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# Practical Bench Comparison of BBL CHROMagar Orientation and Standard Two-Plate Media for Urine Cultures

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**A total of 1,023 urine samples sent for routine culture were plated onto sheep blood and MacConkey agars and a BBL CHROMagar Orientation (CO; Becton Dickinson, Cockeysville, Md.) plate, and the results were compared. Of these, 250 urine samples (24%) grew >10,000 CFU of one or two putative pathogens/ml and 773 showed no growth (NG), mixed growth of <10,000 CFU/ml, or three or more strains (mixed). The CO and conventional medium results agreed completely for 595 cultures with NG or <10,000 CFU/ml. An additional 178 urine samples yielded clinically insignificant differences. Both medium sets essentially agreed on quantities and identification for 400 single-pathogen cultures and 9 mixed cultures. With the caveat that CO cannot differentiate *Klebsiella*, *Enterobacter*, and *Serratia* spp., enteric pathogens were identified only by morphology and color on CO. Direct visual differentiation of group B streptococci from lactobacilli is not possible, but lactobacillus cells always exhibited easily recognizable morphology on Gram stain. Of 108 paired organism susceptibility results encompassing 2,268 drug-pathogen combinations, there were 3% errors and only 1% very major errors. Use of CO allowed a >50% reduction in inoculation time and a >20% reduction in work-up time. For our laboratory, with 50% "no growth" and ca. 25% significant results (50% *Escherichia coli*), CO allowed time and workup cost savings for a majority of cultures. A cost analysis (time and supplies for our laboratory) showed that if CO is used alone, the break-even level for CO pricing is $1.78; if CO and blood agar are both used, the break-even pricing for CO is $1.53.**

Urine samples are among the most numerous of specimen types sent for microbiology studies. The labor expended in the workup of mixed cultures, which may not be clinically relevant, and the fact that many clinicians treat urinary tract infections (UTI) empirically have prompted many laboratories to explore methods to limit the time and expense of urine culture processing (6). Although screening methods have been studied exhaustively, none has been universally accepted, especially for the complex microbiology of cultures from institutionalized patients (3, 17). Chromogenic agars have recently been developed to facilitate recognition of species directly on primary media (1, 4, 5, 7, 9, 16, 18, 19, 20). CHROMagar Candida (Becton Dickinson Microbiology Products [BD], Cockeysville, Md.) has been widely adopted, including in our laboratory, and several studies have indicated its utility (8, 10, 14, 15, 18). Given our positive experience with CHROMagar Candida, we sought to evaluate CHROMagar Orientation (CO [BD]), a modification of a chromogenic agar developed for urine cultures, in a routine laboratory setting (7, 16). We compared culture results and the ability to perform automated antimicrobial susceptibility testing directly from the original CO plate, and we did a cost comparison (time and materials) with our standard two-plate method for routine urine cultures.

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## MATERIALS AND METHODS

**Specimen handling and microbiological interpretation.** Consecutive urine samples sent for routine culture were plated by paratechnical personnel with a 0.001 calibrated platinum loop onto three plate media: tryptic soy agar with 5% sheep blood (BAP), MacConkey agar, and CO (all from BD). Among the 1,023 urine samples evaluated, 53% were clean catch voided, 37% were obtained via straight catheter, 9% were collected through the port of an indwelling Foley catheter, and the remaining 1% were suprapubic taps (which were also evaluated for low colony count growth [results not shown]). The same streaking technique, a single line down the center of the plate and a second side-to-side streak across the width of the plate from top to bottom, was used for all media. Plates were incubated in air at 35°C overnight and placed onto the routine benches for reading, along with all other cultures, with actual incubation times ranging from 10 to 24 h. In reality, CO plates were difficult to interpret if read after less than 16 h of incubation. Plates were reread after another overnight incubation, but additional isolates were rarely seen. The results reported here represent the comparison between media read on day 1. Clinical laboratory scientists (CLSs) placed the CO plates in a separate pile and read them at a different time from the routine media reading; results were recorded on special CO workcards. The standard protocol in our laboratory for routine urine cultures requires that up to two potential pathogens detected at >10,000 CFU/ml are considered clinically significant; they are identified, and susceptibility tests performed if appropriate. Three or more potential pathogens at >10,000 CFU/ml are usually reported as "mixed culture," with a descriptive comment about the organisms (there are rare exceptions for which a third organism, such as *Staphylococcus aureus*, is also reported). Skin flora, lactobacilli, and other nonpathogens are described morphologically. Only the conventional plates were used for patient result reporting. Rapid methods, including spot indole, pyrrolidonyl-β-naphthylamide aminopeptidase (PYR), oxidase, coagulase, and smell, as described by the National Committee for Clinical Laboratory Standards (NCCLS M35-A), were used for identification of appropriate isolates, such as *E. coli*, *Enterococcus* spp., and *Pseudomonas aeruginosa*, and conventional methods were used for the identification of all other cultures (12, 13). A Vitek 2 (bioMerieux-Vitek, Hazelwood, Mo.) was used for identification (where necessary), and susceptibility testing of gram-negative enteric bacilli and a MicroScan Walkaway (Dade-Behring MicroScan, Sacramento, Calif.) was used for susceptibility testing *Pseudomonas*, staphylococci, and enterococci. If clinically significant organisms were seen on CO, they were independently identified, and susceptibilities were determined by the same methods. The results were reviewed the day that reports were finalized,

