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Erythrocyte Morphology and Its Disorders

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Abstract

Blood cell morphology is a key tool in laboratory haematology. Erythrocyte morphology points to possible aetiopathogenetic events in several primary and secondary haemopathies. Despite advances in medical technology and laboratory automation, red cell morphology remains a basic aspect of haematological evaluation. The human erythrocytes are discoid (bi-concave), about 7–8 μm (size of the nucleus of a small lymphocyte) in diameter, with a central area of pallor (which occupies a third of the red cell diameter) and is well haemoglobinised in the outer two thirds of the red cell diameters, without any inclusions. Deviations from the normal in terms of size, shape, colour, distribution or presence of inclusion bodies suggests possible disease processes. This chapter is therefore dedicated to morphologic description of the human erythrocytes, a study of possible abnormalities, its underlying pathophysiology and the associated differential diagnosis in humans.

Keywords: red cell, erythrocytes, morphology, haematology, red cell disorders, peripheral blood film

1. Introduction

Erythrocytes are the major cellular component of the circulating blood. Roughly, erythrocytes in circulation average about 5 million cells per cubic millimetres of blood. With an average life span of about 100–120 days, erythrocyte production and senescence is maintained in constant equilibrium. Any imbalances affecting production or destruction of red cells result in red cell disorder. In essence, red cells are maintained at a constant volume in the body, depending on several factors. Physiologic factors such as age, sex, altitude, smoking status or pregnancy account for slight inter-individual and intra-individual variations. Typically, there are different measures of red cell counts and they include red cell mass, red cell volume, red cell count, haematocrit and haemoglobin concentration. Red cell volume or mass is expected to fall within an interval of mean \pm 2 SD within a specified population for a person's age, sex and race.

Beyond count anomalies (quantitative abnormalities), morphologic aberrations (qualitative abnormalities) are highly relevant in clinical evaluation of red cell diseases. Normally, a red cell has a round form, shaped like a disc, well-haemoglobinised cytoplasmic rim with a central pallor covering inner third of the red cell. Deviations in morphology (size, shape, colour, contents/inclusion or distribution) may be associated or perhaps diagnostic of disease entities. For instance, a blood

picture with paucity of red cells, numerous red cell fragments, increased polychromatic red cells suggests a micro-angiopathy or fragmentation syndrome.

This chapter aims to discuss principles of red cell morphology, as well as describe red cells in terms of morphology, identify morphologic abnormalities associated with different disease conditions.

2. Principles of erythrocyte morphology

Circulating red cells are formed from bone marrow stem cells. Stem cells are pluripotent; they self-replicate and differentiate to specialized cells in circulation through different lineages. Red cells are formed from the myeloid stem cell lineage (colony forming unit—granulocytes, erythroid, myeloid and megakaryocytes). The earliest recognizable red cell precursor in the bone marrow is the pronormoblast. The pronormoblast undergoes series of maturation to become the orthochromatic normoblast. Upon extrusion of its nucleus, the late normoblast becomes the shift reticulocytes, which is released into the circulation. Finally, DNA remnants and other chromatin materials in the reticulocytes is removed by the pitting action of the spleen, hence the mature red cells.

Erythrocytes cannot be seen with the naked eyes. Typically, morphology of red cells is performed on peripheral blood smears, once there is an indication. Erythrocyte morphology is either indicated by a clinical request or laboratory flags. Examples of clinical indications for peripheral blood film/erythrocyte morphology are listed in **Table 1**.

Erythrocyte morphology may also be indicated when significant deviations from the normal are seen in the laboratory during blood work (full blood count) irrespective of a clinical request. For instance, a significantly reduced haemoglobin level with low MCV and raised RDW may suggest iron deficiency anaemia. This is an indication for red cell morphology and other ancillary investigation for iron deficiency.

Blood for peripheral blood film is collected through venipuncture. Anticoagulant of choice is the potassium EDTA. Specimens should be analysed as

A clinical request for a PBF may be prompted by the following indications:
• Unexplained anaemia, leucopenia or thrombocytopenia
• Unexplained leukocytosis, lymphocytosis or monocytosis
• Unexplained jaundice or haemolysis
• Features of congenital haemolytic anaemias such as splenomegaly, jaundice or bone pains
• Suspected chronic or acute myeloproliferative disease, e.g. Chronic myeloid leukaemia
• Suspected organ failure such as renal disease, liver failure
• Features of hyperviscosity syndrome as in paraproteinaemias, leukaemic hyperleucocytosis, polycythaemia
• Severe bacterial sepsis and parasitic infections
• Advanced malignancies with possible bone marrow involvement
• Suspected cases of nutritional anaemia
• Suspected chronic lymphoproliferative such as chronic lymphocytic leukaemia
• Advanced lymphoma with leukaemic spills
• Evaluation of disseminated intravascular coagulopathy and other red cell fragmentation syndromes

Table 1.
Clinical indications for peripheral blood film.

