FOX INSTRUCTIONS FOR USE

The Food Xplorer (FOX) is an in-vitro diagnostic test based on Enzyme-Linked Immunosorbent Assay (ELISA) for the semi-quantitative determination of specific immunoglobulin G (IgG).

INTENDED USE

The Food Xplorer (FOX) is a semi-quantitative in vitro diagnostic test for the determination of specific immunoglobulin G (IgG) against 286 food antigens. The Food Xplorer is suitable for use with serum or plasma. FOX can be used to support dietary clarification and to monitor dietary compliance. Furthermore, FOX can be used in case of reasonable suspicion of immunologically caused food intolerance. Before starting a diet based on FOX results, it is important to discuss the results with a physician or a dietician. The processing of FOX should always be carried out by professionally trained personnel and/or medical professionals.

SUMMARY AND EXPLANATION OF THE TEST

Type III pattern Food Allergy is a delayed hypersensitivity reaction presenting a whole-body systemic response involving serveral organ systems. Gastrointestinal tract dysfunction are central to the pathogenesis of a variety of systemic manifestations. Due to the high permeability of the intestinal wall, food proteins may cross the intestine barrier unnaturally, exposing them to the immune system and ultimately triggering inflammatory reactions. The associated symptoms occur in a delayed manner and range from gastrointestinal symptoms and joint pain to migraines. Delayed type allergic reactions to food is mediated by IgG antibodies. By detecting the food-specific IgG antibodies in human serum, the triggering antigens can be easily identified and the symptoms alleviated by avoiding the corresponding food.

All major type III food sources are covered by FOX. A complete list of FOX food extracts can be found at the bottom of this instruction. Link

Important information for the user!

For the correct use of FOX, it is necessary for the user to carefully read and follow these instructions for use. The manufacturer assumes no liability for any use of this test system which is not described in this document or for modifications by the user of the test system.

PRINCIPLE OF THE PROCEDURE

FOX is a solid-phase immunoassay test based on Enzyme-Linked Immunosorbent Assay (ELISA). Food extracts, which are coupled to nanoparticles, are deposited in a systematic fashion onto a solid phase forming a macroscopic array. First, the particle bound proteins react with specific IgG that is present in the patient's sample. After incubation, non-specific IgG is washed off. The procedure continues by adding an enzyme labelled anti-human IgG detection antibody which forms a complex with the particle bound specific IgG. After a second washing step, substrate is added which is converted to an insoluble, coloured precipitate by the antibody-bound enzyme. Finally, the enzyme-substrate reaction is stopped by adding a blocking reagent. The amount of precipitate is proportional to the concentration of specific IgG in the patient sample. The lab test procedure is followed by image acquisition and analysis using the ImageXplorer device. The test results are analysed with MADx's RAPTOR SERVER Analysis Software and reported in IgG classes.

SHIPMENT AND STORAGE

The shipment of FOX takes place at ambient temperature conditions. Nevertheless, the kit must be stored immediately upon delivery at 2-8°C. Stored correctly, FOX and its components can be used until the indicated expiration date.

Kit reagents are stable for 12 months after opening (at the indicated storage conditions).

WASTE DISPOSAL

Dispose the used FOX cartridge and unused kit components with laboratory chemical waste. Follow all national, state, and local regulations regarding disposal.

GLOSSARY OF SYMBOLS

The meaning of the symbols stays the same, regardless of colour.

Warning (GHS pictogram)
Refer to safety data sheet for details

Catalogue number
Indicates the manufacturer's catalogue number so that the medical device can be identified.

Contains sufficient for <n> tests
Indicates the total number of IVD tests that can be performed with the IVD.

Do not use if package is damaged
Indicates a medical device that should not be used if the package has been damaged or opened.

CE mark

Batch code
Indicates the manufacturer's batch code so that the batch or lot can be identified.

Consult instructions for use
Indicates the need for the user to consult the instructions for use.

Manufacturer
Indicates the medical device manufacturer, as defined in EU Directives 90/385/ EEC, 93/42/EEC and 98/79/EC.

In vitro diagnostic medical device
Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

Do not re-use
Indicates a test that is intended for one use, or for use on a single patient during a single procedure.

Temperature limit
Indicates the temperature limits to which the test (reagents) can be safely exposed to.

Use-by date
Indicates the date after which the test (reagents) is not to be used.

