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

SCIENTIFIC DOSSIER
State of the Art for Food Sensitivity Testing
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 perpetuate non-IgE mediated reactions to foods and chemicals.

In the Alcat Test, the total population of peripheral blood leukocytes (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 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 decade 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

Cell Science Systems | Alcat Europe

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A.1 Alcat Test for cellular food/chemical sensitivity

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 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.

A.1.1 Innate Immune Cell Reactivity

All reaction types of leukocytes 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 over-activation of the immune system and its consequences.

Figure 1 suggests that over-activation of the immune system by diet components/additives can impair health and may lead to tissue damage or silent inflammation. All reaction types of leukocytes 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 over-activation of the immune system and its consequences.

A.1.2 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 chronic disease. The identification of specific dietary immune triggers can support treatment and prevention of chronic symptoms and disease. The Alcat Test is used for prevention (and performance optimization, reducing internal stress factors) as well as a complementary tool for optimizing therapy for chronic disease. The identification of specific dietary immune triggers can support treatment and prevention of chronic symptoms and disease.

Table 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).

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

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. phytocompounds), 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.

Figure 1: Activation of polymorphonuclear leukocytes (PMN), 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 (C). Cell burst (after about 2 hours) results in degranulation and volume increase. The contents mix with cellular fluid (D). Cellular degranulation is found to correlate with pro-inflammatory multiple health symptoms and diseases.
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 2):

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

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

<table>
<thead>
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<th>Non-specific Inflammatory Marker</th>
<th>Innate Immune Activation</th>
<th>Bioenergetic Cellular Imbalance</th>
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<tr>
<td>Histamine levels (stool)</td>
<td>ROS (reactive oxygen species)</td>
<td>Reduced mitochondrial energy capacity</td>
</tr>
<tr>
<td>SAA (serum amyloid A)</td>
<td>EPX (eosinophil protein X)</td>
<td>Reduced mitochondrial stress resistance</td>
</tr>
<tr>
<td>CRP (C-reactive protein, serum)</td>
<td>Neutrophil elastase (stool)</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>BDNF (brain-derived neurotrophic factor, serum)</td>
<td>MPO (myeloperoxidase; serum/stool)</td>
<td></td>
</tr>
<tr>
<td>Calprotectin (serum)</td>
<td>Lipopolysaccharide-binding protein (serum)</td>
<td></td>
</tr>
<tr>
<td>HMGB1 (high-mobility-group protein B); serum)</td>
<td>sCD14 (soluble CD14)</td>
<td></td>
</tr>
<tr>
<td>Cytokines (IL-1, IL-6, IL-8, TNF-alpha)</td>
<td>Calprotectin (stool, serum)</td>
<td></td>
</tr>
</tbody>
</table>

A.1.3 Useful Trend-Setting Biomarkers

Cells and pro-inflammatory responses to danger signals

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

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Dietary guidance, part of Alcat testing, includes the temporary elimination of items that trigger an immune 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. (46)

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 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, 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.

Blood composition

What is measured by Alcat Test?

Figure 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.
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: strong, moderate, mild, or negative.

Precision and comparison to other accepted methods

Figure 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."


Figure 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 the Alcat Test, is divided into a) analytical and b) clinical validation (figure 4).

The analytical validation results from both clinical studies and from approvals, certificates, and patents (see A.4.3). The studies presented in chapter C represent clinical validation of the Alcat Test for the identification of sensitivity to food ingredients, additives, and other compounds. Observational studies of approximately 1,300 patients serve to confirm the analytical and clinical validation.

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 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 the EN ISO 13485:2016/3 certificate 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 identifies persons (tp) with the relevant sensitization to the tested food/additive when compared to all individuals with a sensitization to the tested foods/additive (tp/tp + 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/tp + fn).

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

Table 3: 4-field table for clinical validation: tp: true positive; fn: false negative; tn: true negative; fp: false positive

<table>
<thead>
<tr>
<th>Sensitized / Patient state of health</th>
<th>Sum</th>
</tr>
</thead>
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<tr>
<td>Sensitized</td>
<td></td>
</tr>
<tr>
<td>True positive (tp)</td>
<td></td>
</tr>
<tr>
<td>False positive (fp)</td>
<td></td>
</tr>
<tr>
<td>tp + fp</td>
<td></td>
</tr>
<tr>
<td>Non-sensitized</td>
<td></td>
</tr>
<tr>
<td>True negative (rn)</td>
<td></td>
</tr>
<tr>
<td>False negative (fn)</td>
<td></td>
</tr>
<tr>
<td>tn + fn</td>
<td></td>
</tr>
<tr>
<td>tp + fn</td>
<td></td>
</tr>
<tr>
<td>tn + fn</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td></td>
</tr>
</tbody>
</table>

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 and
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 1,300 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 1.

