(54) Title: CANCER SCREENING TEST

(57) Abstract

The disialyted disaccharide beta-D-Gal(1 → 3)-D-Gal-NAc, which specifically binds with peanut agglutinin (PNA) or is oxidized by galactose oxidase, has been discovered in the body fluids of patients with cancer and precancer. Because of the presence of beta-D-Gal(1 → 3)-D-GalNAc also on neuraminidase-treated erythrocytes of the ABO type, their competitive binding with PNA has been exploited to develop a hemagglutination inhibition assay. Additional methods of simple detection of this disaccharide include a latex agglutination test, enzyme-avidin-biotinylated PNA, immunochemical detection of the disaccharide, and a galactose oxidase strip test. This rapid, simple and inexpensive assay is designed to test the presence of beta-D-Gal(1 → 3)-D-GalNAc the body fluids and has the potential for screening populations for cancer and precancer.
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CANCER SCREENING TEST

DESCRIPTION OF THE INVENTION

1. Technical Field of the Invention

This invention relates to a screening test for cancer by detecting the presence of the disaccharide beta-D-Gal-(1->3)-D-GalNAc in body fluids.

2. Background Art

Most of the cancers of the human are caused by environmental carcinogens which are excreted through the large intestine and the urinary tract via the stool and urine respectively. Thus the excreted carcinogens, causing cancer elsewhere, are likely to cause the same biochemical changes in the large intestine and the bladder.

Cancer of the colon, cecum, and rectum (large intestinal cancer) is a major cause of morbidity and
mortality in Western society. Large intestinal cancer is the second most common form of cancer in males and the third most common in females in the United States, with 138,000 new cases and 60,000 related deaths in 1985. Colon or rectal cancer eventually develops in approximately 6% of the U.S. population, and it has been estimated that some six million Americans who are alive in 1986 will die of it. Because there is currently no effective method for treating large intestinal cancer, the American Cancer Society's (ACS) guidelines recommend large intestinal cancer screening, an opinion which is also supported by others at the National Cancer Institute (NCI).

Assays for enzymatic activity in colonic biopsy material are invasive, have a high cost, and entail the use of complicated procedures in a well equipped central laboratory, which make these assays unsuited as screening tests.

Based on the observation that large tumors are associated with bleeding, the fecal occult blood test is the current empirical screening test for cancer of the large intestine. The indirect nature of this test, which detects bleeding rather than cancer or other physiological changes which are more closely related to cancer, gives it an inherent inaccuracy because not all tumors bleed, nor are all bleedings due to cancer. Cancers that are large enough to bleed are more than likely to be advanced, so that the fecal occult blood test is not well suited for early cancer detection. Furthermore, not every malignant change in the large intestine will be bleeding at the time the test is applied, nor will such bleeding necessarily be detected
in the stool, especially if the site of bleeding is in
the proximal segments of the colon.

The fecal occult blood test gives very high false
negatives and false positive results. Indeed, a
recently completed mass screening test involving 45,668
people reported by Winchester et al. (1983, CA
33:333 - 343) demonstrated that a mere 4.3% were
positive. A false positive fecal occult blood test can
easily occur, inter alia, because of ingestion of
certain foods and drugs.

The desialylated disaccharide beta-D-Gal-(1->3)-D-
GalNAc, also known as T-antigen, has been recently
demonstrated to be expressed in the malignant and
premalignant colonic epithelia in humans and in
experimental animals but absent in the normal
epithelia. Peanut (Arachis hypogaea) agglutinin (PNA)
has specificity for T-antigen. Following neuraminidase
treatment, erythrocytes of the human ABO type are
agglutinated by PNA due to binding with cell surface
T-antigen. At stoichiometric concentrations, PNA
barely causes the red blood cells to agglutinate and fails
to do so if a solution containing the T-antigen
is added to the system, leading to agglutination
inhibition.

While T-antigen has been reported to be associated
with a number of different types of cancer tissue, it
has not heretofore been reported to be present in
extracellular body fluids which have accordingly not
been employed as a sample to test for the presence of
T-antigen associated with cancer or precancer.
Disclosure of the Invention

Accordingly, it is a general object of the present invention to provide a diagnostic test for cancer that does not suffer from the above-indicated deficiencies of the prior art.

