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Evaluation of the DipStreak, a New Device with an Original Streaking Mechanism for Detection, Counting, and Presumptive Identification of Urinary Tract Pathogens

Claudio Scarparo,* Paola Piccoli, Paolo Ricordi, and Mariuccia Scagnelli

Laboratory of Clinical Microbiology and Virology, S. Bortolo Hospital, Vicenza, Italy

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DipStreak is a new urine culture device with two types of agar attached back-to-back on a plastic paddle. It combines dip-slide technology and an original streaking inoculation mechanism, allowing for bacterial counting and colony isolation. The performance of the DipStreak device with two different medium formulations, CHROMagar and MacConkey media in study A and UriSelect 3 and MacConkey media in study B, was evaluated and compared to that of the reference streak method by using plates of cystine-lactose-electrolyte-deficient (CLED) agar, tryptic soy agar with 5% sheep blood, and UriSelect 3 medium. In study A, 2,000 urine specimens were processed and 511 cultures were found positive. The DipStreak device and the UriSelect 3 and CLED medium plates gave the same detection rate, 99.7%. For the direct identification of *Escherichia coli*, *Proteus mirabilis*, and *Enterococcus* sp. isolates, the DipStreak device and the UriSelect 3 medium plate showed overall sensitivities of 97 and 93.4%, respectively. In study B, 3,000 urine specimens were processed and 714 cultures were found positive. The DipStreak device and the UriSelect 3 and CLED medium plates gave detection rates of 99.4, 99.9, and 99.2%, respectively. For the direct identification of *E. coli*, *P. mirabilis*, and *Enterococcus* sp. isolates, the DipStreak device and the UriSelect 3 medium plate showed overall sensitivities of 88 and 94.4%, respectively. In conclusion, the DipStreak device with both medium formulations represents an attractive and excellent screening method for the reliable detection, counting, and presumptive identification of urinary tract pathogens. It enables bedside urine inoculation and provides a valid means of transporting the sample back to the laboratory, decreasing drastically the rate of false-positive results due to bacterial overgrowth and reducing associated costs.

Urinary tract infection (UTI) is one of the most common acute infectious diseases. It is responsible for a significant share of the workload in many clinical microbiology laboratories (5, 9, 15). A standard method for quantitative urine culturing is the surface streak plate method with calibrated disposable loops. This method allows the isolation of colonies suitable for identification and for antimicrobial susceptibility testing (2, 13).

Delay in transport and improper storage conditions may, alone or combined, limit the quality of the final laboratory report due to the fact that urine is an excellent culture medium and the bacteria present in it may grow rapidly at room temperature.

DipStreak (Novamed, Jerusalem, Israel) is a new urine culture device that combines dip-slide technology and an original streaking inoculation mechanism (two medium surfaces), allowing bacterial counting and colony isolation. This device can be easily inoculated at the collection site and then sent to the laboratory, thus decreasing bacterial overgrowth.

UriSelect 3 (US3) medium (Sanofi Diagnostic Pasteur, Paris, France) and CHROMagar Orientation (CHR) medium (Becton Dickinson, Cockeysville, Md.), two chromogenic media, allow the detection, counting, and presumptive identification of the most common microorganisms involved in UTIs. The identification is based on enzyme detection by means of chromogenic substrates incorporated into the agar. Several chromogenic media have been developed and, due to their reported excellent performance, they can replace conventional primary plating media for the detection of uropathogens (1, 4, 6, 7, 8, 10, 11, 12, 14).

The main objective of this study was to evaluate the UTI diagnostic performance of the DipStreak device with two different chromogenic medium configurations and to compare this performance to that of the reference streak method (calibrated loop) by using plates of US3 medium, cystine-lactose-electrolyte-deficient (CLED) agar (Becton Dickinson), and tryptic soy agar (TSA) with 5% sheep blood (Becton Dickinson).

(These results were partially presented at the 101st General Meeting of the American Society for Microbiology, Orlando, Fla. [C. Scarparo, P. Piccoli, P. Ricordi, and M. Scagnelli, Abstr. 101st Gen. Meet. Am. Soc. Microbiol. abstr. C-20, p. 151, 2001]).

MATERIALS AND METHODS

Urine specimens. In study A, 2,000 routine urine specimens were used for microbiological screening. A total of 876 specimens were collected from outpatients, and 1,124 specimens were collected from patients in different departments of our hospital as follows: 161 from the nephrology and kidney transplant unit, 137 from hematology, 101 from geriatrics, 97 from pediatrics, 92 from metabolic diseases, 90 from obstetrics and gynecology, 89 from intensive care units, 82 from urology, 80 from internal medicine, 62 from surgery, and 133 from other departments. Most of the urine specimens (1,707 from clean-catch midstream urine and 262 from indwelling bladder catheters) were collected in sterile plastic contain-

* Corresponding author. Mailing address: Laboratory of Clinical Microbiology and Virology, S. Bortolo Hospital, Viale Rodolfi, 37, Vicenza, I-36100 Italy. Phone: 39-444-993507. Fax: 39-444-993963. E-mail: claudio.scarparo@tin.it.