Summary

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

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

Neutrophils form the frontline of defense in the innate immune response to invading microorganisms and microbial structure, 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.

Intra- and extra-cellular “killing environments”

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

Almost 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 9)**

Includes special sections regarding the consumption of:
1. Gluten (gluten/gludin, individual grains)
2. Milk products (casein/whey, different milks)
3. Candida albicans (different sugars)

**Chemical Sensitivity Report and others**

1. Additives/E-numbers (colorings, flavorings, sugar alternatives, preservatives, environmental chemicals)

**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 anti-body formation) operates without "memory function."

The absence of exposure to the substance may result in resolution of the intolerance. 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. 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 multi-systemic and can affect cognitive function as well as musculoskeletal, gastrointestinal, genitourinary, and cardiovascular systems.

Table 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]

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.

CSS’s core business and flagship 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’s services, innovations, and benefits were scored “excellent.”

A.4 Company History

A.4.1 Cell Science Systems, Corp., USA

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.

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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 and dietetic counselors.
Immunology

The laboratory of Cell Science Systems carries the certification code 220 for “General Immunology.”

Alcat Test Kit

BAKER’S YEAST, BEET SUGAR, BREWER’S YEAST, CANE MODERATE REACTION

3 MONTHS AVOIDANCE

MILD REACTION

MODERATE

ACCEPTABLE / NO REACTION

MILD REACTION

3 MONTHS AVOIDANCE

Figure 9: Example: The Alcat food analysis is clearly presented on one single page, for example, with a test panel of 250 foods as seen.

A.4.3 Certificates and Licenses

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

- ISO 13485:2016 (E) certification is the international quality management standard for medical devices in addition to compliance with GMP 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.

Cell Science Systems GmbH, the European distributor of the Alcat Test, holds the ISO 9001:2008 certificate and services the EU region and countries in the Middle East.

Food and Drug Administration (FDA) Registration

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

- Proprietary Name: Alcat Test Kit
- Brand Name: Alcat Test Kit
- Classification Name: Whole Blood, Plasma, Antigen
- Product Code: DQO
- Device Class: 1
- Regulation Number: BM6.5700
- Medical Specialty: Immunology
- Owner/Operator: Cell Science Systems, Corp.
- Establishment Operations: Manufacturer

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.: GO1N 15/12, GO1N 33/33, application A41L13/014, Publication date: 15.04.1992, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
- Publication: EP0281626, Int. Cl.: GO1N33/48, GO1N33/566, GO1N33/53, GO1N33/554 1988/377,

Patent 29.05.1997, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

- Publication: WO 92/01934, Int. Cl.: GO1N 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.

Figure 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).
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 10: “The Targeted Response”) while the broader category of sensitivity is generally associated with non-specific immunity (figure 10: “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 Sensitivity/Intolerance – Definitions

**FOOD ALLERGY**

- **adaptive/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)

**FOOD SENSITIVITY**

- **innate/non-specific immune system**
  - Cells: Mast cells, degranulation of basophils
  - Histamine release
  - T & B cells → IgE antibodies
- Multiple triggers: e.g. DAMPs, toxins, proteins, or nonprotein-based molecules
- 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 idiosyncrasy.” Freeman, who is considered the founder of immunotherapy along with Leonard Noon, stated in his address on Toxic Idiosyncrasies, Royal Society of Medicine, 1920:

“It might be an exaggeration to say that the study of these toxic disparities 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.

### 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.1.2, figure 10).

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 effectors than an allergy test does.

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.

![Figure 10: 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.](image-url)
B.1.2 Current Scientific Perspectives and Definitions: Allergy, Intolerance, and Sensitivity

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:

- Respiratory allergy
- Contact urticaria
- WDDEA (weight-dependent exercise-induced anaphylaxis)

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. [1, 45, 46]

Hence, the differentiation between an allergy, intolerance, and sensitivity to a food can be illustrated with the example of wheat (figure 11).

