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Summary Report:

Investigation and comparison of broad spectrum antioxidant activity by proprietary phytonutrient health nutraceuticals against major free radicals found within the human body

1. Introduction & Summary:

Consumers of dietary supplements often seek products with antioxidants within their formulations due to the known ability of antioxidants to quench free radicals. However, there is often confusion regarding strength, spectrum of activity, absorption and bioavailability of antioxidant nutritional formulations. It is generally well accepted that ingested antioxidants from fruits, vegetables and other such substances are beneficial to human health, but the same cannot be said for antioxidant dietary supplement formulations. This is likely related to the research supported notion that broad spectrum antioxidant activity is present from multiple phytonutrients that are present within natural fruits and vegetables, not just single isolated nutrients. This mechanism is related to cofactor synergy which exists in natural foods, an explanation in which full mechanistic understanding is still developing. Due to the interest in the potential ability of ingested antioxidants to alter the harmful physiologic effects of free radicals, intense research has explored antioxidant activities of various nutritional substances and combinations. Research has also sought to identify compound combinations which have broad spectrum activity against multiple free radical agents, thus promoting broad spectrum antioxidant defense.

Antioxidants are substances which can interact with free radicals and render them harmless through electron donation, thus potentially protecting the body from oxidative harm. Free radicals are reactive oxygen species (oxidants), generated internally and externally, that can have adverse effects on normal physiological function of the human body when out of homeostatic balance and process. Free radicals are known agents of cellular damage at the membrane, mitochondrial and DNA levels. The adverse effect of free radicals on biologic systems is described generally as Oxidative Stress (OS).

This study undertook investigation of the anti-oxidant activity of phytonutrient dietary supplements containing verified essential RDA nutrients and proprietary blends of fruits, vegetables, aloe vera gel and green tea epigallocatechin gallate (EGCG) catechin complexes (VIBE Original Energy & Life™ and the VIBE 2.0 Cardiac & LIFE™) against multiple free radical agents, including: peroxy (water and lipid soluble), hydroxyl, peroxylnitrite, singlet oxygen and ferric free radicals. The investigational substances were sent for third party independent analysis (Brunswick Labs, MA). Antioxidant activity was measured by oxygen radical absorbance capacity (lipophilic and hydrophilic) (ORAC total), hydroxyl radical averting capacity (HORAC), peroxylnitrite radical averting capacity (NORAC), superoxide radical averting capacity (SORAC) and ferric reducing antioxidant capacity (FRAP) assays.

The results demonstrated broad spectrum antioxidant activities against all free radical species tested for both formulations and are likely related to the inclusion of multiple phytonutrient components within the formulations, not solely a single isolated nutrient. While *in vitro* results do not always

correlate to *in vivo* activity, the Eniva VIBE nutraceuticals represent over-the-counter (OTC) dietary supplements which have demonstrated *in vitro* antioxidant activity against a broad range of free radicals known to exist in the human body and stand as a consumer option for those seeking products verified for antioxidant activity.

2. STUDY METHODOLOGY:

Methodology Synopsis:

Sealed and identified Eniva VIBE nutraceuticals (VIBE Original Energy & Life™ and VIBE 2.0 Cardiac & Life™) were sent for third-party independent *in vitro* analysis (Brunswick Labs, MA) for anti-oxidant capacity evaluation. The goal was to quantify their abilities to quench a variety of free radical species generated via various validated methodologies (please see addendum report for specific test methodology references). The free radicals tested represent major radical species in the human body:

Test	Radical
ORAC-ROO (hydro)	Water-soluble peroxy radicals
ORAC-ROO (lipo)	Fat-soluble peroxy radicals
N-ORAC (ONOO-)	Peroxynitrite radical
H-ORAC (HO-)	Hydroxyl Radical
S-ORAC (O-)	Singlet oxygen (measured in SOD equivalence result)
*FRAP (FeIII)	Ferric Ion

* Although not a major endogenously produced free radical species, iron derivatives represent ingested pro-oxidants from the diet. Within the human biologic system, ionic iron can act as a significant pro-oxidant, resulting in oxidative stress to biologic systems.

1. Test Material:

This study undertook the investigation of the anti-oxidant activity of a phytonutrient dietary supplement formulation of verified essential RDA nutrients and proprietary combinations of fruit, vegetable and aloe vera gel concentrated components, also with a green tea epigallocatechin gallate (EGCG) catechin complex. This is identified as Eniva VIBE 2.0 Cardiac & Life. Nutrient content and validated essential nutrients on file.

