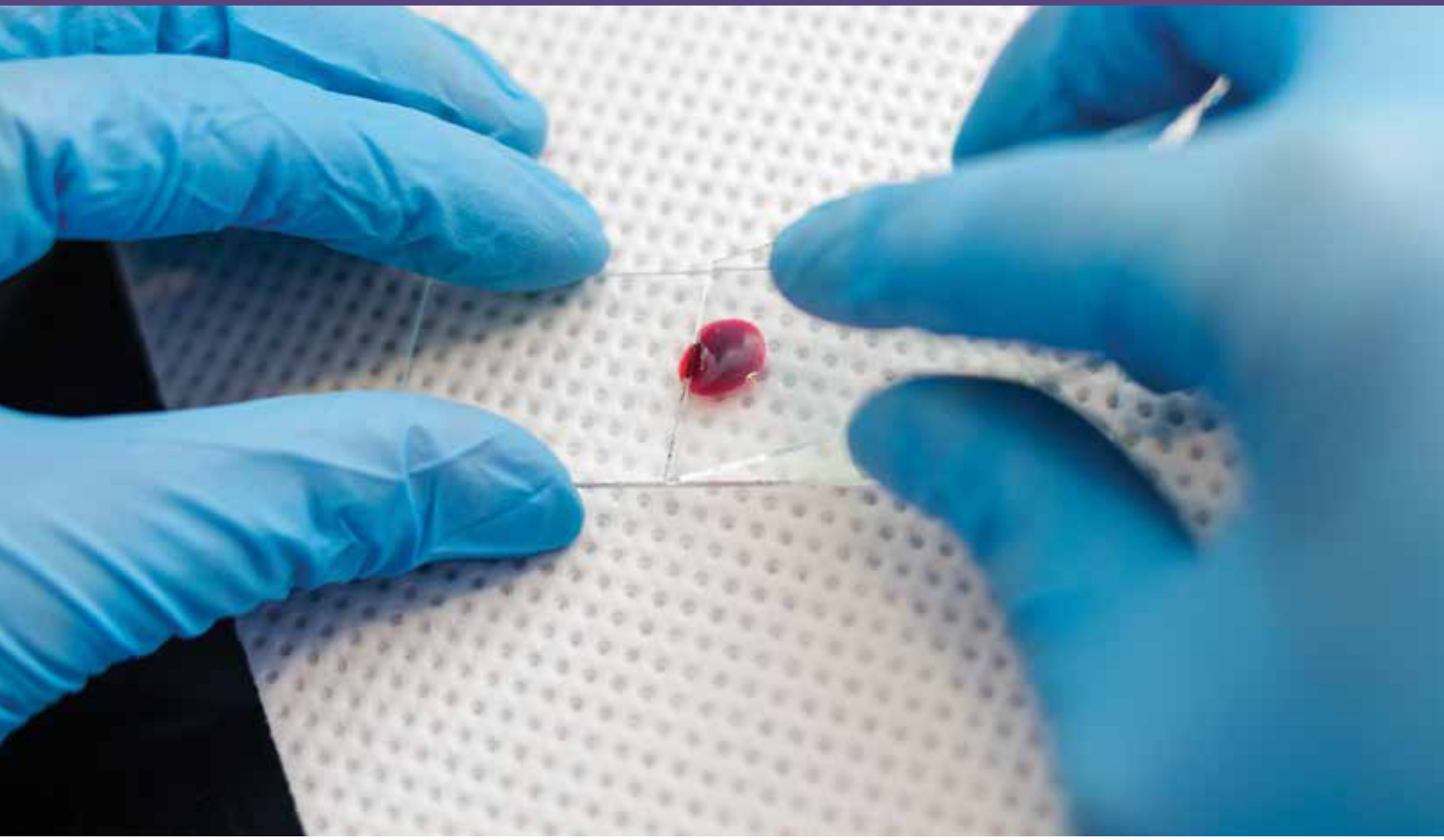

SKILLS CHECK

Manual Blood Smear Preparation and Review

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Abstract

Manual blood smear review is a quality assurance step in veterinary diagnostics, complementing automated hematology analyzers by providing insights into blood samples that machines might miss. Manual blood film review involves using a high-quality blood smear to validate automated results, assess cell morphology, and detect abnormalities such as parasites or unusual cell forms. Despite its value, manual review can be hindered by time constraints, limited resources, and varying levels of preparation and interpretation expertise. Addressing these challenges through improved workflow practices and targeted training can enhance the accuracy and efficiency of hematology testing, ensuring comprehensive patient care through both automated and manual analysis.

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Take-Home Points

- A well-prepared blood smear should start with a thicker area near the back of the slide and be spread to a thin, feathered edge.
- The manual blood smear review process involves a quick yet thorough survey of the blood smear to identify additional details that machines might overlook.
- Time, personnel, and expertise can be barriers to manual blood smear review but can easily be overcome.

Manual blood smear review is an essential quality assurance step used in veterinary diagnostics, offering insights that automated systems alone may not provide.[1-3] Manual review enables veterinary nurses to scrutinize cell morphology, estimate platelet counts, and detect abnormalities that automated analyzers might miss. Hands-on manual review is particularly useful for troubleshooting blood abnormalities, whether investigating possible platelet clumping, identifying unusual cell shapes, or distinguishing between various types of white blood cells (WBCs).

The expertise of skilled veterinary nurses with regard to recognizing the subtle and often complex changes is vital for accurate diagnostics. By complementing automated hematology testing with a manual review, veterinary nurses ensure a more thorough evaluation, enhancing diagnostic accuracy and patient care.

BLOOD SMEAR PREPARATION AND STAINING

A high-quality blood smear is an essential component of manual hematology analysis, providing the foundation for accurate blood smear review. A well-prepared blood smear (**VIDEO 1**) should be an even layer of blood spread across the slide, culminating in a monolayer, an area where the cells are arranged in a single layer and are neither overlapping nor too sparse (**FIGURE 1**), which is necessary for accurate cell morphology assessment and counting. The smear should start with a thicker area near the back of the slide, tapering to a thin, feathered edge. Achieving this delicate balance requires precision and practice (**BOX 1**).

Several challenges can affect the quality of a blood smear:

- Inconsistency among blood samples (e.g., smears that are too thick or too thin) leading to poorly defined smears that are difficult to interpret (**TABLE 1**)
- Blood characteristic variations among species
- Lack of experience (those out of practice may find it challenging and time-consuming to achieve the desired results)[1]

Standard staining techniques enhance the visibility and differentiation of blood cells under a microscope.

VIDEO 1
Preparing a blood smear

FIGURE 1. Unstained blood smear. Note the iridescent color of the monolayer.

BOX 1

Creating a Diagnostic-Quality Blood Smear

Creating the Smear

1. Begin with a fresh EDTA blood sample.
   - When collecting blood, ensure the correct ratio of blood to anticoagulant. Overfilling or underfilling the tubes could result in clotting or diluting the sample.[3]
   - If the sample has been refrigerated, allow it to warm to room temperature. Blood cells will clump in refrigerated samples.[3]
2. Mix the sample well by gently inverting the blood tube 8–10 times. If the sample is left to sit, the cells will settle to the bottom of the tube, resulting in uneven distribution of cells within the sample.[4]
3. For the blood slide (the slide on which the blood is placed), use quality slides that are free of chips or defects in the glass. Using a pipette, wooden applicator stick, or microhematocrit tube, add a drop of blood onto the end of the blood slide.
4. Hold the spreader slide (the slide used to create the smear) at a 30°–40° angle (adjust as needed) in front of the drop.[4,5]
5. While anchoring the blood slide, back the spreader slide into the blood drop and pause to allow the drop to spread horizontally.[4,5]
6. Push the spreader slide forward with a quick, smooth motion (**FIGURE A**). Do not change the angle of the slide while spreading, and do not lift it until the smear is complete.[4,5]

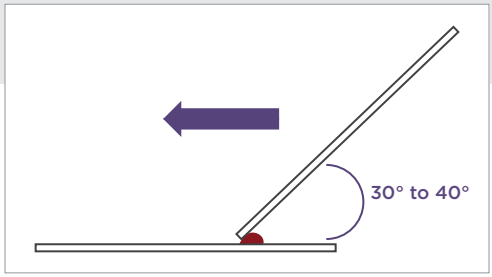

FIGURE A. Side view of blood smear technique.

Staining the Smear

1. Before staining, be sure the blood smear is completely dry and labeled correctly.
2. After drying, follow the manufacturer's instructions, beginning with the fixative solution (typically 95% methanol), followed by the eosin solution, and finally the methylene blue solution. Rinsing between the solutions is optional.[4,5]
3. Commonly, slides are fixed in methanol for a minimum of 60 seconds. On average, the smear is exposed to the eosin and methylene blue solutions for 30 seconds each. The timing of stain exposure can be adjusted depending on the age of the stains or the thickness of the smear.
4. Rinse the slide with distilled water and allow it to dry.

