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DIAGNOSTIC COMPOSITION FOR THE QUANTITATIVE DETERMINATION OF GLUCOSE

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This invention relates to new and improved diagnostic compositions. Particularly the invention relates to diagnostic compositions which are useful for the quantitative determination of glucose in fluids, particularly body fluids, such as urine, plasma, blood, and the like.

The detection of glucose in urine as well as the determination of its concentration therein is of great importance for diabetic patients who must control their diets so as to regulate their sugar intake and who must frequently be guided in this regard by a regular check on urine glucose. But beyond its usefulness in regular urine testing on known diabetics by both patients and physicians, this glucose indicator may also be used efficiently in routine urinalyses in hospitals and physicians' offices, in diabetes detection screening programs, in the differentiation of glucosuria from other meliturias, and the like.

Because early diagnosis and continued control are so important in diabetes, a urine-sugar test, to be of greatest value, must be conveniently rapid, simple enough for any patient to learn with ease, accurate enough to serve the clinician, and sensitive enough to reflect variations in the patient's condition. Moreover, the reagent composition must be adequately stable.

Procedures for the detection of sugar in urine are well known in clinical chemistry. One such procedure utilizes Benedict's copper reduction test in tablet form (U.S. Patent No. 2,387,244), still another involves a test which depends on the action of a double enzyme system (U.S. application Ser. No. 514,395, filed June 9, 1955, by Alfred H. Free and assigned to the assignee of the present invention). These tests have greatly contributed to the art of diagnosing biological fluids for glucose.

I have now found a novel and highly useful glucose-detecting means which represents an important improvement in the area of determining glucose in various materials including body fluids, such as urine. This improved technique utilizes a diagnostic composition which is quantitative for glucose.

Specifically, I have now found a unique combination of a glucose oxidase system with a novel indicator system which offers a superior means for testing biological fluids for their glucose content. This unique combination comprises an enzyme system having glucose oxidase activity, a material having peroxidative activity, a primary indicator material which is capable of being preferentially oxidized to a colored form in the presence of hydrogen peroxide and the material having peroxidative activity and, as a secondary indicator, a material or materials which is capable of reducing the preferentially oxidized primary indicator material while simultaneously becoming colored. Thus the secondary indicator material blends with the preferentially oxidized primary indicator material to finally yield distinctly different colors which are quantitatively indicative of the concentration of glucose present originally in the fluid being tested. A simple color chart based on the colors developed may be readily prepared for each combination of indicator materials.

The principles underlying the basic reactions of enzyme tests for glucose are well known. Glucose oxidase catalyzes the aerobic oxidation of glucose to produce gluconic acid (gluconolactone) and hydrogen peroxide. In this reaction hydrogen removed from the glucose combines with atmospheric oxygen to form the hydrogen peroxide.

This reaction may be represented by the following schematic equation:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$

A substance having peroxidative activity (such as horseradish peroxidase, iodide and molybdate salts, blood, etc.), in turn, is capable of inducing the oxidation of a class of indicators by the hydrogen peroxide formed in the conversion of glucose to gluconolactone. This latter reaction may be represented by the following schematic equation:

$$H_2O_2 + \text{oxidizable dye} \xrightarrow[\text{peroxidative activity}]{\text{Substance having}} \text{oxidized dye} + H_2O$$

(color change)

Using, as an example, materials known to the art, glucose oxidase, peroxidase and o-tolidine, the reaction proceeds as follows:

The glucose oxidase reacts with the glucose present in the material being tested and there is formed, as indicated above, gluconic acid and hydrogen peroxide. The hydrogen peroxide in the presence of the peroxidase oxidizes the o-tolidine to its colored form, thus indicating the presence of glucose.

The previously described compositions of the prior art have the disadvantage that at a glucose concentration of about 0.5% and above the indicators assume such an intense color or pigmented density as to render colorimetric discrimination beyond normal or average visual perception. The composition of the present invention, on the contrary, makes use of a more accurately controllable coloration as set out in detail below.

