AUTOMATION IN MICROBIOLOGY

Abstract

Automation is a process where a machine or equipment can function without human interference or minimal human interference. Microbiology is one of the branches which are labor intensive. Automated techniques in a laboratory have changed the pathways for microbiologists to detect pathogenic organisms. It has influenced all the branches of clinical microbiology: bacteriology, virology, mycology, and parasitology. With automation, a large amount of manual labor involved in processing bacteriological samples is removed. On the other hand, appropriately trained staff are required to operate automated machines. The laboratory's workflow is changed by allowing continuous flow processing instead of batch processing. This improves the quality of a laboratory. The manual errors linked with different batches are reduced as the machines work in a steady and consistent manner. The turnaround time of the laboratory is also reduced providing better patient and healthcare services. The time to report a negative report in addition to a positive one, is also significantly reduced which is equally important for the patient. All these benefits come along with a heavy financial burden for the healthcare system. This is one of the major drawbacks of the automated systems. The advantages of automated systems strongly outweigh the disadvantages associated with them; hence, they are being implemented more and more in the healthcare system.

Keywords: Automation, Clinical Microbiology, Improved Quality, Reduced Turnaround Time

Author

Dr. Ankita Mohanty
Senior Resident
Department of Microbiology
JIPMER, Puducherry, India.
ankitamohanty0211@gmail.com

I. INTRODUCTION

Clinical microbiology has been one of the versatile branches of the medical sciences. Interesting milestones include the recognition of new pathogenic organisms, the development of the latest techniques for detecting them, and the introduction of automated techniques for their identification and antibiotic susceptibility results. Automation has been largely taking over manual techniques in diagnostic laboratories.

Webster's Dictionary defines automation as the "automatically controlled operation of an apparatus, process, or system by mechanical or electronic devices that take the place of human organs of observation, effort, and decision"[1].

This chapter covers automation in various branches of clinical microbiology: bacteriology, virology, mycology, and parasitology along with its strengths and limitations.

II. AUTOMATION IN BACTERIOLOGY

The introduction of automated systems took place in the 1970s for the first time. It included the BACTEC series which could detect bacterial growth using broth-based principles in blood samples. The concept of MALDI was first noted by researchers in the 1990s. The total laboratory automation (TLA) system based on culture (BD Kiestra™) was noted for the first time in 2006. In 2012, the Copan Company introduced the TLA system (WASPLab™). [2]

The BD Kiestra TLA system components include the SorterA (to store up to 48 different media types and distribution), the BarcodA (barcoding), the InoqulA (specimen processing and inoculation), the ReadA compact (normal atmosphere and carbon dioxide incubators with digital imaging system) and the Ergonomic A (workbenches). The modules are connected by a bidirectional Proceed A conveyor system.

WASPLab consists of the WASP (Walk Away Specimen Processor for specimen processing and inoculation) and incubators (normal atmosphere and carbon dioxide) which are connected by a unidirectional conveyor system (Table 1) [3]

Table 1: Laboratory Automation Configurations

System	BD Kiestra TLA	BD Kiestra WCA	Copan WASP Lab
Specimen processors	One to two (SorterA,BarcodA,InoquIA)	One (SorterA,BarcodA,InoquIA)	One to two WASP
Incubators	One to six	One to three	One to three (single/double capacity)
Integrated Workbenches	1-12	Not Applicable	Not Applicable
Number of media types	Upto 48	12	9-18

1. **Initial Processing:** All samples received are taken for processing without delay. Appropriate culture media is selected and streaking is done with the calibrated metallic loops, plates are sent for incubation through the conveyor belt.

