National Standard Operating Procedure for Complete Blood Count (CBC) Using 3-Part Hematology Analyzer (Horiba ABX Micros ES 60)

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Acronyms:

CBC	Complete Blood Count
EDTA	Ethylenediaminetetraacetic Acid
QC	Quality Control
IQC	Internal Quality Control
WBC	White Blood Cells
DIFF	Differential White Blood Cell
RBC	Red Blood Cells
HGB	Hemoglobin
HCT	Hematocrit
PLT	Platelets
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
ICSH	The International Council for Standardization in Haematology

1. Purpose

This procedure provides standardized instructions for performing Complete Blood Count (CBC) analysis using the Horiba ABX Micros ES 60 automated hematology analyzer in medical laboratories under the Ministry of Health.

2. Scope

This document is applicable for all medical laboratories operating the Horiba ABX Micros ES 60 hematology analyzer for CBC testing under MOH and other collaborative governmental and non-governmental health institutions.

3. Definitions:

1. **Complete Blood Count (CBC):** A standardized hematological test that quantitatively measures the cellular components of blood, including hemoglobin concentration, red blood cell count and indices, white blood cell count (with or without differential), and platelet count, performed using validated automated hematology analyzers in accordance with ICSH recommendations.
2. **Differential white cells count (DIFF):** A blood test that measures the percentage or number of each of the five types of white blood cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils)
3. **Quality control (QC)**: the process of monitoring a test during its development to ensure it meets predefined standards and customer expectations

4. Procedure

4.1. Clinical background:

Complete Blood Count (CBC) is an essential and widely used hematological test that quantitatively assesses cellular components of blood including red blood cells, white blood cells, and platelets. The CBC reflects the functional status of bone marrow and related hematopoietic tissues.

CBC parameters are critical for diagnosing and monitoring diseases such as anemia, infections, inflammation, hematologic malignancies, and systemic disorders. Key red cell indices (MCV, MCH, MCHC) provide insights into red cell size and hemoglobin content, while white blood cell differentials assist in identifying immune status and hematologic abnormalities. Variations outside reference ranges guide further clinical evaluation and intervention.

4.2. Principle:

The ABX Micros ES 60 uses automated cell counting and sizing to analyze whole blood specimens. Each blood cell suspended in a conductive diluent acts as an insulator, momentarily increasing electrical resistance as it passes through a narrow aperture between two submerged electrodes. This change produces an electronic pulse; the number of pulses corresponds to cell count, and the amplitude of each pulse is proportional to the cell's volume.

The electrical pulses are converted from analog to digital signals and processed by an embedded system. The analyzer uses two dedicated chambers: one for red blood cell (RBC) and platelet (PLT) analysis, and another for white blood cell (WBC) and hemoglobin (HGB) measurement.

Hemoglobin concentration is measured spectrophotometrically after lysing the red cells to release hemoglobin, which is converted into a measurable form. This dual approach provides comprehensive complete blood count parameters, including cell counts, size distributions, and hemoglobin concentration.

Component Measured	Principle of Technology	Description
WBC, RBC, PLT	DC Impedance Method (Direct Current) with dual aperture volumes	The analyzer quantitatively measures blood cells using the DC impedance method. Cells pass through apertures where changes in electrical resistance generate pulses proportional to cell volume. Separate counting apertures and dilution ratios are used for RBC/PLT and WBC to enhance accuracy. This method provides enumeration and sizing of blood cells including white blood cells, red blood cells, and platelets.
HGB	Spectrophotometry (non-cyanide hemoglobin method)	Hemoglobin concentration is measured by lysing the blood sample with a dedicated reagent that releases hemoglobin, converted to methemoglobin.

Component Measured	Principle of Technology	Description
		The absorbance of this complex is measured spectrophotometrically at a specific wavelength to determine hemoglobin concentration accurately without the use of toxic cyanide reagents.
3-Part Differential (LYM, MON, GRA, LYM%, MON%, GRA%)	DC Impedance Measurement Combined with Specialized Reagent Lysis and Volume Discrimination	White blood cells are differentiated into lymphocytes, monocytes, and granulocytes by their impedance characteristics following selective lysing and measurement of cell volume distribution. The system computes both absolute counts and percentages for these subtypes, enabling detailed leukocyte profiling beyond a 3-part differential.

4.3. Pre – analytical stage:

4.3.1. Sample Requirements:

1) Collect whole blood via venipuncture into K2-EDTA or K3-EDTA anticoagulant tubes, ensuring the anticoagulant-to-blood ratio is maintained as specified by manufacturers.
2) Specimen minimum volume should be 1 mL. As the analyzer use 50 µL for the analysis.
3) Mix tubes gently with 10 inversions immediately post-collection to prevent clot formation.
4) Reject specimens with visible clots or incorrect volume.
5) Process fresh specimens preferably within 8 hours; store samples between 2–8°C if delay is unavoidable up to 48 hours for stable parameters.

