COMPOSITIONS, DEVICES, AND METHODS OF IBS SENSITIVITY TESTING

[0001] This application claims priority to our U.S. provisional patent application with the serial number 62/079783 filed November 14, 2014 which is incorporated by reference herein in its entirety.

Field of the Invention

[0002] The field of the invention is sensitivity testing for food intolerance, and especially as it relates to testing and possible elimination of selected food items as trigger foods for patients diagnosed with or suspected to have irritable bowel syndrome.

Background

[0003] The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0004] Food sensitivity, especially as it relates to irritable bowel syndrome (IBS), often presents with chronic abdominal pain, discomfort, bloating, and/or change in bowel habits and is not well understood in the medical community. Most typically, IBS is diagnosed by elimination of other pathological conditions (*e.g.*, bacterial or protozoan infection, lactose intolerance, etc.) that may have similar or overlapping symptoms. However, IBS is often quite diverse with respect to dietary items triggering symptoms, and no standardized test to help identify trigger food items with a reasonable degree of certainty is known, leaving such patients often to trial-and-error.

[0005] While there are some commercially available tests and labs to help identify trigger foods, the quality of the test results from these labs is generally poor as is reported by a consumer advocacy group (*e.g.*, http://www.which.co.uk/news/2008/08/food-allergy-tests-could-risk-your-health-154711/). Most notably, problems associated with these tests and labs were high false positive rates, high false negative rates, high intra-patient variability, and inter-laboratory variability, rendering such tests nearly useless. Similarly, further inconclusive and highly variable test results were also reported elsewhere (Alternative Medicine Review, Vol. 9, No. 2,

2004: pp 198-207), and the authors concluded that this may be due to food reactions and food sensitivities occurring via a number of different mechanisms. For example, not all IBS patients show positive response to food A, and not all IBS patients show negative response to food B. Thus, even if an IBS patient shows positive response to food A, removal of food A from the patient's diet may not relieve the patient's IBS symptoms. In other words, it is not well determined whether food samples used in the currently available tests are properly selected based on the high probabilities to correlate sensitivities to those food samples to IBS.

[0006] Many have made efforts to select food items or allergens to include in the test panel for immunoassay tests. For example, US Patent Application No. 2007/0122840 to Cousins discloses selection of 29 food allergens that are included in the test panel for ELISA assay. The 29 food allergens are selected based on the frequency of IgG positivity in preliminary experiments with a larger panel of food allergens. However, Cousins fails to teach any quantitative and/or statistical analysis for the selected antigens and as such fails to provide any rationale for the selection. Indeed, Cousin's method to select 29 food allergens for test panel has been criticized that the selection is rather arbitrary. For example, Croft criticized in a paper titled "IgG food antibodies and irritating the bowel", published in Gastroenterology, Vol. 128, Issue 4, p.1135–1136, that Cousin's method is not clear whether the quantity and range of food antibodies being measured are similar or completely different to non-IBS patients or non-food intolerant patients because it lacks controls (normal or non-IBS control subject). Thus, it is at best unclear if Cousins achieves any improvement with respect to false positive and false negative results.

[0007] For another example, US Patent Application No. 2011/0306898 to Stierstorfer discloses selection of 41 food substances as test materials on skin patches. The 41 food substances are selected based on chemical compounds included in the food substances (e.g., vanillin, cinnamic aldehyde, sorbic acid, etc.). The food substances are tested on IBS patients or IBS-suspected patients for allergic contact dermatitis. However, Stierstorfer also fails to disclose how the false positive or false negative food allergens are eliminated and whether the food allergens are selected based on the gender stratification among IgG positivity results.

[0008] All publications identified herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to

be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

[0009] Thus, even though various tests for food sensitivities are known in the art, all or almost all of them suffer from one or more disadvantages. Therefore, there is still a need for improved compositions, devices, and methods of food sensitivity testing, especially for identification and possible elimination of trigger foods for patients identified with or suspected of having IBS.

Summary of The Invention

[0010] The inventive subject matter provides systems and methods for testing food intolerance in patients diagnosed with or suspected to have irritable bowel syndrome. One aspect of the invention is a test kit with for testing food intolerance in patients diagnosed with or suspected to have irritable bowel syndrome. The test kit includes a plurality of distinct food preparations coupled to individually addressable respective solid carriers. The plurality of distinct food preparations have an average discriminatory p-value of ≤ 0.07 as determined by raw p-value or an average discriminatory p-value of ≤ 0.10 as determined by FDR multiplicity adjusted p-value.

[0011] Another aspect of the invention includes a method of testing food intolerance in patients diagnosed with or suspected to have irritable bowel syndrome. The method includes a step of contacting a food preparation with a bodily fluid of a patient that is diagnosed with or suspected to have irritable bowel syndrome. The bodily fluid is associated with a gender identification. It is especially preferred that the step of contacting is performed under conditions that allow IgG from the bodily fluid to bind to at least one component of the food preparation. The method continues with a step of measuring IgG bound to the at least one component of the food preparation to obtain a signal, and then comparing the signal to a gender-stratified reference value for the food preparation using the gender identification to obtain a result. Then, the method also includes a step of updating or generating a report using the result.

[0012] Another aspect of the invention includes a method of generating a test for food intolerance in patients diagnosed with or suspected to have irritable bowel syndrome. The method includes a step of obtaining test results for a plurality of distinct food preparations. The test results are based on bodily fluids of patients diagnosed with or suspected to have irritable

bowel syndrome and bodily fluids of a control group not diagnosed with or not suspected to have irritable bowel syndrome. The method also includes a step of stratifying the test results by gender for each of the distinct food preparations. Then the method continues with a step of assigning for a predetermined percentile rank a different cutoff value for male and female patients for each of the distinct food preparations.

[0013] Still another aspect of the invention includes a use of a plurality of distinct food preparations coupled to individually addressable respective solid carriers in a diagnosis of irritable bowel syndrome. The plurality of distinct food preparations are selected based on their average discriminatory p-value of ≤ 0.07 as determined by raw p-value or an average discriminatory p-value of ≤ 0.10 as determined by FDR multiplicity adjusted p-value.

[0014] Various objects, features, aspects and advantages of the inventive subject matter will become more apparent from the following detailed description of preferred embodiments, along with the accompanying drawing figures in which like numerals represent like components.

Brief Description of The Drawings

[0015] Table 1 shows a list of food items from which food preparations can be prepared.

[0016] Table 2 shows statistical data of foods ranked according to 2-tailed FDR multiplicity-adjusted p-values.

[0017] Table 3 shows statistical data of ELISA score by food and gender.

[0018] Table 4 shows cutoff values of foods for a predetermined percentile rank.

[0019] Figure 1A illustrates ELISA signal score of male IBS patients and control tested with white wheat.

[0020] Figure 1B illustrates a distribution of percentage of male IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with white wheat.

[0021] Figure 1C illustrates a signal distribution in women along with the 95^{th} percentile cutoff as determined from the female control population tested with white wheat.

[0022] Figure 1D illustrates a distribution of percentage of female IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with white wheat.

[0023] Figure 2A illustrates ELISA signal score of male IBS patients and control tested with cocoa.

[0024] Figure 2B illustrates a distribution of percentage of male IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with cocoa.

[0025] Figure 2C illustrates a signal distribution in women along with the 95^{th} percentile cutoff as determined from the female control population tested with cocoa.

[0026] Figure 2D illustrates a distribution of percentage of female IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with cocoa.

[0027] Figure 3A illustrates ELISA signal score of male IBS patients and control tested with rye.

[0028] Figure 3B illustrates a distribution of percentage of male IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with rye.

[0029] Figure 3C illustrates a signal distribution in women along with the 95^{th} percentile cutoff as determined from the female control population tested with rye.

[0030] Figure 3D illustrates a distribution of percentage of female IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with rye.

[0031] Figure 4A illustrates ELISA signal score of male IBS patients and control tested with black tea.

[0032] Figure 4B illustrates a distribution of percentage of male IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with black tea.

[0033] Figure 4C illustrates a signal distribution in women along with the 95^{th} percentile cutoff as determined from the female control population tested with black tea.

[0034] Figure 4D illustrates a distribution of percentage of female IBS subjects exceeding the 90^{th} and 95^{th} percentile tested with black tea.

[0035] Figures 5A-5B illustrate distributions of IBS subjects by number of foods that were identified as trigger foods at the 90^{th} percentile and 95^{th} percentile.

[0036] Table 5 shows raw data of IBS patients and control with number of positive results based on the 90^{th} percentile.

[0037] Table 6 shows statistical data summarizing the raw data of IBS patient populations shown in Table 5.

[0038] Table 7 shows statistical data summarizing the raw data of control populations shown in Table 5.

[0039] Table 8 shows statistical data summarizing the raw data of IBS patient populations shown in Table 5 transformed by logarithmic transformation.

[0040] Table 9 shows statistical data summarizing the raw data of control populations shown in Table 5 transformed by logarithmic transformation.

[0041] Table 10 shows statistical data of an independent T-test to compare the geometric mean number of positive foods between the IBS and non- IBS samples.

[0042] Table 11 shows statistical data of a Mann-Whitney test to compare the geometric mean number of positive foods between the IBS and non- IBS samples.

[0043] Figure 6A illustrates a box and whisker plot of data shown in Table 5.

[0044] Figure 6B illustrates a notched box and whisker plot of data shown in Table 5.

[0045] Figure 7 illustrates the ROC curve corresponding to the statistical data shown in Table 12.

[0046] Table 12 shows statistical data of a Receiver Operating Characteristic (ROC) curve analysis of data shown in Tables 5-11.

[0047] **Table 13** shows statistical data of performance metrics in predicting IBS status among female patients from number of positive foods.

[0048] **Table 14** shows statistical data of performance metrics in predicting IBS status among male patients from number of positive foods.

