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Automated Diagnostic Techniques in Medical Microbiology

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Chapter 1
Desires Objectives, Purposes of Automation in Clinical Microbiology

Vikramjeet Singh and Jyotsna Agarwal

Abstract The technology which process microbiology samples without human assistance and better ergonomics is termed as automation. We are experiencing a new revolution of artificial intelligence via automation. Globally, researchers and microbiologists are upgrading their laboratories from conventional methods to total laboratory automation considering the increased awareness about impact of infectious diseases, rising sample load and lack of expertise of skilled manpower resources. This chapter discusses various purposes and defined objectives of automation.

Keywords Automation · Artificial intelligence · Ergonomics · Turnaround time · Quality improvement

1.1 Introduction

Infectious diseases are considered the top global killer in all age groups of the population. Early clinical diagnosis and prompt treatment can save many lives. In the past 5–7 years, conventional manual processes used for culturing samples from bacterial infections have been observed to be time taking and clinicians lose pivotal time duration to reach correct diagnostic decisions. Decade's old manual methods are now being replaced by automated laboratory methods for prompt identification and culturing of infectious agents. Usually, routine diagnostics methods on average can take anywhere from 48 to 96 hours to final reporting of pathogens and their susceptibility testing. This delay can lead to incorrect and inappropriate empirical antibiotic therapy for a longer duration, prevention of early targeted antimicrobial therapy, and generating a pool of multidrug-resistant pathogens leading to therapeutic failure. The question arises, what has taken so long for the laboratories belonging

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to clinical microbiology to progress towards automation in clinical microbiology? Truly speaking, change from traditional to automation technique faces hard resistance due to the complexity of testing, increase initial cost in set up, and the need for the human acceptance and training of workforce. However, as time has progressed in recent years, there is a revolution for automation in clinical microbiology. In recent years, laboratory automation is progressively replaced many traditional processes, causing reduced turnaround time for bacterial and fungal culture and identification, ease of laboratory work, and helping in effective therapeutic options. Frankly, considering automation has been long used throughout the clinical chemistry and clinical hematology areas of diagnostic laboratories, the same pace for the upgradation or automation of clinical microbiology laboratories has yet to be achieved. Clinical samples related to medical microbiology are much more complicated than samples received in chemistry and hematology. Automation in medical microbiology needs a better understanding of normal human flora, commensals, and pathogens [1]. The newer aspects in microbiology laboratory automation wish to focus on and address the problems faced by patients and clinicians regarding testing time and they demand less turnaround time for prompt decisions for the practice of clinical evidence-based medicine in order to save as many lives. Artificial intelligence (AI) enhances the quick and quality analysis along with accuracy and avoidance of error and introduces a level of automation examples involve microscopy of wet mounts and stained smears, colony counting, and microbial characterization and matching – each of these parameters are based on improvements in image analysis by automated artificial intelligence. These emerging AI tools have tremendous potential to reshape various aspects of rapid microbiology within the healthcare sector for patient benefit [2].

1.2 Desires and Objectives of Automation

Automation in microbiology is not simply using big machines and automating the manual conventional methods of clinical specimen processing and then incubating those processed cultures. On a global vision, automation should be a multilevel approach working at the same time to involve processing samples which includes rapid identification, prompt decision on antimicrobial susceptibility, and of utmost importance is patient care with a decrease in turnaround time and communicating information. For this reason, automation was claimed as “a paradigm shift in clinical microbiology, representing the beginning of the future” [2, 3].

Automation in clinical microbiology is like a system that aims to refine the quality of work, reduce the turnaround time and cost, efficiently manage increasing sample load, and compensate for the reduction in skilled manpower resources. When the sample load keeps constantly rising in our laboratory and to counter it we hire manpower resources to meet the demand. However, if there is a lack of trained,

knowledgeable, and qualified staff with issues of increased sample load, maybe it's high time to think about microbiology laboratory automation in order to maximize the potential and efficiency of the workforce for quality control results. Also, the crowding of instruments of diagnostic tests in microbiology laboratories poses great safety risks for the working staff many times when infections and communicable pathogens are increasing. Increased sample loads can be countered by high-throughput culture, isolation, and screening automated procedures which allow a parallel and cost-efficient processing of a large number of samples in less time. Today the inception of total laboratory automation in medical microbiology is providing an innovative approach from receiving of clinical samples to the development and transmission of the final patient report [3, 4].

