

## Validation Study of Inter-Laboratory Haematology Results in Enugu, Nigeria.

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### Abstract

**Background:** A reliable and reproducible Medical Laboratory (Haematology) result needs internal and external quality control within the Medical Laboratory facilities and participation in external proficiency testing program such as this validation checks of inter-laboratory haematology results in Enugu – Nigeria.

**Objectives:** This study was done to assess haematology laboratory performance and uniformity of results through comparison of manual and automated methods by participating laboratories in reporting test samples sent for complete blood counts and CD4–T-cell counts.

**Materials and Method:** A total number of 10 subjects (males and females) were selected for this research study. Blood samples were collected from each of these subjects and were aliquoted each and distributed to 11 laboratories for analysis. The result generated from each laboratory was subjected to robust statistical analysis.

**Results and Discussions:** The result revealed a significant statistical difference ( $P < 0.05$ ) in M1 cells (Neutrophils, Lymphocytes, eosinophils, Basophils and monocyte) when the mean and standard deviation of automated methods of NONAH and UNTH were compared. There were no significant difference ( $p > 0.05$ ) observed in white blood cell count, lymphocytes count, mean cell haemoglobin concentration, total white blood cell count, Granulocytes, Haemoglobin, Haematocrit, mean cell volume, platelets count, Red blood cell count, and CD4 – cell counts between the automated techniques. There was a significant difference ( $p < 0.05$ ) in the mean lymphocyte counts, Haemoglobin concentration and mean cell haemoglobin concentrations when manual and automated methods were compared. These inverse correlations existing in the correlation of different results gotten from different laboratory facilities and different methods of haematological analysis may pose great diagnostic challenges in the clinical management / treatment of various disease conditions such as iron deficiency anaemia, etc.

**Conclusion:** Report showed that inter-laboratory differences exist in Haematology.

**Keywords :** Automated, Haematology, Lymphocytes, Haemoglobin.

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### I. Introduction

The need for accuracy and precision of laboratory result is vital in diagnosis and treatment of various disease conditions. A reliable and reproducible report from a laboratory needs internal and external quality control within the laboratory and participation in external proficiency testing programs[1]. The importance of inter-medical laboratory results, as they are the keys to the achievement of acceptable levels of accuracy and precision. Lewis and Burges had in their work “Quality Control in Haematology Inter- medical laboratory reports in Britain,” suggested that periodic inter- medical laboratory trials, at a national level, in conjunction with regular individual intra- medical laboratory quality control procedures, are necessary in order to achieve acceptable levels of accuracy and precision[2]. The World Health Organization (WHO) has defined quality assurance as the total process whereby the quality of laboratory assurance can be guaranteed. It has been summarized as the: Right Results, at the Right Time, on the Right Specimen, from the Right Patient, with results interpreted based on correct reference data, and at the Right Price[3]. Quality assurance according to WHO comprises internal quality control and external quality control. This research paper is emphatic on external quality control in Haematology. Quality assurance must be an integral part of laboratory philosophy and practice as only then can we begin to use our scarce resources of trained personnel and expensive materials to the best advantage[4]. The term quality control covers that part of quality assurance, which primarily concerns the control of errors in the performance of tests and verification of test result quality control must be practical, achievable and affordable. Quality control can be applied internally and also as part of external quality assurance. Internal Quality Control involves a set of procedures undertaken by the staff (within a laboratory) for continuously and concurrently assessing the laboratory work so that results produced meet the expectations of the customers, while External Quality Assessment is a system of objectively assessing the laboratory

performance by an outside agency. This assessment is retrospective and periodic but is aimed at improving the internal quality control. Quality Control involves the process of monitoring the characteristics of the testing system. This is done by studying control samples along with patients' samples and analyzing the results with appropriate statistical methods to establish accuracy and precision, which are benchmarks for determining conformance and hence acceptability of results [5]. It also involves taking any necessary corrective actions to bring the results into conformance [5]. Quality assurance denotes a system for continuously improving reliability, efficiency and utilization of products and services [6]. Quality laboratory result is influenced not only by factors within the laboratory but also by factors outside the laboratory such as specimen collection, transportation, time and etc. Furthermore, reports by [1] and [7] show that inter-laboratory differences exist in Haematology. Results of all scientific observation are subject to variance and this also includes observations in Haematology laboratory [6].

