US 20050136495A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2005/0136495 A1

Boone et al. (43) Pub. Date: Jun. 23, 2005

(54) METHOD AND APPARATUS FOR DISTINGUISHING CROHN'S DISEASE FROM ULCERATIVE COLITIS AND OTHER GASTROINTESTINAL DISEASES BY DETECTING THE PRESENCE OF FECAL ANTIBODIES TO SACCHAROMYCES CEREVISIAE

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(21) Appl. No.: **11/059,711**

(22) Filed: **Feb. 16, 2005**

Related U.S. Application Data

(62) Division of application No. 10/280,564, filed on Oct. 25, 2002, now Pat. No. 6,872,540.

(60) Provisional application No. 60/335,812, filed on Oct. 26, 2001.

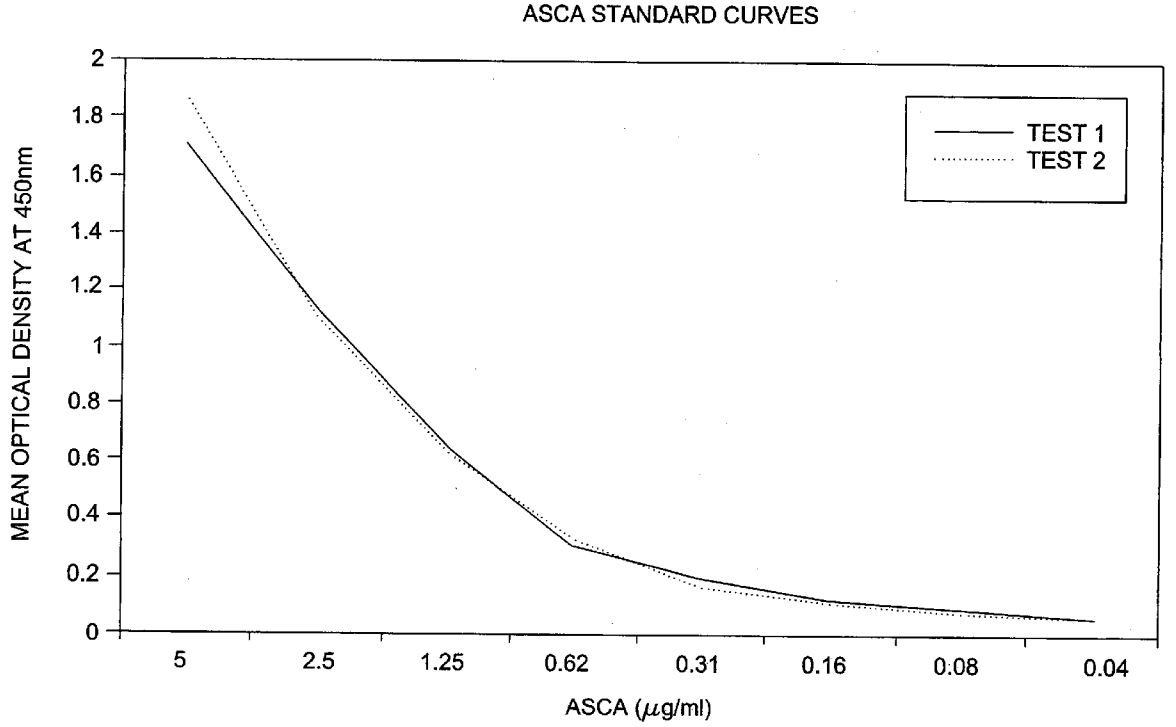
Publication Classification

(51) **Int. Cl.**[7] **G01N 33/53**; G01N 33/569

(52) **U.S. Cl.** **435/7.31**

(57) **ABSTRACT**

A method and apparatus for the differentiation of Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome, using the presence of fecal anti-Saccharomyces cerevisiae antibodies (ASCA) as a marker for Crohn's disease are provided. The apparatus includes an enzyme-linked immunoassay or other immunoassay that utilizes antibodies specific to human immunoglobins for the measurement of total endogenous ASCA in a human fecal sample. The method and apparatus may be used by healthcare providers to distinguish Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome.



