Evaluation of the Cult-Dip Plus dip slide method for urinary tract infection

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Abstract

Aim—To evaluate the Cult-Dip Plus (Merck, Germany), a bacteriological culture test for detecting uropathogens.

Methods—Cult-Dip Plus consists of Brolacin (CLED) and MacConkey agar, each containing methylumbelliferylglucuronide (MUG). Using 1022 urine samples, this product was compared with the routine method of calibrated loop inoculated CLED and blood agar for screening urine for uropathogens. The MUG test for identifying *Escherichia coli* was also evaluated.

Results—Compared with the routine method, Cult-Dip Plus has a sensitivity, specificity, positive predictive value and negative predictive value of 88.3%, 98.0%, 91.9%, and 97.1%, respectively. The MUG test correctly identified 92% of *E coli* isolates with a sensitivity, specificity and positive predictive value of 91.6%, 95.2%, and 93.6%, respectively.

Conclusion—Cult-Dip Plus appears to be an alternative method to the calibrate loop method for detecting uropathogens. The MUG test permits rapid, reliable and inexpensive identification of *E coli*.

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Acute urinary tract infections are a major health problem affecting an estimated 10–20% of women at some point during their lifetime.¹ In healthy men the prevalence of urinary tract infections is less than 0.1%. As many as 42% of all hospital acquired infections are of the urinary tract.²

In both compromised and uncompromised hosts *Escherichia coli* is the micro-organism most commonly associated with urinary tract infections. Others, such as *Staphylococcus* sp, *Enterococcus* sp, *Pseudomonas* sp, and other species belonging to the *Enterobacteriaceae* are also significant causes of urinary tract infections; however, their prevalence may vary depending on the age and condition of the host. Isolation and rapid identification of micro-organisms causing urinary tract infections is important for determining the causative organism and because it affects the appropriate selection or continuation, or both, of antimicrobial therapy.

Urine dip slides are immersible plastic paddles containing agar medium on each side. Dip slides have previously been shown to be cost-effective, easy to use devices that provide

results comparable with those obtained using standard plate streaking methods.^{3–7} Dip slides that incorporate substrates for the detection of bacterial enzymes could result in the early identification of uropathogens. Kilian and Bulow⁸ showed that *E coli* was one of the few bacteria to produce the enzyme B-glucuronidase. Hydrolysis of 4-methylumbelliferyl-B-D-glucuronide (MUG) by B-glucuronidase produces 4-methylumbelliferon—a product which fluoresces under ultraviolet light.

Cult-Dip Plus is a new urine dip slide consisting of Brolacin (cystine, lactose, electrolyte deficient (CLED)) and MacConkey agar, each containing MUG. We evaluated the Cult-Dip Plus for recovery of uropathogens and the MUG test for rapid identification of *E coli*.

Methods

The MUG test is based on detection of fluorescence from release of 4-methylumbelliferon following hydrolysis of the parent compound by B-glucuronidase. Cult-Dip Plus was supplied by BDH (Toronto, Ontario, Canada). Blood and CLED agars were purchased from PML Microbiologicals (Edmonton, Alberta, Canada).

We prospectively analysed 1022 urine samples from different patients submitted over a two month period. The semiquantitative plate culture method described by Clarridge *et al*⁹ was used as the reference method for determining bacteriuria. For routine culture, 0.001 ml urine was delivered to each blood and CLED agar plate using a calibrated disposable loop (Simport, Quebec, Canada). This method detects ≥ 1000 colony forming units/ml (cfu/ml).

Following this, the Cult-Dip Plus dip slide was inoculated by either immersing the slide in the urine (minimum 30 ml required) or when the volume of urine was such that the paddle could not be immersed, by flooding both sides of the slide with urine using a sterile disposable pasteur pipette (5 ml). This method detects 100 cfu/ml. Colony counts of 1–10 colonies detected by Cult-Dip Plus were considered negative so as to standardise the colony count comparisons between both methods. After inoculation, culture plates/dip slides were incubated for 18–24 hours at 35–37°C.

