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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Diagnostic agent.

We, BOEHRINGER MANNHEIM G.M.B.H. (formerly C. F. BOEHRINGER & SOEHNE G.M.B.H.), of Mannheim-Waldhof, Germany, a Corporation organised under the laws of Germany, do hereby declare the invention, for which we pray, that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with a new and improved diagnostic agent for the detection of components in body fluids and is also concerned with the production of this new diagnostic agent.

The detection of components in body fluids is of ever increasing importance in medical diagnosis. The detection procedures have already been considerably simplified by the use of test tablets and test papers but many of the tests of this type leave a lot to be desired with regard to their actual use, as well as with regard to their reliability. Furthermore, the large scale production thereof frequently gives rise to difficulties. This applies particularly to the processes for the determination of components in cloudy and strongly coloured liquids, such as full blood.

It is known to coat conventional test papers with a semi-permeable membrane and subsequently to hydrophobe them. However, these test papers can only be produced by several impregnations of filter papers which not only results in technical difficulties from the point of view of production but also increases the cost consider-

ably. Since, with an increasing number of process steps, the possible number of production errors increases, either the reliability of a test paper is reduced or additional intermediate controls are necessary, which are, of course, expensive.

For the determination of the hydrogen ion concentration in cloudy, muddy or strongly coloured liquids and fluids, it is also known to use cellulose films containing indicators (c.f. P. Wulff, Kolloid Z., 40, 341/1926). These cellulose films are very swellable, hydrophilic and non-water-resistant membranes which, in addition, are difficult to produce from a technical point of view. Thus, the indicator-containing cellulose solutions must be forced through slits into an acid bath and must subsequently be subjected to a further treatment until there are no longer any acid residues in the film.

We have now, surprisingly, found that diagnostic agents for the detection of components in body fluids, which satisfy all requirements, can be produced when all the reagents necessary for a detection reaction are introduced into a solution or dispersion of those film-forming substances which can be made into films which are resistant to hydrolysis and are substantially non-swellable in aqueous media. For this purpose, the film-forming mixture is applied either to a non-retaining substrate or to a substrate to which the film adheres firmly, whereafter the coated substrate is dried. In the first case, the film can subsequently be removed from the substrate, whereas in the second case, the substrate serves as a carrier for

[Price 4s. 6d.]

Price 25p

SPECIFICATION AMENDED - SEE ATTACHED SLIP

the film so that, in the case of this variant, the film can be mechanically less stable and thinner.

Consequently, the diagnostic agent according to the present invention for the detection of components in body fluids comprises a water-resistant film in which the reagents for the detection reaction are substantially uniformly distributed, the film being, if desired, applied to a substrate.

As film-forming agents, there can be used those which are soluble in organic solvents or which can be dispersed in water, such as cellulose ethers, cellulose esters, cellulose acetate phthalate, polyvinyl esters, polyvinyl acetals, polyacrylic esters, polyacrylamide, polyamides and other film-forming, natural and synthetic polymers, as well as mixtures thereof. Ethyl cellulose, acetyl cellulose, polyvinyl acetate, polyvinyl propionate, polyvinyl butyral and rubber latex have proved to be especially useful.

As carriers for coating with the films, it is preferred to use synthetic resin foils but it is also possible to use, for example, glass, wood, paper, metal foils and other carriers.

Apart from the reagents necessary for the detection reaction, the film-forming masses can also contain conventional fillers and adjuvants, such as chalk, titanium dioxide, silica gel, starch, talc, pearl white, metal soaps and cellulose powder, as well as suitable thickening agents, emulsifiers, dispersion agents and plasticisers. By the addition of pigments, the films can also be rendered opaque. The films can also be applied to a coloured carrier material. In this manner, the reagent foils, in contradistinction to completely transparent foils, can be compared directly with colour charts without porcelain tiles or the like being necessary.

If small amounts of wetting agents are added to the film-forming solutions or dispersions, these can be uniformly coated on to hydrophobic synthetic resin foils without the other properties of the diagnostic agents according to the present invention thereby being substantially changed.

