

[54] **DIAGNOSTIC TEST STRIP FOR THE DETECTION OF COMPONENTS OF BODY FLUIDS**

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[58] Field of Search **23/230 B, 253 TP; 252/408, 252/186, 400 A; 195/103.5 R**

[56] **References Cited**

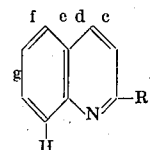
UNITED STATES PATENTS

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[57] **ABSTRACT**

Test strips are provided for detecting even small amounts of blood or other peroxidatively active substances in body fluids; the strips comprising a carrier containing a hydroperoxide, a chromogen, and as an activator, a compound of the formula



(1)

wherein R₁ is a hydrogen atom or a methyl radical and benzene and/or pyridine rings are fused on at least one of the positions indicated with c, (d,e), f and g, provided that two adjacent rings must not simultaneously each contain a cyclic nitrogen atom, and wherein the aromatic compounds (1), apart from the positions indicated by H and R₁, can be substituted by lower alkyl radicals which together can also form a hydroaromatic ring.

29 Claims, No Drawings

DIAGNOSTIC TEST STRIP FOR THE DETECTION OF COMPONENTS OF BODY FLUIDS

The present invention is concerned with a test strip for the sensitive and rapid detection of very small amounts of blood and of other peroxidate-active substances in body fluids.

The detection of small amounts of blood, which are invisible to the naked eye, in urine, feces or vomit is very important for the diagnosis of hemorrhages in the stomach, intestines and urinary tract. Such hemorrhages are caused, for example, by tumors, ulcers and inflammations of the corresponding organs. Furthermore, free hemoglobin can also occur in the urine and plasma due to the influence of certain hemolytic toxins in the blood. Blood and hemoglobin are peroxidate-active, i.e., they liberate oxygen from hydroperoxides and transfer it to certain acceptors. Other peroxidate-active substances occur in leukocytes and bacteria. The detection of these substances is important for the diagnosis of diseases and infections of the kidney and urinary tract. Myoglobin, which is also peroxidate-active, is found in the urine, for example, after a cardiac infarct. Furthermore, blood occurs especially frequently in the urine when calculi are present in the bladder or kidneys.

Their peroxidate action is especially suitable for a sensitive detection of all of these substances. The oxygen liberated from a hydroperoxide is hereby transferred to a chromogen which is oxidized to a colored substance and thus the presence of the peroxidate-active substance is indicated. This reaction has been used for quite a long time in medicinal and forensic analysis, especially for the detection of blood. The reaction is, as a rule, carried out in a test tube or as a spot test, hydrogen peroxide usually being employed as the hydroperoxide. As chromogen, there is preferably used benzidine, o-tolidine or leuko malachite green.

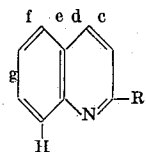
Rapid tests are usually absorbent carriers, preferably papers, which have been impregnated with all of the reagents necessary for the detection reaction. After simply dipping them into a body fluid, they show a color reaction. Because of the great importance which rapid tests have recently achieved, they have been developed in various ways for the detection of blood in body fluids.

Since, for the detection of blood, the sensitivity of the rapid test is of decisive importance and, furthermore, it is also desirable, if possible, to include leukocytes and bacteria which are less peroxidate-active, various attempts have already been made to increase the sensitivity of the known detection reactions by means of additives. Thus, for example, German Pat. No. 1,242,905 describes test papers which, as activating additives, contain certain quinoline derivatives, preferably quinine. It is certainly asserted that the sensitivity can be considerably increased with the mentioned additives but, according to our own investigations, it is practically impossible also to detect leukocytes and bacteria with the test strips improved in this manner.

The activating action of quinoline per se on the blood detection reaction has been known for a long time (see *Zeitschrift f. gerichtl. Med.*, 12, 216/1928); however, this, as well as its simple derivatives, are liquid or volatile and, therefore, cannot be used for a rapid test.

The instant invention provides test strips for the detection of peroxidate-active substances with a hitherto unachievably high sensitivity.

