Review Article

Role of Peripheral Blood Smear Examination in Modern Diagnostics

Abstract

Even in the modern age of automation and molecular testing, blood smear examination (PBS) remains one of the most pragmatic and useful diagnostic tests in haematology. The direct visualisation of blood cell morphology through the examination of stained blood films cannot be replaced; there will be instances where automated haematology analysers would miss important diagnostic clues. Examining a peripheral smear is indispensable when correlating unusual results from plaque automation analyses and when exploring diagnoses such as anaemia, leukaemia, infections, and platelet disorders. Recently, advances in digital microscopy and artificial intelligence (AI) have improved accuracy and efficiency of interpreting PBS and are education tools as well. We will advocate for the relevance of peripheral blood smear examination in the current age without neglecting the diagnostic uses, technological developments, and relevance into education for laboratory professionals. Keywords: peripheral blood smear, haematology, cell morphology, digital microscopy, automation, diagnostics.

Introduction

A peripheral blood smear (PBS) is one of the oldest, yet most informative tests we perform in haematology. Its worth endures in the current milieu of high-throughput analysers, flow cytometry, and molecular diagnostics, because it affords us a direct microscopic view of red cells, white cells and platelets; details that cannot even be gleaned from the best of current medical technology. In the routine practice of hematology, PBS serves as an adjunct to and confirmation of automated testing, assists in clarifying instrument flags, and provides that critical morphological context that represents an appointed collection of CBC numbers and converts it into relevant clinical information. In instances where analyser outputs and histograms do not correlate, flags suggest blasts or fragments, or pre-analytical conditions have impacted the indices, the PBS serves as the final arbiter, situating interpretation in what actually presents on the slide.

On a well-prepared, well-stained smear, red blood cell morphology yields rich diagnostic clues. Cell size and haemoglobinisation reveal microcytosis and hypochromia in iron deficiency or thalassaemia, and macrocytosis in B12/folate deficiency, liver disease, hypothyroidism, alcohol use, certain drugs, or reticulocytosis. Shape changes (poikilocytosis) are equally instructive: spherocytes suggest autoimmune haemolysis or hereditary spherocytosis; schistocytes point to thrombotic microangiopathy or mechanical haemolysis; target cells occur in thalassaemia, liver disease, and haemoglobinopathies; sickle cells indicate sickle cell disease; elliptocytes/ovalocytes are seen in hereditary elliptocytosis or iron deficiency; acanthocytes (spur cells) accompany severe liver disease, abetalipoproteinaemia, or post-splenectomy states; echinocytes (burr cells) arise in renal disease or from drying artefact; teardrop cells (dacrocytes) reflect myelofibrosis or marrow infiltration; and rouleaux correlates with hypergammaglobulinaemia or paraproteinaemia. Distribution patterns matter too: cold

agglutinins produce RBC clumping and spurious indices (elevated MCHC, variable MCV), whereas true rouleaux stacks disperse on saline. Polychromasia signals a reticulocyte response; nucleated RBCs imply marrow stress, severe haemolysis, or hypoxia. Inclusions further refine interpretation: Howell–Jolly bodies in hyposplenism or megaloblastic change; Pappenheimer bodies (siderotic granules) in iron overload or post-splenectomy; basophilic stippling in thalassaemia, lead poisoning, or disordered haem synthesis; Cabot rings in severe dyserythropoiesis; and, with supravital staining, Heinz bodies and HbH inclusions indicating unstable haemoglobins or oxidative injury (e.g., G6PD deficiency). These features, synthesised with indices such as MCV, MCHC, RDW, and reticulocyte counts, help separate mixed pictures (e.g., concurrent iron deficiency and B12 deficiency) from single-cause anaemias

