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ORIGINAL ARTICLE

Comparative Study upon Protein Effect on Hemoglobin and Hematocrit with Conductivity based Point of Care Device and Optical Methods

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ABSTRACT

The conductivity based testing provides rapid assessment in patients including the need of transfusion for H/H assay can be influenced by plasma protein concentration. There are conductivity and optical methods for measurement of hemoglobin and hematocrit value. The H/H optical results were compared in respect by core laboratory hematology analyzer. The whole anti-coagulated blood was centrifuged for RBC sedimentation and removal of plasma to serve as source of protein for mixing studies. A series of reconstituted samples was prepared with varying H/H and protein levels. There samples were diluted with saline Ringer's solution to avoid hemodilution in clinical practice. The H/H results obtained with the Hemocue instrument correlated exactly with those of the GenS analyzer at protein concentrations from 0.7 to 6.2 g/dl. The correlation was unaffected with hemodiluted blood. The H/H results obtained with the POCT instrument gave slightly less correlation with those of the GenS analyzer over this protein range. However, the iSTAT-1 results were generally lower than the GenS results, with discrepancies up to 2 g/dL for hemoglobin values and up to 4% for hematocrits at the lowest protein concentration. Therefore, it is recommended that H/H testing in patients with suspected hypoproteinemia or substantial hemodilution should be tested with an optical method.

Key words: *point-of-care-testing, blood conductivity, hemoglobin, hematocrit*

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INTRODUCTION

The point-of-care testing (POCT) devices are currently available (P St Luis, 2001) that provide blood hemoglobin and hematocrit (H/H) determination in rapid patient assessment as in the case of blood transfusion (Connely, *et al* 1996; Lardi, *et al* 1998). These conductivity-based procedures can be influenced by plasma composition, including electrolyte and protein concentrations (Connely, *et al* 1996; Papadea, *et al* 2002). The iSTAT-1 POCT device uses conductivity to estimate the hematocrit of blood samples and calculates the hemoglobin concentration by a simple equation (Durrart, *et al* 2003). This instrument is accurate for several studies (Papadea, *et al* 2002), especially in patients undergoing significant hemodilution as during cardiopulmonary bypass surgery (Durart, *et al* 2003). The studies showed that variations in plasma composition can substantially influence the H/H results, with plasma protein having the greatest effect (Connely, *et al* 1996).

These studies analyzed actual patient blood samples with few data points at the extremes of H/H and protein levels that are present in critically adult populations (Papadea, *et al* 2002; Blunt, *et al* 1998). There a series of reconstituted whole blood samples were generated with varying H/H levels and plasma protein concentrations. The reconstituted

samples were assayed for H/H using iSTAT-1 (conductivity method) and Hemocue (optical method). These H/H results obtained by these POCT devices were compared to those obtained by use of the GenS hematology analyzer that is the primary method in core clinical laboratory.

MATERIALS AND METHODS

All materials and procedures for point of-care testing (POCT) were obtained from the manufacturers and were used in accordance with directions. Saline (NaCl, 9 g/L) and lactated Ringer's solutions and Lithium-heparin Vacutainer tubes were used under process.

Table 1: Sample reconstitution design

Group No.	Tube No.	RBCs (ml)	Plasma (ml)	Saline or Ringer's Soln. (ml)
1	1	1.2	0.8	0.0
	2	1.2	0.4	0.4
	3	1.2	0.2	0.6
2	4	0.9	0.8	0.3
	5	0.9	0.4	0.7
	6	0.9	0.2	0.9
3	7	0.6	0.8	0.6
	8	0.6	0.4	1.0
	9	0.6	0.2	1.2
4	10	0.5	0.8	0.7
	11	0.5	0.4	1.1
	12	0.5	0.2	1.3
5	13	0.4	0.8	0.8
	14	0.4	0.4	1.2
	15	0.4	0.2	1.4
6	16	0.3	0.8	0.9
	17	0.3	0.4	1.3
	18	0.3	0.2	1.5