Detailed Description

[0049] The inventors have discovered that food preparations used in food tests to identify trigger foods in patients diagnosed with or suspected to have IBS are not equally well predictive and/or associated with IBS/IBS symptoms. Indeed, various experiments have revealed that among a wide variety of food items certain food items are highly predictive/associated with IBS whereas others have no statistically significant association with IBS.

[0050] Even more unexpectedly, the inventors discovered that in addition to the high variability of food items, gender variability with respect to response in a test plays a substantial role in the determination of association or a food item with IBS. Consequently, based on the inventors' findings and further contemplations, test kits and methods are now presented with substantially higher predictive power in the choice of food items that could be eliminated for reduction of IBS signs and symptoms.

[0051] The following discussion provides many example embodiments of the inventive subject matter. Although each embodiment represents a single combination of inventive elements, the inventive subject matter is considered to include all possible combinations of the disclosed elements. Thus if one embodiment comprises elements A, B, and C, and a second embodiment comprises elements B and D, then the inventive subject matter is also considered to include other remaining combinations of A, B, C, or D, even if not explicitly disclosed.

[0052] In some embodiments, the numbers expressing quantities or ranges, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported

significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Unless the context dictates the contrary, all ranges set forth herein should be interpreted as being inclusive of their endpoints and open-ended ranges should be interpreted to include only commercially practical values. Similarly, all lists of values should be considered as inclusive of intermediate values unless the context indicates the contrary.

[0053] As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

[0054] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0055] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0056] In one especially preferred aspect, the inventors therefore contemplate a test kit or test panel that is suitable for testing food intolerance in patients where the patient is diagnosed with

or suspected to have irritable bowel syndrome. Most preferably, such test kit or panel will include a plurality of distinct food preparations (*e.g.*, raw or processed extract, preferably aqueous extract with optional co-solvent, which may or may not be filtered) that are coupled to individually addressable respective solid carriers (*e.g.*, in a form of an array or a micro well plate), wherein the distinct food preparations have an average discriminatory p-value of ≤ 0.07 as determined by raw p-value or an average discriminatory p-value of ≤ 0.10 as determined by FDR multiplicity adjusted p-value. As used herein, processed extracts includes food extracts made of food items that are mechanically or chemically modified (e.g., minced, heated, boiled, fermented, smoked, etc.).

[0057] In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, and unless the context dictates the contrary, all ranges set forth herein should be interpreted as being inclusive of their endpoints and open-ended ranges should be interpreted to include only commercially practical values. Similarly, all lists of values should be considered as inclusive of intermediate values unless the context indicates the contrary.

[0058] While not limiting to the inventive subject matter, food preparations will typically be drawn from foods generally known or suspected to trigger signs or symptoms of IBS. Particularly suitable food preparations may be identified by the experimental procedures outlined below. Thus, it should be appreciated that the food items need not be limited to the items described herein, but that all items are contemplated that can be identified by the methods

presented herein. Therefore, exemplary food preparations include at least two, at least four, at least eight, or at least 12 food preparations prepared from cocoa, tea (e.g. green, black, etc.), oat, cabbage, cow milk, onion (e.g. yellow, white, maui, etc.), honey, rye, corn, yeast, wheat (e.g. red, white, etc.), soybean, egg, tuna, lemon, pineapple, cucumber, orange, halibut, walnut, grapefruit, cane sugar, chicken, blueberry, or shrimp (e.g. US Gulf white, Thai, Tiger, etc.). Additionally contemplated food preparations are prepared from Crab (e.g. Dungeness, Blue, Alaskan King, etc.), Barley, Strawberry, Pork, Rice (e.g. Brown, White, etc.), Beef, Cashew, Codfish, Potato, White Sesame, Broccoli, Almond, Turkey, Scallop, and/or Salmon. Still further especially contemplated food items and food additives from which food preparations can be prepared are listed in **Table 1**.

[0059] Using bodily fluids from patients diagnosed with or suspected to have irritable bowel syndrome and healthy control group individuals (*i.e.*, those not diagnosed with or not suspected to have irritable bowel syndrome), numerous additional food items may be identified. Preferably, such identified food items will have high discriminatory power and as such have a p-value of ≤ 0.15, more preferably ≤ 0.10, and most preferably ≤ 0.05 as determined by raw p-value, and/or a p-value of ≤ 0.10, more preferably ≤ 0.08, and most preferably ≤ 0.07 as determined by False Discovery Rate (FDR) multiplicity adjusted p-value.

[0060] Therefore, where a panel has multiple food preparations, it is contemplated that the plurality of distinct food preparations has an average discriminatory p-value of ≤ 0.05 as determined by raw p-value or an average discriminatory p-value of ≤ 0.08 as determined by FDR multiplicity adjusted p-value, or even more preferably an average discriminatory p-value of ≤ 0.025 as determined by raw p-value or an average discriminatory p-value of ≤ 0.07 as determined by FDR multiplicity adjusted p-value. In further preferred aspects, it should be appreciated that the FDR multiplicity adjusted p-value may be adjusted for at least one of age and gender, and most preferably adjusted for both age and gender. On the other hand, where a test kit or panel is stratified for use with a single gender, it is also contemplated that in a test kit or panel at least 50% (and more typically 70% or all) of the plurality of distinct food preparations, when adjusted for a single gender, have an average discriminatory p-value of ≤ 0.07 as determined by raw p-value or an average discriminatory p-value of ≤ 0.10 as determined by FDR multiplicity adjusted p-value. Furthermore, it should be appreciated that other

stratifications (*e.g.*, dietary preference, ethnicity, place of residence, genetic predisposition or family history, etc.) are also contemplated, and the PHOSITA will be readily appraised of the appropriate choice of stratification.

[0061] The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0062] Of course, it should be noted that the particular format of the test kit or panel may vary considerably and contemplated formats include micro well plates, a microfluidic device, dip sticks, membrane-bound arrays, etc. Consequently, the solid carrier to which the food preparations are coupled may include wells of a multiwall plate, a microfluidic device, a (*e.g.*, color-coded or magnetic) bead, or an adsorptive film (*e.g.*, nitrocellulose or micro/nanoporous polymeric film), a chemical sensor, or an electrical sensor, (*e.g.* a printed copper sensor or microchip). In some embodiments, it is also contemplated that a suitable solid carrier for molecular absorption and signal detection by a light detector (e.g., surface plasmon resonance, etc.) can be used.

[0063] Consequently, the inventors also contemplate a method of testing food intolerance in patients that are diagnosed with or suspected to have irritable bowel syndrome. Most typically, such methods will include a step of contacting a food preparation with a bodily fluid (e.g., whole blood, plasma, serum, saliva, or a fecal suspension) of a patient that is diagnosed with or suspected to have irritable bowel syndrome, and wherein the bodily fluid is associated with a gender identification. As noted before, the step of contacting is preferably performed under conditions that allow IgG (or IgE or IgA or IgM) from the bodily fluid to bind to at least one

component of the food preparation, and the IgG bound to the component(s) of the food preparation are then quantified/measured to obtain a signal. Most preferably, the signal is then compared against a gender-stratified reference value (e.g., at least a 90th percentile value) for the food preparation using the gender identification to obtain a result, which is then used to update or generate a report. Preferably, the report can be generated as an aggregate result of individual assay results.

[0064] Most commonly, such methods will not be limited to a single food preparation, but will employ multiple different food preparations. As noted before, suitable food preparations can be identified using various methods as described below, however, especially preferred food preparations include cocoa, tea (e.g. green, black, etc.), oat, cabbage, cow milk, onion (e.g. yellow, white, maui, etc.), honey, rye, corn, yeast, wheat (e.g. red, white, etc.), soybean, egg, tuna, lemon, pineapple, cucumber, orange, halibut, walnut, grapefruit, cane sugar, chicken, blueberry, or shrimp (e.g. US Gulf white, Thai, Tiger, etc.). Additionally contemplated food preparations are prepared from Crab (e.g. Dungeness, Blue, Alaskan King, etc.), Barley, Strawberry, Pork, Rice (e.g. Brown, White, etc.), Beef, Cashew, Codfish, Potato, White Sesame, Broccoli, Almond, Turkey, Scallop, and/or Salmon, and/or items of Table 1. As also noted above, it is generally preferred that at least some, or all of the different food preparations have an average discriminatory p-value of ≤ 0.07 (or ≤ 0.05, or ≤ 0.025) as determined by raw p-value, and/or or an average discriminatory p-value of ≤ 0.10 (or ≤ 0.08, or ≤ 0.07) as determined by FDR multiplicity adjusted p-value.

[0065] While it is preferred that food preparations are prepared from a single food items as crude extracts, or crude filtered extracts, it is contemplated that food preparations can be prepared from mixtures of a plurality of food items (e.g. a mixture of citrus comprising lemon, orange and lime, a mixture of crabs comprising blue crab, king crab and Dungeness crab, a mixture of wheat comprising a white wheat and red wheat, a mixture of shrimp comprising US Gulf white, Thai and Tiger shrimps, etc). In some embodiments, it is also contemplated that food preparations can be prepared from purified food antigens or recombinant food antigens.

[0066] As it is generally preferred that the food preparation is immobilized on a solid surface (typically in an addressable manner), it is contemplated that the step of measuring the IgG or

other type of antibody bound to the component of the food preparation is performed via an immunoassay test (e.g., ELISA test, antibody capture enzyme immunoassay, other types of antibody capture assays, etc.)