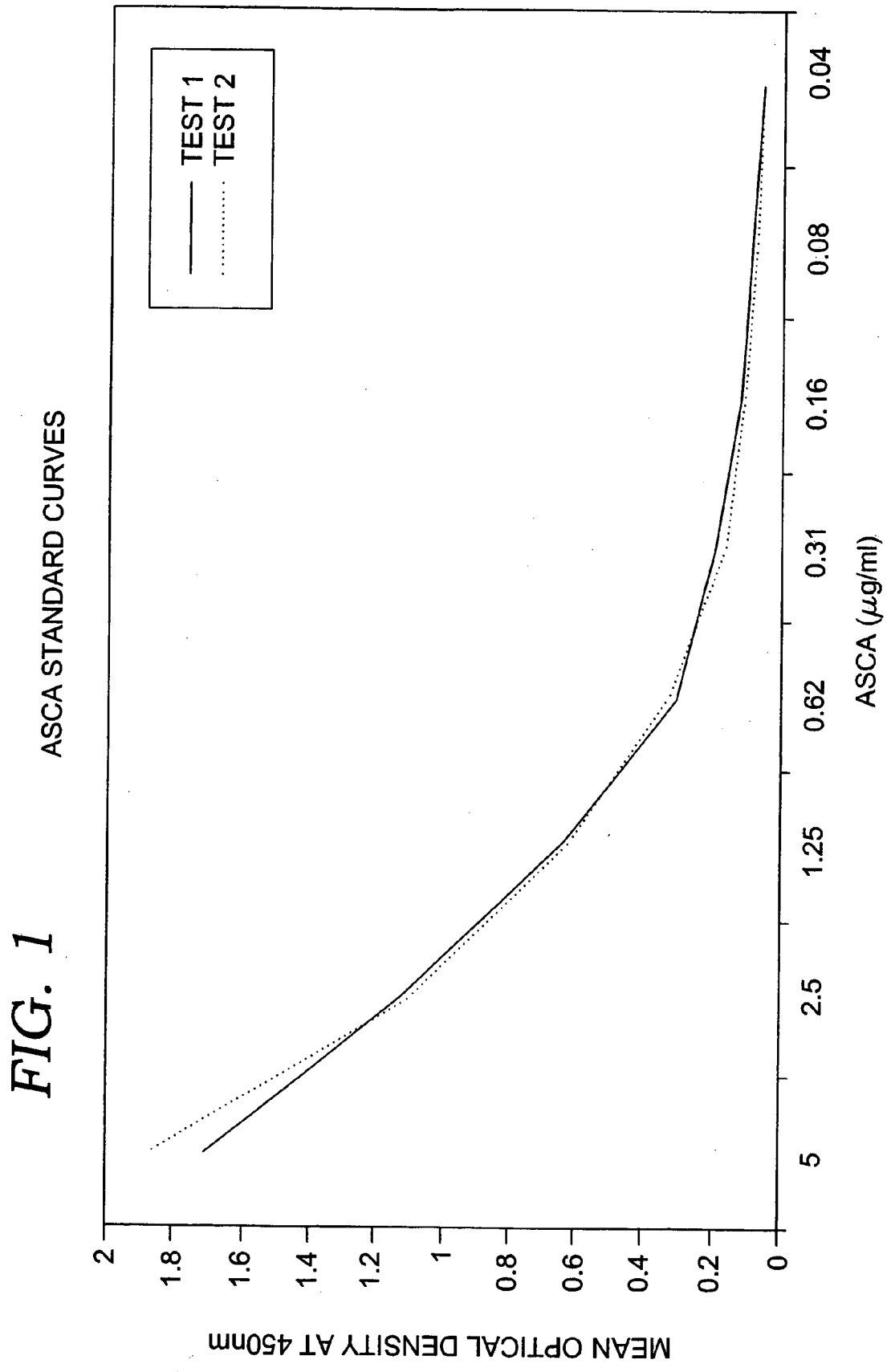

ASCA STANDARD CURVES
TEST 1
TEST 2
MEAN OPTICAL DENSITY AT 450nm
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5
2.5
1.25
0.62
0.31
0.16
0.08
0.04
ASCA (μg/ml)

FIG. 1

METHOD AND APPARATUS FOR DISTINGUISHING CROHN'S DISEASE FROM ULCERATIVE COLITIS AND OTHER GASTROINTESTINAL DISEASES BY DETECTING THE PRESENCE OF FECAL ANTIBODIES TO SACCHAROMYCES CEREVISIAE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. application Ser. No. 10/280,564 filed on Oct. 25, 2002 which claims the benefit of priority to U.S. Provisional Application No. 60/335,812 filed on Oct. 26, 2001, the entirety of the disclosures of which are hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

FIELD OF THE INVENTION

[0003] A method and apparatus for the differentiation of Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome, using the presence of fecal anti-*Saccharomyces cerevisiae* antibodies (ASCA) as a marker for Crohn's disease are provided. The apparatus includes an enzyme-linked immunoassay or other immunoassay that utilizes antibodies specific to human immunoglobulins for the measurement of total endogenous ASCA in a human fecal sample. The method and apparatus may be used by healthcare providers to distinguish Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome.

BACKGROUND OF THE INVENTION

[0004] It is estimated that at least one million Americans suffer from Inflammatory Bowel Disease (IBD). IBD is characterized by a chronic inflammatory response that results in histologic damage to the intestinal lining. IBD comprises two known clinical subtypes, Crohn's Disease (CD) and ulcerative colitis (UC). CD may involve the entire gastrointestinal tract and include inflammation extending into the transmural mucosa whereas UC affects solely the large bowel and includes inflammation of the innermost lining. Due to the differences between them, these two distinct diseases require a rapid differential diagnosis for optimal treatment. Conventional methods for differentiating between these clinical subtypes of IBD utilize multiple endoscopy examinations and histological analysis. These methods, however, do not permit quick differential diagnosis as each may require years for a diagnosis to be confirmed. As a result, methods are needed for the rapid differential diagnosis of CD and UC.