The present diagnostic test embodies the use of an enzyme system having glucose oxidase activity, a material having peroxidative activity, a primary indicator material which is oxidized to its colored form in the presence of hydrogen peroxide and the material having peroxidative activity, and a secondary indicator material which is oxidized by the preferentially oxidized primary indicator and assumes its own particular colored form to produce a blended color which is a separate and distinct color rather than one merely showing a change of intensity or depth of hue of one and the same color. The preferentially oxidized primary indicator material may be thus partially or completely returned to its reduced or colorless form.

Although the principle or mode of action as well as the interaction of this combination of materials making up the novel indicating system is not yet fully understood, it appears that the primary dye component of the system is preferentially oxidized. This oxidized dye is then completely or partially reduced by the secondary indicator substance whereby the color intensity of the preferentially oxidized primary indicator dye is diluted. The reducing secondary indicator substance assumes a color and blends therewith to form a new color which may be readily distinguishable by the average eye. The concentration of glucose may be correlated with each such distinguishable blended dye color and is thus readily and quantitatively determinable by reference to a simple color chart.

In one of the preferred examples the test composition comprises glucose oxidase, peroxidase, o-tolidine and a secondary indicator dye or dyes such as those specifically mentioned in Example 1, below, namely dl-β-(3,4-dihydroxyphenyl) alanine, p-aminobenzoic acid and chlorpromazine which produces blended colors which are distinct colors for predetermined concentrations of glucose in urine, i.e. distinct colors at 0.1, 0.25, 0.50, 1.0 and 2.0 percent of glucose present . When this composition is contacted with a glucose-containing fluid, it appears that the o-tolidine is preferentially oxidized by the hydrogen peroxide. Thereafter, the supplementing oxidizable

secondary color indicator or indicators which reduce the preferentially oxidized o-tolidine upon oxidation produce their own color change while diluting the o-tolidine color produced. This results in separate and distinct colors rather than changes in intensity of one color for each of the specific glucose concentrations correlated to 0.1%, 0.25%, 0.50%, 1.0% and 2.0% of glucose.

As will be pointed out in more specific detail below, these secondary indicator materials are oxidized *only* by the oxidizable primary indicator material. For example in a system using glucose oxidase, horseradish peroxidase and the dl-β-(3,4-dihydroxyphenyl) alanine, no color is obtained when contacted with urines containing as much as 2% glucose. However, when a preferentially oxidized primary indicator material such as ortho-tolidine is added to the above composition, the first color observed is that characteristic of the ortho-tolidine. As the concentration of glucose in the fluid is increased the color obtained with this combination of indicators changes from blue through blue-green, light blue, dark blue to black.

The invention will now be illustrated in greater detail, but not limited, by the following examples:

EXAMPLE 1

Preparation of the impregnating solution

A gelatin solution was prepared by dissolving 4.8 gm. of gelatin in 100 ml. of boiling water. Then a buffer solution was prepared by dissolving 7.4 gm. of anhydrous citric acid and 32.6 gm. of sodium citrate in 100 ml. of water. 12 ml. of the buffer solution to which was added 0.010 gm. of dl-β-(3,4-dihydroxyphenyl) alanine, then was added with stirring to 10 ml. of the gelatin solution. A solution of 1 gm. of o-tolidine dihydrochloride in 50 ml. of water to which when solution was complete 50 ml. of 95% ethyl alcohol was added was then prepared and 10 ml. of this solution was added to the gelatin-buffer solution. Then an enzyme solution of 2.4 gm. of glucose oxidase having an activity of about 3000 units per gm. and 0.05 gm. of peroxidase were added to 50 ml. of water and 10 ml. of this enzyme solution were added to the gelatin-buffer-orthotolidine solution.

Preparation of reagent strips

Bibulous strips, such as filter paper cut into narrow strips, are dipped into the impregnated solution so that through the process of submersion and capillary attraction about ½ inch of the strip at one end is completely impregnated. These strips are then dried in a drying tunnel or in a forced draft oven. It will be understood that other porous or absorbent materials such as small sticks of wood, etc. and other methods of applying the impregnating solutions and of drying the impregnated sticks may likewise be used.

