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DIAGNOSTIC DEVICE FOR VARIOUS SUGARS

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ABSTRACT OF THE DISCLOSURE

A diagnostic test indicator strip having an aqueous liquid absorbent ion-exchange-effective cellulose derivative body-fluid test sample absorption portion and contiguous therewith an indicator portion impregnated with peroxidase, an oxidizable chromogen and an oxidase specific to a sugar for which the body fluid is being tested.

This invention is that of a diagnostic test device for detecting the presence of individual specific sugars such as may occur in any of the various animal body fluids such as urine, blood serum or plasma.

More specifically the invention is that of such a diagnostic test device composed (i) at least in part of a body-fluid-absorbent ion exchange cellulose derivative, one portion of which, conveniently called the unchanged portion, is to serve to absorb a test quantity of the body fluid (more often urine) for the particular sugar, and (ii) having a contiguous other portion impregnated with a reactive-indicator composition which when wetted with the body fluid coming from the first portion reacts with the specific sugar tested for, with resulting color change manifesting its presence. The reactive-indicator composition comprises peroxidase, an oxidizable chromogen forming a differently colored oxidation product in presence of the peroxidase, and an oxidase specific to the particular sugar concerned, e.g. galactose oxidase or glucose oxidase.

Also part of the invention is the method of testing an animal body liquid for the presence of an individual sugar which might occur in it, e.g. galactose or even low level presence of glucose, for example, from about 5 milligrams of glucose to as little as about 0.5 milligram per 100 milliliters, by immersing an unchanged portion of a strip of the above-mentioned ion exchange cellulose derivative in the test body fluid, allowing the fluid to flow from that first wetted portion by capillary attraction into the neighboring portion of the strip impregnated with the reactive-indicator composition containing peroxidase and the oxidase specific to the sugar being tested for, and observing whether an indicative color change occurs in the indicator-impregnated portion within a sufficient time for that.

Thus, by using galactose oxidase as the oxidase in the reactive-indicator composition, the invention enables detecting the presence of the metabolic disorder galactosemia.

A further part of the invention is (a) the discovery that in bacteriuria the bacteria consume glucose so that very little and practically no glucose is present in the urine, and (b) the provision of a diagnostic test device or strip using glucose oxidase as the oxidase in the reactive-indicator composition and enabling detecting glucose at a level of from about 0.5 to about 3 milligrams per 100 milliliters of the body fluid particularly urine and thus showing the presence of bacteriuria or even a latent case of it.

As to some sugars, certain detection methods are known, involving the influence of specific oxidases of the particular sugars which are sought to be detected, as well as techniques for producing, by means of oxidation, a change of the light absorption of an indicator toward the visible

range of the spectrum. These methods may be adapted to a certain extent to use, for example, with ordinary filter paper strips impregnated with peroxidase, an oxidase pertinent to the specific sugar, and a chromogen indicator.

Unfortunately, these methods and means have involved limitations which seriously restrict their use. For example, such hitherto used type of test strips serve satisfactorily when used on aqueous solutions of exclusively the particular sugar which it is desired to detect. That is so as to both glucose and galactose according to Rorem et al., Analytical Biochemistry, volume 3 (1962), pages 230 to 235.

However, when tried with more complex liquids such as the biological waste fluid, urine, the utility of the earlier technique must be varied in its application and steps from one type of sugar to another. Moreover, undesirable variations in result occur even from sample to sample with individual sugars. Thus, while it may be possible to detect glucose in urine by an indicator-impregnated ordinary filter paper strip, yet that cannot apply to galactose because no reliable results are obtainable with it.

The wide variations observed in detecting sugars in various urine samples stem from the simultaneously present in them varying amounts of species specific compounds, residues, or metabolites of the digested foods and/or drugs taken.

A conventional glucose test strip may indicate amounts of glucose corresponding to from 30 to 60 mg. per 100 ml. It may also give positive detection down to 20 mg. per 100 ml., but also may give negative results up to 130 mg. per 100 ml. As most diabetic cases show urinary glucose levels of the order of above 80 mg. per 100 ml., the conventional glucose test strip is useful as a bedside test for that.

However, that conventional strip cannot be used for screening within the normal range of the non-diabetic population, 95.6% of whom show levels below 20 mg. per 100 ml. That conventional strip definitely then cannot be used to differentiate between a normal individual and one with a latent bacterial infection of the urinary tract and thus showing a glucose level below 3 mg. per 100 ml. of urine.