Another object of the present invention is to provide a diagnostic test for cancer which does not give a high percentage of false positive and false negative readings.

A further object of the present invention is to provide a diagnostic test for precancer or cancer which detects a biochemical change directly associated therewith.

An additional object of the present invention is to provide such a test which can detect cancer in its early stages prior to development of a bleeding tumor.

A more particular object of the present invention is to provide a kit by means of which such a test can be conducted outside of a hospital or medical laboratory setting.

In a currently preferred embodiment, the present invention provides such benefits in a test for large intestinal cancer.

Upon study of the specification and appended claims, further objects, features and advantages of the present invention will become more fully apparent to those skilled in the art to which this invention pertains.
BEST MODE FOR CARRYING OUT THE INVENTION

Briefly, the above and other objects, features and advantages of the present invention are attained in one aspect thereof by providing a method for detecting the presence of precancer or cancer in a human, which comprises obtaining a sample of body fluids; assaying said sample to quantitatively detect the presence of the desialyted disaccharide beta-D-Gal-(1->3)-D-GalNAc therein; and diagnosing the presence and degree of precancer or cancer based upon the amount of the disaccharide detected in the body fluid.

In a simple embodiment, the assay may be performed by use of a hemagglutination inhibition test, preferably by reacting the body fluid with a precise amount of peanut agglutinin or other specific binding moiety for the disaccharide and then detecting the presence of unbound moiety. The reactant moiety can be immobilized onto a water-insoluble support, such as a membrane filter or solid beads of latex, plastic, glass, etc. In order to increase the sensitivity of the method, the reactant moiety can first be biotinylated in a conventional manner.

The complex can be detected by any of various suitable techniques, either directly or indirectly, e.g. immunologically, enzymatically, oxidatively-reductively, etc. Presently preferred is the formation of a complex with avidin conjugated to a suitable marker, e.g. fuchsin or other dyes, radioactive labelling, fluorescent dyes such as fluorescein
isothiocyanate or Rhodamine B, luminescent dyes such as luciferol, luminol, biotin, etc.

The presence of the disaccharide is readily detected by agglomeration of sensitized beads which have been coated with PNA, e.g. glass, agarose, polystyrene, latex, etc. A preferred method for detecting the presence of the complex is by selectively oxidizing the sugar moiety of the disaccharide, e.g. with galactose oxidase, and detecting the presence of the oxidized sugar therein.

Adsorption of the body fluid sample onto a protein-capturing membrane filtering material has numerous advantages. For one thing, these samples appear to be quite stable and can be assayed days or weeks after being stored at or below room temperature without any significant deviation in results. In a preferred embodiment of the invention, a kit is provided which comprises separate containers of galactose oxidase, a protein-capturing membrane filter, basic fuchsin, and optionally deionized distilled water. Preferably the galactose oxidase is in lyophilized form, especially when impregnated onto a porous support such as filter paper, which can then be wetted and contacted directly with the body fluid sample on the membrane filter, then visualized by staining with fuchsin.

BRIEF DESCRIPTION OF THE DRAWINGS

Referring to the annexed drawings, like or corresponding reference characters refer to like or corresponding parts in the several figures, wherein:
Figure 1 is a photograph of a microtitration plate in which hemagglutination test is performed by mixing PNA, mucus, and T-antigen activated erythrocytes;

Figure 2 is a schematic representation of the principle of the large intestinal cancer assay of this invention;

Figure 3 is a schematic representation of the biotinylated PNA-Avidin enzyme assay of this invention.

**DETAILED DESCRIPTION**

This large intestinal cancer assay test detects a specific biochemical change in large intestinal mucus associated with cancer of the large intestine. The present inventors have discovered that the desialyted disaccharide beta-D-Gal*- (1->3)-D-GalNAc, also known as T-antigen, is absent in the body fluids of normal individuals but present in the relevant body fluids of patients with cancer and precancer. The inventors then developed various techniques for the detection of this sugar moiety in a rapid, simple and inexpensive manner. These newly developed techniques were then tested to screen individuals for large intestinal diseases including cancer.