Important Note

FOX KIT COMPONENTS

Each component (reagent) is stable until the date stated on each individual component's label. It is not recommended to pool any reagents from different kit lots.

FOX kit components REF 80-5001-01	**Content**	**Properties**
FOX Cartridge	5 Blisters á 10 FOX for 50 analyses in total.	Ready for use. Store at 2-8°C until expiry date.Opened blister is stable for 12 months at 2-8°C.
FOX Sample Diluent	1 bottle á 30 mL	Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 12 months at 2-8°C.
FOX Washing Solution	4 x conc. 1 bottle á 250 mL	Store at 2-8°C until expiry date. Dilute 1 to 4 with purifed water before use (250 ml Washing Solution + 750 ml purified water). Allow reagent to reach room temperature before use. Opened reagent is stable for 12 months at 2-8°C.
FOX Detection Antibody	1 bottle á 30 mL	Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 12 months at 2-8°C.
FOX Substrate	1 bottle á 30 mL	Ready for use. Store at 2-8°C until expiry date. Allow reagent to reach room temperature before use. Opened reagent is stable for 12 months at 2-8°C.

Solution		
FOX Stop Solution	1 bottle á 10 mL	Ready for use. Store at 2-8°C until expiry date. Allow stop solution to reach room temperature before use. Opened reagent is stable for 12 months at 2-8°C. May appear as a turbid solution after prolonged storage. This has no effect on results.

REQUIRED EQUIPMENT FOR PROCESSING AND ANALYSING FOX

- Arrayholder (optional)
- Lab Rocker (inclination angle 8°, required speed 8 rpm)
- ImageXplorer or MAX 45k analyser
- Incubation chamber (WxDxH – 35x25x2 cm)
- RAPTOR SERVER Analysis Software
- PC/Laptop
- For fully automated test procedure with FOX, a MAX 45k instrument and a PC/Laptop with Internet connection is required. Please refer to 16-IFU-01-EN-xx for details about the autmated FOX test procedure using the MAX 45k instrument.

Required equipment, not provided by MADx:

- Purified Water
- Pipettes & tips (100 µL & 1000 µL). Dilution tubes (e.g. 1.5ml Eppendorf Tubes)

Maintenance services according to manufacturer's instructions.

HANDLING OF FOX ARRAYS

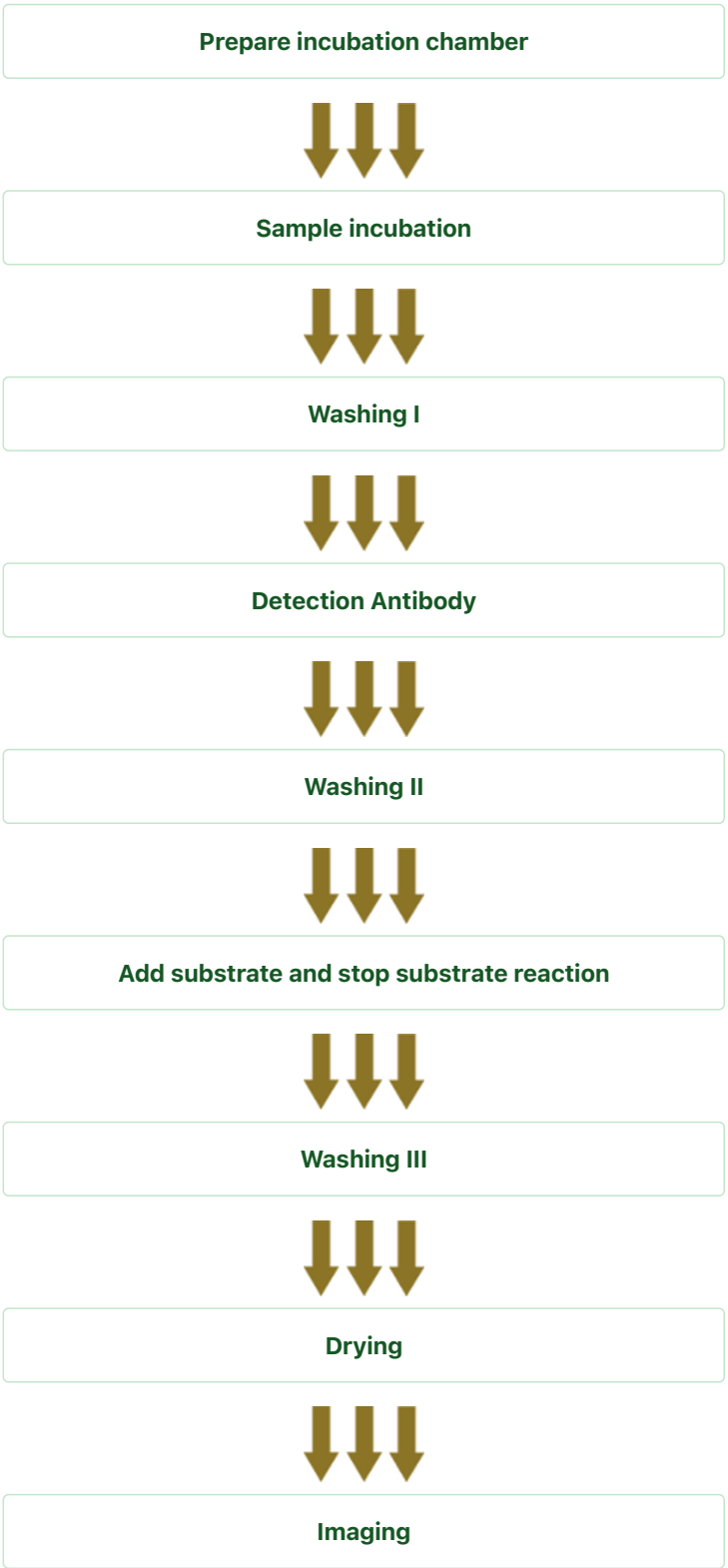
Do not touch the array surface. Any surface defects caused by blunt or sharp objects can interfere with the correct readout of the results. Do not acquire FOX images before array is completely dry (dry at room temperature).