Considered broadly, the diagnostic device of the invention comprises a strip of a body-fluid-absorbent ion-exchange-effective cellulose derivative, an unchanged portion thereof to enable absorbing a test quantity of an animal body fluid to be tested for a particular sugar, a contiguous other portion of said strip connected with said first portion to enable test body fluid absorbed by it to flow by capillary attraction from said first portion to said second portion, the latter being impregnated with a reactive-indicator composition comprising peroxidase, an oxidizable chromogen convertible by the presence of the peroxidase to a distinctly differently colored oxidation product, and an oxidase specific to the particular sugar to be tested for, which indicator composition upon being wetted by test body fluid coming from said first portion, will react therewith and after a sufficient time for color change to occur, will change to the different color corresponding to the presence of the particular sugar in the event that said body fluid being tested contains the sugar sought for.

Thus, the diagnostic device of the invention advantageously is made in the form of a filter paper-like strip of the ion exchange cellulose derivative, with the strip having an intake absorption zone into which the test body fluid to be tested initially is taken up and absorbed and through which it has to pass before it reaches and is absorbed in the reactive-indicator composition impregnated zone wherein the sugar, if present, is detected by the color reaction change.

The strip of the test device of the invention is prepared

from an ordinarily water-absorbable and thus body-fluid-absorbent, sheet of an ion-exchange-effective cellulose derivatives, that is to say a cellulose derivative which possesses ion exchange properties. Such derivatives are known and available with cationic or anionic exchange properties resulting from reacting cellulose with alkaline or acid reactants to introduce onto the cellulose molecule basic or acid substituents respectively.

Cellulose phosphate, cellulose sulfate, and carboxymethyl cellulose exemplify the acidic or cationic type. The anionic or basic type is illustrated by aminoethyl cellulose, N,N-diethylaminoethyl cellulose (designated DEAE), and "Ecteola"-cellulose (obtained by treating cellulose with epichlorhydrin and triethanolamine).

While it is advantageous for the ion-exchange-effective cellulose material to be the carrier for the reactive-indicator composition, the cellulose-comprising carrier also can be incorporated with other types of ion exchange materials, for example, "Amberlite SB–2" (of Rohm & Haas Company) or "Dowex 2x8" (of Dow Chemical Company). The strong anion exchangers give superior results in the determination of galactose.

It is beneficial to include in the reactive-indicator composition for a commercial embodiment of the invention a hydrophilic colloid such as a polyethylene glycol of average molecular weight, say, from about 500 to 40,000, or egg or other albumin, and others to enhance the uniformity of the dispersion and stabilize and protect the composition and its constituents for long periods of storage and shelf-life without loss of activity and sensitiveness.

The method of testing an animal body fluid for presence in it of an individual sugar then comprises immersing in a test sample of the body fluid the test-sample-absorption portion of the ion-exchange-comprising cellulose material until the body fluid passes through that portion and by capillary attraction continues on into the second zone or portion impregnated with the reactive-indicator composition to wet it and keep it so for a time sufficient for the corresponding color change to occur if the sugar sought for is present in the body fluid.

Glucose oxidase is the oxidase included in the reactive-indicator composition of the diagnostic device for use in the embodiment of the method of the invention for testing for the presence of bacteriuria whether active or latent.

The invention thus includes the method of testing whether a subject is ill with bacteriuria, at any stage from latent to active, by processing a test sample of a body fluid from such subject by immersing in a test sample of said fluid part of the fluid-test-sample-absorption-portion of a diagnostic test strip of the invention, wherein glucose oxidase is the oxidase used in the reactive-indicator composition, for a sufficient time to allow the sample fluid to pass through the ion-exchange-effective sample-absorption-portion and on into and through the reactive-indicator portion of the test strip, and allowing the fluid which has passed into the reactive-indicator portion to remain in contact with its composition for a sufficient time for a color change to occur in its chromogen if glucose is present in the body fluid sample.

The test strip wherein glucose oxidase is the oxidase used in the reactive-indicator composition enables detecting glucose present in an animal body fluid in the range from about 5 mgs. to as little as about 0.5 mg. per 100 milliliters. The invention thus also includes the method of testing an animal body fluid to detect in it glucose present even within the just noted range, by subjecting a test sample of such body fluid to the procedure described in the just preceding paragraph.

The diagnostic test device and method of the invention avoid the adverse influence of the species specific compounds, residues, or metabolites from foods and/or drugs, whose presence in urine interfered with the detection of sugars by the prior devices.