Essentially, the present invention comprises test strips comprising as the activator, an aromatic compound of the general formula:



(1)

wherein R_1 is a hydrogen atom or a methyl radical and benzene and/or pyridine rings are fused on at least one of the positions indicated with c , (d,e) , f and g , provided that two adjacent rings must not simultaneously each contain a cyclic nitrogen atom, and wherein the aromatic compounds (1), apart from the positions indicated by H and R_1 , can be substituted by lower alkyl radicals which together can also form a hydroaromatic ring.

Those compounds of general formula (1) are preferred in which R_1 is a hydrogen atom. By lower alkyl radicals, there are to be understood alkyl radicals containing up to four carbon atoms and, when two alkyl radicals together form a hydroaromatic ring, 5- and 6-membered rings are preferred.

In the following, there are given examples of the most important groups of compounds coming within the scope of general formula (1); the individual compounds are all known from the literature:

1. Benzoquinolines

- 1.1 benzo [c] quinoline (phenanthridine)
 - 2-methylphenanthridine
 - 6-methylphenanthridine
 - 2-ethylphenanthridine

- 1.2 benzo [f] quinoline
 - 3-methylbenzo [f] quinoline
 - 1,3-dimethylbenzo [f] quinoline
 - 1,2-tetramethylenebenzo [f] quinoline
 - 1,2-trimethylenebenzo [f] quinoline

- 1.3 benzo [g] quinoline
 - 4-methylbenzo [g] quinoline
 - 2,4-dimethylbenzo [g] quinoline

2. Dibenzoquinolines.

- 2.1 dibenzo [c,f] quinoline (benzo [a] phenanthridine)
- 2.2 dibenzo [c,d,e] quinoline (4-azapyrene, thebenidine)

3. Pyridoquinolines

- 3.1 pyrido [2,3-f] quinoline (1,7-phenanthroline)
 - 2-methyl-1, 7-phenanthroline
 - 2,8-dimethyl-1,7-phenanthroline
- 3.2 pyrido [3,2-f] quinoline (4,7-phenanthroline)
 - 3-methyl-4,7-phenanthroline
 - 3,8-dimethyl-4,7-phenanthroline
 - 1,3,4,8-tetramethyl-4,7-phenanthroline
- 3.3 pyrido [2,3-f] quinoline (1,6-anthrazoline)
 - 2,7-dimethyl-1,6-anthrazoline

Consequently, according to the present invention, there is provided a test strip for the detection of peroxidate-active substances in body fluids, comprising a carrier containing a hydroperoxide, at least one chromogen and, as activator, a compound of general formula (1).

It was not to have been foreseen that the modification according to the present invention, of the known activator quinoline would lead to such an extraordinary increase in effectiveness. It is important for the present invention that only anelation of certain positions of the quinoline system enables an increase of activity to be achieved. Thus, for example, in the case of anelation of the [b]- and [h]-positions of the quinoline, i.e., in the case of acridine or of benzo [h] quinoline, the activity is reduced in comparison with quinoline. The *b* and *h* positions being the next logical sides of the compound of general formula (I).

The effectiveness of the compounds used as activators according to the present invention is difficult to explain; if, as might be regarded as being obvious, it were due to complex formation with the peroxidate-active substances, then, for example, *o*-phenanthroline, which is known to be a strong complex former, should also have a strong activating effect. However, this is not the case, whereas *m*- and *p*-phenanthroline are very effective activators.

Surprisingly, the compounds of general formula (I) do not increase the sensitivity of peroxidases of vegetable origin, for example horseradish peroxidase, but act specifically on peroxidate-active substances of human and animal origin. It is thus possible selectively to detect leukocytes, blood and blood components and bacteria in feces or in vomit in the presence of vegetable peroxidases. In this case, myoglobin is detected with about the same degree of sensitivity as hemoglobin.

The sensitivity of the detection reaction is increased by some of the compounds of general formula (I) to such an extent that even in urine it is possible to detect individual erythrocytes which are visible on the test paper as colored dots. Thus, for example, by means of phenanthridine, it is possible to produce a test paper with which it is still possible clearly to detect 5 erythrocytes per mm³ urine. This corresponds to a blood dilution of 1:1,000,000.

