Evaluation of the WASPLab software to Automatically Read CHROMID CPS Elite Agar for Reporting of Urine Cultures

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Abstract

Urine cultures are among the most common specimens received by clinical laboratories and generate a major share of the laboratory workload. Chromogenic agar can expedite culture results, but technologist review is still needed. In this study, we evaluated WASPLab™ software to interpret urine specimens plated onto CHROMID® CPS® Elite (CPSE) agar. Urine specimens submitted for bacterial culture were plated onto CPSE agar with a 1µl loop using the WASP™. Each plate was imaged after 0 and 18h of incubation and colonies were enumerated by color using the WASPLab™ software and a technologist reading from an HD monitor. Results were reported as negative if <10 colonies/plate were detected. LIS time stamps were used to measure time to result. A total of 1,581 urine cultures were tested. Sensitivity and specificity of the software was 99.8% and 68.5%, respectively, which included 2 Manual Positive/Automation Negative (MP/AN) and 170 Manual Negative/Automation Positive (MN/AP) results. Of the 170 MN/AP specimens, 116 were caused by microcolonies missed by the technologist. The remaining MN/AP were cause by either count differences near the 10-colony threshold (n=43) or count differences >50 CFU (n=11). Use of both CPSE agar and WASPLab software improved time to result for urine culture reducing the average time to result by 4h42m for negative specimens and 3h28m for positive specimens compared to standard of care testing. These data demonstrate that use of CPSE agar and automated plate reading has the potential to improve turnaround time while maintaining high sensitivity and reducing urine culture workload.
Introduction

Urinary tract infections (UTIs) are some of the most common bacterial infections, with estimates of up to 150 million cases a year worldwide (1, 2). In the United States (US) alone, UTI symptoms account for almost 1% of all clinic visits and the societal costs associated with these infections are calculated to cost the US approximately $3.5 billion a year based on healthcare costs and lost productivity (3, 4). The high occurrence of UTIs and the ease of specimen collection creates a large burden on clinical laboratories, accounting for 24-80% of all cultures set up, dependent upon the laboratory's serviced population (5).

Laboratory diagnosis of UTIs is based on urinalysis including several markers such as nitrite, leukocyte esterase, and microscopic urinalysis white blood cell count; however, bacterial culture is necessary for definitive diagnosis of symptomatic and asymptomatic UTIs (6, 7). Interpretation of bacterial culture is dependent upon the bacterial burden, diversity, and clinical scenario including assignment of uncomplicated vs. complicated infection. Complicated UTIs are typically defined as a patient with a structural or functional urinary tract abnormality or altered immune system whereas uncomplicated UTIs are characterized as females of child-bearing age with symptoms of dysuria or urgency, but lacking systemic symptoms (8). For uncomplicated UTIs, current Infectious Disease Society of America (IDSA) guidelines define that a bacterial burden of $10^4$ CFU/mL as significant (9). This threshold is reduced to $10^3$ CFU/mL when specimens are tested from patients with complicated UTIs due to their increased risk and likelihood of disseminated or severe infection (10).

Bacterial burden is a key factor for interpretation, however accurate interpretation and diagnosis requires differentiation of active infections versus contamination or colonization. The majority of uncomplicated UTIs are caused by a single pathogen, so many laboratory guidelines recommend against full identification and reporting of cultures containing 3 or more possible pathogens at $>10^4$.
CFU/mL as these are indicative of poor-quality specimens (5, 8, 11). Furthermore, specimens that contain a majority of skin and genital flora should also be disregarded as poor specimen collection. The most common UTI pathogens include *E. coli, K. pneumoniae, Enterococcus* spp., *P. mirabilis*, and *P. aeruginosa* (12).

Due to the high volume of cultures and technical expertise needed to properly report urine cultures, selective and chromogenic media have been developed to improve workflow and reduce overall cost. Several chromogenic agars are FDA-cleared and commercially available to aid in detection of potential uropathogens and studies comparing these media to traditional culture with blood and MacConkey agar have found equitable sensitivity (13). Automation in the clinical laboratory has developed significantly over the years, beginning with automated streaking instrumentation and culminating in total laboratory automation (TLA) that inoculates and streaks plates, images, and incubates cultures. The two most prominent automation systems are the WASPLab (Copan Diagnostics, Brescia IT) and the BD Kiestra (Becton Dickinson, Franklin Lakes, NJ), both of which have been reported to improve consistency in plating and free up technologist time to perform other duties (14, 15). Image analysis software has been added to these systems and has demonstrated high sensitivity for detecting growth on chromogenic agar for patient screening (16-18).

In this study, the performance of the WASPLab™ chromogenic detection module (CDM) software to aid in initial segregation of negative vs non-negative urine cultures using CHROMID® CPS® Elite (CPSE) agar (bioMérieux, Marcy l’Etoile, France) was evaluated. CPSE agar is a chromogenic agar designed specifically for use with urines culture that has the capability to directly identify *E. coli* and presumptively identify *Enterococcus, Enterobacteriaceae*, and members of the *Proteae* (now *Morganellaceae*) group based on the use of different chromogens. Images of urine specimens plated to CPSE agar were analyzed by the CDM algorithm and compared to a technologist’s interpretation as the
gold standard. Performance of bacterial counts, color, and turnaround time (TAT) was compared between manual and software reporting.

Materials and Methods

Specimen Processing

Enrollment of urine specimens occurred during January 2017 through April of 2017 at Wisconsin Diagnostic Laboratories, which services a primarily adult population for Froedtert hospital and the surrounding clinics and long-term care facilities. All testing was performed on clean catch specimens from both in and outpatients that fit the laboratory policy for bacterial culture (<24 hour for unpreserved, <72 hour preserved). These specimens included both unpreserved and preserved (boric acid) collections. All plating was performed by the WASP™ using a 1μL loop and plates were transferred to the WASPLab™ for incubation and imaging. Testing was performed in parallel with standard of care (SOC) testing.

Manual Scoring of Chromogenic Plates and urine culture

Multiple technologists were used in the study and each were trained on how to read CPSE agar prior to the initiation. In addition, a reading guide was placed next to the bench for quick access when reporting results. All testing was integrated into the SOC testing, which followed the laboratories standard practices. Briefly, urine cultures imaged at 18 hours are populated into a WASPLab workstation list. Technologists view all images on a HD monitor and preliminarily reporting negative cultures and pull any plates that need further workup. In this study, the technologist read both BAP and MAC as well as the CPSE agar. Specimens are worked up 24/7 and have two technologists during first shift (06:30-15:30). During second and third shift a single technologist performs the preliminary image review and follow-up work. Results are recorded into the LIS and plates that need further workup are
sent to a stack on the WASPLab. In batches, technologists work these cultures up which include:

- creating purity plates, performing MALDI, and setting up AST (if possible). No additional work-up was
- required for CPSE agar after colony counts were recorded (image review). Culture results as well as the
- time to reporting for preliminary and final MALDI ID were obtained from the LIS time stamp. Final SOC
- results included laboratory expert rules to differentiate between poor collection or contamination.

Rules used were multiple pathogens detected and growth of normal genital flora. These are defined
- as identification of 3 or more gram-negative organisms and growth over the 10 CFU threshold, but
- contain skin contaminants such as *S. epidermidis* or *Corynebacterium*, respectively. All technologists
- were blinded to the software results.

**Digital Analysis of Chromogenic Media**

Two images were taken of the media, one immediately after inoculation (time point 0) and then
- again after 18 hours of incubation. Using the CDM image analysis software, each paired image was
- reviewed as previously published with some differences to the algorithm (17). In this revised version of
- the software, multiple HSV (Hue, Saturation, Value) color ranges were incorporated to differentiate
- between *E. coli* (red to burgundy), *Proteeae* (now *Morganellaceae*) (light brown to dark brown),
- *Enterococcus* spp. (turquoise), and KESC (*Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Citrobacter*
- spp.) group (green to blue green). Furthermore, the software analyzed the pixel differences to define
- and count individual colonies to report the number of each organism present. Analysis was performed
- automatically after the 18-hour image was taken and the results, including time read and colony count
- based on color, were recorded. The software also reported out counts of microcolonies, which are
- defined as colonies with a diameter ≤0.2mm.

**Discrepant Analysis**
Software results were compiled and sent to the laboratory at the end of the study. This limitation required all discrepant analysis to be performed with only plate images as all cultures had been discarded. Images of discordant specimens were sent to the laboratory for a second review. Discrepancies in the data were classified in three distinct categories, which included: growth of microcolonies not detected by the technologist, count difference near limit of significance, and count difference ≥50 colonies. As specimens were decarded, confirmatory identification was not possible.

Statistical Analysis

The software reported out colony count (based on chromogen color) and time analysis was completed, which were compared to manual reads as the gold standard. Sensitivity and specificity were calculated using standard methods and McNemar’s test (19). These calculations were performed for both growth/no growth and detection of colonies at the threshold level. Ninety-five-percent confidence intervals (CI) were calculated by using the efficiency score method (20). Statistical significance for time to result was performed using an unpaired 2-tailed Student T test.

Results

Specimen characterization and prevalence

A total of 1,581 specimens were evaluated that contained both a CPSE agar software result and a technologist result to allow for a complete comparison. Enrolled specimens consisted of approximately a 3:1 ratio for unpreserved and preserved urines. Of these 1,581 specimens, 566 (35.8%) contained <10 colonies (382 no growth) based on technologist reporting. Another 13.3% of specimens were considered insignificant due to multiple pathogens or normal genital flora detected. The remaining 50.9% of the specimens were considered significant for pathogens following the policies defined by the laboratory.
Performance of the Software to read CPSE plates.

Images read by the technologist and software were recorded as either positive (≥10K CFU/mL) or negative (<10K CFU/mL). Comparisons were then resulted into 4 categories, Manual Positive/Automation Positive (MP/AP), Manual Negative/Automation Negative (MN/AN), Manual Negative/Automation Positive (MN/AP), or Manual Positive/Automation Negative (MP/AN).

Comparisons consisted of 1,013 that were MP/AP, 396 that were MN/AN, 170 that were MN/AP, and 215 specimens that were MP/AN (Table 1). These data resulted in a sensitivity of 99.8% and a specificity of 69.9%.

The 172 discrepant images were sent back to the laboratory for a second review to determine the reason for the discrepancy (Table 2). Causes of MN/AP specimens were observed to fall into 3 separate categories consisting of growth of microcolonies (≤0.2mm diameter), bacterial counts near the limit of significance (≥10 CFU), and count differences that were >50 colonies. The majority of the MN/AP results were due to the presence of microcolonies, which occurred in 68.2% (116/170) of MN/AP specimens. Differences in manual vs software counting near the significance limit occurred in 25.3% (43/170) MN/AP specimens while specimens containing >50 colony count difference occurred in 6.5% (11/170). The only trend observed in these 11 specimens was the presence of colonies with no color, which may have been missed or not reported by the technologist. The software’s definition of microcolonies often required zooming in on images to detect manually, which may have been why these were often missed. A version of the software that ignored these microcolonies was used to re-analyze the dataset, which improved specificity of the reading to 90.0%. However, further studies are needed to determine what organisms these microcolonies were and if they are important for patient care.
For the 2 MP/AN specimens, the software reported 5 and 6 colonies from the CPSE agar and the technologist counted 102 and 104 colonies, respectively. Upon further review of the CPSE agar images, 3 and 4 colonies were observed on the two CPSE plates (Figure 1A-C). The final report for one specimen stated that multiple organisms present, which indicates likely contamination. The other specimen’s final report was resulted as >100k *P. aeruginosa* with 5k gram positive cocci in chains. It is unclear as to why the initial manual read for the CPSE agar reported greater than 100 colonies (Figure 1D). Upon zooming into the image on an HD monitor, small dust like spots could be observed as either brown or colorless. These are also present on the T0 images. It is possible that technologists interpreted these as colonies and reported out as either no color or brown. Alternatively, the technologist could have reported from the blood agar plate that had small colonies at >100 CFU (Figure 2E). A final possibility is that plates were recorded after removal from the incubator. Both specimens were reported 10 hours after the images were taken, which could result in different growth, not captured in the picture.

