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Impact of Introduction of the BD Kiestra InoqulA on Urine Culture Results in a Hospital Clinical Microbiology Laboratory

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This study compared results from plating urine specimens with the BD InoqulA instrument using a 10-μl inoculum with results from cultures plated manually with a 1-μl loop for comparable 2-month periods. The positivity rates, turnaround times for positive cultures, and BD Phoenix identification and antimicrobial susceptibility test results were comparable for both time periods. We experienced no problems with culture interpretation as the result of moving to the 10-μl inoculum.

In contrast to the 1-μl urine specimen routinely plated in U.S. and Canadian laboratories, many European labs (1, 2, 3), as well as laboratories in other parts of the world, historically have plated 10-μl urine specimens. The BD Kiestra InoqulA (BD Kiestra B.V., Drachten, The Netherlands) utilizes a pipetting device with a disposable pipette that dispenses a minimum volume of 10 μl. As a result, routine urine specimens plated with the BD InoqulA generally utilize a 10-μl inoculum volume, although volumes up to 100 μl can be dispensed if needed for a specific laboratory protocol/application. Urine cultures represent a significant percentage of routine bacteriology cultures in most clinical microbiology laboratories, and consequently, any changes to urine culture protocols merit significant attention. For a laboratory that is transitioning from plating 1 μl of urine with a manual loop to 10 μl with the BD InoqulA, it would be important to assess any impact(s) that this change might have on culture metrics, including positivity rates, ability to obtain isolated colonies, and time(s) to results. This study, designed to compare urine culture results obtained for a fixed time period prior to the installation of a BD InoqulA when plating for specified urine specimen types, was performed manually using a 1-μl loop with a time period post-BD InoqulA installation comparable to that when plating was performed with the BD InoqulA using a 10-μl inoculum volume. On the basis of the repeated experiences of European laboratories (1, 2, 3), we hypothesized that we would obtain sufficient isolated colonies with the BD InoqulA 10-μl inoculum so as to not adversely affect time to completion of cultures or positivity rates compared to results obtained with a 1-μl inoculum volume.

All urine specimens for the pre-BD InoqulA installation and post-BD InoqulA installation time periods were plated on MacConkey agar and Trypticase soy agar with 5% sheep blood with incubation of all plates at 35°C to 37°C in 5 to 7% CO_2. Only clean-catch urine specimens or urine collected from indwelling catheters was included in this study. All cultures were examined the day following inoculation, and cultures with no growth on day 1 were reincubated for one additional day. Cultures were worked up similarly during the preinstallation and postinstallation time periods using standard Reading Hospital microbiology laboratory protocols, with workup performed by the microbiology technologist(s) routinely assigned to the urine bench. As appropriate, for positive results, organism identification and antimicrobial susceptibility testing were performed with the BD Phoenix instrument (BD Diagnostics, Sparks, MD). Nearly all specimens for which a BD Phoenix antimicrobial susceptibility test was set up also had a BD Phoenix identification panel set up. The only rapid/spot tests routinely performed in this laboratory are for identification of *Aerococcus* spp. and for serotyping of beta-hemolytic streptococci. Any required supplemental organism identifications (e.g., spot indole test) and/or antimicrobial susceptibility testing (Etests) were performed per routine laboratory protocols.

Retrospective urine culture results were reviewed for a 2-month period prior to installation of the BD InoqulA instrument (1 μl of urine plated manually with a calibrated loop) and for a 2-month period 3 months post-BD InoqulA installation (10 μl of urine plated with the BD InoqulA instrument). Culture result metrics extracted from Cerner Classic LIS 015 using Discern Explorer included specimen source, time and date of receipt, organism(s) identification and quantity, completion time and date, BD Phoenix instrument completion time and date, and received-to-completion time for culture and for each BD Phoenix instrument result.