In use, an impregnated test strip is dipped into the liquid specimen to be tested. The reagents of Example 1 give a positive reaction in about 10 to 60 seconds and with urines containing 0.1% to 2.0% of glucose give the following distinct colors:

At 0.1% glucose—a pink color
At 0.25% glucose—a pinkish-rose color
At 0.50% glucose—a rose color
At 1.0% glucose—a rose-purple color
At 2.0% glucose— a purple color

These are distinct colors which are clearly distinguishable by the average eye. The contributions of the primary and secondary indicator constituents blend to give these final colors. Thus the dihydroxyphenyl alanine contributes a tan tinge, while the o-tolidine yields a blue color. When dipped in urine containing no glucose, the strips undergo no color change. A simple color chart based on the above distinct color series may be conveniently prepared for use in testing for urine glucose with each example herein given.

EXAMPLE 2

Example 1 was repeated exactly, eliminating the preferentially oxidizable primary indicator material, that is to say the o-tolidine dihydrochloride. When test strips were prepared with this composition as described in Example 1 and used to test urines containing up to 2.0% glucose, no color change was observed whatsoever.

A comparison of the results of Example 1 and Example 2 above shows that the indicating system to which the instant invention is directed is not merely a combination of indicators as is suggested by the prior art. Rather the compositions of this invention are directed toward a novel indicating system wherein the secondary indicator is oxidized, with the concomitant color change *only* by the preferentially oxidized primary indicator. The combination of the hydrogen peroxide (formed by the interaction of glucose oxidase and glucose), and the substance having peroxidative activity (horseradish peroxidase) does not oxidize the secondary indicator material. However when oxidized o-tolidine is present, it is reduced by the secondary indicator material which thereby becomes oxidized and exhibits its characteristic color change. It is the formation of separate and distinct colors or color blends, which lend themselves to ready duplication on a color chart which makes the compositions of the instant invention so advantageous as a quantitative test. These distinct colors are formed by combinations of the primary color indicator and the secondary indicator and are not merely shades of varying intensity of one color.

EXAMPLE 3

Example 1 was repeated substituting for the (dl-β-3,4-dihydroxyphenyl alanine) 0.100 gm. of chlorpromazine hydrochloride.

This composition gave the following colors with concentrations of glucose in urine indicated:

0.1% glucose—pink color
0.25% glucose—light pink color
0.50% glucose—light green color
1.00% glucose—medium green-blue color
2.00% glucose—dark green-blue color

EXAMPLE 4

Example 3 was repeated except that the o-tolidine was omitted from the formulation. Test strips made from this formulation gave no color with urine containing as much as 2.0% glucose.

In the follow examples, the amount of the secondary indicators shown was substituted for the dl-β-(3,4-dihydroxyphenyl) alanine of Example 1. All the other ingredients of the composition and the procedure for preparing the test strips were the same.

EXAMPLE 5

	Gm.
2,4 dinitrophenol	0.050

This composition gave a color ranging from yellow for 0.1% glucose, through green for 0.5% glucose to blue for 2.0% glucose. Intermediate colors were yielded by intermediate glucose concentrations.

EXAMPLE 6

	Gm.
N,N-dimethyl-p-phenylenediamine sulfate	0.100
p-Aminobenzoic acid	0.400

This composition gave distinct colors as follows: at 0.1% glucose a tan pink, at 0.5% glucose a rose, and at 2.0% glucose a purple.

EXAMPLE 7

	Gm.
N,N-dimethyl-p-phenylenediamine sulfate	0.250

The colors obtained were as follows: at 0.1% glucose a tan color, at 0.5% glucose a rose color and at 2.0% glucose a blue color.

EXAMPLE 8

	Gm.
3,3'-diaminobenzidine dihydrochloride	0.100

The colors obtained with this composition were: at 0.1% glucose a tan, at 0.5% glucose a brown and at 2.0% glucose a greenish brown.

EXAMPLE 9

	Gm.
p-Amino dimethylaniline monohydrochloride	0.050

The colors obtained with this composition were: at 0.1% glucose a pink, at 0.5% glucose a rose and at 2.0% glucose a purple. With the time interval extended to 5 or more minutes after dipping, the purple color became very much darker with the 2.0% glucose test.

