

Determining Total Antioxidant Capacity of Serum or Plasma in the ABEL® Total Antioxidant Capacity (TAC) Assay with Pholasin® and Peroxynitrite.

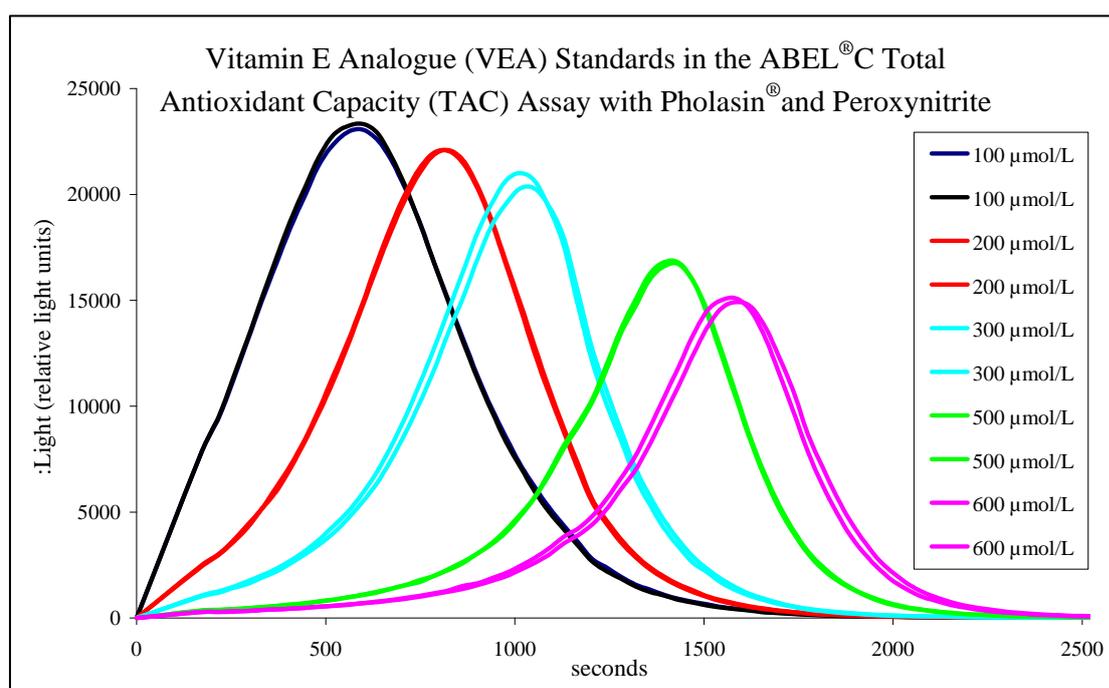
Samples of serum can be collected into coated tubes from finger prick blood. There are a number of suppliers of such tubes but we prefer those supplied by Sarstedt. The tubes containing clotted blood can be sent by post to our laboratory for analysis or the assays can be performed in your own laboratory with kits purchased from Knight Scientific. If venous blood is being collected for other tests then a small serum tube can be used to collect a sample for testing.

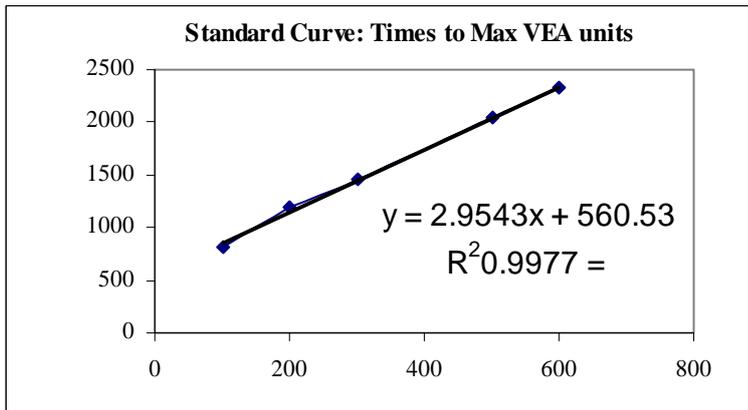
Total antioxidant capacity (TAC) of 5 μ L samples of serum or plasma is determined in the ABEL® antioxidant assay with Pholasin® and peroxynitrite (Knight Scientific Limited, Plymouth UK). In this assay antioxidants compete with Pholasin® for peroxynitrite. The effect of this competition is a reduction in light emitted by Pholasin® and an increase in the time at which the maximum peak of light occurs (time-to-max) with highest antioxidant capacity corresponding to lowest light signals and longest times-to-max. The TAC of the sample is derived from the equation of the linear regression of a set of Vitamin E analogue (VEA) (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) standards (VEA concentration against time).and is expressed as μmolL^{-1} VEA.

