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Automation in Clinical Microbiology

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In recent years, while automation has steadily spread throughout the clinical chemistry and clinical hematology areas of diagnostic laboratories, the clinical microbiology laboratory has largely been excluded from this trend. Although continuous monitoring blood culture systems, automated microbial identification and automated antimicrobial susceptibility testing systems are widely utilized in microbiology laboratories, microbiology specimen processing and culture workup, in particular, remain largely manual tasks, and indeed few changes to the methods used to perform these tasks have occurred for many years. While we acknowledge that some larger microbiology laboratories utilize urine-plating instrumentation, most microbiology laboratories have little to no automation in their specimen processing areas. With the exception of some laboratories in Western Europe, Australia, and the middle-eastern nations; still fewer laboratories have implemented some version of total laboratory automation (TLA).

Driven by a variety of factors, we believe that the level and degree of automation in the clinical microbiology laboratory is poised for a dramatic change. While it would probably be an overstatement to suggest that a tsunami of automation is sweeping towards the microbiology laboratory, we do believe it accurate to state that a "wave" of automation is coming to the microbiology laboratory and that this change will occur much more rapidly than most laboratorians may suspect, and, moreover, the changes associated with selection and implementation of microbiology automation solutions will place significant management and financial challenges upon laboratory leadership. Of the primary drivers of automation, standardization of identification methods to matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and the adoption of liquid microbiology transport have allowed microbiology laboratories to simplify collection and identification systems, creating a work-flow that can be optimized with automation

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For the purposes of this article, the use of the term automation in clinical microbiology laboratories excludes blood culture systems, automated microbial identification and automated antimicrobial susceptibility testing systems, and rather refers specifically to microbiology specimen processing instruments and microbiology TLA solutions.

In this article, we will review historical impediments for implementation of automation in the microbiology laboratory and discuss the reasons why we believe that attitudes towards automation are changing. In addition, we will review each of the currently available microbiology processing instruments and total microbiology automation solutions.

Historical Impediments to Automation in Microbiology.

Several real or perceived factors have contributed to the current dearth of automation in clinical microbiology labs. These include the ideas that: microbiology is too complex to automate; no machine can replace a human in the microbiology laboratory; automation is too expensive for microbiology laboratories; and microbiology laboratories are too small to automate.

Microbiology too complex to automate. In comparison to chemistry and hematology areas of the laboratory, where most specimens are blood or urine-based and utilize a limited selection of tube sizes, microbiology specimens are much more complex. Microbiology specimen types include blood, sterile body fluids, tissues, urine, catheter tips and other prosthetic devices, and lower respiratory tract specimens, among others. Moreover, microbiology specimens are collected and transported utilizing a wide variety of devices including urine transport tubes, varieties of swab collection devices, sterile containers for tissues, stool specimens, aspirates, and prosthetic material, lower respiratory tract collection devices, and more, not to mention the occasional Mason jar and dessert-topping container. An additional aspect of microbiology complexity is the variation in the manner in which specimens are processed. Specimens can be

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concentrated, macerated, digested, decontaminated, sonicated prior to plating or plated directly, and plating can be quantitative, semi-quantitative, or non-quantitative. A last aspect of the complexity of microbiology specimen processing is related to media. In additional to tubed media of various sizes, plates from different manufacturers vary in height and the geometry of the lids that some manufacturers utilize to facilitate plate stacking.

No machine can replace a human in the microbiology laboratory. A long standing mantra is that humans are generally considered capable of performing tasks faster than machines, and machines can't think. The perception has persisted that machines can't exercise the critical decision-making skills required to process microbiology specimens.Specifically, human observation of organism growth on agar plates is still considered essential by many. While machines are programmable, humans are more flexible.

Cost of automation. Automation has historically been considered too expensive for microbiology. It simply has not been viewed as cost-effective. Although justified for chemistry and hematology, the relative specimen and test volumes for microbiology are much lower, making automation seemingly less attractive.

Microbiology labs are too small for automation. Most microbiology laboratories have been considered to be too small for automation. This sentiment has been that, while automation may have a place in the very largest microbiology labs, it does not have a place in the "average-sized" laboratory. Because these labs are small, any automation would be underutilized

Winds of Change.

In our opinion, several driving forces have emerged that are changing attitudes about automation in microbiology laboratories. These relate to overall changes in the laboratory industry, growing shortages of trained personnel, declining reimbursement, a growing demand

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for improved quality, and two very important technological innovations-the introduction of liquid-based swab transport devices and the emergence of MALDI-TOF technology.