Laboratory automation provides safety to working technicians/residents but also provides a better ergonomic environment to them, which is one of the core objectives of automation. The better working efficiency and traceability of specimens with automation are unmatched. With the advent of laboratory automation, prompt processing of specimens can be performed without delay, once received in the laboratory, digital barcode imaging utilization can help in the selection of appropriate media for individual specimens, culture plate labelling, and finally inoculation and incubation of the culture media can be done simultaneously. Automated calibrated pipettes can increase the accuracy of specimen inoculation and in case of high sample load automated streaking of multiple culture plates can be done simultaneously in less time.

Another objective of automation is the rapid detection of emerging diseases as these diseases produce great challenges to the medical profession, which we have seen in the COVID-19 Pandemic. Many opportunistic emerging and re-emerging infections demand the necessity for many progressive and rapid innovations in automated and molecular methods. Hospital inpatients demand shorter lengths of stay. For this reason, clinicians and patient attendants raise the demand for rapid tests with less turnaround time for clinical microbiology assays, thereby improving prompt patient care and early discharge from the hospital. In life-threatening conditions like sepsis, meningitis, etc. it is an utmost priority to save many and culture the specimens at the earliest possible as every hour of delay accounts for a faster approach towards the death of suffering patients and the necessities of automation [5].

1.3 Purposes and Benefits of Automation in Clinical Microbiology

Major benefits of automation in clinical microbiology are improved productivity and efficient workforce, consistency and uniformity across the system, and calibration of test methods leading to improved quality of laboratory testing [5, 6].

1.3.1 Efficiency

In a microbiology lab, medical residents/laboratory technicians dedicate maximum part of their daily work hours doing repetitive work at frequent intervals that can be automated, such as manual sorting of the culture media plates depending on the sample to be processed, transferring the plates to oven and incubator, uniform streaking of culture media plates, and performing AST (antimicrobial susceptibility test). With the involvement of automation methods, a larger number of samples can be processed at the same time without increasing staff and without any delay. Duties like reading processed culture plates with growth and interpreting AST require the expertise of a medical microbiologist. So if automated machines are continuously doing the work of moving and sorting the plates around, doing streaking, and performing minimum inhibitory concentration-based AST testing, the medical microbiologists can focus on other important things. The use of conveyor systems and rolling belt-based systems helps in the downward sorting of samples after processing in a much more precise manner for identification and AST methods. Also, accurate and consistent test results in terms of quality and quantity are ensured by electronic validation and authentication, which is affected by variations in technicians performing the test [5, 7].

1.3.2 Advantage of Uninterrupted Incubation Period

In traditional microbiology laboratory workflow, positive growth culture plates spend a duration of 2–3 h outside on the working table of the laboratory (for observing and reading colonies, gram staining of colonies, biochemical identification, AST processing, etc.) along with the continuous opening and closing of incubator doors many times. All of these events drastically affect the recovery of microorganisms, especially the fastidious ones or slow growers. Also increases the chances of contamination in such culture plates. In automated machines, plates are uninterruptedly incubated in an optimal atmosphere with a continuous electrical supply. In automated machines when plates are viewed on high-resolution displays and monitors, different types of colonies won't be missed and identified promptly for necessary decision-making regarding antimicrobials to be administered. The uninterrupted incubation in automated systems also promotes the growth of fastidious organisms at a much faster rate than traditional methods [6].

1.3.3 Reduction in Turnaround Time (TAT)

Earlier with the traditional method it took a minimum 2 days for the growth of fastidious organisms in a specialized environment and then a further 2 days more for AST testing. These fastidious organisms are prone for causing severe infections like pneumonia, meningitis, etc. However, with the inclusion of automation, the turnaround time decreases by almost 48 h in the case of these organisms. This is possible

due to uninterrupted incubation, which helps reduce TAT to final reports leading to patient healthcare benefits. These reduced TATs can play a pivotal role in life-threatening infections like sepsis, pneumonia and meningitis which can be promptly treated with appropriate antibiotics with correct dosing and frequency. This further benefits the patient by decreasing the duration of hospital stay and reducing costs [7].

