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Original Article

Comparison of CPS ID 3 and CHROMagar Orientation chromogenic agars with standard biplate technique for culture of clinical urine samples

Jen-Chyi Chang[1,2], Mei-Lin Chien[2], Hung-Mo Chen[2], Jing-Jou Yan[1,2], Jiunn-Jong Wu[1,2]

[1]Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University; and [2]Division of Microbiology, Department of Pathology, National Cheng Kung University Hospital, Tainan, Taiwan

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Background and Purpose: Chromogenic agars have been developed to recognize frequently occurring microorganisms directly on primary cultures, thus reducing the daily workload in a clinical microbiology laboratory. We compare two chromogenic agars, CHROMagar Orientation (CO) and CPS ID 3 (CPS3), with routine media (biplate technique using trypticase soy blood agar and eosin methylene blue agar) for the isolation, enumeration and identification of organisms in urinary tract infection (UTI).

Methods: The clinical significance of the urine samples was categorized as probable UTI, possible UTI, no UTI (negative), or contaminated according to the culture result. Discrepancy analysis with the categories of minor error, major error and very major error was used to compare the culture media.

Results: Of 1386 urine specimens, the consistencies in clinical significance of CO and CPS3 to routine media were 90.7% and 89.8%, respectively. For the enumeration of microorganisms, 524, 514, and 521 clinically significant isolates were isolated on routine media, CO, and CPS3, respectively. Of the 524 significant isolates on routine media, results for 473 and 474 isolates agreed on CO and CPS3, respectively. Approximately 91.9% of *Escherichia coli* and 100.0% of *Enterococcus* spp. could be identified directly on CO media, while 97.5% of *E. coli* and 94.4% of *Enterococcus* spp. could be identified on CPS3 media.

Conclusion: The use of CO and CPS3 as single media is promising for clinical urine culture.

Key words: Agar; Chromogenic compounds; Culture media; Urine

Introduction

The diagnosis of urinary tract infections (UTIs) by performing urine cultures contributes greatly to the daily workload in a clinical microbiology laboratory [1,2]. Therefore, any reduction of the workload while maintaining high-quality performance would be desirable. Traditionally, a non-selective medium, such as trypticase soy blood agar (BP), and a selective and differential medium, such as eosin methylene blue agar (EMB), are used when culturing urine specimens. Identification of bacterial isolates is accomplished based on the appearance of colonies, Gram stain reaction, and results of biochemical tests. The first chromogenic medium that could identify *Escherichia coli* directly in primary urine culture was introduced in 1979 [3]. Thereafter, many chromogenic agars have been developed to recognize frequently occurring microorganisms directly on primary media [4-11], and their performance has been evaluated previously [1,12,13]. In a comparison of the chromogenic agars CHROMagar Orientation (CO) [Becton Dickinson Microbiology, Cockeysville, MD, USA] and CPS ID 2 (bioMérieux, Marcy l'Etoile, France), CO showed higher organism recovery rates, greater ability to detect mixed culture, and more accurate identification of organisms than CPS ID 2 [14]. Recently, CPS ID 3

Corresponding author: Dr Jiunn-Jong Wu, Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, No. 1, University Road, Tainan 70101, Taiwan.
E-mail: jjwu@mail.ncku.edu.tw

(CPS3; bioMérieux, Marcy l'Etoile, France) has been developed in order to improve on the performance of CPS ID 2. The purpose of this study was to compare the use of CO and CPS3 plates with the use of traditional routine media for recovery, enumeration, and identification of organisms in urine cultures.