[0005] Serological methods for the differential diagnosis of CD and UC are known in the art. For example, it is known in the art to use the presence of serum anti-*Saccharomyces cerevisiae* antibodies (ASCA) to diagnose CD. See Main et al., Antibody to *Saccharomyces cerevisiae* (baker's yeast) in Crohn's disease, BMJ Vol. 297 (Oct. 29, 1988); Broker et al., A Murine Monoclonal Antibody Directed Against a Yeast Cell Wall Glycoprotein Antigen of the Yeast Genus *Saccharomyces,* FEMS Microbiology Letters 118 (1994), 297-304. It is further known in the art to use the presence of serum ASCA to diagnose clinical subtypes of UC and CD in patients presenting with established diagnoses. For example, U.S. Pat. No. 5,968,741 discloses utilizing the presence of serum ASCA to diagnose a medically resistant clinical subtype of UC in patients presenting with an established diagnosis of UC. Similarly, U.S. Pat. No. 5,932,429 discloses utilizing the presence of serum ASCA to diagnose a clinical subtype of CD in patients presenting with an established diagnosis of CD.

[0006] Each of the above-mentioned serological methods utilizing ASCA as a marker has a number of drawbacks. For instance, each method requires an invasive procedure such as a finger prick or the like to obtain a serum sample. Further, each method utilizes only serum antibodies that are not required to cross the intestinal wall and the serum antibodies may not be accurate indicator for the proper diagnosis.

SUMMARY OF THE INVENTION

[0007] A method for testing a fecal sample, the method comprising: obtaining a fecal sample from a person; and determining the amount of anti-*Saccharomyces cerevisiae* antibodies in the sample.

[0008] A method for testing a fecal sample, the method comprising: obtaining a fecal sample from a person; and determining the presence of anti-*Saccharomyces cerevisiae* antibodies in the sample, wherein the presence of fecal anti-*Saccharomyces cerevisiae* antibodies is used to aid in the differentiation of Crohn's disease from other gastrointestinal illnesses such as, ulcerative colitis and irritable bowel syndrome (IBS).

[0009] An assay for determining the concentration of endogenous anti-*Saccharomyces cerevisiae* antibodies, the assay comprising: obtaining a human fecal sample; diluting the fecal sample; contacting the sample with extract of *Saccharomyces cerevisiae* to create a treated sample; contacting the treated sample with enzyme-linked polyclonal antibodies to create a readable sample; determining the optical density of the readable sample at 450 nm; generating a purified anti-*Saccharomyces cerevisiae* antibodies standard curve; and comparing the optical density of the readable sample to the standard curve to determine the concentration of endogenous anti-*Saccharomyces cerevisiae* antibodies in the fecal sample.

[0010] A diagnostic assay for diagnosing Crohn's disease by determining the level of endogenous anti-*Saccharomyces cerevisiae* antibodies, the assay comprising: obtaining a human fecal sample; diluting the sample; contacting the sample extract *Saccharomyces cerevisiae* to create a treated sample; contacting the treated sample with enzyme-linked polyclonal antibodies to create a readable sample; adding an enzyme substrate for color development and determining the optical density of the readable sample at 450 nm to determine whether the readable sample contains an elevated level of endogenous anti-*Saccharomyces cerevisiae* antibodies as compared to a reference value for healthy control subjects.

[0011] A kit for diagnosing Crohn's disease by testing a fecal sample from a person to be diagnosed, the kit comprising: one or more microassay plates, each the plate containing extract *Saccharomyces cerevisiae;* enzyme-linked polyclonal antibody to human anti-*Saccharomyces cerevisiae* antibodies; and enzyme substrate for color development.

[0012] Additional aspects of invention, together with the advantages and novel features appurtenant thereto, will be

set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned from the practice of the invention. The objects and advantages of the invention may be realized and attained by means, instrumentalities and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0013] **FIG. 1** is a graphical representation of a standard curve of purified anti-*Saccharomyces cerevisiae* antibodies.

DETAILED DESCRIPTION OF THE INVENTION

[0014] A method and apparatus for the differentiation of Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome, using the presence of fecal anti-*Saccharomyces cerevisiae* antibodies (ASCA) as a marker for Crohn's disease are provided. The apparatus includes an enzyme-linked immunoassay or other immunoassay that utilizes antibodies specific to human immunoglobulins for the measurement of total endogenous ASCA in a human fecal sample. The method and apparatus may be used by healthcare providers to distinguish Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome. The particular embodiments described herein are intended in all respects to be illustrative rather than restrictive. Alternative embodiments will become apparent to those skilled in the art to which the present embodiment of the invention pertains without departing from its scope.

