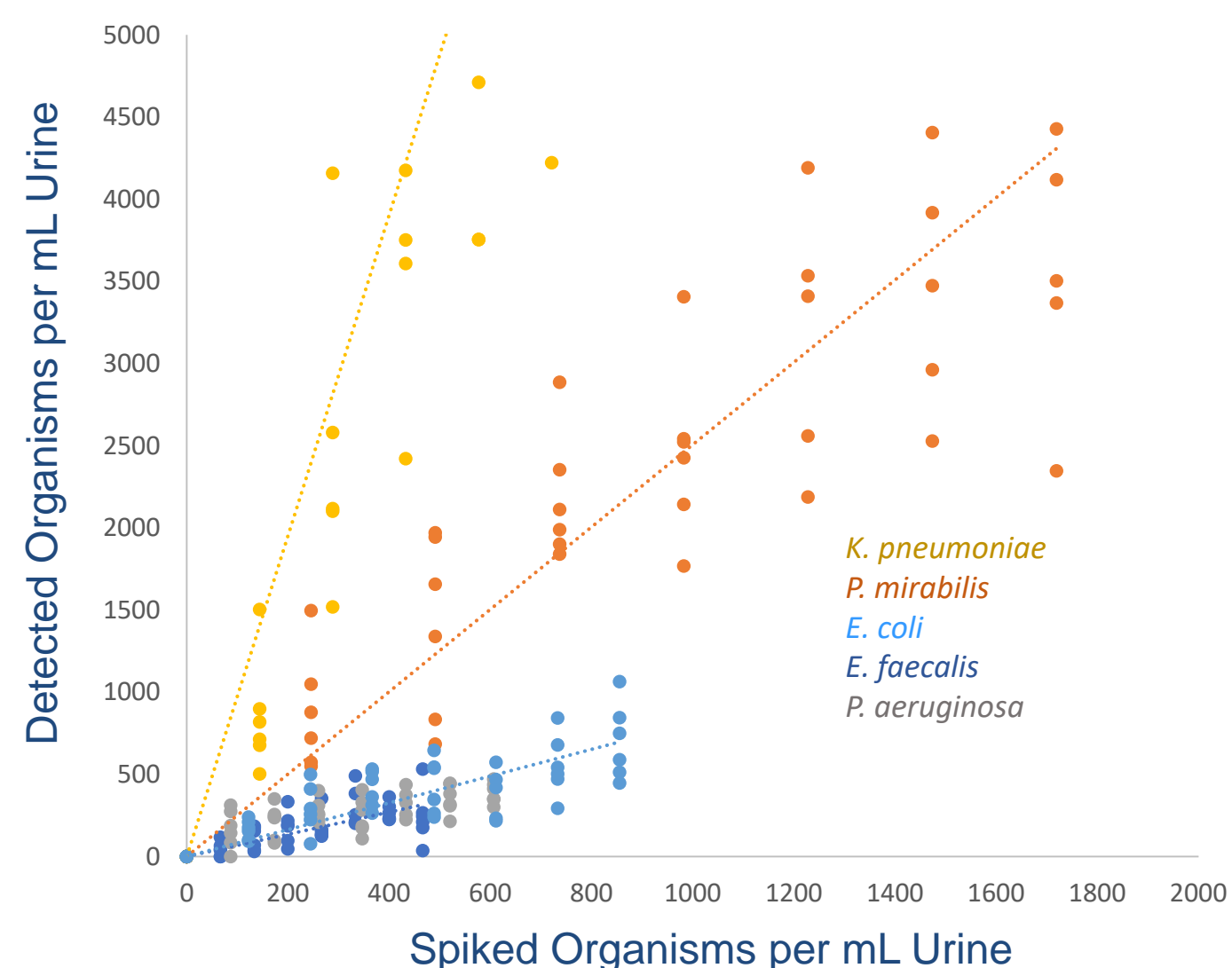
**Material/Methods:** We developed a PCR test, the Acuitas® AMR Gene Panel u5.47 (RUO), for semi-quantitative detection of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *E. faecalis* plus 47 antibiotic resistance genes which can be used in conjunction with statistical algorithms of the Acuitas Lighthouse® Software to predict phenotypic resistance to 13 antibiotics commonly prescribed for these pathogens. Test sample is primary urine specimens or bacterial isolates and time to result is 2.5 hours using automated DNA extraction and real-time multiplex PCR.

