Eur J Clin Microbiol Infect Dis (2000) 19:694–698 © Springer-Verlag 2000

Article

Clinical Evaluation of a Novel Chromogenic Agar Dipslide for Diagnosis of Urinary Tract Infections

P. Yagupsky, M. Rider, N. Peled

Abstract The performance of a novel dipslide (DipStreak; Novamed, Israel) consisting of chromogenic agar (Uriselect 3; Sanofi Pasteur, France) and blood agar media was evaluated prospectively and compared to that of conventional urine culture for the diagnosis of urinary tract infection. A total of 1070 clean-catch urine specimens obtained from 251 hospitalized patients and 819 outpatients were processed. The overall performance of the DipStreak was as follows: sensitivity, 95.7%; specificity, 99.2%; agreement, 89.8%; accuracy, 98%; positive predictive value, 98.5%; and negative predictive value, 97.7%. A total of 270 urine specimens were positive by both DipStreak and conventional culture. The chromogenic agar allowed rapid identification of organisms in 211 (78.1%) cultures, while isolates in the other 59 (21.9%) cultures remained unidentified. The results indicate that the DipStreak device coupled with the Uriselect 3 agar represents a convenient and accurate method for inoculation of urine specimens, quantitation of bacteria, diagnosis of significant bacteriuria, and presumptive identification of isolates.

Introduction

The laboratory diagnosis of urinary tract infection (UTI) is based on quantitation of organisms and identification of the isolate [1]. Both goals are achieved by performing streaking dilution of the urine specimen on solid agar media. After incubation, this procedure enables enumeration of colonies, each one representing a viable organism in the original urine sample, and provides individual colonies that are suitable for identification and antimicrobial susceptibility testing [1]. The reliability of this method as a diagnostic tool, however, strongly depends on the transport conditions and the time required to transport the specimen to the laboratory because urine is an excellent culture medium and delayed plating may result in false-positive results [2]. To obviate this inconvenience, dipslide devices that enable bedside inoculation of the urine have been developed [3–6]. Experience gained with these culture devices has demonstrated that dipslides provide accurate quantitative information on the concentrations of organisms in urine [3–6].

P. Yagupsky (✉), M. Rider, N. Peled
Clinical Microbiology Laboratory, Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva 84101, Israel
e-mail: pavloj@bgumail.bgu.ac.il

In the past few years, several chromogenic media have been introduced in clinical microbiology practice to allow the presumptive identification of a wide array of microorganisms on a single medium by means of distinct colony colors. This technology is based on the incorporation of chromogenic substrata into a peptone- and tryptophan-rich culture medium. The presence of organism-specific enzymatic activity is revealed by color changes in the substrata. Recent studies have shown that chromogenic agar enables the identification of a variety of common pathogens, including those that cause UTI [7, 8]. The present study was conducted to evaluate the performance of a novel dipslide (DipStreak; Novamed, Israel) device containing chromogenic agar (Uriselect 3; Sanofi Pasteur, France) for the diagnosis of UTI.

Materials and Methods

The DipStreak device consists of two types of agar media attached back-to-back on a plastic paddle and housed in a transparent plastic tube. A ring with elongated plastic prongs is attached to the end of the paddle so that there are prongs on each side of the slide (Figure 1). The ends of the prongs are dipped into the urine, and upon reinsertion into the plastic tube, the prongs touch the agar surfaces, resulting in streak dilution of the sample and enabling isolation of single colonies in the range of

