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(54) Title: BIOMARKERS

(57) Abstract: The invention relates to a method of identifying or monitoring irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD) comprising detecting one or more volatile organic compounds (VOCs) from a sample from a subject. Typically the sample is faecal material or urine. The VOC may be one or more of ethyl esters of propanoic or butanoic acids, propanoic acid, butanoic acid, butanoic acid methyl ester, propanoic acid methyl ester, 3-methyl butanoic acid, 1-butanol, 1-propanol, indole, 2-methyl propanoic acid, 1-pentanol, hydroxy urea, methyl cyclobutane, (R)-(-)-2-amino-1-propanol, 2-hydroxy-propanamide, acetic acid hydrazide, 2-pentanone, pyrrole, 1-butoxy-2-propanol, 2-butanone, dimethyl disulphide, 2-methyl-1,3-dioxolane and sulphur dioxide.



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Biomarkers

The invention relates to methods of identifying irritable bowel syndrome (IBS) or inflammatory bowel diseases (IBD) in a subject, comprising detecting the presence or absence or one or more volatile organic compounds (VOCs) in a sample of biological fluids (urine, blood) or faecal material from the subject.

IBD is a group of diseases associated with the colon and small intestine. Patients with IBS, or spastic colon, have similar symptoms to those with potentially more serious IBD such as Crohn's disease (CD) and ulcerative colitis (UC). All of these diseases require invasive investigation for correct diagnosis. Examination of volatile organic compounds (VOCs) from faecal samples has allowed the identification of biomarkers for individual diseases, allowing the development of non-invasive techniques for the diagnosis of such diseases.

The inventors have found that it is possible to identify suitable markers in biological fluids or faecal samples from subjects.

The invention provides a method of identifying or monitoring irritable bowel syndrome (IBS) or an inflammatory bowel disease (IBD) comprising detecting one or more volatile organic compounds (VOCs) in a sample such as biological fluid or faecal material from a subject. The biological fluid may be urine, blood or serum. The use of urine has been found to be particularly useful in the identification of the biomarkers. Blood and breath material were less able to identify biomarkers capable of identifying or discriminating between the different diseases, than faecal material.

By comparing the VOCs pre-treatment and post treatment, the progression or treatment of the disease may be followed.

Biological fluids and faecal samples produce several hundred different candidate compounds which can be detected by gas chromatography/mass spectroscopy, have been selected using various criteria:

Most abundance.

Most probable based on knowledge of samples associated with the diseases.

Visual inspection of subsets of chromatograms.

Further statistical analysis was carried out to identify the best compounds

The selection process identified eight compounds which showed significance between groups based on Kruskal-Wallis analysis of variance.

Typically the compounds are selected from one, two, three or more of ethyl esters of propanoic or butanoic acids, propanoic acid, butanoic acid, butanoic acid methyl ester, propanoic acid methyl ester, 3-methyl butanoic acid, 1-butanol, 1-propanol, indole, 2-methyl propanoic acid, 1-pentanol, hydroxy urea, methyl cyclobutane, (R)-(-)-2-amino-1-propanol, 2-hydroxy-propanamide, acetic acid hydrazide, 2-pentanone, pyrrole, 1-butoxy-2-propanol, 2-butanone, dimethyl disulphide, 2-methyl-1, 3-dioxolane and sulphur dioxide.

These preferred compounds are selected from one or more of ethyl esters propanoic or butanoic acids, butanoic acid, butanoic acid methyl ester, 3-methyl butanoic acid, 1-butanol, 1-propanol and indole.

The subject may be human or non-human

The method of the invention can identify the likelihood that the subject has the presence or absence of IBS or IBD, and can discriminate between IBD and IBS. The method may also discriminate between different IBDs such as the method identifies the subject as having Crohn's disease (CD) or ulcerative colitis (UC).

The concentration of butanoic acid, butanoic acid ethyl ester, propanoic acid ethyl ester and optionally one or more of 1-butanol, 1-propanol and indole, may be determined in a sample of faeces. These compounds may be used to show the presence of CD.

The concentration of propanoic acid and butanoic acid and optionally one or more of 2-methyl propanoic acid and 1-pentanol may be measured in faeces. The presence of these are indicative of IBS.

The concentration of hydroxy urea, methyl cyclobutane, (R)-(-)-2-amino-1-propanol and 2-hydroxy propanamide may be measured in faeces. These are indicative of UC.