Cell component	Colour
Chromatin (including H-J bodies)	Purple
Cytoplasm with RNA and nuclear Remnants (e.g. polychromasia, basophilic stippling's)	In polychromatic red cells, RNA produces a blue colour, which offsets the pink colour imparting a purple tinge Basophilic stippling: appears as blue granules dispersed within the cytoplasm in a punctate appearance
Mature red cells	Pink

Table 2.
Romanowsky staining characteristics of red cell components.

soon as possible, preferably within 2 hours of blood collection. Samples not analysed immediately should be stored at 2–6°C in a refrigerator, or the blood smear should be made, dried and fixed, for subsequent staining with Romanowsky dyes.

Asides automated slide makers, the commonest method for preparation of peripheral blood film is the slide ‘wedge’ or push technique. This technique typically requires microscope slides, pipette/blood dropper, spreader slide and the blood specimen to be analysed. Standard precautions must be observed to prevent transmission of infectious pathogens such as human immunodeficiency virus and hepatitis viruses.

Quality control measures will include ensuring proper anticoagulant: blood ratio, sample processing/analysis within sample viability period and adequate mixing of the blood before smearing. Each slide must be labelled with at least two patient identifiers such as name and laboratory, and date of procedure. Once the smear is air-dried in about 5–10 minutes, fixation of the blood tissue is another very important step. Fixation helps to preserve the architecture of the cells, which ensures good morphology. A dried slide should be fixed within 4 hours of preparation, preferably in the first hour.

For routine morphology, the glass slides are stained with Romanowsky dyes. Romanowsky dyes are differential stains composed of both acidic and basic components. The acidic component is eosin and the basic part is azure B or polychrome methylene blue. Examples of Romanowsky stains include Leishman stain, Jenner, Wright stain, May-Grunwald-Giemsa stain and Giemsa stain. Generally, the eosin part of the dye binds to the basic component of the cell such as the haemoglobin molecules in the red cell and stains it pink. The basophilic part of the dye binds to the acidic component of the cells such as the nucleus and stains it blue. Other components of the cells appear in different colour shades that contrasts and compares with the dye. The term, azurophilic is used to describe a neutral to sky-blue colour shade. For instance, the cytoplasm of a neutrophil is described as azurophilic in colour. Furthermore, the characteristic staining quality of different red cell components is presented in **Table 2**.

Staining procedure and the stain contact time depends on the type of dye in use. Staining protocols are contained in standard laboratory texts and reagent manuals. Red cell morphology should be examined at the monolayer region of the film which is 2–4 × 10 fields from the feathered edge. In this place red cells are randomly distributed with most lying singly and only a few overlapping. If area is too thin, the RBCs will appear flat with no central pallor. If too thick, false rouleaux may be reported and morphology may be difficult to evaluate because red cells are packed.

3. Red cell morphologic disorders

The haemato-morphologist reviews the red cell morphology under the compound microscope and notes any significant abnormalities for reporting/diagnosis

in light of patient clinical context. Red cell morphology is evaluated in terms of size, shape, colour, distribution and intra cytoplasmic inclusions. In general, red cells have a fairly uniform variation in size, with a red cell distribution width of 11–15% in normal individuals. Abnormal variations in sizes and shape are termed anisocytosis and poikilocytosis, respectively [1].