## **II. Materials And Methods**

### **Ethical Clearance**

Approval for this research work was obtained from the Ethics Committee of the college of Health Sciences and Technology of University of Nigeria Enugu Campus, Enugu, Nigeria.

### **Study Area (Site)**

A total number of eleven (11) Medical Laboratories were selected within Enugu metropolis and Ituku – Ozalla. These laboratories were selected based on three criteria viz:

- (a) Strategic location; (North, South, East and Western Enugu, Nigeria).
- (b) Ownership (Privately owned; mission or faith-based and government owned)
- (c) Class (Secondary and Tertiary health institution laboratories)

Based on the above factors, the following facilities were selected:

- 1- University of Nigeria Teaching Hospital Laboratory, Ituku Ozalla – (Tertiary Hospital Laboratory)
- 2- National Orthopedic Hospital Laboratory, Enugu (Tertiary Health Laboratory)
- 3- 82 – Division Military Hospital Laboratory, Enugu (Secondary Health Facility)
- 4- Enugu State University Teaching Hospital Laboratory (Tertiary Health Facility)
- 5- Annunciation Catholic Specialist Hospital Laboratory, Emene (Secondary/Faith-Based)
- 6- Ntasi Obi Specialist Hospital Laboratory, Trans-Ekulu (Secondary/Faith-Based Facility)
- 7- Mother of Christ Catholic Specialist Hospital Laboratory, Ogbete, Enugu (Secondary/Faith-Based Facility)
- 8- Eastern Nigerian Medical Center, Uwani (Private Health Institution)
- 9- McChuks Laboratory, Agbani Road (Private facility)
- 10- Zenith Laboratory, Abakpa Nike (Private facility)
- 11- Benedictus Laboratory, New Haven, Enugu (Private facility).

### **Sample collections**

11.0mls of blood samples were collected from each of the 10 subjects (males and females) selected for this research work into EDTA bottles, of which five (5) out of the ten (10) subjects were patients attending Ntasi Obi Ndi N'afufu Specialist Hospital, Enugu, and the other five (5) subjects were healthy staff of Ntasi Obi Ndi N'afufu Specialist Hospital, Enugu.

Each of these blood samples were divided into 11 aliquots and distributed to the 11 selected Laboratories on the same day for analysis, taking special measure to maintain sample integrity. A total number of 110 samples were analyzed.

## **III. Methods**

Samples were prepared according to international standard organization (ISO) guidelines. The performance of individual laboratory was assessed using robust  $Z$  – score and specimens correlation curves which is an indicator of the acceptability of the test results.

Two automated systems – Sysmex Haematology Analyzer (KX-21N) and Abarcus Junior Haematology Analyzer were used by the University of Nigeria Teaching Hospital Laboratory, Ituku Ozalla and Ntasi Obi Specialist Laboratory, (NONAH) respectively for full blood count. The CD4-cell count was done using Partek Cyflow machines by both UNTH and NONAH laboratories. Annunciation Specialist Hospital used Hemocue machine for haemoglobin estimation while other facilities used manual haemoglobin cyanide method. All the facilities used microhaematocrit method for haematocrit estimation. Platelet count was done using 1% ammonium oxalate.

#### **Automated Full Blood Count Method.**

The full blood count was carried out following the standard operating procedures as given by the manufacturers of Sysmex, KX-21N and Abarcus Junior respectively.

#### **CD4 – Cell Automated Count Method**

After proper mixing, 20µl of samples were added to 20µl of the standard antibody in a precipitin tube, Mixed and incubated for 15minutes in the dark. The reactions were stopped after the count check beads have been estimated according to standard. The result was given in cells/µl.

