(54) Title: SIMPLE TEST FOR DETECTING CARCINOEMBRYONIC ANTIGEN

(57) Abstract

A rapid, simple, sensitive and reliable method for detecting fecal carcinoembryonic antigen in stool, indicative of colorectal cancer, is described. The invention is based in part on the discovery that previous methods of removing coarse and gelatinous contaminants from a stool and liquid mixture resulted in removing a significant amount of the CEA. By not removing macromolecules smaller than about 1,000 MW, preferably 5,000 MW, a significant portion of the CEA will remain in the filtered liquid for detection.
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SIMPLE TEST FOR DETECTING CARCINOEMBRYONIC ANTIGEN

Background of the Invention

This invention relates to detection of colorectal or large bowel cancer.

It has been reported that the presence of carcinoembryonic antigen (CEA) in the stool is an indicator of cancerous condition in the large bowel. However, known methods of detecting CEA have various drawbacks, and are not widely used, e.g., some require preparation of purified CEA from fecal samples, a procedure in which much CEA is lost. A need exists for a rapid, simple, sensitive, and reliable method for detecting fecal CEA.

Summary of the Invention

In general, the invention features a method for detecting CEA in a stool sample, by the steps of:

(a) mixing said stool sample with an aqueous buffer to yield a liquid mixture;

(b) filtering said liquid mixture to remove coarse and gelatinous contaminants, but not macromolecules smaller than about 1,000 MW; and

(c) detecting said carcinoembryonic antigen in said filtered mixture.

The invention is based in part on my discovery that previous methods of removing coarse and gelatinous contaminants from a stool and liquid mixture resulted in removing a significant amount of the CEA. By employing a filtration method which avoids removal of coagulated proteins (i.e., avoids removal of macromolecules smaller than about 1,000 MW, more preferably 5,000 MW), a significant portion of the CEA remains in the filtered liquid for detection. This also reduces interference from related, potentially interfering macromolecules likely to be present in stool samples. The method detects CEA, which is a heterogenous protein, without the necessity of purifying CEA prior to detection.
Any filtration medium which is effective in removing coarse and gelatinous contaminants and macromolecules can be used. A filter having an average pore size equivalent to that of a Whatman # 40 filter is preferred.

The method preferably includes a step of diluting the filtered liquid mixture at least 100-fold, v/v, prior to detecting the carcinoembryonic antigen. The dilution step provides further sensitivity by decreasing interference by other macromolecules. Preferably, the filtered liquid mixture is diluted at about 1:100 - 1:1,500, with a dilution of about 1:700 - 1:1,200, more preferably about 1:1,000, being most preferred.

The method is preferably carried out without pretreatment of the subject's bowel prior to obtaining of the sample; such pretreatment can change the measured CEA amount. Phosphate buffered saline (PBS) is preferably used as the diluent in the method of the invention. This diluent will decrease denaturation of CEA which could occur with the use of other diluents.

In preferred embodiments, the method employs between about 0.5 and 5 grams of stool sample; and the detecting step is carried out using an immunometric assay, e.g, a radioimmunoassay, or an enzyme-linked-immunoassay.

The invention can be carried out using a dipstick coated with anti-CEA monoclonal antibody, which is dipped sequentially in the filtered liquid mixture and then in a colored developing solution, the appearance of color on the dipstick being indicative of the presence of CEA in the sample. The invention can also be carried out by dipping the coated dipstick in the filtered and diluted mixture and then in a colored developing solution, the appearance of color on the dipstick being indicative of the presence of CEA in the sample.
The present method of stool CEA offers the following additional advantages.

a) The use of random, freshly obtained stool avoids the need for diet restriction, cathartics, or enemas.

b) The buffer solution is devoid of any preservatives such as sodium azide or phenols and the like.

c) There is no need for high precision filtration requiring the use of micropore filters of specific porosity, because filtration involves only separating gelatinous and coarse contaminants.

d) Centrifugation, dialysis, periodic acid, and high heat (80°C) treatment are not necessary; these steps can affect the physicochemical nature of the antigen molecules and interfere with the highly sensitive RIA or EIA assays.

