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## **Comparative Assessment of Red Blood Cell Morphology in Anaemic Children**

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### **Abstract**

Anaemia (haemoglobin level < 11 g/dl) is a health burden among preschool children and women of child bearing age and affects over 27% of the World population. Anaemia results from reduction in the functional haemoglobin or red blood cell numbers or mass leading to decreased oxygen carrying capacity characterized by clinical features such as; skin pallor, fatigue, shortness of breath, congestive heart failure, jaundice and tachycardia. Evaluating and interpreting red blood cell morphology provides key information in the differential diagnosis of Anaemia. However, the current standard Peripheral thin blood method of assessing red blood cell morphology is highly technical and time consuming. There was need to carry out assessment into the alternative Automated Complete Blood Count method to aid in the selection of the reliable assay. The objective of the study was to compare between Peripheral blood thin film and Automated Complete Blood Count morphologically classified Anaemia in children. The study was cross-sectional and employed simple random sampling technique. Blood samples were obtained from the participants, assessed for red blood cell morphology by Automated Complete Blood Count and Peripheral thin blood film. Data was analyzed using SPSS and a paired t-test used to test for the statistical significance.

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Results show no significant difference in the scores for Peripheral thin blood film (M=25.5, SD=11.82) and Automated Complete Blood Count (M=25.5, SD=12.66)  $t(3) = 0.00$ ,  $p = 1.000$ . Automated Complete Blood Count is a method of choice in assessing red blood cell morphology and evaluating Anaemia. The study recommends assessment into various Automated Complete Blood Count models available in the market to aid in the selection of most reliable one.

**Keywords:** Anaemia; Red Blood Cell Morphology; Assessment; Complete Blood Count; Peripheral Thin Blood Film.

## **1. Introduction**

Anaemia (haemoglobin level < 11 g/dl) is a global health problem affecting over 1.93 billion people (27% of the World population), and of which 89% of the affected population is in the developing countries [5]. Anaemia distresses mostly preschool children and women of child bearing age and is associated with increased morbidity and mortality [5]. Red blood cells contain haemoglobin protein whose primary role is to deliver oxygen to tissues and return carbon dioxide to the lungs for elimination from the body [1]. Reduction in the functional haemoglobin or red blood cell numbers or mass may result into Anaemia characterized by decreased oxygen carrying capacity. The resulting tissue hypoxia is responsible for the clinical signs and symptoms of Anaemia which include; skin pallor, fatigue, shortness of breath, congestive heart failure, jaundice and tachycardia [1]. Evaluation and interpretation of Red blood cell morphology provides key information in the differential diagnosis of Anaemia and systemic diseases. Red blood cell shape, size, color and inclusions are influenced by environment of the cells and their metabolic status. During erythropoiesis, red cells rely on the biochemical precursors in plasma for haemoglobin and membrane synthesis [4]. The erythrocyte membranes contain cytoskeletal proteins and phospholipids which play important roles in maintaining cell morphology [4]. In Anaemic patients, normocytic blood picture is indicative of blood loss, suppressed production of erythrocytes or haemolysis [2]. Changes in interaction of cytoskeletal proteins and distribution of phospholipids can cause alteration in the red cell membranes resulting into microcytic or macrocytic Anaemia [2]. Microcytosis is a diagnostic likelihood of Anaemia due to thalassemia, chronic disease, iron deficiency and lead poisoning [3]. Macrocytosis helps to guide on the megaloblastic and non-megaloblastic causes of Anaemia [1]. In addition, the terms red blood cell color intensity and size of the central pallor reflect amount of haemoglobin in the population of red blood cells. Erythrocytes that appear disc shaped with a central pallor occupying approximately one-third of the cell's diameter contain normal content of haemoglobin and are considered normochromic. Erythrocytes with a central pallor larger than expected are considered hypochromic. Erythrocytes that lack central pallor due to increased haemoglobin concentration are termed as hyperchromic [1]. In Uganda, the standard method of assessing red blood cell morphology is qualitative examination of cells on a well-made Peripheral thin blood smear [1, 4]. However, this qualitative method is time consuming and also requiring high technical experience. The alternative method is Complete Blood Count (CBC), which involves quantitative measurement of red blood cell indices (MCV, MCH and MCHC) [4]. Apparently, there was no study carried out to compare Anaemia assessment methods in Uganda. Therefore, objective of the study was to compare between Peripheral blood thin film and Automated CBC morphologically classified Anaemia.

