

DOSSIER

SCIENTIFIC BASIS OF THE ALCAT TEST



**"The Doctor of the future will give no medicine,
but will instruct his patient in the care of the human frame,
in diet, and in the cause and prevention of disease."**

(Thomas Edison, 1902)

ALCAT

TABLE OF CONTENTS

A. INTRODUCTION.....	4
A.1. WHAT IS THE ALCAT TEST?	4
A.1.1 Basic Principle	4
A.1.2 Measurement Methodology.....	4
A.2 SCIENTIFIC BACKGROUND: THE ROLE OF THE INNATE IMMUNE SYSTEM	5
A.2.1 Structure of the Human Immune System.....	5
A.2.2 Food Intolerance and Innate Immune Defense.....	5
A.2.3 Molecular Processes of Granulocyte Activation.....	7
A.2.4 Bowel, Immune System and Intolerance	8
A.2.5 Understanding Allergy and Intolerance	9
A.3 HISTORY	11
A.3.1 Immunology of Allergy and Intolerance.....	11
A.3.2 Corporate History.....	12
A.4 CASE STUDIES.....	13
A.4.1 Case Study 1 – Skin.....	13
A.4.2 Case Study 2 – Pancreatitis	13
A.4.3 Case Study 3 – Rheumatoid Arthritis / severe overweight	13
B. STUDIES.....	14
B.1 DOUBLE-BLIND STUDIES AS A TOOL FOR CLINICAL EVALUATION.....	14
B.2 USING DOUBLE-BLIND STUDIES TO EVALUATE THE ALCAT TEST FOR FOOD INTOLERANCES	14
B.3 DOUBLE-BLIND STUDIES OF THE ALCAT TEST	15
B.3.1 Two Basic Studies With Food – UK 1988.....	15
B.3.2 Double-Blind Study of Food Additives - Denmark 1996.....	16
B.4 ALCAT TEST VALIDATION STUDIES.....	17
B.5 FURTHER SUBJECT-SPECIFIC INTERNATIONAL CLINICAL STUDIES OF THE ALCAT TEST	18
B.6 RESPONDING TO UNJUSTIFIED CRITICISM OF THE ALCAT TEST	26
B.7 SCIENTIFIC CONTEXT AND CURRENT RESEARCH	29
B.7.1 Innate Immunity and Inflammation	29
B.7.2 Synergism Between Innate and Specific Immunity.....	30
B.7.3 Interaction Between Genetically and Enzymatically Related Intolerance	31
B.8 DIFFERENTIATION OF THE ALCAT TEST FROM OTHER TEST METHODS.....	31
B.8.1 IgG testing – The Protective Role of Immunoglobulin G, a Natural Physiological Reaction	31
C. APPROVALS, CERTIFICATES, GRANTS, PATENTS RELATED TO THE ALCAT TEST	32
D. SUMMARY AND CONCLUSIONS.....	34
APPENDIX 1. PRACTITIONER AND PATIENT TESTIMONIALS.....	35
APPENDIX 2 - REFERENCES.....	38
IMPRESSUM.....	40

Dear Colleague,

The Alcat Test is now considered the, "gold standard" laboratory method for identification of non-IgE mediated reactions to foods, chemicals, and other categories of substances. As there is currently no single molecule or antibody, serum protein, or gene, that reflects substance induced activation of innate immunity, we target the immune cells to give us such information. And, throughout the many years of its enthusiastic use by clinicians and practitioners we have continued to engage in R&D 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, the, "impedance method" remains the most reliable and accurate.

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

In the Alcat Test, the total population of peripheral WBC's is incubated, in physiologic buffers and solutions, with a battery of approximately as many as 360 different individual substances. The reaction of WBC's 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 many technical and clinical studies and reports.

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. Likewise, unlike, "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 possible dietary and environmental triggers of inflammation. Using the test results, the practitioner or a nutritional counselor provided by our sister company, PreviMedica (PreviMedica.com) is able to counsel the patient on achieving a more healthful diet and lifestyle, which can also have 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 Soc. 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.



A handwritten signature in blue ink, appearing to read 'Roger Deutsch', written over a light blue horizontal line.

Roger Deutsch, CEO

Cell Science Systems | Alcat Europe

A. INTRODUCTION

A.1. What is the Alcat Test?

A.1.1 Basic Principle

The Alcat Test is a lab based immune stimulation test in which a patient's WBC's are challenged with various substances including foods, additives, colorings, chemicals, medicinal herbs, functional foods, molds and pharmaceutical compounds. The patient's unique set of responses help to identify substances that may trigger potentially harmful immune system reactions.

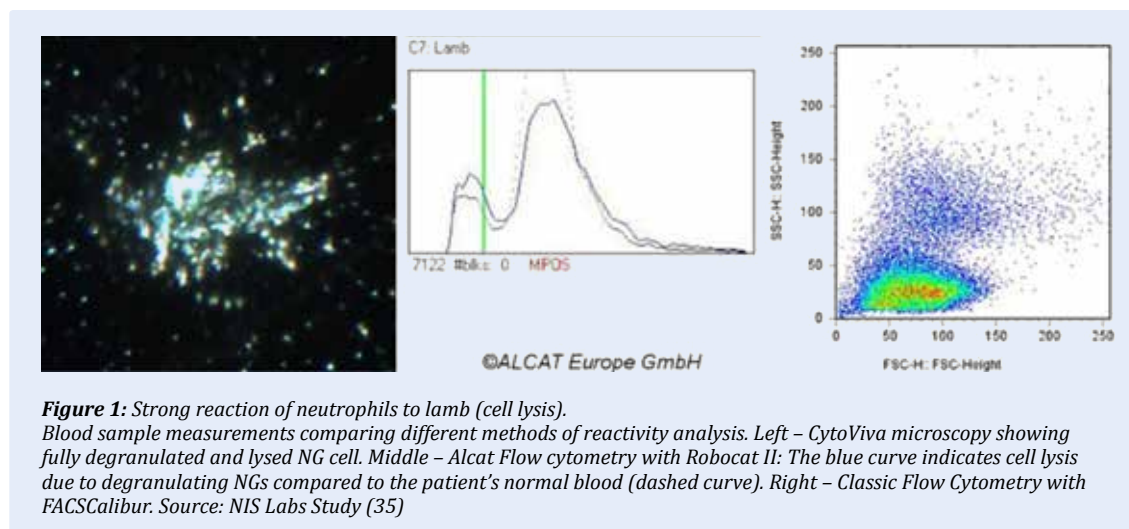
The Alcat Test objectively classifies a patient's response to each test substance as reactive, borderline or non-reactive. Based on these classifications, a customized elimination/rotation diet may be designed to effectively eliminate the specific triggers of chronic immune system activation. By reducing this ongoing burden – and in particular, by reversing the sustained and destructive inflammation it produces – normal body functions and immune system balance can be improved.

A.1.2 Measurement Methodology

Cellular changes occurring in response to Alcat Test challenges are detected using a device that employs the principles of flow cytometry and specific impedance. This automated process is based on the principle that as cells pass through an electric field their electrical resistance (technically referred to as impedance) is related to their size. If the patient's blood reacts to an Alcat Test challenge, some of the cells will be destroyed and some will change in volume in response to immune system reactions (see Figure 1, left). By comparing the number and distribution of cell sizes present in the patient's normal blood with the distribution following an Alcat Test challenge, the degree of reactivity to the test substance can be objectively determined.

Figure 1 illustrates three different methods of measuring a subject's reaction to a test challenge – in this example an extract derived from lamb. Natural Immune System Labs (B.4, 35) examined blood cells after exposure to the lamb extract using 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.

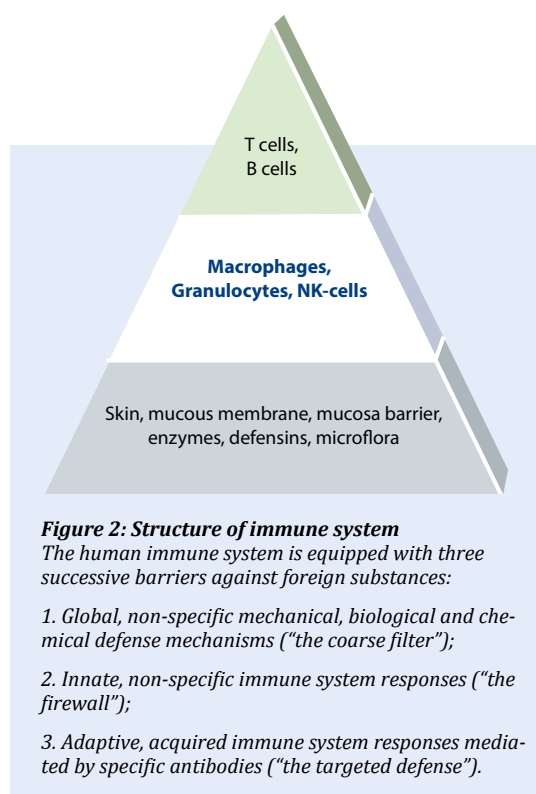


A.2 Scientific Background: The Role of the Innate Immune System

A.2.1 Structure of the Human Immune System

The human immune system consists of three complementary layers of protective and defensive mechanisms:

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



1. “The Coarse Filter”

The first layer of the immune system acts like a coarse filter, capturing, destroying or deactivating potential pathogens by mechanical and biological means. In addition to the protective surface barrier of the skin, many other systems provide a first line of defense against potentially harmful microorganisms and substances. These include mucus, which can trap and help expel invaders; lytic enzymes in saliva including lysozyme and phospholipase A2; body fluids such as tears and urine that flush microorganisms and other substances out of the system; hydrochloric acid in the stomach; antimicrobial peptides produced by the skin and respiratory tract such as β -defensin; and IgA antibodies in the intestines.

2. “The Firewall”

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

In particular, neutrophils act as “first responders,” offering an acute, non-specific defense against encroaching pathogens. Neutrophils release aggressive inflammatory mediators, free radicals and oxidative enzymes, enabling the body to quickly and effectively combat invading pathogens. Neutrophils are vigilant; they continually patrol the body, and by sensing specific molecular patterns can differentiate between endogenous or harmless bacteria and those that are exogenous and potentially pathogenic. In circulating blood, 70% to 80% of white blood cells – leukocytes – are neutrophils. Their overwhelming preponderance clearly indicates their importance. Further, when required, neutrophils can extravasate from blood vessels into the surrounding tissues in response to molecular signals of infection, damage or danger.

3. “The Targeted Defense”

The third level of the immune response – the adaptive immune system – allows the body to recognize unfamiliar pathogens and variable antigens and rapidly respond by inventing and producing novel antibodies. The adaptive kicks in if the innate system’s capabilities are exceeded and more targeted approaches are required. It has the capacity to remember its responses, and this remarkable ability helps to protect against reinfection and also enables adaptation to new or modified pathogens.

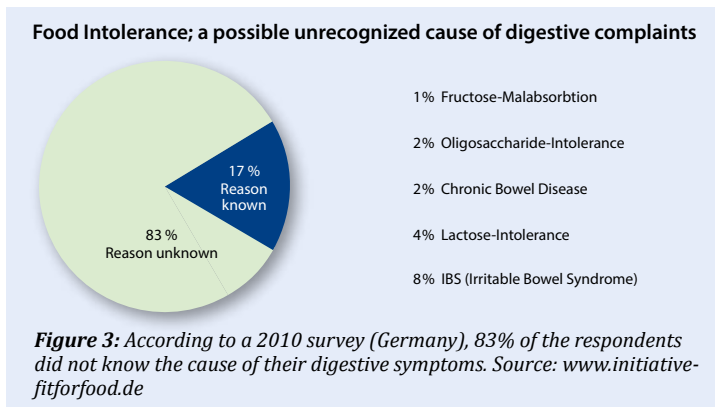
The innate and the adaptive immune systems can work together and/or independently of one another. Ideally, their well-coordinated interactions enable the body to address a wide array of challenges, all the while, without attacking harmless substances or self structures. However, the ideal is not always realized and reliable identification of immune triggers, and the avoidance, where possible, can be of great advantage.

A.2.2 Food Intolerance and Innate Immune Defense

Food intolerance is much more common than true food allergy. However, many people are unaware that they suffer from food intolerance because their symptoms may vary widely and often appear hours,

or even days, after the causative agent is consumed. (see Figure 3 survey, FET e.v. 2010)

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



The complement system is a coordinated cascade of serum proteins that attach to the outer membranes of pathogens. This process, called opsonization, targets the pathogen for destruction and makes it easier for phagocytic white blood cells to engulf it. Opsonization does this by neutralizing the invading cell's negative electrical charge, which would otherwise repel the natural negative charge of the immune system cell. Complement facilitated defense can also be achieved through the use of a different set of proteins (specifically C5-C9) to create a membrane attack complex that perforates the pathogen's cell membrane, effectively destroying it. Since complement protein binding is nonspecific, the body must also produce serum proteins and membrane-bound molecules that protect its own cells from its destructive actions.

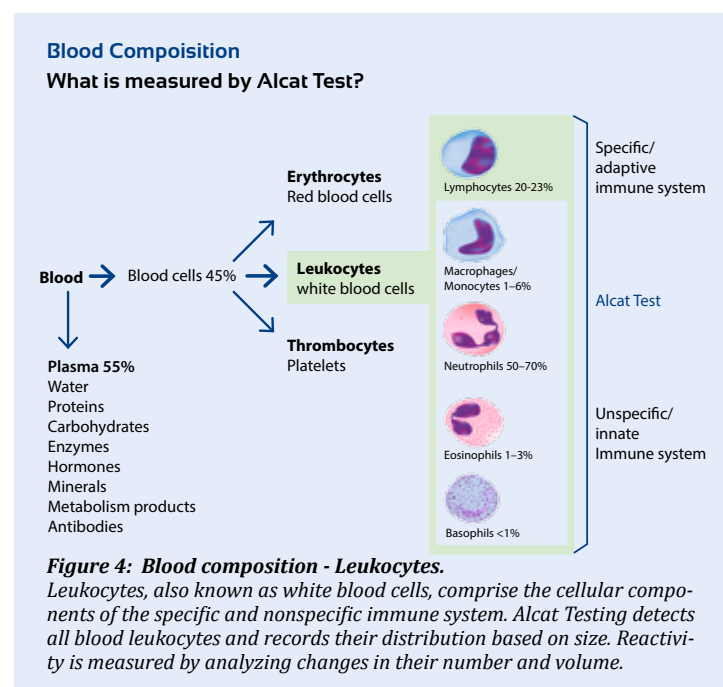
Cell fragments and other debris must be continuously removed from the body. This is accomplished by the process of phagocytosis – which literally means cell eating process. Neutrophils comprise the largest population of phagocytic cells of the innate immune system, representing 90-95% of granular white blood cells in the blood. The other granulocytes – monocytes, eosinophils and basophils – occur in much smaller numbers. Moreover, chronic activation of the innate immune system results in persistent inflammation and oxidative stress – factors that produce and perpetuate disease symptoms.