#### **Microhaematocrit Method**

Plain capillary tubes 75mm in length and internal diameters of about 1mm were used for the anticoagulated blood samples. The blood was allowed to enter the tubes by capillarity, leaving at least 15mm unfilled. The tubes were then sealed by heating the dry end of the tube rapidly in a pilot light of a Bunsen burner, combined with rotation. They were centrifuged at 3,000 rpm (revolution per minute) for 5 minutes. The haematocrit value was measured using a reading device accompanied by a magnifying glass. A tachometer was used to measure the speed of centrifugation[8].

#### **Cyanmethaemoglobin Method**

20µl of blood sample was added to 4ml of diluent (Drabkin's Station). The tube containing the solution was stopped with a rubber bung and inverted several times. It was then allowed to stand at room temperature for a sufficient period of time to ensure the completion of the reaction. Meanwhile, a standard curve was prepared because of the number of samples to be analyzed. This was prepared using a secondary reference solution. The optical density of the solution was measured against a blank of cyanideferricyanide reagent, in the same standard solution diluted with the reagent 1 in 2, 1 in 3 up to 1 in 5. the optical density of the individual dilutions was plotted against the concentration of the solution. The line of best fit was drawn which passed through the origin ensuring linear calibration of the spectrophotometer. The readings of subsequent tests were read off from the graph [9].

#### **Total White Blood Cell Count (WBC) or Leucocyte Count**

A 1 in 20 dilution of blood was made by adding 20µl of blood to 0.38ml of diluting fluid (Turk's solution) in a 75x10mm glass or plastic tube.

After tightly corking the tube, the suspension was mixed by rotating in a cell - suspension mixer for at least 1 minute. The Neubauer counting chamber was filled with the suspension by means of a Pasteur pipette. The red cells were lysed by the diluting fluid but the leucocytes remain intact, their nuclei stained deep violet - black. Using x10 objective lens or x40 objective lens to view, white blood cells were counted and recorded.

#### **Calculation:**

$$\text{Count (per litre)} = \frac{\text{No. of cells counted}}{\text{Volume Counted}} \times \text{Dilution} \times 10^6$$

#### **Manual Differential White Cell Count**

All the Medical Laboratory centers used the standard Leishman staining technique to stain their well-made thin blood films. Differential leucocyte count cells were counted using x100 oil immersion lens, in a strip running the whole length of the film. Using a cell counter, a total of 100 different leucocytes were counted and reported per percentage of each type of cell.

#### **Platelet Count**

Manual counts are still necessary if there are a significant proportion of giant platelets[8]. All participating facilities performed manual platelet counts by microscopic examination of diluted whole blood. The diluent consist of 1% ammonium oxalate in which the red cell lysed.

A 1 in 20 dilution of the blood in the diluent adding 0.1ml of blood to 1.9ml of the diluent, the suspension was mixed on a mechanical mixer for 10 – 15 minutes.

The Neubauer counting chamber was filled with the suspension using a Pasteur pipette. The counting chamber was placed in a moist Petri dish and left untouched for at least 20 minutes to give time for the platelets to settle.

The preparation was examined with x10 objective and x40 objective lens.

The small highly refractile particles of platelets numbers were counted in one or more areas of 1mm<sup>2</sup> and calculated thus:

**Calculation:**

$$\text{Count (per litre)} = \frac{\text{No. of cells counted}}{\text{Volume Counted (ml)}} \times \text{Dilution} \times 10^6$$

**IV. Statistical Analysis**

The data for all parameters from all facilities were expressed in their common comparable units. The mean, standard deviation, Z score, ANOVA and P-values (Level of significance) were calculated.