Methods

Inoculation and incubation

In total, 1386 urine samples sent to the Clinical Microbiology Laboratory, National Cheng Kung University Hospital (NCKUH), Tainan, Taiwan, from January 2, 2006 to April 7, 2006 for routine culture were included. These samples comprised 1270 midstream, clean-catch voided (V) samples and 116 catheterized (T) samples. All inoculation procedures were performed by a senior medical technologist. The 1270 V samples were plated in parallel on BP/EMB biplate (BP/EMB; Becton Dickinson Microbiology), CO, and CPS3 with calibrated disposable 1 μL loop (Becton Dickinson Microbiology). The 116 T samples were plated in parallel on BP/EMB with calibrated disposable 1 μL and 10 μL loops (Becton Dickinson Microbiology), and on CO and CPS3 with calibrated disposable 1 μL loop. A semi-quantitative technique was used to streak the plates. A loopful of urine was inoculated onto each medium by making a straight line down to the center of the plate, and then the plate was streaked for isolation by making a series of close perpendicular streaks through the original line. The chromogenic plates, CO and CPS3, were incubated in ambient air at 35°C overnight, and were read by a senior medical technologist. The BP/EMB plates were incubated in 5% CO_2 in an incubator, and were read and processed by standard protocol in the laboratory with involvement of several medical technologists.

Criteria for clinical significance of isolates

If up to 2 microorganisms were recovered from a sample, an isolate quantitated at ≥100,000 colony-forming units (CFU)/mL in V samples, or ≥1000 CFU/mL in T samples, was considered clinically significant. For BP/EMB plates, all clinically significant isolates showing growth were identified. Conventional methods and the VITEK® system (bioMérieux, Marcy l'Etoile, France) were used for identification. Colonies that grew on chromogenic agar were recorded with regard to colony count, color and size, and occasionally Gram stain result. Growth on BP/EMB served as a reference.

Interpretation and categorization of growth

Culture results were interpreted according to the relevance of UTI at NCKUH [15]. If only one clinically significant isolate was recovered, the sample was considered probable UTI. If only 2 clinically significant isolates were recovered, the sample was considered possible UTI. If up to 2 microorganisms were recovered, and the growth for each microorganism was less than the significance level, the sample was considered negative for UTI. If more than 2 species of microorganisms were recovered, the sample was considered contaminated. A sample considered negative for UTI or contaminated on CO or CPS3, with a result of probable UTI or possible UTI on BP/EMB, was categorized as very major error. A sample considered probable UTI or possible UTI on CO or CPS3, with a result of UTI or contaminated on BP/EMB, was categorized as major error. A sample considered probable UTI, possible UTI, negative for UTI, or contaminated on either CO or CPS3, while simultaneously having a result of possible UTI, probable UTI, contaminated or negative for UTI on BP/EMB, respectively was categorized as minor error.

Colony on CO and CPS3

According to manufacturers' recommendations and previous reports [11,16], colonies were identified as follows: *E. coli* — pink colonies on CO and pink to burgundy colonies on CPS3; *Enterococcus* spp. — small blue colonies on CO and small turquoise colonies on CPS3; *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. and *Citrobacter* spp. (KESC group) — large blue colonies on CO and large green colonies on CPS3; *Proteeae* — beige colonies with brown halo on CO and light brown to dark brown colonies on CPS3; *Streptococcus agalactiae* — pin-like light blue colonies on CO and small violet colonies on CPS3; *Candida* spp. — white colonies on CO and CPS3; *Staphylococcus saprophyticus* — pink opaque colonies on CO; *Staphylococcus aureus* — yellow colonies on CPS3. Colonies whose appearance did not conform to any of these descriptions were recorded as 'other'.