Neutrophils have a very short life span and are extremely aggressive after they are activated. They can destroy pathogens in many ways, primarily by bombarding them with cytotoxic oxygen and nitrogen radicals; through the production of acids such as hypochlorous acid; or through the activation of proteolytic enzymes including myeloperoxidases. (See A.2.3.)

As an inflammatory cascade arises, the immune system stimulates granulocyte activity through the activation of pattern recognition receptors located in the cell membranes and in the cytoplasm of immune system cells. (See Figure 4.) These cells contain a very large number of cytoplasmic granules – small membrane-bound vesicles that encapsulate infection-fighting substances including pro-inflammatory acids. Notably MPO is emerging as an important marker for CVD.

Frequently, these immune system cells undergo degranulation and lysis, producing a measurable change in their shape and also in the number of intact cells remaining. At the same time, cells communicate with one another and with other parts of the immune system, facilitating their coordinated response.

Alcat Testing is based on the understanding that food intolerances arise from activation of cellular responses of the innate immune system – the Firewall. Instead of perceiving them as harmless, the immune system responds to specific foods and other substances as though they were threats requiring aggressive targeting, which, in fact, they may be. For example, the CEO of Cell Science Systems reacts to cucumbers on the test. If he inadvertently consumes too much cucumber he will experience strong GI spasms and pain several hours later. Thus it would appear that activation of the innate immune system may, on the one hand, cause damage to otherwise healthy bystander cells through a host of inflammatory pathways; and, detect substances that might be of harm to the physiology through other non-inflammatory pathways, as yet, varied and unknown.



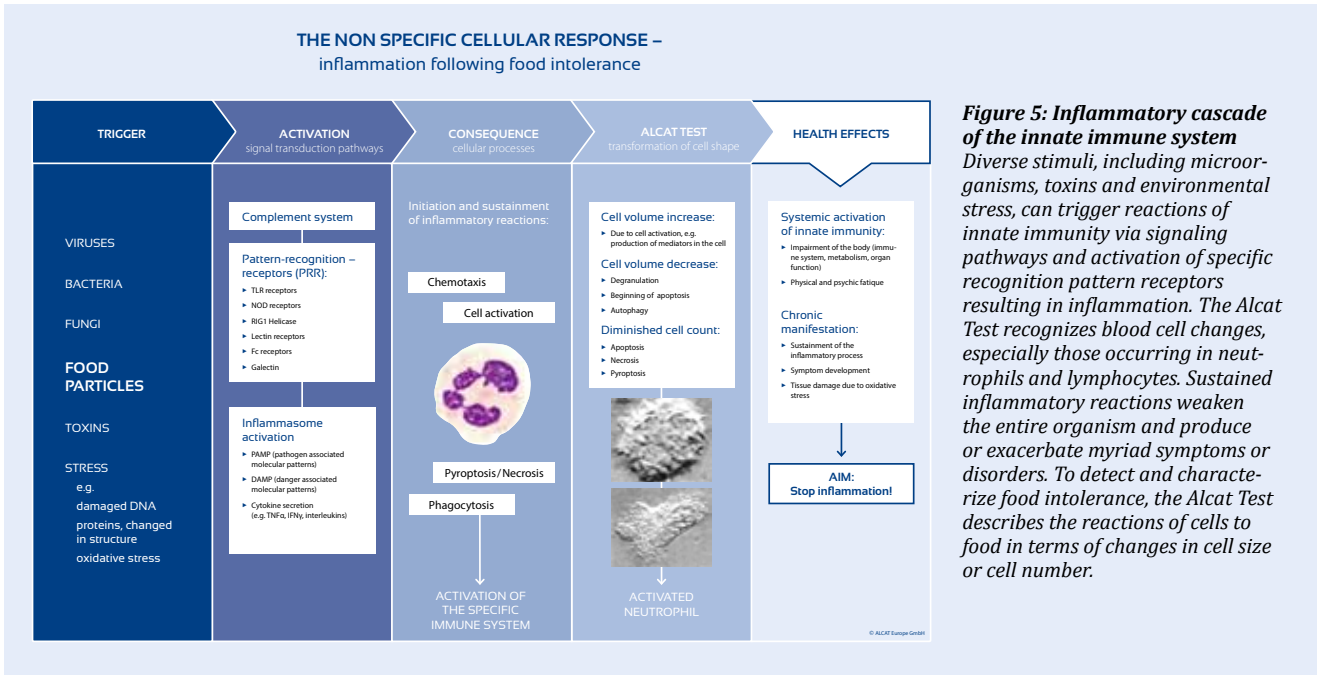


Figure 5: Inflammatory cascade of the innate immune system
Diverse stimuli, including microorganisms, toxins and environmental stress, can trigger reactions of innate immunity via signaling pathways and activation of specific recognition pattern receptors resulting in inflammation. The Alcat Test recognizes blood cell changes, especially those occurring in neutrophils and lymphocytes. Sustained inflammatory reactions weaken the entire organism and produce or exacerbate myriad symptoms or disorders. To detect and characterize food intolerance, the Alcat Test describes the reactions of cells to food in terms of changes in cell size or cell number.

Neutrophils play an especially important role in the body's defense against perceived invaders. However, sustained activation of these immune cells is destructive, promoting effects ranging from micro-inflammation of intestinal tissue to the type of chronic, widespread inflammation responsible for numerous disorders including gastrointestinal complaints, metabolic disorders, skin diseases, obesity, respiratory

diseases, neurological and musculoskeletal disorders. (see B.6, Tab. 1.)
The effects of chronic inflammation are often gradual but insidious, and we now appreciate that their long-term consequences are often severe and possibly life-threatening. For example, chronic intestinal inflammation increases the risk of cancer.

A.2.3 Molecular Processes of Granulocyte Activation

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

Gluten, found in grains like wheat, rye, barley and spelt is quite sticky. Gluten contains a 33 mer peptide, comprised of many proline and glutamine residues, yielding a structure that is indigestible by pancreatic and brush border enzymes. In fact, the familiar texture of bread arises from the creation of countless sticky, stretchy bubbles of gluten as carbon dioxide is released from fermenting yeast cells.

The gluten peptide, indigestible as it is, may or may not trigger lymphocyte activation depending upon genetic antecedents, particularly, HLA DQ2 and DQ8, which encode for MHC II molecules on antigen presenting cells. APC presentation of gluten to lymphocytes in the lumen of the gut sets the stage for celiac disease (i.e., crypt cell hyperplasia and destruction of villi). Fortunately, celiac is fairly rare, but it should

be screened for testing for DQ2/8 alleles has almost 100% negative predictability. However, sensitivity to gluten can produce similar symptoms, does not require these genetic antecedents, and is mediated by the innate immune system. Alcat testing is indicated for detection of gluten and other grain and substance (i.e., FODMAPS) induced sensitivity.

Other prominent receptors include CLRs (C-type lectin receptors), RLRs (RIG-I-like receptors) and NODs (NOD-like receptors). The latter participate in the formation of the inflammasome – a multi-protein complex identified in 2002 as a particularly active component of innate immunity responsible for the promotion of pro-inflammatory cytokines including Interleukin-1B and Interleukin-18. The inflammasome therefore plays a key role in initiating inflammatory processes (See Inflammasome B.7).

After a neutrophil is activated, one of its internal enzyme families, NADPH-Oxidase, converts oxygen into highly reactive forms including hydrogen peroxide, the same substance we keep in our first aid kits to disinfect wounds. These reactions take place within

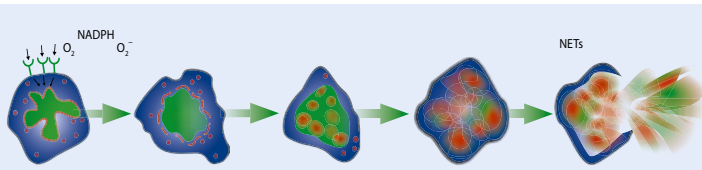


Figure 6: Activation of neutrophils and NET formation.

In response to activation of signaling molecules of the innate immune system, the enzyme NADPH oxidase converts oxygen into highly reactive forms (step 1), dissolving the nuclear membrane and granules. (steps 2) The contents mix with cellular fluid (step 3), and the cell membrane breaks down after about 2 hours, ejecting the NET and engulfing nearby pathogens (step 4).

the neutrophil's cytoplasmic granules as they dissolve and release their contents into the cell's interior. When these compounds combine with the cell's other components, the cell swells to bursting, expelling the reactive compounds that formed in the cell to fight the invading substance. The released compounds include reactive oxygen species, oxidative enzymes, and inflammatory mediators (Fig. 6).

The activated cell dies as a consequence of one of three possible pathways; necrosis, apoptosis or; if the formation of "NET's are involved, pyroptosis. Pyroptosis is a special form of apoptosis, often referred to as programmed cell death, a natural process associated with activation of the inflammasome.

Researchers at the Max Planck Institute for Infection Biology in Berlin have shed light on a unique mechanism of cell lysis that's triggered when neutrophils attack: the ejection of neutrophil extracellular traps, commonly called NETs.

NETs are composed of a mixture of reactive cell contents and neutrophil DNA (Fig. 6, 7). Thus, pathogenic bacteria are literally captured in the NET where they are immobilized and rendered harmless. Professor Arturo Zychlinsky of the MPI-IB Berlin explains, "In the NETs nearly as many bacteria die as are digested by neutrophils." The dying cells sacrifice themselves for

A.2.4 Bowel, Immune System and Intolerance

Whether the bowel is healthy and able to fulfill its important tasks depends on several factors including the physical integrity of the intestinal barrier; the vitality and appropriate diversity of the intestinal microecology, characterized by the presence of beneficial commensal microflora; and the biochemical properties of the intestinal environment.

The composition of the bowel's resident microbial flora has a huge impact on whether nutrients can be properly broken down, and absorbed or whether dysfunctions arise including gas formation, the reduced degradation of biogenic amines etc.

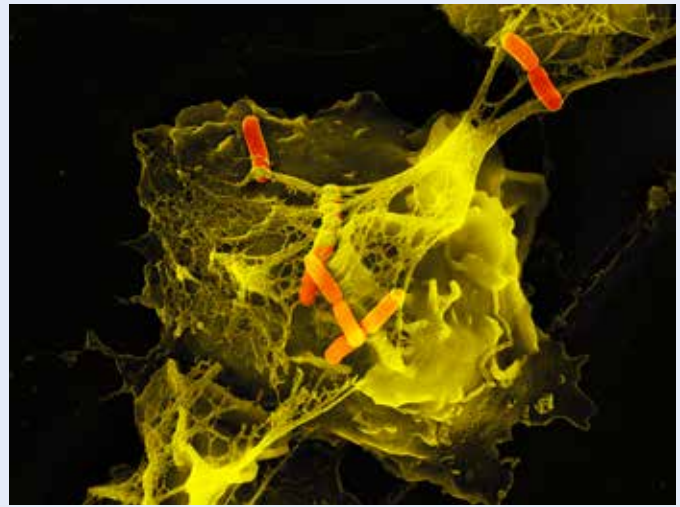


Figure 7: Neutrophils exhibit a unique defense mechanism by expelling NETs (Neutrophil Extracellular Traps)

Colored scanning electron microscopy image showing an activated neutrophil ejecting a neutrophil extracellular trap, or NET, to capture *Shigella* species bacteria (orange rods). NETs are a mixture of reactive cell contents including enzymes, reactive oxygen molecules and neutrophil DNA. NETs can rapidly disarm and immobilize pathogens until they can be removed by macrophages.

the greater good, transforming strands of their own DNA into protective traps. This is fine unless, due to a deficiency in an enzyme called, DNase, whereby the NET's cannot be removed from the circulation by the monocyte/macrophage system; or, with respect to apoptosis or pyroptosis, the neutrophil DNA is not adequately cleared, perhaps owing to an overwhelming of the monocyte/macrophage system, or, another defect in the immune system, such as C1q deficiency, the DNA from the activated cell can itself become immunogenic, as observed in both Lupus and rheumatoid arthritis. Thus chronic activation of the innate immune system may lead to development of autoimmune disorders.

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

The Intestinal Barrier and the Gut Associated Immune System (GALT – Gut Associated Lymphoid Tissue)

Damage to the intestinal barrier may lead to a wide array of health problems. Before exiting the intes-

tines for passage into the body, nutrient contents from food are meant to be broken down into specific functional units. When properly metabolized, these intermediate nutritional products are less likely to directly antagonize the immune system. However, when larger, improperly metabolized food particles (including small peptides, fatty acid complexes, oligosaccharides and mineral complexes that should be processed by specific functional transporters in the intestinal wall) are instead exposed directly to the cells of the immune system, where they may provoke destructive reactions.

An increasing number of studies confirm a close association between dysfunctions of the intestinal barrier and conditions such as obesity and fatty liver disease. The causes of altered interstitial permeability have not yet been completely elucidated. For example, it remains unclear whether a disrupted intestinal barrier is the trigger of a disease process, or its result. Perhaps its both. Widely acknowledged triggers include industrially modified foods and stresses or imbalances in the population of intestinal flora caused by alcohol, drugs, poor diet and fungal infection as well as inflammation. (See Figure 8.)

The intestinal wall comprises approximately 400 square meters (equivalent to about 4300 square feet) of complex structured epithelial tissues that perform numerous vital functions. It must enable the efficient absorption of nutrients from ingested foods and fluids while simultaneously preventing the entry of bacteria on the one hand and tolerating harmless antigens on the other. The intestinal wall continually negotiates the exchange of macromolecules between the external environment and the inside of the body.

Intestinal epithelial cells form a dense cell structure secured with extra “locks” that provide extremely tight junctions. Narrow strips of membrane proteins (e.g., claudins, CLDNs, occludins, and OCLNs) (1) between epithelial cells create a paracellular diffusion barrier and provide for proper cellular polarity, a prerequisite for directional mass transport.

