Evaluation of DipStreak Containing CNA-MacConkey Agar: A New Bedside Urine Culture Device

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Abstract

Background: Many bedside urine culture devices have been developed with the aim of reliability, simplicity and use in both the physician's office and the clinical laboratory.

Objective: To compare a novel bedside urine culture device (DipStreak®, Novamed Ltd., Israel) comprising a combination of MacConkey and Columbia CNA blood agar with conventional seeding on the same culture media.

Methods: A total of 1,000 urine specimens sent to our microbiology laboratory were simultaneously processed by both methods. Results were evaluated after 24 and 48 hours incubation at 37°C.

Results: Altogether, 171 (17.1%) and 124 (12.4%) specimens were defined as positive by the conventional method using cutoff values of 10^4 colony-forming units/ml and 10^5 CFU/ml respectively; 178 specimens (17.8%) were defined as contaminated. The sensitivity, specificity, positive and negative predictive values of DipStreak for urinary tract infection were 98.8%, 98.6%, 96% and 99.6% respectively, using a cutoff value of 10^4 CFU/ml, and 99.3%, 99.2%, 96% and 99.8% respectively, using cutoff value of 10^5 CFU/ml. Full agreement between both techniques was 95%.

Conclusions: The agreement rate between DipStreak and conventional seeding was remarkably high. These results suggest that DipStreak in the agar combination tested in this study is a useful and precise tool for diagnosing urinary tract infections.

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In the last few years many bedside urine culture devices have been developed [1–4]. The goal of the designers was to develop a reliable, simple, user-friendly device that could be used both in the physician's office and the clinical laboratory. In-hospital laboratories that serve community clinics face logistic problems involved with optimal transportation of the urine specimen from the clinic to the lab. The major concern therefore is how to assure accurate and reliable results from urine specimens that have been hours on the way in variable conditions of transportation.

DipStreak® (Novamed Ltd., Jerusalem, Israel) is a urine culture device designed for isolating and enumerating bacteria in urine. The device comprises a plastic paddle with two types of agar strips attached back-to-back, enclosed in a transparent plastic tube. A ring with elongated prongs is attached to the end of the paddle so that there are prongs on each side of the slide. The ends of the prongs are dipped into the urine sample. Upon reinsertion into the plastic tube, the prongs are prevented from moving, and the agar surfaces are inoculated with urine as the paddle passes over the prongs. The result is a series of streaks of decreasing inoculum concentration that permit isolation of single colonies, even with a high number of organisms/ml. The device is then incubated overnight for culture evaluation the following day. The manufacturer offers a variety of agar combinations. In the present study, we evaluated the MacConkey agar/CNA (Columbia sheep blood agar supplemented with 10 μg/ml colistin/15 μg/ml nalidixic acid) combination.

The aim of our study was to evaluate an alternative agar combination employing DipStreak, a novel urine sample device. With most of the other devices on the market it is difficult to achieve an effective isolation of bacterial colonies. DipStreak introduces a new approach, whereby its unique design enables good colony isolation at 10^4–10^5 CFU/ml levels. DipStreak differs from other similar devices such as Diaslide [5], in that, being a closed system, it protects the agar from drying out and avoids dripping of residual urine.

Materials and Methods

Of the 1,000 urine samples included in this study, 738 (73.8%) arrived from outpatient clinics and 262 (26.2%) from hospitalized patients. Outpatient specimens were sent in a refrigerated container and arrived at the laboratory on the same morning of voiding.

A 1 μl inoculum was streaked using disposable bacteriological loops (Quad-Loops, Miniplast, Kibbutz Ein Shemer, Israel) on each of three 90 mm plates:

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CFU = colony-forming units

MacConkey agar (CM109, Oxoid, UK), CNA (CM331, Oxoid, supplemented with 5% sheep blood, 10 μg/ml colistin and 15 μg/ml nalidixic acid), and SBA (5% sheep blood supplemented Columbia agar base, CM331). The inoculum was seeded on the center of the plate and then spread perpendicularly in order to allow colony counting [6].

