US 20240053336A1

(19) **United States**

(12) **Patent Application Publication**

SHOME et al.

(10) **Pub. No.: US 2024/0053336 A1**

(43) **Pub. Date: Feb. 15, 2024**

(54) **ANTI-MICROBIAL ANTIBODY SIGNATURES OF INFLAMMATORY BOWEL DISEASE AND USES THEREOF**

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(21) Appl. No.: **18/448,685**

(22) Filed: **Aug. 11, 2023**

Related U.S. Application Data

(60) Provisional application No. 63/397,799, filed on Aug. 12, 2022.

Publication Classification

(51) **Int. Cl.**

G01N 33/569 (2006.01)

G01N 33/68 (2006.01)

(52) **U.S. Cl.**

CPC ***G01N 33/56911*** (2013.01); ***G01N 33/686*** (2013.01); *G01N 2800/065* (2013.01)

(57) **ABSTRACT**

The present invention relates anti-microbial antibody signatures of inflammatory bowel disease and their use in early and accurate diagnosis of disease including ulcerative colitis (UC) and Crohn's disease (CD).

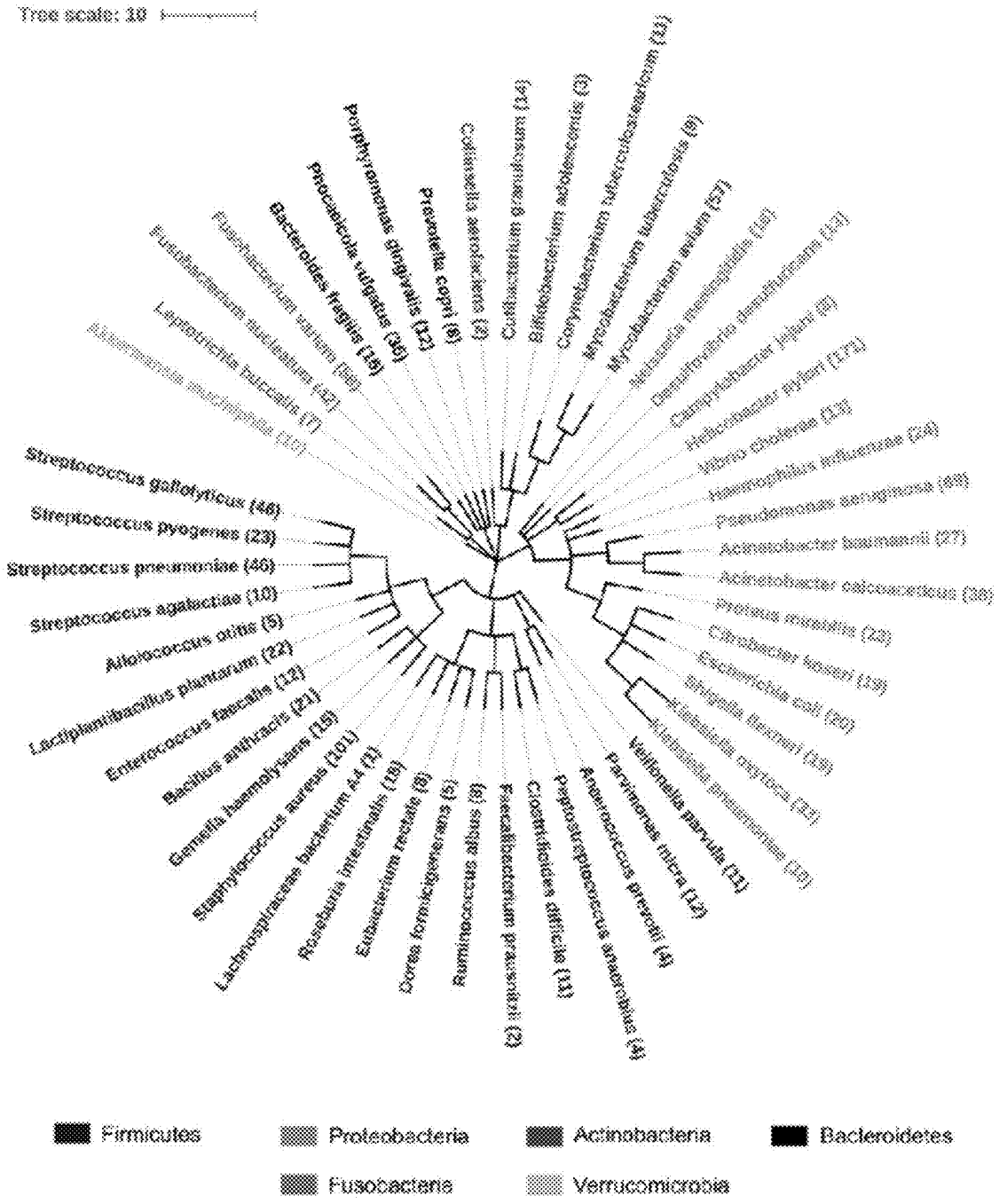
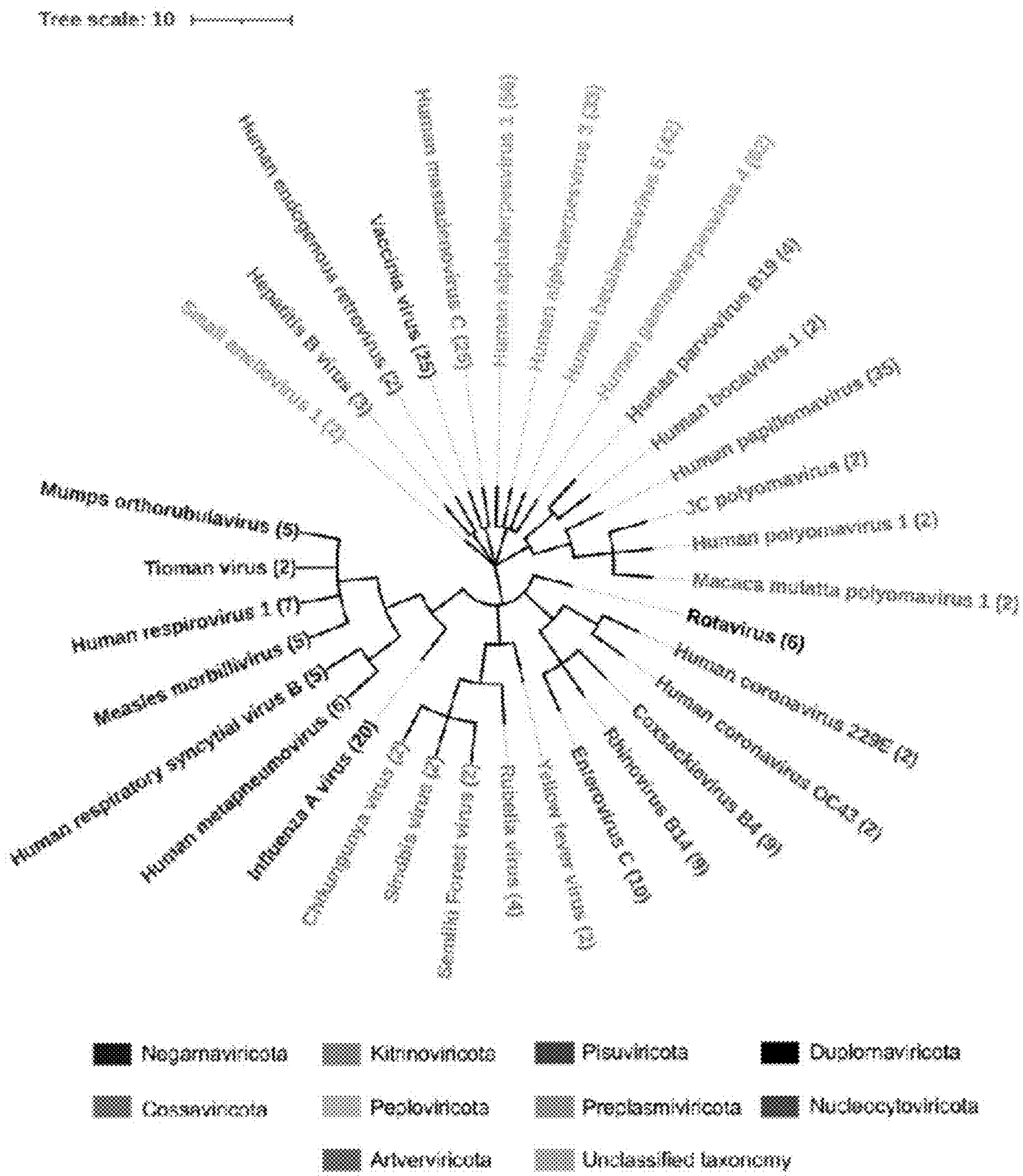

Tree scale: 10
Streptococcus gallolyticus (46)
Streptococcus pyogenes (23)
Streptococcus pneumoniae (46)
Streptococcus agalactiae (10)
Aerococcus urinae (5)
Lactiplantibacillus plantarum (22)
Enterococcus faecalis (12)
Bacillus anthracis (21)
Gemella haemolysans (15)
Staphylococcus aureus (101)
Lachnospiraceae bacterium A4 (1)
Roseburia intestinalis (18)
Eubacterium rectale (8)
Dorea formicigenerans (6)
Ruminococcus sp. (6)
Fusobacterium prausnitzii (2)
Clostridioides difficile (11)
Peptostreptococcus anaerobius (4)
Parvimonas micra (12)
Veillonella parvula (21)
Akkermansia muciniphila (18)
Verrucomicrobia
Fusobacteria
Proteobacteria
Actinobacteria
Bacteroidetes
Firmicutes

FIG. 1A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Hepatitis B virus (3)
Vaccinia virus (35)
Human parvovirus B19 (4)
Human bocavirus 1 (2)
Human papillomavirus (35)
JC polyomavirus (2)
Human polyomavirus 1 (2)
Macaca mulatta polyomavirus 1 (2)
Rotavirus (6)
Human coronavirus 229E (2)
Human coronavirus OC43 (2)
Coxsackievirus B4 (9)
Rhinovirus B14 (9)
Enterovirus C (10)
Yellow fever virus (3)
Rubella virus (4)
Influenza A virus (20)
Human metapneumovirus (6)
Human respiratory syncytial virus B (5)
Measles morbillivirus (5)
Human respirovirus 1 (7)
Tioman virus (2)
Mumps orthorubulavirus (5)
Negarnaviricota
Kitrinoviricota
Pisuviricota
Duplornaviricota
Cossaviricota
Peploviricota
Preplasmiviricota
Nucleocytoviricota
Artverviricota
Unclassified taxonomy