Colony counts were also performed differentiating colony color by both the technologist and the software. Technologists only counted up to 99 CFU and anything past this number was reported as >100 CFU. The software was not limited to a set amount and reported total count on each plate. To evaluate the accuracy of the software to differentiate colony forming units, only plates that contained 1-99 colonies (technologist counts) were compared. Results were batched into 7 categories which included: no difference (range 0), colony counts differences between 1-10 CFU (range +/- 1-10), differences in counts between 10-50 (range +/- 10-50), and count differences >50 (range +/- 50-99) (Figure 2). Results in the positive values indicate more colonies counted by the software and counts reported as negative values indicate plates that had more colonies from the manual read. Plates containing >100 colonies were removed from these calculations as it would not be an equal comparison due to the human limitations.
Comparing colony counts demonstrated the high accuracy of the software to differentiate colonies (Figure 2). Out of 954 remaining specimens, 44.4% (424/954) fell within a difference of 10 or fewer CFU. In general, the software reported a higher number of colonies, 823 vs 47 compared to manual analysis. The trend in overcalling is expected as a technologist likely underestimate counts as the difference between 30-99 colonies is not clinically relevant and would have little effect on patient care.

Data analysis was also performed to determine the difference between growth and no growth on the plates. Growth was defined as any single colony, with microcolonies reporting by the software turned off as they were undetectable without magnification by the technologist. The software and manual read results were concordant in 88.9% of specimens (1406/1581). Of the 175 discordant specimens, only 6 were manual growth, automation no growth. All but 1 of these had counts below 10 colonies. The remaining manual growth, automation no growth specimen had 15 colonies lacking pigmentation and the final report was reported as multiple organisms present. Further review of this discordant specimen found that the software could detect 16 microcolonies. The majority of the automation growth, manual no growth specimens had less than 10 colonies on the plate (66.7%). The remaining discrepant specimens consisted of 18.7% with growth between 10-99 and 14.6% that had bacterial counts ≥ 100 colonies. Two trends appeared from the ≥100 colony discrepant specimens. One appears to be possible reporting errors on the technologist as the final report had results that were not entered into the LIS properly. The other group was observed to have small light blue colony that was missed by the technologists. Based on the CPSE package insert, blue colonies fall into the KESC group, but no further definitions for small colonies are described.

Evaluating time to result between conventional culture, CPSE agar, and software analysis.
Time of reporting was collected to determine the effects that the software and CPSE agar had on TAT. These readouts included the start of culture, defined as the first T0 image that was taken by the WASPLab, time the software recorded a result, time the technologist viewed the CPSE agar, and time of identification (ID) from our SOC workup. The natural workflow of the laboratory was included as a part of the study design so that the normal functions of the laboratory, such as workload, phone calls, and other distractions were considered. Time to reporting was calculated separately for positive and negative cultures.

There was a high variability in TAT for manually read cultures with results ranging from 18:23 (hh:mm) to 106:11. Results over 30 hours were rare and the majority clustered around 24 hours from initial plating (Figure 3). Conventional culture averaged a final time to result at 24:18 for negative cultures and a final time to ID result of 26:08 for positive cultures (Figure 3). Manual reading of the CPSE agar reduced the TAT for negative specimens, which averaged 24:03 (P < 0.05). Positive specimens using CPSE agar were reported on average faster than negatives at 23:15; however, these differences were likely caused by outliers that may have increased the average time to result in negative specimens. Resulting of positive specimens were significantly shorter than standard time to ID (P <0.01), but this value would only be useful for *E. coli* only cultures as no work up is needed. The median TAT for conventional and CPSE agar was 23:33 and 23:37 for negative specimens and 25:07 and 22:17 for positive specimens.

Significant improvements in TAT were observed for positive and negative specimens when comparing software analysis to conventional testing and manual reading of CPSE agar (P < 0.01). Overall, the software had an average TAT of 18:44 with a median of 18:17 when analyzing negative specimens and an average of 18:40 and median of 18:18 for positive specimens (Figure 3). Compared to manual reporting of the CPSE agar, this resulted in a median reduction in TAT of 03:28 for positive
specimens and 04:42 for negative specimens. Reduction was even greater when compared to conventional culture with a median reduction of 06:23 for positive specimens and 04:48 for negative specimen results. These calculations were performed using all positive specimens, but only E. coli positive specimens would be possible to report using software alone. Comparing time of result for E. coli only specimens, found similar results with a median difference of 03:01 compared to CPSE agar and 06:28 compared to SOC identification.

