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## COMPOSITION AND METHOD OF ASSAYING FOR KETONE BODIES

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### FIELD OF THE INVENTION

The present invention relates to a composition and  
method of determining the presence or concentration of  
D- $\beta$ -hydroxybutyrate (DHBBA) in particular, and of ketone  
bodies in general, in a test sample. More particularly, the  
present invention relates to a new and improved method of  
assaying a liquid test sample, such as urine, whole blood,  
blood plasma or blood serum, for ketone bodies, specifically  
D- $\beta$ -hydroxybutyrate, by utilizing a stable and sensitive  
indicator reagent composition. The enzyme-based indicator  
reagent composition undergoes a detectable or measurable  
response upon contact with a test sample containing D- $\beta$ -  
hydroxybutyrate. The indicator reagent composition of the  
present invention provides a more accurate and sensitive  
assay for D- $\beta$ -hydroxybutyrate, and therefore ketone bodies,  
by effectively resisting the interfering affects of common test  
samples components, like glutathione and ascorbate ion, on  
the indicator dye. Accordingly, the improved sensitivity  
achieved by the stable indicator reagent composition of the  
present invention provides an improved method of assaying  
for ketone bodies, like D- $\beta$ -hydroxybutyrate, in a test  
sample, like a biological fluid, such as whole blood, blood  
serum, blood plasma, or urine.

### BACKGROUND OF THE INVENTION

The body usually completely metabolizes fats to carbon  
dioxide and water. However, if an inadequate amount of  
carbohydrate is present in the diet, or if a defect in carbo-  
hydrate metabolism or absorption is present, the body then  
metabolizes increasing amounts of fatty acids. When large  
amounts of fatty acids are metabolized, fatty acid utilization  
is incomplete. Therefore, the intermediate products of fat  
metabolism appear in the blood and are excreted in the urine.  
These intermediate products are termed ketone bodies; and  
include acetoacetic acid, acetone, and  $\beta$ -hydroxybutyric  
acid. In addition, stress, physical exercise and diabetes can  
cause an accelerated decomposition of fats and oxidation of  
fatty acids to increase the concentration of  $\beta$ -hydroxybutyric  
acid, acetone and acetoacetic acid in the blood and urine.  
Accordingly, the assay for ketone bodies in a biological fluid  
can be helpful in the diagnosis, treatment and monitoring of  
diabetes.

The concentration of ketone bodies present in the urine  
and blood of a healthy individual is very low to negligible.  
Whenever increased amounts of fats are metabolized, such  
as when the carbohydrate intake is restricted or when the diet  
is rich in fat, the concentration of ketone bodies can increase.  
If an excess amount of ketone bodies is present in the blood,  
the condition is termed ketosis; and if an excess of ketone  
bodies is present in the urine, the condition is termed  
ketonuria. Ketonuria also is observed from the restricted  
carbohydrate intake that occurs with fevers, anorexia, gas-  
trointestinal disturbances, fasting, starvation, cyclic vomit-  
ing, pernicious vomiting of pregnancy, cachexia, postanes-  
thesia, and as a result of certain neurologic disorders. In  
general, all three ketone bodies are present in the urine of  
individuals with ketonuria in the relative proportions of 20%  
acetoacetic acid, 2% acetone, and 78%  $\beta$ -hydroxybutyric

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acid. Acetone and  $\beta$ -hydroxybutyric acid are derived from  
acetoacetic acid.

Diabetes mellitus is the most important disorder associ-  
ated with ketosis or ketonuria. Diabetes mellitus is a disorder  
of glucose metabolism, and, in insulin-deficient diabetes,  
usually the juvenile-onset type, glucose metabolism is suf-  
ficiently impaired such that fatty acids are utilized to meet  
the energy requirements of the body. If diabetes mellitus is  
untreated, or is inadequately treated, excessive amounts of  
fatty acids are metabolized. Consequently, ketone bodies  
accumulate in the blood, i.e. ketosis, and are excreted in  
urine, i.e. ketonuria. In addition, ketone bodies are excreted  
from the body in combination with normal basic ions,  
thereby reducing in the carbon dioxide combining power of  
the body and causing systemic acidosis, i.e. increased acid-  
ity of the blood. Progressive diabetic ketosis causing dia-  
betic acidosis can lead to coma, and eventually death. The  
term ketoacidosis is frequently used to designate the com-  
bined ketosis and acidosis conditions associated with dia-  
betes.

Thus, detection of ketosis or ketonuria in an individual  
with diabetes mellitus is important and often indicates that  
a change in insulin dosage or other management procedures  
is necessary. Therefore, during periods of acute infections,  
surgery, gastrointestinal disturbances, or stress, and when-  
ever the management routine does not adequately control the  
disease, the blood or urine of a diabetic individual should be  
checked for the presence of ketone bodies.

Usually, the presence of ketone bodies has been detected  
by assaying urine. In ketonuria, the acetoacetic acid, acetone  
and  $\beta$ -hydroxybutyric acid are excreted in the urine. Con-  
sequently, an assay procedure that detects or measures the  
presence of one of the three ketone bodies usually is  
satisfactory for the diagnosis of ketonuria. Although specific  
tests exist for the determination of each of the ketone bodies,  
the specific tests usually are not used because the methods  
are more cumbersome, less reliable and less sensitive than  
the general assay for ketone bodies.

For example, the nitroprusside ion,  $(\text{Fe}(\text{NO})(\text{CN})_5)^{-2}$ ,  
interacts both with acetone and acetoacetic acid in the  
presence of alkali to produce a purple-colored compound.  
Thus, sodium nitroprusside assays are specific to acetoacetic  
acid and acetone and do not detect  $\beta$ -hydroxybutyric acid.  
This nitroprusside interaction forms the basis of a number of  
different prior art assays, such as Rothera's test and Legal's  
test. The present day reagent strip method is the simplest  
technique for determination of ketonuria. The reagent strip  
is impregnated with sodium nitroprusside and alkaline buff-  
ers. The strip is dipped into fresh urine, then compared to the  
color chart after exactly 15 seconds. The chart has six color  
blocks indicating negative, trace (5 mg/dL), small (15  
mg/dL), moderate (40 mg/dL), large (80 mg/dL), or (160  
mg/dL) concentrations of ketones, and ranging in color from  
buff to lavender to maroon. However, the nitroprusside assay  
method does not measure the concentration of  $\beta$ -hydroxy-  
butyrate, the major ketone body. Accordingly, the assay  
result can be a misleading determination of the total amount  
of ketone bodies in the urine.

For example, studies have shown that assaying urine for  
acetoacetate by the nitroprusside method failed to detect  
ketonuria in from about 50% to about 60% of diabetic  
individuals actually having ketonuria. Coupling this fact of  
misdiagnosis with the fact that assaying urine for ketones  
already is a delay in detecting blood ketosis, makes it  
obvious that a urine assay for acetoacetate is not sufficient to  
monitor the onset of ketosis in a diabetic individual. Accord-