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4th Edition



Previously Known as
The Short Textbook of Medical Laboratory for Technicians

The Short Textbook of MEDICAL LABORATORY SCIENCES

Satish Gupte

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Section

9 AUTOMATION

Section Outline

- Automation in Histopathology

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CHAPTER 10

Automation in Histopathology

AUTOMATION IN HISTOPATHOLOGY

Histopathology is the microscopic study of tissues including abnormalities of tissue, of biopsy study, disease in specimens and cancer study of tissue plus cells.

In automation of histopathology technician automate the tissue for automatically prepare tissue sample for laboratory testing. It is done by fixing, dehydration, cleaning and infiltrating them with paraffin. In practice of evaluating digital images of histology slides is becoming more and more popular. Actually automated histological analysis is moving towards automated digital image system and is associated image analysis solution in near future.

In fact automation involves work that is totally digital to examine digitalized slides for pathological use optical microscope is attached with monitors. Microscopes are fitted with photo of entire slide image. It is more effective as well as efficient process. In medical analysis it provides lower cost, high efficiency, and accuracy.

Benefits of Automation in Histopathology

- By processing the biopsies and larger specimens simultaneously, it shortens processing times and speed up productivity.
- The peaks and troughs can be removed by approaching to automated working.
- Here specimens are continuously supplied after processing and embedding.
- It provides uninterrupted workflow and it is possible with less number of workers that too throughout day.

TYPES OF AUTOMATED MACHINES IN HISTOPATHOLOGY

- Microtome
- Automated tissue processor

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Fig. 10.1: Microtome.

- Tissue embedding machine
- Tissue floater/waterbath
- Automatic tissue stainer
- Cover slipper

Microtome (Fig. 10.1)

- Tissues are cut and processed by cutting thin sections in microtome.
- Thickness of section is 50–100 nm (ultra microtomy). However common range is 5–10 micron.
- These sections are stained followed by their examine using microscope.

Automated Tissue Processor

- It is exchange of fluids using series for fixed time.
- It may be linear tissue transfer process where tissue (block) are transported in basket.
- Different reagents are kept in fixed containers.
- Electric timers should be to regulate the amount of time submerged specimens spend in carts reagent containers agitation is caused by tissue basket rotating vertically.

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- Other type is exchange of fluids using series of solutions for predefined amount of time and controlled environment.

Tissue Embedding Machine

- Using mold, molten is used to surround tissue.
- This medium is solidified to form blocks for slicing tissue into thin sections.
- Three functions of embedding are to provide support to tissue, to avoid tissue distortion during cutting and to preserve the tissue for archival usage.

Tissue Floater/Water Bath

- It is a stage between cutting paraffin sections and maintaining them on slides is called floating bath water bath.
- It is made up of heat resistant glass with or without an insulated cover or insulated metal.
- Heater is used to maintain water temperature.
- Thermostat is provided to automatically regulate the water bath's temperature.
- The water in water bath is kept at 40 to 50°C.
- A warm water bath enables tissue to relax and smooth out before being mounted on a glass slide.

Automatic Tissue Stainer

Auto steer's advantages include time saving, decrease of repetitive motions no bending and twisting, etc. On the contrary H&E stain requires 25 minutes to dry this stains. Linear are batch Stainer where slides are sequentially dipped into staining solution after being loaded into this device. One slide at a time in linear design slide holders are clipped to carrier mechanism which is connected to slide holder. Linear slide racks are moved through staining solution baths as their are multiple slides.

Cover Slipper

- Picking up a cover glass is done with a suction device from a stack of cover glasses.
- Apply pressure to cover glass.
- Air bubbles are removed from the cover glass capillary action after it has been placed on the slide.

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AUTOMATION IN CLINICAL BIOCHEMISTRY

Automation is the mechanization of the steps in a procedure. It not only saves labor but also allows reliable quality control measures to imposed strongly. The automation in clinical biochemistry laboratory has advantages, including the increased accuracy less discrepancies in results, better quality control, reduced subject errors and use of smaller quantities of samples and reagents.

CLASSIFICATION OF AUTOMATION

The automated system is grouped into following types:

Continuous Flow Analysis (Figs. 10.2 and 10.3)

This is the first automated system introduced by Technicon (USA) in 1960s which has since been followed by other systems. In this system, liquid specimen, reagents and diluents are pumped through a system of continuous flow tubing. Samples are introduced in a sequential manner, following each other through the same network. A series of air bubbles separate and clean the media. The commonly used models are SMA 6/60 and 12/60, which process 60 specimens per hour and report the results of 6 and 12 parameters simultaneously. This system is ideal for larger laboratories.

