Validation of Direct Inoculation of Urinary pathogens from Alere Orientation Agar to Vitek 2 and Phoenix Identification panels and to Vitek MS (MALDITOF) (with Susceptibility Testing).

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Abstract

Background: Chromagenic agar offers a faster approach to identification (ID) of pathogens from mixed cultures such as urine cultures. Unfortunately, these pathogens must still be subcultured before being tested on automated ID and susceptibility (AST) systems. The purpose of this study is to validate direct inoculation of urine pathogens from Alere Orientation agar into Vitek2, Phoenix and Vitek MS instruments.

Methods: One hundred urines know to contain a wide range of pathogens were inoculated onto Orientation agar and incubated at 35 ° C for 18h. Colonies of the appropriate chromagenic were tested by Phoenix (ID), Vitek MS (ID) and Vitek2 (ID and AST). Results of ID and AST were compared to those obtained by the laboratory testing the original sample. Essential and categorical agreement was calculated for each antimicrobial and percentage correct for identification results.

Results: Ninety-six percent, 97%, and 100% of identification results were correct for Phoenix, Vitek2 and Vitek MS respectively Categorical agreement for all 35 antimicrobials tested was greater than 90% except for cefepime (30% minor errors (me)), piperacillin (20% me), ticarcillin (20% me) and ticarcillin-clavulanate (20% me,10% very major) and all occurred with *P.aeruginosa*. All essential agreements were greater than 90%.

Conclusion: Direct inoculation of urinary pathogens from Alere Orientation agar for ID using Vitek2, Phoenix and Vitek MS is a reliable method to reduce time and costs of ID of many common organisms. Direct inoculation for AST testing using Vitek2 is a reliable method except with *P.aeruginosa*. The lower than expected EA and CA for this group of organisms may be due to the small number tested (n=10) and further testing of this group is needed.

Objective

Chromagenic agar offers a faster approach to identification (ID) of pathogens from mixed cultures such as urine cultures. Unfortunately, these pathogens must still be subcultured before being tested on automated ID and susceptibility (AST) systems. The purpose of this study is to validate direct inoculation of urine pathogens from Alere Orientation agar into Vitek2, Phoenix and Vitek MS instruments.

Materials and Methods

One hundred urines know to contain a wide range of pathogens(Table 1) were inoculated onto CHROMagar Orientation media and incubated at 35 ° C for 18h. Isolated colonies from the chromagenic medium (Figure 1) were tested by Phoenix (ID), Vitek MS (ID) and Vitek2 (ID and AST). Results of ID and AST were compared to those obtained by the laboratory testing the original sample. Essential agreement (EA) (within ± one log dilution) and categorical agreement (CA) (S/I/R) was determined for each organism / antimicrobial combination as well as percentage correct identification results.

Performance Criteria:
- ≥ 90% Essential and Categorical Agreement
- ≤ 1.5% Very Major Errors (VM)
- ≤ 3% Major Errors (M)
- ≤ 10% Minor Errors (m)

Materials and Methods

Table 1. Organisms Tested

Organism Tested	No. tested
E. coli	20
Klebsiella sp.	16
Citrobacter sp.	10
Proteus and Providencia sp.	11
P. aeruginosa	10
S. aureus	10
Enterococcus sp.	10
E. cloacae	7
M. morganii	4
S. marcescens	1
H.alvei	1

