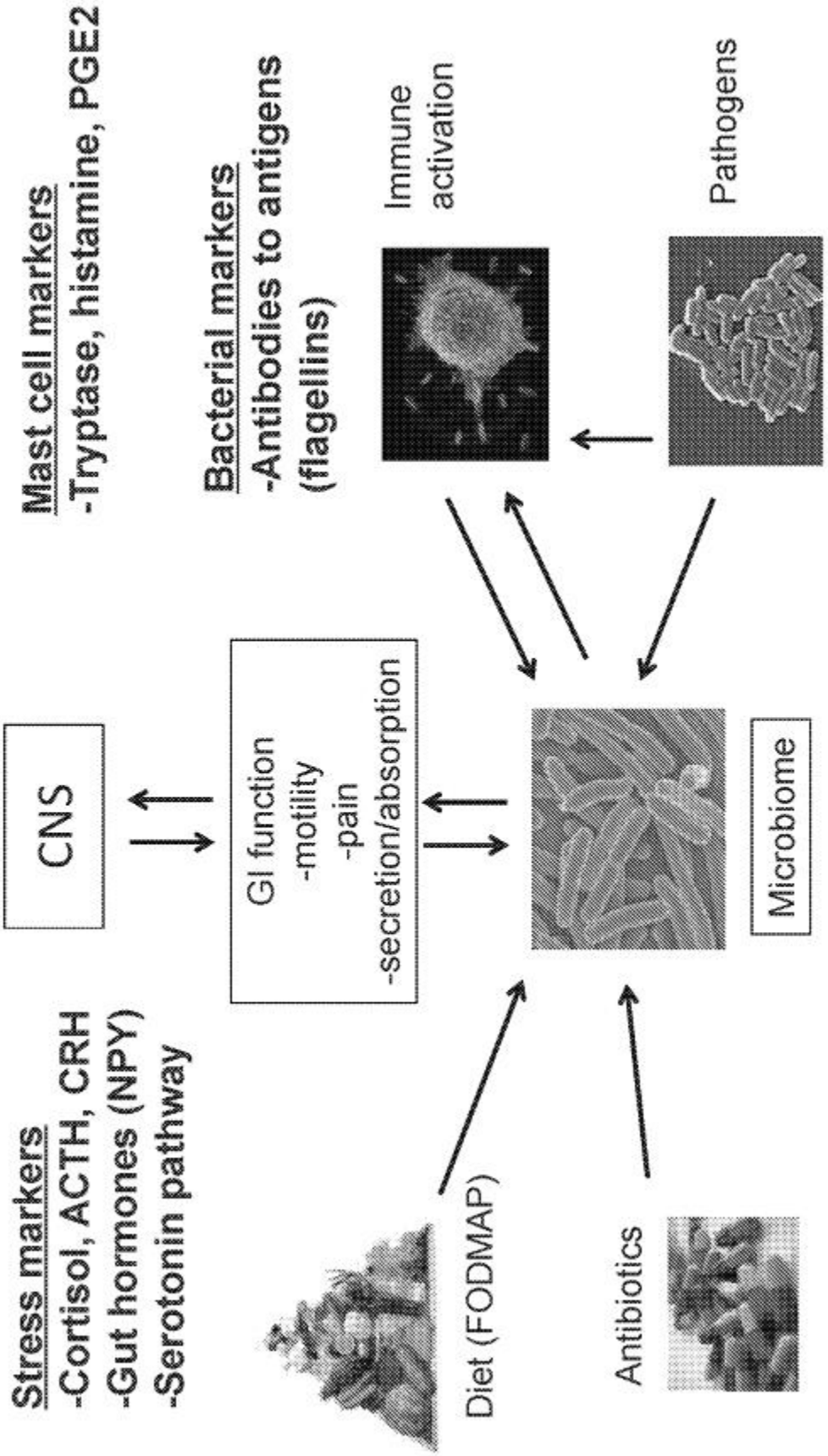

Stress markers
-Cortisol, ACTH, CRH
-Gut hormones (NPY)
-Serotonin pathway
CNS
GI function
-motility
-pain
-secretion/absorption
Mast cell markers
-Tryptase, histamine, PGE2
Bacterial markers
-Antibodies to antigens
(flagellins)
Immune activation
Pathogens
Diet (FODMAP)
Antibiotics
Microbiome

FIG. 1

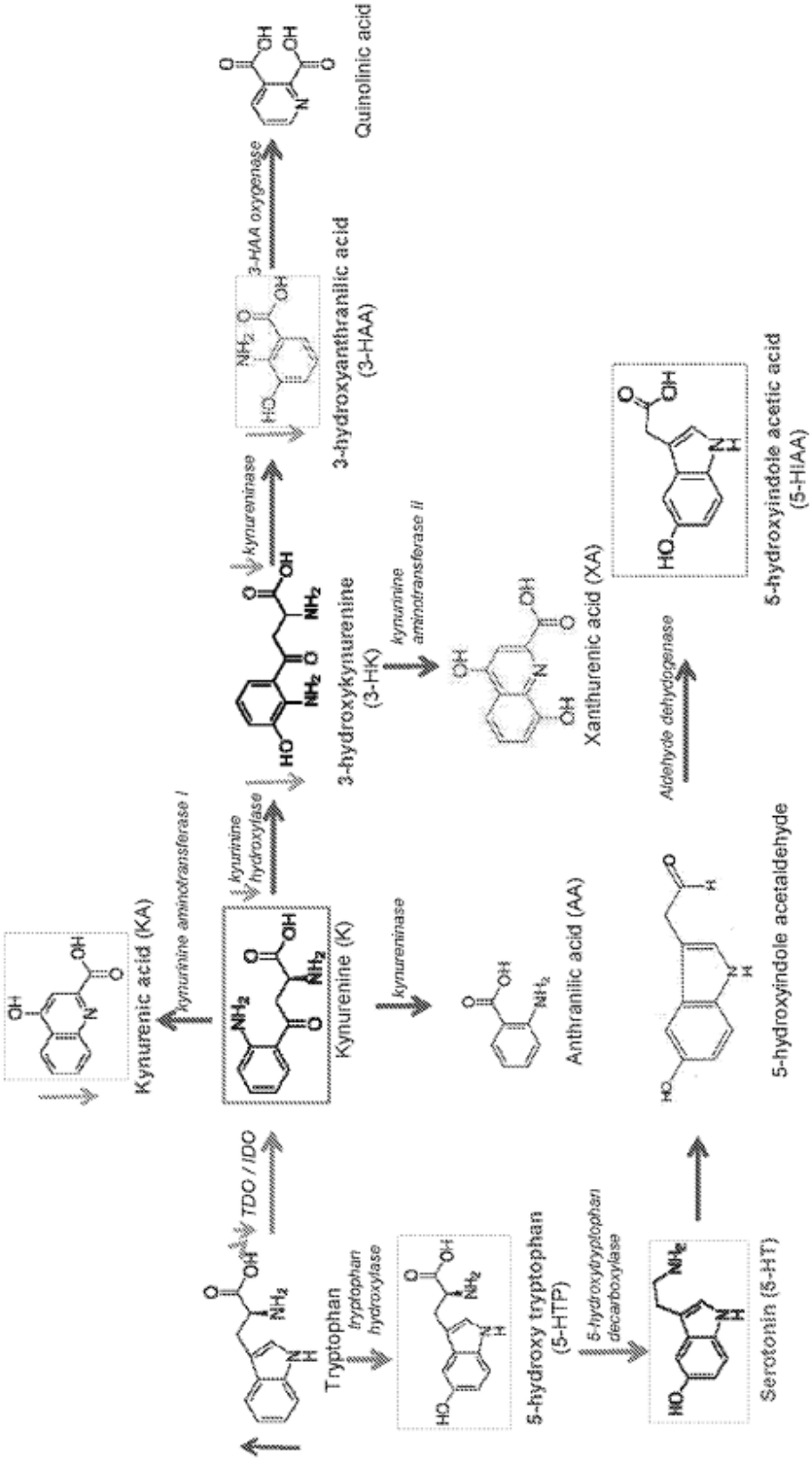

Kynurenic acid (KA)
kynurenine aminotransferase I
Kynurenine (K)
kynurenine hydroxylase
3-hydroxykynurenine (3-HK)
kynureninase
3-hydroxyanthranilic acid (3-HAA)
3-HAA oxygenase
Quinolinic acid
kynureninase
Anthranilic acid (AA)
kynurenine aminotransferase II
Xanthurenic acid (XA)
Tryptophan
tryptophan hydroxylase
5-hydroxy tryptophan (5-HTP)
TDC / IDO
5-hydroxytryptophan decarboxylase
Serotonin (5-HT)
5-hydroxyindole acetaldehyde
Aldehyde dehydrogenase
5-hydroxyindole acetic acid (5-HIAA)

