



NUT ZOOMER



Nut Zoomer

Peptide level identification of nut sensitivity



1(866) 364-0963
support@vibrant-wellness.com



www.vibrant-wellness.com



1360 Bayport Ave. Ste. B
San Carlos, CA 94070

Final Report Date:	10-22-2018 11:22	Specimen Collected:	11-30-2015
Accession ID:	1512010000	Specimen Received:	12-01-2015 00:00

LAST NAME	FIRST NAME	MIDDLE NAME	GENDER	DATE OF BIRTH	ACCESSION ID
TESTNAME	PATIENT		MALE	1996-10-22	1512010000

PATIENT

Name: PATIENT TESTNAME
 Date of Birth: 1996-10-22
 Gender: Male
 Age: 22

Telephone #: 000-001-0002
 Street Address: 1021 HOWARD AVENUE SUITE B
 City: San Carlos
 State: CA Zip #: 94070

Fasting: FASTING No. of hours: 12.0

PROVIDER

Practice Name: Test Client, MD
Provider Name: Internal2 Test Client2, MD (999999)
 Phlebotomist:
 Street Address: 999999 PRACTICE STREET AVE
 City: SAN CARLOS
 State: CA
 Zip #: 94404
 Telephone #: 666-666-6662
 Fax #: 111-222-0000

For doctor's reference

Vibrant Wellness is pleased to present to you the Vibrant Nut Zoomer, to help you make healthy lifestyle and dietary choices in consultation with your healthcare provider. It is intended to be used as a tool to encourage a general state of health and well-being.

The Vibrant Nut Zoomer is an array of commonly consumed tree nut antigens which offers very specific antibody-to-antigen recognition. The panel is designed to assess an individual's IgG and IgA sensitivity to these antigens at the peptide level.

Interpretation of Report: The summary report provided for Nut Zoomer lists the set of food tree nuts to be avoided based on the calculated results. In the detailed report, the test results of antibody levels to the individual proteins are calculated by comparing the average intensity of the individual protein antibody to that of a healthy reference population. Reference ranges have been established using 192 healthy individuals. The results are displayed as Positive, Moderate or Negative. A Positive result indicates that you have an increased reactivity to the antigen with respect to the reference range. A Moderate sensitive result indicates that you have a moderate reactivity to the food antigen with respect to the reference range. A Negative or no sensitivity result indicates that you have a low reactivity to the food antigen with respect to the reference range. Vibrant utilizes proprietary fluorescent analysis which is designed to assay specific total IgG (subclasses 1, 2, 3, 4) and total IgA (subclasses 1, 2) antibodies. The classification of Positive to Moderate to Negative denotes the level of antibody reactivity detected through this analysis.

The Vibrant Wellness platform provides tools for you to track and analyze your general wellness profile. Testing for Nut Zoomer panel is performed by Vibrant America, a CLIA certified lab CLIA#:05D2078809. Vibrant Wellness provides and makes available this report and any related services pursuant to the Terms of Use Agreement (the "Terms") on its website at www.vibrant-wellness.com. By accessing, browsing, or otherwise using the report or website or any services, you acknowledge that you have read, understood, and agree to be bound by these terms. If you do not agree to accept these terms, you shall not access, browse, or use the report or website. The statements in this report have not been evaluated by the Food and Drug Administration and are only meant to be lifestyle choices for potential risk mitigation. Please consult your physician/dietitian for medication, treatment, or lifestyle management. This product is not intended to diagnose, treat, or cure any disease.

Please Note - It is important that you discuss any modifications to your diet, exercise and nutritional supplementation with your physician before making any changes. To schedule an appointment with Vibrant Clinical Dietitians please call: Toll-Free 866-364-0963.