[0015] The present embodiment of the invention provides immunoassays, including, but not limited to, enzyme-linked immunoassays (ELISAs), that utilize antibodies specific to human ASCA for the measurement of total endogenous ASCA in fecal samples, including feces and mucosal secretions. The assay of the present invention may include, but is not limited to, the following steps: 1) obtaining a fecal sample from a person to be diagnosed, 2) diluting the sample, 3) contacting the sample with extract of *Saccharomyces cerevisiae* to create a treated sample, and 4) contacting the treated sample with enzyme-linked polyclonal antibodies to create a readable sample. Further, the optical density of the readable sample at 450 nm may be determined. The optical density of the readable sample then may be compared to a standard curve generated using purified anti-*Saccharomyces cerevisiae* standard curve to determine the concentration of the endogenous anti-*Saccharomyces cerevisiae* antibodies in the fecal sample. The present embodiment of the invention further provides a kit usable in such immunoassays to aid physicians and other clinical personnel in diagnosing Crohn's disease.

[0016] It will be understood and appreciated by those of skill in the art that a immunoassay such as a lateral flow dipstick that utilizes both monoclonal and polyclonal antibodies to total endogenous ASCA also may be used to diagnose Crohn's disease. Such is contemplated to be within the scope hereof.

[0017] A limited number of cases of ulcerative colitis and IBS may test positive for ASCA. Therefore, it is possible that a diagnosis of Crohn's disease cannot be established solely on the basis of a positive result with the assay of the present embodiment of the invention. However, a positive result with the assay of the present embodiment of the invention will permit the substantial preclusion of a diagnosis of a other gastrointestinal illness, such as IBS or ulcerative colitis.

[0018] The immunoassay of the embodiment of the present embodiment of the invention may be used as an in vitro diagnostic aid for detecting elevated levels of ASCA as a detection marker for Crohn's disease. The immunoassay of the present embodiment of the invention provides a test that is easy to use, simple to read, and accurate for distinguishing Crohn's disease from IBS or ulcerative colitis.

[0019] The following examples are intended in all respects to be illustrative rather than restrictive.

EXAMPLE 1

[0020] In this example using an ELISA method, a fecal sample was obtained and serially diluted 20 fold. 100 μl of the diluted sample was added to a test well of a microassay plate coated with extract of *Saccharomyces cerevisiae* and purified mannan. The sample then was incubated at 37° C. to allow antibodies to *Saccharomyces cerevisiae* to bind to the extract of *Saccharomyces cerevisiae*. Following incubation, anti-human Ig polyclonal antibodies coupled to horseradish peroxidase enzyme (conjugate) were added to the test well and allowed to bind to captured ASCA. Unbound conjugate then was washed from the well and one component substrate (tetra-methyl-benzidene and hydrogen peroxide) was added for color development. Following the substrate incubation, 0.1M sulfuric acid was added to quench the reaction and the optical density (OD) was obtained spectrophotometrically at 450 nm using a single wavelength spectrophotometer.

[0021] The method described above was used in a clinical study to test a total of 86 IBD patients (55.8% males and 44.2% females). The approximate 1 to 1 ratio of males to females was similar to the ratio observed in IBD patient populations. The IBS patient group ranged in age from 19 to 78 years and was 9% male and 91% female. This ratio of males to females (1:10) reflects the increased incidence for IBS in females as seen in patient populations. The healthy control (HC) patient group ranged in age from 20 to 79 years old and was 33.3% male and 66.6% female. A summary of the patient population in the clinical study is shown in Table 1.

TABLE 1

Summary of patient population.

Summary of Clinical Histories (N = 120)	Total Subjects
Total number of IBD patients	86
No. Males	48
No. Females	38
Total number of patients with Crohn's Disease	49
No. Males	26
No. Females	23
Total number of patients with ulcerative colitis	37
No. Males	22
No. Females	15
Total number of patients with irritable bowel syndrome	22
No. Males	2
No. Females	20
Total number of healthy controls	12
No. Males	4
No. Females	8

[0022] In the clinical study, there were 37 ulcerative colitis patients, 49 Crohn's disease patients, 22 irritable bowel

patients, and 12 healthy controls. Fecal samples were collected from each enrolled subject and stored at −70° C. until tested. The optical densities for each sample were determined using the method described above. Results were reported as positive for fecal ASCA if an optical density of greater than or equal to 0.200 was observed. Results were reported as negative for fecal ASCA if an optical density of less than or equal to 0.199 was observed. Other clinical data, such as stool consistency, was also determined. Table 2, below, contains the clinical data and test results for healthy patients that participated in this clinical study. Table 3, below, contains the clinical data and test results for patients with ulcerative colitis patients that participated in this clinical study. Table 4, below, contains the clinical data and test results for patients with Crohn's disease that participated in this study. Table 5, below, contains the clinical data and test results for patients with irritable bowel syndrome that participated in this study.

