

## **The Effect of Storage on Eosinophil Count and Evaluation of Two Major Methods on Eosinophil Count**

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**Abstract:** *The effect of storage on eosinophil count and evaluation of two major methods of counting eosinophil was carried out in Owerri, Imo State. A total of one hundred (100) subjects were used and they were divided as follows. Adult male patients, adult female patients, sick children and healthy adults as control. The blood samples were analysed for eosinophils on the first day of collection, the second and third day after collection by total (direct) and differential (indirect) methods. The results obtained shows that total eosinophil count in a haemocytometer using a special diluting fluid gave a higher result than the differential eosinophil count using stained blood film; that eosinophils degenerated on storage, that the percentage loss was higher in patients than in healthy subjects and that percentage loss was higher in adult female patients than in adult male patients and sick children. In spite of the loss of cells due to storage, reliable eosinophil counts may still be obtained in blood stored overnight at 4°C but probably not after 48 hours of storage. This finding may be of value to staff in very busy medical laboratories where overnight storage of samples may sometimes be inevitable.*

**Keywords:** *Eosinophil count, haemocytometer, healthy subjects and Imo State.*

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### **I. Introduction**

In clinical examination, importance is often attached to abnormally high or abnormally low value of the number of eosinophils circulating in the blood. This applies especially to changes in the numbers of eosinophils in the course of certain diseases like parasitic infection and allergic reactions, or during the administration of drugs.

In our locality, eosinophila is not uncommon feature even in healthy subjects. A recent study (Ukaejiofo, et al, 1979) showed the 95% confidence interval for eosinophil by the differential count in the healthy adult Nigerian male of higher socio-economic class to be 11±1%. This represents a figure much higher than the frequently quoted Caucasian normal values. The reason for this is probably because of the endemicity of parasitic infection in our environment due to fifth and dense vegetation. So in our hospitals, eosinophil count is frequently requested to monitor response to allergic conditions and in patients under treatment for parasitic infections. We have therefore decided to study further the viability of eosinophils in stored blood taken into sequestrene as an anticoagulant.

Several factors have been shown to affect the normal values of blood eosinophil count. These are diurnal variation, age sex and geographical distribution. There is normally considerable diurnal variation in the eosinophil count, and differences amounting to as much as 100% had been recorded. The lowest count are found in the morning (8am -12pm) and the highest is at night (midnight – 4am). (Dacie, 1965). This finding was investigated by (Unrbrand, 1957), who also confirmed that the number of eosinophils fall from morning to noon, rises in the course of the afternoon, and night, reaching a maximum at about midnight to 4am, and then falls to the original value.

The reasons for this variation, apart from the stress are as follows.

1. It was found that the excretion of adrenocortical steroids in the urine shows regular 24 hours variation, it being minimal during sleep and maximal in the morning, (Pincus, 1943). The course of the variation therefore supports the view that the number of eosinophils, not only during stress but also under normal condition, is regulated wholly or in part by adrenocortical steroids.

2. The variation might be related to light, (Archer, 1963) however, this was not at all clear. (Counningham, 1975) observed age and sex difference in children. According to him children under 10 years of age had substantially higher average value than those previously reported for adults. Peak values for boys occurred at 8-10 years, (p<0.01) and for girls, at 6-8 years (p<0.05). On the average, boys had higher eosinophil

count than girls ( $p < 0.05$ ). the values being given as  $0.180 \times 10^9/L$  respective. Some authors had gone further to compare the methods for eosinophil count. According to (Discombe, 1946), it is quicker and more accurate to determine the eosinophil count of blood in a haemocytometer using a special diluting fluid, than to do a differential count using stained films.

(Randolph, and Stanton, 1945) had earlier claimed that the differential count method has a large margin of error and therefore not suitable for the enumeration of eosinophils in the peripheral blood.

Several other authors (Rud, 1947, Spier and Meyer, 1949) also confirmed that the direct count method is preferable because the indirect procedure involves a greater variation in cell counts and is impractical especially when the eosinophil counts are low. However, clumping is often observed in the direct count method if blood is exposed to air for a considerable length of time (Spier, 1952). On the other hand, (Ukajiofo Ayeni & Esan, 1978) recommend the differential (indirect) method, provided the film was made by an experienced hand, and more than one hundred cells surveyed. Most authors have failed to investigate the rate of degeneration of eosinophils in stored blood. Storage above twenty four hours at non temperature may equally affect both the morphology and eosinophil numbers.