FIG. 1B

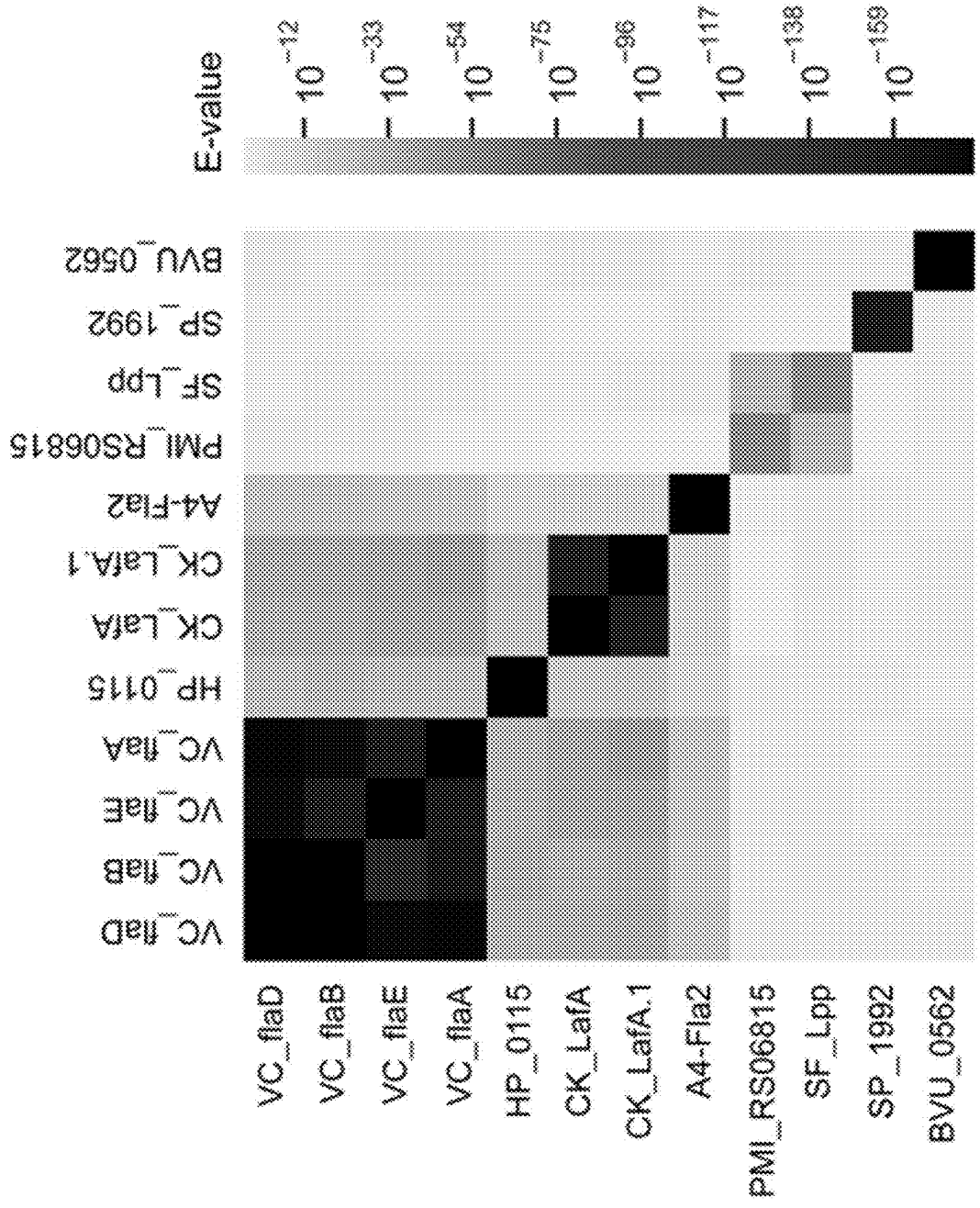
E-value
10^-12
10^-33
10^-54
10^-75
10^-96
10^-117
10^-138
10^-159
VC_flaD
VC_flaB
VC_flaE
VC_flaA
HP_0115
CK_LafA
CK_LafA.1
A4-Fla2
PMI_RS06815
SF_Lpp
SP_1992
BVU_0562