WARNINGS AND PRECAUTIONS

- It is recommended to wear hand and eye protection as well as lab coats and follow good laboratory practices when preparing and handling reagents and samples.
- In accordance with good laboratory practice, all human source material should be considered potentially infectious and handled with the same precautions as Patient samples.
- The sample diluent is partially prepared from human blood sources. The product was tested non-reactive for Hepatitis B Surface Antigen (HBsAg), antibodies to Hepatitis C (HCV) and antibodies to HIV-1/HIV-2.
- Sample diluent and washing solution contain sodium azide as a preservative and must be handled with care. Safety data sheet is available upon request.
- For in vitro diagnostic use only. Not for internal or external use in humans or animals.
- Only personnel trained in laboratory practice should use this kit.
- Upon arrival, check the kit components for damage. If one of the components is damaged (e.g. buffer bottles), contact MADx (support@macroarraydx.com). Do not use damaged kit components, as their use may lead to poor kit performance.
- Do not use reagents beyond their expiry dates.
- Do not mix reagents from different batches.

ELISA ASSAY PROCEDURE

Flowchart of ELISA Assay Procedure



Preparation:

Preparation of samples: Serum or plasma (heparin, citrate, EDTA) samples from capillary or venous blood can be used. Blood samples can be collected using standard procedures. Store samples at 2–8°C for up to one week. Keep serum and plasma samples at -20°C for prolonged storage. Shipment of serum/plasma samples at room temperature is applicable. Always allow samples to reach room temperature before use.

Preparation of Washing Solution: Pure the content of 1 vial of Washing Solution into the washing container of the instrument. Fill destilled water up to the red mark and carefully mix the container several times without generating foam. Store at 2-8°C until expiry date if not in use.

Incubation chamber: Close lid for all assay steps to prevent drop in humidity.

Parameters of Procedure

- Predilute 10 µL sample with 490 µL FOX Sample Diluent in a separate tube and add 500 µl of the mixture onto the FOX Cartridge
- 500 µL FOX Detection Antibody
- 500 µL FOX Substrate Solution
- 100 µL FOX Stop Solution
- 4500 µL FOX Washing Solution

Assay time is approximately 3 h 30 min.

It is not recommended to run more assays than can be pipetted in 8 min. All incubations are performed at room temperature, 20-26°C.

FOX ELISA ASSAY PROCEDURE

All reagents are to be used at room temperature (20-26°C). The assay must not be performed in direct sunlight.

