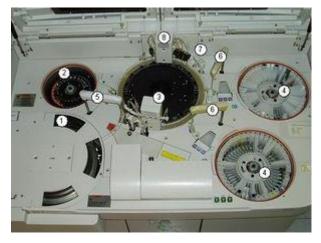# Biochemical analyzers

Biochemical analyzers entered clinical laboratory practice in the 1970s as a continuation of the process of mechanization of laboratory activities. Mechanization elements included the use of piston pipettes and piston valve dispensers to measure biological material and reagent dosing, pouring or flow cuvettes of photometers and varying the degree of mathematical processing of the measured signal (absorbance) of calibrators and samples with printing the final concentration of the measured analyte. Nowadays, in addition to biochemical analyzers used in routine operation of clinical-biochemical laboratory, also portable bench-type analyzers of various types are used. It is used for rapid determination in small laboratory operations, in general practitioners, specialist's offices, or directly at the patient's bedside. The latter application represents a very comprehensive, diverse and constantly evolving POCT (Point of Care Testing) system. A specific feature of POCT analyzers is their simple way of operation, highly developed system of self-diagnostics with a minimum requirement for calibration, inspection and maintenance by laboratory untrained medical personnel. The usual solution is the so-called cartridge system, where the inserted cartridge contains all necessary reagents, calibration and control materials, or other necessary disposable components such as measuring electrodes. POCT analyzers are subject to the so-called supervisory activities of laboratory staff. This is facilitated by their on-line integration into hospital networks, which enables remote administration, monitoring of their function, evaluation of automatically performed control analyzes and transfer of measured data to laboratory and hospital information systems. The price for quick availability and easy operation is significantly higher financial costs compared to classical laboratory examination.

Automatické analyzátory

The automatic analyzers were able to perform the individual steps of biochemical analysis according to a pre-programmed algorithm without operator intervention. It was transport of analytical sample, pipetting, reagent dosing, mixing, incubation, measurement of absorbance changes, calculation of concentration using calibration standards, displaying and printing of the result of biochemical analysis, eventually its transfer in electronic form to laboratory information system. In addition to the basic spectrophotometric principle of measuring absorbance changes, the analyzers use many other measurement principles, such as immunoturbidimetry, chemiluminescence, enzyme-linked immunoassay on microparticles,

fluorescence polarization immunoassay, etc. The standard part of biochemical automatic analyzers is the ion selective electrode (ISE) module for Na^+, K^+ and Cl^-.

Main components of the automatic analyzer:

View of the analyzer workspace.

1. a transport system transporting samples in racks to a serum pipettor,
2. a refrigerated rotor for calibrators, controls and static samples,
3. reaction cuvette rotor housed in a 37 °C water bath,
4. reagents (R1, R2) in barcoded containers; the cooled space is closed by a lid during operation,
5. serum pipettor,
6. reagent pipettor - dispenser,
7. rotary stirrer,
8. reaction cuvettes washing station.

Current automatic biochemical analyzers belong to the generation of selective random access analyzers, which allow free selection of dozens of methods per sample. The hourly performance of different types of analyzers is different. Values range from 100 to several thousand analyzes per hour. The so-called **analyzer clock**, is important for performance. It is the time in seconds after which the reaction cuvette is regularly paused, allowing the pipette to sample another sample. At 4 s, it is 15 tests per minute and 900 tests per hour. Regular function of analyzers is the possibility of preferential analysis in the so-called **statim sample**. The time response to these samples is calculated in minutes. The actions described are handled differently by different manufacturers, sometimes uniquely using patented procedures. With some

simplification in mind, the following is limited to the description of an example of a standard solution.

Analyzer components

The transport system transports the analytical samples from the analyzer inlet to the pipettor working area and after pipetting to the analyzer outlet. It is most often provided by moving the sample racks in a linear or rotary motion. A laser barcode scanner is located at the analyzer input. The barcode uniquely identifies the sample for connection to the patient's data in the laboratory information system and at the same time informs the analyzer about the type of analyzed material (serum, plasma, urine, cerebrospinal fluid, etc.).

The pipettor ensures that the sample is pipetted into the reaction cuvette. The pipetting needle is made of inert material and is equipped with a level sensor (conductivity, capacitance, radiofrequency system), which on contact of the needle with the surface of the sample stops the vertical movement of the pipettor and the sample is sucked just below the surface. Pipetting volumes are in the range of 2−20 μl. To avoid contamination, transfer (cary over), the pipetting needle is washed externally and internally in the wash station. Where very low concentrations are measured, the analyte being measured over a wide concentration range and there is a high risk of contamination with a false positive result, the analyzers use pipette tips that automatically change as the sample passes.

