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EVALUATION OF GLUCOSE MEASUREMENT METHOD USING MULTILAYER FILM ANALYTICAL ELEMENT, DRICHEM 1000®

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INDEXING WORDS

glucose, multilayer film analytical element, whole blood, DRICHEM 1000®

SYNOPSIS

A multilayer film analyzer has been recently[1] developed for a clinician to know the blood glucose levels of his patient. Its accuracy and simplicity in measuring the blood glucose concentrations has been compared with the previously employed wet chemistry. It, with some modifications, has been applied for clinical purposes.[4, 5]

Here we used the multilayer film analysis element to measure glucose concentrations in animal blood and serosal fluid during animal studies, conducted in addition to the clinical study.

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MATERIALS AND METHODS

We used DRICHEM 1000®, which was employed as the incubator-photometer equipment and film elements (GLU-W and GLU-P) from Fuji Photo Film Company.

A multilayer film element is composed of four layers including a spreading layer, a blocking layer, a reagent layer, and transparent support (Fig. 1). The highly porous spreading layer has an active layer surface over which blood spreads quickly and uniformly after spotting. Blood cells and platelets are filtered off through the fibrous structure of this layer, but the plasma diffuses through the blocking layer. The blocking layer blocks hydrophobic substances and macromolecular constituents and, at the same time, serves as a reflector for reflection densitometry and a shield from interference by the colored materials in blood. The reagent layer in which enzymes and color-developing reagents are incorporated as a solid phase provides the analytical basis of glucose measurement by this system. A series of reactions first reported by Trinder[6] occurs in this layer as shown in Fig. 2: oxidation of glucose is catalyzed by glucose oxidase (EC 1.1.3.4), followed by oxidative coupling catalyzed by peroxidase (EC 1.11.1.7).

The procedure for measuring the glucose concentration is shown schematically in Fig. 3. The applied sample (6 µl for whole blood, 10 µl for plasma) is diffused through the reagent layer after the cell components and macromolecules are filtered off in the preceded two layers by the process mentioned above. In the reagent layer, reacting with Trinder reagent during incubation, the sample develops color according to the glucose concentrations contained. Then the sample is illuminated at 45C from below and color density is measured by reflection at 500 nm. The reflection density is converted into plasma glucose concentration (mg/dl) based on the predetermined calibration curve, which is stored in the memory of a microcomputer incorporated in the equipment.

Blood samples were collected from out patients in Kobe University Hospital, and from dogs during animal experiments. Serosal fluid was also collected serially for glucose measurement during in vitro glucose absorption testing using everted sac of rat intestine.

We used AUTO STAT® (Kyoto Daiichi Kagaku K.K.) as the comparison method for plasma glucose analysis.

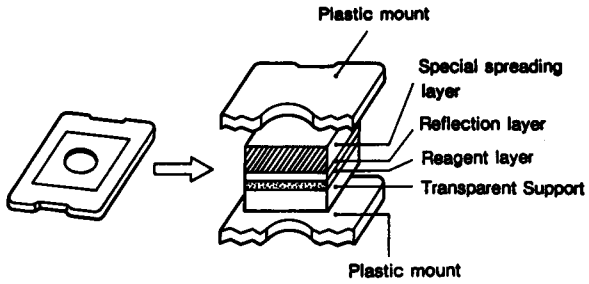


Fig. 1 Basic concentrations of the multilayer-film analysis slide.

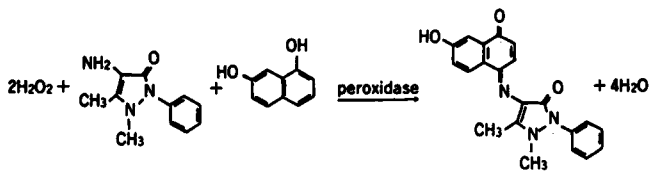


Fig. 2 Sequential enzymatic reaction.

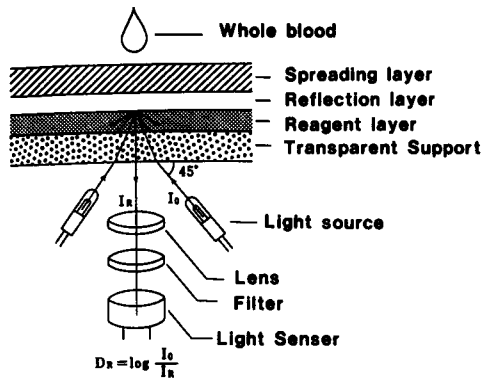


Fig. 3 Schematic diagram of analytical procedure.

RESULTS

Comparison of the DRICHEM system with AUTO STAT using patients' whole blood (n=147) revealed a good correlation (r=0.991) on glucose concentration, as shown in Fig. 4.

The correlation between glucose levels measured with whole blood using GLU-W and those with plasma from the same sample using DRICHEM 1000® GLU-P in diabetic patients (n=102) was good, as

shown in Fig. 5.

Comparison of the results from the expect group with those from the unskilled group using two kinds of blood samples of known glucose concentrations revealed no significant difference. The variation coefficients were 1.4% and 1.7%, respectively.

Fig. 6(A) shows the comparison of DRICHEM with AUTO STAT in measuring glucose concentration using whole blood of dogs (r=0.983). After input of the calibration curve regarding the low viscosity of the sample, the correlation between them improved (r=0.979), as shown on Fig. 6(B).

During the in vitro glucose absorption test using everted sac of rat small intestine (Fig. 7), we were able to measure glucose concentrations serially with a small volume of serosal fluid, with reliable results (Fig. 8).