Industry changes. Changes in the industry are multiple. Overall testing volumes are increasing 10-15% per year, driven in part by an aging population, testing innovations, infection control demands, and the growing challenges placed by detection and identification of multi-drug resistant microorganisms. Consolidation of laboratories, particularly for microbiology testing, continues to increase. Larger laboratories have a greater potential to benefit from lab automation than smaller laboratories. The 24-7 microbiology laboratory is becoming much more common, and automation that can shorten turnaround time is being viewed more favorably. The 24-7 microbiology laboratory also allows cultures to be read following an appropriate incubation time, rather than waiting for the day shift, a scientifically unnecessary delay which can result in delays in turnaround time. Today, in most laboratories, plate reading is primarily a first shift activity. TLA will facilitate reading plates on other shifts as well. Lastly, from a relative perspective, reimbursement is declining and opportunities for enhanced reimbursement in the current health care environment are low.

Personnel shortages. Although recently stabilizing, shortages in trained microbiology technologists are an industry challenge (1). Fewer students are choosing medical technology as a career than occurred a generation ago. Moreover, the number of medical technology training programs has been dramatically declining with the number of graduates declining 50% between 1983 and 2008 (2). The pay for medical technologists is also substandard when compared to some other healthcare professionals. Each of these challenges has resulted in the mean age of the current workforce continuing to increase without sufficient replacement workers for those eligible for retirement.

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Quality issues. Demand by clinicians for new tests continue to grow, not just in total numbers but in the types (width and breath) of testing being performed, driven in part by the clinical utility of many of the newer molecular-based assays for the diagnosis of infectious diseases. The trend to decreasingly shorter lengths of stay for hospital inpatients places increased demand for more rapid turnaround time for infectious disease assays. While sometimes less expensive when performed by a reference laboratory, the longer turnaround time for the reference lab test result drives bringing some of this testing back to the hospital laboratory.

Another aspect of quality is the increasing importance placed on traceability for laboratory testing. Automated specimen processors and TLA solutions provide far greater traceability than when the same testing is performed manually.

Liquid-based microbiology. Traditionally, microbiology swabs have been transported in a device that was designed to keep the specimen associated with the swab during the transport period. The swab itself was used to inoculate media and prepare smears. A paradigm shift occurred with the introduction of liquid based swab transport devices, first with ESwab (Copan, Murietta, CA) and later with other similar products. With these products, the specimen is associated not with the swab but with the liquid phase of the transport device. The presence of the specimen in a liquid-based transport enables inoculation of the specimen and smear preparation with automated liquid-based specimen processors.

MALDI-TOF Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is transforming identification of microorganisms. The technology touts accurate, rapid, and inexpensive identification of microorganisms isolated from clinical specimens. MALDI-TOF procedures are highly amenable to automation because they are technically relatively simple and reproducible. Additionally spotting of target plates and

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138 extraction of proteins can be standardized for most organisms and, when combined with
139 automation, can be performed with minimal staffing.
140 To summarize the challenges currently being faced by microbiology laboratories, they are being
141 asked to perform more testing, both in volume and complexity, cope with increasing shortages of
142 trained microbiology technologists, and do all this in an economic climate where reimbursement
143 is not likely to keep pace with increasing costs.

144 **Requirements for Automation.**

145 For automation to be successful, it will need to be flexible in design, embrace the human element,
146 and adapt to the challenges of specimen diversity. Flexibility acknowledges that one size will not
147 fit all and incorporates an open, expandable architecture that can be adapted to a laboratory's
148 available space and potential future growth. Moreover, flexibility will also require that
149 automation systems embrace diversity of equipment manufacturers. Laboratories may select an
150 automation system from one vendor that best fits their needs while selecting analytical
151 instrumentation from a second and/or third vendor. The capability of integrating equipment from
152 these different manufacturers will be critical to microbiology TLA success.
153 Embracing the human element focuses microbiology technologists on the performance of the
154 most complex tasks, such as selecting colonies for further workup while removing these
155 personnel from tasks, such as plating, that can be performed by an instrument, operated by a less
156 trained individual. It is important to appreciate that automation does not remove decision making
157 for the microbiology technologist; rather, if facilitates decision making and eliminates wasteful
158 activities.
159 Microbiology must move as much as is practical to liquid-based transport devices to facilitate
160 automated plating. One way in which this can occur is replacing traditional wound swabs

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(Culturette-like) with liquid-based swab transports. For those specimen types that will never be in a liquid-based transport, the automated solutions must be able to accommodate the introduction of manually inoculated media into their systems.

