Research article/Raziskovalni prispevek

CHROMOGENIC MEDIA FOR URINE CULTURES CAN BE COST-EFFECTIVE

KROMOGENA GOJIŠČA ZA BAKTERIOLOŠKO PREISKAVO URINA SO LAHKO STROŠKOVNO SMOTRNA

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Abstract

Background *Chromogenic media for diagnostic urinary bacteriology have several advantages over traditional media, such as cysteine-lactose-electrolyte deficient (CLED) medium. Chromogenic media allow for easier recognition of mixed growth, save time, reduce workload and provide higher detection rates. However, the cost of chromogenic media is significantly higher compared to CLED and performance of chromogenic media varies depending on the manufacturer. In the present study, performance, turn-around time and cost of Uriselect4 chromogenic medium was compared to CLED.*

Methods *For performance analysis, 351 midstream urine (MSU) samples from September 2005 to December 2005 were directly plated in parallel on Uriselect4 and CLED agar using the calibrated loop technique. Isolates on Uriselect4 were presumptively identified according to the product insert.*
For cost-effectiveness analysis, we included 1,972 consecutive MSU samples from May 2005 to July 2006. We compared the cost of required materials as well as technologists' or specialists' time for each medium examined.

Results *No significant differences were found between the isolation rates of urinary pathogens on the studied media. The procedure using chromogenic media for uropathogens is slightly cheaper than the procedure using CLED, considering the proportion of bacteriuria positive samples (50.5 %) and the distribution of taxa among isolates (namely Escherichia coli with 59.6 %) observed in our laboratory. At the current isolation proportion in MSU samples processed in our laboratory, the average time to reporting results could be decreased by 0.3 days.*

Conclusions *Use of chromogenic media for urine investigations offers multiple advantages without increasing costs compared to procedures using CLED.*

Key words *laboratory techniques and procedures; urinalysis; urinary tract infections; costs and cost analysis; comparative study; chromogenic compounds; culture media*

Izvleček

Izhodišča *Kromogena gojišča za bakteriološko preiskavo urina imajo številne prednosti pred tradicionalnimi gojišči, kot je gojišče s cisteinom in laktozo ter brez elektrolitov (CLED). Kromogena gojišča omogočajo lažje odkrivanje mešane rasti povzročiteljev okužb urinskih poti ter skrajšajo čas preiskave, zmanjšajo delovno intenzivnost ter imajo večjo stopnjo detekcije mikroorganizmov. Največja ovira pri uvedbi teh gojišč v rutinske postopke je njihova cena, saj je od 2- do 13-krat višja od cene gojišča CLED. Poleg tega se kromogena gojišča različnih izdelovalcev razlikujejo v zmogljivosti. Stroškovna smotrnost zamenjave tradi-*

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cionalnih gojišč s kromogenimi gojišči še ni bila podrobno raziskana. V tej raziskavi smo primerjali zmogljivost, trajanje in stroške postopka s kromogenim gojiščem Uriselect4 in postopka z gojiščem CLED.

Metode *Za analizo zmogljivosti gojišča smo 351 zaporednih vzorcev srednjega curka urina od septembra 2005 do decembra 2005 neposredno cepili na gojišči Uriselect4 in CLED s tehniko kalibrirane zanke. Izolate na gojišču Uriselect4 smo identificirali skladno z navodilom za uporabo.*
V analizo stroškovne smotrnosti smo vključili 1972 zaporednih vzorcev srednjega curka urina od maja 2005 do julija 2006. Primerjali smo stroške potrebnega materiala ter potrebne delovne ure laboratorijskih tehnikov in mikrobiologov.

Rezultati *Med gojiščema nismo odkrili statistično pomembnih razlik v stopnji izolacije povzročiteljev okužb urinskih poti. Postopek z uporabo kromogenega gojišča je nekoliko cenejši od postopka z uporabo gojišča CLED, če upoštevamo delež za bakteriurijo pozitivnih vzorcev srednjega curka urina (50,5 %) in porazdelitev bakterijskih skupin med izolati (zlasti bakterije Escherichia coli z 59,6 % vseh izolatov) v našem laboratoriju. Pri tem deležu za bakteriurijo pozitivnih vzorcev srednjega curka urina se povprečno trajanje preiskave urina z uporabo kromogenega gojišča skrajša za 0,3 dneva.*