The lectin, peanut agglutinin (PNA) specifically binds with T-antigen and causes agglutination of T-antigen activated RBC. Exploiting these characteristics of PNA, initially a simple inhibition assay has been developed wherein T-antigen in a body fluid sample will bind with PNA and, therefore, PNA will not react with RBC and the red cells will
accordingly not agglutinate. This test is very simple and can be performed rapidly. Using microtiter plates, a large number of samples can be screened in a short time. The galactose oxidase test can be done conveniently on a strip of membrane filter.

Unlike the empirical fecal occult blood test which has very high false positive and false negative values, this test is based on specific biochemical abnormality of large intestinal mucus which the present inventors have found to be associated with cancer and precancer. In vivo and in vitro studies of rat colon carcinogenesis demonstrated that certain changes precede the cancer formation (presumably precancerous changes); these are dilatation, distortion and hypercellularity of the colonic crypts as well as change in the nature of mucus secretion.

In a pilot clinical study of the hemagglutination inhibition assay, rectal mucus was obtained from 13 patients with colon cancer and polyps and 42 healthy subjects free of large intestinal cancer during routine digital rectal examination. The mucus on the gloved finger was mixed with phosphate buffered saline (PBS).

A hemagglutination test was performed by mixing PNA, mucus and T-antigen activated erythrocytes (Figure 1). The assay conditions were optimized by using serial dilutions of PNA and a fixed volume of T-antigen activated erythrocytes, since PNA has only a limited binding capacity for T-antigen. It was determined that a PNA concentration of 2.5 mg/ml barely causes hemagglutination of 50 ul T-antigen activated erythrocytes recognizable after 1 hour of incubation which is inhibited by the trace amount of disaccharide present in the mucus of cancer and precancer patients.
Figure 2 is a schematic representation of the principle of this assay. Upper panel: normal mucus glycoprotein (strands) not having recognizable beta-D-Gal-(1→3)-D-GalNAc fails to bind with PNA which in turn binds with T-antigen on activated erythrocytes causing agglutination. The lower panel shows cancer-associated mucus glycoprotein containing beta-D-Gal-(1→3)-D-GalNAc which readily binds with PNA. At optimal PNA concentrations, all binding sites will be blocked by the available disaccharide and therefore none will be available for binding with T-antigen on the erythrocytes; hence, agglutination will be inhibited.

The pilot study using mucus obtained during digital rectal examination from 55 individuals showed an inhibition of agglutination in 8 of 13 patients with cancer and polyps and 14 of 42 with no obvious polyps or cancer. The remaining 28 patients (ages 54-89) were known to be free of any large intestinal cancer and showed no inhibition of agglutination (Table 1). One patient (age 89) with diverticulosis was negative, indicating that diverticulosis and/or old age per se do not necessarily cause an agglutination inhibition reaction. Virtually no false negatives and a low rate of false positive reactions renders this assay more suitable than the fecal occult blood test for population screening.

In comparison to the hemagglutination test, a galactose oxidase strip test described in Example 3 shows a 100% sensitivity (14 of 14 cancer patients) and 91.5% specificity.
A study was also conducted wherein pieces of colon obtained from rats were maintained in explant culture system and exposed to the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Changes in the colonic crypts similar to the early precancerous lesions seen in the azoxymethane (AOM) treated rats were also seen in the colonic epithelium exposed to MNNG, including a shift from sulphomucin to sialomucin. The present inventors' studies on human colon mucosa remote from carcinomas demonstrated that multifocal lesions identical to what has been observed in the carcinogen AOM treated rat and MNNG treated rat colon explant in culture were seen in the human colon away from cancers. Contrary to the popular belief that the colonic epithelium remote from a cancer is normal, it has now been demonstrated that the mucosa away from a cancer shows multiple foci of precancerous changes. This also includes a change in the type of mucin secretion, from normal sulphomucin to sialomucin and normal glycoconjuge to the abnormal glycoconjuge, the latter being also seen in cancerous conditions. Thus, the present invention has demonstrated that large intestinal mucosa, and the secreted mucus remote from a cancer of that organ, does show all of these precancerous changes including secretion of abnormal glycoconjuge. If one subscribes to the "field-effect" theory of carcinogenesis, then the rational behind these observed changes remote from the actual site of the cancer or precancer becomes obvious.

