Laboratory automation impact on antimicrobial resistance

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Antibiotic resistance in common bacterial pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, has significantly limited the therapeutic options available for management of infectious diseases. While the indiscriminant use of broad spectrum antibiotics is a significant contributing factor, a more fundamental problem exists. Diagnostic microbiology test results have historically been available too late to be useful. This is, in part, due to the nature of the test methods and in part due to workflow practices in the laboratory. Thus, patients remain on empiric treatments that are frequently ineffective or unnecessarily too broad spectrum[1,2]. Microscopy and bacterial cultures are mainstays in the microbiology lab, using techniques developed more than 100 years ago. Although microbiologists speak with pride about the 'art' of their science, the clinical value of the diagnostic tests is frequently lost because of the delays in reporting results with these 'traditional' approaches. Fortunately, the practice of clinical microbiology is undergoing a dramatic transformation with the introduction of molecular diagnostics, primarily for rapid diagnosis of infections caused by viruses and difficult to grow bacteria, MALDI-TOF mass spectrometry for identification of bacteria, mycobacteria and fungi, and automation of all practices in bacteriology.

Whereas the impacts of the molecular diagnostics and identification by mass spectrometry on time to results are readily recognised, the transformative impact of automation is only slowly being realised, in part because the full benefits of automation require the laboratory to change their work practices. Processing specimens in the microbiology laboratory can be subdivided into: (1) the initial receipt and processing of the specimen (e.g. preparation of Gram stain and inoculation of plates); (2) the incubation of the inoculated plates; (3) examination of the plates; and (4)

performance of identification tests and antibiotic susceptibility tests. The impact of lab automation will have on improving patient outcomes and combatting antimicrobial resistance is directly related to improving diagnostic accuracy and timeliness of results.

Initial receipt and specimen processing

The traditional approach after receipt of a specimen is to enter the patient demographic information and test requests into the laboratory information system, sort the specimens based on processing priorities, and then inoculate specific enrichment and selective differential culture media based on established laboratory procedures. Because the selection of culture media is defined by the specimen type, the common practice is to process similar specimens in batches. This is efficient for the technologist but less so for processing specimens with a lower priority – processing these specimens may introduce significant delays from the time of receipt to inoculation onto media and subsequent incubation. Additionally, because the processing area and incubators are typically in different areas of the laboratory, it is inefficient for the technologist performing the initial processing to transport the plates to the incubator after each specimen is processed. This is normally done in batches or even at fixed intervals during the day, introducing significant delays between inoculation of plates and incubation. Finally, the inoculation of plates is dependent on the skill of the technologist. Indeed, the traditional approach to inoculation of a plate is to perform a four-quadrant streaking pattern (with ideally the use of a sterile loop for each quadrant) in order to obtain adequate isolation of colonies for further workup. The quality of this process can vary tremendously based on the technical skills of the microbiologists.

The first automated systems focused on the initial processing of specimens because it was recognised that the quality of this step was important for all subsequent work. Full laboratory automation (Figure 1) allows the user to process specimens immediately on receipt in the laboratory and then automatically transport the inoculated plates to incubators by a track system similar to what is used in automated chemistry, haematology, or immunology laboratories. Systems that permit inoculation of multiple plates simultaneously result in more rapid throughput than possible with inoculation of single plates manually. Use of a pipette to dispense the sample rather than the traditional bacteriologic loop ensures
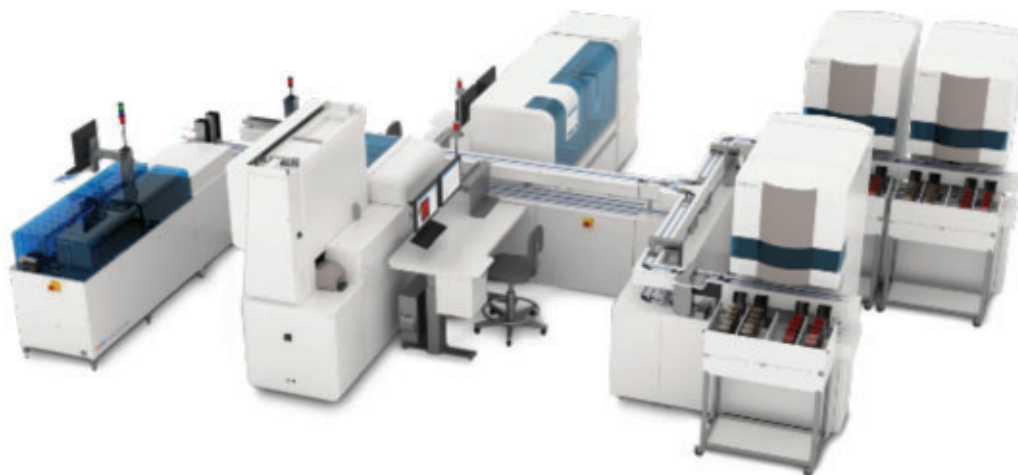
Figure 1. BD Kiestra total laboratory automation platform.

the proper volume is dispensed, and the use of magnetic beads or mechanical loops improves both the reproducibility of the plate inoculation and the ability to obtain isolated colonies[3–6]. Improved specimen throughput, rapid incubation and decreased need for subcultures all directly impact on the time from initial specimen processing to identification and antimicrobial susceptibility testing, critical steps in moving from empiric antibiotic therapy to directed therapy.