Zaključki *Uporaba kromogenih gojišč pri bakteriološki preiskavi urina nudi številne prednosti pred postopki z uporabo gojišča CLED brez dodatnih stroškov.*

Ključne besede *laboratorijske tehnike in postopki; analiza urina; okužbe sečil; analiza stroškov; primerjalna raziskava; kromogene spojine; gojišča*

Introduction

Traditionally, isolation of urinary pathogens is performed on cysteine-lactose-electrolyte deficient (CLED) medium. Recently, chromogenic media for diagnostic urinary bacteriology were developed that provide presumptive identification of common urinary tract pathogens. Their performance was confirmed in several studies.[1-7] The greatest advantages of chromogenic media over CLED are easier recognition of mixed growth, shorter analysis duration, workload reduction and higher detection rates. However, chromogenic media from different manufacturers vary in performance and cost. In particular, the cost of chromogenic media presents a major obstacle for their use in routine practice, since it ranges from 2 to 13-times the cost of CLED. The cost-effectiveness of switching from CLED to a chromogenic medium has not been fully established.[1-5, 8] In the present study, the performance, turn-around time and cost for Uriselect4 chromogenic medium were compared to CLED.

Materials and methods

Specimens

For performance analysis, 351 consecutive midstream urine (MSU) samples from September 2005 to December 2005 were included in the study. Of these, 147 samples were referred from the local hospital and 204 samples were from general practitioners.
For cost-effectiveness analysis, 1,972 consecutive MSU samples from May 2005 to July 2006 were included in the study. Of these, 897 samples were referred from the local hospital and 1,075 samples were from general practitioners.
All samples were transported, refrigerated and processed within 24 hours of sampling.

Media and reagents

We used Uriselect4 agar (#64694) from Bio-Rad (Marnes-La-Coquette, France) at € 276 for 500 g and CLED agar (#40129012) from Biolife (Milano, Italy) at € 41 for 500 g. Both media were supplied in dehydrated form and prepared in the laboratory according to manufacturers' instructions. The preparation process and the prepared plates were routinely checked in our ISO 9001:2000 certified quality assurance system. The plates were used within 14 days of preparation. Kovac's indole reagent (Fluka, Buchs, Switzerland) was used for indole production testing on Uriselect4.

Inoculation of media and incubation

MSU samples were directly plated in parallel on CLED and Uriselect4 using the calibrated loop technique.[9] Samples were inoculated with a 1 µL calibrated plastic disposable loop (Copan, Brescia, Italy) using one plate per sample. All plates were incubated in the air at (35 ± 1) °C for 18–20 hours.

Interpretation and identification

Uriselect4 and CLED plates were read independently of each other by separate qualified specialists. The number of colonies on each medium was recorded. All plates that produced 50 colonies or more (i. e. ≥ 5 × 10^4 colony forming units/ml of urine) of any morphological type and contained ≤ 2 isolates were considered indicative for infection and subjected to further identification. Isolates on Uriselect4 were presumptively identified according to the product insert.

All suspected *Escherichia coli* and *Proteus* spp. colonies on Uriselect4 were tested for indole production on the plate as recommended by the manufacturer. A positive indole reaction for *E. coli* presumptives and negative indole reaction for *Proteus mirabilis* presumptives on Uriselect4 were taken as definitive identification. Further identification of other presumptive colonies from Uriselect4, and all identifications from CLED, were performed using conventional morphological and biochemical methods, namely the indole-methyl red-Voges Proskauer-citrate-double sugar agar-urea agar (IMVC) panel for Enterobacteriaceae.

Statistical methods

Differences between media were compared using McNemar's test.

Calculation of cost

For cost-effectiveness analysis, we compared three different protocols for the isolation of urinary pathogens: (i) CLED as the primary medium, followed by the IMVC panel for confirmation of presumptive Enterobacteriaceae; (ii) Uriselect4 as the primary medium, followed by the IMVC panel for confirmation of presumptive Enterobacteriaceae not already identified on the plate; (iii) Uriselect4 as the primary medium, followed by the ID32GN panel (bioMerieux, Marcy l'Etoile, France, € 2.84 per panel) for confirmation of all presumptive gram-negative bacteria not already identified on the plate.