1.3.4 Quality

Automation minimizes many types of human-based errors (such as improper labeling of the plates, error during identification, preparing incorrect inoculum for AST) and increases the efficiency and quality of the test results. The use of a barcoding system for different sample plates and procedures helps in moving and sorting through the system and less chance of mistakes or missing [8].

1.3.5 Uniformity in Work

For example, when it comes to the streaking of specimens on culture media, we face a lot of variation in streaking patterns from one technician to another. Automation processes dealing with the streaking of samples are done in a very uniform manner. Many automated systems use the rolling bead-based method for streaking the sample. Studies claim that the bead-based rolling method generates more isolated colonies than loop or wire-based methods and reduces the need for sub-culturing [7–9]. Another innovative method that is escalating nowadays in automation is digital imaging in microbiology. Digital imaging helps the laboratory determine at what intervals the culture plates are photographed. The photographic positive culture plates such as ID or AST can be labelled on-screen for processing. Manual processing can be greatly reduced with the involvement of automated digital imaging in workflow. This facilitates early growth detection of microorganisms and shortens the time of identification. All samples from one patient, e.g., urine, blood, and sputum, including multidrug-resistant organism screening, can be evaluated simultaneously with computer-assisted processing and the same result can be communicated to clinicians working at the patient's bedside for better patient outcomes. Lastly, images can be archived or stored as backup and later used for training students/technicians or quality assessment or control programs [6, 9].

1.3.6 Accentuates Teaching Program

Automation in clinical microbiology can help to accelerate the teaching program. Students can observe record and compare digitally imaged and archived culture plates and interpret how the colonies changed from their initial appearance. A

plethora of available images, records, and information will help in the teaching and benefit the learning process [7, 8].

1.3.7 Increase Quality, Reduced Error and Cost Efficiency

Automated instruments like MALDITOF bring quality identification of exotic organisms for example most frequently Gram-positive organism responsible for bloodstream infections is Staphylococcus aureus followed by Coagulase-negative Staphylococcus species (CoNS), however, with the addition of MALDI-TOF, now CoNS can be further identified till species level and helps in differentiation between commensals and actual pathogens. This can also lead to rapid microbiological methods that can help to overcome tedious and time-consuming conventional biochemical processes and error-prone and precision processes. Similarly instruments with artificial intelligence technology will alter the diagnostic landscape of medical microbiology. AI presently focuses on the following algorithms which includes chromogenic detection, morphological detection, and separate colony counting and characters. The establishment of these automated instruments might look expensive approach but down the line the advantage it provides and a number of tests performed in rapid time don't always add on to the cost [10, 11], (Fig. 1.1).

Fig. 1.1 Importance of Automation in Microbiology

1.4 Limitations of Automation in Clinical Microbiology

1.4.1 Not for all Types of Samples

Automation systems are generally most efficient in handling liquid specimens such as urine, blood, pleural fluid, etc. Precious samples requiring specialized processing methods, such as grinding and cutting of organ or tissue biopsies, are not easily incorporated by an automatic system. However, the use of liquid-based specimen transport systems which can dissolve the hard structures and maintains the viability of microorganisms can efficiently changes a very high volume sample, the swab, into a liquid specimen that can be managed easily with an automated processor [11].

1.4.2 It May Not Be for Labs of all Sizes

Small laboratories may fail to allocate the initial investment required for the setup of automation in the microbiology laboratory. The benefits of automation for small to medium sample laboratories in the long run should not be outweighed by increased expenditure for reagent supplies, space requirements, and infrastructure constraints [9–11].