The reagent dispensers operate on the same principle, including level sensors and a washing station to prevent cross-contamination of the reagents. Dosing volumes are programmable to a range corresponding to the volume of the reaction cuvette, eg 20–300 μl. Precise metering of the sample volume and reagent dosing are ensured by piston dispensers that are connected to the end pipettors by tubing. Volumetric changes in the movement of the Teflon pistons are transmitted to the end needles of the pipettors.

The reaction cuvettes vary according to the material used, the shape, the volume and the method of use, either disposable, which is discarded after use or reused after automatic washing. The basic requirement is the UV permeability of the cuvette material, which is achieved by synthetic materials and quartz glass. Gradual miniaturization leads to cuvettes of less than 100 μl.

The incubation bath is the environment in which the reaction cells are located. The incubation temperature, particularly important for determining the catalytic enzyme concentration, is maintained at 37 °C with an accuracy of ± 0.1 °C. A homogeneous temperature environment is ensured in the incubation bath by circulating water, oil or air.

Light source - monochromator - absorption environment - detector. A halogen or xenon lamp is usually used as a light source, after passing through the absorption medium (cuvette), the light spectrum of the continuous spectrum is decomposed by a monochromator (optical lattice) into beams of defined wavelength (monochromatic radiation), which reach the detector, mostly **diode array**. The absorbance changes of the reaction mixture in the cuvette are monitored (recorded) each time the cuvette passes through the optical system beam.

Reagents. By default, automatic biochemical analyzers allow the use of two reagents per method (two-stage methods), 3 to 4 reagents can also be used. Reagents may be liquid-ready to use, less stable reagents prepared by dissolving the powder or tablet form before loading into the analyzer. They are stored in refrigerated rooms to increase their stability and reduce evaporation. The reagent containers are usually labeled with a bar code, which is registered by the analyzer and does not depend on the position of their storage in the reagent compartment. Before dispensing the reagent into the cuvette, the appropriate container is set to the pipetting position of the dispenser.

The stirrer ensures the mixing of the reaction mixture in the cuvette, for example by rotating the stirrer blade immersed briefly in the reaction cuvette.

The washing station aspirates the reaction mixture after completion of the measurement and rinses the cuvette repeatedly with water to finally dry it for further use.

Parameters - definition of methods. Each method has defined parameters: method of measurement (end point, kinetic), wavelength, volume of pipetted sample and dosed reagents, determination of measured points for concentration calculation, measurement of increase or decrease of absorbance, limit values for repeat analysis with larger or smaller sample volume at too high or too low concentration.

Display and transmission of results. The results of the analyzes are continuously displayed on the screen of the analyzer, printed on the analyzer printer, transmitted on-line to the

laboratory information system, or further in electronic form to the hospital information system for patient documentation.

The course of the reaction. The absorbance changes of the reaction mixture in the cuvette are continuously monitored and graphically recorded (reaction monitor). Graphic recording throughout the incubation period is particularly important when determining the catalytic concentration of enzymes, where it is possible to detect substrate depletion at high enzyme activity, non-linear, or otherwise altered course of the reaction.

Error messages, self-diagnostics. All analyzer operations and functions are programmed in the control computer software. The moving parts of the analyzer are usually moved by precision stepper motors. The correct functioning of the moving parts is constantly monitored by means of special sensors that monitor the end position and the time required to reach it. If the set positions and time limits are not observed, the analyzer stops with the corresponding error message. Water in the analyzer is driven by pumps and sucked off by a vacuum which is controlled by solenoid valves.

Today's electronically controlled automatic biochemical analyzers often have sophisticated software. Statistical evaluation of control analyzes in numerical and well-arranged graphical form and tools of internal quality control enabling selection and combination of **Westgard rules** are common. Another software application is, for example, **the reflex mode**, which allows automatic analysis of the pathological value of the ordained test.

A characteristic feature of the development of automated analytical systems in recent years has been the construction of hybrid systems connecting analyzers using different spectrophotometric principles with immunochemical (homogeneous and heterogeneous) immunoanalysis. Both types of analyzers were connected by a different conveyor system to transport biological samples and the measured data were collected in one result sheet.

Recently, manufacturers of automated laboratory systems have focused on developing and manufacturing automated and robotized lines that cover the pre-analytical and post-analytical phases of laboratory processes. So-called **total laboratory automation** became real and relatively accessible.

The pre-analytical process in the laboratory consists of:

- receipt and identification of biological sample,
- centrifugation,
- uncorking,
- aliquoting,
- labeling of aliquots with bar code
- sorting of primary and secondary samples (aliquots),
- on-line connection, direct access to the conveyor system of the analytical part.

Postanalytic process means:

- validation of analytical results,
- electronic transmission to the laboratory information system,
- archiving primary or aliquot samples.

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