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Improving Time To Result with BD Kiestra™ Laboratory Automation Solution: Finding the Optimal Plate Reading Time of Plates with Significant Growth

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Background

The BD Kiestra™ Total Lab Automation (TLA) is an in-vitro diagnostic system which automates specimen processing as well as transport, smart incubation, and time series digital imaging of Petri dishes. These systems typically will provide improved efficiency of the traditional work-intensive microbiology laboratory. In addition, microbiology reporting may be improved with more accurate plate incubation, less chance for sampling errors, and more reproducible colony isolation. With the use of smart incubation and time series digital imaging analysis software, the next phase of traditional micro automation will begin. This will offer a real opportunity for automated plate interpretation with potential for both improved quality and time to reporting for traditional microbiology results. For this study, the BD Kiestra™ ReadA™ Compact combined with the BD Kiestra™ BD Kiestra™ Optis™™ imaging software was used to evaluate the feasibility for early evaluation of urine cultures to allow for early ID/AST processing and thus the potential for significant reduction for time to results. For this study, BD Kiestra™ Optis™ with Urine APP software was used to analyze plates after 12 hours incubation. This would allow the microbiology laboratory for early plate screening to select the cultures with significant growth density for further ID/AST work-up. All negative plates would remain in incubator per normal laboratory protocol. Since many urine culture will be negative, the BD Kiestra™ Optis™ Urine APP would allow selection of positive plates only for review by the laboratory for early processing.

Methods

For this study, 262 clean-catch clinical urine culture were collected using the Vacutainer™ Urinalysis Preservative Tube . All urine samples were processed with BD Kiestra™ InoqulA+TM using 0.01mL inoculum, pre-defined streak method ZigZag Pattern 4, bead streak technology, and testing using the following BD BBL™ TSA II 5% Sheep Blood, MacConkey II, CHROMAgar Orientation, and Columbia CNA Agar with 5% Sheep Blood. For the purposes of this study, images were acquired at 3 (baseline), 8, 12, 16, 18, and 24 hours with incubation in O_2 at 36^{0}C. After 18-24 hours, manual readings by technologists were compared to automated classification of growth on each urine plate: $<10^2$ CFU/ml [No significant growth], 10^2-10^3, 10^3 – 10^4,10^4 – 10^5 and $\geq 10^5$ CFU/ml. Significant isolates were identified using Gram stain, catalase/spot tests, CHROMAgar Orientation, and MALDI-TOF.

OPTIS™ imaging software combined with digital analysis programs were used to provide urine CFU/mL range quantitation at 8, 12 and 16 hours for each urine culture plated media. These early Optis Urine APP results were then compare to the final reported 18 - 24 manual reading.

Results

Table1 summarizes the culture results for the 262 urine samples evaluated for this study.

TABLE 1 Summary of Culture Results for the 262 Urine Samples

Culture Result	Number of Specimens
<100 No Growth	47
>100,000 Candida albicans	2
>100,000 Citrobacter amalonaticus, >100,000 Aerococcus urinae	1
>100,000 Citrobacter freundii	3
>100,000 E.coli	32
>100,000 E.coli, >100,000 Klebsiella oxytoca	1
>100,000 E.coli, 10,000-50,000 Proteus mirabilis	1
>100,000 Enterococcus faecalis	6
>100,000 Enterococcus faecalis, 10,000-50,000 Morganella morganii	1
>100,000 Enterococcus faecalis, 50,000-100,000 Pseudomonas aeruginosa	1
>100,000 Enterococcus faecium	1
>100,000 Escherichia coli, 10,000 to 100,000 Enterococcus faecalis	2
>100,000 Klebsiella pneumoniae	5
>100,000 Klebsiella pneumoniae, 10,000-50,000 Proteus mirabilis	1
>100,000 Proteus mirabilis, >100,000 Enterococcus faecalis	1
>100,000 Proteus mirabilis, 10,000-50,000 Enterococcus faecalis	1
>100,000 Pseudomonas aeruginosa, >100,000 Klebsiella pneumoniae	2
>100,000 Pseudomonas aeruguinosa	1
>100,000 Staphylococcus aureus	1
>100,000 Staphylococcus epidermidis	1
>100,000 Urogenital flora	4
10,000 to 100,000 E.coli	2
10,000 to 100,000 Enterococcus faecalis	9
10,000 to 100,000 Enterococcus faecium	1
10,000 to 100,000 Escherichia coli, 10,000 to 100,000 Enterococcus faecalis	1
10,000 to 100,000 Klebsiella pneumoniae	3
10,000 to 100,000 Staphylococcus epidermidis	1
10,000 to 100,000 Streptococcus agalactiae	1
10,000 to 100,000 Urogenital Flora	27
1,000 to 10,000 Urogenital Flora	2
1,000 to 10,000 Citrobacter Koseri	1
1,000 to 10,000 E.coli	1
1,000 to 10,000 Enterococcus faecalis	5
1,000 to 10,000 Escherichia coli	1
1,000 to 10,000 Streptococcus agalactiae (Strep. group B)	2
1,000 to 10,000 Urogenital Flora	37
1,000-10,000 Streptococcus agalactiae (Strep. group B)	1
100 to 1,000 E.coli	1
100 to 1,000 S.agalactiae, 1,000-5,000 Urogenital Flora	1
100 to 1,000 Staphylococcus saprophyticus	1
100 to 1,000 Urogenital Flora	49
Grand Total	262