1.4.3 Consequences if the System Fails

One of utmost importance is that support and maintenance of automated instruments are essential and manufacturers should provide timely servicing and care of instruments and solve technical complaints within a few hours. The unavailability of spare parts, reagents, and cartridges and the lack of efficient and timely availability of technical experts to fix the issues may prone the laboratory facility for dysfunctional capacity. To counter major failure automation situations, the laboratories should retain a minimal backup laboratory setup such as conventional incubators and traditional test systems [12, 13].

1.4.4 Dependency on Machines

Automation definitely raises the dependency of skilled person resources relying upon instruments for even minutes of work. This complete dependency on automated methods may lead to loss of the trained technical skills of the important procedures in the long run. This dependency creates problems during the processing of urgent samples, which will critically require shifting to traditional methods. The

Table 1.1 Pros and Cons of Automation

Advantages	Disadvantages
1. Liquid samples like urine, blood and body fluids are processed efficiently	1. Standardization requires for organ and tissue biopsy samples processing
2. Decreased turnaround time	2. Dependency on machines, any outrage or failure can shutdown automated laboratory work
3. No expertise needed	3. Evolving techniques can be complex for less experienced and skilled person
4. Uniformity in work	4. Non uniformity can be experienced from small lab to tertiary lab

decreased accuracy in processing of clinical microbiology samples in critical periods may jeopardize the patients' health [13] (Table 1.1).

1.5 Conclusion: Looking to and at the Future

In India, Automation in clinical laboratories is attaining the right pace, presently 25–30% of laboratories (government and private) have a good setup for automation. In future, automation will play a pivotal role by becoming more organized, user-friendly, acceptability to various samples and consolidated as small and independent laboratories become part in the hub-and-spoke model of the central and automated laboratories at every level, for better patient care by providing ergonomically based setups, efficient reporting and effective management of patients at the earliest possible. Automation is the only solution in an era when laboratories have to reduce costs, improve efficiency and quality, prompt and accurate results to inform patient management with less turnaround time. Truly the future of clinical microbiology lies in total laboratory automation.

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Chapter 2
Common Techniques Used for Automated Diagnosis in Medical Microbiology

Tanushri Chatterji, Namrata Khanna, and Tanya Bhagat

Abstract Several manual activities have undergone significant changes by introduction of automated techniques, which have unquestionably improved performance. Various manual tasks have now been partially or entirely replaced by automated and labour-saving instrumentation in clinical laboratories as a result of the widespread and multifaceted advancements in automation technologies. The diagnostic techniques employed in infectious diseases should be rapid, precise, simple and cost-effective. A rapid diagnosis can be crucial in initiating appropriate antibiotic treatment. Increased rate of serious infections by multidrug resistant bacteria, lead to a high incidence of error in antibiotic treatment.

Current chapter elaborates several semi-automated and totally automated techniques, which have been developed for microbiology clinical laboratories.

Keywords Automation · Laboratory diagnosis · Multi-drug resistance Spectroscopy · Genome sequencing · Mass spectrometry

2.1 Introduction

The term automation is defined as the introduction of machines and equipment which speed up the process, lowering errors and reducing human labour also. In medical field automation is implemented in the laboratory of clinical microbiology, pathology, haematology for diagnostic purposes. For the purpose of diagnosis automation has been employed for blood culturing, antibacterial sensitivity testing,

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identification of microoganisms, hormone level determination, haematological analysis etc.

Numerous human activities have undergone significant variation due to automation, which has unquestionably improved system performance [1]. Various manual tasks have now been partially or entirely replaced by automated and labour-saving instrumentation in clinical laboratories as a result of the widespread and multifaceted advancements in automation technologies [2, 3]. The journey of laboratory automation is usually classified according to the complexity of instrument integration, ranging from no automation, partial laboratory automation up to total laboratory automation (TLA) Fig. 2.1.

Over the generations the automation process has speeded up, by adapting autosampler, auto processor and autoanalyzer which deal directly with patient's samples. Furthermore, these were classified into chemical and immunoassays, according to the analyses of these clinical samples. More inclination towards automation contributes in pre-analyses (identification and distinction of samples, centrifugation) as well as post-analyses, which includes storage of samples as an archive for future uses. In this context, different stages of these tests and processes are combined and defined as "total laboratory automation", which is interlinked among the various processes from initiation to end via tracking systems.