FIG. 2

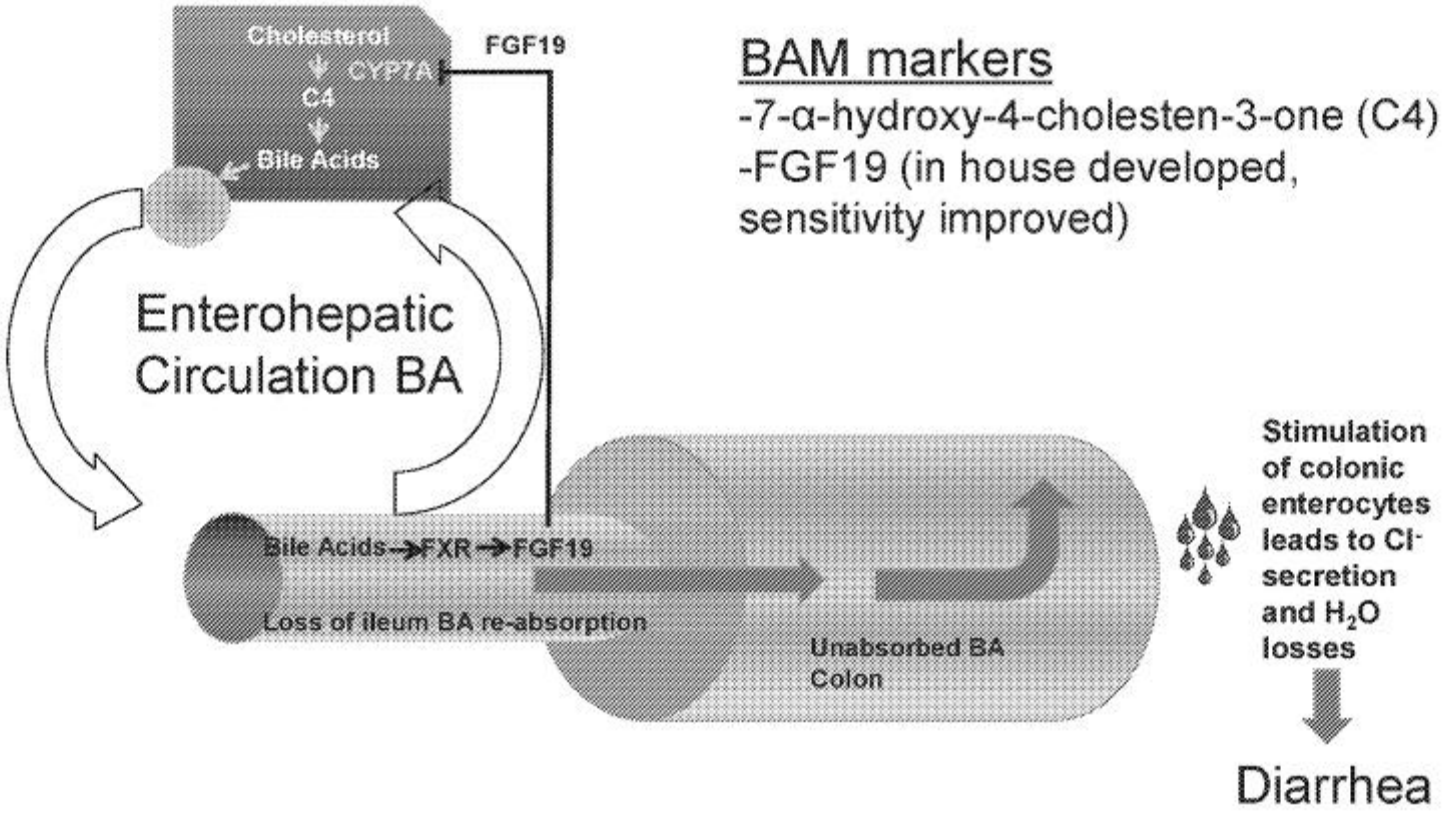

Cholesterol
CYP7A
C4
Bile Acids
FGF19
Enterohepatic Circulation BA
Bile Acids→FXR→FGF19
Loss of ileum BA re-absorption
Unabsorbed BA Colon
Stimulation of colonic enterocytes leads to Cl- secretion and H2O losses
Diarrhea
BAM markers
-7-α-hydroxy-4-cholesten-3-one (C4)
-FGF19 (in house developed, sensitivity improved)

FIG. 3

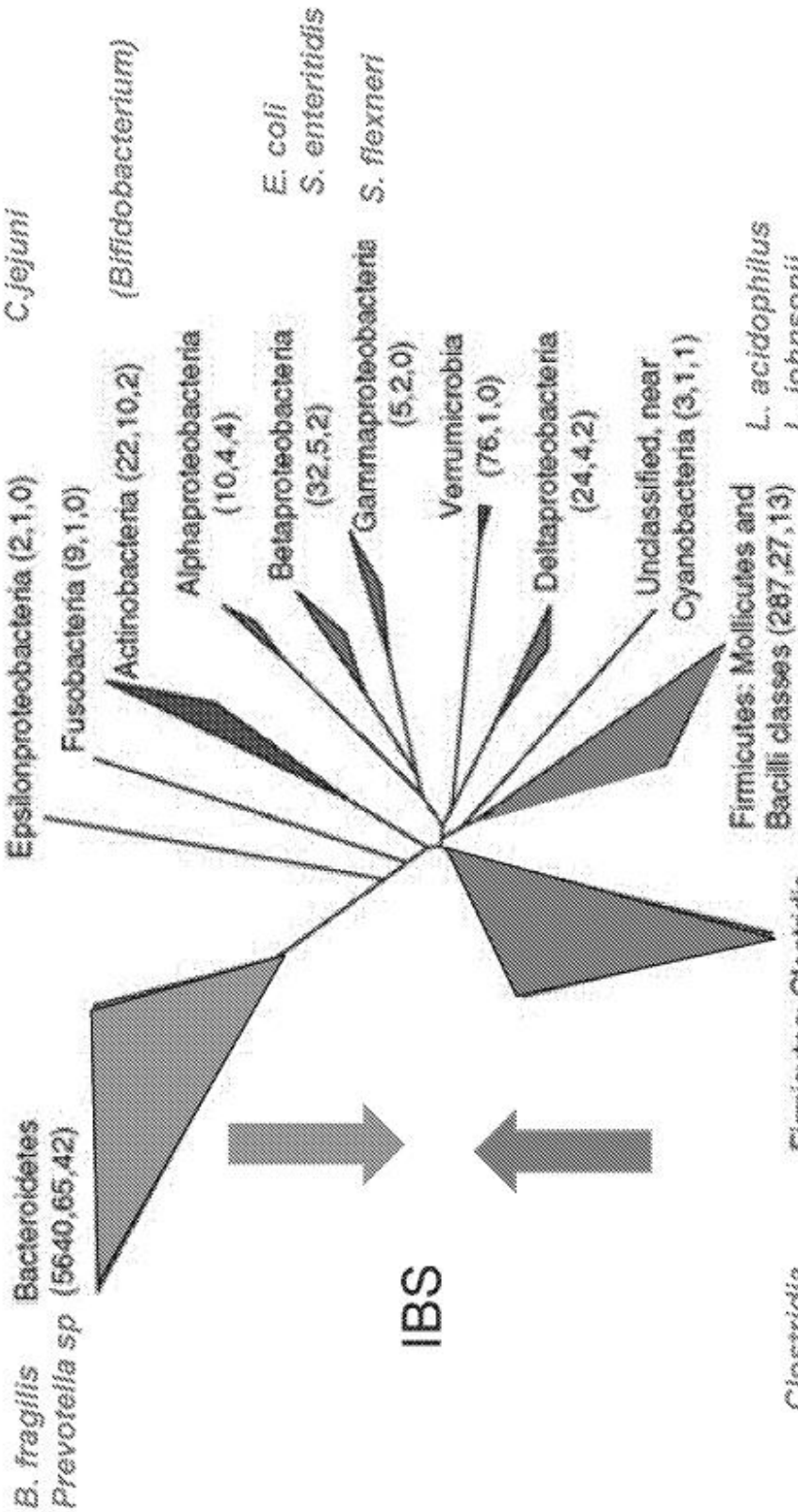

B. fragilis
Prevotella sp
Bacteroidetes (5640,65,42)
Clostridia
Firmicutes: Clostridia classes (5721,274,178)
IBS
Epsilonproteobacteria (2,1,0)
Fusobacteria (9,1,0)
Actinobacteria (22,10,2)
Alphaproteobacteria (10,4,4)
Betaproteobacteria (32,5,2)
Gammaproteobacteria (5,2,0)
Verrumicrobia (76,1,0)
Deltaproteobacteria (24,4,2)
Unclassified, near Cyanobacteria (3,1,1)
Firmicutes: Mollicutes and Bacilli classes (287,27,13)
0.10
C.jejuni
(Bifidobacterium)
E. coli
S. enteritidis
S. flexneri
L. acidophilus
L. johnsonii