The concentration of acetic acid anhydride, 2-pentanone, pyrrole and 2-butoxy-2-propanol and optionally 2-methyl-1, 3-dioxolane may be measured in urine. These are indicative of CD.

The concentration of dimethyl disulphide, 2-methyl-1, 3-dioxolane and optionally one or both of 2-butanone and 2-pentanone may be measured in urine. These are indicative of IBS.

The presence of sulphur dioxide and acetic acid hydrazide and optionally propane may be measured in urine. These are indicative of UC.

One or more or all of the additional compounds shown in Tables 1 to 6 for the respective indications (CD, IBS, and UC) for faeces and urine may additionally be detected.

The amount of the or each compound present is typically determined and compared to a predetermined control. The predetermined control may, for example be a normal amount of the compound that shows the amount of the compound normally found in subjects without IBS or IBD, or compared to subjects known to have IBS or an IBD, such as CD or UC.

Preferably concentrations of butanoic acid methyl ester or propanoic acid methyl ester are determined. These compounds also retained significant differences between groups when results were analysed post treatment. An amount of these compounds above a predetermined amount pre-treatment indicates an IBD, such as CD or UC.

The VOCs are detected in a sample of headspace gas produced by the sample. For example, the headspace gas may be produced by incubating the sample in a sealed container for a predetermined amount of time. The container may be a rigid container, such as a glass container. Alternatively a flexible, expandable container, such as a plastics bag, may be used. Head space samples may be prepared in bags, such as Nalophantm bags.

The containers may be filled with zero grade air, and the VOCs are detected in samples of the air after incubation of the faeces. The faecal sample may be incubated, for example at 37°C for a predetermined amount of time, such as 5 to 30 minutes, e.g. 10 minutes, to allow VOCs to be released into the head space.

The VOCs may be detected and quantified in the samples using any suitable method. For example, a sample of the headspace may be analysed using chromatographic techniques generally known in the art. Preferably the VOCs are detected by thermal desorption-gas chromatography-mass spectroscopy (TD-GC-MS). This utilises a thermal desorption tube to help separate compounds in the head space sample, which are then detected by GC-MS. HPLC/MS (high performance liquid chromatography - mass spectrometry) may also be used to identify samples in, for example, biological fluids.

Similar patterns of metabolite profiles can be obtained and analysed by, for example, principal components analysis, partial least squares analysis and hierarchical cluster analysis.

Metabolomic analysis of bacterial metabolites provides a reliable way of diagnosing gastrointestinal disorders, using HPLC/MS. Using a specific ion (M/z 98SIC), Crohn's disease was classified, using faeces, in 95.24% of cases and using urine of IBS in 81.8% in IBS. Overall, urine analysis by HPLC/MS proved to be most reliable.

The amount of VOC detected may be compared to the amount of a control substance in the sample. For example, samples may be normalised against a deuterated toluene SIC peak.

The invention also provides computers and computer programs for carrying out the methods of the invention.

A preferred aspect of the invention provides computer for use in a method as defined above, comprising a data storage memory, the data storage memory comprising predetermined values of VOCs associated with normal, IBS or IBD, a processor preconfigured to compare one or more values of VOC obtained from a sample of faeces with the predetermined values of VOCs, said computer being connectable to a device for measuring VOCs from a sample of faeces.

Also provided is a computer program, which when executed, will compared a value of VOC obtained by a method as defined above, with a predetermined value of VOC.

The invention will now be described by way of example only with reference to the following figures:

Figures 1: Representative chromatograms for control and each disease group are displayed together to visualise differences in peak heights of compounds.

Figure 2: Examples of two compounds of significance after Kruskal-Wallis test. The box and whisker plots indicate medians and interquartile ranges.

Material and Methods

91 volunteers (28 IBS, 24CD, 19UC and 20 healthy controls) were recruited. Faeces were taken pre-treatment and post treatment for IBD.

Headspace samples were prepared in Nalophan bags filled with zero grade air and incubated (Kalle, Germany) at 37°C for 10 minutes. A 500ml volume of the resulting headspace was drawn into a thermal desorption tube using an air pump. The sample was analysed by TD-GC-MS, using deuterated toluene as a standard marker.