The test device and method of the invention, being particularly applicable to detecting galactose and also low levels of glucose (indicative of bacteriuria) and more often in urine, are described in more detail by embodiments relating to galactose and glucose detection in urine. Accordingly, the invention only is illustrated by, but is not to be restricted to, the following examples:

EXAMPLE 1

DEAE-cellulose test strips for galactose

Diethylaminoethyl cellulose ion exchange paper, which was prepared by treating a bibulose cellulose comprising sheet material wherein the cellulose was converted to its sodium or other alkali metal salt form in known manner with N,N-diethylaminoethyl chloride, washing out the resulting alkali metal chloride, and drying, then was treated by impregnation for 10 minutes in a 0.01 M sodium phosphate buffer solution (to pH 7), water-washed and dried.

A 50 by 50 centimeters sheet of this buffer-treated DEAE cellulose paper then was impregnated with a chromogen solution composed of 0.75 gram of ortho-tolidine dissolved in 50 ml. of methanol, dried in the dark, and cut into strips measuring about 10 by 60 millimeters.

About one-half only of the length of each of these strips then was moistened with about 40 microliters (i.e. μl.) per strip of a buffered mixed enzyme solution composed as follows:

2 ml. of 0.5 M sodium phosphate buffer, pH 6.9,
50 mg. polyethylene glycol, average mol. wt. about 4000,
2 mg peroxidase, and
5 mg. galactose oxidase.

The thus finally impregnated strips were dried in the dark and stored in a dry place.

The portion of the finished diagnostic test strip impregnated with the mixed enzyme composition can be referred to as the reactive-indicator zone or portion, as already indicated. Then the initial portion which is not impregnated with the mixed enzymes and polyethylene glycol or other hydrophilic colloid stabilizer conveniently is called the test-sample-absorption zone or portion.

EXAMPLE 2

"Ecteola"-cellulose test strips

"Ecteola"-cellulose ion exchange paper, prepared in known manner by reacting an alkali metal salt of cellulose with epichlorhydrin and then with triethanolamine, washing and drying, was treated by impregnation for 15 minutes in the 0.01 M sodium phosphate buffer used in Example 1 and dried.

Then a 50 x 50 cm. sheet of this buffer-treated "Ecteola"-cellulose paper was impregnated with the same chromogen solution, dried in the dark, cut into 10 by 60 mm. strips, the lower half of each of which was moistened to the same extent with the same buffered mixed enzyme solution, dried in the dark and stored in a dry place, all as in Example 1.

EXAMPLE 3

Aminoethyl cellulose ion exchange paper test strips

Aminoethyl cellulose ion exchange paper, prepared in known manner by reacting sodium cellulose with aminoethyl chloride, washing with distilled water and drying, was soaked with a buffer solution, for pH 7, and dried. A 50 by 50 cm. sheet of this dried buffered-impregnated ion exchange paper then was treated in entirely the same way as the corresponding sheets and strips cut from them were treated in Examples 1 and 2, to yield dried finished test strips prepared from this aminoethyl cellulose to be stored in a dry place.

EXAMPLE 4

Detecting galactose in urine

A 2 ml. sample of a test subject's urine is placed in a 10 ml. beaker. Into this sample is partly immersed the free end of the test-sample-absorption portion of the diagnostic test strip of any of Examples 1, 2 or 3. Thereby the urine sample initially enters the solely ion-ex-

changing test-sample-absorption zone. During passage of the urine by capillary attraction up through that zone, there are taken up in it those constituents of the urine, which otherwise would interfere with and upset the detection of any galactose.

As a result, the ion-exchanged urine containing the galactose free of these interferants continues by capillary attraction up into the reactive-indicator portion of the test strip. The reaction between the galactose, the enzymes and the chromogen produces a distinct color change in the indicator zone. With the ortho-tolidine a blue to blue-green is produced rapidly with the presence of galactose in the sample.

A urine sample from a subject with galactoseuria and containing about 400 mg. of galactose per 100 ml. provides the identifying color reaction in about 15 seconds. A urine sample containing about 100 mg. of galactose per 100 ml. produces the color reaction within about 1 to 2 minutes. No color reaction is produced by a urine sample devoid of galactose.

EXAMPLE 5

DEAE-cellulose test strips for glucose

DEAE-cellulose ion exchange paper was treated in precisely the same way as in all of the steps of Example 1, except that the 5 mg. of galactose oxidase of its buffered mixed enzyme solution was replaced by 13 mg. of glucose oxidase. Thereby there were produced diagnostic test strips of the invention for use, for example, in testing for bacteriuria.

EXAMPLE 6

"Ecteola"-cellulose test strips for glucose

"Ecteola"-cellulose ion exchange paper was treated in precisely the same way as in all of the steps of Example 5 and yielded finished "Ecteola"-cellulose diagnostic test strips for use like those of Example 5.