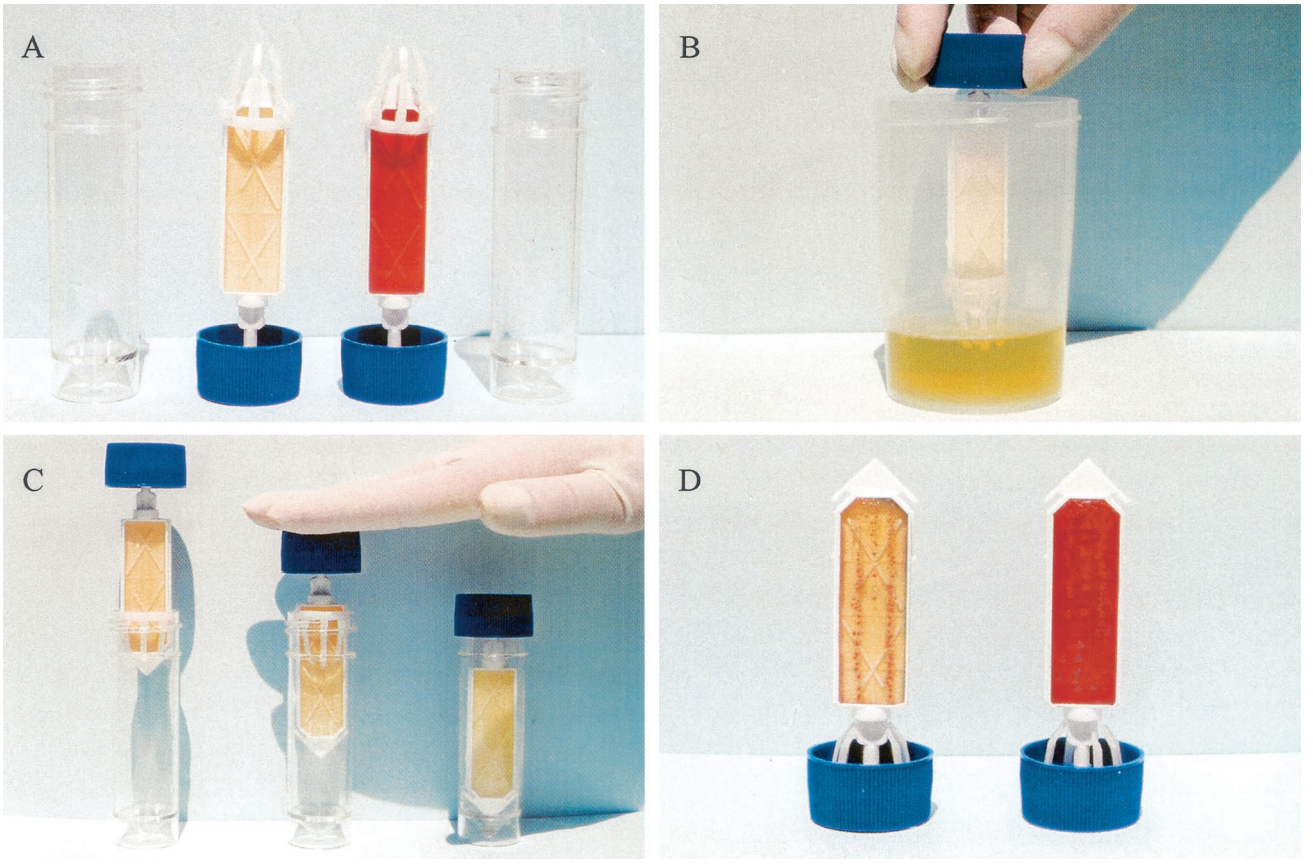

FIG. 1. DipStreak device. (A) DipStreak device with US3 and MacConkey media. (B and C) Inoculation procedures. (D) Detection of an *E. coli* strain on US3 and MacConkey media.

ers, while 31 urine specimens (from pediatric patients) were collected with the application of a sterile plastic adhesive bag.

In study B, 3,000 routine urine specimens were used for microbiological screening. A total of 1,203 specimens were collected from outpatients, and 1,797 specimens were collected from patients in different departments of our hospital as follows: 277 from the nephrology and kidney transplant unit, 223 from hematology, 185 from pediatrics, 156 from geriatrics, 155 from metabolic diseases, 149 from obstetrics and gynecology, 131 from urology, 110 from internal medicine, 104 from surgery, 94 from intensive care units, and 213 from other departments. Most of the urine specimens (2,574 from clean-catch midstream urine and 368 from indwelling bladder catheters) were collected in sterile plastic containers, while 58 urine specimens (from pediatric patients) were collected with the application of a sterile plastic adhesive bag.

Specimens obtained from selected urology and pediatric patients, urine specimens collected by in-and-out (straight) catheterization or suprapubic aspiration, and specimens for which special cultures are recommended (2) were excluded, since the cutoff value that defines a positive culture was below the lower level of detection of the DipStreak device for these specimens.