The composition of the reagents for the detection of components in body fluids is, in principle, the same as in the case of the previously known diagnostic agents. Thus, for example, glucose can be detected with the use of a mixture of glucose oxidase, peroxidase, buffer and colour-forming agent. For the detection of blood in urine, there can be used a mixture of peroxide and a colour-forming agent and urobilinogen can be detected with *p*-dialkylamino-benzaldehydes and a strong acid. As examples of other typical detection reactions, there may be mentioned the Griess nitrite test and the detection of uric acid with the help of uricase-peroxidase and a colour-forming agent.

It was certainly not to have been foreseen that the new diagnostic agents according to the present invention could even be used for the intended purpose since it was to have been assumed that the reagents enclosed in films which are resistant to hydrolysis and are substantially non-swellable in aqueous media, would either not react at all with the fluid to be investigated or would not react quickly enough. Typical hydrophilic and non-water-resistant foils, such as those of cellulose or gelatine, swell in water and are able to take up several times their own weight of water. Nevertheless, in the case of cellulose indicator films, the take up of water in comparison with the absorbency of paper is already reduced to such an extent that the Wulff indicator films must be dipped in the fluid under investigation for at least one minute. In the case of the determination of pH with the help of cellulose reagent films, it only depends upon the migration velocity of the mobile and small hydrogen and hydroxyl ions, whereas in the case of the diagnostic agents according to the present invention, the substantially larger and considerably less mobile molecules of the components to be detected in body fluids must pass through the film material in order to reach the reagents.

It is also surprising that such large and sensitive molecules as those of the enzymes also retain their reactivity in the solid solutions of the synthetic resin films. In addition, it is extremely surprising that, in spite of the improved protection of the reagents from external influences, enzyme reactions requiring oxygen, for example, the detection of glucose with the use of glucose oxidase, peroxidase and a colour-forming agent, also take place quickly and without trouble in the diagnostic agents according to the present invention.

The advantage of the new diagnostic agents according to the present invention, in comparison with the previously known test papers and indicator films, is, in particular, their greatly simplified production. In comparison with the cellulose films, there is the further advantage that a precipitation in an acid bath, in which sensitive reagents would be destroyed or decomposed, is not necessary. From the chemical point of view, test papers and cellulose films consist of the same material, namely, cellulose. Since the reagent films according to the present invention can be made from the most varied materials, the most compatible and best suited material for each special purpose can be selected.

Furthermore, the materials used according to the present invention can be worked up much more easily with constant quality, are more homogeneous and, consequently,

are more dependable. Furthermore, we have found that not only the mechanical stability of the foils but also the stability of the reagents is better than in the case of test papers. Even after reaction has taken place with the body fluid being investigated, the new test film according to the present invention are considerably more stable than the previously used test papers so that the results obtained can, if desired, be evaluated much later or can even be checked after several days. Thus, for example, diagnostic agents according to the present invention for the detection of glucose in blood or urine can be enclosed with a patient's examination report and can be filed away with the documents relating to the findings.

The production of the new diagnostic agents according to the present invention takes place, in the simplest way, by the application of the film-forming solution or dispersion to a suitable substrate and subsequent drying. In the case of the continuous application of narrow film strips on to suitable substrates, preferably on to synthetic resin foils, it is, in addition, possible to apply different reagent mixtures side by side on a substrate. After drying has been carried out, this substrate is cut at right angles to the direction of application to give narrow strips; in other words, there are obtained multi-test strips.

In contradistinction to test papers, the reagent concentration of which cannot be varied as desired because of the absorbency and the saturation limit of the paper, the reagent films according to the present invention can be produced with any desired thickness and with any desired reagent concentration. Since the reagents also cannot be leached out or non-uniformly distributed by chromatographic effects, the fluid to be investigated can, if desired, be left to act upon the film for a comparatively long period of time, a stronger colour reaction thereby being produced. Therefore, the new diagnostic agents according to the present invention can be used not only for the previously known test reactions but also for those which, because of the known disadvantages of test papers, have hitherto not been capable of being made up into the considerably simpler and easier used form of a test strip.

