Comparison of the Falling Drop Method to Existing Hemoglobin Methods

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What is Anemia?

- Definition
  - Reduced oxygen carrying capacity of the blood
- Causes
  - Nutritional deficiency (vitamins [B_{12}/folate], iron)
  - Increased utilization of nutrients (infections, pregnancy, growth)
  - Inherited conditions (thalassemia, hemoglobinopathies, enzyme deficiencies, membrane defects, porphyrias)
  - Autoimmune hemolytic anemias (ITP - IgG, IgM)
  - Somatic mutations
    - Aplastic Anemia, Congenital Dyserythropoietic Anemia, Paroxysmal Nocturnal Hemoglobinuria
  - Malignancies (Leukemias, metastatic malignancies)

Symptoms of Anemia

- Mild Anemia
  - Weakness, fatigue, headache, pallor
- Moderate Anemia
  - Dyspnea, tachycardia, dizziness, irritability
- Severe Anemia
  - Incapacitating fatigue
  - Cognitive deficits
  - Cardiac defects (MI, CHF)
  - Low birth weight
  - Preterm birth
  - Risk of maternal or neonatal mortality

Prevalence of Anemia Worldwide

- Anemia affects 25% of the world's population
- Underdeveloped countries are more affected
  - All deaths from Iron Deficiency Anemia worldwide
    - 1.4% in North America
    - 71% in Africa and parts of Asia
  - Link between anemia and poverty
    - Nutritional deficiencies
    - Increase in parasitic and bacterial infections
      - Bleeding and increased utilization
  - Haiti is the poorest country in the Northern and Western hemispheres
    - In 2017, Haiti was ranked the 17th poorest country in the world

How is Anemia Measured

- RBC
  - Automated CBC
  - Manual RBC count
- Hematocrit
  - Automated CBC (calculated from RBC and Hb)
  - Microhematocrit
- Hemoglobin
  - Automated CBC (spectrophotometric)
  - POC instruments

Limitations to Modern Hb Measurement in Haiti

- Cost
  - Instrument
  - Reagents
  - QC
  - Electricity
- Stable and reliable electricity
  - CBC Instruments
  - Climate control
  - Refrigeration
- Adequate training of lab staff
- Instrument maintenance and repair

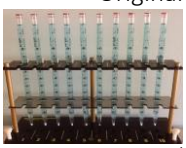
Problem

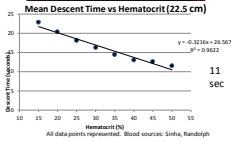
- Anemia is prevalent worldwide
- More prevalent in underdeveloped countries
- Modern methods are expensive, complex, electricity dependent
- Some Haitian labs do not have any electricity for even microhematocrit
- Sickle Confirm requires a Hb measurement to perform
- Need Hb method that is cheap, fast, easy and electricity independent
- Falling Drop Method

Falling Drop Method

- Principle
  - The rate of descent of a drop of blood down a column through a density gradient is proportional to the hemoglobin content in the drop of blood
- Variables to consider
  - Column size (length and diameter)
  - Vertical positioning of column
  - Density gradient solution (inert and dynamic)
  - Blood drop delivery system (reproducible)
  - Blood drop integrity
    - Anticoagulant used in blood collection (oxalate, citrate, EDTA, heparin)
  - Linear relationship between descent time and Hb content
  - Accuracy
  - Reproducibility
  - Reagent stability

Original Falling Drop Method





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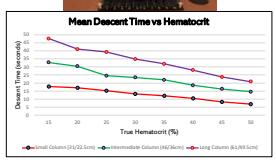

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Materials and Methods

- Samples
  - 20 blood samples from healthy, IRB consented, subjects
    - One EDTA tube for all Hb testing except the falling drop method
    - One-potassium oxalate/sodium fluoride for the falling drop method
  - Each sample was diluted 1:2 in autologous plasma for a total of 40 samples
- Falling Drop Set-up
  - Six-61cm glass columns with 10mm inner-bore diameter
  - Start/stop markings at 49.5cm apart
  - Modified, universal ESR rack to hold tubes
  - Modified pipette tips at mouth of column to stabilize blood drop delivery
- Density Solution
  - 4.58% solution of copper sulfate pentahydrate with specific gravity of 1.015

Materials and Methods

- Falling Drop Method
  - A 44uL drop of blood collected in potassium oxalate/sodium fluoride is applied to the surface of the copper sulfate in the column through a modified pipette tip to stabilize drop delivery
  - Descent time is measured between the markings (49.5cm)
  - Six trials per blood sample tested is applied to the column
  - Average of six trials is compared to the standard curve to determine Hb level
- Correlation of Falling Drop to Other Methods
  - Falling Drop method was correlated to:
    - Sysmex KX-21N, Hemocue 201+, Mission Plus Hb, and WHO Visual
    - Descriptive statistics: Mean and standard deviation
    - Inferential statistics: Correlation Coefficient (r) & Coefficient of Determination (r^2)

Hemoglobin Methods

Sysmex KX-21N

Hemocue 201+

Mission Plus

WHO Visual

Correlation of Sysmex to Other Methods

Method	Mean Hb (g/dL)	Standard Deviation (g/dL)	Sysmex vs Other Methods	Correlation Coefficient (r)	Coefficient of Determination (r^2)
Sysmex KX-21N	12.1	3.0	Sysmex KX-21N vs Sysmex KX-21N	1.0	1.0
Hemocue 201+	12.0	2.9	Sysmex KX-21N vs Hemocue 201+	0.9959	0.9918
Mission Plus	12.1	3.1	Sysmex KX-21N vs Mission Plus	0.9887	0.9775
Falling Drop	12.1	3.1	Sysmex KX-21N vs Falling Drop	0.9647	0.9306
WHO Visual	11.9	3.6	Sysmex KX-21N vs WHO Visual	0.9423	0.8879

Cost Comparison Between 5 Hb Methods

Method	Instrument	Other Start Up Costs	Start up Costs		Reagents	Total Costs	Cost/Test
Sysmex KX-21N	$21,000	$250	$21,250		$250/500	$21,150	$0.50/test
Hemocue 201+	$500	$0	$500		$45/25 tests	$545	$1.80/test
Mission Plus	$100	$0	$100		$60/50 tests	$160	$1.20/test
Falling Drop	$0	$100	$100		$10/500mL = 17 tests	$110	$0.59/test
WHO Visual	$0	$0	$0		$15/150 tests	$15	$0.10/test

Conclusions

- Sysmex is costly, requires stable electricity, maintenance and repairs
- Hemocue and Mission Plus are less expensive but require electricity or batteries
- Falling Drop and WHO Visual are power independent
- Falling Drop correlated well with Sysmex, Hemocue and Mission Plus
- WHO Visual was the least accurate and reproducible
- Falling Drop is a viable alternative to measure Hb w/o power

Thank you!!!!

Questions????