In these two studies, all specimens were processed immediately on receipt and usually within 2 h after collection; if this was not possible, they were shipped to the laboratory under refrigerated conditions, kept at 4°C, and processed within 4 h after collection.

DipStreak device. The DipStreak device consists of two types of agar attached back-to-back to a plastic paddle housed in a closed transparent plastic tube. A ring with three elongated prongs on each side of the slide is attached to the end of the paddle (Fig. 1A). The tips of the prongs are dipped into the urine sample (Fig. 1B). Upon reinsertion into the plastic tube, the prongs are prevented from moving and the agar surfaces are inoculated with the sample as the prongs pass over the paddle (Fig. 1C). The result is a series of streaks of decreasing bacterial concentrations, which allow for the isolation of single colonies in the range of 10^3 to 10^7 CFU/ml (Fig. 1D). According to the manufacturer, the lowest level of detection of this device is 10^3 CFU/ml. The shelf lives of the DipStreak device with CHR or US3 chromogenic medium coupled with MacConkey agar are 8 months at 4°C and 4 months at room temperature.

Culture media. The US3 chromogenic medium, TSA with 5% sheep blood, and CLED agar are sold as prepared plated media; they were directly inoculated under parallel conditions by the surface streak plate method with a calibrated 0.01-ml disposable loop. DipStreak culturing was performed with two different medium formulations, CHR and MacConkey media in study A and US3 and MacConkey media in study B, in accordance with the manufacturers' instructions. The DipStreak device and the conventional cultures were inoculated with urine specimens in the laboratory at the same time and by the same person in order to correctly evaluate and compare the performance of this new method. Cultures were incubated at 35 to 37°C under aerobic conditions for 18 to 24 h.

Detection of antimicrobial agents. To detect the presence of antimicrobial agents in each urine sample, antimicrobial test plates were used. A 6-mm-diameter paper disk was dipped into the urine sample and placed onto a Mueller-Hinton agar plate seeded with a fully sensitive strain of *Staphylococcus epidermidis* (ATCC 12228). The plates were incubated at 35 to 37°C under aerobic conditions and examined after 18 to 24 h for zones of inhibition around the disks. In study A, a total of 243 specimens were found to contain antimicrobial agents. Of these specimens, 127 yielded clinically significant bacteriuria and 116 yielded nonsignificant bacteriuria or no growth. In study B, a total of 425 specimens were found to contain antimicrobial agents. Of these specimens, 203 yielded clinically significant bacteriuria and 222 yielded nonsignificant bacteriuria or no growth.

Microscopy. A microscopic examination and cell counting were performed on well-mixed noncentrifuged urine for each of the urine samples by using Kova slides with 10 counting grids. Each of the 10 chambers holds a standardized 6.6-μl sample. A sample was considered positive for the presence of pyuria when the count was ≥10 leukocytes/μl. Squamous epithelial cells were recorded as few (<5 cells per low-power field), moderate (6 to 15 cells), or numerous (>15 cells). The presence of bacteria was also recorded. Microorganisms were classified for morphology, and the approximate number per high-power field was quantified.

Presumptive identification and counting. US3 and CHR culture media contain specific chromogenic substrates for particular enzymes, such as β-glucuronidase on US3 medium and β-galactosidase on CHR medium, which determine the development of colonies that are colored from pink to bordeaux or that are clear with a pink to bordeaux center, and β-glucosidase enzyme, which determines the development of colonies colored from blue to greenish blue on both media. With these two media, it is also possible to use two supplementary reagents in order to evidence two other enzyme-related activities: tryptophanase (indole test) and tryptophan deaminase. The activity of tryptophan deaminase is characteristic for the *Proteus-Morganella-Providencia* group, and when this activity ranges from medium to strong, spontaneous and clearly visible production of a diffuse brown or orange pigment colors the medium around the colonies on both media, even without the use of a specific reagent ($FeCl_3$).

According to the manufacturers' instructions, the media enable the identification of the following organisms. (i) *Escherichia coli* colonies produce a pink to bordeaux color, and confirmation of the identification must be performed by detection of indole production by using a drop of Kovács reagent (the indole test can be carried out on filter paper). (ii) *Enterococcus* sp. colonies are small and produce a blue to turquoise color, and microscopic examination is required to confirm the morphology (cocci in chains). On US3 medium, the colony color and the microscopic evaluation allow the identification of *Enterococcus* spp., while on CHR medium, identification is confirmed by the L-pyrrolidonyl-β-naphthalamide hydrolysis test. (iii) *Proteus mirabilis* colonies are colorless or beige and surrounded by a brown or orange halo, and confirmation of the identification must be performed by detection of indole production by using a drop of Kovács reagent. *Proteus penneri* isolates are indistinguishable from *P. mirabilis* isolates (both indole negative) on chromogenic media. Nevertheless, *P. penneri* strains are occasionally isolated from urine specimens, and they are chloramphenicol resistant and can be easily distinguished from *P. mirabilis* strains by antimicrobial susceptibility tests.

