

VP01 Hematocrit and Hemoglobin Measurements

Various techniques employed to monitor hematocrit value include micro centrifugation, electrical conductivity, and photometry, the optical probe VP01 employ the spectrophotometric method where light radiation, in the visible and near infrared region, is directed onto blood circulating inside a transparent plastic cuvette. Photo-detector sensors then monitor the radiation reflectively. Radiation spectra are chosen at wavelengths where the metabolite or compounds sought for, either absorbs highly or poorly.

VP01 probe uses solid-state light sources at two different wavelengths for the measurement of Hematocrit and Hemoglobin concentration. The selections of wavelengths are near or at the isobestic points 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 wavelengths, the amount of light adsorption is independent of the amount oxygenated or reduced hemoglobin in the red cells.

The two selected wavelengths are 805 nm and 1450 nm. In the region of 900 to 2000 nm the blood absorption coefficient depend on hematocrit and water, whereas at 805 nm the blood coefficient absorption only depends on hematocrit.

The algorithm for calculating the Ht% from the measured scattered light is shown here below. Even though the wavelengths chosen takes in minimize the effect of blood saturation, a correction for such parameter is taken into account.

$$Ht\% = \left[\left(\frac{\sigma_{805}}{\sigma_{1450}} \right)^3 * \alpha_3 + \left(\frac{\sigma_{805}}{\sigma_{1450}} \right)^2 * \alpha_2 + \left(\frac{\sigma_{805}}{\sigma_{1450}} \right) * \alpha_1 + \alpha_0 \right] + \alpha * Sat\%$$

where σ_{805} and σ_{1450} are the value of scattered light

The algorithm for calculating the Hb% is based on the measurement of Ht% with experimental correction parameters for taking into account the variation cell hemoglobin and not simply apply the "rule of a thumb":
 $Hct \approx 3.0 * Hb$.

$$Hb(g/dL) = \frac{Ht\%}{f(Ht\%, \beta)}$$

where β is a constant obtained from blood calibration

Typically the value of function $f(Ht\%, \beta)$ varies in the range of 2.8 to 3.15 (dl/g).

FACTORS AFFECTING HEMATOCRIT ACCURACY

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

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

IN-VIVO HEMATOCRIT

The VP01 measures a true in-vivo Hematocrit (Hct_{iv}) value by optical transillumination of whole human blood flowing in an extracorporeal circuit. The Hct_{iv} is defined by:

$$\text{Hct}_{iv} = \frac{\text{RBC}_{\text{volume}}}{(\text{RBC}_{\text{volume}} + \text{Plasma}_{\text{volume}})}$$

Where RBC = Red Blood Cell

Because this optical technique does not affect the blood flow or physiology and does not require removal of a blood sample from the flow, it will not incur in errors of other techniques. It measures a true in-vivo Hct, affected by intravascular dosage of heparin and physiologic changes. Techniques which requires aspiration of a blood sample change the sample status to “in-vitro” and introduces at least three potentially significant errors: DILUTION, MEAN CELLULAR VOLUME (MVC) and TECHNIQUES ERRORS.

Hence,

$$\text{Hct} = \text{Hct}_{iv} + \frac{\text{Dilution} + \text{MCV} + \text{Techniques errors}}{\text{in-vitro errors}}$$

IN-VITRO HEMATOCRIT ERRORS

Dilution Errors

Dilution Errors are a direct result of not accounting for diluent volume to the overall blood volume of the sample or imprecise measure of blood volume in the test tube. For example, even with a precise blood sample of 5 ml, the 0.05 ml of EDTA anticoagulant contained in a purple-top test tube leads to a – 0,5 % Hct in-vitro error for a 50% Hct_{iv}.

Dilution Error Potential: -0,5 to -1Hct units

MCV Errors

Changes in the MCV may dramatically affect in-vitro Hct values. Some MCV changes can be related to patient non-compliance (i.e. due to high [Na⁺] intake or Overhydration: [Na⁺] < 135 mEq/L). The most serious MCV error is associated with shrinkage of the RBC's due to effect of the anticoagulant like CPDA1 or EDTA, which is contained in purple-top test tubes. (Red-top test tubes contain no anticoagulant and hence do not produce MCV changes or errors).

An error due to anticoagulants induced red cell shrinkage may result in as much as a 10% Hct change. This is especially true if the sample volume of blood in the test tube is less than required by specification.

Since most Hct determination methods require removal of blood from the in-vivo environment and therefore require use of a diluent or anticoagulant, MCV errors are impossible to avoid unless a red-top tube is used in lieu of an EDTA based purple-top tube. This is due to the functional dependence of Hct on MCV as given below.

Functional dependence of Hct on MCV

The functional dependence of the two most common reference standards for Hct determination: the microcentrifuge and the Coulter Counter (CC Hct), is defined below:

Microcentrifuge Hct (Spun Hct)

of RBC's = number of red Blood Cells

R_v = RBC_{volume} = (MCV)* (# of RBC's)

P_v = Plasma_{volume}

hence

$$\text{Spun Hct} = \frac{1}{\left(1 + \frac{P_v}{R_v}\right)}$$

and therefore:

$$\text{Spun Hct} = \frac{1}{1 + \frac{\text{Pv}}{[(\text{MCV}) * (\# \text{ of RBC's})]}}$$

Coulter Counter Hct .The CC Hct method of determining Hct is based on a known MCV and the number of Red Cells:

$$\text{CC Hct} = \frac{[(\text{MCV}) * (\# \text{ of RBC's})]}{\text{sample volume}}$$

Both standards are MCV dependent: thus both are affected by MCV Errors which are primarily the direct results of EDTA (with respect to heparin) and may range from a minimum Hct reduction of -1.8 Hct units¹ to a 11% change in Hct depending upon relative concentration of EDTA². (Even the use of isotonic agent, to compensate for EDTA induced MCV changes, may not adequately normalize the sample).

