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(54) TEST STRIP AND METHOD FOR ITS USE

(71) We, THYROID DIAGNOSTICS INC., a Corporation organised and existing under the laws of Massachusetts, of 74 Loomis Street, Bedford, Massachusetts, United States of America, 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:

This invention relates to a test strip for the detection of a characteristic of a sample, e.g. the presence or amount of an ingredient thereof, and a method of using it. In a preferred embodiment it relates to a test strip and method for determining the presence or amount of a ligand in, or the ligand binding capacity of, a fluid sample, such as a sample of a biological liquid. In preferred embodiments, the test is for a binding method of assay in which a radioisotope is used as a label.

Test strips have become commonplace in the analysis of various types of liquid samples, e.g. industrial and biological liquids, because of the convenience and speed of use of strips. Test strips designed for detecting various clinically significant substances in biological fluids, such as urine and serum, in particular, have been found to be very advantageous in assisting medical and veterinary diagnosis.

Conventional test strips generally comprise an absorbent or porous matrix incorporating indicator reagents, usually of a colorimetric type. The sample to be tested is contacted with the reagent matrix, such as by momentary immersion where the sample is liquid, and the indicator response is observed after a set period of time. Such test strips have the limitation that where more than one chemical reaction is involved, all of the test reactions must be mutually compatible since they all occur in the presence of each other in the reagent matrix.

Recently, certain test strips have been developed which allow several test reactions to take place in a predetermined sequence. U.S. Patent No. 3,011,874 discloses a test strip representative of this type. The disclosed strip comprises a paper strip partitioned into various transversely extending bands, first a blank band for immersion in a liquid test sample, followed successively by a reaction band, a gas liberation band, a barrier band, and an indicator band. However, there still exists a long-felt and recognized need for further improvement.

First of all, the conventional test strips have limited sensitivity. Because the detectable response produced by the conventional strips is almost always a color change, limited color resolution of the eye, and spectrometers as well, does not allow conventional test strips to detect substances, such as hormones, vitamins, and the like, which appear in body fluids at concentrations below 0.1 mg/ml. Secondly, a relatively large volume must be provided in order to wet the entire reagent matrix of the conventional test strips. The use of the test strip described in the aforementioned U.S. Patent No. 3,011,874 requires a sample volume sufficient to wet by capillary absorption all of the strip up to the barrier band.

The known analytical methods for detecting substances which occur in samples in small amounts are based on the binding affinity of such substances for certain synthetic or naturally produced binding agents. The most commonly used binding assay at the present time is probably the radio-immunoassay in which the substance to be determined competes under controlled conditions with a radiolabeled form of itself for binding to a limited quantity of specific antibody. The proportion of the radiolabeled form that successfully binds to antibody to that which remains in a free state is a function of the amount of the substance under determination in the test sample. Because binding assay methods require

accurate and timed addition of minute quantities of reagents, the state of the art is that of time-consuming and burdensome wet chemistry. The association of binding assay techniques with test devices which attempt to simplify and reduce the cost of such assays have been extremely limited despite the fact that many hundreds of technical papers have appeared in the literature over the past two decades relating to radioimmunoassay approaches alone.

U.S. Patent No. 3,888,629 describes a device wherein the binding reaction takes place in a disc-shaped matrix pad held in a section of a column. Free and bound label are separated by fitting a wash reservoir column section above the pad-containing section and a column section containing an abosrbent material below the pad-containing section. As the wash solution is drawn through the reaction pad, free label is carried along by the wash leaving the bound label behind because of the filtration properties of the reaction pad. This device would be cumbersome and awkward to use and requires several time consuming manipulative steps.

German Offenlegungsschrift No. 2,241,646 discloses a complex automated instrument for performing radioimmunoassays wherein the binding reagents, i.e. the label and specific antibody, and an aliquot of the liquid to be tested are dispensed onto a cellulose strip at discrete locations. After an incubation period, the test areas on the strip are washed by drawing a liquid therethrough by suction, thereby removing free label. The level of radioactivity remaining at each test area is then measured and related to the amount of unknown in the sample. In addition to requiring the use of an expensive and complex instrument, this method requires controlled dispensing of reagents and timing of incubations, the same as the conventional wet chemistry methods.

It is therefore an object of the present invention to provide a test device capable of application to analytical methods wherein a set of sequential test reactions is involved and wherein a minute sample size may be used.

It is a further object of the present invention to provide a test device capable of application to analytical methods having sensitivities below 0.1 mg/ml.

It is an object of a preferred embodiment of the present invention to provide a test device useful in performing binding assays to detect characteristics of fluid samples wherein the user is not required to dispense any of the binding reagents or to carefully time incubation periods and particularly wherein radioactive labels are employed, such as in radioimmunoassays.

The present invention provides a test strip for determining a characteristic of a sample, comprising a length of material capable of transporting a developing liquid therealong by capillarity (hereinafter referred to as a "capillary length"), said strip including

a first end, at which capillary transport begins;

a second end, at which capillary transport ends;

a plurality of zones positioned between said first and second ends, said zones including

a first zone for receiving said sample;

a second zone impregnated with a first reagent capable of being transported along said strip by said developing liquid,

a third zone downstream of said first and second zones and impregnated with a second reagent,

said first and second zones being spaced sufficiently from said first end to permit contact of said first end but not said first and second zones with said developing liquid,

said first reagent being capable of reacting (1) with said sample, (2) with said sample and said second reagent, or (3) with said second reagent in competition with said sample to form a product in an amount dependent on said characteristic being determined,

said second reagent or said material being capable of slowing capillary transport of said product or said first reagent to thereby separate said product from said first reagent, and

said first reagent having a detectable property that provides a label for said first reagent and for said product to permit one of them to be detected; and

a measuring location at or downstream of said third zone,

said measuring location being spaced upstream of said second end a distance preselected so that, when transport of said developing liquid along said strip element is terminated by the leading front thereof reaching said second end, said product and said first reagent have been separated sufficiently to permit the amount of said product or said first reagent to be detected at said location by measuring said detectable property,

whereby, after said sample is placed on said first zone and said first end is dipped into said developing liquid, said developing liquid transports said sample and first reagent to bring about the reaction to form said product, in an amount dependent on said characteristic; said second reagent or material slows transport of either said product or first reagent to spatially separate the two; and the amount of said product or first reagent, and thereby said characteristic, is measured at said measurement location by measuring said detectable

property.

