

LRA by ELISA/ACT® CLINICAL UPDATE # 15

LRA by ELISA/ACT Technology and Answers to Common Questions

This practical overview of Lymphocyte Response Assay (LRA) by ELISA/ACT® describes **the most advanced lymphocyte response, functional delayed allergy / hypersensitivity diagnostic / interpretive procedure. All delayed immune system reactions on as many as 400 lectins, foods, chemicals can be concurrently measured on a small (one ounce) blood sample.** LRA by ELISA/ACT measures all delayed allergy pathways through the lymphocyte subsets specific for the various pathways by uniquely combining ELISA amplification (done on the lymphocyte surface itself) with classical lymphocyte recognition (mitogenic) responses.

With the help of clinical colleagues and to help you, we have put together the following technology overview, its basis in science and medicine and its successful use in practice today.

This overview has four components:

- The immune system's role
- The use of LRA by ELISA/ACT in diagnosis and treatment of autoimmune diseases
- The causes of delayed hypersensitivity
- Outcome results with LRA by ELISA/ACT in treatment-resistant conditions

The Immune System's Role: A Summary

The widely held view of the immune system as an autonomous system, blessed with unlimited capacities, has given way to a recognition that it is an integral sub-system of the neuro-chemical, hormonal and immune complex with both defense and repair roles and finite limits to discharge

these functions when under multiple assaults and distresses.

Concurrently, better understanding of autoimmune illness (AI), and the increasing number of "mystery" diseases linked to anti-self response has clearly tied AI disease to a heightened and/or dysfunctional state of immune system alert.

This evolving body of knowledge has, in turn, revived scientific interest in so-called delayed or hidden immune system reactions to that much larger body of food, chemical, and environmental antigens that are beyond the diagnostic scope of existing tests for IgE or any other single antibody class. The issue is no longer whether food or other delayed "allergies" are a serious threat to health and wellness, but rather how do we accurately measure what happens within the immune system and what do we do about it. For, what we can not measure too often remains clinically obscure and frustrating.

The Evolution of Delayed Hypersensitivity Technology

The amplification powers of Enzyme Linked Immunoabsorbent Assay (ELISA) give us a new diagnostic window on IgG or Humoral (Type II) reactions, but they cannot differentiate between the reactive "bad" antibodies and the protective "good" ones. They further give us no insights into Immune Complex and Cell-Mediated (Types III and IV) reactions. Other new tests (e.g., Raji Cell Assay, Lymphocyte Cytokine Release) emerged to cover these other delayed response pathways individually, but they have all proven to be costly and incomplete at best and often prone to problems of sensitivity, specificity, or reproducibility in clinical practice.

Before 1980, no single diagnostic test measuring all delayed antibody class responses through all delayed pathways had been reduced to practice. (Fig. 1)

ELISA/ACT Biotechnologies LLC (EAB) pioneered cell surface chemistries, high-precision antigen purification techniques, and a specially engineered microtiter plate, all essential for proper high-sensitivity cell response. The synthesis of these developments and years of bench trial and error facilitated the merging of high-amplification ELISA and the advanced cell culture technology of lymphocyte blastogenesis. **LRA by ELISA/ACT was the first (and remains the most advanced) functional lymphocyte response assay, measuring all Humoral, Immune Complex and Cell-Mediated reactions to the broadest possible range of antigens (currently ~400) from a single blood sample.**

Internally, we refer to the results of a complete LRA by ELISA/ACT scan (we run smaller, more targeted test panels on request) as an individual immunologic "fingerprint", distinctive to each patient, yet highly accurate and reproducible in its

INDEX

Background on LRA by ELISA/ACT..	1
The immune system's role: A summary..	1
Use of ELISA/ACT in diagnosis	2
The causes of hypersensitivity	3
Clinicians report on	
LRA by ELISA/ACT	4

indications of strong, mild, or no lymphocytic response to each tested substance. Blinded lab tests in healthy and autoimmune disease patients and other comparative analyses find clinical estimates of sensitivity (i.e. false negative) of <4% and specificity (i.e. false positive) of <2%. Most critical to the practical application of the technology, on-going split sample and other quality control studies show reproducibility of results (i.e. the same “immune fingerprint”) >97%. This means a day-to-day variance of the method of <3%. Physicians familiar with the comparable “quality control” on the standard array of reference lab tests recognize these performance characteristics as exceptional and due to the advanced technique used uniquely in the LRA by ELISA/ACT system. While we *do* have palliative, symptom suppressive therapies for the symptoms of AI, we **need** diagnostic tools with linked **outcome effective treatment plans that address the causes** rather than the consequences of AI. LRA by ELISA/ACT fills this need.

LRA by ELISA/ACT in Practice

In the past three decades we have partially peeled back the secrets of the human immune system. We know that:

1. Our immune system must both defend us against foreign invasion and repair us when wear and tear set in.
2. In AI, a heightened state of alert and deferral of needed repair eventually provokes anti-self responses in tissues that have become more permeable as the blood-tissue barrier has been weakened and breached.
3. Individual immune responses are just that. . . Specific for the individual.
4. Increased intestinal permeability is a common coincident of symptomatic AI.
5. Metabolic disorders (particularly cellular acidosis) are common in progressive AI with common essential factor deficits, including antioxidants and acid-buffering minerals like magnesium and zinc (albeit commonly overlooked in the press of symptom-driven care).