In reviewing the current options available for automation in the microbiology laboratory, we have chosen to divide the automation solutions into two groups: instruments that primarily function as specimen processors and systems that offer total microbiology laboratory automation solutions. Tasks performed by processing instruments can include inoculation of tubes and plated media, subculture of broth cultures, plate streaking, plate labeling, bar coding for specimen tracking, and slide preparation. Total microbiology laboratory solutions generally include the functions of specimen processors and add modules to achieve varying degrees of total microbiology automation.

Microbiology Specimen Processors.

Historically, some laboratories, mainly large reference laboratories, have utilized specimen processing instruments, such as the Inoculab (Dynacon, Canada) (no longer being sold) for plating of urine specimens. Although limited in speed and functionality, the Inoculab was shown to plate specimens more reproducibly than manual plating (3).

The current generation of specimen processors has far more functionality than was found in instruments such as the Inoculab. The four currently available specimen processors are (listed in alphabetical order by manufacturer): Innova (BD Diagnostics, Sparks, MD); InoqulA FA/MI (BD Kiestra B.V., Drachten, Netherlands); PREVI Isola (bioMerieux, Inc., Hazelwood, MO); and WASP (Copan Diagnostics, Murietta, CA). Each of the 4 instruments is capable of automating the processing a variety of liquid-based specimens.

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Innova. The Innova instrument has 5 specimen drawers with each holding up to 40 containers for a maximum capacity of 200 containers (Figure 1). Specimens can be added as they arrive in lab (bar-coded, lid/cap intact). Innova uses a universal decapper that decaps/recaps different sized containers without any manual adjustment. A drawer can only hold a single sized tube at any one time. There are 6 input stacks with a capacity of 45 plates each (270 plates total). Different agar (including bi-plates) can be loaded into each stack or all stacks can hold same type of agar. The Innova includes a full library of traditional streaking patterns, streaked plates are ejected into an output carousel (5 stacks), and can be organized in output stacks by groups so that no sorting is required after streaking. The Innova utilizes reusable 1, 10, 30 µL ni-chrome loops. No disposable supplies are required for specimen plating with the Innova.

InoqulA FA/MI. The InoqulA. FA/MI (Full Automation/Manual Interaction) can be utilized for automated inoculation of liquid specimens and manual plating of other types of specimens (such as wound swabs) as well as for slide preparation (Figure 2). The streaking process is performed using a magnetic rolling bead and up to 5 inoculated plates can be struck out at one time, yielding a throughput of up to 400 plates/h. The instrument holds up to 30 types of plated (including biplates) and 7 types of tubed media. Inoculated plated media can be sorted in up to 4 different cassettes for different atmospheres of incubation. The manual interaction section of the InoqulA FA/MI permits manual inoculation of non-liquid specimens such as catheter tips and wound swabs. Once inoculated, these manually inoculated specimens are struck out with magnetic beads as occurs with liquid-based specimens. A disposable pipette is required for each liquid-based specimen.

Studies have been performed assessing the InoqulA inoculation performance. Kleefstra et al reported that InoqulA produced more isolated colonies than manual plating while also showing

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good reproducibility (4). Rydback et al also reported more isolated colonies with the InoqulA than were obtained with manual plating while also noting significant variation in results between technicians for manual plating (5). Sturm et al reported similar numbers of isolated colonies using InoqulA and manual plating (6).

PREVI Isola. The PREVI Isola instrument has 5 different size racks, one size for each of 5 different diameter specimen tubes (Figure 3). All specimens must be uncapped before being placed on the instrument. There are 5 input cassettes with a capacity of 30 plates each stack (150 plates total). Different agar plates (including bi-plates) can be loaded into each stack or all stacks can hold same type of agar. Streaked plates are ejected into output cassettes (3 stacks, 30 plates each) and can be organized by groups so that no sorting is required after streaking. Two different specimen volumes can be inoculated based on plating protocols. A disposable pipette is required for each specimen and a disposable applicator is required for each plate. The applicator produces a unique radial comb streak pattern, and there are no other streaking-pattern options. Maximum capacity is 180 plates/hour.

Studies have been performed assessing the PREVI Isola inoculation performance. Chapin et al reported a 54% decrease in hands-on-time for Isola compared to manual planting ($p<0.0001$) and that samples with 2 to 3 different organisms were statistically more likely to be properly isolated with the Isola (7). Andrea et al reported that plating only urine and pre-processed stool specimens would result in an approximate savings of $20,000 per year in their laboratory (8). Utilizing feces diluted in saline, Zimmerman and Trampe reported that Isola reduced processing time compared to manual culture while the suitability of Isola and manual plating were judged to be superior or equivalent for 52% and 6% of specimens, respectively (9). Mischnik et al evaluated the performance of Isola on wound specimens using polyurethane swabs in liquid Amies medium

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in comparison to manually plated wound viscose swabs in Amies medium. They reported that the quality of colony growth on culture media for further investigations was superior with Isola inoculated plates compared to manual plating techniques (10).

