Development of a Hemolysis Index for Vanadis® cfDNA NIPT Sample Acceptance

Luo, Zhiwen; Shenoy, Suresh; Donti, Taraka; Prensky Lawrence; Chin, Ephrem; Borandi, P.J; and Hegde, Madhuri

Revvity Omics, Pittsburgh, PA, USA.

BACKGROUND

The arrival of a hemolyzed sample has long been a concern for laboratories as hemolyzed samples are often felt to cause errors in many laboratory tests due to dilution effects, proteolysis, loss of DNA, the increase in the intracellular components, or chemical interference[1] In a review of samples received in laboratories, it was found that up to 3.3% of them were hemolyzed to various degrees[2,3]. The obvious first step is to try to minimize the collection and running of these samples. Some relevant causes for the hemolysis of samples are:[2,4-8]

- Underfilling of tubes[9,10]
- Use of small-gauge needles[10,11]
- Prolonged tourniquet application[7,10,12]
- Difficult draws (unsatisfactory attempts, small or fragile veins, forcing blood into tubes)[1,10,11,13,14]
- Excessive temperatures[15,16] , lack of packing material[17], or delays in shipping[16]
- Excessive tube inversion/shaking[11]

To determine whether such samples are suitable for screening within the Vanadis® system, a study was performed to measure the system's reliability with hemolyzed samples collected in Streck tubes.

METHODS

An initial range-finding study was conducted by mixing completely hemolyzed whole blood from a pool of male volunteers into normal, non-hemolyzed, whole blood from a pool of female volunteers in Streck tubes. The following hemolysis mixtures were prepared: 0.1, 0.2, 0.5, 0.75, 1.0, 1.5, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0 and 50.0 (volume/volume percent levels). Plasmas from these blends were extracted and their cfDNA purified on Vanadis® Extract. The purified cfDNA were converted to "rolling circle products" (RCP) via Rolling Circle Amplification (RCA) on Vanadis® Core. The RCPs for chromosomes 13, 18, 21 and Y were imaged and counted on Vanadis® View. Reference chromosome density (sum of chromosomes 13, 18 and 21) and chromosome Y RCP density were obtained from Vanadis® System Software. Aliquots of cfDNA from Vanadis® Extract were analyzed using microcapillary electrophoresis to quantify sizes and amounts of cfDNA. Aliquots of plasma samples were analyzed by scanning spectroscopy to measure oxyhemoglobin peak at 571 nm. A color chart of plasmas from 0-50% hemolyzed blood was also created. Sample acceptance for use with the Vanadis® system was determined using 3 criteria: relative cfDNA amounts, relative reference chromosome density and Vanadis® Quality Assessment of samples. After determining that a 1% level of hemolysis is acceptable for the Vanadis® cfDNA NIPT in these synthetic mixtures, we examined the performance of the system on pregnant samples by adding hemolyzed whole blood into whole blood from 22 pregnant women, as well as in synthetic trisomy 21 reference materials at a 1% vol/vol level.

RESULTS

The results indicated that cfDNA amounts started to decrease at >2.5% hemolysis (Fig. 1a and 1b), and reference chromosome densities decreased at hemolysis levels > 10% (Fig.2). Vanadis System Software approved samples up to 15% hemolysis (Table 1). Using a color chart of plasmas obtained from 0-50% hemolysis (Fig. 7), a survey of lab operators indicated that we had never received blood samples with greater than 1.5% hemolysis. Therefore, to be conservative, we chose to study the effect of hemolytic material at 1% in whole blood from pregnant women and in T21 reference materials in the Vanadis® cfDNA test. Those results indicate that the presence of 1% hemolytic material had no effect on the reference chromosome densities (Fig. 3) and the chromosome Y/reference chromosome density ratios (a surrogate for fetal fraction in male fetus pregnancies) (Fig. 4). The euploid calls (for chromosomes 13, 18 and 21) and the fetal sex calls remained the same for the 22 pregnancy cases (Table 2). Additionally, the presence of 1% hemolytic material in trisomy 21 reference material had no significant effect on the elevated chromosome 21 ratio scores (Fig. 5 and Table 2).

