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Automated, Rapid and Accurate Semi-Quantitation of Urine Cultures in BD Kiestra™ Laboratory Automation Solution: Using Image Analysis with Expert Systems

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Background

The BD Kiestra™ Total Lab Automation (TLA) is an in-vitro diagnostic system that 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 and improved colony isolation. With the use of smart incubation and time series digital imaging analysis software, the next phase of micro automation will begin with further automation for diagnostic applications. 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™ Optis™ imaging software was used to evaluate the feasibility of automated processing of urine cultures. The objectives were to evaluate for: (1) Automated negative urine prediction and (2) Accurate semi-quantitation of positive urines.

Methods

For this study, 262 clean-catch clinical urine specimens were collected using the Vacutainer™ Urinalysis Preservative Tube. All urine samples were processed with BD Kiestra™ InoqulA+™ using 0.01mL inoculum, pre-defined streak method ZigZag Pattern 4, bead streak technology, and testing using the following BD media: 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) hours, 12, 18, and 24 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 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 for each urine culture plated media. The 0.01mL inoculation (higher than manual loop plating with 0.001mL) plus the highly reproducible bead streaking technology allow for use of both discrete colony counting and colony count estimation based on colony density along the expected streak path to accurately predict CFU/mL range, even for low count specimens.

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 aeruginosa	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

Automated, semi-quantitation of urine specimens using the Optis™ software compared with manual interpretation of growth on 4 agar media is summarized in Table 2.

TABLE 2

24h incubation		Auto-release of negatives (cfu/ml) - Threshold value											
Truth = Manual interpretation 24h		<100			<1000			<10000			<100000		
	N	Acc	Sens (PPA)	Spec (NPA)	Acc	Sens (PPA)	Spec (NPA)	Acc	Sens (PPA)	Spec (NPA)	Acc	Sens (PPA)	Spec (NPA)
Average all media	259.5	95.0%	97.7%	89.4%	96.1%	97.8%	94.0%	95.9%	97.3%	94.9%	97.2%	95.3%	97.8%
TSAII	260	94.6%	98.9%	83.6%	95.8%	100%	90.6%	95.8%	98.9%	94.0%	95.4%	92.2%	96.4%
CHROM	259	94.6%	97.8%	86.5%	95.4%	97.2%	93.0%	94.6%	98.0%	92.4%	97.3%	93.9%	98.4%
CNA	262	94.7%	96.3%	92.2%	94.7%	95.5%	94.0%	93.1%	92.2%	93.4%	96.9%	95.0%	97.1%
MAC	255	96.1%	97.8%	95.1%	98.4%	98.6%	98.4%	100%	100%	100%	99.2%	100%	99.0%

Accurate quantitation at all concentrations of organisms exceeded 95% at all threshold values, with a positive percent agreement (PPA) ranging from 95.3% to 97.8% and negative percent agreement from 89.4% to 97.8%. Significance differences in quantitative estimates were not observed for the 4 media types with the exception of plates classified with no growth (<100 cfu/ml).

Table 3 summarizes the BD Kiestra™ Optis™ Urine APP performance accuracy for automated semi-quantification for the correct CFU/mL range class for each media type. The 5 class range includes $<10^2$ CFU/ml [No growth], 10^2 – 10^3, 10^3 – 10^4,10^4 – 10^5 and $\geq 10^5$ CFU/ml. The 4 class range includes $<10^3$, 10^3 – 10^4,10^4 – 10^5 and $\geq 10^5$ CFU/ml.

TABLE 3

24h incubation		Semi-quantification			
Truth = Manual interpr. 24h		5 classes		4 classes	
	N	strict	pm1	strict	pm1
TSA2	260	84.2%	97.7%	87.7%	99.2%
CHROM	259	83.8%	98.1%	88.8%	98.5%
CNA	262	82.1%	98.5%	86.6%	98.5%
MAC	255	94.5%	99.2%	97.6%	100.0%
average All Media	259.5	86.2%	98.4%	90.2%	99.0%

pm1: +/- one class range score

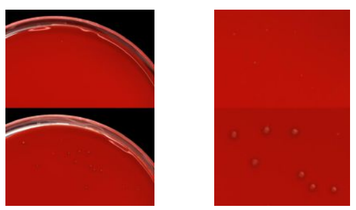
These results show >85% accuracy for all media types based on exact CFU/mL range for $<10^3$, 10^3 – 10^4,10^4 – 10^5 and $\geq 10^5$ CFU/ml. >98% accuracy was achieve based on matching the +/- one CFU/mL range. Slightly lower results were obtained for semi-quantification accuracy based on including the <100 CFU/mL range. This was due mostly to "False Positive" results associated with image software detecting growth not yet visible by human eye as presented in Figure 1.

