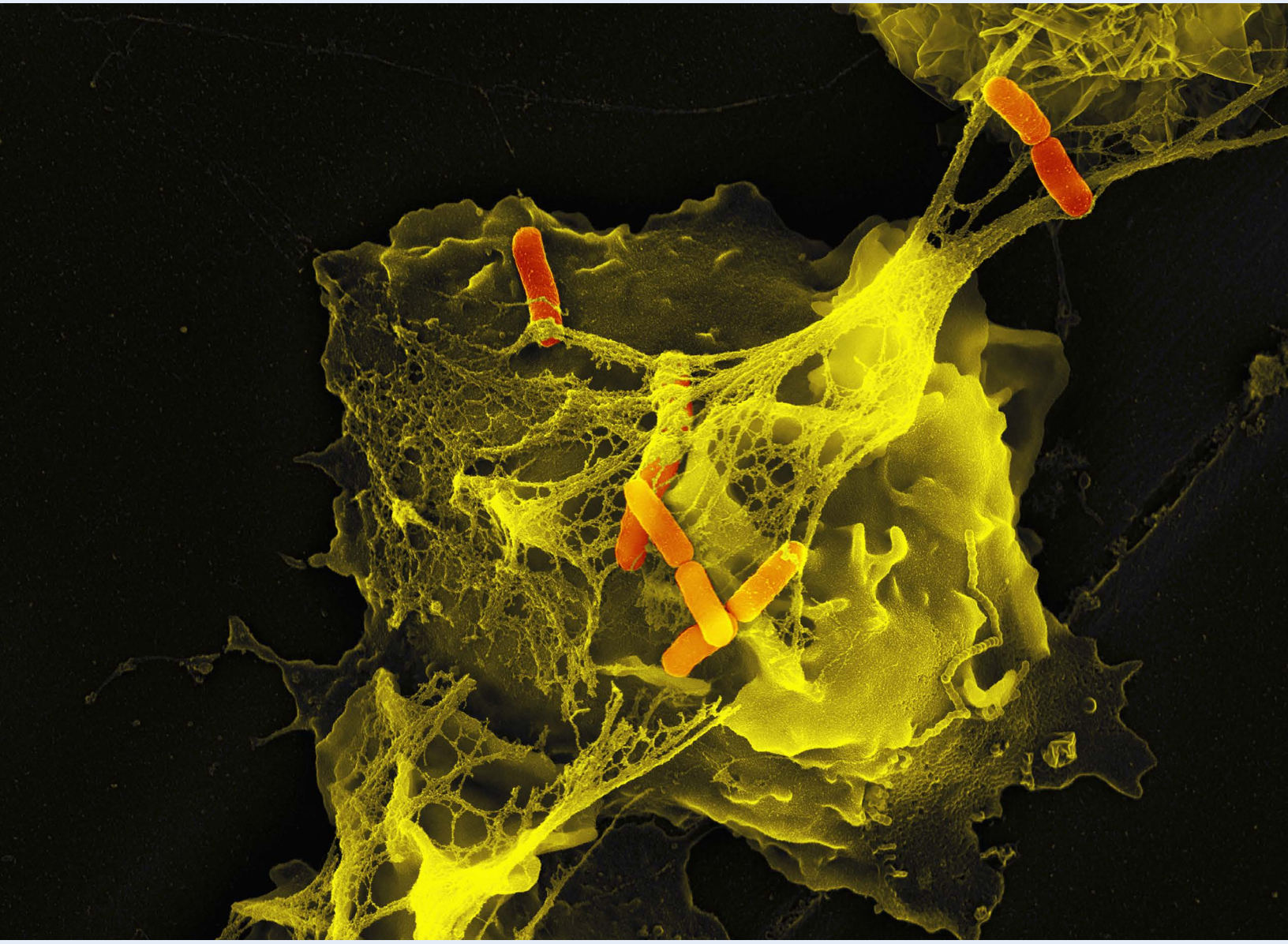
Updated Version 2025

ALCATTEST

Measurement of cellular responses to foods, chemicals, pharmaceuticals, and other substances



SCIENTIFIC DOSSIER

State of the art food and chemical sensitivity testing

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Dear Colleague,

The Alcat Test is a laboratory method for identification of non-IgE mediated reactions to foods, chemicals, and other categories of substances. As there is currently no single biomarker, molecule, antibody, serum protein, or gene, that reflects substance-induced activation of innate immunity, we investigate the immune cells to give us such information. Throughout the many years of its use by clinicians we have continued efforts to seek better and more accurate methods of cellular measurement. After thoroughly researching the latest concepts and techniques in both laser based and automated microscopic technology, it remains clear that the methodology underlying the Alcat Test, i.e. the "impedance method" remains the most reliable and accurate.

The Alcat Test differs from antibody tests. It is a functional response test and captures the final common pathway of many of the pathogenic mechanisms, immunologic, toxic, and pharmacologic, that underlie such non-IgE mediated reactions to foods and chemicals.

In the Alcat Test, the total population of peripheral WBCs is incubated, in physiologic buffers and solutions, with a battery of almost 500 different individual substances. The reaction of WBCs to each substance is analyzed by comparing the test curve, or histogram, derived from each test sample, to a master control. The master control is an average of control curves derived from the same patient sample, identically treated, but not exposed to a test sample. Thus the test is internally controlled and reflects a cellular ex vivo response to the specific test substance. Contained herein are technical reports and studies attesting clinical efficacy.

Since our food and other environmental exposures are often regular; in that people eat similar foods regularly, live and work in the same environment regularly, and take the same herbs and supplements regularly, an immune reaction triggered thereby is equally likely to be regular; i.e., chronic, potentially giving rise to chronic degenerative and metabolic conditions, like overweight and poor blood sugar control. However, despite the persistence of the symptoms, unlike the acute symptom onset associated with "true" allergy (IgE-mediated, Gell & Coombs Type 1 reactivity) such innate immune reactivity may be delayed, thus obscuring the trigger(s).

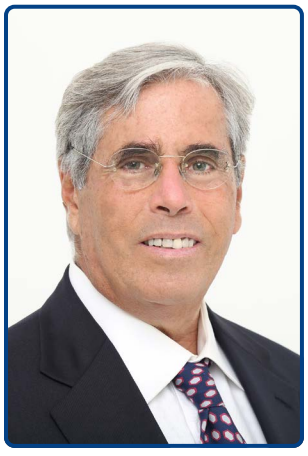
Hence, the Alcat Test frequently reveals clinically significant reactions that don't fall within the conventional definition of allergy. However, it also means that some specific allergic reactions may not overlap with the information revealed by Alcat testing. For this reason, we consider the Alcat Test to be a possible complement to conventional allergy testing, but not a substitute. Thus, if a person suffers from an IgE-mediated allergy they should seek the help of an allergist.

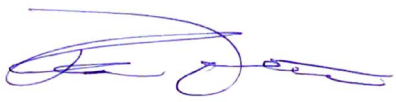
Alcat results offer the practitioner a valuable tool for identifying dietary and environmental triggers of inflammation. Using the test results, the practitioner or a nutrition counselor is able to counsel the patient on achieving a more healthful diet and lifestyle having a profound impact on both health care costs and life quality.

Throughout the 20th century and into the first decades of the 21st century astounding technological development has occurred; but, with that, an increasing prevalence of food and chemical intolerance. The words of Dr. J. Freeman, co-developer of specific immunotherapy at St. Mary's Hospital (London) seem even more apt today than they were while giving an address to the Royal Society of Physicians almost a hundred years ago:

"It might be an exaggeration to say that the study of these toxic idiopathies will open a new field of medicine, but I feel confident that they throw light from a new angle across a very large field of the old medicine."

Whether you work in a new or an old field of medicine, we hope the following pages shed light on areas that are of interest to you.





Roger Deutsch, CEO and owner

Cell Science Systems, Corp. / Cell Science Systems GmbH

A.1 The Alcat Test, a Cellular Test for Food and Chemical Sensitivities

What is the Alcat Test?

The Alcat Test is a leukocyte activation test that detects immune responses to food extracts and additives [2, 3]. This reaction primarily originates from leukocytes that represent the innate immune response. A sustained immune leukocyte response can lead to chronic inflammation, which ultimately negatively affects energy systems and performance of all body cells. A chronically activated immune system is the underlying cause of many chronic diseases. The identification of specific dietary immune triggers can support treatment and prevention of chronic symptoms and disease. Identification and elimination of these triggers may be fundamental to restoring bioenergetic balance and optimal functioning of immune and body cells.

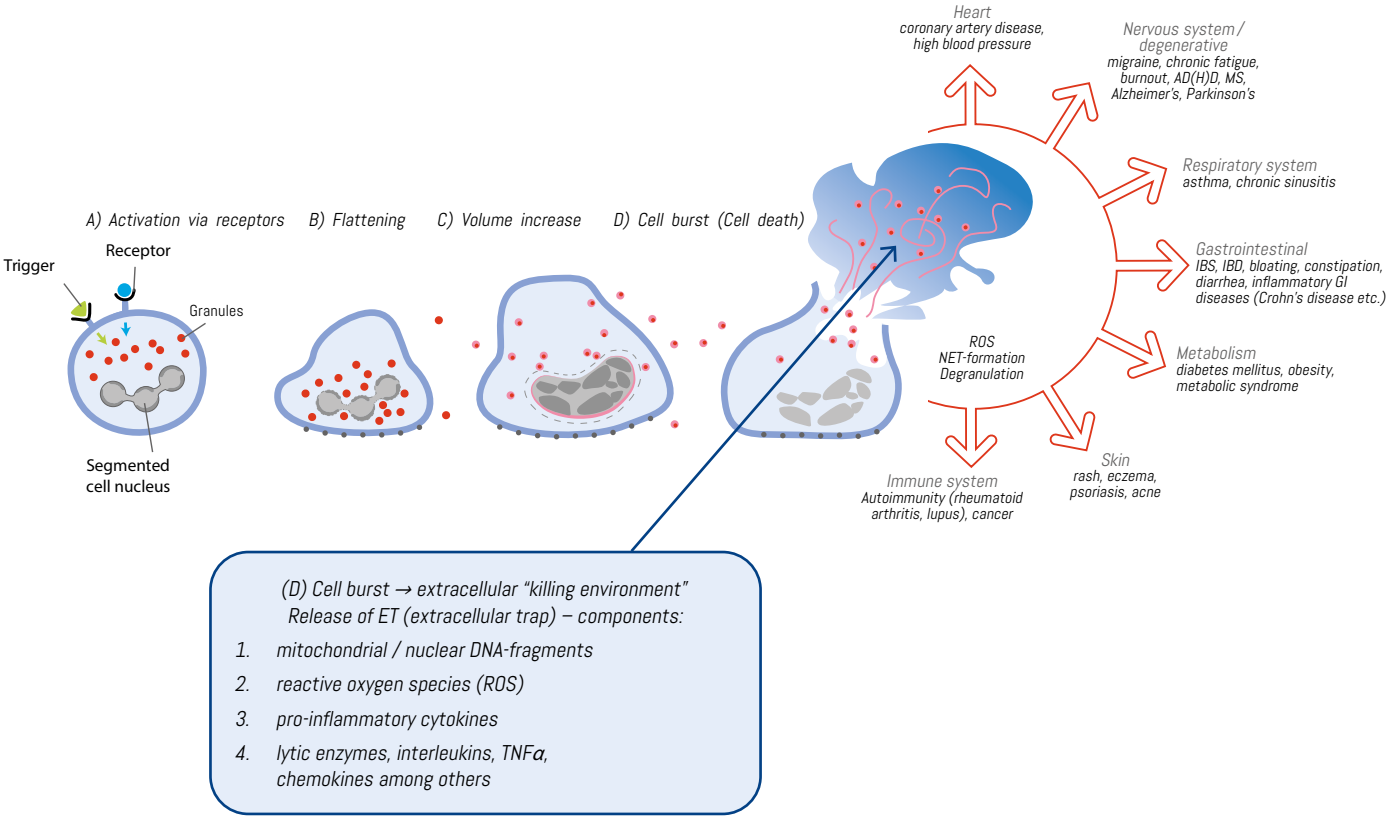


Figure A.1: Activation of polymorphonuclear leukocytes (PMN), seen here: neutrophil. In response to activation of signaling molecules of the innate immune system to various endogenous/exogenous triggers, the enzyme NADPH oxidase converts oxygen into highly reactive forms (A). Flattening and disintegration of the cell membrane and granules (B). Loss of segmentation and volume increase. The contents mix with cellular fluid (B-C). Cell burst results in degranulation and the release of reactive oxygen species (ROS), pro-inflammatory cytokines and NET (neutrophil extracellular trap) formation (D). Cellular degranulation is found to correlate with multiple pro-inflammatory health symptoms and diseases.

A.1.1 Clinical Application

The Alcat Test is used for prevention (and performance optimization, reducing internal stress factors) as well as a complementary tool for optimizing therapy for disorders that correlate with a chronically activated or malfunctioning innate immune system (table A.1).

Table A.1: All aspects and areas of the body can be affected by food sensitivities. The Alcat Test is used for chronic diseases associated with a chronically activated innate immune system (silent inflammation).

Affected Body System	Symptoms (examples)	Reference
• Gastrointestinal disorders	Diarrhea/constipation, bloating, irritable bowel syndrome (IBS), gastritis, ulcerative colitis, Crohn's disease, gastric reflux, malabsorption	[4-6, 47, 48, 50]
• Skin diseases	Eczema, psoriasis, rashes, keratosis pilaris, urticaria, acne	[6-11]
• Neurological and psychological disorders	Migraines, headaches, memory problems, Alzheimer's, chronic fatigue, mood swings, depression (related to the neuroendocrine immune system), ADHD, neuropathy, autism, schizophrenia	[5,6, 14-24, 39-45]
• Respiratory problems	Asthma, chronic cough, wheezing / bronchoconstriction, sinusitis	[6, 9, 10, 12, 25, 26]
• Metabolic diseases / Endocrine disorders	Obesity, diabetes, metabolic syndrome, inability to lose weight, weight loss, arteriosclerosis, thyroid diseases, infertility, irregular menstruation	[6, 27-31, 51, 52]
• Musculoskeletal disorders	Stiff or sore joints, arthritis, tendonitis, non-specific muscle pains	[6, 12, 32]
• Immune system and other comorbidities	Weakened viral immunity, allergies, autoimmune diseases, heart problems, tumors	[4, 6, 32-37]
• Periodontal diseases	Periodontitis	[6, 38, 39]

A.1.2 Innate Immune Cell Reactivity

Figure A.1 suggests that overactivation of the immune system by diet components/additives can impair health and may lead to tissue damage or silent inflammation.

All types of leukocyte reactions include characteristic changes of cell volume and/or number, changes which can be measured with the Alcat Test using precise impedance-flow cytometry. Thus, the aim is to control diet-induced overactivation of the immune system and its consequences.

Direct Cellular Response is a Clear Inflammatory Marker

Alcat does NOT look for one single chemical in the blood or one single antibody or factor, but tries to replicate as closely as possible in the laboratory how the whole leukocyte population responds.

→ Thus, the overall effect of a sensitivity reaction on various complex immune mechanisms and blood cells may be detected.

Immediate first defense

The direct non-specific cellular reaction is the immediate first defense and a key feature of the innate immune system. In this reaction, cells initiate and maintain pro-inflammatory reactions and cascades.

Not protein-dependent

The specific immune system (i.e. antibody formation) is mainly directed to protein agents while the non-specific cellular defense reacts against a broad trigger spectrum (pathogens, danger molecules).

Multiple factors associated with food

Foods also contain **non-proteinaceous molecules** such as fats, carbohydrates, active and vital constituents (e.g. phytochemicals), as well as xenobiotics, additives, and other processing ingredients that can contribute to adverse food reactions and which may not be detected by antibody measurement.

A.1.3 Useful Trend-Setting Biomarkers

Regardless of clinically-manifested diseases, the Alcat Test may also be useful in controlling and monitoring the diet if single or multiple markers are elevated.

Biomarkers may indicate (table A.2):

- Localized microinflammation (e.g. intestine), systemic inflammation
- Activation of the innate immune system (e.g. degranulation), or
- Cellular bioenergetic imbalance (e.g. mitochondrial dysfunction)

Table A.2: Examples of biomarkers in localized (e.g. intestine) and/or systemic inflammation, innate immune activation, and cellular bioenergetic imbalance.

Non-specific Inflammatory Marker	Innate Immune Activation Indicators of direct inflammation (in the intestine)	Bioenergetic Cellular Imbalance
Histamine levels (stool)	Eosinophil granulocytes:	Reduced **mitochondrial energy capacity**
SAA (serum amyloid A)	**EPX** (Eosinophil protein X), **EDN** (Eosinophil-Derived Neurotoxin)	Reduced mitochondrial **stress resistance**
CRP (C-reactive protein; serum)	Neutrophil granulocytes & monocytes:	**Oxidative stress**
BDNF (brain-derived neurotrophic factor; serum)	**Alpha-1-Antitrypsin** (serum), **Lactoferrin** (stool), Lysozyme (stool), **PMN** (Polymorphonuclear Neutrophil) **Elastase** (stool), **MPO** (Myeloperoxidase; serum/stool), **LPS** (Lipopolysaccharide; serum), **ROS** (reactive oxygen species), **sCD14** (soluble CD14), **Calprotectin** (stool, serum)	
Calprotectin (serum)		
HMGB1 (high-mobility-group-protein B1; serum)		
Cytokines (IL-1, IL-6, IL-8, TNF-alpha)		

A.1.4 Basic Testing Principle

The Alcat Test is a leukocyte activation test that measures cellular immune response following exposure to individual food substances at the molecular level. These molecular processes and cellular changes in leukocytes can be detected via means of a measurement methodology known as impedance cytometry.

Cells and pro-inflammatory responses to danger signals

Cellular changes are associated with pro-inflammatory reactions triggered primarily by degranulation processes of granulocytes (e.g. neutrophils), the main fraction of the leukocyte population.

Degranulation processes are an early immune response to danger signals. Granulocyte reactions are thus a clear indicator for the sensitivity to or intolerance of a substance.

Response detection to multiple trigger items

The effect of more than 450 individual items (foods, medicinal herbs, additives, colorings, pharmaceutical agents, molds, etc.) can be analyzed if they adversely affect the immune system.

Dietary guidance, part of Alcat testing, includes the temporary elimination of items that trigger an immune cell response. Ultimately, the aim is to prevent an unnecessary burden to the immune system, support recovery of body functions, and promote immune equilibrium.

A.1.5 Testing Method

Blood sample

Customized test kits can be obtained from Cell Science Systems. The blood sample is received in the laboratory via an expedited courier while the cells are still viable. The effector cells (e.g. neutrophils) have a lifespan of about 5 days. Each patient's sample may differ. Thus, the leukocytes are analyzed within 30-72 hours of blood collection. The integrity of the cells and cell population is controlled prior to each analysis. [40]

Pre-analysis

Each patient's cellular profile is unique. The leukocyte population is mainly composed of neutrophils, eosinophils, basophils, macrophages/monocytes, and lymphocytes. The individual cell types are present in the blood in different quantities (figure A.2).

First a preliminary test is performed to determine cell number and distribution. Next, the patient's diluted whole blood is introduced to the individual test substances to confirm the sample is viable.

Test substances and cell confrontation

Test substances are prepared from organic raw material following strict preparation protocols and quality assurance guidelines. The manufacturing division is an FDA registered medical device establishment and is also ISO EN 13485 certified as a medical device manufacturer. Cell Science Systems Lab is certified and licensed by the U.S. government.

The leukocytes are pipetted into test cassettes using a robotic pipetting system that is especially designed for the Alcat Test. It has an error factor of less than one percent. After incubation, the tray is placed in the instrument for fully automated analysis.

Quality controls

All operations from instrumentation health, to quality control testing, to data integrity verification, are constantly monitored in real time and displayed on screen throughout the laboratory. These systems are HIPAA compliant and provide traceability and validation that all of our processes and procedures are performed according to protocol.

Measuring changes of cell volume/ form and number

The reaction of the leukocytes as immune effector cells of the innate immune response is detected primarily by changes in leukocyte cell volume and/or cell number.

- **Cell volume increase**: When an immune stimulus promotes an effector cell response that involves formation of mediators (particularly PMN cells), changes are usually associated with cell volume increase.
- **Cell volume decrease** is usually reflective of degranulation, onset of apoptosis, or autophagy.
- **Cell number decrease** is indicative of apoptosis, NETosis, necrosis, or pyroptosis

A.1.6 Measurement Technology

Cell changes are detected using specialized automated impedance-flow cytometry, a form of "non-invasive real-time cell monitoring."

Measuring principle

The measuring principle of the ROBOCat II device is based on the fact that during the passage of a cell through an electric field, the resistance (as a function of the cells) will change in proportion to the cell's volume (impedance). The system's processor will capture the change and express it as a pulse where its amplitude is directly proportional to the cell's volume. The number and size distribution of the cells is displayed graphically.

For relative analysis, the degree of deviation of the measurement to the control curve (baseline/negative control) is determined mathematically using computer algorithms. It is then categorized into four response levels: severe, moderate, mild, or negative.

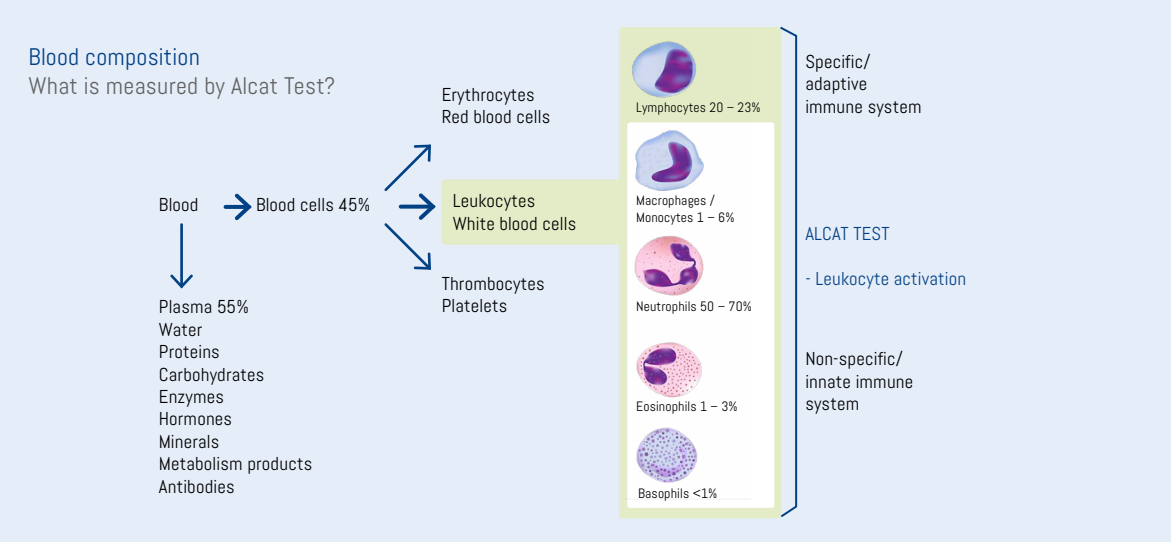


Figure A.2: Blood composition - Leukocytes. Leukocytes, also known as white blood cells, comprise the cellular components of the specific and non-specific immune system. Alcat Testing detects all blood leukocytes and records their distribution based on size. Reactivity is measured by analyzing changes in their number and volume.

Precision and comparison to other accepted methods

Figure A.3 illustrates three different methods of measuring a subject's reaction to a test challenge – in this example is an extract derived from lamb. Natural Immune System Labs examined blood cells after exposure to the lamb extract using the following three methods: CytoViva optical microscopy (left), Alcat ROBOCat II analysis (middle), and conventional FACSCalibur flow cytometry (right).

The two flow cytometry methods – FACSCalibur and Alcat ROBOCat II – show a very close correlation. Each revealed a reduced population of intact cells due to degranulation and lysis and a larger population of cells swollen by internal reactions. This finding is also clearly evident with light microscopy, which vividly shows evidence of cellular lysis. [41]

Why does the Alcat Test use the impedance-flow cytometry as a measurement technology?

The measurement technique for the Alcat Test is a specific combination of flow cytometry and impedance methodology. According to the current state of science, it is used to analyze early cellular responses, and is superior to all other available methods for the analysis of cellular responses. Analysis of cellular responses by conventional flow cytometers can miss early adverse cellular responses. We refer to the work of Prof. M. Cooper, a pioneer in label-free technology for "continuous non-invasive real-time cell monitoring."

(Label-Free Technologies for Drug Discovery, Wiley-Blackwell; Label-free Biosensors: Techniques and Applications. Cambridge University Press).

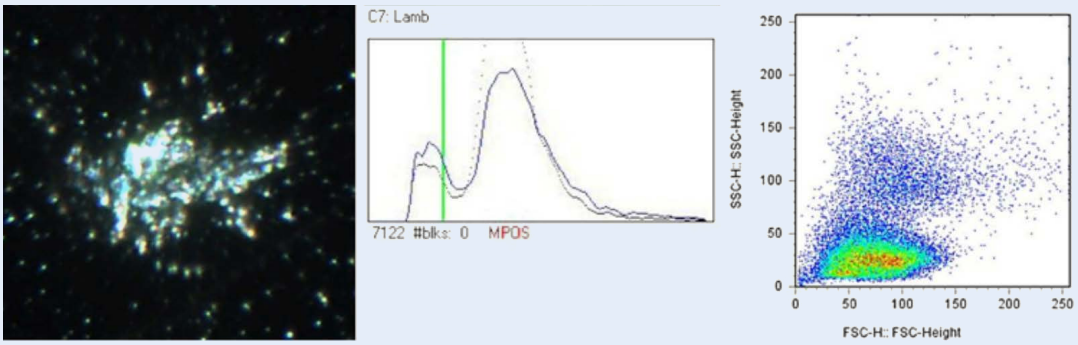


Figure A.3: Strong reaction of neutrophil granulocytes (NG) to lamb (cell lysis). Blood sample measurements comparing different methods of reactivity analysis. Left – CytoViva microscopy showing fully degranulated and lysed NGs. Middle – Alcat flow cytometry with ROBOCat II: The blue curve indicates cell lysis due to degranulating NGs compared to the patient's normal blood (dashed curve). Right – Classic flow cytometry with FACSCalibur. Source: NIS Labs Study. [41]

A.2 Validation of the Alcat Test

Introduction: The gold standard for identifying food sensitivity is oral provocation. Accordingly, only an immunological blood test can approach the gold standard, which measures the effect of food substances on precisely those immune parameters that are responsible for the biological effector function. The validation of an in vitro test, such as is the Alcat Test, is divided into an a) analytical and b) clinical validation.

The analytical validation results from both clinical studies and from approvals, certificates, and patents (See A.4.3 and A.4.4). The studies presented in chapter D represent clinical validation of the Alcat Test for the identification of sensitivity to food ingredients, additives, and other compounds. Observational studies of approximately 1,500 patients serve to confirm the analytical and clinical validation.

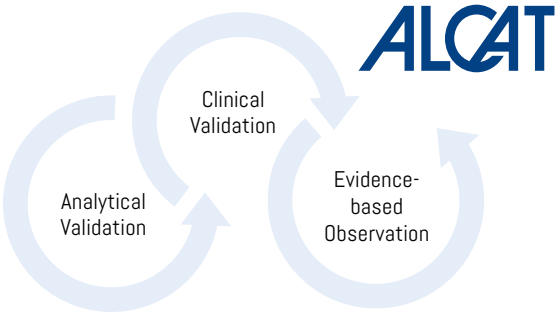


Figure A.4: The Alcat Test is validated analytically and clinically as a food sensitivity test. The efficacy is demonstrated in a meta-analysis of approximately 1,500 documented patients.

A.2.1 Analytical Validation

Analytical Reproducibility, Specificity, and Sensitivity

For analytical validation, three studies were carried out showing a **reproducibility between 92-96%** (appendix).

To validate the Alcat Test analyzer ROBOCat II, a comparison study was performed between a conventional flow cytometer (FACSCalibur) and the ROBOCat II (Alcat analyzer using impedance-flow cytometry). The measurement results of the flow cytometer and the ROBOCat II correlated and additionally were confirmed by microscopic images (CytoViva microscopy), see A.1.6; figure A.3. [41]

Cell Sciences Systems Corp. has CLIA certification and is registered with the FDA (Food and Drug Administration) and thus analytically validated by these entities. Furthermore, the company can provide proof of compliance with current GMP standards (cGMPs) and EN ISO 13485:2016(E) for the production of ROBOCats and test substances (see A.4.3).

A.2.2 Clinical Validation

Clinical Sensitivity & Specificity

The clinical validation of in vitro laboratory methods is mainly characterized in two ways: a) the diagnostic sensitivity and b) diagnostic specificity.

The diagnostic **sensitivity** is the percentage by which the test correctly identified persons (rp) with the relevant sensitization to the tested food/additive when compared to all individuals with a sensitization to the tested foods/additive (rp/(rp + fn). In short: the people with an identified sensitivity are actually recognized as **"True Positive".**

Diagnostic **specificity** reflects the proportion of the detected **"True Negative"** test subjects of the non-sensitized (rn/fp + rn).

The results are entered according to a general consensus in a 4-field table (table A.3).

Table A.3: four-column table for clinical validation: rp: true positive; fp: false positive; rn: true negative; fn: false negative

	Sensitization / Patient state of health		Sum
	Sensitized	Non-sensitized	
Test results positive	True positive (rp)	False positive (fp)	rp+fp
Test results negative	False negative (fn)	True negative (rn)	fn+rn
Sum	rp+fn	fp+rn	rp+fp+fn+rn

Clinical Alcat validation

To assess the clinical sensitivity and specificity of the Alcat Test, **two double-blind placebo-controlled studies** were performed on 154 study subjects:

a) 1988 in England with 58 study subjects

b) 1996 in Denmark with 96 study subjects

The clinical sensitivity and specificity were assessed at 83.4% for foods and 96% for colorings, ≥ 80%, which is remarkably high.

Thus, the determination of clinical sensitivity and specificity of the Alcat Test on 154 subjects was carried out according to the guideline for "Diagnostic validation of the Robert Koch Institute" with the recommended minimum sample size of 120 study subjects (Bundesgesundheitsblatt/ Gesundheitsforschung/Gesundheitsschutz 2008, 51:1353–1356).

Other interesting clinical observations

There was a high correlation between positive reactions and the severity of a disease (e.g. healthy subjects being less reactive vs. those with hay fever/allergic rhinitis, asthma, irritable bowel syndrome being more reactive).

In studies, the **meta-analysis of approximately 1,500 patients** showed that modifying the diet according to Alcat Test results led to positive improvements in many of the common symptoms and chronic diseases listed in table A.1.

Summary

The abstracts of more than 40 studies on the Alcat Test are documented in part D.

The overall study material provides a reliable overview of the Alcat Test as a useful tool in clinical practice for guiding the treatment of patients with food sensitivities and inflammation-related diseases.

A.2.3 Molecular Pathomechanisms

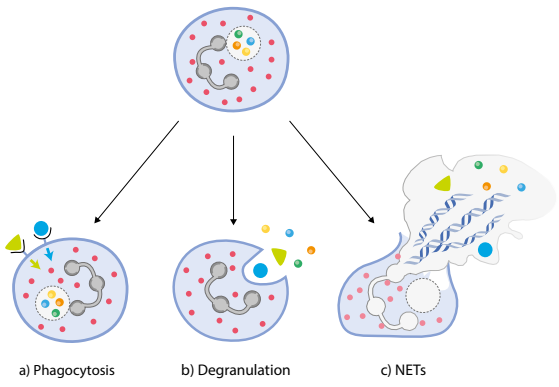


Figure A.5: Link to the Alcat Test measurement; intra and extra-cellular killing mechanisms of immune cells

Pathogens and danger molecules lead to cell activation

In humans, 50–70% of circulating leukocytes are neutrophils and thus the predominant immune cell population in human blood.

Neutrophils form the frontline of defense in the innate immune response to invading microorganisms and microbial structures called **pathogen-associated molecular patterns (PAMPs),** as well as to foreign particles. Analogous to PAMPs are endogenous molecules called **danger/damage-associated molecular patterns (DAMPs)** that also trigger an innate immune response.

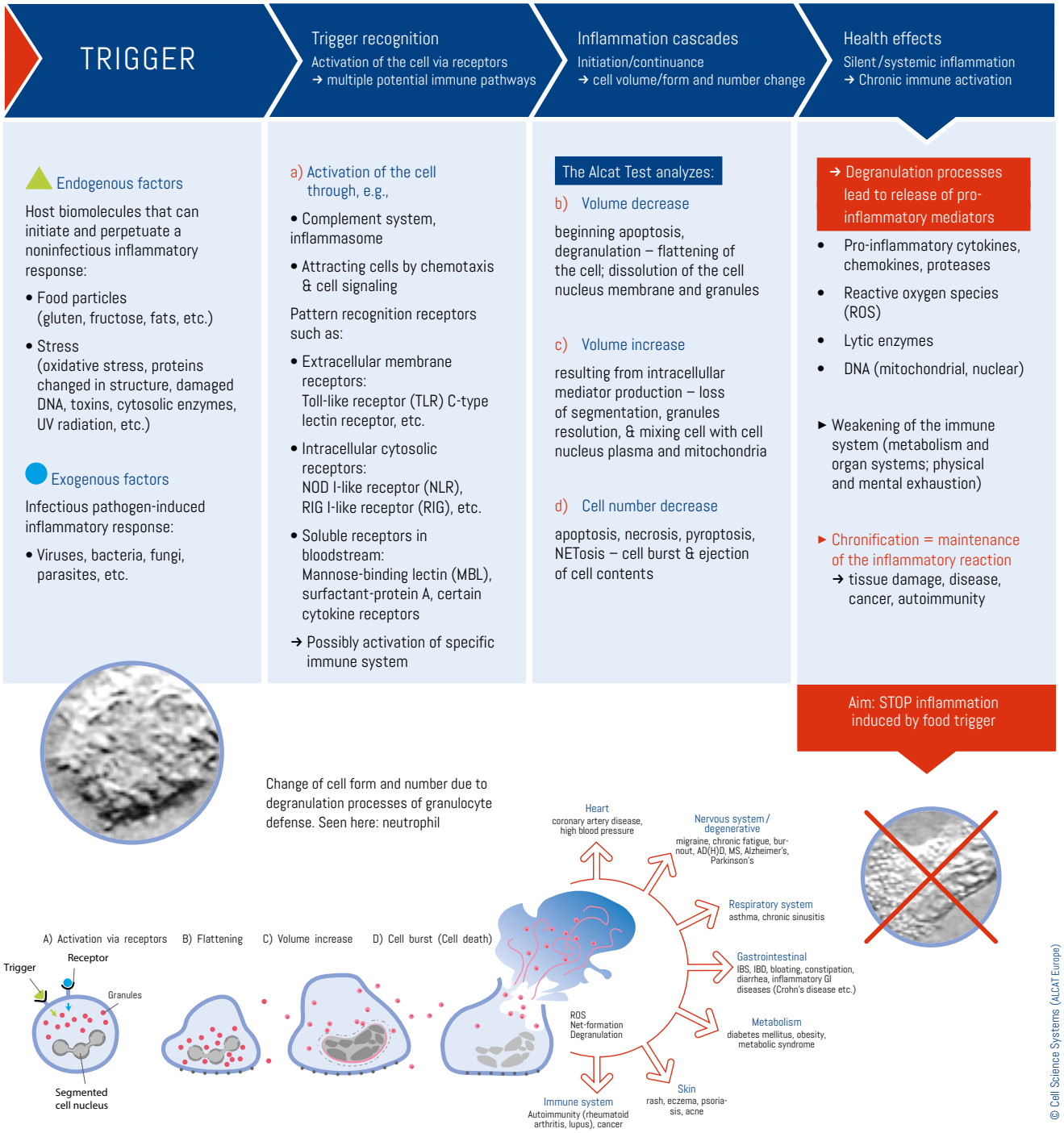


Figure A.6: Inflammatory cascade of the innate immune system

Diverse stimuli, including endogenous factors (DAMPs) and exogenous factors (PAMPs) can trigger reactions of innate immunity via signaling pathways and activation of specific pattern recognition receptors (PRR) resulting in inflammation. The Alcat Test recognizes blood cell changes, especially those occurring in PMN cells (seen here: neutrophil). Sustained inflammatory reactions weaken the entire organism and produce or exacerbate a myriad of symptoms or disorders. To detect and characterize food sensitivity, the Alcat Test describes the reactions of cells to food in terms of changes in cell volume/size and cell number.

Intra- and extra-cellular "killing environments"

Neutrophils use a number of strategies to respond to PAMPs, foreign particles, and DAMPs. Such strategies include phagocytosis, degranulation, inflammasomes (innate immune sensors), and the recently discovered formation of extracellular traps (ET) (figure A.5).

a) During **phagocytosis,** internalized pathogens, particles, or DAMPs are translocated to phagosomes where antimicrobial factors derived from granules and reactive oxygen species (ROS) create a killing environment.

b) The second mechanism, **degranulation,** is similar to phagocytosis. However, during phagocytosis rather than being engulfed, the pathogens, particles, or DAMPs are killed extracellularly by the same antimicrobial factors that are in part released outside the cell.

c) The **neutrophil extracellular traps (NETs)** can be released by neutrophils in a process called **NETosis.** NETs are a special kind of trap formed by condensed chromatin fibers along with antimicrobial factors delivered by the granules.

Double-edged sword defense

Neutrophils act as "double-edged swords" as major effector cells in innate immunity. While they are necessary in defense, over-activation of neutrophils can be fatal.

- In this regard, neutrophils that continuously react to food ingredients/chemicals, either by phagocytosis, degranulation, or formation of NETs, contribute to tissue damage and chronic inflammation. [40-43]

The Alcat Test detects all reaction types

> The Alcat Test detects all three types of leukocyte reactions toward food ingredients/chemicals. These distinct reactions are automatically measured by the ROBOCat II and transferred into a reaction profile (non-reactive, mild, moderate, or severe reactive) using a specific algorithm (figure A.7).
>
> In this context, Yale School of Medicine was first to demonstrate (in 2015) that DNA (acting as DAMPs) and myeloperoxidase of peripheral blood leukocytes were released during certain food reactions and that these items were identified with the Alcat Test. [2, 3]

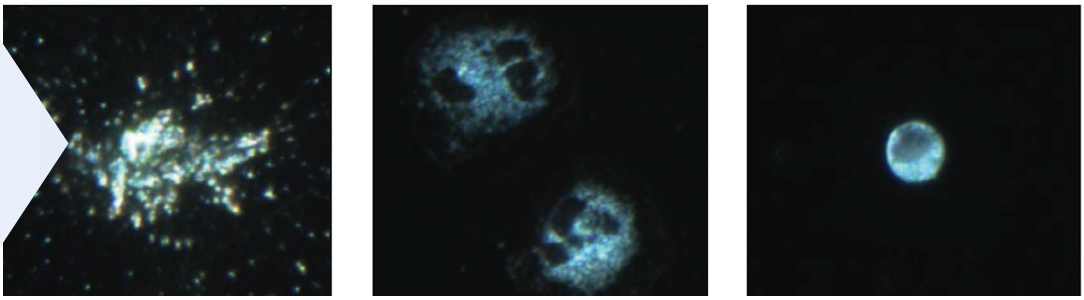
Conclusion

The Alcat Test can demonstrate a clear correlation between a) an Alcat reaction, b) a specific test item, and c) the pathogenic inflammatory effect.

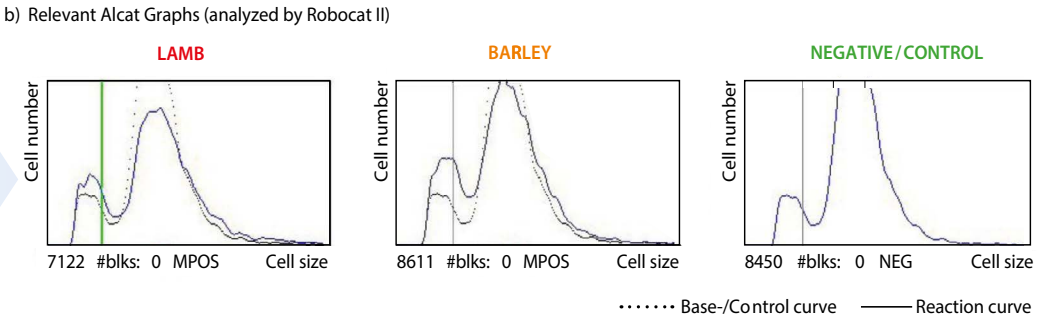
A.3 Alcat Test Results

The Alcat Test analyzes the effect of each individual test substance on the leukocytes. Reactions are displayed on a single page results sheet using a simple color scheme (red, orange, yellow, green) so that the patient receives a structured and easy-to-understand overview of almost 500 test agents.

A) Microscopic picture
of PMN cells after the exposure with reactive and non-reactive food extracts.



B) Alcat Test histogram
(analyzed with ROBOCat II); Comparison of leukocyte reactions in automated process after exposure with the extracts (reaction curves) and without (control curve).



C) Reaction gradient
Classification of tested foods into four color categories according to the measured cell reactivity of the histogram.

- Severe reaction (example lamb): cell burst → recommendation: 6 month elimination
- Moderate reaction (example barley): distinct cell change → recommendation: 3-6 month elimination
- Mild reaction → recommendation: Limit intake
- Non-reactive substances show no cell change (size, number).

Figure A.7: The Alcat Test analyzes the effect of each tested individual substance to the leukocytes. Cellular immune reactions lead to changes in cell volume and cell number, which are displayed in histograms after mathematically computerized algorithm evaluation. Thus, the degree of reaction is exactly calculated and "mirrored" as a snapshot in four reaction degrees: severe, moderate, mild, no reaction.

A.3.1 Alcat Degrees of Reactivity

Cells are not static or unchanging, but instead are living and dynamic entities. It may be said that the Alcat analysis provides a snapshot, "mirroring" these biological processes in four degrees of reactivity (figure A.7).

Influence of epigenetic factors

Some sensitivities are governed by inborn errors of metabolism and may remain fixed for many years or even the entire lifetime. However, most sensitivities are greatly influenced by lifestyle factors. For example, poor nutritional status, stress (emotional, physical), and chronic disease may impact the gastrointestinal and immune systems and impact the cell's degree of reactivity.

More than 500 individual substances can be analyzed with the Alcat Test. Flexible test profiles combining foods, chemical additives, and medicinal herbs are available.

A.3.2 Test Result Overview

Alcat Test results are easy to understand using a clear structure. Follow-up to ensure patient compliance includes detailed personal instructions and optional support of nutritional counselors for implementing dietary changes.

Food Sensitivity Test Report (figure A.9)

Includes special sections regarding the reactivity to:

1. Gluten (gluten/gliadin, individual grains)
2. Casein/Whey (cow's milk, goat's milk, sheep's milk)
3. *Candida albicans* (and various forms of sugar)

Chemical Sensitivity Report and others

1. Additives/E-numbers (colorings, flavorings, sugar alternatives, preservatives, environmental chemicals)
2. Pharmaceutical substances
3. Bioactive agents (e.g. biogenic amines)
4. Molds

Medicinal Herbs/Functional Foods Report

1. Basic: medicinal herbs, functional foods, adaptogens
2. Performance profile (athletes, supplements) (U.S.: Male herb profile)
3. Female herb profile (menopause)

Personalized "Foods to avoid" list

Personalized "Rotation Guide"

Guidance Notes include directory of test items

A.3.3 Elimination Period and Reintroduction of Reactive Foods

For reactive foods, a 3-6 month rotation/elimination period is recommended.

"Reset" of immune reactivity

Unlike allergies, the innate immune system (no antibody formation) operates without "memory function."

The absence of exposure to the substance may result in resolution of the sensitivity. Thus, reactive foods can often be tolerated again after the defined elimination period ("reset"). [20]

A.3.4 Alcat Test Substances /Biological & Chemical Compounds in Today's Foods

Foods are principally derived from vegetable and/or animal sources. However, today numerous chemicals are in our foods as well. They may be intentionally added or may result from natural and chemical processes involved in food production.

Chemicals in food, cosmetics, and environment

In addition, additives such as preservatives, hormones, drug contamination (antibiotics, xenobiotics), flavors, flavor enhancers, spices, colorings, stabilizers, etc. may cause health problems.

Chemicals can also be found in cosmetics, cleaning products, cigarette smoke, pesticides, and exhaust.

Table A.4 provides an overview of possible food contaminants. Even people who eat primarily organic foods are not exempt from exposure to additives and contaminants. Population-based surveys have shown that (multiple) chemical sensitivities occur with a prevalence of 2-13%. Moreover, the researchers suggest that approximately 20% of patients may have sensitivities to chemicals. [44]

It is known that chemical substances can lead to malfunction of biochemical pathways in the body, disruption of the intestinal flora, and to both localized and systemic inflammation resulting in a variety of symptoms.

Chemical substances can cause both physical and psychological symptoms. Symptoms are typically multisystemic and can affect cognitive function as well as musculoskeletal, gastrointestinal, genitourinary, and cardiovascular systems. [44]

Figure A.8: A standard fast food meal may contain more than 100 artificial substances (colorings, preservatives, environmental chemicals, artificial sugars, pesticides).

Table A.4: Potential contaminants in food (Food and Chemical Toxicology (FCT).

▸ Elementary contaminants	such as mercury, cadmium, manganese, arsenic
▸ Mycotoxins	such as aflatoxins, ochratoxin, fumonisins, trichothecenes
▸ Additives	such as sulfites, nitrites / nitrates, benzoic acid
▸ Pesticides	such as organophosphates, carbamates, DDT, pyrethrins
▸ Process contaminants	such as acrylamides, polycyclic aromatic hydrocarbons (PAHs), chloropropane
▸ Allergens	such as peanuts, wheat
▸ Pharmaceuticals from agriculture	such as antibiotics, hormones

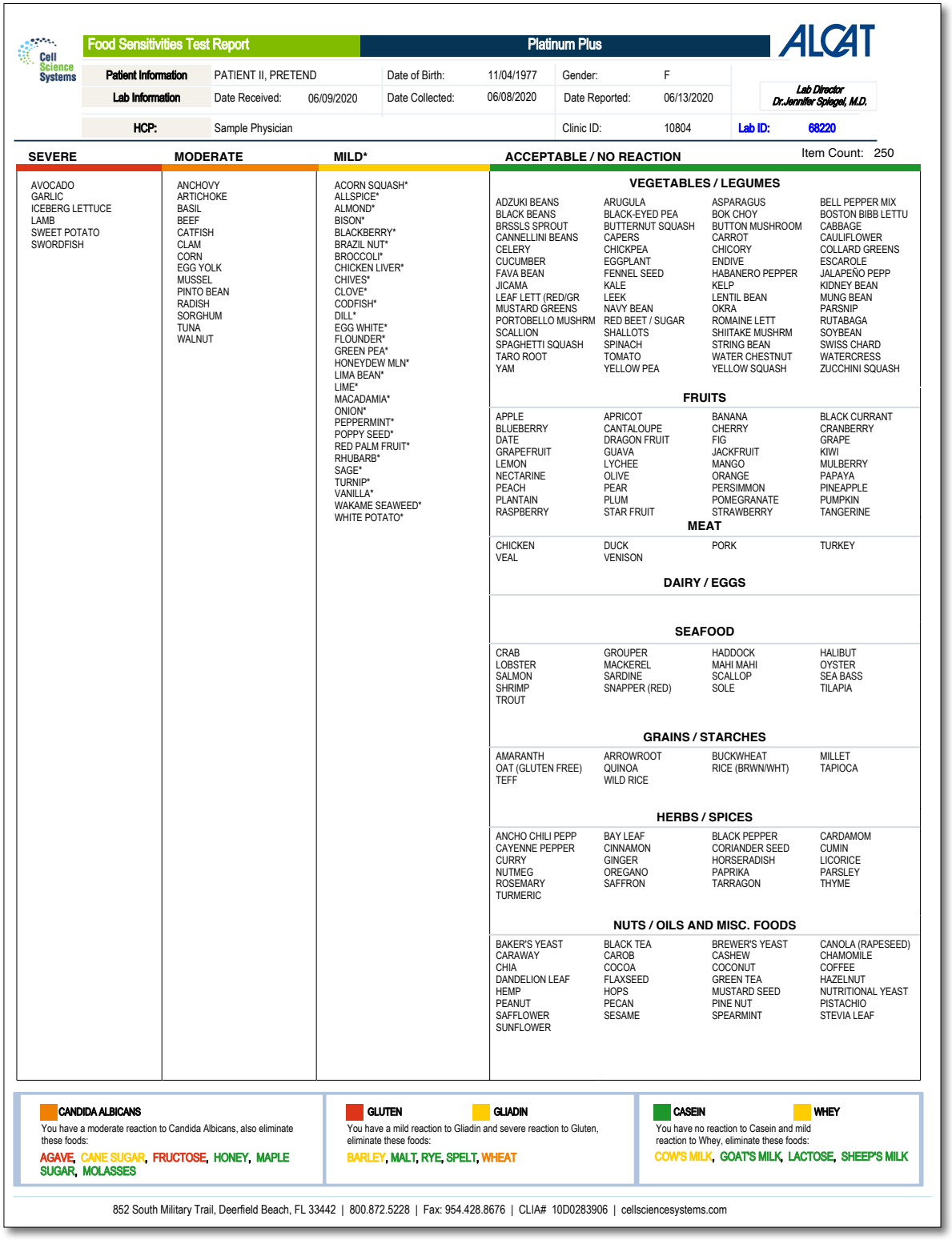


Figure A.9: Example: The Alcat food analysis is clearly presented on one single page, for example, with a test panel of 250 foods as seen here. Individually reactive foods are summarized along with degree of reaction in 4 categories - severe, moderate, mild, no reaction (no. 1). In addition, the analysis includes reactions to specific food components ("blue box") related to the intake of gluten products, dairy, and sugar (no. 2).

A.4 Company History

Cell Science Systems nearly 40 years of research, development, and experience with the Alcat Test to use. The Alcat method is based on the principle that an ongoing immune response of the innate immune system to foods and its ingredients leads to uncontrolled immune activation causing chronic inflammation. The relationship between an overactivated innate immune response and various chronic inflammatory diseases is now recognized.

A.4.1 Cell Science Systems, Corp. , USA

CELL SCIENCE SYSTEMS

Cell Science Systems (CSS) is a specialty clinical laboratory that develops and performs laboratory testing in immunology and cell biology, supporting the personalized treatment and prevention of chronic disease.

Testing includes genetic panels that can guide nutrition and assess disease risk, an array for assessing GI risk and status, hormone levels, and the CSS flagship service, the Alcat Test for identification of food sensitivities.

- The flagship of CSS is the Alcat Test for food and chemical sensitivities. R. Deutsch is one of the pioneers of food and chemical sensitivities testing with involvement in this field since 1986.
- CSS operates a CLIA-certified laboratory and is an FDA inspected and registered, cGMP medical device manufacturer meeting ISO EN13485 standards.
- **2016 "North American Food Intolerance Testing Company of the Year Award:"** independent Frost & Sullivan experts concluded that CSS offers progressive health solutions and great patient benefits for those suffering from inflammation induced or chronic diseases. CSS was awarded an "excellent"score for its services, innovations, and benefits.
- A division of CSS, PreviMedica, provides telehealth and nutrition/lifestyle counseling for customers. PreviMedica offers free test result reviews for health care providers and their patients.
- GOOD MANUFACTURING PRACTICE GMP CERTIFIED · FDA REGISTERED FACILITY · Clinical Laboratory Improvement Amendments · MEDICAL DEVICES QUALITY MANAGEMENT ISO 13485:2016 · CE

A.4.2 Vertically Integrated Production of the ROBOCat II Analyzer and Reagents

- Vertical integration requires registration and compliance with FDA current good manufacturing procedures. This is unusual for a laboratory; laboratories usually purchase their instrumentation from 3rd parties.
- CSS exercises 100% control over the substances that go into the test to control their potency.
- The ROBOCat II, as well as the reagents, are registered with the Food & Drug Administration of the United States and are produced under strictly supervised ISO processes. Thus, the reproducibility of the analysis is ensured and excludes a subjective interpretation.
- **Unique vertically integrated patient tools and nutrition network support;** Utilizing proprietary software tools, PreviMedica provides the services of in-house registered dietitians/nutritionists (RDNs).

A.4.3 Certificates and Licenses

ISO 13485:2016(E), GMP, CE Mark

- ISO 13485:2016 (E) compliance is the international quality management standard for medical devices in addition to compliance with cGMP guidelines.
- The laboratory of Cell Science Systems carries the certification code 220 for "General Immunology."
- The CE mark grants permission to distribute the analysis device in the EU.

Food and Drug Administration (FDA) Registration

Under the FDA's Establishment Registration and Device Listing, Alcat's listing shows the following:

Proprietary Name	ALCAT
Classification Name	Whole blood plasma, Antigen, Antiserum Control
Product Code	DGQ
Device Class	1
Regulation No.	866.5770
Medical Specialty	Immunology
Registered Establishment	Cell Science Systems, Corp.
Registered Establishment No.	1051901
Owner/Operator	Cell Science Systems, Corp.
Owner/Operator No.	9070941
Establishment Operations	Manufacturer

The analyzer used in Alcat Testing has been registered with the FDA. Manufacturing is carried out under cGMPs in compliance with FDA guidelines and subject to inspection by the FDA, separate from inspections of laboratories using the device. Food extracts and medicinal herbs are mainly produced from organic sources.

CLIA License

CLIA refers to the Clinical Laboratory Improvement Act of 1963, amended in 1988. CLIA requires adherence to U.S. federal regulatory standards that apply to all clinical laboratories that perform laboratory tests on human specimens in the United States, except clinical trials and basic research.

The Alcat Test is considered a high complexity test and is required to have systems and processes for monitoring testing equipment, procedures to ensure proper test performance and accurate results, and an overall plan to monitor the quality of all aspects of the laboratory's ongoing operation.

The objective of CLIA is to ensure accuracy, reliability and timeliness of test results regardless. Therefore, the Alcat system has stringent quality control requirements relating to purity of test antigens and standardized processes: temperature control of samples; dilution of chemicals; incubation, maintenance and control of the analyzer; and checking all results regarding conformity (compliance).

A.4.4 Patents

The company, with assistance of Prof. D. H. Sandberg from the University of Miami, was involved in creating the technology referenced in the original U.S. patents, including one apparatus and one method patent (with various continuation-in-part applications and numerous international counterparts).

Both U.S. and foreign patents have been awarded based on the principles, technology, and apparatus that are used in the Alcat Test to measure the degree of reaction between immune cells and antigens.

International Patents:

- Publication: 0 140 379 B1, Int. Cl.: G01N 15/12, G01N 33/53, application 84113101.4, Publication date: 15.04.1992, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
- Publication: EP0281626, Int. Cl.: G01N33/48, G01N33/566, G01N33/53, G01N33/554 1988/37],
- Publication 28.05.1997, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
- Publication: WO 92/01934, Int. Cl.: G01N 33/48, 33/00, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

US Patents:

- 5,147,785: Method and apparatus for measuring the degree of reaction between a foreign entity and white blood cells.
- 4,788,155: Method and apparatus for measuring the degree of reaction between a foreign entity and a subject's blood cells.
- 4,614,722: Method and apparatus for determining reaction between a substance and a blood sample, by comparing the number and/or size-distribution of white blood cells present in the blood sample before and after reaction.

B.1 Food Sensitivity/Intolerance – Definitions

The term "allergy" is often used as a catch-all phrase to describe all of the body's altered and excessive responses to otherwise harmless substances. However, a true allergic reaction is a function of the specific immune system (figure B.1; "The Targeted Response") while the broader category of sensitivity is generally associated with non-specific immunity (figure B.1; "The Firewall"). Findings show that inflammatory cascades, initiated by the cells of the non-specific immune system, play a central role in adverse reactions and are regarded as the cause of systemic/silent inflammation. New studies are published every year elucidating the complex interplay and impact that the microbiome, the immune system, and the intestinal barrier have on health. Consequently, this evolution results in revolutionary insights, broader perspectives, and new definitions.

FOOD ALLERGY adaptive/specific immune system	FOOD SENSITIVITY innate/non-specific immune system
Trigger: Allergen/antigen (mostly protein structures) → specific recognition of antigens (antigen dependent) memory	Multiple triggers: e.g. DAMPs, toxins, proteins, or nonprotein-based molecules Interpretation self/foreign – harmless/dangerous → non-specific (immunological, metabolic, toxic) No memory (or only rudimentary memory)
Cells: Mast cells, degranulation of basophils → Histamine release T & B cells → IgE antibodies	Cells: Cell activation, e.g. neutrophils, eosinophils → release of pro-inflammatory mediators: Cytokines, chemokines, lytic enzymes, reactive oxygen species (ROS), DNA, etc.
Acute immediate and dramatic symptom onset	**Reaction mostly delayed, less dramatic** → subclinical/silent chronic inflammation
Allergy Tests	Alcat Test

B. 1.1 History of the term "allergy"

1906 Baron Clemens von Pirquet, a Viennese pediatrician, introduced the term "allergy" (ancient Greek: allos-ergon/ foreign-reaction). At that time, allergy was very broadly understood as an "altered or adverse reaction."

Allergy

To date, "allergy" is defined as a specific change in the immunity situation in the sense of a pathogenic overreaction to otherwise harmless exogenous substances, which are carried out by antibodies or a reaction of the specific immune system.

In 1963, Gell and Coombs proposed a classification model that distinguished between 4 clinical allergy types, identifying them with the Roman numerals I to IV. In this system, usually allergy of type I or a type IV reaction (delayed immune response) is associated in the context of food.

Type 1 food allergy, or, "classical" food allergy, is triggered by allergen specific IgE antibodies bound to the fc (fragment crystalline) receptors expressed on the membranes of basophils and mast cells. When the inciting allergen binds two or more of these antibodies (at the fragment antibody region) receptor aggregation sets in motion transduction signals that result in the release of preformed mediators, notably histamine, and produce a nearly immediate reaction. A so called, "late phase" involving the synthesis of prostaglandins and leukotrienes perpetuates symptoms.

This classification is based solely on parameters of the specific immune system (involving antibodies).

Sensitivity / Intolerance

The Alcat Test is concerned with functional molecular biological mechanisms and does not analyze allergies/ antibodies. As such, the Alcat Test does not classify its results using the 4 traditional Gell and Coombs categories.

The concept of "sensitivity" was first described by J. Freeman and was defined in relation to food as "toxic idiopathy." Freeman, who is considered the founder of immunotherapy along with Leonard Noon, stated in his address on Toxic Idiopathies, Royal Society of Medicine, 1920:

"It might be an exaggeration to say that the study of these toxic idiopathies will open a new field of medicine, but I feel confident to say that they throw light from a new angle across a very large field of the old medicine."

Thus, malfunctions of the non-specific immune system are responsible for a large share of adverse reactions to foreign substances, those against food and environmental chemicals in particular. Furthermore, the evidence suggests that the non-specific immune system directs the specific immune system in its functions.

Current research in immunology, molecular biology, and biomedicine is making great strides in clarifying the complex relationships and activities of the innate/non-specific immune systems.

Summary:

- The term "allergy" is traditionally attributed to an adverse reaction of the specific immune system.
- On the other hand, adverse reactions not mediated by the specific immune system/antibody formation are defined under the terms "intolerance," "sensitivity," or "hypersensitivity" (B.1., part B.1.2).

FAQ: Allergy test positive and Alcat Test negative – can that be?

Yes. – It is a testament to the high clinical specificity of the Alcat Test.

A classical allergy is usually mediated by the specific immune system via the formation of IgE antibodies. The Alcat Test, on the other hand, reflects the response of the innate immune system. This means a patient with a diagnosed classical allergy has to continue eliminating allergenic foods - even if this food should be negative in the Alcat Test.

Hence, the Alcat Test must be considered a complementary test that assesses fundamentally different immune mechanisms and effector cells than an allergy test does.

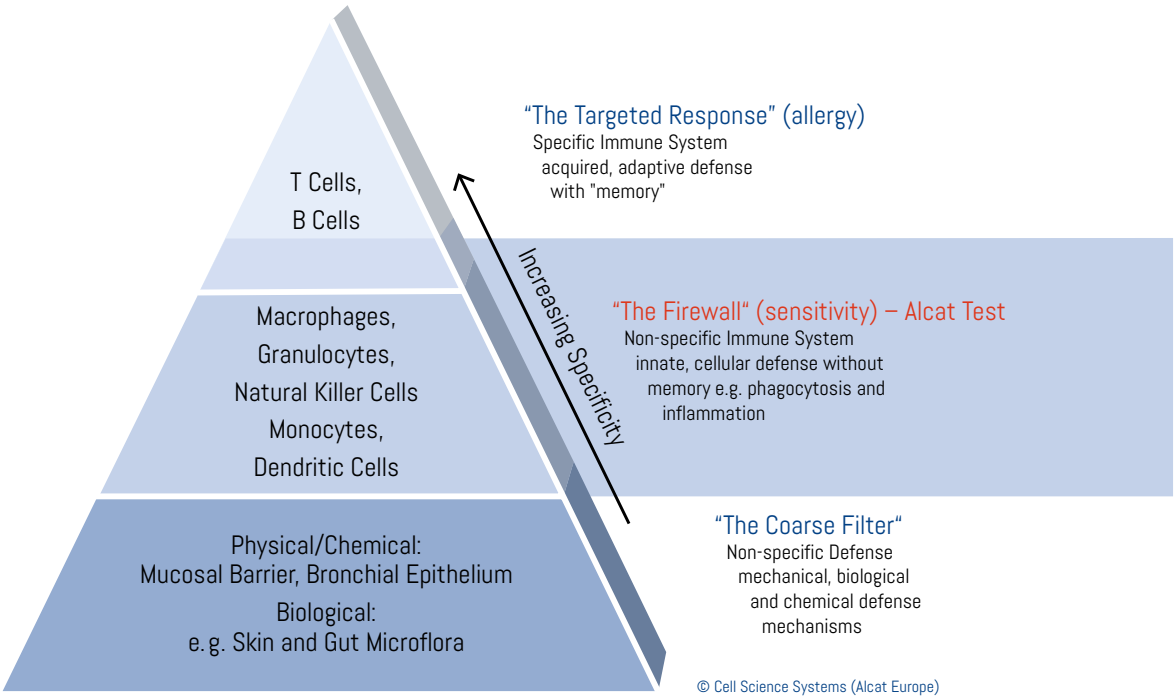


Figure B.1: The fundamental difference between "Allergy" and "Sensitivity/Intolerance" comprises different branches of the immune system and thus, immune pathways. While an allergy involves specific immune parameters (antibodies), sensitivity involves non-specific cellular responses.

Intolerance

= umbrella term for non-classic allergic reactions (non-IgE)

Intolerance

→ genetic/enzyme-related intolerance, etc.

The term "adverse reaction to food" is a general premise referring to all excessive body reactions to food ingredients or additives. Such reactions are subdivided into "classic-allergic" and "non-classic-allergic."

The latter category had been referred to simply as "food intolerance" until recently.

To date, the term "intolerance" is not clearly defined. Within the food intolerance category, a pathophysiological distinction is made between the following types:

Sensitivity (Intolerance) / Hypersensitivity

→ direct cell-mediated (e.g. Alcat Test)

- **Genetic** (e.g. GLUT-5-fructose malabsorption, celiac disease)
- **Pathophysiological;** enzymatic (e.g. lactase deficiency)
- **Pharmacological** (e.g. induced by biogenic amines or bioactive agents)
- **Immunologically**-induced adverse reactions
- **Psychosomatic** symptoms or diseases

Classic allergic	Intolerance	Sensitivity
SPECIFIC Immune system Allergic	SPECIFIC Immune system (Genetic) Autoimmune	NON-SPECIFIC Immune system Systemic/silent inflammation
↓	↓	↓
Wheat allergy	**Celiac disease**	**Non-Celiac Gluten Sensitivity**
• Respiratory allergy • Food allergy • Contact urticaria • WDEIA (wheat-dependent exercise -induced anaphylaxis)	• Classical/Non-classical • Symptomatic • Subclinical • Refractory According to "the Oslo definitions" for Celiac disease and related terms, 2014.	• Gastrointestinal disorders • Skin diseases • Neurological diseases • Respiratory disorders • Metabolic disorders • Musculoskeletal disorders • Immune system and comorbidities

Figure B.2: Pathogenesis of gluten-related disorders manifesting via different immune pathways

A study in 2010 showed celiac disease and non-celiac gluten sensitivity (NCGS) to be separate entities with different mechanisms of pathogenesis.

NCGS was proposed to result from an innate immune response to gluten-containing foods, whereas celiac disease was associated with the adaptive immune response. [B. 1-3, 5,6]

Hence, the differentiation between an allergy, intolerance, and sensitivity to a food can be illustrated with the example of wheat (figure B.2).

NEW definitions

In 2015, Prof. Alessio Fasano proposed a "more precise approach by assigning "non-classic-allergic" reactions to two different entities: food intolerance and food sensitivity." [B. 1, 3]

Prof. A. Fasano: "... Therefore, to avoid further confusion, it is important to clearly define the difference between food sensitivity and food intolerance... According to the United States (US) National Institute of Allergy and Infectious Diseases, [48] food intolerance occurs when the body lacks a particular enzyme to digest nutrients, nutrients are too abundant to be completely digested, or a particular nutrient cannot be properly digested. Therefore, symptoms are exclusively GI and mostly secondary to sugar fermentation by the intestinal microbiota, leading to production of gas that causes abdominal distention, abdominal pain, and irregular bowel movements.

Common examples include lactose intolerance or intolerance to excess fermentable oligo- and disaccharides, monosaccharides, and polyols (FODMAPs).

> *Food sensitivities are immune-mediated reactions to some nutrients; these reactions (intestinal and extra-intestinal) do not always occur in the same way when people ingest that particular nutrient. NCGS (non-celiac gluten sensitivity) is an example of food sensitivity." [B. 1-3, 5,6]*
>
> ► **According to this definition, the Alcat Test can be correctly classified as a "test for food sensitivity."**

B.1.3 Definition of the Alcat Test

It was demonstrated that the definition "sensitivity" is a useful classification term in reference to the Alcat Test. Thus, the Alcat Test can be classified as a Food Sensitivity Test using currently accepted scientific criteria.

Alcat Test definitions

The Alcat Test for Food/Chemical Sensitivity is a leukocyte activation test to identify:

- **"Direct Cell-mediated Food Sensitivity or Intolerance"**
- **"Cellular Pro-inflammatory Food Sensitivity"**
- **"Food Sensitivity Mediated by the Innate Immune System"**

B.1.4 Clinical Complementary Approach using the Alcat Food Sensitivity Test

It was shown so far, that foods can act as immune modulators with positive or negative health impact (table A.1). One of the strongest factors determining whether disease will manifest or not is the epigenetic factor.

Thus, the intake and quality of food is one of the most potent lifestyle factors affecting every living being.

The knowledge that chronic diseases are related to a chronically-activated innate immune system is a relatively new concept. Hence, **not many available laboratory tests are designed to evaluate the corresponding innate target cells.**

Current ongoing research influences therapy

During the 20th century, science was strongly focused on discoveries linked to the specific immune system. The long history of studies related to the role of innate immune cells, from the discovery of phagocytes in 1882 by Nobel Prize winner Ilya Mechnikov until recently, has been largely ignored.

However, current research is providing ongoing new insights that over-activation of the non-specific immune system plays a central role in the development and maintenance of chronic inflammatory diseases. Diseases may not be evident immediately. They may develop over a long period of time before manifestation.

Food sensitivity can follow different immune pathways and symptoms may be delayed. Therefore, it is challenging to identify and, accordingly, is referred to as "hidden or masked allergy" or "diagnostic chameleon."

Since inflammation can affect every system and organ of the body, no single medical specialty looks at this phenomenon comprehensively.

C. Proposed Pathomechanisms of Food Sensitivity

Scientific Background: The Role of the Innate Immune System

Food sensitivities have many causes: a major one of which is associated with innate immune cell activation (the "firewall"), and the resultant bystander tissue damage it promotes, and, most probably, to the immune system's secondary response to the cellular debris that follows. Chronic activation of the innate immune system regardless of the trigger, has been linked to: autoinflammatory conditions, autoimmunity, many cancers, and metabolic disorders. Any organ system may be affected.

C.1.1 Structure of the Human Immune System

The human immune system consists of three coordinated layers of protective and defensive structures:

- An extensive system of mechanical, biological, and chemical barriers that act like a coarse filter
- An innate, non-specific set of immune responses present from birth that act as a firewall
- A set of specific, adaptive immune responses that mature throughout life and provide a targeted defense.

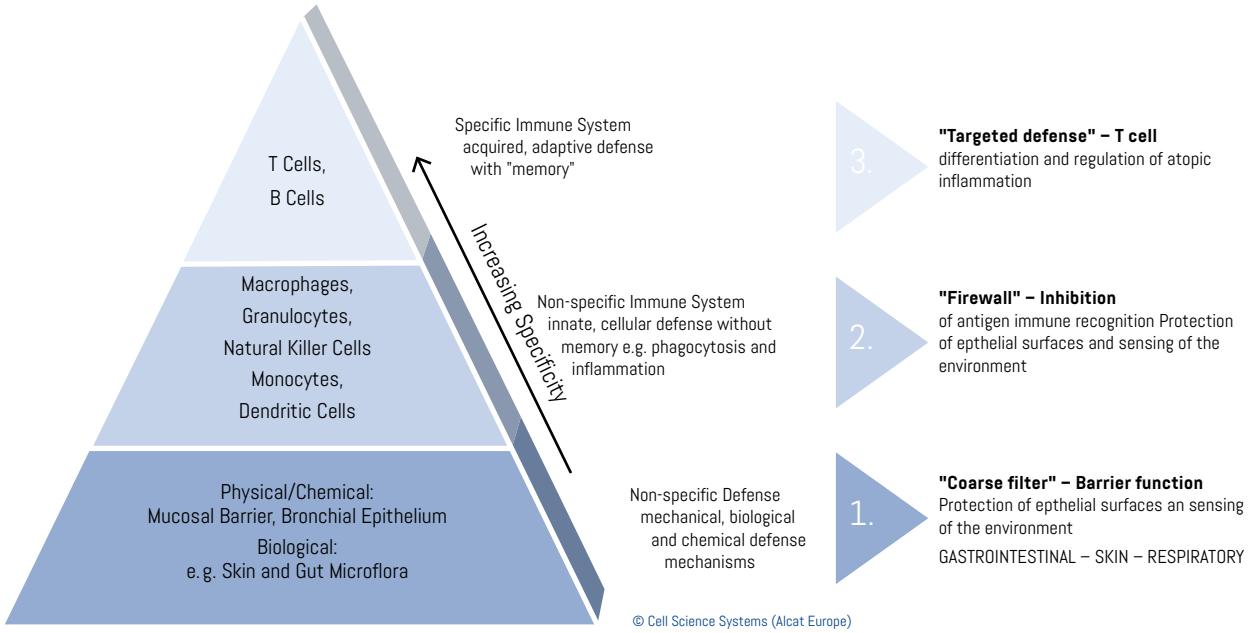


Figure C.1: Structure of the immune system: The human immune system is equipped with three successive barriers against foreign substances:

1. *Global, non-specific mechanical, biological, and chemical defense mechanisms ("the coarse filter")*

2. *Innate, non-specific immune system responses ("the firewall")*

3. *Adaptive, acquired immune system responses mediated by specific antibodies and receptors ("the targeted defense").*

1. "The Coarse Filter"

The first layer of the immune system acts like a "coarse filter" by capturing, destroying, or deactivating potential pathogens by mechanical and biological means.

In addition to the protective barrier of the skin, other systems provide a first line of defense against potentially harmful microorganisms and substances. These include mucus, which can trap and help expel invaders; lytic enzymes in saliva; including, lysozyme and phospholipase A2; body fluids such as tears and urine that flush microorganisms and other substances out of the system; hydrochloric acid in the stomach; antimicrobial peptides produced by the skin and respiratory tract such as β-defensin; and, IgA antibodies in the intestines.

2. "The Firewall"

The second level – the innate or non-specific immune system – evolved to provide protection from a wide variety of potentially harmful microorganisms. The innate immune system acts like a firewall, providing a rapid response against invading pathogens. It initiates defensive actions within moments of exposure and continues to combat invaders long after the initial breach.

> In particular, neutrophils act as "first responders", offering an acute, non-specific defense against pathogens, toxins, and danger molecules releasing inflammatory mediators, free radicals, and oxidative enzymes. Neutrophils continually patrol the body, and by sensing specific molecular patterns discriminate between those that are harmless bacteria and those that are potentially pathogenic or toxic.

In circulating blood, 70% to 80% of white blood cells (leukocytes) are neutrophils (figure A.2). Their overwhelming numbers speak to the importance of their role in the body. Further, when required, neutrophils can extravasate from blood vessels into the surrounding tissues in response to molecular signals of infection, damage, or danger.

Additionally, the complement system is a coordinated cascade of serum proteins that attach to the outer membranes of pathogens. This process, called opsonization, targets the pathogen for destruction and makes it easier for phagocytic white blood cells to engulf it. Opsonization does this by neutralizing the invading cell's negative electrical charge, which would otherwise repel the natural negative charge of the immune system cell.

Complement facilitated defense can also be achieved through the action of a different set of proteins (specifically C5-C9) to create a membrane attack complex (MAC) that perforates the pathogen's cell membrane; or, the membrane of an infected cell, leading to its destruction. Since complement protein binding is nonspecific, the body produces serum proteins and membrane-bound molecules that protect its own cells from its destructive actions.

Cell fragments and other debris must be continuously removed from the body. This is accomplished by the process of phagocytosis – which literally means cell eating process or; efferocytosis. [1]

3. "The Targeted Defense"

The third level of the immune response – the adaptive immune system – allows the body to recognize unfamiliar pathogens and variable antigens and responds by inventing and producing novel antibodies. The adaptive system kicks in if the innate system's capabilities are exceeded and more targeted approaches are required. It has the capacity to remember its responses, and this remarkable ability helps to protect against reinfection and also enables adaptation to new or modified pathogens. The innate and the adaptive immune systems can work together and/or independently of one another. Ideally, their well-coordinated interactions enable the body to address a wide array of challenges, without attacking harmless substances or self-structures. However, this ideal is not always realized; producing allergy, autoinflammation or autoimmune responses. Hence, reliable identification of immune triggers, and the avoidance, where possible, can be of great advantage.

C.1.2 Underlying Pathomechanism of the Alcat Test

Our interfaces protect us against exogenous influences. The largest interfaces are the skin, the airways, and the intestines. In the context of nutrition and food consumption our body has to deal with a vast variety of food and food components including

- Phytochemicals or proteins in a food
- Molds
- Elemental contaminants (e.g. mercury, cadmium, manganese, arsenic)
- Mycotoxins (e.g., aflatoxins, ochratoxins, fumonisins, trichothocenes)
- Additives (e.g., sulphites, nitrites / nitrates, benzoic acid); pesticides (eg, organophosphates, carbamates, DDT, pyrethrins)
- Medicines from agriculture (e.g. antibiotics, hormones)
- Process contaminants (e.g. acrylamides, PAHs, choropropanol)
- Natural toxins (e.g., cyanides, aminoglycosides)
- Contaminating bacteria and others

Thus, the intestinal tract is composed of topological, chemical, and immunological barriers to limit damaging influences from outside. With regard to the immunological barrier, we distinguish, from a historical point of view, the innate and the acquired immune system. The major difference between the innate and the adaptive immune system, therefore, is the memory function of the latter. Today, it is known that both arms of the immune system can communicate with each other and influence each other.

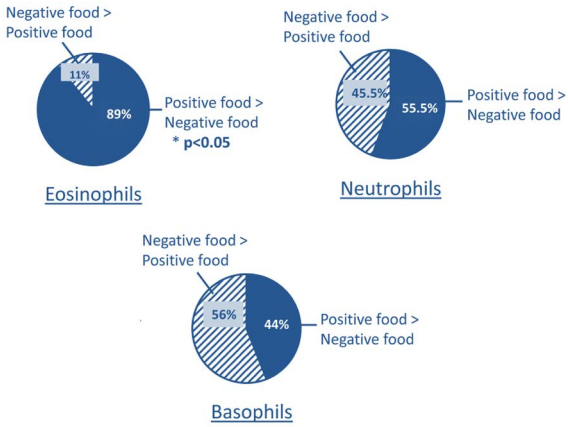
However, food sensitivity is most often associated with reactions of "The Firewall"– the innate, nonspecific mechanisms of the immune system including both the complement system and cellular components.

C.1.3 Underlying interaction between innate immune cells and food components

Target cells of the Alcat Test

Since the Alcat Test focuses on the functionality of the innate immune system, especially the granulocytes, the pathomechanism underlying the interaction between this kind of immune cells and food components and/or contaminants will be considered in detail.

Figure C.2: Yale School of Medicine: "A Leukocyte Activation Test Identifies Food Items which Induce Release of DNA by Innate Immune Peripheral Blood Leucocytes". Target cells of the Alcat Test are innate immune cells: Positive foods result in up-regulation of CD63 in eosinophils to a greater degree than negative foods. [C. 39] (Part D no. 6)



Granulocytes

Granulocytes (figure A.2) are a category of white blood cells formed in the bone marrow and those in the blood can be classified as basophils, eosinophils, and neutrophils. The cell types are named by their distinct staining characteristics using hematoxylin and eosin (H&E) histological preparations. Granules in basophils stain dark blue, eosinophilic components stain bright red, and neutrophilic components stain a neutral pink.

Granulocytes can be found in the bloodstream, with a lifespan of 1 to 5 days [2], and in tissue, where they can survive for up to 7 days (neutrophils) or; in the case of eosinophils for weeks to months. Neutrophils are the most abundant leukocytes, comprising 50-70% of all white blood cells. Eosinophils make up approximately 2-4% of circulating leukocytes and basophils are making only 0.5% of the circulating blood leukocytes. The remainder are lymphocytes and monocytes.

Under physiological conditions, neutrophils are concentrated in the the intestinal tract, spleen, liver, and lungs. With regard to the intestinal tract, however, recent studies show that neutrophils help to regulate the commensal flora. In accordance with the neutrophils the eosinophils are present in the gastrointestinal tract from the stomach through the large intestine under physiological conditions as well [3]. Because eosinophils are present at highest concentration in the small intestines, immediately juxtaposed to the epithelial cells lining the lumen, the potential for direct interactions among eosinophils, microflora, food components and/or contaminants certainly exists under physiological conditions. This might be true for neutrophils as well.

Should the gut become inflamed larger food particles can more easily gain access thereto and directly interact with cells of the innate immune system and trigger their activation.

Neutrophils – first defense

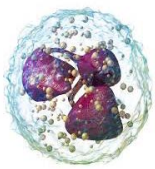


Figure C.3: neutrophilic granulocyte

Neutrophils (C.3) comprise the largest population of phagocytic cells of the innate immune system, representing 90-95% of granular white blood cells in the blood. Neutrophils have long been considered as crude killing machines, particularly trained to attack bacterial or fungal pathogens in wounds or infected tissues. That perspective has fundamentally changed over the last decades, as neutrophils have been shown to exert a lively exchange between other cells of the innate and adaptive immune system. They contribute to major immunomodulation during acute inflammation and subsequent clearance.

Neutrophils have a short life span and are extremely aggressive after they are activated. They can destroy pathogens in many ways, primarily by bombarding them with cytotoxic oxygen and nitrogen radicals; through the production of acids such as hypochlorous acid; or through the activation of proteolytic enzymes including myeloperoxidases or elastase.

As an inflammatory cascade arises, the immune system stimulates granulocyte activity through the activation of pattern recognition receptors located in the cell membranes as well as internal structures; i.e., endosomes and the cytosol (see Figure A.6). These cells contain a very large number of cytoplasmic granules – small membrane-bound vesicles that encapsulate infection-fighting substances including pro-inflammatory acids. Notably, myeloperoxidase (MPO) is emerging as an important marker for cardiovascular disease [4].

Thus, two roles are played by neutrophils:

- As a first line of defense against microorganisms
- As a contributor to the pathogenesis of various illnesses, such as autoimmune, autoinflammatory, and metabolic diseases.

Weapons of neutrophils

Neutrophils, in their function as first line defenders implement different mechanisms. These include, phagocytosis, degranulation, cytokine production, and, the most recently described, neutrophil extracellular trap (NET) production [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16] (figure A.5; A.6).

To fulfill these actions neutrophils are equipped with impressive and various armories of biological weapons. All of these weapons work efficiently on most live material, including, the host's own structures, making the neutrophil a dangerous cell.

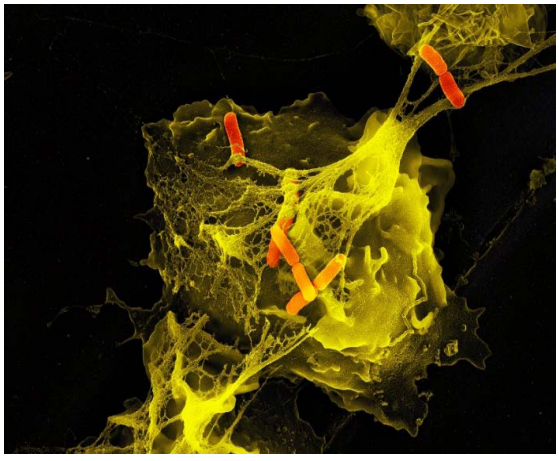


Figure C.4 / Cover image: Neutrophil Extracellular Traps (NETs); A special feature of innate immunity due to strong cellular defense reactions (Neutrophil Cell Trapping Bacteria. Max Planck Institute, Berlin, see cover image).

Although phagocytosis, degranulation, and NET generation has been described initially as an antimicrobial mechanism, recent data suggest that uncontrolled phagocytosis (frustrated phagocytosis) [17], exaggerated degranulation, and especially NET formation could play a more important role in autoimmune and autoinflammatory pathologies, such as vasculitis, lung injury, atherosclerosis, rheumatoid arthritis, thrombosis, gout, systemic lupus erythematosus (SLE), and intestinal damage.

Phagocytosis, degranulation and extracellular traps

Neutrophils are densely packed with secretory granules which are filled with more than 700 proteins. They contain at least three different types of granules [18]:

1) Primary granules, also known as azurophilic granules

2) Secondary granules, also known as specific granules

3) Tertiary granules; and (4) secretory vesicles

The azurophilic (primary) granules contain myeloperoxidase (MPO), the specific (secondary) granules contain lactoferrin, and tertiary granules contain matrix metalloproteinase 9 (MMP9); also known as gelatinase.

During phagocytosis the granule contents are used to kill the pathogen or to eliminate the foreign particle. Nonetheless, the degranulation of granules can be performed independent of phagocytosis. The release of extracellular traps from neutrophils is called NETosis [19].

Neutrophil extracellular traps (image C.4) are DNA structures released due to chromatin decondensation and spreading, and they thus occupy three to five times the volume of condensed chromatin. Several proteins adhere to NETs, including histones and over 30 components of primary and secondary granules, among which are components with high destructive activity such as elastase, myeloperoxidase, cathepsin G, lactoferrin, pentraxin 3, gelatinase, proteinase 3, LL37, and others. [20]

Three models for NETosis are known to date [20, 21]:

1) Suicidal NETosis

2) Vital NETosis

3) Vital NETosis dependent on Reactive Oxygen Species (ROS)

The latter type attracts great interest because, instead of nuclear DNA, mitochondrial DNA is thrown out of the cells. Mitochondrial DNA belongs to the group of damage-associated molecular patterns (DAMPs) which perpetuate inflammation [22].

Eosinophils

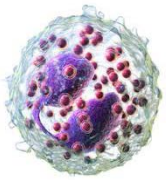


Figure C.5: eosinophilic granulocyte

Human eosinophils have long been associated with allergic and anthelminthic parasite functions [23]. Like neutrophils the understanding of their role in health and disease is changing.

Eosinophils contain approximately 200 cytoplasmic crystalloid granules per cell which belong to two major types of granules, primary and secondary granules, as well as lipid bodies.

These phospholipid bilayer-enveloped granules are central to the functional responses of eosinophils in that granules house preformed stores of four major granule proteins, including eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), which are highly cationic proteins directly toxic to extracellular microorganisms and parasites as well as host tissue.

In addition to the known mechanisms of extracellular secretion of granule proteins (i.e., exocytosis and piecemeal degranulation), a distinct mode of eosinophil degranulation associated with cell death, eosinophil-derived extracellular traps (EETs) and release of free granules; an event termed as EETosis [24, 25].

In contrast to active projection of mitochondrial DNA from live eosinophils in EETs, EETosis-mediated DNA release is accompanied by cell death, and DNA traps spread by a passive process.

Again, as is the case with respect to neutrophils, mitochondrial DNA release by eosinophils is a damage-associated molecular pattern (DAMP) which perpetuates inflammation.

Basophils

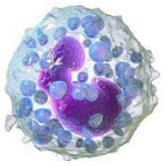


Figure C.6: basophilic granulocyte

Basophils have been recognized as important players for protective immunity against a variety of different endo- and ectoparasites [26].

Basophils represent a relatively rare and short-lived cell type. The cytoplasm of basophils contains a varied amount of granules. Granule contents of basophils are abundant with histamine, heparin, chondroitin sulfate, peroxidase, platelet-activating factor and other substances.

Physiological activation of human basophils lead to granule degranulation as well as the rapid formation of extracellular DNA traps.

C.1.4 Molecular Processes of Granulocyte Activation: PAMPs and DAMPs

Neutrophils are able to detect potential pathogens because they contain numerous pattern recognition receptors. In particular, recent research has focused on TLRs – toll-like receptors – which play an important role in the recognition of pathogens and other substances (table C.1).

The mammalian organism is under constant threat from the environment and prone to harm from danger. Danger represents challenges (physical, chemical, or infectious) that can injure or kill cells and lead to tissue loss and organ dysfunction.

Pathogen-associated molecular patterns, or **PAMPs,** are molecules associated with groups of pathogens that are recognized by cells of the innate immune system [27]. These molecules can be referred to as small molecular motifs conserved within a class of microbes. They are recognized by toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) in humans. A vast array of different types of molecules can serve as PAMPs, including glycans, glycoconjugates, lipoproteins and peptidoglycans.

By analogy with the exogenous pathogen-associated molecular patterns (PAMPs), the endogenous molecules with similar immunologic roles are called **damage-associated molecular patterns (DAMPs).** The term alarmin can be used for those DAMPs that can induce chemotaxis and promote the induction of immune responses [27]. Once outside the cell, extracellular nuclear molecules can induce responses by interacting with pattern recognition receptors (PRRs). These receptors can reside on the cell membrane or inside the cell.

Another source of extracellular DNA with DAMP activity is mitochondria. The human mitochondrion is believed to have evolved from endosymbiont bacteria and thus expresses molecules that resemble bacterial products. As such, mitochondrial molecules have properties that could be variously categorized as DAMPs or PAMPs because they can trigger inflammation.

Thus, the formation of NETs and EETs might act as a danger molecule amplifying the inflammatory response. In this regard, endogenous danger molecules subsequently induce a pro-inflammatory cascade by activating Toll-like receptors (TLRs), upregulating pro-inflammatory mediators and triggering further tissue damage leading to increasing levels of DAMPs. The creation of this pro-inflammatory autocrine loop may result in chronic inflammation or autoimmune disease.

The activated cell dies as a consequence of one of three possible pathways; necrosis, apoptosis or; if the formation of NETs are involved, pyroptosis [28]. Pyroptosis is a special form of apoptosis, often referred to as programmed cell death, a natural process associated with activation of the inflammasome. Researchers at the Max Planck Institute for Infection Biology in Berlin have shed light on

a unique mechanism of cell lysis that's triggered when neutrophils attack: the ejection of neutrophil extracellular traps, commonly called NETs [5]).

NETs are composed of a mixture of reactive cell contents and neutrophil DNA (figures A.1; A.6; C.4). Thus, pathogenic bacteria are literally captured in the NET where they are immobilized and rendered harmless. Professor Arturo Zychlinsky of the MPI-IB Berlin explains, "In the NETs nearly as many bacteria die as are digested by neutrophils." The dying cells sacrifice themselves for the greater good, transforming strands of their own DNA into protective traps.

This is fine unless, due to a deficiency in an enzyme called DNase, whereby the NETs cannot be removed from the circulation by the monocyte/macrophage system; or, with respect to apoptosis or pyroptosis, the neutrophil DNA is not adequately cleared, perhaps owing to an overwhelming of the monocyte/macrophage system, or, another defect in the immune system, such as C1q deficiency, the DNA from the activated cell can itself become immunogenic, as observed in both lupus and rheumatoid arthritis [29].

Thus, chronic activation of the innate immune system may lead to development of autoimmune disorders.

Pattern Recognition Receptors (PRRs) for PAMPs and DAMPs

Neutrophils express a large number of cell surface receptors for the recognition of pathogen invasion and the inflammatory environment. Those include G-protein-coupled chemokine (Formyl-peptide receptors, chemokine receptors, and classical chemoattractant receptors), Fc-receptors, adhesion receptors such as selectins/selectin ligands and integrins, various cytokine receptors, as well as innate immune receptors such as Toll-like receptors, C-type lectins, NOD-like and RIG-like receptors.

Neutrophils also express a number of additional receptors that cannot be grouped into the above categories. Their function is mediated by a number of cell surface receptors which trigger complex intracellular signal transduction pathways that we are only beginning to understand. [30]

Toll-like receptors (TLRs) are the best-studied group of pattern recognition receptors (PRRs). TLRs play a critical role in the initiation of innate immunity through the recognition of pathogen and damage-associated molecular patterns (PAMPs and DAMPs, respectively). To date, 10 human TLRs have been identified. TLR1, TLR2, TLR4, TLR5, and TLR6 reside on the plasma membrane whereas TLR3, TLR7, TLR8, and TLR9 are localized to the endolysosomal compartment.

Thus far, the TLR ligands are classified as PAMPs, conserved structural motifs of bacteria, fungi and viruses; or DAMPs, endogenous proteins which are released upon tissue injury. However, other classes of molecules are being identified which can activate the TLR signaling pathway including but not limited to morphine, glucuronic acid and the ethanol metabolite ethyl-glucuronide, green tea polyphenol epigallocatechin-3-gallate, phenethyl isothiocyanate and parthenolide. It is highly plausible that the whole repertoire of TLR ligands has not been discovered yet.

Table C.1: Summary of different types of Pattern Recognition Receptors

Pattern Recognition Receptors (PRRs)	Occurrence	Examples
Membrane bound	Different host and immune cells	Toll-Like Receptors (TLR), C-Type Lectin Receptor
Cytosolic	Cytoplasm of various host and immune cells	NOD I-Like Receptor (NLR), RIG I-Like Receptor (RIG)
Soluble	Circulate in the blood	Mannose-Binding Lectin (MBL), Surfactant-Protein A

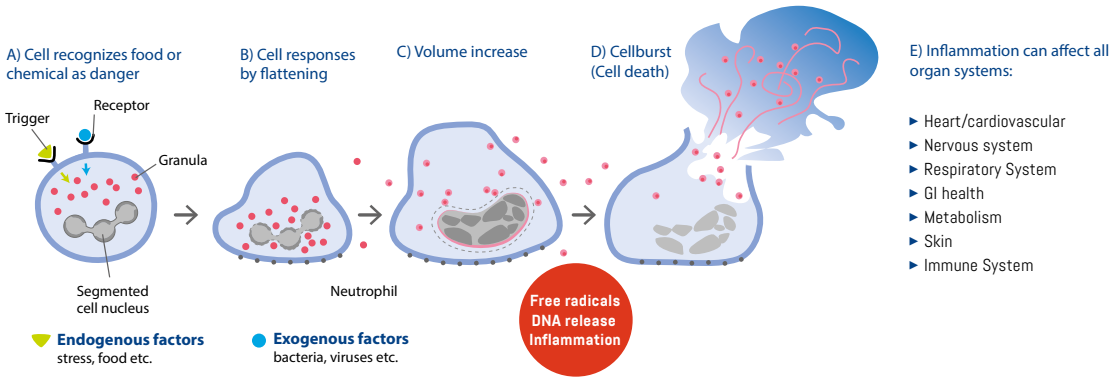


Figure C.7 / A.1: Activation of neutrophil and NET formation.

In response to activation and signaling molecules of the innate immune system, the enzyme NADPH oxidase converts oxygen into highly reactive forms (step A), dissolving the nuclear membrane and granules (step B). The contents mix with cellular fluid (step C), and the cell membrane breaks down after about 2 hours, ejecting the NET and engulfing nearby danger molecules (step D).

C.2 Digestive System, Immunity, and Food Sensitivity/Intolerance

Whether the bowel is healthy and able to fulfill its important tasks depends on several factors including the physical integrity of the intestinal barrier; the vitality and appropriate diversity of the intestinal microecology, characterized by the presence of beneficial commensal microflora; and the biochemical properties of the intestinal environment, including adequate levels of secretory IgA, a protective antibody that excludes allergens from the circulation. The composition of the bowel's resident microbial flora has a huge impact on whether nutrients can be properly broken down, and absorbed or whether dysfunctions arise including gas formation, the reduced degradation of biogenic amines, etc.

Poor diet, stress, corticosteroids, birth control pills, antibiotics, infection, yeast *(Candida albicans),* and a variety of other health and lifestyle factors, can lead to an unfavorable gut ecology, which in turn may cause discomfort or more serious digestive disorders. Figure C.8 shows some of the factors that may promote the emergence of food sensitivity.

Damage to the intestinal barrier may lead to a wide array of health problems. Foods are to be digested and broken down into specific functional units prior to entering the circulation. When properly metabolized, these intermediate nutrients are less likely to directly antagonize the immune system. However, when larger, improperly metabolized food particles (including small peptides, fatty acid complexes, oligosaccharides, and mineral complexes that should be processed by specific functional transporters in the intestinal wall) are instead exposed directly to the cells of the immune system, they may provoke destructive reactions. An increasing number of studies confirm a close association between dysfunctions of the intestinal barrier and conditions such as obesity and fatty liver disease, as well as autoimmunity and cancer.

Figure C.8: Many factors can influence the delicate homeostasis of gut, GALT (gut associated lymphoid tissue) and NEI (neuroendocrine-immune system).

Thus, it is also not surprising that the neuroimmune regulation is effected by chronic immune cell activation. An unbalanced neuroendocrine immune system (NEI) could be an explaining link and indicative for many disorders. (Table A.1.; figure C.9).

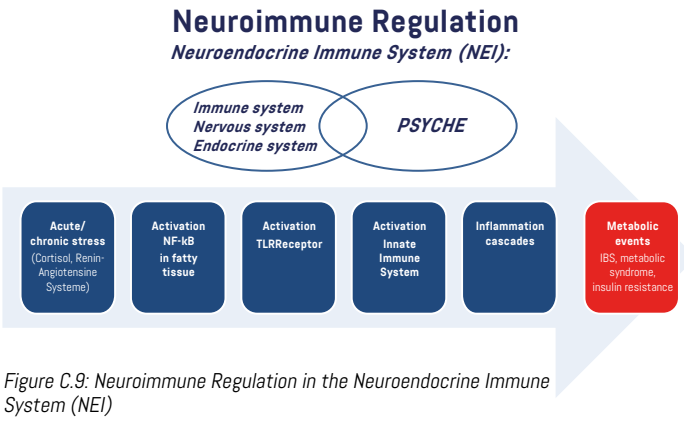


Figure C.9: Neuroimmune Regulation in the Neuroendocrine Immune System (NEI)

The causes of altered interstitial permeability have not yet been completely elucidated. For example, it remains unclear whether a disrupted intestinal barrier is the trigger of a disease process, or its result. Perhaps it is both. Widely acknowledged triggers include industrially modified foods and stresses or imbalances in the population of intestinal flora caused by alcohol, drugs, poor diet, and fungal infection as well as inflammation (see Figure C.8).

The intestinal wall comprises approximately 400 square meters (equivalent to about 4,300 square feet) of complex structured epithelial tissues that perform numerous vital functions. It must enable the efficient absorption of nutrients from ingested foods and fluids while simultaneously preventing the entry of bacteria on the one hand and tolerating harmless antigens on the other. The intestinal wall continually negotiates the exchange of

macromolecules between the external environment and the inside of the body. Intestinal epithelial cells form a dense cell structure secured with extra "locks" that provide extremely tight junctions. Narrow strips of membrane proteins (e.g. claudins and occludins [31] between epithelial cells create a paracellular diffusion barrier and provide for proper cellular polarity, a prerequisite for directional mass transport.

Zonulin is an endogenous protein that modulates the permeability of these cellular junctions [32]. In effect, it acts as a "doorman," by selectively either allowing or disallowing the passage of substances through the intestinal barrier. Recent research suggests that zonulin can be activated by gluten and induce the opening of the intestinal barrier at inappropriate times. By permitting incompletely metabolized macromolecules to pass through the barrier, it plays a potentially crucial role in the course of celiac disease, other inflammatory conditions, and can lead to autoinflammation as well as other manifestations of autoimmunity.

Along with food, the intestinal lumen is exposed to numerous potentially infectious organisms and antigens from other sources. Thus, it's essential that the gut be enclosed and protected by tissues with a high level of immune system intelligence. This specialized tissue is called gut associated lymphoid tissue, or GALT [33]. The GALT is a large lymphoid tissue surrounding the colon which comprises about 70% of the body's total immune system capacity (figure C.10).

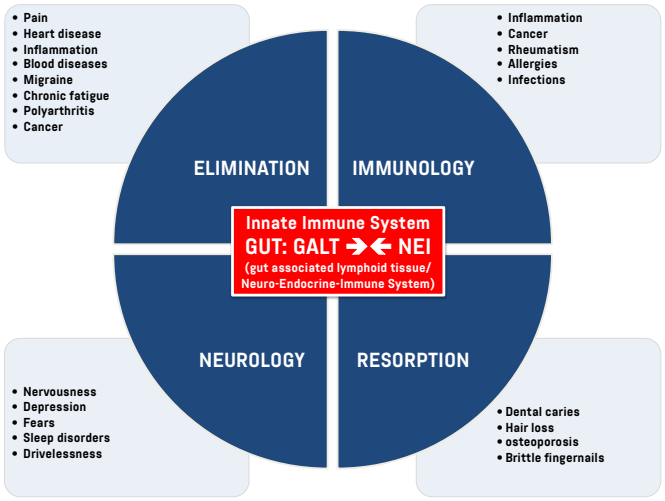


Figure C.10: Summary "Central Role of the Gut for Regulation of Resorption and Elimination as well as connector to the nervous system and the immune system."

In addition to important factors for supporting mucosal immunity in the lumen (e.g., sIgA) the GALT includes a concentration of specific immune cells including intraepithelial T lymphocytes and B cells within the intestinal submucosa, the lamina propria, and Peyer's patches.

The intestinal wall and the GALT jointly manage the balance between the body's tolerance and immune reaction to foreign antigens.

C.3 How is this related to the Alcat Test?

Exogenous pathogens entering the human body lead to defense mechanisms described above. However, nutrients enter the human body in a comparative manner in the gastrointestinal tract and can also act as a DAMP – which means, be interpreted as "danger signal" by the human immune system (figure A.6).

The quantitative and qualitative aspects of nutrients thus have a profound effect on leukocytes. As a result, nutrition affects the incidence, natural progression, and therapeutic response of malignant diseases [34]. There is an interdependence between nutritional patterns and disease in a society consuming predominately the "western diet" [35]. Increasing evidence of the importance of diet, our immune system, and our microbiome continues to mount [36].

C.3.1 Immunological perspective on the Alcat Test

In the past, research has focused on the specific immune system much more than the host's innate immune response against food. Now, more studies show the importance of innate immune responses, including the inflammasome, the "firewall" defense line consisting of neutrophilic, basophilic, and eosinophilic leukocytes, and the fact that innate immune cells are not only activated by pathogens but also by a host of danger signals, including the host's own DNA, RNA, and related proteins and chemicals. [37]

Current research efforts are increasingly focused on the role of DAMPs in disease processes. Clearly a sustained activation of these immune cells is destructive, promoting effects ranging from microinflammation of intestinal tissue to the type of chronic, widespread inflammation responsible for numerous disorders; including, gastrointestinal complaints, metabolic disorders, skin diseases, obesity, respiratory diseases, neurological, musculoskeletal disorders, and autoimmunity.

The effects of chronic inflammation are often gradual but insidious, and we now appreciate that their long-term consequences are often severe and possibly life-threatening. For example, chronic intestinal inflammation increases the risk of cancer and autoimmunity.

C.3.2 The Alcat Test is the only validated food sensitivity test

There are a multitude of commercially available blood tests purporting to diagnose "food allergy" though most remain invalidated. Among the more widely utilized food sensitivity assays are leukocyte activation (LA) tests used to guide dietary advice [38]. A direct relationship between the activation of the innate immune system (Alcat Test) and irritable bowel syndrome has been demonstrated [38].

Previous investigations have linked Alcat Test reactivity with migraine, metabolic syndrome, chronic bowel issues, skin and respiratory disorders, and numerous other inflammatory diseases. (Part D, overview studies and research).

Frequently, these immune cells undergo degranulation and lysis, releasing factors that interact with other parts of the immune system, facilitating a coordinated response. Alcat Testing is based on the concept that food sensitivities occur from activation of cellular responses of the innate immune system – the Firewall. Instead of perceiving them as harmless, the immune system responds to specific foods and other substances as though they were threats requiring aggressive targeting.

The Alcat Test identifies aberrant cellular responses regardless of the pathway that induces the reaction whether immunologic, toxic, or pharmacologic. Also, the Alcat Test detects all three types of neutrophil reactions toward food ingredients/chemicals, i.e suicidal NETosis, vital NETosis, and vital NETosis dependent on ROS. These distinct reactions are automatically characterized as non-reactive, mild, moderate, or severe reactive using a specific algorithm at Cell Science Systems, Corp.

> **In this context, Yale School of Medicine was first to demonstrate (in 2015) that DNA (acting as DAMPs) and myeloperoxidase of peripheral blood leukocytes were released during certain food reactions and that these items were identified with the Alcat Test [39].**

C.3.3 Scientific Context and Current Research

The Alcat Test measures the reactions of peripheral blood leukocytes in response to ex vivo challenge with a battery of individual test substances. A positive reaction is one in which the granulocyte fraction (comprised predominantly of neutrophils) of the leukocytes, is seen to exhibit a shift in size and/or a reduction in cell number. Of note is that the observed size shifts will progress to a decrease in number if provided sufficient time; or, a stronger dose of the test substance.

Researchers at Yale have correlated these reactions with the activation of well-known inflammatory pathways; notably, PKC, Nuclear factor kappa B; as well as the release of DNA [39]. The release of DNA is indicative of a necrotic (resulting from degranulation) or pyroptopic (resulting from NETosis) demise of the reactive immune cells, as opposed to an orderly programmed cell death, apoptosis.

Herein lays the key to potentially understanding the link between an innate immune cell mediated reaction to a food, chemical, or other substance; and, the onset of autoinflammatory and autoimmune conditions, which have now reached pandemic proportions throughout the industrialized world. [40]

Every day, approximately one hundred billion of the short lived neutrophils die through the orderly process of apoptosis. Neutrophils, the most abundant type of leukocyte, survive for approximately five days [2]. During a steady state, one of homeostasis, one hundred billion of these cells will be replaced by new ones each day. The old cells that die in a non-activated state, having undergone apoptosis, will be "silently" removed by macrophages. The process is called "efferocytosis" [1]. "Silently" means inflammation will not occur. In fact, the process actual produces an anti-inflammatory response; and, the adoptive transfer of apoptotic cells is being explored as an anti-inflammation therapy with graft versus host disease, and other inflammatory disorders.

However, in the case of efferocytosis of necrotic cells, the now cell free DNA and other cellular debris, produces an inflammatory and/or auto-immune response. In short, when macrophages consume un-activated neutrophils they remain in a normal, homeostatic state and confer anti-inflammatory effects to surrounding tissue(s). When macrophages consume necrotic cellular debris they are re-programmed into; or, polarized into, an inflammation producing mode.

The key point to note here is that the Alcat test identifies food and other substances that induce cellular necrosis.

The innate immune system can produce either a vicious or virtuous cycle, depending on its level of activation. Chronic activation of the innate immune system has been linked to dozens of inflammatory and auto-immune disorders; and, the production of cell free DNA and other undegraded debris is the major driving factor. Hence, the Alcat Test identifies substances that activate the innate immune system in such fashion as to promote inflammation and autoimmunity.

D.1 Studies and research

Alcat / Research Overview reversed chronological order 2019-1988

The following section provides a comprehensive overview of the scientific research conducted to date on the Alcat Test. Research reviewed below confirms the reproducibility, sensitivity, and specificity of the Alcat Test as a clinical tool for identifying foods and compounds that may trigger immune cell activation and inflammation. Studies include double-blind placebo controlled, molecular pathomechanism assessment, mechanistic/technical studies, and review papers. Conditions studied include gastrointestinal disorders, skin diseases, respiratory problems, metabolic diseases, autism, migraine, and others.

	Study ref. number / title	Author / publication	Year / study type study subjects	Data points
1)	The role of leukocyte activation in suspected Non-IgE excipient-related COVID-19 vaccine reactions: An exploratory hypothesis-driven study of pathogenesis	J. Bellanti, Yen-Chih Huang et al; Allergy Asthma Proc; 2024 Nov 1;45(6):438-446. doi: 10.2500/aap.2024.45.240040 https://pubmed.ncbi.nlm.nih.gov/39517070/	2024 Exploratory pilot study1 n=30	est. 150
2)	Prediabetes Reduction from Food Allergen Elimination—A Controlled Pilot Study	Galveston Clinical Research Center; F. Buck Willis et al; Diabetes 2024;73(Supplement_1):627-P; https://doi.org/10.2337/db24-627-P https://diabetesjournals.org/diabetes/article/73/Supplement_1/627-P/156201/627-P-Prediabetes-Reduction-from-Food-Allergen	2024 Controlled clinical study n=24	est. 6,000
3)	Immune-Based Personalized Elimination Diet for the Treatment of Irritable Bowel Syndrome: A Double-Blind Randomized Sham-Controlled Study	Gastroenterology Institute, Sheba Medical Center; S. Horin; t. Engel et al; The American Journal of Gastroenterology 118(10S):p S454-S455, Oct. 23. | DOI: 10.14309/01.ajg.0000952116.88348.96 https://journals.lww.com/ajg/fulltext/2023/10001/s619_immune_based_personalized_elimination_diet.975.aspx	2023 Double-Blind Randomized Sham-Controlled Study n=68	est. 17,000
4)	Studies of mitochondrial and nuclear DNA released from food allergen-activated neutrophils. Implications for non-IgE food allergy	University Clinic of Leipzig, Georgetown University Medical Center B. König, J. Bellanti et al; Allergy Asthma Proc; 2021 May 1;42(3):e59-e70. doi: 10.2500/aap.2021.42.210021; Ingenta OPEN ACCESS https://pubmed.ncbi.nlm.nih.gov/33980341/	2021 Mechanism study DNA release from innate immune cells n=6	est. 1,500
5)	Alcat Test (food intolerance test): Assessment of its Clinical Utility	Research Service. Río Hortega University Hospital J. San Miguel Rodríguez et al; Journal of Biomedicine and Biosensors, 1(1) (2021): 57-76 https://gpub.org/journal-admin/uploads/articles/jbb114.pdf	2021 Review paper	
6)	Food Allergen Elimination for Obesity Reduction; a Longitudinal, Case-Control Trial	Texas State University Dr. Buck Willis, Galveston Clinical Research; I; Br J Gastroenterol Aug 2020, 2:4 2020; 2(4): 199 - 203. doi: 10.31488/bjg.1000122 https://britishjournalofgastroenterology.com/wp-content/uploads/2020/04/BJG-122.pdf	2020 Medical Weight Loss Study n=94 (group 1-4)	ca. 10,665 group 1 & 2
7)	A Clinical Evidence of a Correlation Between Insulin Resistance and the ALCAT Food Intolerance Test.	University of Camerino (Italy) Pompei P, Grappasonni I, Scuri S, Petrelli F, Traini E, Sorrentino S, Di Fede G; Altern Ther Health Med. 2019 Mar;25(2):22-38. https://www.ncbi.nlm.nih.gov/pubmed/30990791	March 2019 Clinical study n=188 Comparison with DNA	est. 5,000

8)	Effects of Antigen Leukocyte Cellular Activation Test-Based Diet on Inflammation, Body Composition, and Medical Symptoms	Northern University Illinois (NIU) Lukaszuk JM, Shokrani,M, Roy PG, Hoppensteadt J, Umoren J; Alternative and Complementary Therapies VOL. 24, NO. 5 ; 11 Oct 2018 https://doi.org/10.1089/act.2018.29183.jml	Oct. 2018 Clinical; Multiple disorders n=133 DBPC	ca. 30,000
9)	Non-Celiac Gluten Sensitivity in Patients with Severe Abdominal Pain and Bloating: The Accuracy of ALCAT 5	University of Pavia (Italy); Di Stefano M, Pesatori EV, Manfredi GF, De Amici M, Grandi G, Gabriele A, Iozzi D, Di Fede G; Clin Nutr ESPEN. 2018 Dec;28:127-131. doi: 10.1016/j.clnesp.2018.08.017. Epub 2018 Sep 9; https://www.ncbi.nlm.nih.gov/pubmed/30390869	Sept. 2018 Clinical Study n=25 DBPC	1,150
10)	Food Allergen Eliminations for Obesity Reduction: A Comparison Study with Therapeutic Exercise	Texas State University, Galveston Clinical Research, Cardiff Metropolitan University (UK), Royal College of Physicians and Surgeons of Glasgow (UK); Willis BF, Shanmugam R, Curran SA; Sci Nutr Res. Sept 2018; 1(1): 1-6 http://scivisionpub.com/pdfs/food-allergen-eliminations-for-obesity-reduction-a-comparison-study-with-therapeutic-exercise-580.pdf	Sept. 2018 n=17 Comparison study	1,700
11)	A Leukocyte Activation Test Identifies Food Items which Induce Release of DNA by Innate Immune Peripheral Blood Leucocytes	Yale School of Medicine Garcia-Martinez I, Weiss TR, Yousaf MN, Ali A, Mehal WZ; Nutrition & Metabolism volume 15, Article number: 26 (2018) https://pmc.ncbi.nlm.nih.gov/articles/PMC5896029/	Apr. 2018 Molecular pathomechanism n=20	4,000
12)	Efficacy of Individualised Diets in Patients with Irritable Bowel Syndrome: a Randomised Controlled Trial	Yale School of Medicine: Ali A, Weiss TR, Scherban A, Khan A, McKee D, Apollo D, Mehal WZ; BMJ Open Gastroenterology, 2017 Volume 4, Issue 1 https://bmjopengastro.bmj.com/content/4/1/e000164	Sept. 2017 Clinical study; IBS 58 patients DBPC	ca. 12,000
13)	The Alcat Test Predicts the Release of DNA and Myeloperoxidase by Innate Immune Peripheral Blood Leukocytes Via a PKC Dependent Pathway	Yale School of Medicine: Garcia-Martinez I, Weiss TR, Ali A, Mehal WZ; https://cdn.websites.hibu.com/52b7399e6b214e2c8b2e0758e4e932ec/files/uploaded/Yale%20studies%20plus%20NIU.pdf	2016 Molecular pathomechanism n=20	4,000
14)	Food Intolerance: Immune Activation Through Diet-Associated Stimuli in Chronic Disease	Pietschmann N; Alternative therapies in health and medicine Jul-Aug 2015;21(4):42-52 https://www.ncbi.nlm.nih.gov/pubmed/26030116	2015 Review paper	
15)	Food Reactivity on the ALCAT Leukocyte Activation Test is Associated with Upregulation of CD11b on T Cells	Yale School of Medicine: Ayaz G, Mehal WZ, Ali A; The Journal of Alternative and Complementary Medicine; 2014;20(5):A35-A36 https://www.researchgate.net/publication/262146351_Food_Reactivity_on_the_ALCAT_Leukocyte_Activation_Test_Is_Associated_with_Upregulation_of_CD11b_on_T_Cells	2014 pathomechanisms n= 10 DBPC	2,000
16)	Evaluation of ALCAT Test Results in the Non IgE-Mediated Pathology of the Skin	De Amici M, Berardi L, Castello M, Mantegna G, Giunta V, Ronzi G, Vignini M; European Journal of Allergy and Clinical Immunology, 2011;66:226 https://www.slideshare.net/slideshow/evaluation-of-alcat-test-results-in-the-non-ig-e-mediated-pathology-of-the-skin/8634254 and https://alcat.com/wp-content/uploads/2025/07/Evaluation-of-ALCAT-Test-results-in-the-non-IgE-mediated-pathology-of-the-skin.pdf	2011 Clinical; skin 35 patients	1,750
17)	ALCAT Test Results in the Treatment of Gastrointestinal Symptoms	Berardi L, De Amici M, Castello M, Torre C, Giunta V, Legoratto, Vignini M; European Journal of Allergy and Clinical Immunology, 2011 https://alcat.com/wp-content/uploads/2025/07/ALCAT-Test-Results-in-the-Treatment-of.pdf	2011 Clinical; GI disorders 48 patients	2,400
18)	Rational Management of Food Intolerance in Elite Soccer Club	Angelini F, Marzatico F, Stesina G, Stefanini L, Bonuccelli A, Buonocore D, Rucci S, Tencone F; Journal of the International Society of Sports Nutrition 2011, 8 (Suppl. 1):36; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3238170/	2011 Multiple symptoms 8 athletes	800
19)	Utilidad clínica del test AL CAT. Mito o realidad [Clinical utility of the ALCAT test. Fact or fiction?]	Ángel San Miguel et al.; FUEL AND ENERGY ABSTRACTS. 2010;107(1):12-20 https://www.researchgate.net/publication/251563604_Utilidad_clinica_del_test_ALCAT_Mito_o_realidad	2010 Review paper	

20)	ALCAT Test Identifies Food Intolerance in Patients with Gastrointestinal Symptoms	Berardi L, De Amici M, Castello M, Torre C, Giunta V, Legoratto, Vignini M; European Journal of Allergy and Clinical Immunology.2009;64:490 https://alcat.com/wp-content/uploads/2025/07/ALCAT-test-identifies-food-intolerance-in-patients-with-gastrointestinal-symptoms.pdf	2009 Clinical; gastrointestinal 15 patients	750
21)	Food Intolerance in Patients with Cutaneous Diseases: Diagnostic Value of the ALCAT Test	Berardi L, De Amici M, Vignini A, Torre C, Mosca M; European Journal of Allergy and Clinical Immunology. 2009;64:490 https://alcat.com/wp-content/uploads/2025/07/Food-intolerance-in-patients-with-cutaneous-diseases-diagnostic-value-of-the-ALCAT-test.pdf	2009 Clinical: Skin 20 patients	700
22)	The Effect of the ALCAT Test Diet Therapy for Food Sensitivity in Patients with Obesity	Akmal M, Khan SA, Khan AQ.; Middle East J Fam Med. 2009;7(3) https://www.researchgate.net/publication/268337548_The_Effect_of_The_ALCAT_Test_Diet_Therapy_for_Food_Sensitivity_in_Patient's_With_Obesity	2009 weight loss 27 patients	2,700
23)	Comparison of Cellular Changes upon Exposure to Allergens between ROBOCat II versus FACSCalibur-based Methods	Jensen G.; NIS Labs (Natural Immune System) Oregon USA, 2009 https://cellsciencesystems.com/education/research/comparison-of-cellular-changes-upon-exposure-to-allergens-between-robocat-ii-versus-facscalibur-based-methods/	2009 Method comparison	Method validation
24)	The Right Stuff: Use of ALCAT Testing to Determine Dietary Factors Affecting Immune Balance, Health, and Longevity	Deutsch RD; Anti-Aging-Therapeutics, Volume X; 2007 Conference Year, Chapter 8 https://www.researchgate.net/publication/287020093_The_right_stuff_Use_of_ALCAT_testing_to_determine_dietary_factors_affecting_immune_balance_health_and_longevity	2007 Review paper	---
25)	IMS Health Economics and Outcomes Research- Influence of Food Intolerance in Migraines: Final Report of Statistical Results	Immunological Center of Catalunya; Version 3, December 28, 2006. http://whatsmyfoodintolerance.com/media/Influence_of_Food_Intolerance_in_Migraines.pdf	2006 Clinical: Migraine 21 patients	2,100
26)	Technical Study Comparing the Alcat Methodology with Activation of Granulocytes Following Challenge with Zymosan	University of Rome Mele C; 2002	2002 Research paper methodology	Method validation
27)	Ogni intervento comincia a tavola. [Every intervention begins at the table]	University of Rome Mele C; Medici Oggi, Maggio 2002: 210-213. https://alcat.com/wp-content/uploads/2025/07/Ogni-Intervento-Comincia-A-Tavola.pdf	2002 Research paper methodology	
28)	The Controversial Antigen Leukocyte Cellular Antibody Test (ALCAT) a Non-Specific Inhibitory Effect of Alpha Glycoproteins	Kedryna T, Guminska M.; Med Sci Monit 1999; 5(2): BR193-197 https://www.medscimonit.com/abstract/index/idArt/505074	1999 Immunology research paper 37 patients	370
29)	Parexel Medstat Statistical Report- Final Statistical Report: Study of the ALCAT Test in 10 Subjects	Fuglerud P (Norway), Parexel Medstat Statistical Report; Nov. 1999 Not published https://alcat.com/wp-content/uploads/2025/07/Parexel-Medstat-Statistical-Report.pdf	1999 Reproducibility validation; 10 patients DBPC	500
30)	Reproducibility of the Antigen Leukocyte Cellular Antibody Test	University of The Orange Free State in Bloemfontein (South Africa) Neetling WML, Kachelhoffer AM; Jan-April 1998 https://alcat.com/wp-content/uploads/2025/07/Reproducibility-of-the-antigen-leukocyte-cellular-antibody-test.pdf	1998 Reproducibility validation; 10 patients	1,000
31)	Outcome Study in 305 Consecutive Patients Following the Alcat Diet	Hoj, L; Allergy Clinic Charlottenlund, Denmark 1998 (Kopenhagen). Non-Published. Pg. 17-18 https://alcat.com/wp-content/uploads/2025/09/TheRightStuff_E314.pdf	1998 353 patients	35,300
32)	Evaluation of the Cytotoxic Food Test and the ALCAT (antigen leukocyte cellular antibody test)	Pasula MJ; Pol Merkur Lekarski. 1997 Feb; 2(8):154-159. https://www.ncbi.nlm.nih.gov/pubmed/9538667/	1997 Review paper method evaluation	

33)	South African Outcome Study - Randomized Study on 274 Patients	Geldenhuys J; Johannesburg, S. Africa, 1997 Not published	1997 Clinical: Multiple disorders 274 patients randomized	13,700
34)	The Short Term Efficacy of the ALCAT Test of Food Sensitivities to Facilitate Changes in Body Composition and Self-Reported Disease Symptoms: a Randomized Controlled Study.	Kaats GR, Pullin D, Parker LK; Bariatrician.1996;18-23. https://www.researchgate.net/publication/237241520_The_Short_Term_Efficacy_of_the_ALCAT_Test_of_Food_Sensitivities_to_Facilitate_Changes_in_Body_Composition_and_Self-reported_Disease_Symptoms_A_Randomized_Controlled_Study	1996 Clinical: weight loss 100 patients randomized	10,000-15,000
35)	Diagnostic Value of ALCAT Test in Intolerance to Food Additives Compared with Double-Blind Placebo-Controlled (DBPC) Oral Challenges.	Hoj L; Allergy Clinic Charlottenlund (DK); Journal of Allergy and Clinical Immunology, Vol. 97, No. 1, Part 3, January 1996 https://www.researchgate.net/publication/246090959_616_Diagnostic_value_of_ALCAT_test_in_intolerance_to_food_additives_compared_with_double-blind_placebo-controlled_DBPC_oral_challanges	1996 Validation chemicals 92 patients DBPC	1,840
36)	El test Alcat de Sensibilidad a los Alimentos y su Interés en Medicina Estética	Amigo C, Moreno Mercer J, Calderon Gomez J, Cabo Soler JR; 14th Med Day of Esthetical Medicine & Dermatological Survey. Venice, Italy, Sep. 22 – 23, 1995; published in the proceedings; subsequently published in Estetica Medica Numero 40 - March 1996 https://alcat.com/wp-content/uploads/2025/07/El-test-ALCAT-de-sensibilidad-a-los-alimentos-y-su-interes-en-Medicina-Estetica.pdf	1995 Clinical: weight loss 30 obese patients	3,000
37)	ALCAT Test Results in the Treatment of Respiratory and Gastrointestinal Symptoms, Arthritis, Skin and Central Nervous System	Mylek D; Rocz Akad Med Bialymst. 1995, 40(3):625-629. https://www.ncbi.nlm.nih.gov/pubmed/8775317	1995 Clinical: multiple disorders 72 patients	3,600
38)	Prevalence of Food Allergy and Intolerance in Children Based On MAST CLA and ALCAT Tests.	Buczylko K, Obarzanowski T, Rosiak K, Staškiewicz G, Fiszer A, Chmielewski S, Kowalczyk J; Advances in Medical Sciences 1995;40(3):452-456 https://www.ncbi.nlm.nih.gov/pubmed/8775289	1995 Clinical Comparison 56 patients; single-blinded	2,000
39)	Food Intolerance in Patients with Angioedema and Chronic Urticaria: - an Investigation by RAST and ALCAT Test	Hoj L; Allergy Clinic Charlottenlund (DK); European Journal of Allergy and clinical Immunology Supplement 1995; 50:26 https://alcat.com/wp-content/uploads/2025/07/food-intolerance-in-patients-with-angioderma-and-chronic-urticaria-an-investigation-by-RAST-and-Alcat-test.pdf	1995 Clinical comparison 52 patients	5,600
40)	Allergie alimentari. Tecniche diagnostiche a confronto [Food Allergy: Comparison of Diagnostic Techniques]	Mancini S; Fierimonte V, Iacovoni R, Spaini A, Viarani P, Pichi A; Minerva Pediatr. 1995 May;47(5):159-63 (Italian) https://www.ncbi.nlm.nih.gov/pubmed/7643816	1995 Diagnostic comparison 14 patients	112
41)	Autism - A Multidisciplinary Approach to Treatment	Kotsanis CA, Dart L, Harjes C, Miller R; American Academy of Otolaryngic Allergy; https://cellsciencesystems.com/education/research/autism-a-multidisciplinary-approach-to-treatment/	1994 Clinical: autism 12 patients	600
42)	Reproducibility of the ALCAT Test	University of Cape Town Steinman H; Potter P; Johannesburg, South Africa 1994; also 2 letter statements available where the author confirmed reproducibility and improvements of symptoms https://alcat.com/wp-content/uploads/2025/07/Reproducibility-of-the-ALCAT-Test.pdf	1994 Reproducibility validation 12 patients	600
43)	The ALCAT Test: In-Vitro Procedure for Determining Food Sensitivities	Pasula MJ; Folia Med Cracov. 1993;34(1-4):153-157. https://www.ncbi.nlm.nih.gov/pubmed/8175054	1993 Review paper	

44)	The ALCAT Test- A Guide and Barometer in the Therapy of Environmental and Food Sensitivities	Solomon B.; Environmental Medicine. 1992;9 (1 & 2). https://alcat.com/wp-content/uploads/2025/07/The-ALCAT-Test-A-Guide-and-Barometer-in-the-Therapy-of-Envionmental-and-Food-Sensitivities.pdf	1992 Clinical: multiple disorders 172 patients	17,200
45)	Cellular Responses to Food in Irritable Bowel Syndrome - an Investigation of the ALCAT Test	Fell PJ, Soulsby S, Brostoff J; Journal of Nutritional & Environmental Medicine; 1991;2(2):143-149. https://www.researchgate.net/publication/237626374_Cellular_Responses_to_Food_in_Irritable_Bowel_Syndrome_-_an_Investigation_of_the_ALCAT_Test	1991 Clinical: IBS 22 patients DBPC	2,200
46)	Pilot Study into the Effect of Naturally Occurring Pharmacoactive Agents on the Alcat Test	Fell PJ; Annual Meeting of the American Otolaryngic Allergy Association, Sept 27, 1991; Kansas City https://www.researchgate.net/publication/237648208_PILOT_STUDY_INTO_THE_EFFECT_OF_NATURALLY_OCCURING_PHARMACOACTIVE_AGENTS_ON_THE_ALCAT_TEST	1991 Validation biogenic amines 24 patients	336
47)	Pharmacoactive Compounds in Foods – The Effect on the Alcat Test in Healthy Volunteers and Patients Suffering from Migraine	Fell PJ, Brostoff J, Pasula M; AAOA News 9:2:29.	1990 Clinical: Migraine	
48)	Gastrointestinal Complaints Related to Diet	Sandberg DH; Int Pediatr 1990; 5:23-29. https://alcat.com/wp-content/uploads/2025/07/gastrointestinal-complaints-related-to-diet.pdf	1990 Clinical: gastrointestinal 3 patients	40
49)	ALCAT® - "A New Cellular Test For Food Sensitivity".	Fell PJ, Brostoff J, Soulsby S; American In-Vitro Allergy & Immunology Society August 1990 Toronto Canada; https://www.researchgate.net/publication/242123352_ALCATR_-_A_NEW_CELLULAR_TEST_FOR_FOOD_SENSITIVITY	1990 Clinical: gastrointestinal 80 patients DBPC	4,000
50)	Inhibitory Effect of Sodium Cromoglycate on Granulocyte Response to Food Antigens In-Vitro	Fell PJ, Sandberg DH, Pasula MJ; ACAI 45thAnnual Meeting, Nov. 10–14, 1990, San Francisco. published in the proceedings	1990 Immunological study 10 patients	100
51)	Multiple Pathogenic Mechanisms in Food Sensitivity Reactions In-Vitro	Pasula MJ, Puccio S; 4th International Symposium on Immunological and Clinical Problems of Food Allergy. Milan, Italy: November 5-9, 1989. published in the proceedings https://alcat.com/wp-content/uploads/2025/07/Multiple-pathogenic-mechanisms-in-food-sensitivity-reactions.pdf	1989 Immunological study 9 patients	54
52)	Influence of Food Antigens on Volumes of Circulating White Blood Cells and Platelets Aggregation	Brostoff J, Fell PJ; 4th International Symposium on Immunological and Clinical Problems of Food Allergy. Milan, Italy: November 5-9, 1989. published in the proceedings https://alcat.com/wp-content/uploads/2025/07/Influence-of-food-antigens-on-volumes-of-circulating-white-blood-cells-and-platelets-aggregation.pdf	1989 Immunological study 9 patients	54
53)	High Correlation of the Alcat Test Results with Double Blind Challenge (DBC) in Food Sensitivity.	Fell PJ, Brostoff J, Pasula MJ; ACAI, 45th Annual Meeting, Los Angeles, Nov. 12-16, 1988, published in Annals of Allergy https://alcat.com/wp-content/uploads/2025/07/High-Correlation-Alcat-test-Double-Blind-Clinical-study.pdf	1988 Clinical: gastrointestinal 179 patients DBPC	8,950
54)	A Comparison of the Alcat Test for Food Reactions Amongst 2 Population Sub-Groups	Sandberg DH; ACAI 45th Annual Meeting, L.A. Nov. 12-16, 1988, published in Annals of Allergy https://alcat.com/wp-content/uploads/2025/07/A-Comparison-Of-The-Alcat-Test-For-Food-Reactions.pdf	1988 Clinical: multiple disorders 50 patients	450
55)	ALCAT- "A New Test for Food Induced Problems in Medicine?"	Fell PJ, Brostoff J, O'Donnell H, O'Connor A, Charig E; Annual Meeting of the American Academy of Otolaryngic Allergy, 1988; Washington, D.C. https://alcat.com/wp-content/uploads/2025/07/A-new-Test-for-food-induced-problems-in-medicine.pdf	1988 Clinical: multiple disorders 53 patients DBPC	2,650

The Alcat Test Predicts the Release of DNA by Innate Immune Peripheral Blood Leukocytes via a Protein Kinase C (PKC) Dependent Pathway

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Background

The Alcat (antigen leukocyte antibody test) is a commercially available assay that examines the response of peripheral blood immune cells to food antigens, and identifies food items which provide a cellular response (defined as a positive response).[1,2] Clinically, Alcat test results are used to individualize the diets of patients with a diverse range of medical conditions, and are commonly used in complementary/alternative medicine practices. Despite widespread use since 1986, the Alcat test, like many unconventional diagnostic assays,[3] has not been rigorously validated in clinical or immunological studies.

Aim and Hypothesis

The objective of this study was to bridge the gap in understanding between the known morphological and physiochemical (conductance) based changes which are detected by the Alcat test and current paradigms of immune activation.

Hypothesis: We hypothesized that positive foods result in an immune response due to the release of DNA from peripheral blood leukocytes, resulting in an inflammatory response. If this is the case, then positive food items are predicted to release DNA to a greater degree than negative food items.

Methods

The study protocol, consent form and all recruitment materials were approved by the Human Investigation Committee of Yale University (New Haven, **ct**). The study was conducted in accordance with the Declaration of Helsinki.

Eligible participants were healthy females (n=17) and males (n=3), with a mean age of 38 years of age (range 20 to 61 years) with no reported food allergies/sensitivities. Participants provided 5-11 blood samples (20 mL) approximately monthly for 17 months.

The initial blood sample from each subject was collected at Yale University and sent to the sponsor for Alcat testing, resulting in a list of foods categorized as positive, negative or intermediate for each subject. All work apart from the initial Alcat testing was performed in the Section of Digestive Diseases at Yale University.

1. Quantification of plasma total DNA by direct fluorescent PicoGreen staining DNA quantification was performed using the PicoGreen dsDNA kit (Life Technologies), according to the manufacturer's instructions. Each sample DNA was analyzed in two duplicated dilution series. Black microtiter plates were read in a plate reader (BioTek) at an emission wavelength of 520 nm and excitation of 480 nm. The quantification of MPO in supernatants from cells treated with various foods and inhibitors was performed by a standard ELISA using a commercial kit (Human MPO ELISA Kit, Abcam).

2. The quantification of MPO in supernatants from cells treated with various foods and inhibitors was performed by a standard ELISA using a commercial kit (Human MPO ELISA Kit, Abcam).

3. Flow cytometry analysis was performed to assess if a particular immune cell population (neutrophils, eosinophils, and basophils) was activated by the positive food items. PBL cell populations were gated based on the expression of the following surface markers:

Neutrophils: CD66b+ CD16+
Eosinophils: CD66b+ CD16 -
Basophils: CD66b - CD16 - CD123+

The CD63 activation marker (eBioscience) was analyzed in each population. Samples were acquired in a FacsCanto cytometer (Becton Dickinson) and the data were processed using the FacsDiva 12.0 software.

Statistical Analysis

Differences between the 2 groups were compared with Mann-Whitney U test. (GraphPad Prism 6; GraphPad Software Inc,). A P-value of <0.05 was considered significant.

Results

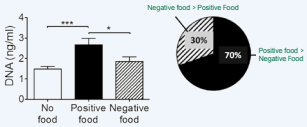

Figure 1: *Positive foods result in greater release of DNA than negative foods*

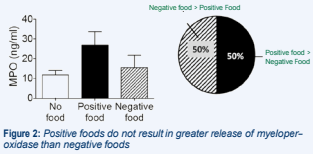

Figure 2: *Positive foods do not result in greater release of myeloperoxidase than negative foods*

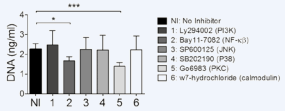

Figure 3: *Protein kinase C and NF-kb inhibition reduce DNA release by positive foods*

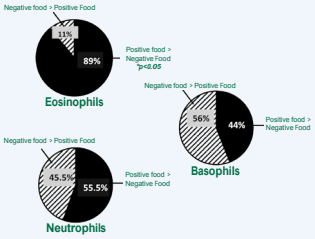

Figure 4: *Positive foods result in up-regulation of CD63 in eosinophils to a greater degree than negative foods*

Conclusion

This is the first demonstration that specific food items can result in release of DNA by peripheral blood leukocytes, and further that these items can be identified by the Alcat test. This may provide a mechanistic rationale for the reported findings of clinical improvement in patients using the Alcat test to guide dietary choices, and provides support for conducting randomized clinical trials of the Alcat test in gastrointestinal and liver diseases known to have dietary associations.

Acknowledgements

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Funding

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1. Altern. Ther. Health Med. 2015;21(4):42-52.
2. Folia Med. Cracov. 1993;34(1-4):153-157.
3. J Alt Comp Med. May 2014, 20(5): A3-A4.

Yale SCHOOL OF MEDICINE

Figure D.1.: Original Yale University poster presentation referring to reference (13) demonstrating a pathomechanism that Alcat Test reactions relate to classical immune mediated inflammatory pathways: Garcia-Martinez I, Weiss TR, Ali A, Mehal WZ; The Alcat Test Predicts the Release of DNA and Myeloperoxidase by Innate Immune Peripheral Blood Leukocytes Via a PKC Dependent Pathway; Yale School of Medicine; https://pmc.ncbi.nlm.nih.gov/articles/PMC5896029/

D.2 Double-Blind Studies as a Tool for Clinical Evaluation

The rigorous evaluation of a drug, diagnostic test, or procedure generally involves conducting double-blind, placebo-controlled (DBPC) oral challenge trials, which are recognized as the most reliable and valid measures of clinical efficacy. Rigorous research methods include the careful selection of subjects based on relevant inclusion and exclusion criteria, collection of sufficient data, and analysis to determine statistically significant outcomes.

D.2.1 Yale University: Recent Double-Blinded Studies to Evaluate the Alcat Leukocyte Activation Test

Scientists from Yale School of Medicine have recently (2016-2018) evaluated the Alcat Test using a rigorous clinical trial and mechanistic studies to shed light on pathomechanism.

The Clinical Study:

(12) Efficacy of Individualized Diets in Irritable Bowel Syndrome: A Randomized Controlled Trial

Citation: Yale School of Medicine: Ali A, Weiss TR, Scherban A, Khan A, McKee D, Apollo D, Mehal WZ; BMJ Open Gastroenterology. 2017; 4(1):e000164. doi:10.1136/bmjgast-2017-000164

Methods:
Parallel group, **double-blind, randomized, controlled clinical trial** in 58 adults with IBS, with moderate or greater severity, at a single academic center. Peripheral venous blood was analyzed by standard Alcat testing against a range of foods; individual foods were reported as intolerant or acceptable for each subject. Subjects received

1) Intervention: individualized dietary guidance (elimination of all intolerant foods and allowance of acceptable foods); or

2) Matched placebo individualized dietary guidance (elimination of acceptable foods and allowance of intolerant foods) and were guided by a registered dietitian during the 4-week intervention period. Outcomes included the IBS Global Improvement Scale (GIS; primary), IBS Symptom Severity Scale (SSS), IBS Adequate Relief (AR), quality of life, and dietary adherence, assessed through 8-weeks.

Results:
55 subjects completed the trial (7/2014–1/2016). T-tests of equality of means were performed ($\alpha = 0.05$), corroborated by nonparametric Wilcoxon rank sum tests of equality of distribution. All outcomes improved in both groups. The intervention group demonstrated significantly larger between-period changes than the placebo group in the GIS (2.04 vs. 1.07; $p = 0.02$) and SSS (126.81 vs. 59.59; $p = 0.02$) at 4-weeks, with significance persisting at 8-weeks. At 4-weeks, there was a greater trend towards lower BMI in the intervention group ($p = 0.08$) and higher AR ($p = 0.24$) vs. the placebo group.

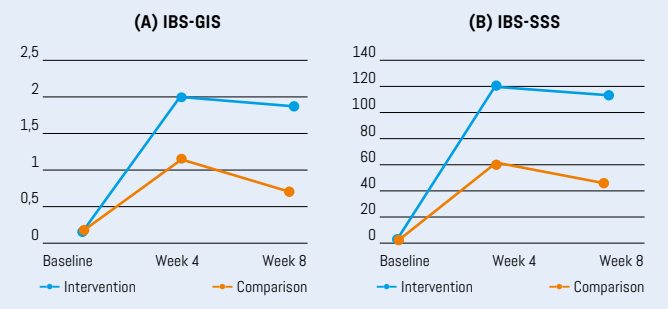


Figure D.1: (6) The double-blinded study shows that patients with irritable bowel syndrome (IBS) improved significantly using the Alcat test (blue) vs. the comparison/placebo group (red).

Conclusion: Individualized dietary plans guided by the Alcat test show efficacy in IBS. Our results have implications in understanding pathophysiological mechanisms, dietary interventions, and the role of food intolerance in IBS.

Pathomechanism referring to the Yale study: Explanations and correlation with clinical outcomes

(11, 13) The Alcat Test Predicts the Release of DNA and Myeloperoxidase by Innate Immune Peripheral Blood Leukocytes via a PKC Dependent Pathway

Citation: Yale School of Medicine;

(11) Garcia-Martinez I, Weiss TR, Yousaf MN, Ali A, Mehal WZ; Nutrition & Metabolism volume 15, Article number: 26 (2018)

(13) Garcia-Martinez I, Weiss TR, Ali A, Mehal (2016) WZ;

Summary: Whole blood samples from 20 healthy volunteers underwent Alcat testing. Other analyses were performed at Yale University (New Haven, CT). Quantification of total DNA and myeloperoxidase (MPO) in the supernatant was performed in the presence and absence of specific inhibitors of key signaling pathways (phosphoinositide 3-kinase, nuclear factor-kβ, c-Jun N-terminal kinase, mitogen-activated protein kinase P38, protein kinase C and calmodulin).

Results: Alcat positive foods gave a higher supernatant DNA content in 53 of 76 (70%), and a higher MPO in 18 of 28 (64%) (significant at $P < 0.05$). PKC inhibitors resulted in inhibition of Alcat positive food stimulated DNA release ($P < 0.05$). Activation of neutrophils, eosinophils, and basophils was identified by established cell surface markers and flow-cytometric analysis. Alcat positive samples resulted in CD63 levels greater than Alcat negative samples in eosinophils in 76% of tests ($p < 0.02$), but only 47% and 41% for neutrophils and basophils respectively (NS).

Conclusion: The Alcat test identifies food items that result in release of DNA2 and MPO3 and activation of peripheral blood innate immune cells in a PKC dependent manner. This demonstrates that the Alcat test identifies food items that result in release of inflammatory markers and activation of innate immune cells.

Scientists from Sheba Medical Center recently confirmed (2023) the effectiveness of the Alcat Test for IBS treatment using a rigorous double blinded clinical trial:

(3) Immune-Based Personalized Elimination Diet for the Treatment of Irritable Bowel Syndrome: A Double-Blind Randomized Sham-Controlled Study

Citation: Gastroenterology Institute, Sheba Medical Center; Horin, Shomron MD; Engel, Tal MD et al; The American Journal of Gastroenterology 118(10S): p S454-S455, October 2023. | DOI: 10.14309/01.ajg.0000952116.88348.96

Methods: Parallel group, randomized, double-blind, 2-arm trial, comparing the efficacy of Alcat-diet versus a "Sham" balanced diet for 8-week treatment of IBS. The primary outcome was defined as a reduction of ≥50 points on the IBS-severity scoring system (IBS-SSS). Secondary outcomes included the raw scores of IBS-SSS, Irritable Bowel Syndrome Quality of Life Questionnaire (IBS-QoL) and the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). IBS-Adequate Response rate and the rates of moderate / substantial improvement on the IBS Global assessment of improvement (IBS-GAI) and the IBS Global Improvement Scale (IBS-GIS) were documented.

Results: 68 patients with IBS-D (44) and IBS-M (24) were enrolled. At baseline, patients in the Alcat-group had a higher median IBS-SSS score compared with the controls (390 [305-435] vs 330 [240-390], respectively, P=0.013). At week 8 of dietary intervention, 30/35 (85.7%) patients in the Alcat-group met the primary outcome, compared with 18/33 (54.5%) of the controls (P=0.005). After 8 weeks, there were higher rates of patients in the Alcat-group who reported a symptomatic improvement (i.e., positive response: 85.7% vs 57.6%, P=0.010 and IBS-GIS: 74.3% vs 42.4%, P=0.008, Δ VAS-IBS abdominal pain score: 49.7±29.8 vs 31.6±28.8, P=0.020) compared with the controls. Alcat-diet was more beneficial for patients who were naïve to dietary-intervention (ΔIBS-SSS≥50 rate: 100% vs 57%, respectively, P=0.010), than patients who had previously tried any dietary-intervention (ΔIBS-SSS≥50 rate: 77% vs 50%, respectively, P=0.148) compared with the Sham-diet. No serious adverse events were reported during follow-up.

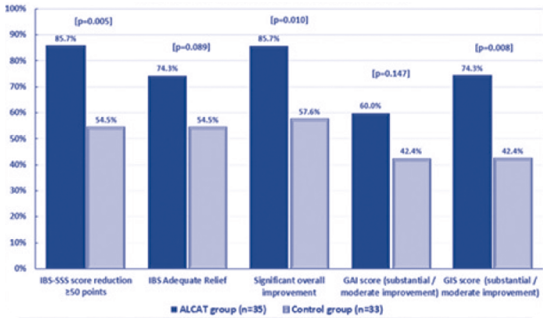
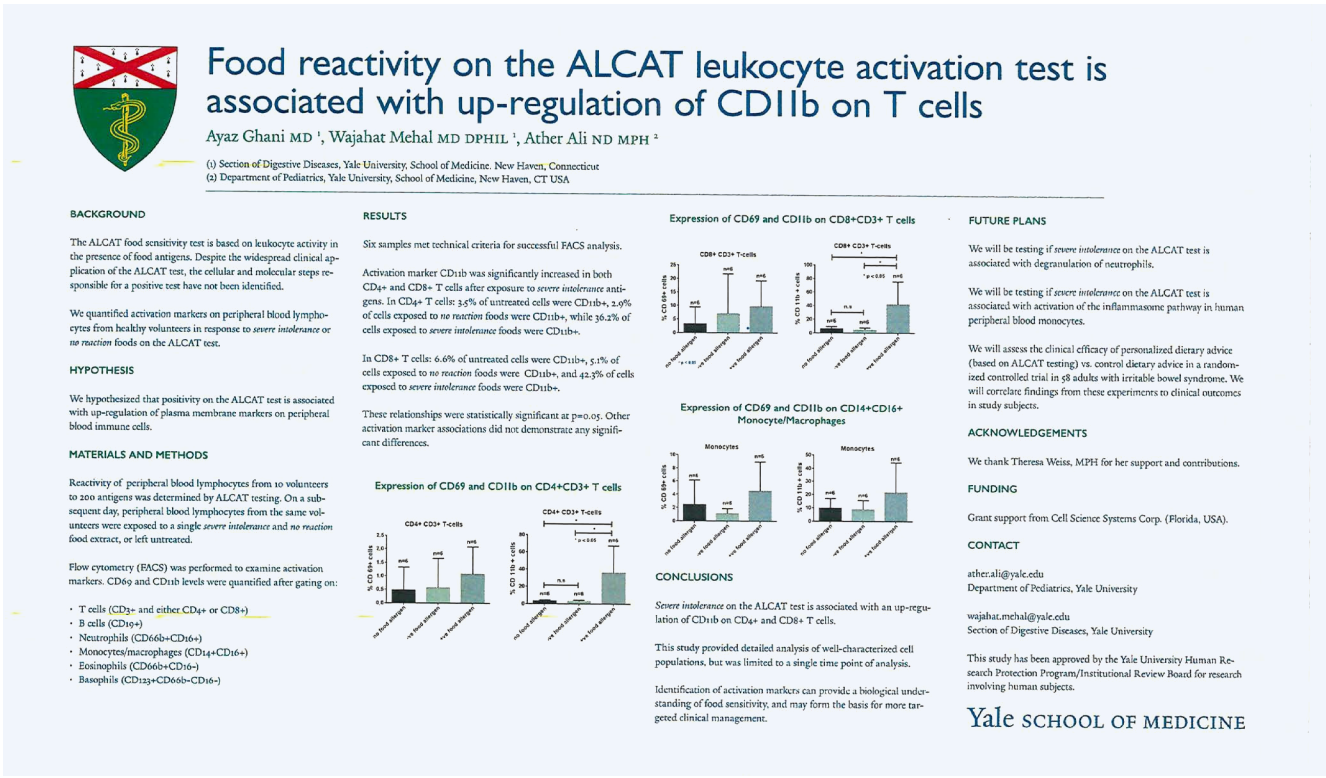


Figure: Study outcome rates at the end of follow-up (8-week time point) divided to the Alcat-based diet group and the Sham-based diet group. Abbreviations: Irritable bowel syndrome, IBS; IBS Symptom Severity Scale, IBS-SSS; IBS global assessment of improvement, IBS-GAI; IBS global improvement scale, IBS-GIS.

(15) Food Reactivity on the ALCAT Leukocyte Activation Test is Associated with Upregulation of CD11b on T Cells.

Citation: Yale School of Medicine: Ghani A, Mehal WZ, Ali A; The Journal of Alternative and Complementary Medicine. 2014;20(5):A35-A36.

Summary: The ALCAT food sensitivity test is based on leukocyte activity in the presence of food antigens, though the underlying cellular and molecular steps have not been fully identified. We quantified activation markers on peripheral blood lymphocytes (PBL) from healthy volunteers in response to "severe intolerance" or "no reaction" foods on the ALCAT test. Methods: Reactivity of PBL from 10 volunteers to 200 antigens was determined by ALCAT testing. On a subsequent day, PBL from the same volunteers were exposed to a single "severe intolerance" and "no reaction" food extract, or left untreated. Flow cytometry (FACS) was performed to examine activation markers CD69 and CD11b after gating on: T cells (CD3+ and either CD4+ or CD8+), B cells (CD19+), neutrophils (CD66b+ CD16+), monocytes/macrophages (CD14+ CD16+), eosinophils (CD66b+ CD16-) and basophils (CD123+ CD66b- CD16-).



Food reactivity on the ALCAT leukocyte activation test is associated with up-regulation of CD11b on T cells

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BACKGROUND

The ALCAT food sensitivity test is based on leukocyte activity in the presence of food antigens. Despite the widespread clinical application of the ALCAT test, the cellular and molecular steps responsible for a positive test have not been identified.

We quantified activation markers on peripheral blood lymphocytes from healthy volunteers in response to *severe intolerance* or *no reaction* foods on the ALCAT test.

HYPOTHESIS

We hypothesized that positivity on the ALCAT test is associated with up-regulation of plasma membrane markers on peripheral blood immune cells.

MATERIALS AND METHODS

Reactivity of peripheral blood lymphocytes from 10 volunteers to 200 antigens was determined by ALCAT testing. On a subsequent day, peripheral blood lymphocytes from the same volunteers were exposed to a single *severe intolerance* and *no reaction* food extract, or left untreated.

Flow cytometry (FACS) was performed to examine activation markers. CD69 and CD11b levels were quantified after gating on:

- T cells (CD3+ and either CD4+ or CD8+)
- B cells (CD19+)
- Neutrophils (CD66b+CD16+)
- Monocytes/macrophages (CD14+CD16+)
- Eosinophils (CD66b+CD16-)
- Basophils (CD123+CD66b-CD16-)

RESULTS

Six samples met technical criteria for successful FACS analysis.

Activation marker CD11b was significantly increased in both CD4+ and CD8+ T cells after exposure to *severe intolerance* antigens. In CD4+ T cells: 3.5% of untreated cells were CD11b+, 2.9% of cells exposed to *no reaction* foods were CD11b+, while 36.2% of cells exposed to *severe intolerance* foods were CD11b+.

In CD8+ T cells: 6.6% of untreated cells were CD11b+, 5.1% of cells exposed to *no reaction* foods were CD11b+, and 42.3% of cells exposed to *severe intolerance* foods were CD11b+.

These relationships were statistically significant at p=0.05. Other activation marker associations did not demonstrate any significant differences.

Expression of CD69 and CD11b on CD4+CD3+ T cells

Expression of CD69 and CD11b on CD8+CD3+ T cells

Expression of CD69 and CD11b on CD14+CD16+ Monocyte/Macrophages

CONCLUSIONS

Severe intolerance on the ALCAT test is associated with an up-regulation of CD11b on CD4+ and CD8+ T cells.

This study provided detailed analysis of well-characterized cell populations, but was limited to a single time point of analysis.

Identification of activation markers can provide a biological understanding of food sensitivity, and may form the basis for more targeted clinical management.

FUTURE PLANS

We will be testing if *severe intolerance* on the ALCAT test is associated with degranulation of neutrophils.

We will be testing if *severe intolerance* on the ALCAT test is associated with activation of the inflammasome pathway in human peripheral blood monocytes.

We will assess the clinical efficacy of personalized dietary advice (based on ALCAT testing) vs. control dietary advice in a randomized controlled trial in 58 adults with irritable bowel syndrome. We will correlate findings from these experiments to clinical outcomes in study subjects.

ACKNOWLEDGEMENTS

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This study has been approved by the Yale University Human Research Protection Program/Institutional Review Board for research involving human subjects.

Yale SCHOOL OF MEDICINE

Results: Six samples met technical criteria for successful FACS analysis. Activation marker CD11b was significantly increased in both CD4+ and CD8+ T cells after exposure to "severe intolerance" antigens. In CD4+ T cells: 3.5% of untreated cells were CD11b+, 2.9% of cells exposed to "no reaction" foods were CD11b+, while 36.2% of cells exposed to "severe intolerance" foods were CD11b+. In CD8+ T cells: 6.6% of untreated cells were CD11b+, 5.1% of cells exposed to "no reaction" foods were CD11b+, and 42.3% of cells ex- posed to "severe intolerance" foods were CD11b+. These relationships were statistically significant at p=0.05. Other activation marker associations did not demonstrate any significant differences.

Conclusion: "Severe intolerance" on the ALCAT test is associated with an upregulation of CD11b on CD4+ and CD8+ T cells. This study provided detailed analysis of well-characterized cell populations, but was limited to a single time point of analysis. Identification of activation markers can provide a biological understanding of food sensitivity, and may form the basis for more targeted clinical management.

Other recent and important double blinded trials

(9) Non-Celiac Gluten Sensitivity in Patients with Severe Abdominal Pain and Bloating: The Accuracy of ALCAT 5.

Citation: University of Pavia (IT); Di Stefano M, Pesatori EV, Manfredi GF, De Amici M, Grandi G, Gabriele A, Iozzi D, Di Fede G;

Clin Nutr ESPEN. 2018 Dec;28:127-131.

Background and aims: Non-Celiac Gluten Sensitivity (NCGS) is a recently proposed clinical condition causing both intestinal and extra-intestinal symptoms, without gastrointestinal lesions, which improve on avoiding gluten intake, in the absence of celiac disease and wheat allergy. The prevalence of this condition is still a matter of debate, in part due to the very recent introduction of an accepted diagnostic test, a double-blind, placebo controlled gluten challenge. However, this is a lengthy and cumbersome procedure, theoretically burdened by a significant reduction of patient compliance. ALCAT 5 is an automated in vitro test evaluating the toxic effect of gluten on neutrophils by the exposure of these cells to a gluten-containing extract of gluten-containing cereals. The test is very simple to perform, the results are rapidly obtained, and might represent, if sufficiently accurate, a promising alternative to diagnose gluten intolerance. The aim of this study was the comparison of ALCAT 5 results with those of a double-blind, placebo-controlled, gluten challenge, in a group of patients with clinically-suspected NCGS.

Methods: Twenty-five patients (M/F 3/22, mean age 32 ± 4 yrs) with severe functional abdominal pain and bloating, who had previously undergone the ALCAT 5 test, were enrolled. All the subjects reported their symptoms on a gluten-containing diet and considered gluten the causal agent. Following the Salerno Experts' Criteria, they underwent a double-blind, placebo controlled trial with gluten vs placebo. A mean value during gluten ingestion >30% of the value during placebo was considered as indicative of gluten sensitivity.

Results: After blinded administration of gluten, 13 out of 25 (52%) patients showed an increase in the severity of abdominal pain, and 11 out of 25 (44%) showed an increase in the severity of abdominal bloating. Considering these two symptoms together, in 16 patients out of 25 (64%), blinded gluten administration induced an increase of abdominal pain and/or bloating. The ALCAT 5 test proved to be positive in 20 and negative in 5 patients. In sixteen patients out of 25 the result of ALCAT 5 agreed with the double-blind trial (64%). In particular, both tests were positive in 14 patients and negative in 2.

Conclusions: In this subgroup of patients, ALCAT 5 could be used to support the clinical suspicion of the presence of NCGS and to address these patients to a blinded gluten challenge.

(8) Effects of Antigen Leukocyte Cellular Activation Test-Based Diet on Inflammation, Body Composition, and Medical Symptoms

Citation: Northern University Illinois (NIU); Lukaszuk JM, Shokrani M, Roy PG, Hoppensteadt J, Umoren J; Alternative and Complementary Therapies VOL. 24, NO. 5; 11 Oct 2018

Summary see Clinical Studies, D.4.2

D.2.2 Introduction: Using Double-Blind Studies to Evaluate the Alcat Test for Food Sensitivity

To confirm the validity, reliability, sensitivity, specificity, and effectiveness of the Alcat Test, it must be shown to compare favorably with an established method for testing food intolerances. Ideally, it should be as valid, reliable, and efficacious as a method generally recognized as the gold standard of diagnostic testing.

In the area of food allergy and food sensitivity, the double-blind, placebo-controlled food challenge (DBPCFC) is the gold standard for diagnostic testing.

In a DBPCFC, certain foods are eliminated from the diet and individually reintroduced at pre-determined time intervals. Because symptoms are often delayed, an objective assessment is the only reliable way to identify a true connection between food consumption and symptoms. In rigorous research, neither the patient nor the doctor knows the composition of the patient's test meal or test results. The evaluation is completed 48 hours later when the doctor determines whether the patient has reacted to the food.

In a DBPCFC study the results of the Alcat Test for food sensitivity were compared to the results of oral provocation to determine whether they were comparable. Following a clinical examination and selection of appropriate study subjects, the Alcat Test was performed. Neither the doctor nor the patient was told the test results. Thus, neither the physician (examiner) nor the subject knew whether the foods used in oral provocation were identified by the Alcat Test as positive (reactive) or negative (non-reactive). Figure D2 displays the study protocol used to compare Alcat Test results with those of the oral challenge. These results are described in greater detail in Alcat – A new cellular test for food sensitivity (49).

The Alcat Test measures the direct effect of food substances on immune cells associated with the symptoms of intolerance. For Alcat Test results to be considered valid they must comport with the results of the oral food challenge test.

Study Design

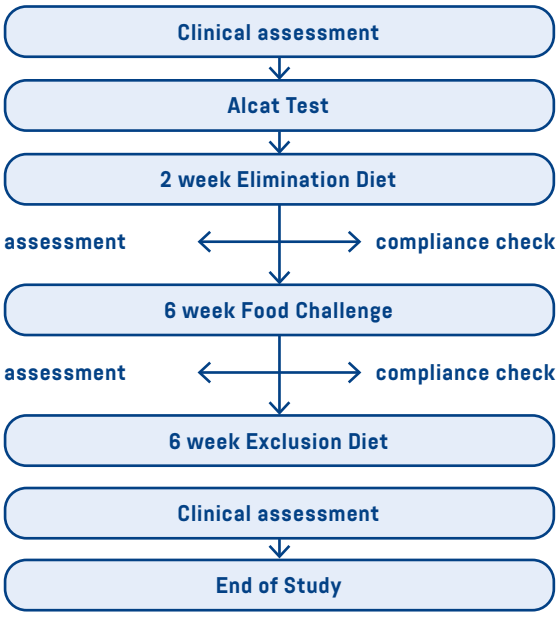

Figure D.2: Study scheme for comparing the results of randomized, double-blind oral challenge studies with the results of the Alcat Test. (49)

C.2.3 Clinical Sensitivity and Specificity of the Alcat Test

Double-Blind Placebo-Controlled Food Challenge Studies (DBPC)

A large DBPC randomized study was conducted in England in 1988, led by Dr. Peter Fell and Prof. Jonathan Brostoff in the allergy clinic of the Health Center, Deddington, Oxfordshire. The Deddington Health Center frequently took part in clinical trials and was able to provide a large pool of appropriate study subjects, namely, patients presenting for at least 3 years with multiple symptoms of indeterminate etiology.

Two successive double-blind studies were conducted following the protocol summarized in the flowchart shown in Figure D.2, above. The pilot study enrolled 58 patients, each suffering from multiple symptoms resistant to conventional diagnosis. The second study enrolled 20 patients diagnosed with irritable bowel syndrome (IBS).

In both studies, patients recorded their symptoms in diaries and followed a two-week elimination diet based on their individual Alcat Test results. The final three days of the elimination period were used to define the baseline condition for the next phase of the study, a six-week period of provocative food challenges in which test substances were rotated weekly. Each week, data from the last three days were used to avoid any carried over effects from the previous week's provocation. Changes in symptoms between the baseline condition and each challenge was evaluated by a clinician.

Results of pilot study and main study:

In the pilot trial, 10 (18.9%) of the 53 enrolled patients discontinued the study due to severe adverse reactions. Of these, 9 patients (90% of discontinued subjects) had a positive Alcat Test result for the substance causing their reaction, thereby demonstrating a 90% agreement between Alcat Test results and symptoms.

In the 43 patients (81.1%) who completed the study, a significant difference between positive and negative provocation was observed (p. value < 0.024). This correlated well with the patients' respective Alcat Test results, providing a nearly 80% level of agreement. The overall clinical assessment, which considered whether patients had experienced noticeable improvement in their IBS symptoms, showed that 50% of patients experienced significant improvement by following the Alcat recommended diet, without taking any additional medication. More than three quarters (77%) had improvement in migraine, 71% saw improvement in eczema and/or urticaria, and 100% had improvement in rhinitis and hay fever symptoms.

It is important to note that IBS is a complex disorder involving many factors in addition to food reactions, and that purely pharmacological treatment of IBS often fails to alleviate symptoms. That 50% of IBS patients in this study experienced significant symptomatic relief by following a purely dietary approach based on Alcat identification of individual food intolerances is an important demonstration of the clinical value of this method.

The second study, which exclusively enrolled patients diagnosed with IBS of at least 3 year's duration who often experienced IBS-associated joint pain, headaches, and lethargy, also showed a very strong correlation between Alcat identified food intolerances and responses to oral provocation. The correlation between substances and symptoms due to experimental provocation was 79.3% positive for substances identified by Alcat as reactive, and 87.5% negative for substances identified by Alcat as non-reactive.

Conclusions (see also A.2.2; clinical validation):

- The average correlation between Alcat Test and DBPCFCs was 83.4%, demonstrating the efficacy of the Alcat Test for detection of incompatible, immuno-reactive food.
- The Alcat Test may be regarded as a valid tool for functional medicine. It objectively predicts the ability of a specific, customized diet to alleviate a wide array of symptoms.
- The Alcat Test makes it easier for the clinician to create a highly effective elimination and exclusion diet individually tailored to the patient.
- The Alcat Test may be used in other clinical situations where food intolerance is suspected.

Additional observations:

In the year following the publication of these two double-blind studies, an additional 179 cases were reported by the same clinic. Clinicians prescribing diets to their patients based on the results of their Alcat Tests continued to report significant improvement in symptoms. For data and tables describing these cases, see ALCAT® - "A New Cellular Test For Food Sensitivity". (49)

Interestingly, IBS patients achieved significant improvement of symptoms in more than 54% of cases. It is striking that in the majority of cases the most significant improvements occurred after 6 months, and in some cases as long as 12 months after the elimination of foods identified by Alcat as reactive. This is the reason that Alcat dietary recommendations advise eliminating reactive substances from the diet for at least 6 months.

The 4 DBPC food challenge studies:

Individual publications on Alcat studies by Fell PJ, Brostoff J, et al; 1988-1992.

(53) High Correlation of the Alcat Test Results with Double-blind Challenge (DBC) in Food Sensitivity

Presentation at the 45th Annual Congress of the American College of Allergy and Immunology, Los Angeles, November 12-16, 1988; published in the Annals of Allergy.

(55) ALCAT – "A New Test for Food Induced Problems in Medicine?"

Presentation at the annual meeting of the American Academy of Otolaryngic Allergy, Washington DC, 1. October 1988.

(49) ALCAT® – A New Cellular Test for Food Sensitivity

Presentation at the annual meeting of the American In-Vitro Allergy & Immunology Society, August 1990, Toronto, Canada.

(45) Cellular Responses to Food in Irritable Bowel Syndrome – an Investigation of the ALCAT Test

Publication of pooled study results in the Journal of Nutritional Medicine, Vol. 2, No. 2, 1991. During this 3 year investigation on the Alcat Test about 120 patients were monitored under DBPC conditions. Additional 179 patients were monitored long-term over a period of 3 years at Deddington Center, London.

D.2.4 DBPC Oral Challenges with Food Additives

In 1996, under the direction of Dr. Lene Hoj at the Allergy Clinic Charlottenlund in Copenhagen, Denmark, a randomized, double-blind, placebo-controlled study of 96 patients was conducted to assess intolerance to food additives. Ten common food colorants and 10 preservatives were tested. Test substances were orally administered in standardized capsules and matching placebo.

The Alcat Test demonstrated high correlation with double-blind, placebo-controlled food additive challenges (DBPC).

The consistency of results between the two methods was 96%.

(35) Diagnostic Value of Alcat Test in Intolerance to Food Additives Compared with Double-Blind Placebo-Controlled (DBPC) Oral Challenges

Presented at the 52nd Annual Meeting of the American Academy of Allergy, Asthma & Immunology, New Orleans, LA. March 15-20, 1996. Publication in the Congress Proceedings of the Journal of Allergy and Clinical Immunology 1996;97:336.

Result: Accuracy of the Alcat Test in identifying true positive and true negative reactions to food additives was 96% as determined by double-blind, placebo-controlled oral challenges.

D.3 Analytical Validation; Pathomechanisms and Technical Studies

D.3.1 Analytical Reproducibility

(42) Reproducibility of the ALCAT Test

Summary: Dr. Paul Potter of the University of Cape Town, South Africa conducted the first Alcat Test reproducibility study in 1994.

The study found high reproducibility (95%) of both positive and negative test results.

Dr. Steinmann, a colleague of Dr. Potter, evaluated and further documented the high reproducibility in a subsequent statement. Dr. Steinmann also found a substantial number of positive responses, particularly in subjects suffering from hay fever (allergic rhinitis), asthma, or IBS. Two patients with GI symptoms experienced dramatic improvement from the elimination diets based on Alcat Test results.

(30) Reproducibility of the Antigen Leukocyte Cellular Antibody test (Alcat) - Statistical Analysis, Summary Statistics, & Scientific Report

Summary: A second Alcat reproducibility study was conducted at the University in Bloemfontein, South Africa by Dr. WML Neetling and Dr. AM Kachelhoffer; Jan-Apr 1998.

The study analyzed 10 consecutive patients. Of these, 2 patients had no prior allergies. The balance reported various symptoms such as migraine, asthma, and IBS. Using the Alcat Test, 1,300 analyses of 4,989 data points were performed, testing responses to 130 antigens.

The study demonstrated 92% reproducibility.

(29) Parexel Medstat Final Statistical Report – Study of the Alcat Test in 10 subjects (500 data points)

Citation Summary: Dr. Per Fuglerud, Parexel, Norway, carried out this study on behalf of AMTL Norge in November 1999 evaluating the reproducibility of Alcat Test results.

Ten (10) subjects were investigated for their reactions to 50 different substances with the Alcat Test. Subjects were divided equally into 2 groups, Cohort A and Cohort B. Each cohort was tested for responses to 50 different substances. Subjects were tested twice and the agreement between their test results was analyzed.

A 97% reproducibility of results was demonstrated in Cohort A and 99% reproducibility in Cohort B. Overall, 983 of 1,000 Alcat Test data points (98.3%) were reproducible under the conditions investigated by the study. The study demonstrated the statistically significant reproducibility of the Alcat Test results.

D.3.2 Mechanistic/Technical Studies

(4) Studies of mitochondrial and nuclear DNA released from food allergen-activated neutrophils. Implications for non-IgE food allergy

Citation: University Clinic of Leipzig, Georgetown University Medical Center; B. König, J. Bellanti et al; Allergy Asthma Proc; 2021 May 1;42(3):e59-e70. doi: 0.2500/aap.2021.42.210021; Ingenta OPEN ACCESS

Summary: Mechanism study DNA release from innate immune cells n=6. This study explored how neutrophils respond to food allergens in non-IgE-mediated food allergies. Researchers found that when neutrophils were exposed to foods that tested positive in leukocyte activation (LA) assays, they released both nuclear and mitochondrial DNA through a process called NETosis. In contrast, foods that tested negative in LA assays did not trigger this response. The release of mitochondrial DNA (mtDNA), a potent inflammatory signal, suggests a possible innate immune mechanism behind non-IgE food allergies. Elevated NET markers like calprotectin and S100A12 supported these findings. The results indicate that measuring mtDNA and NET formation could help identify and understand non-IgE food reactions, though larger studies are needed to confirm clinical relevance.

(23) Study Comparing Alcat Test Results With Flow Cytometry and Microscopy

Background: Gitte Jensen, Ph.D. (Immunology), conducted at NIS Labs (Natural Immune System) in Oregon, USA on behalf of Cell Science System, 2009.

Summary: This study compared the cellular analyses produced by the ROBOCat II device used for Alcat Testing with results produced by conventional laser flow cytometery

(FAS Calibur) and optical microscopy (Cytovita). The study demonstrated that Alcat analyses were comparable to those of laser based flow cytometry and comported with the visual data produced by optical microscopy and concluded that the ROBOCat II device is suitable for the analysis of cell populations and volume changes in WBCs.

Please view A.1.6 Measurement Technology

(26) Technical Study Comparing the Alcat methodology with Activation of Granulocytes Following Challenge with Zymosan

Citation: University of Rome; Mele C; 2002

Summary: This study, conducted by Dr. Cristina Mele of the University of Rome, compared the Alcat method with the activation of granulocytes after zymosan challenge.

Zymosan is derived from yeast cell walls (Saccharomyces cerevisiae) and is used for in vitro immunological evaluation of the phagocytic immune function. If cells are healthy, they respond to a challenge with zymosan by activating NADPH oxidase (through activation of cytochrome B and the cytosolic proteins P47, 67 and 21) to create an "oxidative burst" of superoxide anions. The superoxide anion is detectable and measurable using a luminometer.

Dr. Mele conducted analyzes with aliquots of blood exposed in the course of the Alcat Test to food extracts. She found an inverse correlation in samples that were already reactive in the Alcat method and emitted light. Dr. Mele concluded that these cells' capacity for immune system stimulated reaction was already "spent." This study suggests that the Alcat Test detects substances that activate phagocytes.

(27) Ogni Intervento Comincia a Tavola (Every intervention begins at the table)

Citation: University of Rome; Mele C; Medici Oggi, Maggio 2002: 210-213.

Summary: In this study, it was shown that an elimination diet based on Alcat Test results decreases markers of inflammation. After a period of 4 years, reactive foods were reintroduced. Many previously reactive foods were now tolerated while some remained reactive. Corresponding inflammatory markers (interleukins, TNF, interferon-γ) again increased significantly for these reactive foods.

Increased levels of IL6, TNF and the hepatic synthesis of C-reactive protein prevented weight loss despite consumption of a low-calorie diet because of these metabolic disturbances.

(28) The Controversial Antigen Leucocyte Cellular Antibody Test (ALCAT): a Non-Specific Inhibitory Effect of Alpha Glycoproteins

Citation: Kedryna T, Guminska M; Med Sci Monit 1999; 5(2): BR193-197.

Summary: In this study, the concordance between positive test results obtained from skin tests and Alcat Testing was 80%. The authors hypothesize that false positive Alcat Test results in patients with negative skin tests, as well as false negative Alcat Test results in patients with positive skin tests, occur because glycoproteins in the blood of hypersensitive patients may exert a blocking effect on Alcat results. Thus, some patients might not be even considered as hypersensitive, since glycoproteins tend to yield false negative results.

(40) Allergie alimentari. Tecniche diagnostiche a confronto [Food allergy: comparison of diagnostic techniques]

Citation: Mancini S, Fierimonte V, Iacovoni R, Spaini A, Viarani P, Pichi A; Minerva Pediatr. 1995 May; 47(5):159-63 [Italian]

Summary: Fourteen children affected with allergic disease, from 8 months to 7-years-old, were studied; three children had bronchial asthma, one child bronchial asthma and rhinitis, one child recurrent laryngo-spasm, six children atopic dermatitis, two children diarrhea, one abdominal pain. In all patients, cow's milk and egg were the most common offending foods. Total IgE serum level, IgE serum specific, Prick test and Alcat Test (the test predicts food which would produce a problem) were investigated. A comparison among the different laboratory methods for food allergy diagnosis was analyzed.

A history of their allergic reactions was documented noting a worsening or recurrence of symptoms after introducing cow's milk or ingestion of the basic dietary foods.

In all patients, skin prick tests were performed with food antigens and pollen inhalants. All were tested for total IgE and specific IgE to food and pollen inhalants; simultaneously the Alcat Test was performed on each patient for the following foods: egg, cow's milk, wheat, peanut, beef, orange, soy, and tomato.

There are significant differences between the atopic patients and those in which total IgE was normal, and, in the latter group that the positivity observed with the Alcat is more significant. These findings demonstrate that in children with food intolerances, there are highly reactive foods that exert pathogenic actions though a non-IgE mediated mechanism.

Conclusion: The results of the Alcat Test, designed to find non-IgE mediated reactions to foods, was evidenced in the determination of positive results to major food antigens in the 7 children with non-atopic disorders.

The results of our study have shown that in non-atopic subjects, the Alcat Test in all cases, revealed a positive reactivity to the basic foods in children with clinical manifestations related to a food allergy or intolerance.

In conclusion, the Alcat is also useful for preparing a dietary program which simplifies the job of the clinician

in establishing accurate elimination diets and exclusions, and finally the Alcat results (positive or negative) were correlated with the food challenge test in a considerable number of cases.

(50) Inhibitory Effect of Sodium Cromoglycate on Granulocyte Response to Food Antigens In Vitro

Citation: Fell PJ, Sandberg DH, et al; 47th Annual meeting of the American College of Allergy & Immunology, November 10-14, 1990, San Francisco CA. Published in the Annals of Allergy.

Summary: The aim of this study was to determine whether sodium cromoglycate (also known as sodium cromolyn or SC, used as a drug in the prevention of allergic diseases in the form of drops, inhalers) would inhibit or prevent the cellular response to food antigens as measured by the Alcat Test. Previous studies had shown a stabilizing effect of SC on mast cells (allergy).

Results: The inhibitory effect of SC on WBC degranulation was observed and detected by the Alcat Test. The Alcat Test has both clinical research applications.

(51) Multiple Pathogenic Mechanisms in Food Sensitivity Reactions In-Vitro

Citation: Puccio et al; Study presentation at the 4th International Symposium on Clinical and Immunological Problems of Food Allergy, Milan, Italy, November 1989. Published in the Proceedings.

Summary: The objective of this study was to investigate various pathogenic mechanisms that might operate when the whole blood of 9 food sensitive asthmatic patients was incubated with each of 10 food extracts.

	IgA_1	IgM_1	IgG_1	IgG_4	$C3_2$	$C4_2$	$Spect_3$
Beef	0	1	0	0	2	0	1
Corn	1	0	1	0	0	0	1
Egg	0	2	1	0	0	1	1
Milk	0	2	0	0	2	0	0
Orange	0	1	2	0	1	0	0
Peanut	1	3	0	3	2	0	2
Soybean	0	0	0	0	3	0	1
Tomato	2	1	0	0	3	0	0
Wheat	0	0	1	0	4	0	1
Yeast	0	2	0	0	1	0	0
TOTAL	4	12	5	3	19	1	7

Multiple pathogenic mechanisms in food sensitivity in-vitro: 1. Frequency of Immunoglobulin level change exceeded S.D.2for that food. / 2. Frequency of Complement Activation for that food as measured by the height difference of the rocket peaks / 3. Frequency of spectrophotometry reading of hemolysis exceeded S.D. 2 for that food.

Serum immunoglobulin levels (IgA, IgM, IgG, IgG4) using turbidometrology, were measured to establish baseline levels. Whole blood from the same patients was then incubated, with each of the respective food antigens at 36.6 °C for 60 min. and serum once again separated. A second analysis of Ig levels was made. Activation of C3 or C4 was determined by rocket immune-electrophoresis. Hemolysis was measured by spectrophotometry.

Results were analyzed by calculating percent change from control values in each of the 90 tests per pathogenic mechanism. Scores falling outside the range of 2 standard deviations were: IgA = 4, IgM = 12, IgG = 5, IgG4 = 3. Marked hemolysis occurred in 7 tests; complement activation was seen in 19 for C3 and in 1 for C4. Significant changes in one or more immunoglobulin and complement components occurred in every patient to one or more foods. In most subjects, multiple mechanisms were observed suggesting adverse reactions to foods commonly involve various triggers and pathways.

(52) Influence of Food Antigens on Volumes of Circulating White Blood Cells and Platelet Aggregation

Citation: Brostoff, Fell et al; Study presentation at the 4th International Symposium on Immunological and Clinical Problems of Food Allergy Milano, Italy, 5-9 November 1989, Published in the Proceedings

Summary: Mitogenic properties of peanut and phyto-hemaglutenin were the first evidence that cytoplasm and its surrounding membrane is associated with variable changes of involved cell volume. Such cellular transformation became the principal method for assessment of cellular immunity i.e. "delayed hypersensitivity." Immediate hypersensitivity reactions involving degranulation of mast cells and basophils appear to correlate with involved cell size changes. Formation of cluster of aggregated platelets may be another event mediated by antigenic stimulus. Application of new computerized models allows precise electronic instrumentation to measure volumetric cytodynamics of antigen activation through the Alcat Test System which is designed to objectively determine the direct interaction of food antigens with circulating WBC on the basis of volumetric changes. Alcat Test computer produced histograms depict separate peaks for lymphocytes, polymorphonuclear cells, and a specific area affected by platelet aggregation. Vertical and horizontal dislocation of histograms depict cellular degranulation, cell enlargement, or disintegration upon in-vitro challenge with offending antigen.

Fell and Brostoff reported over 70% correlation between Double Blind Oral Food Challenges and Alcat Test scores based on WBC changes alone (74 positive and 68 negative challenges).

Additional evaluation of Alcat Test histographs was conducted on 9 migraine and 7 urticaria patients. Each of those patients was challenged with 6 foods; 3 positive and 3 negative, according to Alcat Test WBC volume changes.

In an Alcat histogram, platelets are seen in the area under 65 fl. This parameter was examined with the following changes in the area under the curve observed:

- 19 out of 136 antigens tested triggered marked changes of the platelet aggregation region of the graph.
- 10 out of 19 of these reactions correlated with Alcat WBC changes and oral challenge.

See also under clinical validation:
(44) The Alcat Test – A Guide and Barometer in the Therapy of Environmental and Food Sensitivities

D.4 Review papers and Clinical Studies

D.4.1 Review papers

(5) Alcat Test (food intolerance test): Assessment of its Clinical Utility

Citation: Research Service. Río Hortega University Hospital; J. San Miguel Rodríguez et al; Journal of Biomedicine and Biosensors, 1(1) (2021): 57-76

Summary: The antigen leukocyte cellular antibody test (Alcat) is an assay to assess food intolerance in vitro and could be a diagnostic aid at the service of specialists involved in nutrition. With regard to the symptoms associated with food intolerance, it is chronic and groups the clinical manifestations, such as gastrointestinal disorders, abdominal pain and bloating, vomiting and diarrhea, dermatitis and eczema, migraine, fluid retention, chronic fatigue and alterations rheumatics. In the test, cell reactivity is determined by cytometric reading, which is related to the modification of cell number and size. The cell types that the test analyzes and that are the target of the adverse effects caused by intolerance are granulocytes, platelets and lymphocytes. So the test is a test that reproduces in the laboratory a situation equivalent to what actually occurs in the body when we come into contact with food.

(14) Immune Activation Through Diet-associated Stimuli in Chronic Disease

Citation: Pietschmann N; Alternative therapies in health and medicine. Jul-Aug 2015; 21(4):42-52.

Summary: The immune response is a very complex interplay of specific and non-specific branches that have evolved to distinguish between non-dangerous and dangerous or non-tolerated factors. In the past, research has focused on the specific immune system much more than the host's innate non-specific defense. Studies have shown that a key component of the immune response involves activation of the inflammasome. A direct relationship between the presence of the inflammasome and the onset of disease has already been characterized for a variety of chronic and food-related diseases, including arteriosclerosis, metabolic syndrome, and chronic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.

The leukocyte activation (ALCAT test), an immunological blood test for food intolerance reactions, is ideal as a clinical tool to identify and eliminate individual food stimuli that may act as triggers for the cellular non-specific immune response. Although the test is not diagnostic, studies have established that it can be a useful screening tool for the identification of foreign substances that may trigger immune cell activation, particularly of neutrophils, leading to inflammatory disorders.

The ALCAT test, coupled with a targeted diet that is individually tailored according to the test's results, may support immune homeostasis and provide a valuable complementary approach for therapy and overall health.

(24) The Right Stuff: Use of ALCAT Testing to Determine Dietary Factors Affecting Immune Balance, Health, and Longevity

Citation: Deutsch RD; Anti-Aging-Therapeutics Volume X; 2007, Chapter 8

Summary: The aging processes, described by Denham Harmon in the 1950s, are manifesting at earlier ages. Metabolic syndrome, characterized by obesity, diabetes, and cardiovascular disease, has sharply increased in recent years. Many observational studies support the thesis that the deleterious effects exerted by free radicals, upon lipid membranes, DNA, and protein structures, forms the common underlying basis of the many diverse degenerative aging disorders. The inability to tolerate foods and environmental factors induces chronic activation of the innate immune system and gives rise to inflammatory processes. These processes include excess production of reactive oxygen species and the release of preformed and newly synthesized mediators of inflammation.

A simple blood test (the Alcat Test) can be used to identify food and other factors that induce innate immune system activation. This review article describes the relationships between inflammatory processes, degenerative disorders, and dietary factors.

(19) Utilidad clinica del test ALCAT. Mito o realidad

Citation: San Miguel R, Martín B, Armentia A; Fuel and Energy Abstracts; 2010; 107(1):12-20.;

Summary: The ALCAT test is an assay that was developed to assess food intolerance in vitro and is thus used as a diagnostic aid in dietetics and nutrition. The symptoms associated with food intolerance are usually chronic and highly varied and encompass gastrointestinal disorders (such as abdominal pain and bloating), vomiting and diarrhea, dermatitis and eczema, migraine, fluid retention, chronic fatigue, and rheumatic alterations. The ALCAT test

determines cellular reactivity by cytometric reading, which is related to changes in cell number and size. The cell types analyzed by the test (which are the targets of the adverse effects caused by intolerance) are granulocytes, platelets and lymphocytes. Thus, the ALCAT test is an assay that reproduces in the laboratory a situation similar to that occurring in the human body when contact is made with food.

D.4.2 Clinical Studies

(1) The role of leukocyte activation in suspected Non-IgE excipient-related COVID-19 vaccine reactions: An exploratory hypothesis-driven study of pathogenesis

Citation: J. Bellanti, Yen-Chih Huang et al; Allergy Asthma Proc; 2024 Nov 1;45(6):438-446. doi: 10.2500/aap.2024.45.240040

Summary: This study examined whether lipid excipients in mRNA COVID-19 vaccines, such as PEG and ALC-0159, contribute to non-IgE-mediated adverse reactions through leukocyte activation (LA). Using an LA assay, researchers tested 30 participants, including individuals with severe or mild vaccine reactions and unvaccinated controls. Those with serious reactions showed significantly higher rates of leukocyte activation to vaccine excipients compared to controls. The findings suggest that LA responses to excipients may help identify individuals at risk for non-IgE vaccine hypersensitivity. n=30

(2) Prediabetes Reduction from Food Allergen Elimination—A Controlled Pilot Study

Citation: Galveston Clinical Research; F. Buck Willis et al; Diabetes 2024;73(Suppl.1):627-P; https://doi.org/10.2337/db24-627-P

Summary: This controlled clinic pilot study of n=24 participants investigated whether eliminating immune-reactive foods could improve metabolic markers in people with prediabetes. Twenty-four participants followed a standard lifestyle intervention, but half also eliminated foods flagged as reactive by the ALCAT Test. After three months, the food elimination group saw significant improvements: HbA1c dropped by 0.8%, while it increased by 0.7% in the control group. They also experienced reductions in BMI, fat mass, blood pressure, and waist circumference. All improvements were statistically significant.

(6) Food Allergen Elimination for Obesity Reduction; a Longitudinal, Case-Control Trial

Citation: Dr. Buck Willis, Galveston Clinical Research; I; Br J Gastroenterol Aug 2020, 2:4 2020; 2(4): 199 - 203. doi: 10.31488/bjg.1000122

Summary: Medical Weight Loss Study; n=94 (group 1-4); The purpose of this study was to examine separate and combined efficacy of food allergen testing/elimination and/or aerobic-surge exercise bouts with longitudinal outcomes. The changes were significant ($p < 0.0001$) and while the exercise was beneficial, this is the first study to show food allergens as a contributing factor in obesity with longitudinal outcomes (Figure 2). Future studies should examine both immediate evidence from food allergentesting/elimination (90 days) with longer durations (36 months) and more variables (body composition testing) to measure pure efficacy of this combined approach addressing obesity.

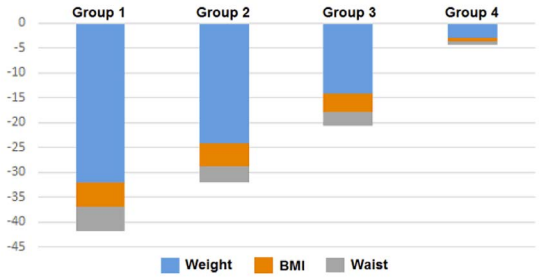

Figure: Stacked Changes after 12-months

Our study confirms that eliminating food allergens and performing aerobic-surge exercise was effective in changing the dependent variables for both male and female subjects. Both the polarity and proximity of the age and gender shift differently from each other's with respect to the standardized body weight (pounds), BMI (kg/m2), and waist circumference (inches).

(7) A Clinical Evidence of a Correlation Between Insulin Resistance and the ALCAT Food Intolerance Test.

Citation: University of Camerino (Italy), Pompei P, Grappasonni I, Scuri S, Petrelli F, Traini E, Sorrentino S, Di Fede G; Altern Ther Health Med. 2019 Mar;25(2):22-38.

Insulin resistance (IR) is defined as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization. Several mechanisms have been proposed as possible causes underlying the development of IR and the IR syndrome. IR occurs as part of a cluster of cardiovascular-metabolic abnormalities commonly referred to as "The Metabolic Syndrome."

This may lead to the development of type 2 diabetes, accelerated atherosclerosis, hypertension, dysmenorrhea, hirsutism, and polycystic ovarian syndrome, depending on the genetic background of the individual developing IR.

Study subjects and aim of study:

The aim of this study was to assess, in **123 female**

and 35 male (mean age, 42 y ± 10.3; range 19-75 y) volunteers) whether IR could be partly related to a dietary sugar intolerance and whether there could be a correlation between the ALCAT intolerance test and a mutation of the TCFTL2 gene (it promotes the transcription of the proglucagone and plays a key role in the development of the Langerhans islands).

Study results:
Results evidenced that subjects with an intolerance to sugar, also showed a statistically significant complete or incomplete alteration of the TCFTL2 genetic test.

Based upon these findings, our study demonstrated that there is a clinical correlation between the ALCAT food intolerance test and the IR. The positivity to the ALCAT test of one of the sugars tested (fructose, sugar cane, and sugar beet) indicates, in the majority of the subjects, the presence of a mutation of the gene TCF7L2 and could contribute to the prevention and treatment of the IR.

(8) Effects of Antigen Leukocyte Cellular Activation Test-Based Diet on Inflammation, Body Composition, and Medical Symptoms

Citation: Northern University Illinois (NIU); Lukaszuk JM, Shokrani M, Ghosh-Roy P, Hoppensteadt J, Umoren J; Alternative & Complementary Therapies VOL. 24, NO. 5; 11 Oct 2018

Summary: The Randomized, placebo-controlled double-blinded, prospective study was conducted for 4 weeks. Study Subjects: n=133 participants; Alcat Test/ Treatment-Group: n=87 participants, elimination diet according to their individual Alcat Test results; Control/ Placebo-Group: n=46 participants, elimination diet according to a sham diet.

A Disease Symptom Inventory (DSI) was completed to see who would qualify for the study; the entire DSI consists of 20 questions. Subjects had to have a "3" (a somewhat severe effect) on at least two of the DSI questions. Researchers set out to recruit individuals with signs of inflammation present, not individuals who were healthy and symptom-free. For example, the initial DSI revealed individuals with gastroesophageal reflux disease (GERD), eczema, anxiety, and other signs that they may be having food intolerance issues. 133 study subjects followed a four-week elimination diet based on the Alcat results (treatment group) or Alcat sham results (placebo group). Blood samples, body composition, and Medical Symptoms Questionnaire (MSQ) were completed day 1 and day 30.

Results:

- **Significant Decrease in Serum Amyloid A Levels (SAA) from day 1 to day 30**

After 30 days on the elimination protocol, the treatment group experienced a statistically significant decrease in serum amyloid A levels (SAA) from day 1 to day 30. Conversely inflammation and SAA scores increased for the placebo group. The results were statistically significant at the 0.001 level.

These remarkable results indicate that there is a significant decrease in inflammation when an individual avoids foods to which they are intolerant (according to the Alcat Test). The study utilized the SAA level as it can be considered a more sensitive marker for systemic inflammation than C-reactive protein (CRP).

- **Weight Loss**

Weight and BMI also decreased during the study. When restricting any type or number of foods, an individual will be more aware of what they are eating, caloric intake will be reduced, and BMI will be expected to decrease. Although both groups in the current study lost weight, the treatment group had a much more significant BMI decrease then did the placebo group.

Significant Improvement of Symptoms according to the MSQ /Medical Symptom Questioner

After the initial DSI was completed, an MSQ was administered looking at head, ears, eyes, nose, throat, overall well-being, energy level, joint pain, and basically how one feels overall. Individuals in the treatment group felt significantly better from day 1 to day 30. The placebo group had the benefit of feeling better but not nearly to the extent that the treatment group reported.

Conclusion: The treatment group had a more significant decline in body fat than the placebo group though both groups lost weight.

Elimination of inflammatory foods according to the Alcat Test show a positive impact on SAA, BMI, and MSQ scores.

Avoiding reactive foods and adhering to Alcat Test results may positively influence inflammatory responses, body composition, and well-being.

All study subjects indicated (in a post-study interview) that they would continue to eliminate reactive foods (as designated on the Alcat Test) because they felt so much better when avoiding those foods.

(16) Evaluation of Alcat Test Results in the Non-IgE Mediated Pathology of the Skin

Citation: University of Pavia; De Amici M, Berardi L, Castello M, Mantegna G, Giunta V, Ronzi G, Vignini M; Study presentation at the 30th Congress of the European Academy of Allergy and Clinical Immunology, Istanbul, Turkey, June 11-15, 2011. (Poster Presentation, Abstract # 553).

Summary: The 35 patients in this study had abnormal skin reactions that were identified by the Alcat Test and resolved with elimination diets.

Results: Two-thirds (66%) showed significant symptom improvement, 31% had some improvement, and only 3% reported no change.

(17) Alcat Test Results in the Treatment of Gastrointestinal Symptoms

Citation: University of Pavia (IT); Berardi L, De Amici M, Castello M, Torre C, Giunta V, Legoratto, Vignini M; Presented at the 30th Congress of the European Academy of Allergy and Clinical Immunology, Istanbul, Turkey, June 11-15, 2011. (Abstract # 552, published in the proceedings).

Summary: The 48 patients in this study suffered from gastrointestinal symptoms. The Alcat Test was provided to the patients and appropriate diets were prescribed.

Results: The majority of patients (71%) experienced significant improvement of their symptoms, 27% had a slight improvement, and only 2% reported no change.

(18) Rational Management of Food Intolerance in Elite Athletes

Citation: Angelini F, Marzatico F, Stesina G, Stefanini L, Bonuccelli A, Buonocore D, Rucci S, Tencone F; Journal of the International Society of Sports Nutrition 2011, 8 (Suppl. 1):36

Summary: A team of investigators led by Fabrizio Angelini studied eight elite European athletes selected for symptoms suspected of being related to food intolerance (e.g. headache, gastrointestinal discomfort, constipation, diarrhea, intestinal bloating, and nausea).

The Alcat Test was performed and test based-elimination diets were prescribed. The athletes were followed for eight months and retested.

Results: There was significant improvement in the athletes' condition and reduction in inflammatory response demonstrated by improvement in body composition, symptoms, and exercise capacity.

(20) Alcat Test Identifies Food Intolerance in Patients with Gastrointestinal Symptoms

Citation: Berardi L, De Amici M, Castello M, Torre C, Giunta V, Legoratto, Vignini M. Allergy: European Journal of Allergy and Clinical

Immunology.2009;64:490; Study presented at the XXVIII Congress of the European Academy of Allergy & Clinical Immunology, Warsaw, PL, June 2009. Published in the European Journal of Allergy and Clinical Immunology, Supplement 90, Volume 64, 2009, pg. 490.

Summary: A 15 patient trial showed that the Alcat Test and recommended elimination diets proved to be effective. In more than half of the patients, symptoms improved significantly.

(21) Food Intolerance in Patients with Cutaneous Diseases: Diagnostic Value of the Alcat Test

Citation: Berardi L, De Amici M; Vignini A, Torre C, Mosca M; Study presented at the XXVIII Congress of the Europe- an Academy of Allergy & Clinical Immunology, 6-10, Warsaw, Poland June 2009. Published in the European Journal of Allergy and Clinical Immunology, Supplement 90, Volume 64, 2009, pg. 490.

Summary: The aim of this study was to evaluate whether food intolerance is associated with cutaneous diseases such as chronic urticaria, angioedema, itching, and dermatitis, and to evaluate the diagnostic value of the Alcat Test in such cases.

A group of twenty (20) patients with cutaneous diseases, presenting with chronic urticaria (1%), itching (2%) or dermatitis (17%), with negative allergic tests (prick and/or RAST) were tested. No subject was under treatment with systemic corticosteroids, antihistamines or anticoagulants. Six patients were lost to follow-up. The remaining patients were Alcat tested and answered a survey about their 2-month elimination diets based on Alcat results.

Conclusion: The majority (86%) exhibited a dramatic improvement in symptoms, while 14% showed no change or did not follow the diet. The Alcat Test appears to have diagnostic value in detecting food intolerance.

(22) The Effect of the Alcat Test Diet Therapy for Food Sensitivity in Patients with Obesity

Citation: Akmal M, Khan SA, Khan AQ; Middle East Journal of Family Medicine. April 2009 - Vol. 7, Issue 3.

Summary: 27 obese patients who experienced no weight loss using caloric reduction diets were tested for intolerance to 100 foods. Significant positive changes in body weight, body fat, and body composition were observed, as well as improvements in related health problems. These results confirmed the value of the Alcat Test and elimination diet in helping address obesity, gastrointestinal reflux, chronic fatigue, headache, and other chronic disorders.

(25) IMS Health Economics and Outcomes Research –Influence of Food Intolerance in Migraines: Final Report of Statistical Results

Citation: Immunological Center of Cataluna, Version 3, December 28, 2006.

Summary: 21 migraine patients who had positive results in the Alcat Test for at least one food were studied.

The number of migraine attacks that occurred while following a 3-month Alcat diet phase that eliminated migraine-provoking foods (phase I) was compared with the number of migraines occurring in patients during the same 3-month period with a control group who did not follow a special diet (Phase II). Nearly half of patients (47.6%) following a diet based on their Alcat Test results

experienced a reduction in the number and intensity of migraine attacks.

Conclusion: A diet that eliminates or otherwise avoids Alcat positive foods reduces the frequency of migraine attacks and their pain intensity and duration.

(31) Outcome Study in 305 Consecutive Patients Following the Alcat Diet

Citation: Hoj L; Observational study conducted in Copenhagen at the Allergy Clinic Charlottenlund, Denmark 1998 unpublished

Summary: The results of 305 consecutive patients who were following diets based on their Alcat Test responses are presented in the table on the left.

Benefits of the Alcat diet were assessed as follows:

- **237 Patients: Significant improvement or nearly symptom free**
- 40 Patients: Some improvement
- 19 Patients: Mild improvement
- 5 Patients: Worsened symptoms
- 4 Patients: Alcat Test diet was not followed

Symptom	Significant Improvement	Moderate Improvement	Mild Improvement	Condition Worsened	Diet not Followed	Percent Improved
Rhinitis/Sinusitis Number of Patients: 37	30	5	1		1	**95**
Atopic eczema Number of Patients: 72	63	5	2	2		**94**
Urticaria Number of Patients: 24	19	1	2	2		**83**
Irritable bowel syndrome Number of Patients: 46	32	8	5	1		**87**
Migraines, headache Number of Patients: 21	20		1			**95**
Polyarthritis Number of Patients: 20	14	3	2		1	**85**
Asthma Number of Patients: 27	19	7	1			**96**
Angioedema Number of Patients: 21	19	2				**100**
Multi-organ-syndrome Number of Patients: 37	21	9	5		2	**75**

(31) Data from this study were not published but the results were made available by Dr. Lene Hoj for training and presentation

(33) South African Outcome Study – Randomized Study on 274 patients

Citation: Dr. Geldenhuys, a practicing physician in 1997 in Johannesburg, described data collected from his patients treated with diets guided by their Alcat Test results.

Summary: This randomized study followed 274 patients with different symptoms who adhered to a diet plan based on their individual Alcat Test results.

The percentage of patients that experienced improvement or complete recovery from their health complaints was as follows:

- 78% Migraine
- 77% Arthritis
- 67% Eczema
- 71% Intestinal cramps
- 71% Chronic fatigue
- 73% Diarrhea/constipation
- 62% Chronic sinusitis

(34) The Short Term Efficacy of the Alcat Test of Food Sensitivities to Facilitate Changes in Body Composition and Self-Reported Disease Symptoms: A Randomized Controlled Study

Citation: Kaats GR, Pullin D, Parker LK; Bariatrician. 1996; 18-23

Summary: This study of 100 obese patients investigated two different dietary changes. At screening, body constitution using underwater displacement (fat-muscle ratio) was recorded for all patients and an Alcat Test was performed to inform patients about their specific food intolerances. Thereafter, patients were randomly divided into two groups. The control group chose their own calorie restricted diets for 4 weeks while the Alcat Test

group followed rotation/elimination diets based on their specific test results. These patients also received dietary counseling. The Alcat group lost significantly ($p < .001$) more weight and body fat than did the control group. The Alcat group also realized body composition improvements characterized by greater increases in lean body mass ($p < .001$) than were observed in the control group.

Results: 98% of the subjects following the Alcat Test eating plan experienced short term improvement in scale weight and/or body composition.

Furthermore, also in contrast to the control group, patients in the Alcat group saw numerous improvements of the individual health complaints they reported at screening in all reported categories.

(36) El test Alcat de Sensibilidad a los Alimentos y su Interés en Medicina Estética

Citation: Amigo C, Moreno Mercer J, Calderon Gomez J, Cabo Soler JR; 14th Med Day of Esthetical Medicine & Dermatological Survey. Venice, Italy, Sep. 22 – 23, 1995; published in the proceedings Estetica Medica Numero 40 - March 1996 (Spanish)

Summary: This study considered 30 patients who previously had difficulty losing weight even though they followed calorie restrictive diets. Weight loss and body composition were compared at 4 weeks following a conventional calorie restrictive diet compared with Alcat Test results.

Results: Nearly all patients lost more weight following the Alcat-based calorie restrictive diet. Furthermore, most patients lost fat and gained muscle mass on the Alcat prescribed diet.

In addition, the patients following Alcat informed diets reported feeling better and having improved energy. Digestive disorders (e.g., bloating and indigestion) and skin problems also improved.

(37) Alcat Test Results in the Treatment of Respiratory and Gastrointestinal Symptoms, Arthritis, Skin and Central Nervous System

Citation: Mylek D; Rocz Akad Med Bialymst. 1995; 40(3): 625-629.

Summary: This study followed 72 patients with complaints thought to be attributable to food intolerance. They were prescribed elimination diets based on their Alcat Test results. The results of the study showed an overall improvement in symptoms (number of patients expressed as a percentage) for

- Arthritis (83%),
- Urticaria, bronchitis, and gastroenteritis (75%),
- Migraine (70%),
- Chronic fatigue syndrome (60%),
- Asthma (50%),
- Atopic dermatitis (49%),
- Rhinitis (47%)
- Hyperactivity (32%).

The investigators concluded that an elimination diet based on this non-invasive method can significantly improve the symptoms of various ailments. In their experience, symptoms resolve in in 50-83% of patients following an Alcat-based diet.

(38) Prevalence of Food Allergy and Intolerance in Children Based on MAST CLA and Alcat Test

Citation: Buczylko K, Obarzanowski T, Rosiak K, Staśkiewicz G, Fiszer A, Chmielewski S, Kowalczyk J; Advances in Medical Sciences 1995;40(3):452-456

Summary: This single-blind study of 56 children aged 5 months to 16 years (average age 7.2 years) with various allergies sought to compare the most common food allergies (using MAST CLA and skin prick test) and the most common food intolerances (using the Alcat Test).

The most common food allergies significantly differed from those described in the literature, i.e., protein and dairy. The most frequently observed food allergies were nuts, peanuts, wheat, and whole grains. Food intolerances in response to apples, tea, and cola were documented.

(39) Food Intolerance in Patients with Angioedema and Chronic Urticaria. An investigation by RAST and Alcat Test

Citation: Hoj L; Presented at the XVI European Congress of Allergy and Clinical Immunology, Madrid, Spain, June 25-30, 1995 and published in the European Journal of Allergy and Clinical Immunology, Supplement, no. 26, Vol. 50, 1995.

Summary: This study aim was to determine whether there was a relationship between angioedema and chronic urticaria and food allergy or intolerance. Fifty two (52) patients were given an Alcat Test for 100 foods, and 18 typical allergens were tested by RAST. In addition, total IgE and eosinophil counts were measured.

Results: All patients were reactive in Alcat Tests against 8-29 foods. Elimination diets based on Alcat Test results produced complete remission in 45 patients and remission of angioedema but not chronic urticaria in 5 patients.

(41) Autism – a Multidisciplinary Approach to Treatment

Citation: Kotsanis CA, Dart L, Harjes C, Miller R; 1994.

Summary: This study was conducted under the direction of Dr. Constantine A. Kotsanis.

Study results were presented at the annual meeting of the American Academy of Allergy Otolaryngology in 1994 and are available on the website of the Institute Kotsianis at http://www.kotsanisinstitute.com/home/autism-treatment

This study of 12 autistic children was the first to examine a combined treatment approach including food allergy/intolerance testing and nutritional intervention with audio therapy. Allergies and intolerances were determined by RAST and Alcat analysis, which informed a complete nutritional profile and metabolism audiograms. Leaky gut syndrome was treated, an AET (Auditory Enhancement Training) performed, and a rotation/elimination diet was prescribed based on Alcat Test results.

The combination of these treatments alleviated many typical symptoms (e.g., hyperactivity, hypersensitivity to the touch, impulsivity) and an improved behavior as evidenced in language, logic, communication, eye contact, memory, and the ability to follow instructions. Interventions based upon inputs from the varying modalities resulted in patient improvement.

(44) The Alcat Test - A Guide and Barometer in the Therapy of Environmental and Food Sensitivities

Citation: Dr. BA Solomon, Environmental Medicine, Vol. 9, Number 2, 1992:2-6.

Summary: The Alcat Test was used in a busy primary care internal medicine practice to identify foods and molds as possible triggers of symptoms in 172 patients.

These methods are cumbersome, and in the case of the cytotoxic test subjective, since it is based on an evaluation performed by a medical technician. The Alcat Test proved to be a fast, accurate method to diagnose and manage diet-related disorders. When the Alcat Test was used to create elimination diets patients experienced significant symptom relief:

A food elimination diet based on Alcat Test results was helpful in one case each of:

- Crohn's disease (80% improvement)
- Alzheimer's disease (80% improvement)
- Attention deficit disorder (60% improvement) in two cases of children
- Hyperactivity (80% improvement)

Dr. Salomon's conclusions were that Alcat Testing has advantages over other forms of allergy testing including:

1. The Alcat is specific to each patient:

A food that is not on the standard IBS or migraine diet, but which triggers a reaction in the patient, may be identified by Alcat.

2. Alcat is quick:

Alcat can test 100 foods in 4 hours. Four-day fasting and oral challenge can take as long as 25 days (at 3 to 4 foods a day).

3. Alcat is simple:

The system measures and counts approximately 4,000-8,000 cells in 6 seconds. Even a trained cytotoxic technician cannot visually identify the subtle cell size changes which are accurately identified by the Alcat System.

4. Alcat panels are predetermined.

5. The observed percentage of correlation for Alcat is:

- 79.3% correlation with positive reacting foods
- 87–92% correlation with positive foods when platelet reacting foods are included
- 87% correlation with non-reacting foods

Clinical condition	Average improvement (%)	Number of cases
Classic migraine	82	9
Common migraine	62	39
Sinus headaches	58	23
Irritable bowel syndrome	71	68
Gastroesophageal reflux	75	8
Inflammatory arthritis	65	31
Degenerative arthritis	44	16
Asthma	30	18
Recurrent sinusitis	59	20
Tension fatigue syndrome	60	97
Depression/anxiety	31	14
Obesity	50	33
Eczema	55	11
Recurrent vaginitis	20	7
Recurrent urinary infection	46	4
Allergic rhinitis	42	108

(46) Pilot Study into the Effect of Naturally Occurring Pharmacoactive Agents on the Alcat Test

Citation: Fell PJ; American Academy of Otolaryngic Allergy Annual Meeting, September 27, 1991, Kansas City, MO. Published in the proceedings.

Summary: The aim of this study was to determine whether the Alcat Test could also detect reactions in whole blood to naturally occurring pharmacoactive substances. If so, this would provide important information about the mechanism of action of the test because these substances do not set in motion allergic or immunological changes. The study considered healthy subjects and migraine patients.

Results: The results showed significant differences between healthy subjects and migraine patients.

Both groups reacted to histamine, however, the migraine patients were significantly more reactive to gluten, tryptamine, octopamine, dopamine, lectin and chlorogenic acid.

By elucidating white cell changes to substances likely to trigger migraine, the Alcat Test has the potential to assess toxic or pharmacological effects of many commonly occurring substances.

(48) Gastrointestinal Complaints Related to Diet

Citation: Sandberg DH, International Pediatrics, Vol. 5 No. 1, 1990:23-9.

Summary: Three case studies of patients with gastrointestinal complaints are presented, illustrating the use of the Alcat Test set, together with analysis (skin end point titration/skin-prick test) to analyze foods intolerance. The author concludes that the use of a combination of the Alcat Test and skin test (skin-prick test) prove to be an efficient diagnostic approach: "The progress in the understanding of interactions between food and GI disorders would go much faster if gastroenterologists separate them from allergies and would devote their talents to the study of this fascinating subject."

The author identifies GI disorders in which food allergy is accepted as having an etiological role:

- Chronic or recurrent diarrhea in infancy
- Recurrent vomiting in infancy
- GI bleeding
- Milk-induced colitis
- Small intestinal enteropathy

He also listed GI disorders in which food allergy or sensitivity may play a role:

- Infantile colic
- Post enteritis syndrome
- Transient gluten intolerance
- Irritable bowel syndrome
- Crohn's disease
- Chronic ulcerative colitis
- Recurrent abdominal pain
- Chronic constipation
- Intussusception
- Migraine-related GI symptoms
- Eosinophilic gastroenteritis

The author explains that before using the Alcat, one documented case (Case #3) obtained the following arrays of costly tests:

1) Complete blood count
2) Erythrocyte sedimentation rate
3) Liver function tests
4) Chemistry profile
5) Serum protein electrophoresis
6) Serum immunoglobulins
7) Alpha-1-antitrypsin
8) Blood folic acid
9) Vitamin B-12 concentrations
10) Vitamins D and E
11) Sweat chloride test
12) Lipoprotein electrophoresis
13) Examination of stools for pathogenic bacteria
14) Occult blood
15) Reducing substances and ova and parasites
16) Routine Urinalysis
17) Xylose Absorption test
18) Abdominal sonography (ultrasound exam)
19) Computerized tomography (CT Scan); abdomen and pelvis
20) Upper GI barium contrast studies with small bowel follow-through
21) Upper GI panendoscopy
22) Lactose breath test

Conclusion: The Alcat Test was performed, accurately diagnosed the food sensitivity, and a diet eliminating the offending foods helped resolve the patient's symptoms. The author's conclusion was that the Alcat test saves costs.

(54) A Comparison of the Alcat Test for Food Reactions among 2 Population Sub-Groups

Citation: Study by Dr. DH Sandberg and Dr. MJ Pasula, 45th Annual Congress of the American College of Allergy and Immunology, Los Angeles CA, November 12-16, 1988, published in the Annals of Allergy.

Summary: The aim of this study was to determine whether the Alcat Test reliably distinguishes between the reactions of healthy volunteers with no signs of food intolerance and those with suspected food sensitivities. The 50 participants consisted of 25 healthy young athletes on a nutritionally balanced diet and an age-matched control group with suspected food intolerances. A panel composed of each of 9 different diet components was investigated using the Alcat Test. The healthy group showed a total of 5 positive responses while the group with suspected food intolerances showed a total of 47 positive results. The results of this study suggest that the Alcat Test can reliably discriminate between healthy subjects and food-sensitive patients.

Appendix 1 Comments on Criticism

OPEN FORUM | Medical Tribune · volume 49 · No. 18 · 6 May 2016

Letter to the editor regarding the validity of the blood test for food sensitivity:

Appendix 1.1 Alcat Test: And again, the defamation of a useful method

Article criticism: "Blood tests for food intolerance are misleading" in the Medical Tribune 12-13 from 1 April 2016 Page 21.

To MEDICAL TRIBUNE

I am writing this reader letter because the information on the Alcat Test should be updated and corrected as the presentation of this test procedure is significantly distorted. In my opinion and according to precise research, those outdated and misinformed statements are based on original opinions papers (not studies!) of some allergists dating back to the 1990s or even much further back and yet still they persist today due to the famous "copy-and-paste." The basic research on leukocytes between 1947 and 1989 is questioned per se.

However, scientific work is more likely to question oneself. Among others, I was engaged in basic IgE-mediated research as well as clinically-oriented research. Thus, I had to deal intensively with the specific adaptive (acquired) branch of the immune system.

My scientific roots, however, lie in the leukocytes, so mainly the innate branch of the immune system. In the meantime, no scientist is questioning that both branches of the immune system are interconnected through biochemical networks. I therefore come to other conclusions in my assessment of the validity of the Alcat Test:

- The Alcat Test is indeed a leukocyte activation test, whereby reactions of leukocytes (the immune system's first line of defense) against individual foods or chemical substances are analyzed.
- The Alcat Test is not a "cytotoxic food test." The innate immune system mediates toxic reactions. However, the pro-inflammatory granulocyte reactions are broader and can also activate immunological or metabolic immune pathways. Granulocytes account for the majority of white blood cells in the body.
- On the subject of "reproducibility," no reference for the source was attached to the article (Vincent Aubert et al.), only a negative opinion. I therefore assume that the statement on reproducibility comes from opinion papers of allergists, in which the author referred to a source from 1993. However, this reference cannot be found. Nevertheless, the negative statement was passed unchecked since 1996 using "copy-and paste" again and again. I have seen the documents of the original scientists of the 1993 source who, in 1994-1995, expressed a very positive response regarding the reproducibility and reliability of the Alcat Test.

Research material on the Alcat Test demonstrates a high degree of reproducibility (https://cellsciencesystems.com/education/research); agreement in the Alcat results of patient blood tests that have been tested several times is higher than 90%. The reproducibility studies also demonstrate a trend towards stronger positive Alcat results in patients with defined symptoms (irritable bowel syndrome, allergic rhinitis).

Yale School of Medicine conducted a study on the clinical value of the Alcat Test. Alcat Test results have been compared with well-known and well-studied immune markers, e.g. the upregulation of surface markers on immune cells, and the test correlated well with established biomarkers. Changes of innate immune cells in the peripheral blood were detected, which correlated with the release of DNA and myeloperoxidase (MPO). The released mediators are known to initiate pro-inflammatory reactions and can be responsible for the development of inflammatory responses in patients.

The provider responsible for the European region has a scientific dossier, which can be requested (info@alcat-europe.com). It explains the test procedure as a non-allergic approach and includes extensive study data abstracts. With my contribution, I would like to promote more openness and professional discussion, as well as more thorough research in order to avoid the defamation of a useful procedure.

Prof. Dr. Brigitte König
Institute for Clinical Microbiology &
Infectious Epidemiology
University Clinic Leipzig

Appendix 1.2 Good Results for Patients with unclear pro-inflammatory symptoms after following the diet change according to the Alcat Test

On the same post:

To MEDICAL TRIBUNE

I would like to share my experiences with the Alcat Test, which I believe was not properly evaluated in the article cited above. I have been a specialist in general medicine since 1998, and my flagship has become, over time, cause-oriented diagnosis. The Alcat Test has been an invaluable diagnostic tool in my practice for at least three years, especially for patients with inflammatory symptoms who have been "diagnosed with unfounded cause or incurable" and were dismissed as having psychological issues versus physiological complications. The results of the dietary change according to the Alcat results are particularly evident in those with gastrointestinal complaints, persistent or periodically recurring eczema, weight problems, blood pressure disorders, migraine, asthmatic complaints, arthritis, idiopathic pain, autoimmune diseases, etc. The elimination of avoidable stressors leads to a reduction in oxidative stress and increased recovery of antioxidant capacity.

The Alcat Test is a leukocyte activation test in which the cells of the patient's blood sample are challenged in vitro with individual dietary foods, additives, phytoceuticals, etc. The cell changes are detected by flow cytometry using the impedance method, that is, the leukocytes pass through an electric field. Cell changes indicate potential pro-inflammatory responses that are classified into four categories (severe, moderate, mild, non-reactive). The evaluation is automated. Based on this, an individual elimination / rotation diet is recommended, as is customary in dietary allergology.

The food analysis is the basis for the basic daily diet and includes recommended foods as well as the reactive tested substances. Chemical additives (environmental chemicals, colorings, preservatives, pharmaceutical substances), phytoceuticals, biogenic amines and molds are also classified according to the traffic color principle. The Alcat Test is provided with detailed, personal guidance as well support from nutritional scientists to help implement dietary changes. No nutritional deficiencies were observed or attributed to the diet change, on the contrary, the diminished oxidative stress led to the recovery of the auxiliary antioxidants.

Current research includes numerous studies on diet-based diseases, their correlation with "silent inflammation," and how these are induced and maintained by the innate immune system.

The Alcat Test measures mainly immediate granulocyte reactions since the leukocytes are only confronted with the antigen for a short time. Characteristically, the defense cells (especially neutrophils or eosinophilic granulocytes) swell, burst, and release their inflammation-promoting "weapons" in order to effectively neutralize a substance interpreted as "dangerous." The cascade-like effects affect the entire body and its immune balance - everyone knows the principle of the flu.

> The Alcat method was positively validated by independent international researchers using double-blinded and reproducibility studies. An overview of study abstracts or a scientific dossier can be requested from the supplier, so that you can form an opinion. "Judge and decide only when you have examined all facts" has become my guiding principle. This test provides an answer for patients who report various complaints associated with consuming a particular food or ingredient (e.g. spices or additive), yet all antibody and histamine tests fail to show results.

Dr. med. Dirk Wiechert
Lesumstotelerstrasse 65
27721 Ritterhude, Germany

Appendix 2 Expert Answers IgG / important differentiations from Alcat

We are often asked about immunoglobulin G tests and its validity with regard to food sensitivity. Although we do not consider it our task to explain different "IgG test" offers, we have the impression that important questions remain unanswered. However, correct scientific understanding about the role and function of IgG seems to be essential for many practitioners to understand the demarcation from the Alcat Test. It is important to us to communicate scientific knowledge truthfully and well-founded.

"Food Sensitivity and the Significance of IgG tests"

Q&A about IgG

We would like to thank Prof. J. Bellanti* from Georgetown University for the approval of this document.

Like any antibody, immunoglobulin G (IgG) reflects an immune parameter of the **adaptive** immune system while the Alcat Test analyzes direct immediate pro-inflammatory responses of immune cells of the **innate** immune system.

Unlike research on IgE (mediator of classical allergy, type I), there are currently no conclusive studies that show that the antigen-specific IgG1-4 antibodies are also an indicator of an adverse reaction to food.

IgG1-4 antibody subclasses – what is it?

In the 1960s it was discovered that two of the five classes of antibodies (IgM, IgG, IgA, IgD, IgE) can be divided into subclasses. The subclasses of human IgG include IgG1, IgG2, IgG3 and IgG4 and those of IgA consist of IgA1 and IgA2. The sequential enumeration from IgG1 to IgG4 corresponds to the order of their discovery and directly correlates with their serum concentrations, with IgG1 containing the largest amounts.

When are IgG antibodies formed and what functions do they have?

IgG1 contains > 50% of total IgG and IgG4 generally <5%. IgG antibodies of subclasses IgG1-3 are primarily induced by microbial antigens (streptococci, pneumococci). IgG1 and IgG3 antibodies are directed primarily to protein antigens, e.g., tetanus and diphtheria toxoids, while IgG2 and IgG4 to polysaccharide antigens, e.g., pneumococcal polysaccharide. As a group, IgG1-3 antibodies are generally of low affinity and specificity, and therefore show high cross-reactivity.

Although the precise role of IgG4 is not known with certainty, it seems to function as an IgE counterpart. One function of IgG4 is thought to provide a protective role in allergies of various causes (e.g. parasitic, food) through its ability to block specific IgE. Since it is known that elevated IgG subclass concentrations are detectable in pathological conditions undergoing chronic antigen stimulation, a polyclonal increase of IgG4 is frequently observed in allergies.

So, IgG4 antibodies can be formed to foods... but what does it mean when IgG4 is present in the blood?

In the context of hypersensitivity to food, a link between the IgE and the IgG4 is not surprising. Both IgE and IgG4 are dependent on a TH2 cytokine profile. Thus, all antigens that induce an IgE response are, in principle, also efficient IgG4 inducers.

In contrast to IgE, food/allergen-specific IgG4 **is not an indicator of a classic food allergy or a food sensitivity, but a natural physiological response of the immune system to food/allergen exposure.**

Therefore, increased IgG4 levels are the expression of a chronic exposure to allergens and a marker of tolerance induction. Thus, a study by Tomicić et al. (one study among many others), shows that **elevated IgG4 levels in childhood are later associated with a tolerance towards food, not with an intolerance** (1-4).

This means that elevated IgG4 levels do not indicate a pro-inflammatory response? – What about the IgG subclasses 1-3?

IgG antibodies of subclasses IgG1-3 are primarily induced by microbial antigens. **It is to be noted that these antibodies are of low affinity and specificity, and therefore show high cross-reactivity, e.g. to food components.** Studies demonstrate that IgG anti-gliadin antibody level is not a valid measurement parameter for sensitivity to gluten in order to accurately diagnose a gluten sensitivity (NCGS) (5). Rather, growing evidence indicates that gluten sensitivity is instead mediated by mechanisms of tissue injury directed by the innate immune system (6).

"Intolerance" or "Allergy III" implies an inflammatory immune reaction.
Are IgG antibodies pathogenic?

Detection of the allergen-specific IgG4 during a specific immunotherapy can be a useful marker for adequate desensitization. In context with food, since IgG4 antibodies are formed only after a very long exposure, the determination of the ratio of IgG4 to total IgG would be preferred to IgG4 alone. However, IgG1-3 subclasses are appear to be indicators of more current exposure to food components. This is demonstrated by a study by Tiikkainen et al. with 62 symptom-free bakers (who showed elevated IgG levels to wheat but no symptoms) (7).

The use of total IgG titers to food in developing a preventive rotational diet is understandable, since the exposure-related IgG levels are usually related to the **main allergens** such as gluten, soy, egg, yeast, or dairy products.

However, the high cross-reactivity of the antibodies and the undifferentiated observation of the different IgG subclasses strongly restrict the diagnostic value of IgG.

Summary IgG Tests

Increased IgG levels are therefore not the same as an inflammatory immune reaction.
- But individual subclasses such as IgG1 or 3 can indirectly induce phagocytosis.

- IgG4 antibodies are considered part of a physiological response of the immune system following exposure to food components and therefore indicate tolerance, not intolerance.
- IgG4 is also referred to as an "anti-IgE antibody" because it blocks IgE antibodies in order to avoid a potentially dangerous classic type I allergic reaction.
- IgG subclasses 1-3 reflect the exposure to antigens primarily of microbial origin. The antibodies are of low affinity and specificity, and high cross-reactivity.
- The IgG subclasses 1-3 can play a role in facilitating phagocytosis. However, this is only one possible immune pathway. Nevertheless, it is the phagocytes (such as neutrophils or eosinophils), which initiate inflammation and release pro-inflammatory mediators – NOT the antibodies.
- **Although antibodies are mainly directed to protein antigens, foods also contain non-proteinaceous molecules (e.g., fat, carbohydrates, biogenic amines, chemical substances) that can contribute to adverse food reactions which may be problematic and which may not be detected by IgG measurement.**
- IgG subclasses 1-3 reflect the exposure to antigens primarily of microbial origin. Therefore, the high cross-reactivity of the antibodies and the undifferentiated observation of the different IgG subclasses strongly restricts the diagnostic value of IgG.

Conclusion:

There is no scientific evidence that antigen-specific IgG antibodies are pathogenic, i.e., are indicators of food intolerance. The results do not have discrimination properties, so that errors and/or misinterpretations are expected (due to the undifferentiated observation of different IgG subclasses).

***Prof. Joseph A. Bellanti**
Professor of Pediatrics & Microbiology-Immunology, Georgetown University Medical Center; Director of the International Center for Interdisciplinary Studies of Immunology
→ https://www.immunologycenter.org/about.html

Appendix 3 Practitioner First Hand Experiences

This appendix contains a selection of first hand experiences from physicians. More can be reviewed on our website.

Robert D. Sheeler, M.D.

Associated Professor Emeritus, Mayo Clinic, Department of Family Medicine; December 1, 2016

Dear Colleague:

I am writing at this time as a Board-Certified Family Medicine specialist in Traditional Western Medicine with over 20 years' experience at Mayo Clinic where I served as the Medical Editor of the Mayo clinic Health Letter and taught at Mayo Medical School. I am also writing as a specialist who is Boarded and Certified in Integrative Medicine, Holistic Medicine and Functional Medicine.

I would like to encourage you to look at the attached reprint from Leaders Magazine; an interview with Roger Deutsch, CEO and Founder of Cell Science Systems, which speaks to the existence of a relatively new technology, the Alcat Test, which has recently been independently validated at Yale School of Medicine. The Yale researchers showed it to be a clinically useful diagnostic tool for the identification of foods and other substances that trigger gastrointestinal and potentially many other illnesses. Its relatively low cost, ease of use, and clinical utility, lead me to believe that its adoption will make a serious dent in healthcare expenditure. It can serve as a viable complement to conventional approaches to treating many common maladies making diagnosis more accurate and thus relieving much patient suffering.

I commend to your attention the leading-edge testing procedures that have been developed and standardized by Cell Science Systems for identifying food sensitivities. There are a number of methods that attempt to test for reactivity to food and other substances. I believe, however, based on the mechanisms involved that the Cell Science System Alcat Test offers distinct advantages in clinical practice. If it were more widely available I feel that the root cause of a number of different autoimmune, gastrointestinal and system-wide disease states could be improved for many patients.

Pradeep Chopra, MD, BROWN Medical School

"I have been practicing medicine for 10 years. I have used the Alcat Test in many refractory and otherwise complicated cases involving an inflammatory component. The vast majority of these patients have experienced significant health attributable to dietary modification based upon Alcat Test findings. I have little doubt that judicious utilization of this intervention bears a highly significant cost: benefit ratio.

In my professional opinion the Alcat Test can be successfully utilized in a broad spectrum of disease conditions cost-effectively. The scientific literature suggests the approximately 70% of chronic degenerative conditions arise from lifestyle factors, which this modality effectively addresses.

I hope that you and other insurance carriers will recognize the important cost saving and quality of life benefits of this approach; benefits that are attained with NO side effects. I can supply case histories and data in support of this if needed. I am also available to discuss these issues personally."

Nathan Goodyear, MD

"I have been practicing medicine for 7 years now. I have been using the Alcat Test for many of my patients. No one test has provided more positive results for my patients.

I have seen many patients lose massive amounts of weight, reduce blood pressure and eliminate medications, improve blood sugar control and eliminate medications, eliminate migraines/headaches, reduce and eliminate acne, rosacea, and other skin conditions, improve asthma, and improve a vast array of gastrointestinal symptoms. Dietary modifications based on Alcat Test results have provided significant benefits in all inflammatory conditions in my clients.

With the ever-increasing cost of medical care, no one test has a better cost/benefit ratio. In 2009, health care costs reached $2.5 trillion. But if you break it down, you see the real benefits. The health of the patient equals cost savings for the insurance company. The obesity epidemic is out of control. Since 1995, the number of states with obese adults exceeding 30% has gone from 1 to 16. Obesity is the doorway to disease. So if we extrapolate that with resultant associated diseases such as diabetes, cardiovascular disease, and cancer, the costs of doing nothing and the benefits of real prevention become evident. Look at a 5-year annual cost window:

	Men	Women
Obesity	$ 2,646	$ 4,879
Diabates	$ 11,744	$ 11,744
Cardiovascular	$ 18,953	$ 18,953
Cancer	$ 29,643	$ 13,925
Total	$ 62,986	$ 49,501

In my professional opinion, the Alcat Test can be successfully utilized in a broad spectrum of disease conditions cost-effectively. One test can really implement lifestyle changes that close the doorway on inflammatory related conditions and diseases."

Mark L. Andrews, MD

"...I have only been able to order the test a limited number of times because insurance does not pay for it, but each time I have been very impressed with how much money the insurance company was likely to save in the years following our test.

I have had a new nurse present with chronic asthma so bad that she couldn't speak in complete sentences. She was taking maximum therapy and was still not in control. After adjusting her diet based on Alcat testing, her asthma cleared completely and she was able to stop all medications. I assume this saved her insurance carrier at least $4,000.00 per year.

Another coworker had testing done because of weight loss, malaise, blisters on his feet and extreme mental obtundation. He had developed sleep apnea and was functioning at an unacceptable level. After adjusting his diet, his weight loss stopped, his rashes cleared and his mental clarity and endurance returned to normal.

Another coworker's testing was done after he started failing college classes and was dealing with untenable ADHD. He was getting ready to start Adderall. For one week after his diet adjustment, he felt like he had the flu. Then his symptoms all cleared and his mentation returned to normal. His grades returned to "A's" and he never needed the Adderall. This was again, a savings of many thousands of dollars to his insurance company over a lifetime.

I have had numerous patients with fibromyalgia that have seen a dramatic improvement after taking the Alcat Test. The degree of recovery seems most closely linked to the degree of compliance with the diet..."

Pauline Harding, MD, Family Medicine Practitioner

"I have been practicing medicine for 35 years. I have used the Alcat Test in many... cases involving an inflammatory component... Examples of the benefits of identifying inflammatory agents include the following: One middle-aged woman with chronic headaches discovered that the aspartame in her toothpaste was the culprit.

- One middle school youngster found that he was highly allergic to a single variety of mold found under the toilet tank in his bathroom.
- One middle-aged male discovered that what appeared to be unexplained acute alcohol intoxication was actually due to a chemical inhaled in his workplace.
- One child's untreatable seizures resolved when he stopped using paper plates and paper cups and milk carton that had a corn-sugar coating.
- One young adult male from out-of-state had resolutions of his longstanding intestinal bleeding upon identifying gluten as the cause of his bowel disease. Two colonoscopies had not identified the issue.
- Several patients diagnosed with multiple sclerosis had resolution of their symptoms after identifying aspartame and/or gluten as the cause of their neurologic symptoms.

... I hope that insurance carriers will recognize the important cost saving and quality of life producing benefits of this approach."

Laura Miles, MD

"I have been using Alcat testing for almost 5 years and it has truly changed lives. With the utilization of this testing and following the elimination diets I have seen numerous cases of severe irritable bowel syndrome completely resolve.

Life-long eczema disappears. Joint pain improves associated with arthritis as well as idiopathic joint pain. People are able to lose weight when they have tried everything else with no results. ADD and ADHD kids are more focused not requiring medication or can at least lower the doses. Crohn's disease disappears. These are just a few of the positive outcomes I have seen. The Alcat Test is one of the most comprehensive tests on the market for food sensitivities and is absolutely essential in treating underlying causes of diseases.

Wouldn't it be more cost effective to pay for this test rather than pay for years and years of unnecessary medications or even surgeries?...".

Lisa Kamphuis, ARNP

"I have been practicing as a Nurse Practitioner for 16 years and have been using the Alcat Test in many seemingly impossible cases which involve inflammation.

Some of these cases include previously assumed fibromyalgia, arthritis, obesity, and irritable bowel syndrome. The majority of these patients have benefitted significantly or completely resolved their diagnoses, which we can attribute solely to dietary changes based on findings of the Alcat Test.

One particular patient reported that he made at least 3 visits to the ER per year and took hundreds of dollars' worth of medications monthly until he made these dietary changes. He has now lost 40 pounds and is happily working out, feeling more energy than ever, and doesn't take any prescriptions. I believe the Alcat has many uses which can save millions of dollars to our healthcare system..."

David Blyweiss, MD

"I wanted you to know the changes that a couple of my patients had in their lives after following the Alcat guidelines.

- One patient, an 11-year-old boy with autism also suffered from a body rash that never went away...it went away after a week of following the diet guidelines.
- Another patient, a 35-year-old woman with frequent urination (20 x/day) and urinary incontinence found that her symptoms resolved completely after 4 weeks on the dietary program changes...this after three other doctors...multiple meds and multiple cystoscopies."

Amy E. Pieczarka, Clinical Dietitian

"I have been a registered dietitian and licensed dietitian/nutritionist, providing medical nutrition therapy for 25 years. Utilizing the Alcat Test has provided my patients information necessary to help manage existing health problems and promote optimal health. I have used the Alcat Test in many complicated cases, which prior to the knowledge obtained from the testing, were seeing no progress despite my suggestions.

The Alcat Test has provided the understanding of what contributes to their health problems, most of which involve an inflammatory component. I believe that utilizing this intervention demonstrates a highly significant cost benefit ratio. It is my professional opinion that anyone would benefit from the Alcat testing. It is cost effective and can be a successful intervention in a broad spectrum of disease conditions.

As a practitioner who focuses on lifestyle change counseling to prevent and help manage inflammatory degenerative conditions, I hope that other insurance carriers will acknowledge the Alcat Test as an important cost saving."

David M. Brady, ND, CCN, DACBN

"I am writing to provide my clinical experience with the Alcat Test over the past 15 years. This novel testing of immunological reaction to exogenous proteins, including food-derived antigens has been extremely valuable in many cases of both acute and chronic illness related to inflammatory and immunological phenomena.

The test has been used in the University of Bridgeport public health clinics, and I have used it extensively in my private practices throughout the years, with great success. In fact, this testing methodology has been the key to resolving many patients' long standing complaints, including dermatologic issues, fatigue, headache, joint and soft-tissue pain, gastrointestinal discomfort, and much more when other diagnostic testing and treatment intervention had failed.

The outcomes research on this test at major academic centers around the world is also impressive and I urge to consider coverage for patients seeking this test at the recommendation of their licensed health care provider."

Jamie Wright, DO, FACOOG, ABAARM

"My name is Dr. Jamie Wright and I am a board certified gynecologist who practices preventive medicine and nutritional medicine.

I support reimbursement for the Alcat Test based on my personal and clinical experience that it is a test that provides the patient and clinician with immediately useful information that will affect the patient's quality of life and reduce the severity of many diseases.

The application of the dietary recommendations based on the Alcat results in decreased weight, obesity, chronic pain, migraine, mood disorders, arthritis, bladder and pelvic pain, and a variety of bowel disturbances because it reduces abnormal immune system activation and inflammation."

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Citations Part D - Overview Studies & Research directly related to the Alcat Test

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Neutrophil cell trapping bacteria. Coloured scanning electron micrograph (SEM) of bacteria (rod-shaped) being trapped by a neutrophil cell. The neutrophil cell (a type of white blood cell) has trapped the bacteria with extruded material that forms a net-like structure called a NET (neutrophil extracellular trap). This method of cellular defense was first discovered in 2004. These are Shigella sp. bacteria, one of the causes of dysentery, a severe intestinal inflammation ("picture of the year 2004", Max Planck Institute, Berlin).

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