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ORIGINAL ARTICLE

Reducing the time between inoculation and first-read of urine cultures using total lab automation significantly reduces turn-around-time of positive culture results with minimal loss of first-read sensitivity

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Abstract

In order to realize the full potential of total laboratory automation (TLA) in the clinical microbiology laboratory, workflows must be optimized to match each laboratory's capabilities, patient population, and staffing model. Using TLA-based digital photography to monitor urine cultures, we sought to improve culture result turn-around-time (TAT) by changing the time at which a culture is first photographed and thus available for analysis/work-up (Pre1) from 18 h (16,391 cultures) to 16 h (53,113 cultures) (with a total of 24-h culture incubation in both time periods); in both time periods, cultures were set up 24/7, and culture work-up occurred during the day shift only. With this change, we observed a significant decrease in time-to-final-result TAT for positive cultures (18 h-Pre1 median: 71.6 h; 16 h-Pre1 median: 61.0 h). This effect was most pronounced for Gram-negative organisms, with a median reduction in time-to-final-result for *Escherichia coli* cultures (51.8% of positive urine cultures) of 14.2 h (18 h-Pre1 median: 77.3 h; 16 h-Pre1 median: 63.1 h). This reduction in TAT was accompanied by a decrease in sensitivity at the Pre1 time point (18 h-Pre1 91.01%; 16 h-Pre1 88.06%), which we also found to vary by species: there was a reduction in sensitivity at the first culture reading of 1 to 2% for cultures with Gram-negative microorganisms, but for some Gram-positive microorganisms (e.g., *Aerococcus urinae* and non-*aureus Staphylococcus* species), there was a reduction in sensitivity at the Pre1 time-point of 3 to 7%. These results can guide workflow decisions for laboratories seeking to implement and/or optimize TLA systems, demonstrating a tradeoff between TAT and the sensitivity of preliminary urine culture results.

Keywords Automation · Urine · *Escherichia coli* · Total laboratory automation

Introduction

Traditionally, the workup of clinical bacterial cultures has required manual inspection of culture plates by a laboratory technologist following "overnight incubation" (i.e., at approximately 24 h intervals)—a practice driven more by the norms of the human workday than by bacterial growth kinetics. This is now changing as total laboratory automation (TLA) systems for culture-based microbiology revolutionize the clinical microbiology laboratory. With TLA, the capture of digital images of culture plates can be automatically performed at any time-point defined by the user [1, 2]. This not only obviates reliance on the 24-h culture cycle, but provides an opportunity to identify growth sooner, potentially impacting patient care by expediting culture workup and reducing result turn-around-time (TAT) [3–7].

Current TLA systems photograph plates by moving plates from an incubator to an adjacent "photo booth" module. While it is not feasible to continuously monitor each individual plate, culture plates are imaged at predetermined intervals that are established for each culture type. Photo-capture of plates at earlier time-points has the potential to lead to earlier identification (and thus earlier reporting) of growth; importantly however, the potential collateral damage of reducing

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the time-to-first-photograph is a reduced ability of the initial photograph to detect all cultures that will eventually become positive (i.e., reduced sensitivity), a tradeoff that will result in a relative delay in time to reporting for a subset of cultures.

Our laboratory began using the BD KiestraTLA (Becton-Dickinson) in 2016, with urine cultures being the first specimen type to be implemented on this system. We have iterated our approach to automated urine culture processing; initially, we programmed the KiestraTLA to capture the first photograph of urine culture plates (a time-point referred to throughout as "Pre1") at 18 h after inoculation. However, we subsequently changed the Pre1 photo-capture time-point to 16 h post-inoculation to optimize the reading of specimens that arrived in the late afternoon within the morning-shift's workflow. Here, our objective was to compare the effect of this change in the Pre1 time-point on the time-to-final-culture-report TAT and sensitivity of the Pre1 interpretation.

Materials and methods

Ethics statement This study was reviewed and approved by the Institutional Review Board at Washington University in St. Louis (IRB ID#: 201801212).