All the samples tested in this study were also seeded onto conventional non-selective sheep blood Columbia agar to check for possible loss (if any) of gram-positive organisms sensitive to nalidixic acid or colistin. At the same time, a Columbia CNA–MacConkey DipStreak device was inoculated for each urine sample according to the manufacturer's instructions. In order to avoid bias in culture results, DipStreak and conventional plates employed in this study were manufactured using the same media source.

All plates and devices were incubated at 37°C overnight and read after 18 hours. Colonies were counted and positive cultures were processed using Microscan Urine Combo 5 and Positive Combo 6 panels (Dade, Sacramento, CA, USA) for organism identification and antibiotic susceptibility testing. Panels were processed in a Microscan Walk-Away 96 instrument. All cultures were incubated for an additional 24 hours and read again for final evaluation.

Sub-culturing was performed in two settings: when non-conclusive organism identification gave a "clue" of mixed culture, or when this was necessary in order to achieve accurate colony isolation and further identification of two different organisms that grew together.

Interpretative criteria

- ***Positive culture:*** one type of organism greater than 10^4 CFU/ml (10 colonies per plate) or two organisms when the colony count of one of them equals or exceeds 10^5 CFU/ml.
- ***Negative culture:*** no growth or any number of organisms below 10^4 CFU/ml.
- ***Mixed (contaminated) culture:*** growth of two different organisms, with a colony count for both between 10^4 and 10^5 CFU/ml, or growth of three or more different organisms in any number or growth of *Lactobacillus* sp., *Corynebacterium* sp. or *Streptococcus viridans* in any number.

In order to facilitate the evaluation of the results obtained in our study we divided the positive cultures into two major groups: cultures that grew a single organism between 10^4 and 10^5 CFU/ml, and cultures that grew in numbers equal to or greater than 10^5 CFU/ml.

DipStreak cultures were evaluated according to the chart provided by the manufacturer: 10^4 to 10^5 CFU/ml are represented by 6 to 20 colonies and $>10^5$ CFU/ml by >20 colonies in the agar strip.

Results

A comparison of the results obtained by conventional seeding and DipStreak results is shown in Table 1. From the 1,000 urine specimens in this study, 171 (17.1%) presented positive cultures using 10^4 CFU/ml as a cutoff number or 124 (12.4%) with a cutoff number of 10^5 CFU/ml. Of those cultures, 167 (16.7%) and 121 (12.1%) respectively were also positive by DipStreak. Of 178 (17.8%) specimens presenting mixed growth by conventional seeding, 176 had identical results when their DipStreak cultures had been evaluated.

Seven of the mixed cultures derived from the conventional seeding required subculturing (0.07% of the total number of cultures), while just three (0.03%) of the DipStreak cultures required this procedure. Using a cutoff of 10^4 CFU/ml, 641 of the 651 negative conventional cultures were negative by the DipStreak method.

Table 2 shows that sensitivity and specificity values for DipStreak versus conventional seeding for both cutoff numbers were all above 98.6%. Also, the positive and negative predictive values were remarkably high: 96–99.6% for 10^4 CFU/ml and 96–99.8% for 10^5 CFU/ml respectively, giving an efficiency of 98.8% and 99.3% for the two levels of interpretation.