### **Justification FOR STUDY**

Considering the increase demand for eosinophil count in the laboratories, this study aims at finding out whether a reliable eosinophil count could be obtained from blood after overnight storage at  $4^{\circ}C$ . Evaluating critically the two major methods for eosinophil counts adopted in the laboratory.

The knowledge obtained from the study will be extremely useful in busy haematology laboratories where an investigator might be compelled by circumstances to store blood on sequestrene overnight to perform counts the next day.

### **Aims And Objectives**

1. To determine eosinophil count using two major Methods.
2. To determine eosinophil count in an overnight Blood specimen.
3. Compare the overnight value with less than 24 Hours values of eosinophil numbers
4. Compare the values obtained from the two different methods of eosinophil count.

## **II. Materials And Methods**

In selecting a method for any quantitative determination, it is usually possible to find one which is, in theory inherently less inaccurate than others, though difficulties of manipulation, speed and accuracy may render it impracticable. Efforts were made to see that both the technical skill and instruments used are reliable.

Thus the method and instruments used, samples studied and technical skill employed were given special consideration with adequate attention paid to the minutest detail. In this chapter therefore, a total of one hundred (100) samples were used.

### **Study Area**

Four groups of subjects were investigated.

- a) Thirty adult male patients in the wards and clinics of university of Nigeria Teaching Hospital, Enugu, whose venous blood samples were sent to the Laboratory for routine blood counts in bottles containing sequestrene as anti-coagulant. Investigations were performed on the day of collection. There was no standardization as regards time for taking the blood.
- b) Thirty adult female's patients also in the wards and clinics of the same hospital as indicated above.
- c) Fifteen patients (children) in the wards and clinics of the same hospital as indicated in (a) above.
- d) Twenty five healthy adult males and females comprising of male blood donors, some female staff of the teaching hospital and some female students of medical laboratory sciences of University of Nigeria, Enugu Campus.

### **Methods**

#### **Total (Direct) Counts**

A 1 in 20 dilution of the blood was made after mixing the blood on a mathurn blood mixer (Mathurn Ltd, UK) for at least five minutes; by adding  $20\mu L$  of the blood to 0.38ml of the diluting fluid. The suspension was mixed for not longer than 30 seconds, and then the chamber was filled using a pasture pipette. The eosinophil was counted after allowing 10 minutes for the cells to settle in a moist atmosphere.

Two sides of the chamber containing sixteen ruled squares each were surveyed for each blood sample, and the average taken. The value was then multiplied by 6.25 per  $\mu L$  as shown above.

**Differential (Indirect) Count**

Thin dry films were prepared at same time as the dilutions were being made by flooding the slide with leishman’s stain. After fixing the film for 2 minutes, the stain was then diluted with double its volume with buffered distilled water, and allowed to stain for 10 minutes. The slide was washed with buffer cleaned and kept vertically to dry.

Two hundred (200) leucocytes were surveyed and the percentage of eosinophils were recorded.

**Sample Collection And Method**

All the blood samples were preserved in a refrigerator at 4<sup>o</sup>c±2<sup>o</sup>c. Counts were repeated on each sample after 24 and 48 hours to assess the viability of the eosinophils in stored blood taken into sequestrene.

**Statistical Analysis**

The means and standard deviations (SD) of eosinophils counted by the two methods were calculated. Preliminary examination of the data showed marked nonnormality which indicated a necessity for transforming the data by taking common logarithm of individual count. This was observed to correct the skewness quite considerably and therefore the main analysis was done using the common logarithm transformations.

**III. Results**

The mean values of eosinophil counts on the first day of collection of samples by the two methods (Direct and Indirect) employed are shown in table 1 for the four groups of subjects. In all the groups examined, the mean values of eosinophils counted by the differential (Indirect) count method were lower than the means obtained by the direct (total) count method. These differences are statistically significant at the 95 percent confidence limit (p<0.95).

**Table 4.1:** Comparison of the total direct count method with differential (Indirect) count method on the first day of collection of sample counts transformed to common logarithm units in four groups of subjects.