Setting Barnes-Jewish Hospital Microbiology Laboratory processes urine culture specimens for Barnes-Jewish Hospital, a 1250 bed, tertiary care academic medical center, as well as four additional hospitals in the Barnes-Jewish Corporation HealthCare System, including three community hospitals and a stand-alone children's hospital. The laboratory processes approximately 80,000 urine cultures annually. Urine cultures are inoculated, incubated, and imaged 24/7, but photographs and subsequent culture workup (including identification (ID) and antimicrobial susceptibility testing (AST)) are only performed on the day shift.

Urine culture procedure All urine specimens collected during the stated timeframe (see section on "Data acquisition and processing") were considered for analysis. The KiestraTLA instrument used by the Barnes-Jewish Hospital Microbiology Laboratory during this study included one InoqulA module, six incubators (three with an O_2 and three with a CO_2 incubation environment) with attached photography modules, and five reading stations, all connected by a double-decker two-way track. Upon arrival in the laboratory, 10 μL of specimen was inoculated onto each of blood and MacConkey agar plates (Remel Inc., Lenexa, KS), using the Kiestra's InoqulA module. A chocolate agar plate was also added for specimens from "sterile" sites (see Table S1). A sterile 5-mm rolling bead was then used to automate spreading of the inoculum over plates in a zig-zag pattern (Kiestra streak pattern 04). Plates were then transferred immediately via the track system to incubators maintained at 35 ± 2 °C (ambient oxygen tension for MacConkey agar and 5% CO_2 for blood agar plates). Plates were monitored by photography, utilizing the front-, side-, and back-lighting features at the indicated time-points. Cultures were photographed again at a second "Pre2" time-point at 24-h total incubation time. Additional workup of positive cultures—which typically included identification by MALDI-TOF MS (Bruker BioTyper, Billerica, MA) and antimicrobial susceptibility testing by disk-diffusion or gradient-diffusion-device methods—was performed manually in accordance with the laboratory's standard operating procedures. A schematic of the urine culture procedure used by the Barnes-Jewish Hospital Microbiology Laboratory during this study can be found in Fig. 1.

Data acquisition and processing Work-card data from the laboratory information system (Cerner Millennium, Cerner Corporation, Kansas City, MO) at Barnes-Jewish Hospital was obtained for all urine cultures performed on the KiestraTLA system between October, 2016 and November, 2017. This time-frame was chosen to minimize the effect of a "burn-in period" for technologists using the KiestraTLA, as we began performing urine cultures on the instrument in June 2016. After removing erroneous and non-patient (e.g., proficiency testing) cultures, 74,660 urine cultures were evaluated. A comprehensive list of urine microorganisms (Table S2) was generated by deleting words from the entire dataset that were unrelated to microbial identity using regular expressions in TextWrangler (Bare Bones Software, Inc., North Chelmsford, MA) followed by removal of duplicate uropathogen types in Microsoft Excel (Microsoft Corporation, Redmond, WA).

Data categorization In categorizing final culture results, any result with a microorganism listed in Table S2 was categorized as "positive;" any culture result that did not contain a microorganism listed in Table S2 was categorized as "negative." To be categorized as "positive," routine cultures had to have grown > 100,000 colony forming units (CFU)/mL, with no more than two colony morphologies present above this colony count threshold. However, this threshold was lower for urine obtained by straight catheter (> 10,000 CFU/mL), urine from pediatric patients (> 10,000 CFU/mL), and sterile specimens (such as suprapubic aspirate specimens) (any growth). Any amount of *Streptococcus agalactiae* was identified and reported from cultures of specimens from reproductive-age women (broadly defined as 14–50 years of age) (see Table S1 for additional specimen details). Cultures that grew above-threshold amounts of three colony morphologies were categorized as "contaminated." Cultures that grew below threshold colony counts for the urine specimen type of any organism (with the exceptions listed above) were categorized as "insignificant." Cultures with final results of "contaminated,"