Results

Clinical relevance of growth

On BP/EMB plates, 27.0%, 4.5%, 58.3% and 10.2% of V samples were categorized as probable UTI, possible UTI, negative for UTI, and contaminated, respectively. For T samples, these respective values were

Table 1. Inter-relationship of outcomes of culture of clean-catch voided urine samples using trypticase soy blood/eosin methylene blue biplate (BP/EMB) and CHROMagar Orientation (CO) media

CO media	BP/EMB media				
	Probable UTI	Possible UTI	No UTI	Contamination	Total
Probable UTI	306	15	9	6	336
Possible UTI	16	39	0	1	56
No UTI	14	0	713	26	753
Contamination	7	3	18	97	125
Total	343	57	740	130	1270

Abbreviation: UTI = urinary tract infection

42.2%, 7.8%, 47.4% and 2.6%. On CO plates, 26.5%, 4.4%, 59.3% and 9.8% of V samples were probable UTI, possible UTI, negative for UTI and contaminated, respectively. For T samples, these respective values were 37.9%, 9.5%, 49.1% and 3.4%. On CPS3 plates, 27.0%, 4.3%, 59.3%, and 9.4% of V samples were categorized as probable UTI, possible UTI, negative for UTI, and contaminated, respectively. For T samples, these respective values were 41.4%, 9.5%, 46.6% and 2.6%. The comparative growth results of V samples on 3 kinds of plates (BP/EMB vs CO, BP/EMB vs CPS3, and CO vs CPS3) are shown in Tables 1, 2 and 3, respectively. The analogous comparative growth results for T samples on the 3 kinds of plates were similar to those for V samples. Calculated errors of clinical relevance for CO and CPS3 are shown in Table 4.

Significant isolates on BP/EMB

Of the 400 V samples categorized as probable UTI or possible UTI on BP/EMB media, 457 significant isolates were recovered. Among these isolates were *E. coli* (n = 187), *Enterococcus* (n = 47), *Klebsiella pneumoniae* (n = 44), yeast (n = 41), *Pseudomonas aeruginosa* (n = 31), *Proteus mirabilis* (n = 18), *Acinetobacter baumannii* (n = 16), Gram-positive bacilli (n = 15), *Enterobacter cloacae* (n = 13), *S. aureus* (n = 8), *Serratia marcescens* (n = 5), *Enterobacter aerogenes* (n = 4), *Citrobacter freundii* (n = 4), *S. agalactiae* (n = 4), unidentified glucose non-fermenter (n = 3) and *Citrobacter koseri* (n = 3), group D streptococci (n = 2), *S. saprophyticus* (n = 2), coagulase-negative staphylococci other than *S. saprophyticus* (n = 2), *Morganella morganii* (n = 2), *Proteus vulgaris* (n = 1), *Providencia stuartii* (n = 1), *Pseudomonas fluorescens* (n = 1), *Pseudomonas putida* (n = 1), *Alcaligenes xylosoxidans* (n = 1), and *Klebsiella ozaenae* (n = 1).

Of the 58 T samples categorized as probable UTI or possible UTI on BP/EMB media, 67 significant isolates were recovered. Among these isolates were yeast (n = 20), *E. coli* (n = 16), *Enterococcus* (n = 12), *K. pneumoniae* (n = 4), *P. aeruginosa* (n = 4), *A. baumannii* (n = 2), *E. aerogenes* (n = 2), unidentified glucose non-fermenter (n = 2), *P. mirabilis* (n = 1), *E. cloacae* (n = 1), *S. aureus* (n = 1), coagulase-negative staphylococci other than *S. saprophyticus* (n = 1), and *Stenotrophomonas maltophilia* (n = 1).

The significant isolates recovered on both BP/EMB and CO or both BP/EMB and CPS3 are shown in Table 5.

Discrepant isolates

Among V samples, 29 and 33 isolates had significant growth on BP/EMB, but insignificant growth on CO and CPS3, respectively. Of the 29 insufficient growth isolates on CO, 7 were Gram-positive bacilli, 5 were yeast, 4

Table 2. Inter-relationship of outcomes of culture of clean-catch voided urine samples using trypticase soy blood/eosin methylene blue biplate (BP/EMB) and CPS ID 3 (CPS3) media

CPS3 media	BP/EMB media				
	Probable UTI	Possible UTI	No UTI	Contamination	Total
Probable UTI	307	18	9	9	343
Possible UTI	14	37	0	3	54
No UTI	15	0	709	29	753
Contamination	7	2	22	89	120
Total	343	57	740	130	1270

Abbreviation: UTI = urinary tract infection

Table 3. Inter-relationship of outcomes of culture of clean-catch voided urine samples using CHROMagar Orientation (CO) and CPS ID 3 (CPS3) media

CPS3 media	CO media				
	Probable UTI	Possible UTI	No UTI	Contamination	Total
Probable UTI	334	5	0	4	343
Possible UTI	0	51	0	3	54
No UTI	1	0	743	9	753
Contamination	1	0	10	109	120
Total	336	56	753	125	1270

Abbreviation: UTI = urinary tract infection

were KESC group, 2 were *E. coli*, 2 were *S. agalactiae*, 2 were enterococci, and 7 were others. Of the 33 insufficient growth isolates on CPS3, 7 were Gram-positive bacilli, 8 were yeast, 4 were KESC group, 2 were *E. coli*, 2 were *S. agalactiae*, 2 were enterococci, and 8 were others. Twenty five and 23 isolates, respectively, were significant on CO and CPS3, but insignificant on BP/EMB due to insufficient growth. Of the 25 insufficient growth isolates on BP/EMB, 11 were Gram-positive cocci, 5 were tiny Gram-positive bacilli, and 9 were others. Of the 23 insufficient growth isolates on BP/EMB, 10 were Gram-positive cocci, 5 were tiny Gram-positive bacilli and 8 were others. All Gram-positive cocci were recovered from samples considered possible UTI on CO and CPS3 and probable UTI on BP/EMB. Among these, significant isolates from the samples on BP/EMB were *E. coli*, *K. pneumoniae* and *P. mirabilis*.

Among T samples, 5 and 4 isolates, respectively, had significant growth on BP/EMB, but insignificant growth on CO and CPS3. Of the 5 insignificant growth isolates on CO, 4 isolates grew in quantities of 1000 CFU/mL on BP/EMB. Of the 4 insignificant isolates on CPS3, 3 isolates grew in quantities of 1000 CFU/mL on BP/EMB. Six and 7 isolates, respectively, were significant on CO and CPS3 but had insignificant growth on BP/EMB. Of these isolates with insufficient growth on BP/EMB, 3 were Gram-positive cocci and recovered simultaneously with *Enterobacteriaceae*.

Discussion

In this study, both CO and CPS3 showed less total errors in V samples than in T samples. This probably resulted from larger inoculum differences in T samples than in V samples — 21 μL and 1 μL, respectively. However, sampling errors cannot be excluded [17]. Because of the lack of criteria for acceptable errors and limited use of semi-quantitative urine culture procedures, Reisner and Austin reported that acceptable standards for the replacement of BP/EMB by CO or CPS3 depended on the individual laboratory's situation [17].

The discrepancies in diagnosis of UTI between CO and CPS3 in V samples and T samples were 0.7% and 3.4%, respectively. Three T samples were considered negative for UTI on CO, but probable UTI on CPS3. The microorganisms of these 3 samples on CPS3 were 1000 CFU/mL *K. pneumoniae*, 1000 CFU/mL *Enterococcus*, and 2000 CFU/mL *E. coli*, respectively. The sample that produced *K. pneumoniae* on CPS3 showed comparable results on BP/EMB. The other 2 samples showed comparable results on BP/EMB and CO but discrepant results on BP/EMB and CPS3. The discrepancies between CO and CPS3 were less than between CO and BP/EMB, and between CPS3 and BP/EMB.