FIG. 2A

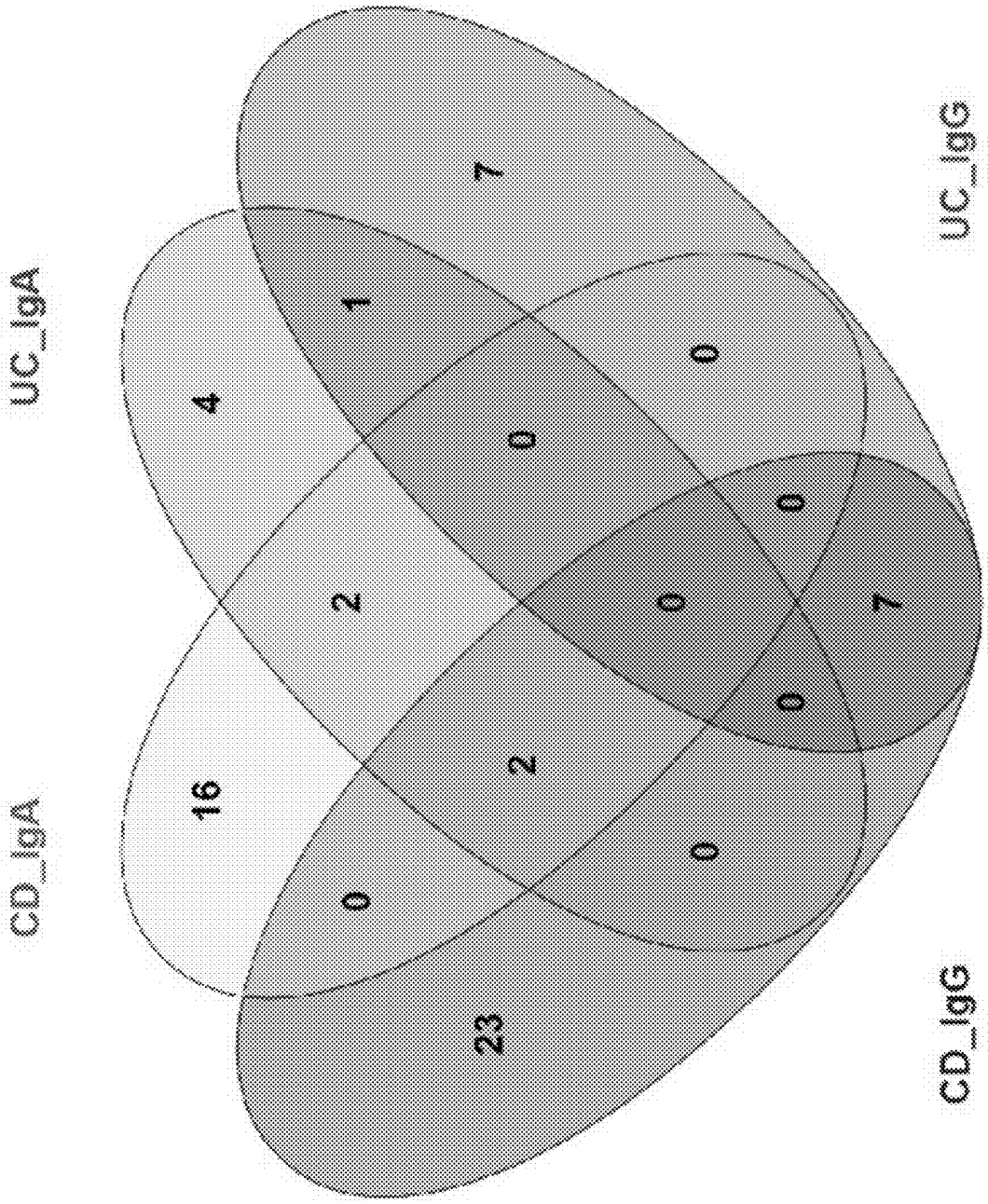

FIG. 2B

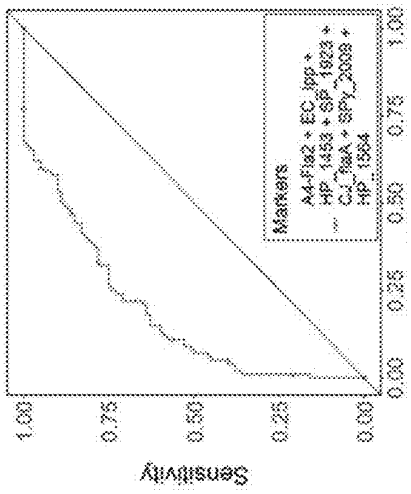

FIG. 3C

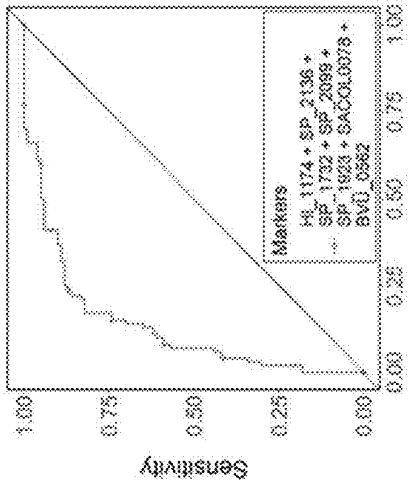

FIG. 3B

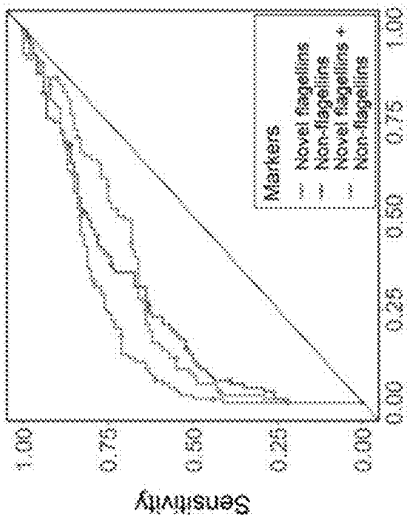

FIG. 3A

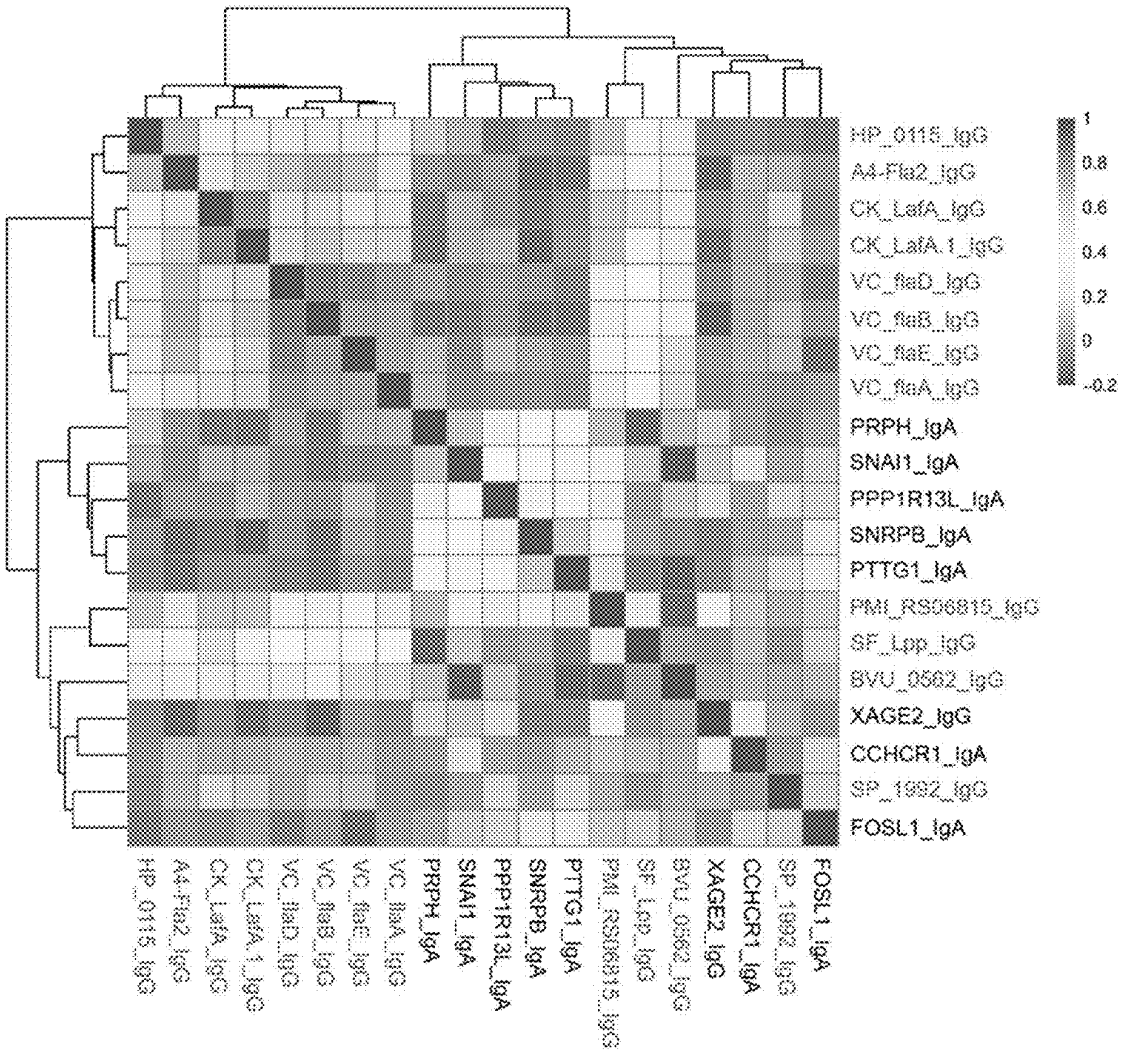
HP_0115_IgG
A4-Fla2_IgG
CK_LafA_IgG
CK_LafA.1_IgG
VC_flaD_IgG
VC_flaB_IgG
VC_flaE_IgG
VC_flaA_IgG
PRPH_IgA
SNAI1_IgA
PPP1R13L_IgA
SNRPB_IgA
PTTG1_IgA
PMI_RS06815_IgG
SF_Lpp_IgG
SVU_0562_IgG
XAGE2_IgG
CCHCR1_IgA
SP_1992_IgG
FOSL1_IgA
1
0.8
0.6
0.4
0.2
0
-0.2

FIG. 4

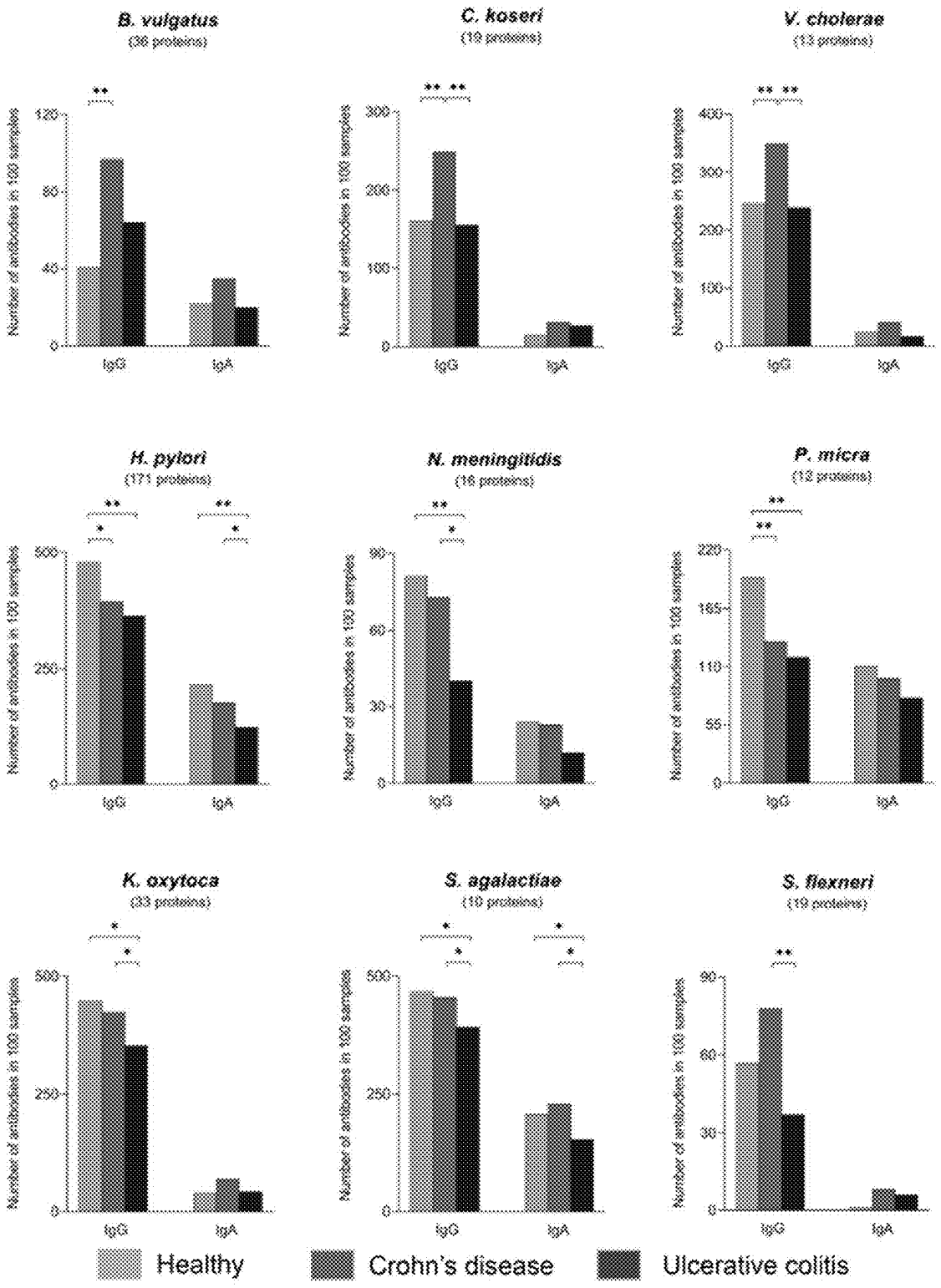
B. vulgatus
(36 proteins)
C. koseri
(19 proteins)
V. cholerae
(13 proteins)
H. pylori
(171 proteins)
N. meningitidis
(16 proteins)
P. micra
(12 proteins)
K. oxytoca
(33 proteins)
S. agalactiae
(19 proteins)
S. flexneri
(19 proteins)
Number of antibodies in 100 samples
IgG
IgA
Healthy
Crohn's disease
Ulcerative colitis

FIG. 5

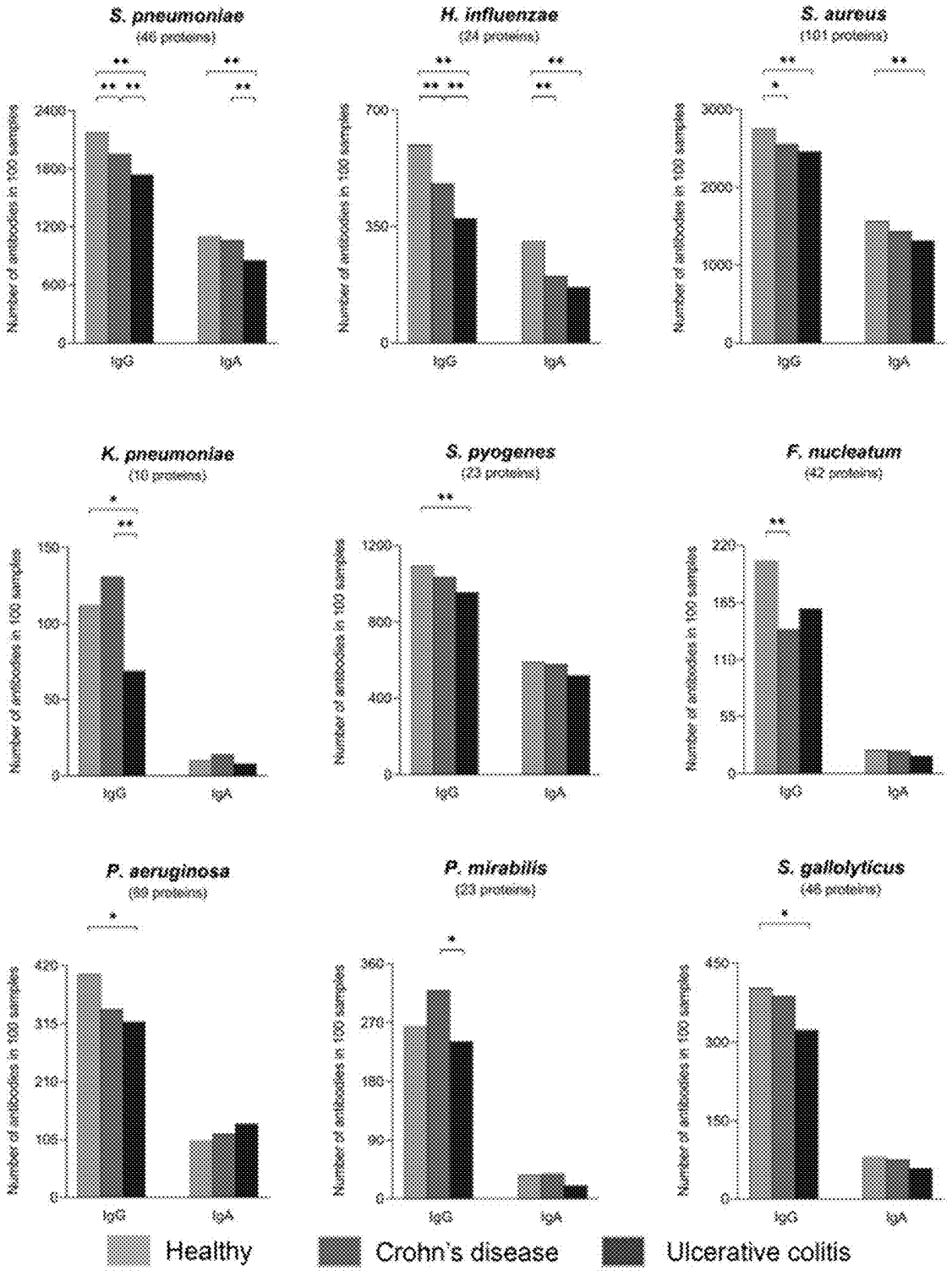

FIG. 5 (continued)

ANTI-MICROBIAL ANTIBODY SIGNATURES OF INFLAMMATORY BOWEL DISEASE AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/397,799, entitled "Anti-Microbial Antibody Signatures of Inflammatory Bowel Disease and Uses Thereof," which was filed Aug. 12, 2022, the entire disclosure of which is hereby incorporated herein by this reference.

