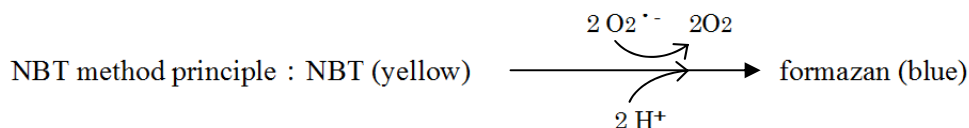


## Superoxide Anion Radical Scavenging Activity of Serum in Diabetic Patients

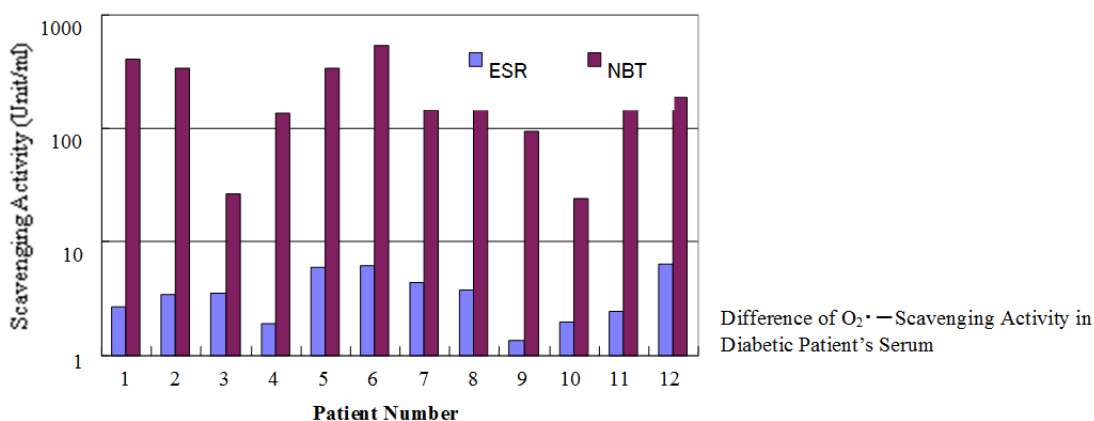
In order to evaluate oxidative stress in biological systems, the antioxidative activity of serum (plasma) is often used. The superoxide anion radical ( $O_2^{\cdot-}$ ) is an important species which causes oxidative stress in biological systems and several methods have been proposed to evaluate scavenging activity. Of these, the ESR spin-trap method is excellent and is unaffected by other factors as it is highly selective detecting only  $O_2^{\cdot-}$ .

When the  $O_2^{\cdot-}$  scavenging activity of serum (plasma) is to be measured, the difficulty in evaluating haemolysis samples using a photo-absorption techniques such as cytochrome-C, is generally recognised. There are other methods which can be influenced by other factors. Here, we show the comparison against the nitroblue tetrazolium (NBT) method.

Under acidic condition, NBT is reduced by  $O_2^{\cdot-}$  to formazan. If a sample has  $O_2^{\cdot-}$  scavenging activity, production of formazan (560nm) is suppressed, therefore, it is possible to evaluate the antioxidation activity.



The following shows the result of  $O_2^{\cdot-}$  scavenging activity of serum from diabetic patients by both ESR and NBT methods. (Refer to ER-070002 for details of the ESR method.) Calibration curves for both methods were made using superoxide dismutase (SOD) as a standard material for quantification. As shown in the figure, there was a significant difference in the results. When we measured the amount of Cu,Zn-SOD and Mn-SOD in these samples by the enzyme immunoassay method, the total volume of SOD was up to 2.5 times greater. The possibility is that with the NBT method, antioxidation activity is over-estimated because of interference from other factors. Here we demonstrate that measurement by ESR, because of its higher selectivity for  $O_2^{\cdot-}$  provides more reliable data.



Reference : Nakai, Ohta, Sakurai, Aoyama, Kamata, Magnetic Resonance in Medicine, **9**, 56-59, Nihon Igakukan (1998)