CHROMagar™ Orientation Media Reduces Urine Culture Workload

Kanchana Manickam¹,², James A. Karlowsky¹,², Heather Adam¹,², Philippe R.S. Lagacé-Wiens¹,²,
Assunta Rendina¹, Paulette Pang¹, Brenda-Lee Murray¹, Michelle J. Alfa¹,²*

¹Diagnostic Services of Manitoba and ²Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada

Running Title: CHROMOGENIC MEDIA REDUCES URINE CULTURE WORKLOAD

* Corresponding author: St. Boniface Hospital, 409 Taché Avenue, L4025, Winnipeg, Manitoba, Canada R2H 2A6 Tel: (204) 237-2105 Fax: (204) 237-7678 e-mail: malfa@dsmanitoba.ca

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

JCM Accepts, published online ahead of print on 30 January 2013
Copyright © 2013, American Society for Microbiology. All Rights Reserved.
Microbiology laboratories continually strive to streamline and improve their urine culture algorithms because of the high volumes of urine specimens they receive and the modest numbers of those specimens that are ultimately considered clinically significant. In the current study we quantitatively measured the impact of the introduction of CHROMagar™ Orientation (CO) medium into routine use in two hospital laboratories and compared it to conventional culture on blood and MacConkey agars. Based on data extracted from our Laboratory Information System from 2006 to 2011, the use of CO medium resulted in a 28% reduction in workload for additional procedures such as Gram stains, subcultures, identification panels, agglutination tests, and biochemical tests. The average number of workload units (one workload unit equals one minute of hands on labor) per urine specimen were significantly reduced ($P < 0.0001$; 95% CI (0.5326 to 1.047) from 2.67 in 2006 (pre-implementation of CO medium) to 1.88 in 2011 (post-implementation of CO medium). We conclude that the use of CO medium streamlined the urine culture process and increased bench throughput by reducing both workload and turn-around-time in our laboratories.
INTRODUCTION

Urinary tract infections are one of the most common infectious diseases for which patients seek medical attention, and although many of these infections are treated empirically, urine cultures account for a significant portion of every clinical microbiology laboratory’s daily workload (1, 2, 3, 4). The laboratory diagnosis of urinary tract infection requires quantitative urine culture on standard agar media. Because only 20-30% of urine samples result in significant growth a considerable amount of time is expended evaluating samples that do not have clinical utility (5). Therefore, any new media or method with the ability to streamline urine culture processing in a meaningful way, such as reducing technologist workload, improving result turnaround times (TATs), or reducing laboratory costs would be welcomed and has the potential to have considerable laboratory impact.

Urine cultures have traditionally been performed using sheep blood agar (BA), a non-selective media, and a selective media such as MacConkey (MAC) agar, cysteine lactose electrolyte-deficient (CLED) agar, or eosin methylene blue (EMB) agar (6). Chromogenic media applicable to urine culture processing and reporting have been commercially available for more than 10 years and offer another option for diagnostic laboratories. Chromogenic media are intended to correctly identify more frequently occurring bacteria and yeasts or organism groups on primary culture with no further testing or a minimum number of confirmatory tests. Substrates present in chromogenic media target specific classes of enzymes produced by certain bacteria and yeasts (7). Target enzymes hydrolyze chromogenic substrates generating colored products which allow for easy identification of specific organisms (7, 8). Chromogenic media have been reported to be an acceptable alternative to traditional media for the isolation of urinary pathogens (1, 9, 10, 11, 12, 13, 14). Chromogenic media may facilitate improved sensitivity of
identification of some gram-positive cocci (e.g., enterococci) in mixed cultures with Enterobacteriaceae and may promote more uniform interpretation of urine culture plates by less experienced bench technologists (1, 10, 13, 15). Chromogenic media may also promote more rapid identification of the etiological agent(s) of infection and may provide clinicians with relevant information regarding their choice of empiric antimicrobial therapy for their patients. Consequently, this may decrease inappropriate use of antibacterial and antifungal agents (1, 4, 16, 17). Published data support performing antimicrobial susceptibility testing on colonies taken directly from CHROMagar™ Orientation (CO) medium (Becton Dickinson, Cockeysville, MD, USA) (1, 14).

Only two previous reports have been published describing an assessment of workload and/or cost savings associated with chromogenic media used for urine cultures (1, 13). D’Souza et al., reported that the use of CO resulted in a >50% reduction in inoculation time and a >20% reduction in work-up time (1). Ohkusu (13) reported the cost associated with identification of gram-negative bacilli using CO medium was reduced 70% compared to identifications generated using the Crystal E/NF system (Becton Dickinson).