The above defined strip comprises a length of a material capable of transporting the developing liquid along the strip, i.e. longitudinally therealong, by capillarity. One of the end portions of the capillary length is designated the first end, or beginning end portion, and the other the second end or terminal end portion, to reflect the fact that the travel of the developing liquid "downstream" along the strip, i.e. after immersion or other contact of the developing liquid with the beginning end portion, terminates when the leading front of the developing liquid reaches the terminal end. It is intended that in use of the strip the sample to be tested should be applied to the capillary length at the sample-receiving location downstream of the first end and upstream of the third zone. The strip have a first zone designated or marked for receiving the sample to be tested. By choice of appropriate dimensions of the capillary length and of spacings between the above-defined constituent parts thereof, it can be arranged that when the developing liquid reaches the end of the capillary length, a detectable response which is a function of the present or amount of the characteristic under determination is obtained at a predetermined upstream measuring location.

The reaction which takes places is not necessarily a chemical one, as explained hereinafter in connection with an embodiment. The following are examples of procedures involving various kinds of reaction:

(a) the first reagent is selected to react with said sample to form an amount of said product that is dependent on said characteristic and

said second reagent is selected to slow transport of the portion of said first reagent unreacted with said sample.

(b) the first reagent is selected to be mixable with said sample and transportable therewith to said third zone and

said second reagent is selected to react in said third zone with at least said sample to form said product; embodiments of procedure (b) include:

(b)(i) the second reagent reacts with said sample and said first reagent in said third zone and

said first reagent is selected to be sufficiently chemically similar to said ingredient as to compete with said ingredient in reacting with said second reagent to form said product, whereby the amount of said product is dependent on the amount of ingredient competing therewith in reacting with said second reagent; preferably the second reagent shows transport of the product; and

(b)(ii) the second reagent reacts with said sample and said first reagent to form said product; preferably transport of the product is slowed by the material of the strip.

The invention also produces a method of detecting the presence or amount of an ingredient or a characteristic of a sample, comprising:

(a) providing a test strip of the invention;

(b) depositing the sample at the sample-receiving location;

(c) immersing the first end of the length of material in a developing liquid transportable through said length by capillarity;

(d) continuing the development until the developing liquid is transported to said second end; and

(e) measuring the amount of a detectable property at said measuring location, the amount of said property being a function of said characteristic of said sample.

Embodiments of the invention will be described hereinafter by reference to the accompanying drawings, in which:

Figures 1, 3, and *5*, respectively, are front plan views of three different forms of the test strip of the present invention.

Figure 2 is a cross-sectional view of the test strip shown in Figure 1 taken along line 2-2.

Figure 4 is a cross-sectional view of the test strip shown in Figure 3 taken along line 4-4.

Figure 6 is a rear plan view of the test strip depicted in Figure 5.

Figure 7 is a cross-sectional view of the test strip shown in Figure 5 taken along line 7-7.

Figure 8 is a perspective view of an assembly comprising the test strip depicted in Figures 5-7 enclosed in a sealed chamber with the strip in contact with a volume of developing liquid.

Figure 9 is a perspective view in partial cross-section of the assembly depicted in Figure 8 inverted and positioned in a well of an instrument for measuring the desired response.

Referring to the drawing, Figures 1 and 2 depict a test strip 10 comprising a capillary length or strip element 11 composed of a material, usually bibulous paper, which is absorbent relative to a selected developing liquid, usually an aqueous solution. Strip element 11 has a beginning end portion 12 and a terminal end portion 16. A sample-receiving portion 13 is designated on strip element 11 by appropriate marking means such as a dried spot of a dye solution. Portions 14 and 15 of strip element 11

constitute second and third zones and contain the first and second reagents respectively. In use, the sample to be tested is dispensed on a first, sample-receiving, zone portion 13 of strip element 11 and beginning end portion 12 is immersed in the developing liquid which then begins to advance along strip element 11 by capillarity towards terminal end portion 16. The developing liquid is selected so that the dispensed sample and the constituents of the reagent means are appropriately combined as the developing liquid transverses strip element 11. When the leading front of the developing liquid reaches terminal end portion 16, a detectable property, related to the characteristic to be determined, is measured at a predetermined measuring location on strip element 11, e.g. at the third zone portion 15. The detectable propery can be, for example, a chemical or physical property. If the detectable property is a physical one, e.g. fluorescence, light absorbance, or radioactivity, that property can be measured on the intact strip element. If the detectable property is a chemical one, e.g. the appearance of a chemical product or the disappearance of a chemical reactant, such property can be observed by adding an indicator to the measuring location on the strip element or by first separating the measuring location from the remainder of the strip element and making appropriate measurements and/or reagent additions. Where the detectable property is measured on the intact strip element, for example at portion 15, it is useful to appropriately mask all of the remaining surface of the strip element to assure that the measured response is only that associated with the measuring location, i.e. portion 15. A masking agent may be used, a shroud which is opaque to the physical property being measured and which has an opening for registry with portion 15. Preferably an indicator means responsive to contact with the developing liquid is incorporated in terminal end portion 16 to signal completion of the test. It is also preferred that the volume of developing liquid into which beginning end portion 12 is immersed be equal to the precise volume of developing liquid that is taken up by strip element 11 upon arrival of the leading edge of the developing liquid at terminal end portion 16, thereby resulting in the automatic termination of the transport of the developing liquid at the proper time.

In one embodiment of the test strip of the present invention for determining a ligand ingredient in a sample, the first reagent comprises a labeled form of said ligand or of a specific binding analog thereof, the ligand and binding analog respectively being capable of reacting in competition with the ligand in the sample, with a second reagent which comprises a specific binding partner of said ligand. Predetermined quantities of the labeled form of ligand and the specific binding partner, the latter in an immobilised form, are incorporated in second and third zones 14 and 15 respectively. In use, as the developing liquid advances along capillary length or strip element 11, the sample and the first reagent are mixed and transported into contact with the immobilised binding partner, whereupon the first reagent and any ligand in the sample compete for binding to the binding partners. The fraction of the first reagent which successfully becomes bound to the binding partners thereby becomes immobilised at portion 15. As the developing liquid advances to terminal end portion 16, the unbound, or free, fraction of the label is transported a distance downstream of portion 15. The amount of label immobilised at portion 15 is then measured appropriately and is related to the amount of ligand in the test sample. It is particularly useful to use as the labeled form of ligand a radioactive form of the ligand or of a binding analog thereof. If the radioactive label is provided by an iodine isotope, an additional advantage of the test strip results in that contamination in the form of radioactive free iodide does not interfere with measurements at portion 15 since the free iodide is transported downstream away from portion 15, towards end portion 16 by the advance of the developing liquid.