3.1 Anisocytosis

Normal red cells (normocytes) are about 7–8 μm in diameter [2]. Reduced size is termed microcytosis. Increase in red cell diameter above normal is called macrocytosis. Red cell sizes form the basis for morphologic or cytometric classification of anaemia. In terms of red cell size, anaemia could be described as microcytic, normocytic or macrocytic. Typically, the normal red cell size is adjudged by comparison with the nucleus of a small lymphocyte. The reference interval for mean red cell volume (MCV) is 80–95 fl [3, 4]. $\text{MCV} > 95 \text{ fl}$ is termed macrocytic. While, red cell size $< 6 \mu\text{m}$ and/or $\text{MCV} < 80 \text{ fl}$ is termed microcytic [5]. Differentials of microcytic anaemias include iron deficiency, thalassemias, sideroblastic anaemia and anaemia of chronic inflammation (20% of cases). Further test such as serum ferritin, total iron binding capacity (TIBC), haemoglobin electrophoresis with quantification helps to differentiate microcytic anaemia [4, 6]. For instance, low serum ferritin, raised TIBC and raised RDW is expected in iron deficiency. A normal or elevated red cell counts with little red cell size variation (RDW) in the presence of microcytosis is suggestive of a thalassaemia.

Normocytic anaemia occurs in acute blood loss, marrow aplasia, anaemia of chronic disease (80% of cases) and anaemias of endocrine origin. Macrocytosis may be oval or round, with specific casual relationships. Oval macrocytes are seen in megaloblastic anaemias (folate/cobalamin deficiencies), myelodysplastic syndrome and drug therapies such as hydroxyurea [7]. Round macrocytes are seen in liver disease and excess alcohol use. MCV may appear falsely normal with the haematology analyser in combined substrate deficiency states. However, the blood picture will reveal marked anisopoikilocytosis. The red cell distribution width (RDW) is a calculated parameter and it measures the individual size variability (heterogeneity) of the red cells. RDW is the percentage coefficient of variation of the individual red cell volumes enumerated by the particle counter [8]. RDW normally ranges between 11.5 and 15.5%. For interpretation purposes, raised RDW is seen in iron deficiency anaemia, megaloblastic anaemia (folate and cobalamin deficiency), haemolytic anaemia, recent blood transfusion, hereditary spherocytosis and sickle cell syndromes [8, 9]. RDW is useful in interpreting apparently normal MCV since it will be quite high in combined micronutrient deficiency state.

3.2 Poikilocytosis

Shape abnormalities, otherwise called poikilocytes are useful pointers to specific diagnosis. It is important to note that poikilocytosis may also occur in vitro (artefactual causes). It is therefore necessary to ensure adequate precautions in reducing pre-analytic and intra-analytic errors that affects morphology. As a reminder, the following quality control measures apply in blood film morphology:

- Blood specimens for PBF are best collected in EDTA bottles through venipuncture.
- Optimal blood: anticoagulant ratio should be observed.

- Samples should be dispatched immediately to the haematology laboratory. Prolonged delay in analysis allows for cellular degeneration, pseudo-thrombocytopenia and artefactual changes [10].
- Blood specimens for morphology are best analysed within 2 hours of collection.

Poikilocytes are categorized as either spiculated or non-spiculated. Spiculated red cells have at least one pointed projection from the cell surface. Examples of spiculated poikilocytes are burr cells, schistocytes (red cell fragments), irreversibly sickled red cells (drepanocytes), acanthocytes and tear drop red cells (dacrocytes). Non-spiculated poikilocytes include target cells, ovalocytes and stomatocytes. Various mechanical, biochemical and molecular mechanisms underlie pathologic changes in red cell shape. Some occur as a result of disturbances in the haematopoietic system. Target cells have an area of central haemoglobinization (termed hyperchromic bull eyes) surrounded by a halo of pallor. Increased red cell surface area to volume ratio in target cells is due to its redundant membrane, which gives rise to the targetoid shape. Target cells (**Figure 1**) are seen in sickle haemoglobinopathies, thalassemias, iron deficiency and post splenectomy state. Tear drop red cells (**Figure 2**) results from abnormal spleen or bone marrow pathology such as primary myelofibrosis when the red cells stretch out in order to navigate its way into the periphery or as a result of stretching from the pitting action of the spleen, when red cells with inclusions such as Heinz bodies navigates the splenic cords into the sinuses [5].

