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JANUARY 2014 ISSUE #366 $7.50
Predictive Biomarkers in Personalized Laboratory Diagnosis and Evidence Based Best Practices Outcome Monitoring

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Predictive biomarkers are a few tests that can now be referenced to goal values whose interpretation can include a lifestyle action plan that enhances functional cost and outcome effectiveness, adding years to life and life to years.

This article addresses:

• what these predictive biomarkers are and why they are valid;
• interpretation of the test results based on goal values;
• how to reduce risk and bring tests value to or nearer to the safer value at least cost and best outcome effectiveness.

The existence of predictive biomarkers is the first conceptual advancement in lab medicine since sensitivity, specificity, and predictive index were introduced a generation ago. Integrative, comprehensive, personalized medicine seeks evidence-based objective predictive biomarkers to determine that both risk and response to therapy can be quantified. Each predictive biomarker is selected for its sensitivity; that is, its accuracy, and its specificity; that is, its lack of false results so that its clinical predictive significance – the product of sensitivity and specificity – is high.

Eight predictive biomarkers are proposed here along with their goal values, including which aspect of the metabolome and the microbiome are most affected, and the genes and epigenetic modulation of genetic expression. Strategies and tactics are presented to enable people to improve upon their biomarker values through virtuous cycles and health-enhancing habits of daily living.

Predictive Biomarkers

Predictive markers are independent and interdependent assessments of health risk and status. Together they cover the 92% of lifetime health that is based on lifestyle habits, or epigenetics, if you prefer. While 8% is genetic, over 9/10 of the quality and quantity of life is determined by the sum of what, how, and when people eat, drink, think, and do.

Predictive biomarker test results provide a comprehensive, accessible, actionable, and personalized plan for health with added value when the goal value and interpretation referenced here are included. Eight functional tests, each predictive of outcome, are useful in monitoring therapeutic responses to any program designed the help the person or evoke healing responses.

Usual (Statistical) Test Results vs. Predictive (Healthy) Goal Value Results

Prior to predictive biomarkers, conventional clinical lab tests provided information about “usual” or “normal” statistical ranges of a particular item analyzed. They are useful for population studies but not clinically as relevant or predictive. By contrast, these specific predictive biomarker tests provide information that extends the concept of “optimum,” or “high-level health,” reference ranges pioneered by Cheraskin and Ringsdorf or the biochemical
individuality concept documented by Roger Williams. The goal values recommended here for each predictive biomarker are designed to improve predictive personal precision in practice and are set to be the least risk or highest gain value for each test. When predictive biomarker tests are at their goal value, all-cause morbidity and mortality are at their best outcome value; quality of life and lifespan are optimized. The specific predictive biomarker tests included have each also been validated on large numbers of people from all ethnic and socioeconomic backgrounds.

Predictive biomarkers referenced to goal values and interpreted with a focus on epigenetic opportunities and lifestyle habit changes can stimulate virtuous behavior cycles, and more cost-effective and outcome-effective care for each individual.

Table 1 gives an overview of the predictive biomarkers here suggested with their clinical significance.

<table>
<thead>
<tr>
<th>Predictive Biomarker Test</th>
<th>Metabolome, Microbiome, Genes, &amp; Epigenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin A1c (Hgb A1c)</td>
<td>Sugar, energy, diabetic risk &amp; insulin resistance; epigenetic metabolic syndrome; syndrome X</td>
</tr>
<tr>
<td>High sensitivity C-reactive protein (hs-CRP)</td>
<td>Epigenetic inflammation, repair ability; calls for immune help; telomere length</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Epigenetic methylation, detox, transport, sulfur cycles</td>
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<tr>
<td>Oxidized LDL/HDL</td>
<td>Epigenetic CVD risk; lipid AO status</td>
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<tr>
<td>8-oxoguanine</td>
<td>DNA oxidative stress; nuclear AO status</td>
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<tr>
<td>Vitamin D</td>
<td>Epigenetic cell talk &amp; adhesion, C, CVD, &amp; AI risks</td>
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<tr>
<td>1st morning (a.m.) urine pH</td>
<td>Metabolic acidosis; mineral status; cell battery</td>
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<td>LRA by ELISA/ACT</td>
<td>Immune tolerance or intolerance; delayed allergies</td>
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</tbody>
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Legend: AI: autoimmune; AO: antioxidant; C: cancer; CVD: cardiovascular diseases; DNA: genetic code; deoxyribonucleic acid; ELISA/ACT: enzyme-linked immunosorbent assay/advanced cell technique; LRA: lymphocyte response assay