It is, of course, obvious that not all of the compounds coming within the scope of general formula (I) possess activating properties of the same degree. Thus, it is possible to adjust the sensitivity of, for example, a blood test in accordance with practical requirements. For example, test strips of increasing activity are obtained when, as activator, there are used the compounds set out in the following and in the given order: *m*-phenanthroline < *p*-phenanthroline < benzo-(*f*)quinoline < phenanthridine.

The sensitivity can be further modified by alkyl substitution; thus, for example, by means of a methyl substituent in a α -position to a cyclic nitrogen atom, the activity is somewhat reduced, whereas otherwise it usually leads to an increase of the activation.

Thus, for example, the sensitivity limit of a test strip which contains a weak activator, for example 2, 8-dimethyl-1,7-phenanthroline, is about 50 erythrocytes/mm³ urine.

The activation agents according to the present invention can be used in amounts of about 0.05–1.0 percent, preferably of 0.2–0.5 percent, per 100 ml. impregnation solution.

Further components of a rapid test for blood are an organic hydroperoxide, an oxidation indicator (chromogen), a buffer and a surface-active agent, as well as, if desired, a phosphoramidate, for example phosphoric

acid trimorpholide, for stabilization, as well as other conventional adjuvants.

As hydroperoxides, there can be used, for example, cumol hydroperoxide or 2,5-dimethyl-hexane-2,5-dihydroperoxide, and as indicators those of the benzidine series, for example, *o*-tolidine, or those of the heterocyclic azines series, for example bis-(*N*-ethyl-quinol-2-one)-azine or (*N*-methylbenzthiazol-2-one)-(1-ethyl-3-phenyl-5-methyltriazol-2-one)-azine (see German Pat. No. 1,648,840).

The indicators can be used in amounts of from 0.05–5 g., preferably of 0.2–1.0 g., per 100 ml. of impregnation solution.

As buffer, there can be used, for example, a citrate, phosphate, phthalate or succinate buffer, the pH value and capacity being so chosen that, after dipping the test strip into a body fluid, a pH value of 4–7, preferably of 5–6, is obtained thereon.

It is also advantageous to add to the formulation small amounts of about 0.05–0.5 g. per 100 ml. of a complex former, for example sodium metaphosphate or ethylene-diamine-tetraacetic acid, falsely positive reactions, which could be due to traces of metals, thereby being avoided.

Since the test strips, due to the relatively large amounts of water-soluble substances present therein, could tend to bleed, it is of practical importance to add a thickening agent to the formulation, for example methyl cellulose and, in particular, gelatine, preferably in an amount of about 0.5–5 g. per 100 ml.

As wetting agent, there is preferably used a long-chained organic sulphate or sulphonate, for example sodium dodecyl-benzene sulphonate, dioctyl sodium sulposuccinate or sodium lauryl sulphate, which, as is known, stabilizes radical cations, such as oxidized *o*-tolidine. The wetting agents can be added to the impregnation solution in amounts of 0.5 to 5 percent, preferably of 1–3 percent.

For the production of the test strips according to the present invention, absorbent carriers, for example filter paper, cellulose or synthetic resin fleeces, can be impregnated with solutions of the reagents in readily volatile solvents. This is preferably carried out in two separate steps. First, impregnation is carried out with a solution which contains a hydroperoxide, wetting agent, buffer and optionally a thickening agent. Thereafter, impregnation is carried out with a solution of an indicator and of an activator of general formula (I).

The test strips according to the present invention are, after drying, cut up into strips and preferably sealed between a synthetic resin film and a fine-meshed material in the manner described in German Pat. No. 2,118,455.

For the detection of peroxidate-active substances in feces, it is also possible to incorporate the activators according to the present invention, together with the reagents, in a water-stable film in the manner described in U.S. Pat. No. 3,630,957. This has the advantage that the surface of the test strip can, for reading off the color reaction, be cleaned simply by wiping it.