Prepare incubation chamber

Open incubation chamber and place paper towels on bottom part. Soak paper towels with purified water until no dry parts of the paper towels are visible.

1. Sample incubation

Predilute 10 µL sample with 490 µL FOX Sample Diluent in a separate tube. Take out the needed number of FOX cartridges and place them into the array holder(s). Add 500 µl of the prediluted sample to the cartridges. Ensure that the resulting solution is spread evenly. Place the cartridges onto the lab rocker and start the serum incubation with 8 rpm for 2 hours. Close incubation chamber before starting the lab rocker. After 2 hours, discharge the patient samples into a collection container. Carefully wipe off droplets from the cartridge using a paper towel.

Avoid touching the array surface with the paper towel! Avoid any carry over or cross-contamination of patient samples between individual FOX cartridges!

1a. Washing I

Add 500 µL FOX Washing Solution to each cartridge and incubate on the lab rocker (at 8 rpm) for 5 minutes. Discharge the washing solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels.

Repeat this step 2 more times.

2. Add detection antibody

Add 500 µL of FOX Detection Antibody to each cartridge.

Make sure that the complete array surface is covered by the FOX Detection Antibody solution.

Place the cartridges into the incubation chamber on the lab rocker and incubate at 8 rpm for 30 minutes. Discharge the detection antibody solution into a collection container. Carefully wipe off remaining droplets from the cartridges using a paper towel.

2a. Washing II

Add 500 µL FOX Washing Solution to each cartridge and incubate on the lab rocker at 8 rpm for 5 minutes. Discharge the washing solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels.

Repeat this step 4 more times.

3+4. Add substrate solution and stop substrate reaction

Add 500 µL of FOX Substrate Solution to each cartridge. Start a timer with filling the first cartridge and proceed with the filling of the remaining cartridges. Make sure that the complete array surface is covered by the substrate solution and incubate the arrays for 8 minutes without shaking (lab rocker at 0 rpm and in horizontal position).

After exactly 8 minutes, add 100 µL of the FOX Stop Solution to all cartridges, starting with the first cartridge to assure that all arrays are incubated for the same time with the Substrate solution. Carefully agitate to evenly distribute the stop solution in the array cartridges, after the stop solution was pipetted onto all arrays. Afterwards discharge substrate/stop solution from the cartridges and wipe off any remaining droplets from the cartridges using a paper towel.

Do NOT SHAKE during substrate incubation!!

4a. Washing III

Add 500 µL FOX Washing Solution to each cartridge and incubate on the lab rocker at 8 rpm for 30 seconds. Discharge the washing solution into a collection container and vigorously tap the cartridges on a stack of dry paper towels.

5. Image analysis

After finishing the assay procedure, air dry the arrays at room temperature until they are completely dry (can take up to 45 min).

The complete drying is essential for the sensitivity of the test. Only completely dried arrays provide an optimal signal to noise ratio.

Finally, the dried arrays are scanned with the ImageXplorer and analysed with RAPTOR SERVER software (see details in the RAPTOR SERVER software handbook). The RAPTOR SERVER Analysis Software is only verified in combination with the ImageXplorer instrument and with the MAX 45k analyzer, therefore MADx does not take any responsibilities for results, which have been obtained with any other image capture device (like scanners).

Assay Calibration

Semi-quantitative evaluation of the test results is based on a heterologous calibration against an IgG reference curve. Curve parameters are determined from the standard features of the test panel. A curve fit is calculated, and the resulting equation applied to transform arbitrary intensity units into quantitative units. The standard curve for each lot is adjusted by in-house reference testing

against serum samples tested on a reference test system for specific IgG against several antigens. Lot specific calibration parameters are encoded in the barcode. FOX sIgG test results are expressed as µg/ml.

Measuring Range

Specific IgG 5.0 - 50.0 µg/ml

QUALITY CONTROL

Record keeping for each assay

According to good laboratory practice it is recommended to record the lot numbers of all reagents used.

Control Specimens

According to good laboratory practice it is recommended that quality control samples are included within defined intervals.

DATA ANALYSIS

For the image analysis of processed arrays, the ImageXplorer or MAX 45k analyzer has to be used. FOX images are automatically analysed using MADx's RAPTOR SERVER Analysis Software and a report is generated summarising the results for the user.