Predictive Biomarker 1: Glycosylated Hemoglobin/Hemoglobin A1c (Hgb A1c)

Hemoglobin A1c (Hgb A1c) most accurately measures average glucose or blood sugar. Fasting and 2-hour postprandial blood sugar have long been measured to get information about moments in time. More recently, insulin and glucose/insulin ratios have been developed to better understand sugar energy metabolism. Hgb A1c better predicts average blood sugar level for the previous 3 months than any other lab test.

This is important because frequently people have unremarkable blood sugar on the particular day that the test is run, but their Hgb A1c is elevated, or conversely stress may increase blood sugar at a moment while Hgb A1c is low, showing that “white coat” blood sugar elevations happen just as do blood pressure elevations. Day-to-day blood sugar levels can be distorted by many pre- and postanalytic variables such as exercise, meal timings, or medications. More importantly, we do not see high blood glucose levels routinely until prediabetes is far advanced. Hgb A1c gives more reliable indication of actual and future risk. Accurate fasting blood sugar values require at least 12 hours of water only prior to the blood draw. In practice, this preanalytic variable is often ignored.

Hgb A1c is a marker of insulin sensitivity and resistance. Elevated Hgb A1c is strongly linked to inflammation as well as chronic, degenerative, autoimmune disease risks.

A Hgb A1c of <5% is the desired or goal value and reflects a 99% probability of living 10 years. The graphs below (Figures 1A and 1B, p. 95) represent the correlation between Hgb A1c levels, blood glucose levels, and 10-year survival probability.

When above goal value, an immunotolerant diet of whole foods enriched with super-foods and targeted full disclosure supplements directed to improve energy, glucose, and insulin balance, is included as part of the interpretation. Being active physically and
mentally is also included in the recommendations designed to evoke healing responses.

**Predictive Biomarker 2: High-Sensitivity C-Reactive Protein (hs-CRP)**

High-sensitivity C-reactive protein (hs-CRP) is one of the most predictive markers of inflammation systemically and particularly in the cardiovascular system. Levels of hs-CRP rise in response to repair need, also known as inflammation. Hs-CRP is more precise and predictive at low levels than CRP. When inflammatory repair deficit persists, often a chronic damaging process is slowly smoldering below the surface that is merely troublesome and not yet disabling progresses. Inflammation burdens the body’s organ systems, especially the immune system, slowly wearing it down, taking a toll on daily quality of life, increasing risk, and reducing survival.

The charts below (Figures 2A and 2B) represent the correlation between hs-CRP, Framingham 10-year CVD risk scores, and 10-year survival probability. Elevated hs-CRP is common in prediabetes and diabetes, reflecting insulin resistance and metabolic syndrome X, a continuum of conditions with increased inflammation and a high risk of cardiovascular complications due to cumulative repair deficits. Elevated hs-CRP levels may indicate a long-term chronic infection or host hospitality due to cumulative repair deficit; that is, inflammation.

As a predictive biomarker, hs-CRP reflects the effectiveness and efficiency of first line innate cellular immune defenses, responsible for neutralizing any sign of infection, repairing daily wear and tear, and identifying and eliminating cancerous cells.

Hs-CRP goal value is <0.5 mg/dl.

If hs-CRP is above goal value, our interpretation includes an immunocompetent diet of whole foods with an emphasis on repair-promoting super-foods, targeted supplementation directed toward adequate systemic repair, as well as mental and physical activities to evoke healing responses.

**Predictive Biomarker 3: Homocysteine**

Homocysteine is an amino acid whose balance with methionine reflects methylation status. Methylation controls many aspect of cell function, including expression of our genetic material; modulates RNA; and helps transport or deposit proteins. When this process is not working properly, homocysteine levels are elevated. Homocysteine reflects a deeper imbalance in two critical important aspects of metabolism, detoxification and methylation within all cells.