Zonulin is an endogenous protein that modulates the permeability of these cellular junctions. In effect, it acts as a “doorman,” by selectively either allowing or disallowing the passage of substances through the intestinal barrier. Recent research suggests that zonulin can be activated by gluten and induce the opening of the intestinal barrier at inappropriate times. By permitting incompletely metabolized mac-



Abb. 8: Multiple factors can promote the emergence of food intolerance.

romolecules to pass through the barrier, it plays a potentially crucial role in the course of celiac disease, other inflammatory conditions, and can lead to auto inflammation as well as other manifestations of auto immunity..

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

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

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

A 2.5 Understanding Allergy and Intolerance

The term “allergy” is often used as a catchall 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 (“The Targeted Response” described earlier) while the broader category of intolerance is generally associated with nonspecific immunity (“The Firewall”).

True allergies are associated with immunological memory – an important feature of the specific immune system. When the body is exposed to an unfamiliar antigen, a complex, adaptive process may result in the formation of novel antibodies. These are literally bioengineered by the specific immune system to selectively bind to the triggering antigen. The creation of new antibodies is a remarkable process that takes place within B lymphocytes through the modification of DNA sequences that govern the shape – and therefore the binding properties – of specific portions of immunoglobulin molecules. Once the DNA regions that encode the new antibody have been successfully programmed, the specific B lymphocytes that produce it rapidly proliferate to generate a therapeutically adequate supply.

After the new antibody has helped neutralize the threat, a small number of these reprogrammed B cells persist in the body. If the same or very similar antigen is encountered at a later date, the immune system can recall the results of its previously successful antibody reprogramming experiments and quickly clone a large population of cells that already know how to make the required antibody. Memory cells therefore constitute a circulating archive of adaptive immunological experience and intelligence. Vaccinations deliberately introduce provocative antigens into the body to stimulate antibody formation and memory, proactively conferring a level of sustained protection without actually having to encounter a dangerous pathogen or reactive substance. Sometimes, this protective inoculation happens naturally. The great “Spanish Flu Epidemic” of 1918 killed tens of millions of people around the world. But most individuals who had contracted a similar but less virulent form of the virus a year or two earlier survived because they were able to rapidly mount a protective immune response based on adaptation to their prior exposure.

Allergy is defined as an altered immune response to normally harmless substances – collectively referred to as allergens – including foods, non-food proteins like latex, and protein-interacting toxins such as those

found in poison ivy and other natural irritants. In the case of repeated contact and binding of the allergen, immediate and pronounced symptoms can arise due to activation of mast cells, mediated by serological factors – particularly by immunoglobulin E (IgE).

Allergens are mainly delivered to the body through the respiratory and the GI tracts, and the skin. Some examples of allergens include pollen grains, dust mites, and bee venom. While there are exceptions, including peanuts, shellfish, and strawberries, most foods do not produce true, IgE-mediated Gell and Coombs type I allergic responses and instead produce the sort of food intolerances we have been discussing.

The symptoms of allergy such as rash, watery eyes, coughing, sneezing, asthma, nausea or vomiting, even including anaphylactic shock, usually follow within minutes of exposure to the triggering allergen. Hence, true, “allergic” reactions, when they occur against a food, produce dramatic and obvious symptoms; and, are therefore, easy to diagnose from a history. It is interesting to note that this so called allergic pathway represents our natural defense against helminthic (worms) infection. Worms are quite large, robust and foreboding, when compared against an immune cell. Hence, the David like immune cell must arouse a dramatic response against such a Goliathian foe. As already mentioned many times, the Alcat test does not detect “true” allergy” but, rather intolerance, or sensitivity, mediated by the innate rather than the specific, branch of the immune system. These reactions are more subtle, symptom onset is delayed and usually more chronic than acute. Hence they are not easily detected and are not usually detected at all by classical allergy tests, be they skin tests or RAST. (tests for IgE mediated, also know as Gell & Coombs Type 1 reactions). Despite the frequent use of the word “allergy” to describe a broad range of symptoms, the prevalence of true allergies in the population is relatively small – only about 2.5%. (See Figure 3.) However, intolerances or sensitivities, which are detected by the Alcat Test, are quite common.

Insert:

The allergy pathway and the role of IgE

1. An antigen capturing and presenting cell (primarily macrophages and dendritic cells) takes up the allergen and degrades it.
2. The allergen binds to a major histocompatibility complex molecule (MHC II molecule) and the MHC/allergen complex migrates to the cell surface membrane.
3. MHC class II molecules present allergens or pathogen components to CD4+ lymphocytes.
4. A T-Helper 2 lymphocyte (a type of lymphocyte that matures in the thymus gland) with a conforming receptor will recognize the antigen peptide/MHC complex.
5. Activation of the T cell requires not only this interaction, but also a co-stimulatory signal, usually involving a B7 molecule on the Antigen Presenting Cell (macrophage) and a CD 28 receptor on the T-cell.
6. The T-cells, once activated, proceed to chemically instruct those few B-lymphocytes that also possess surface receptors conforming to the initiating allergen's peptide sequence.
7. In the case of Type I allergy, the chemical message from T-cell to B-cell is usually mediated by Interleukin 4 (IL4).
8. Activated B-cells mature into plasmacytes, B-cells programmed to rapidly manufacture a specific antibody, and which continue producing antibody molecules specific to the initiating allergen peptides.
9. These cells subsequently clone themselves as rapidly as their genetic machinery and the level of available and utilizable nutrients will allow. Each new cell continues to manufacture and secrete soluble forms of the specific receptor molecule, namely, allergen specific IgE antibodies that conform to the peptide structures of the initiating allergen.
10. T and B lymphocytes that persist in circulation following the resolution of an infection (or allergic exposure) are termed memory cells.
11. T and B cells are notably allergen or pathogen-specific in terms of their recognition mechanisms. Consequently, there will only be a few memory T and B cells that initially recognize the specific antigen. However, these cells are able to rapidly clone themselves and quickly increase their population.
12. Once in circulation, IgE antibodies bind to surface fc receptors on mast cells distributed throughout the connective tissue of the skin and mucosal linings of the respiratory, GI and GU tract. They also bind basophils, as well as other types of cells, in circulation. These cells possess internal pre-formed mediator-containing granules, including histamine.
13. When 2 or more cell-bound IgE antibodies subsequently encounter the specific inciting allergen, their binding and cross-linking induces aggregation of cell membrane receptors, which in turn activates various enzymes and kinases, leading to the activation of gene expression factors such as NF kappa, resulting in the release of these mediators from the mast cells and basophils in a process known as degranulation.
14. Inflammatory processes soon follow and consist of increased blood vessel permeability, mucous secretion, irritation of nerve endings, and smooth muscle constriction – all common traits of a true allergic response.
15. The “late phase” reaction, mediated by newly formed lipid mediators, perpetuates the allergic response. These mediators include products of arachidonic acid metabolism as well as leukotrienes generated via the lipoxygenase pathway; prostaglandins produced through the cyclo-oxygenase pathway; bradykinins; and various other substances.
16. Both pre-formed and newly synthesized lipid mediators, as well as cytokines including IL3, IL5 and granulocyte macrophage-colony stimulating factor, attract inflammatory cells – primarily basophils and eosinophils (but not neutrophils) – to the local site of the reaction.

Table: 1 describes the role of IgE in the pathogenesis of the allergic response.

A.3 History

A.3.1 Immunology of Allergy and Intolerance

Baron Clemens von Pirquet, a Viennese pediatrician, introduced the term allergy at the beginning of the last century. At that time, he understood allergy very broadly as an "altered reaction".

Today, the term allergy describes the immunological overreaction of the body to otherwise harmless antigens. It primarily refers to the classical type I allergy associated with the interaction of antibodies by mast cells (IgE-mediated immediate type).

In 1963, Coombs and Gell proposed a model of allergy that distinguished between 4 clinical types, identifying them with the Roman numerals I to IV. In their system, food allergy was generally assigned to type I ("immediate IgE mediated hypersensitivity response"). However, the Alcat Test is concerned with functional molecular biological mechanisms and does not detect allergies. As such, the Alcat Test does not classify its results using the 4 traditional Coombs and Gell categories. (See B.6).

The concept of “intolerance” was first elucidated in 1920 by John Freeman and was described in relation to food as “toxic idiopathy”. Freeman, who together with Leonard Noon, are considered the founder of immunotherapy, stated,

“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.”
(From an address on Toxic Idiopathies delivered at The Royal Society of Medicine, 1920).

At present, there is no uniform definition of intolerance, which can arise from at least 4 or 5 different sources:

1. Genetic factors, e.g., GLUT-5-fructose malabsorption and celiac disease
2. Enzymatic deficiencies and dysfunctions, e.g., lactase deficiency
3. Immunological reactions
4. Pharmacologically mediated reactions
5. Toxic reactions. All plants contain natural toxins to protect against pests. If we have had numerous generations of exposure to the food then we have most likely evolved the enzymes to detoxify these components; however, today, we eat novel foods, genetically modified foods and food like substances, to which the body is not accustomed.

Because of the intrinsic diversity and complexity of the phenomena associated with intolerance, many different approaches have been developed for its definition, testing and treatment. Naturally, each approach tends to reflect the methods and beliefs of their various proponents.

This diversity of perspectives is welcome. However, many specific approaches continue to be strongly influenced by the framework of classical allergy theory and often fail to include current discoveries in clinical immunological research and certain historically significant lines of research. For example, despite a long history of published research dating back as far as 1917, many approaches fail to take into account the role of the innate immune system and new objective testing methods. They also fail to integrate essential current research concerning the role of the innate immune system and its vital relationship to systemic inflammation.

By continuously integrating emerging knowledge and combining a deeper understanding of molecular system such as pattern recognition receptors and the inflammasome (See B.8, 25) with a growing body of sophisticated clinical observations, we can dynamically reevaluate our long-standing assumptions about immunological mechanisms, deepen our understanding of fundamental processes, and update our approaches to testing treatment.

Broadening our vision to encompass diverse multiple disciplines and integrate emerging research is a continuous process. We remain focused on helping practitioners to work more effectively with their patients, and helping patients deal more effectively with their health challenges.

A.3.2 Corporate History

In 2004 Cell Science Systems, Corporation (CSS) acquired the technology and assets of the organization that invented the Alcat Test. Development of the technology had begun in 1984, completing an initial round of clinical studies in 1988. Since that time, the Alcat Test has been scrutinized in more than 30 different studies and its clinical utility and efficacy have been demonstrated in more than 38 peer-reviewed publications.

Located in Potsdam, Germany, close to Berlin, Alcat Europe GmbH was founded in 2007 to serve the European community with Alcat Testing services, patient and practitioner education and support, and ongoing research.

The organisation is currently establishing a laboratory in Asia.



A.4. Case Studies

This section presents selected case studies in which the Alcat Test was used as a diagnostic tool to identify food intolerance and inform dietary management.

A selection of observational studies and patient reports from Europe and the United States is presented in Appendix 2.0.

A.4.1 Case Study 1 – Skin

- ▶ *Dr. Fiorenzo Angehrn, Director, Klinik Piano, Bienne, Switzerland. Specialist in surgery and phlebology (vein disorders).*

The outer skin is immunologically related to the intestinal mucosa, which may be thought of as the body's inner skin. Therefore, disturbances in the gut may present as blemishes, acne or skin diseases such as eczema, atopic dermatitis and urticaria.

Medial History: Patient with atopic dermatitis at various body sites (Fig. 9).

Treatment: Identification of incompatible foods using the Alcat Test and instituting a corresponding elimination/rotation diet based on test results.

Results: After 14 weeks, the patient was symptom-free. Using a 22 MHz ultrasound device –the Osteoson Collagenoson "ICU" (Minors) – skin thickness and skin density were documented with the corresponding collagen points in the dermis (OHD value).

A.4.2 Case Study 2 – Pancreatitis

- ▶ *Angela Käßner, Berlin (Germany) – Naturopathic practitioner, lecturer, reviewer*

Medial History: Patient, age 47, had a history of two years of recurrent abdominal pain. Gastroscopy, colonoscopy, CT, MRI and conventional laboratory analysis yielded no significant findings and painkillers were ineffective.

Diagnosis and Treatment: Food intolerance, resulting in chronic pancreatitis. IgG test and Alcat Test were performed and an elimination/rotation diet was prescribed based on test results.

Results: Compliance with the Alcat diet produced freedom from pain and symptoms. Following the prescribed diet period, most foods could be reintroduced without adverse reactions. The IgG test did not detect the most important highly reactive foods previously identified by the Alcat Test.

A.4.3 Case Study 3 – Rheumatoid Arthritis / severe overweight

- ▶ *Dr. David Blyweiss, MD – Functional medicine, private clinic, Boca Raton, FL (USA)*

Medical history: Patient, age 54, 5'4" (162 cm), 169 pounds (77) kg was diagnosed with rheumatoid arthritis. Drug treatment with Humira, methotrexate, Enbrel and prednisolone failed to produce pain relief and caused severe side effects.

Symptoms: Joint pain, fatigue, intolerance to cold, insomnia, swollen tongue, pain resulting from rheumatoid swelling in the finger joints.

Laboratory findings: ANA-test negative, HbA1c 5.8%, RA factor 8.7 IU/mL, Vitamin D 25-hydroxy 20.5 ng/ml, Alcat Test identifying a specific food intolerance profile.

Treatment: Strict adherence to elimination/rotation diet based on Alcat Test results.

Results: Significant improvement of symptoms after 6 months. After 9 months, the only remaining pain was in the right hip in the a.m. or after gardening. In addition, 37 pound (17 kg) weight loss and improvement in blood values: HbA1c 5.4%, RA factor 5.6 IU/mL, Vitamin D 24.9 ng/ml

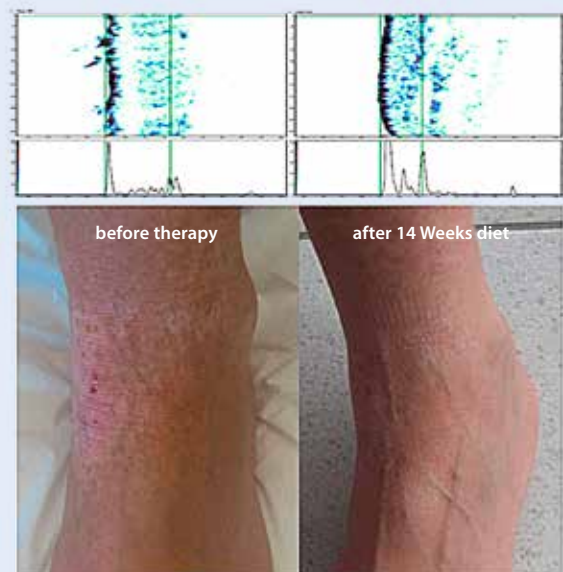


Figure 9: Skin condition before the start of treatment and after 14 weeks.
Up Ultrasound/Collagenoson – below light level imaging

B. STUDIES

B.1 Double-Blind Studies as a Tool for Clinical Evaluation

The rigorous evaluation of a drug, diagnostic test or procedure generally involves conducting randomized, double blind, placebo-controlled trials, which are recognized as the most reliable and valid measures of clinical efficacy. Rigorous research meth-

ods include the careful selection of subjects based on relevant inclusion and exclusion criteria, collection of sufficient data, and analysis to determine statistically significant outcomes.