The unknown VEA equivalent values of the sample (TAC scores) are derived from the equation of the linear regression of the time to max values against concentration of VEA.

The equation for the linear regression of concentrations of vitamin E analogue (VEA) (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) against time at which the maximum light occurs (time-to-max) for each concentration of VEA is obtained using an Excel template produced by Knight Scientific Limited in which times-to-max values from the BMG Lumistar microplate luminometer software are pasted into an appropriate grid.

For example





The equation is:.

$$Y = Ax + B$$

Y is the time to max

TTMax

A = intercept

B = slope

The equation is: $Y = Ax + B$

Y is the time to max (in our results we use the mean of two values for TTMax)

A = intercept

B = slope

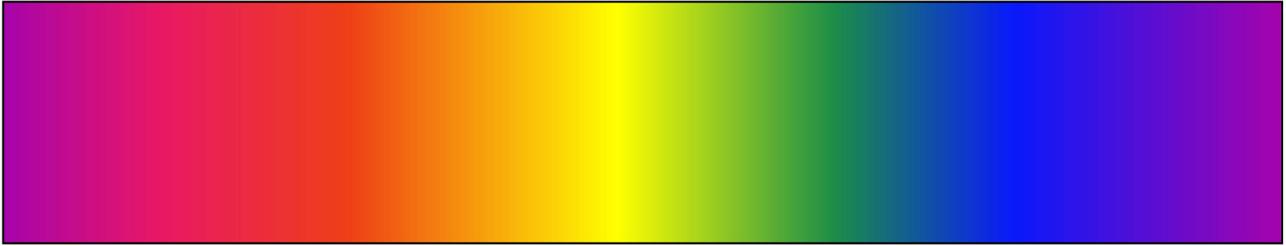
To obtain the unknown VEA equivalent value from the standard curve

$$X = (Y-B)/A$$

Where X = unknown VEA equivalent values (Y, A, and B) are as above

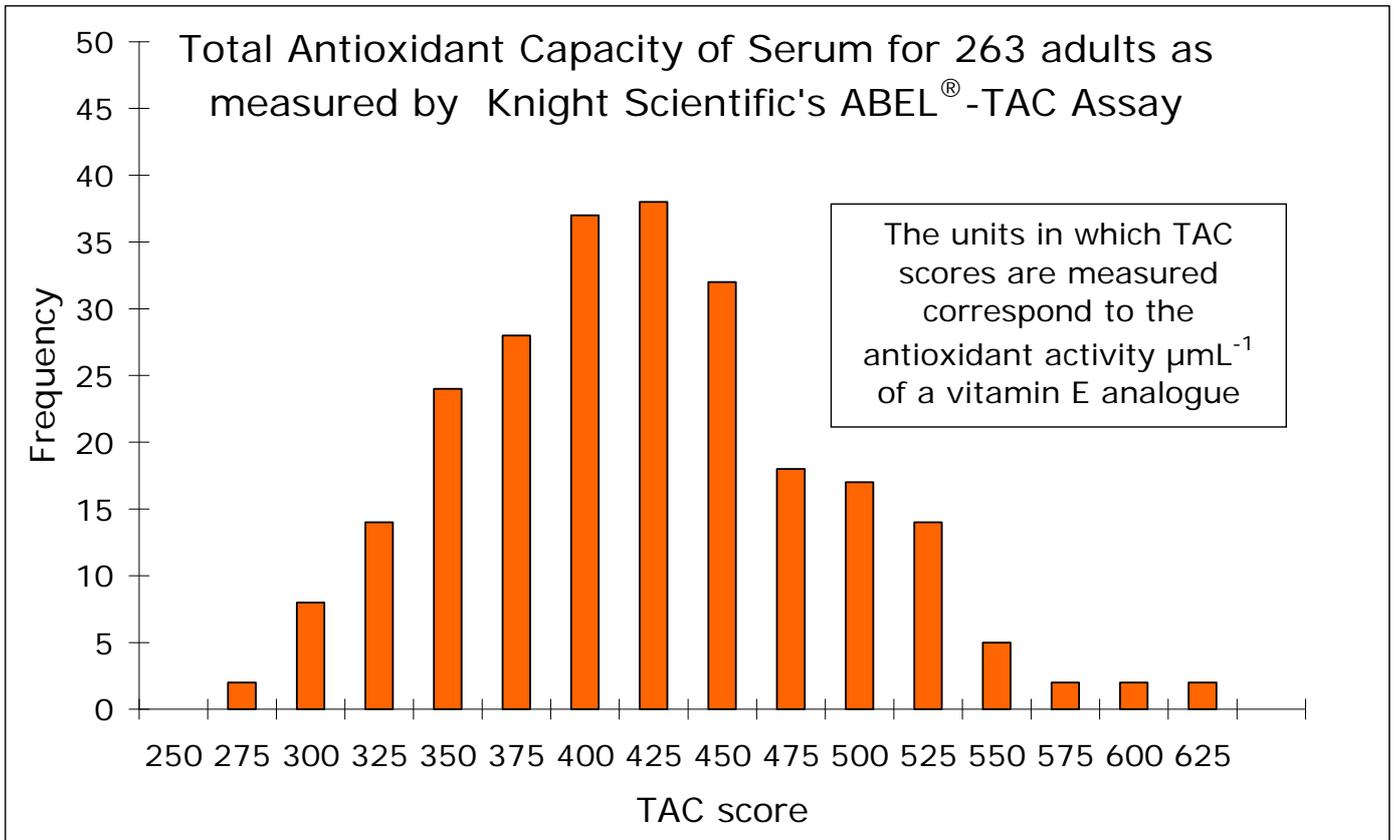
Typical intra and intra assay variability is less than 3% cv.