EXAMPLE 7

Aminoethyl cellulose test strips for glucose

Aminoethyl cellulose ion exchange paper was treated in procisely the same way as in all of the steps of Example 5 and yielded finished aminoethyl cellulose diagnostic test strips for use like those of Example 5.

The hydrophilic colloid stabilizer used in any of Examples 1–3 and 5–7 is not essential to the useful functioning of the diagnostic test strips of the invention, which strips then can be the product of any of the foregoing examples wherein the polyethylene glycol was omitted or was replaced in part or as a whole by any other hydrophilic colloid compatible with the other substances used in treating the ion exchange cellulose. Such other hydrophilic colloids include albumin, bovine serum albumin, glucose-free egg white, methyl cellulose, agar, tragacanth, gum acacia, karaya gum, algin, pectin, pentosans, dextran, gelatin, polyvinylpyrrolidone, gluten sulfate, sodium gluten phosphate, and other similarly effective. The amount to use of any of them is not critical and generally can be from about 25 to about 250% by weight of the galactose oxidase, glucose oxidase, or oxidase of any other sugar for which a diagnostic test strip of the invention is to be prepared.

So also either one of the specific buffers used at each of the two different stages of any of Examples 1–3 and 5–7 can be replaced by the respectively suitable quantity of an applicable concentration of any other compatible buffering agent for providing substantially neutral conditions such as between about pH 6.5 to 7.5 and beneficially generally more nearly 7.0.

Each of the two foregoing possible substitutions is illustrated by:

EXAMPLE 8

Albumin as colloid in diagnostic test strip

DEAE-cellulose ion exchange paper was treated in precisely the same way as in all of the steps of Example 5, except that for the buffered mixed enzyme solution, the 2 ml. of 0.5 M sodium phosphate buffer and the 50 mg. of polyethylene glycol were replaced respectively by:

2 ml. of 1.0 M sodium phthalate buffer pH 6.7, and
20 mg. of albumin.

That resulted in corresponding diagnostic test strips wherein the second buffer is sodium phthalate and the hydrophilic colloid stabilizer is albumin.

In any of the Examples 1–3 and 5–8, the specific ion exchange cellulose paper can be replaced by that used in any of the other examples or by any other ion exchange cellulose paper even one prepared from mixtures of more than one of any of the specific ion exchange cellulose derivatives, all of which may be either anionic or cationic or mixtures of both anionic and cationic cellulose derivatives can be used.

So also in any of those examples or any indicated modifications of any of them, ortho-tolidine as the specific chromogen can be replaced by an equivalent quantity of any other compatible applicable chromogen such as ortho-dianisidine, mesidine, meta-tolidine, benzidine, ortho - methylbenzidine, 4,4′ - diaminodiphenyl, ortho-, meta-, or para-phenylenediamine, 2,3-, 2,4-, 2,5- or 2,6-toluylenediamine, pyrogallic acid, pyrogallol, gallic acid, phloroglucinol, hydroquinone, guaiacol, and others.

EXAMPLE 9

Detecting glucose in urine

Samples of the urine from test subjects were tested with diagnostic test strips of the separate Examples 5 through 8 by the procedure shown by Example 4. These enabled detection of glucose present at a level of from 0.5 to 3 mg. per 100 ml. of urine with the characteristic blue to blue-green color reaction developing with ortho-tolidine within 3 minutes. Levels of glucose above 4 mg. per 100 ml. of urine produced the color reaction within about 1 minute.

In the glucose detection test of Example 9 as well as in the galactose detection test of Example 4, there can be used any other diagnostic test strip of any modifications of the Examples 5 through 8 and of Examples 1, 2 and 3 respectively wherein the ortho-tolidine was replaced by any other of the earlier above indicated applicable chromogens. In each of such substitutions the corresponding color change respectively shown by the specific chromogen used appears if the body fluid sample contains galactose and a test strip of some such modification of Examples 1–3 was used, or if it contains glucose and the test strip was a such modification of Examples 5 through 8.

The diagnostic test device of the invention is referred to broadly as a strip. Such strip can be composed of ion exchange cellulose derivative paper as already described or even of such cellulose derivative fabric. In any event, while it is advantageous that the strip be of filter paper consistency, the term "strip" is used in the appended claims broadly to embrace the diagnostic device whether of filter paper or greater thickness and whether of ion exchange cellulose derivative paper, fabric film, sheet layer or plate.