FIELD OF THE INVENTION

[0002] The invention relates to anti-microbial antibody signatures of inflammatory bowel disease and their use in early and accurate diagnosis of disease.

BACKGROUND OF THE INVENTION

[0003] Inflammatory bowel disease (IBD) represents a group of intestinal disorders that causes chronic inflammation in the digestive tract. The two main clinical phenotypes are ulcerative colitis (UC) and Crohn's disease (CD). The public health burden of IBD is rising globally. Early and accurate diagnosis is key to reducing this burden. Gastroenterologists often use a combination of relatively invasive procedures, like ileocolonoscopy with biopsy for diagnosis, and to determine the disease extent and activity. There is a need for serological biomarkers that can reveal the disease state non-invasively.

SUMMARY OF THE INVENTION

[0004] In some aspects, the disclosure concerns an antibody panel for diagnosing a subject with inflammatory bowel disease (IBD). The antibody panel described herein differentiates IBD from irritable bowl syndrome in a subject exhibit gastrointestinal symptoms. The antibody panel comprising at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, BILF2, CK_flgG, A4-Fla2, BVRF2, and UL139. In some embodiments, the inflammatory bowel disease is Crohn's disease, the antibody panel comprises at least at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2. In certain embodiments, the antibody panel comprises HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA.

[0005] Some embodiments have an antibody panel comprising BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp. Certain antibody panels comprise HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2.

[0006] In some embodiments, the inflammatory bowel disease is ulcerative colitis (UC), and the antibody panel comprises at least at least one antigen selected from the group consisting of: CK_flgG, A4-Fla2, BVRF2, and UL139. In certain embodiments, the antibody panel comprises CK_flgG, A4-Fla2, BVRF2, and UL139. In some implementations, the antibody panel can be used to distinguish between Crohn's disease (CD) and UC.

[0007] Other aspects of the disclosure concern methods of diagnosing IBD in a subject with gastrointestinal distress, the method comprising: (i) providing a biofluid sample from the subject with gastrointestinal distress; (ii) contacting the biofluid sample with at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, BILF2, CK_flgG, A4-Fla2, BVRF2, and UL139; and (iii) determining if the biofluid sample comprises an antibody against the at least one antigen, wherein the presence of the antibody against the at least one antigen diagnoses the subject with gastrointestinal distress with an inflammatory bowel disease. In some embodiments, the biofluid sample is blood or serum. In certain embodiments, the biofluid sample is blood.

[0008] In some embodiments, the biofluid sample is contacted with HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2, wherein the presence of antibodies against HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2 diagnoses the subject with gastrointestinal distress with CD. In certain embodiments, the biofluid sample is in contact with HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA, wherein the presence of HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA diagnoses the subject with gastrointestinal distress with CD instead of UC. In other embodiments, the antibody panel comprises BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp, wherein the presence of BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp diagnoses the subject with gastrointestinal distress with CD instead of UC.

[0009] In some embodiments, the antibody panel comprises HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2, wherein the presence of HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2 diagnoses the subject with gastrointestinal distress with CD instead of UC.

[0010] In other embodiments, the biofluid sample is contacted with CK_flgG, A4-Fla2, BVRF2, and UL139, wherein the presence of antibodies against CK_flgG, A4-Fla2, BVRF2, and UL139 diagnoses the subject with gastrointestinal distress with UC.

[0011] Yet other aspects of the disclosure concern methods of distinguishing the cause of gastrointestinal distress in a subject, the method comprising: (i) providing a biofluid sample from a subject with gastrointestinal distress; (ii) contacting the biofluid sample with at least one antigen selected from the group consisting of: SACOL2509, SACOL2511, SACOL2476, SPy 2009, HI_null, HI_oapA, SP_1479, SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194; (iii) determining the biofluid sample comprises an antibody against the at least one antigen, wherein the presence of the antibody against the at least one antigen diagnoses the subject with gastrointestinal distress with an inflammatory bowel disease. In some embodiments, the biofluid sample is blood or serum. In certain embodiments, the biofluid sample is blood.

[0012] In some embodiments, the biofluid sample is contact with SACOL2509, SACOL2511, SACOL2476, SPy 2009, HI_null, HI_oapA, and SP_1479, the presence of antibodies against SACOL2509, SACOL2511, SACOL2476, SPy 2009, HI_null, HI_oapA, and SP_1479 diagnoses the subject with gastrointestinal distress with CD instead of UC. In other embodiments, the biofluid sample is contact with SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194, the presence of antibodies against SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194 diagnoses the subject with gastrointestinal distress with UC instead of CD.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0014] FIGS. **1**A and **1**B depict the phylogenetic tree of microbes studied with their corresponding number of proteins analyzed. FIG. **1**A shows 50 species of bacteria with 1173 proteins were segregated into 6 phyla. FIG. **1**B shows 33 species of viruses with 397 proteins were segregated into 10 phyla.

[0015] FIGS. **2**A and **2**B depict, in accordance with certain embodiments, the sequence homology of target antigens of validated antibodies and overlap of antibodies among Crohn's disease and ulcerative colitis. FIG. **2**A depicts a heatmap showing sequence homology among target antigens for antibodies with validated performance of ≥14% sensitivity at 96% specificity comparing Crohn's disease (CD) patients with healthy controls. FIG. **2**B shows that CD IgG, ulcerative colitis (UC) IgG, CD IgA and UC IgG represent the overlap of anti-microbial antibodies of IgG and IgA isotypes in CD and UC patients with ≥14% sensitivity at 96% specificity against healthy controls in the discovery set.

[0016] FIGS. **3**A-**3**C depict, in accordance with certain embodiments, the receiver operating characteristic curves to discriminate Crohn's disease, ulcerative colitis, and healthy controls. FIG. **3**A: Receiver operating characteristic (ROC) curve for Crohn's disease (CD) vs healthy controls. Area under the curve (AUC) values of novel anti-flagellin antibodies (HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, VC_flaA) and anti-non-flagellin antibodies (BVU_0562, SP_1992, PMI_RS06815, SF_Lpp) was 0.73 and 0.75, respectively. The AUC value obtained with a combination of novel anti-flagellin and anti-non-flagellin antibodies was 0.81; FIG. **3**B: ROC curve for ulcerative colitis (UC) vs healthy controls. The AUC value obtained with a combination of 7 markers was 0.87; FIG. **3**C: ROC curve for CD vs UC. The AUC value obtained with a combination of 7 markers was 0.82.

[0017] FIG. **4** depicts, in accordance with certain embodiments, a Spearman's rank correlation coefficient heatmap of anti-microbial antibodies and autoantibodies in Crohn's disease patients. The names of anti-microbial antibodies are colored in blue while autoantibodies are colored in black.

[0018] FIG. **5** depicts, in accordance with certain embodiments, comparisons of total number of antibodies in healthy controls, CD and UC at the bacterial species level. The number of proteins displayed on the microbial protein arrays for each species is shown in parenthesis. The statistical significance of the difference in seroprevalence between groups were calculated using Chi-squared test, *P<0.05, **P<0.01.

DESCRIPTION OF THE INVENTION

[0019] Detailed aspects and applications of the invention are described below in the drawings and detailed description of the invention. Unless specifically noted, it is intended that the words and phrases in the specification and the claims be given their plain, ordinary, and accustomed meaning to those of ordinary skill in the applicable arts.

[0020] In the following description, and for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the various aspects of the invention. It will be understood, however, by those skilled in the relevant arts, that the present invention may be practiced without these specific details. It should be noted that there are many different and alternative configurations, devices, and technologies to which the disclosed inventions may be applied. The full scope of the inventions is not limited to the examples that are described below.

[0021] The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a step" includes reference to one or more of such steps.

[0022] As used herein, the term "false positive" refers a test result which incorrectly indicates that a particular condition or attribute is present. Accordingly, the "false positive indicator", in some aspects, refers to a biomarker that indicates the corresponding positive test result incorrectly indicates the presence of a particular condition or attribute, for example, cancer or an autoimmune condition.

[0023] Inflammatory bowel disease (IBD) is caused by a combination of genetic predisposition, faulty immune responses, and environmental factors. The interaction of microbes with the gut mucosa in a genetically susceptible individual and the corresponding immune response play a pivotal role in the initiation and progression of IBD. After birth, a limited diversity microbial community develops into a complex community due to the influence of diet and environmental factors. During the second or third decade of life, a dysbiosis is observed in IBD patients which leads to an imbalance between commensal and potentially pathogenic microorganisms. The healthy gut microbiota predominately comprises Firmicutes and Bacteroidetes, and to a lesser extent, Actinobacteria and Proteobacteria. In IBD, dysbiosis is observed with reduced abundance of Firmicutes and either higher or similar abundance of Proteobacteria. Besides compositional changes, genetic alterations also contribute to gut dysbiosis that leads to disease initiation and progression. For example, NOD2 variants were found in 20%-40% of European and American Crohn's disease (CD) patients. NOD2 encodes an intracellular receptor for the bacterial peptidoglycan muramyl dipeptide, which helps maintain the balance of commensal bacterial flora.

[0024] Immune response to microbes results in the production of antibodies to microbial antigens. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are associated with CD patients, with sensitivities and specificities ranging between 55% to 65% and 80% to 95%, respectively. Perinuclear antineutrophil cytoplasmic antibodies (pANCA) are associated with ulcerative colitis (UC) patients, with sensitivities

and specificities ranging between 50% to 71% and 75% to 98%, respectively. Outer membrane protein of *Escherichia coli* (OmpC) and flagellin (CBir1) antibodies are prevalent in CD patients, with prevalence ranging between 24%-55% and 50%-56%. The number and response magnitude of anti-microbial antibodies have previously been shown to indicate the presence of IBD, its severity and its clinical course; however, the clinical utility of available antibodies in diagnosis and clinical management of IBD patients has been limited. The techniques used to discover the known anti-microbial antibodies associated with IBD are of low throughput and have only been applied to test on small number of candidate microorganisms or microbial antigens.