## **2. Materials and Methods**

### **2.1 Study design and population set up**

Across-sectional study was designed to compare between Peripheral thin blood film and Automated CBC morphologically classified Anaemia in children  $\leq 12$  years who were attending outpatient department Virika hospital, Kabarole district. From May to July 2012, a total of 102 Anaemic children were enrolled into the study

### **2.2 Methods and laboratory analyses**

**CBC;** Venous blood was aseptically drawn from antecubital or great saphenous vein into an EDTA tube (Sarstedt AG & Co, Numbrecht, Germany) from the participant. The tube was gently inverted 8-10 times to enable thorough mixing. A complete blood count (CBC) was performed using the Automated Hemacount Haematology analyser. The red cells were classified as Microcytic-Hypochromic, Macrocytic-Hypochromic, Macrocytic-Normochromic, and Normocytic-Normochromic based on MCV, MCH and MCHC.

**Peripheral thin film;** A thin smear was made from a drop of well mixed venous blood [1]. Following air-drying, the smear was stained by Field technique [2] and examined by Olympus microscope. The red cells were classified as Microcytic-Hypochromic, Macrocytic-Hypochromic, Macrocytic-Normochromic, and Normocytic-Normochromic based on their morphology (size and color).

### **2.3 Ethical consideration**

Written ethical approval was obtained from the Faculty of Research and Ethics Committee (FREC) Department of Mbarara University of Science and Technology. Permission was also sought from Virika hospital administration who allowed the study to take place. Informed consent was sought from all the subjects who participated in the study. All information obtained from the study was kept confidential

### **2.4 Data analysis**

Data was analyzed using SPSS and a paired t-test was used to test for the statistical significance.

## **3. Results**

Out of 102 children, 64 (62.7%) were females while 38 (37.3%) were males. A paired-samples t-test was conducted to compare red blood morphology amongst Anaemic children in Peripheral thin blood film and Automated CBC. There was no significant difference in the scores for Peripheral thin blood film ( $M=25.5$ ,  $SD=11.82$ ) and Automated CBC ( $M=25.5$ ,  $SD=12.66$ )  $t(3) = 0.00$ ,  $p = 1.000$ . Results indicated no significant difference in the scores for Peripheral thin blood film and Automated CBC.

## **4. Discussion**

The cross-sectional study was conducted at Virika hospital, where by 102 Anaemic children were recruited.

Venous blood samples were collected and analyzed by Peripheral thin blood film and Automated CBC. The results suggest that Peripheral thin blood film and Automated CBC produce similar Anaemia findings, an outcome that makes them equally good methods. Assessment of red blood morphologies is a key tool in clinical management and follow up of Anaemic patients [2]. In spite of Peripheral thin blood film being a good method, it is highly technical and time consuming compared to Automated CBC. This makes Automated CBC a method of choice in assessing red blood cell morphology and evaluating Anaemia. However, it is also important to note that different Automated CBC models produce varying results. The study therefore recommends assessment into the available CBC models in the market to aid in the selection of the most reliable one.

## **5. Conclusion**

Automated CBC is a method of choice in assessing red blood cell morphology and evaluating Anaemia.

## **6. Recommendations**

There are a number of Automated CBC models in the market of unevaluated performance. This study recommends assessment into the available systems to aid in the selection of the most reliable model.

## **7. Conflict of interest**

Authors declare no conflict of interest

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## **References**

- [1]. Cheesbrough, M. *District Laboratory Practice in Tropical Countries*, 2<sup>nd</sup> edition. United States of America: Cambridge University press, 2006, pp. 274-286.
- [2]. Jason Ford. "Red Blood Cell Morphology". *International Journal of Laboratory Hematology*. Vol. 35, Mar. 2013. Available: <https://doi.org/10.1111/ijlh.12082>
- [3]. Lockitch G, Berry B, Roland E, Wadsworth L, Kaikov Y, Mirhady F. "Seizures in a 10- week- old infant: lead poisoning from an unexpected source". *Can Med Assoc J*; 1991, **145**: 1465– 8.
- [4]. Mary M. Christopher. "Interpretation of red blood cell morphology (Advanced level)". *World small veterinary association World congress proceedings*. University of California: Davis CA, 2004
- [5]. Kassebaum NJ; GBD 2013 Anaemia Collaborators. "The Global burden of Anaemia". *Hematol Oncol Clin North Am*. 2016 Apr; 30 (2):247-308. doi: 10.1016/j.hoc.2015.11.002. PMID: 27040955
- [6]. Warhurst D. C, Williams J. E." *Laboratory diagnosis of Malaria, PHLS Malaria Reference Laboratory*". London School of Hygiene and Tropical Medicine. *J clini Pathol* 1996; 49:533-538