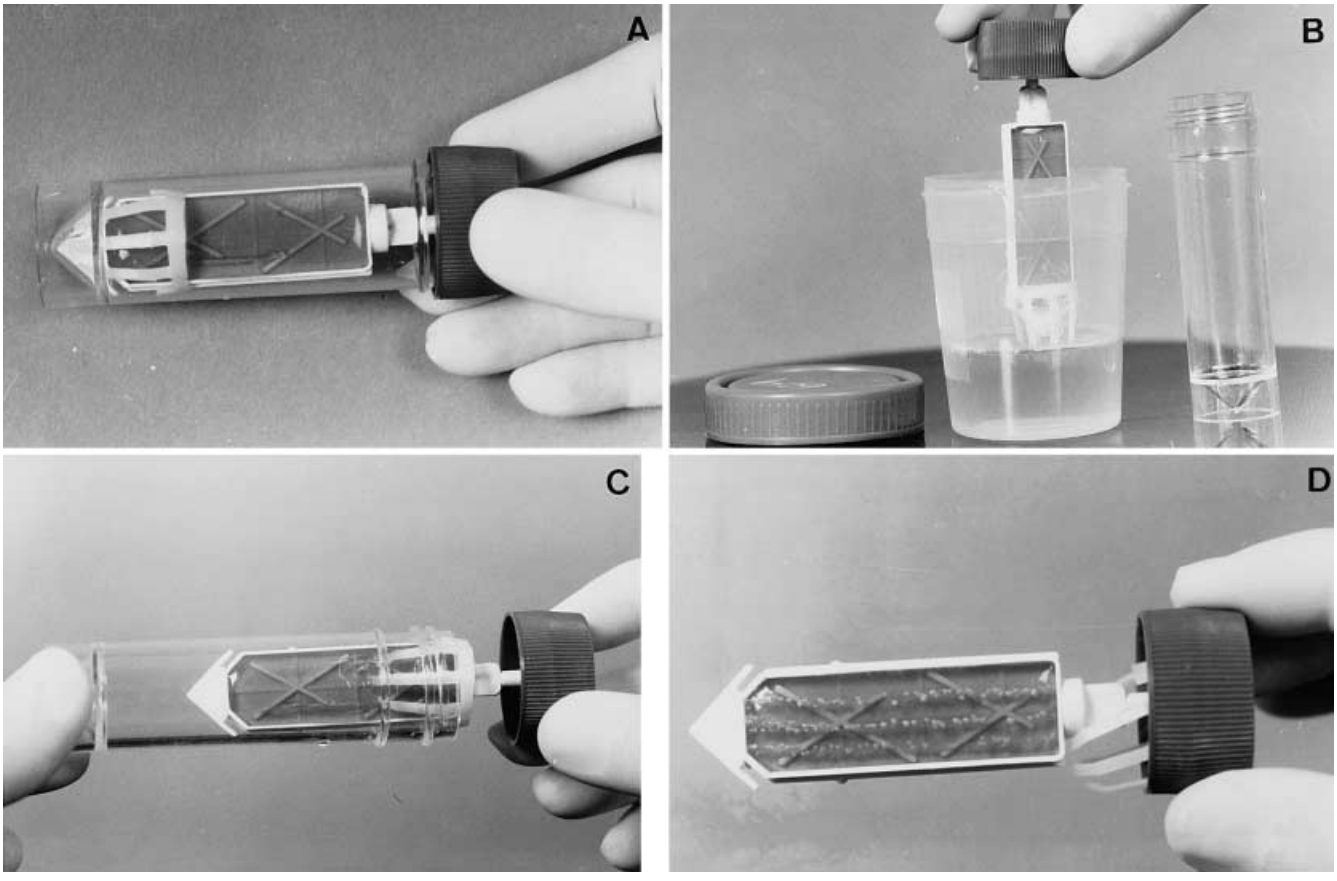

Figure 1 **A**, Design of the DipStreak; **B** and **C**, inoculation procedure; **D**, detection of significant bacteriuria caused by *Candida albicans* (white colonies)

10^3–10^7 cfu/ml. According to the manufacturer, the lower level of detection of the DipStreak device is between 5×10^2 and 5×10^3 cfu/ml. The shelf life of the Uriselect chromogenic agar is 6 months at room temperature. However, because of the combination with a blood-agar medium, the configuration of the DipStreak used in the present study has a shelf life of only 4 months, and it should be kept at 4 °C.

Clean-catch urine specimens obtained from hospitalized patients and outpatients were sent to the Clinical Microbiology Laboratory of the Soroka Medical Center in southern Israel and cultured by the conventional method and the DipStreak technique. Specimens obtained in the course of urologic surgical procedures or by bladder catheterization or by suprapubic aspiration were excluded because the cutoff value to define a positive culture in these specimens is below the lower level of detection of the DipStreak device.