Sample group	total count		differential			Paired T. value	
	X	SD	N	X	SD		
Adult male patients	2.57	55	30	1.17	29	30	2.12
Adult female patient	2.54	55	30	1.17	27	30	2.21
Children	2.63	29	15	1.22	20	15	3.45
Healthy adult	2.61	21	25	1.19	13	25	4.09

- X = mean log count
- SD = standard Deviation of log count
- N = Number of specimen
- X = (p<0.95)

Sample of the four groups of subjects were examined after 24 hours storage by total differential count methods. The mean values obtained are shown in table 2. These results further showed general decrease in the mean value of eosinophils counted by the differential method. These differences are also statistically significant at 95 percent confidence limit. (p<0.95).

**Table 4.2:** Comparison of the total (direct) count method with differential (indirect) count method after 24 hours of collection of samples (counts transformed to common log. Units)

Sample Group	TOTAL			DIFFERENTIAL			PAIRED T VALUE			
	X	SD	N	X	SD	N				
Adult male patient	2.50	0.54	30				1.05	27	30	2.27
Adult female patients	2.38	0.51	30				99	26	30	2.29
Children (patients)	2.56	0.31	15				1.06	26	15	3.20
Healthy adults	2.56	0.22	25				1.07	14	25	5.27

- X = mean log count
- SD = standard Deviation of log count
- N = Number of specimen
- X = (p<0.95)

**TABLE 4.3:** shows the mean values of eosinophils obtained from same samples of the four groups of subjects examined after storing the blood for 48 hours at 48<sup>o</sup>c. It further observed that the mean values obtained by the differential count method were still lower than those obtained by the total count method. These differences are also statistically significant at 95 percent confidence limit (p<0.95).

**Table 4.3** comparison of total count method with differential count method after 48 hours of collection of samples (counts transformed into common log. Units).

Sample Group	TOTAL			DIFFERENTIAL			T VALUE			
	X	SD	N	X	SD	N	PAIRED			
Adult male patient	2.40	.52	30				0.89	.26	30	2.45
Adult female patients	2.23	.49	30				.78	.25	30	2.49
Children (patients)	2.47	.32	15				.89	.22	15	3.51
Healthy adults	.22	0.22	25				.92	.18	25	5.16

X = mean log count  
 SD = standard Deviation of log count  
 N = Number of specimen  
 X = (p<0.95)

Table 4 shows the mean values obtained after eosinophils were counted immediately after collection, and then after 24 hours of collection from the four groups of subjects examined by the direct (total) count method alone. It was observed that the mean values obtained after 24 hours of collection of samples were lower in all the groups of subjects examined. However, when these differences were tested statistically, none of them showed any significant difference of 95 percent confidence limit (p > 0.95).

**Table 4.4:** Comparison of immediate count with 24 hours count by the total direct count method. (counts transformed to common log. Units)

Sample Group	TOTAL			DIFFERENTIAL			T VALUE				
	X	SD	N	X	SD	N	%loss	PAIRED			
Adult male patient	2.57	.55	30				2.50	.54	30	13.6	.086
Adult female patients	2.54	.55	30				2.38	.51	30	32.7	.201
Children (patients)	2.63	.29	15				2.56	.31	15	13.6	.142
Healthy adults	2.61	.21	25				2.56	.22	25	11.2	.148

X = mean log count  
 SD = standard Deviation of log count  
 N = Number of specimen  
 X = (p<0.95)

Eosinophil count was repeated on the same four groups of samples after 48 hours by employing only the count method. The results obtained are shown in table 5. The mean values obtained further showed a general lower mean count in the 48 hour examination than the immediate count. However, these differences are not statistically significant at 95 percent confidence limit (p > 0.95).

**Table 4.5:** Comparison of immediate count with 48 hour count by the total direct count method (counts transformed to common log units).

