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The Reflotron®-system—Principles and Practical Experiences

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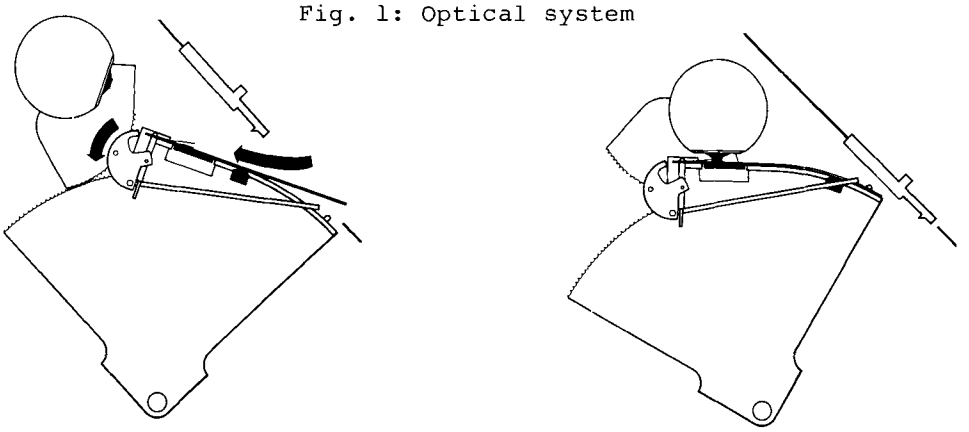
The Reflotron®-system consists of three major parts. First there is the microprocessor driven reflection photometer Reflotron®, second there are the different Reflotron® reagent carriers and third there is a special pipette for applying the sample onto Reflotron® reagent carriers.
The most important specifications for the Reflotron® instrument were:

- The achievement and maintenance of the general measuring temperature of 37°C with a maximum deviation of ± 0.1°C.
- The mechanical start of the reaction
- The control of all time and reaction steps.
- The self-calibration of the optical system and the measurement at the different wave lengths 565, 641 and 950 nm with an accuracy of ± 0.5% remission.
- The possibility to perform kinetic measurements
- The automated calculation and display of the measuring results in either conventional or SI-units.

Fig. 1 explains some of the optical and mechanical parts of the instrument. The cooperation of these two components demonstrates what happens from the moment of applying the sample onto the reagent carrier until the measurement of reflection takes place. The first step is the insertion of the reagent carrier, which is fixed at his upper position by a little pin.

Fig. 1: Optical system



Insertion of reagent carrier

Final measuring position

The next step is closing of the lid of the adapter, the reagent carrier then is fixed at his lower end with another pin.

By movement of the triangle shaped part of the optical system, the reagent carrier or in other words - the magnetic tape is transported along the magnetic reading head, thus transferring all necessary information to the instrument.

In the final position of both, the reagent carrier and the measuring Ulbricht's sphere the measurements can take place at the right time.
Another important feature of the Reflotron® instrument is that it works with two receiver diodes, one is working as an internal standard and thus allows to compensate for intensity differences or aging processes of the emitting diodes.

Just briefly mentioned should be the magnetic code on the rear side of the reagent carriers and the informations given with it.

- Which parameter is going to be analyzed,
- The length of pre-incubation and reaction phase
- Which measuring wavelength is to be used.
- The number of measuring points and intervals.
- Instructions for calculation of results.
- The factors from conventional to SI-units,
- A number of redundancy checks.

The last part of the system is that part of the reagent carrier, which contains the chemistry.

Some of the specifications for the reagent carriers are as follows:
- The clean separation of erythrocytes if blood is used as sample material.
- The separation of interferring constituents or in reverse the addition of activating substances.
- The possibility for any necessary pre-incubation time
- The possibility to start the reaction independent of sample application.
- The possibility to measure universal undiluted samples within the clinically relevant range with reliable sensitivity
- The long-term stability of reagents in dry phase.

One of the most important features of the reagent carriers is the blood-plasma separation. It is achieved by certain glass fibres which have been put into an appropriate geometric order and thus enable the plasma to separate in one direction only and to diffuse into the reaction zones.

This made it possible to replace the plasma separation by centrifugation by a simple diffusing step, integrated directly into the reagent carrier system.

When working with the system, one major handling step is nearly all one has to do. That means one has to apply 30 µl of sample onto the reagent carrier. The next thing one has to do is to insert the reagent carrier into the adapter of the instrument, then to close the lid of the adapter and after 2 or 3 min to read the result, which is automatically displayed by the instrument.