The transition towards automation:

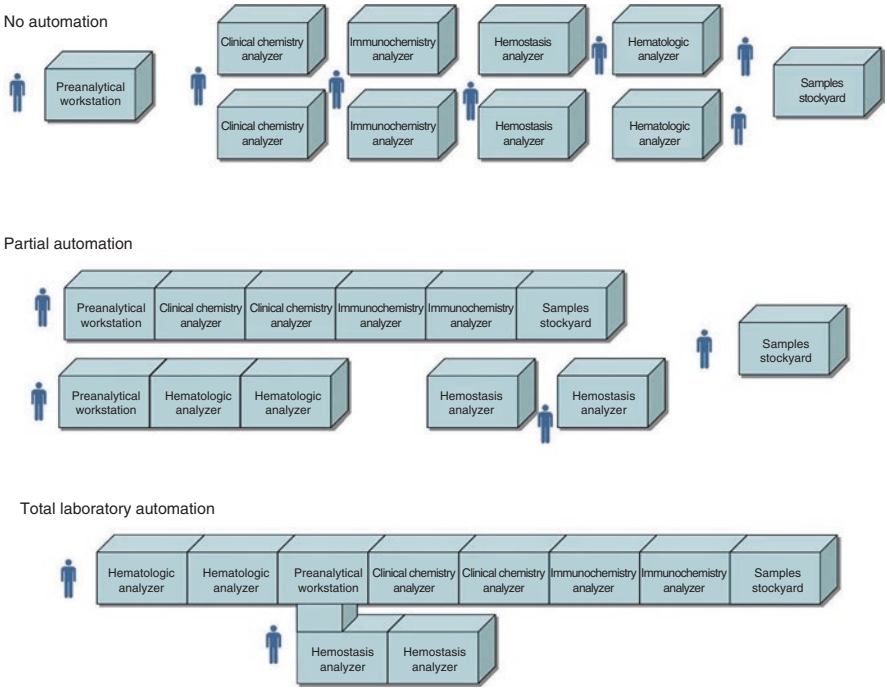

Fig. 2.1 Transition levels of Automation in Medical Microbiology [4]

- Proves cost effective over a long period
- Reduces laboratory congestion
- Improves efficiency
- Improves traceability and sample management (such as repeat, reflex, and add-on testing).
- Enhances accreditation/certification standards.
- Promotes testing quality.
- Requires minimal sample volume.
- Provides greater integration of test results efficiency.
- Reduces biological risks for operators.
- Improves staff requalification and provides job satisfaction.

The conventional diagnostic techniques used as microbiological assays are microscopy, culture and sensitivity, serology and PCR (polymerase chain reaction). Manually performed culture and sensitivity requires 48–72 h time, whereas technical advancement reduces the time to a few hours. In case of PCR, typical primers fail to detect mutations occurring in the genome of an organism, responsible for major genetic disorders, cross reaction and viable count of the pathogens. Their application lacks high sensitivity, rapid culture of viable organisms and accurate identification of microorganisms leading to false reports. The vitality of commonly used automated techniques contributes towards various epidemiological studies, reveals novel uncharacterised and typical microbial species, detects infectious organisms in a short span of time. Therefore, automation in clinical microbiology has become need of the hour for rapid, accurate and better patient management. Thus, transition of traditional diagnostic techniques towards application of automated techniques is being globally accepted [5, 6].

2.1.1 Autoanalyzers

They are broadly classified into the following types:

Semi-automated: These are partially automated i.e. addition of sample and reagents is done manually and the remaining functions like absorbance reading, result analyses, diagnosis etc. are automated. Semi-automated autoanalyzers include Polymerase Chain Reaction (PCR) in differentiating serogroups or serotypes of some bacterial infections, Pulse Field Gel Electrophoresis (PFGE) for analyses of bacterial and viral infections etc.

Automated: These are complete autoanalyzers which facilitate less human interference in a high-throughput mode. They aid in handling the clinical samples, initiating with sample preparation, addition of reagents, diagnosis and finally interpretation of the results, all being processed in an automated manner. Total automation involves the following techniques:

- Flowcytometry for urine analysis
- Identification of bacterial strains mycobacterium strains through BACTEC and VITEK respectively

- Sequencing of Microorganisms
- Automated Fluorescent capillary electrophoresis system
- Automated immunoassays

2.2 Types of Autoanalyzers in Microbiology

Automation in specimen handling and processing is being widely used through InocuLAB (Dynacon, Canada). This equipment performs spread plating of biofluids like urine samples. This provides feasibility in a reproducible manner, avoiding manual labour but lacks in speed and functionality.

2.2.1 Innova Specimen Processor

Currently it is in use for processing varied types of biofluids. This processor facilitates streaking method in environmental friendly manner as there is nothing in it to dispose. It provides dispersion of sample for streaking with variables ranging in 1, 10 and 30 μL of nichrome loops. The processor comprises of 5 sample holding drawers with 6 input stacks which contain 40–200 containers, with a capacity of holding 270 agar plates or other media plates. The streaked plates are ejected and placed in another stack or output carousel.

2.2.2 Radial Comb Streak Pattern

It is quite similar to Innova Specimen processor but varies in capacity of processing the plates i.e.; 180 plates/h. It also possesses conveyor belt with decapper (decap or recap) for loading and unloading of sample tubes with different size ranges.

2.3 Automated Characterization of Multidrug Resistant Microorganisms

Antibiotics are defined as the biochemical compounds that are produced by microorganisms that inhibit the growth of other microbes which act in a very selective way, contrary to other forms of microbial species. The various mechanisms for resisting the effect of antibiotics, as exhibited by these microbes are:

Intrinsic- If the microorganism shows resistance to an antibiotic even before it has been exposed to it, then this phenomenon is known as intrinsic resistance.

Acquired- In this case the microbe has been previously treated with an antibiotic and was responsive, but later it attained resistance to that particular antibiotic. This resistance is being developed due to gene transfer mechanisms (conjugation, transformation or transduction) or mutations.

Co-resistance and cross-resistance mechanisms have aggravated multidrug resistance against various microbial infections [7].

2.3.1 Types of Antibiotic Sensitivity Tests

Bacteria are the most commonly found pathogens causing infection. Therefore, in order to treat these bacterial infections antimicrobial susceptibility testing (AST) is performed in clinical laboratories to figure out effective antimicrobial regimen for treating the patients. The sensitivity and resistance pattern variates due to mutations in the genetic material of bacteria [8, 9]. These strategies include the Kirby-Bauer disk diffusion method and minimum inhibitory concentration (MIC) methods. It is a usual practice to assess the antibiotic sensitivity of bacteria using disc diffusion in agar. The diameter of the zone that inhibits culture growth determines whether an organism is sensitive or resistant, and this dimension is statistically connected with the lowest inhibitory concentration (MIC). Though the process is time consuming and lacks accuracy at times [10]. All laboratories must be meticulous during each stage of the sampling and test process, regardless of the variations in susceptibility test procedures.

In order to get test findings with consistently high levels of accuracy and reliability, the scientists and clinicians opted for automation in identification of bacteria and their antibiotic susceptibility through VITEK 2 Compact [11, 12].

2.3.1.1 VITEK-2 Compact

This automated technique is being widely used for the identification and susceptibility testing of the pathogenic bacteria responsible for causing urine infection, blood infection and food infection etc. Out of other similar systems VITEK system (bioMérieux-Vitek, Hazelwood, Mo.) is noted for accuracy of identification and susceptibility of the bacteria. Initially it was implemented on astronauts for the detection of urinary tract pathogens. From 1979 onwards, it was practiced clinically in laboratories [13, 14]. The working principle is based on metabolic alterations by fluorescence-based approach, which enables identification of pathogenic bacteria within 3 hours. The technique scrutinizes the bacterial growth kinetics and computes MICs through a distinctive algorithm. The upgraded version of the VITEK-2 Compact is technically improvised which automates many procedures that were previously performed with human labour [14]. The workflow of the system is depicted in Fig. 2.2 [15].