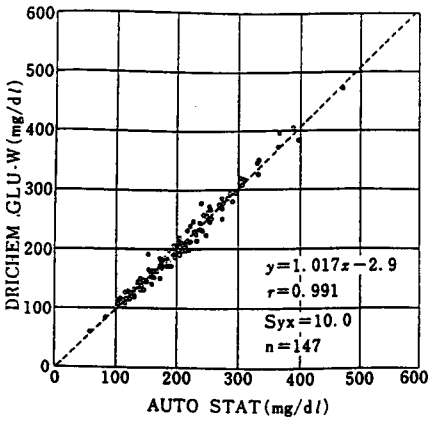


Fig. 4
Correlation between DRICHEM GLU-W and AUTO STAT (routine method).

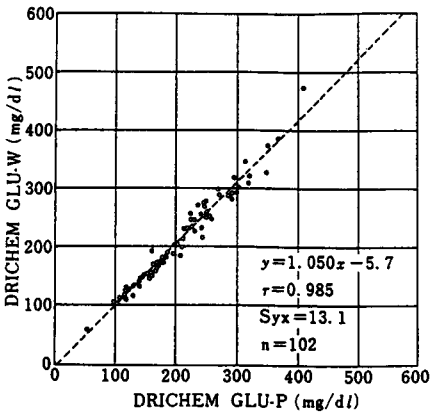
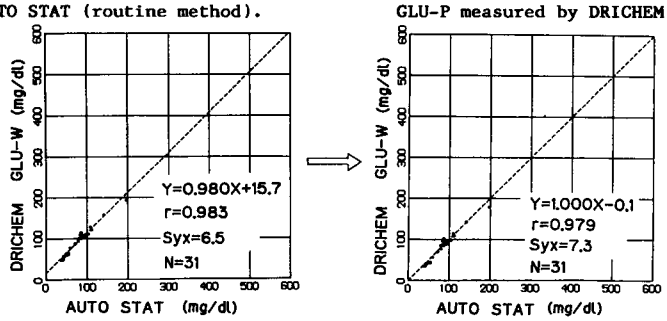


Fig. 5
Correlation between GLU-W and GLU-P measured by DRICHEM.



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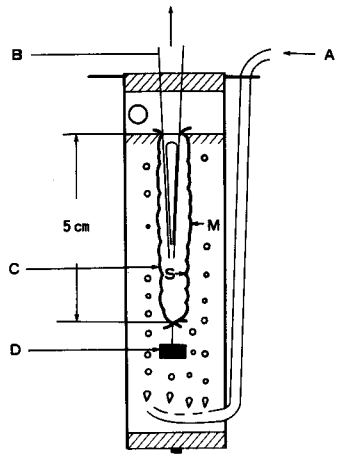

A : Tube for inlet of gas (95%O_2, 5 %CO_2)
B : Tip of Beckman's Autoanalyzer with 3 holes.
C : An everted sac of rat small intestine.
D : Sinker.
S : Serosal side
M : Mucosal side

Fig. 7
Incubation apparatus.

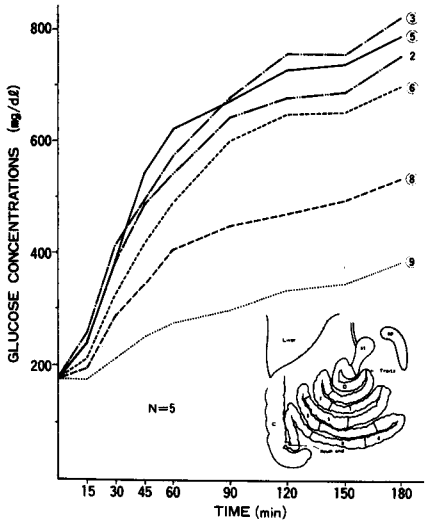

Fig. 8
Time course of glucose concentration of everted intestinal sac (serosal site).

DISCUSSION

Various methods and equipments have been developed for the measurement of glucose concentration in blood, and applied clinically. However, even to day it remains a problem to check blood glucose levels in massive samples, rapidly and accurately at one time.

Wet chemistry using liquid reagents has provided us with a means to measure blood glucose concentrations automatically, in massive samples with accuracy. However, wet chemistry needs time, equipment and labour involving pre- and post-treatment of samples and regents. In addition, the process of calculation of the necessary reagents differes between examiners.

The multilayer film analysis element makes it possible to check blood glucose concentrations rapidly in massive samples, because there is no need to treat the samples or equipment before or after measurement. For one, all the necessary reagents are incorporated into the dry film, and other merits include simplicity and easiness of handling, as well as minimalized differences between examiners. Moreover, there is a good correlation between

this system and wet chemistry using human blood as the specimen, as shown above (see results) and in other reports,[2, 3, 4] which makes it a reliable method for accurate measurement of blood glucose concentration.

As shown above, it may also be possible to apply this method to animal experiments. The blood sample is to some extent different in liquid properties from that of human, but an input in the calibration curve will account for such difference.

As this system requires only a small volume of specimen, it may become a useful method in pediatric fields and in vitro experiments in which frequent sampling is required.

CONCLUSION

We applied DRICHEM to a separate computer system (NEC PC9810) and input the measured data for analysis of clinical study and use in patients, education.

The multilayer film analysis element system can satisfy specifications as a accuracy, simplicity, rapidity, and minimization of sample volume. Accordingly, it will be suitable for clinical use especially in the operating room, in emergency, and at the bed side, and also for animal studies with minor modifications. However, the narrow range of measurable glucose concentrations, error due to viscocity or certain anticoagulants, and size and expense of the equipment are the disadvantages to be corrected.

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