**V. Results**

**Table 4.1:** Mean (D), Standard Deviation (Sd), Z-Score And P – Value (Level Of Significance Of Haematological Parameters Of Automated Methods (Unth And Ntasi Obi Specialist Hospital Labs. Nonah)

Comparable Parameters	NONAH Mean (X) and SD	UNTH Mean (X) and SD	Z – Score and P – value	Level of Significance
WBC	6.3±2.4	6.30±2.4	Z <sub>1</sub> = 0.02 P <sub>v</sub> = 0.93	P > 0.05
Lym	32.0±14.2	35.7±17.1	Z <sub>1</sub> = 0.71 P <sub>v</sub> = 0.52	P > 0.05
GR	15.1±5.5	51.9±14.4	Z <sub>1</sub> = 0.16 P <sub>v</sub> = 0.87	P > 0.05
MI	15.1±5.5	9.5±6.1	Z <sub>1</sub> = 2.17 P <sub>v</sub> = 0.04	P < 0.05
HB	12.6±2.5	12.7±6.1	Z <sub>1</sub> = 0.04 P <sub>v</sub> = 0.97	P > 0.05
HCT/PCV	36.5±6.4	37.4±6.4	Z <sub>1</sub> = 0.32 P <sub>v</sub> = 0.74	P > 0.05
MCV	75.8±6.8	78.9±10.2	Z <sub>1</sub> = 0.81 P <sub>v</sub> = 0.43	P > 0.05
MCHC	34.4±1.5	33.8±12.2	Z <sub>v</sub> = 0.66 P <sub>v</sub> = 0.91	P > 0.05
PTL	218.8±116.0	224.1±114.4	Z <sub>v</sub> = 0.10 P <sub>v</sub> = 0.91	P > 0.05
RBC	4.85±116.9	4.80±0.99	Z <sub>v</sub> = 0.11 P <sub>v</sub> = 0.90	P > 0.05
CD4	561.8±398.4	677.7±407.7	Z <sub>v</sub> = 0.64 P <sub>v</sub> = 0.52	P > 0.05

**Table 4.2:** Mean (D), Standard Deviation (Sd), Z-Score And P-Value Of Automated And Manual Techniques Of Comparable Haematological Parameters Compared

Comparable Parameters	Automated Techniques	Manual	Z – Score and P – value	Level of Significance
White Blood Cells	6.3±2.3	5.4±1.9	Z <sub>v</sub> = 1.76 P <sub>v</sub> = 0.08	P <sub>v</sub> > 0.05
Lymphocytes	34.3±15.4	42.8±13.3	Z <sub>v</sub> = 2.4 P <sub>v</sub> = 0.01	P <sub>v</sub> < 0.05
Haemoglobin	12.7±2.4	12.2±2.0	Z <sub>v</sub> = 0.9 P <sub>v</sub> = 0.01	P <sub>v</sub> < 0.05
Packed Cell Volume/Haematocrit	36.9±6.2	36.7±6.1	Z <sub>v</sub> = 0.15, P <sub>v</sub> = 0.9	P <sub>v</sub> > 0.05
Platelets	221.4±112	225.8±131	Z <sub>v</sub> = 0.11, P <sub>v</sub> = 0.9	P <sub>v</sub> > 0.05
Mean Cell Haemoglobin Concentration	34.1±1.9	33.2±0.7	Z <sub>v</sub> = 3.14, P <sub>v</sub> = 0.002	P <sub>v</sub> < 0.05

**Table 4.3** Mean (D), Standard Deviation (Sd) Anova (F) And P.Value For Each Manual Technique Compared For All Laboratories.