TABLE 2

Clinical data and test results for healthy controls

Donor ID	Sex	Age Range	Previous of chronic GI illness	Stool Consistency	Optical Density	Fecal ASCA
HC1	F	40–49	NO	Solid	0.098	NEGATIVE
HC2	F	40–49	NO	Solid	0.089	NEGATIVE
HC3	M	70–79	NO	Solid	0.095	NEGATIVE
HC4	F	60–69	NO	Solid	0.085	NEGATIVE
HC5	M	70–79	NO	Solid	0.083	NEGATIVE
HC6	F	70–79	NO	Solid	0.076	NEGATIVE
HC7	F	50–59	NO	Solid	0.124	NEGATIVE
HC8	F	40–49	NO	Solid	0.095	NEGATIVE
HC9	F	50–49	NO	Solid	0.111	NEGATIVE
HC10	F	40–49	NO	Solid	0.111	NEGATIVE
HC11	M	50–60	NO	Solid	0.070	NEGATIVE
HC12	M	50–60	NO	Solid	0.054	NEGATIVE

[0023]

TABLE 3

Clinical data and test results for ulcerative colitis patients

Patient ID	Sex	Age	Disease	Stool Consistency	Disease Activity	Optical Density	Fecal ASCA
UC1	F	46	UC	Liquid	ACTIVE	0.184	NEGATIVE
UC2	M	39	UC	Liquid	ACTIVE	0.378	POSITIVE
UC3	F	30	UC	Semi-Solid	ACTIVE	0.193	NEGATIVE
UC4	F	31	UC	Semi-Solid	INACTIVE	0.319	POSITIVE
UC5	F	30	UC	Semi-Solid	ACTIVE	0.114	NEGATIVE
UC6	M	61	UC	Semi-Solid	INACTIVE	0.115	NEGATIVE
UC7	F	68	UC	Liquid	INACTIVE	0.091	NEGATIVE
UC8	F	45	UC	Liquid	ACTIVE	0.356	POSITIVE
UC9	F	21	UC	Semi-Solid	ACTIVE	0.082	NEGATIVE
UC10	F	27	UC	Liquid	ACTIVE	0.161	NEGATIVE
UC11	F	24	UC	Solid	INACTIVE	0.104	NEGATIVE
UC12	F	74	UC	Semi-Solid	INACTIVE	0.091	NEGATIVE
UC13	M	69	UC	Semi-Solid	ACTIVE	0.070	NEGATIVE
UC14	M	19	UC	Solid	INACTIVE	0.088	NEGATIVE
UC15	M	62	UC	Solid	INACTIVE	0.054	NEGATIVE
UC16	F	70	UC	Solid	INACTIVE	0.056	NEGATIVE
UC17	M	23	UC	Liquid	ACTIVE	0.573	POSITIVE
UC18	F	52	UC	Solid	ACTIVE	0.073	NEGATIVE
UC19	M	60	UC	Solid	INACTIVE	0.062	NEGATIVE
UC20	F	52	UC	Liquid	ACTIVE	0.089	NEGATIVE
UC21	M	31	UC	Solid	INACTIVE	0.064	NEGATIVE
UC22	M	44	UC	Semi-Solid	INACTIVE	0.143	NEGATIVE
UC23	F	30	UC	Liquid	ACTIVE	0.110	NEGATIVE
UC24	M	48	UC	Semi-Solid	INACTIVE	0.096	NEGATIVE
UC25	F	37	UC	Liquid	ACTIVE	0.282	POSITIVE
UC26	F	32	UC	Solid	ACTIVE	0.107	NEGATIVE
UC27	F	46	UC	Liquid	ACTIVE	0.199	NEGATIVE
UC28	M	49	UC	Semi-Solid	INACTIVE	0.161	NEGATIVE
UC29	F	42	UC	Solid	INACTIVE	0.080	NEGATIVE
UC30	F	41	UC	Semi-Solid	INACTIVE	0.087	NEGATIVE
UC31	F	43	UC	Solid	INACTIVE	0.070	NEGATIVE
UC32	M	30	UC	Solid	ACTIVE	0.103	NEGATIVE

TABLE 3-continued

Clinical data and test results for ulcerative colitis patients

Patient ID	Sex	Age	Disease	Stool Consistency	Disease Activity	Optical Density	Fecal ASCA
UC33	F	43	UC	Solid	INACTIVE	0.092	NEGATIVE
UC34	F	33	UC	Semi-Solid	INACTIVE	0.075	NEGATIVE
UC35	M	58	UC	Semi-Solid	ACTIVE	0.121	NEGATIVE
UC36	F	32	UC	Semi-Solid	ACTIVE	0.083	NEGATIVE

[0024]

TABLE 4

Clinical Data and test results for Crohn's disease patients.