The following Examples are given for the purpose of illustrating the present invention:

EXAMPLE 1

For the production of a reagent film for the detection of glucose in, for example, urine or full blood, there is prepared a mixture of the following composition:

polyvinyl propionate	
(50% aqueous dispersion)	100.00 g.
o-vanillin-(vanilloyl)-hydrazone	2.50 g.
glucose oxidase	0.06 g.

peroxidase	0.01 g.
colloidal silicic acid	4.00 g.
phosphate buffer 0.4M (pH 5.7)	70.00 ml.
sodium alginate solution (2.5%)	35.00 g.
organic sodium sulphonate ("Rapidnetzer BASF")	1.6 g.
methanol	40.00 ml.

On to a white polyvinyl chloride foil, there is applied a 400 μ thick layer of the above mixture which is subsequently dried with warm air. There is formed a water-insoluble film which gives graduated violet colour reactions with glucose in, for example, urine or blood. The reaction with glucose in blood can be seen as soon as the blood is wiped or washed off the foil. The colour reactions stand out well from the background of the white carrier foil.

EXAMPLE 2

For the production of a reagent foil for the detection of glucose in, for example, urine or full blood, there is prepared a mixture of the following composition:

ethyl cellulose	35.00 g.
o-vanillin-(vanilloyl)-hydrazone	1.7 g.
glucose oxidase	0.04 g.
peroxidase	0.011 g.
colloidal silicic acid	2.00 g.
phosphate buffer 0.4M (pH 5.7)	25.00 ml.
organic sodium sulphonate ("Rapidnetzer BASF")	1.00 g.
methanol	75.00 ml.

On to a white polyvinyl chloride foil, there is applied a 500 μ thick layer of the above mixture which is subsequently dried with warm air. There is obtained a water-insoluble film, the properties of which correspond to those of the film described in Example 1.

EXAMPLE 3

For the production of a reagent film for the detection of blood in urine, there is prepared a mixture of the following composition:

o-tolidine	0.25 g.
strontium peroxide	2.0 g.
tartaric acid	3.75 g.
calcium acetate	7.0 g.
colloidal silicic acid	1.5 g.
sodium carbonate	2.0 g.
polyvinyl acetate	5.0 g.
anhydrous isopropanol	70.0 ml.

On to a polyvinyl chloride foil, there is applied a 500 μ thick layer of the above mixture which is subsequently dried with warm air. There is obtained a water-insoluble film which, upon dipping into blood-containing urine, gives a more or less strong colour, depending upon the blood concentration.

EXAMPLE 4

For the production of reagent films for the detection of urobilinogen in urine, there is prepared a mixture of the following composition:

- p-dimethylamino-benzaldehyde 1.0 g.
 orthophosphoric acid (85%) 20.0 g.
 polyvinyl acetal 20.0 g.
 organic sodium sulphonate
 5 ("Rapidnetzer BASF") 0.5 g.
 methanol 100.0 ml.

On to a polyvinyl chloride foil, there is applied a 300 μ thick layer of the above mixture which is subsequently dried with warm air. There is obtained a water-insoluble film which, upon moistening with urobilinogen-containing urine, shews a more or less dark red colour, depending upon the urobilinogen concentration.

15 EXAMPLE 5

For the production of reagent films for the detection of nitrite in urine, there is prepared a mixture with the following composition:

- 20 α -naphthylamine 1.0 g.
 sulphanilamide 2.5 g.
 trichloroacetic acid 3.0 g.
 polyvinyl butyral 20.0 g.
 methanol 100.0 ml.

25 Onto a polyvinyl chloride foil, there is applied a 400 μ thick layer of the above mixture, which is subsequently dried with warm air. There is obtained a water-insoluble film which, upon moistening with nitrite-containing urine, shews a more or less dark red colour, depending upon the nitrite content.

30 EXAMPLE 6

For the production of a reagent film for the detection of glucose in, for example, urine or full blood, there is prepared a mixture of the following composition:

- polyvinyl propionate
 (50% aqueous dispersion) 45.0 g.
 phosphate buffer 0.4M (pH 5.5) 45.0 ml.
 40 sodium alginate 0.5 g.
 organic sodium sulphonate
 ("Rapidnetzer BASF") 0.6 g.
 3-amino-9-ethyl-carbazole 0.03 g.
 o-tolidine 0.2 g.
 45 glucose oxidase 0.04 g.
 peroxidase 0.02 g.
 methanol 6.0 g.

