

Hematocrit (Hct) determination in hemodialysis is a complex process often resulting in values differing from the circulating in-vivo Hct. As this Tech Note illustrates, typical laboratory or clinic 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 CRIT-LINE Monitor (CLM) measures a true in-vivo hematocrit (Hct_{iv}) value by optical transillumination of whole human blood flowing in an extracorporeal circuit defined by:

$$\text{Hct}_{iv} = \frac{\text{RBC} \times \text{MCV}}{\text{Total Volume}}$$

Where:

RBC = Red Blood Cell Count

MCV = Mean Cell Volume of the Red Blood Cell

Total Volume = The total volume of the sample from which the RBC and MCV were taken

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 the errors of other techniques. It measures a true in-vivo Hct, affected by the intravascular dosage of heparin and physiologic changes. Techniques which require aspiration of a blood sample for Hct determination change the sample status to “in-vitro” and introduce at least three potentially significant errors:

Dilution, Mean Cell Volume (MCV), and Technique Errors. Hence,

$$\text{Hct} = \text{Hct}_{iv} + \frac{\text{Dilution} + \text{MCV} + \text{Technique Errors}}{\text{In-Vitro Errors}}$$

IN-VITRO HEMATOCRIT ERRORS

Dilution Errors

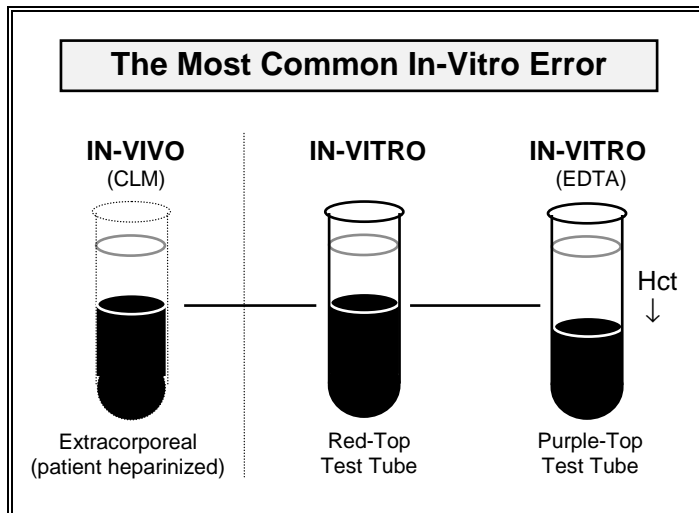
Dilution Errors are a direct result of not accounting for the diluent volume to the overall blood volume of the sample or imprecise measure of blood volume in the test tube. For example, even with a precise blood sample of 5 ml, the 0.05 ml of EDTA anticoagulant contained in a purple-top test tube leads to a 1% (-0.5 Hct) in-vitro error for a Hct_{iv} = 50.

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 the effect of the anticoagulant EDTA that is contained in purple-top test tubes. (Red-top test tubes contain no anticoagulant and hence do not produce MCV changes or errors.)



As shown in the diagram, an MCV error due to EDTA induced red cell shrinkage may result in as much as a 10% Hct change (i.e., a lower Hct value of 45.0 for a Hct_{iv} = 50.0). 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) (Coulter Electronics, Hialeah, Florida), is defined below:

a. Microcentrifuge Hct (Spun Hct)

of RBC's = number of Red Blood Cells
 $R_V = RBC_{\text{volume}} = (MCV) \cdot (\# \text{ of RBC's})$
 $P_V = Plasma_{\text{volume}}$
 hence:

$$\text{Spun Hct} = 1 / \{1 + P_V / R_V\},$$

and therefore:

$$\text{Spun Hct} = 1 / \{1 + P_V / [(MCV) \cdot (\# \text{ of RBC's})]\}$$

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

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

Both standards are MCV dependent; thus, both are affected by MCV Errors which are primarily the direct result 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 the relative concentration of EDTA². (Even the use of an isotonic agent, to compensate for EDTA induced MCV changes, may not adequately normalize the sample.)

MCV Error Potential: - 1.8 to -5 Hct units

MCV Correction

The CLM III is calibrated to “normal” hemodialysis patient blood with a MCV of $91\mu^3$ (“Normal” means normal ranges for sodium and osmolarity). $91\mu^3$ is used because in HemaMetric’s clinical studies, the *mean* pre and post-dialysis MCV was found to be $91\mu^3$ over an intradialytic blood sodium concentration range of 132 (pre) to 137 meq/L (post) and a correlated intradialytic dialysate sodium modeling range of 150 to 140 meq/L respectively. This mean value has been proven to be precisely correlated with hematocrit lab values when sampling technique is consistent.

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.

Deviation of in vivo MCV from this calibration point will be reflected by a difference between measured hematocrit and actual hematocrit.

For precise correlation purposes, at least two capillary tube samples of each CLM III data point should be “spun” and at least two samples sent to a reputable lab for hematocrit cross-validation and MCV determination.

Once the MCV of the sample is known, the following correction can be applied:

$$\text{Hct}_{91} = \frac{91 * \text{Hct}}{91 * \text{Hct} + \text{MCV}(1-\text{Hct})}$$

For example, if the results from the lab indicate that the MCV of a particular blood sample is 93.4 and the hematocrit is 37.8, the MCV is placed into the above formula to generate a Hct value of -0.65. This value is then added to 37.8 resulting in a value of 37.15. This value (37.15) is the value that will be compared to the CLM value that was recorded when the sample was drawn.