A variation of the test strip is illustrated in Figures 3 and 4 of the drawing. Test strip 20 comprises a capillary length or strip element 21 affixed to an inert support strip 22, usually made of a semi-rigid plastic material and having a first end or beginning end portion 23, a second end or terminal end portion 28, and a first, sample-receiving, zone portion 25, which in this embodiment is situated between second and third zone portions 24 and 26. The second zone portion 24 of the strip element contains the label and third zone portion 26 the specific binding partner, which in this embodiment is not immobilised relative to the developing liquid, but rather is transportable thereby. In use, the sample and first reagent are mixed by the advancing developing liquid and competitive binding for the binding partner occurs at portion 26. As the developing liquid advances farther, the resulting first reagent binding partner complexes providing the label are transported along strip element 21 along with the sample and free label; however, such complexes advance at a slower rate than that at which free label is carried. When the leading front of the developing liquid reaches terminal end portion 28, the labeled complexes are disposed at portion 27 while free label has been carried farther towards terminal end portion 28. Measurement can then be made at portion 27 of strip element 21. Alternatively, portion 27 can contain an immobilised agent for the first reagent/binding partner complexes, such as a second

antibody or a protein precipitating agent, in order to ensure localisation of such complexes at portion 27.

A preferred form of the test device of the present invention is illustrated in Figures 5-9 of the drawing. Test device 30 comprises a capillary length or strip element 31 folded widthwise (transversely) over one end of an inert support strip 32. The transverse edge of beginning end portion 33 of strip element 31 is about even, i.e. co-extensive, with the other end of support 32 and the strip is folded so that the transverse edge of terminal end portion 37 of strip element 31 is spaced from the edge of the beginning end portion 33 of support 32. First, sample-receiving, zone portion 34 and second and third zone portions 35 and 36 correspond to portions 13, 14, and 15, respectively, of test strip 10 depicted in Figures 1 and 2. For example, for using test device 30 to detect a ligand in a liquid sample, portion 35 may contain a label and portion 36 an immobilised binding partner. In this embodiment, test strip 30 functions in a similar manner to test strip 10 with the predetermined measuring location being at third zone portion 36.

Figure 8 illustrates an operative mode of a preferred test means 40 of the present invention. After the test sample has been dispensed onto first zone portion 34 of the strip element 31, test device 30 is inserted, with the first end or beginning end portion 33 down, into test tube 41 which contains a volume of a developing liquid 42. The size of test tube 41 and the dimensions of strip element 31 are selected preferably so that the volume of developing liquid 42 is precisely the amount that is taken up by strip element 31 upon arrival of the leading edge of developing liquid 42 at terminal end portion 37 and so that terminal end portion 37 remains out of contact with developing liquid 42 when test device 30 is initially inserted into tube 41. After sample-inoculated test device 30 is inserted into tube 41, friction cap 43 is fitted onto tube 41 to form a sealed chamber to prevent evaporation of developing liquid 42 during the traversal thereof along strip element 31.

Figure 9 depicts a preferred manner of measuring the response at portion 36 of strip element 31. It can be seen that by positioning first, sample receiving, zone 34 and second and third zone portions 35 and 36 appropriately on strip element 31 so that the observation location, i.e. the third zone portion 36, is at or near the fold in the strip observation, especially measurement, of the response can be conveniently accomplished by masking all of strip element 31 other than the portion proximate to the fold. Test means 40 is inserted into shroud 50 which is opaque to the physical property characterising the response of the reagent means. The resulting assembly is inverted and placed into well 52 of instrument means 51 for measuring the response.

While the test strip of the present invention is particularly suited for use in performing binding assays, various other types of assay methods can be carried out using the test device. Conventional colorimetric assays may be carried out by selecting appropriate reagents, approximately positioning the first, second and third zone portions along the strip element, so that the desired color response is obtained at a convenient predetermined measuring location upon completion of the test. In such an assay, the test strip offers the advantage of accommodating sequential test reactions and has a high degree of accuracy because of the use of a precisely dispensed portion of the test sample.

As a further example of the diverse types of assays that can be performed using the test strip of the present invention, there will now be described a test strip for quantitatively determining proteins in a liquid sample. Referring to Figures 3 and 4 of the drawing, strip element 21 is provided with first zone portion 24 and with second and third zone portions 25 and 26 containing respectively, a first reagent, preferably radioactive iodide, and an agent for coupling the first reagent to proteins, thereby providing labeled proteins. An example of such a combination is radioactive iodide as first reagent and 1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril(e.g. that sold as IODO-GEN by Pierce Chemical Co., Rockford, IL.) as second reagent. The capillary advance of the developing liquid mixes the test sample and the first reagent, and when the resulting mixture enters third zone portion 26, the first reagent is chemically coupled to proteins in the sample, forming labeled derivatives. As the developing liquid advances farther, the reacted label (labeled derivatives) and free (unreacted) label are separated by their differing rates of transport along strip element 21 by the developing liquid. Upon complete traversal of strip element 21 by the developing liquid, the label response in the area of strip element 21 at which are disposed the labeled derivatives provides a measure of the protein content of the sample. This type of test strip can also be used to determine a particular protein quantitatively by positioning a measuring location 27 downstream of the second zone and coupling agent-containing portion 26 element a predetermined distance from the measuring location 27 to measure the amount of coupled product transported by said developing liquid when said developing liquid reaches the terminal end portion 28 of the strip element. The measuring location 27, will then contain the labeled derivative of the protein of interest only.

The test device of the present invention can also be designed to determine quantitatively

a first substance which reacts with a second substance to produce hydrogen peroxide. Referring to Figures 5-7 of the drawing, strip element 31 is provided with first zone portion 34 and with second and third zone portions 35 and 36 containing respectively, radioactive iodide as first reagent and the aforementioned second substance as second reagent. For determining quantitatively in a liquid sample, a substrate for an oxidase enzyme reaction which produces hydrogen peroxide, a preferred second reagent (second substance) comprises the oxidase enzyme. The oxidase enzyme is normally specific for the substance to be determined. For example, to detect glucose or cholesterol, the second reagent would comprise glucose oxidase or cholesterol oxidase, respectively. In use, the developing liquid mixes the sample and radioactive iodide. When the mixture contacts the second reagent (second substance) hydrogen peroxide is produced in proportion to the amount of the substance to be determined (the first substance) in the sample, with an attendant proportional conversion of radioactive iodide to radioactive iodine, the latter being strongly adsorbed by strip element 31. Consequently, the iodide, by its reaction with the hydrogen peroxide, provides a label for the glucose or cholesterol etc., in the form of radioactive iodine. As the developing liquid advances, the remaining (unreacted) radioactive iodide is separated from the adsorbed radioactive iodine. The final location of either radioactive species upon completion of the test can be the measuring location. Thus, the travel of a portion of the iodide, which has reacted with the hydrogen peroxide, and is now in the form of free iodine is slowed and eventually stopped, whereby the iodine becomes detectably separated on the strip from the unreacted iodide label.

