

Development of a Microscopic Method to Diagnose Hemoglobin C Conditions for Use in Developing Countries

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BACKGROUND

- HbC vs HbA
- Hemoglobin C disease prevalence
- Symptoms
- Variations in genotypes
- Gold standard of testing
- Why this method is important

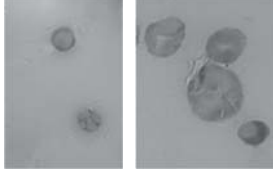
RESEARCH HYPOTHESIS

- If the optimal salt-based solution type, concentration, incubation time and temperature can be determined, then crystal formation will be maximized and the test will be cheap enough and accurate enough to be used clinically in underdeveloped countries to identify HbC and distinguish zygosity.

MATERIALS & METHODS

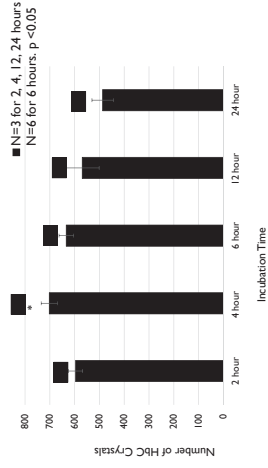
- Preparation
- Blood Samples
- Washed RBCs
- Salt type
- Salt concentrations
- RBC/salt ratio
- Incubation
- Times
- Temperatures
- Staining
- New methylene blue
- Centrifuge
- Transfer to slide
- Counting

MATERIALS & METHODS

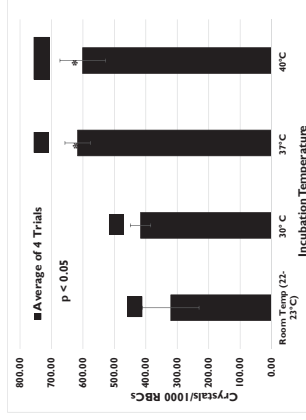


- Per 1,000 RBCs the number of RBCs with crystals are counted and compared
- More crystals equals improved optimization of test procedure

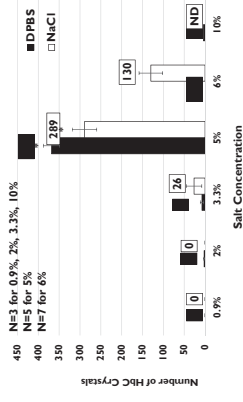
DATA ANALYSES—INCUBATION TIME USING 5% DPBS



DATA ANALYSES—INCUBATION TEMPERATURE USING 5% DPBS



DATA ANALYSES—SALT CONCENTRATION



RELIABILITY/ CREDIBILITY

- Negative Controls:
- SC samples performed in saline to control the salt
 - AA samples performed in hypertonic salt to control sample
- Positive Controls:
- HbSC samples serve as the positive control.
 - HbSC samples consistently form crystals in hypertonic salt

COST ANALYSES

Table 1: Cost Analysis

Product	Size (grams or volume of original item purchased)	Cost (for this size)	Volume or amount/Test	Cost/150 Test
Dulbecco's Phosphate Buffer Saline (10X concentrate)	1 L	\$36.00	\$0.036	\$5.40
New Methylene Blue Paraffin	250 mL 125 x 4"	\$37.30 \$28.50	\$0.012 \$0.038	\$1.80 \$5.70
Total Cost		\$101.80	\$0.086	\$72.90

SUMMARY/NEXT STEPS

- We have produced a procedure that optimizes HbC crystal formation using RBCs from patients with HbSC disease
- It is inexpensive & does not require resources underdeveloped countries do not have
- Relativity fast, simple and accurate
- Upon IRB approval, we will be testing other genotypes to use the method to determine zygosity
- We will implement it in Haiti

THANK YOU