Other error factors due to handling or technique must be considered separately.

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 or due to misapplication of protocol: (*error potential* given in ()’s)

- Operator error (1 to 3 Hct units)
- Calibration problems (1 to 3 Hct units)

- Inappropriate conversion errors: Example: Use of Hemoglobin to determine Hct.

When the mean cell hemoglobin concentrations $\neq 0.33$, then

$$\text{Hct} \neq 3.0 \times \text{Hgb.} \quad (1 \text{ to } 2 \text{ Hct units})$$

Also included as methodology errors are errors specifically associated with microcentrifuge use, all of which may produce individual errors of from ± 1 to 3 Hct units³ unless otherwise noted:

- Trapped plasma volume $(+1.4 \text{ Hct units}^4)$
- “Short cut” spinning with an in-expensive device or abbreviated method $(\pm 2.0 \text{ Hct units})$
- Lack of precision in following microcentrifuge specification for Hct determination. *(If the microcentrifuge specifications presented in the Reference Standards Section are not precisely maintained, the Spun Hct values may be significantly offset.)*
- Capillary tube leakage from porous plug ends
- Settling of red cells in the blood sample before transfer to a capillary tube
- Air pockets in the capillary tube
- Inappropriate reading of the 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. CLM:

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

Sampling Error Potential: $\pm .5$ to 1 Hct units

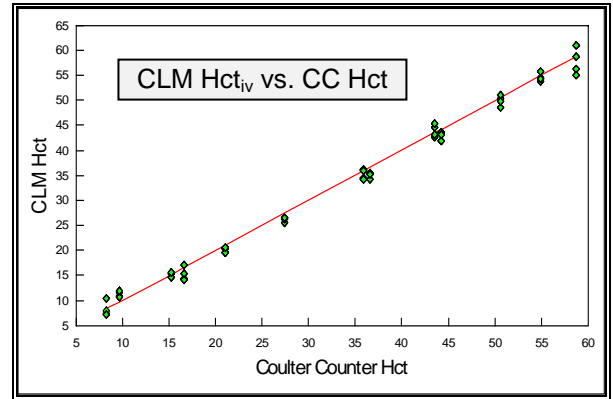
Summary of In-Vitro Errors

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. With extreme care in sampling, use of EDTA as an anticoagulant (use of a Purple-top tube), and precise adherence to standard specifications; Dilution, MCV, and Technique Errors can be minimized. Under such conditions, both CC and Spun Hct can be shown to exhibit excellent correlation with CLM Hct_{iv}.

³Henry, JB; Clinical Diagnosis & Management by Laboratory Methods.

18th Edition, pp 560-561, 1991

⁴Ibid.



REFERENCE STANDARDS

Instruments

The CRIT-LINE Monitor (CLM) has been calibrated to two laboratory standards using in-vivo whole blood (Heparinized only) and sampled from acute and chronic dialysis patients during treatment:

Coulter Counter. The CLM has been calibrated to the Coulter Counter (CC) either using Purple-Top test tubes or normalizing samples to $\text{MCV}=91\mu^3$.

Microcentrifuge. The CLM has also been calibrated to the IEC MB microcentrifuge (International Equipment Company, Needham Heights, MA).

Specifications for Microhematocrit Accuracy.

Accuracy through microcentrifugation of whole blood requires:

- Minimal amounts of heparin anticoagulation in blood samples
- 13,000 g force
- 11,500 RPM
- Spin time: 5 minutes
- Precision micrometer with magnification used for column height measurement—no graphic scales

If the microcentrifuge specifications presented above are not precisely maintained, the Spun Hct values may be significantly offset.

Additional References

Abnormal {Na+} Levels.

The CLM was calibrated using blood samples from patients with serum sodium levels of ~ 137 meq/L. 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 concentrations:

- Blood Bank Blood with {NaCitrate} ≈ 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 CLM measurement have been noted for plasma hemoglobin levels below 5 gm %.

Abnormal Patient Conditions.

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

Bubbles in Sample

Air bubbles trapped in the CRIT-LINE™ Blood Chamber or in the blood sample drawn for comparison will cause poor correlation between the CRIT-LINE Instrument and the reference standard.

TABLE FOR ESTIMATING IN-VITRO HCT ERRORS

ERROR TYPE	CAUSE OF ERROR (refer to text or references for a more complete explanation)	MINIMUM ERROR (note signs)
Dilutional	• Imprecise volume of sampled blood	- 0.5 Hct units
	• Anticoagulant not accounted for in volume	“
MCV	• Purple-top test tubes (EDTA)	-1.8 Hct units
Sampling	• Inexact timing of the sample	+/- 0.5 Hct units
	• No dialyzer blood flow	“
	• Sampling during priming	“
Handling	• Hemolysis of the sample	+/- 1.0 Hct units
	• Contamination of the sample	“
Methodology (General)	• Operator error	+/- 1.0 Hct units
	• Calibration of measurement device	“
Methodology (Spinning Hct)	• Trapped plasma volume (higher Hcts)	+ 1.4 Hct units
	• Capillary tube leakage from porous ends	+/- 1.0 Hct units
	• Settling of RBC's in the sample	“
	• Air pockets in the capillary tube	“
	• Lack of precision (microcentrifuge spec.)	+/- 1.5 Hct units
	• Reading errors: printed scale vs. microcalipers	“
	• “Short-cut” spinning method or device	+/- 2.0 Hct units
Methodology (Conversion)	• Hgb conversion error (Hct \neq 3.0 x Hgb)	+/- 1.0 Hct units