Disadvantages

- The physician may not be interested in all the tests reported by the instrument.

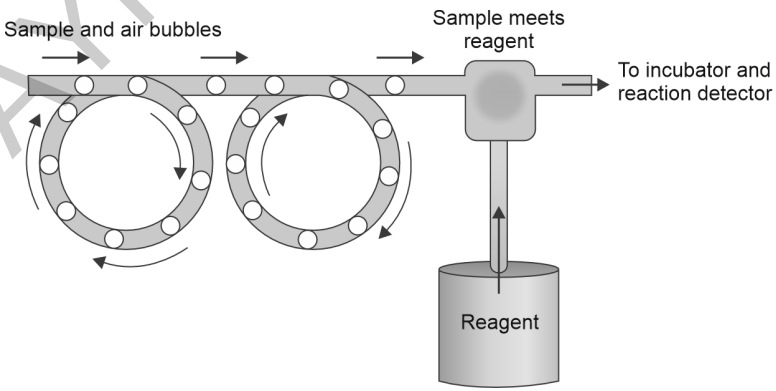

Fig. 10.2: Continuous flow analyzer.

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- The instrument does not report the test, which the physician may be interested in.

Discrete Analysis (Fig. 10.4)

In this system, each sample is separated along with the accompanying reagent. This can be used both for special tests and routine tests. It is ideal for smaller laboratories. The commonly used models for this

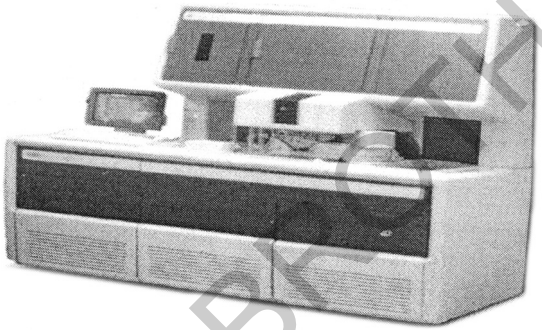

Fig. 10.3: Continuous flow analyzer.



Fig. 10.4: Discrete analyzer.

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system are Clinicon (Boehringer Mannheim) and Auto Pacer (Miles of India Ltd).

Centrifugal Analyzer (Fig. 10.5)

This is the recent automated system used. In this system, the specimens and the corresponding reagents are placed in their places on the special centrifuge head, the machine is run and the force generated by the centrifugation transfers the reagents and the specimen to a cuvette for chemical reaction to occur. The optical measurements are made while the cuvette is in motion (2,500 rpm). It is the most useful in performing enzyme assays.

Random Access Analyzer

Random access analyzers perform analyzes on a batch of specimens sequentially with each specimen analyzed for a different selection of tests. The tests performed in the random access analyzers are selectable through the use of different containers of liquid reagents, different reagent packs, or different reagent tablets depending upon the analyzer. This approach permits measurement of a variable number and variety of analytes in each specimen. Profiles or group of tests are defined for a specimen at the time the requested tests are entered into analyzer by means of a keyboard, by instruction from a



Fig. 10.5: Centrifugal analyzer.

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laboratory information system in conjunction with barcoding on the specimen tube or by operator selection of appropriate reagent packs. Like centrifugal analyzers, random access analyzers incorporate computers and are able to take multiple absorbance or reflectance measurements for each assay.

Steps of Automation in Biochemical Analysis

The steps are divided as below:

- Sample handling
- Sample processing
- Reagent handling
- Analytical procedure
- Reaction analysis or sensing
- Calculation
- **Sample handling and sample processing:** The specimens are manually labeled, centrifuged, and divided into aliquots if tests for more than one workstation have been requested. This manual processing also removes the specimens which are not acceptable for the analysis (e.g., hemolyzed or lipemic specimens, presence of debris) and reviews the request form received with the specimen. Extreme care is needed in this step to avoid mislabeling of patient samples or their aliquots.