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INTRODUCTION

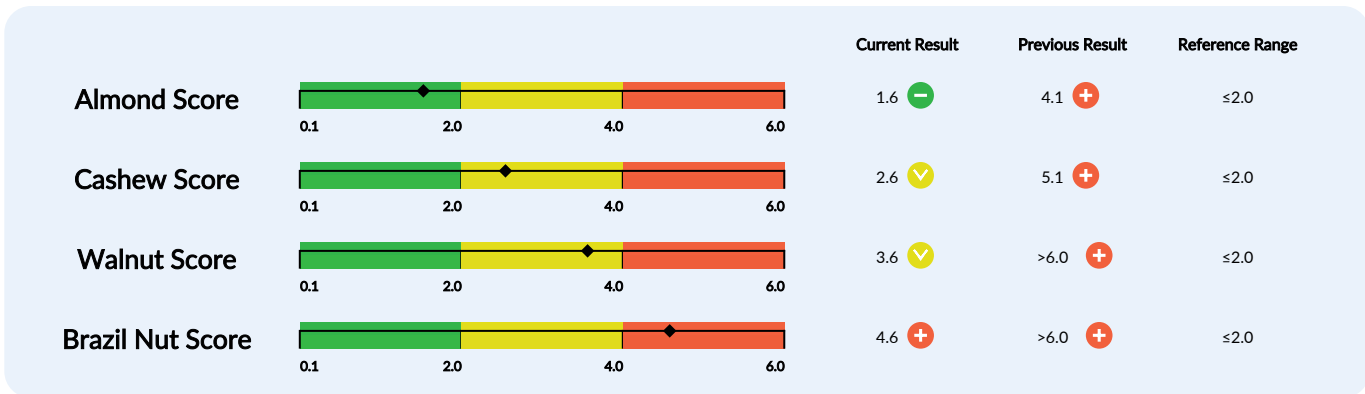
Tree nuts are rich in nutritional value, therefore commonly found in most foods to enhance their taste and potential health benefits. The U.S. Food and Drug Administration (FDA) has recognized nuts as “heart-protective” foods, thus the consumption of these nuts has risen, mainly in developed countries. Unfortunately, the rising consumption of tree nuts has also led to concerns about an increasing number of individuals sensitized to tree nuts, especially in developed countries.¹ As preventive measurements, since 1985, the Codex Alimentarius Commission introduced mandatory labeling of susceptible food ingredients that can cause hypersensitization in individuals, including tree nuts. However, accidental ingestion is common because many restaurant foods routinely use tree nuts as direct and as hidden sources.²

Major varieties of tree nuts that are implicated in causing sensitive reactions include almond, cashew, walnuts, and Brazil nuts. The prevalence of nut sensitivity varies by region and is higher in children.³ Nut sensitivity typically develops by the age of 2 and the number of nuts that an individual is sensitized to can also increase with age. This observation can be attributed to the increase in the number of nuts introduced to children with age.⁴

Routinely, doctors advise to avoid all tree nuts and peanuts if an individual is sensitive to one kind due to possible cross-reactivity. Most people are often sensitive to one or more tree nuts, but not to all of them. Hence, it would be more helpful to find the exact tree nut(s) to avoid and to safely consume the rest. Vibrant’s Nut Zoomer is a simple blood test that assesses nut sensitivity towards different nut varieties with a high degree of sensitivity. Therefore, an individual can avoid certain nuts that he/she is most sensitive to, while including the nuts which are mildly sensitive in rotation diets to safely balance a healthy diet.



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Positive		Moderate		Negative			
IgG	IgA	IgG	IgA				
Cashew Ana o 2 Ana o 3	Almond Pru du 3	Juglans nigra (Black walnut) Jug n 2	Juglans regia (English walnut) Jug r 1 Jug r 5	Almond			
Juglans nigra (Black walnut) Jug n 1 Jug n 4	Cashew Ana o 3	Juglans regia (English walnut) Jug r 4	Brazil Nut Ber e 1	Pru du 1 Pru du 6	Pru du 2	Pru du 4	Pru du 5
Juglans regia (English walnut) Jug r 1 Jug r 2 Jug r 5 Jug r 7	Juglans nigra (Black walnut) Jug n 1 Jug n 2 Jug n 4			Cashew			
Brazil Nut Ber e 1	Juglans regia (English walnut) Jug r 2			Juglans regia (English walnut)			
				Brazil Nut			
				Ber e 2			

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LIFESTYLE CONSIDERATIONS

»» Avoid the offending nut variety or nut containing food:

The primary and important standard of care management is strict avoidance of the reactive nut. The simplest management method is to avoid all nuts. However, it is possible that individuals may become hypersensitive to other nuts during their period of avoidance. Hence, it is best to avoid only the offending nut(s) and consume other nuts that are not sensitive.

»» Follow a physician recommended rotation diet:

A rotation diet will help you to avoid highly sensitive nuts from your diet and include mild to low sensitive nuts once every 4-5 days.