CONCLUSIONS

Testing samples that are below 1% hemolysis does not affect the accuracy of Vanadis® cfDNA NIPT results. Either spectrophotometry (Fig. 6) or a color comparison chart (Fig. 7) can be used to preferentially remove those samples that are highly hemolyzed from the workflow.

REFERENCES

1.G. Lippi, G. Salvagno, M. Montagnana, G. Brocco, G. Guidi, Influence of hemolysis on routine clinical chemistry testing, Clinical Chemistry and Laboratory Medicine 44(3) (2006) 311-316.
2.Kroll MH, Elin RJ. Interference with clinical laboratory analyses. Clin Chem 1994;40:1996–2005.
3.Jones BA, Calam RR, Howanitz PJ. Chemistry specimen acceptability. A College of American Pathologists QProbes study of 453 laboratories. Arch Pathol Lab Med 1997;121:19–26
4.G. Lima-Oliveira, W. Volanski, G. Lippi, G. Picheth, G.C. Guidi, Pre-analytical phase management: a review of the procedures from patient preparation to laboratory analysis, Scand J Clin Lab Invest 77(3) (2017) 153-163.
5.D. Giavarina, G. Lippi, Blood venous sample collection: Recommendations overview and a checklist to improve quality, Clin Biochem 50(10-11) (2017) 568-573.
6.G. Lippi, G. Banfi, M. Buttarello, F. Ceriotti, M. Daves, A. Dolci, M. Caputo, D. Giavarina, M. Montagnana, V. Micon, B. Milanesi, A. Mosca, M. Morandini, G. Luca, S.-S.-C. Ital Intersoc, Recommendations for detection and management of unsuitable samples in clinical laboratories, Clinical Chemistry and Laboratory Medicine 45(6) (2007) 728-736.
7.G. Lima-Oliveira, G. Lippi, G.L. Salvagno, G. Picheth, G.C. Guidi, Laboratory Diagnostics and Quality of Blood Collection, J Med Biochem 34(3) (2015) 288-294.
8.Carraro P, Servidio G, Plebani M. Hemolyzed specimens: a reason for rejection or a clinical challenge? Clin Chem 2000;46:306–7.
9.G. Lippi, M. Plebani, S. Di Somma, G. Cervellin, Hemolyzed specimens: a major challenge for emergency departments and clinical laboratories, Critical Reviews in Clinical Laboratory Sciences 48(3) (2011) 143-153.
10.A. Wollowitz, P. Bijur, D. Esses, E. Gallagher, Use of Butterfly Needles to Draw Blood Is Independently Associated With Marked Reduction in Hemolysis Compared to Intravenous Catheter, Academic Emergency Medicine 20(11) (2013) 1151-1155.
11.G. Lippi, G.L. Salvagno, E.J. Favaloro, G.C. Guidi, Survey on the prevalence of hemolytic specimens in an academic hospital according to collection facility: opportunities for quality improvement, Clin Chem Lab Med 47(5) (2009) 616-8.
12.S. Saleem, V. Mani, M.A. Chadwick, S. Creanor, R.M. Ayling, A prospective study of causes of haemolysis during venepuncture: tourniquet time should be kept to a minimum, Ann Clin Biochem 46(Pt 3) (2009) 244-6.
13.I. Munnix, M. Schellart, C. Gorissen, H. Kleinveld, Factors reducing hemolysis rates in blood samples from the emergency department, Clinical Chemistry and Laboratory Medicine 49(1) (2011) 157-158.
14.L. Dugan, L. Leech, K.G. Speroni, J. Corriher, Factors affecting hemolysis rates in blood samples drawn from newly placed IV sites in the emergency department, J Emerg Nurs 31(4) (2005) 338-45.
15.G. Lippi, N. Blanckaert, P. Bonini, S. Green, S. Kitchen, V. Palicka, A.J. Vassault, M. Plebani, Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories, Clin Chem Lab Med 46(6) (2008) 764-72.
16.C.M. Fernandes, A. Worster, K. Eva, S. Hill, C. McCallum, Pneumatic tube delivery system for blood samples reduces turnaround times without affecting sample quality, J Emerg Nurs 32(2) (2006) 139-43.
17.G. Cakirca, H. Erdal, The Effect of Pneumatic Tube Systems on the Hemolysis of Biochemistry Blood Samples, J Emerg Nurs 43(3) (2017) 255-258.