FIG. 4

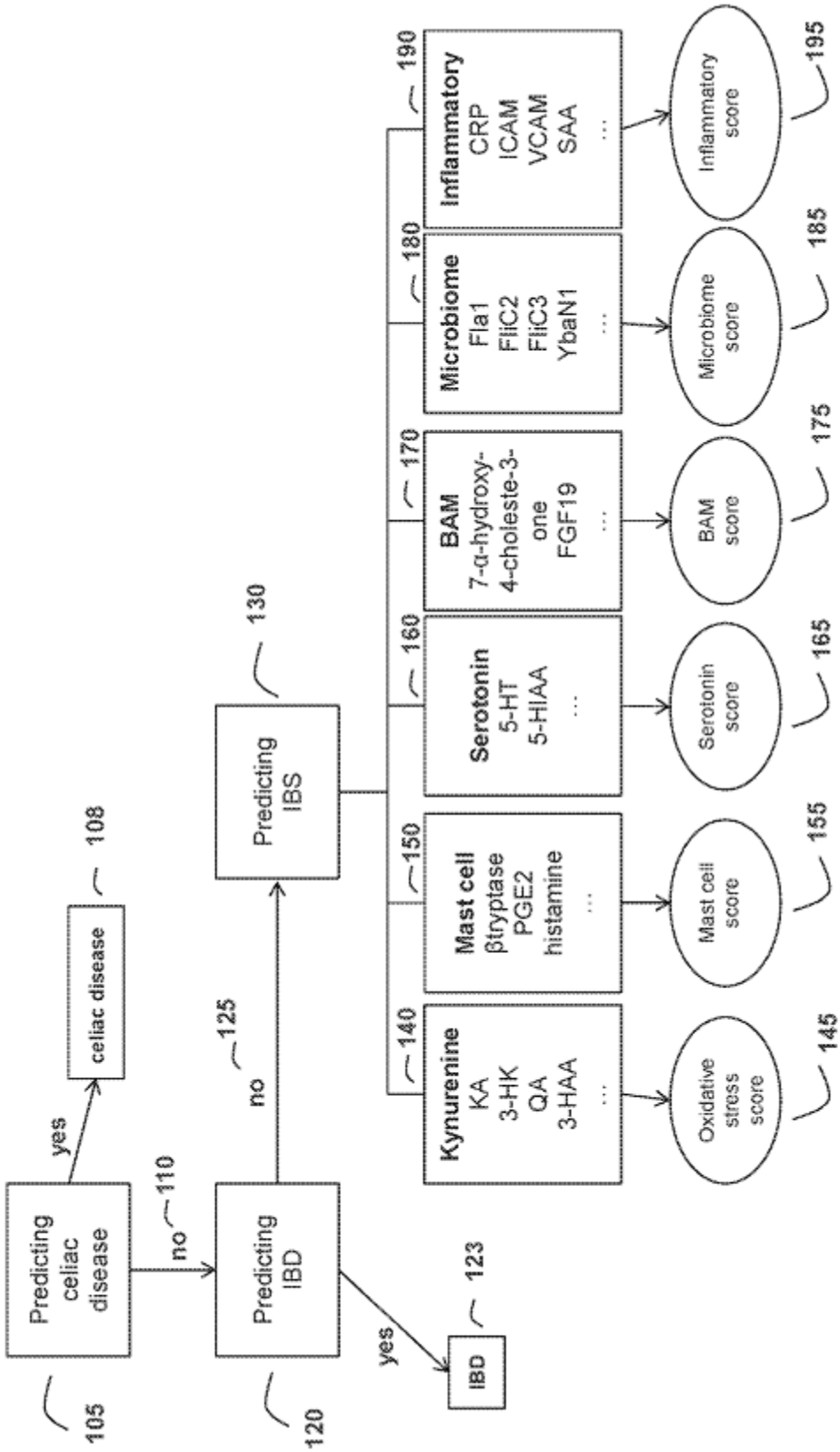

Predicting celiac disease
105
yes
celiac disease
108
no
110
Predicting IBD
120
yes
IBD
123
no
125
Predicting IBS
130
Kynurenine
KA
3-HK
QA
3-HAA
...
140
Mast cell
βtryptase
PGE2
histamine
...
150
Serotonin
5-HT
5-HIAA
...
160
BAM
7-α-hydroxy-4-choleste-3-one
FGF19
...
170
Microbiome
Fla1
FliC2
FliC3
YbaN1
...
180
Inflammatory
CRP
ICAM
VCAM
SAA
...
190
Oxidative stress score
145
Mast cell score
155
Serotonin score
165
BAM score
175
Microbiome score
185
Inflammatory score
195

FIG. 5

FIG. 6A

ROC AUC = 0.843 with bacterial markers + 1 inflammatory marker

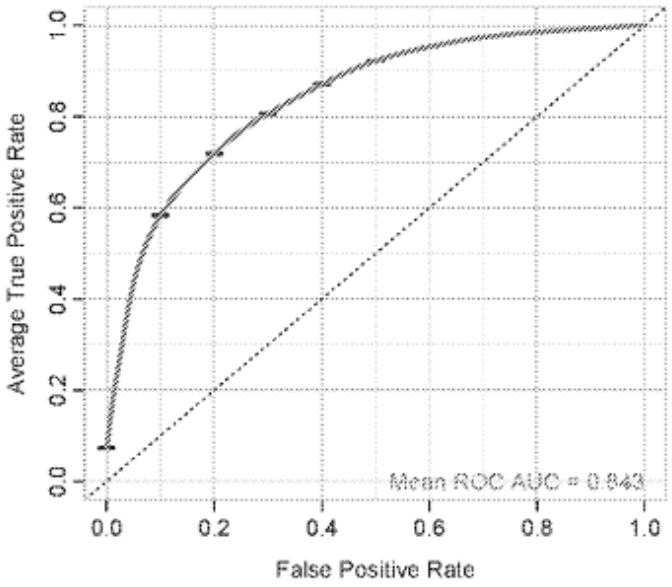
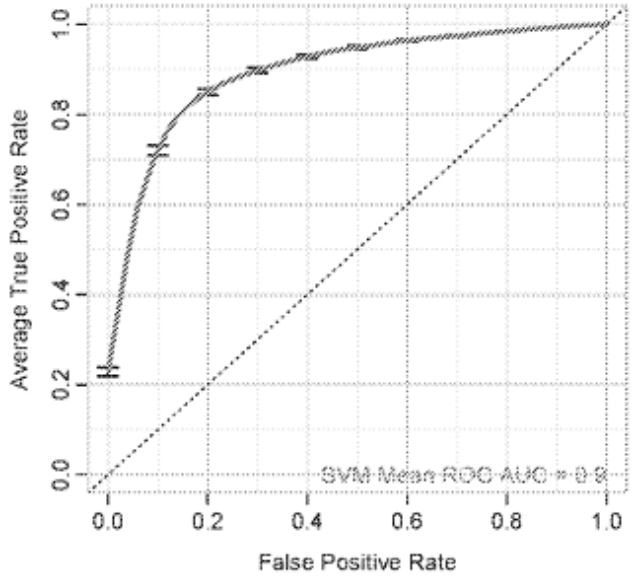


FIG. 6B

ROC AUC = 0.9 with bacterial markers + 1 inflammatory marker + 1 mast cell marker