Most of the cancers of the human are caused by environmental carcinogens which are excreted through the large intestine and the urinary tract via the stool and urine respectively. Thus the excreted carcinogens, causing cancer elsewhere, are likely to cause the same
biochemical changes in the large intestine and the bladder. In a pilot study 50% of patients (4 of 8) with cancer of the neck, breast, kidney, testes and other sites where detected by the rectal mucus test according to the present invention. This is a much higher percentage than in patients with no known cancer. In addition to assaying rectal mucus, other body fluids such as urine, saliva, serum, body cavity fluids, cerebrospinal fluid, etc. appear to behave similarly with respect to containing the altered disaccharide moiety.

In addition to cancer, this test has the power to detect other diseases of the colon including those that carry a high risk of cancer such as fistula, urterosigmoidostomy, Crohn's disease, and ulcerative colitis.

Other properties, such as immobilization of PNA onto a water-insoluble support, immunological detection of the glycoconjugate or oxidation of the sugar moiety and detection by dyes, radio-chemicals, etc. can be exploited to develop additional assays. The use of an antibody directed against this sugar moiety in an immunoassay enables accurate estimation and monitoring of this moiety in rectal mucus as well as other body fluids. In the immunoassays the antibody can be tagged by a radioactive fluorescent or other suitable label for quantitative or semiquantitative detection.

Avidin, a glycoprotein (67,000 MW) has an extraordinarily high affinity for the vitamin biotin. Inasmuch as biotin molecules can be coupled to various proteins (biotinylation), avidin can be conjugated with various markers such as enzymes, dyes, heavy metals,
radioactive isotopes, etc. Avidin has four binding sites for biotin, and many biotin molecules can be incorporated on a given protein. The present inventors have exploited this amplification principle to detect minute amounts (i.e. ng/ml or even pg/ml) of the marker disaccharide beta-D-Gal-(1->3)-D-GalNAc in mucous glycoproteins obtained during digital rectal examination. An assay was developed according to the following rationale: Mucous glycoprotein containing the specific disaccharide will avidly bind to the PNA immobilized on a solid phase. A matrix formed by biotinylated PNA and enzyme-avidin D conjugate will bind to residual disaccharides on the immobilized glycoprotein PNA, while a suitable substrate will amplify the reaction.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever. In the following Examples, the temperatures are set forth uncorrected in degrees Celsius; unless otherwise indicated, all parts and percentages are by weight.

EXAMPLE 1

Hemagglutination Inhibition Test

This technique uses PNA and T-activated RBCs in an agglutination inhibition assay to test for the presence of this sialic acid free terminal sugar residue, utilizing the specific affinity of PNA to bind to the
terminal galactose residue and more preferentially (by an affinity several orders of magnitude greater) to the terminal dimer beta-D-Gal-(1->3)-D-GalNAc as the binding of this lectin requires a free hydroxyl group at C-2. Mucus was obtained from 13 patients with colon cancer and polyps and 42 healthy subjects free of colon cancer during digital rectal examination by inserting the saline lubricated gloved index finger of the physician. The finger containing the mucus was then dipped in a specimen bottle containing 500 ul phosphate buffered saline (PBS) pH 7.2. The finger was rinsed thoroughly in the PBS to allow maximum extraction of the mucus from the glove.

Hemagglutination was performed by using PNA (Vector Laboratories Inc., Burlingame, CA 94010) and T-antigen activated human erythrocytes. Stock PNA solution was made by dissolving lyophilized PNA in PBS to a concentration of 40 ug/ml. Serial twofold dilutions up to 78 ng/ml were made for the assay. Activation of T-antigen on human erythrocytes was performed by washing outdated human Group O blood three times with equal volumes of PBS, centrifuging at 3,000 RPM for 5 minutes and removing of the supernatant. An equal volume of 10% neuraminidase (Vibrio cholera neuraminidase, Behring Diagnostics, La Jolla, CA 92037 USA) was added to the packed, washed erythrocytes and incubated at 37°C for 30 minutes. The erythrocytes were washed again three times and were either used for experiments or stored at 4°C in an equal volume of Alsever's solution for future experiments.