Sample Group	TOTAL			DIFFERENTIAL			T VALUE				
	X	SD	N	X	SD	N	%loss	PAIRED			
Adult male patient	2.57	.55	30				2.40	.55	30	28	.250
Adult female patients	2.54	.55	30				2.28	.49	30	51.6	.299
Children (patients)	2.63	.29	15				2.47	.32	15	26.7	.319
Healthy adults	2.61	.21	25				2.51	.22	25	20.1	.1303

X = mean log count  
 SD = standard Deviation of log count  
 N = Number of specimen  
 X = (p<0.95)

The mean values of immediate eosinophil count and counts obtained from 24 hours samples of the four groups of subjects examined by the differential (indirect) count method alone are shown in table 6. The results showed a lower mean eosinophil count after 24 hours of collection. However, these differences did not attain any statistical significance at 95 percent confidence limit.

**Table 4.6:** Comparison of immediate count with 24 hours count by the differential (indirect) count method (counts transformed to common log units).

TOTAL DIFFERENTIAL

Sample Group	X	SD	N	X	SD	N	%loss	PAIRED	T VALUE		
Adult male patient	1.17	.29	30				1.05	.27	30	25.4	.681
Adult female patients	1.17	.27	30				.99	.26	30	33.7	.453
Children (patients)	1.22	.20	15				1.06	.26	26	28.5	.420
Healthy adults	1.19	.13	25				1.07	.14	25	22.6	.580

X = mean log count  
 SD = standard Deviation of log count  
 N = Number of specimen  
 X = (p<0.95)

In table 7 the mean values of eosinophil counted by the differential count method after immediate and 48 hours of collection of samples from four groups of subjects are shown. The table showed generally lower mean count obtained after 48 hours. However, the differences did not attain any statistical significance at 95 percent confidence limit.

**Table 4.7:** Comparison Of Immediate Count With 48 Hours Count By The Differential (Indirect) Count Method (Counts Transformed To Common Log Units).

Sample Group	TOTAL			DIFFERENTIAL			T VALUE				
	X	SD	N	X	SD	N	%loss	PAIRED			
Adult male patient	1.17	.29	30				.89	.26	30	47.4	.944
Adult female patients	1.17	.27	30				.78	.25	30	58.6	1.00
Children (patients)	1.22	.20	15				.89	.22	15	52.8	.956
Healthy adults	1.19	.13	25				.92	.18	25	43.4	1.12

X = mean log count  
 SD = standard Deviation of log count  
 N = Number of specimen  
 X = (p<0.95)

Table 8 shows a summary of the mean percentage losses in eosinophil counts from 24 hours old and 48 hours old blood samples after counting by the two methods employed. The mean losses by the direct count method after 24 hour storage was:-

Healthy adults	-	11.2%
Adult male patients	-	13.6%
Sick children	-	13.6%
Adult female patients	-	32.7%

By the direct count method, the mean losses after 48 hours storage were:

Healthy adults	-	20.1%
Adult male patients	-	28%
Sick children	-	26.7%
Adult female patients	-	51.6%

By the indirect count method, the mean losses after 24 hours storage were:

Healthy adults	-	22.6%
Adult male patients	-	25.4%
Sick children	-	28.5%
Adult female patients	-	33.7%

The mean losses by the indirect method after 48 hours storage were

Healthy adults	-	43.3%
Adult male patients	-	47.4%
Sick children	-	52.8%
Adult female patients	-	58.6%

**Table 4.8:** Summary of the mean percentage loss of eosinophils in peripheral blood using total and differential methods.

Samples group	% loss by the direct count method		% loss by the indirect count method	
	0.-24hour	0.-48 hour	0.-24 hour	0.-48 hour
Healthy adults	13.6	28	25.4	47.4
Adult male patients	32.7	51.6	33.7	58.6
Sick children	13.6	26.7	28.5	52.8
Adult female patients	11.2	20.1	22.6	43.4

Figure 1 is a bar chart of the mean percentage loss of eosinophils counted by the direct count method. It shows that eosinophil were lost most in adult female patients, the loss was gradual in adult male patients, children (sick), while the least occurred in healthy adults.

The mean percentage loss by the indirect count method is also shown in fig.1. This bar chart shows that eosinophils were lost most in adult female patients; the loss was then gradual in sick children, adult male patients, while the least percentage loss was observed in healthy adults.

