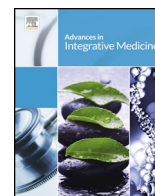




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An exploratory comparative investigation of Food Allergy/Sensitivity Testing in IBS (*The FAST Study*): A comparison between various laboratory methods and an elimination diet

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ABSTRACT

Objective: To determine the ability of several non-IgE mediated food allergy (NIMFA) test methods to identify which foods would elicit symptoms in irritable bowel syndrome (IBS) sufferers.

Methods: This was a correlative, hypothesis generating study. Participants ($n = 4$) with IBS (Rome III >200) provided blood samples for various marketed NIMFA tests (cytotoxic assay, IgG food antigen, electrodermal screening), prior to initiating an Elimination/Challenge diet. Eight foods were challenged following a 4-week elimination diet. Challenge foods were selected from reported reactive foods on test results. Participants and team members were blinded to the test results until study completion. Dietary choices and symptoms were tracked daily and reviewed bi-weekly. The IBS symptom severity scale (IBS-SS) was administered at each visit ($n = 5$).

Results: There was poor agreement, assessed by Fleiss' kappa, between the various test results and those foods, on challenge, elicited a reaction. The sensitivity of the NIMFA tests ranged from 20 to 75%, while the specificity, from 50 to 89%.

Conclusion: None of the NIMFA tests evaluated in this study were found to be diagnostically predictive in the identification of specific foods that exacerbate the gastrointestinal symptoms in our small sample of IBS sufferers.

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1. Introduction

Adverse food reactions are a broad category whose symptoms can be associated with an immune mediated response, either IgE or non-IgE mediated, called food allergy (FA), or non-immune mediated called food intolerance (FI) [1]. Non-IgE mediated FAs (NIMFAs) usually produce delayed reactions that are not life-threatening and are associated with less severe or long-term symptom development such as digestive difficulties or joint stiffness [2].

The gold standard used to confirm a FA is through an elimination/challenge (E/C) diet under controlled circumstances. Many variants of the E/C diet exist [3,4]. The E/C diet involves the elimination of all major potential allergens for a period of between three to six weeks [4]. The challenge phase, which can be blinded, involves consuming several servings of the suspected food for one

day and monitoring for signs and symptoms for up to 72 h. If a food does elicit a reaction, the food is avoided; if no reaction is noted, the food can be consumed.

A high degree of compliance and commitment is required to adhere to an E/C diet and may take months to complete. Efforts to accurately identify NIMFAs and provide patients with specific treatment strategies sooner have spawned the development of more objective and achievable methods to identify NIMFAs [5]. Some of these methods include cytotoxic assays, serum IgG antibody assessment, electrodermal screening (EDS) and applied kinesiology. Evidence for and acceptance of these methods is variable and limited [5–7].

Our current understanding of irritable bowel syndrome (IBS) suggests that FAs may indeed play a role in IBS symptomatology [8,9]. A high percentage of IBS sufferers report symptom exacerbation after the ingestion of various food(s) [10,11]. However, many are not able to pinpoint the specific food(s) or adhere to an E/C diet [11]. Theoretically, increased gastrointestinal (GI) barrier permeability may lead to an increased activation of the immune system as part of the IBS pathogenesis [12]. Studies investigating NIMFA testing in IBS sufferers have found an improvement through the elimination of food(s) identified on these tests [13–20].

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However, no study has compared the predictability of each method to the results of an E/C diet [5]. An understanding of the relative agreement between these tests may help patients and practitioners make better decisions around how to best investigate NIMFAs for IBS.

The objective of this study was to explore whether NIMFA test methods would be helpful in predicting whether foods would elicit an exacerbation of symptoms in IBS sufferers.

2. Methods

This was a correlative, hypothesis generating study conducted from February 27, 2013 to April 24, 2013. The study was funded by a grant from the Canadian CAM Research Fund through the Canadian Interdisciplinary Network for Complementary and Alternative Medicine Research and registered at ClinicalTrials.gov (NCT01791621).

