**AUTOMATION IN MICROBIOLOGY**

**Author**

**Dr. Ankita Mohanty**

Senior Resident

Department of Microbiology

JIPMER

Puducherry-605006

India

Email id: ankitamohanty0211@gmail.com

**ABSTRACT**

Automation is a process where a machine or equipment can function without human interference or minimal human interference. Microbiology has been one of the branches which are labor intensive. Automation in the microbiology laboratory has changed the way in which microbiologists can detect human pathogens. 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

1. **INTRODUCTION**

Clinical microbiology has been one of the dynamic branches of the medical sciences. Interesting developments include the recognization of several new pathogenic organisms, the development of newer 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**

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

The BD Kiestra TLA system components include the SorterA (media storage with a capacity of up to 48 different media types and distribution), the BarcodA (barcoding), the InoqulA (specimen processing and inoculation), the ReadA compact (normal atmosphere and CO2 incubators with digital imaging system) and the ErgonomicA (workbenches). All the modules are connected by a two-way ProceedA conveyor system.

The WASPLab consists of the WASP (Walk Away Specimen Processor for specimen processing and inoculation) and incubators (normal atmosphere and CO2) which are connected by a one-way conveyor system(Table 1) [3]

**Table 1: Laboratory Automation configurations**

|  |  |  |  |
| --- | --- | --- | --- |
| System | BD Kiestra TLA | BD Kiestra WCA | Copan WASP Lab |
| Specimen processors | 1-2(SorterA,BarcodA,InoquIA) | 1(SorterA,BarcodA,InoquIA) | 1-2 WASP |
| Incubators | 1-6 | 1-3 | 1-3 (single or double capacity) |
| Integrated Workbenches | 1-12 | Not Applicable | Not Applicable |
| No.of media types | Upto 48 | 12 | 9-18 |

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 conveyer belt.

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 detection times, reduced rates of contaminant blood cultures, and a reduction in hands-on processing time | Cost and affordabilitySpecific 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 identificationUpdated and dynamic database | High cost |
| MALDI-TOF MS | Examination of the pattern of ribosomal proteins | Fast, accurate, early identification for empirical therapy | High costCannot 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 taking, labor-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 comprise radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and counterimmunoelectrophesis (CIE) for detecting viral antigens or viral antibodies. Biotin-labeled probes to enhance the sensitivity and specificity of the diagnostic techniques have been used.

Developments in molecular diagnosis such as real-time-PCR techniques, nucleic acid sequencing, DNA microarrays, and proteomics have been of great help 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. Newer techniques need to be developed to overcome such challenges.

**IV. AUTOMATION IN MYCOLOGY**

Mycology is one of the least studied areas of microbiology and 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 required, false positives with other fungi |
| Yeast Traffic Light and Quick Fish | PNA-FISH | Rapid TAT from Positive blood culture bottle | Detects limited 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 directly from blood | Detects limited Candida species, High cost |
| SeptiFast Light cycler | Real-time PCR | Detects five common Candida Species and Aspergillus | Detects limited Candida species, High cost |
| Asper Genius | Multiplex Real-time PCR | Detects Aspergillus directly in BAL specimens | A limited number of Aspergillus species are detected, not currently available for clinical use |
| MycAssay Aspergillus | Real-time PCR with molecular beacons | Detects Aspergillus directly in BAL and serum specimens | Not currently available for clinical use |

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 diagnose parasitic infections by reliable and cost-effective methods to prevent disease transmission and chronic illnesses. Microscopy requires technical expertise and is laborious and time-consuming. It lacks sensitivity and a low level of infection is missed. The limitations of microscopy and 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 |
| PCRPolymerase Chain Reaction | Conventional denaturation of DNA | SensitiveSpecific | High rates of contamination |
| RT-PCRReal-time Polymerase Chain reaction | Real-time monitoring of the amplification process | More sensitive and specific.Fewer chances of cross-contamination | High cost |
| Luminex | Bead-based xMAP technology (multianalyte profiling) | DetectsAntigenic diversity | High Cost |
| LAMPLoop-Mediated Isothermal Amplification | Strand Displacement | Extremely high sensitivity and specificity to discriminate single nucleotide differences | High cost |
| RAPDRandom Amplified Polymorphic DNA | Random amplification of the genome | a very simple, fast, and inexpensive, does not require prior knowledge of the DNA sequence or DNA hybridization | Difficult to put to routine use |
| AFLPAmplified Fragment Length Polymorphism | PCR to selectively amplify the groups of restriction fragments of totally digested genomic. | Highly efficient, the possibility of analyzing a large number of bands simultaneously, with extensive coverage of the genome. | Difficult to put to routine use in low and middle-income countries |
| RFLPRestriction Fragment Length Polymorphism |  Restriction enzymes or endonucleases digest PCR products | Suitable for environmental samples because it permits the detection of multiple genotypes in the same sample | Difficult to put to routine use in low and middle-income countries |
| Microsatellites | Short DNA sequences (about 300 base pairs) composed of tandem repeats of one to six nucleotides, with approximately one hundred repeats | Frequent polymorphism, codominant inheritance, high reproducibility, and resolution, can be detected by PCR and easy typing methods. | A high number of microsatellites cause technical difficulties in isolating parasites by PCR. |

**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 broaden the scope of empirical therapy. The quality of the work is improved 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 expert staff for added value tasks (e.g. pre- and post-analytic phase, reading, interpretation, troubleshooting, R&D, microscopy). There is a reduction in overtime payments for employers.

**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 a misuse of tools 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 needed. Good support and maintenance are essential. There is an expensive maintenance budget. There are challenges to identify 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 to a loss of microbiologic knowledge and a decrease in analytical variability

**VII I. CONCLUSION**

 To conclude, Automation is certainly a way ahead for the diagnostic techniques in microbiology, but the aim is not to supersede experienced laboratory personnel but to assist them in their daily tasks. The importance of human mind in the diagnosis of various diseases should not be under estimated. Automated systems are a tool to decrease the amount of unnecessary physical labour involved in the microbiology laboratories. A large number of research as well as new discoveries are always welcome in this medical field.

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