WASP. The WASP (Walk Away Specimen processor) utilizes specimen load and unload conveyors with different sized pallets for different diameter tubes (Figure 4). It uses a universal decapper that decaps/recaps different sized containers without any manual adjustment. There are 9 media silos with a total capacity of 342-370 plates (including bi-plates). Each silo can hold a single type of media or multiple silos can be used for a single media type. The WASP utilizes two Toshiba selective compliant assembly robot arm (SCARA) robots to move specimens and plates. It includes a full library of streaking patterns, and streaked plates can be organized by groups so that no sorting is required after streaking. Two separate cultures can be inoculated to one-half each of a plate and then separately labeled, a practice that is very cost-effective for epidemiological screening cultures. Inoculated plates can be labeled on the side or bottom of the plate. The WASP utilizes reusable 1, 10, 30 µL ni-chrome loops with an automatic loop changer. No disposable supplies are required for specimen plating with the WASP. An optional Gram SlidePrep module is available for slide preparation.

Bourbeau and Swartz evaluated the performance characteristics of the WASP (11). They determined that no cross-contamination occurs during plating of urine transport tubes and ESwabs. They also demonstrated that subculture of LIM broth tubes by the WASP produced results identical to those produced by manual subculture. Lastly, they demonstrated that plating of urine transport tubes by the WASP is highly reproducible (11). Jones et al demonstrated increased detection of *Staphylococcus aureus* nasal colonization using ESwabs plated with the WASP in comparison to manually inoculated wound fiber swabs (12).

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252 The factors to consider in the selection of a microbiology specimen processing instrument were
253 reviewed by Greub and Prod'hom (13). They recommended that the following factors be
254 included in the selection of a particular specimen processing platform: accuracy; capacity;
255 manufacturer's technical support; flexibility (specimen types, loops, inoculation protocols, media
256 options, LIS issues); capacity; flexibility; modularity; and costs (initial, any required disposable
257 supplies, and operational labor).

258 **Microbiology Total Lab Automation (TLA) Solutions**

259 There are currently 3 microbiology TLA solutions in use or in development (listed in
260 alphabetical order by manufacturer): Kiestra TLA (BD Kiestra B.V., Drachten, Netherlands);
261 FMLA® (bioMerieux, Inc., La Balme, France); WASPLab(Copan Diagnostics, Murietta, CA).
262 Certain common elements exist or are envisioned for all 3 systems. These include conveyor/track
263 systems to move plates to and from incubators, digital cameras to capture plate images at
264 specified intervals, automated incubators with digital reading stations, and proprietary software
265 to facilitate these processes. They utilize various versions of computer-driven robotic plate
266 management to automate specimen processing and workup.
267 By adding TLA to automated specimen processing, significant additional benefits can accrue
268 for the microbiology laboratory. Because media is not sitting on a workbench waiting to be read,
269 there is continuous incubation of plated media, rather than intermittent periods of incubation as
270 traditionally occurs in microbiology laboratories. Plate reading can be performed when
271 incubation is adequate on a plate, and is not tied to a traditional lab work schedule. When plates
272 are required for workup, they can be efficiently retrieved, obviating the need to handle multiple
273 stacks of plates.

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Plate image records are retained which facilitates review of growth over time, irrespective of the number of technologists who may work on the culture. With stored image analysis, microbiologists have the ability to compare a patient's culture history, both over time and between different specimens. Lastly, this workflow will facilitate improvement in the quality of supervisory culture review and enhance training of new technologists.

Kiestra TLA. The Kiestra TLA (Total Lab Automation) system was first installed in a clinical microbiology laboratory in 2006, with a total of 38 installations to date (Figure 5). The Kiestra TLA system is comprised of distinct modules linked together by a conveyor/track system which can be combined in various combinations to create the full TLA system. These modules include the SorterA, BarcodA and InoqulA TLA (the specimen processing and streaking modules) (refer to the Specimen Processor Section for more detail on the InoqulA) the ReadA (incubators with digital imaging equipment) and ErgonomicA (workbenches). In 2013, BD Kiestra plans to introduce a new incubator model which will be called ReadA Compact to replace the current ReadA incubators. The open architecture of the Kiestra TLA permits laboratories to use various numbers of ReadA incubators (CO2 or non-CO2), and SorterA, BarcodA and InoqulA instruments depending upon total specimen volumes. Future planned enhancements to the Kiestra TLA system include instrumentation to automate microbial identification and antimicrobial susceptibility testing utiilizing: Automatic Colony Picking by the MalditofA® combined with Bruker's MALDIBiotyper®.