The morphology of white blood cells provides additional clinical significance that automation cannot offer. A left shift with bands and metamyelocytes is usually seen in infection or inflammation. Toxic granulation, Döhle bodies, and cytoplasmic vacuolation are suggestive of acute bacterial sepsis. Reactive (atypical) lymphocytes are often seen in viral infection, including EBV, CMV, and COVID-19; plasmacytoid forms or circulating plasma cells may be seen in some inflammatory or neoplastic situations. Blasts with fine chromatin, nucleoli, and possibly Auer rods may be seen in acute myeloid leukaemia; heavily granulated or atypical promyelocytes should raise concern for acute promyelocytic leukaemia. Hypersegmented neutrophils in the smear provide evidence of megaloblastic anaemia; hypogranular or hypolobated "pseudo–Pelger–Huët" neutrophils may indicate dysplasia or drug effect; mature lymphocytosis with smudge cells may indicate chronic lymphocytic leukaemia; and "hairy" cells, large granular lymphocytes, and prolymphocytes suggest immunophenotyping by flow cytometry is needed. The blood smear may therefore triage cases requiring urgent escalation, examination of the bone marrow, cytogenetics, or molecular testing.

Evaluation of platelets, as noted in the smear, is also useful. A blood smear informs the difference between true thrombocytopenia versus EDTA-induced pseudothrombocytopenia due to platelet clumping or satellitism; confirms the platelet estimate is accurate, an important step to avoid erroneous reporting; and characterizes morphology. Large or giant platelets signify increased turnover or inherited macrothrombocytopenias (e.g. Bernard).

The technique is fundamental to this whole process. You prepare a quality smear quickly on a clean, grease-free slide using the wedge method to get an even monolayer and feathered edge, and then stain it with a buffered Romanowsky method (Wright, Wright-Giemsa, or Leishman) at the correct pH to produce the colours expected (salmon-pink RBCs, lilac granules in neutrophils, orange-red granules in eosinophils, deep purple granules in basophils, blue coloured cytoplasm of lymphocytes, grey-blue colour cytoplasm of monocytes.) Methodical examination at low power to assess distribution and the feathered edge (ability to view the smear tube of the monolayer for RBC morphology and platelet estimate) and then oil immersion for the differential and fine inclusions, and avoid overlooking samples. Being aware of artefacts (stain precipitate which can resemble organisms, slow drying of the smear can cause echinocytosis, water artefact can create vacuolated RBCs, prolonged exposure to EDTA (anticoagulant) can addigitate platelet clumping and the leukocyte degenerative process independent of the blood sample) ensures you maintain accuracy. The thick and thin film when examined for malaria workup provide species-level morphology, while the thick film improves a level of sensitivity both panels can work in hand with PBS findings to not only quantify parasitaemia, but even help with treatment selection.

The practice of today extends the same examination of PBS as a solely manual test to a platform for higher image-based diagnostics and education. Whole-slide scanners create high-resolution digital smears, which can be archived or shared with others for telehaematology consultation, education, or standardisation of teaching and quality.

To summarize, the PBS is integral to diagnostic haematology because it tells the story behind the numbers. It verifies or corrects analyser report, allows the morphology to inform immediate management, detects parasites and inclusions missed by machines and is a remarkable teaching tool. In an increasingly automated world, it is essential that every laboratory professional is trained to make, and report on, high quality, diagnostic blood smears, incorporate these findings with automated results and clinical context, and use AI and digital advancements—all without losing their expert judgement. That combination is what will keep the peripheral blood smear necessary, current, and timeless.

Principle and Preparation of Peripheral Blood Smear

A properly made smear facilitates correct interpretation. The most frequently used method is the wedge technique, whereby a drop of blood is placed toward one edge of a clean microscope slide and, using a second slide, the drop is spread out into a thin, even film at approximately a 30-to-45-degree angle. The cover glass technique is also useful for small volume blood samples or capillary samples. After making the smear, air dry the preparation before staining with Romanowsky stains (Wright, Giemsa, or Leishman). A properly stained smear will allow cellular components to be differentiated visually by color and structure. The final step is to view the stained smear under an oil immersion objective using a light microscope.