Stomatocytes have a fish mouth appearance (slit-like central pallor). They are mostly due to increased red cell permeability, resulting in increased volume. Stomatocytes may be inherited or acquired. Hereditary stomatocytosis is seen in Rh null phenotype. Acquired stomatocytosis is mostly seen with recent excessive alcohol and typically resolves within 2 weeks of alcohol withdrawal. When artefactual, stomatocytes are usually <10% of the red cell population. As the name implies, irreversibly sickled red cells (**Figure 1**) are seen in sickle syndromes. The primary event is intra-erythrocytic haemoglobin precipitation (gelation), with resultant formation of tactoids, which deforms the discoid red cell to sickle or crescent morphology [11]. Burr cells are seen in renal failure and may be artefactual. Artefactual red

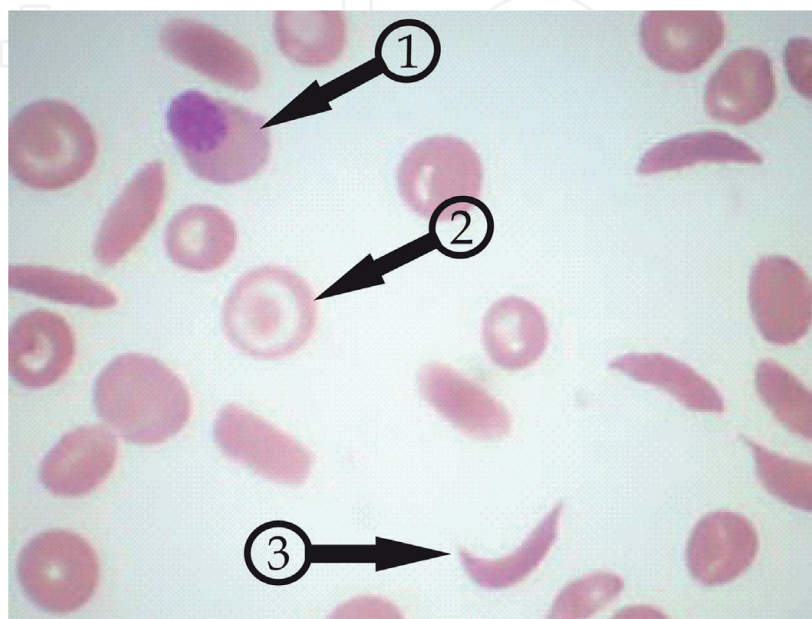


Figure 1.
(1) Nucleated red cell, (2) target cell, and (3) irreversibly sickled red cell.

cells may be caused by poor fixation and high humidity in the laboratory ambience. Artefactual tear drop cells should be suspected if the tails line up in the same direction. **Table 3** itemizes common poikilocytes and its differentials [1, 5, 12–15].

3.3 Anisochromia/polychromasia

Anisochromia depicts increased or decreased haemoglobinization of the red cells. In hypochromic red cells, the central pallor exceeds one third of the diameter. Hypochromia usually follows microcytosis, as seen in iron deficiency states. Hyperchromia (increased haemoglobinisation) is associated with shape abnormalities such as (micro)-spherocytes and sickled red cells. Increased haemoglobinization obliterates central pallor. Occasionally, severe hypochromia is associated with macrocytic red cells, termed leptocytes. Leptocytes are seen in severe iron deficiency, thalassemia and liver diseases [14]. Polychromasia on PBF suggests in-vivo reticulocytosis. Literally, polychromasia means ‘many colours’, i.e. the red cells bear another shade of colour than pink (eosinophilic). Polychromatic red cells are macrocytic (young red cells) and have a bluish tinge. The blue tinge denotes the presence of rRNA which eventually undergo the pitting action of the spleen to become mature circulating red cells [1]. Normally, polychromatic red cells are not obvious on PBF—adult reticulocyte population is about 0.5–2.5% [3]. However, polychromatic red cells in excess of 1–2% in the periphery should be considered significant since normal daily rate of red cell turnover is about 1–2% [16]. In situations of acute haemorrhage, haemolysis, and high altitude, hypoxia induces increased erythroid activity, hence polychromasia. Polychromasia is also seen in extramedullary haemopoiesis due to myeloid metaplasia in reticulo-endothelial tissue. Following haematinic therapy, polychromatic red cells are seen as a response to treatment of micronutrient deficiency [1].

Similarly, in severe situations causing marrow stress, nucleated red cells (erythroblastosis) exit the bone marrow prematurely in order to compensate. Notable causes of erythroblastosis (or normoblastemia) include severe anaemia, asplenic/hyposplenic state as in sickle cell disease, severe hypoxia, marrow replacements or infiltrations and extramedullary haemopoiesis [17, 18]. In neonates, nucleated red cells are normally seen in the periphery [15].