Incubation and imaging of culture plates

Traditionally, inoculated plates are incubated overnight before they are examined. Because plates are inoculated throughout the day and evening, they have been typically incubated 12–24 hours before they are removed from the incubator. Laboratory automation systems allow standardisation of the incubation time for each specimen type (i.e. shorter incubation times for specimens such as urines with rapidly growing organisms, longer incubation times for specimens such as respiratory with slow growing organisms)[7–10]. In other words, incubation is defined by the specimen type and not by the work practices of the technical staff. Indeed, this is a challenge for the laboratory to maximise the value of automation by processing the specimen after it has been incubated for a defined period of time which can occur throughout the day, evening and night.

The common practice in manual processing of cultures is to remove all culture plates when the technical staff arrives in the laboratory, sort the plates by specimen type, and then process the cultures by technologists working with the specific specimens (e.g. urines, respiratory, wounds, stools, etc.). There are some efficiencies realised by having a technologist work with the same specimen type, with each culture processed according to standardised procedures, and the same pathogens commonly isolated. However, the workload is unevenly distributed so it is difficult to assess the efficiency of individual technologists. Additionally, because the

culture plates are not in the lab incubator during the processing, cultures may remain at room temperature for hours thus negating the advantage of incubation in the optimum temperature and atmosphere. With laboratory automation systems, plates remain in the incubator and examination is performed digitally by imaging the plates. Software has been developed to maximise the early detection of growth and optimise the images. Under the optimised incubation conditions, cultures can be examined and subsequent processing (e.g. identification, antimicrobial susceptibility tests) initiated hours earlier than traditional approaches[9,11,12]. Better recovery of organisms such as *S. aureus* and *E. coli* as well as slow growing pathogens has also been reported with these incubation systems[13,14]. The imaging software can also screen cultures for insignificant growth and eliminate further processing of the culture plates. Again, the decreased time to results and improved recovery directly impact on the timely selection of optimum therapy.

Automation of identification and antimicrobial susceptibility tests

The use of MALDI-TOF mass spectrometry has dramatically changed the way organisms are identified, providing increased accuracy, decreased time to results and decreased costs[15–19], particularly when combined with total lab automation[9,11]. Use of molecular diagnostics permits the rapid detection of resistance genes in selected pathogens; however, the presence of a resistance gene informs which drug cannot be used but not which drug can be used. Thus, guidance for the selection of antibiotic treatment currently requires performance of traditional, phenotypic antibiotic susceptibility tests. Traditional susceptibility tests frequently require subcultures to obtain isolated colonies for testing. Automated platforms have been demonstrated to provide improved isolation of colonies thus eliminating the need for a significant number of subcultures, as well as reducing problems with mixed cultures requiring repeat testing.

Conclusions

Because empiric antibiotic therapy is frequently inappropriate, the rapid transition to directed therapy defined by the microbiological culture results is critically important for improved patient outcomes, decreased hospital costs and decreased risk of selecting antimicrobial resistant bacteria. The microbiology laboratory is undergoing a technological transformation with laboratory automation potentially influencing every stage of generating timely test results.

Conflicts of interest

The author is a salaried employee of Becton Dickinson Diagnostic Systems.

Acknowledgement

References

1. Raman, G. *et al*. (2015) Appropriate initial antibiotic therapy in hospitalized patients with gram-negative infections: systematic review and meta-analysis. *BMC Infect. Dis.* **15**, 395–405. doi:10.1186/s12879-015-1123-5

2. Paul, M. *et al*. (2010) Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob. Agents Chemother.* **54**, 4851–4863. doi:10.1128/AAC.00627-10

3. Froment, P. *et al*. (2014) Automated versus manual sample inoculations in routine clinical microbiology: a performance evaluation of the fully automated InoqulA instrument. *J. Clin. Microbiol.* **52**, 796–802. doi:10.1128/JCM.02341-13

4. Croxatto, A. *et al*. (2015) Comparison of inoculation with the InoqulA and WASP automated systems with manual inoculation. *J. Clin. Microbiol.* **53**, 2298–2307. doi:10.1128/JCM.03076-14