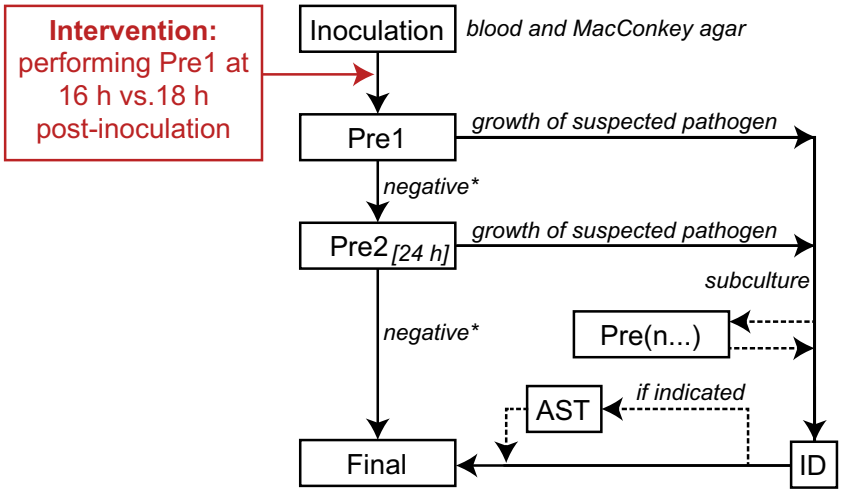

Fig. 1 Urine culture workflow on the KiestraTLA system in the clinical microbiology laboratory at Barnes-Jewish Hospital. Following inoculation, urine cultures were photographed and given a preliminary (Pre1) result. This study compares the impact of performing the Pre1 photograph at 16 h vs. 18 h on the time-to-final result. As shown in the schematic, several additional reads/reports (Pre2, Pre(n …), etc.) can be appended to the culture's record between Pre1 and final depending on the subsequent workup, which could include identification (ID) and antimicrobial susceptibility testing (AST). The asterisk indicates that cultures categorized as "negative" contained either "insignificant," "no growth," or "contaminated" in the diagnostic comment. ID: identification; AST: antimicrobial susceptibility testing

"insignificant," or "no growth" were further categorized as "negative" for the purpose of analysis.

A variety of results were also used in our laboratory for preliminary reporting at the Pre1 time-point. We categorized Pre1 results into four main groups: (1) results containing a species-level identification of a microorganism, (2) results containing the term "Gram negative," (3) results containing the term "pending" meaning that growth was present but too scant or immature for identification, or (4) results containing the terms "insignificant," "no growth," or "contamination," which were collectively categorized as "negative." The percentage of cultures that had a positive final result but a negative Pre1 result was used to calculate the sensitivity [(total positive at final–negative at Pre1) / total positive at final] of the Pre1 read.

Statistical analyses Data was processed and tabulated in Microsoft Excel. Graphs were made using Prism 7 (GraphPad Software, La Jolla, CA). All statistical analyses were performed in Prism and the statistical test used to evaluate significance is specified in the relevant figure legends.

Results

Recovery of microorganisms at a large academic clinical microbiology laboratory using TLA We downloaded work-card data from our laboratory information system (LIS) for all urine cultures performed on the KiestraTLA system between October 2016 and November 2017, resulting in 74,660 patient urine cultures. In total, we identified 69 unique microorganisms, 55 of which were identified to the species level. Many of these were rare: for 21 unique organisms, only a single positive urine culture was identified (Table S2). Only 11 species accounted for > 96% of all positive urine cultures (Fig. 2). As anticipated, the majority of positive cultures grew Gram-negative organisms, with *Escherichia coli* (51.8%), *Klebsiella* spp. (10.9%), and *Proteus mirabilis* (4.4%) being most common. *Streptococcus agalactiae* (11.1%) and *Enterococcus faecalis* (4.7%) were the most common Gram-positive organisms.