Figure 1 is a bar chart of the mean percentage loss of eosinophils counted by the direct

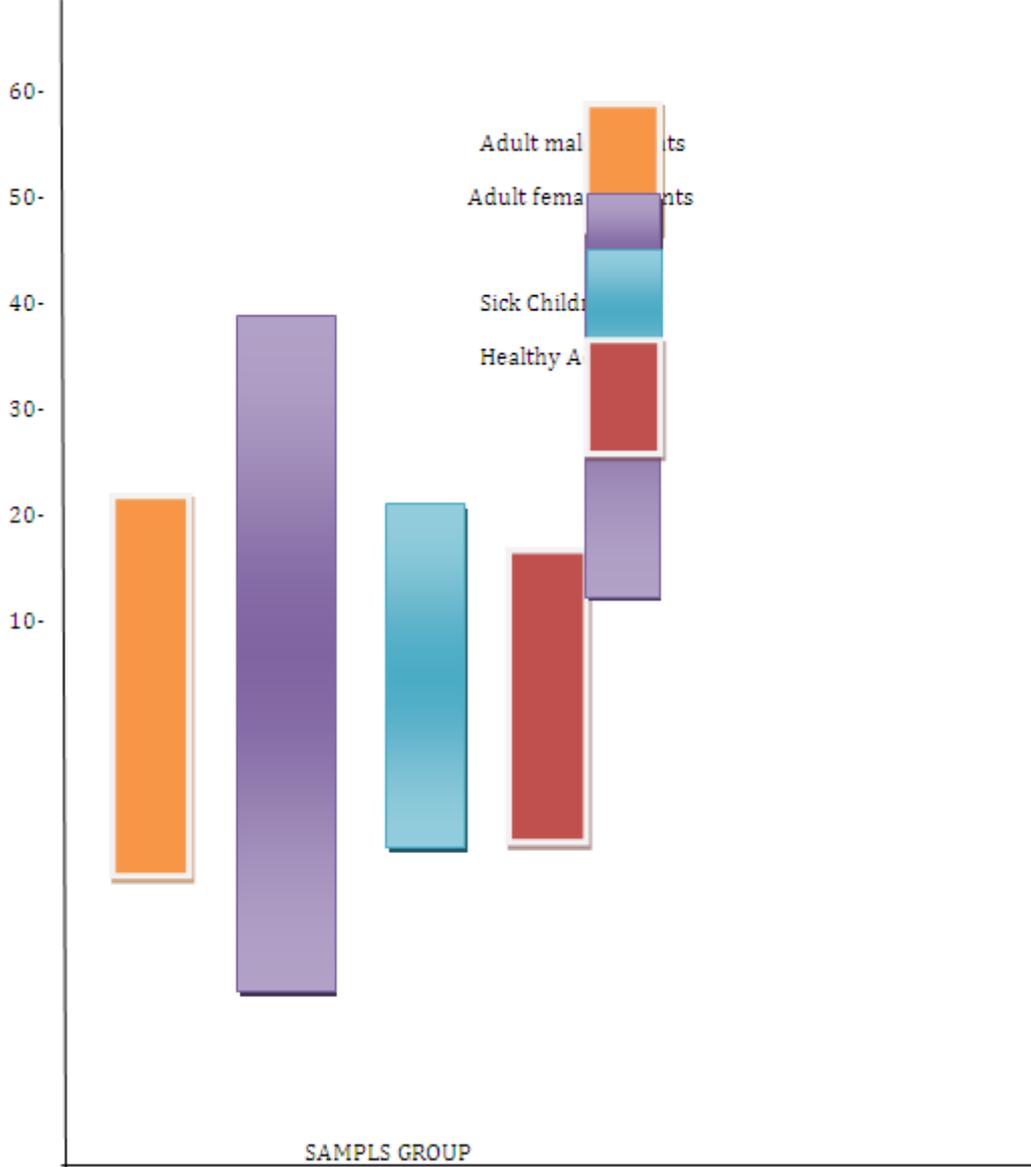


Fig 4.1: Bar chart of the mean percentage losses of Eosinopils by the Direct count methods.

