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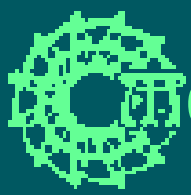

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Evaluation of the DIRAMIC system for detection of urinary tract infections and for *Escherichia coli* identification

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ABSTRACT. The use of the DIRAMIC system for the detection of urinary tract infections (UTI) and the possibility to identify *Escherichia coli* in the same culture media was evaluated. The results from DIRAMIC detection system were compared to counts of colony forming units per milliliter (CFU/ml) of urine inoculated in CLED Medium; 884 urine specimens were processed taking $\geq 10^4$ CFU/ml as criteria of positive urine culture counts. For *E. coli* identification, substrates for the determination of β-glucuronidase and tryptophanase were incorporated to the culture medium and named DETID-Ec. Outputs were compared to those from API RAPIDEC-ur strips. The DIRAMIC system can detect UTI, with a sensitivity and specificity of 82.25 and 94.49%, respectively. It was possible to identify *E. coli* during detection with 87.50% of sensitivity and 95.96% of specificity. The small volumes of culture medium used in the DIRAMIC system as well as the short times for the detection make the system a rapid and economical method for screening UTI. Furthermore, by using DETID-Ec culture medium the time and the number of biochemical tests necessary for the *E. coli* identification are lowered.

Key words: Urine, urinary tract infection, *Escherichia coli*, detection, identification.

RESUMEN. Se evaluó el uso de sistema DIRAMIC en la detección de las infecciones del tracto urinario (ITU) y la posibilidad de identificar *Escherichia coli* en el mismo medio de cultivo. Los resultados de la detección por el DIRAMIC fueron comparados con el recuento de unidades formadoras de colonias por mililitro de orina inoculado (UFC/ml) en Medio CLED; se procesaron 884 muestras tomando como criterio de urocultivo positivo los recuentos $\geq 10^4$ UFC/ml. Para la identificación de *E. coli*, se incorporaron al medio los sustratos para la determinación de la β-glucuronidasa y la triptofanasa y se denominó DETID-Ec, los resultados se compararon con los de tiras API RAPIDEC-ur. El sistema DIRAMIC pudo detectar las ITU con una sensibilidad y una especificidad de 82.25 y 94.49%, respectivamente. Fue posible identificar *E. coli* durante la detección con un 87.50% de sensibilidad y un 95.96% de especificidad. Los resultados de la evaluación del sistema DIRAMIC unido a los pequeños volúmenes de medio de cultivo utilizado y al corto tiempo requerido en la detección, lo convierten en una alternativa rápida y económica para el monitoreo de las ITU. Con el uso del medio de cultivo DETID-Ec, disminuye el tiempo y el número de pruebas bioquímicas necesarias para la identificación de *E. coli*.

Palabras clave: Orina, infección del tracto urinario, *Escherichia coli*, detección, identificación.

INTRODUCTION

UTI are the most common bacterial infections that affect humans and the most frequent urological diseases, in both inpatients and outpatients. For this reason urine is the specimen more frequently processed in clinical microbiology laboratories.[2,4,25] Nevertheless, approximately 20.00% of specimens in urine cultures are positive[6,20] being rather necessary economic and rapid methods for performing this analysis. DIRAMIC is a turbidimetric semiautomatic system developed in the National Center for Scientific Research (DIRAMIC, Cuba) for the detection of UTI just in four hours. This system is being also used in antibiotic susceptibility studies and evaluating microbial quality of raw and pasteurized milk.[8,16]

The microbial enzyme detection indicating the presence of pathogens in clinical specimens has been largely used in microbiological diagnosis, since it allows to save resources and avoid wasting time for results. *Escherichia coli* is the most common microorganism associated with UTI (70.00 – 80.00% of cases).[6,23,25] A wide amount of diagnostic kits use the detection of enzymes β-glucuronidase and tryptophanase as indicators of *E. coli*. On the other hand, demonstration of both enzymes makes possible identification of almost all *E. coli* isolates.[1,3,12,22] The culture medium used for the detection of UTI through the DIRAMIC system was supplemented with the substrates of these enzymes and was named DETID-Ec Medium.

In this survey, the DIRAMIC system and DETID-Ec diagnostic kit (culture medium + revealing reagent of indole) were evaluated in detection of UTI and *E. coli* identification.

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MATERIAL AND METHODS

Clinical specimens

A total of 884 urine specimens from outpatients (557) and inpatients (327) from five hospitals in Havana with symptoms of UTI were processed, 603 specimens from women and 281 from men. These urine samples were obtained mainly by midstream specimens (811), so that the correct method of collection was indicated to the patients; the rest were obtained either by bags applied around the perineum or indwelling catheters (73) using syringes for collection. Specimens were appropriately transported and processed before two hours after collecting.