The two media also enable the presumptive identification of the following organisms. (i) The *Klebsiella-Enterobacter-Serratia* group develops colonies that are colored from metallic blue to blue-green, and microscopic evaluation is required to confirm the morphology (bacilli). (ii) *Morganella*–indole-positive *Proteus*– *Providencia* group colonies are colorless, beige, or blue-green and surrounded by a brown or orange halo, and they have a positive indole reaction. The latter two groups of bacteria, however, require further biochemical tests for correct identification of the species.

CHR medium is also claimed to allow the presumptive identification of *Staphylococcus saprophyticus* on the bases of the light pink color of the colonies and of microscopic examination, which is required to confirm the morphology (cocci).

All other isolates, including those with questionable or discrepant results, were identified to the species level by standard biochemical identification tests and API systems (Biomerieux, Marcy l'Etoile, France).

Colony counts obtained with the DipStreak device. Semiquantitative assessments of bacterial growth obtained with the DipStreak device were performed by following the manufacturer's reference chart. The manufacturer does not specify the amount of urine that is captured on the elongated prongs.

Interpretation of results. Clinically significant bacteriuria was defined as follows: (i) a bacterial colony count of $\geq 10^5$ CFU/ml for one or two species of probable pathogens (the possible presence of other isolates at low concentrations [$\leq 10^4$] was ignored) and (ii) a colony count of 10^3 to 10^5 CFU/ml for one or two species of probable pathogens from symptomatic patients and from patients with indwelling bladder catheters.

Detection of a bacterial colony count of $<10^4$ CFU/ml in a nonselected patient population devoid of clinical information was interpreted as nonsignificant bacteriuria; detection of sterile cultures was evaluated as no growth. These latter cultures were both considered negative. Detection of more than two species of bacteria at low concentrations in a urine specimen was interpreted as a contaminated (mixed) culture.

The performance of the two configurations of the DipStreak device was evaluated and compared to that of US3, CLED, and TSA–5% sheep blood media plates.

TSA with 5% sheep blood was primarily used to evaluate the detection of fastidious uropathogens. It was not used for bacterial counting due to its several disadvantages, such as the minimal differentiation of the organisms recovered and its complete failure to inhibit the swarming of *Proteus* and other motile bacteria.

Reproducibility study. Five dilutions, from 10^3 to 10^7, of *E. coli*, *Enterococcus faecalis*, and *P. mirabilis* were each tested by using six different DipStreak devices with CHR and MacConkey medium formulations. The number of bacteria used for reference was determined by growth on CLED agar plates. Dilutions of the bacteria were prepared in sterile urine, and the samples were tested with the DipStreak device in accordance with the manufacturer's instructions. The data showed that the DipStreak device yielded consistent and reproducible results at all bacterial dilutions.

Statistical analysis. Presumptive rates of identification of bacteria on each chromogenic medium were evaluated by using the chi-square test (Epi Info, version 6.04; Centers for Disease Control and Prevention). The statistical differences in the quantitative counts of bacteria were determined by Student's *t* test. *P* values of ≤ 0.05 were considered statistically significant.

RESULTS

In study A, a total of 2,000 routine urine samples were processed; of these, 511 (25.5%) showed clinically significant growth on at least one of the media.

A total of 574 organisms were isolated, a single bacterial species in 448 specimens and two bacterial species in 63 specimens (Table 1).

The DipStreak device and the US3 and CLED medium plates gave the same detection rate, 99.7%.

Of the 574 clinically important strains detected, 502 gave concordant counts on the two chromogenic media, 68 gave discordant counts with a difference of ≤ 1 logarithm, and 4 gave discordant counts with a difference of ≥ 2 logarithms. With regard to the last discordance, two cultures yielded a double bacterial isolation on the US3 medium plate, but only one strain was isolated on the DipStreak CHR medium (one strain of *Pseudomonas* sp. and one strain of *E. coli* were not distinguished). Two other cultures yielded a double bacterial isolation on the DipStreak CHR medium, but only one strain was isolated on the US3 medium plate (one strain of *Enterococcus* sp. and one strain of *Klebsiella* sp. were not distinguished).

The presumptive identification results obtained with the DipStreak CHR medium and the US3 medium plate are shown in Table 2.

In this study, the DipStreak device and the US3 medium plate showed overall sensitivities of 97 and 93.4%, respectively, for the identification of *E. coli* ($n = 269$), *P. mirabilis* ($n = 48$), and *Enterococcus* sp. ($n = 95$) strains, which were isolated at a rate of 71.8% (on the basis of the color exhibited and a few confirmatory tests performed directly on the colonies). For the presumptive identification of the *Klebsiella-Enterobacter-Serratia-Citrobacter* and indole-positive *Proteus– Morganella– Providencia* groups, which were isolated at a rate of 15%, the DipStreak device and the US3 medium plate showed overall sensitivities of 97.7 and 88.4%, respectively.

Thirty-nine mixed cultures with more than two species were isolated from urine specimens; of these, 35 (89.7%) exhibited the same number and type of isolates on both chromogenic media. With the conventional method, 4 of the 39 specimens yielded mixed cultures, while with the DipStreak method, only two species were isolated.

