AUTOMATED ANALYSERS

-AIM:

To give theoretical background on different types, use, advantages and dis-advantages of automated analysers used in a diagnostic laboratory. A brief account on future trends of automation will also be given.

-What is automation and why do we need to know about it?

-History

-General advantages to automated procedures

-Basic approaches to automated analysers:

a)- Continuous flow analysers

b)- Centrifugal analysers

c)- Discrete auto analysers

d)- Dry chemical analysers

Advantages to automating procedures

-Increase the number of tests performed by one individual in a given time period (short turn around time)….speeds up the result

- Human factor is decreased during the mechanical and repetitive part of an assay as labor is an expensive commodity in Medical laboratories.

-To minimize the variation in results from one individual to another (for accuracy, coefficient of variation is reduced hence the reproducibility increases).

-The quality of patients test results is monitored continuously for improvement of testing process.

-Automation eliminates the potential errors of manual analyses such as volumetric pipetting steps, calculation of results, and transcription of results (human error is reduced).

-Instruments can use very small amounts of samples and reagents subsequently allowing less blood to be drawn from each patient. In addition, the use of small amounts of reagents decreases the cost of consumable.

BASIC APPROACHES WITH AUTOMATED ANALYZERS:

I-Continuous flow analyzers (nearly out of date)

-Liquids (reagents, diluents and samples) are pumped through a system of continuous tubing.

-Samples are introduced in a sequential manner, following each other through the same network. Series of air bubbles at regular intervals serve as separating and media. The internal diameter of the tubing and the rate of flow determine the volumes of sample prior to mixing with the reagents and the turn around time of the result.

-An oil heating bath is used to promote color development or the completion of enzymatic reaction

Principle of detection:

Detection is by measuring absorbency by spectrophotometer through a continuous flow cuvet (cell).

-When there is no sample, the sampler probe is placed in distilled water to avoid blockages and precipitation.

-More sophisticated continuous flow analyzers use parallel single channels to run multiple tests on each sample.

-For single channel machines, results are plotted on a chart recorder.

-For multi channel machines, computer and printers are used to report the results in the appropriate units.

Uses:

-Major use for certain test profiles (e.g. liver function, lipid function).

-Single channel machines may be used for frequently requested independent analysis (e.g. blood glucose, blood total protein).

Disadvantages:

-The machine does not allow test selection; all tests must be performed even if not requested.

-The machine must run continuously even when there are no tests.

-Because of the continuous flow, reagents must be drawn at all times even when there are no tests to perform; which results in reagent wasting. Therefore a good stock of reagents must be available to avoid system malfunction due to reagent depletion.

-The instrument must be closely monitored all the time for air bubbles uniformity; reagent availability and tubing integrity and most important of all carry over problems.

-Multi-channel machines are usually large in size and occupy large space.

II- Centrifugal Analyzer

-Samples and reagents are added in a specially designed centrifugal type cuvet that has three main compartments (see fig).

-Sample is added from the sample cup by auto-sampler into the sample compartment of the centrifugal cuvet.

- The reagent probe into the reagent compartment of the centrifugal cuvet adds Reagent.

- Both sample and reagents are allowed to equilibrate to the reaction temperature.

-Mixing of sample and reagent occurs when the rotor holding the cuvet is spun at high speed (4000 rpm) and then sudden stop. The spinning causes the sample to be added to the reagent while the turbulence caused by sudden stop results in mixing of sample and reagent.

-After mixing, the rotor is spun at 1000 rpm. The reaction mixture is pushed horizontally to the bottom of the cuvet.

Principle of detection:

which has clear transparent sides for spectrophotometric measurement.

Advantages:

- Rapid test performance analysing multiple samples. Batch analysis is a major advantage because reactions in all cuvets are read virtually

simultaneously.

- Use small sample (as small as 2μL).

- Use small reagent volumes (250 μL).

- Can be programmed to carry out many different assay methods.

Disadvantages:

- Only one test type can be performed each time.

- Each cuvet must be uniformly matched to each other to maintain quality handling of each sample

III- Discrete auto analyzers

Principle:

- Non-continuous flow using random access fluid which is a hydrofluorocarbon liquid to reduce surface tension between samples/reagents and their tubing and therefore reduce carry over.

- Discrete analysers have the capability to run multiple tests one sample at a time or multiple samples one test at a time. They are the most versatile analysers.

- Discrete analysis is a separation of each sample and accompanying reagents in a separate container.

- Each sample is treated differently according to the tests requested and programmed by the operator:

E.g. sample 1 glucose, urea, creatinine and electrolytes
sample 2 total protein, albumin, calcium
sample 3 triglycerides, cholesterol
Sample 4 bilirubin, ALT, AST, ALP

- These instruments are heavily dependent on electronic control.

- Sample is aspirated by the auto sampler from the sample cup and placed in the reaction cuvet. Samples are programmed or adjusted to reach a prescribed depth in

those cups to maximize use of available sample.

- Mixing of sample and reagents may be achieved by several methods such as:

    a)-Spinning of the cuvet at high speed followed by sudden stop.
    b)-Introducing the reagent into the cuvet by jet action.
    c)-Introducing air bubbles into the cuvet.

- The reaction chamber temperature is controlled for colour development or enzyme assay to proceed.

-The absorbency of the reaction in the reaction cuvet is read by a spectrophotometer, which is housed in the reaction chamber.

- Computer then calculates the results and produces it in printed format.

- Many of these machines have a Q.C system built in and automatically checks on the results of the Q.C samples to determine whether to accept or reject the results of the run.

- De-proteinisation is not performed to save time.

