

The Usefulness of Chromogenic Media for Qualitative and Semi-Quantitative Diagnostic of Urinary Tract Infections

ELŻBIETA M. STEFANIUK*

Department of Epidemiology and Clinical Microbiology, National Medicines Institute, Warsaw, Poland

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Abstract

The aim of this study was to evaluate the usefulness of chromogenic media for isolation of bacteria from urine and direct identification of UTI pathogens. A total of 100 urine specimens were inoculated on blood agar and MacConkey agar as a reference method and on the following media to be tested: chromID® CPS® Elite (CPSE, bioMérieux), CHROMagar™ Orientation (BioMaxima), BD CHROMagar Orientation Medium (ORI, Becton Dickinson), CHROMagar™ Orientation (ORIE, Graso) and Brilliance UTI Clarity Agar (UTI C, Oxoid). After a 24-hour incubation period, 47 Gram-positive cocci and 62 Gram-negative rods were observed. The specificity and sensitivity of all chromogenic media was 97.3% and 93.5% respectively for qualitative diagnostic; and 81.9% and 81.3% respectively for semi-quantitative diagnostic. The mean PPV and NPV of the chromogenic media were 98.7% and 87.7% for qualitative UTI diagnostic, and 90.9% and 71.9% respectively for semi-quantitative diagnostic.

Key words: chromogenic media, qualitative and semi-quantitative microbiological diagnostic, urinary tract infections (UTI)

Urinary tract infections (UTIs) belong to the most common infections both in community and hospital settings. They account for 10–20% of all infections treated in primary care and 30–40% of infections treated in hospitals. *Escherichia coli* remains the predominant uropathogen (80%) isolated in the acute community-acquired uncomplicated infections. Uncomplicated cystitis and pyelonephritis may also be caused by other species of Enterobacteriaceae rods (*Klebsiella* spp., *Proteus* spp., *Enterobacter* spp.) or, less frequently, by coagulase-negative staphylococci and enterococci. The etiology of UTI also depends on the patient's age, gender and many other factors predisposing them to urinary tract infection as: urinary/fecal incontinence, nephritis, kidney stones, prostate hypertrophy, renal insufficiency, diabetes, kidney empyema, polycystic kidneys and kidney cancer (Pezzlo, 2014; Flores-Mireles *et al.*, 2015; Stefaniuk *et al.*, 2016b).

Urine samples constitute a large share in the daily workload of microbiological laboratories. A combination of blood agars, such as Columbia agar and MacConkey agar, are traditionally widely used for urine culture (Green, 2009; Akter *et al.*, 2014). These are non-selective media capable of supporting the growth of most pathogens. MacConkey agar is able to identify aerobic Gram-negative bacteria by detecting lactose

utilisation, but is not capable for detecting the mixed Gram-negative cultures. The pathogens grow on chromogenic media for UTI diagnostics by forming colonies of a characteristic colour. The pathogen identification is based on chromogenic substrates contained in the media to detect the bacterial enzymes, mainly β -galactosidase, β -glucosidase, β -glucuronidase (the bacteria grow in the form of pink-red or blue-green colonies, respectively) and tryptophan deaminase, which is characteristic for the *Proteae* group of bacteria (*Proteus-Morganella-Providencia*). These pathogens appear on chromogenic media as brownish colonies (Fallon *et al.*, 2003; Pezzlo, 2014; Yarbrough *et al.*, 2016).

The aim of the study was to evaluate the usefulness of chromogenic media in detecting bacteria from urine and identifying UTI pathogens directly. The media were evaluated by using 100 urine specimens sent for routine diagnostic from paediatric and adult patients of two Polish hospitals: the Poviato Hospital in Wołomin, and the Baby Jesus University Hospital in Warsaw, between May and September 2016. Five chromogenic culture media were tested, namely chromID® CPS® Elite (CPSE; bioMérieux, France), BD CHROMagar Orientation Medium (ORI; Becton Dickinson, Germany), Brilliance UTI Clarity Agar (UTI C; Oxoid, USA), CHROMagar™ Orientation (BMagar; BioMaxima,

* Corresponding author: E.M. Stefaniuk, Department of Epidemiology and Clinical Microbiology, National Medicines Institute, Warsaw, Poland; e-mail: elzbieta.stefaniuk@gmail.com

Poland), CHROMagar™ Orientation (ORIE; Graso, Poland). Blood agar (Columbia agar enriched with 5% sheep blood (BAP; Becton Dickinson, Germany) and MacConkey agar (MAC; Becton Dickinson, Germany) served as the reference media. All media were inoculated with a 0.01 ml disposable plastic loop as prescribed (Sharp *et al.*, 2009). The plates were incubated and the bacterial colony characteristics were assessed after 18 to 24 hours at the Department of Epidemiology and Clinical Microbiology (DECM) of the National Medicines Institute in Warsaw, Poland. Additionally, each batch was tested for typical colony appearance and growth with reference strains of *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *K. pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *P. mirabilis* ATCC 29245.