Control inhibition of agglutination was performed by using 0.6 M D(+) Galactose (Sigma Chemical Company, St. Louis, MO 63178). Ninety-six well polyvinyl
microtiter plates (Dynatech, VA) were used, in which each horizontal row of wells was filled with 50 μl of serial dilutions of PNA solutions, beginning from left (78 ng/ml) to right (40 μg/ml). The wells in the test row were given 50 μl of mucus solution followed by incubation at room temperature for 60 minutes to allow for interaction between PNA and the mucus, which is inhibited by the trace amounts of beta-D-Gal-(1→3)-D-GalNAc present in the mucus of patients with precancer and cancer. 50 μl of the T-antigen activated erythrocytes were then added to each well and incubated at room temperature for 1 hour. Figure 2 is a schematic representation of the principle of this assay.

A typical microtiter plate showing the results of this hemagglutination inhibition assay is shown in Figure 1. Wells 3-12 in the top row show control hemagglutination inhibition with serial double concentrations of PNA from left (78 ng/ml) to right (40 μg/ml). Well no. 1 contains 40 μg/ml PNA but no galactose and therefore acts as a positive control for agglutination. Hemagglutination is completely inhibited in Wells No. 3 (78 ng PNA/ml) through 6 (628 ng/ml) as indicated by their doughnut shaped appearance. Wells 10 through 12 show agglutination of erythrocytes while wells 7, 8 and 9 show weak agglutination which could be easily inhibited by trace amounts of the glycoconjugate. Wells 3-12 in the bottom row shows a test mucus sample that has inhibited hemagglutination in all wells. Well no. 1 is a control hemagglutination inhibition as performed by the addition of 0.6 M D(+)galactose to 40 μg PNA/ml.
The results are shown below in Table 1, from which it can be seen that no false negatives and far fewer false positives (33% vs. about 95%) were found than in the conventional fecal occult blood test:

**TABLE 1 - Hemagglutination Inhibition Assay Results***

<table>
<thead>
<tr>
<th>Method</th>
<th>Diagnosis</th>
<th>No. of Cases Showing Agglutination Inhibition</th>
<th>Total Cases</th>
<th>% Positive Test</th>
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<tr>
<td>Direct Smear of resected colon</td>
<td>Cancer</td>
<td>5</td>
<td>5</td>
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<td></td>
<td>Normal</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Rectal Smear from patients</td>
<td>Cancer</td>
<td>3</td>
<td>3</td>
<td>100</td>
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<td></td>
<td>Polyp</td>
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<td>10</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Non-cancer</td>
<td>14</td>
<td>42</td>
<td>33</td>
</tr>
</tbody>
</table>

*Age range 35 - 89 years. "Non-cancer" category includes patients with diverticulosis who are not currently diagnosed to have obvious polyps or cancer. The Fisher exact test for rectal smear cases was significant at p = 0.0052 (two tailed test).

**EXAMPLE 2**

**Latex Agglutination Test**

500 ul of suspended latex beads (15.8 u diameter, Sigma Chemical Co., St. Louis, MO) was centrifuged at 3,000 RPM for 15 seconds and the supernatant was decanted. 500 ug of peanut agglutinin (Vector Laboratories Ltd., Burlingame, CA) was dissolved in 500
ul of carbonate buffer (pH 9.6) and added to the pellet of latex beads. The pellet was resuspended and incubated at 25°C for 2 hours with occasional mild shaking to resuspend the beads and allow a more uniform binding. After incubation, the sample was centrifuged at 3,000 RPM for 15 seconds, the supernatant decanted, and the pellet resuspended in PBS (pH 7.4). Any unbound PNA was washed off by repeating the previous step three times. The final pellet was suspended and diluted 1:10 in PBS.