Table 2 summarizes the Optis Urine APP performance for early selection of positive urine cultures. The use of image analysis enumeration software to predict positive urine results with all media types using various growth threshold were evaluated. Specificity, Sensitivity and Accuracy for urine prediction at thresholds - $<10^2$; $<10^3$, $<10^4$, and $<10^5$ are summarized in this table:

Specificity at 12 hours ranged from 96.2 to 100% for evaluation of plates with >10,000 CFU/mL (all media types). Very few true negative plates would be flagged for review by the Urine APP at 12 hours for further review thus greatly reducing the laboratory effort for early reporting urine cultures.

Sensitivity at 12 hours ranged from 78.4 to 100% for evaluation of plates with >10,000 CFU/mL (all media types). This study shows that >80% of true growth positive plates could be evaluated at 12 hours for possible further ID/AST work-up.

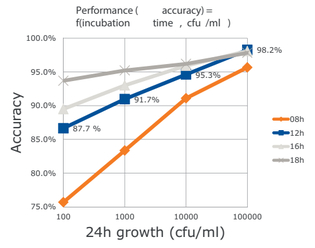
TABLE 2

12h incubation		Auto - release of negatives (cfu /ml) - Threshold value											
Truth = Manual interpretation 24h		<100			<1000			<10000			<100000		
		Spec (NPA)	Sens (PPA)	Acc	Spec (NPA)	Sens (PPA)	Acc	Spec (NPA)	Sens (PPA)	Acc	Spec (NPA)	Sens (PPA)	Acc
Average all media	N	87.6%	85.3%	86.7%	96.4%	85.2%	91.0%	97.8%	86.8%	94.6%	99.6%	94.4%	98.3%
TSA2	260	89.0%	88.2%	88.5%	100.0 %	84.6%	91.5%	100.0 %	89.4%	96.2%	100.0 %	90.6%	97.7%
CHROM	259	85.1%	78.9%	80.7%	95.7%	79.9%	86.9%	96.2%	79.4%	89.6%	100.0 %	89.4%	97.3%
CNA	262	98.0%	76.9%	85.1%	99.3%	78.4%	90.5%	99.5%	78.4%	95.4%	98.8%	100.0 %	98.9%
MAC	255	97.0%	95.6%	96.5%	98.4%	97.2%	98.0%	100.0 %	100.0 %	100.0 %	99.5%	96.1%	98.8%

Considering early incubation times, when present, colonies are smaller in size and usually less contrasted when compared to 24h incubation time, therefore slightly impacting detection accuracy performance (see figure 1). A linear relationship can be observed between performance (accuracy) and the growth's $\log_{10}$ as manually evaluated after 24h incubation.

FIGURE 1

24h growth (cfu /ml)	Accuracy			
	08h	12h	16h	18h
100	75.7%	87.7%	89.5%	93.7%
1000	83.3%	91.7%	93.0%	95.2%
10000	91.1%	95.3%	95.9%	96.2%
100000	95.7%	98.2%	98.1%	97.8%



Organisms that are not detected in the early time points are primarily nonpathogenic urethral flora. Figure 2 depicts the Urine app early detection performance when considering plates with significant growth ($\geq 10^4$ CFU/ml) only, as reported from manual observation at 24h incubation. Results are segregated between growth due to Urine Pathogens only (blue line) and growth due to any species (orange line). It can be observed that Urine pathogens are detected earlier and more efficiently than non-pathogens. Based on this observation, the 10h-14h incubation window seems to be appropriate for an early indication of significant growth.

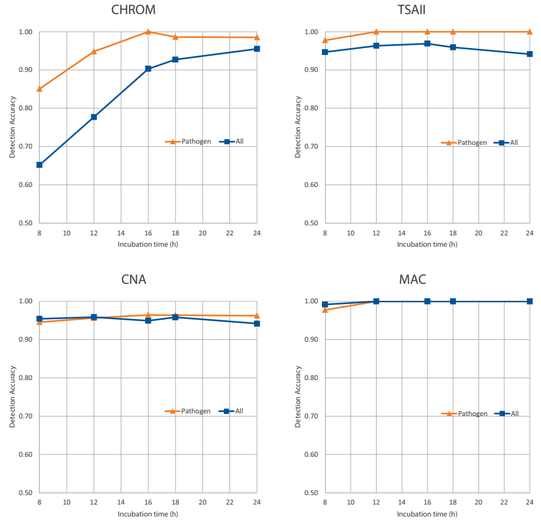


FIGURE 2 - early detection performance (accuracy) for plates showing significant growth ($\geq 10^4$ CFU/ml) after 24h incubation, considering only Uropathogens or All Urine isolates.

Figure 3 - Examples of images acquire during incubation time from 8h to 24h. It can be observed that in case of significant growth, urine pathogens can already be detected as early as 8h - 12h of incubation time.

Conclusions

Use of automated imaging permits detection of urine specimens with significant growth ($\geq 10^4$ CFU/ml) as early as 12 hours of incubation.

Disclosure

Optis™ image analysis software and expert rules product under development; not available for sale or use.