The capillary length of the strip (strip element) may be made of any material which is insoluble in the developing liquid and which is capable of transporting the developing liquid by capillarity. The strip element typically is relatively flexible while having a satisfactory wet strength to stand up under use. Of course, it should be made of a material which will not deleteriously affect the interactions between the developing liquid, the test sample, and the reagent means. A particularly useful material for the strip element is bibulous paper, such as filter paper, since the developing liquid is usually aqueous in nature; however, other materials may also be used, including various felts, cloths, gels, membranes, and films made of natural or synthetic substances including polymers. While the length and width of the strip element may vary widely, its thickness is usually between 0.008 inch (0.2 mm) and 0.04 inch (1.0 mm).

The strip element is preferably affixed to an inert support for mechanical strength. Usually, the strip element and inert support are joined in laminate fashion with both being of approximately equal width. The thickness of the inert support may vary depending on the rigidity of the material of which it is made. Exemplary materials are the various vinyl plastics as well as polyester, polycarbonate, methyl methacrylate polymer, polystyrene, polyethylene, polypropylene, and waxed cardboard. The length of the inert support will vary depending upon the desired configuration of the test device. The inert support may be approximately the same length as the strip element (as shown in Figures 3 and 4 of the drawing) or, as is particulary preferred, may have a length greater than 0.5 times but less than 1.0 times the length of the strip element in order that the transverse edge of the beginning end portion of the strip element may be even (co-extensive) with or slightly extended over one end of the support and the strip element folded (transversely) over the other end of the support with the transverse edge of the terminal end portion of the strip element being a spaced distance from the first mentioned end of the support (as shown in Figures 5-7 of the drawing). The latter mentioned preferred configuration is most advantageous when the orientation of the sample-receiving first zone and the second and third zones are established such that the predetermined measuring location is at or proximate to the fold in the strip element. This allows convenient measurement of the detectable property at the measuring location by masking all but a minor portion of the strip element proximate to the fold.

The terminal end portion of the strip element is preferably incorporated with indicator means responsive to the developing liquid to serve as a signal that the traversal of the strip element by the developing liquid is complete. For example, the indictor means may comprise a colorimetric reagent composition sensitive to a solvent or solute of the developing liquid. Where the developing liquid is aqueous, the indicator means may contain a water sensitive reagent such as cobalt chloride and, in such case, the indicator means may also serve as a stability indicator. Also, the indicator means may comprise all of the components of a colorimetric reaction activated by the developing liquid. For example, where the developing liquid is aqueous, the indictor means may include an acidic or basic material and a pH indicator.

The developing liquid must be capable of traversing the strip element by capillarity and have solvent properties appropriate for the desired combination of the test sample and reagent means during such traversal. Usually, the developing solution is a solvent for

appropriate substances in the test sample, such as a substance to be detected and contains various ancillary agents such as stabilizing agents, preservatives, and inhibitory agents against interfering reactions.

Where a reagent is required to be incorporated in a portion of the strip element in an immobilized state, the immobilization may be accomplished in any conventional manner. For example, the constituent may be immobilized by physical adsorption or chemical coupling to the strip element. An alternative method is to make the constituent insoluble and immovable by the developing liquid such as by associating it by physical or chemical means with a large insoluble particle. For example, where a binding assay test device is involved and one of the reagent constituents is a protein, such as an antibody, such can be immobilized effectively by adsorption onto plastic beads.

The first reagent may be any chemical substance having a detectable property which provides a label for itself or for the product. The label is a detectable characteristic which is unique compared to the other materials involved in carrying out the test. For instance, such label may have fluorescent or distinguishable light absorption properties or reactivity. Particularly preferred are substances which are radioactive, because of the high degree of sensitivity at which such can be detected. Radioactive labels, particularly radioactive iodine such as ^{125}I and ^{131}I, are particularly useful in carrying out binding assays using the test device of the present invention. While radioactive labels incorporated in a ligand or a binding analog thereof elicit a measurable characteristic which is the same no matter whether such ligand or analog is bound to a binding partner or not, it is comtemplated that labels may be provided in a ligand or binding analog giving characteristics which are measurably different when the ligand or analog is in its bound state than when it is in its free state.

As applied to binding assays, the present test strip can be designed to detect any ligand for which there is a specific binding partner. The ligand usually is a peptide, protein, carbohydrate, glycoprotein, steroid, or other organic or inorganic molecule or ion for which a specific binding partner exists in biological systems or can be synthesized. The ligand, in functional terms, is usally selected from the group consisting of antigens and antibodies thereto; haptens and antibodies thereto: and hormones, vitamins, metabolites and pharmacological agents, and their receptors and binding substances. Specific examples of ligands which may be detected using the present invention are hormones such as insulin, chorionic gonadotropin, thyroxine, triiodothyronine, estriol, testosterone, androsterone, equilenin, estrone, progesterone, pregnenolone, cortisol, 17-hydroxydeoxy-corticosterone, and aldosterone; pharmacological agents and their metabolites such as dilantin, digoxin, morphine, digitoxin, barbiturates, catecholamines, glutethimide, cocaine, diphenylhydantoin, meprobamate, benzdiazocycloheptanes, and phenothiazines; antigens and haptens such as ferritin, bradykinin, prostoglandins, hemoglobin, enzymes, myoglobin, and tumor specific antigens; vitamins such as biotin, the B vitamin group, vitamin A, the D vitamins, vitamins E and K, folic acid, and ascorbic acid; binding proteins such as antibodies, thyroxine binding globulin, avidin, intrinsic factor, and transcobalamin; and other substances including pesticides, fungicides, nematocides, living or non-living cells derived from bacteria, protozoa, fungi, viruses, and high order animals, and minerals such as calcium, ferrous and ferric ions, and oxalate, phosphate, and chloride ions.

In addition to the detection of particular substances or groups of substances in a test sample, other characteristics may be determined using the present test strip. For example, ligand binding capacities may be determined where a ligand exists in the sample in a free form and a bound form. The ligand binding capacity of the sample is the amount or percent of exogenous free form ligand that is converted into the bound form when added to the sample (such as triiodothyronine binding capacity as involved in Example 2 hereof). Accordingly, the invention provides a test strip for detecting the binding capacity for a first ligand of a sample which contains a second ligand in free and bound forms, wherein the first reagent includes a labeled form of the first ligand, and the second reagent comprises a binding agent capable of binding said labeled form of first ligand when in one of said free form and said bound form but not when in the other, said labeled form and said binding agent being incorporated in said strip in their respective zones in proportions such that an amount of said product is produced, which is a function of the ligand binding capacity of said sample.

In this embodiment the second reagent does not necessarily react chemically with the sample or first reagent but can have a purely physical reaction thereon, as shown by Example 2.

