The present invention concerns a carrier-bound, multicomponent detection system for the colorimetric determination of esterolytically- and/or proteolytically-active components of body fluids.

The detection of esterolytically- and/or proteolytically-active components of body fluids is of great importance. Especially for the diagnosis of diseases of the kidneys and of the urogenital tract, it is important to detect leukocytes in urine on the basis of their inherent esterolytic and/or proteolytic activity. Carrier-bound detection systems, e.g. in the form of test strips, have proved to be quite especially advantageous herefor because these only need to be dipped briefly into the liquid sample to be investigated and then subsequently, after the removal of the strip from the sample, permit a determination of the number of leukocytes/µl of sample, e.g. on the basis of a coloration, within a short time. There is thus provided a clean, simple and rapid possibility for the detection of such component materials in body fluids without complicated time- and cost-consuming manipulations having to be carried out with or on the sample for this purpose.

From the prior art, there are known, in particular, the so-called multicomponent systems as advantageous detection systems for the colorimetric determination of esterolytically- and/or proteolytically-active components of body fluids, such as e.g. of leukocytes in urine. These are those multicomponent systems which consist of several chemical substances, at least one of which is so chemically changed by esterolytically- and/or proteolytically-active component materials of body fluids that the reaction product reacts directly with one or, in a reaction sequence, with several of the other components of the

detection system to give a coloured substance which can then be determined colorimetrically. The amount of coloured substance formed is a measure for the amount of esterolytically- and/or proteolytically-active component materials in the sample investigated.

Especially suitable for this type of detection of esterolytically- and/or proteolytically-active components in body fluids are e.g. two-component systems in which an ester is first cleaved by the component materials to be detected and the resultant hydroxy compound is then reacted by a further reactive substance to give a coloured material. Carrier-bound multicomponent systems of this principle are known e.g. from European Patent Application 0 039 880.

This Patent Specification concerns, inter alia, carrier-bound detection systems for the determination of esterolytically- and/or proteolytically-active component materials in body fluids, such as e.g. leukocytes in urine, in which an ester cleavable by this component and a diazonium salt as reaction component for the hydroxy compound produced by cleavage of the ester are applied together to the same reagent carrier. The joint presence of all components of the detection system on one and the same reagent carrier appeared to be optimal insofar as it appears to be ensured that the product resulting by reaction with the esterolytically- and/or proteolytically-active component materials can quickly react further to give a coloured end product.

A rapid and complete taking place of the detection reaction forms the basis for a sensitive test. In the case of the above-mentioned detection systems, the detection limit for leukocytes in urine in the case of reading off the value after about 2 minutes lies at about 15 to 50 leukocytes/µl of urine. However, from

the point of view of the physician, it is desirable to have available detection agents which are even more sensitive. Comparatively long waiting periods up to the reading off of the value to be determined are undesirable.

From EP-A-0 064 710, a test agent is known which has a layer retardedly absorbing the liquid sample and a layer which contains the reagents necessary for the detection reaction.

From EP-A-0 244 825 it is known that, in the case of cholesterol tests on redox basis, the separation of a coloured precursor and of an electron transmitter leads to an increased sensitivity and exactitude.

In FR-A-2514511 is described the increasing of the sensitivity of immune tests by carrying out on a test strip.

From EP-A-0 200 540 is known an analytical element for the determination of creatine kinase in which the creatine phosphate and an indicator substance reacting with H_2O_2 are present in separate layers.

Also in EP-A-0 137 521 such a multi-layer analytical element is described in which an oxidase containing layer is also additionally present.

Therefore, the task forming the basis of the invention is to make available for medical diagnosis a carrier-bound, multicomponent detection system for the colorimetric determination of esterolytically- and/or proteolytically-active component materials of body fluids which exceeds the sensitivity of the carrier-bound detection systems known for this purpose without being involved with the disadvantage of a comparatively long waiting time up to the reading off of the value indicated by the detection system.

Consequently, there has been found the initially-defined carrier-bound, multicomponent detection system for the colorimetric determination of esterolytically- and/or proteolytically-active component materials of body fluids, containing a component esterolytically and/or proteolytically cleavable by the component material and a component reacting with the product of this reaction to give a coloured substance, which is characterised in that the components of the detection system are present in different reagent carriers making possible a liquid exchange between them.

As multicomponent detection systems for the colorimetric determination of esterolytically- and/or proteolytically-active component materials of body fluids, there hereby come into question the reagent systems known herefrom from the prior art. Those multicomponent detection systems have proved to be especially advantageous which contain an esterolytically- and/or proteolytically-cleavable ester and a diazonium salt. Such systems have proved their suitability especially for the determination of leukocytes in urine. However, they can also serve for the detection of proteolytic enzymes, such as e.g. elastase, chymotrypsin or trypsin, in aqueous solutions or in body fluids, such as e.g. whole blood, serum, liquor, pancreatic secretion or aqueous faecal extracts.