Evaluated methods

The DIRAMIC system (DIRAMIC, Cuba) harbour as measuring unit a turbidimetric reader calibrated with the Mc Farland turbidity standards. It allows to detect as optical transmittance, the turbidimetric changes produced by the microbial growth in culture media.

The substrates of β-glucuronidase and tryptophanase (4-methylumbelliferyl-β-D-glucuronide and L-tryptophan (OXOID, UK), respectively) were added to the culture medium used for the diagnostic kit of DIRAMIC system. The resulting medium, DETID-Ec, makes possible *E. coli* identification.

Before using DIRAMIC, the system was calibrated with fresh culture media. For urine analysis, 500 µl of urine were inoculated in vials with 4.5 ml of culture medium, a turbidimetric reading was carried out at zero hour and vials were incubated at 37 °C during four hours. Then, a second reading was carried out in order to detect positive specimens by increasing turbidity of cultures; for doubtful results the incubation was lengthened one hour further.

After the infection was detected in a urine specimen, incubation continues up to six hours (that is, one or two hours more). Thus, vials were exposed to ultraviolet light (365 nm) and observed for fluorescence due to 4-methylumbelliferone. Finally, 200 µl of indole detecting reagent were added to detect tryptophanase activity, using the reagent containing p-dimethylaminocinnamaldehyde, which is the most sensitive.[7,13]

Reference methods

The count of CFU/ml in CLED Medium (OXOID, UK) was used as a reference method for the determination of UTI; five µl of urine was delivered to the medium. After 18 h of incubation at 37 ºC, those cultures presenting amounts of $\geq 10^4$ CFU/ml were considered infected specimens.

The identification of all urine isolates was made using API RAPIDEC-ur strips (bioMériux, France). For inoculating strips, homogenized suspensions from several colonies (two-five colonies) showing the same morphology were used. The results are evaluated after two hours of incubation at 37 ºC.

Sensitivity and specificity of the methods

The sensitivity and specificity of both methods were calculated as follows:

$$\% \text{ Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}} \times 100$$

$$\% \text{ Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}} \times 100$$

RESULTS

The results of urine cultures from the 884 urine specimens are shown in Table 1. The evaluation of DIRAMIC system in the detection of UTI generated 139 true positive

Table 1. Comparison of both urinary tract infections detection methods.

	Count of CFU/ml in CLED Medium				
	Positive: 179		Negative: 498		Contaminated: 207
DIRAMIC System	True positive	False negative	True negative	False positive	
Positive: 239	139	—	—	25	75
Negative: 557	—	30	429	—	98
Doubtful: 88	10	—	44	—	34
Sensitivity	82.25%				
Specificity	94.49%				

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results, 25 false positive, 429 true negative and 30 false negative for a sensitivity of 82.25% and specificity of 94.49%. Contaminated samples were not taken into account.

Through the classical method, 179 urine cultures were positive. From them, 190 isolates were totally or partially identified (Table 2). In 11 samples, two different microorganisms were isolated from agar plates; six cases of them, two bacteria produced mixed infection of samples and in the other five, a second bacterium was identified as lactobacillus from normal vagina microbiota.

The results obtained in *E. coli* identification were 74 using DETID-Ec and 80 by API RAPIDEC-ur (Table 3). Other microorganisms, including *E. coli* glucuronidase and/or indole negative, were 105 with DETID-Ec and 99 by API RAPIDEC-ur. The evaluation of DETID-Ec produced 70 true positive results, 4 false positive, 95 true negative and 10 false negative; the sensitivity and specificity were 87.50 and 95.96%, respectively.

Table 2. Identification of bacteria isolated from the 179 positive urine cultures.

Microorganism	Number of isolates (%)
Escherichia coli	80 (42.10)
Escherichia coli glucuronidase negative	9 (4.74)
Escherichia coli indole negative	1 (0.53)
Enterococcus	18 (9.47)
Klebsiella pneumoniae	14 (7.37)
Pseudomonas	11 (5.79)
Staphylococcus aureus	8 (4.21)
Enterobacter-Citrobacter	8 (4.21)
Proteus mirabilis	7 (3.68)
Other microorganisms	34 (17.89)
Total	190 (100)

DISCUSSION

The 20.25% of positive specimens obtained using the reference method are in agreement with reports of some other authors,[6,14,20] also the 3.35% of multiple isolates.[5,6,11] In this survey, 23.42% of the specimens were contaminated, being their quality especially important in the reliability of results by rapid diagnostic methods.

Contaminated specimens must be discarded in the reference method, because in solid culture media it is possible to notice colony morphological differences, so these samples should be reanalyzed. Since in DIRAMIC system reading is made in liquid media, contaminated specimens became distributed among positive, negative or doubtful results. Therefore, contaminated samples taken as doubtful or negative can be discarded, being unnecessary further analysis. For instance, it was observed that the 88.64% of all doubtful results were negative (50.00%), nevertheless they include some colonies, or contaminated specimens (38.64%). In the remaining 11.36% growth indexes reached after four hours hampered positive results.

