

pared with a conventional absorbance damping method. Measurement conditions are as shown in Example 1.

As a conventional absorbance damping method, "Ketone Test A (Sanwa)" manufactured by Sanwa Kagaku Kenkyusho for measuring acetoacetic acid in blood was used according to the attached explanation, and measurement was carried out by means of the automatic analyzer, Hitachi Model 7150.

Pooled normal blood serums to which 50 μ M, 100 μ M and 200 μ M of AcA were added respectively were used as samples, and the assay precision was compared in terms of coefficients of variation in, absorbance to be obtained by measuring the same specimen 10 times concurrently.

The results are shown in Table 3.

In comparison with the absorbance damping method, it was confirmed that the method of the present invention is remarkably precise.

TABLE 3

	Simultaneous Reproducibility		
	50 μ M AcA added serum	100 μ M AcA added serum	200 μ M AcA added serum
absorbance damping method			
n	10	10	10
Max.	0.0200	0.0270	0.0491
Min.	0.0156	0.0238	0.0441
S.D.	0.00148	0.00104	0.00175
Mean	0.01778	0.02521	0.04591
C.V. (%)	8.32	4.13	3.81
Method of the present invention			
n	10	10	10
Max.	0.0575	0.0707	0.0929
Min.	0.0561	0.0675	0.0904
S.D.	0.00046	0.00093	0.00069
Mean	0.05703	0.06915	0.09202
C.V. (%)	0.81	1.34	0.75

EXAMPLE 5

Interference to Measured Values by the Lactate Acid Dehydrogenase in Samples

The absorbance of the pooled normal serum to which 5000 IU/l of lactate dehydrogenase and 2 mM of pyruvic acid or 20 mM of lactic acid were added were compared with the absorbance obtained by the measurement according to the present invention using the pooled serum with no additional component. The results are shown in Table 4. Measurement conditions are as in Example 1.

According to the method of the present invention, even if lactate dehydrogenase enzyme and as its substrate, pyruvic acid or lactic acid exist at a high concentration, no change was observed in the measured absorbance. This shows that their affection is completely avoided.

TABLE 4

	Interference to Measured Values by the Lactate Dehydrogenase in the Samples		
	Pooled serum with noting added	Lactate dehydrogenase and Pyruvic acid (2 mM)	Lactate dehydrogenase Lactic acid (20 mM)
Measured absorbance	0.0477	0.0470	0.0464

EXAMPLE 6

Correlativity

The precision of the measured values of the total ketone body in the present invention was confirmed in terms of the correlativity with known methods. Known methods are as shown below.

1. AcA sample reagent (absorbance damping method)

Manufacturer: Sanwa Kagaku Kenkyusho

Trade name: "Ketone Test A (Sanwa)" for measuring acetoacetic acid in blood

2. 3-HB sample reagent (absorbance increasing method)

Manufacturer: Sanwa Kagaku Kenkyusho

Trade name: "Ketone Test B (Sanwa)" for measuring 3-hydroxybutyric acid in blood

The method of the present invention was carried out under the conditions described in Example 1, and the measurement of the known methods was carried out by means of the automatic analyzer (Hitachi Model 7150) using each of the above reagents according to the attached explanation.

Correlativity between the sum of the results of the measurement according to "Ketone Test A (Sanwa)" and "Ketone Test B (Sanwa)" and the measured values in the present invention is shown in FIG. 5.

According to the results, nearly $Y=X$ and the coefficient of correlation is nearly 1. It is revealed that the method of measurement according to the present invention has a high correlativity with the conventional methods and exhibits very high reliability.

EFFECTS OF THE INVENTION

As shown in the above Examples, the method of assay according to the present invention has an extremely high precision and can be easily applied to an automatic analyzer. Namely, according to the method of the present invention, the assay of the total ketone body, particularly AcA, which has been a problem, has become simple and highly precise. In addition, the assay having high reliability by means of an automatic analyzer has become possible without any pre-treatment of samples.

What is claimed is:

1. A method of assaying the total ketone body in a sample, which is applicable to an automatic analyzer and comprises the steps of:

(1) converting acetoacetic acid in the sample to 3-hydroxybutyric acid with the aid of 3-hydroxybutyrate dehydrogenase in the presence of reduced-type nicotinamide adenine dinucleotide, said reaction being conjugated with another reaction in which the resulting oxidized-type nicotinamide adenine dinucleotide is used as a coenzyme to form its reduced-type;