4.3.2. Materials and Reagents:

Item Type	Product Name (Example/Type)	Purpose
Diluent	ABX Minidil LMG (10 Liters or 20 Liters)	Used for RBC/PLT dilution, sleeving, and rinsing.
Lysing Agent	Minilysebio, ABX Minilyse LMG or ABX Alphalyse	Used to prepare the sample for WBC counting and HGB measurement (by lysing RBCs).
Cleaning Agent	ABX Miniclean (1 Liter), ABX Cleaner (0.5 Liter)	Used for cleaning procedures.
Concentrated Cleaner	ABX Minoclair	Used for Extended Concentrated Cleaning (also 12% active chlorine bleach solution).
Quality Control Material	ABX Mintrol 16 Twin Packs	Used in Quality Control procedure.
Calibrator	ABX Minocal	Used in Calibration procedure.

4.3.3. Safety Precautions:

1) Treat all blood samples and reagents as potentially infectious and handled under standard biosafety protocols.
2) Avoid contact with electrical components and moving parts during operation to prevent injury or equipment damage.
3) Adhere to local regulations for disposal of biohazardous materials and waste.

4.3.4. Instrument Setup and Start-Up

1) Place the instrument on a stable, leveled bench, away from dust, direct sunlight, and vibration.
2) Confirm the environment meets temperature (16-30°C) and humidity (<85%) specifications.

3) Turn on the instrument; perform the automatic startup cycle to validate instrument readiness.
4) Check waste container levels and reagent levels prior to sample analysis.

4.3.5. Physical Reagent Replacement:

1. **Diluent Container:** Unscrew the stopper assembly from the new container using the provided opening tool. Transfer the existing reagent straw from the old container into the new one.
2. **Lyse/Cleaner Bottles:** Install a new reagent straw and the bottle stopper onto the new bottle. Connect the appropriate hydraulic tube: **White tube for Lyse**, **Blue tube for Cleaner**.

Step	Action	Screen Navigation / Input
1.	**Access Maintenance Menu**	Navigate to the **Maintenance menu** from the Main Screen.
2.	**Select Reagents Tab**	Select the **Reag. (tab)**.
3.	**Select Reagent Type**	In **bottle mode**, select the radio button corresponding to the reagent container being replaced (**LYSE**, **DILUENT**, or **CLEANER**).
4.	**Initiate Replacement**	Press the **Replace** button.
5.	**Confirm Selection**	Press **Validate** in the contextual toolbar.
6.	**Access Edit Screen**	In the **Edit reagent screen**, press **Edit** in the contextual toolbar.
7.	**Enter Lot Number**	Press the **Lot field** to activate the keyboard. Enter the **LOT number (Barcode 1)**.
8.	**Enter Reagent ID**	Enter the **ID of the reagent (Barcode 2)**.
9.	**Finalize Registration**	Press **Validate** in the contextual toolbar to permanently save the new lot information.

4.3.6. Post-Replacement Verification

1. **Prime Reagents:** Execute the **Priming of the Reagents** function to ensure the new fluid is fully introduced into the hydraulic lines. (Consult the *Hydraulic Maintenance* section for this specific command).
2. **System Startup:** Run a **Startup Cycle** (recommended after Lyse or Cleaner replacement).
3. **Quality Control:** Run the appropriate **QC material** and verify results are within the established acceptance range.
4. **Background Check:** Run a **Background Check** and ensure parameters (especially PLT) meet acceptable limits before performing patient analysis.

4.4. Analytical stage:

4.4.1. Internal Quality Control:

Quality control allows user to monitor a set of analyses based on known sample values and ranges over a period of months. Three control levels are available for CBC test, which they can be simultaneously active allowing QC on three levels.

One control lot can be active and is used as a reference to trigger alarm.

The following controls are used for Horiba ABX ES60:

- ABX Minotrol 16 Twin pack 2N
- ABX Minotrol 16 Twin pack 2L
- ABX Minotrol 16 Twin pack 2H

4.4.1.1. Download a Control File from Horiba Website:

1. Connect to www.Horiba.com
2. Enter Documentation Database
3. Select Hematology > quality control target
4. In quality control target select Minotrol 16
5. Select instrument name (Micros ES 60)
6. Click on your machine lyse (e.g. Minilysbio)

7. On control target file click the required file in button Download target values column according to your control lot number (file contains lot number, name, expiration name and target value).
8. On control target file click the required file in button Download target values column according to your control lot number (file contains lot number, name, expiration name and target value)
9. Download the control file on the USB flash.
10. Make sure a USB flash is free of any virus

4.4.1.2. Import the Control Lot from the Flash to Instrument:

This procedure is executed via analyzer software to import the new lot number.

1. Access Main screen
2. Quality control menu
3. QC tab
4. Press **create** on QC tab or **edit** on quality control screen
5. Press **Import QC**
6. Insert the USB flash (that containing the control file) in the front slot of the instrument.
7. Wait 5 minutes and press **validate** in the dialog box
8. Select the required control lot or select **All the QC**
9. Press **Import QC** button.

4.4.1.3. Activate and deactivate lot number:

After importing the control lots, it will appear automatically in **pending status. Activate the new lot and** deactivate the old lot (only one control lot can be active at a time).