Patient ID	Sex	Age	Disease	Stool Consistency	Disease Activity	Optical Density	FECAL ASCA
CD1	M	26	CD	Liquid	INACTIVE	1.900	POSITIVE
CD2	M	60	CD	Liquid	ACTIVE	2.849	POSITIVE
CD3	F	66	CD	Liquid	ACTIVE	0.282	POSITIVE
CD4	F	74	CD	Semi-Solid	INACTIVE	0.091	NEGATIVE
CD5	F	25	CD	Solid	INACTIVE	0.162	NEGATIVE
CD6	F	66	CD	Semi-Solid	INACTIVE	1.240	POSITIVE
CD7	M	39	CD	No Data	ACTIVE	1.150	POSITIVE
CD8	F	46	CD	Liquid	ACTIVE	0.160	NEGATIVE
CD9	F	46	CD	Semi-Solid	INACTIVE	0.074	NEGATIVE
CD10	F	56	CD	Solid	ACTIVE	0.406	POSITIVE
CD11	M	56	CD	Solid	ACTIVE	0.168	NEGATIVE
CD12	F	56	CD	Liquid	ACTIVE	0.732	POSITIVE
CD13	M	21	CD	Solid	ACTIVE	1.369	POSITIVE
CD14	M	52	CD	Semi-Solid	INACTIVE	0.136	NEGATIVE
CD15	M	63	CD	Solid	INACTIVE	0.134	NEGATIVE
CD16	M	34	CD	Solid	ACTIVE	0.076	NEGATIVE
CD17	F	45	CD	Semi-Solid	ACTIVE	0.160	NEGATIVE
CD18	M	67	CD	Semi-Solid	INACTIVE	0.059	NEGATIVE
CD19	F	46	CD	No Data	ACTIVE	0.839	POSITIVE
CD20	M	66	CD	Semi-Solid	INACTIVE	0.084	NEGATIVE
CD21	M	63	CD	Liquid	ACTIVE	0.780	POSITIVE
CD21	M	51	CD	Semi-Solid	ACTIVE	3.000	POSITIVE
CD22	M	34	CD	Semi-Solid	ACTIVE	1.447	POSITIVE
CD23	M	21	CD	Solid	ACTIVE	2.757	POSITIVE
CD24	F	78	CD	Semi-Solid	INACTIVE	0.092	NEGATIVE
CD25	F	27	CD	Semi-Solid	ACTIVE	0.979	POSITIVE
CD26	M	40	CD	Liquid	ACTIVE	0.373	POSITIVE
CD27	M	51	CD	Liquid	ACTIVE	0.978	POSITIVE
CD28	M	42	CD	Liquid	ACTIVE	0.089	NEGATIVE
CD29	F	31	CD	Solid	INACTIVE	0.075	NEGATIVE
CD30	F	59	CD	Solid	ACTIVE	0.088	NEGATIVE
CD31	M	35	CD	Semi-Solid	ACTIVE	1.487	POSITIVE
CD32	M	37	CD	Semi-Solid	INACTIVE	1.257	POSITIVE
CD33	F	77	CD	Solid	INACTIVE	0.093	NEGATIVE
CD34	F	40	CD	No Data	ACTIVE	1.762	POSITIVE
CD35	F	38	CD	Liquid	ACTIVE	0.098	NEGATIVE
CD36	M	51	CD	Liquid	ACTIVE	2.326	POSITIVE
CD37	M	38	CD	Semi-Solid	ACTIVE	0.091	NEGATIVE
CD38	M	37	CD	Liquid	ACTIVE	0.372	POSITIVE
CD39	M	59	CD	Semi-Solid	ACTIVE	0.224	POSITIVE
CD40	F	41	CD	Solid	ACTIVE	0.503	POSITIVE
CD41	M	41	CD	Solid	ACTIVE	0.117	NEGATIVE
CD42	M	48	CD	Liquid	ACTIVE	0.115	NEGATIVE
CD43	F	40	CD	Solid	INACTIVE	0.638	POSITIVE
CD44	F	72	CD	Solid	ACTIVE	0.087	NEGATIVE
CD45	F	32	CD	Liquid	INACTIVE	0.911	POSITIVE
CD46	F	24	CD	Liquid	ACTIVE	0.341	POSITIVE
CD47	M	23	CD	Solid	INACTIVE	0.088	NEGATIVE
CD48	F	34	CD	Liquid	ACTIVE	0.599	POSITIVE

[0025]