A total of 1,450 urine cultures were considered negative. (i) Of 1,446 specimens with no growth or colony counts of $\leq 10^4$ CFU/ml, 1,310 (90.6%) yielded the same colony count with both methods. A total of 136 specimens gave discordant counts on at least one medium, producing no growth or yielding a count of $\leq 10^4$ CFU/ml; all results were considered negative. (ii) Four specimens grew *Lactobacillus* spp. with a colony count of $\geq 10^5$ CFU/ml on DipStreak CHR and CLED media but showed no growth on US3 medium. *Lactobacillus* sp. isolates yielded blue to turquoise colonies on CHR medium, similar to

TABLE 1. Number of clinically important isolates in 511 urine samples in study A

Species	No. of isolates	No. of clinically important isolates detected with:											
		DipStreak device with CHR medium				US3 medium plates				CLED medium			
		Total	Pure	$\geq 10^5$ CFU/ml	$<10^5$ CFU/ml	Total	Pure	$\geq 10^5$ CFU/ml	$<10^5$ CFU/ml	Total	Pure	$\geq 10^5$ CFU/ml	$<10^5$ CFU/ml
Candida spp.	10	10	7	10	0	10	7	6	4	10	7	7	3
Citrobacter spp.	9	9	9	9	0	9	9	9	0	9	9	9	0
Escherichia coli	269	268	241	257	11	269	240	249	20	267	242	252	15
Enterobacter spp.	6	6	4	6	0	6	4	6	0	6	4	6	0
Enterococcus spp.	95	95	51	93	2	94	51	75	19	95	51	90	5
Klebsiella spp.	53	53	42	53	0	52	42	50	2	53	42	53	0
PMP group[a]	62	62	48	61	1	62	48	59	3	62	47	61	1
Pseudomonas spp.	30	29	16	23	6	30	17	24	6	30	16	22	8
Serratia marcescens	4	4	3	4	0	4	3	4	0	4	3	4	0
Staphylococcus aureus	12	12	9	11	1	12	9	9	3	12	9	8	4
Staphylococcus spp.	12	12	9	12	0	12	9	11	1	12	9	10	2
Streptococcus agalactiae	12	12	11	12	0	12	11	10	2	12	11	12	0
Total	574	572	450[b]	551	21	572	450[c]	512	60	572	450[d]	534	38

[a] PMP group, *Proteus-Morganella-Providencia* group.
[b] Two cultures yielded a double bacterial isolation on US3 and CLED media, but only one strain was identified on CHR medium.
[c] Two cultures yielded a double bacterial isolation on CHR and CLED media, but only one strain was identified on US3 medium.
[d] Two urine samples with a double bacterial isolation contained two *E. coli* variants, but only one strain was identified on CLED medium.

those of *Enterococcus* sp. isolates, and a microscopic examination was required to confirm the morphology (bacilli). *Lactobacillus* sp. isolates colonize the genitourinary area but are not recognized as a cause of UTIs; consequently, these cultures were considered negative.

For strain identification, a total of 45 subcultures were ob-

TABLE 2. Color of colonies on CHR medium in the DipStreak device and on US3 medium plates in study A

Organism	No. of isolates (total, 574)	Dipstreak device with CHR medium		US3 medium plates	
		No. (%) of isolates with described color	Description of pigment and/or morphology of colonies	No. (%) of isolates with described color	Description of pigment and/or morphology of colonies
Candida spp.	10	10 (100)	White, creamy, convex	10 (100)	White, creamy, convex
Citrobacter spp.	9	8 (88.9)	Metallic blue with or without pink halo	9 (100)	Light green
		1 (11.1)	Colorless		
Escherichia coli	269	258 (96.3)	Pink to red	247 (91.8)	Pink to red
		10 (3.7)	Colorless to beige	22 (8.2)	Colorless to beige
Enterobacter spp.	6	6 (100)	Metallic blue with or without pink halo	6 (100)	Light green
Enterococcus spp.	95	94 (98.9)	Blue to turquoise, small	93 (98.9)	Blue to turquoise, small
		1 (1.1)	Light blue	1 (1.1)	Light blue
Klebsiella spp.	53	53 (100)	Metallic blue with or without pink halo	48 (92.3)	Light green
				4 (7.7)	Blue
Proteus mirabilis	48	48 (100)	Colorless to beige with brown halo	45 (93.8)	Orange to brown with brown halo
				3 (6.2)	Colorless to beige
Proteus, indole positive	14	13 (92.8)	Colorless to beige with brown halo	9 (64.3)	Orange to brown with brown halo
		1 (1.6)	Blue-green with brown halo	2 (14.3)	Colorless to beige
				3 (21.4)	Light green
Pseudomonas spp.	30	26 (89.7)	Transparent, yellow to green, serrated edge, diffuse	27 (90)	Transparent, yellow to green, serrated edge, diffuse
		3 (10.3)	Beige	3 (10)	Beige
Serratia marcescens	4	4 (100)	Blue-green	4 (100)	Light green
Staphylococcus aureus	12	12 (100)	Golden opaque, white, pink	12 (100)	White to beige
Staphylococcus saprophyticus	2	2 (100)	Pink opaque	2 (100)	White
Staphylococcus spp.	10	10 (100)	White to pink	10 (100)	White to pink
Streptococcus agalactiae	12	6 (50)	Light blue, pinpoint	6 (50)	Light blue, pinpoint
		3 (25)	Blue	6 (50)	Colorless to white
		3 (25)	Colorless to white		

