C-191

Evaluation of BluEcoli™: a New Chromogenic Medium for the Isolation and Identification of Urinary Tract Pathogens

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Abstract

The urine culture is the most commonly performed test in the clinical laboratory, and contributes significantly to laboratory expense and workload. Of positive urine cultures, *E. coli* is the etiologic agent most commonly isolated. In fact, *E. coli* is the causative agent in 90 percent of urinary tract infections (UTI) in ambulatory patients and fifty percent of nosocomial UTI.

A novel chromogenic media was evaluated for the ability to isolate and identify *E. coli* from other urinary pathogens without the need for further confirmatory testing. The new urine biplate, contains two formulations; Blood Agar on one side and BluEcoli™, a MacConkey base with an added chromogenic substrate on the other. In addition to exhibiting typical MacConkey reactions, the chromogenic media can readily differentiate *E. coli* from other gram-negative urinary isolates by color change. The chromogenic substrate is cleaved specifically by *E. coli,* which causes the colonies of *E. coli* to turn blue (except for *E. coli* 0157); while non-*E. coli* isolates will be either pink or clear depending on their ability to ferment lactose.

Two separate evaluations were carried-out. The first one consisted of an "in-house" trial involving 125 isolates including 70 clinical *E. coli* strains and 35 other common species of urine pathogens. All isolates were overnight cultures that were tested in parallel with BluEcoli™, CHROMagar Orientation, and MacConkey media without added chromogen as a control. BluEcoli™ demonstrated 100% sensitivity and specificity; with all *E. coli* isolates exhibiting a blue color on the MacConkey based chromogenic media, and as expected, traditional colony morphologies of all non-*E. coli* gram negative organisms were preserved.

The second evaluation was performed by Central Coast Pathology Consultants in which BluEcoli™ was compared in parallel to the routine media used for urine. Of 100 specimens tested, 47 were considered positive for UTIs. 29 of positive cultures were identified as *E. coli* by either conventional methods or automated methods. BluEcoli™ successfully detected all of these isolates without need for any additional testing (spot tests or automated methods). This study suggests that the novel chromogenic medium, BluEcoli™, is accurate and cost effective in the differentiation and identification of *E. coli* from other organisms on primary isolation from urine samples.

Introduction

Diagnosis of urinary tract infections contributes significantly to the daily workload in a microbiology laboratory, therefore any attempts of innovation by reducing the work load and cost are always welcome while maintaining a high quality. For several years, development of culture media containing chromogens and fluorogens has led to the development of a great number of methods for the rapid identification of microorganisms in primary isolation media.

Hardy Diagnostics BluEcoli™ uses beta-D-glucoronidase (GUD) as an indicator for *E. coli* since this enzyme is present in 94-96% of members of this species.(1) Presence of GUD can be measured by using different chromogenic and fluorogenic substrates. Fluorogens are hydrolyzed by GUD yielding fluorescent colonies of *E. coli* under a long-wave (365nm) UV light source, whereas chromogens release chromophores resulting in a simple, visual read out of blue to purple colored colonies of *E. coli*.[1]

E. coli is known as the most common pathogen responsible for great majority of urinary tract infections. Therefore rapid identification and reporting is useful in the direction of therapy. It also streamlines the overall turn-around-time of microbiology laboratory allowing microbiologists to devote more time to problematic cultures.[2,3]

Several chromogenic media have been compared to traditional urine culture media (i.e., Blood and MacConkey Agars) and were found to be at least as good as traditional media for the isolation of uropathogens.[2-5] Overall, studies agree that rapid detection and identification of microorganisms is of high importance in a diverse array of clinical and research settings. By incorporating synthetic enzyme substrates into primary isolation media, enumeration and detection utilizing color reactions can be performed directly on the isolation plate, enhancing the accuracy and performance of the microbiologist by the immediate recognition of *E. coli* isolates, and ruling out clinically insignificant mixed cultures.[3]

Since limited information has been available on the accuracy of using colonies from chromogenic media for susceptibility testing in automated systems, it is recommended that colonies from the Blood Agar side of BluEcoli™ be used for susceptibility testing. However, colonies from the chromogenic side of the biplate may be used for the disk diffusion (Kirby-Bauer) method of susceptibility testing.

Materials and Methods

The "in-house" study

1. The "in-house" comparison consisted of evaluating previously identified clinical isolates from Hardy Diagnostics' collection: *E. coli* (n=70), *P. aeruginosa* (n=5), *E. cloacae* (n=5), *C. freundii* (n=5), *E. aerogenes* (n=5), *P. mirabilis* (n=5), *S. marcescens* (n=5), *K. pneumoniae* (n=3), *S. saprophyticus* (n=2), and 20 other ATCC strains of several species.

2. All isolates were diluted to the equivalent of a 0.5 McFarland from overnight cultures and streaked to BluEcoli™, CHROMagar Orientation, and MacConkey media without added chromogen as a control.

3. The plates were incubated aerobically at 35°C and read after 18 to 24 hours of incubation.

4. *E. coli* appears as blue to purple colored colonies on the chromogenic side of the BluEcoli™ biplate. Other gram-negative rods that grow on the BluEcoli™ will retain their traditional morphology as seen on MacConkey (pink to slight pink for lactose-positive colonies, and colorless for lactose-negative colonies).

5. On the CHROMagar Orientation, *E. coli* appears as violet colored colonies with a deeper purple center ("bull's-eye" appearance). *Citrobacter*, *Enterobacter*, *Serratia*, and *Klebsiella* spp. produce large metallic blue-gray or blue-violet colonies with or without a purple to pink halo. *Proteus mirabilis* produces clear colonies with a diffuse golden-orange to brown pigment around the periphery of each colony. *Pseudomonas* spp. produce colorless, translucent colonies. *Staphylococcus saprophyticus* present as small, opaque colonies that range from light amber to pink.