2. **Incubation of Culture Plates:** The inoculated culture plates are incubated under optimum atmospheric conditions and the growth is monitored by a series of digital images. [2]

Various automated systems are available for blood culture. (Table 2) [4]

Table 2: Automated System for Blood Culture

System	Principle	Advantages	Disadvantages
VersaTREK	Measurement of pressure changes	shorter turnaround time, decreased rates of contamination reports, and reduced manual processing time	Cost and affordability Specific bottles are needed for loading into the machine.
BACTEC	Fluorometry	Same as above	Same as above
BacT/Alert	Colorimetry	Same as above	Same as above
BacT/Alert Virtuo	Colorimetry	In addition, Automatic loading of bottles, continuous monitoring	Same as above

Identification of microorganisms: Different systems are available to identify microorganisms. (Table 3)[5]

Table 3: Automated Systems available for the Identification of Microorganisms

System	Principle	Advantages	Disadvantages
Vitek 2 Automated system	Colorimetric detection of metabolic products	Rapid identification Updated and dynamic database	High cost
MALDI-TOF MS	Examination of the pattern of ribosomal proteins	Fast, accurate, early identification for empirical therapy	High cost Cannot speciate *Salmonella* Cannot differentiate between *Escherichia coli* and *Shigella* species.
Biofire Film Array	Nested PCR	Covers a panel of pathogens, e.g.: Respiratory, Gastrointestinal panel	High cost

III. AUTOMATION IN VIROLOGY

The availability of viral diagnostic centers has always been a challenge for healthcare facilities. Despite the progress in the world of medicine, fewer diagnostic centers are functioning for all viral diseases. Traditional viral diagnostic techniques are time-consuming, labour-intensive, and difficult to access by the common people as well as the clinicians.[6]
In the past, animal inoculation techniques were performed for virus isolation and diagnosis. This was followed by the development of cell and tissue culture techniques which are now mainly confined for research purposes.[7]

Many immunological reactions have been used for the diagnosis in recent years. These methods include radioimmunoassay (RIA), immune histochemistry, counter immuno electrophesis (CIE), immune chromatographic tests, and enzyme-linked immunosorbent assay (ELISA) for detecting viral antigens or viral antibodies. Biotin-labelled probes have been used to enhance the sensitivity and specificity of the diagnostic techniques.

Developments such as real-time PCR techniques, DNA microarrays, proteomics, and nucleic acids sequencing have revolutionized molecular diagnostics in the world of virology.[8]

The introduction of various amplification techniques like the polymerase chain reaction (PCR), ligase chain reaction (LCR), and nucleic acid sequence-based amplification (NASBA), aided in the diagnosis of many more viral diseases. Among them, PCR is the most widely used technique. The details of the molecular techniques are briefly covered in the "Automation in parasitology" section.

The COVID pandemic led to the development of more and more molecular laboratories so that RT-PCR can be performed for its diagnosis and early detection of the disease. This pandemic has been a revolution in viral diagnostic techniques for many countries. Most of the countries, including many low and middle-income countries expedited the process of setting up molecular laboratories for its diagnosis.

Microarray "DNA chips" containing immobilized oligonucleotide probes are being developed to detect a wide number of target molecules from clinical specimens.
Diagnosis of viral infections still remains a challenge as many viral infections like HIV, and HCV cannot be diagnosed during the window period. New techniques need to be developed to overcome such challenges.

IV. AUTOMATION IN MYCOLOGY

Mycology is one of the least studied areas of microbiology; hence, many secrets of the fungi world are yet to be revealed. Traditionally fungal infections were presumptively diagnosed with the help of clinical signs, symptoms, and microscopic techniques including potassium hydroxide mount, and Calcofluor mount. Conventional culture media like SDA has been used and LPCB mount prepared from the growth has been the means of identification for the molds. These methods take weeks for identification which may lead to the worsening of the clinical condition of the patient.

Systemic mycoses still remain a public health threat. Many serological assays like Galactomannan for Aspergillus and Beta Glucan for Candida and other mycoses have aided in the diagnosis of fungi.