Compound identification was achieved automated mass spectral deconvolution and identification system (AMDIS) and the mass spectral library from the National Institute of Standards Technology (NIST v2.0). Many hundred compounds were identified, necessitating the selection of a panel of candidate compounds for statistical analysis, based on:

Most abundance

Most probable based on knowledge of samples associated with the diseases.

Visual inspection of subsets of chromatograms (see, for example, Figure 1).

Additionally, blood and urine were tested. Faecal material provided a better data set to be analysed.

Results

13 compounds were selected for data handling. 8 of those compounds were found to be statistically significant: ethyl esters propanoic or butanoic acids, butanoic acid, butanoic acid methyl ester, 3-methyl butanoic acid, 1-butanol, 1-propanol and indole.

The results of TD- GC-MS for CD in faeces, for example, are shown below:

Table 1

Compound	Retention Time (Mins)	Probability (%)
2-hydroxy-propanamide	6.54	67.5
Butanoic Acid, ethylester	14.87	92.5
1-butoxy-2-propanol	18.67	88.4
4-methyl-phenol	22.92	41.0
Tetradecane	22.87	52.3
1-decylsulphonyl-d-Mannitol	22.81	7.7
Dimethyldisulphide	13.92	97.7
<i>1-propanol</i>	<i>9.08</i>	<i>96.1</i>
<i>Butanoic Acid</i>	<i>16.14</i>	<i>91.3</i>
<i>3-methyl-butanoic acid</i>	<i>17.18</i>	<i>84.1</i>
<i>4-O-beta-D-galactopyranosyl-alpha-D-Glucopyranose</i>	<i>21.03</i>	<i>6.04</i>
<i>Indole</i>	<i>29.65</i>	<i>35.2</i>
<i>Indolizine</i>	<i>29.70</i>	<i>22.6</i>

Kruskal-Wallis tests for controls, IBS, CD and UC, all pre-treatment, for methyl esters of butanoic acid and propanoic acid is shown in Figure 2.

GC-MS was repeated for faecal material from IBS patients compared to normal patients.

Table 2

Metabolite	RT (Min)	Probability via NIST %
Benzeneethanamine,2-fluoro-á,3-dihydroxy-N-methyl	6.461	20.3
Propanoic acid	13.82	80.5
1-Pentanol	14.76	62.8
d-Glycero-d-ido-heptose	15.83	6.7
Butanoic acid	16.00	89.4
d-Mannitol,1-decylsulfonyl	22.77	19.7
1-Pentanol,4-amino-	5.42	33.5
Borane,compd. with dimethylamine	7.18	40.3
Propanoic acid,2-methyl-	15.13	63.4
Butanoic acid,3-methyl-	17.28	15.6
Hexanoic acid (but also mannopyranoside and glycerol)	18.05	13.0
d-Glycero-d-ido-heptose	19.66	6.5

This shows that propanoic acid and butanoic acid are significant markers, together with to a lesser extent 2-methyl propanoic acid and 1-pentanol.

It shows that IBS may be distinguished from normal and CD patients by the markers identified.

Table 3 shows faecal material from ulcerative colitis (UC) patients compared to normal patients.

Table 3

Metabolite	RT (Min)	Probability via NIST %
(R)-(-)-2-Amino-1-propanol	4.40	59.0
Hydroxyurea	5.32	84.6
2,5-Dimethoxy-4-(methylsulfonyl) amphetamine	6.22	24.3
Benzeneethanamine,2-fluoro-á,3-dihydroxy-N-methyl	7.16	13.0
2-Heptanol,6-amino-2-methyl-	7.98	15.1
Cyclobutane,methyl-	8.10	67.2
1-Pentanol,4-amino-	8.23	37.0
dl-3-Aminobutyric acid	12.78	6.26
Boronic acid, ethyl-	13.68	26.6
Hexanoic acid	15.83	12.6
Propanesulfonylacetoneitrile	18.23	19.5
1-Hepten-4-ol	27.50	15.8
Methane,dipropoxy-	29.59	19.5
Propanamide,2-hydroxy-	6.61	57.8
2,3-Dihydrofuran	8.98	16.6
à-Aminoisobutyronitrile	9.37	12.3
2-Trifluoroacetoxystodecane	11.86	6.57
1,3,4-Oxadiazole,2-(acetyloxy)-2,5-dihydro-2,5,5-trimethyl-	15.24	14.1
Cyanoacetylurea	17.38	8.29
Butanenitrile,3-methyl-	19.67	38.2
1-Propanamine,N,2-dimethyl-N-nitroso-	26.87	34.5

UC patients exhibited a different pattern of VOCs. Significant components included hydroxy urea, methyl cyclobutane, (R)-(-)-2-Amino-1-propanol and 2-hydroxy-propanamide.