»» Always read advisory labels before consumption:

The Food Allergy Labeling and Consumer Protection Act of 2004 mandates that manufacturers in the United States must include the presence of any of the eight most common hypersensitive food antigens, including nuts, in the food label. However, manufacturers may occasionally change their recipes or use different ingredients for varieties of the same product, thus reading the label before every consumption may be helpful to avoid accidental consumptions. It is advised to avoid any products that do not have an ingredient list.

»» Some unexpected sources of nuts include:

Baking mixes, cereals, crackers, barbecue and pesto sauces, dressings and gravies, flavored coffees, frozen desserts, natural flavorings and extracts (e.g., pure almond extract), salads, snack foods, some alcoholic beverages. Tree nut oils, such as walnut and almond, are sometimes used in lotions, hair care products, and soaps.

»» Use alternative nutrition sources to enrich your diet

Nut Variety	Nutrients	Alternative Food Sources
Walnut	Monounsaturated and polyunsaturated fats (PUFAs), omega-3 fatty acids, iron, selenium, calcium, zinc, vitamin E, and some B vitamins.	Hummus (a good source of proteins), sunflower seeds, chia seeds, pumpkin seeds, squash seeds, flaxseed
Cashew	Monounsaturated and polyunsaturated fatty acids, protein, magnesium, copper, proanthocyanins, selenium, riboflavin, pantothenic acid, thiamin, and niacin	Sunflower seeds, pumpkin seeds, flaxseeds, hempseeds, beans such as chickpeas or soy beans
Almond	Fiber, protein, magnesium, vitamin E, manganese, and antioxidants	Sunflower seeds, chia seeds, pumpkin seeds, squash seeds, flaxseed
Brazil Nuts	Monounsaturated fats, selenium, magnesium, zinc, calcium, vitamin E, and some B vitamins	Fish, ham, sunflower seeds, chia seeds, pumpkin seeds, squash seeds, flaxseed

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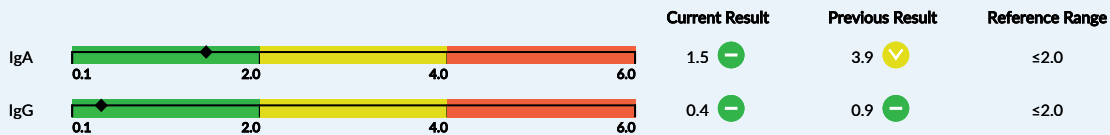
Almond Antigens:



Protein Family	Antigens
PR-10 family	Pru du 1
PR-5 family	Pru du 2
prolamin family	Pru du 3
profilin family	Pru du 4
60S acidic ribosomal protein P2 family	Pru du 5
cupin family	Pru du 6

Pru du 1

Pru du 1 is a common antigen group found in almond that belongs to the pathogenesis related-10 proteins (PR-10) family with Bet V-1 homology. Pru du 1 proteins have an intracellular defense mechanism that provides protection against pathogenic constraints, fungal or bacterial infections, and stressful environmental conditions. In almond, seven Pru du 1 isoforms have been identified. They are commonly labile proteins and in general can suffer unfolding during the cooking process. The boiling process (wet processing) causes the destruction of their conformation, reducing their ability to trigger immune reactions in sensitized individuals. PR-10 proteins from almond are analogous to those found in apple, pear, sweet cherry, and apricot.¹



Pru du 2

Pru du 2 is a group of PR-5 family proteins in almond with thaumatin-like protein (TLP) homology. They provide the plant protection against pathogenic infections, osmotic stress (osmatins), and fungal proteins. Pru du 2 in almond contains five putative isoforms. Unlike Pru du 1, these proteins are not significantly destroyed by the usual food processing methods, since they are resistant to proteases, pH, or heat-induced denaturation acquired due to the special structural configurations.¹



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Pru du 3

Pru du 3 proteins belong to the prolamin superfamily with nonspecific lipid-transfer protein (nsLTP) homology. These proteins possess an internal hydrophobic core that functions as the binding site for lipids to facilitate the transference of fatty acids, phospholipids, glycolipids, steroids etc., between membranes. Besides lipid transport and assembly, they also intervene in the defense of plants against fungal and bacterial activities. This family contains three isoforms. Pru du 3 proteins usually accumulate in the outer epidermal layer, hence thought to be responsible for the stronger sensitivity of the peels in comparison to the inner layer of the nut. They have the ability to refold to their functional conformation after cooling hence resistant to thermal treatments. In addition, they are very resistant to abrupt pH changes, and pepsin digestions. Similar nsLTP proteins are present in diverse fruits and seeds such as apple, peach, plum, sweet cherry, and apricot, implicating a probable cross-reactivity among them.¹