2. Test Methodologies:

Antioxidant activity was evaluated by validated testing methodologies for evaluating the the ORAC, FRAP and SOD scores. Assays performed via standard methodologies at Brunswick Labs, MA.

- a. **Peroxy Radicals** (water and hydrosoluble): Quantification through oxygen radical absorbance capacity (ORAC). Reported in micromole Trolox equivalents/liter.
- b. **Hydroxyl Radicals**: Quantification through hydroxyl radical averting capacity (HORAC). Reported in micromole Trolox equivalents per liter.
- c. **Peroxynitrite radicals**: Quantification through peroxynitrite radical averting capacity (NORAC). Reported in micromole Trolox equivalents per liter.
- d. **Iron ferric radical**: Ferric reducing antioxidant capacity (FRAP). Reported in micromole Trolox equivalents per liter.

e. Superoxide radical: superoxide radical averting capacity (SORAC). Reported in kilounits Superoxide Dismutase(SOD) equivalence per liter.

Results:

1. Antioxidant activity:

The antioxidant activity of the test material was evaluated against 6 major free radical known to exist in the human body. Please find the results summarized in table 1.

Table 1: Summary of broad spectrum antioxidant activity values of tested formulations.

Test Sample	ORAC-ROO hydro (umolTE/L)	ORAC-ROO lipo (umolTE/L)	H-ORAC (HO-) (umolTE/L)	N-ORAC (ONOO-) (umolTE/L)	F-RAP (FeIII) (umolTE/L)	SOD (kunitsSO Deq/L)
VIBE Original Energy & Life™	92,485	2,901	9,412	9,952	57,341	918
VIBE 2.0 Cardiac & Life™	150,734	3,012	15,944	15,880	76,794	14,878

Figure 1: Comparison of ORAC-ROO Hydro Values between test materials.