The reagent systems mentioned in Europen Patent Application 0,039,880 are quite especially advantageously usable according to the invention. For this purpose, at least one esterolytically- and/or proteolytically-cleavable ester and at least one diazonium salt of the there-described type are applied to separate reagent carriers.

Besides the components of the detection system engaging directly chemically in the detection reaction,

such as e.g. ester and a diazonium salt, this can also contain still further adjuvants, such as e.g. buffer substances, wetting agents, stabilisers and/or activators. Such adjuvants are also known e.g. from European Patent Application 0,039,880. However, they do not comprise components of the detection system in the meaning of the present invention.

As reagent carriers, there can be used all materials conventionally employed herefor, such as e.g. absorbent or swellable, porous or non-porous materials or also those which, in the case of contact with the liquid to be investigated, dissolve therein. By way of example, there may be mentioned cellulose, filter paper, synthetic fibre or glass fibre fleece, polyvinyl ester, polyamide films or also films of e.g. xanthan gum.

Carrier-bound detection agents according to the invention contain the components of the detection system in different reagent carriers. The reagents engaging directly chemically in the detection reaction are only mixed with one another after the contacting with the liquid to be investigated by going at least partly into solution. The different reagent carriers are thereby admittedly separate but nevertheless are so arranged that a liquid exchange between them is possible. In a preferred embodimental form of the carrier-bound detection system according to the invention, e.g. the reagent carriers containing the particular components of the detection system, such as e.g. ester and diazonium salt, are arranged above one another quasi as stack. For the better handling of this reagent carrier stack, these can be applied e.g. to a synthetic material carrier, such as a stiff film. The fixing of the stack on to the synthetic material carrier can take place, for example, by enmeshing, by

edge adhesion or by sticking of a reagent carrier on to the synthetic material carrier and punctate adhesion of the further reagent carrier on to the previous one. For a better absorption of the liquid to be investigated, an empty fleece can, for example, also be placed below such a reagent carrier stack.

As materials for a reagent carrier lying directly on a synthetic material carrier, there are especially well suited synthetic material films, such as polyester or polyamide films, or absorbent materials, such as e.g. a cellulose fleece. For reagent carriers arranged thereover, there are especially advantageous, for example, fabrics, thin transparent porous materials, transparent films or materials which dissolve in the liquid to be investigated. By contact with the liquid to be investigated, for example by dipping in of such the carrier-bound detection agents or by measured application of the liquid to be investigated, the components of the detection system present in the reagent carriers are brought at least partly into solution, totally mixed together by the dissolving procedure and finally the detection reaction initiated in the case of the presence of esterolytically- and/or proteolytically-active component materials.

Surprisingly, it has been shown that the carrier-bound detection agent according to the invention is more than 100% more sensitive than the previously known agents. In the case of a waiting time of 2 minutes, 5 leukocytes/µl of sample can be determined colorimetrically. Values above 10 leukocytes/µl of sample can even already be read off 1 minute after wetting of the carrier-bound detection agent.

The invention is to be explained in more detail by the following Example. However, it is not to be regarded as limited thereto.

Example 1.
Sensitivity of the test for the detection of leukocytes in urine.

A) Carrier-bound detection system of the prior art according to Figure 1 with single impregnation with regard to ester and diazonium salt:

A paper fleece 23 SL of the firm Schleicher & Schüll, Dassel, Federal Republic of Germany, was impregnated in the following manner with the adjuvants and components of the detection system:

a) Pre-impregnation with an impregnation solution which, in 1 l of water, contained 65 g boric acid and 21 g sodium hydroxide and had been adjusted with 1N hydrochloric acid to pH 8.0. Subsequent drying of the impregnated fleece.

b) Main impregnation of the buffer-impregnated fleece produced according to a) with a solution which, in 1 l of ethanol, contained 0.35 g 2-methoxy-4-(N-morpholino)-benzene-diazonium tetrachlorozincate, 0.78 g 3-(N-toluene-4'-sulphonyl-L-alanyloxy)-indole, 81.3 g phosphoric acid trimorpholide and 21.7 ml decanol and subsequent drying.

The so impregnated fleece was cut up into squares of 6 x 6 mm and the so produced reagent carriers (1) each fixed by means of a nylon mesh (2) NY75HC (filament thickness 60 µm) of the firm Züricher Beuteltuchfabrik AG, Zürich, Switzerland, on to a 12.6 cm long, 6 mm wide and 1 mm thick polystyrene strip (3). For this purpose, the nylon mesh was partly melted with the synthetic resin strip by heating to about 175°C.

