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ORIGINAL ARTICLE

Red Blood Cell Count: Brief History and New Method

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ABSTRACT

Introduction: Red blood cells (RBCs) were observed under microscope by Jan Swammerdam in 1658. The RBC count was done in 1852 by Professor Karl Vierordt from Germany for the first time almost after 192 years. After this, the other scientists have also done RBC count with different methods. Hayem used a new diluting fluid (Hayem's diluting fluid) for RBC count which is used even today. The present methods have many errors mainly due to sampling, diluting, and pipetting.

Materials and methods: In this study, the modified method of RBC counting has been shown. The RBC counting was done using hemoglobinometer pipette and modified diluting fluid – Hayem's fluid mixed with Leishman's stain (HFLS) RBC diluting fluid – in the ratio of 97.5 mL of Hayem's RBC diluting fluid and 2.5 mL of Leishman's stain to make 100 mL. Amber colored glass bottle top dispenser was used to dispense 2 and 4 mL of diluting fluid into the glass test tubes .With aseptic precautions, 10 and 20 µL of blood samples were collected by using the hemoglobinometer pipette (marked with black marker pen to get accurate measurement for 10 µL) from finger prick with sterile needle. The blood samples were added to the glass test tubes containing HFLS RBC diluting fluid and mixing was done with a glass stirrer. With the help of glass capillary tube, Neubauer chambers were charged and observed under microscope.

Results: Red blood cells were seen better. The RBCs retained their shape and size even after 96 hours when the blood samples mixed with HFLS RBC diluting fluid were kept at room temperature.

Conclusion: Red blood cells were seen better with this method and diluting and charging errors were minimized.

Keywords: Glass bottle top dispenser, Hayem's red blood cell diluting fluid, Hemoglobinometer pipette, Leishman's stain, Red blood cell count.

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INTRODUCTION

Red blood cell (RBC) count is done as a part of complete blood examination for diagnostic and prognostic purposes. The RBC count is also one of the hematological experiments in physiology performed by students. Presently, the students are using hemocytometer for doing total RBC count, which contains Neubauer's chamber and the RBC pipette. The dilution fluid used is Hayem's fluid, which is colorless. The principal of this method is based on diluting the blood with Hayem's fluid and then counting the RBCs.

Historical Background

Jan Swammerdam, a Dutch naturalist, was the first person to see RBCs under the microscope in 1658, followed by Antoni van Leeuwenhoek in 1695. In next 150 years, in medical history, despite the availability of stains like iodine, suffron, and ammonia carmine for staining tissues and cells, there was no advances made in knowledge of morphology of blood cells or counting of blood cells.[1,2]

Professor Karl Vierordt from University of Tubingen performed the first blood counts in 1852 and laid the foundation of laboratory study of hematology as an aid to clinical diagnosis.[3] He used a glass capillary of known diameter whose capacity could be measured and the blood was blown on a slide covered with a thin smear of albumen. Micrometer was used to do the RBC count under microscope. Later, he improved the method by diluting the blood with a solution of gum arabic and his methods were fairly accurate. In 1855, Cramer made an improvement by pipetting diluting fluid and blood separately into a mixing vessel. He used the principle of capillary space using a glass slide and two strips of glass of equal and known thickness to count the RBCs. Pierre-Carl Joseph Potain invented the diluting pipette, similar to the RBC pipette used at present.[3] Louis-Charles Malassez devised a method of RBC counting in a length of capillary tube (elliptical in cross section).[3,4] Dreyfuss gave Georges Hayem the title, Father of Hematology.[4] At present, Hayem's RBC diluting fluid is still the most commonly used fluid to do RBC count.[5]

Sir William Richard Gowers modified the counting chamber with the principle of ruling the floor of the counting chamber.[6,7] Richard Thoma made an important improvement in the counting chamber. Thoma pipette is the common diluting pipette used today. Later, Thoma devised a separate leukocyte pipette.[8] Alferow devised

a counting chamber with a detachable coverslip and this was filled by capillary action.[9] McMunn[3] in 1903 used photography as an aid to accurate counting. Burker modified the counting chamber. Max Levy Company from Philadelphia modified the hemocytometer by making it from a single block of glass.