Molecular assays have also recently led to a decrease in turnaround time for the diagnosis of fungal infections. (Table 4)[9]

Table 4: Molecular Assays for Fungal Identification

Assay	Principle	Advantages	Disadvantages
AccuProbe	Chemiluminescence	Identifies Dimorphic fungi like *Coccidiodes*, *Blastomyces,* and *Histoplasma* rapidly.	The growing culture is required, other fungi give false positive results
Yeast Traffic Light and Quick Fish	PNA-FISH	Rapid TAT from culture bottles of blood samples that are positive	Detects restricted Candida species, requires a fluorescent microscope
BioFire Film Array	Nested multiplex PCR	The Meningitis panel detects Cryptococcus directly from CSF	Detects limited Candida species, High cost
T2 candida	PCR with Nuclear magnetic resonance	Can detect Candida from blood samples directly	Detects limited Candida species, High cost
SeptiFast Light cycler	Real-time PCR	Detection of the common Candida Species (5 species) and Aspergillus	Detects limited Candida species, High cost
Asper Genius	Multiplex Real-time PCR	In Broncho Alveolar lavage specimens, Aspergillus can be identified directly.	A restricted number of Aspergillus species are identified, unavailable for clinical use presently
MycAssay Aspergillus	Real-time PCR with molecular beacons	In Broncho Alveolar lavage and serum specimens, Aspergillus can be identified directly.	Unavailable for clinical use presently

Automation is an upcoming means for the detection of fungal infections. It replaces the mechanical task involved in the culture of various fungi. It paves the way for the development of the field of mycology.

V. AUTOMATION IN PARASITOLOGY

Parasitic diseases are a threat to public health in tropical countries and underdeveloped countries. Conventionally, the diagnosis of parasites is carried out in the laboratory by stool microscopy. The parasite is identified by comparing its appearance with the known forms.

It is important to detect infectious parasitic agents by authentic and economical methods to prevent disease transmission and chronic illnesses. Microscopy requires expert technologists and is laborious and time-consuming. It has limited sensitivity and patients with low parasitic load are missed. The disadvantages of microscopy and rapid tests like antigen detection tests led to the development of molecular diagnostics, but their use is restricted due to the high cost. (Table 5)[10]

Table 5: Molecular Diagnostics in Parasitology

Technique	Principle	Advantages	Disadvantages
PCR Polymerase Chain Reaction	Conventional denaturation of DNA	Sensitive Specific	High rates of contamination
RT-PCR Real-time Polymerase Chain reaction	Real-time monitoring of the amplification process	More sensitive and specific. Fewer chances of cross-contamination	High cost
Luminex	Multianalyte profiling based on the xMAP technology using beads	Detects Antigenic diversity	High Cost
LAMP Loop-Mediated Isothermal Amplification	Strand Displacement	Very high sensitivity and specificity which differentiates single nucleotide differences	High cost
RAPD Random Amplified Polymorphic DNA	Random amplification of the genome	Simple, rapid, and cost-effective, no knowledge of DNA sequencing or DNA hybridization technology is needed.	Difficult to put to routine use
AFLP Amplified Fragment Length Polymorphism	PCR specifically amplifies the restriction fragment groups from the digested genome.	Highly efficient, A large number of bands are analyzed together with a wide coverage of the genome.	Difficult to put to routine use in low and middle-income countries
RFLP Restriction Fragment Length	Restriction enzymes or endonucleases digest PCR products	Appropriate for environmental samples as it allows	Difficult to put to routine use in low and middle-

Polymorphism		the identification of many genotypes in a sample.	income countries
Microsatellites	Short DNA sequences (approximately 300 base pairs) consisting of 1-6 nucleotide tandem repeats, with almost a hundred repeats	Recurrent polymorphism, inheritance having shared dominance, high duplicity, and resolution, can be detected by PCR and easy typing methods.	The isolation of parasites by PCR is hindered by the presence of microsatellites.

