Upsala J Med Sci 91: 165–168, 1986

Principles of the Leo Microcuvette System

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In order to minimize technical problems in performing analyses in practical clinical chemistry we have developed a system of disposable microcuvettes. The origin of the idea goes back to 1975-1977 when two laboratory engineers in Kristanstad, Sven-Erik Nilsson and Jan Lilja, wanted to improve the precision of blood hemoglobin determination in primary health care.

At that time the instrumentation was based on simple analogue photometers of varying age and manufacturing brands using different equipment for dispensing reagents and blood samples. Since several years the central hospital laboratory had a quality control and service system for primary health care in the whole county. Many hours were laid down on technical errors. Of course these were necessary to correct, but the problems themselves often seemed unnecessary and trivial.

Most errors were related to sample handling, i.e. measuring and dilution. The reagent could be inactive or handled in the wrong way or the volume of reagent incorrectly dispensed. Most faults in the photometers were due to incorrect adjustment, because the regulation knobs had been touched accidentally. As there were few electronic components at that time, these errors were rare, usually only a bad lamp. However, the instruments could be very sensitive to interference due to static electricity or varying net voltage.

The basic demands for an innovative system were rather clear:

-- no dilutions or volume measurements
-- good stability
-- no daily adjustment and calibration of the measuring instrument
-- simple handling

A film technique for hemoglobin determination proved to implicate technical problems. Even though complicated algorithms may be handled by microproces-

sors, Lamberth-Beer's law is much simpler to handle for measuring purposes. Instead we launched another idea, a disposable plastic cuvette for photometry absorbance with "built-in" reagents and a possibility for sampling of blood directly into the measuring cuvette. The old Sicca technique for hemoglobin determination may be recognizable in the new system, but the drawbacks are avoided (difficult and non-adjustable calibration, non-disposable equipment, risk of virus transmission).

In the beginning all manufacturers in the plastic industry gave us the same concordant reply that it would be impossible to produce microcuvettes with our demands on high accuracy and reproducibility. We even met humorous comments when asking for a production of several million units per year. They explained to us that the measuring cavity in the hemoglobin cuvette is formed by a metal tongue, which is thick as a stamp - with glue. The moulding plastic has the same viscosity and consistancy as cold syrup. It will hit the metal tounge as if literally shot out of a pistol. Furthermore, the mould will set around the nucleus under a pressure of several tons. The nucleus must then be pulled out of the completed cuvette. With the help of a grant from The Society for Technical Development in Sweden, we could get started and see for ourselves that it was difficult - but maybe not impossible. Since then we have tested a number of cuvette designs. Obviously the choice of design and material is critical.

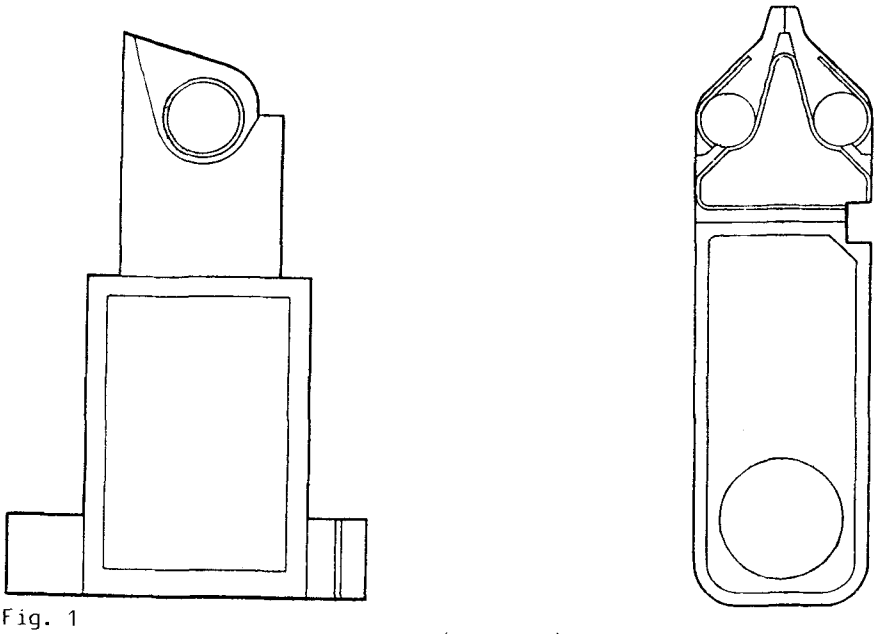

Fig. 1
Disposable microcuvettes. Type I (standard) with one and type II with two measuring cavities