Presumptive identification of isolates using the manufacturer's colour criteria was compared with the biochemical identification of bacteria Vitek 2 Compact system (bioMérieux, Marcy l'Etoile France) as a reference method, but complementary tests such as microscopic examination by Gram stain method, indol, oxidase and catalase detection (Perry, 2017) were also performed. Bacterial growth was recorded semi-quantitatively based on the colony count. All plates were recorded as having no growth (NG), 1, 2, 3, 4 or 5 colony types according to their morphology and colour. The number of each colony type was also recorded to facilitate the identification of pathogens in mixed cultures; 10^2 CFU/ml signified a growth of < 10 CFU on a plate; 10^3 CFU/ml signified 10–99 CFU on a plate, *etc.* (Sharp *et al.*, 2009). The qualitative and quantitative urine culture results were compared with the results of urine culture on BAP and MAC. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the chromogenic media were calculated according to the guidelines of the American Society of Microbiology (ASM) (Sharp and Clark, 2009). For the analysis of the results, all data was entered into an Excel spreadsheet.

In forty-one out of 100 (41%) urine samples no microbial growth (NG) was observed on any of the media used in the study. In the remaining 59 urine samples, 1 to 5 distinct microorganisms were cultured. Sixty-two Gram-negative rods (Enterobacteriaceae $n=61$ and non-fermenting rods $n=1$) and 47 Gram-positive cocci (enterococci $n=22$; staphylococci $n=21$; streptococci $n=4$) were isolated from the urine samples. All of the isolates were screened for their colony colours using the colour criteria provided by manufacturers. Table I shows the analysis of bacterial growth on chromogenic media as compared with the reference method. *E. coli* constituted 53.2% ($n=33$) of the Gram-negative rods collected from urine samples. Differences in *E. coli* growth were observed in three cases (no. 80, 3866 and

4181). *Enterobacter cloacae* was characterized by KES colonies (*Klebsiella*, *Enterobacter*, *Serratia*), while colonies of *Citrobacter freundii* resembled the *E. coli* ones in colour. None of the tested chromogenic media allowed identification of *Acinetobacter* spp. colonies by colour; the colony colour suggested the *Proteae* group.

As regards the Gram-positive cocci group, four streptococcal isolates grew on all media used in this study, but various results were obtained for twelve isolates – four *Enterococcus faecalis* (samples no.: 91, 4181, 12935, 3866) and eight staphylococcal isolates (*S. epidermidis* $n=2$; *S. haemolyticus* $n=2$; *S. hominis* $n=2$ and *S. warnerii* $n=1$). Colonies of *Enterococcus* spp. are similar in colour, their identification is therefore only possible to the level of the genus. Colonies of *Streptococcus* spp. appear on chromogenic media as blue. Streptococci and enterococci are easily distinguishable from each other, since the *Enterococcus* spp. colonies are blue-green and grow larger than streptococci colonies.

Monoculture was recorded in 31 (52.5%) “positive” samples; two morphologically different bacterial isolates were cultured in 12 (20.3%) urine samples; three different strains were found in 7 (11.9%) samples, four – in 7 (11.9%) samples, whereas one cultured sample (1.7%) yielded five different microorganisms. An analysis of the number of samples containing mixed bacterial population showed that all five chromogenic media evaluated in this study allowed for pre-differentiation of pathogen types when compared to the reference method. A direct comparison of these chromogenic media showed minor discrepancies between them in detecting all microorganisms when mixed culture was observed (Table II).

Table III summarises the sensitivity, specificity, PPV and NPV of chromogenic media for UTI diagnostic with qualitative and semi-quantitative colony count (for all pathogens) and the sensitivity for *E. coli* only. The specificity and sensitivity of chromogenic media calculated for the entire study group was 97.3% and 93.5% respectively for the qualitative diagnostic, whereas for the semi-quantitative diagnostic the values mentioned above were 81.9% and 81.3% respectively. The mean value of PPV and NPV of the chromogenic media for the qualitative UTI diagnosis was 98.7% and 87.7% respectively, and for semi-quantitative diagnosis, 90.9% and 71.9% respectively. The difference between the chromogenic media in qualitative and semi-quantitative colony count methods was not statistically significant ($p>0.99$ and $p=0.9$ respectively). The sensitivity main values (%) of chromogenic media for qualitative and semi-quantitative cultures for *E. coli* were 96.0% and 90.6% respectively. In qualitative UTI diagnostic, the ORIE achieved the highest sensitivity in the study, amounting to 97.2%. The CPSE and ORIE media exhibited the highest sensitivity for the semi-quantitative

Table II
Detection of bacteria in mono- and mixed urine culture on chromogenic media for UTI diagnosis.