Drying and staining are just as critical as spreading. Allow the smear to air dry quickly, and avoid blowing, heat, or humid conditions, which all introduce artefacts, such as echinocytes (crenated RBCs) and distorted leukocyte morphology. Romanowsky-type stains (Wright, Wright–Giemsa, or Leishman) are standard because they differentially stain cellular structures. These stains contain methanol, which fixes the smear; therefore, with manual methods on an occasional basis, a brief methanol fixation step can be used before staining if desired. The microscopic examination of blood smears necessitates a systematic approach. It is best to start with low power to assess the overall smear quality, distribution, and feathered edge, where large cells, clumps of platelets, and parasites may gather. Next, examine the monolayer for assessment of red cell morphology and to obtain a platelet estimate, then move to the oil immersion objective (100×) for the differential count and to assess finer detail (e.g., inclusions, granularity, nuclear detail, and subtle shape changes). The carefulness of your microscope setup (clean optics, Köhler illumination, appropriate condenser position, and fresh immersion oil) optimizes resolution and color fidelity, further enhancing interpretive accuracy.

There are several preparation details to avoid artefacts that may alter interpretation. First and foremost, slides should be clean and free of grease; even a thin film of oil can produce poor cell spread, ridges, or holes in the film. The drop size should be consistent; if too much blood is used, a short, thick smear is created. If too little blood is used, an overly thin, short smear is produced. The spread also must be soft and continuous; if you stop moving the spreader and begin again, waves or streaks will form in the smear. Try to prepare smears as quickly as possible after collection; ideally, preparations should be made within 2–3 hours of the blood collection due to EDTA causing platelet clumping, echinocytosis, and/or degenerative changes

in the leukocytes. Capillary samples should be collected using more stringent technique: discard the first drop to prevent contamination with tissue fluid, then collect several drops of blood until the last capillary sample has reached the desired volume. The first drop will be more likely to be contaminated and should not be used for assessment.

If the smear is overly blue, it can be the result of using an alkaline buffer or too much stain; if the smear is overly pink, it may indicate an acidic buffer or under-staining time. Always rinse smears gently with neutral pH water and allow the slide to dry completely prior to microscopy. It is good practice as a periodic preventative measure to filter stains and buffers to remove precipitates that may resemble organisms or inclusions.

Common artefacts and how to avoid

- Dirty or greasy slides: debris and refractile artefacts; clean or replace slides, handle by edges only.
- Stain precipitate: dark granular debris looking like parasites; filter the stain and buffer, do not dry stain on slide.
- Water artefact (hypotonic rinse or humidity): RBC vacuoles/"pale moth-eaten" cells; use buffered water at appropriate pH and allow sample to dry completely before staining.
- Improper angle/speed: tails, waves, or too thick/thin films; use correct angle (30-45°) and push speed according to the hematocrit.
- Slow drying or heat: echinocytes and distorted leukocytes; air dry quickly at room temperature, keep away from heat.
- Prolonged exposure to EDTA: platelet clumps, swollen white blood cells; make smears for samples as soon as possible, and if not able to do so dilute and recollect with citrate if platelet clumping still persists.
- Cold agglutinins: RBC clumping and spurious indices; keep sample warm and prepare smear as soon as possible.
- Platelet clumping at feathered edge: check edge; if the clumping is large enough, report as potential pseudo thrombocytopenia and recollect in citrate if necessary.

Ultimately the quality of the smear is part of diagnostic quality. Clean slides, appropriate size and thickness of film, quick air-drying of the sample, adequate fixation and staining time produces consistent color and morphology and allows confident identification of cell type, abnormal findings, and inclusions. On the other hand, poor technique can create artefacts that mimic disease or are confusing.

Key aspects of smear prep:

Having clean and grease-free slides, proper film size and thickness, and adequate fixation and staining time are all important aspects of smear prep.

Avoiding artefacts caused by contamination or drying.

Interpretation accuracy will be influenced by smear quality since poor quality smears can introduce morphological artefacts or result in misidentification of cells.

Uses for Diagnostics

A peripheral blood smear (PBS) examination is an essential diagnostic technique for many systemic and haematological disorders. It is particularly useful when considering the following situations.

Anaemia

Assessing the morphology of red blood cells (RBCs) as a guide to the classification of anaemia:

• Microcytic hypochromic anaemia: iron deficiency presents with small pale RBCs.

• Macrocytic anaemia: megaloblastic anaemia also presents with large, oval RBCs, in addition to hyper-segmented neutrophils (also part of PBS examination).