IDENTIFICATION OF MICRO-ORGANISMS

Typical lactose fermenting colonies resembling *E coli* were identified by using the Bactident *E coli* test (BDH, Toronto, Ontario, Canada), a

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Table 1 Comparison of 1022 urine specimens isolated by Cult-Dip Plus and routine culture

	Both methods	Cult-Dip Plus	Routine culture
Negative ⁺	761	24	16
Positive*	181	16	24

* Pure isolates in quantities greater than 10 000 cfu/ml.

⁺ Includes those with no visible growth and those with two or more species of micro-organism (mixed).

Table 2 Frequency of uropathogens isolated by Cult-Dip Plus and routine culture

Micro-organism	Both methods	Cult-Dip Plus	Routine method
Gram negative bacilli	131	13	11
Gram positive cocci	38	3	13
Yeast	12	—	—
All genera	181	16	24

Table 3 Summary of uropathogens MUG test positive by either Cult-Dip Plus, Bactident, or both

Micro-organism	Cult-Dip Plus Isolates (No. positive/no. tested (%))		Routine culture (No. positive/no. tested (%))
	Cult-Dip Plus	Bactident	Bactident
<i>E. coli</i>	88/96 (92)	64/68 (94)	70/77 (91)
<i>K. pneumoniae</i>	4/16 (25)	NT	NT
<i>P. mirabilis</i>	2/10 (20)	NT	NT

NT = not tested.

two hour identification system for *E. coli* based on indole formation and B-D-glucuronidase (MUG test). If either of these tests were negative, isolates were identified by Vitek (bio-Merieux Vitek Inc., Hazelwood, Missouri, USA). All other significant micro-organisms recovered by either method were identified by Vitek or by a combination of individual biochemical and/or latex agglutination tests. The dip slide MUG test was read by holding dip slides with significant growth under long wave ultraviolet light (360 nm). Presumptive identification of *E. coli* was confirmed by Bactident. The presence of fluorescence indicated a positive MUG test. Results for isolation of uropathogens and identification of *E. coli* by our routine method(s) and with the Cult-Dip Plus were compared.

Results

Of the 1022 urine samples analysed, 221 (21.6%) had significant findings (>10 000 cfu/ml and pure) by one method or the other; 181 (81.9%) were detected by both methods; 24 (10.9%) by the routine method only; and 16 (7.2%) by Cult-Dip Plus only. Table 1 shows the distribution of the data. With regard to insignificant (mixed with two or more species of micro-organisms or <10 000 cfu/ml) or negative results (no visible growth), 417 (40.8%) urine samples were negative by both methods; 344 (33.7%) had mixed or insignificant counts by both methods and of these, 162 (15.9%) had colony counts that differed between both methods (data not shown). Of the urine samples cultured by Cult-Dip Plus, 97 had <1000 cfu/ml; however, these were scored as no growth when the cut off was adjusted to ≥1000 cfu/ml for comparison with routine culture.

Compared with the calibrated loop method Cult-Dip Plus had a sensitivity of 88.3%, a specificity of 98.0%, a positive predictive value of 91.9%, and a negative predictive value of 97.1% (table 1).

There were 221 significant organisms identified. Table 2 shows the frequency of uropathogens detected by either Cult-Dip Plus, routine culture or both. As expected, *E. coli* was the most frequently recovered pathogen. In all cases where a significant uropathogen was recovered by either Cult-Dip Plus or by routine culture but not by both, the corresponding method had mixed organisms and was not investigated further.

Table 3 shows the results of the MUG test for identification of *E. coli*. Of the *E. coli* isolates, 92% were MUG test positive compared with 25% of *K. pneumoniae* and 20% of *P. mirabilis* isolates recovered by Cult-Dip Plus. None of the remaining isolates were MUG test positive.