Samples of urine have also been assayed.

Table 4 shows GC-MS of urine from CD patients compared to controls.

Table 4

Metabolite	RT (Min)	Probability via NIST %
1,3-Dioxolane,2-methyl-	10.97	59.3
Fluoro-acetylene; (<i>hydroxyurea</i>)	4.82	63.1; (5.4)
Acetic acid hydrazide	7.83	80.3
Acetamide N-methyl-	10.93	36.9
2-pentanone	12.49	73.4
Dimethyl-disulphide	13.94	44.9
4-heptanone	17.11	48.3
Nonanal	22.10	14.5
1,2,4-Benzenetricarboxylic acid,1,2-dimethyl ester	26.11	11.9
Carbon dioxide or nitrous oxide	4.07	53.0 or 39.5 rsp
3,4-Epoxytetrahydrothiophene-1,1-dioxide	8.13	33.6
2-butanone	9.93	42.6
Pyrrole	15.33	84.3
(Z)-2-Hydroxyimino-3-oxobutyric acid, 1,1-dimethylethyl ester	18.28	25.5
2-Propanol, 1-butoxy-	18.61	89.9
Carbamic acid, phenyl ester	20.20	19.6
d-Mannitol, 1-decylsulfonyl-	24.59	27.4
Furazano[3,4-b]pyrazin-5(4H)-one, 6-(1-pyrrolidiny)-	26.77	13.7
N-Methyl-1-adamantaneacetamide	28.83	14.4

Significant markers included acetic acid hydrazide, 2-pentanone, pyrrole and 1-butoxy-2-propanol.

Table 5 shows urine samples for IBS patients using urine samples. Significant markers observed were dimethyl disulphide, 2-methyl-1,3-dioxolane, and to lesser extent 2-butanone and 2-pentanone.

Table 5

Metabolite	RT (Min)	Probability via NIST %
L-Alanine,3-sulfo-	4.65	33.3
Chlorozotocin or cysteine	5.72	13.6 or 11.5
2-Butanone	9.91	60.4
1,3-Dioxolane, 2-methyl-	10.99	80.3
Disulfide, dimethyl	13.93	90.8
Pterin-6-carboxylic acid	18.63	12.6
Cystine	3.93	13.8
Acetamide, 2-cyano-	4.35	12.2
3,4-Epoxytetrahydrothiophene-1,1-dioxide	8.23	12.4
2-Pentanone	12.44	63.2
Tetraacetyl-d-xylonic nitrile	15.00	20.7
2-Trifluoroacetoxystyrene	17.16	10.0
Z,Z,Z-1,4,6,9-Nonadecatetraene	19.82	6.6
Undecane	20.89	21.8
1-Naphthalenemethanol,1,4,4a,5,6,7,8,8a-octahydro-2,5,58a-tetramethyl-	27.55	5.8
1,4-Benzenediol,2,6-bis(1,1-dimethylethyl)-	29.76	10.3

Table 6 shows UC urine samples.

Table 6

Metabolite	RT (Min)	Probability via NIST %
Benzeneethanimine,25-difluoro-á,3,4-trihydroxy-N-methyl-	3.88	39.0
Ethyne, fluoro-	4.18	32.9
Methyl vinyl ketone	9.73	42.5
Butanal, 3-hydroxy-	11.70	18.6
Butanedial	12.68	9.8
Propane	15.42	59.9
9-Octadecanoic acid (Z)-,phenylmethyl ester	26.25	16.7
Sulphur Dioxide	4.65	88.6
Acetaldehyde	5.27	30.2
Acetic acid, hydrazide	7.85	88.7
Tetraacetyl-d-xylonic nitrile	8.75	13.7
Acetamide,N-methyl-N-[4-[4-fluoro-1-hyxahydropyridyl]-2-butynyl]-	10.70	38.1
2-Hexanone,4-hydroxy-3-propyl-	15.18	13.7
1,4-Benzenediol,2,6-bis(1,1-dimethylethyl)-	26.67	10.0

Sulphur dioxide and acetic acid hydrazide and to a lesser extent propane are found to be significant markers.