• Spherocytes, schistocytes, or bite cells are tell-tale signs of haemolytic anaemia. The underlying pathology can be illuminated by the size, shape and colour of RBCs.

White cell disorders, including leukaemia

Smear analysis helps reveal abnormal blast and white blood cells in leukaemia. It helps in differentiating between myeloid and lymphoid lineages, as well as distinguishing acute from chronic leukaemia. Atypical lymphocytes, toxic granulation, and Dohle bodies are also additional features of infections or inflammatory reactions.

Disorders of the Platelets

In a peripheral blood smear (PBS), assessment of platelet morphology and estimate platelet count can be performed directly. Diagnosis of thrombocytopenia and conditions associated with thromocytopenia, is aided by either the presence of giant platelets or platelet clumping, or by a low platelet count.

Infectious and Parasitic Diseases

PBS is critical for identifying trypanosomes, microfilariae, malarial parasites, and other blood-borne infections. Microscopy remains the standard and dependable method for confirming parasitic infection, despite the existence of rapid antigen tests.

Haematological Cancers and Additional Disorders

The presence of blasts, atypical lymphocytes, plasma cells, or unusual RBC inclusions during smear examination can signify bone marrow disorders or haematological malignancies. In addition, it is useful for identifying hemiparasites, rouleaux, or nucleated red blood cells, all of which the potential for bone marrow stress or systemic disease.

Automated Haematology Analysers and Correlation

Automated haematology analysers have transformed laboratory medicine by allowing rapid and accurate full blood counts, indices, and scatterplots using impedance, optical scatter, and fluorescence-based detection, but they are still measuring instruments rather than observers of morphology and will produce flags when distributions deviate from what is expected. Since analysers are unable to describe the subtle morphology of cells such as target cells, sickle cells, spherocytes, schistocytes, blasts, dysplastic neutrophils, giant platelets, and intracytoplasmic inclusions, flagged or unexpected results require confirmation on a well-prepared, Romanowsky-stained peripheral blood smear (PBS). Microscopically, several of the more

common pitfalls of automation can be addressed. PBS can distinguish true thrombocytopenia from EDTA-induced pseudo thrombocytopenia as manifested by platelet clumping or satellitism at the feathered edge. PBS can confirm or negate suspected blasts and immature granulocytes in cases where there is an analyser flag of "left shift" or "blasts." PBS can also aid in the evaluation of haemolysis patterns when spherocytes, bite and blister cells (oxidative injury/G6PD deficiency), or schistocytes appear in thrombotic microangiopathy. Additionally, PBS can explain erroneous indices due to cold agglutinins (falsely high MCHC/MCV with low RBC count), lipaemia or icterus (haemoglobin interference), nucleated RBCs, giant platelets, and red cell fragments that are above impedance thresholds and distort histograms.

Developments in Technology for Peripheral Smear Analysis

There is a clear transition towards digital and artificial intelligence–driven solutions in peripheral blood smear (PBS) testing, with digital microscopy systems that can now scan entire slides at high resolution, generate whole-slide images, and then archive these for rapid access for teaching, quality assurance, or to enable remote consultation. This virtual microscopy allows for telehaematology workflows in which consultant pathologists and biomedical scientists can review cases from anywhere, support smaller or off-hour laboratories, and standardise review criteria across locations. In addition to the ability to digitally capture the PBS, modern algorithms, ranging from traditional image analysis to full deep learning, can pre-classify white blood cells (WBCs), flag atypical/immature forms, quantify blasts, detect parasites (e.g., malaria), and score red cell and platelet morphology (schistocytes, target cells, and giant platelets). In practice, these tools act as a triage engine that surfaces cases for urgency, helps to reduce inter-observer variability, speeds up differentials, and increases the consistency of reporting by present curated galleries of representative cells for human verification.

Crucially, successful use requires proper validation and validation. Quality of image starts at the bench: a well-made and well-stained smear is a must because pre-analytical and staining artefacts will confound any algorithm. To verify performance, local verification against manual differentials after existing analyser flags is required across stains, scanners and magnifications (typically 60–100× with z-stacking) and with respect to the patient populations. Colour calibration and focus standardisation, LIS connectivity, a secure archive (DICOM-WSI type), as well as well-defined escalation protocols help deliver reliable, auditable workflows. The laboratory should guard against automation bias by including a "human-in-the-loop" for the final classification of indices or critical values and atypical findings as well as develop a program to monitor ongoing quality by undertaking case review and participating in external quality assessment and periodic revalidation after software updates. Governance of data, cybersecurity, and privacy, should also be addressed due to the number of high file sizes and remote accessibility.