EXAMPLE 10

A gelatin solution was prepared by dissolving 4.8 gm. of gelatin in 100 ml. of boiling water. Then a buffer solution was prepared by dissolving 7.4 gm. of anhydrous citric acid and 32.6 gm. of sodium citrate in 100 ml. of water. 12 ml. of the buffer solution to which was added 0.010 gm. of dl-β-(3,4-dihydroxyphenyl) alanine, 0.100 gm. of p-aminobenzoic acid and 0.100 gm. of chlorpromazine hydrochloride then was added with stirring to 10 ml. of the gelatin solution. A solution of 1 gm. of o-tolidine dihydrochloride in 50 ml. of water to which when solution was complete 50 ml. of 95% ethyl alcohol was added was then prepared and 10 ml. of this solution was added to the gelatin-buffer solution. Then an enzyme solution of 2.4 gm. of glucose oxidase having an activity of about 3000 units per gm. and 0.05 gm. of peroxidase were added to 50 ml. of water and 10 ml. of this enzyme solution were added to the gelatin-buffer-orthotolidine solution.

The colors obtained with this composition were: at 0.1% glucose a pink, at 0.25% glucose a pinkish rose, at 0.50% glucose a rose, at 1.0% glucose a rose-purple, at 2.0% glucose a purple.

EXAMPLE 11

Formulation of impregnating solution

10% aqueous solution polyvinyl alcohol (PVA)	ml.	25.0
2,7-diaminofluorene dihydrochloride	gm.	0.5
o-Tolidine dihydrochloride	gm.	0.5
KI	gm.	1.6
$Na_2MoO_4.2H_2O$	gm.	1.0
Phosphate buffer—pH 6.5	ml.	20.0
Gelatin	gm.	0.5
Glucose oxidase	gm.	0.6
Alcohol (95% ethanol)	ml.	15.0
dl-β-(3,4-dihydroxyphenyl) alanine	gm.	0.025

Preparation of impregnating solution

(1) 0.5 gm. of 2,7-diaminofluorene dihydrochloride and 0.5 gm. of o-tolidine dihydrochloride are dissolved in 10 ml. of hot water and to this is added 25 ml. of 10% PVA solution.

(2) 0.5 gm. of gelatin is dissolved in 10 ml. of boiling water. 0.025 gm. of dl-β-(3,4-dihydroxyphenyl) alanine is dissolved in 20.0 ml. of phosphate buffer of pH 6.5 [prepared by dissolving 43.55 gm. of dibasic potassium phosphate (K_2HPO_4) and 39.0 gm. of monobasic sodium phosphate ($NaH_2PO_4.H_2O$) in water and bringing to a volume of 500 ml.] and added to the gelatin solution. To this is added 15 ml. of ethanol and the mixed solution is added to solution number 1.

(3) 1.0 gm. of $Na_2MoO_4.2H_2O$ and 1.6 gm. of KI are dissolved in 5 ml. of water and added to solution number 2.

(4) Finally 0.6 gm. of glucose oxidase are dissolved in 9.0 ml. of water and added to solution number 3. The impregnating solution or mix is now ready for use.

Test strips are then prepared in accordance with the procedure given above in Example 1.

Procedure of testing

In use, an impregnated strip, made as described in the above examples, is dipped into the liquid specimen to be tested. When contacted with urine containing glucose, a test strip will give a positive reaction in about 10 to 60 seconds evidenced by various colors as follows:

When dipped into urine containing no glucose the strips undergo no color change. With urine containing as little as 0.1% glucose, the impregnated strip prepared with the composition of Example 11 changes to a distinctly tan-green color. With urines containing 0.25% of glucose the strip assumes a green color. With urines containing 0.5% of glucose a light blue color hue develops. At 1.0% glucose the blue color becomes a deeper dark blue, while at 2.0% glucose a very deep black color develops. Thus it is seen that with urines containing 0.1 to 1.0 or 2% of glucose distinct color shades or hues extending over the color spectrum develop and not one color of varying intensity, as is the case with prior art enzyme tests. The urine having the higher glucose content also develops the deepest color. A sample color chart based on this phenomenon may be conveniently prepared for use in testing for urine glucose.