The Gram-positive cocci isolates that showed significant growth on CO and CPS but insignificant growth on BP/EMB were probably covered by the large colonies of *Enterobacteriaceae* grown on the

Table 4. Errors of clinical relevance with CHROMagar Orientation (CO) and CPS ID 3 (CPS3) media

Error type	Errors (%)			
	CO media		CPS3 media	
	V sample	T sample	V sample	T sample
Minor error	5.9	4.3	6.5	3.4
Major error	1.3	2.6	1.7	4.3
Very major error	1.9	5.2	1.9	3.4

Abbreviations: V sample = clean-catch voided urine sample; T sample = catheterized urine sample

Table 5. Frequency of significant isolates recovered from urine sample cultures using trypticase soy blood/eosin methylene blue biplate (BP/EMB), CHROMagar Orientation (CO) and CPS ID 3 (CPS3) media

Isolate	No. of colonies recovered (no. expected on CO or CPS3)			
	BP/EMB and CO		BP/EMB and CPS3	
	V sample	T sample	V sample	T sample
Escherichia coli	184 (169)	14 (13)	183 (179)	15 (14)
Enterococcus spp.	42 (42)	12 (12)	42 (40)	12 (11)
KESC group	69 (65)	5 (5)	69 (64)	6 (6)
Klebsiella spp.	43 (43)	3 (3)	43 (43)	4 (4)
Enterobacter spp.	16 (16)	2 (2)	16 (16)	2 (2)
Serratia marcescens	4 (1)	0	4 (1)	0
Citrobacter spp.	6 (5)	0	6 (4)	0
Yeast	34 (32)	17 (15)	32 (31)	17 (17)
Pseudomonas spp.	30	4	30	4
Proteeae tribe	18 (17)	0	19 (18)	0
Proteus spp.	15 (15)	0	16 (16)	0
Morganella morganii	2 (1)	0	2 (1)	0
Providencia stuartii	1 (1)	0	1 (1)	0
Acinetobacter baumannii	13	1	13	1
Gram-positive bacilli	7	0	7	9
Staphylococcus aureus	8	1	8 (8)	1 (1)
Staphylococcus saprophyticus	2 (0)	0	2	9
Coagulase-negative staphylococci other than *S. saprophyticus*	1	1	1	1
Streptococcus agalactiae	2 (2)	0	2 (2)	0
Group D *Streptococcus*	2	0	2	0
Unidentified glucose non-fermenter	2	2	2	2
Alcaligenes xylosoxidans	1	0	1	0
Stenotrophomonas maltophilia	0	1	0	1
Total	415	58	413	61

Abbreviations: V sample = clean-catch voided urine sample; T sample = catheterized urine sample

same BP/EMB plates. Our results do not agree with previous reports that suggest that chromogenic media are unfavorable for the growth of some Gram-positive bacteria [18]. In contrast, the CO and CPS3 media enabled Gram-positive cocci detection probably by color difference or by slight inhibition of growth of large colonies of *Enterobacteriaceae*. For T samples, it was easier to fail growing isolates in quantities of 1000 CFU/mL on CO and CPS3 media, which were inoculated with 1-μL samples, than on BP/EMB, which was inoculated with a total sample of 11 μL.

In this study, approximately 31.5% of V samples and 50.0% of T samples were categorized as probable or possible UTI. Samples from catheterized patients showed higher rates of UTI detection than clean-catch voided samples. These data illustrate the importance of proper instruction of patients to the reliability of results from V sample collection. In addition, the recovered microorganisms were quite different in V and T samples, especially with regard to yeast and *E. coli*. It would be interesting to explore the reasons for these discrepancies.

To summarize, CO and CPS3 were equivalent to BP/EMB with regard to the diagnosis of UTI and the recovery and enumeration of urinary tract pathogens. However, the CO and CPS3 media are attractive for the following reasons: (a) frequently observed pathogens in UTI, *E. coli* and *Enterococcus* spp., can be identified directly; (b) primary isolation can be achieved in a single media where previously 2 were required; and (c) prevention of the overgrowth of some *Enterobacteriaceae*, enabling detection of some Gram-positive cocci, especially in mixed cultures. In addition, the savings of processing time and labor requirements are significant for the microbiology laboratory.

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