In 1921, Dreyer[10] described a modified method to do blood count and he diluted 0.1 mL blood in 19.9 mL diluting fluid to do RBC counting. Many errors in blood cell counting have been described by Abbe, Lyon and Thoma, Berkson, Nouvel, Lavergne, Biggs and McMillan, Lancaster and White.[3,11-14] The above methods are having error due to sampling, diluting, and pipetting. The error in RBC counting is 15 to 30%.[11-15] In recent years, unopette system has been used to do RBC count.[16]

Students often face difficulty in counting the RBCs as they could not see properly because they are not stained. In an earlier study, we have shown that RBCs are better visualized using Hayem's fluid mixed with Leishman's stain (HFLS) as a diluting fluid.[17] In the present modified method, students will find it easy to perform the procedure because of easy dilution method and identification of stained RBCs. A bottle top dispenser is used to deliver each time an accurate predetermined volume of the HFLS diluting fluid into a glass test tube.[18]

MATERIALS AND METHODS

Materials

(1) Hemocytometer (Fig. 1); (2) Hayem's fluid with composition of sodium chloride – 2 gm, sodium sulfate – 4.4 gm, mercuric chloride – 1 gm, distilled water – 400 cc (1 and 2 purchased from Fisher Scientific Qualigens Company); (3) Leishman's stain that contains methylene blue and eosin – 0.15 gm of dry stain slowly made up to 100 mL with acetone-free methyl alcohol (from Ranbaxy Fine Chemicals Limited); (4) Modified diluting fluid (HFLS RBC diluting fluid/Hayem's fluid mixed with Leishman): 390 mL of Hayem's fluid mixed with 10 mL of Leishman's stain, so total volume is 400 mL (in the ratio of 97.5 mL of Hayem's RBC diluting fluid and 2.5 mL of Leishman stain to make 100 mL); (5) Hemoglobinometer pipette (Hb pipette) has a mark for 20 µL. This pipette has been marked with a marker pen for 10 µL; (6) Amber colored glass bottle top dispenser (Fig. 2) with graduations for different volumes; (7) Sterile needle; (8) 5 mL glass test tubes; (9) Glass capillary tubes; and (10) Microscope.

Methods

Modified Method of Total RBC count

- In this method Hb pipette is used instead of RBC pipette to take an accurate volume of blood (10 and 20 µL).
- Hayem's fluid mixed with Leishman's stain RBC diluting fluid is used instead of Hayem's fluid.
- Specific amount of HFLS RBC diluting fluid of 2 and 4 mL is delivered into glass tubes using an amber colored glass top bottle dispenser which has graduations for different volumes. This will reduce the errors in dilution. It will also reduce pipetting and mixing errors which occur with the use of RBC pipette.
- Mixing of the blood with the HFLS RBC fluid can be done with glass stirrer and also by tilting the test tube upside down 4 to 5 times.
- The glass capillary tube is used for charging the Neubauer chamber which reduces the charging error.

The HFLS RBC diluting fluid was mixed well and stored in the amber colored glass dispensing bottle. This mixture is used as diluting fluid. 2 and 4 mL of this diluting fluid were taken in two different glass test tubes and these were kept in a rack. With aseptic precautions finger prick was done and blood was collected with the Hb pipette. 10 µL (with Hb pipette marked for 10 µL) and 20 µL of blood was collected and this blood was transferred to the glass test tubes containing 2 and 4 mL of HFLS RBC diluting fluid respectively. The dilution of blood in both