Conventional cultures were carried out by streaking a 90 mm petri dish divided into halves and containing MacConkey and trypticase soy agar with added 5% sheep blood (blood agar plate; Novamed, Israel) with a calibrated 1 μl disposable loop (Quad-Loops; Miniplast Ein Shemer, Israel). DipStreak cultures were performed using the Uriselect 3 blood agar configuration, following the manufacturer's instructions.

After overnight incubation at 35 °C in aerobic conditions, bacterial colonies growing on the plates were counted. Semiquantitative assessment of bacterial growth in the DipStreak devices was performed following the manufacturer's reference chart. If no growth was observed or the colony count was less than 10 cfu, plates and DipStreak devices were reincubated for an additional 24 h to exclude false-negative results caused by insufficient incubation [9]. Antibiotic susceptibility testing of organisms yielding significant bacteriuria (see below) was performed on colonies growing on the blood agar medium.

Organisms growing at a significant level on the plate were also identified to the species level by conventional bacteriological methods and API strips (bioMérieux, France). In the Uriselect 3 medium, presumptive identification of isolates was based on a color scheme and a few simple supplemental tests (Figure 2). The presence of β-glucuronidase was demonstrated by a pink to purple discoloration of colonies and β-glucosidase (esculinase) activity by production of blue to greenish-blue colonies. Tryptophan deaminase activity was detected by a spontaneous orange-brown color of the colony, which was enhanced by depositing a drop of iron perchloride on an isolated colony. To detect tryptophanase (indole production), a drop of Kovac's reagent was deposited on an isolated colony. If the colony turned pink within 15 s, the reaction was considered positive.

To evaluate the performance of the DipStreak device when coupled with the Uriselect 3 medium, the ability of the dipslide to correctly enumerate colonies and the ability of the chromogenic agar to identify uropathogens were evaluated separately, with the results of the conventional culture considered as the gold standard.

For the purposes of the analysis of bacterial quantitation, a simplified report-oriented scheme was used. Significant bacteriuria was defined as growth of $>10^5$ cfu/ml of a single organism or a mixed culture of $>10^5$ cfu/ml of one uropathogen and $<^3$ cfu/ml of other organisms accompanied by nonsignificant growth ($<10^3$ cfu/ml) of other bacteria. Growth of 10^4–10^5 cfu/ml of one