B.2 Using Double-Blind Studies to Evaluate the Alcat Test for Food Intolerances



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 arena of food allergy and food intolerance, the double-blind, placebo-controlled food challenge (DB-PCFC) is the gold standard for diagnostic testing. Certain foods are eliminated from the diet and individually reintroduced at pre-determined time intervals. Because symptoms are often delayed, an objective assessment is the only reliable way to identify a true connection between food consumption and symptoms. In rigorous research, neither the patient nor the doctor knows the composition of the patient's test meal or test results. The evaluation is completed 48 hours later when the doctor determines whether the patient has reacted to the food.

In a double-blind study the results of the Alcat Test for food intolerance 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 10 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*, available at www.alcat.com.

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.

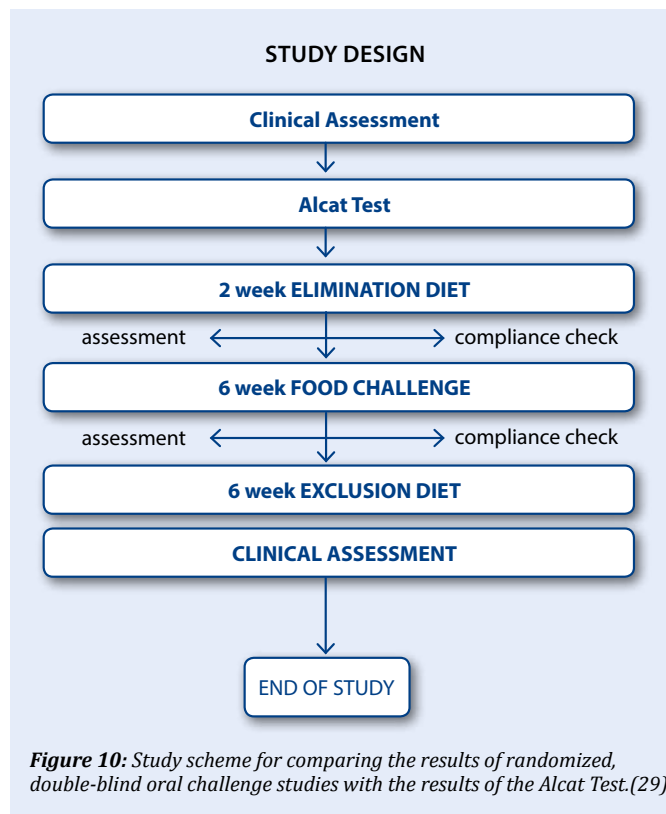


Figure 10: Study scheme for comparing the results of randomized, double-blind oral challenge studies with the results of the Alcat Test.(29)

B.3 Double-Blind Studies of the Alcat Test

B.3.1 Two Basic Studies With Food – UK 1988

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

Two successive double-blind studies were conducted following the protocol summarized in the flowchart shown in Figure 10, above. The pilot study enrolled 53 patients, each suffering from multiple symptoms resistant to conventional diagnosis. The second study enrolled 22 patients diagnosed with irritable bowel syndrome (IBS).

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

Results of pilot study and main study:

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

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

It is important to note that IBS is a complex disease 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:

- ▶ 1. *The average correlation between Alcat Test and DBPCFCs was 83.4%, demonstrating the efficacy of the Alcat Test for detection of incompatible, immunoreactive food.*
- ▶ 2. *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.*
- ▶ 3. *The Alcat Test makes it easier for the clinician to create a highly effective elimination and exclusion diet individually tailored to the patient.*
- ▶ 4. *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.(29)

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

About the study directors:

Dr. Peter Fell

At the time of the study, Dr. Fell was director of the Allergy Centre in Deddington, Oxfordshire. A clinical pharmacologist, Dr. Fell served as director of Fisons, which today is part of Thermo Fisher Scientific. Dr. Fell was responsible for obtaining FDA approval for the use of disodium chromoglycate-based therapies in the United States.

Prof. Dr. Jonathan Brostoff

Dr. Brostoff was the founder and professor of Allergy and Environmental Health Department at University College, London, Director of the Centre for Allergy Research and leader of the Diagnostic Immunology Laboratory of the University College, London Hospital. Presently, he is Professor Emeritus of Allergy and Environmental Health at King's College, London. Professor Brostoff is considered an expert in the field of allergy and food intolerance and is the author of several books including Food Allergy and Intolerance and Food Allergies and Food Intolerance.

The results of the two studies described here were presented at several professional conferences and were subsequently published.

Individual publications on Alcat studies by Dr. Peter Fell, Professor Jonathan Brostoff, et al, 1988-1992.

▶ ***1. High Correlation of the Alcat Test Results with Double-blind Challenge (DBC) in Food Sensitivity (27)***

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

▶ ***2. Alcat a new test for food induced problems in medicine? (28)***

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

▶ ***3. Alcat® – a new cellular test for food sensitivity (29)***

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

▶ ***4. Cellular responses to food in irritable bowel syndrome – an investigation of the Alcat Test (30)***

Publication of pooled study results in the Journal of Nutritional Medicine, Vol. 2, No. 2, 1991

B.3.2 Double-Blind Study of Food Additives - Denmark 1996

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

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

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

▶ ***Diagnostic Value of Alcat Test in intolerance to food additives compared with double-blind placebo-controlled (DBPC) oral challenges (31)***

Presented at 52nd Annual Meeting of the American Academy of Allergy, Asthma & Immunology, New Orleans, LA. March 15-20, 1996. Publication in the Congress Proceedings of the Journal of Allergy and Clinical Immunology 1 (3).

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

B.4 Alcat Test Validation Studies

Multiple studies have been carried out in different areas and published in peer-reviewed journals.

► 1. *Reproducibility of the Alcat Test (32)*

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 Harris, a colleague of Dr. Potter, evaluated and further documented the high reproducibility in a subsequent statement (see Appendix 2).

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.

► 2. *Reproducibility of the Antigen Leukocyte Cellular Antibody test (Alcat) – Statistical Analysis, Summary Statistics & Scientific Report (33)*

Summary: A second Alcat reproducibility study was conducted at the University in Bloemfontein, South Africa by Dr. WML Neetling and Dr. AM Kachelhoffer, January to April, 1998.

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

The study demonstrated 92% reproducibility.

► 3. *Parexel Medstat Final Statistical Report – Study of the Alcat Test in 10 subjects (34)*

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

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

Congruence: The study demonstrated 78% agreement between test results in Cohort A and 86% agreement in Cohort B.

Reproducibility: A 97% reproducibility of results was demonstrated in Cohort A and 99% reproducibility in Cohort B.

Overall, 983 of 1,000 Alcat Test data points (98.3%) were reproducible under the conditions investigated by the study. The study demonstrated the statistically significant reproducibility of the Alcat Test results.

► 4. *Study Comparing Alcat Test Results With Flow Cytometry and Microscopy (35)*

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

Summary: This study compared the cellular analyses produced by the Robocat II device used for Alcat Testing with results produced by conventional laser flow cytometry (FASCalibur) and optical microscopy (Cytovita). The study demonstrated that Alcat analyses were comparable to those of laser based flow cytometry and comported with the visual data produced by optical microscopy and concluded that the Robocat II device is suitable for the analysis of cell populations and volume changes in WBC's.

B.5 Further Subject-Specific International Studies of the Alcat Test

Multiple studies have been carried out in different areas and published in peer-reviewed journals. Key publications are summarized below.

► 1. *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.

► 2. *Alcat Test Results in the Treatment of Gastro-intestinal 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.

► 3. *Rational Management of Food Intolerance in Elite Athletes*

Citation: Angelini et al. Rational Management of Food Intolerance in an Elite Soccer Club. *Journal of the International Society of Sports Nutrition* 2011, 8 (Suppl 1):36.

Summary: A team of investigators led by Fabrizio Angelini studied eight elite European athletes selected for symptoms suspected of being related to food intolerance (e.g., headache, gastrointestinal discomfort, constipation, diarrhea, intestinal bloating, and nausea). The Alcat Test was performed and test based-elimination diets were prescribed. The athletes were followed for eight months and retested. There was significant improvement in the athletes' condition and reduction in inflammatory response demonstrated by improvement in body composition, symptoms, and exercise capacity.

► 4. *Alcat Test Identifies Food Intolerance in Patients with Gastrointestinal Symptoms*

Citation: Berardi et al. Study presented at the XXVIII Congress of the European Academy of Allergy & Clin-

ical 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.

► 5. *Food intolerance in patients with cutaneous diseases: diagnostic value of the Alcat Test*

Citation: Berardi, L¹; De Amici, M¹; Vignini, A²; Torre, C¹; Mosca, M². Food intolerance in patients with cutaneous diseases: diagnostic value of the Alcat Test. Study presented at the XXVIII 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.

¹ Foundation IRCCS Policlinico S. Matteo, Department of Pediatrics, Pavia, Italy

² Foundation IRCCS Policlinico S. Matteo, Department of Dermatology, Pavia, Italy

Summary: The aim of this study was to evaluate whether food intolerance is associated with cutaneous diseases such as chronic urticaria, angioedema, itching, and dermatitis, and to evaluate the diagnostic value of the Alcat Test in such cases. A group of twenty (20) patients with cutaneous diseases, presenting with chronic urticaria (1%), itching (2%) or dermatitis (17%), with negative allergic tests (prick and/or RAST) were tested. No subject was under treatment with systemic corticosteroids, antihistamines or anticoagulants. Six patients were lost to follow-up. The remaining patients were Alcat Tested and answered a survey about their 2-month elimination diets based on Alcat results. The majority (86%) exhibited a dramatic improvement in symptoms, while 14% showed no change or did not follow the diet. The Alcat Test appears to have diagnostic value in detecting food intolerance.

► 6. *The Effect of the Alcat Test Diet Therapy for Food Sensitivity in Patients with Obesity*

Citation: Akmal et al., *Middle East Journal of Family Medicine*. April 2009 - Vol. 7, Issue 3.

Summary: 27 obese patients who experienced no weight loss using caloric reduction diets were tested for intolerance to 100 foods.

Significant positive changes in body weight, body fat and body composition were observed, as well as improvements in related health problems.

These results confirmed the value of the Alcat Test and elimination diet in helping address obesity, gastrointestinal reflux, chronic fatigue, headache and other chronic disorders.

► *7. IMS Health Economics and Outcomes Research—Influence of Food Intolerance in Migraines: Final Report of Statistical Results*

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

Summary: 21 migraine patients who had positive results in the Alcat Test for at least one food were studied. The number of migraine attacks that occurred while following a 3-month Alcat diet phase eliminating 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.

► *8. A Comparison of the Alcat Test for Food Reactions Amongst 2 Population Sub-Groups*

Citation: Study by Dr. DH Sandberg and Dr. MJ Pasula, 45th Annual Congress of the American College of Allergy and Immunology, Los Angeles, CA November 12-16, 1998, published in the Annals of Allergy.

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

► *9. 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 under water displacement (fat-mus-

cle ratio) was recorded for all patients and an Alcat Test was performed to inform patients about their specific food intolerances. Thereafter, patients were randomly divided into two groups. The control group chose their own calorie restricted diets for 4 weeks while the Alcat Test group followed rotation/elimination diets based on their specific test results. These patients also received dietary counseling. The Alcat group lost significantly ($p < .001$) more weight and body fat than did the control group. The Alcat group also realized body composition improvements characterized by greater increases in lean body mass ($p < .001$) than were observed in the control group. 98% of the subjects following the Alcat Test eating plan experienced short term improvement in scale weight and/or body composition.

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

► *10. 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. 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). Skin problems also improved..

► *11. Prevalence of food allergy and intolerance in children based on MAST CLA and Alcat Tests*

Citation: Buczylo et al., Roczn Akad Med Bialymst. 1995; 40(3):452-456.

Summary: This single-blind study of 56 children aged 5 months to 16 years (average age 7.2 years) with various allergies sought to compare the most common food allergies (using MAST CLA and skin prick test) and the most common food intolerances (using the Alcat Test). The most common food allergies significantly differed from those described in the literature, i.e., protein and dairy. The most frequently observed food allergies were nuts, peanuts, wheat and whole grains. Food intolerances in response to apples, tea and cola were documented.

► 12. Outcome Study in 353 Consecutive Patients Following The Alcat Diet

Citation: Observational study conducted in Copenhagen at the Allergy Clinic Charlottenlund, Denmark 1998 unpublished).

Summary: The results of 353 consecutive patients who were following diets based on their Alcat Test responses are presented in the table on the left.

Symptom	Significant Improvement	Some Improvement	Mild Improvement	Condition Worsened	Diet not Followed	Percent Improved
Rhinitis/Sinusitis Number of Patients: 37	30	5	1		1	95
Atopic eczema Number of Patients: 72	63	5	2	2		94
Urticaria Number of Patients: 24	19	1	2	2		83
Irritable bowel syndrome Number of Patients: 46	32	8	5	1		87
Migraines, headache Number of Patients: 21	20		1			95
Polyarthritits Number of Patients: 20	14	3	2		1	85
Asthma Number of Patients: 27	19	7	1			96
Angioedema Number of Patients: 27	19	2				100
Multi-organ-syndrome Number of Patients: 40	21	9	5		2	75

Benefits of the Alcat diet were assessed as follows:

- 313 Patients: Completely or nearly symptom free.
- 13 Patients: Little or no improvement.
- 18 Patients: Worsened symptoms.
- 9 Patients: Alcat Test diet was not followed.

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

► 13. Alcat Test Results in the Treatment of Respiratory and Gastrointestinal Symptoms, Arthritis, Skin and Central Nervous System

Citation: Mylek et al., Roczniki Akad Med Białymst. 1995; 40(3): 625-629.

Summary: This study followed 72 patients with complaints thought to be attributable to food intolerance. They were prescribed elimination diets based on their Alcat Test results. The results of the study showed an overall improvement in symptoms (number of patients expressed as a percentage) for arthritis (83%), urticaria, bronchitis, and gastroenteritis (75%), migraine (70%), chronic fatigue syndrome (60%), asthma (50%), atopic dermatitis (49%), rhinitis (47%) hyperactivity (32%).