Staining highlights various cell components, allowing for detailed morphologic examination and accurate diagnosis. Most commonly used are Romanowsky-type stains (e.g., Wright-Giemsa stain), which consist of a fixative, an eosin solution, and methylene blue.[3] A "clean" and "dirty" set of stains should be included in the laboratory. Clean stains are reserved for blood smears and clean cytology (e.g., joint fluid aspirates). Dirty stains should be used for urine samples, purulent samples, ear samples for cytology, or other preparations for which contamination of the stain solutions is likely.[6]

MANUAL BLOOD SMEAR REVIEW

Manual blood smear review is an integral part of the diagnostic workflow and serves as quality assurance by complementing and verifying the results provided by automated hematology analyzers.[1,2] The primary intent of manual review is to ensure accuracy and reliability by providing a manual check of the autogenerated results. The process involves a quick yet thorough scan of the blood smear to identify additional details that machines might overlook (e.g., specific cell morphologies, the presence of blood parasites) (**BOX 2, VIDEOS 2 AND 3**).

Blood smear review does not need to be time-consuming. The process is designed to be efficient, focusing on a rapid examination of key areas of the smear. When a structured approach is used, the blood smear review can provide a layer of certainty without adding significant time to the diagnostic process.

Challenges and Solutions

Although blood smear review is a vital component of

TABLE 1 Blood Smear Preparation Troubleshooting

PROBLEM	SOLUTION
Smear appears long and narrow	Allow a longer pause when backing the spreader slide into the smear. Allow the blood to spread side to side, to begin with a broader base.[4]
Smear is wide and rectangular	The speed of the spreader slide is too slow, mimicking a "snow plow" movement. After pausing at the drop, move the spreader slide forward quickly.[4]
Smear is short and thick and the monolayer is narrow	Lower the angle of the spreader slide.[4]
Smear has holes	Holes result from lipids in the blood sample itself (lipemia), oils on the surface of the slide, or platelet clumping. Use new, clean slides. Fasting a patient before blood collection can help avoid lipemia.[4]
Smear has tails	Tails result from particles in the sample or on the slide. Be sure the slide surfaces are clean. Examples of sample issues include platelet clumping, which can be confirmed microscopically. Premature lifting of the spreader slide can also produce tails, as the larger elements in the blood are not pushed out to the feathered edge when the spreader slide loses contact.[4]
Patient's blood is concentrated (thicker than that of a healthy patient)	Viscosity of blood can affect how it smears. Increase the time allotted for the blood drop to spread horizontally before pushing the spreader slide forward. Lowering the spreader slide's angle may also help improve smear quality.[3]
The patient is anemic (Blood is thinner than that of a healthy patient. It may be described as "watery" and will spread quickly.)	Take care to not apply a drop that is too large. When pushing the spreader slide forward, increase the speed to help create the bullet shape and a desirable monolayer. Increasing the angle of the spreader slide will also help. Note: Because of the lower concentration of red blood cells, the distribution of cells within the monolayer will appear more spread out, which is to be expected and is not a result of poor smear technique.[4]

hematologic diagnostics, it is not without its challenges. Common barriers faced by veterinary nurses include a need for more resources (e.g., time, personnel, expertise).[1] In busy veterinary practices, blood smear review can be perceived as time-consuming or tedious, leading to its underuse. Limited personnel can also place additional strain on staff, making it challenging to allocate time to perform the task.[1] In addition, lack of confidence or expertise in performing manual analyses can further slow the process.[1]

VIDEO 2
Manual review of a blood smear from a 10-year-old male neutered poodle cross.

VIDEO 3
Manual review of a blood smear from a 5-year-old female spayed domestic shorthair cat with mild anemia.

Addressing barriers involves practical solutions that can enhance workflow and efficiency. Veterinary assistants can play a pivotal role by preparing and staining blood smears, thus freeing up veterinary nurses to focus on examining the prepared slides. A collaborative approach enhances time management and ensures that blood smear review is incorporated into the daily routine. Gaining confidence and expertise in blood smear review comes with practice; examining a variety of blood smears from healthy and sick patients can significantly improve the veterinary nurse's ability to swiftly distinguish between normal and abnormal findings.[1]

SUMMARY

By combining the results of manual blood smear review with those of automated hematology, veterinary practices can ensure that CBCs are comprehensive. This additional layer of quality assurance is essential for providing thorough and accurate patient care.

Regularly examining normal and abnormal samples will enhance expertise and efficiency at analyzing blood

BOX 2

Analyzing the Blood Smear[5]

100× magnification: Feathered edge and monolayer (**FIGURE A**)

1. Examine the feathered edge for cell clumping. Larger clumps of cells will be pushed to this area of the smear.
2. Move to the monolayer and examine cell density and red blood cell arrangement. Agglutination or rouleaux patterns, if present, will be visible.