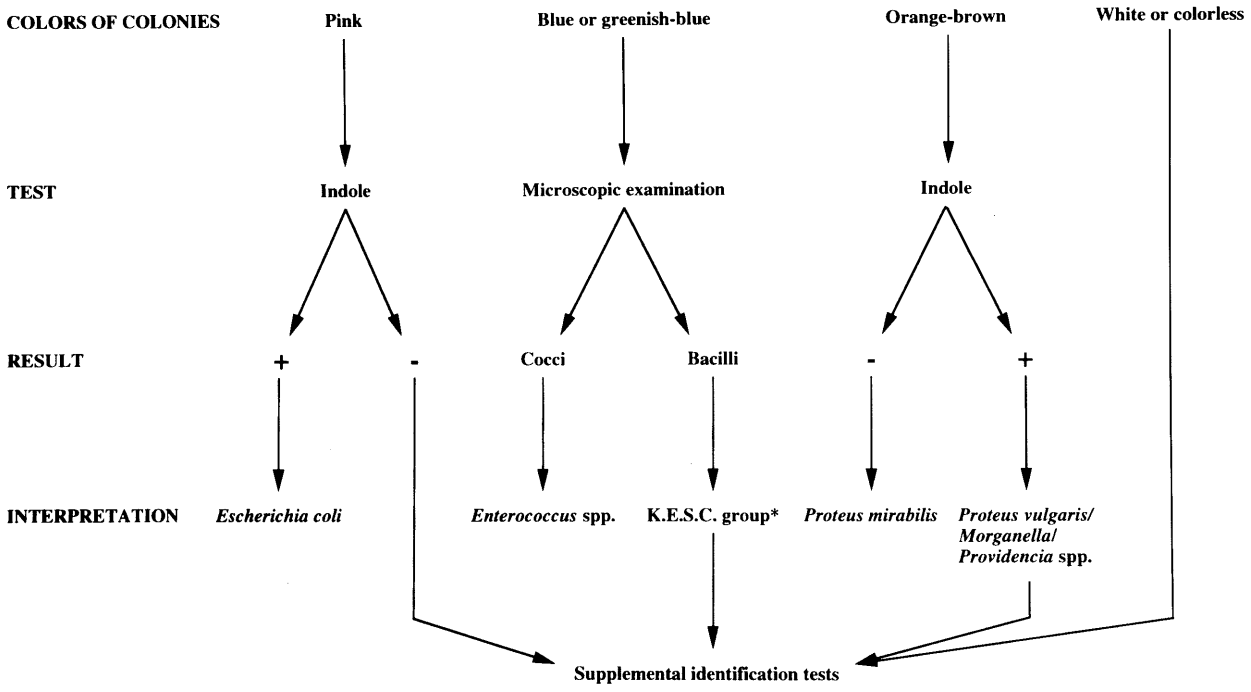

Figure 2 Identification scheme for the Uriselect 3 medium. K.E.S.C. group, *Klebsiella/Enterobacter/Serratia/Citrobacter* spp.

or two organisms indicated the need for a repeat culture. According to recommended protocols, the significance of growth of a single organism at a 10^4–10^5 cfu/ml level needs to be evaluated based on clinical information [1]. In the present study, sufficient clinical data were usually not available, and therefore we chose to classify this result in the repeat culture category to avoid overdiagnosis of UTI. Detection of either sterile cultures or growth of $<10^4$ cfu/ml was interpreted as nonsignificant bacteriuria.

Any difference in the culture results between significant bacteriuria and nonsignificant bacteriuria was considered a major discrepancy. Discrepancies between nonsignificant bacteriuria and repeat culture or between repeat culture and significant bacteriuria were considered minor. This simplification was adopted to facilitate the comparison between the two methods. It was assumed that comparing culture results of a large number of specimens would result in a wide range of quantitative discrepancies (for instance, $>10^5$ cfu/ml by one method and $>10^4$ cfu/ml by the other, or recovery of 2 different isolates at the level of 10^3 and 10^5 cfu/ml by one method and 10^2 cfu/ml and 10^4 cfu/ml by the second method, etc.), making it cumbersome to compare the performance of the two culture methods.

For those cultures yielding significant bacteriuria by both methods, the capability of the chromogenic agar to correctly identify the isolate was determined. Identification of gram-negative organisms as gram-positive or vice versa was considered a major discrepancy, and discrepancies within the gram-negative or gram-positive groups of bacteria were considered minor.

Results

A total of 1070 urine specimens were processed, of which 251 (23.5%) were obtained from hospitalized patients and the remaining 819 from outpatients. Comparison of culture results obtained by both methods and formulae used to calculate the performance of the DipStreak are summarized in Figure 3. Five hundred nine (47.6%) were consistent with nonsignificant bacteriuria, 182 (17%) required repeat culture, and 270 (25.2%) were consistent with significant bacteriuria by both methods (agreement, 89.8%). One hundred nine (10.2%) minor discrepancies but no major discrepancies were found. The overall performance of the DipStreak was as follows: sensitivity, 95.7%; specificity, 99.2%; agreement, 89.8%; accuracy,