RESULTS

FOX is a semi-quantitative ELISA method for specific IgG. Specific IgG antibodies are expressed as IgG response units (µg/ml). MADx's RAPTOR SERVER Analysis Software automatically calculates and reports sIgG results semi-quantitatively as classes (low, intermediate and highly elevated).

LIMITATIONS OF THE PROCEDURE

The IgG concentrations determined with the FOX test system show the degree of sensitization of the patient to the food antigens investigated. A correlation between the level of the determined IgG concentrations and the occurrence or severity of clinical symptoms cannot be deduced.

The results obtained must always be interpreted in combination with the full clinical symptoms. A definitive clinical diagnosis should only be made in conjunction with all available clinical findings by medical professionals and shall not be based on results of a single diagnostic method only.

False-positive test results may occur due to cross-reactivity of the tested antigen with epitopes of other antigens. Antigenic epitopes may be lost during the production of food extracts, which may lead to false negative results. IgG antibodies to food antigens that occur only during industrial preparation, food preparation or digestion may not be detectable in the patient sample.

EXPECTED VALUES

The close association between antigen specific IgG antibody levels and type III food allergy symptoms is well known and is described in literature (see section "References" below). Each sensitized patient will show an individual IgG profile when tested with FOX. The IgG response with samples from healthy individuals will be below 10.0 µg/ml for single food antigens when tested with FOX. Good laboratory practice recommends that each laboratory establishes its own range of expected values.

PERFORMANCE CHARACTERISTICS

All performance characteristics of the FOX test were determined according to the current CLSI guidelines.

Precision (lot-lot variation)

The lot-to-lot variation was determined on three cartridge lots in three separate runs. Multi-sensitized samples were included in the study. The study comprised 867 antigen/sample combinations covering 121 individual antigens over the entire measuring range.

Concentration - µg/ml	Intra CV%	Inter CV%	Total CV%
10.0 - 19.9	6.9	11.2	9.1
≥20.0	3.1	5.5	4.3
≥10.0	4.8	7.9	6.3

Repeatability (within-run precision)

In the repeatability study, multi-sensitized samples were tested 10 times by the same operator on different days. The study comprised 862 antigen/sample combinations covering 115 individual antigens over the entire measuring range.

Concentration - µg/ml	Total CV%
10.0 - 19.9	11.3
≥20.0	5.4
≥10.0	7.2

Analytical Sensitivity

Analytical sensitivity is a measurement of the accuracy of a test at low concentrations of the analyte. The limit of detection (LOD) of the FOX test is below 5.0 µg/ml for all food extracts.

Interference

There is no detectable interference with bilirubin, triglycerides and hemoglobin at normal physiological concentrations.

WARRANTY

The herein presented performance data were obtained using the procedure outlined in this Instructions for Use. Any change or modification in the procedure may affect the results and MacroArray Diagnostics disclaims all warranties expressed (including the implied warranty of merchantability and fitness for use) in such an event. Consequently, MacroArray Diagnostics and its authorized distributors shall not be liable for damages indirect or consequential in such an event.

Antigen list FOX

Vegetables: Artichoke, Arugula, Avocado, Bamboo sprouts, Broccoli, Brussels sprouts, Cabbage, Caper, Carrot, Cauliflower, Celery bulb, Celery stalk, Chard, Chicorèe, Chinese cabbage, Chives, Cucumber, Eggplant, Endive, Fennel, Garlic, Green cabbage, Horseradish, Kiwano, Kohlrabi, Lamb's lettuce, Leek, Nettle leaves, Olive, Onion, Parsnip, Pok-Choi, Potato, Pumpkin (Butternut), Pumpkin (Hokkaido), Radicchio, Radish, Red beet, Red cabbage, Romanesco, Savoy, Shallot, Spinach, Sweet potato, Tomato, Turnip, Watercress, White Asparagus, White cabbage, Wild garlic, Zucchini

Fish & Seafood: Abalone, Atlantic cod, Atlantic herring, Atlantic redfish, Carp, Caviar, Cockle, Common mussel, Crab, Eel, European anchovy, European pilchard, European plaice, Gilt-head bream, Haddock, Hake, Lobster, Mackerel, Monkfish, Noble crayfish, Northern pike, Northern prawn, Octopus, Oyster, Razor shell, Salmon, Scallop, Sepia, Shrimp mix, Sole, Squid, Swordfish, Thornback Ray, Trout, Tuna, Turbot, Venus clam