Recruiting occurred between January–February 2013, using online advertisements through the Canadian College of Naturopathic Medicine's (CCNM) website and social media channels; also, print advertisements were placed in various locations. Interested individuals were screened for eligibility by two naturopathic doctors via email, telephone or an in-person interview.

Inclusion criteria were men and women aged 18–64, diagnosed with IBS based on the ROME III criteria, moderate to severe IBS based on the irritable bowel severity scoring system (IBS-SS >200) [21], not currently eliminating any foods from the diet and women who agreed to use birth control measures throughout the study. Exclusion criteria were women who were breastfeeding, pregnant or open to becoming pregnant during the study; participants currently using natural, over-the-counter or prescription medication for IBS treatment unwilling or unable to undergo a 2-week washout period; those with celiac disease; currently experiencing severe gastrointestinal symptoms; previously diagnosed with Crohn's disease or ulcerative colitis; immune-compromised individuals; recent (<1 month) dietary modifications to address IBS symptoms; history of major or complicated gastrointestinal surgery; severe endometriosis; and/or malignancy or current use of chemotherapy or radiation therapy. If celiac disease had not previously been ruled out in a potential participant, the Celiacure™ biocard test (Glutenpro, Mississauga, Ontario, Canada), a Health Canada approved celiac screening test, was used to rule out celiac disease prior to confirming participation in the study.

The diet used in this study involved the elimination of potential food allergens for 4 weeks, followed by the systematic reintroduction of certain foods for 4 weeks. At the first visit, participants were given a handout outlining the diet, which was reviewed in detail, and various resources to help facilitate compliance. All participants were asked to start the diet on March 4, 2013, after 4 days of familiarizing themselves with the elimination diet protocol.

The challenge phase included the reintroduction of eight foods. These eight foods were chosen by a study team member not directly involved with patient care. Two criteria were used in determining the foods for the challenge: (1) the foods needed to be common to the North American diet, and (2) laboratory test results. Food items were selected where only *some* of the study participants' laboratory results showed reactivity, where *none* of the study participants' showed reactivity or *all* of the participants' showed reactivity.

Participants were instructed to consume a large portion of each challenge food for at least two meals on the assigned day. Participants documented and rated any reaction(s) for an additional two days. If a participant had a reaction to a reintroduced food, they were instructed to wait until symptoms stabilized prior to continuing. Each food was allotted a minimum of three days for reintroduction, and all participants followed the

same challenge food sequence. If a participant had a known severe reaction to a challenge food, they were permitted to skip that food and move to the next. Compliance was monitored via review of the participants' dietary records at each visit. Participants demonstrating <90% compliance with dietary restrictions were withdrawn from the study.

Five different laboratory tests were evaluated for each participant: IgG antibody detection, cytotoxic assay, EDS and food intolerance testing (FIT). Tests were chosen based on market availability and reflect tests commonly advertised as identifying NIMFAs. Samples were obtained on Day 1, and mailed or couriered to respective laboratories the same day.

All samples were obtained by a trained healthcare practitioner under conditions that are compliant with provincial regulatory standards. A brief description of each of the laboratory assessments methods is provided below.

2.1. Cytotoxic assay

The cytotoxic assay (CYTO), an *in vitro* test, assesses the reactivity to 150 items via mediator release from leukocytes, and evaluates type III and IV hypersensitivity reactions. Whole blood is incubated with a food/chemical and, after a specific time, the mediator released into the extracellular fluid is indirectly detected by measuring changes of solid/liquid ratio in the sample. Per manufacturer's instructions, whole blood was taken via venipuncture from the antecubital crease using standard phlebotomy techniques and centrifuged. Reactions were reported as follows: reactive, moderately reactive, or non-reactive. For this study, reactive and moderately reactive items were considered.