[0025] As shown in the examples below, an innovative protein microarray technology, namely Nucleic Acid Programmable Protein Array, was used to conduct a large-scale comparative profiling of anti-microbial antibodies in CD and UC patients and healthy controls. 1570 microbial proteins from the microbial protein collection (DNASU.org) from 50 bacteria and 33 viruses were selected based on preliminary studies and review of the literature, and they were displayed on microarrays and probed against 100 CD, 100 UC and 100 healthy control serum samples.

[0026] The microbiomic study performed in the Examples identified antibody signatures that can aid in the accurate diagnosis of IBD. Antibody responses to novel non-flagellin antigens with elevated prevalence in CD patients compared with healthy controls were identified. Many anti-microbial antibodies with lower prevalence in UC patients relative to healthy controls were also identified. The antibody panels disclosed herein could distinguish CD vs control, UC vs control and CD vs UC with AUCs of 0.81, 0.87, and 0.82, respectively.

[0027] This is an improvement from previously disclosed antibody panels. Lichtenstein et al. (Lichtenstein et al., "Combination of genetic and quantitative serological immune markers are associated with complicated Crohn's disease behavior." *Inflamm Bowel Dis,* 2011, 17: 2488-2496) reported an integrated serological (ASCA-IgA, ASCA-IgG, anti-OmpC, anti-CBir1, anti-I2, pANCA) and genetic (SNP8, SNP12, SNP13) marker panel with an AUC of 0.80 to distinguish CD vs control. A panel of serological markers (ASCA-IgA, ASCA-IgG, ANCA, pANCA, OmpC, and CBir1) built by Plevy et al. (Plevy et al., "Combined serological, genetic, and inflammatory markers differentiate non-IBD, Crohn's disease, and ulcerative colitis patients." *Inflamm Bowel Dis,* 2013, 19: 1139-1148) yields an AUC of 0.78 to distinguish CD vs UC. The antibody panels disclosed herein have comparable or better performance in IBD diagnosis or distinguishing CD from UC subtypes. A stronger anti-microbial antibody response with more aggressive disease in both CD and UC patients. Additionally, the anti-microbial antibodies and autoantibodies have different reactivity patterns in CD patients.

[0028] The results in the Examples also provide interesting insight into its pathogenesis. Antibody responses to proteins from *Bacteroides vulgatus, Proteus mirabilis, Shigella flexneri* and *Streptococcus pneumoniae* were elevated in CD patients. *B. vulgatus* has been reported to induce colitis in IBD-susceptible mice. *P. mirabilis* in gut can induce inflammation in cells and a colitis mouse model and has been associated with CD pathogenesis. Thus, the results in the Examples suggest that *B. vulgatus* and *P. mirabilis* may also play a role in human CD development. Reduced antibody responses was observed in UC patients to several genera of the Firmicutes phylum including *Parvimonas micra, Streptococcus pyogenes, S. aureus*, which were often reduced in abundance in UC patients' gut microbiota. For several genera belonging to Proteobacteria phylum, such as *Haemophilus influenzae, Helibacter pylori, Klebsiella oxytoca*, overall reduced antibody responses were observed; however, their abundance in the gut microbiota of UC patients has been reported to be either increased or remained the same compared with healthy controls.

[0029] Beyond exposure alone, antibody response requires functional immunological interaction between a microorganism and the host; however, anti-microbial antibodies by themselves do not prove causality. As such, source microorganisms whose antibodies show significant changes between IBD patients and healthy controls warrant future confirmation and functional assessment in causing IBD. A4-Fla2 flagellin included in the study showed IBD-specific prevalence with performance similar to that reported in the literature. Several antibodies to flagellins with higher prevalence in CD patients relative to healthy controls were also identified.

[0030] Previous studies mostly focused on antibodies with higher prevalence in IBD patients. The unbiased data-driven approach revealed the existence of many anti-microbial antibodies with higher prevalence in healthy controls relative to CD and especially UC patients. The reduction observed in CD and UC patients may be attributed to the dysbiosis and reduced diversity of gut microbiota in CD and UC patients. It is also possible that the reduction in anti-microbial antibodies in some CD and UC patients was in part because of immunosuppressive therapies they received. The greater number of antibodies having high prevalence in CD patients compared with UC patients indicates stronger anti-microbial humoral immunity in CD than in UC, which is consistent with reports in the literature that most known anti-microbial antibodies, such as ASCA, anti-OmpC, anti-Cbir1, and anti-I2, had higher prevalence in CD patients than in UC patients. This agreement, together with comparable performance of anti-flagellin antibodies in this study and that reported in the literature, suggests that the results reflect the microbial association of IBD etiopathology. However, the use of samples from patients with established disease and the lack of information on immunosuppressive therapies of these patients limited the interpretation of the results.

[0031] The association between anti-microbial antibody prevalence and various disease classifications was studied based on Montreal classification and surgery history, and more antibodies were found with significantly higher prevalence in patients with more aggressive disease behaviors relative to those with milder disease behavior. More antibodies with significantly higher prevalence in colonic CD patients relative to those in ileal CD patients were also found this the analysis. These results were consistent with previous reports that increasing diversity and magnitude of anti-microbial immune response was correlated with increased frequency of penetrating and/or structuring disease behavior. It is known that the colon has a microbial density of 1011-1012 anaerobic bacteria/gram while the ileum is colonized by 107-108 anaerobic bacteria/gram. Kleessen et al. (Kleessen et al., Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. *Scand J Gastroenterol,* 2002, 37: 1034-1041) found higher

percentage of bacterial invasion of mucosa in colon compared to ileum. CD patients requiring surgery usually had more severe disease compared with those who did not need surgery. Stronger anti-microbial immune response in patients with severe CD or UC suggests a higher abundance of the source microorganisms for the target antigens of the differential antibodies and/or a stronger more conducive immune microenvironment at the disease site in severe disease.

[0032] Both autoantibodies and anti-microbial antibodies associated with IBD have been reported. One popular hypothesis for the autoantibody elicitation is molecular mimicry, where anti-microbial antibodies cross react with human proteins. However, minimal correlation was found between the anti-microbial antibodies and the autoantibody profiles in the same set of CD samples. The lack of correlation suggests that IBD-specific autoantibodies and anti-microbial antibodies are elicited independently through different underlying mechanisms, and cross-reactivity may play less of a role in eliciting CD-associated autoantibodies. The breakdown of immune tolerance to human proteins might have occurred due to the damaged gut epithelial cells and the faulty immunological microenvironment partly caused by microbial infections. In addition, the elicitation of autoantibodies may be associated with the infections of multiple microorganisms, and the correlation with individual anti-microbial antibodies may not be great.

[0033] Strengths of the study include the broadest analysis to date of IgG and IgA antibodies against individual antigens from many different microorganisms in both CD and UC patients and the use of a two-stage approach with discovery and independent validation of antibody markers. There are some limitations to the study. Except for a few microbes, the number of proteins studied for each species is small, which might limit the interpretation of antibody response in IBD at the species level. Furthermore, many samples used in studies were collected from patients with established disease.

[0034] Accordingly, disclosed herein are anti-microbial antibody signatures of IBD, in particular CD and UC. These anti-microbial antibody signatures aid the early detection and diagnosis of IBD and can distinguish between IBD and irritable bowls syndrome (IBS), which is not an inflammatory condition. The anti-microbial antibody signatures of CD are antibodies against the antigens of Bacteroidetes *vulgatus* (BVU_0562) and *Streptococcus pneumoniae* (SP_1992). The levels of these antibodies were elevated in CD patients relative to healthy controls. The anti-microbial antibody signatures of UC are antibodies against the antigen of *Streptococcus pyogenes* (SPy 2009). The levels of these antibodies were found to be elevated in healthy controls relative to UC patients.

[0035] Also disclosed are antibody panels developed using these anti-microbial antibody signatures of IBD. Patients with severe disease had higher prevalence of anti-microbial antibodies. There was minimal correlation among the occurrence of autoantibodies and anti-microbial antibodies in CD patients. Subgroup analysis revealed that penetrating CD behavior, colonic CD location, CD patients with a history of surgery, and extensive UC exhibited highest antibody prevalence among all patients.

[0036] In some embodiments, the antibody panel for diagnosing a subject with IBD comprises at least one antigen, at least two antigens, at least three antigens, at least four antigens, or at least five antigens selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, BILF2, CK_flgG, A4-Fla2, BVRF2, and UL139. Some embodiments have an antibody panel comprising BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp. Certain antibody panels comprise HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2.

[0037] Where the IBD is Crohn's disease, the antibody panel comprises at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2. In certain embodiments, the antibody panel for diagnosing a subject with CD comprises HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA.

[0038] Where the IBD is ulcerative colitis, the antibody panel comprises at least one antigen selected from the group consisting of: CK_flgG, A4-Fla2, BVRF2, and UL139. In certain embodiments, the antibody panel comprises CK_flgG, A4-Fla2, BVRF2, and UL139.

[0039] In some embodiments, the antibody panel may be associated with an array or an enzyme-linked immunosorbent assay (ELISA). In some embodiments, the binding of the antibody to the antigen is detected using a secondary antibody, capable of binding to the antibody of interest, linked to a colorimetric detection system such as fluorescent dyes or enzyme substrate that generate a chemiluminescent signal. In some embodiments, the antigen is immobilized on the surface of a substrate using a coupling agent. The biofluid sample is then contacted with the antigen containing substrate. After contact any unattached material may be washed away from the panel.

[0040] Methods of diagnosing IBD in a subject with gastrointestinal distress are also disclosed. In one aspect, the method comprises: (i) providing a biofluid sample from the subject with gastrointestinal distress; (ii) contacting the biofluid sample with at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, BILF2, CK_flgG, A4-Fla2, BVRF2, and UL139; and (iii) determining if the biofluid sample comprises an antibody against the at least one antigen, wherein the presence of the antibody against the at least one antigen diagnoses the subject with gastrointestinal distress with IBD. In some embodiments, the biofluid sample is blood or serum. In certain embodiments, the biofluid sample is blood.

[0041] In some embodiments, the biofluid sample is contacted with HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2, wherein the presence of antibodies against HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2 diagnoses the subject with gastrointestinal distress with CD. In certain embodiments, the biofluid sample is in contact with HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA, wherein the presence of HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA diagnoses the subject with gastrointestinal distress with CD instead of UC. In other embodiments, the antibody panel comprises BVU_0562, SP_1992, PMI_RS06815, and

SF_Lpp, wherein the presence of BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp diagnoses the subject with gastrointestinal distress with CD instead of UC.