In most instances, the test reactions can be carried out successfully at room temperature but, in general, the temperature at which the test is performed may range from about 3°C to about 45°C with the reaction rate being generally directly related to the temperature.

The test sample may be a solid material but usually is a naturally occurring or artificially

formed liquid suspected of containing or known to contain the substance or characteristic to be determined. The present test strip is particularly suited to assay biological fluids, or dilutions or other treatments thereof, such as serum, plasma, urine, and amniotic, cerebral, and spinal fluids. Solid matter, such as tissue or cells, or gases may be tested also by reducing them to a liquid form such as by dissolution of the solid or gas in a liquid or by liquid extraction of a solid.

The present invention will now be illustrated, but is not intended to be limited, by the following Examples. "WHATMAN" and "QUSO" are Registered Trade Marks.

Example 1

Test for Thyroxine in Serum

A. Preparation of Test Strips

A sheet of filter paper (Whatman #17 from W. & R. Balston Ltd. Maidstone, Kent, England) was cut into strips measuring 188 mm in length and 6 mm in width. Each paper strip was folded widthwise (transversely) over one end of a plastic strip 0.015 inch (0.38 mm) thick, 4 inches (102 mm) long, and 1/4 inch (6.3 mm) wide, in such a fashion that one end of the paper strip (designated the beginning end) was about even with one end of the plastic strip, leaving the other end of the paper strip (designated the terminal end) about 3/4 inch (19 mm) short of the same end of the plastic strip on the reverse side thereof. The folded paper strip was affixed to the plastic strip by means of double-faced adhesive tape.

The following liquid mixtures were prepared:

Mixture A - A mixture of:

(1) 0.2 ml goat antiserum raised against a conjugate of thyroxine and human serum albumin (the antiserum was raised in a manner similar to that described in *J. Clin. Endo. 33*: 509-16 (1971));

(2) 0.4 ml of an aqueous suspension of polystyrene beads containing 10% solids consisting of beads with an average diameter of 0.11μ (micron) (supplied by Sigma Chemical Co., St. Louis, MO);

(3) 2.4 ml of a 100 ml aqueous solution containing 694 mg potassium dihydrogen phosphate, 509 mg disodium phosphate heptahydrate, 200 mg thimerosal (obtained from K&K Labs, Plainview, NY) and 0.5 mg crystal violet;

(4) 0.2 ml of 5% aqueous lauryl sulfate;

(5) 20 μl (microliters) of an aqueous solution containing 500 mg gentamicin manufactured by Schering Corp., Bloomfield, NJ per ml; and

(6) a trace (about 1 μl) of silicone antifoam (AF 60 emulsion grade manufactured by General Electric and obtained from Harwick Standard Chemical Co., Boston, MA).

Mixture B - A mixture of:

(1) 20 microcuries ^{125}I-labeled thyroxine (obtained from Cambridge Radiopharmaceuticals Corp., Billerica, MA) in 0.2 ml of 50% propylene glycol (specific activity of about 600 microcuries per mg);

(2) 3 ml of a 100 ml aqueous solution containing 5.32 g sodium barbital, 1.44 ml 2 *N* hydrochloric acid, 400 mg thimerosal, 10 mg disodium ethylenediamine tetra-actetate, and 0.5 mg crystal violet;

(3) 8 μl of an aqueous solution containing 50 mg gentamicin per ml; and

(4) 8 μl of an aqueous solution containing 250 mg human serum albumin per ml.

Mixture C - An aqueous solution containing 400 mg/100 ml thimerosal and 10 mg/100 ml of red dye Ponceau S.

Mixture D - An aqueous solution containing 0.05 M hydrochloric acid and 50 mg/100 ml bromocresol purple.

The above liquid mixtures were then applied dropwise to the paper strips as follows:

Mixture	Volume (μl)	Point of application measured from beginning end of strip (mm)
A	20	108
B	20	80
C	10	60
D	10	185

The strips were allowed to dry at room temperature.

A developing fluid was prepared to consist of 100 ml of an aqueous solution containing 5.32 g sodium barbital, 1.44 ml 2 *N* hydrochloric acid, 400 mg thimerosal, and 10 mg disodium ethylenediamine tetraacetate.

Referring to Figures 5-7 of the drawing, the resulting test strips 30 each included (a) first, sample-receiving zone, portion 34 (point of application of Mixture C) indicated by the dried

spot of the red dye Ponceau S, (b) second zone, portion 35 (point of application of Mixture B) including radiolabeled thyroxine, (c) third zone, portion 36 (point of application of Mixture A) including immobilized antibody to thyroxine, and (d) terminal end portion 37 (point of application of Mixture D) including, as indicator means, a combination of an acid and a pH indicator which produces a color change upon contact by an alkaline liquid.

B. Assay Method

Ten (10) µl of a serum sample to be tested is applied to the sample receiving portion 34 of a test strip 30. The test strip 30 is then placed, with the beginning end portion 33 down, in a test tube 41 (Figure 8) containing 1 ml of the developing solution. The size of the test tube is selected so that the developing solution contacts the test strip 30 only at its beginning end portion 33, with no contact with its terminal end portion 37 (as depicted in Figure 8). The test tube is capped and allowed to stand at room temperature until a color change from yellow to blue is observed at terminal end portion 37 (about one hour). At such time, all of the developing liquid in the test tube will have been drawn up into the strip element 31 of the test strip 30. The test tube is then inverted, all but 1/2 inch (12.2 mm) of the test strip measured from the fold of the paper strip is shrouded by inserting each tube into a length of 5/8 inch (16 mm) O.D. copper tubing, and the tube is placed into the counting well 52 (Figure 9) of an In-V-Tron 200 gamma counter (manufactured by Nuclear Systems, Inc., Garland, Texas) to measure the amount of gamma radiation emitted at and proximate to the third zone 36 of the test strip.

C. Principle of Test

As the developing liquid is transported up strip element 31 by capillarity, the serum sample applied at first zone portion 34 is encountered first. The advancing developing liquid carries the serum sample, including any thyroxine present therein, to the second zone portion 35. Endogenous non-radioactive thyroxine is mixed with labeled thyroxine as the developing liquid advances towards the fold of strip element 31. The thyroxine mixture is carried over the fold in strip element 31 by the developing liquid and then comes into contact with antiserum to thryoxine which is immobilised at third zone portion 36. As the thyroxine mixture moves through the portion 36, endogenous non-radioactive thyroxine and labeled thyroxine compete for antibody binding sites. Once the thyroxine mixture has passed through third zone portion 36, the amount of labeled thyroxine bound to immobilized antibody, and thereby itself immobilized at portion 36, is inversely related to the amount of thyroxine present in the serum sample. Complete traversal of strip element 31 by the developing liquid is indicated by a color change in terminal end portion 37 which results upon wetting by the developing liquid.