2.2. IgG antibody assessment

The IgG antibody assessment (IGG1), is an *in vitro* test that measures the IgG antibody reactivity to 96 items via an Enzyme-Linked Immunosorbent Assay (ELISA) and evaluates either a type II or III hypersensitivity response [22]. Per manufacturer's instructions, a whole blood spot sample was taken from the participant's finger using a lancet, and blood subsequently drawn onto an absorbent card. Results are classed into seven categories of increasing reactivity: no reaction, very low, low, moderate, high, very high and extremely high. For this study, the reactivity categories of low and above were considered.

2.3. IgG antibody assessment with Genarray™

The IgG Antibody assessment II (IGG2), is an *in vitro* test method evaluated reactivity to 200 items, in a manner similar to IGG1; however, the selected laboratory utilizes an assay system called Genarray™, which is purported to increase the sensitivity [23,24]. Per manufacturer's instructions, whole blood spot sample was taken from the participant's finger and blood drawn onto an absorbent card. Reactions were reported as red (IgG >30 U/ml), yellow (IgG between 24 and 30 U/ml) and green (IgG <24 U/ml). For this study, reactions categorized as yellow and red were considered.

2.4. Electrodermal screening

Electrodermal screening (EDS) is a non-invasive, bio-energetic evaluation of the body's response to different stressors via the measurement of changes in the skin's electrical activity at designated acupuncture points. The system was configured as follows: a laptop with appropriate software, a brass tube and a probe, both individually attached to the laptop via separate cables (BEST system, software version 1.6, Biomeridian, Salt Lake City, Utah USA). The laptop contained software with digitized frequencies of

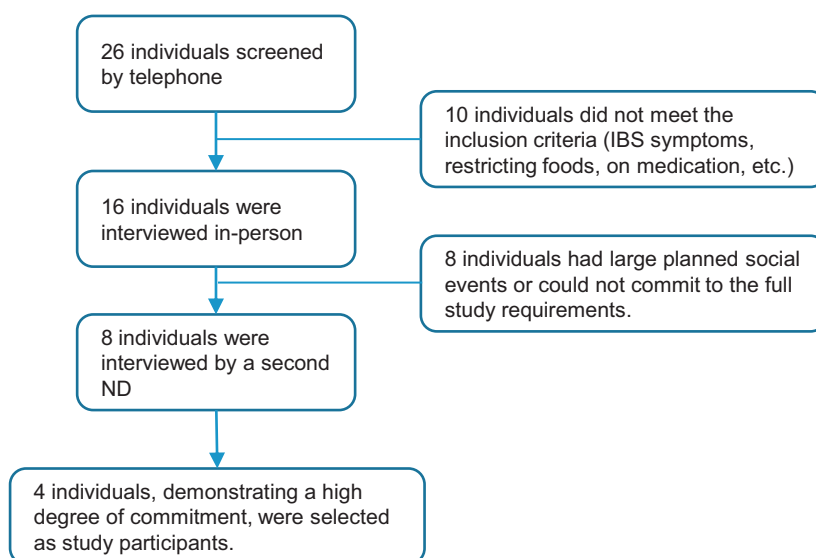


Fig. 1. Study participant flow chart.

231 items, the ability to determine and record the skin's electrical resistance to a digitized frequency and print a report. The participant holds the brass tube in one hand while the EDS technician applies a probe to the specific allergy point on the opposite hand, thus completing the circuit and allowing a resistance reading to be taken [25]. Once baseline measures are established, the digitized frequency of each item was introduced into the circuit, one by one, and the resistance recorded. A scale of 0–100 graduations is used, a reading of between 45 and 55 indicates balanced response to the food item, 0–44 indicates a weakened response to the food item, and 56–100 indicates a stressed response to the food item. For this study, food items with results above 56 were considered as reactive; there were no items that tested below 45. The EDS technician that performed all of the EDS testing had 18 years' experience in the field.

2.5. Food intolerance testing (FIT)

Food intolerance testing (FIT), is an *in vitro* test method developed by Dr. Carroll, ND and is similar to EDS testing in that it uses electric frequency as the variable in identifying reactive foods [25]. Each blood sample is exposed to certain foods in contact with a reagent. Small fluctuations in the electric current are detected and indicate an incompatibility between the food and the blood. Following manufacturer's instructions, a whole blood spot sample was drawn onto an absorbent card. The sample is placed in an electric circuit and exposed to various foods. Ten different food groups are tested individually, as well as, combinations of food groups (e.g. sugar consumed within 8 h of fruit). Only the reactionary food groups or food combinations are reported. In this study, the reactive food group(s) were considered.