[0067] Viewed from a different perspective, the inventors also contemplate a method of generating a test for food intolerance in patients diagnosed with or suspected to have irritable bowel syndrome. Because the test is applied to patients already diagnosed with or suspected to have irritable bowel syndrome, the authors do not contemplate that the method has a primary diagnostic purpose for IBS. Instead, the method is for identifying triggering food items among already diagnosed or suspected IBS patients. Such test will typically include a step of obtaining one or more test results (e.g., ELISA, antibody capture enzyme immunoassay) for various distinct food preparations, wherein the test results are based on bodily fluids (e.g., blood saliva, fecal suspension) of patients diagnosed with or suspected to have irritable bowel syndrome and bodily fluids of a control group not diagnosed with or not suspected to have irritable bowel syndrome. Most preferably, the test results are then stratified by gender for each of the distinct food preparations, a different cutoff value for male and female patients for each of the distinct food preparations (e.g., cutoff value for male and female patients has a difference of at least 10% (abs)) is assigned for a predetermined percentile rank (e.g., 90th or 95th percentile).

[0068] As noted earlier, and while not limiting to the inventive subject matter, it is contemplated that the distinct food preparations include at least two (or six, or ten, or 15) food preparations prepared from food items selected from the group consisting of cocoa, tea (e.g. green, black, etc.), oat, cabbage, cow milk, onion (e.g. yellow, white, maui, etc.), honey, rye, corn, yeast, wheat (e.g. red, white, etc.), soybean, egg, tuna, lemon, pineapple, cucumber, orange, halibut, walnut, grapefruit, cane sugar, chicken, blueberry, or shrimp (e.g. US Gulf white, Thai, Tiger, etc.). Additionally contemplated food preparations are prepared from Crab (e.g. Dungeness, Blue, Alaskan King, etc.), Barley, Strawberry, Pork, Rice (e.g. Brown, White, etc.), Beef, Cashew, Codfish, Potato, White Sesame, Broccoli, Almond, Turkey, Scallop, and/or Salmon, and/or items of Table 1. On the other hand, where new food items are tested, it should be appreciated that the distinct food preparations include a food preparation prepared from a food items other than cocoa, tea (e.g. green, black, etc.), oat, cabbage, cow milk, onion (e.g. yellow, white, maui, etc.), honey, rye, corn, yeast, wheat (e.g. red, white, etc.), soybean, egg, tuna,

lemon, pineapple, cucumber, orange, halibut, walnut, grapefruit, cane sugar, chicken, blueberry, or shrimp (e.g. US Gulf white, Thai, Tiger, etc.).. Regardless of the particular choice of food items, it is generally preferred however, that the distinct food preparations have an average discriminatory p-value of ≤ 0.07 (or ≤ 0.05, or ≤ 0.025) as determined by raw p-value or an average discriminatory p-value of ≤ 0.10 (or ≤ 0.08, or ≤ 0.07) as determined by FDR multiplicity adjusted p-value. Exemplary aspects and protocols, and considerations are provided in the experimental description below.

[0069] Thus, it should be appreciated that by having a high-confidence test system as described herein, the rate of false-positive and false negatives can be significantly reduced, and especially where the test systems and methods are gender stratified or adjusted for gender differences as shown below. Such advantages have heretofore not been realized and it is expected that the systems and methods presented herein will substantially increase the predictive power of food sensitivity tests for patients diagnosed with or suspected to have IBS.

Experiments

[0070] General Protocol for food preparation generation: Commercially available food extracts (available from Biomerica Inc., 17571 Von Karman Ave, Irvine, CA 92614) prepared from the edible portion of the respective raw foods were used to prepare ELISA plates following the manufacturer's instructions.

[0071] For some food extracts, the inventors found that food extracts prepared with specific procedures to generate food extracts provides more superior results in detecting elevated IgG reactivity in IBS patients compared to commercially available food extracts. For example, for grains and nuts, a three-step procedure of generating food extracts is preferred. The first step is a defatting step. In this step, lipids from grains and nuts are extracted by contacting the flour of grains and nuts with a non-polar solvent and collecting residue. Then, the defatted grain or nut flour are extracted by contacting the flour with elevated pH to obtain a mixture and removing the solid from the mixture to obtain the liquid extract. Once the liquid extract is generated, the liquid extract is stabilized by adding an aqueous formulation. In a preferred embodiment, the aqueous formulation includes a sugar alcohol, a metal chelating agent, protease inhibitor, mineral salt,

and buffer component 20-50 mM of buffer from 4-9 pH. This formulation allowed for long term storage at -70 °C and multiple freeze-thaws without a loss of activity.

[0072] For another example, for meats and fish, a two step procedure of generating food extract is preferred. The first step is an extraction step. In this step, extracts from raw, uncooked meats or fish are generated by emulsifying the raw, uncooked meats or fish in an aqueous buffer formulation in a high impact pressure processor. Then, solid materials are removed to obtain liquid extract. Once the liquid extract is generated, the liquid extract is stabilized by adding an aqueous formulation. In a preferred embodiment, the aqueous formulation includes a sugar alcohol, a metal chelating agent, protease inhibitor, mineral salt, and buffer component 20-50 mM of buffer from 4-9 pH. This formulation allowed for long term storage at -70 °C and multiple freeze-thaws without a loss of activity.

[0073] For still another example, for fruits and vegetables, a two step procedure of generating food extract is preferred. The first step is an extraction step. In this step, liquid extracts from fruits or vegetables are generated using an extractor (e.g., masticating juicer, etc) to pulverize foods and extract juice. Then, solid materials are removed to obtain liquid extract. Once the liquid extract is generated, the liquid extract is stabilized by adding an aqueous formulation. In a preferred embodiment, the aqueous formulation includes a sugar alcohol, a metal chelating agent, protease inhibitor, mineral salt, and buffer component 20-50 mM of buffer from 4-9 pH. This formulation allowed for long term storage at -70 °C and multiple freeze-thaws without a loss of activity.

[0074] Blocking of ELISA plates: To optimize signal to noise, plates were blocked with a proprietary blocking buffer. In a preferred embodiment, the blocking buffer includes 20-50 mM of buffer from 4-9 pH, a protein of animal origin and a short chain alcohol. Other blocking buffers, including several commercial preparations, were attempted but could not provide adequate signal to noise and low assay variability required.

[0075] ELISA preparation and sample testing: Food antigen preparations were immobilized onto respective microtiter wells following the manufacturer's instructions. For the assays, the food antigens were allowed to react with antibodies present in the patients' serum, and excess serum proteins were removed by a wash step. For detection of IgG antibody binding, enzyme labeled

anti-IgG antibody conjugate was allowed to react with antigen-antibody complex. A color was developed by the addition of a substrate that reacts with the coupled enzyme. The color intensity was measured and is directly proportional to the concentration of IgG antibody specific to a particular food antigen.

[0076] Methodology to determine ranked food list in order of ability of ELISA signals to distinguish IBS from control subjects: Out of an initial selection (e.g., 100 food items, or 150 food items, or even more), samples can be eliminated prior to analysis due to low consumption in an intended population. In addition, specific food items can be used as being representative of the a larger more generic food group, especially where prior testing has established a correlation among different species within a generic group (most preferably in both genders, but also suitable for correlation for a single gender). For example, Thailand Shrimp could be dropped in favor of U.S. Gulf White Shrimp as representative of the "shrimp" food group, or King Crab could be dropped in favor of Dungeness Crab as representative of the "crab" food group In further preferred aspects, the final list foods is shorter than 50 food items, and more preferably equal or less than of 40 food items.

[0077] Since the foods ultimately selected for the food intolerance panel will not be specific for a particular gender, a gender-neutral food list was necessary. Since the observed sample was imbalanced by gender (*e.g.*, Controls: 22% female, IBS: 64% female), differences in ELISA signal magnitude strictly due to gender were removed by modeling signal scores against gender using a two-sample t-test and storing the residuals for further analysis. For each of the tested foods, residual signal scores were compared between IBS and controls using a permutation test on a two-sample t-test with 50,000 resamplings. The Satterthwaite approximation was used for the denominator degrees of freedom to account for lack of homogeneity of variances, and the 2-tailed permuted p-value represented the raw p-value for each food. False Discovery Rates (FDR) among the comparisons, were adjusted by any acceptable statistical procedures (e.g., Benjamini-Hochberg, Family-wise Error Rate (FWER), Per Comparison Error Rate (PCER), etc.).

[0078] Foods were then ranked according to their 2-tailed FDR multiplicity-adjusted p-values. Foods with adjusted p-values equal to or lower than the desired FDR threshold were deemed to have significantly higher signal scores among IBS than control subjects and therefore deemed

candidates for inclusion into a food intolerance panel. A typical result that is representative of the outcome of the statistical procedure is provided in **Table 2**. Here the ranking of foods is according to 2-tailed permutation T-test p-values with FDR adjustment.

[0079] Notably, the inventors discovered that even for the same food preparation tested, the ELISA score for at least several food items varied dramatically, and exemplary raw data are provided in **Table 3**. As will be readily appreciated, data unstratified by gender will therefore lose significant explanatory power where the same cutoff value is applied to raw data for male and female data. To overcome such disadvantage, the inventors stratified the data by gender as described below.

[0080] Statistical Method for Cutpoint Selection for each Food: The determination of what ELISA signal scores would constitute a "positive" response was made by summarizing the distribution of signal scores among the Control subjects. For each food, IBS subjects who had have observed scores greater than or equal to selected quantiles of the Control subject distribution were deemed "positive". To attenuate the influence of any one subject on cutpoint determination, each food-specific and gender-specific dataset was bootstrap resampled 1000 times. Within each bootstrap replicate, the 90th and 95th percentiles of the Control signal scores were determined. Each IBS subject in the bootstrap sample was compared to the 90th and 95% percentiles to determine whether he/she had a "positive" response. The final 90th and 95th percentile-based cutpoints for each food and gender were computed as the average 90th and 95th percentiles across the 1000 samples. The number of foods for which each IBS subject was rated as "positive" was computed by pooling data across foods. Using such method, the inventors were now able to identify cutoff values for a predetermined percentile rank that in most cases was substantially different as can be taken from **Table 4**.