Fig. 1: Materials in conventional method

Fig. 2: Materials in new method

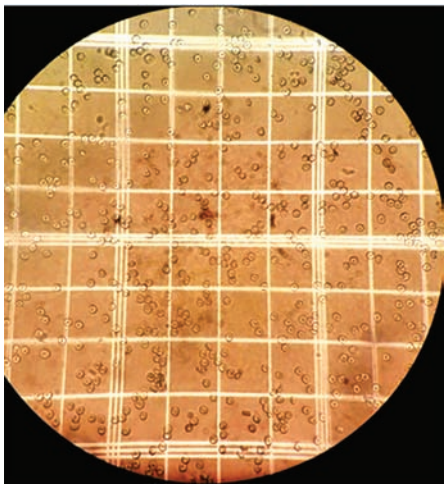

Fig. 3: Magnification 40× for 10 µL

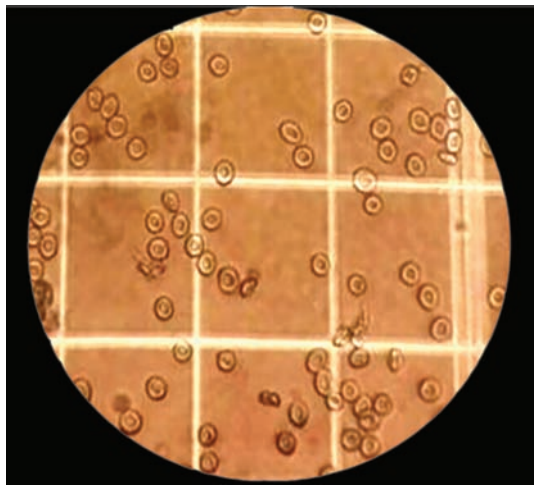

Fig. 4: Enlarged view

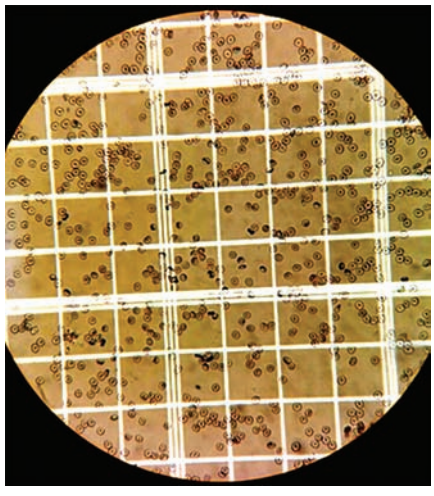

Fig. 5: Magnification 40× for 20 µL

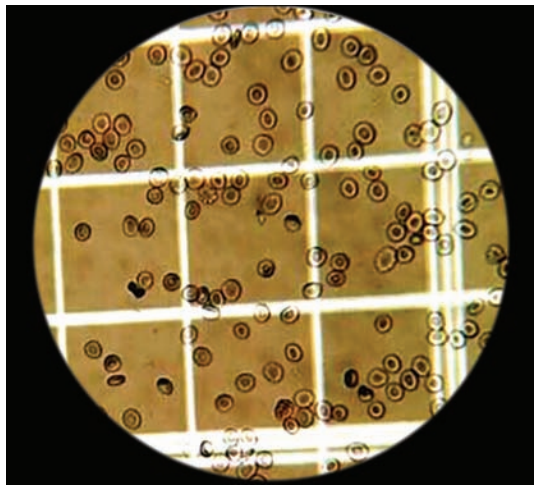

Fig. 6: Enlarged view

the glass test tubes was 1:201. With the help of capillary tube the fluid containing blood sample mixed with HFLS RBC diluting fluid was taken and the charging of the Neubauers chamber was done. Under 10× and 40× magnification RBCs were observed.

RESULTS

The RBCs were seen under 10× and 40× magnification and were seen clearly. Their shape and size were normal. The RBC count was done under 40× magnification. They were seen after taking sample (blood mixed in the HFLS RBC diluting fluid) and charging Neubauer's chamber at 24, 48, 72, and 96 hours. The RBCs retained their shape and size throughout the study period (even after 96 hours). There was no clumping of RBCs (Figs 3 to 6).

