HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Hematology)		
Document:	Internal Policy and Procedure		
Title:	Examination of Peripheral Blood Smear		
Applies To:	All Hematology Staff		
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1. PURPOSE:

1.1 Evaluate RBC morphology (cell size, shape, and consistency).
1.2 Identify types and percentages of WBC.
1.3 Determine if an adequate supply of platelet is present in the blood.
1.4 Check results when abnormalities show up on the automated blood counter.
1.5 Confirm results when the doctor suspects abnormal WBC, RBC, and or platelets are present.

2. DEFINITIONS:

N/A

3. POLICY:

3.1 The criteria needed to be met for performing of blood smears.

4. PROCEDURE:

4.1 **Specimen requirement:**
- 4.1.1 Specimen Type: Venous blood
- 4.1.2 Tube Type: K-EDTA (Lavender) Tube
- 4.1.3 Amount Required: 1.8 ml
- 4.1.4 Delivery Arrangement: As soon as possible and should not exceed 1 hour from collection time.
- 4.1.5 Temperature Restriction: 37°C
- 4.1.6 Stability: 2 hours at RT
- 4.1.7 Unacceptable Specimen:
  - 4.1.7.1 Clerical Error
  - 4.1.7.2 Clotted

4.2 **Blood Smear will be done for the CBCs that meet the following criteria:**
- 4.2.1 Hemoglobin < 8.0 gm or > 18 gms% (excluding newborns).
- 4.2.2 Any MCV < 70 or > 105.
- 4.2.3 WBC<3,000 or > 20,000. 6.5. Lymphocytes > 50% (excluding children < 3)
- 4.2.4 Lymphocytes > 50% (excluding children < 3)
- 4.2.5 Any immature or unidentifiable cell.
- 4.2.6 Platelet count < 80,000 or > 600,000.

4.3 **Standard Procedure for Making Blood Smear:**
- 4.3.1 Place not more than 10 uL drop of blood about 1 cm from the frosted end of a clean slide that is on a flat surface.
- 4.3.2 With the thumb and forefinger of the right hand hold the end of a second slide (Spreader) against the surface of the first slide at an angle of 30-45 degrees.
- 4.3.3 Draw it back to contact the drop of blood. Allow the blood to spread and fill the angle between the two slides.

4.3.4 Push the "Spreader "slide at a moderate speed forward until all of the blood has been spread into a moderate thin film.

4.4 **Manual Staining of Peripheral Blood Smear:**

4.4.1 Make a thin blood smear
4.4.2 Air dry
4.4.3 Fix in methanol for 2 minutes
4.4.4 Dip in Giemsa (Working Solution) stain jar for 30 minutes
4.4.5 Wash with tap water
4.4.6 Allow to dry

4.5 **Steps in the Examination of Blood Smear:**

4.5.1 Scan the smear at low power field to :
4.5.1.1 Locate the optimal area for examination at oil immersion field.
4.5.1.2 Check the feathered edge.
4.5.1.3 Evaluate the distribution of WBC on the smear (To check that leucocytes are uniformly distributed throughout the and that excessively concentrated at the feather edge).

4.6 **Note :**

4.6.1 In some ill- made smears, most of the leucocytes are dragged to the end of the smear. In this case, prepare another smear and move the spreader slide more quickly which should ensure that WBC are adequately.
4.6.2 Perform the differential count (Oil immersion field).
4.6.3 Assess PLT numbers (HPF). Average in 10 high-power field multiplied by 15000 = PLT count /uL of blood.
4.6.4 Perform morphology (Assessment of blood cells).

4.7 **Test limitation:**

4.7.1 The film should not cover the entire surface of the slide.
4.7.2 In a good film, there is a thick portion and a thin portion and a gradual transition from one to the other.
4.7.3 The film should have a smooth, even appearance and be free from ridges, waves, or holes.
4.7.4 The end of the smear should be smooth and even
4.7.5 The edge of the "Spreader" must be absolutely smooth. If it is rough , the film will have ragged tails and will show many leucocytes)

5. MATERIALS AND EQUIPMENT:

5.1 **Preparation of Stains:**

5.1.1 Wrights stain : Ready to use It is recommended to filter all stains before use

5.2 **Preparation of Buffer (PH6.8DW):**

5.2.1 1- Stock Solution A
5.2.1.1 KH2PO4 : 9.1 gm
5.2.1.2 D.Water : 1000 ml
5.2.2 Stock Solution B
5.2.2.1 Na2HPO4 : 11.9 gm
5.2.2.2 D.Water : 1000 ml
5.2.3 Then prepare as:
5.2.3.1 Stock Solution A :50.8 ml
5.2.3.2 Stock Solution B : 49.2 ml

5.3 **Prepare fresh buffer every day (Working Solution):**

5.3.1 Giemsa Stain 25 ml
5.3.2 Buffer Water 100 ml
5.3.3 Mix well
5.3.4 Use for 2-3 days

5.4 Leishman Stain: Ready to use

6. RESPONSIBILITIES:

6.1 Clinical pathologist

7. APPENDICES:

N/A

8. REFERENCES:

8.1 McPherson RA and Pincus MR. Henry's Clinical Diagnosis and Management by Laboratory Methods.
8.2 21st Ed. Philadelphia, Pa: WB Saunders: 2007:461-2.
8.3 Sweet Haven Publishing Services, Copyright @ 2004 Sweet Haven Publishing Services

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Fatma Hassan Ahmed	Clinical Pathologist		January 06, 2025
Reviewed by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 08, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 09, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 12, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025