Fruits: Apple, Apricot, Banana, Blackberry, Blueberry, Cherry, Cranberry, Date, Elderberry, Fig, Gooseberry, Grape, Grapefruit, Kiwi, Lemon, Lime, Lychee, Mango, Melon, Mulberry, Nectarine, Orange, Papaya, Passion fruit, Peach, Pear, Physalis, Pineapple, Plum, Pomegranate, Raisin, Raspberry, Red currant, Strawberry, Tangerine, Watermelon

Spices: Anise, Basil, Bay leaf, Caraway, Cardamom, Cayenne pepper, Chili (red), Cinnamon, Clove, Coriander, Cumin, Curry, Dill, Fenugreek, Ginger, Juniper berry, Lemongrass, Majoram, Mint, Mustard, Nutmeg, Oregano, Paprika, Parsely, Pepper (black/white/green/red/yellow), Rosemary, Sage, Tarragon, Thyme, Turmeric, Vanilla

Cereals & Seeds: Amaranth, Barley, Buckwheat, Chickpea, Corn, Durum, Einkorn, Emmer, Gluten, Hempseed, Lineseed, Lupineseed, Malt (barley), Millet, Oat, Pine nut, Polish wheat, Poppy seed, Pumpkin seed, Quinoa, Rapeseed, Rice, Rye, Sesame, Spelt, Sunflower, Wheat, Wheat bran, Wheat gliadin, Wheatgrass

Novel Foods: Almond milk, Aloe, Aronia, Baobab, Chia seed, Chlorella, Dandelion root, Ginkgo, Ginseng, Greater burdock root, Guarana, House cricket, Maca root, Mealworm, Migratory locust, Nori, Safflower oil, Spirulina, Tapioca, Wakame, Yacòn root

Egg & Milk: Buffalo milk, Buttermilk, Camel's milk, Camembert, Cottage cheese, Cow's milk, Egg white, Egg yolk, Emmental, Goat cheese, Goat milk, Gouda, Mozzarella, Parmesan, Quail egg, Sheep cheese, Sheep milk

Meat: Beef, Boar, Chicken, Duck, Goat, Horse, Lamb, Ostrich, Pork, Rabbit, Stag, Turkey, Veal, Venison

Nuts: Almond, Brazil nut, Cashew, Coconut, Coconut milk, Hazelnut, Kola nut, Macadamia, Pecan nut, Pistachio, Sweet chestnut, Tigernut, Walnut

Coffe & Tea: Chamomile, Cocoa, Coffee, Hibiscus, Jasmine, Moringa, Peppermint, Tee (black), Tee (green)

Legumes: Green bean, Lentil, Mung bean, Pea, Peanut, Soy, Sugar pea, Tamarind, White bean

Edible Mushrooms: Boletus, Chanterelle, Enoki, French horn mushroom, Oyster mushroom, White mushroom

Others: Agar agar, Aspergillus niger, Baker's yeast, Brewer's yeast, Elderflower, Honey, Hops, M-Transglutaminase (meat glue), Cane sugar, Cross-reactive Carbohydrate Determinants

References

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3. Serological investigation of IgG and IgE antibodies against food antigens in patients with inflammatory bowel disease. HY Wang et al. 2019; World Journal of Clinical Cases
4. The Value of Eliminating Foods According to Food-specific Immunoglobulin G Antibodies in Irritable Bowel Syndrome with Diarrhoea. H Guo et al. 2012; The Journal of International Medical Research
5. Prevalence of IgG-mediated food intolerance among patients with allergic symptoms. Shakoor et al. 2016; Annals of Saudi medicine
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7. Chronic Food Antigen-specific IgG-mediated Hypersensitivity Reaction as A Risk Factor for Adolescent Depressive Disorder. R Tao et al. 2019; Genomics, Proteomics & Bioinformatics
8. Diet restriction in migraine, based on IgG against foods: A clinical double-blind, randomised, cross-over trial. Alpay et al. 2010; Cephalalgia

The connection between food intake, elevated IgG levels and chronic disorders has been described in peer reviewed publications and case studies. Nonetheless, this connection is still debated in the scientific community and a consensus has not been reached so far.

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Quick Guide