B) Carrier-bound detection system according to Figure 1 with separate impregnation with regard to the ester and diazonium salt:

A paper fleece 23 SL of the firm Schleicher & Schüll, Dassel, Federal Republic of Germany, was impregnated in the following manner with the adjuvants and components of the detection system:

a) Pre-impregnation with an impregnation solution which, in 1 l of water, contained 65 g boric acid and 21 g sodium hydroxide had been adjusted with 1N hydrochloric acid to pH 8.0. Subsequent drying of the impregnated fleece.

b) Impregnation of the buffer-impregnated fleece produced according to a) with a solution which, in 1 l of ethanol, contained 0.78 g 3-(N-toluene-4'-sulphonyl-L-alanyloxy)-indole, 81.3 g phosphoric acid trimorpholide and 21.7 ml decanol and subsequent drying.

c) Impregnation of the fleece impregnated according to a) and b) with a solution which, in 1 l of water, contained 0.37 g 2-methoxy-4-(N-morpholino)-benzene-diazonium tetrachlorozincate and 0.22 g tartaric acid and subsequent drying.

Analogously to A), a carrier-bound detection system was produced according to Figure 1 with the so impregnated paper fleece.

C) Detection system according to the invention (Fig.2):

I) A paper fleece 23 SL of the firm Schleicher & Schüll, Dassel, Federal Republic of Germany, was impregnated in the following way:

a) Pre-impregnation with an impregnation solution which, in 1 l of water, contained 65 g boric acid and 21 g sodium hydroxide and had been adjusted to pH 8.0 with 1N hydrochloric acid. Subsequent drying of the impregnated fleece.

b) Main impregnation of the buffer-impregnated fleece produced according to a) with a solution which, in 1 l of ethanol, contained 0.78 g 3-(N-toluene-4'-sulphonyl-

L-alanyloxy)-indole, 81.3 g phosphoric acid trimorpholide and 21.7 ml decanol and subsequent drying.

II) A nylon fabric NY 20 HC (filament thickness 20 µm) of the firm Züricher Beuteltuchfabrik AG, Zürich, Switzerland, was impregnated with a solution which, in 1 l of water, contained 3.78 g 2-methoxy-4-(N-morpholino)-benzene-diazonium tetrachlorozincate and 2.25 g tartaric acid and subsequently dried.

The impregnated paper fleece from I) was cut into squares of 6 x 6 mm and gave the reagent carrier (1). The nylon fabric produced in II) was cut up into squares of the same size and gave reagent carrier (4). The reagent carriers (1) and (4) were fixed as a stack by means of a nylon mesh (2) NY75HC (filament thickness 60 µm) of the firm Züricher Beuteltuchfabrik AG, Zürich, Switzerland, on to a 12.6 cm long, 6 mm wide and 1 mm thick polystyrene strip (3). The nylon mesh itself was fixed on to the synthetic material strip by partial melting by heating to about 175°C.

D) Leukocyte-containing solutions were produced by making up leukocyte-free urine with leukocytes isolated from blood. The following concentrations were present for the testing of the detection system: 0.5, 10, 20 and 40 leukocytes/µl of urine.

The test strips produced in A), B) and C) were dipped into the leukocyte-free and leukocyte-containing urine samples and, after 2 minutes, read off visually with regard to the colour formation or measured remission-photometrically. The following results were obtained with the detection systems described in A), B) and C):

number of leukocytes/µl of sample	colour formation in the case of detection system			increase of the remission measured with Urotron RL-9 in remission units (Boehringer Mannheim GmbH)		
	A	B	C	A	B	C
0	-	-	-	0	0	0
5	-	-	+	0	0	4
10	-	-	+	0	0	9
20	+	+	+	6	5	17
40	+	+	+	14	13	31

Whereas in the case of the detection systems A and B (prior art) 2 minutes after sample contact the limit of detection for leukocytes lay at about 20 leukocytes/µl of sample, with the detection system C according to the invention there were already indicated 5 leukocytes/µl of sample.

Already after 1 minute, with the detection system C there can be detected a concentration of 10 leukocytes/µl of sample visually or by means of remission measurement. In the case of this short time, with detection systems A and B the limit of detection lay at more than 20 leukocytes/µl.

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Patent Claims

1. Carrier-bound multi-component detection system for the colorimetric determination of esterolytically- and/or proteolytically-active components of body fluids containing a component cleavable esterolytically and/or proteolytically by the component material and a component reacting with the product of this reaction to give a coloured substance, characterised in that the components are present in different reagent carriers making possible a liquid exchange between them.

2. Detection system according to claim 1, characterised in that, as components, at least one esterolytically and/or proteolytically cleavable ester and at least one diazonium salt are applied to separate reagent carriers.

3. Process for the detection of leukocytes in urine, comprising

- bringing into contact of urine with at least one esterolytically and/or proteolytically cleavable component and at least one diazonium salt and
- reaction of the component cleaved by the leukocytes with the diazonium salt, characterised in that the diazonium salt is dissolved at least partly from a first reagent carrier and the cleavable component from a second reagent carrier and the components are mixed with one another.