Performing the Pre1 read at 18 versus 16 h: impact on culture sensitivity Like many clinical microbiology laboratories, our laboratory only reads urine cultures during the day shift (approximately 7:00 AM to 3:30 PM). We also only perform identification and antimicrobial susceptibility testing during the day shift. However, cultures are processed (i.e., inoculated, incubated, and imaged) upon arrival in the laboratory 24 h per day, 7 days per week (see Table S3 for details). Therefore, although plates are automatically photographed by our TLA system at predetermined intervals, photographs are not reviewed until the earliest opportunity on the day shift. In our particular setting, we receive a large number of cultures at the end of the day shift, and preliminary analysis of timing between specimen receipt and the Pre-1 read suggested that time to culture result reporting might be optimized by changing from 18 to 16 h for the Pre-1 read. Given the robust culture growth at the 18 h read, we anticipated that this change would not have a negative impact on culture interpretation. Thus, in an attempt to improve our workflow and turn-around-time (TAT), we modified the time at which preliminary (Pre1) photographs were taken from 18 h post-inoculation to 16 h post-inoculation for all urine cultures performed on the

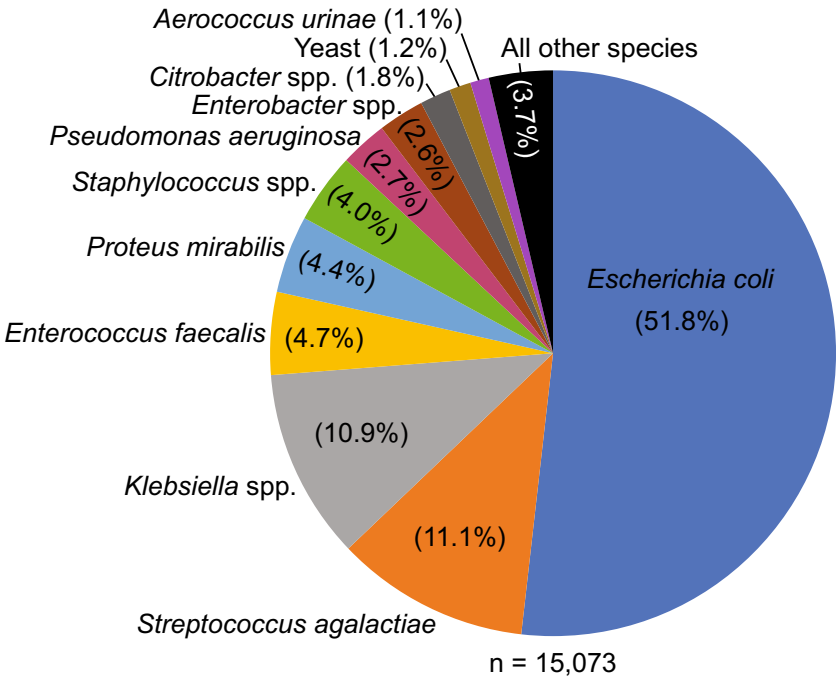

Fig. 2 Distribution of microorganisms isolated from patient urine cultures on the KiestraTLA system at Barnes-Jewish Hospital

KiestraTLA. To assess the impact of this change, we compared results from cultures submitted on October, 2016 to January, 2017 (18 h-Pre1: 16,391 cultures) to those submitted from March, 2017 to November, 2017 (16 h-Pre1: 53,113 cultures). This change was implemented during the month of February 2017, and so, data from this month was not included in either the 18 h-Pre1 or 16 h-Pre1 analysis as a "wash out" period, but was included in analyses where data from the entire study period was considered.

Our primary concern was that this change would impact the sensitivity of our Pre1 results, i.e., we would detect a lower percentage of positive urine cultures at the Pre1 read. Indeed, we found this to be the case: 88% of all positive urine cultures were detected at the 16 h-Pre1 read, which was a modest decrease from the 91% of positive urine cultures detected at the 18 h-Pre1 read (Table 1, Table S4). On a per-species basis, this trend also held true, with our ability to detect a positive culture at the Pre1 read decreasing by approximately 1–3% when the Pre1 was performed at 16 h versus 18 h, although this trend was more significant for Gram-negative ($p = 0.0312$) than Gram-positive species ($p = 0.31$) (Fig. 3). Conversely, the percentage of urine cultures given a "positive" Pre1 read (i.e., cultures given a genus-level identification or containing the terms "Gram-negative" or "pending") that were later changed to a "negative" final read (i.e., "insignificant growth," "no growth," or "contaminated") decreased from 1.9 to 1.4% with the change in performing Pre1 was performed at 16 h versus 18 h.