The iSTAT-1 POCT device uses a conductivity-based method to measure blood hematocrit (Durward, *et al* 2003). There whole blood is introduced by capillary action into a single-use microfabricated biosensor cartridge. The measured conductivity after correction for electrolyte concentration is inversely related to the blood hematocrit. Blood hemoglobin value is calculated by the following equation: hemoglobin (g/dl) = hematocrit (decimal fraction) x 34. The Hemocue POCT device measures hemoglobin by an optical-based method (Hemocue Method). There whole blood is introduced by capillary action into a single use cuvette and sodium deoxycholate is added to lyse the red blood cells (RBC). The hemoglobin that is released is converted by sodium nitrite to methemoglobin, which together with sodium azide generates azide methemoglobin.

The GenS analyzer counts and sizes cells by detecting and measuring changes in electrical resistance when blood cells in a conductive liquid pass through a small pore (Coulter 1999). The blood hematocrit is calculated from the RBC count and the mean corpuscular volume (MCV), using the following equation: hematocrit (%) = (RBC x MCV)/10. The blood hemoglobin concentration is determined optically following RBC lysis, based on transmittance measurements (%T) using the following equation: hemoglobin (g/dl) = constant x log10 (reference %T/sample %T).

The whole blood sample of patients were obtained by venipuncture into Vacutainer tubes with lithium-heparin anticoagulant and immediately centrifuged in a bench-top centrifuge at 3,200 rpm for 10 min at room temperature. The supernatant plasmas, which were removed and transferred to a 50 ml conical polypropylene tube, served as a pooled source of protein for sample reconstitution studies following addition of approximately two volumes of 0.9% saline. The RBC pellets were transferred to and pooled in 50 ml conical

polypropylene tubes after addition of saline to generate various samples with varied H/H and protein contents (Table 1).
The reconstituted samples were diluted with either saline or lactated Ringer's solutions to mimic hemodilutions occur in clinical practice. The reconstituted samples were analyzed as in the POCT, Hemocue (hemoglobin), and GenS (hematocrit and hemoglobin).

RESULTS AND OBSERVATION

Hemoglobin and hematocrit results obtained with the two POCT instruments and the GenS analyzer and the protein concentrations of the samples are listed in Tables 2 and 3.

Table 2: Comparison of hemoglobin and hematocrit in saline diluents

Tube	*i*stat-1 Hct (%)	*i*stat-1 Hb (g/dl)	Gen S Hct (%)	Gen S Hb (g/dl)	Hemocue Hb (g/dl)	Protein (g/dl)	*i*STAT-Gen S Hct (%)	*i*STAT-Gen S Hb (g/dl))
1	51	17	48.2	17.1	17.5	6.5	2.8	-0.1
2	50	17	48.3	17.2	17.7	3.5	1.7	-0.2
3	48	16	48.5	17.1	17.6	2.0	-0.5	-1.1
4	34	12	33.7	12.1	12.8	5.2	0.3	-0.1
5	36	12	36.5	13.0	13.4	2.8	-0.5	-1.0
6	33	11	36.1	13.0	13.4	1.5	-3.1	-2.0
7	24	8	24.8	8.9	9.3	4.2	-2.8	-0.9
8	22	7	24.8	8.8	9.1	2.2	-4.6	-1.8
9	20	7	24.6	8.7	9.0	1.1	-2.1	-1.7
10	18	6	20.1	7.2	7.4	4.1	-2.6	-1.2
11	17	6	20.6	7.3	7.6	2.0	-5.9	-1.3
12	15	5	20.9	7.3	7.6	1.1	-0.6	-2.3
13	15	5	15.6	5.6	5.9	3.8	-3.5	-0.6
14	13	<	15.5	5.9	6.1	2.8	-3.9	NC
15	13	<	16.9	6.0	6.4	0.9	-1.4	NC
16	10	<	11.4	4.2	4.4	3.6	NC	NC
17	<10	<	11.1	4.3	4.5	1.8	NC	NC
18	<10	<	12.8	4.6	4.8	0.8	NC	NC

Table 3: Comparison of hemoglobin and hematocrit results with Ringer's solution diluents