EXAMPLE 12

Example 11 was repeated leaving out of the formulation the preferentially oxidized primary indicators, o-tolidine hydrochloride and 2,7-di-aminofluorene dihydrochloride.

Strips prepared from this formulation gave no color with urines containing as much as 2.0% glucose indicating that the secondary indicator, dl-β-(3,4-dihydroxyphenyl) alanine was not oxidized by the combination of the hydrogen peroxide formed (from the glucose oxidase system) and the substance having peroxidative activity (the potassium iodide-sodium molybdate combination).

In place of the potassium iodide and sodium molybdate it is also possible, and therefore contemplated, to use other equivalent iodide and molybdate salts. For example, other iodides, such as sodium and ammonium iodides and other molybdates, such as potassium and ammonium molybdates may also be used. In lieu of the 2,7-diaminofluorene, o-tolidine, o-dianisidine, leucoindophenols, etc. are other satisfactory primary indicators.

EXAMPLE 13

A further embodiment of this invention wherein a urohemin mixture is used to catalyze the oxidation of sensitive primary indicators such as 2,7-diaminofluorine, o-tolidine, and the like can be readily prepared by taking the composition of Example 1 and for the enzyme solution there given substituting the following:

2.4 gm. of glucose oxidase having an activity of about 3000 units per gm. and 0.050 gm. of urohemin are added to 50 ml. of water.

The remaining ingredients are as given in Example 1.

Test strips are then prepared in accordance with the procedure recited in Example 1.

In use, an impregnated test strip, made as described in the above examples, is dipped into the liquid specimen to be tested. When contacted with urine containing glucose, a test strip will give a positive reaction in about 10 to 60 seconds evidenced by various colors spread over the color spectrum (and not intensities of a single color) as follows:

With urines containing 0.1% to 2.0% glucose in steps of 0.1, 0.25, 0.5, 1.0 and 2.0% glucose present, color changes of distinguishable hue extending over the color spectrum are developed by the indicators of this invention corresponding for example to tan, green, blue, black,

etc. respectively. Urines having the higher glucose content produce the deeper color and accordingly:

At 0.1% glucose—a pink color
At 0.25% glucose—a pink-rose color
At 0.50% glucose—a rose color
At 1.0% glucose—a rose purple color
At 2.0% glucose—a purple color

are the corresponding indicator colors developed in the test strips. When dipped in urine containing no glucose, the strips undergo no color change. A simple color chart based on this phenomenon may be conveniently prepared for use in testing for urine glucose.

Test solutions containing 0.1, 0.25, 0.50, 1.0 and 2.0% of glucose in urine were prepared. A prior art test strip gave slight color changes below 0.5% glucose content but at 0.5% glucose intense color formation blanked out any further visual distinction. Strips prepared in accordance with Examples 1 through 13 resulted in the data set out in Table I.

TABLE I

	0.1	0.25	0.5	1.0	2.0
Prior art strip	no color	no color	dark purple	dark purple	dark purple.
Example:					
1	pink	pinkish rose	rose	rose purple	purple.
2	(1)	(1)	(1)	(1)	(1)
3	pink	light pink	light green	medium green-blue	dark green-blue.
4	(1)	(1)	(1)	(1)	(1)
5	yellow	yellow green	green	green-blue	blue.
6	tan pink	pinkish rose	rose	rose purple	purple.
7	tan	tan rose	rose	rose blue	blue.
8	tan	tan brown	brown	light greenish br	greenish brown.
9	pink	pinkish rose	rose	rose purple	purple.
10	pink	pinkish rose	rose	rose purple	Do.
11	pale yellow	yellow green	light blue	dark blue	black.
12	(1)	(1)	(1)	(1)	(1)
13	pink	pinkish rose	rose	rose purple	purple.

[1] No color change.

From the detailed examples it is evident that additives are incorporated in the reagent composition such as suitable protective, thickening, wetting, suspending agents and the like as well as inert dyes to impart thereto a uniform color background.

Furthermore, it is important to include an appropriate buffer system to maintain a desired pH range in the presence of urine. Although the pH range may vary between 4 to 7.5, an especially preferred pH is 5.2.