Performing the Pre1 read at 18 versus 16 h: impact on turn-around-time To examine the impact of performing the Pre1 read at 18 h vs 16 h, we first examined the TAT reported for all cultures and found that overall TAT increased with Pre1 reading at 16 h (median: 37.3 h) compared to Pre1 reading at 18 h (median: 36.4 h) (Fig. 4). Further analysis revealed that this increase in TAT was heavily influenced by negative cultures (18 h-Pre1 median: 34.4 h; 16 h-Pre1 median: 35.5 h), which make up 79.8% of all cultures performed in our laboratory. In contrast, a significant decrease in TAT was observed for positive cultures (18 h-Pre1 median: 71.6 h; 16 h-Pre1 median: 61.0 h; $p < 0.0001$). When examined on a per-species basis, performing Pre1 reads at 16 h resulted in significantly reduced TAT for the most common microorganisms recovered in urine cultures, with the exception of yeast (Fig. 4, Table S5). For Gram-negative species in particular, this difference was dramatic and much greater than the 2-h difference in Pre-1 culture reading time: for example, the median TAT for a culture growing *E. coli* was reduced by a median of 14.2 h (18 h-Pre1 median: 77.3 h; 16 h-Pre1 median: 63.1 h).

Discussion

Urinary tract infections (UTIs) are among the most common reasons for women to seek healthcare in the USA and account for approximately 1% of all ambulatory encounters [8]. Consequently, urine specimens are one of the most common specimen types submitted to clinical microbiology laboratories for analysis. Although treatment for UTI is typically empiric, expedited culture results can inform early optimization of antimicrobial therapy. A small but growing body of literature demonstrates that implementation of TLA can quantifiably improve many aspects of culture-based

Table 1 Urine cultures evaluated

	Culture result	Total. [No. (%)]	18 h-Pre1 [No. (%)]	16 h-Pre1 [No. (%)]
1	Total cultures evaluated	74,660	16,391	53,113
2	Final: negative[a]	59,587 (79.80)	12,922 (78.83)	42,533 (80.08)
3	Final: positive	15,073 (20.19)	3469 (21.24)	10,580 (19.92)
4	Gram negative at Pre1	10,149 (67.33)	2194 (63.25)	7243 (68.46)
5	Pending at Pre1	3197 (21.21)	957 (27.59)	2045 (19.33)
6	Negative[a] at Pre1	1687 (11.19)	312 (8.99)	1263 (11.94)
7	Sensitivity of Pre1 read[b]	88.81%	91.01%	88.06%

For lines 2 and 3, percentage of total is shown; for lines 4, 5, and 6, percentage of positive cultures is shown

[a] Includes cultures with diagnostic comments containing insignificant, no growth, or contamination

[b] Sensitivity = (final: positive-negative at Pre1) / final: positive

microbiology compared to conventional methods, including improved recovery of fastidious organisms present in urine specimens [9, 10]. In addition, TLA can result in a reduction in TAT for both microorganism identification and antimicrobial susceptibility testing results, each of which can inform antibiotic prescription practices [3, 4, 6, 11]. At the same time, studies also suggest that adoption of TLA does not guarantee improvements in laboratory performance—optimization of laboratory workflow is needed to maximize the potential benefits of TLA [5, 12].

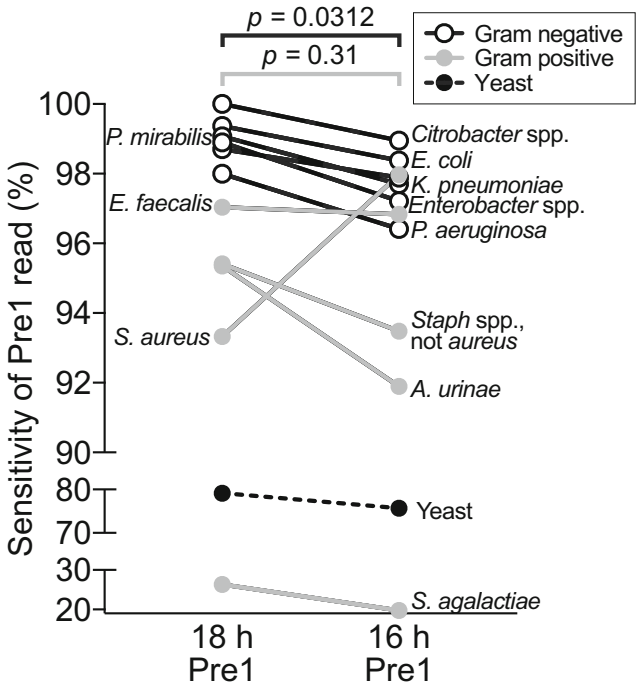

Fig. 3 Performing Pre1 read at 16 h versus 18 h results in reduced sensitivity for early detection of Gram-negative bacteria. The percentage of cultures that were negative at Pre1 but ended up with a positive final result was used to calculate the sensitivity of the Pre1 read for the twelve most common uropathogens, at both the 18 and 16 h time-point. Statistical analyses were performed separately for Gram-negative and Gram-positive species using the Wilcoxon matched-pairs signed rank test

Our results suggest that, even within the same laboratory using the same: TLA instrumentation/setup; number of employees dedicated to the work-up of urine specimens; and laboratory standard operating procedures, small modifications to optimize the workflow can have an outsized impact. Specifically, we show that reducing the time at which a urine culture is first imaged for workup to begins (Pre1) from 18 to 16 h reduced the median time TAT of the final microbiology report for positive cultures by 10 h—a difference that may have important clinical implications, especially in the inpatient setting where many antibiotics are dosed every 8 or 12 h. This effect was even more dramatic for some of the most common Gram-negative uropathogens, such as *E. coli*, *K. pneumoniae*, and *P. mirabilis*. A substantial fraction of this decrease in TAT is likely related to our laboratory's practice of only reading culture results on the day shift and the time of day many urine specimens are received in the laboratory. This minor modification resulted in a larger number of specimens being evaluated the day following receipt in the laboratory, rather than nearly 2 days later. While reading cultures 24/7 would likely reduce the impact of this change on TAT, this was not feasible during the time period of this study and is not commonly practiced in many clinical laboratory settings.

Importantly, the reduction in TAT that we observed incurred a concomitant cost in sensitivity, i.e., a higher percentage of cultures that were positive at the final read were called negative at the Pre1 read performed at 16 h compared to 18 h. This reduction in sensitivity varied by species. While variations in the microbial burden inherent to clinical specimens likely of each clinical specimen likely plays a role [7], the growth rate intrinsic to each species is likely a more important factor: cultures of more rapidly growing Gram-negative bacteria showed a decrease in Pre1 sensitivity between 18 and 16 h of only 1 to 2% while the sensitivity of the Pre1 read decreased by 3 to 7% for cultures of slower-growing Gram-positive species like *A. urinae* and *S. agalactiae*. When considered as a tradeoff, we suggest that the significant reductions in TAT overall far outweigh the accompanying decrease in Pre1 read sensitivity for these less commonly recovered taxa. Logically, this tradeoff exists at every time-point that a