This marker reveals cellular function or dysfunction in regard to sulfur metabolism and methyl group migration at the most basic cell level. High homocysteine is associated with risks from heart disease and cancer to Alzheimer’s disease and osteoporosis. Homocysteine measures all-cause morbidity and mortality; this test is an important predictor of long-term survival (Figures 3A and 3B, p. 96).

The good news is that elevated homocysteine levels reflect an imbalance usually easily correctable with diet and supplements. It is also encouraging to know that as homocysteine levels come back into a more normal range, risk is reduced after as short as a few months at the new, healthier levels after deferred repair as been completed.

Homocysteine goal value is <6 µmol/L.

If homocysteine levels are above the goal value, our interpretation includes an immunotolerant whole-foods diet with an emphasis on sulfur-rich super-foods, targeted supplementation including methylation nutrients, along with mental and physical activities to evoke healing responses.

Additional five predictive biomarkers are discussed below that add independent predictive value to the above set of three. These are:

- oxidized LDL/HDL and 8-oxo-guanine for antioxidant status
- vitamin D level for cell communication
- pH for cell acidity and mineral reserves
- immune tolerance and intolerance by lymphocyte response assay (LRA)

**Predictive Biomarker 4: Oxidized LDL/HDL**

Oxidized LDL/HDL is a highly reliable indicator of oxidative stress, antioxidant status, and additionally cardiac risk. While traditional LDL cholesterol levels have been in use for a while to predict heart disease,
it is well documented now that the oxidized LDL test is a superior blood lipid test to identify risk among apparent healthy men and women. Although the test has only recently become available commercially, the technology has been used in research studies for more than 10 years, and at least 400 papers have been published evaluating oxidize LDL cholesterol.

The test measures the health of DNA in our mitochondria, the “engines” within each cell that make energy. Are we burning up DNA faster than needed due to stress, toxic exposures, or poor diet? In essence, oxidized LDL is a measure of the health of our cells. Impaired function of the mitochondria is one of the central failures in practically every form of chronic illness. By being able to measure the health of our cells, we are measuring an important end point for chronic illness. This test answers the question, do we have increased stress in this critical area and, if so, how much stress?

Oxidized LDL is a preferred measure of oxidative stress and associated cardiac and metabolic syndrome risk with a healthy value of ~0 when adequate antioxidant protection is provided. If oxidized LDL/HDL levels are above the goal value, the interpretation includes an immunotolerant whole-foods diet with an emphasis on super-foods and antioxidant nutrients, along with mental and physical activities to evoke healing responses.

Predictive Biomarker 5: 8-Oxoguanine (8-Hydroxyguanine, 8-Oxo-Gua, or OH8-Gua)

Along with oxidized LDL, testing for 8-oxoguanine provides important information about oxidative stress and its effects on DNA. The test is highly regarded as a measure of oxidative stress and well supported in the research literature.

This indicator focuses on the acceleration of aging due to potential DNA damage and is an effective way to evaluate the success of an intervention, whether it involves dietary change or antioxidant nutrients. Tracking the results of this test provides an indication of:

1. risks due to oxidative stress in the DNA genetic code;
2. benefit or lack of benefit from therapies over time.

When antioxidant levels are sufficient, that prevents oxidative damage from free radicals. Healthy levels of antioxidants such as ascorbate mean efficient energy production in the cells.

The goal for 8-oxoguanine that we suggest is a value of <5.3 ng/mg of creatinine, indicating adequate antioxidant protection.

Both 8-oxoguanine and oxidized LDL/HDL indicate higher levels of inflammation, repair deficit, and oxidative stress throughout the body, factors that underlie almost any form of chronic illness.

If 8-oxoguanine levels are above the goal value, the interpretation includes an immunotolerant whole-foods diet with an emphasis on super-foods and antioxidant nutrients, along with mental and physical activities to evoke healing responses.

Predictive Biomarker 6: Vitamin D

It is estimated that anywhere from 30% to 100% of Americans, depending upon their age and community living environments, are deficient in Vitamin D. Vitamin D levels play a significant role in numerous systems in the body, including immune and neurological regulation and bone health. When levels of this nutrient are low, it increases the risk of cancer, heart disease, autoimmune disorders, and psychiatric and mood problems.