In assessing the impact of Kiestra TLA in their laboratory, Bentley et al reported a reduced culture turnaround time and an increase in the laboratory production index (LPI) (samples/staff member/day) from 37.35 pre-Kiestra implementation to 75.90 post-Kiestra implementation (2.03

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296 fold increase) (14). In another laboratory, Humphrey et al reported a 2.6 fold increase in their
297 LPI following the introduction of the Kiestra TLA (15).

298 **FMLA®**. The bioMerieux FMLA® (Full Microbiology Lab Automation) system is currently
299 under development (Figure 6). Components of the FMLA system include the PREVI Isola (refer
300 to the Specimen Processor Section for more detail on the PREVI Isola) and the SIS (Smart
301 Incubator System) linked together by a conveyor/track system. The SIS will be available in CO2
302 and non-CO2 atmospheres and include image analyzers. A key component of the FMLA is
303 MYLA software, a microbiology middleware solution, which links together FMLA components
304 while integrating various information systems and microbiology instruments. An eventual goal
305 of bioMerieux is to integrate Vitek MS (MALDI-TOF instrument) into the FMLA system while
306 automating the preparation of colonies/suspensions required for antimicrobial susceptibility
307 testing and the Vitek MS.

308 **WASPLab**. The WASPLab was first installed in a clinical laboratory in 2012 (Figure 7). The
309 components of the WASPLab system include the WASP (refer to the Specimen Processor
310 Section for more detail on the WASP) and CO2 and non-CO2 incubators, linked together by a
311 conveyor-track system and middleware. Similar to the FMLA incubators and the ReadA
312 Compact, the WASP Lab features incubators that assign each plate a unique address or "shelf".
313 Because each plate has an individual location, technologists operating the system can request
314 the instrument to send the plates for manual review with little delay. Each incubator also
315 includes an image acquisition station that captures plate images using a variety of light sources
316 and at a variety of angles at programmable time intervals. Plates with detectable growth can be
317 reloaded on the WASP where automated broth inoculation and Kirby-Bauer disk dispensing can

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be performed. The WASP instrument can be modified to permit MALDI-TOF Target Plate Seeding with either the Bruker MALDI-TOF plate or the bioMerieux Vitek MS plate.

The WASP lab can also be connected to an Inpeco (Switzerland) sorting station and track which will sort chemistry, hematology and microbiology tube specimens based on the appropriate test. The Inpeco track will connect all sections of the laboratory to a single distribution system which routes specimen containers to the appropriate laboratory section.

Microbiology Automation Research Needs

The scientific literature assessing the benefits of microbiology automation is sparse. As noted by the references for this article, there are few peer-reviewed publications, with most of the presentations in abstract form. While the benefits of microbiology automation can often be inferred, well-performed studies are needed to accurately assess the financial, operational, and clinical impacts of incremental or total laboratory automation in the microbiology laboratory.

While there is evidence that automated processing instruments produce more isolated colonies than manual plating, there remain questions to be answered related to the downstream benefit of having more isolated colonies on the primary culture plates. What is the measureable impact on labor and supplies for culture workup? What is the effect on the time to organism identification and antimicrobial susceptibility testing result? Is there an effect on the time to final reporting of the culture?

There have been limited studies examining productivity increases following implementation of total microbiology laboratory automation. Additional studies are warranted to assess potential benefits in different types and sizes of laboratories. More complex questions can be raised

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regarding the benefits of total microbiology laboratory automation. What is the measureable impact of TLA on labor and supplies for culture workup? What is the effect on the time to organism identification and antimicrobial susceptibility testing result? What is the effect on the time to final reporting of the culture? What is the impact of earlier examination of plates because the images indicate there is sufficient growth for ID/ASTs?

Lastly and perhaps most importantly, studies are needed to assess the clinical impact of what, we assume, will be more rapid organism identifications and more rapid antimicrobial susceptibility test results. How will patient care be impacted by faster test results? It is reasonable to assume that the clinical benefits will vary depending upon the patient population assessed, inpatient vs. outpatient, intensive care unit patient vs. non-intensive care unit inpatients. Consequently, we envision that a series of studies will be required to properly assess outcome measures in varying situations.

In summary, we believe that we are entering an age of monumental change for the clinical microbiology laboratory. While a precise assessment of full impact of these changes is in its infancy, there is no doubt in our minds that the benefits of automation on laboratory efficiency and indirectly on clinical care will be profound.

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408 Figure 1: Innova

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