

VP01 Oxygen Saturation Measurement Technology

Hemoglobin Saturation (sO_2) determination in extra-corporeal circulation is a complex process often resulting in values differing from the circulating in-vivo sO_2 .

As this Technical Note illustrates, typical laboratory or clinical based in-vitro sO_2 values can only be compared to in-vivo values when the errors associated with the in-vitro process are significantly reduced or eliminated.

The following information identifies factors that may affect the observed accuracy of the reference standard and VP01 measurements.

The VP01 is calibrated for oxygen saturation measurement using the OSM3 CO-Oximeter (RADIOMETER A/S Copenhagen) as the Reference standard and with blood with physiological CarboxyHemoglobin and Methemoglobin levels.

VP01 probe uses solid-state light sources at two different wavelengths for the measurement of sO_2 . The measure is based on ratiometric principle of measured scattered light at two wavelengths.

One wavelength is selected at an isobestic point of reduced hemoglobin or oxyhemoglobin to eliminate the effect of variable blood oxygenation. At an isobestic wavelength, the extinction coefficient, ϵ , or scattered light coefficient σ (in case of VP01) is the same for both reduced and oxygenated hemoglobin. Thus, at the isobestic wavelength, the amount of light adsorption is independent of the amount oxygenated or reduced hemoglobin in the red cells. The second wavelength is chosen at a point of the spectra where there is a great variation in light absorbance for both reduced and oxygenated hemoglobin.

The two selected wavelengths are 805 nm and 660 nm.

The algorithm for calculating the sO_2 from the measured scattered light is shown here below. In order to compensate the effect of hematocrit variation, a correction for such parameter is taken into account.

$$sO_2\%(Ht\%) = \left[\left(\frac{\sigma_{805}}{\sigma_{660}} \right)^2 * \alpha_2 + \left(\frac{\sigma_{805}}{\sigma_{660}} \right) * \alpha_1 + \alpha_0 \right]$$

where σ_{805} and σ_{660} are the value of scattered light

Reference Standard for Oxygen Saturation

Symbols and definitions

Concentration of total hemoglobin

ctHb is the concentration (c) of total hemoglobin (tHb) in blood. Total hemoglobin, in principle, includes all types of hemoglobin:

Hemoglobin (HbA) - normal adult hemoglobin is a complex protein containing iron and capable of transporting oxygen in the blood.

Deoxyhemoglobin (HHb) - unoxygenated (formerly called "reduced") hemoglobin.

Oxyhemoglobin (O₂Hb) - oxygenated hemoglobin, containing four molecules of oxygen per hemoglobin molecule.

Carboxyhemoglobin (COHb) - hemoglobin bound to carbon monoxide, a bond about 210 times stronger than the

oxygen-hemoglobin affinity; prevents normal transfer of oxygen and carbon dioxide in the blood.

Methemoglobin (MetHb) - hemoglobin molecule whose iron is in the oxidized, ferric state; useless for respiration; found in the blood after poisoning with acetanilide, potassium chlorate and other substances.

Sulfhemoglobin - hemoglobin in combination with sulphur. The very rare and non-oxygen-carrying Sulfhemoglobin is not included in the reported ctHb.

Fetal hemoglobin (HbF) - the major type of hemoglobin in the developing fetus. The oxygen dissociation curve for fetal hemoglobin is shifted to the left compared to adult hemoglobin.

The concentration of total hemoglobin may be expressed as:

$$ctHb = cO_2Hb + cHHb + cCOHb + cMetHb$$

The systematic symbol for arterial blood is ctHb(a). The analyzer symbol may be tHb or ctHb.

Reference ranges

ctHb(a) reference range (adult):

Male: 8.4-10.9 mmol/L (13.5-17.5 g/dL)

Female: 7.4-9.9 mmol/L (12.0-16.0 g/dL)

Oxygen Saturation

Definition

sO₂ is oxygen saturation (sometimes called functional saturation) and is defined as the ratio between the concentrations of O₂Hb and HHb + O₂Hb:

$$sO_2 = \left[\frac{cO_2Hb}{cHHb + cO_2Hb} \right] \times 100$$

sO₂, as defined above, will immediately tell if more oxygen can be carried by hemoglobin – or whether an increase in pO₂ will increase only the physically dissolved oxygen.

The systematic symbol for arterial blood is sO₂(a). The analyzer symbol may be sO₂.

Reference ranges

sO₂(a) normal range (adult): 95-99 %

Hemoglobin fraction of total hemoglobin (fractional Oxyhemoglobin)

Definition

FO₂Hb is defined as the ratio between the concentrations of O₂Hb and tHb (cO₂Hb/ ctHb). It is calculated as follows:

$$FO_2Hb = \left[\frac{cO_2Hb}{cHHb + cO_2Hb + cCOHb + cMetHb} \right] \times 100$$

The systematic symbol for arterial blood is FO₂Hb(a).