[0081] Typical examples for the gender difference in IgG response in blood with respect to wheat is shown in **Figures 1A-1D,** where Figure 1A shows the signal distribution in men along with the 95th percentile cutoff as determined from the male control population. Figure 1B shows the distribution of percentage of male IBS subjects exceeding the 90th and 95th percentile, while Figure 1C shows the signal distribution in women along with the 95th percentile cutoff as determined from the female control population. Figure 1D shows the distribution of percentage

of female IBS subjects exceeding the 90th and 95th percentile. In the same fashion, **Figures 2A-2D** exemplarily depict the differential response to cocoa, **Figures 3A-3D** exemplarily depict the differential response to rye, and **Figures 4A-4D** exemplarily depict the differential response to black tea. **Figures 5A-5B** show the distribution of IBS subjects by number of foods that were identified as trigger foods at the 90th percentile (5A) and 95th percentile (5B). Inventors contemplate that regardless of the particular food items, male and female responses were notably distinct.

[0082] It should be noted that nothing in the art have provided any predictable food groups related to IBS that is gender-stratified. Thus, a discovery of food items that show distinct responses by gender is a surprising result, which could not be obviously expected in view of all previously available arts. In other words, selection of food items based on gender stratification provides an unexpected technical effect such that statistical significances for particular food items as triggering food among male or female IBS patients have been significantly improved.

[0083] Normalization of IgG Response Data: While the raw data of the patient's IgG response results can be use to compare strength of response among given foods, it is also contemplated that the IgG response results of a patient are normalized and indexed to generate unit-less numbers for comparison of relative strength of response to a given food. For example, one or more of a patient's food specific IgG results (e.g., IgG specific to Dungeness crab and IgG specific to egg) can be normalized to the patient's total IgG. The normalized value of the patient's IgG specific to Dungeness crab can be 0.1 and the normalized value of the patient's IgG specific to egg can be 0.3. In this scenario, the relative strength of the patient's response to egg is three times higher compared to Dungeness crab. Then, the patient's sensitivity to egg and Dungeness crab can be indexed as such.

[0084] In other examples, one or more of a patient's food specific IgG results (e.g., IgG specific to shrimp and IgG specific to pork) can be normalized to the global mean of that patient's food specific IgG results. The global means of the patient's food specific IgG can be measured by total amount of the patient's food specific IgG. In this scenario, the patient's specific IgG to shrimp can be normalized to the mean of patient's total food specific IgG (e.g., mean of IgG levels to shrimp, pork, Dungeness crab, chicken, peas, etc.) . However, it is also contemplated

that the global means of the patient's food specific IgG can be measured by the patient's IgG levels to a specific type of food via multiple tests. If the patient have been tested for his sensitivity to shrimp five times and to pork seven times previously, the patient's new IgG values to shrimp or to pork are normalized to the mean of five-times test results to shrimp or the mean of seven-times test results to pork. The normalized value of the patient's IgG specific to shrimp can be 6.0 and the normalized value of the patient's IgG specific to pork can be 1.0. In this scenario, the patient has six times higher sensitivity to shrimp at this time compared to his average sensitivity to shrimp, but substantially similar sensitivity to pork. Then, the patient's sensitivity to shrimp and pork can be indexed based on such comparison.

[0085] Methodology to determine the subset of IBS patients with food sensitivities that underlie IBS: While it is suspected that food sensitivities plays a substantial role in signs and symptoms of IBS, some IBS patients may not have food sensitivities that underlie IBS. Those patients would not be benefit from dietary intervention to treat signs and symptoms of IBS. To determine the subset of such patients, body fluid samples of IBS patients and non- IBS patients can be tested with ELISA test using test devices with 24 food samples.

[0086] **Table 5** provides exemplary raw data. As should be readily appreciated, data indicates number of positive results out of 24 sample foods based on 90^{th} percentile value. From the raw data shown in Table 5, average and standard deviation of the number of positive foods was computed for IBS and non-IBS patients. Additionally, the number and percentage of patients with zero positive foods was calculated for both IBS and non-IBS. The number and percentage of patients with zero positive foods is about 5 fold lower in the IBS population than in the non-IBS population (6% vs. 38%, respectively). Thus, it can be easily appreciated that the IBS patient having sensitivity to zero positive foods is unlikely to have food sensitivities underlying their signs and symptoms of IBS.

[0087] **Table 6** and **Table 7** show exemplary statistical data summarizing the raw data of two patient populations shown in Table 5. The statistical data includes normality, arithmetic mean, median, percentiles and 95% confidence interval (CI) for the mean and median representing number of positive foods in the IBS population and the non-IBS population.

[0088] **Table 8** and **Table 9** show another exemplary statistical data summarizing the raw data of two patient populations shown in Table 5. In Tables 8 and 9, the raw data was transformed by logarithmic transformation to improve the data interpretation.

[0089] **Table 10** and **Table 11** show exemplary statistical data of an independent T-test (Table 10, logarithmically transformed data) and a Mann-Whitney test (Table 11) to compare the geometric mean number of positive foods between the IBS and non- IBS samples. The data shown in Table 10 and Table 11 indicates statistically significant differences in the geometric mean of positive number of foods between the IBS population and the non-IBS population. In both statistical tests, it is shown that the number of positive responses with 24 food samples is significantly higher in the IBS population than in the non-IBS population with an average discriminatory p-value of ≤ 0.001. These statistical data is also illustrated as a box and whisker plot in **Figure 6A**, and a notched box and whisker plot in **Figure 6B**.

[0090] **Table 12** shows exemplary statistical data of a Receiver Operating Characteristic (ROC) curve analysis of data shown in Tables 5-11 to determine the diagnostic power of the test used in Table 5 at discriminating IBS from non- IBS subjects. When a cutoff criterion of more than 2 positive foods is used, the test yields a data with 72.4% sensitivity and 72.2% specificity, with an area under the curve (AUROC) of 0.771. The p-value for the ROC is significant at a p-value of <0.0001. **Figure 7** illustrates the ROC curve corresponding to the statistical data shown in Table 12. Because the statistical difference between the IBS population and the non-IBS population is significant when the test results are cut off to positive number of 2, the number of foods that a patient tests positive could be used as a confirmation of the primary clinical diagnosis IBS, and whether it is likely that food sensitivities underlies on the patient's signs and symptoms of IBS. Therefore, the above test can be used as another 'rule in' test to add to currently available clinical criteria for diagnosis for IBS.

[0091] Method for determining distribution of per-person number of foods declared "positive": To determine the distribution of number of "positive" foods per son and measure the diagnostic performance, the analysis was performed with 24 food items from the Table 1, which shows most positive responses to IBS patients. The 24 food items includes Cocoa, Black Tea, Oat, Cabbage, Cow's Milk, Yellow Onion, Honey, Rye, Corn, Yeast White Wheat, Soybean, Egg,

Tuna, Lemon, Pineapple, Cucumber, Orange, Halibut, Walnut, Grapefruit, Cane Sugar, Chicken, US Gulf White Shrimp. To attenuate the influence of any one subject on this analysis, each food-specific and gender-specific dataset was bootstrap resampled 1000 times. Then, for each food item in the bootstrap sample, sex-specific cutpoint was determined using the 90th and 95th percentiles of the control population. Once the sex-specific cutpoints were determined, the sex-specific cutpoints was compared with the observed ELISA signal scores for both control and IBS subjects. In this comparison, if the observed signal is equal or more than the cutpoint value, then it is determined "positive" food, and if the observed signal is less than the cutpoint value, then it is determined "negative" food.

[0092] Once all food items were determined either positive or negative, the results of the 48(24 foods x 2 cutpoints) calls for each subject were saved within each bootstrap replicate. Then, for each subject, 24 calls were summed using 90^{th} percentile as cutpoint to get "Number of Positive Foods(90^{th})," and the rest of 24 calls were summed using 95^{th} percentile to get "Number of Positive Foods(95^{th})." Then, within each replicate, "Number of Positive Foods (90^{th})" and "Number of Positive Foods (95^{th})" were summarized across subjects to get descriptive statistics for each replicate as follows: 1) overall means equals to the mean of means, 2) overall standard deviation equals to the mean of standard deviations, 3) overall medial equals to the mean of medians, 4) overall minimum equals to the minimum of minimums, and 5) overall maximum equals to maximum of maximum. In this analysis, to avoid non-integer "Number of Positive Foods" when computing frequency distribution and histogram, the authors pretended that the 1000 repetitions of the same original dataset were actually 999 sets of new subjects of the same size added to the original sample. Once the summarization of data is done, frequency distributions and historgrams were generated for both "Number of Positive Foods(90^{th})" and "Number of Positive Foods(95^{th})" for both genders and for both IBS subjects and control subjects using programs "a_pos_foods.sas, a_pos_foods_by_dx.sas".

[0093] Method for measuring diagnostic performance: To measure diagnostic performance for each food items for each subject, we used data of "Number of Positive Foods(90^{th})" and "Number of Positive Foods(95^{th})" for each subject within each bootstrap replicate described above. In this analysis, the cutpoint was set to 1. Thus, if a subject has one or more "Number of Positive Foods (90^{th})", then the subject is called "Has IBS." If a subject has less than one

"Number of Positive Foods (90^{th})", then the subject is called "Does Not Have IBS." When all calls were made, the calls were compared with actual diagnosis to determine whether a call was a True Positive(TP), True Negative(TN), False Positive(FP), or False Negative(FN). The comparisons were summarized across subjects to get the performance metrics of sensitivity, specificity, positive predictive value, and negative predictive value for both "Number of Positive Foods(90^{th})" and "Number of Positive Foods(95^{th})" when the cutpoint is set to 1 for each method. Each (sensitivity, 1-specificity) pair becomes a point on the ROC curve for this replicate.

[0094] To increase the accuracy, the analysis above was repeated by incrementing cutpoint from 2 up to 24, and repeated for each of the 1000 bootstrap replicates. Then the performance metrics across the 1000 bootstrap replicates were summarized by calculating averages using a program "t_pos_foods_by_dx.sas". The results of diagnostic performance for female and male are shown in Table 13 (female) and Table 14 (male).