[0042] In some embodiments, the antibody panel comprises HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2, wherein the presence of HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, S P 1992, and BILF2 diagnoses the subject with gastrointestinal distress with CD instead of UC.

[0043] In other embodiments, the biofluid sample is contacted with CK_flgG, A4-Fla2, BVRF2, and UL139, wherein the presence of antibodies against CK_flgG, A4-Fla2, BVRF2, and UL139 diagnoses the subject with gastrointestinal distress with UC.

[0044] Yet other aspects of the disclosure concern methods of distinguishing the cause of gastrointestinal distress in a subject. The method comprises: (i) providing a biofluid sample from a subject with gastrointestinal distress; (ii) contacting the biofluid sample with at least one antigen selected from the group consisting of: SACOL2509, SACOL2511, SACOL2476, SPy 2009, HI_null, HI_oapA, SP_1479, SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194; (iii) determining the biofluid sample comprises an antibody against the at least one antigen, wherein the presence of the antibody against the at least one antigen diagnoses the subject with gastrointestinal distress with an inflammatory bowel disease. In some embodiments, the biofluid sample is blood or serum. In certain embodiments, the biofluid sample is blood.

[0045] In some embodiments, the biofluid sample is contact with SACOL2509, SACOL2511, SACOL2476, SPy 2009, HI_null, HI_oapA, and SP_1479, the presence of antibodies against SACOL2509, SACOL2511, SACOL2476, SPy 2009, HI_null, HI_oapA, and SP_1479 diagnoses the subject with gastrointestinal distress with CD instead of UC. In other embodiments, the biofluid sample is contact with SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194, the presence of antibodies against SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194 diagnoses the subject with gastrointestinal distress with UC instead of CD.

EXAMPLES

[0046] The present invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application, as well as the Figures, are incorporated herein by reference in their entirety for all purposes.

I. Anti-Microbial Antibody Profiling in IBD on Microbial Protein Arrays

[0047] IgG and IgA anti-microbial antibody profiles of 100 CD and 100 UC patients and 100 age-gender matched healthy controls (Table 1) against 1570 microbial antigens including 1173 antigens from 50 different bacteria and 397 antigens from 33 different viruses using the protein microarray platform (FIG. **1**, Table 2). This study provided a representative overview of the anti-microbial antibody response in IBD patients (FIG. **5**). The numbers of IgG antibodies against bacterial proteins from Bacteroidetes *vulgatus* (*B. vulgatus*) and *Citrobacter koseri* (*C. koseri*) were significantly higher in CD patients compared with those in healthy controls (Chi-square test, $P<0.01$) (FIG. **5**). On the contrary, the numbers of IgG antibodies against proteins from several bacteria, such as *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus* influenza (*H. influenzae*), *Staphylococcus aureus* (*S. aureus*), *Helicobacter pylori* (*H. pylori*) and *Parvimonas micra* (*P. micra*) were significantly lower in CD and UC patients compared with those in healthy controls (Chi-square test, $P<0.05$) (FIG. **5**). Overall, fewer IgA anti-microbial antibodies were found than IgG antibodies. The numbers of IgA antibodies against *S. pneumoniae, H. influenzae, S. aureus*, and *H. pylori* were significantly lower in UC patients compared with those in healthy controls (Chi-square test, $P<0.01$). On the other hand, anti-viral IgG and IgA antibodies showed heterogenous prevalence with no clear trend of differences among CD, UC, healthy controls. Therefore, the analysis focused on anti-bacterial antibodies.

TABLE 1

Clinical information of the samples

	Discovery set			Validation set		
	CD	UC	HC	CD	UC	HC
N	50	50	50	50	50	50
Gender (female, male)	29, 21	29, 21	29, 21	28, 22	28, 22	28, 22
Age (median ± SD)	41 ± 17.66	44 ± 17.25	42 ± 18.47	39.5 ± 17.49	44.5 ± 17.23	39.5 ± 16.02
Disease behavior (B1/B2/B3)	9/10/6			16/8/2		
Disease location (L1/L2/L3/L4)	12/6/7/0			12/7/7/0		
Disease extent (E1/E2/E3)		0/32/18			0/34/16	
Surgery (Yes, No)	24, 25	8, 42		22, 27	7, 42	

Fischer's exact test P value is equal to 1 for the gender difference among CD, UC and HC in both discovery and validation set. Kruskal-Wallis test P value for the age difference among CD, UC and HC in discovery and validation set were 0.3159 and 0.1737 respectively. CD: Crohn's disease; UC: Ulcerative colitis; HC: Healthy control.

TABLE 2

Bacteria and viruses studied on the microbial protein arrays.

	Strain	Phylum	Number of proteins
Bacteria	*Helicobacter pylori*	Proteobacteria	171
	Staphylococcus aureus	Firmicutes	101
	Pseudomonas aeruginosa	Proteobacteria	69
	Fusobacterium varium	Fusobacteria	58
	Mycobacterium avium	Actinobacteria	57
	Streptococcus gallolyticus	Firmicutes	46
	Streptococcus pneumoniae	Firmicutes	46
	Fusobacterium nucleatum	Fusobacteria	42
	Acinetobacter calcoaceticus	Proteobacteria	38
	Phocaeicola vulgatus	Bacteroidetes	36
	Klebsiella oxytoca	Proteobacteria	33
	Acinetobacter baumannii	Proteobacteria	27
	Haemophilus influenzae	Proteobacteria	24
	Proteus mirabilis	Proteobacteria	23
	Streptococcus pyogenes	Firmicutes	23
	Lactiplantibacillus plantarum	Firmicutes	22
	Bacillus anthracis	Firmicutes	21
	Escherichia coli	Proteobacteria	20
	Shigella flexneri	Proteobacteria	19
	Citrobacter koseri	Proteobacteria	19
	Roseburia intestinalis	Firmicutes	18
	Bacteroides fragilis	Bacteroidetes	16
	Neisseria meningitidis	Proteobacteria	16
	Gemella haemolysans	Firmicutes	15
	Cutibacterium granulosum	Actinobacteria	14
	Desulfovibrio desulfuricans	Proteobacteria	13
	Vibrio cholerae	Proteobacteria	13
	Parvimonas micra	Firmicutes	12
	Enterococcus faecalis	Firmicutes	12
	Porphyromonas gingivalis	Bacteroidetes	12
	Veillonella parvula	Firmicutes	11
	Clostridioides difficile	Firmicutes	11
	Corynebacterium tuberculostearicum	Actinobacteria	11
	Streptococcus agalactiae	Firmicutes	10
	Klebsiella pneumoniae	Proteobacteria	10
	Akkermansia muciniphila	Verrucomicrobia	10
	Mycobacterium tuberculosis	Actinobacteria	9
	Campylobacter jejuni	Proteobacteria	8
	Eubacterium rectale	Firmicutes	8
	Ruminococcus albus	Firmicutes	8
	Prevotella copri	Bacteroidetes	8
	Leptotrichia buccalis	Fusobacteria	7
	Dorea formicigenerans	Firmicutes	5
	Alloiococcus otitis	Firmicutes	5
	Anaerococcus prevotii	Firmicutes	4
	Peptostreptococcus anaerobius	Firmicutes	4
	Bifidobacterium adolescentis	Actinobacteria	3
	Faecalibacterium prausnitzii	Firmicutes	2
	Collinsella aerofaciens	Actinobacteria	2
	Lachnospiraceae bacterium A4	Firmicutes	1

II. Antibodies Distinguishing CD from Healthy Controls

[0048] The prevalence for individual anti-microbial antibodies between CD patients and healthy controls were compared. Samples were randomly and evenly split into discovery and the validation sets (Table 1). For antibodies with elevated prevalence in CD patients, 13 IgG antibodies passed the criteria (sensitivity ≥14% at 96% specificity) in both discovery and validation sets (Table 3). Anti-A4-Fla2 IgG, a well-studied anti-bacterial flagellin antibody in CD, had the best performance with 47% sensitivity at 96% specificity in the full sample set (Table 3). Beside the flagellins, antibodies to four novel target antigens from *B. vulgatus* (BVU_0562), *P. mirabilis* (PMI_RS06815), *S. flexneri* (SF_Lpp) and *S. pneumoniae* (SP_1992) (Table 3) were found with no significant sequence homology to flagellins (FIG. **2**A).

[0049] Surprisingly, 12 validated IgG antibodies showed elevated prevalence in healthy controls relative to CD patients (Table 4). Among these 12 antibodies, anti-bacterial antibodies performed better in differentiating CD patients from healthy controls than anti-viral antibodies (Table 4). Antibody against SPy_2009, an anchoring protein located in the cell wall of *Streptococcus pyogenes* (*S. pyogenes*), had the highest sensitivity of 24% at 96% specificity in healthy controls relative to CD patients. Seven validated IgA antibodies showed higher prevalence in healthy controls relative to CD patients (Table 6).

TABLE 3

Sensitivities of validated IgG antibodies comparing Crohn's disease and ulcerative colitis with healthy controls in the discovery, validation, and the entire set at 96% specificity.

		Antigen	Protein name	Organism	Discovery	Validation	Entire
Crohn's disease	Bacteria	HP_0115	Flagellin B	*H. pylori*	28	48	38
		BVU_0562	Uncharacterized protein	*B. vulgatus*	26	22	25
		CK_LafA	Lateral flagellin	*C. koseri*	20	22	21
		CK_LafA.1	Lateral flagellin	*C. koseri*	16	26	24
		A4-Fla2	Flagellin	*L. bacterium* A4	40	54	47
		PMI_RS06815	Hypothetical protein	*P. mirabilis*	14	16	15
		VC_flaD	Flagellin	*V. cholerae*	24	18	19
		VC_flaB	Flagellin	*V. cholerae*	28	22	24
		VC_flaE	Flagellin	*V. cholerae*	26	28	23
		VC_flaA	Flagellin	*V. cholerae*	20	22	21
		SF_Lpp	Outer membrane lipoprotein	*S. flexneri*	14	18	14
		SP_1992	Cell wall surface anchor	*S. pneumoniae*	20	16	18
	Virus	BILF2	Glycoprotein BILF2	Human herpesvirus 4	18	18	18
Ulcerative colitis	Bacteria	CK_flgG	Flagellar basal-body rod protein	*C. koseri*	14	16	15
		A4-Fla2	Flagellin	*L. bacterium* A4	22	16	18
	Virus	BVRF2	Capsid scaffolding protein	Human herpesvirus 4	14	16	14
		UL139	Membrane glycoprotein UL139	Human herpesvirus 5	14	20	17

TABLE 4

Sensitivities in discovery, validation, and the entire set at 96% specificity for validated IgG antibodies with higher prevalence in healthy controls relative to CD patients.