False positive results are in correspondence with contaminated specimens or with low colony numbers in CLED Agar plates ($<10^4$ CFU/ml). On the other hand, false negative results were those where growth indexes did not reach required values, possibly due to low multiplication velocities of microorganism, as well as intrinsic characteristics or presence of any inhibitory growth substance in urine.

The high specificity achieved (94.49%), together with small volumes of culture medium used and short times in analysis make this system a valuable alternative for screening a higher amount of specimens in any clinical microbiology laboratory. Despite of a sensitivity not so high (82.25%), the use of the system as screening allows to discard just in few hours (four – five hours) negative specimens and therefore focusing work and resources in positive cases.

Table 3. Comparison of both identification methods.

DETID-Ec	API RAPIDEC-ur			
	E. coli: 80		Other microorganisms: 99	
	True positive	False negative	True negative	False positive
E. coli: 74	70	—	—	4
Other microorganisms: 105	—	10	95	—
Sensitivity 87.50%				
Specificity 95.96%				

Results refers to the 179 positive urine cultures in Petri dishes, not for the 190 isolates from these specimens. The four specimens with *E. coli* isolated in mixed infections were considered as "*E. coli*".

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As other commercial systems, sensitivity was higher when the threshold was $\geq 10^5$ CFU/ml (86.67%), but the necessary specificity of screening detection systems fell down to 92.81%. So, the use of one or the other threshold must be choice according to specific laboratory policies.

Regarding identification, the combinations for the six cases of mixed infection were as follows: two *E. coli* - Enterococcus, two *Staphylococcus aureus* - Enterococcus, one *E. coli* - *Staph. aureus* and one *E. coli* - Pseudomonas.

Although *E. coli* was the most frequent microorganism isolated (47.37%), its incidence as the main pathogen in urinary sepsis was rather low, compared to those reports from various authors indicating this bacterium as responsible for more than 70.00% of the cases. It should be considered that a great number of specimens in this survey belongs to patients with anatomical or functional abnormalities in their urinary tracts or with underlying illnesses, where some other microorganisms play a more important role.[6,19,25]

The identification method used as control detected 9 *E. coli* glucuronidase negative strains. Since isolation cultures medium CLED Agar contains lactose, β-glucuronidase activity is lowered or even inhibited in microorganisms growing there because of the acid produced during fermentation.[2,3] This fact may be the cause of the high percentage of glucuronidase negative strains (10.00%). This kind of strains is reported as "other microorganisms" by DETID-Ec diagnostic kit. They must be subsequently identified as *E. coli* by conventional biochemical tests. So, glucuronidase negative and false negative isolates can be correctly identified but to a larger extent.

Moreover, the use of the DETID-Ec diagnostic kit makes possible to discard with 95.96% of specificity the microorganisms different from *E. coli* in infected urine specimens. The three false positive results observed were, according to RAPIDEC-ur strips, one *Staph. aureus* - Enterococcus, one *Klebsiella oxytoca* and one *Proteus vulgaris* - Morganella-Providencia. Previous surveys have reported the presence of β-glucuronidase activity in some strains of Enterococcus and some other members of *Enterobacteriaceae* different from *E. coli* that are also urinary pathogens.[3,17,21] The ability to produce indole from tryptophan is also present in *Kl. oxytoca* and *Pr. vulgaris*.[9,18]

The occurrence of 10 false negative results obviously affected sensitivity. The results may be explained as insufficient incubation times for a right expression of the enzyme β-glucuronidase in assays and consequently a lack of release detectable amounts of 4- methylumbelliferone. Some other authors have observed the need of incubating some isolates overnight for fluorescence expression.[15,17,24] Furthermore, rests of antibiotics in specimens from previous therapies possibly delay the activity of β-glucuronidase.[10] Detection of trytophanase activity was unaffected.

The 87.50% of the *E. coli* isolated were correctly identified in six hours using the DETID-Ec diagnostic kit, with a great specificity, being valuable for this purpose. In addition identification of *E. coli* by means of β-glucuronidase and tryptophanase activities detection represents a promising and economical option. Since rapid results in *E. coli* identification depend on microbial concentration, positive specimens having contaminants at low concentration can be detected through DETID-Ec.

In conclusion, rapid detection of UTI and identification of *E. coli* in direct urine specimens were possible with the use of DIRAMIC system and the DETID-Ec diagnostic kit. These functions are fulfilled with an acceptable sensitivity and high specificity. The reduction of workload, saving resources and time are benefits of the use of DIRAMIC system and DETID-Ec diagnostic kit.

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