On to a white polyvinyl chloride foil, there is applied a 300 μ thick layer of the above mixture, which is subsequently dried in warm air. There is obtained a water-insoluble film which gives a colour reaction with glucose, for example, in blood in the range of 50-250 mg.%. When a drop of blood is placed on the test strip and then again removed after about one minute, then, depending upon the glucose concentration, the strip is coloured orange to blue-green.

EXAMPLE 7

- 60 90 g. of a 50% aqueous dispersion of polyvinyl propionate (commercially available under the Registered Trade Mark "Propiofan"), 1.3 g. of the sodium salt of a polymannuronic acid ("Algipon"), 70 ml. of a 0.5M citrate or phosphate buffer with a

pH value of 5.5, 2 g. of organic sodium sulphonate ("Texapon P", 320 mg. glucose oxidase, 300 mg. peroxidase and 300 mg. 1-methoxy-4-hydroxy-naphthalene in 20 ml. methanol are stirred to give a homogeneous mixture. This mixture is then applied to a foil with a layer thickness of 300 μ and dried. When a drop of blood or serum is placed upon a film produced in this manner and then again removed after about one minute, then the film is coloured blue, the strength of the colour depending upon the concentration of the glucose.

EXAMPLE 8

45 g. of a polyvinyl propionate dispersion ("Propiofan"), 35 g. of a 1.85% solution of polymannuronic acid ("Algipon") in 0.5M phosphate buffer with a pH value of 5.5, 1 g. of organic sodium sulphonate ("Texapon"), 0.075 g. peroxidase, 0.15 g. glucose oxidase, as well as 0.045 g. 3-amino-9-ethyl-carbazole and 0.25 g. o-tolidine dissolved in 6 ml. methanol, are stirred to give a homogeneous slurry. 0.50 ml. of a 0.5% methanolic solution of Sico-Fettblau 50401 N (Siegle & Co., Stuttgart-Feuerbach) are then also added thereto. The mixture so prepared is applied to a synthetic resin foil at a layer thickness of 300 μ and dried. When, to a reagent film produced in this manner, there are applied samples of urine with various glucose concentrations, these allowed to react for a short time and then again wiped off, then in the case of a glucose concentration of 50 mg.% the film is coloured bright pink, at a concentration of 250 mg.% is coloured dark orange-pink, at a concentration of 500 mg.% is coloured green and at a concentration of 1000 mg.% is coloured dark green. Thus, a clearly graduated colour change occurs.

EXAMPLE 9

For the production of a reagent film for the detection of urea, there is prepared a mixture of the following composition:

- polyisobutylene dispersion
 ("Oppanol B", 60%) 22.50 g.
 sodium alginate solution (1.85%)
 in 0.15M phosphate buffer
 (pH 6.8) 19.50 g.
 urease 0.25 g.
 Brilliant Yellow (available from
 E. Merck A.G., Darmstadt) 0.02 g.
 organic sodium sulphonate
 ("Texapon P") 0.50 g.

On to a white foil, there is applied a 300 μ thick layer of the above mixture, which is subsequently dried with warm air. There is obtained a film which gives orange colour reactions with urea in, for example, urine, serum or full blood. The reaction with urea, for example, in blood can be seen when the blood is dropped on to the foil and then wiped off after 45 seconds. There are obtained, depending upon the urea con-

tent, colour graduations from yellow-orange via orange to red-orange.