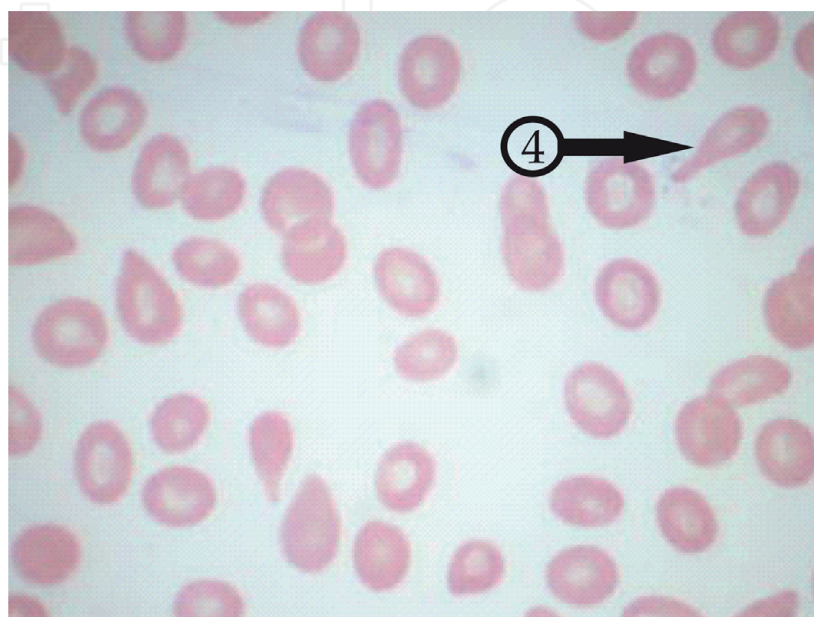


Figure 2.
(4) Tear drop red cell.

Red cell shapes	Differential diagnosis
Irreversibly sickled red cells (drepanocytes)	Sickle cell syndromes (SS, SC, S-β-thalassemia)
Target red cells (codocytes) Target cells (codocytes, Mexican hat cells)	Sickle cell disease, haemoglobin C trait, haemoglobin CC disease, thalassemia's, iron deficiency, liver disease (cholestasis), asplenia
Fragmented red cells (schistocytes, helmet cells, keratocytes)	Thrombotic micro-angiopathic haemolytic anaemias such as disseminated intravascular coagulopathy (DIC), thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome.
Pencil cells	Iron deficiency
Stomatocytes	Artefact (due to slow drying in humid environment), liver disease, alcoholism, Rh-null disease, obstructive lung disease
Elliptocytes	Hereditary elliptocytosis (>25%)
Bite cells (degmacytes)	G6PD deficiency, oxidative stress, unstable haemoglobin's, congenital Heinz body anaemia
Basket cells (half ghost cells/blister cells)	Oxidant damage, G6PD deficiency, unstable haemoglobin's
Spherocytes	Hereditary spherocytosis, ABO incompatibility, autoimmune haemolytic anaemia (warm antibody type), severe burns
Teardrop red cell (dacrocytes, lacrymocytes)	Idiopathic myelofibrosis, myelophthisic anaemia, thalassemia's

Table 3.
Red cell shape anomalies and associated diseases.

3.4 Other red cell abnormalities

Other morphologic abnormalities include presence of inclusion bodies and pathologic distribution of red cells on the smear. A mature erythrocyte lacks inclusion bodies. Red cell inclusion bodies include nuclear products RNA/DNA, haemoglobin or iron pigments. Some, such as haemoglobin H inclusions and Heinz bodies can only be appreciated with supravital staining. Red cell inclusions result from oxidant stress, severe infections and dyserythropoiesis (maturation defects). Basophilic stipplings or punctuate basophilia are denatured RNA fragments dispersed within the cytoplasm. Basophilic stipplings may be fine, blue stipplings or coarse granules. They are non-specific and are generally related to disorders in haem biosynthetic pathways [1, 19]. Differentials include haemoglobinopathies (thalassemias), lead or arsenic poisoning, unstable haemoglobins, severe infections, sideroblastic anaemia, megaloblastic anaemia and a rare inherited condition, pyrimidine 5' nucleotidase deficiency [1, 10, 20].