The analyzer symbol may be O₂Hb or FO₂Hb.

Reference ranges

FO₂Hb(a) reference range (adult): 94-98 %

Measured saturation

An Oximeter is a spectrophotometer designed to measure blood oxygen saturation. Each type of hemoglobin

molecule (i.e., HHb, O₂Hb, COHb and MetHb) has its own light absorption spectrum. Oximeters contain light sources at selected wavelengths that correspond to the absorption spectra of the hemoglobin molecules to be measured. Thus, a basic Oximeter that can measure sO₂ needs to determine the absorption at only two wavelengths, one for HHb and one for O₂Hb.

This is the technology implemented into VP01. However, this technology can give misleading estimates of the oxygen content of blood in the presence of elevated levels of COHb and MetHb.

To obtain FO₂Hb, an Oximeter must use at least four wavelengths (one each for HHb, O₂Hb, COHb and MetHb). At present, such Oximeters require blood samples from the patient.

The relation between FO₂Hb and sO₂ is:

$$FO_2Hb = sO_2 \times (1 - FCOHb - FMetHb)$$

It is important to know that "oxygen saturation" as measured by VP01 is not FO₂Hb, but sO₂.

The equation given above expresses the relationship between FO₂Hb and sO₂. ***Thus, if no abnormal hemoglobins (dyshemoglobins) are present, the fraction of oxygenated hemoglobin equals the oxygen saturation, expressed as a fraction.***

The difference between the two can be seen from the example below.

Note that this primarily is useful when used in relation to ctHb.

ctHb = 8 mmol/L

cHHb = 0.5 mmol/L

cCOHb = 3 mmol/L ~ 30 %

cO₂Hb = 6.5 mmol/L

$$FO_2Hb = \frac{6.5}{6.5 + 0.5 + 3} \times 100\% = 65\%$$

$$sO_2 = \frac{6.5}{6.5 + 0.5} \times 100\% = 92,8\%$$

Calculated saturation

Most blood gas analyzers without a CO-oximeter provide a readout for saturation. However, the value is then calculated rather than measured. The calculation is complex and takes into account the various factors that can affect the shape of the Oxyhemoglobin dissociation curve. The mathematical description and the variables in this vary for different brands of analyzer.

Because of the assumed values in the mathematical model, if other types of hemoglobin are present in significant quantities, or 2,3-DPG is not normal, the estimation of sO₂ will be inaccurate. Clinically important errors can result from using estimated sO₂ in other calculations, such as those for shunt fraction and oxygen content¹.

It is discouraged to make an estimation of sO₂ from a measurement of pO₂ and vice versa using a standard ODC.

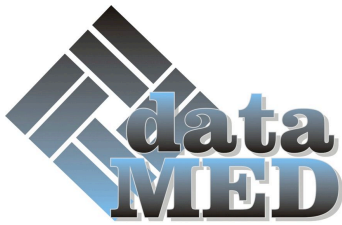
All in all, the most reliable sO₂ is by the measurement by a CO-oximeter. This also gives the advantage that FO₂Hb, FCOHb and FMetHb can be reported as well.

FACTORS AFFECTING BLOOD SATURATION ACCURACY

Other Sat% determination Technique

Using a standard other than the OSM3 CO-Oximeter may influence comparison results.

¹ Hess D, Elser RC, Agarwal N. The effect of measured versus calculated hemoglobin oxygen saturation, carboxyhemoglobin and methemoglobin on the pulmonary shunt calculation. *Respir Care* 1984; 29: 1001-05.



Elevated Methemoglobin and Sulfhemoglobin levels

High concentrations of Methemoglobin or Sulfhemoglobin cause spectral interference affecting both the OSM3 and the VP01 measurements. For example, patients with hereditary methemoglobinemia may present methemoglobin levels above 10%. Moreover, while rare, elevated Sulfhemoglobin levels will cause similar spectral interference. Certain drugs may cause an increase in concentration of both methemoglobin and Sulfhemoglobin.^{2,3}

General Precautions

Bubbles in sample

Air bubbles trapped in the cuvette or in the blood sample drawn for comparison will cause poor correlation between the VP01 and the reference standard.

² Davidshon I, Henry JB (Todd-Stanford) *Clinical Diagnosis by Laboratory Methods*, 15th ed. , Philadelphia: W.B. Saunders Co. 112-113, 1974.

³ Young DS, et al.: "Effect of drugs on Clinical Laboratory Tests", *Clin Chem* 21: 329 D, 1975