Discussion

The goal of both chromogenic agar and automation is to reduce the work load on the clinical microbiology laboratory and improve TAT for patient results. Other studies have looked at the effects on TAT for CPSE agar using only manual reading and reporting. One study evaluated 200 urine specimens using the CPSE agar and found that the time to ID for E. coli was significantly reduced (2.7-hour reduction) when comparing to bacterial culture on blood agar and MALDI-TOF MS for identification (21). The study also evaluated hands on time and consumable usage and found that CPSE agar required 30 seconds less hands on time (on average) and used 1 less swab and biochemical test per specimen. Their data suggested that in a medium size laboratory performing an average of 300 urines a day over 900 technologist hours would be saved annually. Furthermore, The TAT differences observed in the Yarbrough et. al. study and the current study are likely the minimum expected differences due to the method of identification. Both studies used MALDI-TOF MS, which allows identification in minutes or in less than a few hours when batch testing is performed. As MALDI-TOF MS is among the quickest methods used for identification (spot biochemicals are quicker, but not always used in labs with MALDI for final ID), laboratories that rely on slower automated ID systems, such as the Phoenix system (BD, Sparks, MD, USA) the VITEK 2 system (bioMérieux, Marcy l’Etoile, France), or Microscan (Beckman
Coulter, CA, USA should expect a larger difference in TAT as these assays require a minimum of 6-8 hours for ID results.

The addition of software analysis did reduce TAT over that of manual reading; however, there are some limitations to the data analysis that may skew results as compared to the actual impact if implemented into the clinical laboratory. The time of result was calculated based on the time the software analysis was performed, but the requirement of CPSE agar to have additional work-up for species identification of Enterococcus, KESC organisms, and Proteeeae (now Morganellaceae) was not captured. In these cases, additional biochemical testing would need to be performed so the actual time to ID would be similar to the manual read. CPSE agar can directly identify E. coli colonies so an eighteen-hour reporting could be achieved for both negative plates and pure E. coli cultures. In this study, automation negative specimens accounted for 21.6% of all specimens and cultures containing E. coli without other significant organisms accounted for 11.8% of specimens. These cultures combined could allow the software to either presort or auto report 33.4% of urine culture enrolled. An additional 7.3% of specimens could also be reported as significant for E. coli but contained other pathogens with potential clinical significance that would need additional workup. The remaining 59.3% of specimens that do not fall into these categories would require full workup by a technologist.

An unexpected finding during this study was the software detection of microcolonies not detected by the technologists. Unfortunately, analysis was performed after testing was completed so there were no specimens or plates to go back to for identification of these microcolonies. Growth of these slow growing organisms is likely due to the enhanced recovery of microorganism using smart incubators. A recent study in 2018 evaluated recovery of organisms from urine culture pre and post automation (22). Interestingly, the use of smart incubators was able to routinely detect Neisseria gonorrhoeae from blood agar plates more often than standard incubation. Others have found similar...
results, demonstrating increase recovery of gram-positive rods such as \textit{Actinomyces} and \textit{G. vaginalis} \textsuperscript{(23)}. It is possible that these microcolonies may include pathogens so further study is warranted.

Comparisons between technologist and software reporting was performed based on specimens being positive when 10 or greater colonies are identified; however, the software could differentiate between 9 different growth characteristics and was highly accurate in colony counts. Laboratory workflow could be improved by developing software rules that incorporate the laboratory’s standard of care practices. For instance, significant growth could be defined as one or two colony types being ≥10 colonies to remove the need for interpretation of plates that do not contain significant growth of a single colony (i.e. 5 GNR, 7 GPC). In addition, any growth could be reported when source is defined as a catheter to follow IDSA guidelines. Poor collection could also be resulted out when 3 or more colony types are detected at ≥10 CFU/mL. Of the 1,581 specimens tested during this study, 401 (25.4%) could have been determined to contain multiple organisms present using these additional rules. If validated along with reporting significant \textit{E. coli} and negative cultures, these data suggest that 58.8% of all urine cultures could be reported as early as 18 hours post inoculation, which could greatly reduce the workload on a laboratory and allow physicians to make informed treatment decisions earlier.

