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Automated Reticulocyte Count Wins Over Manual Methods

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Abstract
Reticulocytes are immature red blood cells, which contain intracellular Ribonucleic acid (RNA), Mitochondria and Ribosomes. The significance of reticulocyte count in the diagnosis of anaemia cannot be underestimated as it provides vital information about the classification and pathogenesis of anaemia. Reticulocyte count is the index of erythropoietic activity within bone marrow.

The reticulocyte counting methods at clinical laboratoriesare currently divided into manual and automated. The manual reticulocyte counting by microscopy became traditional and hasbeen considered the standard method since 1940, for its simplicity and low cost. Automated reticulocyte count substantially differ from manual method. Because automated methods are counting higher number of cells with precise measurement through specific staining and flow cytometry so it is way ahead superior than manual methods. 30 adult male and female anaemic patients were selected from the Arth Diagnostics laboratory, Rajasthan. Reticulocyte Count by automation was done by Pentra XLR from Horiba Medicals, Japan. Manual reticulocyte count was performed by traditional method with New Methylene Blue dye and light microscopy. We observe from the above table that there is significant difference in the values of manual RC and automated RC. The deviation is large and it varied from -26.0% to 74.9%. The p value of deviation between manual RC and automated RC is significant (p value <0.05).

Pentra XLR from Horiba Medicals, Japan is found to be reliable, dependable and excellent instrument for estimating Reticulocyte Count by automation than its peer methods. It will help anemic patients in terms of better treatment, follow-up and diagnosis in all the stages and types of anaemia.

Introduction

Reticulocytes are precursory to the erythrocyte cells inthe blood. They are anucleated cells, more rounded in shape and 20% greater, in volume, than the erythrocytes. However, when stained with panoptic dyes (Romanowski) they produce polychromatic slides, due to the presence of mature red blood cells with hemoglobin, synthesized during maturation, and reticulocytes with ribonucleic acid residues. These residues are stained with new methylene blue or brilliant cresyl bluedyes which confer the characteristic aspect of reticulum, when observed in optic microscopy. Reticulocyte counting is routinely and widely used in the laboratory to evaluate bone marrow erythropoietic activity. It is of great diagnostic and prognostic value in hemolyticanemias, in acute hemorrhage, in response to iron, folic acid and vitamin B12 therapy, as well as, after chemotherapy or bone marrow transplant[1].

The reticulocyte counting methods at clinical laboratories are currently divided into manual and

automated. The manual reticulocyte counting by microscopy became traditional and has been considered the standard method since 1940, for its simplicity and low cost. However, it presents some inconvenience and limitations, such as lack of accuracy, low reproducibility, time spent in the laboratory routine, lack of quality of the used stains, inappropriate blood films. The observer's visual acuity and patience, the technician's experience to distinguish reticulated cells from other cells with inclusions that also stain with the dye, besides the quality and the resolution power of the microscope are other important factors that affect the accuracy of the manual reticulocyte count[2].

Material and Methods

30 adult male and female anaemic patients were selected from the Arth Diagnostics laboratory, Rajasthan. Reticulocyte Count by automation was done by Pentra XLR from Horiba Medicals, Japan. Manual reticulocyte count was performed by traditional method with New Methylene Blue dye and light microscopy.

Review of Literature

Reticulocytes are immature red blood cells, which contain intracellular Ribonucleic acid (RNA), Mitochondria and Ribosomes. The significance of reticulocyte count in the diagnosis of anaemia cannot be underestimated as it provides vital information about the classification and pathogenesis of anaemia. Reticulocyte count is the index of erythropoietic activity within bone marrow. Hence, reticulocytosis would depict increased erythropoiesis in response to various clinical scenarios like blood loss, haemolysis or post successful therapy in iron, vitamin B12 or folate deficiency states. Similarly, conditions such as untreated nutritional anaemia or bone marrow failure would suppress red cell production and thus the reticulocyte count. Enumeration of reticulocytes can aid in monitoring the response of erythropoietin therapy in chronic renal failure and may also herald post chemotherapy or transplant marrow recovery in aplastic anaemia or malignant disease. Traditionally, reticulocyte quantification had relied upon microscopic techniques but recently automated reticulocyte analysis has become widely available[3]

Discussion

Results are listed in this table

S. No.	PCV	RC	CRC	MANUAL RC	MANUAL PCV	Deviation RC
1	31.90	1.57	1.09	1	34	36.3%
2	29.00	1.90	1.18	1.1	31.5	42.1%
3	33.30	2.27	1.52	1.6	31	29.5%
4	24.60	3.74	1.85	2.8	27.5	25.1%
5	29.40	6.32	3.96	4.3	32.5	32.0%
6	27.30	6.40	1.07	5.1	29.4	20.3%
7	30.50	2.85	1.83	2	28.3	29.8%
8	31.20	2.57	1.64	1.9	33.6	26.1%
9	22.40	0.79	0.35	0.5	17.5	36.7%
10	31.10	2.02	1.24	1.6	33.6	20.8%
11	31.40	1.56	1.01	1.7	33.2	-9.0%
12	30.70	3.50	2.30	3	32.9	14.3%
13	32.70	2.50	1.76	2	35	20.0%
14	28.70	1.81	1.05	1.2	26.3	33.7%
15	29.50	2.98	1.93	2	32.8	32.9%

16	17.10	2.57	0.87	3	20.1	-16.7%
17	33.60	1.36	0.93	0.9	31.2	33.8%
18	28.50	2.65	1.64	1.9	31.5	28.3%
19	30.90	1.92	1.24	2.1	32.5	-9.4%
20	28.60	2.46	1.51	3.1	31.8	-26.0%
21	24.60	0.67	0.33	0.3	27	55.2%
22	28.10	2.52	1.55	1.6	25.3	36.5%
23	12.00	1.46	0.37	0.8	13.9	45.2%
24	28.40	2.39	1.45	0.6	31.9	74.9%
25	26.80	2.41	1.29	1.8	30.3	25.3%
26	14.70	8.12	2.54	9	17.1	-10.8%
27	22.60	1.84	0.90	1.9	25.9	-3.3%
28	30.80	2.43	1.65	2.8	27.1	-15.2%
29	28.10	2.31	1.40	1.8	25	22.1%
30	28.40	1.38	0.85	0.9	25.3	34.8%

We observe from the above table that there is significant difference in the values of manual RC and automated RC. The deviation is large and it varied from -26.0% to 74.9%. The p value of deviation between manual RC and automated RC is significant (p value <0.05).

Conclusion

Reticulocyte enumeration is an important indicator of bone marrow erythropoiesis which is required by clinicians in a number of clinical situations. Because of its diagnostic and therapeutic implications; it is usually the most commonly requested test in the evaluation of anaemia[3].

Automated reticulocyte count substantially differ from manual method. Because automated methods are counting higher number of cells with precise measurement through specific staining and flow cytometry so it is way ahead superior than manual methods. Pentra XLR from Horiba Medicals, Japan is found to be reliable, dependable and excellent instrument for estimating Reticulocyte Count by automation than its peer methods. It will help anemic patients in terms of better treatment, follow-up and diagnosis in all the stages and types of anaemia.

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