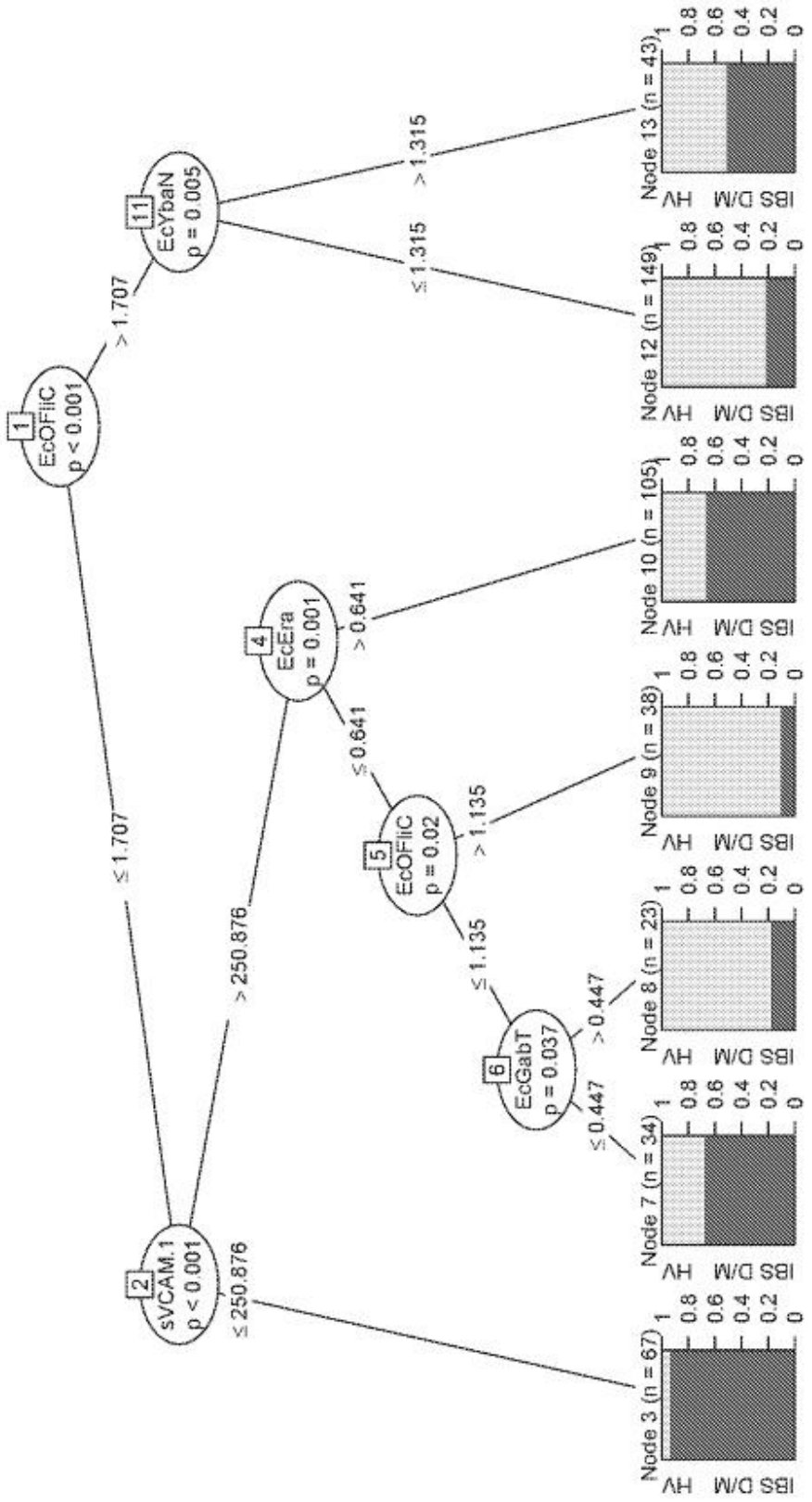

1
EcOFliC
p < 0.001
≤ 1.707
> 1.707
2
sVCAM.1
p < 0.001
≤ 250.876
> 250.876
11
EcYbaN
p = 0.005
≤ 1.315
> 1.315
4
EcEra
p = 0.001
≤ 0.641
> 0.641
5
EcOFliC
p = 0.02
≤ 1.135
> 1.135
6
EcGabT
p = 0.037
≤ 0.447
> 0.447
Node 3 (n = 67)
Node 7 (n = 34)
Node 8 (n = 23)
Node 9 (n = 38)
Node 10 (n = 105)
Node 12 (n = 149)
Node 13 (n = 43)
HV
D/M
IBS
0
0.2
0.4
0.6
0.8
1

FIG. 7

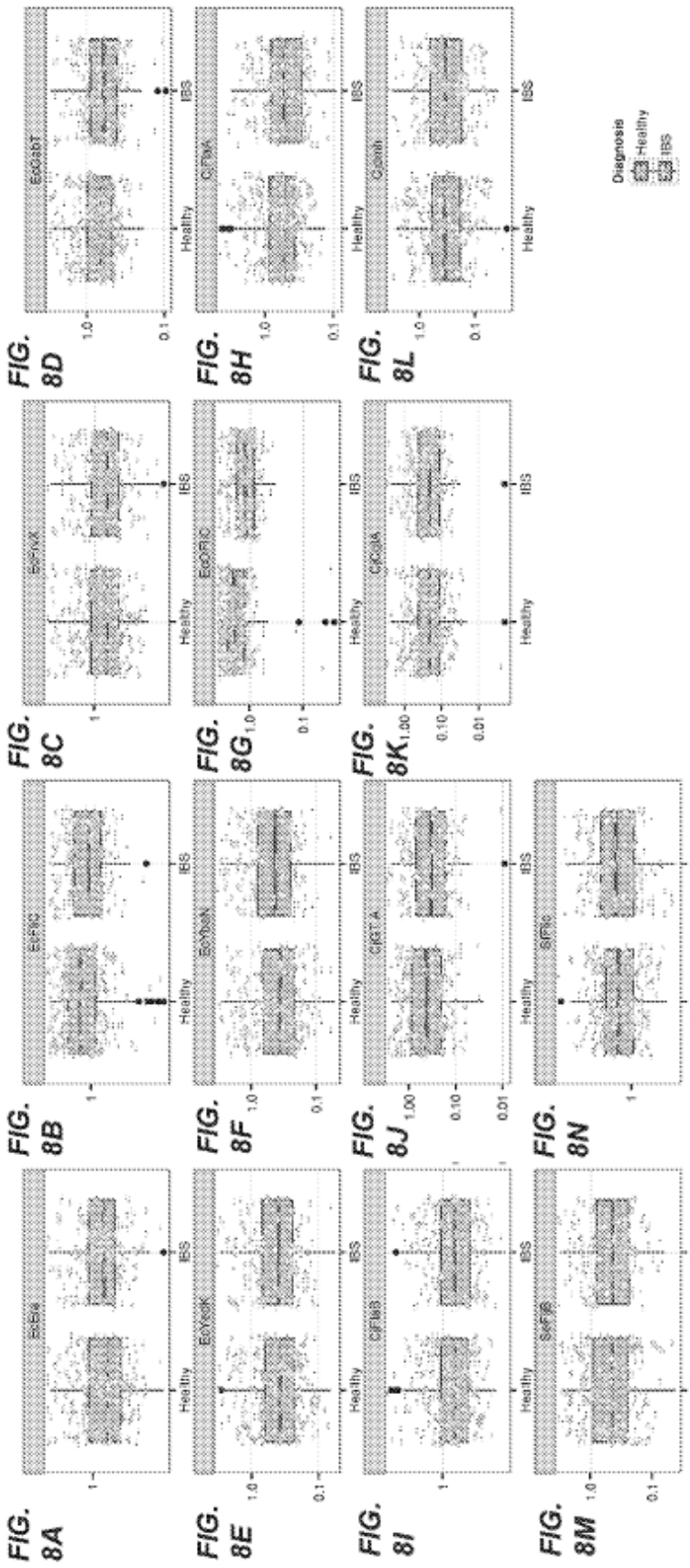

FIG. 8A
EsBst
FIG. 8B
EsFIC
FIG. 8C
EsFnX
FIG. 8D
EsGabT
FIG. 8E
EsYebK
FIG. 8F
EsYbaN
FIG. 8G
EcORID
FIG. 8H
CiTbA
FIG. 8I
EFIB3
FIG. 8J
CiGlT A
FIG. 8K
CiCsiA
FIG. 8L
CiGlnh
FIG. 8M
SeFIB
FIG. 8N
SiFIN6
Healthy
IBS
Diagnosis
Healthy
IBS

FIG. 9A

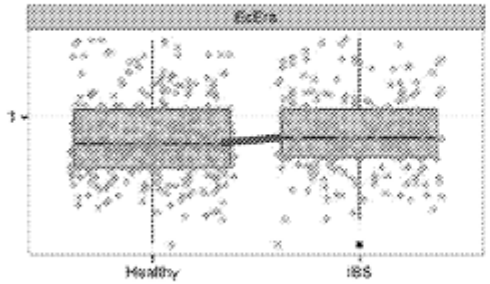


FIG. 9B

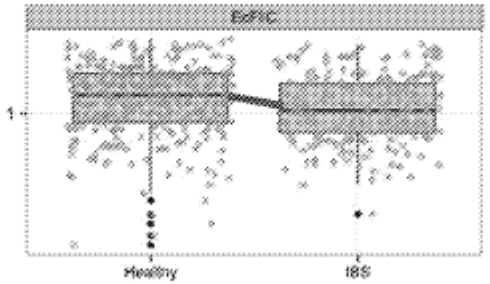
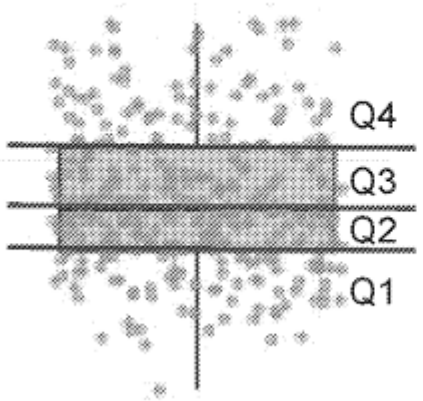


FIG. 9C



1) Logistic regressions including all of the markers.
   Lines represent βs (coefficients from regression/slope).
   Positive for IBS.
   Negative for healthy control.
2) Quartile from healthy controls.
3) Calculate Microbiome Score per subject.