In addition, vitamin D:

• improves type 1 and type 2 diabetes, hypertension, multiple sclerosis, rheumatoid arthritis, and other conditions;
• moderates cell division as a hormone whose function is to provide vital communication links between cells, normalize cell growth, and avoid aggressive cell production;
• improves autoimmune disorders that are quieted by sufficient nutrients;
• reduces brain and nervous system inflammation, particularly important since the brain lacks other regulatory systems to moderate inflammation.

Knowing the status of vitamin D is essential to correct any nutrient depletion. The preferred test of vitamin D involves measuring the metabolite 25-hydroxycholecalciferol (25[OH]D). The predictive goal value range for 25(OH)D is 50 to 80 ng/ml.
If vitamin D levels are below 50 ng/ml, vitamin D drops, 500 IU per drop with rosemary oil, sufficient to bring the vitamin D level into the goal range of 50 to 80 ng/ml, is part of the interpretation.

**Predictive Biomarker 7: First pH After 6-Plus Hours’ Rest in Urine**

The pH level of urine after 6 hours of rest reflects pH throughout the body (Figure 4, p. 96). Levels below 6.5 indicate metabolic acidosis. Low pH also suggests mineral deficits, because minerals are pulled from bone and body fluid during metabolic acidosis to buffer and reduce acids and maintain pH within a health range.

Tiny changes in pH have profound implications for cell metabolism. Life exists poised exquisitely just above the neutral point of 7.0. Levels of pH above 7.5 can indicate catabolic illness in which amino acids are used as energy sources.

Any unusual variation in urinary pH is usually reflected in the first morning urine. This calls for changes in diet and/or nutritional supplements to restore acid–alkaline balance. Simply checking the pH level each day provides ongoing monitoring to see whether pH has been corrected. (fuph.perque.com). This is an important aspect of biochemistry; so if there is an abnormality, that has to be monitored regularly.

The predictive goal value range for urine pH is 6.5 to 7.5 after 6 or more hours of rest, typically first in the morning.

If pH levels are below the goal range, our interpretation includes an immunotolerant diet with alkalinizing whole foods and an emphasis on
**Predictive Biomarkers**

**Figure 2B: High-Sensitivity C-Reactive Protein (hs-CRP) and 10-year survival**

Calculated Framingham 10-Year Risk

![Graph showing the relationship between hs-CRP and Framingham risk](image)

**Figure 3A: Homocysteine Levels and 10-Year Survival**

Homocysteine <6 is *Predictive Biomarker*

![Graph showing the relationship between homocysteine levels and survival](image)

**Figure 3B: Homocysteine Level, CVD, and 10-Year Survival Probability**

Cardiovascular Disease (CVD) vs Probability of Living 10 Years

![Graph showing the relationship between homocysteine levels, CVD, and survival](image)

*Essentially non-detectable.*


super-foods. Targeted supplementation, especially magnesium and choline citrate, along with mental and physical activities to evoke healing responses, are also key.

**Predictive Biomarker 8: Tolerance or Immune Reactivities via LRA by ELISA/ACT Tests**

Conventional therapies for autoimmune conditions involve some combination of immune suppressive therapies. In contrast, the LRA (lymphocyte response assay) by ELISA/ACT determines individual reactive foods or other chemicals that appear to...

*continued on page 99 ➤*
be burdening the immune system and an optional interpretation aimed at restoring immune competence, tolerance, and resilience.

The LRA tests measure all three delayed allergy pathways (Figure 5, p. 96) while avoiding false positives common in other types of delayed allergy tests:

- reactive antibody (IgA, IgM, and IgG)
- immune complexes
- direct T-cell activation

The LRA by ELISA/CT tests are fundamentally different from antibody serology or particle counting tests in that ex vivo LRA tests are substantially more sensitive, specific, and predictive.

Benefits of the LRA compared with other allergy tests include that it is:

1. comprehensive: Only clinical tests of all three delayed allergy pathways at one time (type II, type III, and type IV) able to perform up to 491 cell cultures on just 1 ounce of blood;
2. functional: Identifies only symptom provoking reactive substances, not merely harmful or protective while missing T-cell responses entirely;
3. ex vivo: Unique LRA tests are performed just as they occur inside the body.