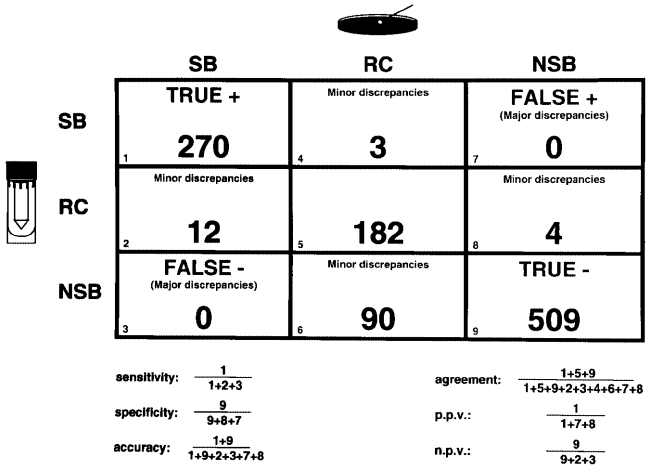

Figure 3 Comparison of results of 1070 urine cultures obtained by DipStreak and conventional plating and formulae used to calculate performance of the DipStreak culture device. SB, significant bacteriuria; RC, repeat culture; NSB, nonsignificant bacteriuria

Table 1 Results of identification of urine isolates by the Uriselect 3 medium

Organism	No. (%) of isolates	No. (%) correctly identified	No. of minor discrepancies	No. of major discrepancies	No. (%) not identified
Escherichia coli	127 (47.0)	110 (86.7)	0	0	17 (13.5)
K.E.S.C. group[a]	48 (17.8)	44 (91.7)	0	0	4 (8.3)
Proteus spp.	29 (10.7)	28 (96.6)	0	0	1 (3.4)
Acinetobacter spp.	16 (5.9)	0 (0.0)	0	0	16 (100.0)
Pseudomonas aeruginosa	9 (3.3)	0 (0.0)	0	0	9 (100.0)
Enterococcus spp.	29 (10.7)	29 (100.0)	0	0	0 (0.0)
Streptococcus agalactiae	5 (1.9)	0 (0.0)	0	0	5 (100.0)
Staphylococcus aureus	2 (0.7)	0 (0.0)	0	0	2 (100.0)
Staphylococcus saprophyticus	1 (0.4)	0 (0.0)	0	0	1 (100.0)
Candida spp.	4 (1.5)	0 (0.0)	0	0	4 (100.0)
Total	270 (100.0)	211 (78.1)	0	0	59 (21.9)

[a] *Klebsiella*/*Enterobacter*/*Serratia*/*Citrobacter* spp. group

98%; positive predictive value, 98.5%; negative predictive value, 97.7%.

Of the 270 urine cultures positive by both methods, the chromogenic agar correctly identified the organism in 211 (78.1%) cultures. Neither major nor minor discrepancies were observed, but 59 (21.9%) isolates remained unidentified (Table 1).

Discussion

In recent decades, many urine culture devices based on a variety of different strategies for inoculating urine onto solid media have been developed. Such devices have incorporated the use of filter paper strips [10], agar-coated pipettes [4], and glass slides [11]. The dipslide approach has gained popularity because it is simple and cost-effective and enables accurate plating of the sample without the need for skilled personnel [3, 5, 6, 11]. Once the urine is applied to the agar, transport conditions of the specimen to the laboratory will not modify the culture results because each multiplying organism yields only a single colony after incubation.

The present evaluation demonstrates that the DipStreak is an accurate dipslide device for the diagnosis of significant bacteriuria, and quantitative culture results obtained by this method are remarkably similar to those of conventional culture. The vast majority of discrepancies were found in urine cultures in which mixed flora at a concentration of $<10^5$ cfu/ml grew in the plates, yet no significant growth was detected by the DipStreak. Although according to the study protocol these results would have required a repeat culture, these results probably represent contamination of the specimen due to a faulty collection technique and/or multiplication of the organism during transport of the urine to the laboratory. This assumption is supported by the relatively large fraction of cultures assigned to the repeat culture category in the study.

The results of the present study also show that use of the chromogenic agar added the advantage of rapid presumptive identification of the isolate in almost 80% of the cases. Overall, *Escherichia coli*, *Proteus* spp., *Enterococcus* spp., and the *Klebsiella*/*Enterobacter*/*Serratia*/*Citrobacter* group represented 233 (86.3%) of the 270 isolates derived from cultures with significant bacteriuria, of which 211 (90.6%) were correctly identified by the chromogenic agar. It should be pointed out that the relative frequency of these pathogens found in this study is similar to that reported in the literature [1, 12].