The purpose of the current study was to add to the limited literature in this area by determining if a reduction in additional test workload, TAT and/or labor costs could be realized by implementing CO medium as the primary medium for urine culture. CO medium was compared to the traditional method of using BA and MAC agar for routine urine cultures in two hospital microbiology laboratories.
MATERIALS AND METHODS

Study sites and timeline. The study was conducted at Diagnostic Services of Manitoba’s two largest clinical microbiology laboratories (Health Sciences Centre and St. Boniface Hospital), which collectively process approximately 65,000 urine specimens per year. Between 2006 and 2011, a number of protocol changes were implemented at both sites to streamline urine culture work-up. In 2006 and 2007, urine cultures were performed using BA and MAC agar split plates. In 2007, the Clinical and Laboratory Standards Institute (CLSI) methods for the abbreviated identification of bacterial organisms (18) were implemented. In 2008, the BA and MAC agar split plates were replaced by CO medium as the primary culture medium following in-house validation by both laboratories.

Workload and turn-around-time assessment following CO implementation. Workload was evaluated at both laboratory sites using their laboratory information systems (Delphi LIS, Sysmex, Auckland, New Zealand) which track workload units (WLUs) automatically. The Standards for Management Information Systems (MIS) have defined one WLU as representing one minute of hands on labor. Data on WLUs related to urine cultures were assessed for a 6 month period each year from 2006 through to 2011. Extra WLUs per urine specimen were assessed prior to and after implementation of CO medium and followed until the end of 2011. WLUs associated with extra plate reading (i.e., urine plates read on two separate days when split plates were used versus a single reading for CO) and antimicrobial susceptibility testing were not included in the calculation. WLU calculations assessed only the extra tests done to identify microorganisms.

To determine average TAT, 240 positive urine specimens (120 urines that grew E. coli and 120 urines that grew Enterococcus species) were randomly selected from the two laboratory
sites in 2007 (pre-implementation of CO medium) and 2009/2011 (post-implementation of CO medium). The TAT for each sample was calculated from time of receipt by the microbiology laboratory to the time identification results were released to the treating physician.

**Urine Culture Protocol.** Urines were inoculated onto BA/MAC agar split plates using a calibrated 0.001 mL loop and streaked manually. CO medium was also inoculated with a calibrated 0.001 mL loop but the plates were streaked using an automated streaking device (ISOplater™, Vista Technology, Edmonton, Canada). All culture plates (BA/MAC agar or CO medium), were incubated at 35-37°C for 24 hours before the first reading. As per the manufacturer’s instructions CO plates were read only once at 24 hours in contrast to the BA/MAC agar split plates which were read at 24 and 48 hours. The number and identification of different organisms grown on CO medium was primarily assessed by colony color and morphology, whereas when using BA/MAC agar split plates other tests and procedures were often required to differentiate between organisms. The additional tests included rapid tests such as Gram stain, rapid biochemical tests, and latex and slide agglutination tests. In addition, tests requiring overnight incubation such as organism subcultures, tube biochemical tests, and commercial Vitek or API identifications panels (bioMérieux, Marcy l’ Etoile, France) were also required. Culture interpretation was based on the number of different organisms, quantitation of organisms, and identification of uropathogens (Table 1).

**Survey of Technologists.** One month after CO medium was implemented in the two laboratories a brief anonymous survey regarding CO medium was performed at one site (St. Boniface Hospital). Those technologists who had experience on the urine bench and had been using CO medium regularly participated in the survey. In 2012, the same survey was performed at both sites to determine if results were consistent after CO medium had been used as the
primary urine culture medium for four years. The questions and breakdown of answers from the
survey are shown in Table 2.

Statistical Analysis. The significance of the differences observed in extra WLU per urine
specimen between pre- and post-implementation of CO medium were determined by two-tailed,
unpaired t-tests. All statistical analyses were done using GraphPad Prism® software, version
5.02 (San Diego, CA, USA).
RESULTS

During the 6 month assessment periods for 2006 to 2011, 187,018 urine samples were analyzed at the two hospital laboratories; in 2006, 2009, and 2011 there were 28,251, 33,750, and 32,125 urine samples, respectively. Overall, 28% fewer additional identification procedures were required when using CO medium as compared to BA/MAC agar split plates. The breakdown of specific tests and the impact of CO medium implementation are shown in Figure 1. The Gram stain was the only procedure that was not affected by the implementation of CO medium. For all tests combined, the average ± standard deviation WLUs per specimen was 2.67 ± 0.24 in 2006, 1.94 ± 0.37 in 2009, and 1.88 ± 0.15 in 2011. There was a statistically significant reduction from 2006 to 2009 ($P = 0.0023$, 95% CI (0.3298 to 1.131)) that was maintained in 2011 ($P < 0.0001$, 95% CI (0.5326 to 1.047)). The average workload reduction after CO medium was introduced was 1.04 WLU per specimen.