WHAT WE CLAIM IS:—

1. Diagnostic agent for the detection of components in body fluids, said agent being a film which is resistant to hydrolysis and is substantially non-swellable in aqueous media and in which reagents for said detection are substantially uniformly distributed.
2. Diagnostic agent according to claim 1, wherein the film is applied to a substrate.
3. Diagnostic agent according to claim 2, wherein the film is firmly attached to the substrate.
4. Diagnostic agent according to claim 2, wherein the film is loosely attached to the substrate and can, if desired, be removed therefrom.
5. Diagnostic agent according to any of the preceding claims, wherein the film is made from a solution or dispersion of one or more natural or synthetic polymers.
6. Diagnostic agent according to any of the preceding claims, wherein the film is made from at least one polymer selected from cellulose ethers, cellulose esters, cellulose acetate phthalate, polyvinyl esters, polyvinyl acetals, polyacrylic esters, polyacrylamide and polyamides.
7. Diagnostic agent according to any of claims 1 to 5, wherein the film is made from at least one polymer selected from ethyl cellulose, acetyl cellulose, polyvinyl acetate, polyvinyl propionate, polyvinyl butyral and rubber latex.
8. Diagnostic agent according to any of claims 2-7, wherein the substrate is glass, wood, paper, metal foil or synthetic resin foil.
9. Diagnostic agent according to any of the preceding claims, wherein the film additionally contains at least one member selected from fillers, thickening agents, emulsifiers, dispersion agents and plasticisers.
10. Diagnostic agent according to any of claims 2-9, wherein the film has been rendered opaque by the incorporation of an appropriate pigment or wherein the film is applied to a coloured substrate.
11. Diagnostic agent according to any of the preceding claims, wherein the film comprises two or more areas containing different reagents so that two or more detection reactions can be simultaneously carried out with one diagnostic agent film.
12. Diagnostic agents according to claim 1, substantially as hereinbefore described and exemplified.
13. Process for the production of a diag-

nostic agent according to claim 1, wherein a solution or dispersion of at least one synthetic or natural, water-resistant, film-forming polymer containing reagents substantially uniformly dispersed therein is applied to a substrate and subsequently dried, whereafter, if desired, the film is removed from the substrate.

14. Process according to claim 13, wherein two or more solutions or dispersions of said polymer, each containing different reagents for different detection reactions, are applied to the substrate adjacent to one another.

15. Process for the production of a diagnostic agent according to claim 1, substantially as hereinbefore described and exemplified.

16. Diagnostic agents, whenever produced by the process according to any of claims 13-15.

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Agents for the Applicants.



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15 substrate.
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- 20 5. Diagnostic agent according to any of the preceding claims, wherein the film is made from a solution or dispersion of one or more natural or synthetic polymers.
- 25 6. Diagnostic agent according to any of the preceding claims, wherein the film is made from at least one polymer selected from cellulose ethers, cellulose esters, cellulose acetate phthalate, polyvinyl esters, polyvinyl acetals, polyacrylic esters, poly-
30 acrylamide and polyamides.
7. Diagnostic agent according to any of claims 1 to 5, wherein the film is made from at least one polymer selected from ethyl cellulose, acetyl cellulose, polyvinyl
35 acetate, polyvinyl propionate, polyvinyl butyral and rubber latex.
8. Diagnostic agent according to any of claims 2-7, wherein the substrate is glass, wood, paper, metal foil or synthetic resin
40 foil.
9. Diagnostic agent according to any of the preceding claims, wherein the film additionally contains at least one member selected from fillers, thickening agents,
45 emulsifiers, dispersion agents and plasticisers.
10. Diagnostic agent according to any of claims 2-9, wherein the film has been rendered opaque by the incorporation of an
50 appropriate pigment or wherein the film is applied to a coloured substrate.
11. Diagnostic agent according to any of the preceding claims, wherein the film comprises two or more areas containing
55 different reagents so that two or more detection reactions can be simultaneously carried out with one diagnostic agent film.
12. Diagnostic agents according to claim 1, substantially as hereinbefore described
60 and exemplified.
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nostic agent according to claim 1, wherein a solution or dispersion of at least one synthetic or natural, water-resistant, film-forming polymer containing reagents substantially uniformly dispersed therein is applied
65 to a substrate and subsequently dried, whereafter, if desired, the film is removed from the substrate.

14. Process according to claim 13, 70 wherein two or more solutions or dispersions of said polymer, each containing different reagents for different detection reactions, are applied to the substrate adjacent to one another. 75

15. Process for the production of a diagnostic agent according to claim 1, substantially as hereinbefore described and exemplified.

16. Diagnostic agents, whenever pro- 80 duced by the process according to any of claims 13-15.

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