TABLE 3. Number of clinically important isolates in 714 urine samples in study B

Species	No. of isolates	No. of clinically important isolates detected with:											
		DipStreak device with US3 medium				US3 medium plates				CLED medium			
		Total	Pure	$\geq 10^5$ CFU/ml	$< 10^5$ CFU/ml	Total	Pure	$\geq 10^5$ CFU/ml	$< 10^5$ CFU/ml	Total	Pure	$\geq 10^5$ CFU/ml	$< 10^5$ CFU/ml
Candida spp.	15	15	10	12	3	15	10	12	3	14	9	12	2
Citrobacter spp.	15	15	11	15	0	15	11	15	0	15	11	15	0
Escherichia coli	420	419	361	401	18	420	360	399	21	415	365	399	16
Enterobacter spp.	12	12	8	12	0	12	8	12	0	12	8	12	0
Enterococcus spp.	136	132	70	117	15	135	70	118	17	136	70	118	18
Klebsiella spp.	70	70	59	66	4	70	57	63	7	70	57	66	4
Ochrobactrum anthropi	1	1	1	1	0	1	1	1	0	1	1	1	0
PMP group[a]	65	65	50	61	4	65	49	61	4	65	48	61	4
Pseudomonas spp.	43	43	27	42	1	43	27	41	2	43	27	41	2
Serratia marcescens	3	3	2	3	0	3	2	3	0	3	2	3	0
Staphylococcus aureus	7	7	6	7	0	7	6	7	0	7	6	7	0
Staphylococcus spp.	8	8	4	8	0	8	4	8	0	8	4	8	0
Streptococcus agalactiae	15	15	14	15	0	15	14	15	0	15	14	15	0
Total	810	805	623[b]	760	45	809	619[c]	755	54	804	622[d]	758	46

[a] PMP group, Proteus-Morganella-Providencia group.
[b] Five cultures yielded a double bacterial isolation on US3 and CLED medium plates, but only one strain was identified on DipStreak US3 medium.
[c] One cultures yielded a double bacterial isolation on DipStreak US3 and CLED media, but only one strain was identified on US3 medium plates.
[d] Five urine samples with a double bacterial isolation contained two E. coli variants, but only one strain was identified on CLED medium.

tained from the DipStreak device, while 24 subcultures were obtained from the US3 medium plate.

In study B, a total of 3,000 routine urine samples were processed, of these, 714 (23.8%) showed clinically significant growth on at least one of the media.

A total of 810 organisms were isolated, a single bacterial species in 618 specimens and two bacterial species in 96 specimens (Table 3).

For the DipStreak device, the US3 medium plate, and the CLED medium plate, the detection rates were 99.4, 99.9, and 99.2%, respectively.

Of the 810 strains detected, 730 gave concordant counts on the two chromogenic media, 74 gave discordant counts with a difference of ≤ 1 logarithm, and 6 gave discordant counts with a difference of ≥ 2 logarithms. With regard to the last discordant counts, five cultures yielded a double bacterial isolation on the US3 medium plate, but only one strain was isolated on the DipStreak US3 medium (four strains of *Enterococcus* sp. and one strain of *E. coli* were not distinguished). Another culture yielded a double bacterial isolation on the DipStreak US3 medium, but only one strain was isolated on the US3 medium plate (one strain of *Enterococcus* sp. was not distinguished). The presumptive identification results obtained on US3 medium (DipStreak and plate formulations) are shown in Table 4.

In this study, the DipStreak device and the US3 medium plate showed overall sensitivities of 88 and 94.4%, respectively, for the direct identification of *E. coli* ($n = 420$), *P. mirabilis* ($n = 54$), and *Enterococcus* sp. ($n = 136$) strains, which were isolated at a rate of 75.3% (on the basis of the color exhibited and a few confirmatory tests performed directly on the colonies). For the presumptive identification of the *Klebsiella-Enterobacter-Serratia-Citrobacter* and indole-positive *Proteus–Morganella– Providencia* groups, which were isolated at a rate of 13.7%, the DipStreak device and the US3 medium plate showed the same overall sensitivity, 93.7%.

Fifty-two mixed cultures with more than two species were isolated from urine specimens; of these, 48 (92.3%) exhibited the same number and type of isolates on both chromogenic media. With the conventional method, 4 of the 52 specimens yielded mixed cultures; the same cultures evaluated by the DipStreak method evidenced the presence of only two species.