  In automated systems, the sample holding containers must be placed in the same order and the laboratory professional must note down the order of the arrangement clearly. Some laboratories prefer to number the sample cups so that if they are misplaced, they can be put into the proper slot. In most automated system, the samples are held in small cups which are placed on a circular tray. The slots are sequentially numbered. An automatic pipette can be used for taking aliquots of specimens into the sample cups. Fresh tip is used for each sample. In continuous flow analysis, the requisite amount of specimen is picked up by the sampler while in others an aliquot of the specimen may have to be manually placed. Probes used in the automated systems for picking up specimen must be thoroughly washed, preferably with a diluent, before the following specimen is drawn. The convenient way is to dispense the specimen and then the diluent to avoid the carry over effect.

  Protein causes major interference in many analyses. In the continuous flow analysis system, dialyzer removes the protein

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from the specimen. However, the dialyzer is not used for protein determination. In other automated system, there is no such step that removes protein from the sample. The other systems use a method that will be least interfered by the presence of protein or they use a more sensitive method or they add appropriate chemicals to remove the protein. Some discrete analyzers read the absorbances with two wavelengths that helps to reduce the effects of protein.

- **Reagent handling:** Most chemical reactions require the combining of reagent and sample in exact amounts. This is called proportioning. In continuous flow analysis, the diameter of the tube regulates the volume of the reagent fluid picked up, the amount of specimen. The dwelling time of the probe inside the specimen container determines the amount of specimen picked up. The rate of flow for all fluids is the same. A single peristaltic pump is used for drawing the fluids. For the discrete system, a single probe may measure the volumes of specimens and reagents but the dwelling time in them varies. Individual automatic dispensers are also used in some discrete systems where syringes and volumetric overflow devices are used to dispense requisite quantities of sample and reagent into test tubes or containers.

  Reagents can be dispensed directly from bulk containers supplied by the manufacturer or reconstituted in the laboratory. Those manufacturers who are able to supply dry reagents or provide the necessary formula for the preparation of reagents within the laboratory, will be the most successful in marketing their goods in the developing countries.
- **Analytical procedure:** Mixing of reagents with the specimen is a vital component of biochemical analysis. In continuous flow system, it is done through glass coils where the liquid rotates and both specimen and reagents fall through one another during their rise and fall through the loop. In the discrete systems, other methods are adopted-vibration, slewing action, centrifugal rotation (in centrifugal analyzers), vortexing, pressing and releasing of plastic bags which receive the fluids and ultrasonic waves.

  Automated incubation is merely a delay station where the test mixture is allowed to react. The chamber where the incubation is held is heated to the desired temperature by the use of heating block, air, water bath or oil bath. To sustain the advantage of speedy

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multiple analyses, the reaction is not taken to completion. Rather, the rate of the reaction can be measured and the values after the completion of the reaction are extrapolated. The instruments may also delay the measurement for a predetermined length of time or present the reaction mixtures for measurement at constant intervals of time. In continuous flow analysis, all measurements are made against a standard and as the procedures are precisely timed, the result of the standard is highly reproducible even if the measurement is made much before the completion of the reaction. In the discrete system, there are a number of delay stations before the readings are taken.

- **Reaction analysis or sensing:** After the reaction is completed, there must be a sensing device (colorimeter) to measure the change, such as development of color. The sensing can be done in the original site where the reaction occurred (internal) or taken into another vessel (external) to make the measurements. In the continuous flow analysis, the reagent stream under analysis flow continuously through the flow cell which acts like a cuvette. The air bubbles are removed by the debubbler before the reacted solution enters the flow cell for photometric measurements. The sensing device converts the optical response into electrical impulses which are then sent to the read out device. The read out device can be the strip chart on which the results are traced or on the digital display.

  Chemical reactions can be monitored either at one time point or at many. Commonly, single point monitoring is used for end point (or mid point) analysis in which the reaction has gone to completion or the instrument extrapolates the value. Multiple point monitoring is done in case of kinetic studies (enzyme activity).
- **Calculation of results:** In the automated system, the results are automatically computed from the response of the sensor and finally printed out in appropriate units. The printing can be done directly on patient's request slip (or reporting slip) or on the laboratory form. In order to avoid transcription errors, it is advisable that multiple copies of the print out should be made so that different copies of the same report can be routed to different destination-financial office, physician, patient's record, laboratory record and others.

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Quality Control in Automated System

Automated systems are not free from errors. In fact, they frequently render a false sense of security among the users and the results may be far from reliable. The system must be frequently subjected to quality control procedures using control sera. Control runs should be done in the beginning of each day and the result is plotted on the quality control chart.