	Current Result	Previous Result	Reference Range
IgA	4.3 +	3.3 ✓	≤2.0
IgG	0.6 -	5.1 +	≤2.0

Pru du 4

Pru du 4 proteins belong to the profilin family with two isoforms. They participate in the binding of a monomeric actin (G-actin) that is responsible for establishing a high-affinity complex with actin, regulating the polymerization of actin into filaments. Pru du 4 proteins have a moderate structural stability, thus can be denatured and undergo subsequent loss of conformational structure by adverse conditions. This labile character and the low levels of this protein make them harder to detect by traditional immunoblot screens and limit the clinical manifestation associated with Pru du 4 to the oral cavity. The cross sensitization is generally expected in peach and sweet cherry attributing to the highest identity and similarity (99 and 98%, respectively) of profilin proteins in these two fruits with almond profilins. In addition, proteins in non related species, such as soybean or olive, also have exhibited over 80% identity and 90% similarity with Pru du 4 antigen.¹

	Current Result	Previous Result	Reference Range
IgA	0.7 -	2.4 ✓	≤2.0
IgG	0.6 -	2.2 ✓	≤2.0

Pru du 5

Pru du 5 is a major almond antigen belonging to 60S acidic ribosomal protein P2 family. They intervene in the elongation step of protein biosynthesis in the plant. P2 proteins seem to be more externally located and subsequently more likely to interact with other cellular components. This protein exhibits 81% identity and 94% homology with the recently described protein ARP60S from tomato, implicating possible cross-reactivity between them.¹

	Current Result	Previous Result	Reference Range
IgA	0.9 -	3.2 ✓	≤2.0
IgG	0.6 -	1.6 -	≤2.0

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Pru du 6

Pru du 6 is referred to as Amandin or almond major protein (AMP) that belongs to the cupin super family, specifically belonging to the 11S seed storage globulin family. They function as storage proteins and are abundant in the nut. Pru du 6 contains two isoforms, Pru-1 and Pru-2. The isoform Pru-1 is highly water-soluble but an elevated thermal stability is gained when both Pru-1 and Pru-2 are assembled into a functional protein (amandin). This protein partially unfolds at temperatures over >94 °C, aggregating to form different structures within foods. Recent studies found that the denaturation process of this type of protein, which consequently decreases their sensitivity, involves the presence of water. Unfortunately, almonds are often thermally treated with low-water systems, such as roasting, that increase the thermal stability of these proteins.¹

	Current Result	Previous Result	Reference Range
IgA	0.6	1.5	≤2.0
IgG	1.0	1.9	≤2.0

Cashew:



Protein Family	Antigens
Vicilin-like 7s globulin protein	Ana o 1
11s globulin protein	Ana o 2
2s albumin protein	Ana o 3

Ana o 1

Ana o 1 is a vicilin-like 7s globulin protein considered to be a major cashew antigen that is expected to form a trimer in native state.⁵ This is a seed storage protein important in seed germination. Ana o 1 is resistant to heat and proteolysis digestion, thus food processing methods may have very little effect on their sensitization characteristics. Some studies showed limited homology between cashew vicilin proteins and peanut vicilins (Ara h 1 etc.), explaining the limited cross-reactivity between cashew and peanut.⁶

	Current Result	Previous Result	Reference Range
IgA	0.4	1.4	≤2.0
IgG	0.2	0.6	≤2.0

Ana o 2

Ana o 2, which is considered to be a major cashew antigen, is an 11s globulin protein with a hexameric structure in nature. It is the most abundant legumin-like cashew protein and represents approximately 50% of the soluble proteins in cashew. These legumin-like proteins are non-glycosylated members of a complex family of proteins with individual subunits. These subunits are either intact precursor proteins or processed acidic and basic subunits that are covalently attached in the seed. Clinical information regarding cross-reactivity between cashews and other legumes has not been well documented.⁷

	Current Result	Previous Result	Reference Range
IgA	1.5	1.4	≤2.0
IgG	>6.0	2.5	≤2.0