and discrepancies were resolved by a research technologist who collected all three plates and subcultured colonies and/or retested as needed. Susceptibility results were considered very major errors if the organism tested from standard medium yielded a resistant result and the corresponding isolate from CO medium yielded a susceptible result. Susceptibility results were considered major errors if the organism tested from the standard medium yielded a susceptible result and the corresponding isolate from CO medium yielded a resistant result. Minor errors were interpretive disparities between either susceptible and intermediate results or between resistant and intermediate results.

**CO.** Developed originally by Alain Rambach, these media incorporate chromogenic substrate compounds that are acted on by bacterial enzymes such as β-galactosidase and β-glucosidase to yield colonies of unique and differential color (11, 19). The product insert for CO states that colony color and growth characteristics can be used to identify certain genera after 20 to 24 h of incubation without further testing: *E. coli* colonies are pale pink to plum; *Enterococcus* spp. are turquoise; *Streptococcus agalactiae* colonies are pale blue (but not very different from *Enterococcus* if read when colonies are young); *Proteus* spp. are pale pinkish orange with a brown halo; *Staphylococcus saprophyticus* colonies are bright pink; other staphylococci and yeast colonies are white; and *Klebsiella*, *Citrobacter*, and *Enterobacter* spp. are similarly dark blue-green. In addition, *P. aeruginosa* colonies on CO are usually recognizable as pale, translucent, olive-colored colonies; they often exhibit a metallic sheen and have the characteristic corn tortilla smell.

**Cost and workload analysis.** Two separate people with stopwatches timed four different paratechnical personnel (ranging from very fast to very slow workers) on nine separate occasions to determine the exact amount of time needed to label and streak one urine culture plate. All other setup activities, including accessioning, removing urine samples from a refrigerator, organizing the work area, flaming loops, and putting items away, were equal for either the CO or the conventional urine inoculation. CLSs were asked to keep accurate records of the time required for them to perform their complete bench workup on the routine plate setup and the CO plates, which were done at separate times. Ten different CLSs recorded a total of 22 days' worth of bench readings to generate an average workup time per system (either the two-plate conventional system or the one-plate CO system). The average hourly salaries plus 25% overhead for paratechnical workers ($27.50) and CLSs ($43.75) in our laboratory were used to determine the labor costs associated with setup and culture workup. An additional cost analysis was performed for laboratories where CLS salaries are in the $50,000/year range and paratechnical personnel salaries are $30,000 annually. Blood agar and MacConkey agar plates cost us $0.25 each. Spot test reagents cost an average of $0.67 each, and spot tests, including preparation time, were found to take 5 min of hands-on time.