Clinically insignificant, fine basophilic stippling may be associated with polychromasia/accelerated erythropoiesis/reticulocytosis. Coarse stipplings are clinically significant and indicates impaired haemoglobin synthesis as seen in megaloblastic anaemia, thalassemias, sideroblastic anaemias and lead poisoning [1, 19]. Unlike other basophilic inclusions such as Howell jolly bodies and Pappenheimer bodies which tend to be displaced to the periphery, basophilic stipplings are diffusely dispersed throughout the red cell cytoplasm. Howell jolly bodies (**Figure 3**) are DNA remnants seen in post-splenectomy patients, anatomical or functional asplenia. Siderotic granules or Pappenheimer bodies appear purple on Romanowsky stain, blue on Perl's stain and are seen in disorders of iron utilization like sideroblastic anaemias.

Parasites such as *Plasmodium* spp. or *Babesia* spp. may also be seen on peripheral blood smear [21]. Both parasites invade the red cells. Their identification requires

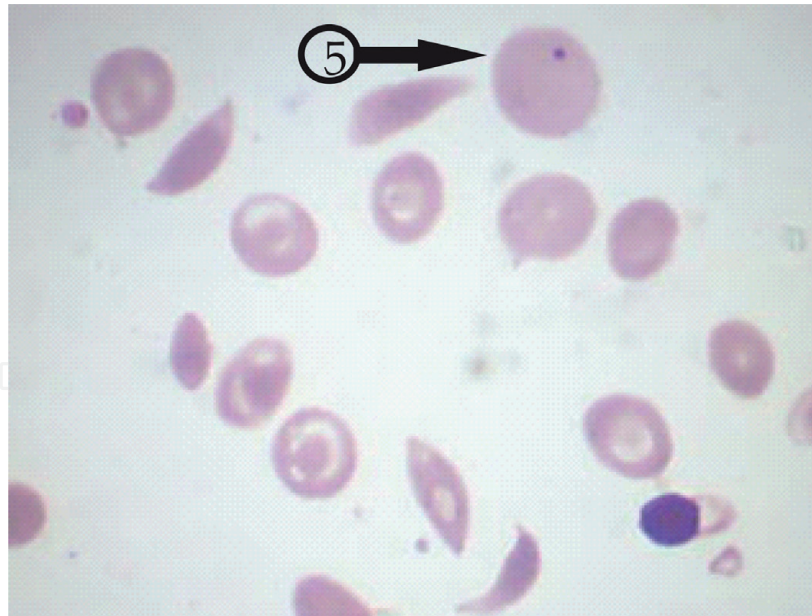


Figure 3.
(5) Howell Jolly body (in a 36-year-old lady with sickle cell disease).

some level of knowledge and experience. Several species of *Plasmodium* spp. exist. *Plasmodium* spp. may exist in different forms such as ring forms (trophozoites), gametocytes and schizonts. *Babesia* spp. appear in small ring forms (like *Plasmodium falciparum*) but schizonts and gametocytes are not formed [1, 21]. Unlike *Plasmodium* spp., *Babesia* spp. do not produce pigments. However, *Babesia* spp. may appear in groups outside the erythrocyte. Clinical history and travel history is also helpful in differentiating the two parasites. Other red cell inclusions such as Heinz bodies and haemoglobin H inclusions can only be appreciated with supravital staining (reticulocyte preparations). Heinz bodies are denatured haemoglobin (seen in oxidant injury, G6PD deficiency). Haemoglobin H inclusions are seen in alpha-thalassemias giving rise to the characteristic 'golf ball' appearance of the erythrocytes [1, 11, 12].

Rouleaux formation refers to stacking of red cells like coins in a single file. Rouleaux is seen in hyperproteinaemias. Elevated plasma fibrinogen or globulins reduces the zeta potential (repulsive force) between circulating red cells, facilitating their stacking effect. Rouleaux is associated with myeloma/paraproteinaemias, other plasma cell disorders as well as B cell lymphomas. On the other hand, agglutination refers to clumping or aggregation of red cells into clusters or masses and is usually antibody mediated [1]. Agglutination of red cells may be seen in cold haemagglutinin disease and Waldenstrom's macroglobulinaemia [1, 11]. Agglutination is associated with falsely reduced red cell count and high MCV. Pre warming the specimen with heating block helps to disperse the red cells prior to making of a blood smear and automated cell counts.

4. Conclusion

Red cell morphology is crucial in evaluating anaemias and several blood disorders. Good quality smear, with proper Romanowsky/special staining, coupled with the expertise of an haemato-morphologists (haematologists/haematology pathologists) remains highly valuable in patient care.

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