[0095] Of course, it should be appreciated that certain variations in the food preparations may be made without altering the inventive subject matter presented herein. For example, where the food item was yellow onion, that item should be understood to also include other onion varieties that were demonstrated to have equivalent activity in the tests. Indeed, the inventors have noted that for each tested food preparation, certain other related food preparations also tested in the same or equivalent manner (data not shown). Thus, it should be appreciated that each tested and claimed food preparation will have equivalent related preparations with demonstrated equal or equivalent reactions in the test.

[0096] It should be apparent to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not

expressly referenced. Where the specification claims refers to at least one of something selected from the group consisting of A, B, C .... and N, the text should be interpreted as requiring only one element from the group, not A plus N, or B plus N, etc.

Abalone	Cured Cheese	Onion	Walnut, black
Adlay	Cuttlefish	Orange	Watermelon
Almond	Duck	Oyster	Welch Onion
American Cheese	Durian	Papaya	Wheat
Apple	Eel	Paprika	Wheat bran
Artichoke	Egg White (separate)	Parsley	Yeast (S. cerevisiae)
Asparagus	Egg Yolk (separate)	Peach	Yogurt
Avocado	Egg, white/yolk (comb.)	Peanut	
Baby Bok Choy	Eggplant	Pear	**FOOD ADDITIVES**
Bamboo shoots	Garlic	Pepper, Black	Arabic Gum
Banana	Ginger	Pineapple	Carboxymethyl Cellulose
Barley, whole grain	Gluten - Gliadin	Pinto bean	Carrageneenan
Beef	Goat's milk	Plum	FD&C Blue #1
Beets	Grape, white/concord	Pork	FD&C Red #3
Beta-lactoglobulin	Grapefruit	Potato	FD&C Red #40
Blueberry	Grass Carp	Rabbit	FD&C Yellow #5
Broccoli	Green Onion	Rice	FD&C Yellow #6
Buckwheat	Green pea	Roquefort Cheese	Gelatin
Butter	Green pepper	Rye	Guar Gum
Cabbage	Guava	Saccharine	Maltodextrin
Cane sugar	Hair Tail	Safflower seed	Pectin
Cantaloupe	Hake	Salmon	Whey
Caraway	Halibut	Sardine	Xanthan Gum
Carrot	Hazelnut	Scallop	
Casein	Honey	Sesame	
Cashew	Kelp	Shark fin	
Cauliflower	Kidney bean	Sheep's milk	
Celery	Kiwi Fruit	Shrimp	
Chard	Lamb	Sole	
Cheddar Cheese	Leek	Soybean	
Chick Peas	Lemon	Spinach	
Chicken	Lentils	Squashes	
Chili pepper	Lettuce, Iceberg	Squid	
Chocolate	Lima bean	Strawberry	
Cinnamon	Lobster	String bean	
Clam	Longan	Sunflower seed	
Cocoa Bean	Mackerel	Sweet potato	
Coconut	Malt	Swiss cheese	
Codfish	Mango	Taro	
Coffee	Marjoram	Tea, black	
Cola nut	Millet	Tobacco	
Corn	Mung bean	Tomato	
Cottage cheese	Mushroom	Trout	
Cow's milk	Mustard seed	Tuna	
Crab	Oat	Turkey	
Cucumber	Olive	Vanilla	

Table 1

Ranking of Foods according to 2-tailed Permutation T-test p-values with FDR adjustment

Rank	*Food*	*Raw p-value*	*FDR Multiplicity-adj p-value*
1	Cocoa	0.0000	0.0000
2	Black Tea	0.0001	0.0020
3	Oat	0.0002	0.0032
4	Cabbage	0.0004	0.0032
5	Cows Milk	0.0004	0.0032
6	Yellow Onion	0.0006	0.0041
7	Honey	0.0008	0.0044
8	Rye	0.0010	0.0044
9	Corn	0.0010	0.0044
10	Yeast	0.0012	0.0047
11	White Wheat	0.0015	0.0055
12	Soybean	0.0020	0.0066
13	Egg	0.0022	0.0069
14	Tuna	0.0029	0.0084
15	Lemon	0.0036	0.0096
16	Pineapple	0.0045	0.0103
17	Cucumber	0.0046	0.0103
18	Orange	0.0046	0.0103
19	Halibut	0.0057	0.0120
20	Walnut	0.0062	0.0125
21	Grapefruit	0.0085	0.0161
22	Cane Sugar	0.0174	0.0316
23	Chicken	0.0184	0.0321
24	Blueberry	0.0218	0.0363
25	US Gulf White Shrimp	0.0230	0.0367
26	Dungeness Crab	0.0346	0.0533
27	Barley	0.0440	0.0652
28	Strawberry	0.0555	0.0793
29	Pork	0.0976	0.1312
30	Brown Rice	0.0984	0.1312

Rank	*Food*	*Raw p-value*	*FDR Multiplicity-adj p-value*
31	Beef	0.1067	0.1377
32	Cashew	0.1375	0.1718
33	Codfish	0.1741	0.2111
34	Potato	0.2443	0.2825
35	White Sesame	0.2472	0.2825
36	Broccoli	0.2589	0.2876
37	Almond	0.3174	0.3432
38	Turkey	0.4028	0.4240
39	Scallop	0.7149	0.7332
40	Salmon	0.9352	0.9352

Table 2

Basic Descriptive Statistics of ELISA Score by Food and Gender Comparing IBS to Control

				ELISA Score			
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
F	Almond	Control	26	0.227	0.124	0.100	0.565
		IBS	46	0.358	0.474	0.078	3.065
		Diff (1-2)	_	-0.132	0.387	_	_
	Barley	Control	26	0.255	0.144	0.093	0.725
		IBS	46	0.450	0.361	0.118	1.676
		Diff (1-2)	_	-0.195	0.302	_	_
	Beef	Control	26	0.170	0.081	0.086	0.439
		IBS	45	0.190	0.090	0.072	0.467
		Diff (1-2)	_	-0.020	0.087	_	_
	Black Tea	Control	26	0.179	0.075	0.069	0.307
		IBS	46	0.272	0.086	0.115	0.508
		Diff (1-2)	_	-0.093	0.083	_	_
	Blueberry	Control	26	0.425	0.190	0.233	1.061
		IBS	46	0.480	0.143	0.239	0.867
		Diff (1-2)	_	-0.055	0.162	_	_
	Broccoli	Control	26	0.220	0.127	0.118	0.620
		IBS	46	0.280	0.174	0.106	1.042
		Diff (1-2)	_	-0.059	0.159	_	_
	Brown Rice	Control	26	0.236	0.118	0.082	0.449
		IBS	46	0.253	0.136	0.101	0.690
		Diff (1-2)	_	-0.018	0.130	_	_
	Cabbage	Control	26	0.285	0.161	0.086	0.642
		IBS	46	0.432	0.173	0.132	1.033
		Diff (1-2)	_	-0.147	0.169	_	_
	Cane Sugar	Control	26	0.377	0.272	0.070	0.835
		IBS	46	0.521	0.234	0.107	0.975
		Diff (1-2)	_	-0.143	0.248	_	_
	Cashew	Control	26	0.249	0.277	0.080	1.528
		IBS	46	0.286	0.215	0.081	1.183
		Diff (1-2)	_	-0.037	0.239	_	_
	Chicken	Control	25	0.123	0.064	0.056	0.314
		IBS	46	0.156	0.097	0.062	0.579
		Diff (1-2)	_	-0.033	0.087	_	_
	Cocoa	Control	26	0.345	0.151	0.142	0.656

				ELISA Score			
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
		IBS	46	0.587	0.349	0.208	2.030
		Diff (1-2)	_	-0.242	0.294	_	_
	Codfish	Control	26	0.202	0.081	0.083	0.392
		IBS	46	0.182	0.180	0.048	1.069
		Diff (1-2)	_	0.020	0.152	_	_
	Corn	Control	26	0.416	0.221	0.114	0.923
		IBS	47	0.562	0.333	0.146	1.686
		Diff (1-2)	_	-0.146	0.298	_	_
	Cow Milk	Control	26	0.676	0.538	0.074	2.212
		IBS	46	1.191	0.845	0.134	3.035
		Diff (1-2)	_	-0.515	0.750	_	_
	Cucumber	Control	26	0.168	0.083	0.079	0.317
		IBS	46	0.211	0.071	0.088	0.460
		Diff (1-2)	_	-0.043	0.075	_	_
	Dungeness Crab	Control	26	0.321	0.187	0.107	0.757
		IBS	46	0.390	0.226	0.082	1.285
		Diff (1-2)	_	-0.068	0.213	_	_
	Egg	Control	26	0.336	0.296	0.060	1.119
		IBS	46	0.903	0.858	0.115	3.274
		Diff (1-2)	_	-0.567	0.710	_	_
	Grapefruit	Control	25	0.154	0.088	0.069	0.458
		IBS	46	0.203	0.148	0.085	1.014
		Diff (1-2)	_	-0.049	0.130	_	_
	Halibut	Control	26	0.246	0.125	0.093	0.544
		IBS	46	0.348	0.198	0.103	0.941
		Diff (1-2)	_	-0.101	0.175	_	_
	Honey	Control	26	0.584	0.306	0.152	1.463
		IBS	46	0.805	0.364	0.200	1.638
		Diff (1-2)	_	-0.220	0.344	_	_
	Lemon	Control	26	0.282	0.157	0.080	0.635
		IBS	45	0.444	0.297	0.120	1.567
		Diff (1-2)	_	-0.162	0.255	_	_
	Oat	Control	26	0.282	0.253	0.071	1.116
		IBS	47	0.693	0.680	0.086	2.934
		Diff (1-2)	_	-0.411	0.567	_	_
	Orange	Control	26	0.222	0.119	0.080	0.549
		IBS	46	0.313	0.166	0.106	1.044
		Diff (1-2)	_	-0.091	0.151	_	_