TABLE 5

Clinical data and test results for irritable bowel syndrome patients

Patient ID	Sex	Age	Disease	Stool consistency	Disease Activity	Optical Density	Fecal ASCA
IBS1	F	56	IBS	Semi-Solid	ACTIVE	0.132	NEGATIVE
IBS2	F	48	IBS	Solid	ACTIVE	0.103	NEGATIVE
IBS3	F	30	IBS	Solid	ACTIVE	0.073	NEGATIVE
IBS4	F	31	IBS	Solid	ACTIVE	0.074	NEGATIVE
IBS5	F	72	IBS	Semi-Solid	ACTIVE	0.079	NEGATIVE
IBS6	F	47	IBS	Solid	ACTIVE	0.088	NEGATIVE
IBS7	F	19	IBS	Semi-Solid	ACTIVE	0.105	NEGATIVE
IBS8	F	58	IBS	Semi-Solid	ACTIVE	0.107	NEGATIVE
IBS9	F	40	IBS	Solid	ACTIVE	0.065	NEGATIVE
IBS10	F	33	IBS	Semi-Solid	ACTIVE	0.065	NEGATIVE
IBS11	F	78	IBS	Solid	ACTIVE	0.071	NEGATIVE
IBS12	F	74	IBS	Semi-Solid	ACTIVE	0.063	NEGATIVE
IBS13	F	50	IBS	Semi-Solid	ACTIVE	0.052	NEGATIVE
IBS14	F	39	IBS	Solid	ACTIVE	0.079	NEGATIVE
IBS15	F	54	IBS	Solid	ACTIVE	0.080	NEGATIVE
IBS16	M	49	IBS	Semi-Solid	ACTIVE	0.238	POSITIVE
IBS17	M	53	IBS	Solid	ACTIVE	0.123	NEGATIVE
IBS18	F	34	IBS	Solid	ACTIVE	0.091	NEGATIVE
IBS19	F	43	IBS	Solid	ACTIVE	0.075	NEGATIVE
IBS20	F	35	IBS	Solid	ACTIVE	0.075	NEGATIVE
IBS21	F	51	IBS	Semi-Solid	ACTIVE	0.081	NEGATIVE
IBS22	F	40	IBS	Solid	ACTIVE	0.083	NEGATIVE

[0026] There were a total of 49 patients with Crohn's disease and 37 with ulcerative colitis. In the Crohn's disease group, a total of 55.1% patients were positive for fecal ASCA. In the ulcerative colitis group, 13.5% were positive. Of the 22 IBS patients, a single patient (4.6%) was positive for fecal ASCA. All 12 healthy controls were negative. A summary of positive results for fecal ASCA is shown in Table 6.

TABLE 6

Summary of positive results for Crohn's disease, ulcerative colitis, active IBS, and healthy controls

Total Assessments N = 120	Total	Fecal ASCA Positive	Fecal ASCA Negative
Total IBD (Crohn's disease and ulcerative colitis)	86	37.2% (32)	62.8% (54)
Total Crohn's Disease	49	55.1% (27)	44.9% (22)
Total Ulcerative Colitis	37	13.5% (5)	86.5% (32)
Total Active IBS	22	4.6% (1)	96.4% (21)
Total Healthy Controls	12	0	100.0% (12)

[0027] When distinguishing Crohn's disease from ulcerative colitis, fecal ASCA exhibited a sensitivity of 55.1% and specificity of 86.5%. The predictive positive and negative values were 84.4% and 59.3%, respectively, and the correlation was 68.6% as shown in Table 7.

TABLE 7

Statistical evaluation using the presence of fecal ASCA to distinguish Crohn's disease from ulcerative colitis

N = 86	Crohn's disease	Ulcerative colitis
Fecal ASCA positive	27	5
Fecal ASCA negative	22	32

TABLE 7-continued

Statistical evaluation using the presence of fecal ASCA to distinguish Crohn's disease from ulcerative colitis

Sensitivity	55.1%
Specificity	86.5%
Predictive Positive Value	84.4%
Predictive Negative Value	59.3%
Correlation	68.6%

[0028] When distinguishing Crohn's disease from ulcerative colitis, irritable bowel syndrome and healthy controls, fecal ASCA exhibited a sensitivity of 55% and a specificity of 91.6%. The predictive positive and negative values were 82% and 75%, respectively, and the correlation was 77% as shown below in Table 8.

TABLE 8

Statistical evaluation using fecal ASCA to distinguish Crohn's disease from ulcerative colitis, irritable bowel syndrome/healthy controls

N = 120	Crohn's disease	UC/IBS/Healthy Controls
Fecal ASCA positive	27	6
Fecal ASCA negative	22	65
Sensitivity		55.1%
Specificity		91.6%
Predictive Positive Value		81.8%
Predictive Negative Value		74.7%
Correlation		76.7%

[0029] The mean optical densities for each group were obtained and differences were tested for statistical significance using a two-tailed t-test giving a p-value result. Of the

33 patients that tested positive for fecal ASCA, there were 27 CD, 5 UC, and 1 IBS. Sensitivity, specificity and overall correlation were 55.1%, 91.5% and 76.7%, respectively. ASCA-positive CD showed a higher mean±SD A450 of 1.183±0.794 as compared to 0.382±0.113 for UC and the single A450 of 0.0.091±0.0.038 for IBS. There was a significant difference between CD and all other subject groups. A summary of the statistical analysis is listed in Table 9.