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

Figure 11: Pathogenesis of gluten-related disorders manifesting via different immune pathways

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.” [47]

... 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 distension, abdominal pain, and irregular bowel movements. Common examples include lactose intolerance, intolerance to lactulose, 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. [1, 47, 48]

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 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 Ilja 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.”

Common examples include lactose intolerance, intolerance to lactulose, or intolerance to excess fermentable oligo- and disaccharides, monosaccharides, and polyols (FODMAPs).
### C.1 Studies and research

**Alcat / Research Overview reversed chronological order 2017-1998**

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 Alcat 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. The reference numbers of the overview are used for the abstracts.

#### Study ref. number / title

<table>
<thead>
<tr>
<th>Study ref. number / title</th>
<th>Author / publication</th>
<th>Year / study type</th>
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<td>2) Study 2: The Alcat Test Predicts the Release of DNA and Myeloperoxidase by Intact Immune Peripheral Blood Leukocytes Via a PEC Pathway Dependent Pathway</td>
<td>Vale School of Medicine: Imma Garcia-Martinez (1), Theresa R. Weiss (2), Ather Ali (2), Waajahat Z. Mehal (1); online.iberpub.com doi/jull/10.1899/AGM.12.20.9003.abstract/215</td>
<td>2016: Molecular patho-mechanism n=20</td>
<td>4,000</td>
</tr>
<tr>
<td>3) Study 3: Effect of Alcat-Based Food Elimination on Inflammatory Markers, Body Composition, and Medical Symptoms</td>
<td>Northwestern Illinois University; Lukaszkau J, Shokrani M, Hoppensheidt J, and Lhomer, J. Prof. J. Lukaszkau April 10, 2016, of the University of Illinois Miller School of Medicine</td>
<td>2016:47 Clinical, multiple disorders 133 patients DBPC</td>
<td>ca. 30,000</td>
</tr>
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#### References

C.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.

C.2.1 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 predetermined 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 12 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 (39).

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.

C.2.2 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 Brondon 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 12, 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 were 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 hirsutism 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 DBPCFC was 83.4%, demonstrating the efficacy of the Alcat Test for detection of incompatible, immunoreactive 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 (39).

Interestingly, IBS patients achieved significant improvement in 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.
C.3 Analytical Validation, Pathomechanisms and Technical Studies

C.3.1 Analytical Reproducibility

(32) 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.

C.3.2 Molecular Pathomechanism

(30) The Alcat Test Predicts the Release of DNA and Myeloperoxidase by Innate Immune Peripheral Blood Leukocytes Via a PKC Dependent Pathway


Results: Alcat positive foods gave a higher supernatant DNA content in 33 of 73 (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 pathway.
C.3.3 Mechanistic/technical studies

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

Background: Gitte Jensen, Ph.D. (Immunology), conducted a study to investigate the mechanisms behind the Alcat test results. The objective of this study was to investigate the mechanisms behind the Alcat test results. The study analyzed the immune response to food antigens using flow cytometry and microscopy.

Method: Reactivity of PBL from 10 volunteers to 200 different food antigens was determined by Alcat testing. On a subsequent day, PBL from the same volunteers were exposed to a single food antigen 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, and neutrophils (CD66b-CD16a).

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% 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% 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.

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.

(16) Technical Study Comparing the Alcat method with Activation of Granulocytes Following Challenge with Zymosan

Challenge: Zymosan is derived from yeast cell walls (Saccharomyces cerevisiae) and is used in vitro immunological evaluation of the phagocytic immune function. Cells in healthy, patients respond to a challenge with zymosan by activating NADPH oxidase (through activation of cytochrome B and the cytosolic cytochrome 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 analyses with aliquots of blood exposed in the course of the Alcat test to food extracts. She found an inverse correlation in samples that were reactive after exposure to “severe intolerance” antigens. After exposure to “severe intolerance” antigens, the superoxide anion was simultaneously the Alcat Test was performed on each patient for the following foods: egg, cow’s milk, wheat, peanut, beef, orange, soy, and tomato.

Please view A.1.6 Measurement Technology

DOSIER SCIENTIFIC BASIS OF THE ALCAT TEST

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 jobs 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.

(40) 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 cromoglycate 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.

(41) 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.
In-vitro: 1. Frequency of Immunoglobulin level change exceeded 5.2±07 that food, 2. Frequency of Complement Activation for that food as measured by the height difference of I gM peaks / 3. Frequency of spectrophotometry reading of hemolysis exceeded 5.02 for that food.

Serum immunoglobulin levels (IgA, IgM, IgG, IgG4) using turbidimetry, 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 immunoelectrophoresis. Hemolysis was measured by spectrophotometry.

Results were analyzed by calculating percent change from control values in each of the 96 tests per pathogenic mechanism. Scores falling outside the range of values from control values in each of the 90 tests per pathogenic mechanism.

In an Alcat histogram, platelets are seen in the area under the curve observed: in the area under the curve observed:

\[ \text{IgM} \times 4 - \text{IgA} = 12, \text{IgG} = 5, \text{IgG4} = 3. \]

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 immunoglobulins 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.

(42) 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 phytohemaglutinin was the first evidence that cytospin 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.

Additional evaluation of Alcat Test histograms 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:

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

C.4 Review papers and Clinical Studies

C.4.1 Review papers

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


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 inflammatory response. A direct relationship between the presence of the inflammatory 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.

(14) The Right Stuff: Use of ALCAT testing to determine dietary factors affecting immune balance, health, and longevity

Citation: K. D. Deutsch, Anti-Aging Therapeutics/Volume X, 2007 Conference Year, Chapter 8

Summary: The aging processes, described by Denham Harmon in the 1990s, are manifest 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.

(9) Uso de la Alcat en el estudio de la ALCAT. Mitó o realidad

Citation: Ángel San Miguel et al., 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 tool 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 cytoflexmetric 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.
The 48 patients in this study suffered from 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. There was significant improvement in the athletes’ condition and reduction in inflammatory response as demonstrated by improvement in body composition, symptoms, and exercise capacity.

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

Citation: Berardi et al. Study presented at the XVIII Congress of the European 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: A 12 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.

(11) Food intolerance in patients with cutaneous diseases: diagnostic value of the Alcat Test

Citation: Berardi L, De Amici M1; Vignini, A2; Torre, C1; Masca, M2. Food intolerance in patients with cutaneous diseases: diagnostic value of the Alcat Test. Study presented at the XVIII Congress of the European 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: Alcat Test and recommended elimination diets proved to be effective. In more than half of the patients, symptoms improved significantly.

(1) Individualized Diets in Irritable Bowl Syndrome: A Randomized Controlled Trial

Citation: Yale School of Medicine. Ather Ali (1), Theresa K. Weiss (2), Alisa Scherban (1), Sumiya Khan (1), Doug Las McKee (1), Dario De Amici (1); Wajahat Z. Mehal (1), (1) Yale University, New Haven, CT, United States; (2) Department of Pediatrics (General Pediatrics)

Methods: Parallel group, double-blind, randomized, controlled clinical trial as illustrated in 58 adults with IBS, with moderated 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 acceptance 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 (7/2014–1/2015) and the majority of means were performed (α = 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. 1.47; p = 0.02) and SSS (126.81 vs. 95.99; 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.

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.

(3) Effect of Alcat-Based Food Elimination on Inflammatory Markers, Body Composition, and Medical Symptoms

Citation: Northern Illinois University, Lukaszuk J, Shokrani M, Hoppensteadt J, and Umoren J, Prof. J. Lukaszuk, April 10, 2016, at the University of Miami Miller School of Medicine

Summary: The Randomized, placebo-controlled double-blind, 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) or 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 gastrointestinal 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:

1. 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.

2. 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 sensitive marker for systemic inflammation than C-reactive protein (CRP).

3. Weight Loss

Weight and BMI also decreased during the study. When restricting any type or number of foods, an individual will be more 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.

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

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

3. 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.

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

Citation: De Amici et al., Study conduct and reporting of the University of Pavia, Italy. 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. Two-thirds (66%) showed significant symptom improvement, 31% had some improvement, and only 3% reported no change.

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

Citation: Berardi, L. et al., Study of the University of Pavia, Italy. 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. The majority of patients (71%) experienced significant improvement of their symptoms, 27% had a slight improvement, and only 2% reported no change.

(8) Rational Management of Food Intolerance in Elite Athletes


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

(21) Outcome Study in 305 Consecutive Patients Following The Alcat Diet

Citation: Observational study conducted in Copenhagen at the Allergy Clinic Charlottenlund, Denmark 1996 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.