## RESULTS

**Cultures.** Our laboratory receives ca. 22,000 urine samples for culture annually, one-fourth of which yield clinically significant results. For the 1,023 urine samples evaluated in the present study, 595 cultures (58.1%) yielded no growth or <10,000 CFU/ml, and another 178 cultures (17.4%) yielded mixed cultures or mixed skin (diphtheroids, staphylococci) or vaginal (lactobacilli) organisms. Of the 250 (24.4%) urine samples that yielded clinically significant results, 116 (46.4%) included *E. coli*, 108 of which were pure cultures. For 199 urine cultures (80% of all positive results), the interpretation of growth on CO and standard media was identical (Table 1). The remaining 51 cultures (Table 1), 40 of which yielded a pure culture (Table 2), yielded different interpretations on the two types of media (Tables 2 and 3). In some cases, organisms were not detected on one of the media types; in other cases, the colony counts differed or the numbers of different strains recognized on the plates differed. Eleven clinically significant organisms were not detected on conventional media but were seen on the CO plate, whereas six organisms detected on standard plates were missed on CO. Four cultures growing high numbers of yeast colonies were not detected on standard plates and would have been reported as "no growth." Three group B streptococci seen on the BAP would have been called "skin flora" on CO. The CO plate was correct for 69% of the urine samples for which results did not match. For all species recognized and named by the reader on both media for which BD has an identification claim (full identification for *E. coli* and *Enterococcus*, presumptive identification for *S. agalactiae*, *S. saprophyticus*, the *Klebsiella-Enterobacter-Serratia* group, and the *Proteus-Morganella-Providencia* group), there were no identification errors; i.e., organism names based on colony color and morphologies on CO were in 100% agreement with these organisms as identified from colonies on conventional media.

TABLE 1. Exact matches and disagreements between CHROMagar and conventional plates

| Organism | No. of specimens with agreement | No. of specimens for which results failed to match |
|---|---|---|
| *Escherichia coli* | 99 | 17 |
| *Enterococcus* species | 23 | 10 |
| *Klebsiella* species | 16 | 1 |
| Yeast species | 12 | 4 |
| Group B streptococci | 6 | 4 |
| Coagulase-negative staphylococci | 6 | 1 |
| *Citrobacter* species | 5 | 0 |
| *Pseudomonas aeruginosa* | 5 | 0 |
| *Enterobacter* species | 4 | 2 |
| *Staphylococcus saprophyticus* | 3 | 0 |
| *Proteus* species | 3 | 0 |
| *Staphylococcus aureus* | 2 | 0 |
| *Stenotrophomonas maltophilia* | 2 | 0 |
| Viridans group streptococci | 2 | 1 |
| *Morganella morganii* | 1 | 0 |
| Mixed (two species) | 10 | 11 |
| Total | 199 | 51 |

**Susceptibilities.** A total of 2,268 drug-organism combinations were compared, with the organism results as shown in Table 4. Many of the errors (two major and eight minor) occurred with *Klebsiella* species and nitrofurantoin. Discrepancies also seemed to be more common with *P. aeruginosa* isolates, although there were no specific antibiotics that showed more problems than others. The other errors were distributed randomly among species and drugs. Among disparities, testing from routine media (reference method) yielded 14 resistant results (25% of all discordant results) that showed intermediate results for the same organism from the CO plate. Most of the minor errors were interpretive differences that involved only one dilution difference in MIC, probably within the experimental error of the systems.

**Cost and workload analysis.** We determined that labeling and inoculating one plate took an average of 38 s for our paratechnical staff. For the 1,023 urine samples evaluated, use of CO only would save the cost of labor for streaking one plate (10.8 h at $27.50 for paratechnical personnel salary and overhead), a total of $297, and the cost of the two standard plates ($0.50 each) for a total of $511.50. Annualized to 20,000 urine samples, the cost avoidance accrued in our accessioning area by inoculating only one plate was $15,805. In areas where the

TABLE 2. Pure culture results for which CHROMagar and conventional plates failed to agree on interpretation

| Organism | No. of occasions | Medium with correct interpretation | Description of inconsistency |
|---|---|---|---|
| *E. coli* | 1 | Blood agar-MacConkey | *K. pneumoniae* recovered on CO but not BAP; *E. coli* seen on BAP not recovered on CO[a] |
| *E. coli* | 5 | Blood agar-MacConkey | CFU <10,000 on CO[a] |
| Group B streptococci | 3 | Blood agar | Growth on CO called mixed skin flora |
| Viridans streptococci | 1 | Blood agar | Reported as "skin flora" from CO; questionable identification of viridans streptococci as a uropathogen; CO might have been correct |
| Enterococci | 2 | Blood agar | Low CFU on CO or PYR[b] test result equivocal on CO |
| *E. coli* | 6 | CO | More than one colony type worked up on routine media; all were the same organism with identical susceptibilities |
| *E. coli* | 5 | CO | CFU below threshold on BAP-MacConkey[a] |
| Yeast species | 4 | CO | Not detected or reported from BAP |
| Enterococci | 7 | CO | Lower CFU on BAP[a] or called "mixed flora" on BAP |
| Group B streptococci | 1 | CO | CFU below threshold on BAP[a] |
| *Enterobacter* species | 1 | CO | CFU below threshold on BAP[a] |
| Other species | 4 | CO | Not detected on BAP[a] |

