Peripheral Blood Smear (PBS)

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SUPERSEDES: Procedure titled _____

Objective

The objective of the Peripheral Blood Smear test is to follow up the auto differential with abnormalities or flag positive reflux to examine the morphology of blood cells—red blood cells (RBCs), white blood cells (WBCs), and platelets—under a microscope to help diagnose hematological disorders.

Principle

In this test, a thin film of blood was spread on a glass slide, air-dried, stained with Wright-Giemsa stain then examined microscopically. It allowed for the evaluation of cell size, shape, color, distribution, and any abnormal features.

Materials

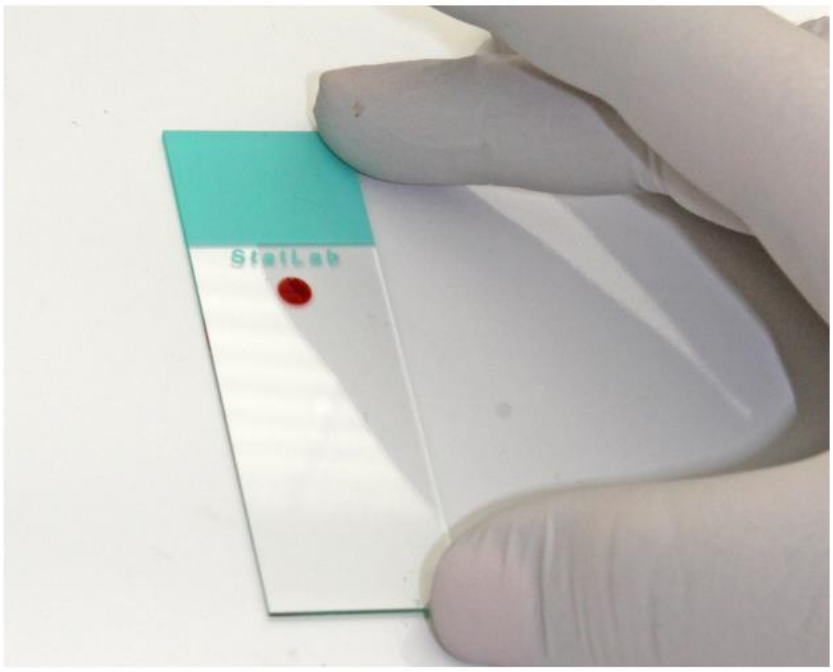
• Fresh EDTA-anticoagulated blood sample

- Glass microscope slides
- Spreaders
- Wright-Giemsa stain
- Microscope
- Immersion oil
- Gloves and PPE