Although the test device itself may comprise the reagent composition in the form of a tablet, powder or other embodiment, I prefer to impregnate the reagent composition on bibulous base materials or carriers, such as strips or sticks of filter paper, by dissolving the components in a suitable solvent, impregnating the bibulous strips with the resulting solution, and drying the impregnated test strips. Embodying the diagnostic composition in the form of test sticks insures ease and simplicity of test procedure.

Although the specific examples herein given illustrate certain preferred embodiments of the invention, a number of variations are possible in the reagent materials. For example, any enzyme having glucose oxidase activity can be used. Likewise a number of other substances or materials having peroxidative activity may be substituted for those given in the examples. Among the materials having peroxidative activity may be included many organic and inorganic preparations. Thus various plant peroxidases, such as horseradish peroxidase, potato peroxidase may be used. In place of potassium iodide and sodium molybdate, other iodides, such as sodium and ammonium iodides and other molybdates such as potassium and ammonium molybdates may also be used. In addition to the urohemin mixture specifically given in Example 13 a number of other porphyrin substances having peroxidative activity may be used. Thus in the metalloporphyrins, although hemin is preferred, various complex-forming compounds which activate certain other metalloporphyrins not operable per se, can be used therewith, such as 2-aminobenzothiazole, pyridine, bipyridyl, bipyridylpyridine, nicotinic acid or the like. Other substances which are not enzymes but have peroxidative activity include such compounds as iron sulfocyanate, iron tannate, ferrous ferrocyanide, potassium chromic sulfate, and others. Also such substances having peroxidase activity as normal whole blood, red blood cells alone, lyophilized whole blood and like substances may be used.

Among the primary indicator substances, other than o-tolidine, which are capable of being oxidized in the presence of hydrogen peroxide and a material having peroxidative activity are 2,7-diaminofluorene, o-dianisidine, leucoindophenols, etc. Substances capable of being used as secondary indicators, that is, capable of reducing the oxidized primary indicator and thereby undergoing a color change themselves are: p-aminobenzoic acid, thymol, resorcinol, etc.

In addition to the compositions described in the above examples illustrating optimum contents, variations in amounts of the four major components embodied in this invention are permissible as shown by the following chart:

(1) Enzyme system having glucose oxidase activity	4000–16,000 units.
(2) Material having peroxidative activity	0.1–1.5 gm.
(3) Primary indicator	0.2–1.0 gm.
(4) Secondary indicator	0.010–1.00 gm.
(5) Buffer	0.1–0.3 molar (pH 4.0–7.5).

In addition various additives may also be incorporated in the reagent composition as suitable protective, thickening and wetting agents as well as inert dyes to impart a uniform color background to the composition.

The impregnated solutions prepared in accordance with any of the above examples as well as test strips impregnated with any of these solutions were found to be very stable.

In summary, this invention pertains to a diagnostic composition for the detection of glucose in fluids, and especially body fluids such as urine, consisting of a bibulous material such as a strip which has been impregnated with a composition comprising an enzyme system having glucose oxidase activity, a substance having peroxidative activity, such as horseradish peroxidase, an iodide-molybdate salt, a urohemin, normal whole blood, red blood cells alone, lyophilized whole blood and like substances having peroxidative activity, a primary color forming indicator (such as 2,7-diaminofluorene, o-tolidine, etc.), which is preferentially oxidizable in the presence of hydrogen peroxide and said substance having peroxidative activity and in combination as a secondary indicator a compound which is capable of reducing the oxidized primary indicator substance and assuming a color dependent upon the concentration of peroxide (and hence

of glucose), which blends with and reduces the preferentially oxidized color forming primary indicator, the combination of both colors serving to form a clear, sharp new color having specific color shades or hues extending over the color spectrum (as contrasted to the production of varying intensities of a single shade or one color). These colors form a clear visible index of the glucose concentration present in the original fluid tested and allow a simple color chart based on this phenomenon to be conveniently prepared for use in determining the concentration of glucose present in a tested sample.