Number of isolates in a clinical specimen	Number of clinical specimens (urine)	Number of all isolates in all clinical specimens	Total number of isolates (% of the number cultured on reference media)				
			Chromogenic media				
			ChromID CPS Elite (CPSE, bioMérieux)	BD CHROMagar Orientation Medium (ORI, Becton Dickinson)	Brilliance UTI Clarity Agar (UTI C, Oxoid)	CHROMagar™ Orientation (BioMaxima)	CHROMagar™ Orientation (ORIE, Graso)
1	31	31	30 (96.8%) [†]	29 (93.6%) [†]	31 (100%)	30 (96.6%) [†]	31 (100%)
2	12	24	23 (95.8%) [†]	22 (91.7%) ^{†,‡}	22 (91.7%) [§]	23 (95.8%) [§]	24 (100%)
3	7	21	21 (100%)	18 (85.7%) ^{†,‡,††}	17 (81.0%) ^{†,‡,§}	19 (90.5%) [†]	20 (95.3%) [†]
4	7	28	25 (89.3%) ^{†,‡}	25 (89.3%) ^{†,‡,‡}	27 (96.4%) [†]	25 (89.3%) ^{†,‡,‡}	26 (92.6%) ^{†,‡}
5	1	5	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)

[†] – no growth of *Staphylococcus* spp. cultured on sheep blood-enriched Columbia agar with a colony count of 10² CFU/ml (clinical specimens no. 78, 79, 89, 3855, 3873, 12929, 12935)

[‡] – no growth of *Staphylococcus* spp. cultured on sheep blood-enriched Columbia agar with a colony count of 10³ CFU/ml (clinical specimen no. 4181)

[§] – no growth of *Enterococcus* spp. cultured on sheep blood-enriched Columbia agar with a colony count of 10² CFU/ml (clinical specimens no. 12935, 4181, 3866)

[¶] – no growth of Gram-negative rods cultured on sheep blood-enriched Columbia agar or MacConkey agar with a colony count of 10² CFU/ml (clinical specimens no. 3866, 80)

^{††} – no growth of Gram-negative rods cultured on sheep blood-enriched Columbia agar and MacConkey agar with a colony count of 10³ CFU/ml (clinical specimen no. 4181)

Table III
Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) of chromogenic media for UTI diagnosis.

Parameters	Chromogenic media					Mean value
	ChromID® CPS® Elite (CPSE, bioMérieux)	BD CHROMagar Orientation Medium (ORI, Becton Dickinson)	Brilliance UTI Clarity Agar (UTI C, Oxoid)	CHROMagar™ Orientation (BioMaxima)	CHROMagar™ Orientation (ORIE, Graso)	
Qualitative cultures for all detected pathogens (p > 0.99)						
Sensitivity (%)	94.7	91.7	89.6	94.4	97.2	93.5
Specificity (%)	97.7	95.5	97.7	97.7	97.7	97.3
Positive predictive value (PPV) (%)	98.9	98.0	98.4	99.0	99.1	98.7
Negative predictive value (NPV) (%)	89.4	82.4	85.7	87.8	93.3	87.7
Qualitative cultures of <i>E. coli</i>						
Sensitivity (%)	96.8	90.1	100	93.3	100	96.0
Semi-quantitative cultures for all detected pathogens (p = 0.9)						
Sensitivity (%)	85.2	80.2	75.0	81.0	85.1	81.3
Specificity (%)	85.7	82.4	91.3	82.7	82.4	84.9
Positive predictive value (PPV) (%)	91.5	90.0	92.3	90.0	90.5	90.9
Negative predictive value (NPV) (%)	76.4	67.7	72.4	69.4	73.7	71.9
Semi-quantitative cultures of <i>E. coli</i>						
Sensitivity (%)	93.1	84.0	95.2	87.1	93.6	90.6

urine culture (85.2% and 85.1% respectively). The UTI C and CPSE media had the highest PPV for the semi-quantitative method, achieving 92.3% and 91.5% respectively, whereas BMagar had the lowest PPV of all (90.0%). The CPSE and ORIE media had the highest NPV for the semi-quantitative method, achieving 76.4% and 73.7% respectively, whereas the lowest NPV of all (70.0%) was recorded for BMagar and ORI.

A detailed analysis of the semi-quantitative assay of different bacteria species on chromogenic media when compared to the BAC and MAC reference media demonstrated a tendency for decreased colony count on some media, yet the differences were not statistically significant. The count of *Staphylococcus* spp. was lower on all chromogenic media. Enterococcal growth was inhibited in three of the five tested media (UTI C, BMagar and

ORI). A diminished colony count (CFU/ml) of Enterobacteriaceae was observed on ORI and UTI C media.

