

The Accuracy and Precision of the HS-Omega-3 Index® Test
William S. Harris, PhD
Chief Scientific Advisor, OmegaQuant Analytics

The HS-Omega-3 Index is the EPA+DHA content of red blood cells expressed as a percent of total identified fatty acids. It is measured using a proprietary methodology developed over several years of research.

Fatty acids are identified by comparison with a commercially-prepared, weighed, standard mixture consisting of 24 fatty acids characteristic of red blood cell membranes. Using the standard, we adjust for minor run-to-run and instrument-to-instrument variations in the analysis. This helps to keep analytical variability low.

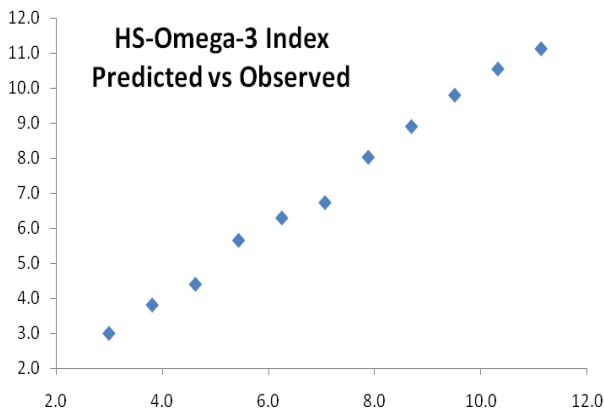


Fig. 1. The correlation between the expected and observed values for the HS-Omega-3 Index. This shows that across the clinically significant range of values, the test gives accurate results. $R=0.999$; $p<0.0001$.

Precision: Current coefficients of variation are 1.4% at mean HS-Omega-3 Index of 8.7%, and 3.5% at a mean Index of 2.5%.

Accuracy: The observed HS-Omega-3 Index value matches precisely the expected value when mixtures of red blood cells with different amounts of EPA+DHA are analyzed (Figure 1).

Sensitivity: The method can detect very low levels of FAs, e.g., it can reproducibly detect the trans-trans isomer of linoleic acid at 0.01% of total FAs.

Linearity

In addition, although the test is typically performed with 25 μL of RBCs, the response is linear down to as little as 1 μL (Figure 2).

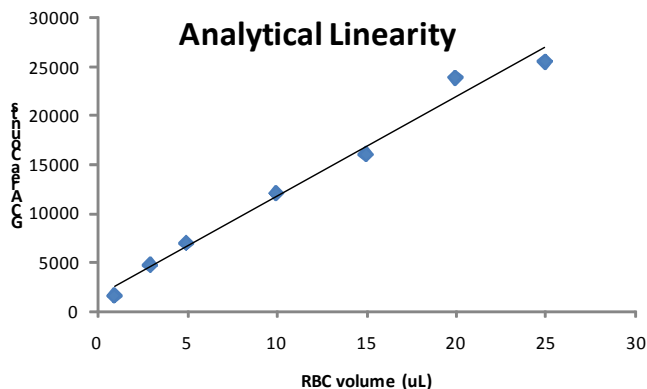


Figure 2. Linearity of response of the HS-Omega-3 Index to increasing amount of RBC sample analyzed. The test is routinely done with 25 μL of packed RBCs.

Biological stability (reliability). In an unpublished study with 37 cardiac rehabilitation patients studied on a stable diet, the mean HS-Omega-3 Index did not change over 18 months ($6.4 \pm 1.6\%$ vs. $6.2 \pm 1.9\%$, $p=0.60$). In another study with 20 healthy volunteers studied 7 times over 6 weeks, the within-subject coefficient of variation (CV) for RBC, whole blood and whole plasma were: $4.1\% \pm 1.9\%$, $6.7\% \pm 4.0\%$, and $15.9\% \pm 6.4\%$, respectively.