ABEL-TAC Scores



0 - 300 301 - 350 351 - 400 401 - 450 451 - 500 500 - 550+

Scores	0 - 300	- 7%
Scores	301 - 350	- 12%
Scores	351 - 450	- 42%
Scores	451 - 550	- 31%
Scores	550 - 600	- 5%
Scores	above 600	- 3%



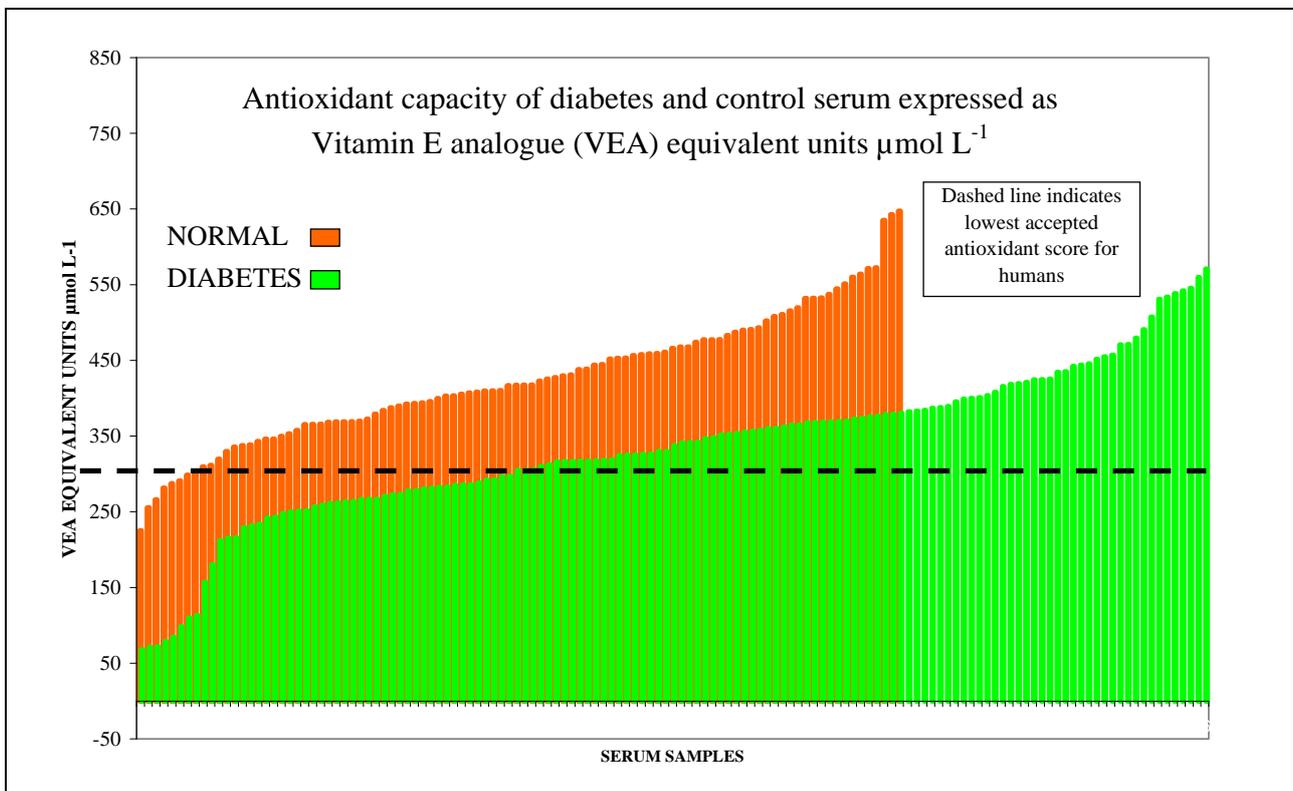
Scores below 325 should be investigated further. Considerations first would be poor diet but it is also considered that very low antioxidant capacity (especially in the low 200s) might indicate the

presence of undiagnosed type 2 diabetes. Scores above 550 may be due to excellent diet or possible oxidative stress. We would apply serum with very high scores to another ABEL antioxidant assay of KSL as a first step in identifying whether these samples scoring very high are possibly abnormal. Current research is being carried out by KSL in collaboration with the Peninsula Medical School to identify possibly causes for these very high scores some of which are thought to involve the liver and others a result of oxidative stress leading to increased concentrations of uric acid in the blood.