TABLE 9

Summary of the Mean and P values of Optical Densities for Fecal ASCA

Test Group	Mean Optical Density	Standard Deviation	Optical Density Range	P Value
CD	1.183	0.794	0.341–3.000	CD vs UC, IBS, HC P < 0.005
UC	0.382	0.113	0.382–0.113	CD vs UC P < 0.05
IBS	0.091	0.038	0.052–0.238	CD vs IBS P < 0.005
HC	0.091	0.019	0.054–0.124	CD vs HC P < 0.005

EXAMPLE 2

[0030] In this example, the sensitivity of the fecal ASCA test was determined using serial two fold dilutions of highly purified ASCA antibodies. For the analysis, standard curves were generated using the kit dilutent. The test was consistently positive at a concentration of 0.62 μg/mL as determined by a cutoff absorbency value of ≧0.200. Individual results are shown below in Table 10. The standard curves are shown in **FIG. 1**.

TABLE 10

Standard curves generated using purified ASCA antibodies

Purified ASCA Antibodies (μg/mL)	Test 1	Test 2	Mean	Std Dev
5.00	1.702	1.856	1.779	0.108
2.50	1.117	1.099	1.108	0.012
1.25	0.634	0.624	0.629	0.007
0.62	0.303	0.329	0.316	0.018
0.31	0.191	0.164	0.177	0.019
0.16	0.115	0.113	0.114	0.001
0.08	0.090	0.077	0.083	0.009
0.04	0.063	0.065	0.064	0.001

EXAMPLE 3

[0031] In this example, tests were conducted to determine what type of immunoglobulins (antibodies) were present in a fecal sample and in serum. The immunglobulin typing was done for human IgA, human IgA_{sec}, human IgD, human IgM, and human IgG. The immunoglobulin typing was done on a fecal sample from 6 Crohn's disease patients and 2 ulcerative colitis and on a serum control sample using pre-absorbed Ig-type specific conjugates. The serum control sample was obtained from a patient with a confirmed allergy to *Saccharomyces cerevisiae*.

[0032] Of the Crohn's disease patients, 5 patients exhibited a response to IgA and IgA_{sec}, 4 patients exhibited a response to IgM and a single patient exhibited a response to IgG. Of the 2 ulcerative colitis patients, a single patient reacted with the Ig conjugate. The serum control only exhibited a response to individual immunoglobulins IgM and IgG. A response to IgA and IgA_{sec} occurred the fecal samples but not in the control serum sample. A summary of results are shown in Table 11.

TABLE 11

A Summary of Immunoglobulin Typing of ASCA in a Human Fecal sample and a Serum Control

Patient Number	Disease	IgA Conjugate	IgA_{sec} Conjugate	IgD Conjugate	IgM Conjugate	IgG Conjugate	Ig Conjugate
1	Crohn's Disease	+	+	–	+	+	+
2	Crohn's Disease	+	+	–	+	–	+
3	Crohn's Disease	–	–	–	–	–	–
4	Crohn's Disease	+	+	NO DATA	+	–	+
5	Crohn's Disease	+	+	NO DATA	–	–	+
6	Crohn's Disease	+	+	NO DATA	+	–	+
7	Ulcerative Colitis	–	–	–	–	–	–
8	Ulcerative Colitis	–	–	–	–	–	+
Serum Control	Yeast Allergy	–	–	–	+	+	+

[0033] In summary, the present embodiment of the invention provides a method and apparatus for the differentiation of Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome, using the presence of fecal anti-*Saccharomyces cerevisiae* antibodies (ASCA) as a marker for Crohn's disease. The apparatus includes an enzyme-linked immunoassay or other immunoassay that utilizes antibodies specific to human immunoglobins for the measurement of total endogenous ASCA in a human fecal sample. The method and apparatus may be used by healthcare providers to distinguish Crohn's disease from other gastrointestinal illnesses, such as ulcerative colitis and irritable bowel syndrome. The present embodiment of the invention has been described in relation to particular embodiments which are intended in all respects to be illustrative rather than restrictive. Alternative embodiments will become apparent to those skilled in the art to which the present embodiment of the invention pertains without departing from its scope.

[0034] From the foregoing, it will be seen that this embodiment of the invention is one well adapted to attain all the ends and objects hereinabove set forth together with other advantages which are obvious and which are inherent to the method.

[0035] It will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims.

Having thus described the invention, what is claimed is:

1. A kit for diagnosing Crohn's disease by testing a fecal sample from a person to be diagnosed, the kit comprising:

one or more microassay plates, each the plate containing extract *Saccharomyces cerevisiae;*

enzyme-linked polyclonal antibody to human anti-*Saccharomyces cerevisiae* antibodies; and

enzyme substrate for color development.

2. The kit as recited in claim 1, further comprising purified human anti-*Saccharomyces cerevisiae* antibodies as a positive control.

3. The kit as recited in claim 1, further comprising a stop solution for quenching the reaction.

4. The kit as recited in claim 2, further comprising a stop solution for quenching the reaction.

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