[a] Most discrepancies were based on differences in CFU counts. Observations of urine plating by paratechnical personnel showed varying expertise, and this likely accounts for most of the discrepancies.
[b] PYR; L-pyrrolidonyl β-naphthylimide aminopeptidase.

paratechnical personnel salaries are in the $30,000/year range (as they are in most other areas of the country), the annualized cost avoidance for a laboratory processing 20,000 urine samples would be $13,800. Of course, the cost of the CO plate must be added back when overall costs are calculated.

A conservative estimate of savings generated in the culture interpretation part of the urine culture during the study time period included avoiding 178 rapid spot tests for organisms that can be identified visually on the CO plate (*E. coli*, *S. saprophyticus*, and *Enterococcus* sp.; Table 5) and avoiding additional workups of organisms seen as different on conventional media but recognized as identical on CO. The savings of labor for each spot test (5 min CLS time at $43.75/h) and reagents ($0.67) came to $768 during the study, which can be annualized to $15,015. An additional savings of 20 s per culture accumulated from the difference between looking at two separate media (conventional system) and examining only one plate (CO). This savings was worth $4,860 annualized. The conservative overall estimated savings in our laboratory for 1 year, processing 20,000 urine samples, was $35,680. If CLS salaries are in the $50,000/year range (as they are in less costly areas of the country), the annualized savings for the same number of urine samples would be $28,142. A laboratory in Northern California could make the change from two conventional plates to CO alone and remain cost neutral if the CO plates cost $1.78 each. A laboratory with lower salaries, as

TABLE 3. Mixed-culture (i.e., two to three different types of pathogen) results for which CHROMagar and conventional plates failed to agree on interpretation

| Organisms | No. of occasions | Medium with correct interpretation | Description of inconsistency |
|---|---|---|---|
| *Proteus* sp. and *Klebsiella oxytoca* | 1 | Blood agar-MacConkey | Not detected on CO[a] |
| *Escherichia coli* and *Proteus* sp. | 1 | Blood agar-MacConkey | *Proteus* not detected on CO[a] |
| *Escherichia coli* and *Klebsiella oxytoca* | 1 | Blood agar-MacConkey | *Escherichia coli* not detected on CO[a] |
| *Leclercia* sp. and *Klebsiella oxytoca* | 1 | Blood agar-MacConkey | *Klebsiella oxytoca* not detected on CO[a] |
| *Escherichia coli*, *Pseudomonas aeruginosa*, and enterococci | 1 | CO | *Escherichia coli* not detected on BAP[a] |
| *Escherichia coli* and *Proteus* sp. | 1 | CO | Two morphotypes worked up on bench that turned out to be the same species on CO |
| *Escherichia coli* and *Staphylococcus saprophyticus* | 1 | CO | CFU of *Escherichia coli* was below threshold on BAP[a] |
| *Klebsiella pneumoniae* and *Proteus mirabilis* | 1 | CO | Reported as "mixed flora" by routine medium cultures |
| *Enterobacter cloacae* and viridans streptococci | 1 | CO | Two morphotypes of *E. cloacae* worked up on bench that turned out to be the same species on CO |
| *S. aureus*, *Enterococcus* sp., and group B streptococci | 1 | CO | *Enterococcus* sp. not detected on BAP |
| *E. faecalis*, *S. aureus*, and yeast sp. | 1 | CO | *Enterococcus* sp. not detected on BAP; lower CFU on conventional media, reported as "mixed flora" |

[a] Most discrepancies were based on differences in CFU counts. Observations of urine plating by paratechnical personnel showed varying expertise, and this likely accounts for most of the discrepancies.