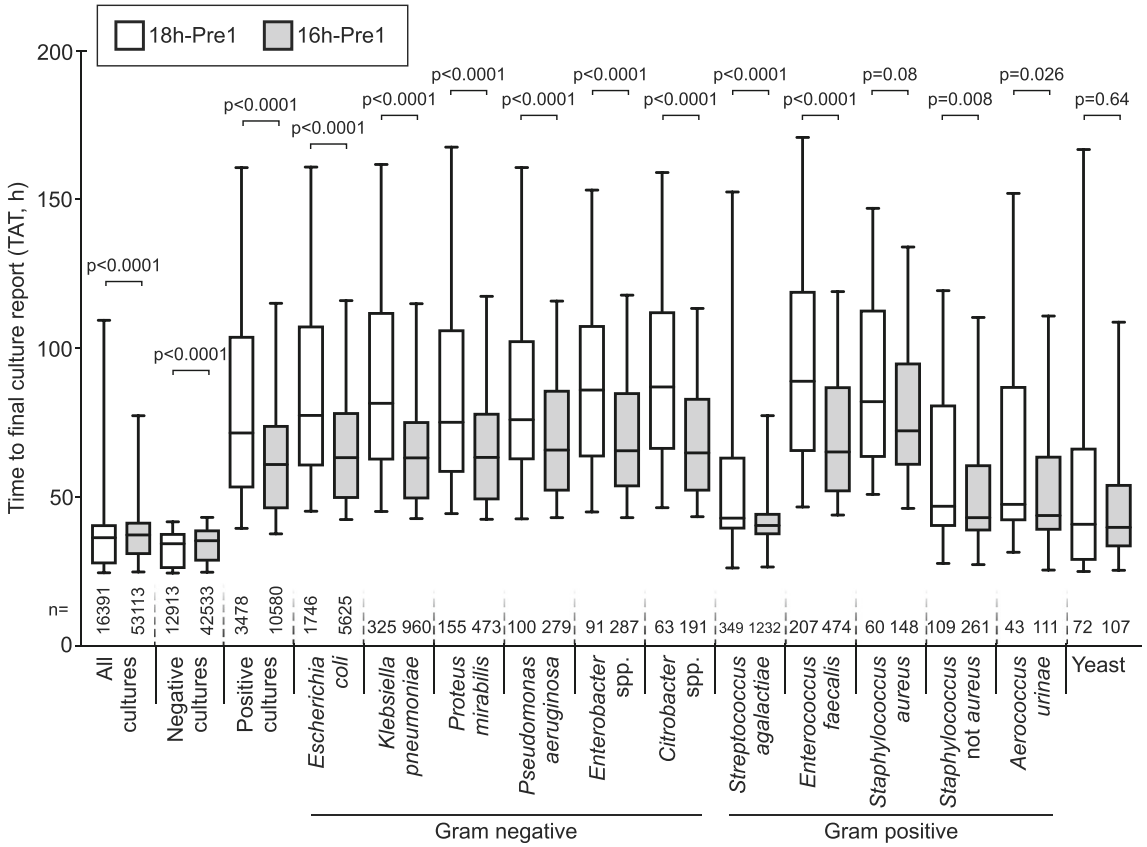

Fig. 4 Performing Pre1 read at 16 h versus 18 h results in reduced time-to-final-culture-report for positive cultures. Box and whisker plots show the median with boxes extending to the 25th and 75th percentiles. Whiskers demarcate the 5th and 95th percentiles. Mann-Whitney tests were used to assess for significance between 18 h and 16 h for each condition

culture could theoretically be examined. However, it is likely that each laboratory will need to optimize reading times to account for differences intrinsic to local microbes, methods for microorganism identification, TLA configurations, and workforce constraints. For example, in contrast to our study in which every culture plate was read at least twice, a recent study with the explicit goal of reducing laboratory workload demonstrated a similar tradeoff between sensitivity and TAT at 20 h versus 24 h using a single reading time-point. If a reduction in TAT is the sole objective, it may be possible to decrease the preliminary read time-point further without significant losses in sensitivity [13], but the practicality of this approach may vary widely by laboratory. The time at which "Final" photographs are taken will also likely need to be optimized for each laboratory and may need to be tailored to ensure detection of slowing-growing organisms such as *S. agalactiae*. In our hands, incubation of urine cultures for 24 h prior to discard as negative is needed to maximize sensitivity.