2.6. Intestinal permeability (IP)

The intestinal permeability (IP) test evaluates the integrity of the intestinal barrier via the urinary excretion of lactulose and mannitol. It is not used to identify a specific reactive food or food groups. The measurement of urinary lactulose, mannitol, and the lactulose/mannitol ratio are used as indicators of malabsorption and IP, respectively [26]. Mannitol, a small sugar molecule, is readily absorbed through the epithelial membrane, while lactulose, a large sugar molecule, is only slightly absorbed. IP sample collection was performed at home by the participants prior to the start of the elimination phase and before commencing the

challenge phase. The participants were provided with an IP kit (Doctor's Data Inc., St. Charles, IL, USA) and a sugar solution (compounded by Smith's Pharmacy, Toronto, Ontario, Canada). Following an overnight fast, participants collected their first morning urine into a container then drank the sugar solution. All urine was subsequently collected for six hours into a second container and sent to the laboratory via courier. Results reported are: lactulose %recovery, mannitol %recovery and lactulose/mannitol ratio. An elevated lactulose/mannitol ratio is indicative of increased IP, while a low percentage of mannitol recovery may indicate malabsorption [26].

The IBS-SS is a validated questionnaire that was used to monitor IBS symptoms at each visit. This validated questionnaire incorporates measures of pain, distension, bowel dysfunction and quality of life [21]. Participants were required to track their food intake and symptoms on a daily basis throughout the study. A detailed flow chart of the study is included in Fig. 1.

Ethics approval was granted on April 2, 2012 by the Research Ethics Board at the CCNM according to the ethical standards set forth in the 1975 Helsinki Declaration (REB Approval number: 2012-1). All participants gave informed consent to participate in the trial.

2.7. Handling of laboratory tests

The laboratories that conducted the testing on the blood samples were not advised of this study, nor were the samples identified as originating from CCNM. Other study team member accounts and practice location addresses were used to submit the samples. Participants were given fictitious names to ensure confidentiality and these were used throughout the study.

All test results were forwarded directly to the Chief Naturopathic Medical Officer at the CCNM, via the other team members, for safekeeping and to maintain blinding of the team. One study team member, not involved with patient care, was given access to these results to select the eight challenge food items.

These methods were employed in order to help facilitate objectivity and blinding where possible.

2.8. Statistical analysis

Doctor's Data IP results report the percentage of lactulose and mannitol recovered and lactulose to mannitol ratio. The difference between each participant's pre and post elimination phase results

were determined. Mann Whitney *U* was used to determine whether the change was significant.

The IBS-SS were determined by totaling the participant's responses to the questionnaire at each visit. The difference in the scores at two points: between pre/post elimination phase and start/completion of the study were calculated. Mann Whitney *U* was used to determine whether the changes were significant.

The 55 food items common to all of the NIMFA tests were used as a basis. For each participant, the reactive foods among these 55 were summarized across all laboratory tests. Cohen's kappa was used to determine the agreement between each set of pairings of laboratories. Stats Direct (version 2.7.7 Altrincham, Cheshire, UK) was used for the nonparametric statistics and Cohen's kappa calculations. A significance level of $p < 0.05$ was established.

The response of each participant to each challenge food and the corresponding NIMFA laboratory results were summarized. Fleiss' kappa was calculated comparing the results of the participants' reactive foods to each of the laboratory results by participant. The Fleiss' kappa calculator, available at www.Statstodo.com, was used for this calculation [27].

3. Results

Participants were recruited between January 9, 2013 and February 14, 2013. Interested participants were screened for the inclusion criteria and their commitment to following an E/C diet. Participants were initially excluded because they failed to meet the inclusion criteria or were not prepared to commit to the study. Supplementary Data Figure 1 provides the details of the selection

Table 1
Characteristics of the participants.