				ELISA Score			
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
	Pineapple	Control	26	0.924	0.853	0.098	3.467
		IBS	47	1.624	1.015	0.206	3.721
		Diff (1-2)	_	-0.700	0.961	_	_
	Pork	Control	26	0.392	0.266	0.107	1.285
		IBS	46	0.466	0.283	0.064	1.248
		Diff (1-2)	_	-0.074	0.277	_	_
	Potato	Control	26	0.209	0.104	0.075	0.441
		IBS	46	0.266	0.089	0.087	0.474
		Diff (1-2)	_	-0.057	0.095	_	_
	Rye	Control	26	0.138	0.054	0.073	0.299
		IBS	47	0.249	0.193	0.080	1.248
		Diff (1-2)	_	-0.111	0.159	_	_
	Salmon	Control	26	0.230	0.140	0.102	0.684
		IBS	46	0.196	0.100	0.058	0.444
		Diff (1-2)	_	0.034	0.116	_	_
	Scallop	Control	25	0.283	0.205	0.086	1.025
		IBS	46	0.277	0.173	0.102	0.860
		Diff (1-2)	_	0.005	0.185	_	_
	Soybean	Control	26	0.508	0.228	0.210	0.849
		IBS	46	0.658	0.230	0.252	1.101
		Diff (1-2)	_	-0.150	0.229	_	_
	Strawberry	Control	26	0.145	0.059	0.060	0.259
		IBS	46	0.176	0.056	0.075	0.370
		Diff (1-2)	_	-0.031	0.057	_	_
	Tuna	Control	26	0.588	0.297	0.202	1.375
		IBS	46	0.859	0.431	0.181	1.875
		Diff (1-2)	_	-0.271	0.388	_	_
	Turkey	Control	26	0.248	0.110	0.072	0.583
		IBS	46	0.267	0.110	0.112	0.691
		Diff (1-2)	_	-0.019	0.110	_	_
	US Gulf White Shrimp	Control	26	0.563	0.325	0.188	1.693
		IBS	45	0.834	0.459	0.210	2.135
		Diff (1-2)	_	-0.271	0.415	_	_
	Walnut	Control	26	0.194	0.070	0.096	0.315
		IBS	46	0.273	0.123	0.135	0.944
		Diff (1-2)	_	-0.079	0.107	_	_
	White Sesame	Control	26	0.705	0.524	0.190	2.663
		IBS	46	0.734	0.393	0.126	1.967

				ELISA Score			
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
		Diff (1-2)	_	-0.029	0.444	_	_
	White Wheat	Control	26	0.228	0.125	0.106	0.576
		IBS	47	0.427	0.355	0.096	1.872
		Diff (1-2)	_	-0.198	0.295	_	_
	Yeast	Control	25	0.963	0.624	0.157	2.364
		IBS	46	1.291	0.844	0.247	3.438
		Diff (1-2)	_	-0.327	0.775	_	_
	Yellow Onion	Control	26	0.570	0.442	0.105	1.497
		IBS	46	0.911	0.439	0.129	1.791
		Diff (1-2)	_	-0.341	0.440	_	_
M	Almond	Control	89	0.335	0.391	0.077	2.342
		IBS	29	0.361	0.341	0.069	1.442
		Diff (1-2)	_	-0.026	0.379	_	_
	Barley	Control	89	0.419	0.430	0.110	2.242
		IBS	29	0.525	0.499	0.092	1.935
		Diff (1-2)	_	-0.106	0.448	_	_
	Beef	Control	73	0.184	0.127	0.081	0.979
		IBS	27	0.222	0.102	0.078	0.555
		Diff (1-2)	_	-0.038	0.121	_	_
	Black Tea	Control	89	0.209	0.088	0.080	0.522
		IBS	29	0.242	0.076	0.118	0.395
		Diff (1-2)	_	-0.033	0.086	_	_
	Blueberry	Control	89	0.425	0.228	0.216	2.031
		IBS	29	0.517	0.207	0.278	1.424
		Diff (1-2)	_	-0.092	0.223	_	_
	Broccoli	Control	89	0.242	0.204	0.096	1.747
		IBS	29	0.263	0.194	0.133	1.116
		Diff (1-2)	_	-0.021	0.201	_	_
	Brown Rice	Control	89	0.237	0.123	0.081	0.714
		IBS	29	0.288	0.122	0.090	0.554
		Diff (1-2)	_	-0.051	0.122	_	_
	Cabbage	Control	89	0.322	0.173	0.089	0.873
		IBS	29	0.409	0.192	0.105	0.878
		Diff (1-2)	_	-0.087	0.178	_	_
	Cane Sugar	Control	89	0.375	0.255	0.076	1.097
		IBS	29	0.446	0.230	0.098	0.804
		Diff (1-2)	_	-0.071	0.249	_	_
	Cashew	Control	89	0.230	0.157	0.078	1.152

			ELISA Score				
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
		IBS	29	0.291	0.167	0.072	0.686
		Diff (1-2)	_	-0.062	0.160	_	_
	Chicken	Control	88	0.134	0.069	0.055	0.339
		IBS	29	0.172	0.087	0.055	0.385
		Diff (1-2)	_	-0.037	0.074	_	_
	Cocoa	Control	89	0.399	0.198	0.158	1.386
		IBS	29	0.623	0.294	0.209	1.310
		Diff (1-2)	_	-0.224	0.225	_	_
	Codfish	Control	89	0.198	0.191	0.072	1.759
		IBS	29	0.146	0.053	0.071	0.325
		Diff (1-2)	_	0.053	0.169	_	_
	Corn	Control	89	0.414	0.240	0.137	1.185
		IBS	29	0.618	0.330	0.183	1.310
		Diff (1-2)	_	-0.204	0.265	_	_
	Cow Milk	Control	89	0.805	0.621	0.095	2.416
		IBS	29	1.309	0.946	0.128	3.525
		Diff (1-2)	_	-0.504	0.713	_	_
	Cucumber	Control	89	0.167	0.069	0.070	0.343
		IBS	29	0.197	0.070	0.079	0.322
		Diff (1-2)	_	-0.030	0.069	_	_
	Dungeness Crab	Control	89	0.342	0.175	0.104	1.047
		IBS	29	0.431	0.249	0.082	0.992
		Diff (1-2)	_	-0.089	0.195	_	_
	Egg	Control	89	0.407	0.385	0.071	1.799
		IBS	29	0.609	0.681	0.094	2.589
		Diff (1-2)	_	-0.202	0.474	_	_
	Grapefruit	Control	88	0.182	0.100	0.063	0.613
		IBS	29	0.260	0.185	0.058	0.658
		Diff (1-2)	_	-0.079	0.126	_	_
	Halibut	Control	89	0.284	0.153	0.105	0.751
		IBS	29	0.379	0.267	0.112	1.477
		Diff (1-2)	_	-0.095	0.187	_	_
	Honey	Control	89	0.644	0.358	0.210	2.059
		IBS	29	0.911	0.557	0.227	2.635
		Diff (1-2)	_	-0.267	0.415	_	_
	Lemon	Control	89	0.358	0.239	0.091	1.254
		IBS	29	0.503	0.375	0.082	1.709
		Diff (1-2)	_	-0.146	0.278	_	_

				ELISA Score			
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
	Oat	Control	89	0.384	0.378	0.066	1.972
		IBS	29	0.745	0.666	0.101	2.614
		Diff (1-2)	_	-0.361	0.464	_	_
	Orange	Control	89	0.265	0.152	0.079	0.831
		IBS	29	0.358	0.252	0.076	1.126
		Diff (1-2)	_	-0.093	0.181	_	_
	Pineapple	Control	89	1.053	0.915	0.123	3.617
		IBS	29	1.354	0.883	0.287	3.126
		Diff (1-2)	_	-0.301	0.908	_	_
	Pork	Control	89	0.388	0.219	0.110	1.216
		IBS	29	0.462	0.244	0.126	1.057
		Diff (1-2)	_	-0.075	0.225	_	_
	Potato	Control	89	0.249	0.170	0.069	1.408
		IBS	29	0.253	0.125	0.100	0.578
		Diff (1-2)	_	-0.004	0.161	_	_
	Rye	Control	89	0.178	0.100	0.079	0.610
		IBS	29	0.237	0.129	0.088	0.651
		Diff (1-2)	_	-0.060	0.108	_	_
	Salmon	Control	89	0.206	0.132	0.073	0.897
		IBS	29	0.229	0.185	0.114	1.065
		Diff (1-2)	_	-0.022	0.147	_	_
	Scallop	Control	88	0.294	0.187	0.095	0.973
		IBS	29	0.320	0.222	0.067	1.161
		Diff (1-2)	_	-0.026	0.196	_	_
	Soybean	Control	89	0.523	0.292	0.175	1.653
		IBS	29	0.715	0.490	0.187	2.583
		Diff (1-2)	_	-0.191	0.351	_	_
	Strawberry	Control	89	0.151	0.058	0.062	0.311
		IBS	29	0.161	0.053	0.077	0.252
		Diff (1-2)	_	-0.010	0.057	_	_
	Tuna	Control	89	0.725	0.369	0.183	1.752
		IBS	29	0.899	0.459	0.213	1.952
		Diff (1-2)	_	-0.174	0.393	_	_
	Turkey	Control	89	0.252	0.109	0.100	0.711
		IBS	29	0.265	0.099	0.109	0.510
		Diff (1-2)	_	-0.013	0.107	_	_
	US Gulf White Shrimp	Control	89	0.709	0.366	0.226	1.982
		IBS	29	0.797	0.422	0.222	1.550