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

EXAMPLE 1

Filter paper is successively impregnated with the following solutions and dried at 40°C.:

Solution 1

1.2M citrate buffer, pH 5.25	35.0 ml.
ethylenediamine-tetraacetic acid, disodium salt	0.1 g.
dioctyl sodium sulphosuccinate	2.0 g.
2,5-dimethylhexane-2,5-dihydroperoxide (about 70%)	1.6 g.
phosphoric acid trimorpholide	12.7 g.
ethanol	30.0 ml.
distilled water	ad 100.0 ml.

Solution 2

o-tolidine	0.3 g.
phenanthridine	0.2 g.
toluene	ad 100.0 ml.

A white test paper is obtained which, upon dipping into a blood-containing urine, becomes green colored after about 5 to 20 seconds. If the erythrocytes are intact, then the paper is green flecked. If hemolysis has taken place or if free hemoglobin is present in the urine, then the paper becomes uniformly green colored. The sensitivity is about 5 erythrocytes/mm³ or the corresponding amount of hemoglobin. A smaller number of intact erythrocytes can, under certain circumstances, still bring about individual green dots on the test paper. The sensitivity with regard to myoglobin corresponds to that for hemoglobin.

Leukocytes and bacteria are also detected when intact, by flecking, or when lysed, by a uniform coloration.

EXAMPLE 2

When, in Solution 1 of Example 1, instead of 2,5-dimethyl-hexane-2,5-dihydroperoxide, there is used an equimolar amount of diisopropyl-benzene hydroperoxide and the phenanthridine in Solution 2 is replaced by one activator listed hereinafter, then test papers are obtained, the sensitivity of which towards blood, leukocytes and bacteria, is of approximately the same order as the test paper of Example 1:

2-methyl- or 2-ethyl-phenanthridine; benzo [f] quinoline; 1,2-tetramethylenebenzo [f] quinoline; benzo [g] quinoline; 4-methyl-benzo [g] quinoline; dibenzo [c,d,e] quinoline; dibenzo [c,f] quinoline; pyrido [3,2-f] quinoline; 3-methyl-4,7-phenanthroline; or pyrido [2,3-f] quinoline.

EXAMPLE 3

Filter paper is impregnated with the following solutions and dried at 40°C.:

Solution 1

1.2M nitrate buffer, pH 5.25	40.0 ml.
ethylenediamine-tetraacetic acid, disodium salt	0.1 g.
dioctyl sodium sulphosuccinate	2.0 g.
gelatine	2.0 g.
2,5-dimethylhexane-2,5-dihydroperoxide (about 70%)	1.6 g.
ethanol	30.0 ml.
distilled water	ad 100.0 ml.

Solution 2

o-tolidine	0.3 g.
3-methylbenzo [f] quinoline	0.2 g.
toluene	ad 100.0 ml.

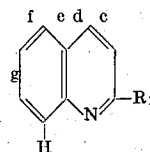
The test paper obtained is about ten times less sensitive than the test papers according to Examples 1 and 2 (about 50-100 erythrocytes/mm³ in 30-60 seconds).

Test papers of similar sensitivity are obtained when, instead of 3-methylbenzo [f] quinoline, there is used an equimolar amount of one of the following activators: 1,3-dimethylbenzo [f] quinoline; 2,4-dimethylbenzo [g] quinoline; 6-methylphenanthridine; 3,8-dimethyl-4,7-phenanthroline; 2,8-dimethyl-1,7-phenanthroline; or 2,7-dimethyl-1,6-anthrazoline.

It will be understood that the specification and examples are illustrative but not limitative of the present invention and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

What is claimed is:

1. Test strip for the detection of peroxidatively-active substances in body fluids, comprising a carrier containing a hydroperoxide, at least one chromogen and, as an activator, a compound of the formula



(1)

wherein

R₁ is hydrogen or methyl; and benzene and/or pyridine rings are fused on at least one of the positions indicated with c, (d,e), f and g, provided that two adjacent rings must not simultaneously contain a cyclic nitrogen atom, and

wherein

said compound can be substituted, other than in the positions indicated by H and R₁, by lower alkyl radicals, which radicals together can also form a hydroaromatic ring.