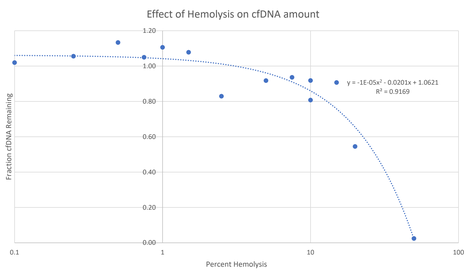


Figure 1a. cfDNA amounts start decreasing at >2.5% hemolysis

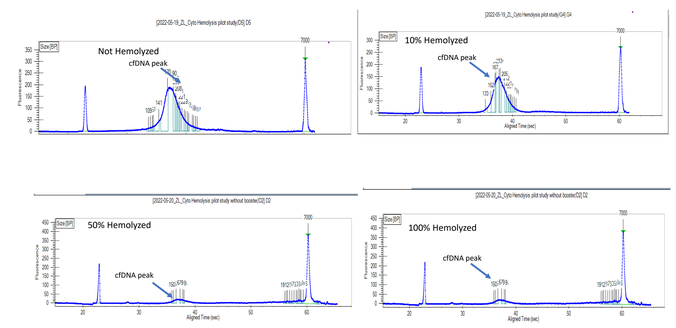


Figure 1b. Loss of cfDNA demonstrated by microcapillary electrophoresis.

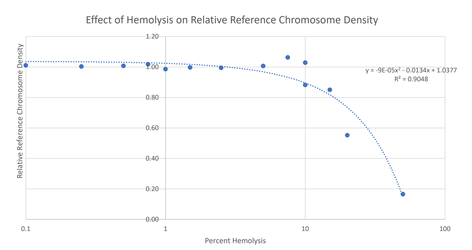


Figure 2. Reference Chromosome Density start decreasing at >10% hemolysis

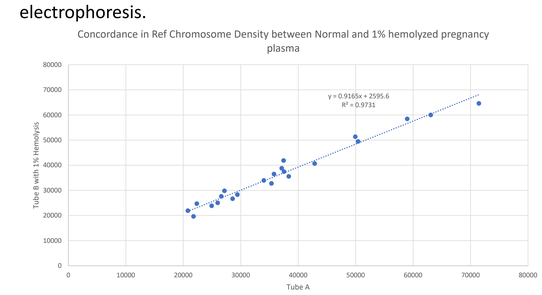


Figure 3. Tubes A from 22 pregnancy plasmas were analyzed using the Vanadis cfDNA assay. Prior to plasma separation of the corresponding blood from tubes B, 100 ul of the blood was removed and hemolyzed. Plasma from the hemolyzed blood were added back to plasma from their un-hemolyzed counterparts at 1% vol/vol on the day of the Vanadis cfDNA assay for the tubes B. Comparison of the reference chromosome densities of the tube A (normal) and tube B (1% hemolyzed) showed good concordance

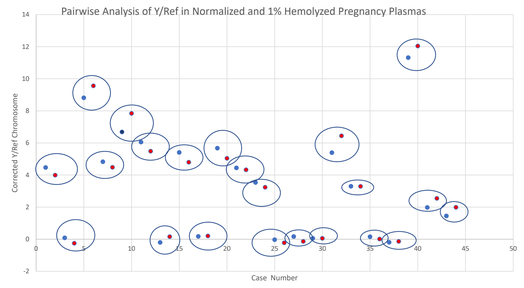


Figure 4. Presence of 1% hemolytic material in blood from pregnant women does not adversely affect fetal sex determination calls