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

► 14. Food Intolerance in Patients with Angioedema and Chronic Urticaria. An investigation by RAST and Alcat Test

Citation: Study by Dr. Lene Hoj, presented at the XVI European Congress of Allergy and Clinical Immunology, Madrid, Spain, June 25-30, 1995 and published in the European Journal of Allergy and Clinical Immunology, Supplement, no. 26, Vol. 50, 1995.

Summary: This study aim was to determine whether there was a relationship between angioedema and

chronic urticaria and food allergy or intolerance. Fifty two (52) patients were given an Alcat Test for 100 foods, and 18 typical allergens were tested by RAST. In addition, total IgE and eosinophil counts were measured.

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

► 15. Multiple pathogenic mechanisms in Food sensitivity Reactions in-vitro

Citation: Puccio et al., study presentation at the 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.

Serum immunoglobulin levels (IgA, IgM, IgG, IgG4) using turbidometry, were measured to establish baseline levels. Whole blood from the same patients was then incubated, with each of the respective food antigens at 36.60C 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.

Result Summary

	IgA 1	IgM 1	IgG 1	IgG 4	C3 2	C4 2	Spect 3
Beef	0	1	0	0	2	0	1
Corn	1	0	1	0	0	0	1
Egg	0	2	1	0	0	1	1
Milk	0	2	0	0	2	0	0
Orange	0	1	2	0	1	0	0
Peanut	1	3	0	3	2	0	2
Soybean	0	0	0	0	3	0	1
Tomato	2	1	0	0	3	0	0
Wheat	0	0	1	0	4	0	1
Yeast	0	2	0	0	2	0	0
TOTAL	4	12	5	3	19	1	7

1 Frequency of Immunoglobulin level change exceeded S.D. 2 for that food. 2 Frequency of Complement Activation for that food as measured by the height difference of the rocket peaks 3 Frequency of spectrophotometry reading of hemolysis exceeded S.D. 2 for that food.

Results were analyzed by calculating percent change from control values in each of the 90 tests per pathogenic mechanism. Scores falling outside the range of 2 standard deviations were: IgA = 4, IgM = 12, IgG = 5, IgG4 = 3. Marked hemolysis occurred in 7 tests; com-

plement activation was seen in 19 for C3 and in 3 for C4. Significant changes in one or more immunoglobulin and complement components occurred in every patient to one or more foods. In most subjects, multiple mechanisms were observed suggesting adverse reactions to foods commonly involve various triggers and pathways.

► 16. Influence of Food antigens on Volumes of Circulating White Blood Cells and Platelets 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 phytohemagglutinin was the first evidence that cytoplasm and it's 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. 1 Alcat Test computer produced histograms depict separate peaks for lymphocytes, polymorphonuclear cells, and a specific area effected by platelet aggregation. Vertical and horizontal dislocation of histograms depict cellular degranulation, cell enlargement, or disintegration upon in-vitro challenge with offending antigen.

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

Additional evaluation of Alcat Test 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.

▶ 8 antigens showed platelets reactivity without WBC changes of which 6 correlated with Double Blind Challenge and 2 did not.

The area of the Alcat Histogram showing platelet aggregation appears to depict an additional and possible independent mechanism in delayed reactions to foods which seems to add useful information to the WBC histograms.

▶ *17. 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: The Alcat Test was used in a busy primary care internal medicine practice to identify foods and molds as possible triggers of symptoms in 172 patients. Earlier methods for the identification of triggers consisted of a cytotoxic test with microscopic

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

A food elimination diet based on Alcat Test results was helpful in one case each of Crohn's Disease (80% improvement), and Alzheimer's Disease (80% improvement), and in two cases of children with attention deficit disorder (60% improvement) and hyperactivity (80% improvement).

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

1. **The Alcat is Specific to Each Patient** – a food that is not on the standard IBS or migraine diet, but which reactions with the patient, may be identified by Alcat.
2. **Alcat is Quick** – Alcat can test 100 foods in 4 hours. Four-day fasting and oral challenge can take as long as 25 days (at 3 to 4 foods a day).
3. **The Alcat is Simple** – the system sizes and counts approximately 4,000 – 8,000 cells in 6 seconds. A trained cytotoxic technician cannot visually identify the subtle cell size changes which are accurately identified by the Alcat System.
4. Alcat panels are predetermined.
5. The observed percentage of correlation for Alcat is:
 - ▶ 79.3% correlation with positive reacting foods.
 - ▶ 87 – 92% correlation with positive foods when platelet reacting foods are included.
 - ▶ 87% correlation with non-reacting foods.

Clinical condition	Average improvement (%)	Number of cases
Classic Migraine	82	9
Common Migraine	62	39
Sinus Headaches	58	23
Irritable Bowel Syndrome	71	68
Gastroesoph. Reflux	75	8
Inflamm. Arthritis	65	31
Inflamm. Arthritis	44	16
Asthma	30	18
Recurrent Sinusitis	59	20
Tension Fatigue Syndrome	60	97
Depression/Anxiety	31	14
Obesity	50	33
Eczema	55	11
Recurrent Vaginitis	20	7
Recurrent Urinary Infection	46	4
Allergic Rhinitis	42	108

► *18. Pilot Study into the Effect of Naturally Occurring Pharmacoeactive Agents on the Alcat Test*

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

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

The results showed significant differences between healthy subjects and migraine patients. Both groups reacted to histamine, however, the migraine patients were significantly more reactive to gluten, tryptamine, octomine, dopamine, lectin and chlorogenic acid. By elucidating white cell changes to substances likely to trigger migraine, the Alcat Test has the potential utility to assess toxic or pharmacological effects of other commonly occurring substances.

► *19. Inhibitory Effect of Sodium Cromoglycate on Granulocyte Response to Food Antigens In Vitro*

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

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

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

► *20. Gastrointestinal Complaints Related to Diet*

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

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

and would devote their talents to the study of this fascinating subject."

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

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

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

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

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

1. Complete Blood Count
2. Erythrocyte Sedimentation Rate
3. Liver Function tests
4. Chemistry Profile
5. Serum protein Electrophoresis
6. Serum immunoglobulins
7. Alpha-1-Antitrypsin
8. Blood Folic Acid
9. Vitamin B-12 Concentrations
10. Vitamins D and E
11. Sweat Chloride test
12. Lipoprotein Electrophoresis
13. Examination of Stools for Pathogenic Bacteria
14. Occult Blood
15. Reducing Substances and Ova and Parasites
16. Routine Urinalysis
17. Xylose Absorption test
18. Abdominal Sonography (ultrasound Exam)
19. Computerized tomography (CT Scan) Abdomen and Pelvis
20. Upper GI Barium Contrast Studies with Small Bowel Follow-through
21. Upper GI Pan endoscopy
22. Lactose breath test

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

► 21. *South African Outcome Study of Dr. Ian Geldenhuys*

Citation: Dr Geldenhuys, a practicing physician in 1997 in Johannesburg, described data collected from his patients treated with diets informed 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

► 22. *Allergie alimentari. Tecniche diagnostiche a confronto [Food allergy: comparison of diagnostic techniques]*

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

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

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

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

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

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

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

In conclusion, the Alcat is also useful for preparing a dietary program which simplifies the job of the clinician in establishing accurate elimination diets and exclusions, and finally the Alcat results (positive or negative) were correlated with the food challenge test in a considerable number of cases.

► 23. *Technical Study Comparing The Alcat Methodology With Activation of Granulocytes Following Challenge With Zymosan*

Summary: This study, conducted by Dr. Cristina Mele of the University of Rome, compared the Alcat method with the activation of granulocytes after zymosan challenge. Zymosan is derived from yeast cell walls (*Saccharomyces cerevisiae*) and is used for in vitro immunological evaluation of the phagocytic immune function. If cells are healthy, they respond to a challenge with zymosan by activating NADPH oxidase (through activation of cytochrome B and the cytosolic proteins P47, 67 and 21) to create an "oxidative burst" of superoxide anions. The superoxide anion is detectable and measurable using a luminometer.

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

► 24. *Autism – a multidisciplinary approach to treatment*

Citation: Kotsanis, CA et al. 1994.

Summary: This study was conducted under the direction of Dr. Constantine A. Kotsanis. Study results were presented at the annual meeting of the American Academy of Allergy Otolaryngology in 1994 and are available on the website of the Institute Kotsanis at <http://www.kotsanisinstitute.com/home/autism-treatment>

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

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

► 25. *Controversial Antigen Leucocyte Cellular Antibody test (Alcat): A Nonspecific Inhibitory Effect of Alpha Glycoproteins*

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

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

► 26. *Ogni Intervento Comincia a Tavola*

Citation: Mele Cristina, *Medici Oggi*, Maggio 2002: 210-213

Summary: In this study it was shown that an elimination diet based on Alcat Test results decreases markers of inflammation. After a period of 4 years, reactive foods were reintroduced. Many previously reactive foods were now tolerated while some remained reactive. Corresponding inflammatory markers (interleukins, TNF, interferon- γ) again increased significantly for these reactive foods. Increased levels of IL6, TNF and the hepatic synthesis of C reactive protein prevented weight loss despite consumption of a low calorie diet because of these metabolic disturbances.



B.6 Responding to Unjustified Criticism of the Alcat Test

Some critics have dismissed the utility and validity of the Alcat Test on various grounds. The arguments presented by critics are almost always based on recycled incorrect and misleading information and are mostly only opinions:

- ① *Nahrungsmittelallergie und -unverträglichkeit: Bewährte statt nicht evaluierte Diagnostik, Deutsches Ärzteblatt 2005; 102(27); Kleine-Tebbe, Jörg; Lepp, Ute; Niggemann, Bodo; Werfel, Thomas.*
- ② *In-vitro-Diagnostik von Nahrungsmittel-Allergien" J. Kleine-Tebbe, T. Fuchs, U. Lepp, B. Niggemann, J. Saloga, I. Vieluf, S. Vieths, T. Werfel, T. Zuberbier, L. Jäger. Allergo J 2001; 10: 333–339.*
- ③ *Unproven techniques in allergy diagnosis. J Invest Allergol Clin Immunol 2005; Vol. 15(2): 86-90; B. Wüthrich.*
- ④ *The Alcat Test – inappropriate in testing for food allergy in clinical practice. S Afr Med 1992, 81: 384.; Potter, PC., Mullineux, J. Weinberg, E.G., Haus, M., Ireland, P. Buys, C., Motala, C.*

In this section, we review these articles and address their criticisms, and clarifying the most common misrepresentations of Alcat technology.

- ① *"Nahrungsmittelallergie und -unverträglichkeit: Bewährte statt nicht evaluierte Diagnostik", Deutsches Ärzteblatt 2005; 102(27); Kleine-Tebbe, Jörg; Lepp, Ute; Niggemann, Bodo; Werfel, Thomas.*

Summary: In this article the authors critique a number of different test methods. The Alcat Test is incorrectly characterized as a cytotoxic test and is presented as being unable to detect food allergies. In addition, the Alcat Test is criticized as showing inadequate reproducibility of results.

Clarification 1:

Contrary to the authors' claims, the Alcat Test is not a cytotoxic assessment.

Cytotoxic testing has been dismissed as being without merit by the American Academy of Allergy and Immunology has been classified by the FDA as worthless. The article cites studies conducted between 1947 and 1976, a substantial number of years prior to the development of the Alcat Test. These studies are not applicable to Alcat technology which uses a completely different approach. Hence, the criticisms of cytotoxic testing have no bearing on the validity of Alcat Testing.

The premise of cytotoxic testing is that mixing white blood cells in vitro with an antigen to which the sub-

ject is allergic will induce observable cellular injury.

The Alcat Test is not based on cytotoxic cellular injury. Rather, Alcat technology measures the responses of relevant immune cells to specific substances derived from foods, additives, molds, drugs and other categories. The objective, quantitative measurement of cellular changes is accomplished through impedance method based flow cytometry (see A.1.2.) to determine changes in cell size and count. The Alcat Test provides an automated, computer-based and, objective in vitro methodology.

The authors' mischaracterization of Alcat Technology is further demonstrated by the fact that the Alcat technology has been awarded patents (see section C). According to patent law, a patent is only granted if the discovery or invention satisfies all of the following conditions:

- ▶ The invention is useful
- ▶ It represents a novel approach
- ▶ It utilizes principles and methods that are not obvious

Prior art must be disclosed, which means that the US and numerous other patent offices were informed, through the patent application, detailed information about the cytotoxic test and made the scientific determination that the Alcat Test was different from the cytotoxic test in a way which would not be obvious even to persons skilled in this particular area of blood test. To be granted a patent, documented evidence must also establish that the invention actually works as described. By reviewing Alcat technology and granting it patent protection, the United States Patent and Trademark Office, and others, have accepted the validity of Alcat technology.

Clarification 2:

The Alcat Test is not an allergy test and should therefore not be criticized for failing to detect and measure food allergies.

The authors are indeed correct that the Alcat Test is not suitable to detect food allergies. However, the purpose of the Alcat Test is to detect intolerances, not allergies, for which it is both clinically useful and scientifically valid. The detection of IgE-mediated type 1 allergy is not the intent of the Alcat Test.

To help avoid these all-too-common misunderstandings, the manufacturers of Alcat technology have taken great pains to explicitly describe the distinctions between food allergy and food intolerance in the context of the Alcat Test.

Clarification 3:

The article offers criticisms based on sources that have no relationship to the Alcat Test.

The authors criticize a perceived lack of reproducibility of test results and dismiss the Alcat Test as a subjective evaluation. In doing so, they make reference to another article, *Niggemann, B., Grüber, C.: Unproven diagnostic procedures in IgE-mediated allergic diseases, Allergy 2004;59:806-808.*

The cited article is concerned with methods of evaluating IgE-mediated type 1 allergic reactions, which do not apply to the Alcat Test. It also refers to the cytotoxic assays such as Bryan's test, described in an earlier section. These methods, irrespective of their value, have nothing in common with the Alcat Test and their assessment should have no bearing on the evaluation of Alcat technology. The Alcat Test results are automated, objective and highly reproducible. (See B.4).

Nine of 10 sources that present evidence against the validity of cytotoxic testing were written between 1947 and 1980, prior to the development of Alcat technology, which began in the mid-1980s. Studies demonstrating the reproducibility and consistency of Alcat Test results and their consistent correlation with clinical symptoms were first conducted in 1989 (comparisons B.2-B.5).