Fasting vs Fed. We studied the effect of consuming a large meal on the EPA+DHA content of RBCs, whole blood, and in plasma using the 20 subjects studied above. Compared to fasting values, a sample collected 3-4 hours after a meal did not significantly affect EPA+DHA in RBCs (-1%), but it did lower the whole blood value by about 5% ($p=0.03$). The meal had a marked effect on the EPA+DHA level in plasma, lowering it by 11% ($p<0.05$). This is due to the dilution of plasma EPA+DHA with non-omega-3 FAs contained in the test meal.

Consistency with external labs: In an international laboratory cross validation study conducted at OmegaQuant Analytics and OmegaMetrix (Munich, Germany), the HS-Omega-3 Index value in the former was $8.5 \pm 3.4\%$ compared to $8.8 \pm 3.5\%$ at the latter. This confirms that the test is transferrable to other labs and that accuracy and precision can be achieved when identical methodologies are employed. Different methodologies cannot guarantee similar results.

Calculation of the HS-Omega-3 Index based on Dried Blood Spot EPA+DHA levels. The DBS method for determining the HS-Omega-3 Index was validated by comparing the two metrics in blood samples collected from 106 subjects. DBS cards and blood tubes were collected. The latter were immediately processed for RBCs which were frozen at -80°C ; the former were express shipped to various cities around the country, and from there, sent by regular US mail

back to OmegaQuant for routine analysis. The correlation between the EPA+DHA content of the two metrics was very high ($r=0.96$; $p<0.0001$), and the 95% confidence interval for the DBS-estimated value was $\pm 1\%$ (Figure 3).

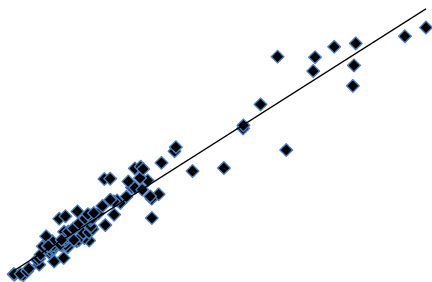


Figure 3. Correlation between the EPA+DHA content of dried blood spots (DBS) and the (RBC-based) HS-Omega-3 Index. DBS samples were collected from 106 subjects, shipped via standard US mail, and compared to RBC samples frozen immediately after collection. $R=0.96$, $p<0.0001$.

Protection of EPA and DHA against oxidative loss by antioxidant treatment. The long-chain omega-3 fatty acids that comprise the HS-Omega-3 Index (EPA and DHA) are highly polyunsaturated and therefore very susceptible to oxidative degradation. At OmegaQuant Analytics, we use a proprietary antioxidant treatment to diminish this natural process in samples collected as dried blood spots on filter paper. In the experiment illustrated in Figure 4, blood samples were placed on non-treated and treated filter papers and analyzed in triplicate at baseline and after 1, 2, and 3 days of storage at room temperature. Whereas there was an 8.3% loss of EPA+DHA in the untreated cards, the treated cards retained baseline values, decreasing by a non-significant 1.8% at 3 days.

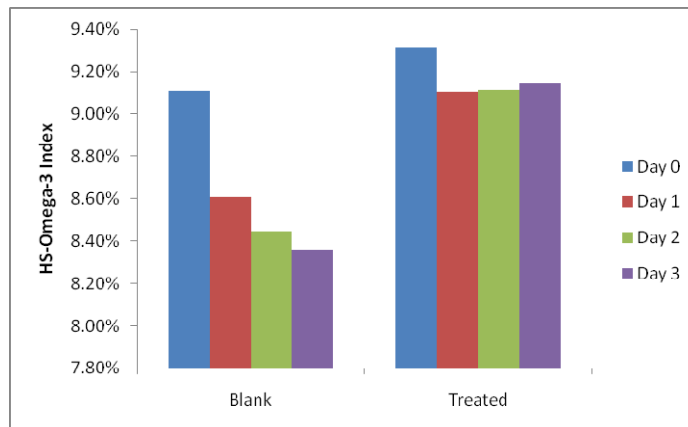


Figure 4. Effects of treatment with an antioxidant on the loss of EPA+DHA over time in blood samples collected on filter papers.