Microbiology laboratories always attempt to reduce the turnaround time and the cost of pathogens identification and their susceptibility testing (Strauss and Bourbeau, 2015). In recent years, chromogenic culture media have been widely used in clinical microbial diagnostic for detection and preliminary identification of pathogens and antimicrobial resistance mechanisms (Fillius *et al.*, 2003; Kuch *et al.*, 2009; Stefaniuk *et al.*, 2016a; Yarbrough *et al.*, 2016). In our study, apart from the results obtained using the three chromogenic media used all over the world (CPSE, ORI and UTI C), we present results obtained using two media manufactured in Poland: BMagar – BioMaxima and ORIE – Graso, which are offered for a lower price. Moreover, along with the qualitative analysis of the cultures obtained on these media, a thorough qualitative analysis of the specimens of the urine samples was conducted. Many studies have shown identical or even better results regarding the detection and identification of UTI pathogens using various chromogenic media over conventional media (Aspevall *et al.*, 2002; Scarparo *et al.*, 2002). The interpretation of mixed cultures of urines is difficult. To diagnose the mixed urine cultures properly, a laboratory needs additional information about the patient. As earlier studies have suggested (Price *et al.*, 2016), chromogenic media are more effective in preventing the swarming of *Proteus* species when compared to conventional media: blood agar or MacConkey agar. For this reason chromogenic media have enjoyed great popularity. Our research adds to this knowledge by demonstrating the utility of chromogenic media in mixed culture detection. It was shown, however, that depending on the media type, colonies of the same isolate might take different colours. Thus, in the vast majority of cases a colony colour only indicates a microbial group rather than a species. This claim is supported by our findings related to the Enterobacteriaceae, in particular *E. coli*, *E. cloacae* and *C. freundii*, as well as *A. lwoffii* isolates. Similarly, the results of our semi-quantitative assay along with the diminished colony count on chromogenic media, point to the need to use chromogenic media as “auxiliary” to the conventional method based on blood agar and MacConkey agar.

ASM recommends culturing urinary samples for colony count on blood agar media only. Other media can be used for semi-quantitative tests, after being evaluated for possible inhibition of bacteria growth. MAC and chromogenic media can limit the growth of bacteria but according to the reference values, the recovery usually amounts to no less than 30%, (Pasek, 1999; Sharp and Clark, 2009; Sharp *et al.*, 2009). Such results were observed for *E. coli* no. 3866 and 4181 and *S. epidermidis* no. 4181. In other cases (*E. coli* no. 80;

E. faecalis – no. 4181, 12935 and 3866) the lack of growth was associated with the limitations of the method. The 10 µl loop allows the detection of colony counts between 100 and 1000 CFU/ml. In urine containing 10² CFU/ml, one loopful transferred onto an agar medium may only contain one colony per plate. When the bacteria are unevenly distributed in the sample, or the density of bacteria is low, bacterial growth on agar plates may not be detected (Chan, 2016). However, many authors concluded that chromogenic media might potentially be used as the single medium for the culture of urine samples (Chang *et al.*, 2008; Jolkkonen *et al.*, 2010). Ojanen T. *et al.* (2016) analysed the results of quantitative cultures of urine samples from laboratories participating in the External Quality Assessment (EQA) in Finland to evaluate the reliability of quantitative urine culture. Among laboratories routinely using chromogenic media, up to 87% obtained the correct results. Interlaboratory comparative studies have shown that the growth of *S. agalactiae* on chromogenic media is significantly better compared to the conventional, non-chromogenic media.

Due to the high number of urine culture specimens processed annually, laboratories are constantly seeking ways to improve their effectiveness. Automated instruments for dispensing the urine samples onto culture plates have been introduced. Due to the high prices of new technical solutions, they are only utilised in big laboratories that diagnose a large number of specimens. In small laboratories, the basic research methods and techniques in UTI diagnostics, such as urine culture and counting colonies are still used. Using chromogenic media appears to be a good alternative to conventional microbial diagnostic of UTI, yet, the user has to know the limitations of chromogenic media. The benefits of using chromogenic media in UTI diagnostics will most likely be seen in a non-hospital laboratory, because the most frequently isolated species from uncomplicated UTI infections is *E. coli* (Pezzlo, 2014; Rigail *et al.*, 2015). Stefaniuk *et al.* (2016b) pointed out that in Poland *E. coli* was responsible for 80.6% of cases of uncomplicated infections and 65.8% of the complicated ones.

In conclusion, all the chromogenic media tested in our study are attractive and easy-to-use screening media that considerably reduce the daily workload and the number of identification kits required.

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