D. Results

The assay procedure was run in duplicate on two (2) serum samples having known thyroxine contents (Thyroid Profile Control Sera from Oxford Laboratories, Inc., Foster City, CA). The results were as follows:

Type of Serum Control	Normal Control	Elevated Control
Lot Number	14221	14222
Stated Thyroxine Content	6.2±0.6 µg/100 ml	13.9±0.7 µg/100ml
Counts/min (thousands)	13.71, 13.51	9.56, 9.70
	av. 13.61	av. 9.63

These data indicate that the amount of labeled thyroxine resulting at and proximate to the fold of the test strip after traversal of the strip element by the developing liquid is an inverse function of the amount of thyroxine present in the serum sample tested.

Example 2

Test for the Triiodothyronine Binding Capacity of Serum

A. Preparation of Test Strips

Blank test strips were prepared by cutting paper strips and folding them over and affixing them to plastic strips in the manner described in Part A of Example 1.

The following liquid mixtures were prepared:

Mixture E - A mixture of:

(1) 9.12 g citric acid monohydrate,

(2) 14.12 g trisodium citrate dihydrate, and

(3) deionized water sufficient to make a total volume of 1 liter.

Mixture F - An aqueous solution containing 10 mg of red dye Ponceau S per 100 ml.

Mixture G - A mixture of:

(1) 100 microcuries ^{125}I-labeled triiodothyronine (obtained from Cambridge Radiopharmaceuticals Corp., Billerica, MA) in 1 ml of 50% propylene glycol;

(2) 20 ml of a 100 ml aqueous solution containing 5.32 sodium barbital, 1.44 ml 2 *N*

hydrochloric acid, and 1 mg crystal violet;
(3) 50 µl of an aqueous solution containing 50 mg gentamicin per ml; and
(4) 50 µl of an aqueous solution containing 250 mg human serum albumin per ml.
Mixture H - A mixture of:
(1) 50 mg hydroxypropyl guar gum (Jaguar HP-11 brand from Stein-Hall Specialty Chemical, New York, NY);
(2) 2 g microfine precipitated silica ("QUSO" 32 brand from Philadelphia Quartz Co., Valley Forge, Pennsylvania);
(3) 0.1 ml of an aqueous solution of 500 mg crystal violet per 100 ml; and
(4) 150 ml of Mixture E.
Mixture J - An aqueous solution containing 0.02 M sodium hydroxide and 50 mg/100 ml bromcresol purple.

The above liquid mixtures were then applied dropwise to the paper strips as follows:

Mixture	Volume (µl)	Point of application measured from beginning end of strip(mm)
F	10 µl	35
G	10 µl	54
H	30 µl	108
J	10 µl	185

The strips were allowed to dry at room temperature.

Mixture E was used as the developing liquid.

Referring to Figures 5-7 of the drawing, the resulting test strips 30 each included (a) first, sample-receiving , zone portion 34 (point of application of Mixture F) indicated by the dried spot of the red dye Ponceau S, (b) second zone portion 35 (point of application of Mixture G) including radiolabeled triiodothyronine, (c) third zone portion 36 (point of application of Mixture H) including immobilized silica capable of adsorbing free triiodothyronine but not triiodothyronine bound to serum proteins, and (d) terminal end portion 37 (point of application of Mixture J) including, as indicator means, a combination of an alkali and a pH indicator.

B. Assay Method

The same procedure as described in Part B of Example 1 was followed.

C. Principle of Test

As the developing liquid is transported up strip element 31 by capillarity, the serum sample applied at portion 34 is encountered first. The advancing developing liquid carries the serum sample to the second zone portion 35 where it is mixed with labeled triiodothyronine. The thyroxine binding globulin present in the serum sample binds an amount of the labeled triiodothyronine proportional to the degree of its unsaturation. When the resulting mixture is passed through third zone portion 36, free labeled triiodothyronine, i.e. that not bound to thyroxine binding globulin, is adsorbed by the immobilized silica particles. Upon complete traversal of strip element 31 by the developing liquid, the amount of labeled triiodothyronine immobilized at portion 36 is directly related to the percent saturation of thyroxine binding globulin in the serum sample.

D. Results

The assay procedure was run in duplicate on two (2) serum samples having known triiodothyronine binding capacities (expressed as T-3 percent uptake) (control sera from Lederle Diagnostics, Pearl River, NY).

The results were as follows:

Type of Serum Control	"RIA Control I"	"RIA Control II"
Lot Number	2945-301	2946-301
Stated T-3 Percent Uptake	42.0	68.9
Counts/min(thousands)	10.35, 11.49	18.60, 18.02
	av. 10.92	av. 18.31

These data indicate that the amount of labeled triiodothyronine resulting at and proximate to the fold of the test strip after traversal of the strip element by the developing liquid is a direct function of the percent saturation of triiodothyronine binding proteins in the serum sample tested.

Example 3

Test for Folic Acid and Analogues Thereof in Serum

A. Preparation of Test Strips

Blank test strips were prepared by cutting paper strips and folding them over and affixing them to plastic strips in the manner described in Part A of Example 1.

The following liquid mixtures were prepared:

Mixture K - A 150 ml aqueous solution containing 100 mg Baker gelatin (obtained from Doe and Ingalls Co., Medford, MA), 500 mg 2-amino-2-(hydroxymethyl)-1-,3-propanediol, 100 mg ascorbic acid, 67 mg sodium azide and 0.383 ml 1 *N* sodium hydroxide.

Mixture L - A 20 ml volume of Mixture K containing 20 mg beta-lactoglobulin (obtained from Sigma Chemical Co., St. Louis, MO).

Mixture M - A 0.5 ml volume of Mixture K containing 1.2 microcuries of ^{125}I-labeled folic acid (obtained from Diagnostic Biochemistry Inc., San Diego, CA).

The above liquid mixtures and Mixture D from Example 1 were then applied dropwise to the paper strips as follows:

Mixture	Volume (μl)	Point of application measured from beginning end of strip (mm)
L	20	92
M	10	80
D	10	185

A light pencil mark was also made on each paper strip at a point 67 mm from the beginning end thereof. The strips were allowed to dry at room temperature.

Mixture K was used as the developing liquid.

Referring to Figures 8 and 9 of the drawing, the resulting test strips 30 included (a) first sample-receiving zone portion 34 (indicated by the pencil mark), (b) second zone portion 35 (point of application of Mixture M) including radiolabeled folic acid, (c) third zone portion 36 (point of application of Mixture L) including beta-lactoglobulin, and (d) terminal end portion 37 (point of application of Mixture D) including, as indicator means, a combination of an acid and a pH indicator.