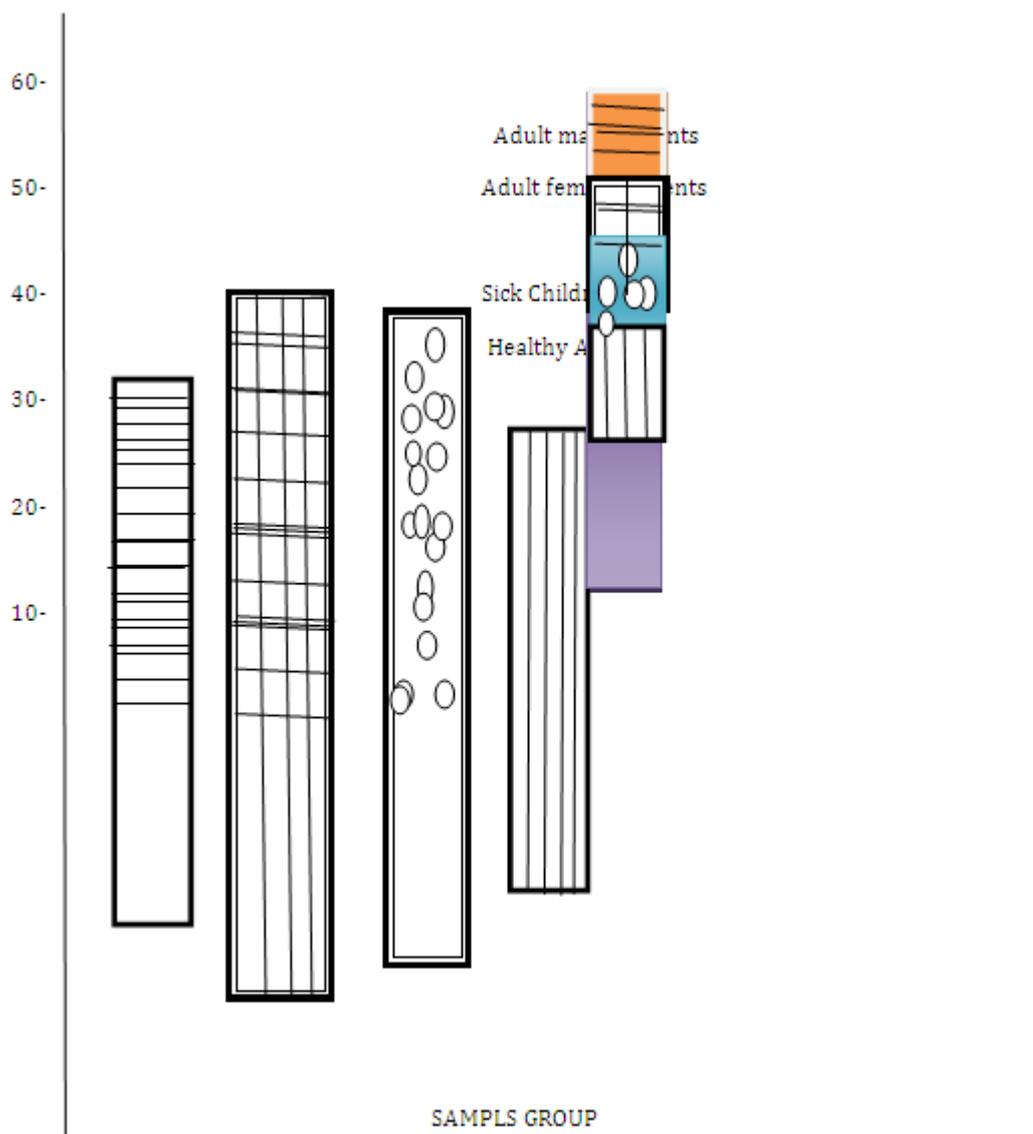


Fig 4.2: Bar chart of the mean percentage losses of Eosinophils by the Indirect count methods.

#### IV. Discussion

**Direct and indirect count:**-The results confirm that there are statistical differences between mean values of eosinophils obtained from differential leucocyte count (Indirect method) in a well made blood film and those from the wet preparation (Direct) method at 95 percent confidence limit ( $P < 0.95$ ). This agrees with previous reported studies by Discombe (1946), who affirmed that it is quicker and more accurate to determine the eosinophil count of blood in a haemocytometer using a special diluting fluid than to do a differential count using stained blood films. This finding is also in keeping with the similar observations by others (Rud, 1947, Spier & Meyo, 1949), that the direct counting procedure is cell counts and is impracticable especially when the eosinophil counts are low. The results also agree with the findings observed by Randolph and (Stanton, 1945) who claimed that differential count method has a large margin of error and therefore not suitable for the enumeration of eosinophil in the peripheral blood. There appears to be some variations in the results of the direct count method, but these are not more than those obtained by the indirect count method. This is because when the same blood sample was charged on to a haemocytometer several different times, different values were obtained. The variations are not significant because this was taken care of by taking the mean values of the numbers of eosinophils after charging the chamber for several times. In seven (7) of the subjects examined, no eosinophil was seen by the differential count method after surveying 200 (two hundred) leucocytes on the first day of collection of samples, but with the direct count method, about  $6-19 \times 10^9/L$  cells were counted in each of the 7 subjects.