In total, 105 *E. coli* isolates were recovered as significant uropathogens. Of these, 88 were recovered by both methods, eight by Cult-Dip Plus only and nine by routine culture only. Of the 99 *E. coli* isolates recovered by Cult-Dip Plus, 96 underwent a MUG test (three were not viewed for fluorescence and were not analysed further) (table 3). Of the 96 tested, 88 (92%) were MUG test positive. Detection of fluorescence was similar for both media on the dip slide. Thirty one of the 99 isolates recovered by Cult-Dip Plus were identified by Vitek and 68 were identified by Bactident. Of the 68 isolates tested by Bactident, 64 (94%) were MUG test positive. Of the eight *E. coli* isolates detected by Cult-Dip Plus only and which were MUG test negative, five were tested by Bactident and three were MUG test positive (see discussion). Overall, the Cult-Dip Plus MUG test had a sensitivity of 91.6% and a specificity of 95.2% and a positive predictive value of 93.6% for identifying *E. coli*.

Of the 95 *E. coli* isolates recovered by routine culture, 77 were tested by Bactident and 70 (91%) were MUG test positive (table 3). Of the 70 MUG test positive isolates, 63 (90%) were also positive by the Cult-Dip Plus MUG test. Of the seven remaining isolates, five were Cult-Dip Plus MUG test negative and the remaining two were untested. Of the 19 routine cultures that the Bactident MUG test were not performed on, 16 were Cult-Dip Plus MUG test positive and three were not tested.

Discussion

The dip slide was introduced in 1967^{10 11} and represented a further development of the dip-spoon method.¹² It was originally seen as an inexpensive, easy to use and convenient system that could be used at the bedside by non-laboratory personnel and still provide reliable and clinically meaningful results.⁵ Many laboratories use dip slides as the method of choice for detecting uropathogens. Kennon and Soderdahl¹³ showed that the dip slide was a cost-effective alternative to conventional urine culture methodology.

Rosenberg *et al*⁷ found that the sensitivity, specificity and positive predictive value of dip slide versus routine culture was 98.8%, 95.7% and 97.2%, respectively, and 97.0%, 98.3% and 98.3%, respectively, for the diaslide urine culture device versus the calibrated loop routine culture method. Our findings of sensitivity, specificity and positive predictive values of 88.3%, 98.0% and 91.9%, respectively, are slightly lower than those reported by Rosenberg *et al*. This difference could be explained by the differences in the two dip slides used in these studies. Alternatively, it could be explained by the difference in urine specimens used in the evaluation: Rosenberg *et al* prescreened urine samples for catalase activity in order to increase the proportion of positive cultures tested and we did not. Rosenberg *et al* state that evaluations using non-screened urine samples should be performed in addition to studies using prescreened urines.

The data showing significant findings detected in urine specimens by one method only (table 1) require further discussion. As previously stated, in all instances where one method (but not both) had significant findings, the corresponding method was scored as having mixed or insignificant findings. Some of these specimens may have been interpreted differently in another laboratory. Given that dip slides sample a larger volume of urine, they probably detect micro-organisms present in lower numbers (that is, contaminants) along with those associated with urinary tract infections. There is no universally accepted protocol for the interpretation of urine cultures. Rather, established guidelines⁹ are widely used; however, interpretation of these guidelines in some clinical situations may vary from one laboratory to another. Therefore, sensitivity and specificity of product evaluations as highlighted here may be different if different interpretative criteria are applied. Laboratories interested in changing their current methodology might best be served by performing evaluations of their own to determine performance of new methodology under their own interpretative criteria.

Ellner and Papachristos³ compared urine dip slides with routine culture and found that the urine dip slides gave equivalent results to those obtained by conventional culture. The percentage of specimens from their evaluations with significant (dip slide, culture: 18.6, 15.3), negative (40.3, 31.4) and insignificant (41.2, 43.3) findings was similar to the results from this current evaluation. Additionally, the percentage and overall distribution of the uropathogens detected in this study is similar to that of Ellner and Papachristos.