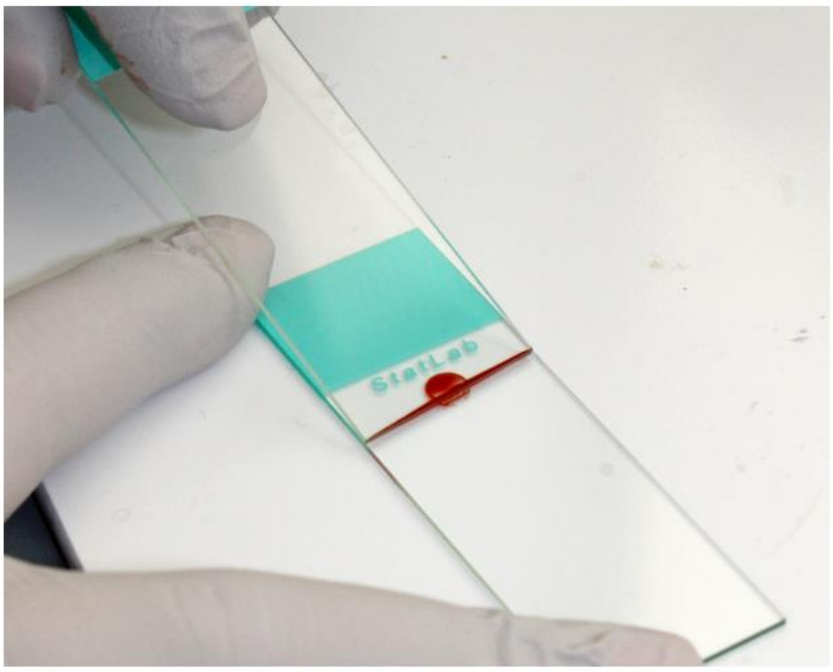
Procedure

1. A drop of blood was placed near one end of a clean glass slide using capillary tubes or 2.5 uL

2. Mix blood thoroughly, place a small drop of blood (2.5-4 uL) using a spreader slide at a 30–45° angle, quickly and evenly by holding the top and bottom edges of the slide with the thumb of your non-dominant hand spread across the slide to form a feathered edge



3. Using your dominant hand, place the edge of the other slide at an approximately 35-45º angle on the first glass slide, in front of the blood drop. Using gentle pressure, gently pull the second slide back into the blood drop and allow the blood to spread to the edge of the slide.



4. To spread the blood, rapidly but gently push the top slide forward through the remainder of the slide. It is important to keep gentle, equal pressure throughout the whole process, and do not lift the top slide before it reaches the edge of the bottom slide. A feathered edge should be present.
5. The slide was air-dried completely.

Using DIP STAIN KIT AND REAGENTS (16/ 128 OZ.) DIP STAIN KIT VDS-100 (16 OZ.), Order fro Mckassin or online at www.volusol.com composed of the following:
Dip Stain I (Methanol), Dip Stain II and Dip Stain III

6. Dip slide or rack of slides 5 times for 1 second each dip into solution I. Allow excess to drain into jar or dish and blot edge on absorbent paper. If smears cannot be stained within 4-6 hours of preparation, they should be fixed with dip stain I.

7. Dip slide or rack of slides 5 times for 1 second each dip into solution II. Allow excess to drain into jar or dish and blot edge on absorbent paper.

8. Dip slide or rack of slides 5 times for 1 second each dip into solution III. Allow excess to drain into jar or dish and blot edge on absorbent paper**.**

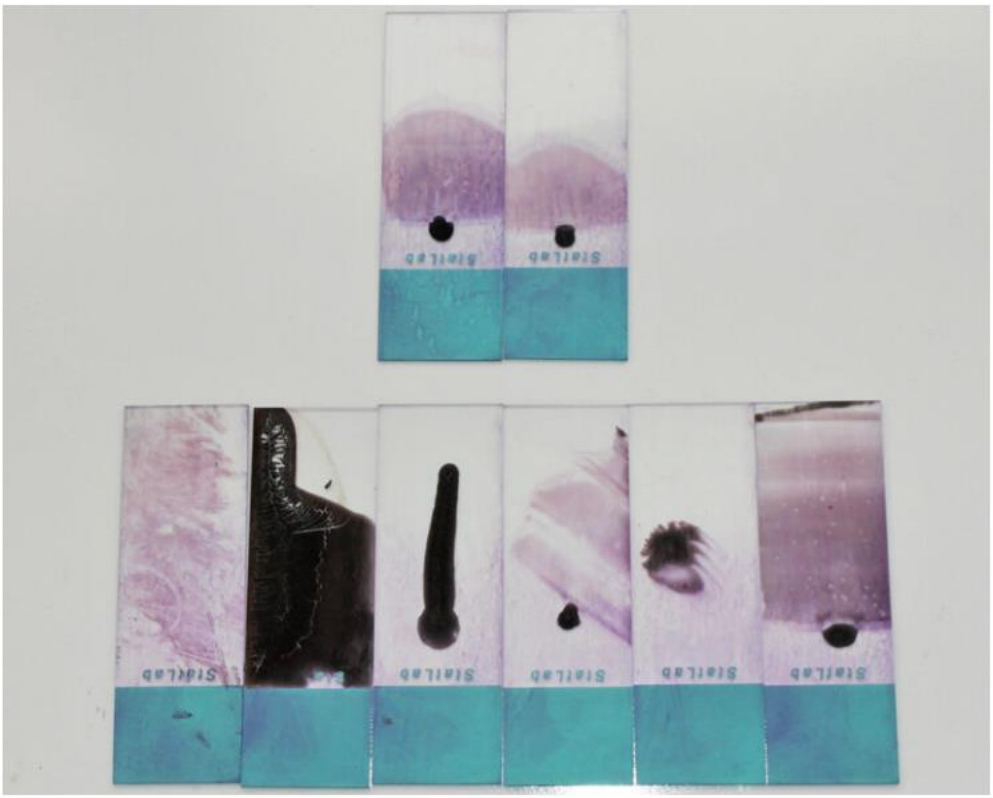
9. Rinse slide or rack of slides by dipping or swishing in distilled or deionized water. Air dry slides or use warm air blower before mounting with oil and reading.