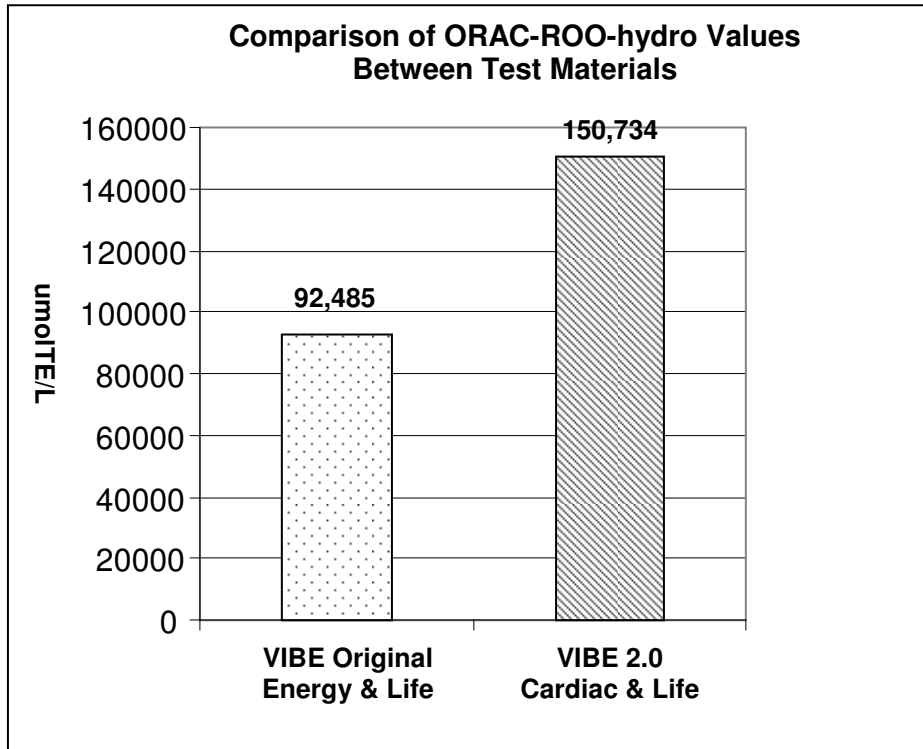


Figure 2: Comparison of ORAC-ROO Lipo, H-ORAC, N-ORAC between test materials

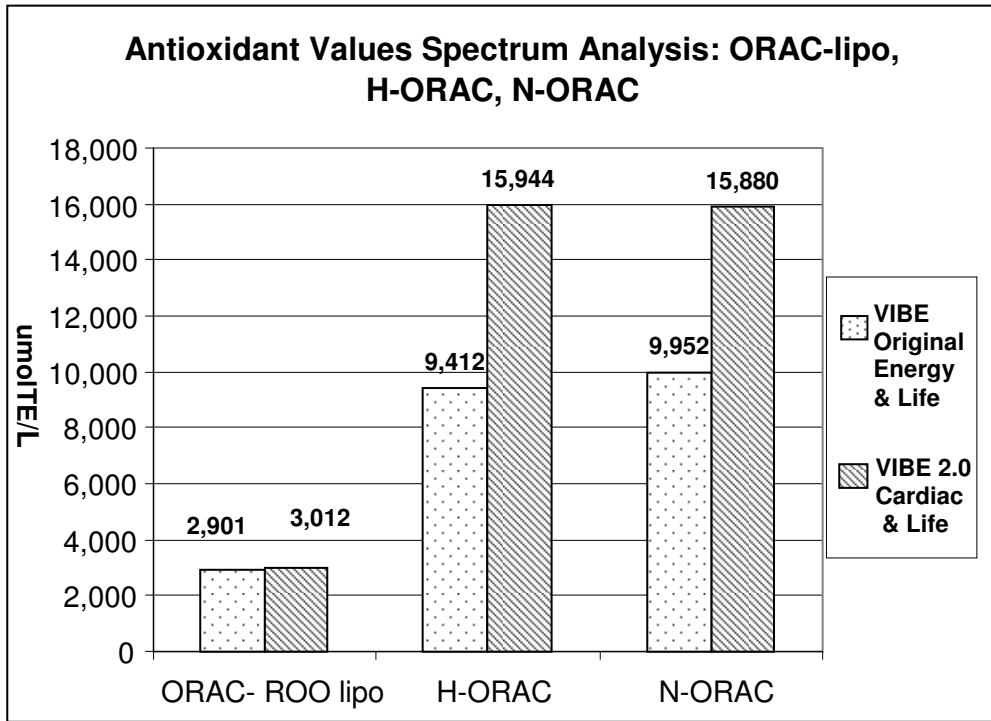


Figure 3: Comparison F-RAP values between test materials.