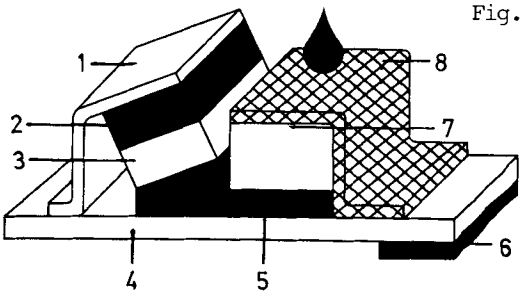


Fig. 2: Basic construction principle of reagent carrier

1 transparent protective layer
2 Indicator layer
3 Reagent layer
4 Support
5 Plasma reservoir
6 Magnetic tape
7 Plasma separation layer
8 Protective layer

Fig. 2 shows the construction principle of the reagent carriers. In detail from the right - first the plasma separation system, protected by a red mesh. This is followed by a zone, which contains or can contain auxiliary reagents like ascorbic acid oxidase or N-acetyl cystein. Next to it is the plasma reservoir and on the left there are the reaction zones which is e.g. in case of the Reflotron® BUN, separated by a hydrophobic mesh. Finally all is covered by a transparent foil, through which the optical measurements are being made.

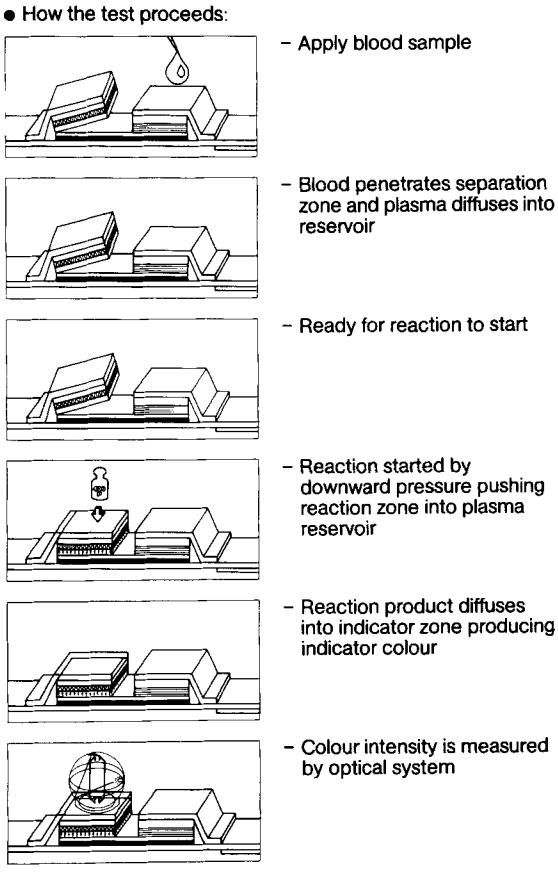


Fig. 3: Functioning of reagent carrier

Fig. 3 demonstrates how in six steps the main functioning principles of the Reflotron® reagent carriers can be illustrated.
The finally measured colour intensity is calculated into results by the means of calibration functions given to the instrument's software by the magnetic code mentioned before.
For setting up the calibration curves human sample material like serum or plasma is measured at different concentrations.

The concentration of the analyte is determined by well-known clinical chemistry methods and plotted versus the percentage remission values obtained with Reflotron®. This leads to a type of function which can mathematically be described with equations which are related to the equations derived from the Kubelka Munk theory.

If the equation is expressed in terms of concentration, this form of equation in general describes a hyperbola function. Although this type of equation fits well to the calibration curves of a number of Reflotron® reagent carriers, it is however better to use a more generalized type of equation, since it allows the introduction of a correction term and thus makes it easier to describe sigmoid shaped function curves.

$$C = A_0 + A_1R + A_2R^{-1} + A_3e^{n \cdot R}$$

First experiences have been encouraging and the results of a recently completed international multicenter study have shown the precision to be less than 5 per cent in the case of most of the evaluators and the methods investigated. The accuracy of the Reflotron®-system has shown to be good by comparison with established wet chemistry methods and the performance of the tests is not affected by variations in hematocrit (up to 50%), icterus, hemolysis or lipaemia.

At the moment the parameters
- Glucose
- Hemoglobin
- GGT
- Cholesterol
- Triglycerides and
- Urea

are ready for introduction.
Under evaluation are the following four parameters:
- AST
- ALT
- Uric acid and
- Creatinine

and amongst others under development are
- CK
- Amlyase
- LDH
- Bilirubin

just to mention a few of them.