Table 1. Comparison of DipStreak versus conventional culture results of 1,000 urine specimens

DipStreak	Conventional seeding			
	Positive	Negative	Mixed	Total
Cutoff 10^4 CFU/ml				
Positive	167	8	2	177
Negative	2	641	0	643
Mixed	2	2	176	180
Total	171	651	178	1,000
Cutoff 10^5 CFU/ml				
Positive	121	5	2	128
Negative	1	691	0	692
Mixed	2	2	176	180
Total	124	698	178	1,000

Table 2. Comparison of the quality of results obtained with DipStreak and conventional seeding

	Sensitivity	Specificity	PPV	NPV	Efficiency	Full agreement
Cutoff 10^4	98.8%	98.6%	96.0%	99.6%	98.8%	98.4%
Cutoff 10^5	99.3%	99.2%	96.0%	99.8%	99.3%	98.8%

PPV = positive predictive value, NPV = negative predictive value

Table 3. Relative distribution of organisms isolated by both methods

Organism	Conventional seeding No. (%)	DipStreak No. (%)
Escherichia coli	69 (37.3)	69 (36.6)
Enterococcus faecalis	44 (23.8)	47 (25.0)
Klebsiella pneumoniae	25 (13.5)	23 (12.2)
Staphylococcus coag negative	15 (8.1)	15 (8.0)
Pseudomonas aeruginosa	*7 (3.8)*	*7 (3.7)*
Proteus mirabilis	7 (3.8)	7 (3.7)
Other gram negative	13 (7.0)	13 (7.0)
Other gram positive	4 (2.2)	4 (2.2)
Candida sp.	1 (0.5)	1 (0.5)
Total	185 (100)	186 (100)

Finally, in view of the number of cultures that were evaluated as either mixed or contaminated, we calculated the rate of full agreement. "Full agreement" values (the sum of the total of true positive, true negative and true mixed cultures, expressed as a percentage of the total number of cultures) were 98.4% and 98.8% for cutoff values of 10^4 CFU/ml and 10^5 CFU/ml respectively. A total of 185 organisms were isolated from 171 true positive conventional cultures and 186 organisms from 177 positive DipStreak cultures [Table 3]. *Escherichia coli* (37.3 and 36.6%), *E. faecalis* (18.4 and 19.6%) and *Klebsiella pneumoniae* (13.5 and 12.2%) represented the most frequently isolated bacteria. No significant differences were observed in the kind of organisms isolated by both methods; positive cultures presenting colony counts above 10^4 CFU/ml grew the same organism by both methods in all cases. The count of gram-positive organisms on SBA and CNA plates was comparable in all the cases.

Discussion

The results presented in this study show that cultures performed with DipStreak correlate very well with those using conventional agar plate seeding. This suggests that the DipStreak device may become a satisfactory substitute for conventional urine cultures. Although the rationale of the DipStreak is its benefit of bedside or physician bench inoculation, this point was not tested in the present study.

As a first approach we compared the results of both methods of inoculation that were performed in the laboratory at the same time. Urine sample inoculation at the bedside level could lead to faster laboratory results, saving hospitalization time and costs. These facts have to be proved in further studies.

Until now, most bedside urine sampling devices employed MacConkey agar on one side of the paddle and CLED or blood agar on the other side. The seeding of urine specimens on selective (MacConkey) and non-selective (CLED or blood agar) media in accordance with existing protocols has the advantage of objectively representing the amount and characterization of gram-negative microorganisms. For gram-positive microorganisms, however, this is not always the case since growth on non-blood supplemented media is not optimal and in mixed growth cultures gram positives would usually be overgrown by gram-negative organisms. In such cases, enumeration of streptococci is not objective at best. In order to obtain the gram-positive microorganisms in pure culture and proceed with their identification a re-streak has to be performed, which means that an extra day is lost to laboratory processing.

The advantage derived from colistin/nalidixic acid-supplemented blood agar for gram-positive organisms was described by Fung et al. [7] and adopted by other leading microbiologists like Pezzlo and coworkers [8,9]. Not only is the growth of streptococci more luxurious, but the hemolysis they produce is an important feature in their recognition and preliminary identification. In our opinion, this advantage has to be proved in further studies comparing DipStreak devices containing different agar combinations.

The results obtained in this study suggest that DipStreak, in the agar combination tested here, is a useful and precise tool in the diagnostic of urinary tract infection.

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We do not weep because we are sad; we are sad because we weep.
William James, American psychologist (1842–1910)