## Limit-of-Detection for Spiked Organisms in Urine (colony forming units per milliliter urine)

<i>E. coli</i>	611 to 856
<i>K. pneumoniae</i>	433 to 1010
<i>P. mirabilis</i>	246 to 1719
<i>P. aeruginosa</i>	434 to 607
<i>E. faecalis</i>	267 to 467

## Species Accuracy (isolates correctly identified)

<i>E. coli</i>	93 of 94 (99%)
<i>K. pneumoniae</i>	94 of 95 (99%)
<i>P. mirabilis</i>	87 of 87 (100%)
<i>P. aeruginosa</i>	96 of 96 (100%)
<i>E. faecalis</i>	79 of 79 (100%)



## Lighthouse Predictions of Phenotypic Resistance for 35 FDA/CDC Isolates and 13 Antibiotics (321 predictions of resistance)

	True Positives	False Positives	True Negatives	False Negatives	Agreement	Sensitivity	Specificity	PPV	NPV
<i>E. coli</i> (n=10)	98	1	14	6	94%	94%	93%	99%	70%
<i>K. pneumoniae</i> (n=10)	84	0	21	15	88%	85%	100%	100%	58%
<i>P. aeruginosa</i> (n=10)	42	0	0	0	100%	100%		100%	
<i>P. mirabilis</i> (n=5)	8	0	15	17	58%	32%	100%	100%	47%
Combined	232	1	50	38	88%	86%	98%	100%	57%

	True Positives	False Positives	True Negatives	False Negatives	Agreement	Sensitivity	Specificity	PPV	NPV
Ampicillin	10	0	1	3	79%	77%	100%	100%	25%
Aztreonam	17	1	1	3	82%	85%	50%	94%	25%
Cefazolin	18	0	3	4	84%	82%	100%	100%	43%
Cefepime	15	0	2	3	85%	83%	100%	100%	40%
Cefotaxime	16	0	5	4	84%	80%	100%	100%	56%
Ceftazidime	21	0	5	5	84%	81%	100%	100%	50%
Ceftriaxone	16	0	5	4	84%	80%	100%	100%	56%
Ciprofloxacin	26	0	4	0	100%	100%	100%	100%	100%
Gentamicin	21	0	7	4	88%	84%	100%	100%	64%
Levofloxacin	26	0	4	0	100%	100%	100%	100%	100%
Tobramycin	25	0	5	2	94%	93%	100%	100%	71%
Trimeth/Sulfa	14	0	6	5	80%	74%	100%	100%	55%
Imipenem	7	0	2	1	90%	88%	100%	100%	67%
Combined	232	1	50	38	88%	86%	98%	100%	57%

**Results:** Lower limit of detection for each pathogen is approximately 1000 organisms per milliliter of urine based on parallel culture plate counts. The test accurately detected pathogens with semi-quantitation over  $10^3$  to  $10^6$  organisms per milliliter of urine. No species cross-reactivity was detected with 39 other pathogens commonly associated with urinary tract infections, although cross-reactivity is expected for some species of *Shigella*, *Klebsiella variicola* and *Proteus vulgaris* with the assays for identification of *E. coli*, *K. pneumoniae* and *P. mirabilis*, respectively. The Acuitas Lighthouse Software accurately predicted antibiotic resistance for 35 highly resistant FDA/CDC isolates across 13 antibiotics with excellent positive predictive values. Prediction accuracy for *P. mirabilis* was subsequently improved by adding several previously excluded mechanisms into the Acuitas Lighthouse Software.

**Conclusions:** Acuitas AMR Gene Panel u5.47 (RUO) rapidly detects a comprehensive set of pathogens and resistance genes in Gram-negative bacilli with high sensitivity and specificity without unexpected cross-reactivity. Test results can be used to predict phenotypic resistance when analyzed by the statistical prediction algorithms of the Acuitas Lighthouse Software. Rapid and accurate recognition of antibiotic resistance mechanisms can improve antibiotic use and outcomes in patients with Gram-negative bacterial infections.