				ELISA Score			
Sex	*Food*	*Diagnosis*	*N*	*Mean*	*SD*	*Min*	*Max*
		Diff (1-2)	_	-0.089	0.380	_	_
	Walnut	Control	89	0.216	0.110	0.095	0.839
		IBS	29	0.244	0.085	0.101	0.428
		Diff (1-2)	_	-0.029	0.104	_	_
	White Sesame	Control	89	0.620	0.375	0.112	1.855
		IBS	29	0.744	0.412	0.153	1.970
		Diff (1-2)	_	-0.124	0.385	_	_
	White Wheat	Control	89	0.290	0.250	0.095	1.681
		IBS	29	0.463	0.400	0.130	1.561
		Diff (1-2)	_	-0.173	0.293	_	_
	Yeast	Control	88	0.940	0.624	0.172	3.157
		IBS	29	1.481	0.788	0.416	2.892
		Diff (1-2)	_	-0.541	0.668	_	_
	Yellow Onion	Control	89	0.558	0.418	0.094	1.672
		IBS	29	0.760	0.417	0.098	1.507
		Diff (1-2)	_	-0.203	0.418	_	_

Table 3

Upper Quantiles of ELISA Signal Scores among Control Subjects as Candidates for Test Cutpoints in Determining "Positive" or "Negative"

Top 24 Foods Ranked by Descending order of Discriminatory Ability using Permutation Test

			Cutpoint	
Food Ranking	*Food*	*Sex*	*90th percentile*	*95th percentile*
1	Cocoa	F	0.544	0.587
		M	0.581	0.721
2	BlackTea	F	0.281	0.296
		M	0.337	0.379
3	Oat	F	0.647	0.813
		M	0.880	1.187
4	Cabbage	F	0.507	0.573
		M	0.542	0.644
5	CowsMilk	F	1.373	1.611
		M	1.872	2.133
6	YellowOnion	F	1.109	1.214
		M	1.142	1.328
7	Honey	F	1.022	1.189
		M	1.111	1.422
8	Rye	F	0.209	0.237
		M	0.313	0.400
9	Corn	F	0.755	0.835
		M	0.774	0.904
10	Yeast	F	1.811	2.014
		M	1.883	2.102
11	WhiteWheat	F	0.409	0.477
		M	0.492	0.704
12	Soybean	F	0.778	0.806
		M	0.891	1.076
13	Egg	F	0.794	0.932
		M	0.988	1.270
14	Tuna	F	1.054	1.208
		M	1.276	1.472
15	Lemon	F	0.533	0.585
		M	0.705	0.885
16	Pineapple	F	2.139	2.646
		M	2.651	3.030
17	Cucumber	F	0.289	0.305

			Cutpoint	
Food Ranking	*Food*	*Sex*	*90th percentile*	*95th percentile*
		M	0.265	0.301
18	Orange	F	0.389	0.456
		M	0.483	0.589
19	Halibut	F	0.451	0.497
		M	0.506	0.600
20	Walnut	F	0.288	0.297
		M	0.319	0.387
21	Grapefruit	F	0.267	0.333
		M	0.328	0.380
22	CaneSugar	F	0.739	0.775
		M	0.746	0.834
23	Chicken	F	0.214	0.249
		M	0.250	0.275
24	Blueberry	F	0.676	0.807
		M	0.630	0.787

Table 4

IBS Population	
Sample ID	# of Positive Results based on 90th Percentile
IBS-3	5
IBS-5	0
IBS-11	1
IBS-12	9
IBS-14	0
IBS-18	0
IBS-19	1
IBS-23	11
IBS-24	1
IBS-30	9
IBS-33	8
IBS-35	7
IBS-38	6
IBS-40	3
IBS-42	6
IBS-43	2
BRH-698596	4
BHR-698597	9
BRH-698598	4
BRH-698599	18
BRH-698600	3
BRH-698621	12
BRH-698622	7
BRH-698623	5
BRH-698624	1
BRH-698625	9
BRH-774496	17
BRH-763476	0
BRH-768030	0
IBS-2	1
IBS-4	1
IBS-6	1
IBS-7	9
IBS-8	9
IBS-9	1
IBS-10	14
IBS-13	19
IBS-15	9
IBS-16	1
IBS-17	9
IBS-20	16
IBS-21	23
IBS-22	20
IBS-25	2

Non-IBS Population	
Sample ID	# of Positive Results based on 90th
BRH-768035	0
BRH-768034	1
BRH-768033	1
BRH-768032	1
BRH-768031	1
BRH-763484	0
BRH-768029	10
BRH-768028	8
BRH-763510	4
BRH-768036	0
BRH-768037	1
BRH-768038	1
BRH-768039	0
BRH-768040	0
BRH-768041	1
BRH-768042	1
BRH-768043	5
BRH-768044	3
BRH-768055	1
BRH-768054	1
BRH-764371	0
BRH-768056	2
BRH-764372	2
BRH-764377	5
BRH-764378	2
BRH-763531	1
BRH-764329	0
BRH-763533	0
BRH-763529	0
BRH-763553	12
BRH-763528	0
BRH-763509	0
BRH-763517	2
BRH-763500	0
BRH-764332	0
BRH-764338	1
BRH-764337	3
BRH-764341	1
BRH-764340	1
BRH-764342	0
BRH-764347	1
BRH-764343	5
BRH-774498	1
BRH-768027	12

IBS Population	
IBS-26	16
IBS-27	8
IBS-28	10
IBS-29	8
IBS-31	4
IBS-32	2
IBS-34	0
IBS-36	3
IBS-37	5
IBS-39	19
IBS-41	5
IBS-44	5
BRH-698601	10
BRH-698602	3
BRH-698603	13
BRH-698604	10
BRH-698605	8
BRH-698606	4
BRH-698607	2
BRH-698608	4
BRH-698609	1
BRH-698610	1
BRH-698611	3
BRH-698612	3
BRH-698613	12
BRH-698614	11
BRH-698615	4
BRH-698616	5
BRH-698617	11
BRH-698618	6
BRH-698619	9
BRH-698620	6

No of observation	76
Average # of	6.63
SD	5.54

Non-IBS Population	
BRH-768000	1
BRH-774499	12
BRH-774502	4
BRH-774504	8
BRH-767999	0
BRH-764350	0
BRH-763534	0
BRH-763506	0
BRH-774495	2
BRH-764353	0
BRH-764355	0
BRH-764356	0
BRH-764361	1
BRH-764368	1
BRH-768053	2
BRH-764370	1
BRH-764346	0
BRH-768052	0
BRH-764335	10
BRH-774510	2
BRH-774511	0
BRH-768001	2
BRH-768007	0
BRH-768008	3
BRH-767995	0
BRH-767992	3
BRH-767991	0
BRH-764344	2
BRH-764386	0
BRH-763513	7
BRH-763530	5
BRH-764345	1
BRH-764336	0
BRH-764352	4
BRH-764360	0
BRH-764339	4
BRH-763527	17
BRH-764334	1
BRH-764349	0
BRH-764380	0
BRH-764366	0
BRH-763526	19
BRH-764351	2
BRH-763503	3
BRH-764365	3
BRH-764381	0
BRH-763523	0
BRH-774500	3

Non-IBS Population	
BRH-774501	1
BRH-774505	6
BRH-774503	2
BRH-774494	0
BRH-774493	0
BRH-774492	1
BRH-774491	0
BRH-764357	2
BRH-764358	0
BRH-768045	0
BRH-768047	1
BRH-768048	8
BRH-768049	12
BRH-768051	0
BRH-768050	5
BRH-774506	11
BRH-774507	0
BRH-774509	0
BRH-774512	0
BRH-774513	4
BRH-774514	1
BRH-764359	3
BRH-763524	1

No of observation	115
Average # of	2.37
SD	3.67

Table 5

Variable	IBS IBS

Sample size	76
Lowest value	0.0000
Highest value	23.0000
Arithmetic mean	6.6316
95% CI for the mean	5.3651 to 7.8980
Median	5.0000
95% CI for the median	4.0000 to 8.0000
Variance	30.7158
Standard deviation	5.5422
Relative standard deviation	0.8357 (83.57%)
Standard error of the mean	0.6357
Coefficient of Skewness	0.9423 (P=0.0017)
Coefficient of Kurtosis	0.3684 (P=0.4053)
D'Agostino-Pearson test for Normal distribution	reject Normality (P=0.0051)

Percentiles		95% Confidence Interval
2.5	0.0000	
5	0.0000	0.0000 to 1.0000
10	1.0000	0.0000 to 1.0000
25	2.0000	1.0000 to 3.1043
75	9.0000	8.8957 to 11.6255
90	15.8000	11.0000 to 19.0000
95	18.7000	15.2303 to 22.7004
97.5	19.6000	

Table 6

Variable	Non_IBS Non-IBS
Sample size	115
Lowest value	0.0000
Highest value	19.0000
Arithmetic mean	2.3652
95% CI for the mean	1.6879 to 3.0426
Median	1.0000
95% CI for the median	1.0000 to 1.0000
Variance	13.4444
Standard deviation	3.6667
Relative standard deviation	1.5502 (155.02%)
Standard error of the mean	0.3419
Coefficient of Skewness	2.3537 (P<0.0001)
Coefficient of Kurtosis	5.8546 (P<0.0001)
D'Agostino-Pearson test for Normal distribution	reject Normality (P<0.0001)

Percentiles		95% Confidence Interval
2.5	0.0000	
5	0.0000	0.0000 to 0.0000
10	0.0000	0.0000 to 0.0000
25	0.0000	0.0000 to 0.0000
75	3.0000	2.0000 to 4.0000
90	8.0000	5.0000 to 11.8759
95	11.7500	8.0000 to 16.1360
97.5	12.0000	

Table 7

Variable	IBS_1 IBS 1

Back-transformed after logarithmic transformation.