Gender	Number	Mean age (years)	Age range	IBS subtype
Male	1	45	45	IBS-D
Female	3	28.3	28–32	IBS-D, IBS-M
Total	4	32.5	28–45	

Abbreviations: IBS-D: irritable bowel syndrome, predominantly diarrhea type; IBS-M: irritable bowel syndrome, mixed type.

process. Table 1 summarizes the characteristics of the participants. A 90% compliance threshold was established and met by all. Early attention to compliance was useful during implementation of the elimination diet.

Table 2 summarizes the results of the intestinal permeability (IP) testing. Improvements in mannitol absorption, a measure of malabsorption, were seen. This suggests that following an elimination diet for 4 weeks could improve gut health as measured by IP, though this warrants further investigation. Fig. 2 graphically depicts the IBS-SS over the study. The largest change in IBS-SS occurred between baseline and the elimination phase, with a $45.4 \pm 27.8\%$ decrease in IBS-SS; however, this result was not significant ($p = 0.06$). The variability in IBS-SS during the challenge phase could be confounded by the timing of the visits, since participants would have been challenging a particular food on a visit day thereby subtly influencing the IBS-SS. One participant (Fast4) was no longer experiencing IBS symptoms by the end of the study.

Each laboratory tested a variable number of items. Several categories of results are presented (Table 3). The EDS method

Table 2
Intestinal permeability results: pre and post elimination phase.

	Pre elimination phase			Post elimination phase			Difference post–pre		
	Lactulose	Mannitol	Lactulose/ mannitol ratio	Lactulose	Mannitol	Lactulose/ mannitol ratio	Lactulose	Mannitol	Lactulose/ mannitol ratio
	(%recovered)	(%recovered)		(%recovered)	(%recovered)		(%recovered)	(%recovered)	
Reference range ^a	0.1–0.6%	2–24.5%	≤ 0.05	0.1–0.6%	2–24.5%	≤ 0.05			
Study participants									
Fast1	0.73	24.3	0.03	0.59	19.2	0.03	–0.14	–5.1	0.0
Fast2	1.00	33.3	0.03	0.59	19.3	0.03	–0.41	–14.0	0.0
Fast3	0.60	40.1	0.02	0.96	22.3	0.04	0.36	–17.8	0.02
Fast4	0.46	33.0	0.01	0.95	28.7	0.03	0.49	–4.3	0.02
<i>p</i>							0.94	0.06	0.28

^a Source: Doctor's data laboratory report.

Table 3
Summary of laboratory test results.

	Laboratory and analysis methods				
	CYTO	IGG1	IGG2	EDA	FIT
	Cytotoxic assay	IgG ^d	IgG	EDS	FIT
Items tested ^a	120	96	122	231	10 food groups
No. of reactive items					
Average	15.7	10	9.7	87.7	1
Range	8–25	3–27	4–16	75–97	1
Percentage	13.1%	10.4%	8.0%	37.9%	10%
Food only items ^b	90	96	105	174	10 food groups
Average	13	10	9.75	52.75	1
Range	6–18	3–27	4–16	45–61	1
Percentage	14.4%	10.4%	9.3%	30.3%	10%
Common foods ^c	55	55	55	55	10 food groups
Average	7	4	5.5	16	1
Range	5–9	1–11	2–10	11–20	1
Percentage	12.7%	9.6%	10%	29%	10%

Abbreviations: EDS: electrodermal screening; FIT: food intolerance testing; IgG: immunoglobulin G; No.: number.

^a This section summarizes the results on the total number of items tested, which could be food and/or chemical substances.

^b This section summarizes the results of the total number of food only items tested.

^c This section summarizes the results for the 55 common food items across all of the tests.

^d One participant's results is not available due to missing sample.