In addition, the method of the present invention does not require the extraction and purification of CEA molecules. Furthermore, it provides a quantitative determination of randomly obtained stool CEA content as a screening test for any pathologic condition of the large bowel which results in CEA being secreted or discharged into the bowel lumen.

Other features and advantages of the invention will be apparent from the following detailed description thereof, and from the claims.

Detailed Description of the Invention

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials
are now described. Unless mentioned otherwise, the
techniques employed or contemplated herein are standard
methodologies well known to one of ordinary skill in the
art. The materials, methods and examples are
illustrative only and not limiting.

The preferred embodiment of the method for
determining CEA in stool samples is now described, and
includes the following steps.

1. A fresh stool sample, formed or liquid, is
randomly obtained in a dry and clean receptacle without
any prior chemical or physical preparation of the bowels.

2. The stool specimen is thoroughly stirred with
a glass rod to ensure uniform mixing of any CEA which
might have come into contact with only a part of the
surface of the feces. Liquid stool is shaken in a clean
glass container for the same purpose.

3. A one gram specimen of the above homogenized
stool, whether formed or liquid, is placed in a separate
clean dry glass beaker.

4. A phosphate buffered saline solution (PBS) of
pH 7.2 and 0.15 molarity is prepared by dissolving about
20 grams of NaCl, 0.5 grams of KCl and 14.3 milliliter of
a solution of Na₂HP₀₄ and 0.5 grams of KH₂PO₄, in 1000 cc
of distilled water.

5. Twenty-five milliliters of this PBS solution
are poured into the beaker of step 3 containing one gram
of the stool homogenate and thoroughly mixed to allow
thorough dissolution of the stool sample in the buffer
solution.

6. All of the stool specimen dissolved in the
buffer solution is passed through a commercially
available ashless filter paper of fast filtering speed
designed for retention of coarse and gelatinous
precipitates (for example: Whatman # 40 filter paper,
Catalog No. 1440 125, Whatman Paper Ltd, Maidstone, England).

7. The filtrate is either used for assay or stored at -20°C until ready to be used for assay.

8. For radioimmunoassay (RIA) of CEA, the filtrate is first brought to room temperature (about 22° - 24°C) and diluted 1:1000, volume to volume, with the above mentioned PBS solution.

9. 50 µl of the diluted filtrate are then utilized in a two-site radioimmunometric assay (sandwich principle) based on the use of commercially available monoclonal antibodies having specific binding affinity for CEA (such as, available from Byk -Sangtec Co. Diagnostica, D-6057 Dietzenbach 2, Germany). The CEA moiety of the filtrate will interact with the anti-CEA monoclonal antibody coated on a suitable support such as polystyrene beads, tubes, microliter plates or the like. Free or unreacted non-specific CEA is then washed away while specifically reacted portion is determined by an 125-I-conjugated moiety, according to standard methods. Final CEA concentration in the filtrate is then calculated from the CPM obtained for the 50µl sample used in the assay.

10. As an alternative to step 9, 50µl of the diluted filtrate is incorporated in a two-site standard ELISA (enzyme-linked immunosorbent assay) system using the antibodies mentioned above for RIA and the final concentration obtained through optical absorbance at 405 or 492 nanometers. Other conventional immunoassay formats can be used as well.

11. Final concentration of CEA in the stool is calculated by correcting for dilution factors employed in steps 5 and 8 and the results expressed in nanograms CEA per gram stool.