The VP01 is calibrated to “normal” MCV ranges (from 80fL-to 100fL)³.

MCV Error Potential: -1,8 to -5 Hct units

Technique Errors

Technique errors are categorized as Handling, Methodology or Sampling Errors.

These errors may be cumulative and therefore may offset or even trivialize MCV errors. At best, technique errors are not negligible.

Handling Errors

Handling errors may result from:

- Hemolysis of the sample
- Contamination of the sample

Handling Error Potential: ± 1 to 3 Hct units

Methodology Errors

Methodology errors may be equipment related due to misapplication of protocol:

- Operator error (1 to 3 Hct units)
- Calibration Problems (1 to 3 Hct units)
- Inappropriate conversion errors (e.g. use of hemoglobin to determine Hct). When the mean cell hemoglobin concentration about 0.33, then

$$\text{Hct} \neq 3.0 * \text{Hb}$$

Also included, as methodology errors are errors specifically associated with microcentrifuge use, all of which may produce individual errors from ± 1 to 3 Hct units⁴ unless otherwise noted:

- Trapped plasma volume (+ 1.4 units)
- “Short cut” spinning with an in-expensive device or abbreviated method (± 2.0 Hct units)
- Lack of precision in following microcentrifuge specification for Hct determination
- Capillary tube leakage from porous plug ends

¹ Gotch F, et al.: “Comparison of Conductivity measured hematocrit to microhematocrit”. ASAIO Trans 37:M138-139, 1991.

² Brittin G, et al.: “Elimination of error in hematocrit produced by excessive EDTA”. Tech Bull Regist Med Technol 39:246-249, 1969.

³ UIHC Pathology Handbook. University Pathology consortium.

⁴ Henry JB; “Clinical Diagnosis&Recommendations for Reference Method for the Packed Cell Volume. ICSH Standard 2001. International Council for the Standardization in Hematology. Laboratory Hematology 7: 148-170, 2001 Carden Jennings Publishing Co. Ltd.

- Setting of red cells in the blood sample before transfer to a capillary tube
- Air pockets in the capillary tube
- Inappropriate reading of meniscus (e.g. including buffy coat or use of an inappropriate scale in lieu of microcalipers)

Methodology Error Potential: ± 1 to 5 Hct units

Sampling Errors

Sampling errors are specifically related to sampling for comparison vs. VP01:

- Exact VP01 Hct_{iv} value not noted at sample time
- Sampling during priming of extracorporeal circuit
- Sample during period of no blood flow

Sampling Error Potential: ± 0.5 to 1 Hct units

Summary of In-Vitro errors

In general, Dilution, MCV and Technique Errors are inherent to in-vitro Hct determination and cannot be ignored. The overall potential error can be as high as ± 5 Hct units.

Reference and Standards

Instruments

The VP01 has been calibrated to Microcentrifuge Standard using ex-vivo whole blood from Donors with “normal” MCV range (from 80fL-to 100fL) and SBE (Standard Base Excess) controlled into the range from -10 mEq/L to +10 mEq/L.

Specification for Microhematocrit accuracy.

Accuracy through microcentrifugation of whole blood requires:

- Minimal amounts of heparin anticoagulation in blood samples
- 15,290 g force
- 12000 RPM
- Spin time: 7 minutes
- Precision micrometer with magnification used for column height measurement

Additional Reference

Abnormal [Na⁺] levels

Changes in [Na⁺] adversely affect the microcentrifuge derived Hct values according to the following relation: 12 mEq/L increase in [Na⁺] \approx 1 Hct unit decrease due to MCV changes

Sources of abnormal sodium concentration:

- Blood Bank with (Na Citrate) \approx 165 mEq/L
- Normal saline as a diluent \approx 154 mEq/L
- Overhydration [Na⁺] < 137 mEq/L

Hemolysis

Hemolysis may affect hematocrit determination, although no changes in the VP01 measurement have been noted for plasma hemoglobin levels below 3 g%

Abnormal Patient conditions

The VP01 has not been tested for all the possible blood conditions. Some of these conditions include sickle cell anemia, macrocytic anemia and hyperlipidemia. Certain drugs and/or medications may cause idiopathic hyperlipidemia such as the prostaglandins (e.g. Alprostadil) and the intralipids given intravenously. These

conditions may cause an offset in Hct measurements

In the table below are listed the ranges of MCV related to the most common pathology

Condition	MCV (fL) Mean and Approx. Range
Normal	89 (82-100)
Iron deficiency anemia)	74 (53-93)
Anemia of chronic disease	86 (70-95)
Thalassemia Minor	68 (56-75)
Thalassemia Major	(48-72)
Hemoglobin H disease	70 (53-88)
HGB E trait, AE	73 (71-78)
HGB E disease, EE	64 (58-76)
HGB C disease, CC	74 (55-93)
Hereditary sideroblastic Anemia	77 (49-104)
Idiopathic sideroblastic anemia	104 (83-118)