Summary of Clarifications:

The Alcat Test represents an advanced technology for detecting the non-allergic reactions associated with intolerance. It is not based on microscopic (subjective) observation of cells nor on the detection of IgE-mediated (type 1) allergy reactions. Alcat is based on the objective, automated analysis of innate immune system cell responses associated with inflammatory reactions in the body.

- ② *"In-vitro-Diagnostik von Nahrungsmittel-Allergien" J. Kleine-Tebbe, T. Fuchs, U. Lepp, B. Niggemann, J. Saloga, I. Vieluf, S. Vieths, T. Werfel, T. Zuberbier, L. Jäger. Allergo J 2001; 10: 333 – 339*

Article 2 is a position paper presented by the German Society of Allergology and Clinical Immunology (DGAI) and the Medical Association of German Allergists (ÄDA). The paper perpetuates the same misunderstandings of the Alcat Test described in the previous section, namely, confusing both the intended use, and the methodology of the Alcat Test with that of IgE and the cytotoxic test, respectively.

- ③ *„Unproven techniques in allergy diagnosis“, J Invest Allergol Clin Immunol 2005; Vol. 15(2): 86 – 90; B. Wüthrich*

The primary criticism of this paper is the author's claim that the utility and validity of the Alcat Test is not supported by study data.

Clarification 1:

The usefulness and functionality of the Alcat Test has been clearly demonstrated in 35 studies.

Blinded clinical studies have demonstrated the reproducibility of Alcat Test results. In addition, numerous scientific studies have established its effectiveness in clinical practice.

While it is true that the numbers of subjects in the trials are relatively small, the pronounced effects has enabled the studies to achieve statistical relevance.

Noteworthy is that more than 1,300 subjects have participated in Alcat Test studies. Different studies at different times in different countries and diverse clinical settings have consistently produced substantially similar results. This consistent reproducibility offers further evidence of the fundamental reliability and validity of the Alcat Test.

Clarification 2:

The authors offer the criticism that leukocyte testing is not suitable to detect allergic reactions.

As previously explained, the Alcat Test is not designed to detect allergies. In support of his position, the author cites a prior publication critical of food allergy testing via leukocyte reactions that was published more than 30 years old (Lehman CW. The leukocytic food allergy test: a study of its reliability and reproducibility. Effect of diet and sublingual food drops on this test. Ann Allergy. 1980;45:150-158.). However, even at the time the cited article was published, many positive studies had already demonstrated that non-IgE-mediated intolerance responses could be effectively characterized through a proper analysis of leukocyte reactions.

(See relevant studies at <http://preventivecare.com/shared/KeyStudies.pdf>)

Clarification 3:

Current research on the intricacies of the innate immune system shows that the complex mechanisms of cellular defense play a significant role in the activation, promotion and maintenance of inflammatory processes.

Given the enormous progress over the past decade in fundamental biological research, as well as the emergence of new discoveries and advanced methods in immunology and molecular biology, older studies must always be read carefully to determine whether they are still relevant. Many new insights have emerged since the publication of these studies that have expanded knowledge of the extraordinarily complex relationships involved in activating the diverse components of the immune system. It is almost always the case that these complex immune system interactions rely on multiple, interacting biochemical signals to initiate and sustain reactions. Hence, an objective, reproducible whole cell measurement technology may confer significant benefits.

④ *"The Alcat TEST – inappropriate in testing for food allergy in clinical practice" S Afr Med 1992, 81: 384.; Potter, PC., Mullineux, J. Weinberg, E.G., Haus, M., Ireland, P. Buys, C., Motala, C.*

Although indexed in Medline/PubMed, this article is not available online nor is it accessible through the South African Medical Journal archive. Cell Science Systems Corp. and Alcat Europe GmbH have sought unsuccessfully to acquire a copy of the paper from the first author, Dr. Paul Potter. Unfortunately, without access to the article, no detailed critique can be

However, Dr. Potter and a colleague, Dr. Harris Steinman, later conducted an Alcat-reproducibility study which concluded that the Alcat Test did, in fact, provide reproducible and reliable data. In addition, the study highlighted the deficiencies of the previous study (cf. B.4). These findings were further evidenced in personal correspondence between Drs. Potter and Steinmann.

Further comments and conclusions

The incorrect use of the Alcat Test to diagnose food allergy has always been the main source of criticism. The Alcat Test is not intended to detect allergies; yet, this criticism continues to circulate, particularly on the Internet.

Hopefully, as more practitioners and patients come to understand the broad range of non IgE mediated adverse reactions to foods and other substances, and the applicability of the Alcat Test and experience its remarkable consistency and clinical effectiveness across a broad range of health complaints, more patients will be able to benefit.

The Need for New Classification Systems

We often attempt to understand new things in terms of our existing classification systems. In the context of food intolerance, most people turn either to Type I, II, III and IV immunological reactions described in 1963 by Gell and Coombs, or some type of non-immunological reaction such as the cellular injury associated with cytotoxic testing. As reviewed in the previous section, most criticisms of the Alcat Test arise from the attempt to understand it in terms of more familiar but outdated classification systems.

In summary, the Alcat Test does not detect allergic reactions. Rather, it's based on an objective, automated assessment of how cells of the innate immune system – primarily neutrophils – react to in vitro challenges from extracts of specific foods, additives, molds, drugs and other potentially provocative substances.

The Alcat Test should therefore be understood as a complementary method of analysis. Ideally, existing classification systems will be expanded to include the insights of current and emerging immunological research.



B.7 Scientific Context and Current Research

B.7.1 Innate Immunity and Inflammation

The role of the innate immune system in the promotion of chronic inflammation and its relationship to disease has been a subject of intense research in recent years.

The following diagram shows an overview of the complex chain of molecular interactions involved in inflammation.

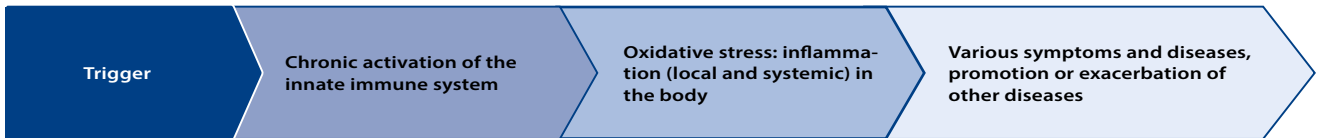


Figure 11: While the adaptive immune system has been extensively studied for many decades, the innate immune system – previously regarded as far more primitive – has only recently received considerable attention.

During the last decade it has been acknowledged that the innate immune system has been enormously underestimated and that its activities impact many essential processes in the body. Specifically, research has recently focused on the inflammasome – a multi-protein complex discovered in 2002 – that is an extremely active component of innate immunity. The inflammasome is responsible for the maturation of pro-inflammatory cytokines (specifically IL-1 β and IL-18) and plays a key role in initiating inflammatory processes.

Chronic inflammation in turn, is now regarded as one of the primary causes of many different diseases (see Tab. 1, Fig 11).

The inflammasome is involved in all of the following processes (25):

- ▶ Antimicrobial defense.
- ▶ The immune response of the mucosal membranes, especially in the gut, which can critically alter the composition of intestinal microflora.

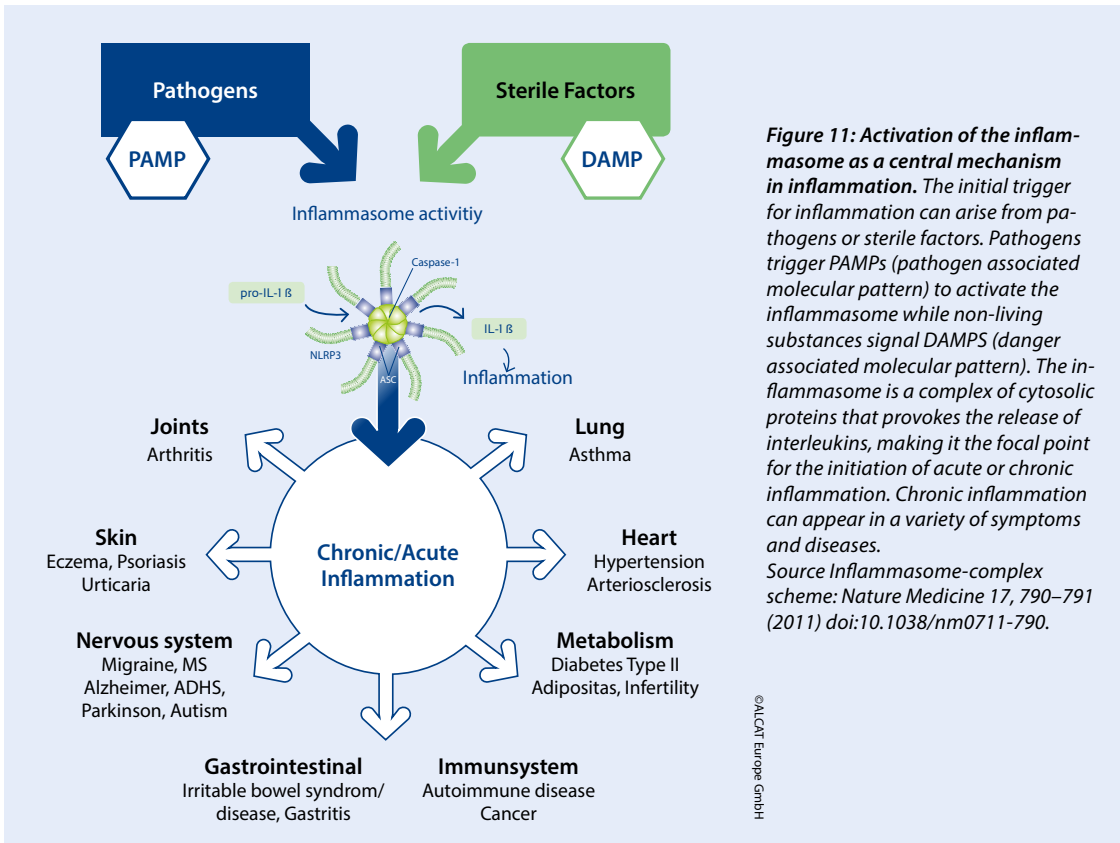


Figure 11: Activation of the inflammasome as a central mechanism in inflammation. The initial trigger for inflammation can arise from pathogens or sterile factors. Pathogens trigger PAMPs (pathogen associated molecular pattern) to activate the inflammasome while non-living substances signal DAMPs (danger associated molecular pattern). The inflammasome is a complex of cytosolic proteins that provokes the release of interleukins, making it the focal point for the initiation of acute or chronic inflammation. Chronic inflammation can appear in a variety of symptoms and diseases. Source *Inflammasome-complex scheme: Nature Medicine 17, 790–791 (2011) doi:10.1038/nm0711-790.*

©ALCAT Europe GmbH

- ▶ Expression and activation of metabolic syndrome – the comorbid occurrence of obesity, metabolic dysregulation, insulin resistance and chronic inflammation – and the effect this has on insulin signalling in the islet of the pancreas.
- ▶ Inflammation associated deconstruction of insulin producing β -cells, promoting the damaging transition from obesity to type 2 diabetes.
- ▶ The formation of atherosclerotic plaque.

The inflammasome can be activated by certain exogenous substances, such as calcium phosphate crystals, asbestos, alum, uric acid crystals and silica. Activation also may be triggered by a variety of naturally occurring endogenous signals including reactive oxygen radicals.

Inflammasome activating substances interact either with PAMPs (pathogen associated molecular patterns) in the case of microbe-induced reactions and DAMPs (danger associated molecular patterns) in response to sterile substances. (See Figure 11).

All of the processes involved in inflammasome signal transmission and other activities have not yet been fully elucidated. It is clear, however, that neutrophils (and other cells measured by the Alcat Test) and inflammasomes are closely related and can be activated either by endogenous or exogenous substances.

Until recently, the operational principle of the Alcat Test was based, at least in part, on prevailing theories concerning the function and role of the innate immune system. These assumptions were supported by the effectiveness of the Alcat method documented through empirical observation and research studies. Some of the more subtle, underlying immunological correlations have not yet been fully explained. Current research is now focused on explaining critical molecular processes.

Table 1 enumerates some of the disorders that may be related to chronic activation of the innate immune system and its oxidative and inflammatory consequences. Supporting literature is described in Appendix 2 in the documents referenced.

System	Condition	References
Gastrointestinal disorders	<i>Diarrhea/constipation, bloating, irritable bowel syndrome (IBS), gastritis, gastric reflux, and associated malabsorption and/or nutrient deficiencies</i>	(1) (2) (3)
Skin diseases	<i>Eczema, psoriasis, rashes, keratosis pilaris, urticaria</i>	(4)
Neurological and mental disorders	<i>Migraines, headaches, memory problems, Alzheimer's, chronic fatigue, mood swings, depression (related to the neuroendocrine immune system), ADHD, neuropathy, autism, schizophrenia</i>	(5) (6) (7) (8) (20) (21)(22)
Respiratory problems	<i>Chronic cough, wheezing / bronchoconstriction, sinusitis</i>	(23) (24)
Metabolic diseases Endocrine / hormonal disorders	<i>Obesity, diabetes, metabolic syndrome, inability to lose weight, weight loss, thyroid diseases, infertility, irregular menstruation</i>	(9) (10) (11) (12) (13)
Muscular-skeletal disorders	<i>Stiff or sore joints, arthritis, tendonitis</i>	(14)
Immune system and other comorbidities favored	<i>Weakened viral immunity, allergies, autoimmune diseases, heart problems, tumors</i>	(15) (16) (17) (18) (19)

Table 1. Disorders Potentially Related to Chronic Activation of the Innate Immune System

B.7.2 Synergism Between Innate and Specific Immunity

In order to be optimally effective, the non-specific and specific immune defenses work hand in hand. In the example of celiac disease - the reaction to the gliadin component of gluten containing grains– it is easy to observe an adverse reaction to food that includes activation of specific immunity. T cells of celiac disease patients react with a specific antigen, which leads to a strong release of cytokines and attracts cells of the innate immune system. This results in injury of local tissue resulting from the release of toxic substances.

A similar feedback between specific and innate immune response may be observed when activation of the innate immune system increases the expression of key recognition molecules (such as B7 molecules on antigen presenting cells). Their enhanced expression provides a secondary signal for the activation of T helper cells.