An important limitation of this study is that our results are based upon an analysis of data collected before and after an intervention, but of note, instrument factors such as the KiestraTLA hardware and imaging software and the laboratory staffing model were consistent throughout the study. The study has many strengths, especially that it represents real-world data on a laboratory cost-neutral solution and includes a very large sample size.

In conclusion, we show that reducing the time at which urine cultures are first photographed in a TLA system can have an outsized impact on the average TAT of the final culture report; however we also demonstrate that such a change can reduce the sensitivity of the initial read, ultimately resulting in a longer TAT for a small subset of cultures. As TLA becomes more widespread in the realm of clinical microbiology, the tradeoff between TAT and sensitivity demonstrated here will be an important consideration as individual laboratories seek to maximize the benefits offered by TLA.

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Compliance with ethical standards

Ethics statement This study was reviewed and approved by the Institutional Review Board at Washington University in St. Louis (IRB ID#: 201801212).

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References

1. Greub G, Prod'hom G (2011) Automation in clinical bacteriology: what system to choose. Clin Microbiol Infect 17:655–660
2. Bailey A, Burnham C-A, Ledeboer N (2018) Clinical microbiology is growing up: the Total Laboratory automation revolution. Clin 2017:274522
3. Dauwalder O, Landrieve L, Laurent F, de Montclos M, Vandenesch F, Lina G (2016) Does bacteriology laboratory automation reduce time to results and increase quality management. Clin Microbiol Infect 22:236–243
4. Mutters NT, Hodiamont CJ, de Jong MD, Overmeijer HP, van den Boogaard M, Visser CE (2014) Performance of Kiestra total laboratory automation combined with MS in clinical microbiology practice. Ann Lab Med 34:111–117
5. Yarbrough ML, Lainhart W, McMullen AR, Anderson NW, Burnham CD (2018) Impact of total laboratory automation on workflow and specimen processing time for culture of urine specimens. Eur J Clin Microbiol Infect Dis 37(12):2405–2411
6. Theparee T, Das S, Thomson RB (2018) Total Laboratory automation and matrix-assisted laser desorption ionization-time of flight mass spectrometry improve turnaround times in the clinical microbiology laboratory: a retrospective analysis. J Clin Microbiol 26:56(1)
7. Burckhardt I, Last K, Zimmermann S (2019) Shorter incubation times for detecting multi-drug resistant Bacteria in patient samples: defining early imaging time points using growth kinetics and Total Laboratory automation. Ann Lab Med 39:43–49
8. Foxman B (2014) Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin N Am 28:1–13
9. Lainhart W, Burnham CA (2018) Enhanced recovery of fastidious organisms from urine culture in the setting of Total Laboratory automation. J Clin Microbiol 26:56(6). https://doi.org/10.1128/JCM.00546-18
10. Klein S, Nurjadi D, Horner S, Heeg K, Zimmermann S, Burckhardt I (2018) Significant increase in cultivation of Gardnerella vaginalis, Alloscardovia omnicolens, Actinotignum schaalii, and Actinomyces spp. in urine samples with total laboratory automation. Eur J Clin Microbiol Infect Dis 37:1305–1311
11. De Socio GV, Di Donato F, Paggi R, Gabrielli C, Belati A, Rizza G, Savoia M, Repetto A, Cenci E, Mencacci A (2018) Laboratory automation reduces time to report of positive blood cultures and improves management of patients with bloodstream infection. Eur J Clin Microbiol Infect Dis. https://doi.org/10.1007/s10096-018-3377-5
12. Burckhardt I, Horner S, Burckhardt F, Zimmermann S (2018) Detection of MRSA in nasal swabs-marked reduction of time to report for negative reports by substituting classical manual workflow with total lab automation. Eur J Clin Microbiol Infect Dis 37:1745–1751
13. Graham M, Tilson L, Streitberg R, Hamblin J, Korman TM (2016) Improved standardization and potential for shortened time to results with BD Kiestra™ total laboratory automation of early urine cultures: a prospective comparison with manual processing. Diagn Microbiol Infect Dis 86:1–4