Advantages of Automation

- Increase the number of tests by one person in a given period of time
- Minimize the variations in results from one person to another
- Minimize errors found in manual analyses—equipment variations—pipettes
- Use *less* sample and reagent for each test

Disadvantages of Automation

- Vary with the instrument type
- High initial cost: Generally cost of equipment, maintenance, amount of quality control (QC)
- Also technologist must be kept knowledgeable and careful in setup and operations
- Decreases work opportunities of man power.

AUTOMATION IN DIAGNOSTIC MICROBIOLOGY

The last few years have seen exciting advances in finding out new ways of identifying microorganisms within a few minutes of arrival of a specimen in the laboratory. Specific microbial components may be detected by countercurrent immunoelectrophoresis also designated as CIE. Another nice method for identification of microorganism is gas liquid chromatography (GLC) in which specific metabolic products are detected.

Microcalorimetry may be used to find out rapidly the number of bacteria in liquid specimen like urine, by detecting their heat output.

Luminescent biometry is again a useful method in which amount of bacterial adenine triphosphate is detected and thus the number of bacteria in a given amount of liquid can be known by measuring the brightness of flash of light when that amount is mixed with

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standard preparation of luciferase (lighting system of fire of flies). Radiorespirometry using C^{14} glucose in basal nutrient medium with different antibiotic is used for antibiotic sensitivity and thus recommending appropriate antibiotic for an infection in only 3 hours' time. Additionally following methods are discussed:

- **Gas chromatography:** The primary use in microbiology is to detect metabolic by-products of bacterial growth. Gas chromatography is useful in the identification of anaerobic bacteria and analysis of body fluids for the compounds that indicate the presence of microorganisms (sensitive and rapid procedure). Methods such as frequency modulated electron capture gas chromatography and pyrolysis gas chromatography are under investigation as a means to identify various bacteria and fungi (or their metabolic end products) from specimen source. Cell wall fatty acid analysis and whole cell carbohydrate study by gas liquid chromatography offer much promise. High pressure liquid chromatography is extremely useful for assay of antimicrobial agents in serum and body fluids.
- **Bactometer:** It is an instrument capable of detecting changes in electric impedance as a result of growth and metabolic activity of bacteria in a suitable growth medium. This has been primarily evaluated for use in detecting clinically significant number of bacteria in urine and blood. Bottles of appropriate broth containing specimen is used. Wire electrodes are kept in this bottle. These wire electrodes are inserted through rubber stopper of bottles for connection to bactometer. Impedance changes are continuously monitored permitting detection within hours.
- **Measurement of radiolabeled compounds:** The instrument in wide use in clinical microbiology is the Bactec system. This instrument measures the release of CO_2^{14} labeled substrates that result from bacterial metabolism. It employs sampling device and an ionization chamber. Specimens are inoculated into the bottles of broth medium containing C^{14} labeled substrate. Depending on the instrument model, bottles are monitored either automatically or manually for measurement of CO_2. The instrument converts this into growth index. This unit of measurement is recorded through light or on meter of instrument as well as on a paper tape. Bactec has a number of other capabilities like identification of *Mycobacterium, Neisseria*, etc.

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- **Autobac multi-test system:** This instrument consists of an incubator shaker, photometer system, curvette information device and remote printer. Each chamber in a disposable cuvette contains a different substrate (or inhibitor) or antibiotic. Identification is based primarily on the pattern of growth and/or substrate utilization in the various chambers plus reaction patterns for various organisms are contained in the computer data base. This instrument is designed to perform antibiotic sensitivity results, identification of both fermentative and nonfermentative organisms after relatively short period of time.
- **MS-2:** Abbot MS-2 is the instrument with multifunction capabilities. It consists of control module and an analysis module. Besides susceptibility testing, the system can also identify the Enterobacteriaceae. The major advantage of the MS-2 system is its level of automation and results are provided within 5 hours. The disadvantage is its limitation to the identification of only the Enterobacteriaceae.
- **Dynatech MIC-2000:** It is a semiautomated system for preparing and performing mircobroth antibiotic susceptibility and identification tests. The system consists of 96-channel dispenser for accurately delivering a volume of liquid, such as antibiotic dilution from test tubes to a 96-microtiter plate (the MIC plate) and an inoculator with 96 pins for accurately transferring liquid inoculum from disposable inoculum tray to the 96 wells of MJC plate. An illuminated magnifying viewer to assist in discriminating between wells with and without visible growth is also included.
- **Replican system:** Replican system is a commercially available replica plating method for the identification of Enterobacteriaceae. This system consists of a variety of biochemical and antibiotics containing device, a viewing table allowing visual inspection and electronic recording of individual reactions, and a computer terminal complete with hard-copy printout. This device allows the simultaneous inoculation of up to 36 organisms on a single agar plate. It is considered to be efficient, economical and effective laboratory tool for identification of organisms.
- **Automicrobic system:** It is designed for the detection, enumeration identification and drug susceptibility testing of microorganisms in clinical specimens, with all its functions to be performed simultaneously or consecutively by the same