TABLE 4. Results of comparison between susceptibilities performed on colonies from CO and standard laboratory agars (BAP or MacConkey)

| Organism groups | No. of[a]: | | | |
|---|---|---|---|---|
| | Pathogen-organism events | Very major errors | Major errors | Minor errors |
| *Escherichia coli* | 630 | 0 | 0 | 8 |
| *Klebsiella* species | 378 | 0 | 4 | 15 |
| *Enterococcus* species | 336 | 0 | 0 | 5 |
| *Staphylococcus* species | 252 | 0 | 0 | 1 |
| *Pseudomonas aeruginosa* | 189 | 0 | 2 | 5 |
| Other pathogens[b] | 483 | 0 | 3 | 14 |
| Total | 2,268 | 0 | 9 (0.4) | 48 (2) |

[a] Percentages are given in parentheses.
[b] Includes *Enterobacter cloacae* (four strains), *Proteus mirabilis* (one strain), and *Serratia marcescens* (one strain).

described above, would require CO plates to cost $1.41 to remain cost neutral. If the laboratory wished to continue to plate a BAP along with a CO plate, urine cultures would remain cost neutral if CO were available for $1.53 each in Northern California and $1.16 each in areas with lower average salaries.

## DISCUSSION

CO was easy to use and, even if technologists had difficulty directly identifying all of the organisms claimed by the manufacturer, the most common urine isolate, *E. coli*, was reliably identified by colony morphology alone. Mixed cultures were more easily recognized, and unnecessary and results-delaying additional workups were avoided. In the majority of instances (69%) in which results were discrepant, the CO results were correct. Many of the inconsistent results could be explained by an inoculum effect; we learned that inoculation of urine cultures was not being done properly by all paratechnical personnel, and thus we recommend that supervisors should review technique and perform direct observation for proficiency of the setup protocol often throughout the year. The detection of clinically relevant numbers of yeast on several cultures that would have been reported as "negative" from conventional media (Table 2) was not expected, since we always incubate conventional urine cultures for at least 2 days.

Group B streptococci were difficult to identify readily during the initial phases of the study, but a simple Gram stain or wet preparation easily distinguished them from lactobacilli, which share similar colony morphology on CO. As CLSs became more familiar with CO, they were better able to recognize group B streptococci, enterococci, and lactobacilli, all of which have small pale blue colonies on CO. In fact, unlike lactobacillus colonies on BAP, lactobacilli on CO always exhibited typical long rod-like morphology so that unnecessary additional identification steps were avoided. L-Pyrrolidonyl β-naphthylimide aminopeptidase could quickly differentiate enterococci from group B streptococci and lactobacilli on CO. One lot number of CO yielded false-negative PYR results for enterococci, but all subsequent lot numbers performed well.

TABLE 5. Culture interpretation workload savings with CO

| Organism and spot test | No. of matched positives | No. of discordant positives |
|---|---|---|
| *E. coli* spot indole | 103 | 3 |
| *Enterococcus* sp. PYR | 27 | 11 |
| *K. pneumoniae* ID panel | 0 | 1 |
| *S. saprophyticus* disk | 3 | 1 |
| Coagulase-negative staphylococci | 0 | 1 |

As have other researchers, we found that CO was reliable, easy to use, resulted in cost savings, primarily in labor savings, that could be significant over time (1, 4, 5, 11, 16, 19, 20). Our study evaluated all CO plates on the first day, after 16 h of incubation, whereas other studies also included readings from the second day. Although some studies included a cost analysis, ours was the only study thus far to also evaluate the utility of performing susceptibility tests directly from CO. In fact, it is likely that the numbers of mixed susceptibilities that must be repeated on isolated colonies, delaying final results reporting, will be reduced with regular use of CO. Finally, although we have not directly evaluated the aspect of more rapid reporting of results that CO allows, we can infer based on the work of others, such as Barenfanger et al. (2), that there are clinical and financial benefits gained from rapid reporting of clinically significant results. The study by Barenfanger et al. included a large number of urine culture results, since these comprise the majority of samples sent for microbiological studies to most laboratories.

In summary, the benefits of CO include more rapid turnaround time for result reporting, decreased labor involved in interpretation, cost savings on reagents and labor for both inoculation and resulting, and ease of use. However, interpretation of CO does require practice, and many microbiologists have years of experience with familiar media and may not welcome any change; we recommend that a laboratory phase in the use of CO gradually, possibly in conjunction with a BAP until CLSs become comfortable with the new agar and are ready to abandon the BAP to achieve the most time and cost savings. Even if used alone, no clinically significant pathogens would be missed on CO.

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