B. Assay Method

The same procedure as described in Part B of Example 1 was followed.

C. Principle of Test

As the developing liquid is transported down strip element 31 by capillarity, it entrains first the serum sample and then the radiolabeled folic acid. The folic acid and its analogues present in the sample and the radiolabeled folic acid become mixed as the developing liquid advances to the third zone portion 36. Beta-lactoglobulin has a limited ability to bind folic acid and its analogues and therefore as the sample/label mixture passes through third zone portion 36, a fraction of the amount of folic acid and its analogues in the mixture become bound to beta-lactoglobulin. As the developing liquid advances upward and over the fold in strip element 31, the beta-lactoglobulin/folic acid or analogue complexes formed are transported along strip element 31 but more slowly than the free folic acid and its anologues. Third zone portion 36 of strip element 31 is positioned such that upon complete traversal of strip element 31 by the developing liquid, substantially all of the beta-lactoglobulin/labelled folic acid complexes that have formed are disposed at or proximate to the fold of strip element 31. Free labeled folic acid meanwhile has been transported to the terminal end portion 37. Therefore, the amount of radiolabeled folic acid resulting at and proximate to the fold of strip element 31 is inversely related to the amount of folic acid and its analogues in the serum sample.

D. Results

The assay procedure was run in duplicate on two (2) serum samples having known folate contents (control sera from Lederle Diagnostics, Pearl River, NY). The results were as follows:

Type of Serum Control	"RIA Control I"	"RIA Control II"
Lot Number	2945-301	2946-301
Stated Folate Content (as determined by three different methods)		
1st Method	4.0 mg/ml	3.8 mg/ml
2nd Method	3.6 mg/ml	2.9 mg/ml
3rd Method	2.5 mg/ml	2.2 mg/ml
Counts/min(thousands)	5.92,6.06 av. 5.99	8.49, 8.02 av. 8.26

These data indicate that the amount of labeled folic acid resulting at and proximate to the fold of the test strip after traversal of the strip element by the developing liquid is an indirect function of the amount of folate in the serum sample tested.

WHAT WE CLAIM IS:

1. A test strip for determining a characteristic of a sample, comprising a length of material capable of transporting a developing liquid therealong by capillarity, said strip including

a first end, at which capillary transport begins;

a second end, at which capillary transport ends;

a plurality of zones positioned between said first and second ends, said zones including a first zone for receiving said sample,

a second zone impregnated with a first reagent capable of being transported along said strip by said developing liquid,

a third zone downstream of said first and second zones impregnated with a second reagent,

said first and second zones being spaced sufficiently from said first end to permit contact of said first end but not said first and second zones with said developing liquid.

said first reagent being capable of reacting (1) with said sample, (2) with said sample and said second reagent, or (3) with said second reagent in competition with said sample, to form a product in an amount dependent on said characteristic being determined,

said second reagent or said material being capable of slowing capillary transport of said product or said first reagent to thereby separate said product from said first reagent, and

said first reagent having a detectable property that provides a label for said first reagent and for said product to permit one of them to be detected; and

a measuring location at or downstream of said third zone,

said measuring location being spaced upstream of said second end a distance preselected so that, when transport of said developing liquid along said strip element is terminated by the leading front thereof reaching said second end, said product and said first reagent have been separated sufficiently to permit the amount of said product or said first reagent to be detected at said location by measuring said detectable property,

whereby, after said sample is placed on said first zone and said first end is dipped into said developing liquid, said developing liquid transports said sample and first reagent to bring about the reaction to form said product, in an amount dependent on said characteristic; said second reagent or material slows transport of either said product or first reagent to spatially separate the two; and the amount of said product or first reagent, and thereby said characteristic, is measured at said measurement location by measuring said detectable property.

2. A test strip according to claim 1 further comprising an inert support to which said strip is affixed.

3. A test strip according to claim 2 wherein said support is a sheet laminated to said strip, the support sheet having a width about the same as that of said strip and a length greater than one-half of, but less than the length of said strip, the transverse edge of said first end being about co-extensive with one end of said support and said strip being folded transversely over the other end of said support so that the transverse edge of said second end is spaced from said first end.

4. A test strip according to claim 3 wherein said third zone is at or proximate to the fold of said strip.

5. A test strip according to any preceding claim further provided with indicator means at said second end, responsive to contact with said developing liquid for indicating when the developing has reached the second end.

6. A test strip according to claim 1 for quantitatively determining a ligand ingredient in a sample, wherein said first reagent comprises a labeled form of said ligand or of a specific binding analog thereof, the ligand and binding analog respectively being capable of reacting in competition with the ligand in the sample, with a second reagent which comprises a specific binding partner of said ligand, said labelled form and said binding partner being incorporated in said strip in their respective zones in proportions such that an amount of said product is produced which is a function of the amount of said ligand in said sample.

7. A test strip according to claim 6 wherein said labelled form is a radioactive form of said ligand or of said binding analog respectively.

8. A test strip according to any one of claims 1 to 5 for determining quantitatively protein(s) in a liquid sample, wherein said second reagent comprises an agent for coupling said first reagent to protein(s) to provide labelled proteins.

9. A test strip according to claim 8, for determining a particular protein quantitatively, in which said measuring location is positioned to measure the amount of coupled product transported by said developing liquid when said developing liquid reaches said second end.

10. A test strip according to claim 8 or 9 wherein said first reagent is radioactive iodide.

11. A test strip according to claim 1 for quantitatively determining in a liquid sample a first substance which reacts with a second substance to produce hydrogen peroxide, wherein said first reagent comprises radioactive iodide and said second reagent comprises said second substance.

12. A test strip according to claim 11 for quantitatively determining in a liquid sample a substrate for an oxidase enzyme reaction which produces hydrogen peroxide, wherein said second substance comprises said oxidase enzyme.

13. A test strip according to any one of claims 1 to 5 for detecting the binding capacity for a first ligand of a sample which contains a second ligand in free and bound forms, wherein the first reagent includes a labeled form of the first ligand, and the second reagent comprises a binding agent capable of binding said labeled form of the first ligand when in one of said free form and said bound form but not when in the other, said labeled form and said binding agent being incorporated in said strip in their respective zones in proportions such that an amount of said product is produced, which is a function of the ligand binding capacity of said sample.

14. A test strip substantially as hereinbefore described with reference to and as illustrated in Figures 1 and 2, 3 and 4 or 5 to 7 of the accompanying drawings.

15. A test strip according to claim 1 substantially as described in any one of the Examples.

16. A test device comprising a sealable chamber in which a test strip according to any preceding claim is held upright therein with said first end of said length of material immersed in said developing liquid.