In addition, a reduction in TAT for reporting the identification of the most common gram-negative (E. coli) and gram-positive (Enterococcus species) uropathogens was observed (Figure 2). Following the implementation of CO medium, the TAT for reporting E. coli and Enterococcus isolates decreased by approximately six hours (20% reduction) and four hours (12.5% reduction), respectively (Figure 2), however, this reduction was not statistically significant ($P > 0.5$) for either uropathogen.

Improved throughput on the urine bench was also documented at both hospital laboratory sites. Historically, there were two technologists scheduled daily to complete work at the urine bench in each laboratory. However, after implementation of CO medium, the workload could be completed by a single technologist in each laboratory. The survey completed by the bench
technologists supported this finding as 97% of technologists felt CO medium improved efficiency on the urine bench (Table 2).
DISCUSSION

Chromogenic media may be used by clinical laboratories for more rapid identification of several common pathogens causing urinary tract infections (1, 7, 8, 9, 10, 11, 12, 14). Our study demonstrated that CO medium as the primary culture medium is advantageous in the microbiology laboratory to streamline identification and reduce workload of urine cultures. Bench throughput was improved because of easier detection of polymicrobial cultures and the workload was reduced as many of the extra tests associated with conventional culture methods were no longer required (Figure 1). A similar finding was also reported by Sharmin et al., (15) who performed media comparison studies on 400 urine samples and plated to BA and MAC agar, CLED agar, and a chromogenic media. They concluded that the chromogenic media considerably reduced workload and minimized the use of conventional identification tests. Unlike our study, the reductions in workload that they reported were based on subjective assessment rather than quantitative data taken from an LIS to objectively evaluate workload and TAT as we did. Perry et al., (7) and Ciragil et al., (16) also suggested that chromogenic media reduces the need for extra reagents and decreases the labor associated with processing urine cultures. Our data support the findings of these investigators.

D’Souza et al. (1) tested a total of 1,023 urine samples sent for routine culture by plating them on BA and MAC agar as well as on CO medium. Results from all media were compared and a reduction in work-up time of >20% was reported when using CO medium. Further, Sekikawa et al. (4) stated that the initial assessment of colony morphology and enumeration of growth on a chromogenic media plate was easier, faster, and more reliable compared to conventional culture methods. Data collected from our staff survey (Table 2) found 94%-100% of technologists surveyed felt CO medium was reliable at distinguishing mixed cultures and
improving efficiency on the urine bench. In addition, the majority of technologists surveyed indicated they were confident in reporting the most common uropathogens (i.e., *E. coli* and *Enterococcus* species) from CO medium, without the use of additional identification tests.

We observed a reduction in TAT for the identification and reporting of results for *E. coli* and *Enterococcus* species but the reduction failed to achieve statistical significance (Figure 2). The lack of statistical significance is likely a reflection of the small sample size (120 isolates of each species in both study periods) used to assess differences in TAT. This data was obtained by performing a manual search. To do this for all urine specimens was beyond the capabilities of the LIS. A reduction in TAT was expected as it has been reported by de Vasconcelos et al. that using chromogenic media for rapid identification of *Candida* species in urine reduced TAT by 24-48 hours (17).

Since the use of CO medium reduces the need for further subculture and extra confirmatory tests there is an overall reduction in reagent use, suggesting CO medium is a cost effective replacement for conventional urine culture methods (3). Ohkusu (13) estimated $3,850 in savings per year when using a chromogenic agar system in their laboratory. Results of our study extend Ohkusu’s findings as we found there was an average reduction of 1.04 WLU per specimen after CO medium was implemented. Therefore, if a laboratory processed 100,000 urine specimens per year, use of CO medium would result in an approximate reduction of 104,000 WLUs, which represents approximately one equivalent full time bench technologist. Thus the use of CO medium not only decreases supply costs but also reduces labor costs in the microbiology laboratory.

In conclusion, our quantitative data demonstrated that CO medium significantly reduced workload in the microbiology laboratory compared to BA/MAC agar split plates and should be
considered as an alternative to conventional culture methods for detecting and reporting uropathogens.
ACKNOWLEDGMENTS

We would like to thank Becton Dickinson Microbiology for providing the laboratories with CHROMagar™ Orientation media during the preliminary studies. We are grateful to the Clinical Microbiology technologists from Health Sciences Centre and St. Boniface General Hospital in Winnipeg MB, Canada, who performed the work for this study and provided the feedback regarding the use of CHROMagar™ Orientation media.