Figure 1

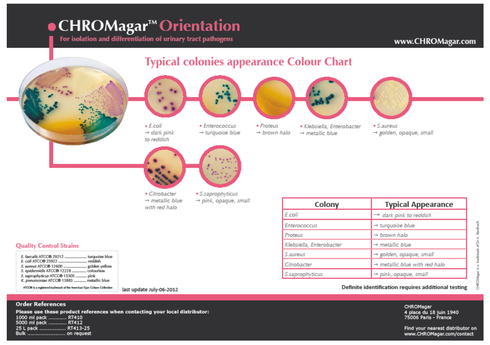

Table 2. Discrepant or Low Discrimination Identification Results

Lab Identification	Phoenix	Vitek2	Vitek MS
Citrobacter sp	*C. amalonaticus*	*No identification*	*C. amalonaticus*
C. freundii	*C. braakii*	*C. freundii*	*C. freundii*
P. aeruginosa	*P. aeruginosa*	*P.aeruginosa low discrim*	*P. aeruginosa*
P. aeruginosa	*P. aeruginosa*	*P. aeruginosa/putida*	*P. aeruginosa*
S. anginosus	*Not Done*	*S. anginosus*	*Several choices including S. anginosus*
C. freundii	*C. braakii*	*C. freundii*	*C. freundii/youngae*
H. alvei	*H. alvei*	*Yokonella regensburgei*	*H. alvei*
M. morganii	*Salmonella sp*	*M. morganii*	*M. morganii*
E. cloacae complex	*C. braakii*	*E. cloacae complex*	*Enterobacter cloacae/asburiae*

Results

Table 2. Categorical and Essential Agreement

Antibiotics	No. Tested	Categorical Errors	Organism	Categorical Agreement (%)	Essential Agreement (EA) (+/-DD)	Organism	EA %
Amikacin	80	2 minor	*P. aeruginosa (2)*	97.5	80		100
Amoxacillin-Clavulanic acid	70	1 minor	*P. mirabilis*	98.6	69	*P. mirabilis*	99
Ampicillin	80			100	80		100
Benzylpenicillin	20			100	20		100
Cefalothin	70	2 minor	*P. aeruginosa, E.coli*	97	70		100
Cefazolin	70	1 minor	*P. mirabilis*	98.6	70		100
Cefepime	10	3 minor	*P. aeruginosa (3)*	70	10		100
Cefixime	70	2 minor	*P. aeruginosa, E.coli*	97	70		100
Cefoxitin	70	1 minor	*E. coli*	98.6	70		100
Ceftazidime	80	2 minor	*P. aeruginosa (2)*	97.5	80		100
Ceftriaxone	70	1 very major	*E. cloacae*	98.6	69	*E. cloacae*	99
Ciprofloxacin	100	1 minor	*P. aeruginosa*	99	100		100
Clindamycin	20			100	20		100
Colistin	10			100	10		100
Ertapenem	70			100	70		100
Erythromycin	20			100	20		100
Gentamicin	80			100	80		100
Imipenem	10	1 minor	*P. aeruginosa*	90	10		100
Levofloxacin	20			100	20		100
Linezolid	20			100	20		100
Meropenem	80			100	78	*P. mirabilis (2)*	98
minorocycline	10			100	10		100
Moxifloxacin	20			100	20		100
Nitrofurantoin	90	3 minor	*Klebsiella sp (2), Enterococcus sp*	97	90		100
Oxacillin	10			100	10		100
Pefloxacin	10	1 minor	*P. aeruginosa*	90	10		100
Piperacillin	10	2 minor	*P. aeruginosa (2)*	80	100		100
Piperacillin-Tazobactam	70	1 minor	*E. coli*	98.6	68	*K. oxytoca, E.coli*	97
Quinupristin-dalfopristin	20			100	20		100
Rifampin	10			100	10		100
Trimethoprim-Sulfamethoxazole	90	1 major	*P. mirabilis*	99	90		100
Tetracyclin	90	1 minor	*K. oxytoca*	99	89	*K. oxytoca*	99
Ticarcillin	10	2 minor	*P. aeruginosa (2)*	80	10		100
Ticarcillin-Clavulanic acid	10	2 minor, 1 very major	*P. aeruginosa (3)*	70	10		100
Tigecycline	20			100	20		100
Tobramycin	90			100	90		100
Vancomycin	20			100	20		100

Results:

Ninety-six (96%) percent, 97%, and 100% of identification results were correct for Phoenix, Vitek2 and Vitek MS respectively. Categorical agreement for all 35 antimicrobials tested was greater than 90% except for cefepime (30% minor errors (me)), piperacillin (20% me), ticarcillin(20% me) and ticarcillin-clavulanate (20% me,10% very major) and all occurred with *P.aeruginosa*. All essential agreements were greater than 90%.

Discussion and Conclusions

Direct inoculation of urinary pathogens from CHROMagar Orientation media for ID using Vitek2, Phoenix and Vitek MS is a reliable method to reduce time and costs of ID of many common organisms. Direct inoculation for AST testing using Vitek2 is a reliable method except with *P.aeruginosa*. The lower than expected CA for this group of organisms may be due to the small number tested (n=10) and further testing of this group is needed.