What is claimed is:

1. A composition for detecting glucose in a fluid which comprises an enzyme system having glucose oxidase activity, a substance having peroxidative activity, a primary indicator material which is preferentially oxidized in the presence of peroxide and said substance having peroxidative activity and changes color thereupon and a secondary indicator material which will reduce a portion of the oxidized primary indicator material and undergo a color change to produce with the preferentially oxidized primary indicator material distinct and different colors corresponding to different concentrations of glucose.

2. A composition for detecting glucose in a fluid which comprises a bibulous material containing impregnated therein a mixture which comprises an enzyme system having glucose oxidase activity, a substance having peroxidative activity, a primary indicator material which is preferentially oxidized in the presence of peroxide and said substance having peroxidative activity and changes color thereupon and a secondary indicator material which will reduce a portion of the oxidized primary indicator material and undergo a color change to produce with the preferentially oxidized primary indicator material distinct and different colors corresponding to different concentrations of glucose.

3. A composition for detecting glucose as defined in claim 2 wherein the components are adherent to an inert solid support material.

4. A composition for detecting glucose as defined in claim 2 wherein the components are impregnated on bibulous paper strips.

5. A diagnostic composition for detecting glucose which comprises glucose oxidase, a substance having peroxidative activity selected from the group consisting of horseradish peroxidase, metalloporphyrin, urohemin, an iodide-molybdate mixed salt, blood, red blood cells, lyophilized whole blood, lyophilized blood cells and mixtures of lyophilized blood and lyophilized plasma, a primary indicator substance preferentially oxidizable in the presence of peroxide and the substance having peroxidative activity and changing color thereupon, said indicator substance being selected from the group consisting of 2,7-diaminofluorene, o-tolidine, o-dianisidine and leucoindophenol, and a secondary indicator material which will reduce a portion of the preferentially oxidized primary indicator substance and undergo a color change to produce with the preferentially oxidizable primary indicator substance distinct and different colors corresponding to different concentrations of glucose, said secondary indicator material being selected from the group consisting of dl-β-(3,4-dihydroxyphenyl) alanine, chlorpromazine dihydrochloride, 2,4-dinitrophenol, N,N-dimethyl-p-phenylenediamine sulfate, and 3,3'-diaminobenzidine dihydrochloride.

6. A diagnostic composition for detecting glucose which comprises glucose oxidase, urohemin, 2,7-diaminofluorene and dl-β-(3,4-dihydroxyphenyl) alanine.

7. A diagnostic composition for detecting glucose which comprises glucose oxidase, potassium iodide, sodium molybdate, 2,7-diaminofluorene and dl-β-(3,4-dihydroxyphenyl) alanine.

8. A diagnostic composition for detecting glucose which comprises glucose oxidase, horseradish peroxidase, o-tolidine and dl-β-(3,4-dihydroxyphenyl) alanine.

9. A diagnostic composition for detecting glucose which comprises glucose oxidase, red blood cells, o-dianisidine and 2,4-dinitrophenol.

10. A diagnostic composition for detecting glucose which comprises glucose oxidase, metalloporphyrin, 2,7-diaminofluorene and N,N-dimethyl - p - phenylenediamine sulfate.

11. A diagnostic composition for detecting glucose which comprises bibulous paper strips impregnated with a composition comprising:

10% aqueous solution polyvinyl alcohol (PVA)	ml.	25.0
2,7-diaminofluorene dihydrochloride	gm.	0.5
o-Tolidine dihydrochloride	gm.	0.5
KI	gm.	1.6
$Na_2MoO_4.2H_2O$	gm.	1.0
Phosphate buffer—pH 6.5	ml.	20.0
Gelatin	gm.	0.5
Glucose oxidase	gm.	0.6
Alcohol (95% ethanol)	ml.	15.0
dl-β-(3,4-dihydroxyphenyl) alanine	gm.	0.025

12. A composition for detecting glucose as defined in claim 2, containing a protein degradation product and a buffer for maintaining the pH of the aforesaid mixture within the range 4.0 to 7.5 in the presence of urine.

13. A method of determining the amount of glucose present in a fluid which comprises bringing the fluid into contact with the glucose indicator defined in claim 2.

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