VI. STRENGTHS OF AUTOMATION

The increased number of samples processed in a short span of time leads to decreased hospitalization time and risks of contracting nosocomial infections. This leads to improvement in the treatment facilities as well as broadens the scope of empirical therapy. The quality of the work is improved and the batch-to-batch variability is decreased; hence the results are reproducible. As the plating methods are standardized, there is an improved yield of isolated colonies of the pathogenic organism, followed by improved growth and reduced time to results (Identification and Antimicrobial Susceptibility Testing). Due to the presence of a barcode on each sample and report, the results can be traced easily in the future. There is minimal scope for errors in sample preparation, handling of media plates, and broth/sample switching. As the resources have to be supplied once, there is a decrease in the labor needed, hence it is labor-saving. The repetitive tasks like plate inoculation and incubation are performed mechanically. This leads to the availability of knowledgeable staff for valuable tasks (e.g., Analysis of reports, interpretation, root cause analysis of the problems faced, Research and Development, microscopy). There is a benefit for employers due to lessened overtime pay for employees.

VII. LIMITATIONS OF AUTOMATION

There is an unmet need for laboratory staff to adapt to automation (e.g., staff shifts, training, 24/7). There can be an abuse of the machines as all the information is computerized and hence open for cybercrimes. Employers have higher expectations for increased productivity. As all the information is stored in the memory of a computer, the crash of the system can lead to the loss of a vast amount of data, hence backups are required. Immense support and maintenance are mandatory which is costly. There are challenges in identifying unusual/new species (Automated systems cannot identify some of them perfectly). There is a risk of contamination of specimen processors and incubators (e.g., fungus spores, biosafety class 3 microorganisms). As most of the analysis is performed by the system, this can lead the microbiologic knowledge to suffer and a reduction in analysis capacity.

VIII. CONCLUSION

To conclude, Automation is certainly a way ahead for diagnostic techniques in microbiology, but the aim is not to supersede skilled laboratory personnel but to help them in their daily chores. The importance of the human mind in the diagnosis of various diseases should not be underestimated. Automated systems are a tool to decrease the amount of

unnecessary physical labor involved in microbiology laboratories. A large number of research as well as new discoveries are always welcome in this medical field.

REFERENCES

[1] Stager' and CE, Davis JR. Automated Systems for Identification of Microorganisms [Internet]. Vol. 5, CLINICAL MICROBIOLOGY REVIEWS. 1992. Available from: https://journals.asm.org/journal/cmr
[2] Cherkaoui A, Schrenzel J. Total Laboratory Automation for Rapid Detection and Identification of Microorganisms and Their Antimicrobial Resistance Profiles. Vol. 12, Frontiers in Cellular and Infection Microbiology. Frontiers Media S.A.; 2022.
[3] Croxatto A, Prod'hom G, Faverjon F, Rochais Y, Greub G. Laboratory automation in clinical bacteriology: What system to choose? Vol. 22, Clinical Microbiology and Infection. Elsevier B.V.; 2016. p. 217–35.
[4] Halperin AV, del Castillo Polo JA, Cortes-Cuevas JL, Cardenas Isasi MJ, Ampuero Morisaki M, Birch R, et al. Impact of Automated Blood Culture Systems on the Management of Bloodstream Infections: Results from a Crossover Diagnostic Clinical Trial. Microbiol Spectr. 2022 Oct 26;10(5).
[5] Sastry A, Pruyadarshi K, Deepashree R. Essentials of Antimicrobial Stewardship. first. JAYPEE; 2023. 242–257 p.
[6] Cobo F. Editorial Automation in the Virology Laboratory: Contribution to Improve The Viral Diagnosis. Vol. 6, The Open Virology Journal. 2012.
[7] Hsiung GD. Diagnostic Virology: From Animals to Automation. Vol. 57, THE YALE JOURNAL OF BIOLOGY AND MEDICINE. 1984.
[8] Read SJ, Burnett D, Fink CG. Molecular techniques for clinical diagnostic virology. J Clin Pathol. 2000;53(7):502–6.
[9] Wickes BL, Wiederhold NP. Molecular diagnostics in medical mycology. Vol. 9, Nature Communications. Nature Publishing Group; 2018.
[10] Alp B. Molecular techniques for the study and diagnosis of parasite infection. Vol. 17, Review Article The Journal of Venomous Animals and Toxins including Tropical Diseases. 2011.