Tube	*i*stat-1 Hct (%)	*i*stat-1 Hb(g/dl)	Gen S Hct (%)	Gen S Hb (g/dl)	Hemocue Hb (g/dl)	Protein (g/dl)	*i*STAT-Gen S Hct (%)	*i*STAT-Gen S Hb (g/dl))
1	50	17	48.0	16.9	17.5	6.2	2.0	0.1
2	48	16	47.3	16.9	17.3	3.4	0.7	-0.9
3	47	16	47.4	16.8	17.2	1.9	-0.4	-0.8
4	33	11	32.4	11.6	12.0	5.2	0.6	-0.6
5	34	12	35.7	12.7	13.1	2.6	-1.7	-0.7
6	33	11	35.8	12.7	13.1	1.4	-2.8	-1.7
7	23	8	23.9	8.5	9.0	4.2	-0.9	-0.5
8	21	7	24.3	8.6	9.0	2.1	-3.3	-1.6
9	20	7	24.2	8.6	8.9	1.1	-4.2	-1.6
10	19	6	20.1	7.2	7.6	4.0	-1.1	-1.2
11	17	6	20.9	7.1	7.4	2.1	-3.0	-1.1
12	16	5	19.9	7.1	7.4	1.1	-3.9	-2.1
13	15	5	16.6	6.0	6.3	3.9	-1.6	-1.0
14	NA	NA	NA	NA	NA	NA	NA	NC
11	4	4	15.9	5.7	5.9	0.8	-4.9	-1.7
10	3	3	11.9	4.3	4.6	3.6	-1.9	-1.3
10	<10	<	11.9	4.3	4.5	1.7	NC	NC
10	<10	<	11.7	4.2	4.4	0.9	NC	NC

Table 4: Comparison of hemoglobin and hematocrit results obtained with applied instruements

Method (y)	Method (x)	Diluent Solution	S y/x	r	Bias
Hemocue Hb	GenS Hb	Saline	0.1065	0.999	0.34
Hemocue Hb	GenS Hb	Ringer	0.0899	1.000	0.33
*i*STAT Hb	GenS Hb	Saline	0.7019	0.975	-1.02
*i*STAT Hb	GenS Hb	Ringer	0.6371	0.978	-0.91
*i*STAT Hct	GenS Hct	Saline	2.0111	0.979	-1.50
*i*STAT Hct	GenS Hct	Ringer	1.5993	0.986	-1.20

The hemoglobin results obtained with the Hemocue POCT instrument were very close to those of the primary method over the broad range of protein concentrations and hematocrit levels due to no effect of hemodilution with either saline or Ringer's solution. In contrast, H/H results with the iSTAT-1 POCT instrument were deviate up to 2 g/dl for hemoglobin and up to 4% for hematocrit at the lowest protein and hemoglobin concentrations, regardless of used hemodiluent. Hemoglobin concentrations in the Hemocue instrument correlated well as obtained through the GenS analyzer (Table 4).

Bland-Altman analysis revealed a constant and relatively negligible positive bias of 0.3 g/dl for hemoglobin results obtained with the Hemocue instrument versus the primary method (Table 4). This analysis is really gives insight about bias in hemoglobin and hematocrit value measured through hemocue and GenS analyser with regression relation into their measurements.

The low level of bias obtained with this optical-based method was unaffected when hemodilution was performed with either the saline or lactated Ringer's solutions. The standard errors of estimate (Sy/x) were relatively low for hemoglobin results obtained with the Hemocue instrument, yielding range of limit from low to high values of 0.50 g/dl for saline and 0.41 g/dl for Ringer's diluted samples. Substantially higher standard errors of estimate (Sy/x) and overall negative bias were observed for H/H results obtained using the iSTAT-1 POCT instrument. Although use of Ringer's solution as the diluent decreased the apparent effect of low protein concentration on the results of the conductivity-based method, the range of limit from low to high values for H/H results obtained with the iSTAT-1 were several fold higher than those with the Hemocue.