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Ana o 3

Ana o 3 is a 2s albumin protein found in cashew nuts. It's a storage protein responsible for providing nutrition during the seed germination. Several studies showed that sensitization to Ana o 3 can be predictive of adverse immune reactions to cashew, thus can be used as a powerful clinical diagnostic marker in cashew sensitivity.⁸

	Current Result	Previous Result	Reference Range
IgA	>6.0 +	>6.0 +	≤2.0
IgG	>6.0 +	>6.0 +	≤2.0

Walnut:



Protein Family	Antigens
Prolamin superfamily	Jug n 1 Jug r 1 Jug r 3
Cupin superfamily	Jug n 2 Jug r 2 Jug r 4
11S globulin protein	Jug n 4
Profilins family	Jug r 5 Jug r 7
Vicilin protein	Jug r 6

Jug n 1

Jug n 1 is an antigen identified in black walnut, classified as a 2S albumin protein belonging to the prolamin superfamily. They are seed storage proteins playing an active role in germination of seeds with additional antifungal properties. These proteins show a high structural homology with other high 2S albumins from nuts, namely 96% with Jug r 1 (Juglans regia), 87% with Car i 1 (Carya illinoensis) and 57% with Cor a 14 (Corylus avellana) attributing to the cross-reactivity seen among different nuts.⁹

	Current Result	Previous Result	Reference Range
IgA	>6.0 +	6.0 +	≤2.0
IgG	>6.0 +	4.1 +	≤2.0

Jug n 2

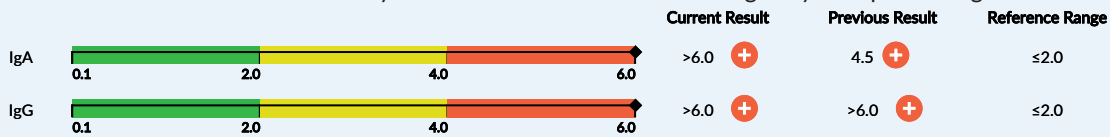
Jug n 2 is a vicilin-like protein belonging to the cupin superfamily. They are considered important seed storage proteins, playing a role as nitrogen donors during seed germination and acting as plant protection proteins. Jug n 2 exhibits high sequence identity with other antigenic vicilins, namely 92% with Car i 2, 46% with Cor a 11 and with Ses i 3. In addition, some studies showed that it also exhibited 84% and 83% of sequence homology with pea and tobacco glyceraldehydes, thus implicating a possible cross-reactivity.⁹

	Current Result	Previous Result	Reference Range
IgA	4.3 +	1.9 -	≤2.0
IgG	3.4 ✓	2.4 ✓	≤2.0

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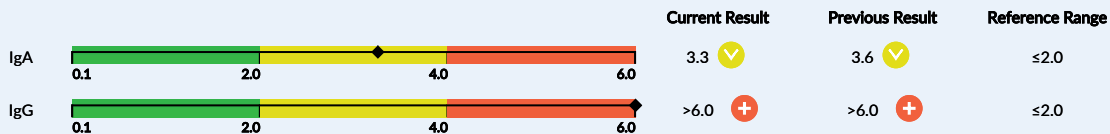
Jug n 4

Jug n 4 is a 11S globulin in black walnut, responsible for adverse immune reactions in sensitized individuals. This protein has not been characterized in detail due to purification difficulties. But several studies have shown that it is a heat-resistant protein, hence the structure or the sensitivity characteristics would not be changed by food processing methods.¹⁰



Jug r 1

Jug r 1 is a class I antigen identified in English walnut, classified as a 2S albumin protein belonging to the prolamin superfamily. They actively function in seed germination and plant protection. These 2S albumins present high pH and thermal stability, mostly attributed to their compact and globular conformational structure, which is probably responsible for the resilient sensitivity related to them. In addition, native Jug r 1 proteins reveal high resistance to enzymatic activity (trypsin/chymotrypsin) at basic pH (8.0), although they progressively lose their ability to sensitization in acidic conditions (pH 1.3) in the presence of pepsin. Jug r 1 shows high sequence identity with other 2S albumins, namely with Ana o 3 from cashew nut (identity of 55% and similarity of 91%) and with Car i 1 from pecan nut (identity of 88% and similarity of 92%).⁹