Weighted quartile sum score:
Σ β*quartile over all markers

FIG. 10A

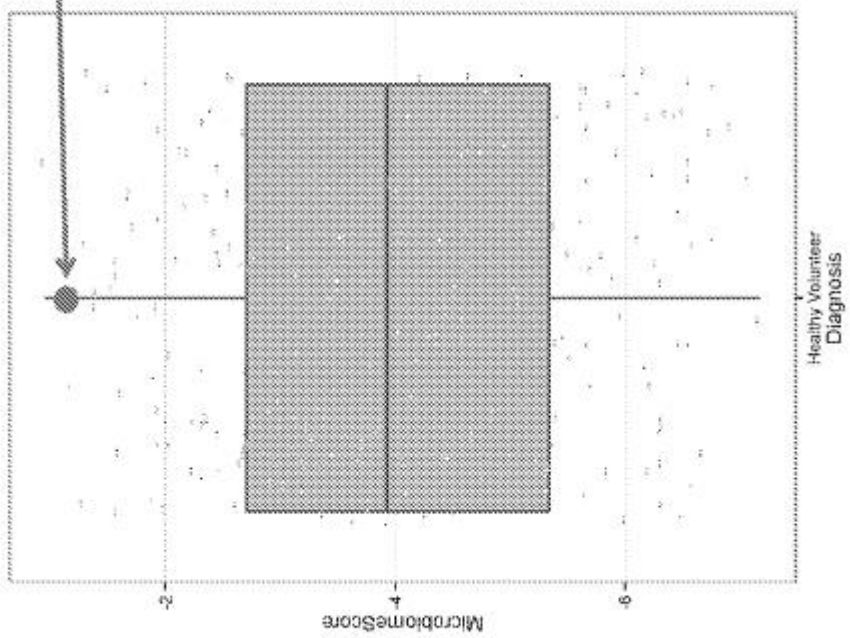


FIG. 10B

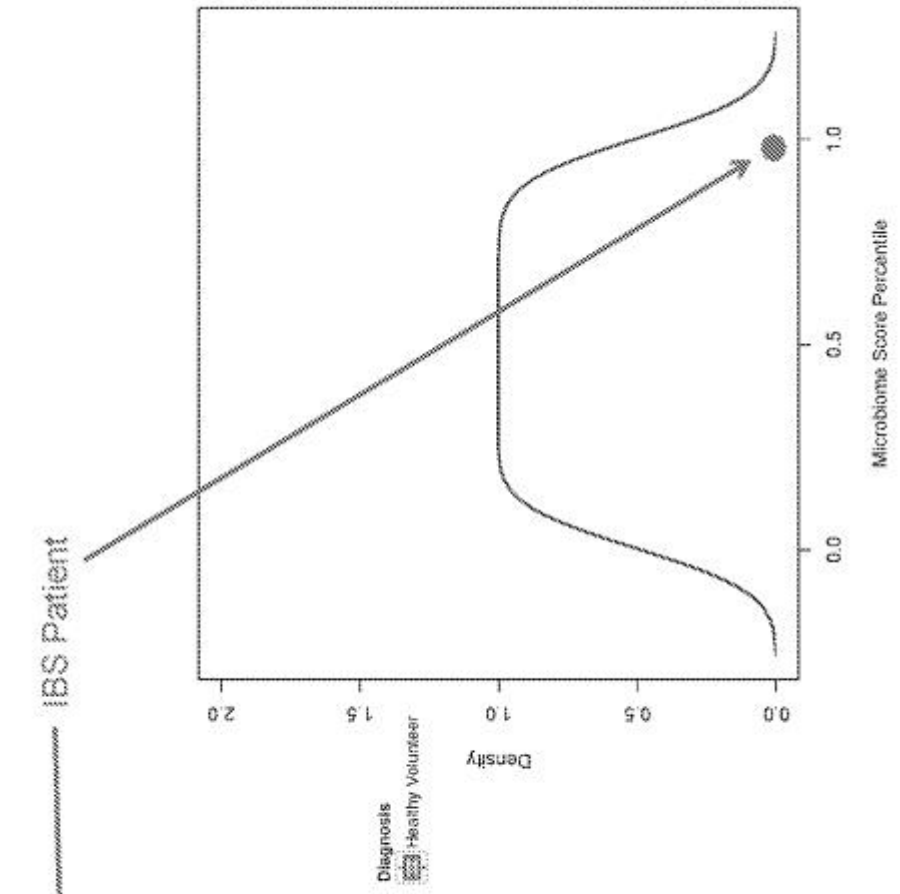


FIG. 11B

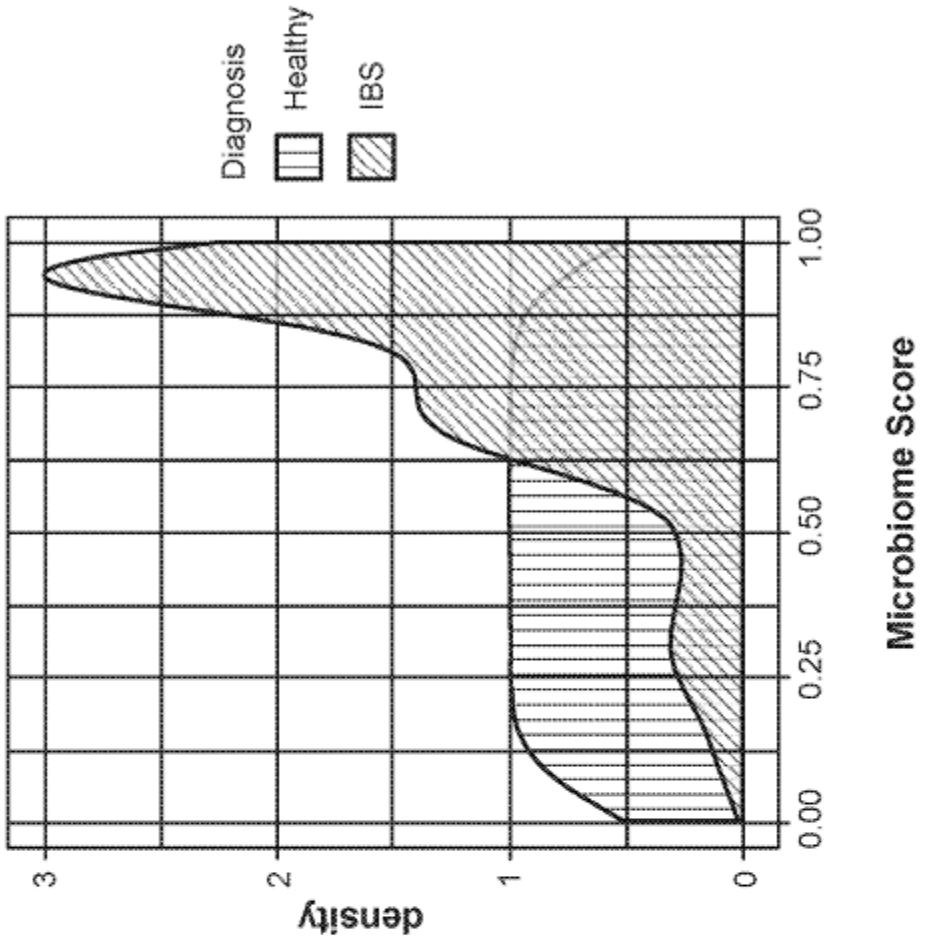


FIG. 11A

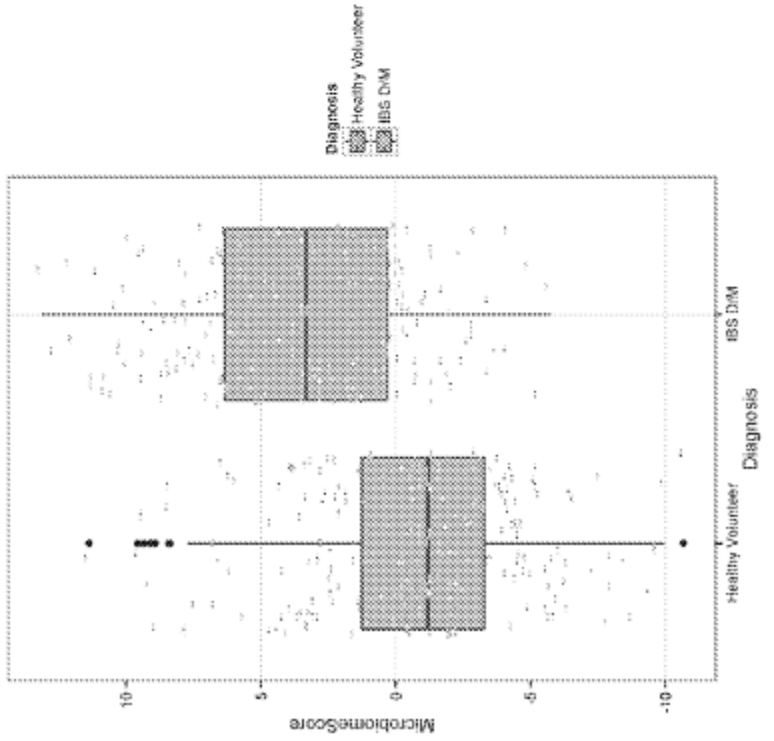


FIG. 12A

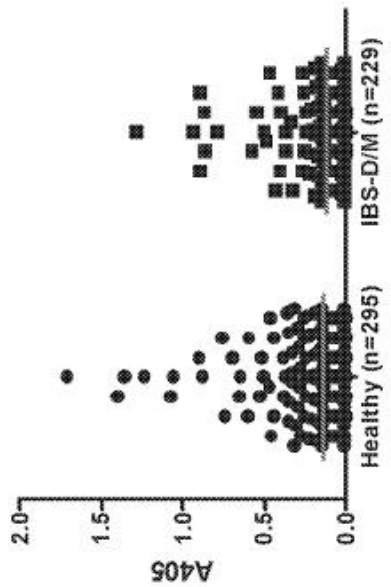


FIG. 12B

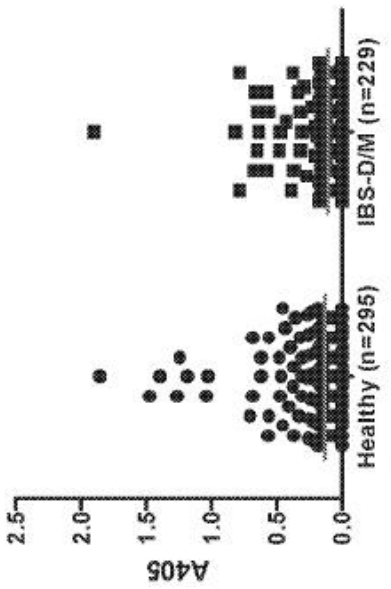


FIG. 12C

FIG. 12D

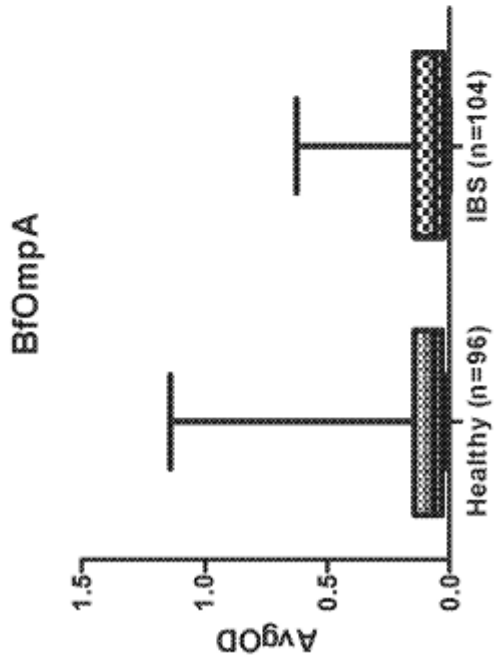


FIG. 12E

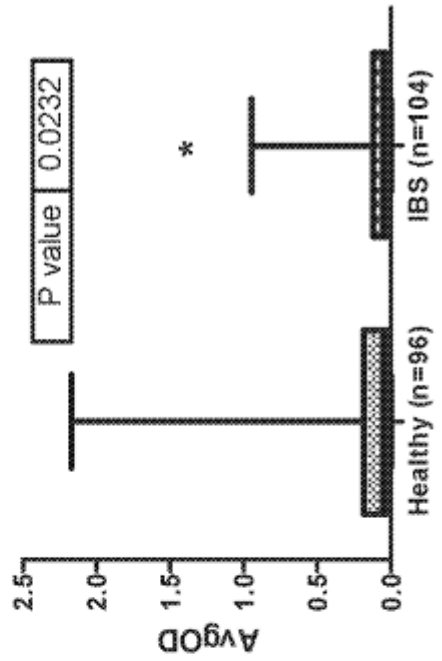


FIG. 13A

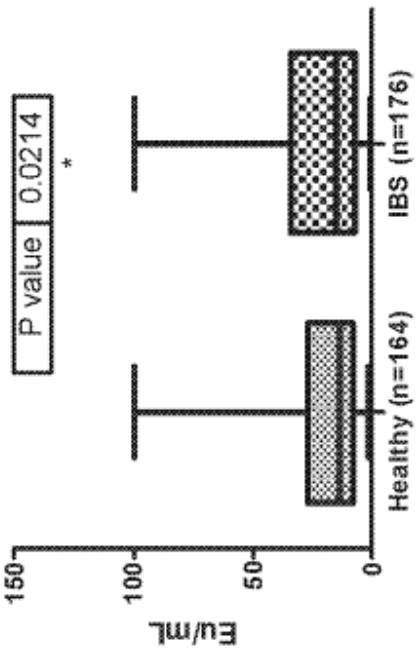


FIG. 13B

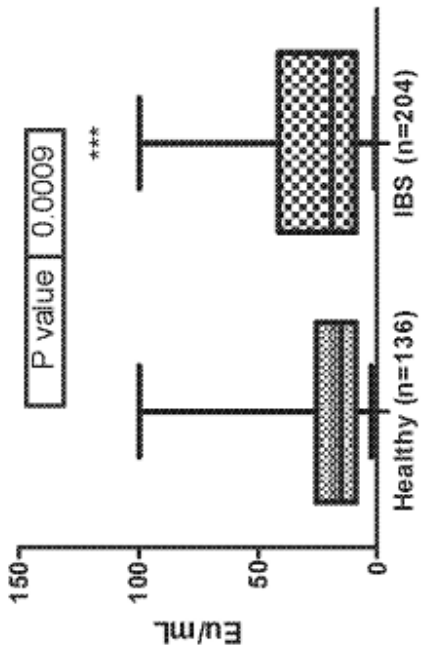


FIG. 13C

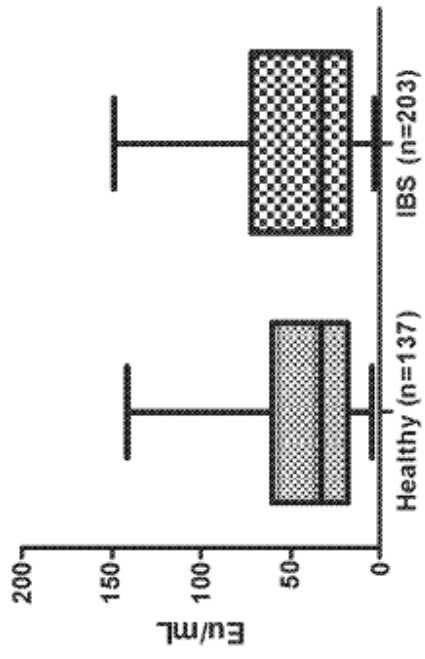


FIG. 13D

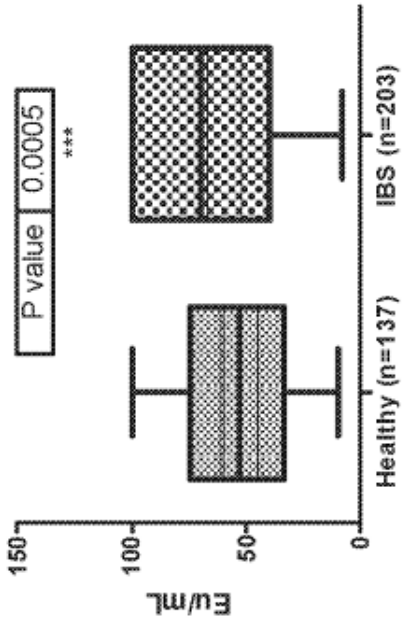


FIG. 13E

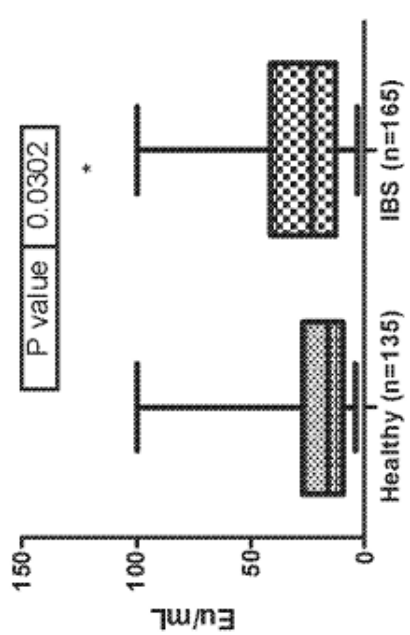


FIG. 13F

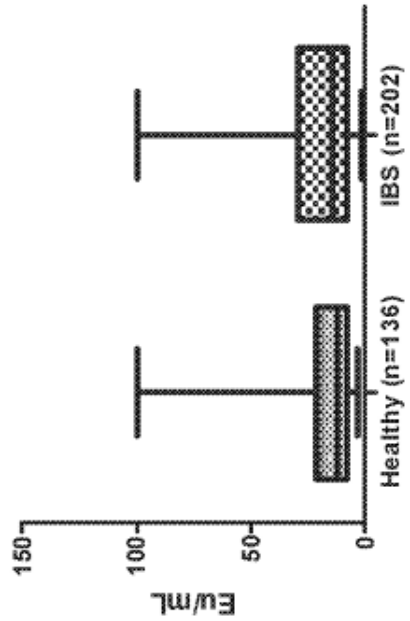


FIG. 13G

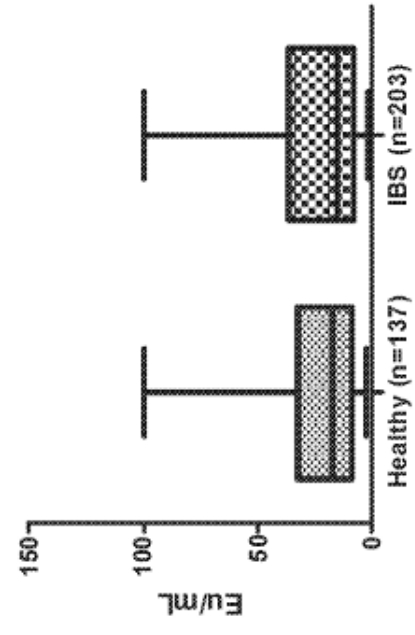


FIG. 13H

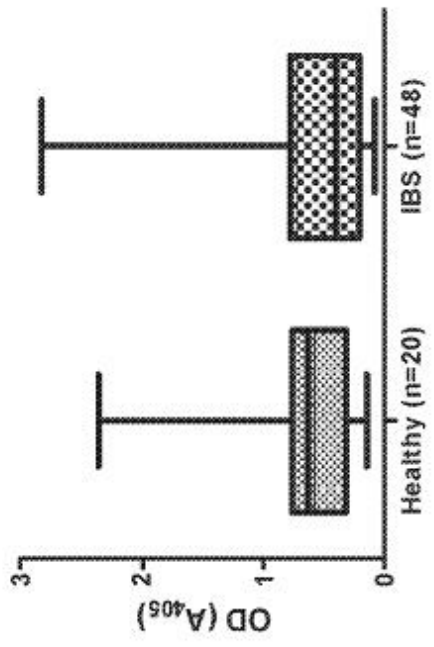


FIG. 13I

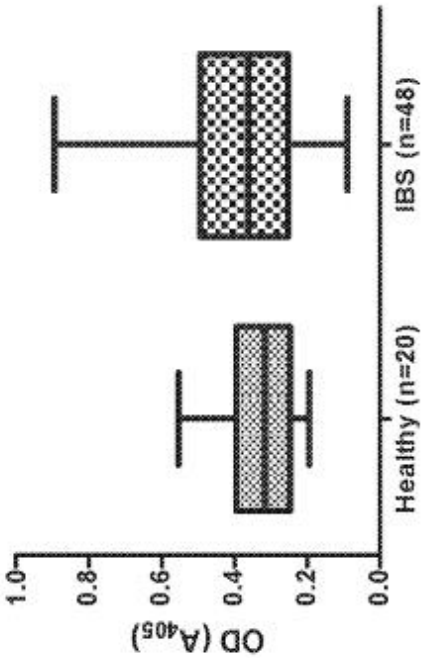


FIG. 13J

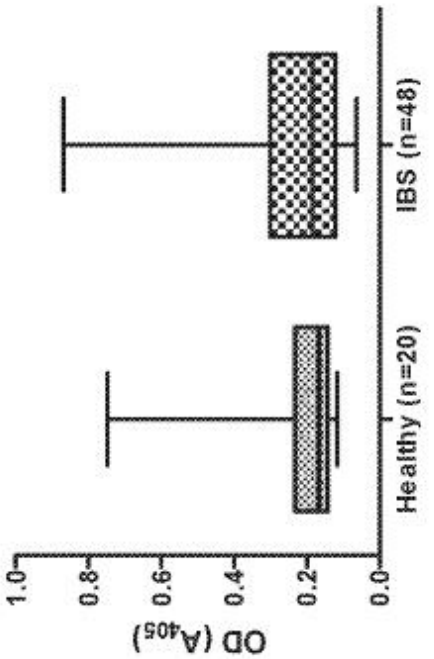


FIG. 13K

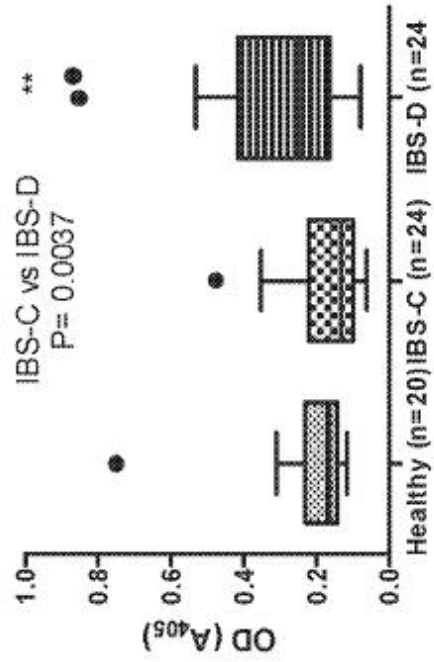


FIG. 14A

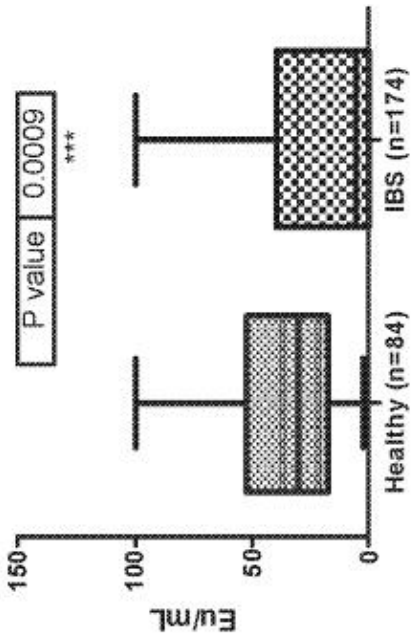


FIG. 14B

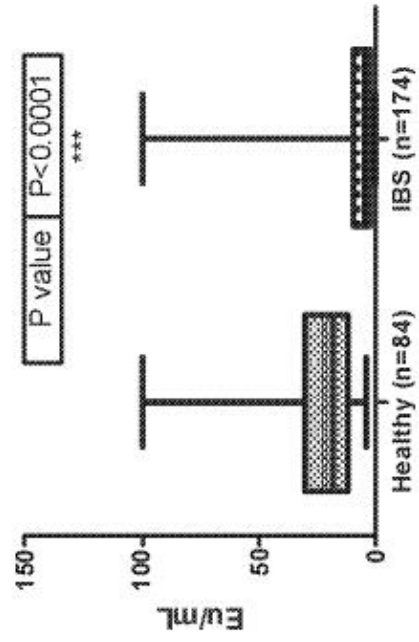


FIG. 14C

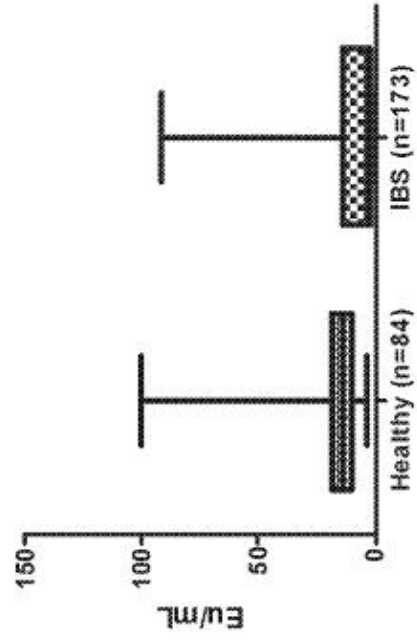


FIG. 14D

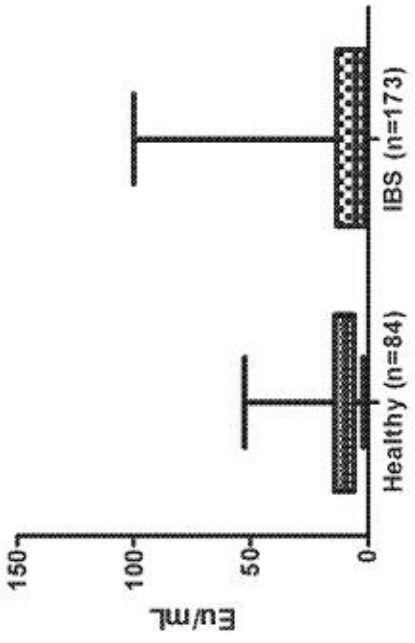


FIG. 14E

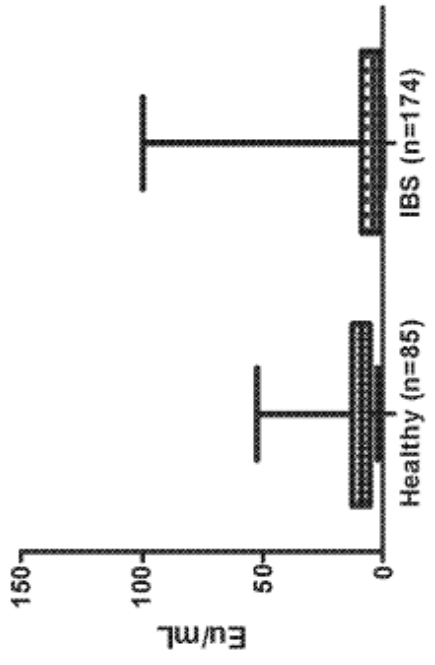


FIG. 14F

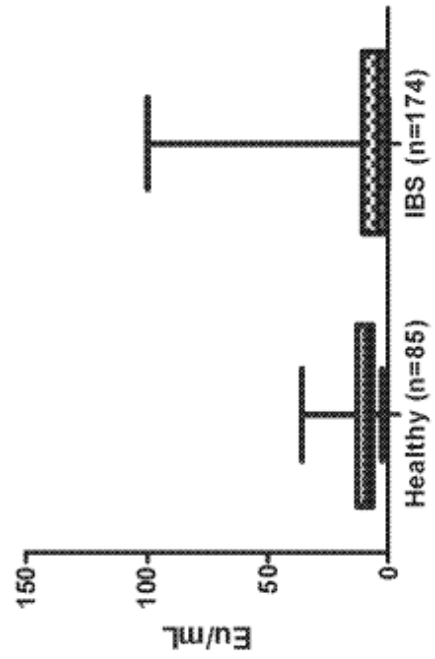


FIG. 14G

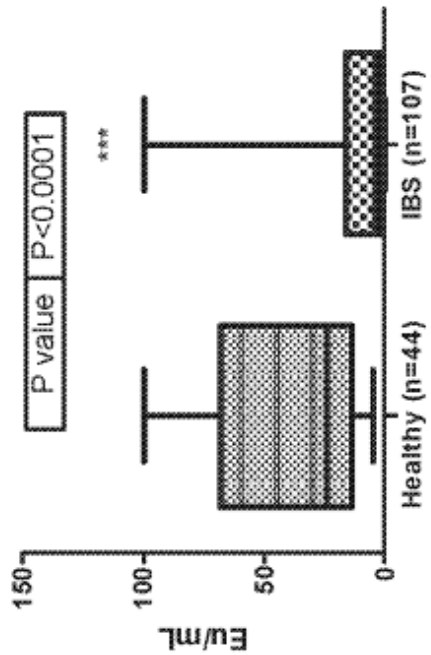


FIG. 15A

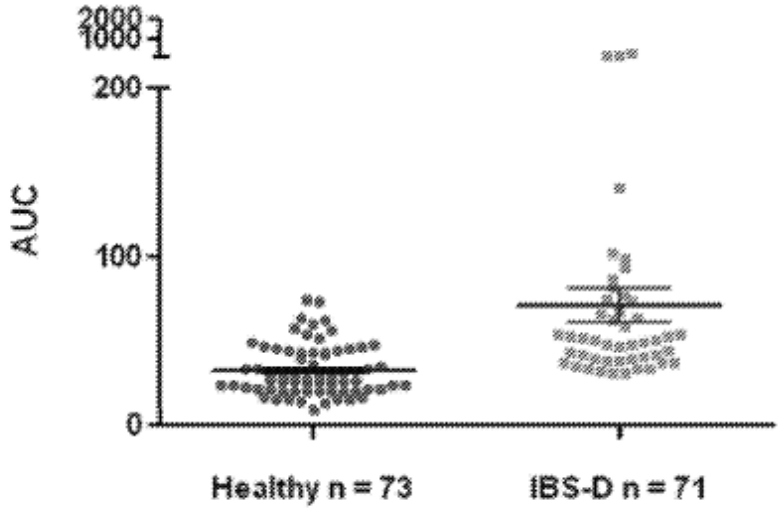


FIG. 15B

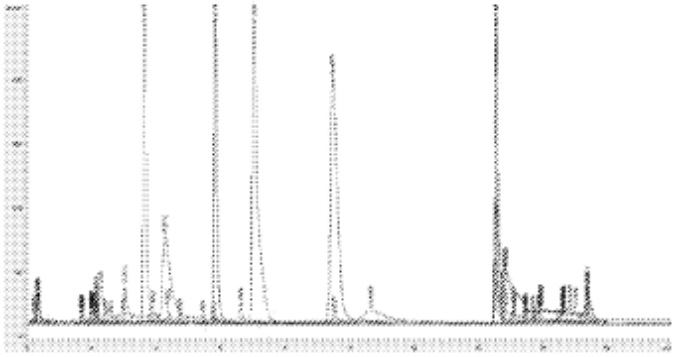


FIG. 15C

	Number of subjects	Mean 5 HT in nM
Healthy	73	33 ± 10
IBS-D	47	55 ± 10

IBS - D	5-HT in nM
Q 1	38.55
Q 2	49.05
Q 3	64.9
Q 4	140.6

Indicates Serotonin dysfunction

FIG. 16A

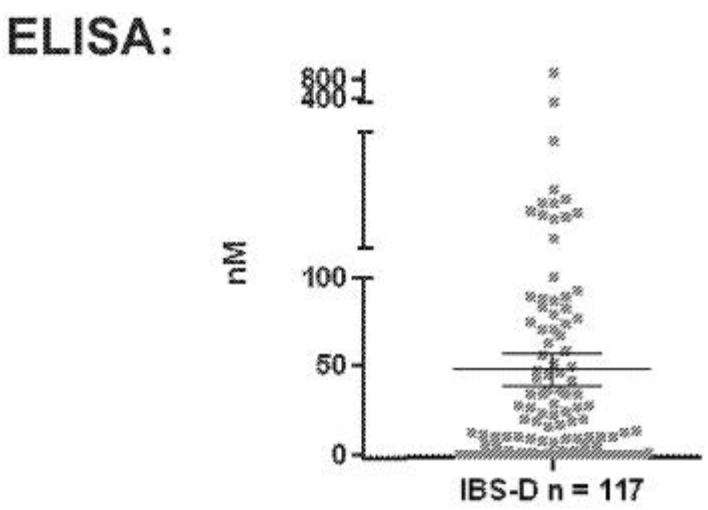


FIG. 16B

	Number of subjects	Mean 5 HT in nM
Healthy	122	23 ± 10
IBS-D	117	50 ± 20

IBS - D	5-HT in nM
Q 1	10.34
Q 2	33.96
Q 3	75.32
Q 4	198.64

Indicates Serotonin dysfunction