5. Iversen, J. *et al*. (2016) Comparative evaluation of inoculation of urine samples with the Copan WASP and BD Kiestra InoqulA instruments. *J. Clin. Microbiol.* **54**, 328–332. doi:10.1128/JCM.01718-15

6. Yarbrough, M.L. *et al*. (2018) Impact of total laboratory automation on workflow and specimen processing time for culture of urine specimens. *Eur. J. Clin. Microbiol. Infect. Dis.* **37**, 2405–2411. doi:10.1007/s10096-018-3391-7

7. Graham, M. *et al*. (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. doi:10.1016/j.diagmicrobio.2016.06.020

8. Burckhardt, I. *et al*. (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. doi:10.1007/s10096-018-3308-5

9. Theparee, T. *et al*. (2018) Total laboratory automation and MALDI-TOF improve turnaround times in the clinical microbiology laboratory: a retrospective analysis. *J. Clin. Microbiol.* **56**, e01242-17. doi:10.1128/JCM.01242-17

10. Burckhardt, I. *et al*. (2019) Shorter incubation time 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. doi:10.3343/alm.2019.39.1.43

11. Mutters, N.T. *et al*. (2014) Performance of Kiestra total laboratory automation combined with MS in clinical microbiology practice. *Ann. Lab. Med.* **34**, 111–117. doi:10.3343/alm.2014.34.2.111

12. Fröding, I. *et al*. (2017) Rapid EUCAST disc diffusion testing of MDR *Escherichia coli* and *Klebsiella pneumoniae*: inhibition zones for extended spectrum cephalosporins can be reliably read after 6 h of incubation. *J. Antimicrob. Chemother.* **72**, 1094–1102.

13. Lainhart, W. and Burnham, C. (2018) Enhanced recovery of fastidious organisms from urine culture in the setting of total laboratory automation. *J. Clin. Microbiol.* **56**, JCM.00546-18. doi:10.1128/JCM.00546-18

14. Klein, S. *et al*. (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. doi:10.1007/s10096-018-3250-6

15. Perez, K.K. *et al*. (2014) Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic resistant gram-negative bacteremia. *J. Infect.* **69**, 216–225. doi:10.1016/j.jinf.2014.05.005

16. Vlek, A.L.M. *et al*. (2012) Direct MALDI TOF MS improves appropriateness of antibiotic treatment of bacteremia. *PLoS One* **7**, e32589. doi:10.1371/journal.pone.0032589

17. Delport, J.A. *et al*. (2017) MALDI-TOF short incubation identification from blood cultures is associated with reducded length of hospitalization and a decrease in bacteremia associated mortality. *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 1181–1186. doi:10.1007/s10096-017-2906-y

18. Beganovic, M. *et al*. (2017) Effect of MALDI-TOF MS alone versus combined with real-time antimicrobial stewardship interventions on time to optimal antimicrobial therapy in patients with positive blood cultures. *J. Clin. Microbiol.* **55**, 1437–1445. doi:10.1128/JCM.02245-16

19. Patel, T.S. *et al*. (2017) Cost analysis of implementing MALDI TOF MS plus real time antimicrobial stewardship intervention for blood stream infections. *J. Clin. Microbiol.* **55**, 60–67. doi:10.1128/JCM.01452-16

Biography

Dr Patrick R Murray received his PhD degree in Microbiology at UCLA, postgraduate training in Clinical Microbiology at the Mayo Clinic in Rochester MN, and was director of the Clinical Microbiology Laboratories at Barnes Hospital and Professor of Medicine and Pathology at Washington University from 1976–1999. In 1999 he joined the faculty of the University of Maryland School of Medicine and in 2001 he accepted the position of Senior Scientist and Chief of Microbiology at the National Institutes of Health. In July 2011 he retired from the NIH and accepted his current position at BD Diagnostics as Vice President, Worldwide Scientific Affairs. He is a fellow of the American Academy of Microbiology and the Infectious Disease Society of America, member of the CLSI Consensus Committee for Antimicrobial Susceptibility Test Standards, former Chairman of the American Board of Medical Microbiology, former Editor-in-Chief of the ASM Manual of Clinical Microbiology from 1990 to 2010, former Editor of the Journal of Clinical Microbiology, and currently serves on numerous editorial boards. He has authored more than 275 research articles and 20 books. He is the recipient of numerous awards including the ASM Award for Research in Clinical Microbiology (1993), ASM Award for Leadership in Clinical Microbiology (2002), ASM Founders Distinguished Service Award (2010), and ABMM/ABMLI Professional Recognition Award (2011), as well as the Pasteur Lifetime Achievement Award (2007), NIH Clinical Center Director's Awards for Patient Care (2006) and Research (2010), and NIH Director's Award for Research (2007).