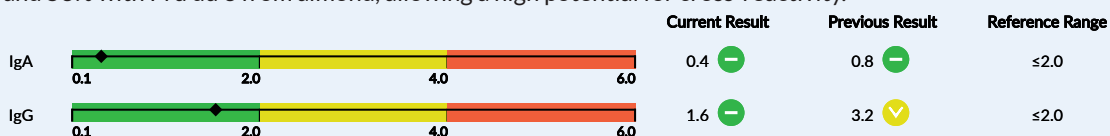
Jug r 2

Jug r 2 is a vicilin-like protein belonging to the cupin superfamily. They are seed storage proteins with additional antifungal activity. These proteins exhibit considerable thermal stability, which allows them to maintain their conformations at temperatures below 70–75°C. Studies showed that these proteins are stable after most of the food processing methods, including gamma-irradiation (1–25 kGy), microwave (500 W, 1 and 3 min), roasting (138°C and 160°C–30 min; 168°C and 177°C–12 min), and frying (191°C, 1 min). Jug r 2 exhibits a sequence identity of 97% with Jug n 2 (black walnut), 70% with Ara h 1 (peanut), 47% with Cor a 11 (hazelnut), 43% with Ses i 3 (sesame), and 38% with Pis v 3 (pistachio).⁹



Jug r 3

Jug r 3 is a nsLTP protein belonging to the prolamin superfamily. The biological function of this protein is related to the transport of different classes of lipids (fatty acids, phospholipids, glycolipids and sterols) through membranes. These nsLTP are mainly located in the outer epidermal layers of the nut. Jug r 3 exhibit a high degree of homology with other fruits and nuts, mainly 80% in peach and apricot, 60% with Cor a 8 from, 59% with Pru av 3 from cherry, 57% with Ara h 9 from peanut, and 53% with Pru du 3 from almond, allowing a high potential for cross-reactivity.⁹



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Jug r 4

Jug r 4 is classified as an 11S globulin (legumin-type) that belongs to the cupin superfamily. They function mainly as seed storage proteins. In general, legumins present a high thermal stability responsible for preserving their conformational structures at temperatures below 94°C, and great resistance to proteolysis, which enable them to retain their adverse effects along the gastrointestinal system. The general food processing methods such as heating (roasting, blanching and autoclaving) and radiation (gamma and microwave) have less impact on immunoreactivity of Jug r 4 protein. This protein shows homologous similarities, mainly 95% identity with Car i 4 from pecan nut, 72% with Cor a 9 from hazelnut, 57% with Ana o 2 from cashew nut, and Pis v 5 from pistachio, 54% with prunin-2 that is part of Pru du 6 antigen from almond, and 51% with Ber e 2 from Brazil nut.⁹

	Current Result	Previous Result	Reference Range
IgA	0.3	2.2	≤2.0
IgG	2.4	1.7	≤2.0

Jug r 5

Jug r 5 is an English Walnut antigen that belongs to the Profilins family. These are actin-binding proteins involved in the dynamic turnover and restructuring of the actin cytoskeleton. In general, profilins are sensitive to heat denaturation and gastric digestion, suggesting their involvement in immune reactions that are usually confined to moderate symptoms.⁹

	Current Result	Previous Result	Reference Range
IgA	3.9	2.4	≤2.0
IgG	5.2	0.6	≤2.0

Jug r 6

Jug r 6 is a natural vicilin protein found in English walnut kernel. Like other vicilin proteins, the native state of the protein has a complex trimeric structure. Jug r 6 displays mild thermal stability, but, similar to Ara h 1, was susceptible to immediate proteolysis. Jug r 6 is susceptible to cross reactions with walnut, hazelnut, sesame, and pistachio due to the common epitopes found among them, mainly in Cor a 11, Ses i 3, Pis v 3, and Jug r 2.¹¹

	Current Result	Previous Result	Reference Range
IgA	0.7	1.2	≤2.0
IgG	1.5	4.1	≤2.0

Jug r 7

Jug r 7 is a profilin protein found in English Walnut. In general, profilins function in regulating intracellular transport, cellular movement, and signaling. These proteins have intermediate stability when subjected to heating, ultrahigh pressure, and enzymatic treatment.¹²

	Current Result	Previous Result	Reference Range
IgA	0.9	1.3	≤2.0
IgG	>6.0	>6.0	≤2.0