The "start time" for the culture was defined as the time when the specimen was accessioned and the test was ordered (not plated) in the microbiology laboratory; there was no change in this process between the two phases of the study. The actual inoculation time was not recorded or captured. The BD Phoenix result was defined as the time that the BD Phoenix instrument finalized the result. For most specimens, this BD Phoenix result included the biochemical identification and antimicrobial susceptibility test result. When both biochemical identification and an antimicrobial susceptibility test were performed on an isolate, the BD Phoenix instrument was programmed to not release the identification until the susceptibility testing was completed. The culture completion time was the time when a technologist electronically finalized the culture result. Since culture work-up is performed on only one shift in this laboratory, there was always a difference between the time of reporting of the BD Phoenix result and the time of the final culture report. For example, a BD Phoenix result may have been finalized at 3:00 a.m., whereas the culture was not

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TABLE 1 Key culture metrics pre- and post-BD InoqulA installation

Parameter	Pre-BD InoqulA installation			Post-BD InoqulA installation			Postinstallation/ preinstallation difference	
	No. of specimens	% of total specimens	95% multinomial CI	No. of specimens	% of total specimens	95% multinomial CI	95% CI adjusted for multiple comparisons	*P* value
Total no. of urine specimens	9,356			8,125				
Positive results	2,491	26.62	25.71, 27.55	2,126	26.17	25.19, 27.15	−0.85, 1.77	0.504
Single pathogen recovered	1,223	13.07	12.04, 14.13	1,132	13.93	12.80, 15.07	−2.20, 0.47	0.101
Two results reported with 1 or 2 pathogens	1,268	13.55	12.52, 14.61	994	12.23	11.10, 13.37	0.01, 2.62	0.010[a]
Negative results	6,865	73.39	72.47, 74.30	5,999	73.83	72.86, 74.82	−1.76, 0.86	0.514
No growth	1,710	18.28	17.24, 19.33	1,750	21.55	20.41, 22.68	−4.83, −1.70	<0.001[a]
Multiple organisms[b]	69	0.74	0.00, 1.79	96	1.18	0.05, 2.32	−0.85, −0.07	0.003[a]
Sap P[c]	4,063	43.43	42.39, 44.48	3,615	44.49	43.36, 45.63	−3.00, 0.87	0.16
Sap PN[d]	781	8.35	7.31, 9.40	406	5.00	3.86, 6.13	2.39, 4.32	<0.001[a]
Single organism of $<10^4$/ml	242	2.59	1.55, 3.64	132	1.62	0.49, 2.76	0.40, 1.53	<0.001[a]

[a] Statistically significant at a *P* of ≤0.05.
[b] Multiple-organism result reported when there were three potential uropathogens or possible uropathogens and there was not a predominant organism.
[c] Sap P result reported when two or more species of Gram-positive skin or urogenital flora of any quantity were isolated or one species that was considered urogenital flora was isolated at $<10^5$ CFU/ml.
[d] Sap PN result reported when there were two or more skin or urogenital flora organisms along with a Gram-negative rod that was present at $<10^4$ CFU/ml or a low quantity of Gram-negative rods with clearly predominant saprophytic organisms.

finalized until 10:20 a.m. When one BD Phoenix ID antimicrobial susceptibility test was performed on a specimen, it was designated BD Phoenix 1, and if two BD Phoenix ID antimicrobial susceptibility tests were performed on a specimen, they were designated BD Phoenix 1 and BD Phoenix 2.

The criteria utilized to define a positive or negative result for the purposes of this study did not change between the two time periods compared in this study. A positive culture was defined as the presence of 1 or 2 potential uropathogens at a concentration of $\geq 10^4$ CFU/ml. Positive results included some specimens with one predominant uropathogen with lesser quantities (>1 $\log_{10}$ difference) of mixed Gram-positive or mixed Gram-positive and Gram-negative urogenital flora. Infrequently, a specimen with a pure culture of 10^3 CFU/ml of a uropathogen with a positive urinalysis result was also treated as a positive result.

For positive results reported as having one result reported, most had a BD Phoenix result for a uropathogen, such as *Escherichia coli*, for which both an identification and antimicrobial susceptibility test could be performed with the BD Phoenix instrument. Those results without a BD Phoenix result were from a uropathogen, such as a group B streptococcus, *Aerococcus* spp., or yeast species, for which there was no BD Phoenix result for either biochemical identification or antimicrobial susceptibility test.