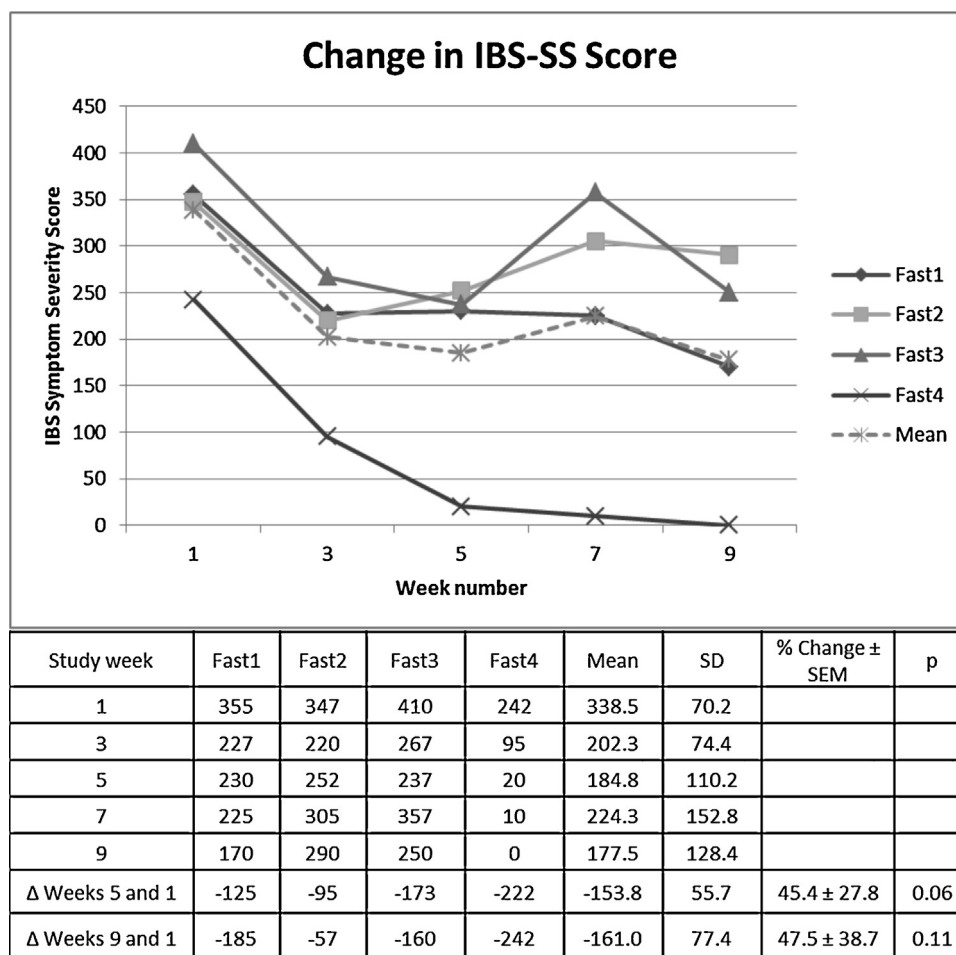


Fig. 2. Irritable bowel syndrome severity scores (IBS-SS) over the course of the study, by participant.

identified the highest number of items at 30.3%. One participant's sample for IGG1 testing was lost during shipping. An analysis of the agreement between each pair of laboratory tests was also performed. Cohen's kappa scores (Table 4) indicate very poor agreement between sets of tests on the 55 common foods reported on across all of the tests. The highest degree of agreement was found between IGG1 and IGG2, both of which evaluated IgG levels.

Table 4
Comparison of the level of agreement between the sets of laboratory test results.

Laboratory and analysis methods Cohen's kappa (CI _{95%} interval)				
CYTO	IGG1	IGG2	EDS	
Cytotoxic assay	IgG ^a	IgG	EDS	
CYTO	1.00	0.009 (−0.143 to 0.162)	−0.024 (−0.156 to 0.108)	0.108 (−0.005 to 0.222)
IGG1		1.00	0.422 (0.270 to 0.574)	−0.0130 (−0.145 to 0.119)
IGG2			1.00	0.0223 (−0.087 to 0.132)
EDS				1.00

Abbreviations: CI_{95%}: 95% confidence interval; IgG: immunoglobulin G; EDS: electrodermal screening.