The coordination of the two branches of the immune system comprises complex synergistic mechanisms. It is often observed that treatment of allergy also reduces food intolerance and conversely, the effective management of food intolerance improves allergic conditions. However, this does not mean that patients

with already proven classic allergies to certain foods may disregard those findings because these allergens have been reported as non-reactive by the Alcat Test. In these instances patients must consider both types of test results. Nevertheless, intolerance reactions to food are mediated primarily via the innate immune system.

B.7.3 Interaction Between Genetically and Enzymatically Related Intolerance

Patients who already suffer from an enzymatically or genetically related intolerance, as lactase deficiency, GLUT-5-fructose malabsorption or celiac disease, may also have an immune-mediated reaction against the same foods and other substances. In a genetic

intolerance, the practitioner should always keep in mind that genetic defects do not necessarily have to present phenotypically; they may appear with variable symptoms. What matters is what happens on the epigenetic level and the individual tolerance limit. A fructose malabsorption may, for example, be due to the fact that the GLUT-5-transport system is simply overwhelmed.

Another salient example is the work of Professor Fasano to differentiate and distinguish celiac disease, potential celiac disease and gluten sensitivity, in which the same genetic preconditions may lead to different phenotypes and severity of expression, depending on still unknown co-factors, but, certainly involving factors related to gut micro-ecology. (1).

B.8 Differentiation of the Alcat Test from Other test Methods

The Alcat Test is not meant to detect or diagnose dysfunctions or properties of the specific immune system. **These specific immune system functions include, but are not limited to:**

- ▶ Classic IgE-antibody mediated, type 1 food allergies
- ▶ Other antibodies, e.g. those used in IgG testing
- ▶ Lymphocyte transformation testing (LTT)

Others:

- ▶ Intolerances caused by enzymatic or genetic predispositions, such as lactose intolerance by lactase deficiency, fructose malabsorption, hereditary fructose intolerance, and celiac disease may not be detected by the Alcat Test as it is performed today.

B.8.1 IgG testing – The Protective Role of Immunoglobulin G, a Natural Physiological Reaction

In addition to the Alcat Test, immunoglobulin G (IgG) is sometimes tested to help identify food intolerances. In these cases, the presence of IgG antibodies in blood against the test food is presumed to be a marker for intolerance. However, IgG primarily serves a protective function in the body. Frequent and intense exposure to an antigen favors an IgG response. By contrast, small, irregular exposures to an allergen typically lead – as in the case of an allergy – to an IgE response. In the course of immunotherapeutic treatment of classical allergies, symptoms are relieved by progressively lowering the levels of allergen-specific IgE while increasing the levels of allergen specific, blocking, IgG antibody.

Thus, IgG antibodies do not promote allergy nor do they serve as a clear indicator of an adverse reaction to a specific food. IgG antibodies form complexes with excess food antigens in the blood and help the monocyte/macrophage system eliminate them, without causing symptoms. Their presence indicates a shift in the T-cell response towards a Th1-dominated immune response. Therefore, high IgG titers are more likely to correlate with repeated exposure to an antigen but not necessarily with a sensitive reaction against them. While it's true that people may develop a high level of protective IgG antibodies against some foods to which they are intolerant, the high IgG level is present because of the exposure and is therefore not a marker for the intolerance.

Hence, IgG testing leads to unnecessary food avoidance. The Alcat Test, however, analyzes the directly observable adverse reaction of the immune cells to food. Some providers of IgG tests advertise that they differ in their methodology by means of “reference antibodies” between the natural IgG and those formed due to incompatibility. A detailed description of how this is accomplished or studies detailing and confirming the methodology are not presented.

According to the current state of science, it is assumed that IgG, particularly IgG4 antibody tests, are not suitable to reliably detect food intolerances. (See position paper of the European Academy of Allergology and Clinical Immunology (EAACI) [26b].)

C. APPROVALS, CERTIFICATES, GRANTS, PATENTS RELATED TO THE ALCAT TEST

Registered with the U.S. Food and Drug Administration (FDA)

The Analyzer used in Alcat Testing has been registered with the FDA. Manufacturing is carried out under cGMPs in compliance with FDA guidelines and subject to inspection by the FDA, separate from inspections of laboratories using the device. In addition, the production facility voluntarily maintains EN ISO 13485 certification, and instrumentation and reagents are CE marked, thus permitting sale and shipment of instrumentation and reagents to other countries. 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
Classification Name:	DGQ
Device Class:	1
Regulation Number:	866.5700
Medical Specialty:	Immunology
Owner/Operator:	Cell Science Systems, CORP.
Establishment Operations:	Manufacturer

In addition, Cell Science System Corp., the owner and operator of the Alcat Test, holds the following certificates and licenses:

ISO N13485:2003 – Certification and CE-Mark

ISO N13485:2003 certification is the international quality management standard for medical devices in addition to compliance with the GMP guidelines. The Laboratory of Cell Science Systems carries the certification code 220 for "General Immunology."

The CE mark grants the permission to distribute the analysis device in the EU.

Alcat Europe GmbH, the European distributor of the Alcat Test, holds ISO certification 9001:2008

CLIA-License

CLIA refers to the Clinical Laboratory Improvement Act of 1963 and amended in 1988. CLIA are U.S. federal regulatory standards that apply to all clinical laboratories that perform laboratory tests on/human specimens in the United States, except clinical trials and basic research. The Alcat Test is considered a high complexity test and as such is required to have systems and processes for monitoring testing equipment, procedures to ensure proper test performance and accurate results, and an overall plan to monitor the quality of all aspects of the laboratory's ongoing operation.

No warning letters or adverse events have ever been reported. The laboratory undergoes on-site surveys at least every two years.

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). These two patents were awarded for measuring the degree of reaction between antigens and leukocyte cellular antibodies. A third patent application has been filed. Both U.S. and Foreign patents were awarded.

Under Section 101 of the U.S. Patent Act, a Patent can only be awarded if the discovery is:

- ▶ Useful
- ▶ Novel
- ▶ Non-obvious

Because patents must provide documented evidence that the invention actually works as described, and since the U.S., European, and other International Patent & Trademark Offices have awarded patents for the Alcat System, these bodies have deemed the Alcat Test system to be a valid, novel and useful.



International patents:

- ▶ Publications.: 0 140 379 B1, Int. Cl.: GO1N 15/12, GO1N 33/53, Application. 84113101.4, Publikationsdatum: 15.04.1992, Gültigkeit in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
- ▶ Publications.: EPO281626, Int. Cl.: GO1N33/48, GO1N33/566, GO1N33/53, GO1N33/554 [1988/37], Publication 28.05.1997, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
- ▶ Publications.: WO 92/01934, Int. Cl.: GO1N 33/48, 33/00, valid in AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE
- ▶ 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 measuring the degree of reaction between antigens and leucocyte cellular antibodies.

Grants from the EU and the ILB (Investitionsbank des Landes Brandenburg):

Four grant-funded programs and an extensive scientific research and development grant

Since 2007, four grant projects have been approved by the Investment Bank of the State of Brandenburg (ILB) and the EU. Among these is a comprehensive scientific research and development project. The grant was approved only after a thorough examination by qualified personnel and an objective third party expert opinion on the Alcat System's feasibility, innovation, content and basic science. The grant, "Development of an innovative instrument for the comprehensive analysis of cell activity to optimize immune function – Immuncat" (project number 801 43 729) is intended to develop an innovative, new laboratory testing method for lymphocyte proliferation to detect immune and nutritional deficits, as well as other application; and, enhancement of Alcat intolerance testing methodologies.



D. SUMMARY AND CONCLUSIONS

This white paper represents a fundamental review of available data describing the science and clinical utility of the Alcat Test method. It is intended to serve as a source of information about the scientific foundations and clinical applications of Alcat technology and demonstrates an ongoing commitment to transparency. It also aims to stimulate professional, interdisciplinary communication and an exchange of ideas about the many manifestations of food intolerance and associated therapies.

Both, immune- as well as non-immune mediated food intolerance and associated inflammatory conditions often go unrecognized as the underlying cause of many different symptoms and health complaints. Their consequences may be long-term and reflected in a wide range of mild to serious disorders such as metabolic syndrome, neurological damage and immune compromise. The treatment of various inflammatory disorders may offer a valuable, complementary therapy approach using dietary modification. Disorders and symptoms detected in connection with a permanently activated congenital immune response and consequent oxidative stress and inflammation may include (see chapter B.7.1 and bibliography appendix 2):

- ▶ Gastrointestinal complaints
- ▶ Skin diseases
- ▶ Neurological and mental disorders
- ▶ Respiratory diseases
- ▶ Metabolic diseases
- ▶ Endocrine disorders
- ▶ Musculoskeletal and Joint disorders
- ▶ Immune system and other comorbidities

In the treatment of various inflammatory diseases, dietary modification offers a valuable, complementary therapeutic approach.

The Alcat Test has demonstrated that it is functional, sensitive, reproducible and useful. Section B presents the results of Alcat Test research. The Alcat method is validated by:

- ▶ 3 double-blind, randomized, placebo-controlled, clinical trials
- ▶ 4 reproducibility studies
- ▶ Extensive clinical studies published in more than 30 peer-reviewed publications
- ▶ Independent, international studies documenting similar excellent results

The Alcat Test identifies patient specific leukocyte reactions to substances in food and the environment, which can interfere with the immune balance and health.

More than **30 scientific studies** and a selection of case studies from different medical fields show that an individual rotation and elimination diet based on the Alcat Test analysis can alleviate reactive substances, and in some cases symptoms completely resolve.

Common criticisms of the Alcat Test were presented in section B.6. The incorrect classification of the Alcat Test as an allergy test accounts for much of the criticism. Outdated studies from 1947-1980 (prior to the invention of the Alcat Test) have been cited to cast doubt on the Alcat Test methodology but, also, do not apply. The mistaken notion of the Alcat Test as a “cytotoxic test” was laid to rest. It is understandable that misconceptions arose as the Alcat technology has allowed us to peer into immunological phenomena which has hitherto been uncategorized.

Current research on leukocyte reactions – especially in granulocytes – and the current understanding of inflammatory pathways of the innate immune system are briefly summarized in the sections A.2, A.3 and B.7.

Both Cell Science Systems, Corp. and Alcat Europe GmbH place great importance on the scientific method for **pursuing high-quality research** (see Section C). The Alcat method has received 6 patents. The granting of patents is tantamount to scientific validation as the functionality and benefits of an invention must both be demonstrated. In addition to the requisite double-blind studies, Alcat Test laboratories are voluntarily certified and the analyzer is registered with the U.S. FDA. Alcat Europe GmbH was awarded 4 ILB/EU project grants, including one for an extensive research and development project.



Appendix I. Practitioner First Hand Experiences

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

Pradeep Chopra, MD, BROWN Medical School

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

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

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

Nathan Goodyear, MD

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

I have seen many patients lose massive amounts of weight, reduce blood pressure and eliminate medications, improve blood sugar control and eliminate medications, eliminate migraines/headaches, reduce and eliminate acne, rosacea, and other skin conditions, improve asthma, and improve a vast array of gastrointestinal symptoms.

Dietary modifications based on Alcat Test results have provided significant benefits in all inflammatory conditions in my clients.

With the ever-increasing cost of medical care, no one test has a better cost/benefit ratio. In 2009, health care costs reached \$2.5 trillion. But if you break it down, you see the real benefits. The health of the patient equals cost savings for the insurance company. The obesity epidemic is out of control. Since 1995, the number of states with obese adults exceeding 30% has gone from 1 to 16. Obesity is the doorway to disease. So if we extrapolate that with resultant associated diseases such as diabetes,

cardiovascular disease, and cancer, the costs of doing nothing and the benefits of real prevention become evident. Look at a 5-year annual cost window:.

	Men	Women
Obesity	2.646 \$	4.879 \$
Diabetes	11.744 \$	11.744 \$
Cardiovascular	18.953 \$	18.953 \$
Cancer	29.643 \$	13.925 \$
Total	62.986 \$	49.501 \$

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

Mark L. Andrews, MD

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

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

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

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

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

**Pauline Harding, MD,
Family Medicine Practitioner**

"I have been practicing medicine for 35 years. I have used the Alcat Test in many... cases involving an inflammatory component. The vast majority of these patients have experienced significant health benefits attributable to dietary modification and avoidance of environmental toxins and allergens based upon Alcat Test findings. ... Examples of the benefits of identifying inflammatory agents include the following:

One middle-aged woman with chronic headaches discovered that the aspartame in her toothpaste was the culprit.

One middle school youngster found that he was highly allergic to a single variety of mold found under the toilet tank in his bathroom.

One middle-aged male discovered that what appeared to be unexplained acute alcohol intoxication was actually due to a chemical inhaled in his workplace.

One child's untreatable seizures resolved when he stopped using paper plates and paper cups and milk carton that had a corn-sugar coating.

One young adult male from out-of-state had resolutions of his longstanding intestinal bleeding upon identifying gluten as the cause of his bowel disease. Two colonoscopies had not identified the issue.

Several patients diagnosed with multiple sclerosis had resolution of their symptoms after identifying aspartame and/or gluten as the cause of their neurologic symptoms.

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

Laura Miles, MD

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

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

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

Lisa Kamphuis, ARNP

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

Some of these cases include previously assumed fibromyalgia, arthritis, obesity, and irritable bowel syndrome. The majority of these patients have benefitted significantly or completely resolved their diagnoses, which we can attribute solely to dietary changes based on findings of the Alcat Test. One particular patient reported that he made at least 3 visits to the ER per year and took hundreds of dollars worth of medications monthly until he made these dietary changes. He has now lost 40 pounds and is happily working out, feeling more energy than ever, and doesn't take any prescriptions. I believe the Alcat has many uses which can save millions of dollars to our healthcare system..."

David Blyweiss, MD

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

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

Amy E. Pieczaraka, Clinical Dietician

"I have been a registered dietitian and licensed dietitian/nutritionist, providing medical nutrition therapy for 25 years. Utilizing the Alcat Test has provided my patients information necessary to help manage existing health problems and promote optimal health.

I have used the Alcat Test in many complicated cases, which prior to the knowledge obtained from the testing, were seeing no progress despite my suggestions. The Alcat Test has provides the understanding of what contributes to their health problems, most of which involve an inflammatory component. I believe that utilizing this intervention demonstrates a highly significant cost benefit ratio.

It is my professional opinion that anyone would benefit from the Alcat testing. It is cost effective and can be a successful intervention in a broad spectrum of disease conditions.

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

David M. Brady, ND, CNN, 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 year, with great success. In fact, this testing methodology has been the key to resolving many patient's long standing complaints, including dermatologic issues, fatigue, headache, joint and soft-tissue pain, gastrointestinal discomfort, and much more when other diagnostic testing and treatment intervention had failed.