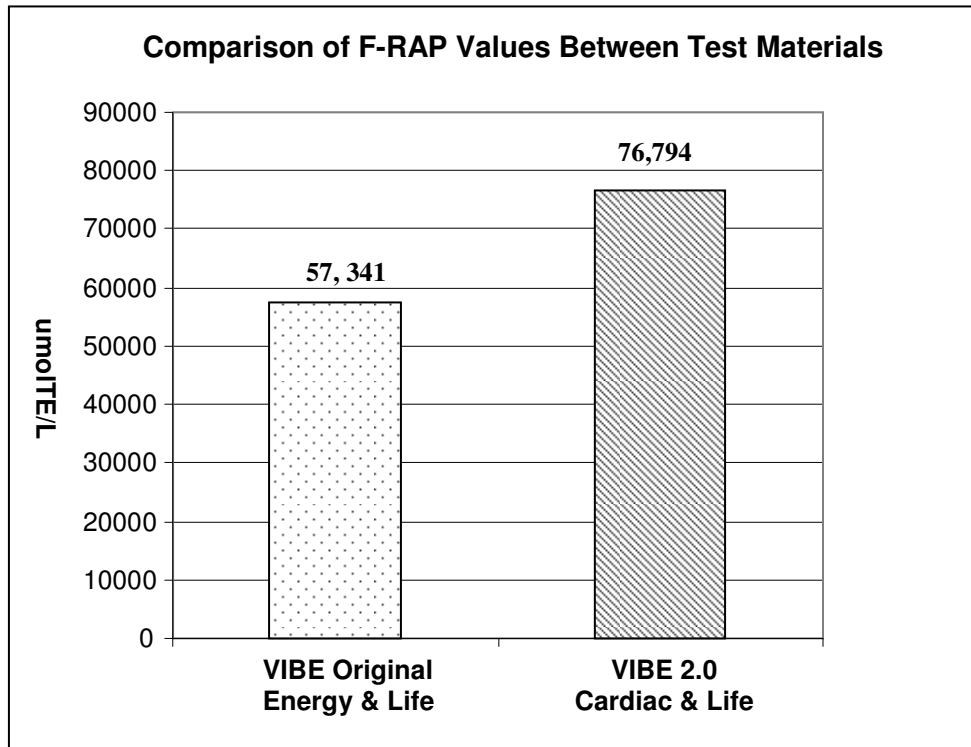
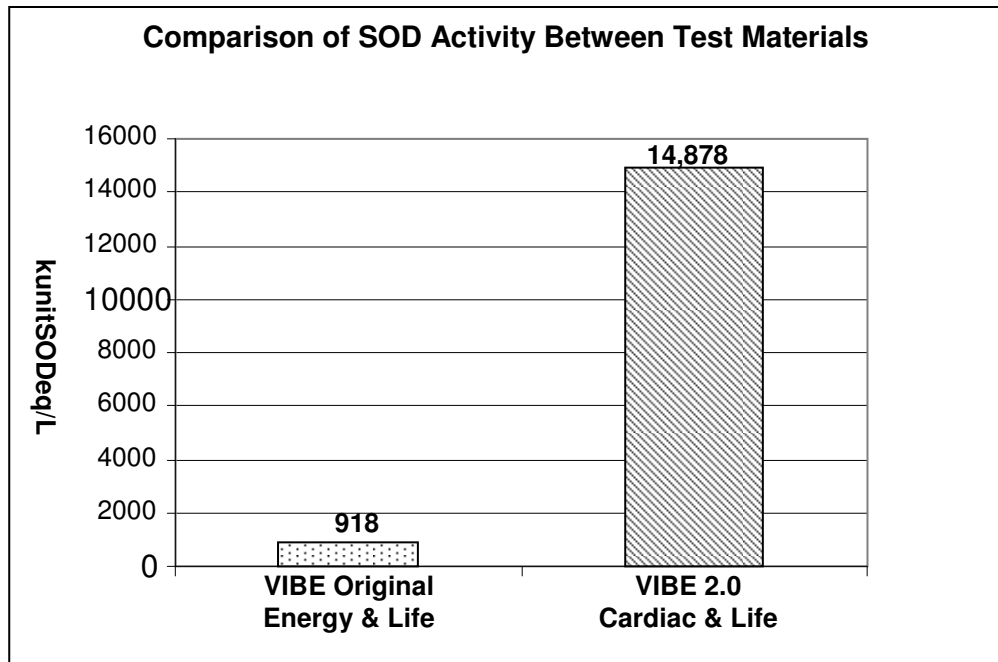


Figure 4: Comparison SOD activity between test materials.



4. Conclusions:

Eniva VIBE Original Energy & Life™ and Eniva VIBE 2.0 Cardiac & Life™

1. The test materials demonstrated significant broad *in vitro* antioxidant activity against all free radicals generated.
2. The *in vitro* free radical species tested against represent major free radicals within the human body known to play a role in aging and several degenerative conditions.
3. The broad antioxidant activity of the test materials is likely related to the multi-ingredient formulation of essential nutrients and additional phytonutrients, versus a single nutrient alone.
4. The Eniva VIBE 2.0 Cardiac & Life™ formulation had the highest antioxidant capacity values in all tests as compared to Eniva VIBE Original Energy & Life™.