We included the costs of required materials and technologists' or specialists' time (€ 15/h and € 26/h, respectively). The cost of materials included isolation media, Petri dishes, sterile inoculating loops, confirmation reagents needed for presumptive colonies of Enterobacteriaceae**,** enterococci and *Pseudomonas aeruginosa.* The cost of work-up included the technologists' time needed for preparation of media, plating of samples, inoculating of confirmation media, preparing gram stains, administration work at receipt and reporting; as well as the specialists' time needed for reading of plates, colony counting, reading of confirmation media and reading gram stains.

Costs incurred for confirmation of presumptives were included in proportion to the isolation frequency of particular organisms within samples included in the cost-effectiveness analysis.

We also determined the theoretical turn-around for each medium by applying durations of laboratory operations to the above mentioned isolation frequencies. The laboratory operations included in the turn-around analysis were: plating of samples, plate analysis, counting, inoculating of confirmation media, preparing gram stains, administration work at receipt and reporting, and the incubation periods required.

Results

Performance of Uriselect4

Nineteen (5.4 %) of the 351 MSU samples had incomplete data about their processing and, as a result, were excluded from the performance analysis. From 151 bacteriuria positive MSU samples, 144 isolates were recovered on Uriselect4 and 145 isolates were recovered on CLED. On each medium, two of these isolates were yeasts. Three MSU samples exhibiting mixed growth were detected. Table 1 shows the distribution of isolates between both media. Statistical analysis indicated no significant difference between the proportion of isolations from CLED vs. Uriselect4 ($p > 0.05$).

Table 1. *Results of plating mid-stream urine samples on cysteine-lactose-electrolyte deficient (CLED) medium and Uriselect4 chromogenic medium.*

Razpr. 1. *Rezultati nacepljanja vzorcev urina iz srednjega curka na gojišče s cisteinom in laktozo ter brez elektrolitov (CLED) in kromogeno gojišče Uriselect4.*

		Uriselect4		*p* value
		≥ 1 isolate	Non-significant	
CLED	≥ 1 isolate	134	9	> 0.05
	Non-significant	8	181	

Isolates that were cultured from Uriselect4, but not CLED, were enterococci (5 samples), *P. mirabilis* (2 samples), *Klebsiella* spp. (1 sample) and group B streptococci (1 sample). Some isolates of *E. coli* (4 samples), *P. aeruginosa* (2 samples), enterococci (2 samples), coagulase-negative staphylococci (2 samples) were cultured only from CLED and not Uriselect4.

Sensitivity of the media were calculated based on the proportion of strains recovered from each medium. One-hundred-fifty-four isolates were recovered from either CLED or Uriselect4. The media sensitivity was 93.5 % and 94.2 % for Uriselect4 and CLED, respectively.

All but one (98.9 %) of the 87 *E. coli* isolates from Uriselect4 produced colonies of the expected color. The atypical isolate produced a cream colored colony. There were no other cases in which the final identification of isolates did not conform to the colony appearance stated in the Uriselect4 product insert.

Only 9 MSU samples gave a result above bacteriuria threshold and below the upper detection limit of the agar plates (200 CFU/plate), so no conclusions could be confirmed regarding the correlation of CFU levels between CLED and Uriselect4.

Costs

Uriselect4 plates cost significantly more than CLED plates; however Uriselect4 has the ability to satisfactorily identify *E. coli* and *P. mirabilis* on the plate, thereby avoiding the cost of the identification procedure for these organisms. Therefore, the cost of the Uriselect4 procedure for urinalysis depends greatly on the frequency distribution of major urinary pathogens. MSU samples used for cost-effectiveness analysis included 50.5 % samples with ≥ 1 isolate. The most frequently isolated taxa from these samples were included in the cost-effectiveness analysis and had the following isolation frequencies: *E. coli* (59.6 %), enterococci (15.3 %), *P. aeruginosa* (6.3 %). *P. mirabilis*

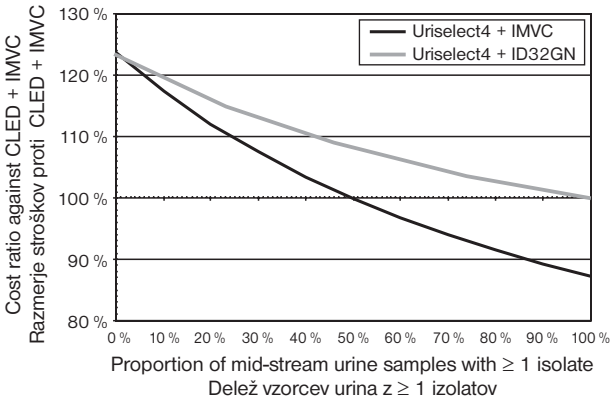

Figure 1. *Cost ratio of procedure using Uriselect4 followed by IMVC panel for confirmation of presumptive Enterobacteriaceae or ID32GN panel for confirmation of presumptive gram-negative bacteria against the procedure using cysteine-lactose-electrolyte deficient (CLED) medium followed by IMVC panel for confirmation of presumptive Enterobacteriaceae.*

Sl. 1. *Razmerje stroškov postopka z gojiščem Uriselect4, kateremu sledi potrjevanje izolatov, sumljivih za enterobakterije na IMVC, ali potrjevanje izolatov, sumljivih za gramnegativne bakterije na ID32GN testu proti postopku z gojiščem s cisteinom in laktozo ter brez elektrolitov (CLED), kateremu sledi potrjevanje izolatov, sumljivih za enterobakterije na IMVC.*

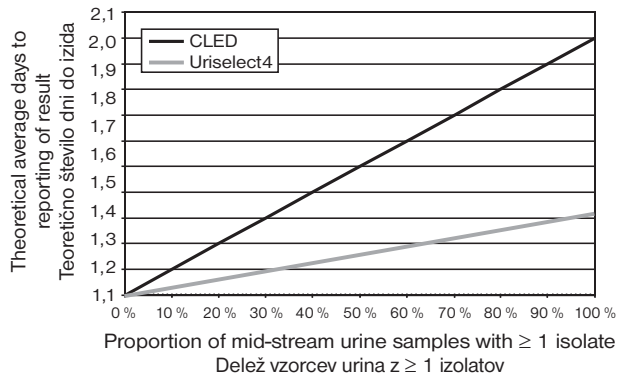

Figure 2. *Theoretical average time to reporting of results against the proportion of mid-stream urine samples with ≥ 1 isolate for the procedure using Uriselect4 or cysteine-lactose-electrolyte deficient (CLED) medium.*

Sl. 2. *Teoretično povprečno trajanje preiskave do rezultata v odvisnosti od deleža vzorcev urina srednjega curka z ≥1 izolatom za gojišče Uriselect4 in gojišče s cisteinom in laktozo ter brez elektrolitov (CLED).*

(5.6 %), *Klebsiella* spp. (4.4 %), *Proteus vulgaris* (1.9 %), *Morganella* spp. (1.4 %), *Enterobacter* spp. (1.0 %).

We constructed a model for urine analysis costs that takes this frequency distribution of isolates into account, and allows for a variable proportion of MSU samples with ≥ 1 isolate. The cost ratio of two different Uriselect4 procedures vs. the CLED procedure is shown in Figure 1. The CLED and Uriselect4 procedures depicted are described in the Materials and Methods section.

Our model shows that the procedure using Uriselect4 followed by IMVC panel for confirmation of presumptive Enterobacteriaceae not already identified on the plate, costs the same as the procedure using CLED followed by IMVC panel for confirmation of all presumptive Enterobacteriaceae, when the proportion of MSU samples with ≥ 1 isolate is higher that 48.5 %. The theoretical turn-around time differs between the two media, since *E. coli* and *P. mirabilis* are identified on Uriselect4 at the same time the plate is read. An additional 24 h for identification of these organisms is necessary on CLED. Figure 2 shows the theoretical average turn-around time for Uriselect4 and CLED procedures.

Discussion

Presumptive identification of urinary pathogens and detection of mixed cultures on CLED medium as well as other traditional media is time-consuming and requires extensive experience in clinical microbiology. The introduction of novel chromogenic compounds into clinical bacterial diagnostics promised an easier and faster identification of isolates, as well as enhanced detection of mixed cultures. The chromogenic compounds used in media for urinary pathogens act as substrates for specific bacterial enzymes, allowing for easier differentiation of taxa and better visualization of mixed cultures.