400× magnification: Monolayer (**FIGURE B**)

1. Validate the white blood cell (WBC) count. While examining the results of automated hematology, briefly confirm the WBC differential. For example, if the test results report 75% neutrophils, then neutrophils should represent 3 out of every 4 WBCs seen on manual examination.
2. Examine morphology of erythrocytes, leukocytes, and platelets. If needed, examine any suspicious abnormalities (e.g., toxic neutrophils, Heinz bodies, parasites, macroplatelets) at 1000× magnification.
3. If suspected, confirm the presence of band neutrophils or nucleated red blood cells.

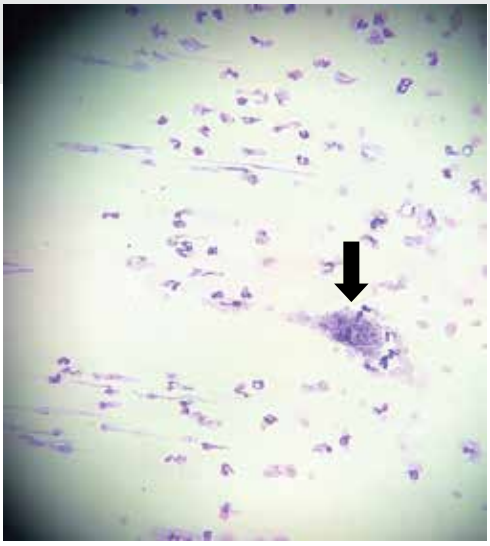

FIGURE A. Microscopic review at 100× magnification. Scan the feathered edge for cell clumping. Note the platelet clump to the right of the field **(arrow)**.

1000× magnification: Monolayer (**FIGURE C**)

1. Validate the platelet count by counting the average number of platelets per field. On average, 7–10 platelets per oil-immersion field are expected for healthy patients.
2. Examine any suspicious or more subtle morphologic findings.

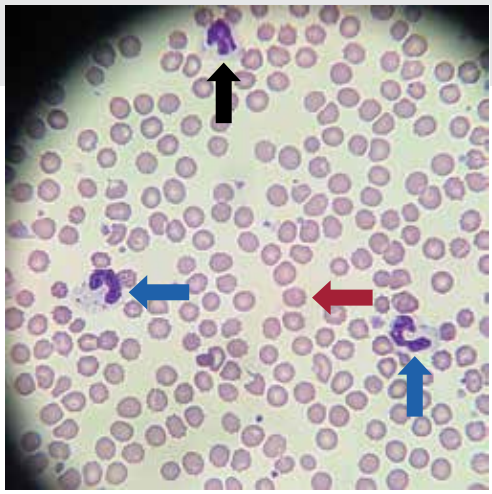

FIGURE B. Microscopic review at 400× magnification. Scan the monolayer for cell morphology. Note the 3 toxic neutrophils **(black and blue arrows)**, band neutrophils **(blue arrows)**, and echinocytes (**red arrow**).

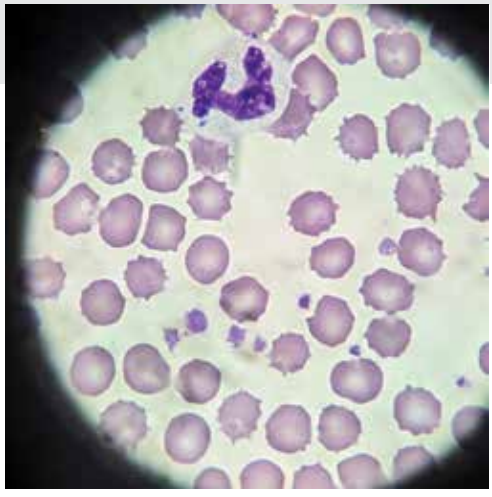

FIGURE C. Microscopic review at 1000× magnification. Scan the monolayer to validate the platelet count.

smears. Familiarizing yourself with cell morphology and pathologic findings will sharpen your skills and improve your ability to quickly identify key features. Practice makes perfect—each review hones your proficiency and confidence, leading to more accurate and efficient diagnostics. TVN

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Brianne is a 2002 graduate of the animal health technology program at Olds College, Alberta, Canada. Her work experience includes mixed animal rural practice for 6 years, after which she moved to a faculty position at the Lakeland College veterinary technician program. In 2016, Brianne obtained VTS certification in clinical pathology and is the president of the Association of Veterinary Clinical Pathology Technicians in 2024. In 2021, Brianne completed a master's degree in advanced practice in veterinary nursing through the University of Glasgow. Since pursuing her passion in veterinary clinical pathology, Brianne has enjoyed opportunities to work with other veterinary nurses/technicians within Canada and internationally.