The values obtained by the two methods employed after 24 hours of storage were statistically significant at 5 percent confidence limit. There was also statistical difference in the mean values obtained by the

two methods after 48 hours. After 24 hours of storage, no cell was seen in four (4) of the subject by the direct count method, while none was seen in thirteen (13) of the subjects by the differential count. Again, after 48 hours storage, no cell was seen in sex (6) of the subjects by the direct count method, while none was seen in twenty seven (27) of the subjects after counting by the differential method. This observation therefore agrees with that of (Rud 1947), (Spier & Meyer, 1949) who reported the direct count method as being preferable to the indirect method in patients with low eosinophil count.

The results of this project and others cited above appear to differ from the results of (Ukajejifo et al., 1978) who observed that there was no significant statistical difference in the two methods employed at 95 percent confidence limit. They obtained no counts with the direct count method in eight (8) of their subjects while there were presence of few cells with the indirect count.

It has been observed that counts derived from percentage differentials made on stained films are subject to substantial error because the eosinophils are sticky cells that do not spread out evenly (Archer, 1963). Consequently, as the number of the cells decrease the proportionate size of the counting error increases. Although the only remedy for this is to count more cells and therefore to examine larger blood specimens, it may not correct the error, therefore eosinophil count by dilution may be preferred, whether counted on a chamber or by electronic machine (Archer, 1963).

On difficult that accompanied this work was the clumping of the eosinophil in a few cases when the diluted whole blood had stood for up to 15 minutes before counting. However, by applying the principle suggested by (Spier, 1952), this was corrected by not allowing the dilution to stand for longer than five minutes before changing the counting chamber. This clumping phenomenon was also observed by (Ukajejifo, et al., 1978).

### **Eosinophil Degeneration**

It has been of considerable interest to study the rate of degeneration of eosinophil from the peripheral blood stored in common laboratory anticoagulant. The results obtained from this study showed that eosinophils actually degenerated when stored in anticoagulants at 4<sup>0c</sup>. This study shows that eosinophils degenerated more in patients than healthy subjects. These findings are in keeping with similar observation by (Ukajejifo et al., 1979). One of the reasons for this high loss in patients may be due to their clinical state. More importantly, it could be due to the poor handling of the specimens before reception for investigation. It was also observed that the loss was higher in adult female patients than in adult male patients. The loss observed in stick children was considerably low. The difference in the percentage loss between adult female patients and adult male patients, even with that in sick children will be further investigated.

The result also showed that more cells were lost from the 48 hour-old samples than from 24 hour-old samples. This may be due to the general degenerative changes that occur in leucocytes during over night storage. (Dacie, 1985) suggested that nuclear lobe of granulocytes become separated and their cytoplasmic margin become ragged or less well defined; small vacuoles appear in the cytoplasm. Thee appearance were probably marks of cell-degeneration and they increase with time. The last observation from the result of this study showed that there was higher loss of eosinophil from both the 24 and 48 hour-old blood samples by the differential count method than by the direct count method. This may also provide a line for further investigation.

### **V. Conclusion**

The results of this study have helped us to evaluate critically the methods for enumeration of eosinophils. Although eosinophils can be enumerated by the two methods employed, it has been observed however that the direct count method appears to yield a higher result even in patients with low eosinophil count, and in blood samples stored in sequentrene at 4<sup>0c</sup> for about 0-48 hours. The study has also confirmed that although is observed percentage loss of in stored blood; this loss is not statistically significant at 95 percent confidence limit.

Conversely, when counting eosinophil from overnight samples of blood but the direct count method, the diluted whole blood should not be mixed vigorous to avoid further damages to the stored cells.

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