****NOTE: Tone and depth of color may be adjusted as follows 1) Too blue—decrease 1 dip in solution III. 2) Too red—decrease 1 dip in solution II. 3) Too dark—decrease by 1 or 2 dips in both solution II and III. 4) Too light—increase by 1 or 2 dips in both solution II and III is required. Always dip slide in solution I a minimum of 5 (1 second dips). For bone marrow smears***

10. After rinsing and drying, the slide was examined under the microscope under low and oil immersion power.

Examine Slide Quality

The top two slides are examples of proper blood smears.
The bottom row displays poorly prepared blood smears.



Troubleshooting

Problem	Solution
Too thick or too short	Try decreasing the angle of the spreader slide or decreasing the size of the initial blood drop.
Too thin or too long	Try increasing the angle of the spreader slide or decreasing the size of the initial blood drop.
Streaking	Try cleaning the edge of the spreader slide.

EXPECTED RESULTS: The reaction of cytoplasm to staining is subject to many variables. Since the majority of staining occurs during the buffering stage, the variable of greatest magnitude is the resultant pH of the stain/buffer mixture at the cellular surfaces. The overall color of the red blood cells is a guide to stain quality and should be used in adjusting staining times for desired results.
ERYTHROCYTES: Pink-tan with degrees of chromasia.
WBC'S: Nuclei with bright, bluish-purple chromatin, light blue nucleoli.
LYMPHOCYTES: Clear blue cytoplasm, red-purple granules may be present.
MONOCYTES: Mosaic of pink and blue cytoplasm, azure granules usually present.
NEUTROPHILS: Light purplish-pinkish or lavender granules in cytoplasm.
EOSINOPHILS: Bright red or reddish-orange granules in cytoplasm.
BASOPHILS: Deep purple and violet-black granules in cytoplasm.
PLATELETS: Clearly demarcated red-purple granules in light blue cytoplasm.
STORAGE AND EXPIRATION:
Store stains at room temperature (70-77.9 °F/ 20-25.5 °C). Maximum intended shelf life is printed on the label.
Expected Results
• RBCs: Mild anisopoikilocytosis; normochromic, normocytic
• WBCs: Neutrophils 65%, Lymphocytes 30%, Eosinophils 3%, Monocytes 2%
• Platelets: Adequate, normal morphology
• No parasites seen

Peripheral Blood Smear used for
• Diagnosed types of anemia (e.g., iron deficiency, megaloblastic, anemia of chronic disease or related to hemoglobinopathy)
• Identified leukemias, infections, or parasitic infestations
• Evaluated platelet count and morphology
• Detected blood parasites (e.g., Plasmodium spp.)
REFERENCES:

1. **Kit insert of DIP STAIN KIT AND REAGENTS (16/ 128 OZ.) DIP STAIN KIT VDS-100 (16 OZ.),**
2. Wright, J.H., Med. Res., 7 138 (1902)
3. Clinical Diagnosis by Laboratory Methods, 15th ed., Davidsohn, I., and Henry, J.B., Saunders, 1974.
4. Wintrobe, M.M., 7th ed., Lea & Febiger, Philadelphia: Clinic Hematology.