Claims

1. A method of identifying or monitoring irritable bowel syndrome (IBS) or an inflammatory bowel disease (IBD) comprising detecting one or more volatile organic compounds (VOCs) in a sample from a subject, preferably of faecal material or urine.
2. A method according to claim 1, wherein the VOC is selected from one or more of ethyl esters of propanoic or butanoic acids, propanoic acid, butanoic acid, butanoic acid methyl ester, propanoic acid methyl ester, 3-methyl butanoic acid, 1-butanol, 1-propanol, indole, 2-methyl propanoic acid, 1-pentanol, hydroxy urea, methyl cyclobutane, (R)-(-)-2-amino-1-propanol, 2-hydroxy-propanamide, acetic acid hydrazide, 2-pentanone, pyrrole, 1-butoxy-2-propanol, 2-butanone, dimethyl disulphide, 2-methyl-1,3-dioxolane and sulphur dioxide.
3. A method according to claims 1 or 2 wherein the method identifies the subject as having Crohn's disease (CD), IBS or ulcerative colitis (UC).
4. A method according to any preceding claim, wherein the amount of the or each compound present is determined and compared to a predetermined control.
5. A method according to any preceding claim, wherein concentrations of butanoic acid methyl ester or propanoic acid methyl ester above a predetermined amount in a sample of faeces indicate an IBD, such as CD.
6. A method according to claim 5, wherein the concentration of butanoic acid, butanoic acid ethyl ester, propanoic acid ethyl ester and optionally one or more of 1-butanol, 1-propanol and indole is also determined.
7. A method according to claims 1 to 4, wherein the concentrations of propanoic acid and butanoic acid, and optionally one or more of 2-methyl propanoic and 1-pentanol are measured in a sample of faeces.

8. A method according to claims 1 to 4, wherein the concentration of hydroxy urea, methyl cyclobutane, (R)-(-)-2-amino-1-propanol and 2-hydroxy propanamide and optionally 2-methyl-1,3-dioxolane are measured in a sample of faeces.
9. A method according to claims 1 to 4, wherein the concentration of acetic acid anhydride, 2-pentanone, pyrrole and 2-butoxy-2-propanol are measured in a sample of urine.
10. A method according to claims 1- to 4, wherein the concentration of dimethyl disulphide, 2-methyl-1,3-dioxolane and optionally one or both of 2-butanone and 2-pentanone are measured in a sample of urine.
11. A method according to claims 1 to 4, wherein the concentration of sulphur dioxide and acetic acid hydrazide, and optionally propane are measured in a sample of urine.
12. A method according to any preceding claim, wherein the amount of the VOCs in the samples indicates the presence of IBS, CD or UC in the subject.
13. A method according to any preceding claim wherein the VOCs are detected in a sample of headspace gas produced by the sample.
14. A method according to claim 13 wherein the headspace gas is produced by incubating the sample in a sealed container for a predetermined amount of time.
15. A method according to any preceding claim wherein, the VOCs are detected by thermal desorption-gas chromatography-mass spectroscopy (TD-GC-MS).
16. A computer for use in a method according to any preceding claim, comprising a data storage memory, the data storage memory comprising predetermined values of VOCs associated with normal, IBS or IBD, a processor preconfigured to compare one or more values of VOC obtained from a sample of faeces with the predetermined values of VOCs, said computer being connectable to a device for measuring VOCs from a sample of faeces.
17. A computer program, which when executed, will compare a value of VOC obtained by a method according to claims 1-15, with a predetermined value of VOC.

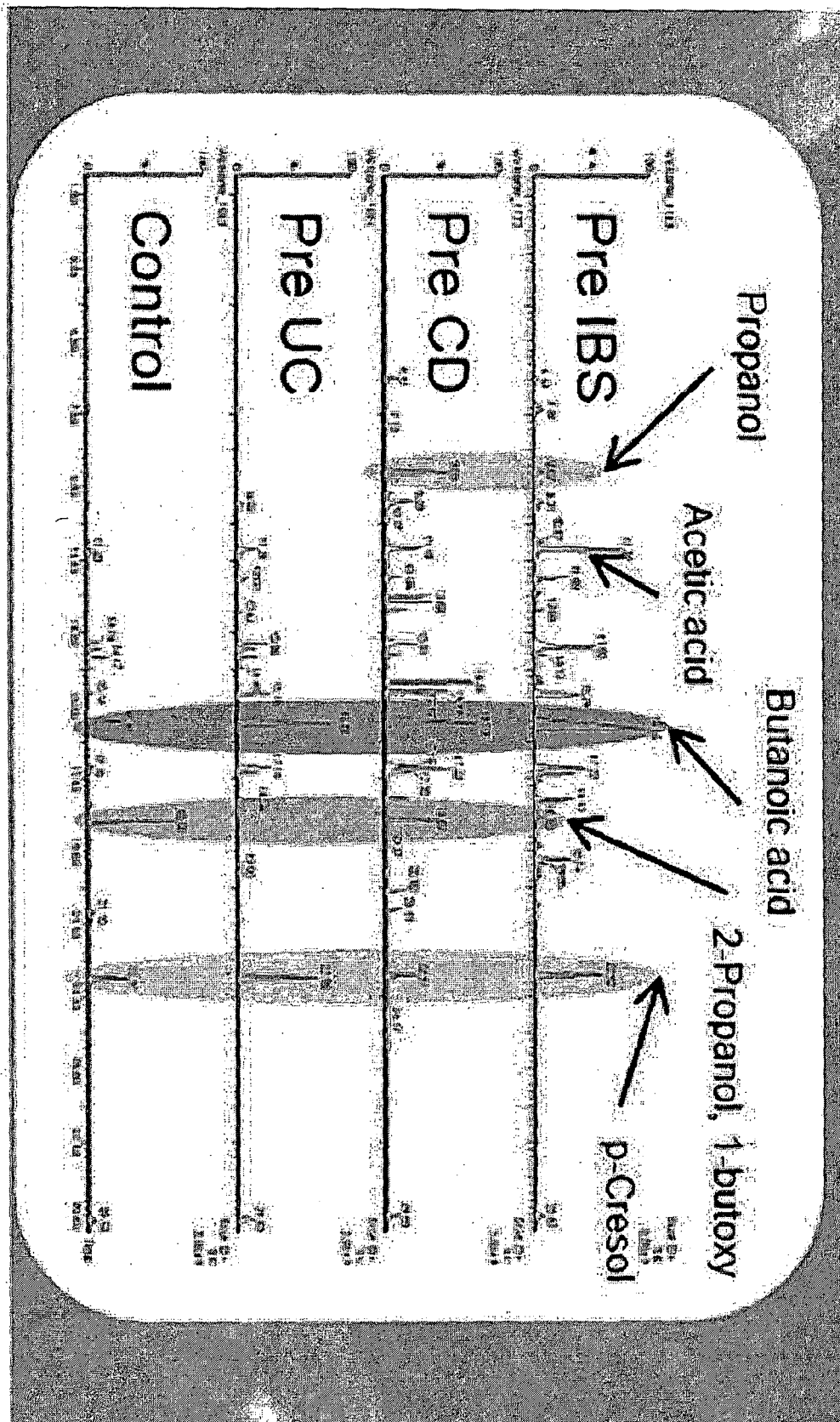


Figure 1

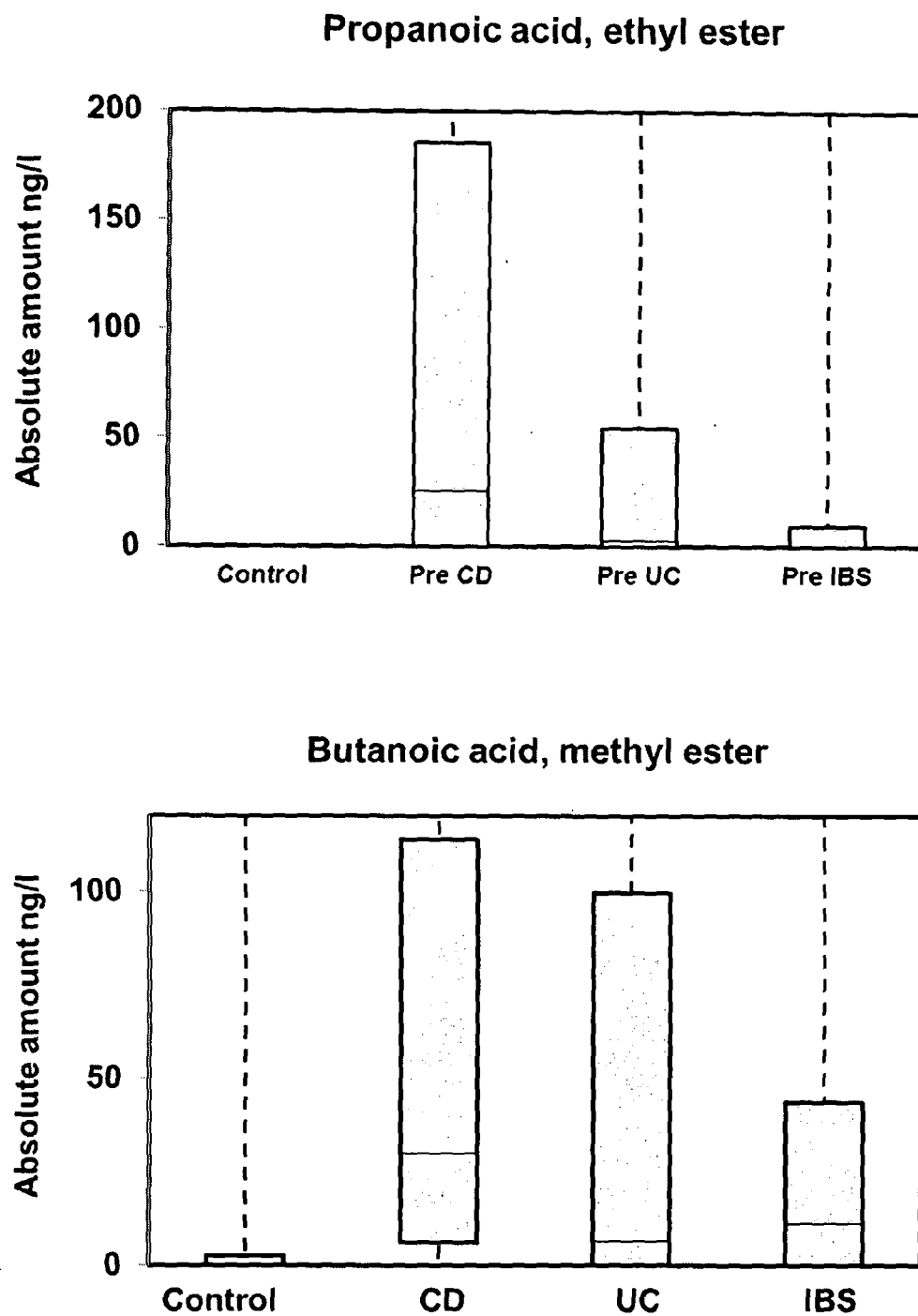


Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/050582

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N33/62 G01N33/64 G01N33/84 G01N33/98 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/182291 A1 (PIMENTEL MARK [US]) 31 July 2008 (2008-07-31)	1,3,4, 12-17
Y	paragraphs [0015], [0029], [0032]; claims 8-12; example 4 -----	2,7-11
Y	GARNER CATHERINE E ET AL: "Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease", FASEB JOURNAL, FED. OF AMERICAN SOC. FOR EXPERIMENTAL BIOLOGY, US, vol. 21, no. 8, 1 June 2007 (2007-06-01), pages 1675-1688, XP002509123, ISSN: 0892-6638, DOI: 10.1096/FJ.06-6927COM abstract; figure 2; tables 2-5 ----- -/-	2,7-11
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 20 April 2012	Date of mailing of the international search report 16/07/2012	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Schmidt, Harald	

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2012/050582

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/066944 A2 (SLIMAK KAREN M [US]) 12 August 2004 (2004-08-12) page 10 - page 17; table 1 -----	1-4,7-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2012/050582

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4, 7-17(all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4, 7-17(all partially)

method of identifying or monitoring irritable bowel syndrome (IBS), and computer or computer program for use in said method

2. claims: 1-17(partially)

method of identifying or monitoring Crohn's disease (CD), and computer or computer program for use in said method

3. claims: 1-17(partially)

method of identifying or monitoring ulcerative colitis (UC), and computer or computer program for use in said method

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2012/050582

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008182291	A1	31-07-2008	NONE

WO 2004066944	A2	12-08-2004	EP 1617955 A2 25-01-2006
			US 2007059454 A1 15-03-2007
			WO 2004066944 A2 12-08-2004