2. Test strip as claimed in claim 1, wherein R₁ is formula (I) is hydrogen.

3. Test strip as claimed in claim 1, wherein R₁ in formula (I) is methyl.

4. Test strip as claimed in claim 1, wherein said compound contains a benzene ring fused on at least one of the c, (d,e), f or g positions.

5. Test strip as claimed in claim 1, wherein said compound contains a pyridine ring fused on at least one of the f or g positions.

6. Test strip as claimed in claim 1, wherein said compound contains a benzene ring fused to at least one of the c, (d,e), f or g positions and a pyridine ring on one of the f and g positions.

7. Test strip as claimed in claim 1, wherein said compound is substituted in at least one available ring position by a lower alkyl radical of from one to four carbon atoms.

8. Test strip as claimed in claim 7, wherein there are two such alkyl radicals linked together to form a 5 or 6-membered hydroaromatic ring.

9. Test strip as claimed in claim 1, wherein said compound is a benzo [c] quinoline.

10. Test strip as claimed in claim 1, wherein said compound is a benzo [f] quinoline.

11. Test strip as claimed in claim 1, wherein said compound is a benzo [g] quinoline.

12. Test strip as claimed in claim 1, wherein said compound is a dibenzoquinoline.

13. Test strip as claimed in claim 12, wherein said dibenzoquinoline is dibenzo [c,f] quinoline.

14. Test strip as claimed in claim 12, wherein said dibenzoquinoline is dibenzo [c,d,e] quinoline.

15. Test strip as claimed in claim 1, wherein said compound is a pyridoquinoline.

16. Test strip as claimed in claim 15, wherein said pyridoquinoline is a pyrido [2,3-f] quinoline.

17. Test strip as claimed in claim 16, wherein said pyridoquinoline is a pyrido [3,2-f] quinoline.

18. Test strip as claimed in claim 16, wherein said pyridoquinoline is a pyrido [2,3-g] quinoline.

19. Test strip as claimed in claim 1, wherein said compound is phenanthridine.

20. Test strip as claimed in claim 1, wherein said compound is selected from the group consisting of

2-methyl- or 2-ethyl-phenanthridine

benzo [f] quinoline

1,2-tetramethylenebenzo [f] quinoline

benzo [g] quinoline

4-methylbenzo [g] quinoline

dibenzo [c,d,e] quinoline

dibenzo [c,f] quinoline

pyrido [3,2-f] quinoline

3-methyl-4,7-phenanthroline

pyrido [2,3-f] quinoline

3-methylbenzo [f] quinoline

1,3-dimethylbenzo [f] quinoline

2,4-dimethylbenzo [g] quinoline

6-methylphenanthridine

3,8-dimethyl-4,7-phenanthroline

2,8-dimethyl-1,7-phenanthroline, and

2,7-dimethyl-1,6-anthrazoline

21. Test strip as claimed in claim 1, wherein the carrier is an absorbent material impregnated with the reagents.

22. Test strip as claimed in claim 1, wherein the carrier is a water-stable film with the reagents incorporated therein.

23. Test strip as claimed in claim 1, wherein the reagent solution used for the production thereof contains 0.05-1 percent of activator per 100 ml. of solution.

24. Test strip as claimed in claim 1, wherein the reagent solution used for the production thereof contains 0.2-0.5 percent of activator per 100 ml. of solution.

25. Test strip as claimed in claim 1, wherein the reagent solution used for the production thereof contains 0.05-5 g. of chromogen per 100 ml. of solution.

26. Test strip as claimed in claim 1, wherein the reagent solution used for the production thereof contains 0.2-1.0 g. of chromogen per 100 ml. of solution.

27. Test strip as claimed in claim 1, wherein the reagent solution used for the production thereof contains a buffer and or a complex former, a thickener or a wetting agent.

28. Method of detecting small amounts of blood in a body fluid which method comprises contacting a test sample of the body fluid with a test strip as claimed in claim 1 and observing the color formation thereon as an indication of the absence or presence of blood in said sample.

29. Method of detecting small amounts of peroxidatively active substances in a body fluid which method comprises contacting a test sample of the body fluid with a test strip as claimed in claim 1 and observing the color formation thereon as an indication of the absence or presence of peroxidatively active substances in said sample.

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