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A Microplate Method for Determination of Glucose in Plasma

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For batch analysis of plasma glucose in large number of samples, a microplate method was developed. After one volume of plasma and three volume saline were mixed in microplate, 10 μl mixture was transferred to another microplate and 250 ul hexokinase reagent was added by a robotic diluter (Tecan 8000). This reaction mixture was incubated for 5 minutes at room temperature to reach the end point of the reaction. The glucose concentration in this reaction mixture was then determined by an ELISA reader at 340nm (refernce 620nm). The within-run and between-run coefficients of variation for this method were 3.7-5.5% and 5.5-7.2%, respectively. Analytical recovery of glucose ranged from 96% to 99%. The test was linear up to 300 mg/dl and the detection limit was estimated to be around 7.2 mg/dl. The correlation of this method and the routine method (Gilford 103 system) was good. This rapid and sensitive method could measure more than 500 specimens per hour. It is suitable to be used for mass screening and epidemiological study of diabetes mellitus.

INTRODUCTION

Since measurement of absorbance in determination of plasma glucose in routine clinical analysis is relatively slow (approx. 3-5 samples/min), a microplate method for fast determination of blood glucose in plasma was developed. The determination of glucose in plasma was based on the hexokinase reaction (1-5). The method had good precision and the results correlated well with those determined with a routine method in which the same reaction is used. In our laboratory, this technique has been used successfully for more than one year.

MATERIALS AND METHODS

Equipments

ELISA reader from SLT (Lab Instruments Ges. m. b. H., Salzburg, Austria), Tecan robotic diluter (model 8000) from Tecan (Landhaus Holgass, Hombrechtikon, Switzerland) and Gilford 103 systems from Ciba Corning Diagnostic Corp.

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