Several researchers have previously demonstrated equal or superior performance of various chromogenic media over traditional media for identification of urinary tract pathogens.[1-3, 5, 6, 8] Their findings suggest that the chromogenic media studied can be used as a single primary isolation medium for urine samples.

We did not find any significant differences between isolations of urinary pathogens on the studied media. We agree that Uriselect4 can be used as a single primary isolation medium for MSU samples. However, we isolated only two yeasts and, thus, are unable to conclude whether Uriselect4 can be successfully used for isolation of these organisms. Our results do not agree with the findings of a previous author, who found that chromogenic media for urinary pathogens have poor growth support for gram-positive bacteria.[3]

Only one *E. coli* isolate on Uriselect4 had a colony appearance that was discordant with the product insert. The proportion of *E. coli* not exhibiting β-galactosidase activity in our study was comparable to results obtained in other studies.[1] Failure to detect group B streptococci on CLED in one sample confirms the idea that chromogenic urinary media better support the growth of more fastidious strains of *Streptococcus agalactiae* as well as low numbers of this organism compared to traditional media.[1]

In previous studies, chromogenic media detected significantly more mixed growth than CLED agar.[6] Some authors warn when the concurrent presence of mul-

tiple isolates is found on a plate, Kovac's indole reagent should be avoided because tryptophanase metabolites can spread in the medium and reach indole-negative colonies.[2, 5] In our study, only three MSU samples with detected mixed growth did not allow media comparison from this viewpoint.

Uriselect4, like most other chromogenic media for urinary pathogens, contains chromogenic substrates for β-galactosidase and β-glucosidase. In addition, the presence of tryptophan allows for the detection of tryptophan deaminase activity (TDA) as well as tryptophanase activity (production of indole) after adding a drop of Kovac's indole reagent to a colony on the plate. The medium's greatest contribution to the speed of identification is the ability to identify *E. coli* and *P. mirabilis* immediately after reading the plate. As a result, the need for subculturing and performing multiple biochemical tests to identify these organisms is eliminated. *E. coli* is identified by the presence of β-galactosidase activity (pink colonies), absence of β-glucosidase activity and a positive indole reaction. *P. mirabilis* is identified by the absence of β-galactosidase and β-glucosidase activity, presence of TDA (brown precipitate in agar surrounding the colony) and a negative indole reaction.

Based on this information, and the fact that *E. coli* is the predominant species in MSU samples followed closely by *P. mirabilis* isolates, using chromogenic media is potentially cheaper than using traditional media. However, cost-effectiveness depends on the actual proportion of MSU samples with ≥ 1 isolate and the proportion of *E. coli* and *P. mirabilis* isolated in each laboratory. Other researchers speculated that the speed and reliability of identification on chromogenic media may render them cost-effective, although the price of chromogenic media is much higher than traditional media.[1-5, 8] Nevertheless, a detailed cost-benefit study was not performed.

In contrast to the general opinion that chromogenic urinary media are costly, we have shown that the procedure using chromogenic media for uropathogens is cheaper than the procedure using CLED, considering the proportion of bacteriuria positive samples and the distribution of taxa among isolates observed in our laboratory. In our laboratory, the proportion of MSU samples with ≥ 1 isolate is 50.5 % (61.9 % for samples from the local hospital and 38.6 % for samples from general practitioners). In this context, the procedure incorporating Uriselect4 costs practically the same as the procedure using CLED. Even if we upgraded the identification of all presumptive gram-negative bacteria not already identified on chromogenic agar to ID32GN panel, the additional overall cost would be 8.1 %. We consider this to be acceptable, considering the wide coverage of taxa identified by the ID32GN panel. At the current isolation proportion in MSU samples processed in our laboratory, the average time to reporting results could be decreased by 0.3 days.

In conclusion, we believe that the use of chromogenic media, like Uriselect4, in urine investigations, offers multiple advantages without increasing costs in comparison to procedures using CLED. Chromogenic media have equal or greater sensitivity of detection, superior detection of mixed cultures as a result of better visualization of different species, and enable shorter times to reporting results. Chromogenic media also provide much more useful information regarding appropriate antibiotic therapy after only 24 hours compared to CLED. Use of these media also allow for substantial upgrading of biochemical identification procedures without a significant rise in total costs.

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