It is obvious that digital and AI-augmented PBS platforms can improve efficiency, reproducibility, training, and access, especially regarding rare morphologies and coverage after hours; however, we should expect this technology to be supportive as opposed to a replacement of human expertise from haematologists and laboratory technologists. Expert supervision is still essential to integrate morphologic findings into the clinical context, finding the 'edge cases', detecting new therapy related changes, or needing to provide any sort of judgement.

Table 1 : Comparison between Manual and Automated Methods

Parameter	Manual Peripheral Smear	Automated Hematology Analyzer
Morphology assessment	Excellent, visual interpretation	Limited to numerical indices
Detection of parasites	Yes	No
Detection of abnormal cells	High sensitivity	May miss atypical forms
Speed	Slow	Very fast
Cost	Low	High
Requirement of expertise	Skilled observer needed	Minimal training

The Value of Education

A peripheral blood smear (PBS) is a key skill to learn for students of medical laboratory science and early career practitioners, as it allows students to visualize for themselves cellular morphology and apply abstract haematological concepts to real patient pathology. During PBS prep, staining, and systematic review of blood films, learners are able to practice crucial bench skills, including mixing, drop size, spread angle, monolayer selection, stain-buffering and timing, learn visual patterns to detect changes in RBC, WBC, and platelets, and recognize artefact versus disease—an important touchstone for quality and patient safety. Petscopic microscopes were previously considered more appropriate with simulacrathic interventions to enhance the process, but there are viable alternatives; PBS can also serve as an educational tool supporting experiential learning and case-based learning: students relate finding under the microscope to CBC indices and analyser flags, create differential diagnosis process to formulate brief interpretative comments—further developing clinical reasoning and communication. The whole slide digital microscopy technology has further enhanced this experience; designers create annotation or "hot spots" raise students attention to specifics, and from remote devices, students have unique access to case libraries (normal and complex pathology overview) and they can self-assess in quizz formats built by spacing repetition and access entities or smears at their own rate—making this type of microscopic teaching experiences possible from anywhere. PBS with competency-based learning rubrics, check lists, assessment through OSCE/OPSE, increased diagnostic reasoning, better critical appraisal of data in blood films, agreement between observers to report out and more self-confidence and any and all quality feedback, incorporates each of the ideas outlined above.

Restrictions and Difficulties

Although evaluating a peripheral blood smear (PBS) has the most value, there are limitations that educators and laboratories must actively lessen. First, interpretation is subjective; each observer can decide differently depending on their training, experience and fatigue - there will be variability unless the morphology is linked to clear criteria of standards, reference atlases, and scheduled external quality assessment. Second, it is time-consuming to perform manual reviews versus automated counts; completing the workflow of preparing, staining, and systematic review of each smear with a time consuming process, particularly if urgent cases

arise within the same workflow. Technical miss-steps are another barrier to accuracy: smear quality (poor thickness/thin films, streaks, uneven feathered edges), stains (i.e. poor pH, staining with precipitates, poor over/under staining, and time and temperature between the edta collection to the beginning of preparation of the smear (temperature decay), as well as artefacts due to humidity, pre-analytical variables such as platelet clumping or cold agglutinins, cell degeneration or integrity for smear processing, mis-set up of microscope for focus or illumination (optical artefacts), and presentation of morphology change of clumpy plates. Each of these issues may mislead representation and include pathology, and preanalytical variables may cling to the smear, potentially influencing interpretation.