Identifying the patient’s specific sensitivities and delayed allergies that burden the immune system is a clinical breakthrough. Patients often experience a dramatic improvement in quality of life as a result of the individualized treatment plan, which includes an alkalinizing diet and targeted supplementation. After 6 months, a reevaluation of progress is recommended with three possible outcomes:

1. If patient is in remission, a guided gradual reintroduction of previously reactive items 1 per week is advised, ingesting an item 3 times in the first week because of amnestic responses.
2. If patient is better but not yet well, repeat LRA tests and treatment guidance every 6 months until in sustained remission. Since digestion and detoxification, among other systems, take time to recover, it is common for people to lose some reactive items and acquire new ones. A repeat program starting from a healthier base is likely to further improve health and sustain remission.
3. If patient reports following instructions carefully and avoiding reactive items but is still not better, look for toxins or hormone-disrupters that might inhibit to recovery. If a person reports making best efforts at following the program and does not report improvement, repeat testing is not indicated.

Healthy immune tolerance means no delayed allergic LRA reactions. Highly healthy people are tolerant. People can restore tolerance as part of a proactive prevention lifestyle. If reactions are found by LRA tests, our approach includes substitution of reactive foods along with an Alkaline Way whole-foods diet with an emphasis on super-foods, targeted supplementation to promote repair, and mental and physical activities to evoke healing responses. LRA by ELISA/ACT cell cultures are reproducible within less than 3% when different readers read split samples on different days.

**Discussion and Conclusion**

The eight predictive biomarkers discussed here are presented for use in clinical practice based on their predictive goal value and translated into years or decades of life at risk, retainable, or recoverable. Every health practice can benefit from learning about the value of personalized medicine and effective proactive prevention.

While each of the predictive biomarkers is predictive, when four or more are interpreted together, their predictive power increases, covering the 92% of lifetime health determined by choice and habit. While each biomarker is a separate marker of certain aspects of physiology, human systems are interdependent and usually consistent. When a biomarker shows higher risk, sooner rather than later is the time to take action and bring that marker back to or toward the goal value.

By example, hemoglobin A1c is highly predictive of certain aspects of sugar, insulin, energy metabolism, weight, metabolic syndrome, diabetes, cardiovascular, and other chronic diseases. Hs-CRP is highly predictive for other aspects of cardiovascular and chronic disease, particularly in regard to repair deficits that present as clinically as inflammation and to the person as stiffness or pain. Homocysteine rounds out
the remainder of cardiovascular risks as they relate to methylation, sulfur metabolism, detoxification, and epigenetic modulation.

Predictive biomarker goal values are best on best outcome and least risk variables that do not depend on age or gender. The usual or statistically normal ranges for homocysteine, for example, are usually based on age and gender. This means that there are more unhealthy people in the population as it ages. Age-conditional usual lab ranges often drift toward the less well with advancing age. Age, however, is a contingent variable. The significant variable is how many unhealthy people are present at each age. An important difference: chronology is fixed and most of function is choice.

Every test has a standard deviation or range within which the value exists. Typical variance for most classic ELISA based tests is 20% or more. This means that a value of 6 from a given specimen will cluster values around 6 with a large range of values from 4.8 to 7.2. The typical lab test variance is 20%. The more narrow the variance, the more predictive is the observed value. For predictive biomarker tests, a variance of 5% or less is desirable. For example, if the “true” value is 6 and the test technology allows for better precision and as a result a 3% variance, a single test value actually exists between a narrow range of 5.92 and 6.18. Improvement in preanalytic variables by reducing interfering substances, improved control of analytic conditions, use of improved curve fitting particularly at the lower end of the test range, often where the accuracy of measurement is most important. As a result, the predictive significance of any specific value becomes much greater. Such improvements in precision have been accomplished in higher-complexity tests such as lymphocyte response assay cell cultures (e.g., LRA by ELISA/ACT). As with all tests and particularly homocysteine, attention to details makes for better clinical results and improved human outcomes, particularly when therapy or management is based on lab results. Colleagues such as Alan Gaby have long pointed out that the best of tests done poorly or whose meaning is misunderstood by practitioners is unhelpful to the client. Mark Twain summed up the issue as follows: “Be careful about reading health books. You could die of a misprint.” Those of us who are clinical, analytical, methodological, and metrological have an obligation to point out strengths and limitations of tests where results drive therapies or predict outcomes.