5. Discussion:

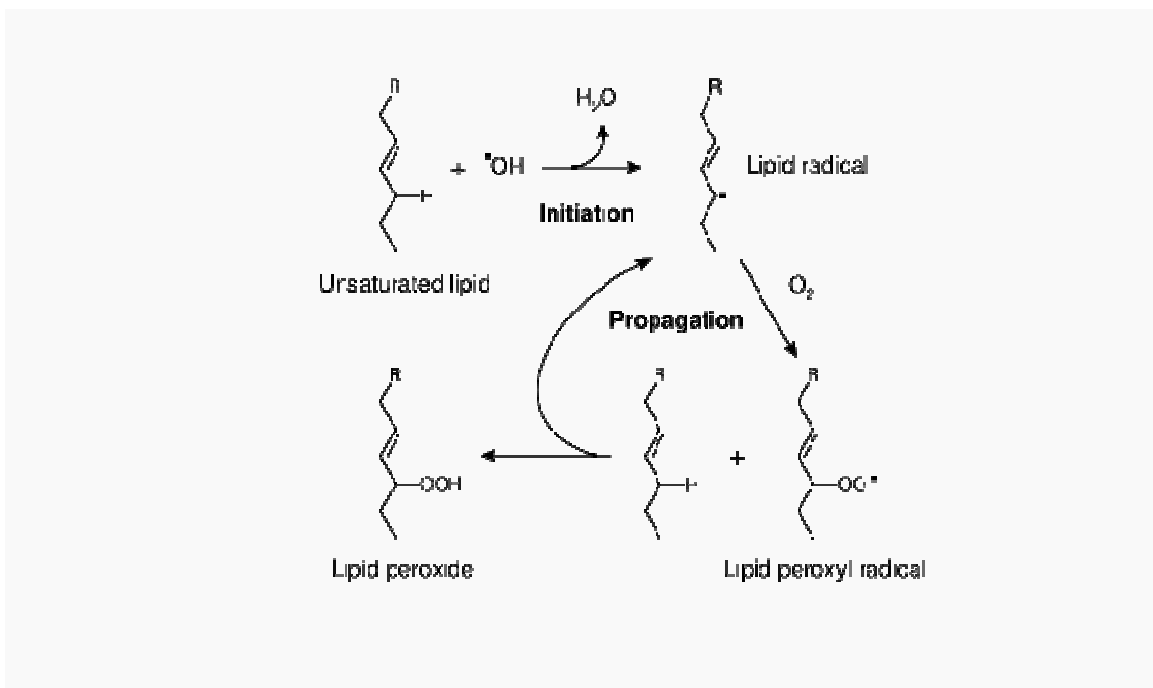
With the attention paid to antioxidants in recent research reports and the common press, consumers are seeking products which contain antioxidant substances. Unfortunately, many of these products do not offer any form of validated testing as to the strength of the antioxidants present, nor to their activity against various forms of free radicals which are found within the human body. While *in vitro* (laboratory) testing does not always equate to *in vivo* (in body) activity, it is argued here that manufacturers of dietary supplements should, at a minimum, be testing their products for *in vitro* antioxidant activity and

making this data readily accessible to consumers. However, this is currently not the standard practice for the nutritional marketplace industry.

The above results suggest broad antioxidant capacity for the tested materials against a variety of free radical species, including the peroxy (water and lipid soluble), hydroxyl, peroxynitrite, singlet oxygen and ferric free radicals. This broad spectrum of activity is likely related to the multi-nutrient formulation of essential nutrients, fruit, aloe and vegetable concentrates and the green tea EGCG catechin complex found within both products. In comparison, we find the VIBE 2.0 Cardiac & Life formulation™ higher in all antioxidant activities as compared to the VIBE Original Energy & Life Formulation. This is likely related to the manufacturer's reported higher content of fruit, vegetable and green tea EGCG catechin complexes within the product.

It is well known that within the biologic system of the human body there exists the presence of various forms of ROS free radicals. The majority of these are generated via metabolic mechanisms. In examining the results, the highest antioxidant activity was found against the peroxy radical for both test materials, a key ROS that is well known to propagate cellular membrane and lipid damage (see Pictoral 1).

Pictoral 1: Lipid peroxidation initiation and propagation.



As well, ingested substances can also contribute to the generation of free radicals. One such substance is iron, which in its ionic form is a known pro-oxidant and contributor to the atherosclerotic process. Of interest in the presented test results are the Eniva formulations' demonstrated activity against this ferric ion. Additionally, the formulations showed activity against singlet oxygen. This would imply superoxide dismutase enzyme like activity, a key human antioxidant defense enzyme. These two specific results are intriguing because of the relation to specific human health issues. It should also be noted that both formulations tested do not possess mega-dosing of any RDA essential nutrient and the Vitamin E and Vitamin C content of both formulations were 30 International Units and 120 milligrams, respectively.

While sole *in vitro* ORAC and similar antioxidant activity testing values cannot be extrapolated to bioavailability and absorption, they do provide verification of antioxidant activity -- a likely first step in the process for evaluating substances which may have biologic impact. In an era where there is often much hype and advertising around antioxidant supplement products, steps must be taken to provide the consumer with data that, at a minimum, offers some degree of validation to the claim of “antioxidant” formulation.

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