Conclusion

The peripheral blood smear (PBS) remains a cornerstone of diagnostic haematology because it adds irreplaceable visual context to the numerical output of automated analysers. Even as impedance, optical scatter, and fluorescence technologies deliver rapid and precise full blood counts and indices, only direct microscopy can reveal the nuanced morphology that turns numbers into diagnoses: schistocytes supporting thrombotic microangiopathy; spherocytes, polychromasia, and nucleated red cells in haemolysis; macro-ovalocytes with hypersegmented neutrophils in megaloblastic states; sickle cells or target cells in haemoglobinopathies; blasts and Auer rods in acute leukaemia; dysplastic neutrophils or platelets suggesting myelodysplasia; giant platelets in inherited or reactive macrothrombocytopenias; EDTA-induced platelet clumping causing pseudothrombocytopenia; and blood-borne parasites such as malaria or Babesia. In practice, laboratories pair analyser outputs with reflex smear-review rules so that flagged or discordant results are confirmed visually, artefacts are recognised, and genuinely critical findings are escalated without delay.

Aside from its historical use, the PBS is now a fully integrated part of modern laboratory workflows. A high-quality smear - prepared quickly, on a clean, grease-free slide; spread to create a smooth monolayer and feathered edge; and stained using a well-buffered Romanowsky method - is the starting point for secure interpretation. Review under the microscope then supplements the automated data by explaining unusual histograms, separating out spurious indices from cold agglutinins or lipaemia, and correlating the recognized morphology with the clinical history. Many laboratories use standardized reporting language and grading scales (e.g. ICSH- aligned vernacular and 1+ to 4+ for RBC shape abnormalities) to promote less subjective report and to instantiate inter-observer agreement. Clear escalation criteria - such as suspicious acute promyelocytic leukaemia morphology, an abundance of schistocytes, or high parasitaemia - facilitate timely clinical action, while quality systems, stain QC, and routine competency checks help to secure the quality of the work. Hence, the PBS can be seen as both a diagnostic safeguard and a source of clinically meaningful commentary that is lost in a purely numerical report.

The area is also changing with the influence of digital pathology and artificial intelligence. Whole-slide imaging allows smears to be scanned and archived at high resolution, which allows telehaematology, remote second opinions, standardised teaching sets, and even efficient quality audits. On top of those images, AI-assisted algorithms can pre-classify white blood cells, auto flag blasts or atypical lymphoid populations, quantify schistocytes, autocrine giant platelets, and detect parasites, effectively triaging cases to allow human expertise to focus on the areas where attention is most warranted. Once the AI interventions are validated and monitored, they mitigate inter-observer variability, increase throughput, and improve

reproducibility, while still maintaining a “human-in-the-loop” for final sign out, and for atypical or out-of-distribution cases. Implementation and successful adoption of AI requires a rigorous local verification system for the various stains and optical magnifications, colour calibration, laboratory information system connectivity, secure archiving and attention to privacy and cyber security, while still thorough re-validation of after the program undergoes software updates or algorithm changes to meet regulatory or accredited standards. In summary, digital and AI capabilities expand the PBS area from a manual method into an image-based diagnostic platform that augments, rather than replaces, the expertise of human, professional judgement through image analysis.

The PBS is also a key tool from a pedagogical and professional development perspective. Preparing and evaluating smears is a useful activity to develop technical bench skills, pattern recognition, and clinical reasoning skills for learners undertaking to differentiate artefact from disease and relate morphology to analyser flags and characteristics of patient data. Use of digital slides and web platforms allows educators to provide interactive case-based teaching, develop a curated library of normal and uncommon cases, annotate for “hot spots,” and use formative quizzes and OSCE-style assessments that help learners articulate and strengthen their clinical reasoning and confidence. Participation in external quality assessment schemes and internal peer review processes, additionally sharpen consistency and support a reflective practice culture. Therefore, it is important for all laboratory-based professionals to be competent in preparation of PBSs and interpretation of their findings, comfortable with making remote review using digital technology, and cognizant of when to provide further testing to the clinician or escalate significant results.

In summary, the PBS has transformed from a purely manual test into a useful, multi-functional "pillar of haematology" that serves to bridge laboratory quantitative counting, applied morphology, digital imaging and clinical decision-making. The PBS is more than just a count of cells; its ability to shed light on data which reveal patient circumstance and care, while able to do remotely facilitate collaboration, education and AI-assisted triage, makes it a valuable diagnostic tool as we work towards an increasingly automated world.

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