DISCUSSION

There hemoglobin results obtained through Hemocue POCT instrument and GenS hematology analyzer were similar. The constant bias of 0.3 g/dl as observed for the Hemocue hemoglobin concentrations in this study are in agreement with other studies (Lardi, *et al* 1998; Rippman, *et al* 1997). These both methods are optical-based methods for hemoglobin determination and thus showed concordance with reconstituted blood samples after dilution in clinical settings.

The H/H value determined by conductivity instruments were generally lower than those obtained with the GenS instrument due to hypoproteinemia. In cardiopulmonary bypass (CPB) patients, H/H testing with the iSTAT-1 instrument may be performed in a CPB mode that automatically corrects hematocrit (~3%) for the decreased plasma protein levels typically associated with CPB hemodilution (Connely, *et al* 1996). There CPB mode substantially correct for differences in protein-based conductivity. In a comparison of samples obtained during CPB surgery, use of the CPB mode on the i-STAT instrument increased the correlation coefficient for hemoglobin and hematocrit from 0.46 to 0.95 and from 0.74 to 0.98, respectively (Schneider, *et al* 1997). It should be noted that the CPB mode corrects H/H results based on constant positive adjustment of hematocrit. This hematocrit adjustment is incapable of reflecting a variety of protein concentrations.

It has been recommended that more restrictive transfusion guidelines be adopted with haemoglobin concentrations between 7 and 10 g/dl (McFarland 1994). The study of McFarland suggested that H/H results may be decreased by low protein levels through conductivity-based method. There analytical limitations are fundamental to clinical reasoning and needful special attention in the case of critical patients (Blunt, *et al* 1998; Vander Linden 2001). Therefore, only non-conductive methods are preferable for H/H testing in patients with suspected hypoproteinemia or substantial hemodilution.

REFERENCES

1. Blunt M.C., Nicholson J.P. and Park G.R. (1998): Serum albumin and colloid osmotic pressure in survivors and nonsurvivors of prolonged critical illness. Anaesthesia, 53:755-761.
2. Connelly N.R., Magee M. and Kiessling B. (1996): The use of the i-STAT portable analyzer in patients undergoing cardiopulmonary bypass. J Clin Monit, 12: 311-315.
3. Coulter GenS (1999): Hematology Analyzer Operating Manual, Beckman-Coulter, Inc., Brea, CA.
4. Durward A., Mayer A., Skellett S., Taylor D., Hanna S., Tibby S.M. and Murdoch I.A. (2003): Hypoalbuminaemia in critically ill children: incidence, prognosis, and influence on the anion gap. Arch Dis Child, 88: 419-422.
5. Implementation Guide and Procedure Manual (1999): i-STAT Corporation, East Windsor, NJ.
6. Lardi A.M., Hirst C., Mortimer A.J. and McCollum C.N. (1998): Evaluation of the Hemocue for measuring intra-operative haemoglobin concentrations: a comparison with the Coulter Max-M. Anaesthesia, 53: 349-352.
7. McFarland J.G. (1999): Perioperative blood transfusions: indications and options. Chest, 115(Suppl 5): 113S-121S.
8. Papadea C., Foster J., Grant S., Ballard S.A., Cate J.C., Southgate W.M. and Purohit D.M. (2002): Evaluation of the iSTAT portable clinical analyzer for point-of-care testing in the Point-of-care testing: effect of protein on Hb and Hct intensive care units of a university children's hospital. Ann Clin Lab Sci., 32: 231-243.
9. Rippmann C.E., Nett P.C., Popovic D., Seifert B., Pasch T. and Spahn D.R. (1997): HemoCue, an accurate bedside method of hemoglobin measurement? J Clin Monit, 13: 373-377.
10. Schneider J., Dudziak R., Westphal K. and Vettermann J. (1997): The i-STAT analyzer. A new hand-held device for the bedside determination of hematocrit, blood gases, and electrolytes. Anaesthesist, 46: 701-714.
11. St-Louis P. (2000): Status of point-of-care testing: promise, realities, and possibilities. Clin Biochem., 33: 427-440.
12. VanDer Linden P. (2001): Transfusion strategy. Eur J. Anaesthesiol, 18: 495-498.