DEVELOPMENT OF A MICROSCOPIC METHOD TO DIAGNOSE HEMOGLOBIN C CONDITIONS FOR USE IN DEVELOPING COUNTRIES

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Abstract

Hemoglobin C is the second most prevalent hemoglobinopathy worldwide behind sickle cell anemia (HbS). HbC disease (HbCC) produces only mild symptoms, but is a life-threatening disorder if inherited with HbS (HbSC). HbS and HbC are most prevalent in countries underequipped to diagnose its presence through the Gold Standard of electrophoresis. This study aims to create a simple, inexpensive method to identify the presence of Hemoglobin C using limited resources. The hypothesis is that when blood is incubated in a hypertonic salt solution, Hemoglobin C will crystalize intracellularly and become visible microscopically when stained with New Methylene Blue. The method was optimized by modifying the salt type, salt concentration, incubation time, and incubation temperature. The optimized method uses a 5x Dulbecco's phosphate buffered saline at 37° C for 4 hours. Blood samples with the HbSC genotype yielded 702 HbC crystals for every 1000 RBCs counted. Future studies will include blood samples of AC, CC, SC, and AA genotypes to determine if the number of crystals that form using this method will differentiate genotypes. If so, this inexpensive, simple, and relatively rapid method can be used to identify patients with HbC and potentially determine genotype.

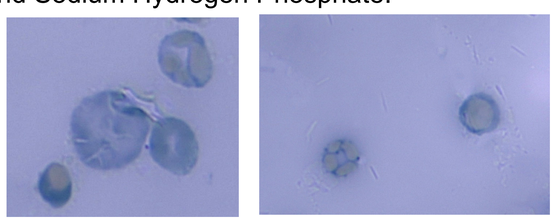
Introduction

Although homozygous Hemoglobin C (CC) is not as severe as Hemoglobin S (sickle cell disease), it causes anemia and can reduce quality of life. However, when HbC is inherited with Hemoglobin S the compound heterozygous condition (HbSC) can cause symptoms similar to that of Sickle Cell Disease.[1]The Gold Standard for diagnosing hemoglobinopathies is electrophoresis.[2] Other diagnostic methods include isoelectric focusing and gene amplification and sequencing.[2] However, many of the countries in which these diseases are present lack the resources to perform these procedures to include reliable electricity to operate sophisticated instruments, sufficient funds, and technical expertise.

Methods

Aliquots from de-identified samples of HbSC blood were washed 3 times using 0.85%. A 1:3 ratio of packed RBCs to hypertonic salts of various types and concentrations were incubated for between 2-24 hours, at temperatures between 22-40^{0}C. Following incubation, the cells were stained using New Methylene Blue. Slides were made and the number of crystals present per 1000 RBCs were counted. Salts that were tested included; Sodium Chloride, Dulbecco's Phosphate Buffer Saline, Calcium Chloride, Magnesium Chloride, Potassium Chloride, and Sodium Hydrogen Phosphate.

Figure 1: Intracellular HbC crystals.