For positive specimens reported as having two results reported, some had two BD Phoenix results; some had one BD Phoenix result, and some no BD Phoenix result. An example of a specimen with two results reported but with only one BD Phoenix result might be a uropathogen, such as *E. coli*, for which both identification and antimicrobial susceptibility tests could be performed with the BD Phoenix, while the second organism without a BD Phoenix result was either something not worked up due to lack of clinical significance, such as mixed organisms at low concentration, or a uropathogen, such as a group B streptococcus, *Aerococcus* spp., or yeast species, for which there was no BD Phoenix result. For those specimens with two results reported but with no BD Phoenix result, one result was something not worked up due to lack of clinical significance, such as mixed organisms at low concentration or a uropathogen, while the second result was a uropathogen such as a group B streptococcus, *Aerococcus* spp., or yeast species, for which there was no BD Phoenix result.

The classification of a negative result included the following culture results: no-growth specimens; multiple organisms (three potential uropathogens or possible uropathogens with no predominant organism); two or more species of Gram-positive skin or urogenital flora of any quantity isolated or one species that is considered urogenital flora at $<10^5$ CFU/ml (here termed Sap P); two or more skin or commensal urogenital organisms along with a Gram-negative rod at $<10^4$ CFU/ml or a small quantity of Gram-negative rods with clearly predominant saprophytic organisms (here termed Sap PN); 10^3 CFU/ml of a uropathogen with a negative urinalysis result; or 10^2 CFU/ml of any organism.

To facilitate the transition to reading cultures with a larger number of colonies produced with the 10-μl BD InoqulA instrument compared to the previously manually plated 1-μl inoculum, quality control organisms of known concentrations were plated with the BD InoqulA instrument, inoculum concentrations were verified by plate counts, pictures were taken of plates with 10^3-, 10^4-, and 10^5-CFU/ml inocula, and instructional teaching aids were produced from these pictures and provided to the technologists to serve as quantitation reference guides.

Statistical analysis for the percentages of results pre- and post-BD InoqulA installation (Table 1) compared the pre- and postinstallation proportions for each category and subcategory, taking into account the difference in sample size in the pre- and postinstallation periods. The 95% multinomial proportion confidence intervals (CI) were computed. The *P* values for proportion differences were computed using the proportion test(s) routine in R set for a two-sided comparison with an α of 0.05, adjusted for multiple comparisons when appropriate. For the pre- and post-BD InoqulA installation times to culture, the time to BD Phoenix 1 results, and the time to BD Phoenix 2 results (Tables 2 and 3), 95% confidence intervals were computed using the bootstrap per-

TABLE 2 Time to culture results pre- and post-BD InoqulA installation

Parameter	Pre-BD InoqulA Installation			Post-BD InoqulA Installation			*P* value
	No. of results	Median time to result (h)	Median 95% CI (h)	No. of results	Median time to result (h)	Median 95% CI (h)	
Positive results	2,491	45.83	(45.50, 46.18)	2,126	45.81	(45.39, 46.10)	0.235
One pathogen reported	1,223	44.68	(44.28, 44.97)	1,132	44.47	(43.92, 44.83)	0.209
With BD Phoenix	1,181	44.65	(44.25, 44.93)	1,067	44.47	(43.90, 44.83)	0.202
Without BD Phoenix	42	45.61	(43.55, 47.67)	65	44.60	(42.23, 46.75)	0.838
Two results reported	1,268	47.25	(46.67, 47.83)	994	47.97	(47.13, 48.82)	0.742
No BD Phoenix result	57	43.75	(42.98, 45.47)	141	43.55	(42.30, 44.80)	0.967
One BD Phoenix result	1,042	46.41	(45.95, 46.86)	730	47.50	(46.73, 48.40)	0.040
Two BD Phoenix results	169	68.65	(66.60, 70.43)	123	65.72	(64.38, 67.58)	0.130
Negative results	6,865	40.60	(40.45, 40.78)	5,999	39.38	(39.18, 39.60)	<0.001
No growth	1,710	40.43	(40.07, 40.80)	1,750	39.76	(39.42, 40.15)	0.063

centile technique and the pre- and postinstallation time distributions were compared for every category using a Wilcoxon test with an α of 0.05, adjusted for multiple comparisons when appropriate.

