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, $\varepsilon$, or scattered light coefficient $\sigma$ (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.

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

where $\sigma_{805}$ and $\sigma_{1450}$ 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":

$$Hct = 3.0 * Hb$$

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

where $\beta$ 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 (Hctiv) value by optical transillumination of whole human blood flowing in an extracorporeal circuit. The Hctiv is defined by:
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} + \text{Dilution} + \text{MCV} + \text{Techniques errors}

\text{in-vitro errors}

**IN-VITRO HEMATOCCRIT 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 measurement 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 -1 Hct 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

\[ R_v = \text{RBC}_{\text{volume}} = (\text{MCV})^\ast (\# \text{ of RBC's}) \]

\[ P_v = \text{Plasma}_{\text{volume}} \]

hence

\[ \text{Spun Hct} = \frac{1}{1 + \frac{P_v}{R_v}} \]

and therefore:
Spun Hct = \( \frac{1}{1 + \left[ \frac{Pv}{(MCV) \times (\# \text{ of RBC's})} \right]} \)

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

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

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 units\(^1\) to a 11% change in Hct depending upon relative concentration of EDTA\(^2\). (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)\(^3\).

\[\text{MCV Error Potential: -1.8 to -5 Hct units}\]

**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

\[Hct = 3.0 \times Hb\]

Also included, as methodology errors are errors specifically associated with microcentrifuge use, all of which may produce individual errors from ± 1 to 3 Hct units\(^4\) 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

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\(^3\) UIHC Pathology Handbook. University Pathology consortium.

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 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 ± 5Hct 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 in 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⁺] = 1 Hct unit decrease due to MCV changes

Sources of abnormal sodium concentration:
- Blood Bank with (Na Citrate) ≈ 165 mEq/L
- Normal saline as a diluent ≈ 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

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