^a One participant's results is not available due to missing sample.

Table 5 summarizes the number of participants that each NIMFA laboratory test indicated was reactive. The number of participants that were identified as reacting to a particular food item varied. For example, IGG1 identified only one participant as having a reaction to bananas, however, with respect to wheat, IGG2 identified that all 4 participants would react to wheat, while IGG1 only 2, CYTO only 1 and EDS, 3 participants. Table 5 also summarizes, for each participant, the foods that, on challenge, elicited GI reactions (diarrhea, constipation, abdominal bloating, pain, etc.). One participant did not actually challenge coffee since this individual had a prior known strong reaction to this food. All participants had a reaction to at least one food on challenge, although these often did not correspond with those identified by the NIMFA tests. Table 6 summarizes the results of the predictability statistics for the NIMFA tests using Fleiss' kappa as a measure of the amount of agreement between raters. The participant's reaction and each laboratory results were considered raters for each food. Kappa scores range between 0 and 1.00 for both positive and negative agreement (i.e. a score of −1.00 indicates full disagreement between tests). A kappa of less than 0.20 is considered poor agreement [28]. Poor agreement was found between the laboratory test results and the foods that the participants identified as eliciting a reaction.

Sensitivity measures the ability to correctly identify those patients with the disease [29]. The tests in our study demonstrate poor sensitivity. CYTO, IGG1 and IGG2 tests will miss ~70% of the patients that would have a positive reaction to a food on challenge. Specificity measures the ability to correctly identify those patients without the disease [29]. We found the tests in our study to have

Table 5

Summary of laboratory results and participant results for the 8 challenge foods.

Challenge foods	Laboratory and analysis methods					Study participants			
	CYTO	IGG1	IGG2	EDS	FIT	Fast1	Fast2	Fast3	Fast4
	Cytotoxic	IgG ^a	IgG	EDS	FIT				
Banana	1	1	0	0	0	X	X		
Beef	1	0	0	4	0	X		X	X
Coffee	4	0	0	4	0	X ^b		X	
Egg	0	1	2	1	0	X	X		
Milk	2	1	2	4	2	X	X	X	
Potato	1	0	0	1	2	X		X	
Tomato	0	1	0	4	0	X		X	X
Wheat	1	2	4	3	0	X	X	X	
Total	10	6	8	21	4	8	4	6	2

Note: An “X” in the cell indicates a positive reaction on food challenge. Abbreviations: EDS: electrodermal screening; FIT: food intolerance testing; IgG: immunoglobulin G.

^a One participant's results is not available due to missing sample.

^b This participant did not challenge coffee due to a previous known reactivity.

Table 6

Summary of the predictive statistics of each laboratory and test methods based on food challenge results.

Test abbreviation	Laboratory and analysis methods				
	CYTO	IGG1	IGG2	EDS	FIT
	Cytotoxic Assay	IgG	IgG	EDS	FIT
Fleiss kappa rating score	−0.072	−0.063	0.057	0.156	−0.067
(CI _{95%})	(−0.203 to 0.059)	(−0.242 to 0.116)	(−0.074 to 0.188)	(0.026 to 0.287)	(−0.198 to 0.064)
Sensitivity (%)	20.0	29.0	30.0	75.0	20.0
Specificity (%)	58.0	80.0	83.0	50.0	89.0
PPV	44.4	66.7	75.0	71.4	75.0
NPV	30.4	44.4	41.7	54.5	40.0

Abbreviations: CI_{95%}: 95% confidence interval; EDS: electrodermal screening; FIT: food intolerance testing; IgG: immunoglobulin G.

better specificity than sensitivity. However, in the case of tests IGG1 and IGG2, 20% of the patients will experience a reaction despite the test predicting that they would not be reactive. The positive predictive value answers the question, “how likely is it that this patient will have a reaction to the food with a positive test result?” [29], while the negative predictive value, answers the questions “how likely is it that this patient will not have a reaction with a negative test result?” [29]. In our study of IBS sufferers, these tests are not highly predictive from a diagnostic perspective.