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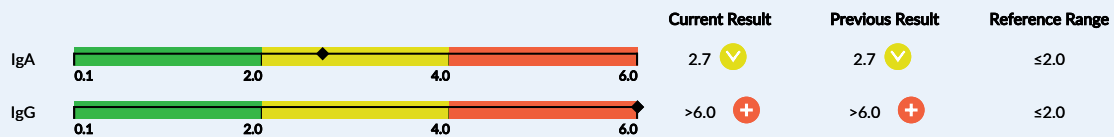
Brazil Nuts :



Protein Family	Antigens
2s albumin protein family	Ber e 1
11S globulin protein	Ber e 2

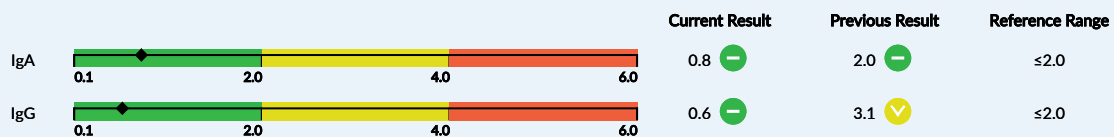
Ber e 1

Ber e 1 protein belongs to the water soluble 2s albumin protein family. It is a major storage protein in Brazil nuts and provides nutritional components during seed germination. It was the first plant antigen transferred from one plant to another (transgenic soybeans). Ber e 1 contains a rich amount of sulphur-containing amino acids, contributing to its pH and chemical and thermal resistance. Ber e 1 has sequence similarities with other 2s globulin proteins often found in many seeds, such as cotton, cocoa bean, sunflower seed, rape seed, castor bean, English walnut (Jug r 1), mustard seed (Sin a 1), and sesame seed (Ses i 2), hence possible cross reactivity could be expected among them.¹³



Ber e 2

Ber e 2, also known as excelsin, is an 11S globulin protein found in Brazil nuts. It is a seed storage protein that is responsible for providing nutrients during seed germination. Similar 11s seed storage proteins also include Ara h 3 in peanuts, Cor a 9 in hazelnuts, Jug r 4 in walnuts, Ana o 2 in cashew nuts, Sin a 2 in mustard seeds, the globulin in sesame seeds, and glycinin in soybeans, implicating possible cross-reactivity among them.¹⁴



Key Terms/Glossary

Nut sensitivity

Immune reactivity mediated by IgG and IgA antibodies.

Allergen

A protein alternatively known as the antigen that can invoke an immune response

Superfamily

A taxonomic category that ranks above family and is comprised of families sharing a set of similar nature or character.

IgG

Immunoglobulin G is a type of antibody produced against an antigen

IgA

Immunoglobulin A is a type of antibody produced against an antigen

Peptide sequence

Short sequence of amino acids

Isoforms

Two or more functionally similar proteins that have a similar but not identical amino acid sequence

Cross-reactivity

An antibody directed against one specific antigen (allergen) is successful in binding with another, different antigen

Globulin protein

The globulins are a family of globular proteins which are insoluble in pure water, but dissolve in dilute salt solutions

Polymerization

A process of reacting monomer molecules together in a chemical reaction to form polymer chain networks

Conformation

Structural arrangement

Germination

The process by which an organism grows from a seed or similar structure

Citations/Sources

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Risk and Limitations

This test has been developed and its performance characteristics determined by Vibrant America LLC., a CLIA certified lab. These assays have not been cleared or approved by the U.S. Food and Drug Administration.

Allergen-specific IgE assays do not demonstrate absolute positive and negative predictive values for allergic disease. Clinical history must be incorporated into the diagnostic determination. Quantification of specific IgG and IgA antibodies is not FDA-recognized diagnostic indicator of allergy.

Tree Nut Zoomer testing is performed at Vibrant America, a CLIA certified laboratory and utilizes ISO-13485 developed technology. Vibrant America has effective procedures in place to protect against technical and operational problems. However, such problems may still occur. Examples include failure to obtain the result for a specific antigen due to circumstances beyond Vibrant's control. Vibrant may re-test a sample in order to obtain these results but upon re-testing the results may still not be obtained. As with all medical laboratory testing, there is a small chance that the laboratory could report incorrect results. A tested individual may wish to pursue further testing to verify any results.

The information in this report is intended for educational purposes only. While every attempt has been made to provide current and accurate information, neither the author nor the publisher can be held accountable for any errors or omissions.

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