What is claimed is:

1. A diagnostic test indicator for detecting in an animal body fluid the presence of a sugar such as may occur in such body fluid, which comprises a body-fluid absorbent ion-exchange-effective cellulose derivative bibulous strip including (i) a body fluid test sample absorption portion, and (ii) contiguous with said sample absorption portion and extending therefrom a reactive-indicator portion impregnated with a reactive-indicator composition comprising peroxidase, an oxidizable chromogen convertible to a differently colored oxidation product, and an oxidase specific to the sugar to be tested for.

2. A test indicator as claimed in claim **1**, wherein said

ion-exchange-effective strip comprises the anionic type ion-exchange material.

3. A test indicator as claimed in claim 1, wherein the chromogen is ortho-tolidine.

4. A test indicator as claimed in claim 1, wherein the oxidase is galactose oxidase.

5. A test indicator as claimed in claim 4, wherein the cellulose derivative is N,N-diethylaminoethyl cellulose.

6. A test indicator as claimed in claim 4, wherein the cellulose derivative is aminoethyl cellulose.

7. A test indicator as claimed in claim 4, wherein the cellulose derivative is "Ecteola"-cellulose obtained by treating an alkali metal salt of cellulose with epichlorhydrin and then with triethanolamine, and washing out the alkali metal chloride.

8. A test indicator as claimed in claim 1, wherein the oxidase is glucose oxidase.

9. A test indicator as claimed in claim 8, wherein the cellulose derivative is N,N-diethylaminoethyl cellulose.

10. A test indicator as claimed in claim 8, wherein the cellulose derivative is aminoethyl cellulose.

11. A test indicator as claimed in claim 8, wherein the cellulose derivative is "Ecteola"-cellulose obtained by treating an alkali metal salt of cellulose with epichlorhydrin and then with triethanolamine, and washing out the alkali metal chloride.

12. The method of testing an animal body fluid to detect therein the presence of a specific sugar which might occur in said fluid, which method comprises immersing in a test sample of said fluid the fluid test sample absorption portion of a body-fluid-absorbent ion-exchange-effective cellulose derivative bibulous test strip for a time sufficient to allow said fluid to pass through the remainder of said ion-exchange-effective sample absorption portion and from there to pass into a contiguous and continuing therefrom reactive-indicator portion of said bibulous strip, which latter portion is impregnated with a reactive-indicator composition comprising peroxidase, an oxidizable chromogen convertible to a differently colored oxidation product, and an oxidase specific to the sugar being tested for; and allowing said body fluid which had passed up from said ion-exchange portion to said reactive-indicator portion to remain in contact with the reactive-indicator composition for a time sufficient for a color change to occur in its chromogen if the specific sugar is present in the body fluid sample.

13. The method as claimed in claim 12, wherein the sugar being sought for in the test is galactose and the oxidase in the reactive-indicator composition is galactose oxidase.

14. The method as claimed in claim 12, wherein the sugar being sought for in the test is glucose and the oxidase in the reactive-indicator composition is glucose oxidase.

15. The method of testing an animal body fluid to detect therein the presence of glucose in the range from about 5 milligrams per 100 milliliters of said fluid to as little as about 0.5 milligram per 100 milliliters, which method comprises subjecting said fluid to the method as claimed in claim 12 and wherein the oxidase in the reactive-indicator composition is glucose oxidase.

16. The method of testing whether a subject is ill with bacteriuria at any stage from latent to active, which method comprises processing a test sample of a body fluid from that subject by the method as claimed in claim 15.

References Cited

UNITED STATES PATENTS

2,850,359	9/1958	Worthington et al.	23—253(TP)X
3,005,714	10/1961	Cooper	23—253(TP)X
3,050,373	8/1962	Collins	23—253TP
3,092,465	6/1963	Adams, Jr.	23—253TP
3,104,209	9/1963	Scott	23—253(TP)X
3,235,337	2/1966	Artis	23—253TP
3,362,886	1/1968	Rupe	23—230Bio

OTHER REFERENCES

Calmon et al., "Ion Exchangers in Organic and Biochemistry," pp. 335–338, 527, 534, 581, 738, Interscience Publishers, Inc., New York, 1957.

C. A. 53:16416d (1959).

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23—230, 253, 253TP; 210—25

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,598,704 Dated August 10, 1971

Inventor(s) Arne Lennart Dahlqvist

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 3, line 13, column 4, line 40, column 5, line 32, and column 7, lines 12 and 23, '"Ecteola"', each occurrence, should read -- ECTEOLA --. Column 5, line 9, "The" should read -- There --; line 57, "other" should read -- others --.

Signed and sealed this 30th day of May 1972.

(SEAL)
Attest:

EDWARD M.FLETCHER,JR.
Attesting Officer

ROBERT GOTTSCHALK
Commissioner of Patents

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