LRA by ELISA/ACT Technologies at Work

- A no damage blood sampling technique based on an all plastic vacutainer system with direct anticoagulation technique that effectively puts the cells in suspended animation. This is a modification of the Born and Cross technique developed by Jaffe and Deykin for doing functional platelet activity studies and further modified by Jaffe for lymphocyte autologous culture. With proper courier transport at a temperature of 4°-8°C, the whole blood sample is stable for at least 72 hours within which identical results are obtained whenever the cell culture is done.

Among other studies, three simultaneously drawn samples were divided: One was read fresh, one was kept in the refrigerator for 72 hours before being read, and the third was sent across country -- to Phoenix in the summer and to Alaska in the winter -- and then sent back to the lab for analysis. Over a year’s such exhaustive testing, identical results (within the 3% variance of the method) were obtained on each of the three samples. While this technique is different from the usual “vacutainer” approach, it is the only way to protect from activation

Fig. 1. Clinical tests of immune responses. LRA by ELISA/ACT tests replace 3 or more assays by measuring all delayed pathways in a functionally meaningful way. This avoids the clinically false positives tests that ELISA IgG tests measure because they don’t distinguish reactive from protective antibody.

- Autoimmune illnesses include most rheumatoid connective tissue diseases (arthritis, Sjogren’s syndrome, lupus/SLE, scleroderma), asthma, inflammatory bowel disease (IBS, Crohn’s disease, and ulcerative colitis), diabetes (DM), multiple sclerosis (MS), migraine headaches, chronic fatigue syndrome, immunotoxic infertility, ITP, Grave’s hyperthyroidism, Hashimoto’s thyroiditis, pemphigus, eczema and psoriasis, fibromyalgia, myofascial and related chronic pain syndromes.

What Measured	Reactions	Humoral	Immune Complex	Cellular
IgE (‘RAST’)	Y	N	N	N
IgA	N	Y (only IgA)	N	N
IgM	N	Y (only IgM)	N	N
IgG (‘Generalist’)	N	Y (only IgG)	N	N
IgG1	N	Y (IgG1)	N	N
IgG2	N	Y (IgG2)	N	N
IgG3	N	Y (IgG3)	N	N
IgG4	N	Y (IgG4)	N	N
Immune Complex	N	N	Y	N
Raji Cell Assay	N	N	Y	N
Lymphocyte Cytokine release	N	N	N	Y
LRA by ELISA/ACT	N	Y	Y	Y

of the blood clotting coagulant system, the complement immunologic activation, and cell cytokine activation.

- LRA by ELISA/ACT tests depend upon the ability to perform a **brief cell culture** and detect amplified results. A unique microtiter system has been developed that meets this need. A purified substance (antigen) is coated as a monolayer on the surface of an optically clear 48 multi-well plate. Two plates have the surface geometry of the more common 96 well microtiter systems and fit standard ELISA readers. However, through technological advances that keep background activity low and true reaction signal high, a novel one-step ELISA procedure has been developed. This avoids the five or more steps of other ELISA procedures, each of which adds its own intrinsic margin of error. By comparison, LRA by ELISA/ACT achieves consistent day-to-day variance of <3% while other serum ELISA tests achieve 15-20% variance (due to the 3% error added at each step of the procedure). Reaction plates are coated and produced under strict quality control directly by EAB. While other delayed sensitivity cell responses tests have been researched, LRA by ELISA/ACT is the first system to employ lymphocyte responses and the first reliable assay system for detecting these time-delayed, late-phase reactions.

- **Antigen purification** is part of LRA by ELISA/ACT technologies. A novel, volatile buffer is used to suspend the antigens. This allows for homogeneous coating of the reaction plates and even deposition of reactive materials. Of the 400 items offered as part of the LRA by ELISA/ACT tests system, all are purified and produced by EAB, and over 150 are unique to LRA by ELISA/ACT testing.

- **LRA by ELISA/ACT tests quality control** include negative internal controls such as buffer alone or saline, which ought not to induce cell reactions, and positive internal controls, like phorbol ester and poke weed mitgoen, which ought to strongly activate cells. Thus, before any test is done, EAB technicians verify that neither preactivation of cells nor cell unresponsiveness is present. When

unsatisfactory specimens are obtained, a retest is requested without additional charge to the physician.

- **Computerized “smart system” results** reporting achieves rapid results with typical turnaround time from phlebotomy to return of test results being seven days (including one day of courier transport for each of the specimen and report). While this is an impressive response time for any ELISA test, it is especially so for a cell culture procedure. In addition, a unique one-page patient information questionnaire provides enough additional information to develop a recommended treatment plan to accompany the results if requested. Many physicians find this helpful in going beyond the test results to a new level of treatment based on substituting for immunotoxic reactors (those substances that test positive on the LRA by ELISA/ACT tests) and stimulating recovery through a plan designed to evoke the human healing response.

