THE PERIPHERAL BLOOD FILM

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Preparing the Peripheral Blood Film

Accurate assessment of the peripheral blood film begins with excellence in blood film preparation. Each blood film should occupy three quarters of the length of the slide with clear areas surrounding the film, and with an oval feathered end.

Preparation involves placing a drop of well-mixed, ethylenediaminetetraacetic acid (EDTA) anticoagulated blood at one end of the slide. A second slide is used as a spreader blade to spread the blood across the base slide width, and to “push” the blood along the base slide’s length before lifting the spreader blade to leave an oval feathered edge. A spreader slide with a smooth edge and a base slide that is clean, dust and oil free, are critical.

Miniprep/Hemoprep instruments may be helpful in standardizing blood film production where multiple individuals are involved in slide preparation. These “automated” slide makers may spread the blood across the entire slide width and may not leave clear areas at film sides.

Common artifacts of slide preparation include:
- Streaks – consider whether slides are dirty or if push slide is chipped
- Fat droplets – consider oil on base slide
- Irregular margins

Blood Film Staining

Peripheral blood films are traditionally stained using Romanowsky type differential stains. Many Romanowsky type stain combinations are now commercially available.

Automated bucket stainers may employ two or more stains to provide a differential staining effect. Platen type stainers require premixed stain. Intensity varies with the length of stain and rinse time. Manual staining may employ two or more stain types and intensity again varies with stain time and concentration.

Staining intensity should adequately colorize red blood cells (RBCs), leaving an area of visible central pallor in normal RBCs. Leukocyte nuclear morphology should be visible with areas of light (euchromatin) and dark (heterochromatin), visible in most cell nuclei. Very dark stain may obscure the normal open or vesicular chromatin pattern of immature cells making blast cell identification difficult. Platelets should be readily visible with fine violet granules visible in most.
Common stain artifacts include:

- Stain precipitate
- Stain debris
- Excessive water on the slide or in the stain producing refractile edges on erythrocytes

Daily review of stain quality and intensity against a known normal well stained and coverslipped slide may be helpful in preventing a gradual darkening of the stain which could obscure the critical morphologic characteristics.

**Scanning the Blood Film**

Once prepared and well dried, the film is reviewed first using a low (10X) power objective. This scan should include review for:

- Film quality
- Stain quality
- Cell distribution
- Platelet, white blood cell (WBC) or red blood cell (RBC) clumping or agglutinates
- Large microorganisms or cells at the periphery and in tails of the film
- Rouleaux
- Immature cells such as suspected nucleated red blood cell or immature leukocytes/myeloid precursors

**Cell Estimation**

White blood cell estimates may be performed as a check on the automated cell count for flagged Complete Blood Count (CBC) specimens and as a general review of WBC distribution prior to differential cell counting. Platelet estimates are also a useful check on automated platelet counts. Routine performance of platelet and WBC estimates ensures competence with this process when instrument counts are inaccurate or erroneous.

Calculation of a “field” or “estimate” factor for WBC and platelet estimation is required for each microscope type used in the laboratory. This procedure ensures that the factor utilized in estimating a platelet or WBC count accounts for the field size of the particular microscope and objective lens.

Following is an example of a method for calculating the field factor:

1. Using the 100X oil immersion object, count the number of platelets in 10 fields.
2. Calculate the average number of platelets per field and record.
3. Divide the platelet count by the average number of platelets per field to obtain the field factor for that blood film.
4. Repeat steps 1, 2 and 3 for each of 31 blood films.
5. Calculate the average field factor by totaling the 31 individual blood film field factors and divide by 31.
6. Round to the nearest whole number.

A similar strategy may be used for calculating the field factor for determining WBC count estimates but would usually be done using a low power or 10X objective.
Following the determination of the field factors, the estimation of WBC and platelet counts from the peripheral film can be accomplished as follows:

<table>
<thead>
<tr>
<th>WBC Estimates</th>
<th>Platelet Estimates</th>
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<tbody>
<tr>
<td>• Find the appropriate viewing area of the blood film</td>
<td>• Count the number of platelets in 10 or more high power (100X objective) fields (HPF)</td>
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<tr>
<td>• Add the total number of WBC including smudge cells in four or more low power (10X objective) fields (LPF)</td>
<td>• Repeat the procedure in thin or thick areas of the film if the distribution is uneven</td>
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<tr>
<td>• Divide the total number of cells by the number of fields viewed to determine the average number</td>
<td>• Determine the overall average number of platelets/HPF by dividing the total number by the number of fields viewed</td>
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<tr>
<td>• Divide the average/LPF by the field factor to determine the WBC estimate</td>
<td>• Multiply the average number of platelets by the established field factor to determine the platelet estimate</td>
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Artifactual or spurious estimates may arise in the setting of very high or very low hemoglobin concentrations. A high hemoglobin may promote counting being performed in the “thin” area of the smear, resulting in an underestimating of the count. A low hemoglobin may result in an overestimate due to the tendency to count in the “thick” regions of the slide.

Platelet estimates and WBC estimates are generally expected to check to within 15% of a normal automated count. At very low or very high WBC counts, the accuracy compared with the automated counter may vary.

**Red Cell Morphology Review**

Ideally, within a laboratory, standardized definitions and reporting format should be employed. The College standards specify that red cell morphology shall be graded in a standardized fashion on all blood films, according to written criteria.

Many texts and atlases (see references) provide excellent definitions and clinical relevancy discussions.

It is important to remember when grading or estimating total numbers of poikilocytes, that not all morphologic subtypes are of equal importance. For example, schistocytes and spherocytes are important and clinically meaningful when present even in small numbers. Target cells and elliptocytes, conversely may only be important if very prominent and frequent. Cell morphology grading should take into account these distinctions.

When reporting RBC morphology it is also important to take into account relevant information from the automated cell counter. When the MCV is low, a recheck of the film is warranted before reporting macrocytes as the apparent macrocytes are likely to be normochromic normocytic cells on a background of profound microcytosis. Similarly, if the MCV is high, a review of the film prior to reporting microcytes may be advisable.
Differential Cell Counting:

Generally, because of the number of cells counted on an automated differential cell counter, there is a greater likelihood of accuracy. Most automated counters enumerate thousands of cells versus a typical 1 – 2 hundred-cell manual differential cell count. Understanding the methodology of differential cell counting and instrument flagging is important in deciding which films should be reviewed. There must be standard, written criteria for blood film and differential cell count review based on instrument parameters, whenever an automated differential is utilized.

For laboratories with a low testing volume, it may be appropriate to review some or all films even when criteria for release of automated differentials are met, to assist with the assurance of ongoing technologist competency in manual differential reporting.

The manual differential in this setting, may serve as a check on the automated differential in some cases, while in others, the automated differential provides a check on manual differential performance accuracy.

Particularly in the setting of fragile cells such as lymphocytes, the manual differential may underestimate the lymphocyte number and therefore spuriously increase the relative number of neutrophils.

When performing a manual differential it is important to:

- Use a standard review pattern to avoid recounting the same cells
- Check the accuracy of all technologists regularly expecting +/- 12% accuracy for cell identification versus a gold standard derived by consensus or automated differential counter.

Summary

The peripheral blood film remains a very useful tool in hematological assessment. Attention to detail in slide preparation, staining and standard criteria for cell estimates, morphology and differential reporting enhance the value of results provided to the physician.
References:


3. M.A. Chichak, Personal communication.


5. Fraser, M., Cell Estimates for WBC, *Medical Laboratory Science (University of Alberta) Teaching Syllabus*.