Of 2,234 negative specimens with no growth or colony counts of $\leq 10^4$ CFU/ml, 1,976 (88.4%) yielded the same colony count with the DipStreak device and the US3 and CLED medium plates. A total of 258 specimens gave discordant counts on at least one medium, producing no growth or yielding a count of $\leq 10^4$ CFU/ml; all results were considered negative.

For the identification of strains, a total of 83 subcultures were obtained from the DipStreak device, while 42 subcultures were obtained from the US3 medium plate.

With respect to the DipStreak device with both US3 and CHR medium formulations, the observation of bacterial growth on MacConkey agar (colorless or pink to red colonies are produced, depending upon the ability of the isolate to ferment lactose) increased the accuracy of presumptive identification, particularly when white or beige colonies were grown on chromogenic media.

Confluent growth is often obtained when the bacterial count is $> 10^7$ CFU/ml, making the detection and isolation of urinary pathogens particularly difficult when two or more colonies are present.

Two cultures in study A and five cultures in study B that yielded a double bacterial isolation contained two *E. coli* variants. These strains were not distinguished on CLED medium or TSA with 5% sheep blood, but they were clearly identifiable due to the different color characteristics of the colonies both with the US3 medium plate and with the DipStreak device. Furthermore, these two variants of *E. coli* showed some differences in susceptibility patterns that resulted in a change of category interpretation and some differences in the standard

TABLE 4. Color of colonies on US3 medium in the DipStreak device and on US3 medium plates in study B

Organism	No. of isolates (total, 810)	DipStreak device with US3 medium		US3 medium plates	
		No. (%) of isolates with described color	Description of pigment and/or morphology of colonies	No. (%) of isolates with described color	Description of pigment and/or morphology of colonies
Candida spp.	15	15 (100)	White, creamy, convex	15 (100)	White, creamy, convex
Citrobacter spp.	15	12 (80)	Light green	12 (80)	Light green
		2 (13.3)	Light blue	2 (13.3)	Light blue
		1 (6.6)	Colorless	1 (6.6)	Colorless
Escherichia coli	420	356 (85)	Pink to bordeaux	390 (92.9)	Pink to bordeaux
		63 (15)	Colorless to beige	30 (7.1)	Colorless to beige
Enterobacter spp.	12	12 (100)	Light green	12 (100)	Light green
Enterococcus spp.	136	129 (97.7)	Blue to turquoise, small	134 (99.3)	Blue to turquoise, small
		3 (2.3)	Light blue to green	1 (0.7)	Light blue
Klebsiella spp.	70	69 (98.6)	Light green	69 (98.6)	Light green
		1 (1.4)	Blue	1 (1.4)	Blue
Ochrobactrum anthropi	1	1 (100)	Beige	1 (100)	Colorless
Proteus mirabilis	54	52 (96.3)	Orange to brown with brown halo	52 (96.3)	Orange to brown with brown halo
		2 (3.7)	Colorless to beige	2 (3.7)	Colorless to beige
Proteus, indole positive	11	9 (81.8)	Orange to brown with brown halo	9 (81.8)	Orange to brown with brown halo
		2 (18.2)	Colorless to beige	2 (18.2)	Colorless to beige
Pseudomonas spp.	43	43 (100)	Transparent, yellow to green, serrated edge, diffuse	43 (100)	Transparent, yellow to green, serrated edge, diffuse
Serratia marcescens	3	2 (66.7)	Blue-green	2 (66.7)	Blue-green
		1 (33.3)	Colorless	1 (33.3)	Colorless
Staphylococcus aureus	7	7 (100)	White to beige	7 (100)	White to beige
Staphylococcus saprophyticus	1	1 (100)	White	1 (100)	White
Staphylococcus spp.	7	7 (100)	White to pink	7 (100)	White to pink
Streptococcus agalactiae	15	8 (53.3)	Light blue, pinpoint	8 (53.3)	Light blue, pinpoint
		6 (40)	Blue to violet	6 (40)	Blue to violet
		1 (6.7)	Colorless	1 (6.7)	Colorless

biochemical profiles used to identify them correctly. These differences confirmed the presence of two different *E. coli* strains responsible for UTIs.

The US3 and CHR chromogenic media inhibited the swarming of *Proteus* and other motile bacteria and supported the growth of all uropathogens detected on TSA with 5% sheep blood.

DISCUSSION

The DipStreak device, with US3 or CHR and MacConkey media, combines the advantages of conventional cultures with dip-slide technology and allows bacterial counting, isolation, and presumptive identification at the species or group level with a simple and easy inoculation step.

Since urine is an excellent medium for bacterial growth, the conditions and the time required to transport a sample to the laboratory are critical for the production of accurate laboratory data. The DipStreak device bypasses these problems for the following reasons. (i) It can be easily and correctly inoculated at the collection site even by unskilled personnel and safely conveyed to the laboratory. (ii) When a urine sample is inoculated onto an agar surface, each multiplying bacterium yields only a single colony on subsequent incubation, thus avoiding problems relating to false-positive results associated with bacterial overgrowth.