Figure 1 presents example for BD Kiestra™ BD Kiestra™ Optis™ Urine APP considered as "False Positive" at 24 hours when compared to manual review but matching manual reading at 48 hours.

FIGURE 1

A) TSAII example of "False Positive" at 24 hours (top row) with matching truth at 48h hours (bottom row). Colonies can be seen at 24h but are likely to be missed by manual inspection of the plates.

Media	24 h manual read	48 h manual Read	Optis result
TSAII	$<10^2$	10^3 - 10^4	10^3 - 10^4



B) BD BBL CHROMagar example of "False Positive" at 24 hours with matching truth at 48h hours. Tiny colonies detected by the app at 24 hours incubation (top row and confirmed at 48h hours, bottom row). These tiny colonies are likely to be missed during 24h manual reading unless contrasted against black background.

Media	24 h manual read	48 h manual Read	Optis result
CHROM	$<10^2$	10^4 - 10^5	10^3 - 10^4

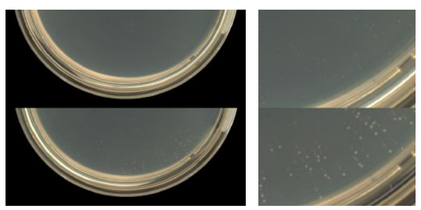
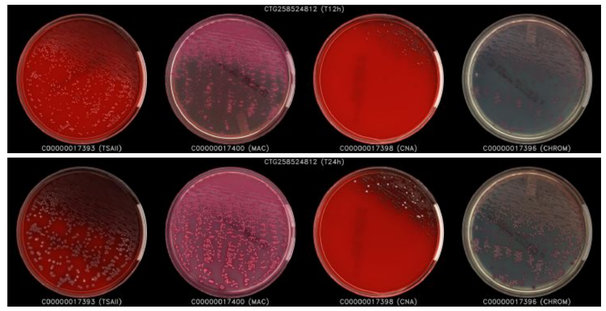
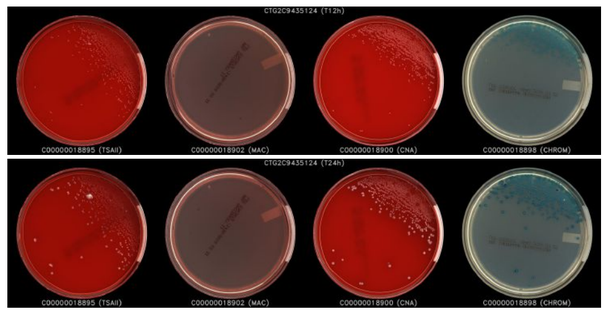


Figure 2 Examples of plated media sets (all media used for study) showing various types of positive cultures after 12h and 24h incubation

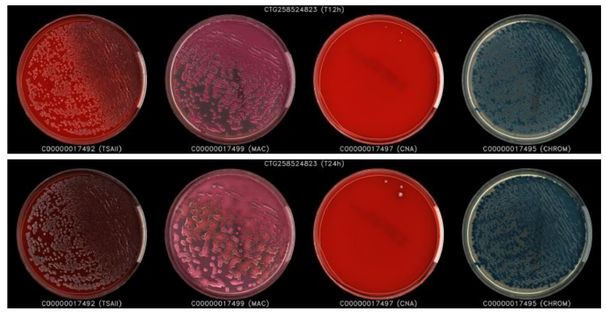
A) >100,000 E.coli, specimen #CTG258524812 (after 12h, first row and 24h incubation, second row)



B) >100,000 Enterococcus faecalis, specimen #CTG2C9435124 (after 12h, first row and 24h incubation, second row)



C) >100,000 Klebsiella pneumoniae, specimen #CTG258524823 (after 12h, first row and 24h incubation, second row)



Conclusions

The BD Kiestra™ System combined with BD Kiestra™ BD Kiestra™ Optis™ image analysis technology and expert rules provides a rapid, accurate and automated method for semi-quantitation of urine growth for negative urine reporting and grouping of positive urine specimens for more efficient review.

Disclosure

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