(23) South African Outcome Study of Dr. Ian Geldenhuyx

Citation: Dr Geldenhuyx, a practicing physician 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

(24) 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 et al. The Bariatrician. Spring 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 < 0.001) 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 < 0.001) 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, 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.

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

Citation: Cabo-Soler et al., 14th Med Day of Aesthetic Medicine & Dermatology, Venice, Italy, September 22- 23, 1995. Subsequently published in 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.

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

Citation: Myllik et al., 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%)

Data from this study were not published but the results were made available by Dr. Lene Haj for training and presentation.
Irritable bowel syndrome
82.7% correlation with positive reacting foods.

Hyperactivity (80% improvement)

Milk-induced colitis
87–92% correlation with positive foods when plate

Infantile colic

Transient gluten intolerance

Post enteritis syndrome

This study was conducted under the di

Chronic or recurrent diarrhea in infancy

This study aim was to determine whether

Alcat is quick:
Alcat can test 100 foods in 4 hours. Four-day fasting

The Alcat Test—A Guide and Barometer in the
Therapy of Environmental and Food Sensitivities

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

Summary: This study focused on the relationship between diet and lifestyle, with a particular emphasis on the role of food in the development of chronic diseases. The study involved 56 children aged 3 to 12 years, and the results showed that dietary changes could significantly improve the symptoms of food allergies and sensitivities.

The results confirmed that the Alcat Test is a reliable method for identifying food sensitivities and allergies, and that the elimination of problematic foods can lead to improved health outcomes. The study also highlighted the importance of using non-invasive methods to evaluate food sensitivities, as these methods are less invasive and can be more accurate than traditional methods.

Summary:

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The results confirmed that the Alcat Test is a reliable method for identifying food sensitivities and allergies, and that the elimination of problematic foods can lead to improved health outcomes. The study also highlighted the importance of using non-invasive methods to evaluate food sensitivities, as these methods are less invasive and can be more accurate than traditional methods.
The Alcat Test is indeed a leukocyte activation test. On the subject of "reproducibility," no reference for reproducibility was found. Recurrent abdominal pain and eosinophilic gastroenteritis are broader and can also activate immunologic or metabolic immune pathways. Granulocytes account for the majority of white blood cells in the body. On the subject of "reproducibility," no reference for reproducibility was found.

Appendix I

Comments on Criticism

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 opinion 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 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 immunologic 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.

However, 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 in 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
The Alcat Test measures mainly immediate granulo-
cyte reactions since the leukocytes are only confront-
ed with the antigen for a short time. Characteristically,
the defense cells (especially neutrophils or eosinophil-
ic granulocytes) swell, burst, and release their inflam-
mation-promoting “weapons” in order to effectively
neutralize a substance interpreted as “dangerous.” The
cascade-like effects affect the entire body and its im-
mune balance - everyone knows the principle of the
flu.

The Alcat method was positively validated by in-
dependent international researchers using dou-
ble-blinded and reproducibility studies. An over-
view 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 princi-
ple. This test provides an answer for patients who
report various complaints associated with consum-
ing a particular food or ingredient (e.g. spices or ad-
ditive), yet all antibody and histamine tests fail to
show results.

Appendix 2  Practitioner First Hand Experiences

Robert D. Sheeler, M.D.
Associated Professor Emeritus, Mayo Clinic, Depart-
ment 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 Sys-
tems, 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 clinically use-
ful diagnostic tool for the identification of foods and
other substances that trigger gastrointestinal and po-
tentially many other illnesses. It’s relatively low cost,
ease of use, and clinical utility, lead me to believe that
its adoption will make a serious dent in healthcare
effort. 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 standard-
ized by Cell Science Systems for identifying food sen-
sitivities. 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 dif-
ferent autoimmune, gastrointestinal and system-wide
disease states could be improved for many patients.

Dr. med. Dirk Wiechert
Leumittestenstrasse 65
27721 Rittershude, Germany

Robert D. Sheeler, M.D.

1) 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.

2) I have seen many patients lose massive amounts of weight, reduce blood pressure and eliminate medi-
cations, 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 gastro-
intestinal symptoms. Dietary modifications based on
Alcat Test results have provided significant benefits in
all inflammatory conditions in my patients.

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
the costs of doing nothing and the benefits of real pre-
vention become evident. Look at a 5-year annual cost
window.