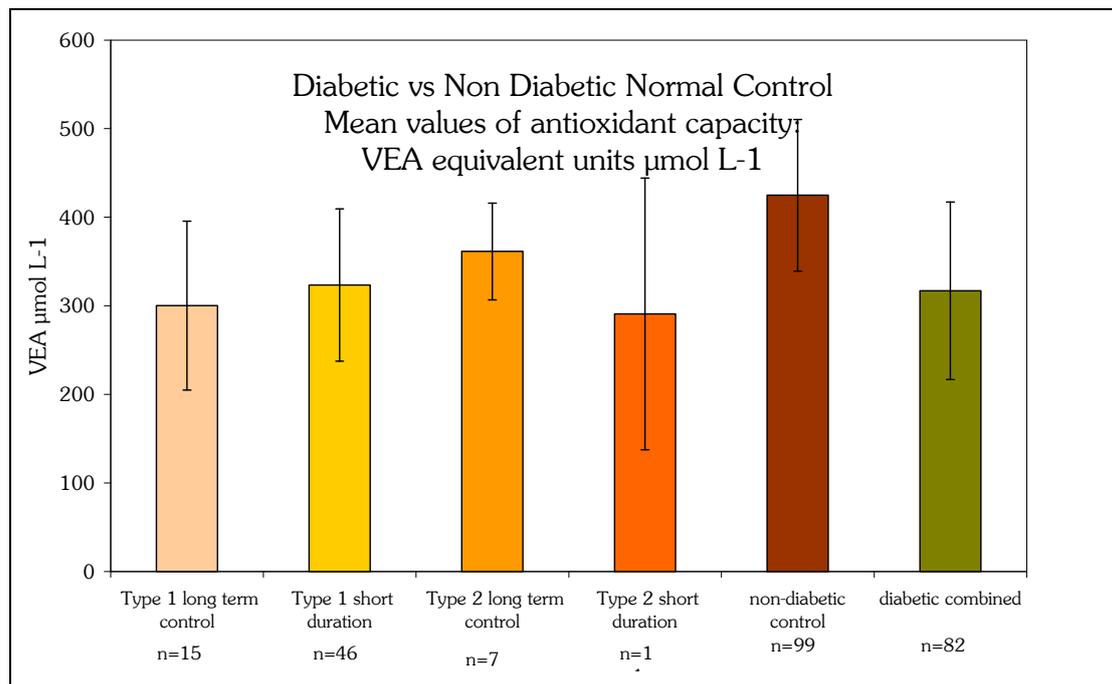
A very important value of this test is that it is very difficult to cheat. For example, individuals who think they eat a diet poor in antioxidants might, just before the test, eat a lot of fruit or drinks high in antioxidants. While small increases in antioxidants are measured soon after eating, it would not be possible to shift a score of say 300 to 400 in just a few days. If someone has a very low score then improvements can be seen in a few weeks after instigating a diet rich in antioxidants. Such diets should also include proteins and good quality oils such as extra virgin olive oil which is high in phenolic antioxidants, vitamin E and mono-unsaturated fatty acids. Fats are needed to absorb vitamins A,D,E and K and carotenoids. And without proteins in the diet it will be difficult for protein antioxidants such as albumin and antioxidant enzymes to be produced in the body.

The scores are relatively stable, day to day but do increase immediately after eating. Repeat testing should therefore be under the same conditions and preferably before eating. People with good antioxidant scores should be encouraged to have the test repeated every two to three months (or more frequently). If an individual, at a repeat test, has a significantly lower score and claims not to have changed his diet then this should be noted and the test repeated after a week. If the score still remains low, or decreases further, then this person should be considered as a possible candidate for pre-type 2 diabetes.

The chart below are the antioxidant scores obtained In a pilot study of serum from diabetic patients compared to serum from non-diabetic. A score of 300 was used to denote very low antioxidant scores.



The scores above were further analysed



The scores from figure 2 were further grouped:

- Type 1 and Type 2 diabetes of short duration
- Type 1 and Type 2 diabetes long term under control
- Non diabetic control
- Diabetic scores combined.

Dr Jan Knight
Knight Scientific Limited
15 Wolseley Close Business Park
PLYMOUTH
PL2 3BY
Tel 01752 565 676
Fax 01752 561672
jan@knightscientific.com
May 2007