For testing the mucus sample collected during digital rectal examination, 10 ul of mucus in PBS was added to an equal amount of the latex beads and placed on a glass slide. After five minutes of incubation at 25°C, the slide was read. An agglutination of beads indicating presence of the disaccharide beta-D-Gal-(1->3)-D-GalNac was read as positive for cancer, whereas no agglutination after 5 minutes indicated absence of the disaccharide and hence cancer free status.

EXAMPLE 3

Galactose Oxidase Strip Test

This technique uses the ability of galactose oxidase to oxidize the C-6 hydroxyl group of both galactose and N-acetyl galactosamine residues of the desialyted beta-D-Gal-(1->3)-D-GalNac to D-galactohexodialdose, then tests for the presence of this oxidized product using basic fuchsin reagent.

Mucus samples were obtained by digital examination with the gloved index finger lubricated with normal
saline. The mucus on the examining finger was smeared on the scored side of a piece of membrane filter and sandwiched in waxed paper supplied with Metrical membrane filter 0.45 μm, (Gelman Sciences, Inc., Ann Arbor, MI 48106). The specimens were left to dry for 2 hours at room temperature then saturated with 100 U/ml galactose oxidase Type V, (Sigma Chemicals Co., St. Louis, MO 63178) in 0.1 M potassium phosphate buffer pH 7.0 and kept in a moist atmosphere at room temperature for 2 hours. The membrane filters were washed in deionized distilled water for 1 min., and placed in Schiff's reagent for 15 min., then placed in running tap water for 10 min. Galactose oxidase (100 U/ml) was lyophilized on a cellulose filter prior to the test. The impregnated test strip was saturated with deionized distilled water just before the test, placed under the membrane filter (unscored side) and wrapped tightly in a small Parafilm™ sheet. Magenta coloration of the strip indicates positive reaction; no coloration is negative.

The results are shown below in Table 2, from which it can be seen that no false negatives and far fewer false positives (8.5% vs. about 95%) were found than in the conventional fecal occult blood test:

<table>
<thead>
<tr>
<th>Method</th>
<th>Diagnosis</th>
<th>Activity</th>
<th>Total Cases</th>
<th>% Positive</th>
</tr>
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<tbody>
<tr>
<td>Rectal</td>
<td>Cancer &amp;</td>
<td>14</td>
<td>14</td>
<td>100%</td>
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<td>Smear</td>
<td>Polyps</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>from patients</td>
<td>Non-Cancer</td>
<td>5</td>
<td>59</td>
<td>8.5%</td>
</tr>
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EXAMPLE 4

Biotinylated Peanut Agglutinin

Avidin-Enzyme Assay

PNA dissolved in carbonate buffer (pH 9) to a final concentration of 100 ng/ml was used to coat the microtiter wells. 10 ng PNA in 100 ul buffer were placed in each well and incubated at 37°C for 2 hours. The wells were then washed off with phosphate buffered saline (PBS) pH 7.4, after which 100 ul of test mucus (dissolved in PBS) was added to microtiter wells and the mixture incubated at 37°C for 1 hour. The wells were then washed three times with PBS to remove 100 ul of unbound mucus. Biotinylated PNA (1 ug/ml) was incubated for an additional hour at 37°C in order to bind with residual beta-D-Gal-(1→3)-D-GalNAc (if any). The wells were washed three times with PBS to wash off unbound biotinylated PNA. Avidin-D-alkaline phosphatase (Vector Corporation, Burlingame, CA) was then added (100 ul/well, 1:50 dilution) to the wells and incubated for 1 hour at 37°C. Following 2 washes with PBS and 3 washes with bicarbonate buffer (pH 9.8), the substrate p-nitrophenyl phosphate (1 mg/ml) was added to the wells (100 ul/well). Optical absorbance at 405 nm was read after 30 minutes incubation at 37°C. Mucus from known cancer patients were positive while non-cancer patients were negative.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those specifically used in the examples. From the foregoing description,
one skilled in the art to which this invention pertains can easily ascertain the essential characteristics thereof and, without departing from the spirit and scope of the present invention, can make various changes and modifications to adapt it to various usages and conditions.