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instrument. This system consists of a diluent dispenser, injector or filling module incubator reader, computer and terminal printer. It uses a novel array of highly selective media in which a mixture of substrates and inhibitors is used to permit the growth of one or group of closely related microorganisms, to concurrently inhibit growth of any other organisms present, and to enumerate the total number of organisms in the sample. The advantages of automicrobic system are its versatility, its rapidity of results with urine samples, its identification potential and its degree of automation. This system can handle up to 240 specimens simultaneously and all of the operations are automatic.

- **Genetic probes:** A labeled (usually P^{32}) sequence of DNA or RNA is used to detect specific DNA sequence. The nucleotide sequence of the test organism is taken out by enzyme (endonuclease), fragmented transferred to a nitrocellulose sheet and the fragments separated by electrophoresis. The specific DNA (labeled) sequence is added to the paper, where it hybridizes with a fragment which has the same sequence, and detected by autoradiography.

MOLECULAR TECHNIQUES IN MICROBIOLOGY

INTRODUCTION

It is a new area in applied medical microbiology. This in fact offers immense help in the form of molecular tests in establishing laboratory diagnosis of enormous infectious diseases. Apart from this, it provides information about prognosis of infectious diseases and response of treatment. Surprisingly molecular tests have replaced conventional culture, etc. Molecular test are convincingly accepted because conventional tests are slow, insensitive, expensive, time consuming or not always available at all and so on.

AMPLIFIED TESTS

- **Polymerase chain reaction (PCR):** This technique offers synthesis of unlimited quantity of the target nucleic acid sequence. This technique is susceptible to contamination causing false positivity. Basic PCR is described on page 317 apart from basic PCR, other PCR are discussed briefly as under:

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- *RT-PCR:* This technique is used to amplify RNA. Here complementary DNA (cDNA) is formed from RNA with the help of an enzyme reverse transcriptase. Subsequently, cDNA is amplified by PCR.
- *Nested PCR:* By this technique both sensitivity and specificity of PCR is increased considerably. In this technique two pairs of amplification primers are required. One of the primers is used in the first round of PCR to amplify needed sequence. The amplified material so obtained is subjected to second round of PCR with the second set of primers. Actually, it anneals to the sequences found in the first of material.
- *Multiplex PCR:* In this case two or more sets designed for amplification of different targets are used. It may help in amplification of more than one target sequence in clinical specimens. As a matter of fact multiplex PCR is less sensitive than PCRs with single set of primers.

- **Transcription mediated amplification (TMA):** It is in fact an isothermal RNA amplification technique. In this technique RNA target is reversely transcribed into cDNA. Now RNA copies are formed with RNA polymerase. In about 2 hours time 109 fold amplification of the target RNA can be synthesized. Advantages of TMA include: (1) there is no need of thermal cycle, (2) there are very less possibility of contamination. TMA uses a reverse transcriptase enzyme with endogenous RNase H activity and T7 RNA polymerase.
- **Nucleic acid sequence based amplification (NASB):** It is a again isothermal RNA amplification based technique. Here RNA target is reverse transcribed in to cDNA and then RNA copies are produced with the help of RNA polymerase. In a way this technique resembles with TMA. It does not require thermal cycles. Here AMVRT, RNase H and T7 bacteriophage, RNA polymerase are used in NASB. Needles to add that NASB based kits are available for detection and quantitation of HIV-1 RNA and CMV RNA.
- **Ligase chain reaction (LCR):** Ligase chain reaction is in fact probe amplification technique. Here amplified products have only a sequence present in initial probes.

In ligase chain reaction a nick is formed when two oligonucleotides probe hybridize adjacent to one another in each side of denatured

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ISBN 978-93-6616-155-6

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