For single analyses (e.g. hemoglobin) we mainly use cuvettes of type I. If a reference is needed or if one can combine two analyses in a natural way, we use cuvette type II. It is also possible to make special designs for very small sample volumes or for introduction of a special substance in the cavities. This will delay the fluid access through the inlet, which will make possible a two step addition of reagents.

The cuvettes are pressure moulded in polystyrene, either in one part or in two parts joined together with ultrasonic welding. Other types of plastic may be used, but price and properties are favourable for polystyrene. In general plastic cuvettes moulded in one part have a somewhat better exactness for small depths, but may cause trouble during reagent filling. Our minimum cuvette depth is 0.20 mm for welded cuvettes and 0.13 mm in a one-piece mould (e.g. the hemoglobin cuvette). The thickness tolerance is $\pm$ 0.001 mm. All four surfaces in the optic eye must be smooth. Complicated cuvettes cannot be moulded in one piece as there must be a nucleus during moulding, which should then be pulled out of the cavity.

Already the plastic raw material, i.e. the plastic cuvette without reagent, is quality control tested for function, depth and other measurements. Every individual cuvette is measured in the production line with the help of an internal standard (i.e. a coloured fluid), which does not interfere with the final measurement.
For quality assessment, imprecision and inaccuracy are evaluated together. The limits for acceptance for hemoglobin cuvettes are $\pm$ 1.5 per cent for inaccuracy and 1.5 per cent for coefficient of variation. Of course, these extremes cannot be accepted together. A high CV can only be accepted if inaccuracy is accordingly low. More than 95 per cent of the cuvettes are better than 2.5 per cent deviation from the reference level.

The reference method for blood hemoglobin determination is the methemoglobin cyanide method, thoroughly investigated by van Kampen and Zijlstra (1). A similar azide technique was suitable for the microcuvette system (2). This method is a chemical analogue of the cyanide method, as both anions contain nitrogen, which will bind to a stable $iron^{III}$-complex in hemoglobin. For oxidizing hemoglobin to methemoglobin we exchanged ferricyanide against nitrite, which initiates an auto-oxidation of hemoglobin. Nitrite can penetrate the red cell membrane, starting methemoglobin formation already before hemolysis of the red cell membrane occurs. The affinity of hemoglobin is somewhat lower for azide than for cyanide, but the reaction time may be

brought down to 30 - 45 seconds and the final result displayed within 60 seconds.

Speed of reagent dissolution is very important for kinetic determinations and also for endpoint determinations in order to avoid long reaction times. If the reagent itself is not highly soluble, a more soluble substance may be admixed in order to disrupt the reagent layer.

All problems cannot be solved by changing cuvette construction or reagent composition. Therefore the photometer forms an important part of the total system. It should be simple to handle and include automatic blanking. Furthermore, it may be equipped with a turbidity compensation. This is carried out with the help of a two-wavelength algorithm, where the reference wavelength must be outside the colour range of the final reaction product. The hemoglobin photometer has such a function with a reference wavelength of 880 nm. Not only scratches and dust particles in the cuvette will be compensated for, but also turbidity originating in the sample to be tested, e.g. hyperlipemic blood or serum.

REFERENCES

1. van Kampen, E.J. & Zijlstra, W.G.: Standardization of hemoglobinometry. II. The hemoglobincyanide method. Clin Chim Acta 6: 538-544, 1961

2 Vanzetti, G.: An azide-methemoglobin method for hemoglobin determination in blood. J Lab Clin Med 67: 116-126, 1966