	NONAH	ANC	BEN	EAST	HANSA	MOC H	NAT	ZENITH	ANOVA	Level of Significa nce
WBC	6.1±2.9	4.3±1.8	5.9±1.3	4.7±1.6	5.1±1.4	6.4±2.1	5.7±1.7	5.1±1.7	F = 1.8 Pv = 0.12	Pv > 0.05
LYM	43.6±8.1	43.6±18.9	38.1±10.2	37.9±8.0	50.6±9.2	41.8±9.4	49.1±2.0	39.6±15.0	F = 1.25 Pv = 0.28	Pv > 0.05
NEUT	50.5±8.1	53.7±18.6	50.0±14.8	60.3±7.1	48.1±9.6	5.3±7.3	50.0±1.9	52.9±16.3	F = 0.81 Pv = 0.55	Pv > 0.05
EOS	2.7±2.1	1.9±0.2	2.3±1.0	0.9±0.1	0.5±0.5	1.1±0.1	0.9±0.1	2.3±0.2	F = 2.3 Pv = 0.06	Pv>0.05
BASO	0.9±0.5	1±0.3	0.0±0.0	0±0.0	0.0±0.0	0.4±0.0	0.0±0.0	0.3±0.04	F = 2.22 Pv = 0.5	Pv > 0.05
PLT	236.6±133.8	215±135	--	--	--	--	--	--	--	--
HB	12.5±1.8	12.6±2.1	10.8±1.1	12.3±2.3	12.3±2.3	12.9±2.3	12.1±2.1	12.9±1.9	F = 1.56 Pv = 0.16	Pv > 0.05
PCV/ HCT	37.6±57	38.2±6.3	32.5±3.2	34.2±5.6	37.3±7.0	38.9±6.9	36.6±6.4	38.5±6.3	F = 1.36 Pv = 0.24	Pv > 0.05
MON O	2.4±1.8	0.9±0.1	2.0±0.0	0.6±0.08	0.6±0.5	0.8±0.2	0.0±0.0	4.1±1.2	f = 12.4 Pv = 0.000	Pv < 0.05

## VI. Discussion

This study was designed to generate a validation check and to carry out a pilot inter-laboratory quality control to assess the accuracy and precision results generated in haematology laboratories in Enugu, Nigeria. From this research study, a significant statistical difference ( $P < 0.05$ ) was observed in M1 cells (mixed cells) (Neutrophils, Lymphocytes, eosinophils, Basophils and monocyte) (Table 4.1) when the mean and standard deviation of automated methods of NONAH and UNTH were compared. This could be due to the incomplete differentiation and counting of leucocytes on the one hand as other evaluations hitherto demonstrated the mobility of XE-2100 (Sysmex analyzer) to give a differential count on certain samples, such as patients with either very high white blood cell counts or lipaemic samples such as those seen in liver patients or on Total Parenteral Nutrition (TPN), gave a total but not differential white blood cell count [11]. There were no significant difference ( $p > 0.05$ ) observed in white blood cell count, lymphocytes count, mean cell haemoglobin concentration, total white blood cell count, Granulocytes, Haemoglobin, Haematocrit, mean cell volume, platelets count, Red blood cell count, and CD4 – cell counts between the automated techniques. This is in agreement with works of Cenci and Maconi, (2005) which state that the use of the sysmex XE – 2100 analyzer could be used as a fast, cost effective, reliable indicator of sepsis in a paediatric setting etc. [12]. In table 4.2, there was a significant difference ( $p < 0.05$ ) in the mean lymphocyte counts, Haemoglobin concentration and mean cell haemoglobin concentrations when manual and automated methods were compared. While the total white blood cell count, packed cell volume/Haematocrit and platelet count showed no significant difference ( $p > 0.05$ ) between automated and manual methods comparison. The differences in Mean Cell Haemoglobin Concentration when manual and automated methods were used could be due to the passage of 2 or more cells at a time through the orifice of the automated machine, (coincidence cell passage) which the machine might sense as a single cell of approximately the sum of their individual values; or due to intracellular haemoglobin concentration which also affects the size of the voltage pulse. High haemoglobin concentrations result in overestimates of cell size; or it could be due to red cells with abnormal shapes which produce voltage pulses which are not proportional to cell volume, e.g. spherical red cells (spherocytes) produce voltage pulses approximately 1.5 times greater than expected [13]. In table 4.3, it was observed that only monocyte counts showed significant difference ( $p < 0.05$ ) when the mean of all manual techniques were compared across all facilities. This difference could be due to technical errors or poor skills.

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