	Antigen	Protein name	Organism	Discovery	Validation	Entire
Bacteria	HP_1564	ABC transporter substrate-binding protein	*H. pylori*	14	16	16
	SACOL0985	MAP domain-containing protein	*S. aureus*	16	14	14
	AUO97_RS08350	hypothetical protein	*A. baumannii*	14	14	13
	SACOL1164	complement convertase inhibitor Ecb	*S. aureus*	14	16	14
	SPy_2009	LPXTG-anchored fibronectin-binding protein FbpA	*S. pyogenes*	38	22	24
	HI_0162	hypothetical protein	*H. influenzae*	18	16	16
	PA_exoT	T3SS effector bifunctional cytotoxin exoenzyme T	*P. aeruginosa*	14	14	13
	AB185_RS23245	type VI secretion system effector Hcp	*K. oxytoca*	18	18	19
	AB185_RS19385	Hcp family type VI secretion system effector	*K. oxytoca*	20	16	19
Virus	null	capsid protein, partial	Rhinovirus B14	14	22	17
	null	nucleocapsid protein	Human coronavirus	32	16	18

III. Antibodies Distinguishing UC from Healthy Controls

[0050] For anti-microbial antibodies with elevated prevalence in UC patients relative to healthy controls, 4 IgG antibodies passed the criteria in both discovery and validation sets (Table 3). Antibodies to A4-Fla2 IgG and a flagellin from *C. koseri* had a sensitivity of 18% and 15% respectively. For IgG antibodies with higher prevalence in healthy controls relative to UC patients, 32 antibodies got validated (Table 5). Source microorganisms for the target antigens of these 32 antibodies were enriched for *S. pneumoniae, S. aureus*, and *H. influenzae* (2-sample proportion test, $P<0.05$). 2.7% of the proteins on the microbial protein microarray were from *S. pneumoniae* while 18.7% of antigens for validated antibodies were from *S. pneumoniae,* 6.1% of the proteins on the microarrays were from *S. aureus* while 18.7% of antigens for validated antibodies were from *S. aureus*, and 1.4% of the proteins on the microarrays were from *H. influenzae* while 12.5% of antigens for validated antibodies were from *H. influenzae*. Nine validated IgA antibodies showed higher prevalence in healthy controls relative to UC patients (Table 6).

[0051] Fewer anti-viral antibodies than anti-bacterial antibodies were validated comparing CD or UC patients with healthy controls (Table 3, Table 4 and Table 5). Anti-viral antibodies to Rhinovirus B14, Enterovirus C, Influenza A virus, Human metapneumovirus had higher prevalence in healthy controls compared with CD and UC patients (Tables 4 and 5).

TABLE 5

Sensitivities in discovery, validation, and the entire set at 96% specificity for validated IgG antibodies with higher prevalence in healthy controls relative to UC patients.

	Antigen	Protein name	Organism	Discovery	Validation	Entire
Bacteria	SACOL0858	extracellular matrix protein-binding adhesin Emp	*S. aureus*	20	14	11
	SACOL1140	LPXTG-anchored heme-scavenging protein IsdA	*S. aureus*	16	20	16
	SACOL0078	phosphatidylinositol-specific phospholipase C	*S. aureus*	14	20	16
	SACOL2197	MAP domain-containing protein	*S. aureus*	16	16	14
	PMI_RS02875	peptidoglycan-associated lipoprotein Pal	*P. mirabilis*	14	16	15
	SPy_2191	lytic transglycosylase domain-containing protein	*S. pyogenes*	16	22	20
	SPy_cfa	CAMP factor pore-forming toxin Cfa	*S. pyogenes*	18	20	16
	HI_0256	outer membrane protein assembly factor BamC	*H. influenzae*	18	18	15
	HI_null	cell envelope integrity protein TolA	*H. influenzae*	14	18	15
	HI_0162	hypothetical protein	*H. influenzae*	16	20	18
	HI_1174	outer membrane beta-barrel protein	*H. influenzae*	16	16	16
	PM_null	InlB B-repeat-containing protein	*P. micra*	14	20	14
	SP_1732	Stk1 family PASTA domain-containing Ser/Thr kinase	*S. pneumoniae*	24	32	24
	SP_2136	choline-binding protein PcpA	*S. pneumoniae*	22	32	23
	SP_0785	membrane-fusion protein	*S. pneumoniae*	16	32	19
	SP_0366	oligopeptide ABC transporter, oligopeptide-binding protein AliA	*S. pneumoniae*	14	22	17
	SP_1923	pneumolysin	*S. pneumoniae*	32	34	33
	SP_0377	choline-binding protein CbpC	*S. pneumoniae*	20	28	22
	SACOL1869	serine protease SplA	*S. aureus*	16	18	16
	AB185_RS27465	peptidoglycan-associated lipoprotein Pal	*K. oxytoca*	16	14	15
	SACOL2291	CHAP domain-containing protein	*S. aureus*	24	18	15
Virus	PVgp1	capsid protein VP1	Enterovirus C	14	20	18
	null	capsid protein, partial	Rhinovirus B14	14	24	18
	null	polyprotein	Rhinovirus B14	34	36	28
	null	polyprotein	Coxsackievirus B4	22	24	18
	N	Nucleoprotein	Human metapneumovirus	14	28	18
	F	fusion glycoprotein	Human metapneumovirus	16	24	20
	null	fusion protein	Human respiratory syncytial virus B	14	18	16
	PA	Polymerase acidic protein	Influenza A virus	26	20	23
	PVgp1	genome polyprotein	Enterovirus C	20	32	28
	NP	nucleoprotein	Influenza A virus	14	20	17
	M1	matrix protein 1	Influenza A virus	22	32	25

TABLE 6

Sensitivities in discovery, validation, and the entire set at 96% specificity for validated IgA antibodies with higher prevalence in healthy controls relative to CD patients (Top) and UC patients (Bottom).

		Antigen	Protein name	Organism	Discovery	Validation	Entire
Crohn's disease	Bacteria	SACOL2509	fibronectin-binding protein FnbB	*S. aureus*	28	18	17
		SACOL2511	fibronectin-binding protein FnbA	*S. aureus*	18	22	19
		SACOL2476	staphylopine-dependent metal ABC transporter substrate-binding protein CntA	*S. aureus*	18	14	12
		SPy_2009	LPXTG-anchored fibronectin-binding protein FbpA	*S. pyogenes*	30	20	21
		HI_null	cell envelope integrity protein TolA	*H. influenzae*	18	18	17
		HI_oapA	opacity-associated protein OapA	*H. influenzae*	16	14	15
		SP_1479	polysaccharide deacetylase family protein	*S. pneumoniae*	18	20	20
Ulcerative colitis	Bacteria	SACOL1868	serine protease SplB	*S. aureus*	18	14	13
		SACOL2509	fibronectin-binding protein FnbB	*S. aureus*	22	18	18
		HI_oapA	opacity-associated protein OapA	*H. influenzae*	14	18	17
		SP_0366	oligopeptide ABC transporter, oligopeptide-binding protein AliA	*S. pneumoniae*	16	16	13
		SP_0346	capsular polysaccharide biosynthesis protein Cps4A	*S. pneumoniae*	20	16	18

TABLE 6-continued

Sensitivities in discovery, validation, and the entire set at 96% specificity for validated IgA antibodies with higher prevalence in healthy controls relative to CD patients (Top) and UC patients (Bottom).

Antigen	Protein name	Organism	Discovery	Validation	Entire
SP_0336	penicillin-binding protein 2X	*S. pneumoniae*	14	16	15
SP_1479	polysaccharide deacetylase family protein	*S. pneumoniae*	18	18	14
SP_0377	choline-binding protein CbpC	*S. pneumoniae*	20	16	18
SACOL2194	hyaluronate lyase HysA	*S. aureus*	20	18	19

IV. Comparison of Anti-Microbial Antibody Response Between CD and UC

[0052] 46 IgG and 22 IgA validated anti-microbial antibodies with higher prevalence in CD patients compared to UC patients were found, while 28 IgG and 9 IgA validated anti-microbial antibodies with higher prevalence in UC patients compared to CD patients were found. There was minimal overlap of the target antigens of these validated IgG and IgA antibodies (FIG. **2**B).

V. Multivariate Analysis to Distinguish CD, UC, and Healthy Controls

[0053] A multi-antibody panels that could distinguish CD vs control, UC vs control, and CD vs UC with an area under the curve (AUC) of 0.81, 0.87, and 0.82 respectively was built. For CD vs control, antibodies against novel flagellins (HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, VC_flaA) had an AUC of 0.73, antibodies against non-flagellins (BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp) had an AUC of 0.75 and the combined AUC of antibodies against novel flagellins and non-flagellins was 0.81 (FIG. **3**A). For UC vs control, a combination of seven antibodies, four against *S. pneumoniae* and one each against *S. aureus, H. influenzae* and *B. vulgatus* had an AUC of 0.87 (FIG. **3**B). For CD vs UC, combination of seven antibodies, two against *H. pylori* and one each against *E. coli, S. pneumoniae, S. pyogenes, C. jejuni* and *L. bacterium* A4 had an AUC of 0.82 (FIG. **3**C).

VI. Subgroup Analysis

[0054] The association of CD behavior (B1, B2, B3), CD location (L1, L2, L3), and UC extent (E1, E2, E3) were investigated based on the Montreal classification with the anti-microbial antibody prevalence. Fourth quartile odds ratio were calculated for each antibody between the two classification groups and compared the number of antibodies with significant odds ratio (P value <0.05) in each group. B3 (penetrating) had the highest prevalence of antibodies followed by B2 (stricturing) and B1 (non-stricturing, non-penetrating) (Table 7). For CD location, L2 had the highest prevalence of antibodies followed by L3 (ileocolonic) and L1 (Table 7). For UC extent, E3 (extensive UC) had higher prevalence of antibodies compared to E2 (left sided UC). In addition to the Montreal classification, subgroup analysis was also performed based on the surgery history of CD patients. Patients who had surgery possessed higher prevalence of antibodies compared to those without surgery (Table 7).

TABLE 7

Subgroup analysis of inflammatory bowel disease patients

Classification	Comparison	Number of antibodies with OR > 1	Number of antibodies with OR < 1	Two sample proportion test
Disease behavior	B1 vs B2 (P < 0.05)	0	32	P < 0.001
B1: non-stricturing, non-penetrating	B2 vs B3 (P < 0.05)	0	19	P < 0.001
B2: stricturing	B1 vs B3 (P < 0.05)	2	41	P < 0.001
B3: penetrating				
Disease location				
L1: ileal	L1 vs L2 (P < 0.05)	0	38	P < 0.001
L2: colonic	L2 vs L3 (P < 0.05)	9	5	P = 0.131
L3: ileocolonic	L1 vs L3 (P < 0.05)	5	16	P < 0.001
Disease extent				
E2: left sided UC; E3: extensive UC	E2 vs E3 (P < 0.05)	11	39	P < 0.001
Surgery in CD patients	No vs Yes (P < 0.05)	6	25	P < 0.001

[0055] For each comparison, the number of antibodies with significant difference in prevalence between two classifications were counted based on odds ratio (OR)>1 and OR<1. The difference in total number of antibodies for each comparison were computed using two sample proportion test. CD: Crohn's disease; UC: Ulcerative colitis.