In the two studies done here, the inoculation of the DipStreak device and conventional cultures with urine specimens was performed in the laboratory at the same time and by the same person in order to correctly evaluate and compare the performance of this new method with regard to detection rates and colony counts.

The availability of a variety of agar combinations attached back-to-back to the plastic paddle allows the DipStreak device to satisfy different laboratory requirements. Several reports have already demonstrated that chromogenic media yield the same performance for detecting common urinary tract pathogens as the combination of two standard primary plating media (such as TSA with 5% sheep blood and MacConkey agar), currently used in many laboratories (1, 4, 6, 7, 8, 10, 11, 12, 14). In the present investigation, the DipStreak device was evaluated with two different chromogenic agar formulations coupled with MacConkey agar and was compared with US3 medium plates and conventional media.

The results of study A showed similar performances for the three media with respect to detection rates; however, when the quantitative counts in Table 1 were compared, the DipStreak CHR medium seemed to yield somewhat higher counts than the other methods, placing many samples in the range of greater than 10^5 CFU/ml. In reality, an accurate analysis of these data revealed the following results. (i) These samples yielded counts in the range of 1×10^5 to 3×10^5 CFU/ml on the DipStreak CHR medium and counts in the range of 5×10^4 to 8×10^4 CFU/ml on the other media. (ii) Only five samples yielded higher counts with the DipStreak device and lower counts on both US3 and CLED media, while in all the other instances, the higher counts obtained with the DipStreak

device were in agreement with the counts obtained on one of the other two media. (iii) These five samples yielding higher counts with the DipStreak device were obtained from symptomatic patients, and three of them were found positive for the presence of pyuria by microscopic examination. (iv) When a pairwise comparison of mean bacterial quantitative counts was performed, no statistically significant differences were found for DipStreak CHR medium versus US3 medium ($t = 1.157$; $P = 0.249$) or for DipStreak CHR medium versus CLED medium ($t = 1.592$; $P = 0.113$).

The results of study B showed similar performances for the three media with regard to detection rates and colony counts. When a pairwise comparison of mean bacterial quantitative counts was performed, no statistically significant differences were found for the DipStreak device versus the other two media.

The use of chromogenic media has the advantage of allowing for rapid presumptive identification of strains at the species level (*E. coli*, *P. mirabilis*, and *Enterococcus* spp.) or at the group level (*Klebsiella-Enterobacter-Serratia-Citrobacter* and indole-positive *Proteus– Morganella– Providencia*). These properties were confirmed for 84.6 and 80.6% of the isolates on CHR medium in the DipStreak device and on U3 medium plates, respectively, in study A and for 79.6 and 84% of the isolates on US3 medium in the DipStreak device and on US3 medium plates, respectively, in study B.

Presumptive identification of *E. coli* strains was better on US3 medium plates (92.9%), which evidenced β-glucuronidase enzyme activity, than on the same chromogenic medium in the DipStreak device (85%). This difference is statistically significant ($P < 0.001$) and, in our opinion, may be attributable to different preparations. The observation of lactose-fermenting isolates on MacConkey agar increased the accuracy of presumptive identification. On the contrary, better presumptive identification of *E. coli* strains was observed on CHR medium in the DipStreak device (96.3%), which evidenced β-galactosidase enzyme activity, than on US3 medium plates (91.8%). The higher sensitivity of β-galactosidase enzyme activity than of β-glucuronidase enzyme activity for *E. coli* identification was recently reported (1, 6).

Only the chromogenic media allowed for the detection of two *E. coli* strains in the same urine specimen, not only because of different color characteristics (in two instances on CHR medium and in five instances on US3 medium) but also because of different drug susceptibilities. Correct differentiation of two different strains is particularly important in order to establish suitable antibiotic treatment.

Two studies evaluating the DipStreak device have been reported so far (3, 16). Despite the limited number of specimens and positive cultures, which characterized the previous studies, the results of these three studies attest to the excellent performance of the DipStreak device.

Growth on the DipStreak media is concentrated along three well-defined streaking lines. When the bacterial count is $<10^7$ CFU/ml, isolated colonies can easily be picked from the agar paddle of the DipStreak device and prepared for identification and susceptibility testing. Confluent growth is often observed when the bacterial count is $\geq 10^7$ CFU/ml, probably due to the smaller surface area of agar available; in this study, subcultures were needed for the isolation of bacterial strains in the event of mixed cultures. This was the only drawback of the DipStreak device observed in our two studies.

In conclusion, the use of the DipStreak device with both chromogenic medium formulations and MacConkey agar represents an attractive and excellent screening method for the reliable detection, counting, and presumptive identification of urinary tract pathogens in both pure and mixed cultures. This closed, safe, and easy-to-use system allows for bedside inoculation of urine specimens and for the proper transport of samples to the laboratory, thus reducing the rate of expensive false-positive results. Although a detailed cost-benefit study was not carried out in this evaluation, it seems clear that the DipStreak device with US3 or CHR medium is a time-saving method that can reduce the daily workload and allow for more efficient use of available resources without compromising clinical performance.

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