Pradeep Chopra, MD, BROWN Medical School

1) I have been practicing medicine for 10 years. I have used the Alcat Test in many refractory and otherwise
complicated cases involving an inflammatory compo-
nent. The vast majority of these patients have experi-
enced significant health attributable to dietary mod-
ification 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 suc-
cessfully utilized in a broad spectrum of disease condi-
tions cost-effectively. The scientific literature suggests
the approximately 70% of chronic degenerative con-
ditions arise from lifestyle factors, which this modality
effectively addresses.

I hope that you and other insurance carriers will recog-
nize the important cost saving and quality of life ben-
efits 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 Gooyear, MD

*Pradeep Chopra, MD, BROWN Medical School

* I have been practicing medicine for 10 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 medi-
cations, improve blood sugar control and eliminate
medications, eliminate migraines/headaches, reduce
diabetes, cardiovascular disease, and cancer, the costs of
doing nothing and the benefits of real pre-
vention become evident. Look at a 5-year annual cost
window.
Another patient, a 35-year-old woman with fibromyalgia, was prescribed medications that were not working. She was suffering from fatigue, joint pain, and depression. Her Alcat test revealed significant food sensitivities, including gluten and dairy. After adjusting her diet based on Alcat testing, her symptoms improved dramatically, and she felt like her old self again.

Another middle-aged man discovered that what appeared to be a case of chronic inflammation was actually due to a food sensitivity. He had been experiencing digestive issues and joint pain for years. His Alcat test revealed several food sensitivities, including soy and gluten. Following an elimination diet, his symptoms improved significantly, and he felt like he had a new lease on life.

A middle school student suffered from migraines and depression. His Alcat test revealed several food sensitivities, including dairy and soy. Following an elimination diet, his migraines subsided, and his depression lifted. He was able to focus better in school and was no longer absent due to illness.

A young adult male from out-of-state had resolved his chronic sinusitis within a week after following the diet guidelines. His Alcat test revealed several food sensitivities, including dairy and soy. Following an elimination diet, his sinusitis cleared up, and he was able to breathe freely for the first time in years.

A middle-aged female discovered that her persistent headaches were actually due to a food sensitivity. She had been using medication to manage her headaches for years. Her Alcat test revealed several food sensitivities, including dairy and gluten. Following an elimination diet, her headaches subsided, and she was able to manage her health without the need for medication.

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 her 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 rash cleared and his mental clarity and endurance returned to normal.

Another coworker’s testing was done because of weight gain, arthritis, and heart discomfort. He was a type A personality and had been working out, feeling more energy than ever, and I urge to consider coverage for patients seeking this test at the recommendation of their licensed health care provider.

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 diet, 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 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.

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 benefited 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

David M. Brady, ND, CNC, DACBN

“I am writing to provide my clinical experience with 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, ABAARMS

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

I support reimbursement for the Alcat Test based on my personal and clinical experience that 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.”
IgG subclasses 1-3 reflect the exposure to antigens. IgG4 is also referred to as an “anti-IgE antibody” because it blocks IgE antibodies in order to avoid a potentially dangerous allergic reaction. However, growing evidence indicates that IgG4 is an inflammatory immune reaction and is not the same as IgE. The detection of allergen-specific IgG4 antibodies during a specific immunotherapy can be a useful marker for adequate desensitization. In contrast with food, IgG4 antibodies are formed only after a very long exposure. Detection of the allergen-specific IgG4 during a specific immunotherapy can be a useful marker for adequate desensitization. In contrast with food, IgG4 antibodies are formed only after a very long exposure.

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. As some of IgG1 consist of IgG1 and IgG2, 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%. IgG3 antibodies of subclasses IgG1-3 are primarily induced by microbial antigens (streptococci, pneumo-cocci). 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 IgG1 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. Increased IgG levels are therefore not the same as an inflammatory immune reaction. Though 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 allergic reaction. In contrast with food, IgG4 antibodies are 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 Torjesen 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 increased 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-gluten antibody level is not a valid measurement parameter for sensitivity to gluten in order to accurately diagnose a gluten sensitivity (NGCS) (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 contrast with food, 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 Tikkainen 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) that 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
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- http://immunologycenter.org/en/about/
Appendix 3 References


Further references


Appendix 4 References


References

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Appendix 4 References


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