Industrial Applicability

As can be seen from the present specification and examples, the present invention is industrially useful in providing easy, simple and inexpensive technique for detection of specific changes accompanying cancer with essentially no false negative and very low false positive reactions, in addition to detecting premalignant conditions and lesions. The simplicity and low cost of the tests as well as the ability to store its components, including the specimens, open the door for its use as a practical screening test for various cancers and for diseases of the colon and rectum.
WHAT IS CLAIMED IS:

1. A method for detecting the presence of precancer or cancer in humans, which comprises:
   a) obtaining a sample of a body fluid;
   b) assaying said sample to quantitatively detect the presence of the disaccharide beta-D-Gal(1->3)-D-GalNAc therein; and
   c) diagnosing the presence and degree of precancer or cancer based upon the amount of the disaccharide detected in the fluid.

2. A process according to claim 1 wherein the assay is performed by use of an agglutination inhibition test.

3. A process according to claim 2 wherein the disaccharide is detected by reacting the body fluid with an approximately stoichiometric amount of peanut agglutinin.

4. A process according to claim 3 wherein the disaccharide is detected by reaction with a specific reactant therefor which is immobilized onto a water-insoluble support.

5. A process according to claim 1, wherein the disaccharide is assayed by detecting the presence of biotinylated peanut agglutinin.

6. A process according to claim 5, wherein the complex is detected by reacting it with avidin which is conjugated with a marker.
7. A process according to claim 1, wherein the disaccharide is detected by agglomeration of sensitized beads.

8. A process according to claim 1, wherein the sugar moiety of the disaccharide is oxidized and the oxidized sugar moiety is determined.

9. A process according to claim 8, wherein body fluid is adsorbed onto a protein- or lipid- capturing membrane filter.

10. A process according to claim 8, wherein galactose oxidase is used to oxidize the sugar moiety in the body fluid.

11. A process according to claim 1, wherein the body fluid is preserved at room temperature for at least several days prior to assaying the sample.

12. A process according to claim 1, wherein the body fluid is large intestinal mucus or urine.

13. A process according to claim 1, wherein the disaccharide is detected immunochemically.

14. A diagnostic kit for detecting the presence of large intestinal cancer, which comprises a container comprising separately packaged galactose oxidase, a protein-capturing membrane filter, and basic fuchsin.

15. A diagnostic kit according to claim 14, wherein at least one of the galactose oxidase and basic fuchsin is microencapsulated.
1. A method for detecting the presence of precancer, cancer or other diseases of the large intestine or bladder which carry a high risk of cancer in humans, which comprises:
   a) obtaining a body fluid sample of large intestinal mucus from the rectum of a patient or of urine from the bladder of a patient;
   b) assaying said sample to detect the presence of the disaccharide marker beta-D-Gal(1→3)-D-GalNAc therein; and
   c) diagnosing precancer or cancer of the large intestine or bladder based upon the presence of the disaccharide detected in the mucus or urine, respectively.

2. A process according to claim 1, wherein the disaccharide is detected immunochemically.

3. A process according to claim 1, wherein the disaccharide is detected quantitatively.

4. A process according to claim 1, wherein the body fluid sample is said large intestinal mucus.

5. A process according to claim 1, wherein the body fluid sample is urine.

6. A process according to claim 1, wherein the assay is performed by use of peanut agglutinin in an agglutination inhibition test.

7. A process according to claim 6, wherein the disaccharide is detected by reacting the urine or mucus with an approximately stoichiometric amount of peanut agglutinin.

8. A process according to claim 6, wherein the disaccharide is detected by reaction with peanut agglutinin which is immobilized onto a water-insoluble support to form a complex of the disaccharide and the peanut agglutinin.

9. A process according to claim 8, wherein the complex is assayed by reaction with biotinylated peanut agglutinin.
10. A process according to claim 8, wherein the complex is detected by reacting it with avidin which is conjugated with a marker.

11. A process according to claim 1, wherein the disaccharide is detected by the agglomeration of sensitized beads.

12. A process according to claim 1, wherein the sugar moiety of the disaccharide is oxidized and the oxidized sugar moiety is detected.

