

mmol/L DHBA in a whole blood assay, and from 0 mmol/L to about 60 mmol/L DHBA in a urine assay.

The dry phase test strip assays of the present invention for DHBA are most useful for assays performed at home, in private physician laboratories and in emergency rooms. For example, the dry phase test strip assay for DHBA in whole blood could replace urinary ketone test strip assays or tablet assays in the emergency room diagnosis of ketoacidosis. In addition, besides the greater convenience in using blood rather than urine in the emergency room, the whole blood test for DHBA also would diagnose alcoholic ketoacidosis. Such a result is extremely important because in cases of alcoholic ketoacidosis, the conversion of acetoacetate to β -hydroxybutyrate is essentially complete. Therefore, urinary tests for acetoacetate usually are negative. As a result, the diagnosis of alcoholic ketoacidosis often is missed.

Therefore, the indicator reagent composition of the present invention, comprising a thiol-responsive indicator dye; LADH; DHBA dehydrogenase; D,L-lipoamide; and NAD is sufficiently stable and selective in reactivity to provide an accurate and sensitive DHBA assay. The indicator reagent composition of the present invention also undergoes a more spectacular color transition in response to the concentration of DHBA in a test sample. In general, therefore, an indicator reagent composition of the present invention demonstrates an ability for DHBA dehydrogenase and LADH to interact with their respective substrates sequentially; demonstrates improved stability and selectivity and therefore eliminates the development of an interfering background color in the test pad due to an interaction between the indicator dye and an interferent in the test sample; and increases the useful life of the test strips because of the stability of the indicator dye.

Obviously, many modifications and variation of the invention as hereinbefore set forth can be made without departing from the spirit and scope thereof and therefore only such limitations should be imposed as are indicated by the appended claims.

I claim:

1. A method of determining the presence or concentration of D- β -hydroxybutyrate in a test sample consisting essentially of:

(a) contacting the test sample with a composition consisting essentially of D- β -hydroxybutyrate dehydrogenase, nicotinamide adenine dinucleotide, a disulfide reductase system consisting essentially of a single disulfide substrate and a disulfide reductase, and a thiol-responsive indicator dye; and

(b) determining the presence or concentration of D- β -hydroxybutyrate in the test sample from the intensity and degree of a color change of the composition.

2. The method of claim 1 wherein the intensity and degree of the color change is determined visually or instrumentally.

3. The method of claim 1 wherein the presence or concentration of D- β -hydroxybutyrate is determined by a dry phase assay.

4. The method of claim 1 wherein the test sample is a biological fluid.

5. The method of claim 4 wherein the biological fluid is whole blood, blood plasma, blood serum or urine.

6. The method of claim 5 wherein the presence or concentration of D- β -hydroxybutyrate in urine is determined in the range of from 0 mmol/L to about 60 mmol/L.

7. The method of claim 5 wherein the presence or concentration of D- β -hydroxybutyrate in whole blood, blood plasma or blood serum is determined in the range of from 0 mmol/L to about 10 mmol/L.

8. The method of claim 1 wherein the D- β -hydroxybutyrate dehydrogenase is present in an amount ranging from about 50 units to about 5000 units.

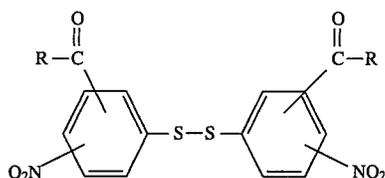
9. The method of claim 1 wherein the nicotinamide adenine dinucleotide is present in a concentration ranging from about 10 mM to about 500 mM.

10. The method of claim 1 wherein the disulfide substrate is present in a concentration ranging from about 10 mM to about 200 mM, and the disulfide reductase is present in an amount ranging from about 100 units to about 2000 units.

11. The method of claim 1 wherein the thiol-responsive dye is present in a concentration ranging from about 10 mM to about 200 mM.

12. The method of claim 1 wherein the disulfide reductase system includes D,L-lipoamide as the disulfide substrate and lipoamide dehydrogenase as the disulfide reductase.

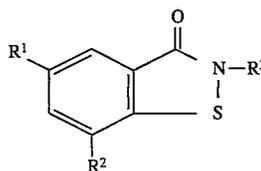
13. The method of claim 1 wherein the thiol-responsive dye is Ellman's reagent, an amide derivative of Ellman's reagent or an ester derivative of Ellman's reagent, wherein amide and ester derivative of Ellman's reagent is represented by the structural formula



wherein R is the residue of an alcohol or an amine; or a combination thereof.

14. The method of claim 1 wherein the thiol-responsive dye is a 2,2'-dipyridyl disulfide or a 4,4'-dipyridyl disulfide.

15. The method of claim 1 wherein the thiol-responsive dye is an isobenzothiazolone compound having the formula:



wherein at least one of the R_1 and R_2 substituents is selected from the group consisting of nitro, arylazo, substituted arylazo, benzylideneamino and substituted benzylideneamino; and the R_3 substituent is selected from the group consisting of alkyl, carboxyalkyl, hydroxyalkyl, aminoaryl, heteroaryl, carboxyheteroaryl, hydroxyheteroaryl, amino-heteroaryl, hydroxy, alkoxy, amino and substituted derivatives thereof.

16. The method of claim 15 wherein when the R_1 substituent is nitro, arylazo, substituted arylazo, benzylideneamino or substituted benzylideneamino, the R_2 substituent is hydrogen.

17. The method of claim 15 wherein when the R_2 substituent is nitro, arylazo, substituted arylazo, benzylideneamino or substituted benzylideneamino, the R_1 substituent is hydrogen.

18. The method of claim 1 wherein the composition has a pH in the range of from about 7 to about 8.5.

19. A method of determining the presence or concentration of d- β -hydroxybutyrate in a test sample consisting essentially of:

(a) contacting the test sample with an indicator reagent composition consisting essentially of: