

($P<0.001$) (Figure 1A). There were no significant differences in anti-CdtB levels among non-IBS subjects (F-test $P=0.25$).

[0197] Anti-vinculin levels were significantly higher in IBS subjects when compared to all non-IBS subjects (1.3334 ± 0.02 (95% CI 1.30-1.37) compared to 1.01 ± 0.06 (95% CI 0.89-1.12)) ($P<0.001$) (Figure 2A). Differences in anti-vinculin levels among non-IBS subjects were not statistically significant (F-test $P=0.08$).

ELISA comparisons between groups (w/ Celiac Patients)

[0198] Using optical density levels, anti-CdtB antibody levels in D-IBS subjects (2.53 ± 0.69) were significantly higher than healthy subjects (1.81 ± 0.73), Crohn's disease (1.72 ± 0.81), ulcerative colitis (1.54 ± 0.68) and celiac disease (2.23 ± 0.70) ($P<0.001$) (Figure 1B). There were no differences in anti-CdtB levels between healthy subjects and IBD subjects ($p=0.23$); however, subjects with celiac disease had higher anti-CdtB levels than all other non-IBS groups ($p<0.001$).

[0199] Anti-vinculin levels were also significantly higher in D-IBS subjects (1.34 ± 0.85) when compared to healthy subjects (0.81 ± 0.59), Crohn's disease (1.05 ± 0.91), ulcerative colitis (0.96 ± 0.77) and celiac disease (1.07 ± 0.98) ($P<0.0001$) (Figure 2B). Differences in anti-vinculin levels among non-IBS subjects were not statistically significant.

Sensitivity and specificity analyses (w/o Celiac Patients)

[0200] Receiver operating characteristics (ROC) were used to assess the utility of anti-vinculin and anti-CdtB levels in differentiating IBS subjects from non-IBS, IBD and healthy individuals. Figures 3A and 4 demonstrate the ROC curves for these two tests when comparing IBS subjects to all non-IBS subjects and to IBD subjects, respectively. The anti-CdtB test performed better than anti-vinculin and appeared equally discriminating of IBS from all non-IBS subjects and from the IBD group alone. In subgroup analysis, there appeared to be no difference based on the type of IBD (data not shown). The ROC curve for both anti-CdtB and anti-vinculin show that D-IBS can be discriminated from healthy subjects based on this test.

[0201] The optical density (OD) levels for each test were then used to determine the ideal threshold for identification of D-IBS. Tables 2A and 3A demonstrate some potential optical density thresholds for the identification of IBS based on sensitivity, specificity and likelihood ratio. For D-IBS, a higher specificity even when associated with a lower sensitivity

CLAIMS

1. An *in vitro* or *ex vivo* method of distinguishing diarrhoea predominant irritable bowel syndrome (D-IBS) from both inflammatory bowel disease (IBD) and celiac disease comprising:
 - 5 providing a biological sample from a subject desiring a diagnosis to distinguish D-IBS from both IBD and celiac disease, in which the biological sample is whole blood, blood plasma, blood serum, stool, intestinal fluid or aspirate, or stomach fluid or aspirate; detecting in the biological sample, levels of anti-vinculin and anti-CdtB (cytolethal distending toxin B) antibodies; and
 - 10 making a diagnosis that the subject has D-IBS but does not have IBD and does not have celiac disease if the level of anti-vinculin antibodies is significantly higher than an established control level of anti-vinculin antibodies, the level of anti-CdtB antibodies is significantly higher than an established control level of anti-CdtB antibodies, or both levels of anti-vinculin antibodies and anti-CdtB antibodies are significantly higher than
 - 15 the established control levels of anti-vinculin antibodies and anti-CdtB antibodies; in which the established control level of anti-vinculin antibodies is a level of anti-vinculin antibodies from subjects without D-IBS; and in which the established control level of anti-CdtB antibodies is a level of anti-CdtB antibodies from subjects without D-IBS.
- 20 2. A method according to Claim 1, in which the biological sample is whole blood, serum, or plasma.
3. A method according to Claim 1, in which detecting in the biological sample comprises using enzyme-linked immunosorbent assay (ELISA).
4. A method according to Claim 1, in which detecting in the biological sample comprises
- 25 using immunohistochemistry, flow cytometry, fluorescence *in situ* hybridization (FISH), radioimmuno assays, or affinity purification.
5. A method according to Claim 1, in which the anti-vinculin antibodies are capable of binding specifically to an epitope on vinculin or SEQ ID NO: 7.

6. A method according to Claim 1, in which the anti-CdtB antibodies are capable of binding specifically to an epitope on CdtB of *Campylobacter jejuni* or SEQ ID NO: 5.

7. A method according to Claim 1, in which the diagnosis of D-IBS is made if the level of anti-vinculin antibodies is significantly higher than the established control level of anti-
5 vinculin antibodies.

8. A method according to Claim 1, in which the diagnosis of D-IBS is made if the level of anti-CdtB antibodies is significantly higher than the established control level of anti-CdtB antibodies.

9. A method according to Claim 1, in which the diagnosis of D-IBS is made if both levels
10 of anti-vinculin antibodies and anti-CdtB antibodies are significantly higher than the established control levels of anti-vinculin antibodies and anti-CdtB antibodies.

10. A method according to Claim 1, in which the established control level of anti-vinculin antibodies, anti-CdtB antibodies, or both is an optical density measurement.

11. An *in vitro* or *ex vivo* method of selecting a treatment for diarrhoea predominant irritable
15 bowel syndrome (D-IBS) comprising:

providing a biological sample from a subject desiring a diagnosis to distinguish D-IBS from both IBD and celiac disease, in which the biological sample is whole blood, blood plasma, blood serum, stool, intestinal fluid or aspirate, or stomach fluid or aspirate;

detecting in the biological sample, levels of anti-vinculin and anti-CdtB (cytolethal
20 distending toxin B) antibodies;

making a diagnosis that the subject has D-IBS but does not have IBD and does not have celiac disease if the level of anti-vinculin antibodies is significantly higher than an established control level of anti-vinculin antibodies, the level of anti-CdtB antibodies is significantly higher than an established control level of anti-CdtB antibodies, or both
25 levels of anti-vinculin antibodies and anti-CdtB antibodies are significantly higher than the established control levels of anti-vinculin antibodies and anti-CdtB antibodies;

in which the established control level of anti-vinculin antibodies is a level of anti-vinculin antibodies from subjects without D-IBS;

in which the established control level of anti-CdtB antibodies is a level of anti-CdtB antibodies from subjects without D-IBS; and

selecting an D-IBS treatment for the subject if D-IBS and not IBD or celiac disease is diagnosed.

- 5 12. A method according to Claim 11, in which the biological sample is whole blood, serum, or plasma.
13. A method according to Claim 11, in which detecting in the biological sample comprises using enzyme-linked immunosorbent assay (ELISA).
14. A method according to Claim 11, in which detecting in the biological sample comprises
10 using immunohistochemistry, flow cytometry, fluorescence *in situ* hybridization (FISH), radioimmuno assays, or affinity purification.
15. A method according to Claim 11, in which the anti-vinculin antibodies are capable of binding specifically to an epitope on vinculin or SEQ ID NO: 7.
16. A method according to Claim 11, in which the anti-CdtB antibodies are capable of
15 binding specifically to an epitope on CdtB of *Campylobacter jejuni* or SEQ ID NO: 5.
17. A method according to Claim 11, in which the established control level of anti-vinculin antibodies, anti-CdtB antibodies, or both is an optical density measurement.
18. A method according to Claim 11, in which the diagnosis of D-IBS is made if the level of anti-vinculin antibodies is significantly higher than the established control level of anti-
20 vinculin antibodies.
19. A method according to Claim 11, in which the diagnosis of D-IBS is made if the level of anti-CdtB antibodies is significantly higher than the established control level of anti-CdtB antibodies.
20. A method according to Claim 11, in which the diagnosis of D-IBS is made if both levels
25 of anti-vinculin antibodies and anti-CdtB antibodies are significantly higher than the established control levels of anti-vinculin antibodies and anti-CdtB antibodies.