Results

Figure 2: Crystal formation in DPBS versus NaCl

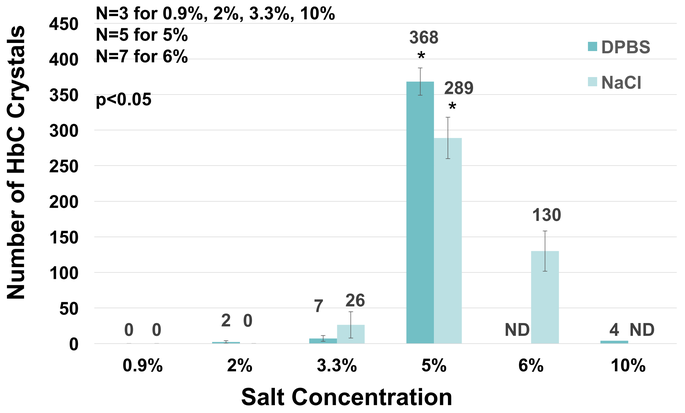


Figure 3: Crystal formation in different incubation time

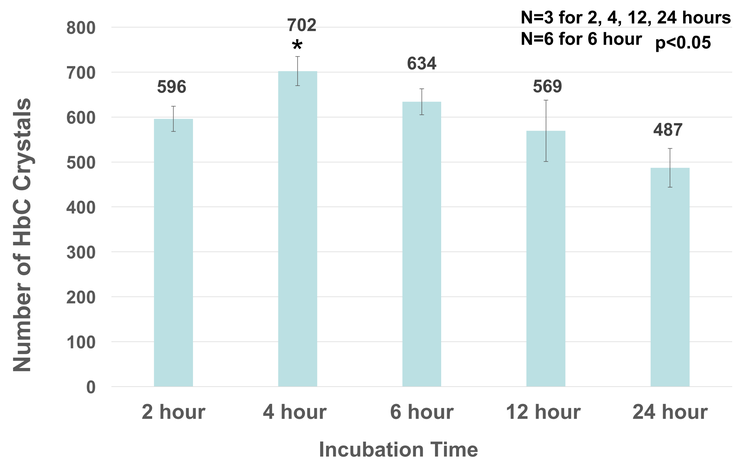


Figure 4: Crystal formation for different incubation temperature

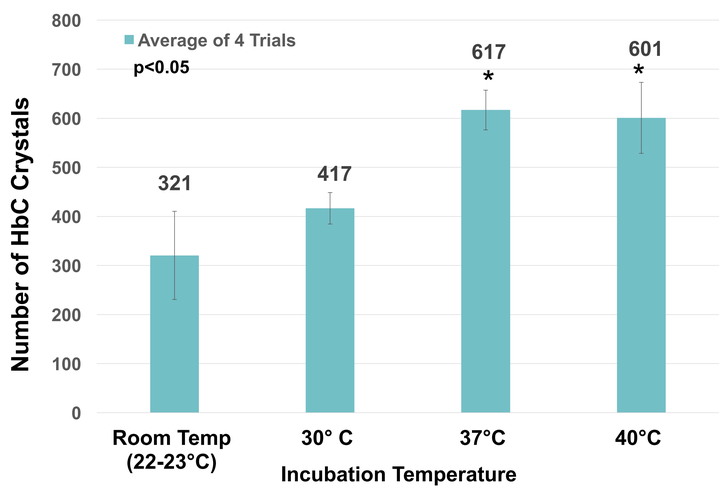


Table 1: Cost Analyses

Product	Size	Cost (for this size)	Volume or amount/Test	Cost/150 Test
DPBS) (10X concentrate)	1 L	$36.00	$0.036	$5.40
New Methylene Blue	250 mL	$37.30	$0.012	$1.80
Parafilm	125' x 4"	$28.50	$0.038	$5.70
Total Cost		$101.80	$0.086	$12.90

Discussion

HbSC blood was used because HbCC patients do not get treatment making HbCC samples difficult to obtain without IRB approval. The number of crystals increased with increasing concentration of NaCl and DPBS up to 5% with DPBC being consistently higher. One-way ANOVA was significant (<0.05) and post Hoc testing using pairwise comparisons showed that the negative control (0.9%), 2%, and 3.3% was significantly different than 5% solution. Samples above and below 5% produced fewer crystals presumably due to insufficient dehydration to induce crystallization. Optimal crystal formation in 5% DPBS was produced at 4 hour incubation time between 37^0 and 40^{0}C. These data were significant producing a p value of <0.05 using an ANOVA. Using post-hoc testing, the 4 hour incubation time was statistically different from 24 hours and 37°C and 40°C were statistically different than 22^{0}C and 30^{0}C.

Conclusion

HbC crystallization can be induced at high levels (600-700 crystals/1,000RBCs) in HbSC blood samples using a 5% DPBS incubated for 4 hours at 37^0-40^{0}C. Pilot data using AC samples produced crystals of 100/1,000 RBCs. Based on these data we speculate that the method may differentiate zygosity. Future studies are planned to test the method against 5 genotypes (AC, CC, SC, AA, and SS) to determine if the method can predict zygosity. If so, the low cost and simplicity makes it ideal for developing countries.

Acknowledgements

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References

[1] McKenzie, Shirlyn B. and Williams, J. Lynn. *Clinical Laboratory Hematology.* Pearson Education, Inc., 2010. Pgs. 221-223.

[2] Wajcman, Henri and Moradkhani, Kamran. Abnormal haemoglobins: detection & characterization. *Indian Journal of Medicine*. 2011 Oct; 134(4): 538-546.