VII. Correlation of Anti-Microbial Antibodies and Autoantibodies in CD Patients

[0056] Novel autoantibodies in CD patients using the same set of CD patients and healthy controls have been previously reported. Both IgG and IgA autoantibodies and anti-microbial antibodies were profiled in all 100 CD and 100 healthy controls. It is interesting to note the antibodies showing differences for autoantibodies were mostly IgA, but the anti-microbial antibodies were mostly IgG. Anti-SNRPB_IgA had the highest sensitivity of 20% at 96% specificity among all autoantibodies compared with 47% sensitivity at 96% specificity for the best performing anti-microbial antibody, anti-A4-Fla2_IgG.

[0057] The novel autoantibodies and validated anti-microbial antibody profiles were compared to determine if correlation existed between their reactivity. Overall, high correlation between autoantibodies and anti-microbial antibodies in CD patients was not observed (FIG. **4**). Anti-microbial antibodies formed two clusters, one with anti-flagellin antibodies, and the other with SF_Lpp IgG and PMI_RS06815 IgG. Five autoantibodies, PRPH_IgA, SNAI1_IgA,

PPP1R13L_IgA, SNRPB_IgA and PTTG1_IgA, formed a cluster. The remaining antibodies had relatively unique reactivity patterns.

VIII. Materials and Methods

[0058] a. Patients and Samples

[0059] All the serum samples were acquired from Serum Biobank at Mayo Clinic with approval from institutional review board. CD patients were randomly selected, followed by age and gender matched healthy controls and UC patients. The samples (100 CD, 100 UC and 100 controls) were divided evenly into two non-overlapping discovery and validation sets randomly (Table 1). Disease status for study participants was assessed by clinicians at Mayo clinic.

[0060] b. Microbial Protein Array Fabrication

[0061] Of the 1570 microbial proteins analyzed, 1173 proteins were from 50 different species of bacteria, 397 proteins were from 33 different species of viruses and the remaining proteins were autoantigens. These proteins were selected from a large collection of microbial antigens (DNASU.org) with reference to the anti-microbial antibody studies on other diseases (unpublished data). Microbial protein arrays were fabricated as described earlier. Briefly, plasmids with genes of interest cloned in the pANT7_cGST expression vector were obtained from the DNASU plasmid repository, prepared, and printed into silicon nanowells using a piezoelectric dispensing system to produce microbial protein arrays. On the day of experiment, proteins were freshly expressed from printed plasmids using an in-vitro transcription and translation protein expression kit (Fisher Scientific) and captured by anti-GST antibody co-printed in each nanowell. After expression, microarrays were incubated with 1:100 diluted serum samples. The case and control serum samples were randomized while profiling on microarray to reduce bias. IgG and IgA anti-microbial protein antibodies were detected by Alexa-647 goat anti-human IgG (H+L) and Cy3 goat anti-human IgA (Jackson ImmunoResearch). After washing and drying, the microarrays were scanned in a Tecan PowerScanner and the raw fluorescence intensity data were extracted using the ArrayPro Analyzer Software. Raw fluorescence intensity of each protein on the microarray was divided by the median intensity of all the proteins on the microarray for normalization. The normalized value was termed as Median Normalized Intensity (MNI) and used for all analysis. Seropositivity of antibody for a particular antigen was defined as MNI≥2 as have been done for other studies.

[0062] c. Statistical Analysis

[0063] Pairwise comparisons of numbers of IgG or IgA antibodies for each bacterial species among the 3 subject groups were performed using Chi-squared tests to assess statistical significance (FIG. **5**). For each pairwise comparison, the Chi-squared P values were adjusted using the FDR (false discovery rate) method to reduce the likelihood of false positives. In addition to the multiple comparison adjustment at the antibody level, adjustment was performed at the species level.

[0064] For univariate analysis between two comparison groups, sensitivity was calculated for one group at the 96th percentile of the other group or the MNI of 2, whichever was larger. Antibodies with ≥14% sensitivity in the discovery set were selected as candidates for further validation. If an antibody had ≥14% sensitivity at 96% specificity in both discovery and validation sets, then it was considered as a "validated marker. Venn diagram for the overlap of microbial antigen targets were plotted using Venny.

[0065] A three-stage approach was used to build the multi-antibody panels. In the first stage, all candidate biomarkers that passed the criteria above, i.e., sensitivity was greater or equal than 14% at 96% specificity were selected. Next, the minimum redundancy maximum relevance algorithm was applied to further select biomarkers that were possibly the most important and least correlated. In the third stage, a logistic regression model was used to fit the selected biomarkers from the first two stages and generated its receiver operating characteristic curve and AUC value to evaluate the model's discriminatory performance between CD, UC, and healthy controls.

[0066] Pair-wise subgroup comparisons based on the Montreal classification were performed for the odds ratio (OR) of each antibody using the seropositivity threshold defined as the maximum of MNI 2 and the 75th percentile of all samples. Chi-squared tests were used to test global significance between all groups with a slight modification by adding 0.5 to each cell of the table to avoid zero cell counts. P values from the chi-squared method were adjusted for each pair of comparisons and for all candidate biomarkers. The number of antibodies with significant difference in prevalence among classifications were counted based on OR>1 and OR<1 for each pair of classification of CD behavior, CD location, UC extent and the surgery history of CD patients. The difference in total number of antibodies with significant difference between classification groups were computed using two sample proportion test. A subgroup analysis for the UC patients based on the surgery history because most (84 out of 100) had no surgeries (Table 1).

[0067] Spearman's rank correlation analysis was performed to assess the correlation between autoantibody and anti-microbial antibody reactivity for CD patients and healthy controls. The R "pheatmap" package was used to generate the heatmap for correlation coefficients.

[0068] d. Bioinformatics Analysis

[0069] The NCBI Taxonomy browser was used to find the taxonomical details of all the bacteria and viruses used in the study. The taxa were downloaded as phylip tree file and was used as an input in interactive tree of life software. Two phylogenetic trees were created for bacteria and viruses with different colors distinguishing the phylum.

[0070] For sequence homology analysis, a pair-wise BLAST analysis was carried out on the antigen protein sequences of validated antibodies for CD vs healthy control analysis. E-values were used to generate a heatmap using Python Seaborn package.

What is claimed is:

1. An antibody panel for diagnosing a subject with inflammatory bowel disease, the antibody panel comprising at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, BILF2, CK_flgG, A4-Fla2, BVRF2, and UL139.

2. The antibody panel of claim **1**, wherein the inflammatory bowel disease is Crohn's disease, the antibody panel comprises at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2.

3. The antibody panel of claim 2, wherein the antibody panel comprises HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA.

4. The antibody panel of claim 2, wherein the antibody panel comprises BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp.

5. The antibody panel of claim 2, wherein the antibody panel comprises HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2.

6. The antibody panel of claim 1, wherein the inflammatory bowel disease is ulcerative colitis, the antibody panel comprises at least at least one antigen selected from the group consisting of: CK_flgG, A4-Fla2, BVRF2, and UL139.

7. The antibody panel of claim 6, wherein the antibody panel comprises CK_flgG, A4-Fla2, BVRF2, and UL139.

8. A method of diagnosing inflammatory bowel disease in a subject with gastrointestinal distress, the method comprising:

providing a biofluid sample from the subject with gastrointestinal distress;

contacting the biofluid sample with at least one antigen selected from the group consisting of: HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, BILF2, CK_flgG, A4-Fla2, BVRF2, and UL139; and

determining if the biofluid sample comprises an antibody against the at least one antigen, wherein the presence of the antibody against the at least one antigen diagnoses the subject with gastrointestinal distress with an inflammatory bowel disease.

9. The method of claim 8, wherein the biofluid sample is contacted with HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2, wherein the presence of antibodies against HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2 diagnoses the subject with gastrointestinal distress with Crohn's disease.

10. The method of claim 9, wherein the biofluid sample is in contact with HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA, wherein the presence of HP_0115, CK_LafA, CK_LafA.1, VC_flaD, VC_flaB, VC_flaE, and VC_flaA diagnoses the subject with gastrointestinal distress with Crohn's disease instead of ulcerative colitis.

11. The method of claim 9, wherein the antibody panel comprises BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp, wherein the presence of BVU_0562, SP_1992, PMI_RS06815, and SF_Lpp diagnoses the subject with gastrointestinal distress with Crohn's disease instead of ulcerative colitis.

12. The method of claim 9, wherein the antibody panel comprises HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2, wherein the presence of HP_0115, BVU_0562, CK_LafA, CK_LafA.1, A4-Fla2, PMI_RS06815, VC_flaD, VC_flaB, VC_flaE, VC_flaA, SF_Lpp, SP_1992, and BILF2 diagnoses the subject with gastrointestinal distress with Crohn's disease instead of ulcerative colitis.

13. The method of claim 8, wherein the biofluid sample is contacted with at least one of CK_flgG, A4-Fla2, BVRF2, and UL139, wherein the presence of antibodies against CK_flgG, A4-Fla2, BVRF2, and UL139 diagnoses the subject with gastrointestinal distress with ulcerative colitis.

14. The method of claim 13, wherein the biofluid sample is contacted with CK_flgG, A4-Fla2, BVRF2, and UL139.

15. The method of claim 8, wherein the biofluid sample is blood or serum.

16. A method of distinguishing the cause of gastrointestinal distress in a subject, the method comprising:

providing a biofluid sample from a subject with gastrointestinal distress;

contacting the biofluid sample with at least one antigen selected from the group consisting of: SACOL2509, SACOL2511, SACOL2476, SPy_2009, HI_null, HI_oapA, SP_1479, SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194; and

determining the biofluid sample comprises an antibody against the at least one antigen, wherein the presence of the antibody against the at least one antigen diagnoses the subject with gastrointestinal distress with an inflammatory bowel disease.

17. The method of claim 16, wherein the biofluid sample is contact with SACOL2509, SACOL2511, SACOL2476, SPy_2009, HI_null, HI_oapA, and SP_1479, the presence of antibodies against SACOL2509, SACOL2511, SACOL2476, SPy_2009, HI_null, HI_oapA, and SP_1479 diagnoses the subject with gastrointestinal distress with Crohn's disease instead of ulcerative colitis.

18. The method of claim 16, wherein the biofluid sample is contact with SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194, the presence of antibodies against SACOL1868, SACOL2509, HI_oapA, SP_0366, SP_0346, SP_0336, SP_1479, SP_0377, and SACOL2194 diagnoses the subject with gastrointestinal distress with ulcerative colitis instead of Crohn's disease.

19. The method of claim 16, wherein the biofluid sample is blood or serum.

20. The method of claim 16, wherein the biofluid sample is blood.

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