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

Jamie Wright, DO, FACOOG, ABAARM

"My name is Dr. Jamie Wright and I am a board certified gynecologist who 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 effect 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."



Appendix 2 - References

- (1) Sapone, A. et al: Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Medicine*, 9:23; 2011
 - (2) Berardi et al: Alcat test identifies food intolerance in patients with gastrointestinal symptoms, Clinical Study 2011, Poliklinik Pavia, Italien
 - (3) Fell, P. et al: Cellular Responses to Food in Irritable Bowel Syndrome – An Investigation of the Alcat Test. *Journal of Nutritional Medicine*, 2, 143-149; 1991
 - (4) Berardi L. et al: Food intolerance in patients with cutaneous diseases: diagnostic value of the Alcat test. Clinical Study 2011, Department of Dermatology, Pavia, Italien
 - (5) Samaroo D. et al: Novel immune response to gluten in individuals with schizophrenia. *Schizophr Res*, 118:248-55; 2010
 - (6) Dickerson F. et al: Markers of gluten sensitivity in acute mania: A longitudinal study. *Psychiatry Res*, 196:68-71; 2012
 - (7) Rashtak et al: Serology of celiac disease in gluten sensitive ataxia or neuropathy; Role of deamidated gliadin antibody. *J Neuroimmunol* 2011; 230:130-134
 - (8) Vitte J et al: Oxidative stress levels in circulating neutrophils is linked to neurodegenerative diseases. *J Clin Immunol*, 24(6):683-92; 2004
 - (9) Oodegard JI et al: Connecting Type 1 and Type 2 Diabetes through Innate Immunity. *Cold Spring Harb Perspect Med*, 2(3); 2012
 - (10) Duncan BB et al: Chronic activation of the innate immune system may underlie the metabolic syndrome. *Sao Paulo Med J*, 119:3; 2001
 - (11) Lee MS. Role of innate immunity in diabetes and metabolism: recent progress in the study of inflammasomes. *Immune Netw* 11:95-9; 2011
 - (12) Tremellen K & Tunc O. Macrophage activity in semen is significantly correlated with sperm quality in infertile men. *Int J Androl* 33:823-31, 2010
 - (13) Bastard JP et al: Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw*, 17(1):4-12; 2006
 - (14) Miesel R et al: Priming of NADPH oxidase by tumor necrosis factor alpha in patients with inflammatory and autoimmune rheumatic diseases. *Inflammation*, 20(4):427-38; 1996
 - (15) Fitzpatrick AL et al: Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol* 165:14-21; 2007
 - (16) Taskalova-Hogenova et al: Involvement of innate immunity in the development of inflammatory and autoimmune diseases. *Ann N Y Acad Sci*, 1051:787-98; 2005
 - (17) Zhonghua et al: Changes of neutrophil myeloperoxidase in coronary circulation among patients with acute coronary syndrome. 33:1106-8; 2005
 - (18) Wan-Wan L et. Al: A cytokine-mediated link between innate immunity, inflammation and cancer. *J Clin Invest*, 117(5):1175-1183; 2007
 - (19) Poon BY et al: alpha(4)-integrin mediates neutrophil-induced free radical injury to cardiac myocytes. *J Cell Biol* 152(5):857-66; 2001
 - (20) Samaroo D, et. al. Novel immune response to gluten in individuals with schizophrenia. *Schizophr Res*. 2010 May; 118(1-3):248-55.
 - (21) Jyonouchi H et al: Children with autism spectrum disorders (ASD) who exhibit chronic gastrointestinal (GI) symptoms and marked fluctuation of behavioral symptoms exhibit distinct innate immune abnormalities and transcriptional profiles of peripheral blood (PB) monocytes. *Journal of Neuroimmunology*, 238:73-80; 2011
 - (22) Jyonouchi H. Food allergy and autism spectrum disorders: is there a link?. *Curr Allergy Asthma Rep*. 9:194-201; 2009
 - (23) Holgate ST., Innate and adaptive immune response in asthma, *Nat Med* 18:673-83; 2012
 - (24) Finn and Bigby: Innate immunity and Asthma, *Proc Am Thorac Soc Vo* 6:260-265, 2009
 - (25) Strowig et al.: Inflammasomes in Health and Disease. *Nature Vol* 481:278-286
 - (26a) Special edition „Fortbildung und Praxis für den Hausarzt“ (engl: further education and practice for the family doctor) as supplement of the journal “Der Allgemeinarzt“ (engl: the doctor), text written by Dr. Imke Reese, Kirchheim publishing house, Mainz, Germany 2008.
 - (26b) Stapel SO et al: testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. *Allergy*, 63:793–796; 2008
 - (26c) http://www.mpiib-berlin.mpg.de/forschung/zellulaere_mikrobiologie
- Overview of Alcat Test studies (as described in Sections B.3 - B.5 plus others studies):**
- (Most of these can be download from www.alcat.com)
- Double-blind studies:**
- (27) High correlation of the Alcat Test Results with Double-Blind Challenge (DBC) in Food Sensitivity
by Dr. P. Fell, presented at the 45th Annual Congress of the American College of Allergy and Immunology, Los Angeles, CA: November 12-16, 1988 and published in the *Annals of Allergy*.
 - (28) Alcat a new test for food induced problems in medicine?
Fell et al., presented at the annual meeting of the American Academy of Otolaryngic Allergy, Washington DC, 1st Oct 1988.
 - (29) Alcat® – a new cellular test for food sensitivity
Fell, Brostoff & Soulsby, presentation of study and results at the annual meeting of the American In-Vitro Allergy & Immunology Society, August 1990, Toronto, Canada.
 - (30) Cellular responses to food in irritable bowel syndrome – an investigation of the Alcat Test
Fell, Soulsby & Brostoff, publication of the summarized study results, *Journal of Nutritional Medicine*, Vol. 2, No. 2, 1991.
 - (31) Diagnostic Value of Alcat Test in intolerance to food additives compared with double-blind placebo-controlled (DBPC) oral challenges L. Hoj, *J Allerg Clin Immun* 1 (3); 1996
- Reproducibility Studies:**
- (32) Reproducibility of the Alcat Test
P. Potter, H. Steinmann et al., University of Cape Town, South Africa, 1994
 - (33) Reproducibility of the Antigen Leukocyte Cellular Antibody test (Alcat)—Statistical Analysis, Summary Statistics & Scientific Report“, University of the Range Free State in Bloemfontein, Südafrika, Dr. WML Neetling and Dr. AM Kachelhoffer von Januar-April, 1998.

- (34) Parexel Medstat Final Statistical Report – Study of the Alcat Test in 10 subjects, Dr. Per Fuglerud, Parexel Norwegen, Nov. 1999
- (35) Study Comparing Alcat test Results With Flow Cytometry and Microscop, Dr. Gitte Jensen, NIS Labs (Natural Immune System) Oregon, USA, 2009

Other Alcat Studies

- (36) Evaluation of Alcat Test Results in the Non-IgE Mediated Pathology of the Skin“ DeAmici et al., Study performance and study report at the University of Pavia, Italy. Presented at the 30th Congress of the European Academy of Allergy and Clinical Immunology, 11-15 June 2011 – Istanbul, Turkey. (Poster Presentation, Abstract #553)
- (37) Alcat Test Results in the Treatment of Gastrointestinal Symptoms“ Berardi L. et al., Study performance and study report at the University of Pavia, Italy. Presented at the 30th Congress of the European Academy of Allergy and Clinical Immunology, 11-15 June 2011 - Istanbul, Turkey. (Poster Presentation, Abstract #552)
- (38) Rational management of food intolerance in an elite soccer club“ Angelini et al., Journal of the International Society of Sports Nutrition 2011, 8(Suppl 1):36
- (39) Alcat Test Identifies Food Intolerance in Patients with Gastrointestinal Symptoms“ Berardi et al., Report of the XXVIII Congress of the European Academy of Allergy & Clinical Immunology, European Journal of Allergy and Clinical Immunology, Supplement 90, Volume 64, 2009, pg. 490.
- (40) Food Intolerance in Patients with Cutaneous Diseases: Diagnostic Value of the Alcat Test“ Berardi et al., Report of the XXVIII Congress of the European Academy of Allergy and Clinical Immunology, European Journal of Allergy and Clinical Immunology, Supplement 90, Volume 64, 2009, pg. 490.
- (41) The Effect of the Alcat Test Diet Therapy for Food Sensitivity in Patients with Obesity“ Akmal et al., Middle East Journal of Family Medicine. April 2009 - Vol. 7, Issue 3.
- (42) IMS Health Economics and Outcomes Research—Influence of Food Intolerance in Migraines: Final Report of Statistical Results“ Immunological Center of Catalonia, Version 3, December 28, 2006.
- (43) Ogni intervento comincia a tavola, Mele Cristina, Medici Oggi, Maggio 2002: 210-213
- (44) Controversial antigen leucocyte cellular antibody test (Alcat): a nonspecific inhibitory effect of alpha glycoproteins, Kedryna & Guminska, Med Sci Monit 1999; 5(2):BR193-197.
- (45) A Comparison of the Alcat Test for Food Reactions Amongst 2 Population Sub-Groups“ Study presentation of Dr. DH Sandberg et al., 45th Annual Congress of the American College of Allergy and Immunology, Los Angeles, CA: November 12-16, 1998, published in the Annals of Allergy.
- (46) Outcome Study in 353 Consecutive Patients Following The Alcat Diet“, Study of Dr. Lene Hoj in Copenhagen at the Allergy Clinic Charlottenlund, Denmark 1998. Non-Published.
- (47) Evaluation of the cytotoxic food test and the Alcat (antigen leucocyte cellular antibody test). Pol Merkuriusz Lek. 1997 Feb;2(8):154-9.
- (48) South African Outcome Study. Randomized study with 274 patients, Dr. Jan Geldenhuys, Johannesburg, South Africa, 1997
- (49) Technical Study Comparing The Alcat Methodology With Activation Of Granulocytes Following Challenge With Zymosan, Study of Dr. Christina Mele at the University of Rome.
- (50) The Short Term Efficacy of the Alcat Test of Food Sensitivities to Facilitate Changes in Body Composition and Self-Reported Disease Symptoms: A Randomized Controlled Study“ Kaats et al. in Am J of Bariatric Med, Spring 1996: 18-23.
- (51) El test Alcat de sensibilidad a los alimentos y su interés en Medicina Estética Cabo-Soler JR. Alcuni Particolari Della Dieta In Medicina Estetica (Comments On Diets In Esthetic Medicine). Abstract of 14th Med Day of Esthetical Medicine & Dermatological Survey. Venice, Italy, Sep. 22-23, 1995. Published in the proceedings.
- (52) Prevalence of food allergy and intolerance in children based on MAST CLA and Alcat Tests“ Buczylo et al., Rocznik Akad Med Białymst. 1995; 40(3):452-456.
- (53) Alcat Test Results in the Treatment of Respiratory and Gastrointestinal Symptoms, Arthritis, Skin and Central Nervous System“ Mylek et al., Rocznik Akad Med Białymst. 1995; 40(3): 625-629.
- (54) Food Intolerance in Patients with Angioedema and Chronic Urticaria. An investigation by RAST and Alcat Test. Study of Dr. Lene Hoj, presented at the XVI European Congress of Allergy and Clinical Immunology, Madrid, Spain: June 25-30, 1995, published in European Journal of Allergy and Clinical Immunology, Supplement No. 26, Vol. 50, 1995.
- (55) “Autism – a multidisciplinary approach to treatment“, Kotsanis et al. 1994. Study conducted under the direction of Dr. Constantine Kotsanis, results presentation at the annual meeting of the American Academy of Otolaryngic Allergy 1994. Study data available for download at www.kotsanisinstitute.com/home/autism-treatment
- (56) The Alcat Test: in vitro procedure for determining food sensitivities“ Pasula MJ., Folia Med Cracov. 1993;34(1-4):153-7.
- (57) The Alcat Test—A Guide and Barometer in the Therapy of Environmental and Food Sensitivities Dr. BA Solomon, Environmental Medicine, Vol. 9, Number 2, 1992:2-6
- (58) Pilot Study into the Effect of Naturally Occurring Pharmacologic Agents on the Alcat Test. Fell, PJ. American Otolaryngic Allergy Association Annual Meeting, September 27, 1991, Kansas City, MO. Published in the proceedings.
- (59) Inhibitory Effect of Sodium Cromoglycate on Granulocyte Response to Food Antigens In-Vitro. 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 proceedings.
- (60) Gastrointestinal Complaints Related to Diet, DH Sandberg, International Pediatrics, Vol. 5 No. 1, 1990:23-9.
- (61) Pharmacologic Compounds in Foods - The effect on the Alcat Test in Healthy volunteers and patients suffering from Migraine Fell PJ, Brostoff J, et al., AAOA News 9:2:29.
- (62) Multiple Pathogenic Mechanisms in Food Sensitivity Reaction In-Vitro. Puccio SG. et al., 4th International Symposium on Immunological and Clinical Problems of Food Allergy, Milan, Italy. November 5-9, 1989. Abstract Symposium Book, pg. 37.
- (63) Influence of Food antigens on Volumes of Circulating White Blood Cells and Platelets Aggregation Studien-präsentation beim 4. Symposium on Immunological and Clinical Problems of Food Allergy, Mailand, Italien, 5.-9. November 1989.

Special thanks to Dr. rer. nat. S. Slazenger for her efforts in the preparation of this document.

Cell Science Systems GmbH
– Alcat Europe

August-Bebel-Str. 68
14482 Potsdam
Germany

T +49 (0)331 740088-0
F +49 (0)331 740088-29
E info@alcat-europe.com
W www.alcat-europe.com
www.alcat.com

Cell Science Systems Corp.
– Alcat Worldwide

852 South Military Trail
Deerfield Beach, FL 33442
USA

T +1 954 426-2304
E info@alcat.com
W www.alcat.com
www.previmedica.com

Alcat Thailand co., Ltd.

No. A2-402, 4th floor 3808,
Rama 4th Rd. Prakanong
Klontoey, Bangkok 10110
Thailand

T +66 (0)2-339-3840-1
E info@alcat.co.th
W www.alcat.com.co.th



Registered Medical
Device Manufacturing
Establishment

EN 13485

Alcat Test® is a registered trademark of Cell Science Systems GmbH – Alcat Europe and Cell Science Systems, Corp.
Copyright © 2014 by Cell Science Systems GmbH – Alcat Europe

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, photocopying, recording, scanning, or otherwise. Permission from the copyright owner is required for any republication or redistribution.