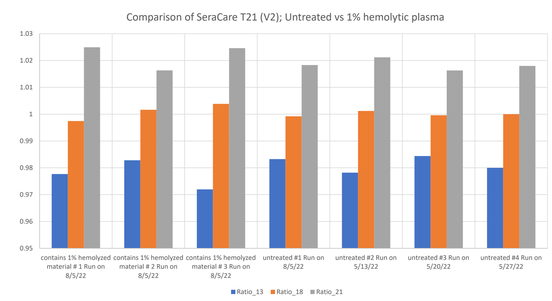
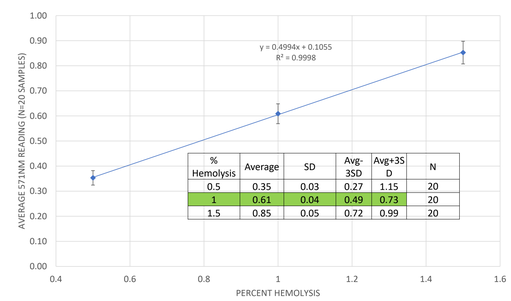


Figure 5. Presence of 1% hemolytic material in SeraCare T21 Reference Materials did not affect elevated T21 ratio scores adversely



% Hemolysis	Average	SD	Avg-3SD	Avg+3SD	N
0.5	0.35	0.03	0.27	1.15	20
1	0.61	0.04	0.49	0.73	20
1.5	0.85	0.05	0.72	0.99	20

Figure 6. Spectrophotometry of plasma at 571 nm indicates that a reading of 0.73 or higher could be used as sample rejection criterion for red-tinted plasma samples.

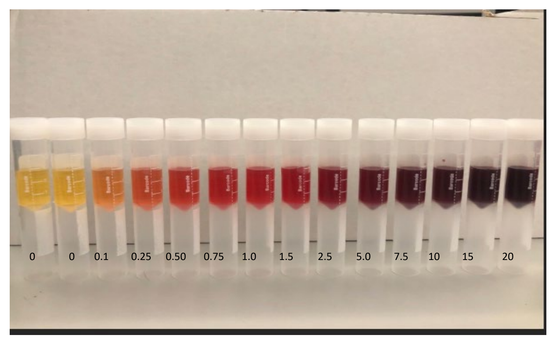


Figure 7. Color chart of plasma from various levels of hemolyzed blood. Our lab has never received samples with >1.0% hemolysis

Percent Hemolysis	Relative cfDNA (Area)	Rel Ref Chrom Density (Normalized)	Vanadis QA PASS RATE
0	1		9 of 9
0.1	1.02	1.01	3 of 3
0.25	1.06	1.00	3 of 3
0.5	1.13	1.01	3 of 3
0.75	1.05	1.02	3 of 3
1	1.11	0.99	3 of 3
1.5	1.08	1.00	3 of 3
2.5	0.83	0.99	3 of 3
5	0.92	1.01	3 of 3
7.5	0.94	1.06	3 of 3
10	0.92	1.03, 0.88	6 of 6
15	0.91	0.85	3 of 3
20	0.54	0.55	2 of 3
50	0.03	0.17	0 of 3
100	0.10	0.37	0 of 3

Table 1. Range finding experiments using synthetic blends of hemolyzed and whole blood indicated that up to 1% level hemolysis was a conservative acceptable level of hemolyisis for Vanadis cfDNA NIPT

Concordance between Normal Pregnancy Plasma with and without 1% hemolytic material				Reference Material Performance
Reference Chromosome Density	Corrected Y/Reference Chromosome Density	Euploid Calls	Fetal Sex Calls	Elevated chr_21 ratio in SeraCare T21 V2 (with 1% plasma from hemolyzed blood)
r=0.9371 (22 paired samples)	Yes, 22 of 22	Yes, 22 of 22	Yes, 22 of 22	Yes, 3 of 3 had elevated chr_21 ratio

Table 2. The presence of 1% hemolytic material did not affect the reference chromosome density, the euploidy calls and the fetal sex determination in pregnancy plasmas. The performance of SeraCare T21 reference material was also not affected by the presence of % hemolytic material