13. A process according to claim 12, wherein galactose oxidase is used to oxidize the sugar moiety.

14. A process according to claim 1, wherein the urine or mucus is absorbed onto a water-insoluble substrate.

15. A process according to claim 14, wherein the water-insoluble substrate is a protein- or lipid-capturing membrane filter.

16. A process according to claim 1, wherein the body fluid is preserved at room temperature for at least several days prior to assaying the sample.

17. A process according to claim 16, wherein the body fluid is large intestinal mucus.

18. A diagnostic kit for detecting the presence of precancer, cancer or other diseases of the bladder or large intestine according to the process of claim 1, which comprises a container comprising separately packaged galactose oxidase, a water-insoluble support capable of absorbing said urine or mucus, and basic fuchsin.
19. A diagnostic kit according to claim 18, wherein the basic fuchsin is packaged as Schiff's reagent.

20. A diagnostic kit according to claim 18, wherein at least one of the galactose oxidase and basic fuchsin is microencapsulated.
HEMAGGLUTINATION INHIBITION ASSAY FOR Gal-GalNAc IN RECTAL MUCUS

NORMAL

Normal mucus without β-D-Gal (1→3)-D-Gal NAc

'T' antigen exposed erythrocytes are agglutinated

Mucus with β-D-Gal (1→3)-D-Gal NAc binds peanut agglutinin

'T' antigen exposed erythrocytes are not agglutinated

PRECANCER & CANCER

FIG. 2
TEST FOR Gal-GaINAC
BY ENZYME ASSAY

1. Bind PNA to microtiter wells
2. Add test mucus to wells
3. Wash
4. Add biotinylated PNA enzyme-Avidin D conjugate
5. Wash
6. Add Substrate
7. Measure Product

FIG. 3
INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/01701

I. CLASSIFICATION OF SUBJECT MATTER
According to International Patent Classification (IPC) or to both National Classification and IPC:
IPC (4): G01N 33/546, 33/566, 33/574; C12Q 1/26
U.S. Cl.: 436/501, 534; 435/25

II. FIELDS SEARCHED

<table>
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<th>Classification System</th>
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<td>U.S.</td>
<td>435/7, 14, 25, 810</td>
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<td>436/501, 518, 533, 534, 64, 94, 808, 813, 827</td>
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Documentation Search other than Minimum Documentation to the extent that such documents are included in the fields searched:

III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, 14 with indication, where appropriate, of the relevant passages</th>
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<tr>
<td>Y</td>
<td>Proceedings of the National Academy of Science USA, Vol. 79, issued March 1982 (Washington, D.C., USA), C.R. Boland et al., &quot;Alterations In Human Colonic Mucin Occurring With Cellular Differentiation and Malignant Transformation&quot;, 2051-2055 see 2053, last line of column 1 to line 6 of column 2, page 2054, paragraph bridging columns 1 and 2 and page 2055, lines 12-21.</td>
<td>1-13</td>
</tr>
<tr>
<td>Y</td>
<td>US, A, 4,334,017 (PLOTKIN) 08 June 1982, see column 1, lines 61-67, column 2, lines 49-54, column 6, lines 26-53 and claim 12.</td>
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* Special categories of cited documents: 13
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 3
05 OCTOBER 1987
International Searching Authority 1
ISA/US

Date of Mailing of this International Search Report 5
19 OCT 1987

Signature of Authorized Office 26
Esther M. Keppinger

Form PCT/ISA/210 (second sheet) (October 1981)
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<tr>
<td>Y</td>
<td>US, A, 571,382 (ADACHI) 18 February 1986, see column 2, lines 47-53, column 3, lines 29-39 and column 6, lines 60-68.</td>
<td>1-13</td>
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<td>Y</td>
<td>M. Hjelm et al, &quot;Methods In Enzymatic Analysis&quot;, Volume 3, published 1974, by Academic Press, Inc. (New York, N.Y., USA), see page 1282, lines 23-33 and page 1286, paragraph 3.</td>
<td>8-10</td>
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