A total of 9,356 urine study specimens were plated manually with a 1-μl calibrated loop during June and July 2013 (pre-BD InoqulA installation), and 8,125 urine study specimens were plated with a 10-μl inoculum with the BD InoqulA instrument during January and February 2014 (post-BD InoqulA installation). BD InoqulA instrument installation and implementation occurred in October-November 2013.

Table 1 summarizes the culture result rate metrics for the two time periods. The parameters culture positivity rate and culture negativity rate, Sap P, and single pathogen of $\geq 10^4$ CFU/ml recovered were all essentially unchanged for the two time periods. Significant decreases were noted for the percentage of specimens with two results reported, Sap PN, and single organisms of $<10^4$/ml, while significant increases were noted in the percentages of specimens showing no growth and multiple-organism results.

Table 2 summarizes culture turnaround time (TAT) metrics (e.g., time to final result) for the two time periods. The only TAT metrics from the two time periods that were significantly different were decreases in the TAT for negative results and no growth results and an increase in the TAT for results with two results reported with a single BD Phoenix result.

Table 3 summarizes TAT metrics for BD Phoenix antimicrobial susceptibility test results. There were no significant differences between the two time periods for any of the metrics assessed.

The microbiology technologists noted that the cultures plated with the BD InoqulA instrument were easier to read than the loop-plated cultures. In particular, the streaking patterns produced by the BD InoqulA instrument were more consistent than the streaking patterns produced in the past with manual streaking. Posttraining, no difficulties were reported with the transition from reading to workup of plates with a 1-μl inoculum compared to that of plates with a 10-μl inoculum.

The standard plating volume for noninvasive urine specimens (clean-catch, indwelling catheter) is either 1 μl or 10 μl. While one reference recommends either a 1-μl or a 10-μl inoculum (4), another reference recommends a 1-μl inoculum (5). Although widely utilized, plating of 1 μl of urine has been shown to result in a significant lack of precision and accuracy, particularly for specimens plated manually with a calibrated loop (1, 2, 6, 7).

Even when a 10-μl inoculum is utilized for manual loop inoculation, there is a notable lack of precision compared to that of automated instrument inoculation. Froment et al. compared manual inoculation of seeded specimens and clinical urine specimens with results obtained by using the BD InoqulA instrument, using a 10-μl inoculum for each method (2). The BD InoqulA instrument resulted in more colony types recovered and isolated (up to 11% and 17%, respectively) than the manual method. BD InoqulA instrument results were also much more precise than results obtained by 15 technicians who plated seeded specimens by the manual loop method. These authors concluded that compared to manual culture, the BD InoqulA instrument improved the quality and standardization of the isolation, contributed to a better overall workflow, shortened the time to results, and provided more accurate results for polymicrobial specimens (2). Croxatto et al., also using a 10-μl inoculum for manual loop inoculation and the BD InoqulA instrument, reported a 3- to 10-fold-higher yield of discrete colonies with the BD InoqulA instrument than with manual loop inoculation (3).

While the BD InoqulA instrument has been shown to be more accurate and produces more isolated colonies than manual loop inoculation, these studies of the BD InoqulA instrument as noted have been performed with a 10-μl inoculum for both the BD InoqulA instrument and the manual method (2, 3). For a laboratory such as ours that is transitioning from manual loop plating of 1 μl of urine to the BD InoqulA instrument with a 10-μl inoculum, it is reasonable to anticipate greater accuracy. It is not so clear, however, how the 10-fold increase in urine specimen volume would affect plate reading and reporting metrics. Hence, this study had a goal of assessing positivity rate and time-to-detection metrics from pre-BD InoqulA and post-PD InoqulA time periods.