LRA by ELISA/ACT tests provide a new generation of functional cell tests combining innovation, value-added precision, and actionable results based on the results from the widest range of antigens and epitopes tested by any immunology lab in the world.

How LRA by ELISA/ACT accomplishes this testing breakthrough: The power of full function testing and its value in clinical practice.

1. Measuring all delayed allergy / hypersensitivity pathways: A functional cell culture technique in which lymphocytes sensitized in the patient allows them to respond in autologous cell-rich plasma. This means that all the plasma, cellular, and cytokine/interleukin elements are present as they are in the body. Thus, reactive IgA, IgM, and IgG can all be detected (Humoral or Type II immune responses). In addition, immune complex, Type III, and direct cell activation, Type IV, responses can be determined concurrently. Perhaps most important is this system’s functional ability to distinguish helpful immunologic memory (protective IgG) from long

standing hypersensitivity (reactive IgG). This differs from the serum IgG assays that are unable to make this distinction and are therefore subject to clinically false-positive results from the cell-based LRA by ELISA/ACT functional immunoassays.

2. Outcome significance of the results:

In treatment-resistant AI illnesses, the clinical outcome results have been encouraging, including results of three-to-five-year case follow-ups. Here is what physicians say about LRA by ELISA/ACT in practice:

- “I have been using EAB and the LRA by ELISA/ACT for three years. It has proven to be, time and time again, the most accurate and clinically relevant diagnostic laboratory test for determination of food and chemical sensitivities. I frequently perform the LRA by ELISA/ACT as a first line diagnostic for my patients’ problems. My patients are impressed not only with the test results but with the included literature explaining the LRA by ELISA/ACT results and the recommendations provided. Many people have been helped from the knowledge gained through the LRA by ELISA/ACT. I include my wife and myself to that list. We both suffered from food allergies and were able to eliminate the offending foods and experience significant improvement in our health.”

The LRA by ELISA/ACT has produced at least two “miracle” cures.

1. A 45-year-old woman suffering from 20 years of chronic depression and obesity who after avoiding allergic foods lost 15 pounds, was off of all medications, and feeling better than she had felt all her life. One year later she has lost 75 pounds and is feeling wonderful with not a hint of depression.

2. A 42-year-old male with 15 years of daily intractable migraine headaches had been diagnosed and treated in major headache research facilities with no success. After two months following the LRA by ELISA/ACT program, he no longer has a headache unless he eats an offending food, and then it is only mild and controllable with aspirin.

- “I highly recommend the LRA by ELISA/ACT as a major tool in a physician’s diagnostic armory.”

Richard Cohen, MD, Hanover, MA

• “Every chronically ill person ought to have the LRA by ELISA/ACT test. There is nothing that compares for cost effectiveness or outcome results.”

Ralph Golan, MD, Seattle, WA.

• “LRA by ELISA/ACT is a true breakthrough in both laboratory diagnostics and clinical medicine. It is the reference standard in delayed allergy testing. I have featured it in my column in *Alive Magazine* and in my book on health published by Lewellan Press. ELISA/ACT makes management of problem patients much more effective and successful.”

Zoltan Rona, MD, Toronto, Canada

• “LRA by ELISA/ACT has been a primary part of my diagnosis of multiple sclerosis patients for over five years. After struggling for years to understand which specific foods trigger symptomatic reactions in which individuals, ELISA/ACT is a breath of fresh air. It gives me and my patients a meaningful starting place for modification of diet to improve their conditions. I believe hypersensitivity testing ought to be part of every MS patient’s diagnostic evaluation.”

Robert Soll, MD, Ph.D.,

Arlington, VA, neurologist & author,

MS: Something can be done & you can do it

• “Since 1985 LRA by ELISA/ACT has been my choice for detecting food, chemical, and preservative sensitivities. While no test is perfect, this test provides my patients and my staff the most useful information available. For results, choose LRA by ELISA/ACT.”

George Mitchell, MD, Washington, DC

Summary: The completeness of testing all delayed immune pathways, the reproducibility of the LRA by ELISA/ACT assays, and the practical clinical implications (therapy by substitution for immunoreactive items with targeted essential nutrient supplementation to correct deficits and allow the repair process to engage) provide a fundamental clinical tool.

Advances in and relevance of immunology in both basic and clinical science highlighted in *Scientific American* (9/93) is devoted entirely to “Life, Death, and the Immune System,” an article on AI describing advances that validate the

clinical fundamentals of the LRA by ELISA/ACT functional immunology tests and treatment plans. More importantly, the article shows that only a complete survey of immune responses through a functional test like the LRA by ELISA/ACT provides the needed information to determine the specific foreign invaders that amplify and/or are the cause of chronic self-attacking illnesses that have become our country’s greatest source of sufferings and health-care costs.

Contact

If you have any questions or would like more information about LRA by ELISA/ACT tests, please contact our Client Services Department at 800-553-5472.