The main drawback of the chromogenic agar was the growth of white colonies that remained unidentified. These colonies represented a wide array of different organisms, especially *Acinetobacter* spp. and *Pseudomonas aeruginosa*. These bacteria are especially frequent among patients with complicated UTI and nosocomial infections and are less frequent among uncomplicated cases of community-acquired infection [12]. The use of the chromogenic agar was intended to enable identification of the isolate after overnight incubation without the need to perform biochemical tests, thereby saving 24 h and reagents. If the chromogenic medium fails to identify the isolate (white colonies), precise speciation of the organism can still be performed using conventional biochemical tests. It is suggested that use of the oxidase test may contribute to the discriminatory power of the identification system by revealing the presence of *Pseudomonas aeruginosa* and by decreasing the fraction of unidentified uropathogens.

Today, health care is increasingly being provided by medical group practices, health maintenance organizations, and private practices [13]. In these settings, transport of urine specimens to distant central laboratories for culture poses logistical problems that may result in an unacceptable rate of false-positive results. On the other hand, cost-effective identification of organisms in

urine cultures by conventional methods may be difficult to achieve in small community laboratories. The results of the present study suggest that the DipStreak device coupled with the Uriselect 3 medium may be of particular value for the diagnosis of significant bacteriuria ($>10^5$ cfu/ml) and presumptive identification of the causative organisms in ambulatory patients. Decisions concerning the convenience of the use of chromogenic agar for primary plating of urine specimens, however, should take into account additional considerations such as the proportion of urine specimens with nonsignificant growth processed for which the use of chromogenic agar is superfluous, and the local costs of culture media and biochemical tests.

References

1. Clarridge JE, Johnson JR, Pezzlo MT: Cumitech 2B. Laboratory diagnosis of urinary tract infections. American Society for Microbiology, Washington DC (1998) pp 1–6
2. Ascher AW, Sussman M, Waters WE, Davis RM, Chick S: Urine as a medium for bacterial growth. Lancet (1966) ii:1037–1041
3. Ferguson J, Tanner J, Miller JM: Evaluation of a new, semiquantitative screening culture device for urine specimens. Journal of Clinical Microbiology (1995) 33:1351–1353
4. Kennon WG, Soderdahl DW: Dipslide urine cultures and cost containment. Surgery Gynecology & Obstetrics (1982) 155:807–808
5. McAllister TA: The day of the dipslide. Nephron (1973) 11:123–133
6. Rosenberg M, Berger SA, Barki M, Goldberg S, Fink A, Miskin A: Initial testing of a novel urine culture device. Journal of Clinical Microbiology (1992) 30:2686–2691
7. Samra Z, Heifetz M, Talmor J, Bain E, Bahar J: Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. Journal of Clinical Microbiology (1998) 36:990–994
8. Carricajo A, Boiste S, Thore J, Aubert G, Gille Y, Freydiere AM: Comparative evaluation of five chromogenic media for detection, enumeration and identification of urinary tract pathogens. European Journal of Clinical Microbiology & Infectious Diseases (1999) 18:796–803
9. Microorganisms encountered in the urinary tract. In: Baron JE, Peterson LR, Finegold SM (eds): Bailey & Scott Diagnostic Microbiology. Mosby-Year Book, St. Louis (1994) p 255
10. Leigh DA, Williams JD: Methods for detection of significant bacteriuria in large groups of patients. Journal of Clinical Pathology (1964) 17:498–503
11. Cohen SN, Kass EH: A simple method for quantitative urine culture. New England Journal of Medicine (1967) 277:176–180
12. Stamm WE, Hooton TM: Management of urinary tract infections in adults. New England Journal of Medicine (1993) 329:1328–1334
13. Robinson A, Marcon M, Mortensen JE, McCarter YS, LaRocco M, Peterson LR, Thomson RB Jr: Controversies affecting the future practice of clinical microbiology. Journal of Clinical Microbiology (1999) 37:883–889