Potential conflicts of interest: In 2009, Dr. Lagacé-Wiens accepted a travel grant from CHROMagar, Paris, France, to present data on a chromogenic ESBL medium at an international conference. He has had no financial dealings with them since. The remaining authors report no conflicts of interest relevant to this article.
REFERENCES


### TABLE 1. Criteria used to interpret urine cultures

<table>
<thead>
<tr>
<th>Culture interpretation</th>
<th>Culture results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Significant growth</strong></td>
<td>Growth at $\geq 10^4$ CFU/ml of 1-2 uropathogens</td>
</tr>
<tr>
<td></td>
<td>Growth at $\geq 10^4$ CFU/ml of 1-2 uropathogens at a 10-fold higher level than non-uropathogens</td>
</tr>
<tr>
<td></td>
<td>Growth at $\geq 10^5$ CFU/ml of a pure culture of any organism</td>
</tr>
<tr>
<td><strong>Insignificant growth</strong></td>
<td>Growth at $&lt; 10^4$ CFU/ml of any organism(s)</td>
</tr>
<tr>
<td></td>
<td>Growth at $\geq 10^4$ CFU/ml of $\geq 2$ non-uropathogens</td>
</tr>
<tr>
<td><strong>No growth</strong></td>
<td>$&lt; 10^3$ CFU/ml</td>
</tr>
<tr>
<td><strong>Possible contamination</strong></td>
<td>Growth at $\geq 10^4$ CFU/ml of $\geq 3$ organisms</td>
</tr>
</tbody>
</table>

*Interpretation of culture results was based on the routine urine culture protocol used by Diagnostic Services of Manitoba (DSM) clinical microbiology laboratories. This table was taken and modified from Kadkhoda et al., 2011 (19).*
<table>
<thead>
<tr>
<th>Question</th>
<th>Number of Responses (% of total)</th>
<th>2008 Data</th>
<th>2012 Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1. Do you routinely (&gt;80% of the time) have sufficient growth on CO medium for susceptibility testing on Day 1 (i.e., subculture not required before testing) for:</td>
<td></td>
<td></td>
<td>18/18 (100%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>18/18 (100%)</td>
<td>0</td>
<td>34/34 (100%)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>11/18 (61%)</td>
<td>7/18 (39%)</td>
<td>16/34 (47%)</td>
</tr>
<tr>
<td>Klebsiella Enterobacter Serratia group</td>
<td>18/18 (100%)</td>
<td>0</td>
<td>34/34 (100%)</td>
</tr>
<tr>
<td>Other Gram-negative bacilli</td>
<td>18/18 (100%)</td>
<td>0</td>
<td>34/34 (100%)</td>
</tr>
<tr>
<td>Other Gram-positive cocci</td>
<td>16/18 (89%)</td>
<td>2/18 (11%)</td>
<td>18/34 (53%)</td>
</tr>
<tr>
<td>2. Do you encounter difficulties with any spot/rapid tests that are performed from CO medium?</td>
<td>1/18 (6%)</td>
<td>17/18 (94%)</td>
<td>5/34 (15%)</td>
</tr>
<tr>
<td>3. Does CO medium produce consistent color for E. coli and Enterococcus to allow you to report the organism without additional tests?</td>
<td>17/18 (94%)</td>
<td>1/18 (6%)</td>
<td>17/18 (94%)</td>
</tr>
<tr>
<td>Question</td>
<td>2008 Data</td>
<td></td>
<td>2012 Data</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>-----------</td>
<td>----------------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>≤10%</td>
<td>11-50%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>8. What percentage of the time do you need to subculture organisms from CO medium before starting workup?</td>
<td>17/18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(94%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. To help identify organisms that will be “listed” in a final report, what percentage of the time do you need to do a Gram stain?</td>
<td>6/18</td>
<td>4/18</td>
<td>7/34</td>
</tr>
<tr>
<td></td>
<td>(34%)</td>
<td>(22%)</td>
<td>(21%)</td>
</tr>
</tbody>
</table>
FIG 1. Impact of CHROMagar™ Orientation (CO) medium on workload for identification tests for pre-implementation (2006) and post-implementation (2009, 2011) periods. CO medium was implemented in 2008. * indicates there was a statistically significant (P < 0.05) reduction in average wlu/urine specimen observed when 2006 was compared to either 2009 or 2011. Bars indicate ± standard deviation. The WLUs per procedure were: 3.0 for Gram stain, 1.5 for Subcultures, 1.0 for Rapid biochemicals, 1.5 for Tube biochemicals, 5.0 for Vitek ID panels and 6.0 for API ID panels, 4.0 for Latex agglutination, and 1.0 for Slide agglutination.
FIG 2. Turnaround time (TAT) for identification of *E. coli* and *Enterococcus* species pre-implementation and post-implementation for CHROMagar™ Orientation (CO) medium. *P* values were not significant for either uropathogen. Bars indicate ± standard deviation.
Most common uropathogens

Turnaround time (TAT) (hours)

- E. coli
- Enterococcus

- 2007
- 2009
- 2011