Materials and Methods (continued)

The Clinical Trial

1. The BluEcoli™ plates were supplied to Central Coast Pathology Consultants and were evaluated from March, 2005 to May, 2005.

2. 100 consecutive urine cultures specimens were processed according to the laboratory's regular protocol (Blood/MacConkey) without deviation.

3. Along with the laboratory's in-house method, the specimen was also inoculated on both sides of the BluEcoli™ biplate using calibrated loops.

4. After 18 to 24 hours of incubation period, the positive cultures were identified by spot tests and by the Vitek™ automated method if necessary.

5. *E. coli* appears as blue to purple colored colonies on the chromogenic side of the BluEcoli™ biplate. Other gram-negative rods that grow on the chromogenic side will retain their traditional morphology as seen on MacConkey (pink to slight pink for lactose-positive colonies, and colorless for lactose-negative colonies).

Results

Results of the "in-house" evaluation

Table 1. Overall results of the "in-house" evaluation

	BluEcoli™	CHROMagar Orientation
	Color reaction	Color reaction
E. coli (n=70)	Blue	Red-Violet
E. cloacae (n=5)	Pink, Lactose +	Blue
E. aerogenes (n=5)	Pink, Lactose +	Blue
C. freundii (n=5)	Pink, Lactose +	Blue
S. marcescens (n=5)	Pink, Lactose +	Blue
K. pneumoniae (n=3)	Pink, Lactose +	Blue
P. mirabilis (n=5)	Clear, brown	Clear colony with brown halo
P. aeruginosa (n=5)	Clear, greenish opalescence	Clear, translucent
S. saprophyticus (n=2)	No growth	Pink

- As shown in Table 1, both methods successfully detected all the *E. coli* isolates evaluated.
- *Enterobacter/Citrobacter/Serratia/Klebsiella* presented similar colony morphologies in each method.
- Besides *E. coli*, all species tested presented their traditional MacConkey morphology on the chromogenic side of BluEcoli™

Results of the clinical evaluation

- 100 consecutive urine specimens were evaluated.
- 47 were considered positive for UTI.
- 29 were identified as *E. coli*.
  - 9 isolates could have been reported as *E. coli* by using spot tests as described in the CLSI document M35-A.
  - 20 isolates were considered atypical *E. coli* isolates, in that they were either indole negative or non-hemolytic on the blood side and needed further confirmation by the Vitek instrument.
  - All 29 isolates were detected by BluEcoli™ within 18 to 24 hours without the need for further confirmation.
- All other remaining positive specimens (n=18) needed further tests for final identification (Vitek, latex agglutination, spot tests).
  - These 18 isolates showed their traditional MacConkey morphology on the BluEcoli™.

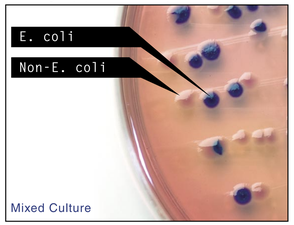
For more information on BluEcoli™ contact Andre Hsiung, MS (email: hsiunga@hardydiagnostics.com)

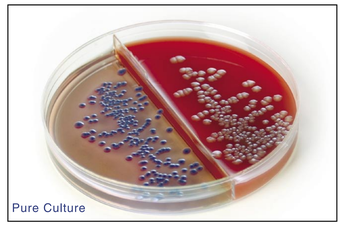
Results (continued)

Table 2. Cost Analysis. Traditional method (Blood/MacConkey) versus BluEcoli™

	Blood/MacConkey			BluEcoli		
		Cost* ($)	Total cost ($)		Cost* ($)	Total cost ($)
Plates	100	1.28 ea.	128.00	100	2.18 ea.	218.00
Negative cultures	53			53		
Positive cultures	47			47		
Positive cultures for *E. coli*	29			29		
E. coli identified by spot tests	9			0		
Positive cultures other than *E. coli*	18			18		
Required ID panel	38	5.65 ea.	214.70	18	5.65 ea.	101.70
Required susceptibility panel	47	6.35 ea.	298.45	47	6.35 ea.	298.45
Labor time - Reading plates and spot tests	1.5 hr.	30/hr.	45.00	0.3 hr.	30/hr.	9.00
Labor time set up automated method**	3.2 hr.	30/hr.	96.00	2.4 hr.	30/hr.	72.00
Misc. cost of spot tests (indole, oxidase)			23.44			
Total cost			805.59			699.15

* List prices were used for calculation.
** Assuming setup speed of 8 cards every 20 minutes.





Discussion

Both "in-house" and the clinical trial show that BluEcoli™ can accurately detect *E. coli* without further confirmation.

BluEcoli™'s ability in *E. coli* detection is equivalent to CHROMagar Orientation.

Many strains of *E. coli* can be atypical and cannot be identified based on spot indole alone. They may be indole negative, lactose negative, or non-hemolytic on blood agar.

According to this study, 20 of 29 (69%) *E. coli* isolates could not be identified based on spot tests as specified in the CLSI document M35-A and needed further confirmatory tests. All of these were correctly identified by BluEcoli™.

Besides elimination of additional testing, BluEcoli™ also helped reduce the time and labor costs, as shown in the Table 2.

Mixed cultures can be easily spotted by BluEcoli™. These cases may be clinically relevant or not, depending on the clinical correlation.

BluEcoli™ presented 100% sensitivity and 100% specificity in both "in-house" and clinical evaluations.

Conclusion

Since *E. coli* is responsible for majority of urinary tract infections, BluEcoli™ can be used as a reliable and cost effective tool for the detection of this species in urine specimen.

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