In our opinion, the most important rate metric assessed, the positivity rate, was unchanged in the pre-BD InoqulA and post-BD InoqulA time periods, indicating that the technologists appropriately adjusted colony count algorithms for the change from a 1-μl inoculum to a 10-μl inoculum. The increase in multiple-organism reports is likely the result of a combination of better separation of colonies with the BD InoqulA instrument than was obtained with the manual method and a contribution of a larger specimen inoculum. The increase in no-growth results from the pre-BD InoqulA time period to the post-BD InoqulA time period is more perplexing, and we can offer no explanation for this result. Importantly, none of the statistically significant rate metric changes (multiple organisms, Sap PN, no growth, or single organisms at $<10^4$/ml) is clinically significant.

In assessing the TAT for the two time periods, most metrics were not significantly affected. We note the reduction in TAT of about 1 h for negative results and can offer no explanation for this change. While we are aware of no protocol or personnel change that could have produced this reduction, we cannot exclude a change in laboratory workflow. It is clear that moving from manual plating, where specimens can be processed individually, to an instrument where batching of specimens is convenient would offer the potential for differences in inoculation time. However, it is our opinion that any differences in setup time were minimal. In practice, when manual plating was performed routinely, it was not unusual for the technicians to wait until a small batch of urine specimens was available before plating, much as occurs with the use of the BD InoqulA instrument.

Ideally, we would have preferred to perform this study as a side-by-side study, comparing results of manual plating with results obtained with the BD InoqulA instrument for the same specimens. This approach was not practical for several reasons, most importantly our desire to plate sufficient numbers of specimens to try to identify any significant differences in results between the two methods and the two specimen volumes. By comparing 2-month periods, we were able to sufficiently increase the "*n*" as to make a meaningful analysis possible, with over 8,000 specimens in each data set. Between the pre-BD InoqulA and post-BD InoqulA test periods, we are aware of no changes in personnel or protocols that could potentially have influenced our results. Consequently, we are comfortable that the results are valid and facilitate an accurate assessment of urine culture results for these two time periods.

A last but important point is that by moving from a 1-μl routine urine inoculum volume to a 10-μlone, a laboratory can now routinely detect as few as 10^2 CFU/ml in patients as opposed to a limit of sensitivity of 10^3 CFU/ml when 1 μl is plated. As noted by Stamm and colleagues, for women with acute urethral syndrome, the best diagnostic criterion was $\geq 10^2$ bacteria/ml (8). Kubik and McCarter noted that the recent literature has suggested that lower levels of bacteriuria (10^2 to 10^4 CFU/ml) should be considered positive for urinary tract infections in patients with symptoms of cystitis (9). Utilizing a precise, reproducible 10-μl inoculum volume with the BD InoqulA instrument thus increases both test accuracy and sensitivity.

In conclusion, our results indicate that there were no clinically significant differences in results obtained for urine cultures during this study, which compared manual loop plating of 1 μl of urine with BD InoqulA instrument plating of 10 μl of urine. Moreover, the microbiology technologists found that the plates inoculated with the BD InoqulA instrument were more easily read.

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TABLE 3 Time to BD Phoenix susceptibility results pre- and post-BD InoqulA installation

Parameter	Pre-BD InoqulA installation				Post-BD InoqulA installation				*P* value for:	
	No. of results	Median time (h) to BD Phoenix result 1	Median CI (h) result 1	Median time (h) to BD Phoenix result 2	Median CI (h) result 2	No. of results	Median time (h) to BD Phoenix result 1	Median CI (h) result 1	Median time (h) to BD Phoenix result 2	Median CI (h) result 2	Result 1	Result 2
One result reported	1,181	32.50	(31.95, 33.1)			1,067	32.30	(31.73, 32.77)			0.552	
Two results reported (one BD Phoenix result)	1,042	34.30	(33.43, 34.86)			730	34.80	(34.06, 35.77)			0.073	
Two results reported (two BD Phoenix results)	169	46.50	(42.38, 50.07)	59.07	(57.28, 61.33)	123	50.00	(43.23, 51.20)	56.92	(55.77, 59.13)		0.167

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