- Kinetic rather than endpoint methodologies are used (minimize protein error and give more accurate results)

- Some of these machines have the ability to store or file patient results.

- Some can be connected to lab or mainframe computers.

Uses:

-Analytes that can be measured by reflectance photometry include; glucose, BUN, ammonia, bilirubin, uric acid, cholesterol, triglycerides, total calcium, total protein, albumin, creatinine, phosphorus, and serum enzymes e.g. Kodak Ektachem.

Advantages:

Assay by reflectance photometry offers advantages:

-The storage requirements for reagents are minimal since no wet reagents are required.

-No pipetting steps are needed as the manufacturing company prepares the slides.

-No sample dilution is required and 10 or 11 μl of sample per test is used.

Dis-advantages:

- Since each sample is in a separate reaction container, uniformity of quality must be maintained in each cuvet so that a particular sample quality is not affected by the cuvet it is placed in.

IV-Dry Chemical Analyzers

Principle:

- Dry chemical methods utilize reagent slides that are composed of several layers which may include:
    1-spreading layer
    2-scavenger layer
    3-reagent layer(s)
    4-plastic or support layer

- The reagent layer(s) contains; enzymes, dye precursor, and buffers necessary for the analysis of a specific component.

- Sample, control, or standard is deposited on the spreading layer.

- Selected components are allowed to penetrate to the reaction layer(s), which in turn activate the dehydrated reagents.

- A chemical reaction is initiated to produce a colour.

- Light is passed from beneath the support or plastic layer and is directed through the reagent layer (s).

- As the light hits the white spreading layer, some of the light reflects back through the reagent layer(s) to a photocell.

- The amount of reflected light, which is in proportion to colour intensity, is used to determine the concentration of the analyte.

- A dry chemical method to determine sodium, potassium, chloride, and carbon dioxide (electrolytes) has been introduced which employs ion-selective electrodes (ISE) that are joined by a paper bridge:

    - A drop of reference sample and a drop of patient sample are deposited, each on its respective electrode.

    - The samples interact with coated reagent layers to create a pair of electrochemical half-cells.

    - The drops spread toward one another across the paper bridge, meeting at the center and forming a stable liquid junction.

    - A voltmeter measures the potential difference of the two half-cells, which is then used to determine the concentration values.

Uses

- Widely used in many clinical laboratories.

- Many offer the ability for the operator to include his own test procedures (open system).

- Examples; The Hitachi group of analyzers (Hitachi 717, Hitachi 917), The Technicon RA 1000.

Advantages

- Uses dry chemistry hence incurring minimum storage costs.

- ISE has a major advantage which allows a sample to be analysed for electrolytes separately even when the analyser is analysing a batch of other samples for various other tests.

Disadvantages

-Samples with abnormal high protein may introduce significant errors (sample dilution may be necessary).

AUTOMATED ANALYSERS

Important steps in automated analysis

1/- Specimen preparation and identification

- Mostly it’s a manual process in many laboratories.

- Sample must be centrifuged, transferred in analyser cup urgently to avoid delay in the testing process.

- Whole blood may be used to handle serum or plasma OR robotics may be introduced to handle the samples.

- The sample must be properly identified and its location in the analyser must be monitored through out the test.

- Sophisticated bar code system could be a solution to this problem. This label is affixed to primary collection tube. It contains patients’ demographics and also may include test requests.

- Bar code labeled tubes are transferred to the loading zone of the analyser where the bar code is scanned and the information is stored in the computer memory.

2/- Specimen measurement and delivery

- Round circular carousels or rectangular racks are used to hold standards, controls and patient specimens to be pipetted into the reaction chambers of the analyzers.

- The slots in the trays are numbered to ease sample identification.

- The racks move at pre-selected speed and samples are analyzed with the aid of computer which allows the aspiration only in slots containing sample cups.

- Volume of sample to be pipetted in each cuvet is determined by min and max indentation in the cuvet.

- With instruments measuring electrolytes, the CO_2 present in the samples will be lost to the atmosphere resulting in lower CO_2 values. Manufacturers have devised lid covers or individual cuvet caps that could be pierced to minimize this evaporation effect.

3/- Reagent system and delivery

- Reagents could be dry or liquid.

- Dry reagents could arrive as lyophilised powder or in a tablet form (some instruments have a tablet crusher on board).

- Delivery of reagents is analyzer type dependant (refer to the types of analyzers for more details).

4/- Chemical reaction phase

- This phase mainly consists of *mixing*, *separation*, *incubation* and *reaction*

time.

5/- Measurement phase

- UV light, fluorescent, flame photometry, ion-selective electrodes, gamma counters and luminometers are various methods for measuring product formed.

- Most common methods are still visible and UV spectrophotometer.

- Analyzers that measure light need monochromators to produce specific wavelength, classically filter wheels have been used to separate light, which are usually computer controlled.

6/- Signal processing and data handling

- Accurate calibration is essential to obtain reliable results

- This requires proper use of standards, which then reflect the data on standard curve according to which the sample results are interpreted.

- After the calibration has been performed and the chemical or electrical analysis of the specimen is either in progress or completed, the instruments computer goes into data acquisition and calculation mode.

- This process may involve signal averaging which may involve hundred of data pulses per second.

FUTURE TRENDS IN AUTOMATION

-Automation will continue to evolve.

-System integration and miniaturization with more technologically advanced computer power will persist to accommodate more portable analyzers for more precise testing.

-Automated analyzers will have artificial intelligence where by the computer will “think” or make decisions if sufficiently programmed with infinite scenarios of data.

-Spectral mapping or multiple wavelength monitoring, with high-resolution photometers and polychromators will become standard.