Digital image analysis for the clinical microbiology laboratory continues to improve, but how it is integrated into the workflow will be dependent upon the laboratory leadership and internal validations. The software is highly flexible and can be set up to either allow minimal or moderate changes to the laboratory’s workflow. Implementation of the software could be used for rapid segregation of growth or no growth cultures. Technicians could then batch view negative specimens and result these out in groups up to 40 specimens per click. This minimalistic adoption should allow technologists to focus their time on positive or complex specimens. Alternatively, the software could be integrated to maximize automation. In this approach, the software would automatically report out negative cultures or cultures
pure for *E. coli* infections. Technologists would then only view and work up positive urine cultures that were not pure for *E. coli*. *E. coli* only cultures could also be sent to work-up stacks for AST testing. In both cases, it would be vital to maintain a minimum of blood culture agar to identify Group B *Streptococcus* as well as some of the emerging pathogens, including *Aerococcus* and *C. urealyticum* (24).

Finally, considerations would be needed for expert ruling of plate interpretation. Plates with multiple pathogens could be ruled as poor collection, which could either be confirmed by a technologist or auto reported. Overall, continued advancements to image analysis software should improve workflow as has been observed for previous automation technologies.

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Table 1. Performance of WASPLab software compared to manual read for 10 or greater colonies.

|                      | MP/APa | MN/ANa | MN/APa | MP/ANa | Total | Sensitivity (95% CI) | Specificity (95% CI) |
|----------------------|--------|--------|--------|--------|-------|-----------------------|----------------------|
| Realtime results     | 1,013  | 396    | 170    | 2      | 1,581 | 99.8 (99-100)         | 69.9 (66-74)         |
| Post Discrepant Analysis | 1,013  | 485    | 54     | 2      | 1,581 | 99.8 (99-100)         | 90.0 (87-92)         |

*a MP/AP Manual Positive, Automation positive; MN/AN Manual Negative, Automation Positive; MN/AP Manual Negative, Automation Positive; and MP/AN Manual Positive, Automation Negative
Table 2. Discrepant analysis of manual negative/automation positive specimens

| Discrepant Category                  | No. of plates |
|-------------------------------------|--------------|
| Counting Microcolonies              | 116          |
| Counts near the limit of detection  | 43           |
| Counts >50 CFU difference           | 11           |

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Figure 1. Images of the 2 Manual Positive Automation Negative specimens. The first specimen is shown in A-C (CPSe, BAP, MAC) and was manually reported as multiple organisms present with 1 pink colony and 100 no color colonies. Automation reported out 6 colonies, 2 pink, 2 no color, and 2 microcolonies. Visual re-examination of the images shows 2 pink and 1 white colony. The second specimen (D-F) was manually reported as >100K * pseudomonas aeruginosa* and 5K gram-positive cocci in chains. Automation reported 2 microcolonies, 2 no color colonies, and 4 turquoise colored colonies. The 4 turquoise colonies can be observed in the re-examination of the image and 7 colonies are seen on the BAP (F). When enlarging both the CPSe and BAP plate images a small dusting of possibly debris or discoloration can be detected, which can also be seen on the T0 image (not shown). It is possible that the technologist misinterpreted this as growth. As plates were discarded by discordant analysis confirmation cannot be confirmed, but the smaller growth is likely the *P. aeruginosa* although it is unclear why no growth was observed on the MAC agar. It is possible that the poor growth on BAP reduced the viability on the MAC plate.
Figure 2. Evaluation of the software’s colony count accuracy for specimens containing 1-100 CFU. Ranges above 0 indicate higher automation counts. Data does not include specimens reported as >100k CFU/mL as the actual manual colony count is unknown.
Figure 3. Distribution of time of results differences for negative and positive specimens between standard of care, manual CPSE, and software analysis. Significance was determined by a 2-tailed paired Student T test (*P=0.046, **P<0.001).