Our study showed that there were fewer urine samples with no growth and more with mixed or insignificant findings by Cult-Dip Plus than by routine culture. These findings are undoubtedly due to the fact that Cult-Dip Plus can detect ≥ 100 cfu/ml compared with ≥ 1000 cfu/ml by routine culture. However, adjusting the cut off for Cult-Dip Plus changes the percentage of specimens with mixed or insignificant findings giving 37.4% for both Cult-Dip Plus and routine culture.

B-glucuronidase activity in the genera of *Escherichiae*, *Salmonella* and *Shigella* was described by Kilian and Bulow in 1976.⁸ Feng and Hartman¹⁴ and Hansen and You-rassowsky¹⁵ used B-glucuronidase actively to identify isolates of *E coli* from water and urine cultures, respectively. Trepeta and Edberg¹⁶ added the fluorogenic substrate 4-methylumbelliferyl-B-D-glucuronide to agar plates and were then able to detect *E coli* by ultraviolet light fluorescence of 4-methylumbelliferon following hydrolysis by B-glucuronidase.

Delisle and Ley¹⁷ and Heizmann *et al*¹⁸ found that 89.5% and 90.5%, respectively, of *E coli* isolates were positive for B-glucuronidase activity. In both of these studies the substrate for detection of B-glucuronidase was incorporated directly into solid media. Our results (91.6%) are consistent with their results. It would be unlikely that 100% of *E coli* isolates would be MUG test positive as previous reports^{8,15} indicate that only 94–97% of isolates were positive for this enzyme.

Our finding of three *E coli* isolates that were negative for fluorescence (MUG test negative) by Cult-Dip Plus were subsequently MUG test positive by Bactident can be explained by previous studies showing that both pH and colour change during lactose fermentation can suppress fluorescence.^{18,19} We have found that there was considerable variability in both the presence and intensity of fluorescence produced by micro-organisms present on the dipstick. This problem is easily overcome by the addition of 1 N NaOH to the colonies of micro-organisms. Changing the pH from acidic to basic intensifies the fluorescence, making detection much easier. It is possible, therefore, that the three *E coli* isolates that were negative for fluorescence on the Cult-Dip Plus and MUG test positive by Bactident were, in fact, isolates that were falsely negative due to failure to intensify fluorescence by adjusting the pH.

In conclusion, urine dip slides are easy to use, reliable and a cost-effective alternative to routine culture methods for detecting significant bacteriuria.^{3,13} The Cult-Dip Plus, with the addition of the substrate for detection of B-glucuronidase, is a convenient product for both recovery of uropathogens and the rapid identification of *E coli*. The MUG test in combination with lactose fermentation and indole formation is reliable for the identification of *E coli*. Given that *E coli* is the pathogen recovered most frequently from patients with urinary tract infections, the Cult-Dip Plus will most likely be a cost-effective alternative to other methods currently in use for rapid identification of this micro-organism in urine specimens. We have calculated in our laboratory that of the 754 *E coli* (data not shown) isolates recovered from urine specimens over a 49 week period, use of Cult-Dip Plus versus our current methodology (plated media and Bactident) would have resulted in a 43% saving per isolate. This saving may be greater or smaller in other laboratories depending on methodology and volume discounts on products. A further addition of tryptophan to the medium would enable a spot

indole test to be performed, thereby expanding the utility of this product.

Bacident is a two hour test for identifying *E coli*. It consists of the MUG test and an indole test. In our laboratory only those micro-organisms with a colonial appearance suggestive of *E coli* are processed by this method. A spot indole test is performed before using this method. In the results section it is noted that not all *E coli* isolates recovered by routine culture were tested by Bacident. These isolates would not have been tested because they were either spot indole negative or did not have a typical colonial appearance, or both.

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