4. Discussion

This is the first study to examine the correlation between the results from several different NIMFA tests with GI symptoms in IBS sufferers after a standardized 4 week elimination diet. The results of our pilot study suggest that these laboratory tests may not be helpful in the identification of the specific foods that exacerbate the GI symptoms in IBS sufferers. The immune based evaluation of the NIMFAs demonstrated the lowest sensitivity. The testing method with the highest sensitivity was the EDS (sensitivity = 75%); however, this method also identified the highest percentage of reactive food items (overall specificity = 50%).

A lack of correlation between laboratory tests and food challenge results was also found in a 1988 study. Zwetchkenbaum and Burakoff used both IgE skin prick testing and IgG serum antibodies (RAST panel) to assess food allergies in 9 IBS patients [30]. Foods that were found to elicit a positive reaction were eliminated from the diet for a two week period, after which an open challenge of each of the reactive foods was conducted. None of the patients experienced an exacerbation of their symptoms on reintroduction of the laboratory identified reactive foods.

Specific food(s) however, would appear to have an impact on GI symptoms in IBS sufferers and a restricted diet can provide symptom improvement. Several studies have investigated the

impact of an exclusion diet on symptom severity, finding an improvement in GI symptoms [11]. This is consistent with our findings. In addition, several studies have been conducted on diets resulting from IgG antibody testing [11,13,14,31–33]. These studies did find a reduction in IBS symptom severity when the laboratory identified reactive foods were eliminated. However, in addition to gaps in reporting and lack of a control/comparator group, no confirmatory investigations were performed to validate which specific food(s) were actually contributing to IBS symptomatology. In short, a healthy diet may improve IBS symptoms better than adhering to the results from NIMFA tests.

Little agreement exists across the NIMFA tests regarding the reactive foods. A number of different reasons are possible to explain the discrepancy. A previous study found that different laboratories source their food antigens from different vendors [34]. Different antigen sources could result in a different composition of proteins and therefore reactive epitopes. A review of the process of IgG food allergy testing by Miller identified other possible issues with the preparation of food antigens [35]. Raw foods can contain other contaminants such as bacteria, fungi, pesticides and organic solvents, which may not be rinsed off in the antigen preparation process. Additionally, each laboratory method assessed different components of the immune system, so perhaps the low level of agreement could be expected given the heterogeneity with which an FA may present.

The pathophysiology of IBS has not been completely elucidated. In order for an immune system reaction to be measured within the blood, this would presuppose that food antigens could breach the intestinal barrier of the GI tract via an increased IP. This increased IP permits small food proteins to pass through the lamina propria, encounter antigen presenting cells to activate either T or B lymphocytes [36]. The IP results from the pre-elimination diet phase of the study suggest that none of the participants had an increased IP that would permit the above mentioned series of

events to occur. This may be another explanation for the poor level of agreement between NIMFA test and the dietary challenge results. Perhaps the symptomatology of IBS results from local GI reactions rather than a systemic reaction, at least in some individuals.

Several limitations in this study need to be kept in mind when interpreting the results. The study sample size was small and this limits the statistical significance and generalizability of the results. We were pragmatic in the design of the challenge phase of the study. Food challenges were not blinded and, due to the time necessary to challenge one food item, not all reactive foods were challenged. Lastly, we sampled the marketplace of different laboratory test vendors and methods, as such, not all available laboratory test vendors were evaluated in this study.

5. Conclusion

None of the NIMFA tests evaluated in this study were found to be diagnostically predictive in the identification of specific foods that exacerbate the gastrointestinal symptoms in our small sample of IBS sufferers. Diet would appear to have a role in IBS symptomatology, since an elimination diet was found to reduce symptom severity. Further investigation into the role of diet and food choices in IBS is warranted.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.aimed.2014.12.002](https://doi.org/10.1016/j.aimed.2014.12.002).

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Supplementary Figure 1: Study Flow