DIP Test for CEA Detection
In accordance with the present invention a simple "dip test" is provided to detect CEA in a stool specimen. This dip test employs a solid support, such as a nitrocellulose film in the shape of a dip stick and the like, coated with a commercially available CEA-specific monoclonal antibody. This monoclonal antibody-coated solid support is dipped in a sample of the filtrate of step (7) described herein above, washed to remove unreacted material, and then reacted in a suitable colorimetric solution, the development of the color being indicative of the presence of CEA in the stool sample.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.
CLAIMS

1. A method for detecting carcinoembryonic antigen in a stool sample, comprising the steps of:
   (a) mixing said stool sample with an aqueous buffer to yield a liquid mixture;
   (b) filtering said liquid mixture to remove coarse and gelatinous contaminants, but not macromolecules smaller than about 1,000 MW; and
   (c) detecting said carcinoembryonic antigen in said filtered mixture.

2. The method of claim 1 wherein macromolecules of smaller than about 5,000 MW are not removed.

3. The method of claim 1 wherein said coarse and gelatinous materials are removed by filtering with a filter having an average pore size of a Whatman # 40 filter.

4. The method of claim 1 wherein said aqueous buffer is phosphate buffered saline.

5. The method of claim 1 wherein said stool sample and said aqueous buffer are mixed in a ratio of about 1 g stool/25 ml aqueous buffer.

6. The method of claim 1 wherein step (a) employs between about 0.5 and 5 grams of stool sample.

7. The method of claim 1 wherein the filtered liquid mixture is diluted at least 100-fold, v/v, prior to detecting said carcinoembryonic antigen.

8. The method of claim 7 wherein the filtered liquid mixture is diluted at about 1:100 - 1:1,500.
9. The method of claim 8 wherein the filtered liquid mixture is diluted at about 1:100 - 1:1,200.

10. The method of claim 9 wherein the filtered mixture is diluted at about 1:1,000.

11. The method of claim 1 wherein said detecting step is carried out using an immunometric assay.

12. The method of claim 11, wherein said immunometric assay is a radioimmunoassay.

13. The method of claim 12, wherein said immunometric assay is an enzyme-linked-immunoassay.

14. The method of claim 1 wherein said detecting step comprises disposing a dip-stick coated with anti-CEA antibody first in the filtered liquid mixture and then in a color developing solution, the appearance of color on the dip-stick being indicative of the presence of CEA.

15. The method of claim 7 wherein said detecting step comprises disposing a dip-stick coated with anti-CEA antibody first in the filtered and diluted liquid mixture and then in a color developing solution, the appearance of color on the dip-stick being indicative of the presence of CEA.
INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00988

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 4

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (S): G01N 33/574, 33/543, 1/00
US CL: 357.7.23, 7.9, 7.92, 436/64, 174, 177, 518

II. FIELDS SEARCHED

Minimum Documentation Searched 6

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Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched 6

CAS ONLINE, BIOSIS, MEDLINE, APS; search terms: cea, carcinoembryonic, fecal, feces, faecal, stool

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

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<th>Category</th>
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<th>Relevant to Claim No. 18</th>
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<td>X/Y</td>
<td>Gut, Volume 27, issued 1986, R.S. Stubbs, et al., &quot;Faecal carcinoembryonic antigen in colorectal cancer patients&quot;, pages 901-905, see the right-hand column of page 901 and the left-hand column of page 902.</td>
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<td>Jpn. J. Cancer Res., Volume 80, issued December 1989, K. Sugano, et al., &quot;Detection of increased Fecal Carcinoembryonic Antigen and its Characterization as a Membrane-bound Form in Colorectal Carcinoma and Other Gastrointestinal Disorders&quot;, pages 1156-1160, see the left-hand columns of pages 1157 and 1158.</td>
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* Special categories of cited documents: 15
   * "A" document defining the general state of the art which is not considered to be of particular relevance
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   * "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
   * "&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2

06 MAY 1992

Date of Mailing of this International Search Report 2

06 MAY 1992

International Searching Authority 1

ISA/US

Signature of Authorized Officer 20

Toni R. Scheiner

Form PCT/ISA/210 (second sheet) (May 1986) B