Sample size	76
Lowest value	0.1000
Highest value	23.0000
Geometric mean	3.7394
95% CI for the mean	2.7247 to 5.1321
Median	5.0000
95% CI for the median	4.0000 to 8.0000
Coefficient of Skewness	-1.3159 (P=0.0001)
Coefficient of Kurtosis	1.3551 (P=0.0481)
D'Agostino-Pearson test for Normal distribution	reject Normality (P<0.0001)

Percentiles		95% Confidence Interval
2.5	0.1000	
5	0.1000	0.1000 to 1.0000
10	1.0000	0.1000 to 1.0000
25	2.0000	1.0000 to 3.0914
75	9.0000	8.8901 to 11.6153
90	15.7878	11.0000 to 19.0000
95	18.6943	15.1985 to 22.6812
97.5	19.5938	

Table 8

Variable	non_IBS_1 non-IBS 1

Back-transformed after logarithmic transformation.

Sample size	115
Lowest value	0.1000
Highest value	19.0000
Geometric mean	0.7278
95% CI for the mean	0.5297 to 1.0001
Median	1.0000
95% CI for the median	1.0000 to 1.0000
Coefficient of Skewness	0.04343 (P=0.8428)
Coefficient of Kurtosis	-1.4006 (P<0.0001)
D'Agostino-Pearson test for Normal distribution	reject Normality (P<0.0001)

Percentiles		95% Confidence Interval
2.5	0.1000	
5	0.1000	0.1000 to 0.1000
10	0.1000	0.1000 to 0.1000
25	0.1000	0.1000 to 0.1000
75	3.0000	2.0000 to 4.0000
90	8.0000	5.0000 to 11.8711
95	11.7418	8.0000 to 16.0070
97.5	12.0000	

Table 9

Sample 1		
Variable	IBS_1 IBS 1	
Sample 2		
Variable	non_IBS_1 non-IBS 1	
Back-transformed after logarithmic transformation.		
	Sample 1	Sample 2
Sample size	76	115
Geometric mean	3.7394	0.7278
95% CI for the mean	2.7247 to 5.1321	0.5297 to 1.0001
Variance of Logs	0.3620	0.5582
F-test for equal variances		P = 0.045
T-test (assuming equal variances)		
Difference on Log-transformed scale		
Difference		-0.7108
Standard Error		0.1025
95% CI of difference		-0.9129 to -0.5087
Test statistic t		-6.937
Degrees of Freedom (DF)		189
Two-tailed probability		P < 0.0001
Back-transformed results		
Ratio of geometric means		0.1946
95% CI of ratio		0.1222 to 0.3100

Table 10

Sample 1	
Variable	IBS_1 IBS 1
Sample 2	
Variable	non_IBS_1 non-IBS 1

	Sample 1	Sample 2
Sample size	76	115
Lowest value	0.1000	0.1000
Highest value	23.0000	19.0000
Median	5.0000	1.0000
95% CI for the median	4.0000 to 8.0000	1.0000 to 1.0000
Interquartile range	2.0000 to 9.0000	0.1000 to 3.0000

Mann-Whitney test (independent samples)

Average rank of first group	127.1382
Average rank of second group	75.4217
Mann-Whitney U	2003.50
Test statistic Z (corrected for ties)	6.410
Two-tailed probability	P < 0.0001

Table 11

Variable	IBS_Test IBS Test
Classification variable	Diag Diag

Sample size		191
Positive group :	Diag = 1	76
Negative group :	Diag = 0	115

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.771
Standard Error[a]	0.0346
95% Confidence interval[b]	0.705 to 0.828
z statistic	7.829
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988
[b] Binomial exact

Youden index

Youden index J	0.4454
95% Confidence interval[a]	0.2976 to 0.5542
Associated criterion	>2
95% Confidence interval[a]	0 to 2

[a] BC_a bootstrap interval (1000 iterations).

Summary Table

Estimated specificity at fixed sensitivity			
Sensitivity	Specificity	95% CI[a]	Criterion
80.00	57.90	43.98 to 74.43	>0.8364
90.00	41.68	30.23 to 52.71	>0.1455
95.00	38.26	27.83 to 46.09	>0
97.50	38.26	27.83 to 46.09	>0
Estimated sensitivity at fixed specificity			
Specificity	Sensitivity	95% CI[a]	Criterion
80.00	62.89	47.37 to 76.32	>3.2
90.00	39.91	15.58 to 59.59	>7.1667
95.00	15.62	5.83 to 32.66	>11.0625
97.50	13.73	3.95 to 29.91	>11.7812

[a] BC_a bootstrap interval (1000 iterations).

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥0	100.00	95.3 - 100.0	0.00	0.0 - 3.2	1.00	1.0 - 1.0		
>0	92.11	83.6 - 97.0	38.26	29.4 - 47.8	1.49	1.3 - 1.7	0.21	0.09 - 0.5
>1	77.63	66.6 - 86.4	61.74	52.2 - 70.6	2.03	1.6 - 2.6	0.36	0.2 - 0.6
>2	72.37	60.9 - 82.0	72.17	63.0 - 80.1	2.60	1.9 - 3.6	0.38	0.3 - 0.6
>3	64.47	52.7 - 75.1	79.13	70.6 - 86.1	3.09	2.1 - 4.6	0.45	0.3 - 0.6
>4	56.58	44.7 - 67.9	83.48	75.4 - 89.7	3.42	2.2 - 5.4	0.52	0.4 - 0.7
>5	48.68	37.0 - 60.4	87.83	80.4 - 93.2	4.00	2.3 - 6.9	0.58	0.5 - 0.7
>6	43.42	32.1 - 55.3	88.70	81.4 - 93.8	3.84	2.2 - 6.8	0.64	0.5 - 0.8

Table 12

Performance Metrics in Predicting IBS Status from Number of Positive Foods Using 90th Percentile of ELISA Signal to determine Positive

Sex	No. of Positive Foods as Cutoff	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Overall Percent Agreement
F	1	0.96	0.35	0.73	0.80	0.74
	2	0.82	0.53	0.76	0.64	0.72
	3	0.77	0.62	0.78	0.60	0.71
	4	0.68	0.67	0.79	0.54	0.68
	5	0.63	0.71	0.80	0.50	0.66
	6	0.57	0.76	0.81	0.50	0.64
	7	0.52	0.81	0.83	0.48	0.62
	8	0.46	0.85	0.84	0.46	0.60
	9	0.41	0.88	0.85	0.45	0.57
	10	0.34	0.88	0.85	0.43	0.54
	11	0.28	0.90	0.85	0.42	0.51
	12	0.21	0.94	0.85	0.40	0.48
	13	0.18	0.94	0.86	0.39	0.46
	14	0.15	0.95	0.89	0.39	0.45
	15	0.13	1.00	1.00	0.39	0.44
	16	0.11	1.00	1.00	0.38	0.43
	17	0.10	1.00	1.00	0.38	0.42
	18	0.07	1.00	1.00	0.38	0.41
	19	0.06	1.00	1.00	0.37	0.40
	20	0.04	1.00	1.00	0.37	0.39
	21	0.03	1.00	1.00	0.37	0.38
	22	0.03	1.00	1.00	0.36	0.37
	23	0.00	1.00	1.00	0.36	0.36
	24	0.00	1.00	1.00	0.36	0.36

Table 13

Performance Metrics in Predicting IBS Status from Number of Positive Foods Using 90th Percentile of ELISA Signal to determine Positive

Sex	No. of Positive Foods as Cutoff	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Overall Percent Agreement
M	1	0.81	0.35	0.29	0.85	0.46
	2	0.71	0.57	0.35	0.86	0.61
	3	0.67	0.68	0.41	0.86	0.68
	4	0.62	0.75	0.45	0.86	0.72
	5	0.53	0.80	0.48	0.84	0.74
	6	0.47	0.85	0.50	0.83	0.76
	7	0.39	0.88	0.50	0.82	0.76
	8	0.30	0.90	0.50	0.80	0.75
	9	0.25	0.92	0.50	0.79	0.75
	10	0.18	0.93	0.43	0.78	0.74
	11	0.14	0.94	0.43	0.77	0.74
	12	0.11	0.95	0.40	0.77	0.75
	13	0.10	0.96	0.43	0.76	0.75
	14	0.07	0.97	0.50	0.76	0.75
	15	0.06	0.97	0.50	0.76	0.75
	16	0.06	0.98	0.50	0.76	0.75
	17	0.05	0.98	0.33	0.76	0.75
	18	0.00	0.98	0.00	0.75	0.75
	19	0.00	0.98	0.00	0.75	0.75
	20	0.00	1.00	0.00	0.75	0.75
	21	0.00	1.00	0.00	0.75	0.75
	22	0.00	1.00	0.00	0.75	0.75
	23	0.00	1.00	0.00	0.75	0.75
	24	0.00	1.00		0.75	0.75

Table 14

What is claimed is:

1. An irritable bowel syndrome (IBS) test kit panel consisting essentially of:

a plurality of distinct IBS trigger food preparations immobilized to an individually addressable solid carrier;

wherein the plurality of distinct IBS trigger food preparations each have an IBS raw p-value of ≤ 0.07 or an IBS false discovery rate (FDR) multiplicity adjusted p-value of ≤ 0.10; and

wherein the IBS test kit panel has at least ten distinct IBS trigger food preparations.

2. The test kit panel of claim 1, wherein the plurality of distinct IBS trigger food preparations each have an IBS raw p-value of ≤ 0.05 or an IBS FDR multiplicity adjusted p-value of ≤ 0.08.

3. The test kit panel of claim 1, wherein the FDR multiplicity adjusted p-value is adjusted for at least one of age or gender.

4. The test kit panel of claim 1, wherein the plurality of distinct IBS trigger food preparations is crude aqueous extracts or processed aqueous extracts.

5. The test kit panel of claim 1, wherein the solid carrier is selected from the group consisting of a microwell plate, a microfluidic device, a dipstick, a membrane-bound array, a bead, an adsorptive film and a sensor.