DISCUSSION

In this modified method, HFLS RBC diluting fluid is used for better visualization of RBCs. Sodium chloride maintains isotonicity. Sodium sulfate maintains the size and shape of the RBCs. The eosin present in the diluting fluid stains the RBCs which helps in their easy identification. Mercury chloride acts as a preservative. For easy and accurate delivery of diluting fluid into the glass test tubes, a dispenser bottle is used. For obtaining an accurate volume of blood, hemoglobinometer pipette is used instead of RBC pipette. Also smaller volume of blood sample (10 µL) can be obtained with hemoglobinometer pipette. There is better mixing of the blood with the diluting fluid in the glass test tube than in the RBC pipette. With the use of glass capillary tube, charging the Neubauer chamber is easy and overflow is avoided.

CONCLUSION

The application of modified method with HFLS RBC diluting fluid is useful. It can be used for optimum visualization and counting of RBCs.

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REFERENCES

1. Hajdu SI. A note from history: the discovery of blood cells. Ann Clin Lab Sci 2003 Spring;33(2):237-238.
2. Bodnar T, Fasano A, Sequeira A. Mathematical models for blood coagulation. In: Bodnar T, Galdi GP, Necasova S, editors. Fluid–structure interaction and biomedical applications. Basel: Springer; 2014 (ISBN 978-3-0348-0821-7). 486p. Chapter 7
3. Verso ML. The evolution of blood-counting techniques. Med Hist 1964 Apr; 8(2):149-158.
4. Verso ML. Some nineteenth-century pioneers of haematology. Med Hist 1971 Jan;15(1):55-67.
5. Hayem, G. Du Sang et de ses alterations anatomiques. Paris: G Masson; 1889, 1074p.
6. Critchley, Macdonald. Sir William Gowers, 1845–1915: a biographical appreciation. London: William Heinemann; 1949.
7. Gowers, WR. On the numeration of blood corpuscles. Lancet 1877;2:797-798.
8. Lyon, JF, Thoma R. Ueber die Methode der Blutkorperzahlung. Virchows Arch Path Anat 1881;84:131.
9. Alferow, S. Nouvel appareil servant a compter exactement les globules sanguins. Arch Physiol Norm et Path 1894:3;269.
10. Dreyer G. A simple procedure for the accurate enumeration of blood cells and bacterial without the use of a counting chamber. Lancet 1921;197(5083): 219-221.
11. Biggs R, Macmillan RL. The error of the red cell count. J Clin Pathol 1948 Nov;1(5):288-291.
12. Ghai CL. The total leucocyte count in a textbook of practical physiology. 8th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2012. p 48. Chapter 1-11.
13. Pal GK, Pal P. The red cell count in text book of practical physiology. 3rd ed. Universities Press (India) Private Limited: Hyderabad;2014. p 44. Chapter 7.
14. Jain AK. Chapter 4: Determination of total erythrocyte (red blood cell) count in manual of practical physiology for MBBS. 4th ed. New Delhi: Arya Publications (Avichal Publishing Company); 2013. p 21.
15. Hepler OE. Haematology in manual of clinical laboratory methods. 4th ed. Springfield: Charles C Thomas Pub Ltd; 1977, pp. 33-37.
16. Freundlich MH, Gerarde HW. A new, automatic, disposable system for blood counts and hemoglobin. Blood 1963 May;21:648-655.
17. Math MV, Kattimani YR, Khadkikar RM, Patel SM, Shanti V, Inamdar RS. Better visualization of red blood cells by adding Leishman's stain to Hayem's fluid. MGM J Med Sci 2015: 2(4):221-222.
18. Cheesbrough M. Equipment for pipetting and dispensing in district laboratory practice in tropical countries, Part 1. 2nd ed. Cambridge: Cambridge University Press; 2009. p 138. Chapters 4-6.