17. A method of detecting a characteristic of a sample, comprising:

(a) providing a test strip defined in any one of claims 1 to 15;

(b) depositing the sample at the sample-receiving location;

(c) immersing, the first end of the length of material in a developing liquid transportable through said length by capillarity;

(d) continuing the development until the developing liquid is transported to said second end; and

(e) measuring the amount of a detectable property at said measuring location, the amount of said property being a function of said characteristic of said sample.

18. A method according to claim 17 wherein a test strip as claimed in claim 5 is used.

19. A method according to claim 17 or 18 wherein the remainder of said strip outside said measuring location is masked for observation of the response.

20. A method according to claim 17 substantially as described in any one of the Examples.

21. The test strip of claim 1 wherein said detectable property is radioactivity.

22. The test strip of claim 1 wherein said first reagent is selected to react with said sample to form an amount of said product that is dependent on said characteristic and said second reagent is selected to slow transport of the portion of said first reagent unreacted with said sample.

23. The test strip of claim 1 wherein

said first reagent is selected to be mixable with said sample and transportable therewith to said third zone and

said second reagent is selected to react in said third zone with at least said sample to form said product.

24. The test strip of claim 23 wherein

said second reagent reacts with said sample and said first reagent in said third zone and said first reagent is selected to be sufficiently chemically similar to said ingredient as to compete with said ingredient in reacting with said second reagent to form said product, whereby the amount of said product is dependent on the amount of ingredient competing therewith in reacting with said second reagent.

25. The test strip of claim 24 wherein said second reagent slows transport of said product.

26. The test strip of claim 25 wherein

said second reagent reacts with said sample and said first reagent to form said product.

27. The test strip of claim 26 wherein transport of said product is slowed by said material.

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1589234 COMPLETE SPECIFICATION

4 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

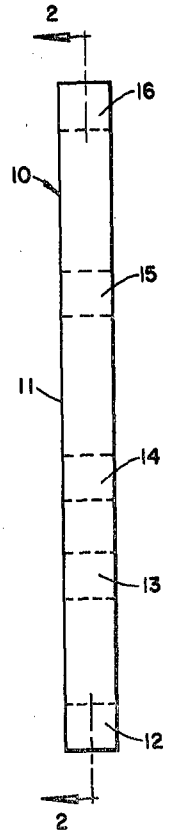

FIG. 1

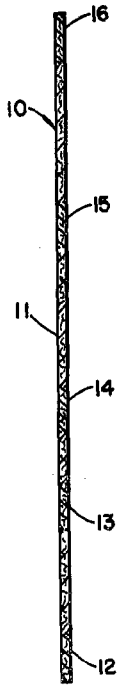

FIG. 2

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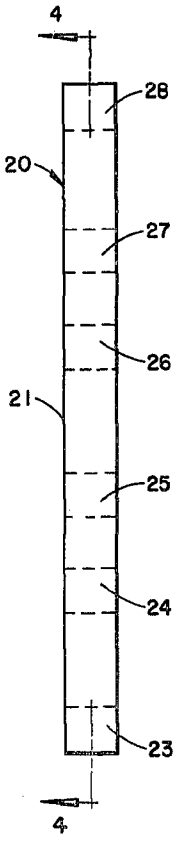

FIG. 3

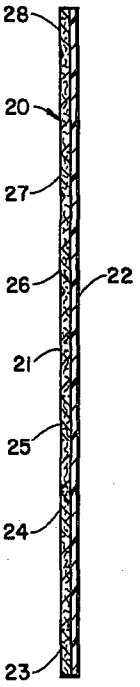

FIG. 4

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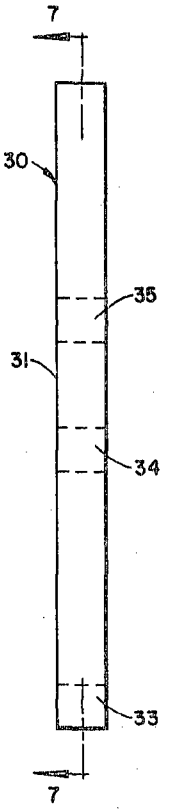

FIG. 5

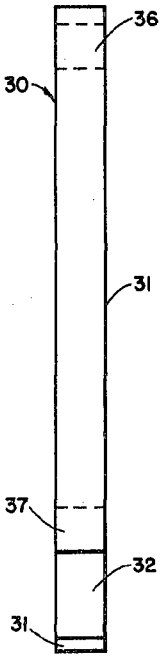

FIG. 6

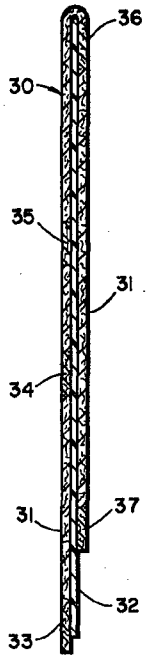

FIG. 7

1589234 COMPLETE SPECIFICATION
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Sheet 4

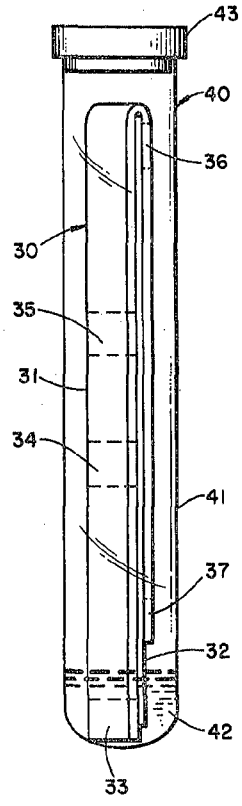

FIG. 8

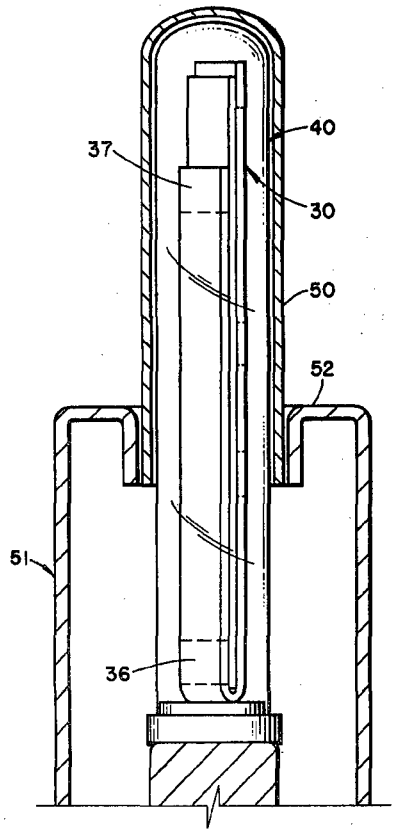

FIG. 9