Separately, two people, each with a true hemoglobin A1c of 8%, one of whom has been diagnosed as a diabetic, both have an equally high risk of a cardiovascular or other health crisis within 10 years. Technically, both are diabetic and only one knows it. This is known as the difference between incidence and prevalence. Many diabetologists today are increasingly diagnosing the degree of diabetes based entirely on accurately performed Hgb A1c tests.

While each of these tests is a predictive biomarker, when they are taken together we can identify both where healthier resilience exists and also where risks that can be reduced are identified. Predictive biomarkers are each based on large-scale, long-term studies including all ethnic, economic, and cultural groups. There are no population exceptions of which we know. The predictive goal value for the biomarkers explained here is based on evidence from many studies covering all ethnic groups over long periods of time in regard to all-cause morbidity and mortality; that is, life expectancy. The probability of living 10 years for these predictive biomarkers is based on large-scale, long-term community-based outcome studies. The Health Studies Collegium includes links to review articles that address aspects of predictive personalized and potentially life-saving tests and what to do about them.

Predictive biomarkers are not age adjusted, because the interpretations of the test results reported here are based on least-risk, best-outcome healthier goal values. Healthy people at all ages have the same lab ranges. As people accumulate age, there are more unhealthy people in each progressive decade. This is why using predictive biomarkers based on goal values is an advancement of the previous statistical normality approach.

While too many people live in denial about their health until some catastrophe occurs, more and more professionals and consumers are using predictive biomarkers to help assure and guide their lifestyles. These proactive consumers of healthier caring are likely to live well and prosper.
Resources
Clinical protocols for these eight predictive biomarkers can be obtained by contacting PERQUE Integrative Health: 800-525-7372; clientservices2@PERQUE.com.
ELISA/ACT Biotechnologies: 800-553-5472; clientservices@ELISAACT.com.
Health Studies Collegium: 800-328-7372; info@4HSC.org.

Notes

Dr. Russell Jaffe received his AB, MD (with senior thesis honors), and PhD (in biochemistry and physiology) from Boston University. Dr. Jaffe served his medical internship at University Hospital and was awarded a the US Public Health Service Officer Commission, assigned to the Clinical Center of the National Institutes of Health, in June 1973. While at the Clinical Center, Dr. Jaffe served his residency in clinical pathology. He is board certified in clinical and subspecialty certified in chemical pathology. Dr. Jaffe remained on the permanent senior staff of the NIH Clinical Pathology Department, where he continued method innovation and was active in collaborative research with the Laboratory of Experimental Atherosclerosis (of the Heart, Lung, and Blood Institute). Concurrently, Dr. Jaffe’s interests in the mechanisms of health and the evoking of human healing responses led him to apprentice in such healing arts as acupuncture; mindfulness; massage; music, art, and color therapy; and a variety of eclectic therapeutic approaches. In addition, Dr. Jaffe performed innovative studies of platelet function and blood clotting in relation to the origins of coronary artery and cardiovascular diseases. Among the tests that he developed are the early colon cancer-screening test using occult blood detection not interfered with by vitamin C consumption, as well as a variety of tests related to the blood clotting and immune defense and repair systems. Dr. Jaffe developed the first method of measuring cell-mediated immunity using a modified ELISA system in a lymphocyte mitogenesis/blasto genesis brief cell culture known as lymphocyte response assays (LRA). This LRA by ELISA/ACT provides an “immunologic fingerprint” of items to which the body is reactive or tolerant.

Dr. Jaffe has contributed over 100 symposium-invited talks, scientific articles, or book chapters. He received the J. D. Lane award for original research from the USPHS, the Merck Sharp and Dohme Excellence in Research Award, and in 2002 the International Research Scientist of the Year, among other recognitions for his investigations. Dr. Jaffe is a fellow of the Health Studies Collegium and director of ELISA/ACT Biotechnologies LLC and PERQUE LLC in Ashburn, Virginia. He may be reached at 800-525-7372 ext. 5101, and rjaffe@ELISAACT.com or rjaffe@PERQUE.com.

Jayashree Mani is a certified clinical nutritionist (CCN). She is experienced in the effective implementation of the comprehensive program described in this article involving these predictive biomarkers, LRA by ELISA/ACT tests, Health Appraisal Questionnaires (HAQ), Alkaline Way diet, and PERQUE nutraceuticals.

Dr. Russell Jaffe

Jayashree Mani

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