1. Access Main screen
2. Quality control menu
3. QC tab
4. Select control and press **view**

5. Press **Active** (when you activate control lot and another one is already activated automatically will return to **pending status)**
6. Press **validate**

4.4.1.4.Running Quality Control samples:

- Before analyzing any patient sample, the quality control analyses for the three levels of control (low, normal and high) must be performed.
- Check control status in the QC tab (if it is same lot number on the analyzer or need to create QC lot number).
- Identify control lot:
  - Main screen
  - Sample identification menu
  - Select **sample ID** field to activate it
  - Read the control barcode label with barcode reader or entered manually
  - Press **Validate**
- Remove the QC from the refrigerator and allow it to warm at room temperature for 15 minutes. (Follow the specific instructions that detailed in package insert).
- Mix sample gently.
- Place the sample in the correct tube holder position.
- Close the tube holder door.
- Make sure that the result is within acceptable values on the control sheet.

NOTE:

- Use manufacturer-approved quality control materials with defined target ranges.
- Run at least three levels of QC samples daily or per shift as per laboratory policy.

- Validate QC results before starting patient samples; rerun or troubleshoot if QC results are out of acceptable ranges. Refer to Internal Quality Control SOP.
- Record QC data electronically or in a designated log for traceability.

4.4.2. Sample Analysis:

1) Mix the sample gently prior to placing it in the tube holder.
2) For CT (Close Tube) model: Place capped sample tube in the holder, close door for cap piercing.
3) Select sample identification via keyboard or barcode reader.
4) Start analysis via software interface and follow on-screen instructions.
5) The analyzer aspirates 10µL blood, performs two dilution steps, counts cells via impedance, and measures hemoglobin photometrically.
6) Results display on the screen and print automatically if configured. Verify absence of alarms.

4.4.3. Calculations:

Parameters like MCH, MCHC, and hematocrit are calculated automatically based on measured values using standard formulas.

4.5. Post – analytical stage:

4.5.1. Result Interpretation

1) Review results against established reference range intervals.
2) Be aware of limitations and interfering substances (e.g. clots, agglutination, lipemia, hemolysis) that may affect accuracy.
3) Investigate abnormal or flagged results with manual methods or repeat testing if necessary. Refer to Table.1 for Specific Parameter Flags, Table.2 for Differential White Blood Cell (DIFF) Flags and Table.3 for General Flags.
4) Report critical or abnormal results promptly following laboratory policy.

4.5.2. Reference Intervals:

	0-1 day		2 days – 90 days		91 days – 1 yr		2-12 yrs		Female 13-99yrs		Male 13-99yrs	
	L	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**
RBC (10^12/L)	4.6	6	3	5.4	3.7	5.3	2.9	5.3	4.1	5.4	4.5	5.8
HGB (g/dL)	12.5	19.5	10	14.1	10.5	13.5	11.5	15.5	11	14.5	11.5	15.5
HCT (L/L)	0.44	0.64	0.31	0.55	0.33	0.39	0.35	0.45	0.34	0.43	0.35	0.45
MCV (fL)	98	118	76	96	70	86	73	95	78	95	78	96
MCH (pg)	31	37	28	36	23	31	24	33	26	33	26	33
MCHC (g/L)	30	36	31	35	31	35	31	35	31	35	31	35
RDW	11.5-16.5											
WBC (10^9/L)	6	22	6	20	6	17.5	4.5	14.5	2.4	9.5	2.2	10
NEUT # (10^9/L)	4.5	12	1	8.5	1.5	8.5	1.4	9	1	4.8	1	5
LYMPH # (10^9/L)	1	6	4	13.5	4	10.5	1.9	9.8	1.2	3.8	1.2	4
MONO # (10^9/L)	0.2	1.6	0.2	1.6	0.2	1.3	0.1	1	0.2	0.5	0.2	0.6
EOS # (10^9/L)	(0-90 d) 0.1-0.6			(91 d – 13yrs) 0.1-0.8			(14-99yrs) 0.1-0.5					
BASO # (10^9/L)	0-0.2											
NRBC # (10^9/L)	(0 day) 0-1				(1 day) 0-0.5				(2-5 days) 0-0.1			
PLT (10^9/L)	(Female 0-99 yrs) 150-450						(Male 0-99 yrs) 140-400					
MPV (fl)	(Female 0-99 yrs) 7-10.5						(Male 0-99 yrs) 7.2-10.5					
RETICS (10^9/L)	(0-14 days) 97-385						(15 days-99 yrs) 20-150					
RETICS %	(0-14 days) 2-7						(15 days-99 yrs) 0.2-2					

Reference ranges for Omani population adopted from SQUH, 1998 approved by MOH

4.5.3. Method Performance Specifications and Limitations:

A. **Analytical Sensitivity (Lower Limit of Detection)**

The analytical sensitivity is defined by the instrument's measuring ranges and background specifications, which establish the effective lower limit of detection.

Parameter	Lower Limit (Reference Range)	Background Limits (Maximum Acceptable)
WBC	1.0×10^3/µL	≤0.1×10^3/µL
RBC	0.2×10^6/µL	≤0.02×10^6/µL
HGB	1 g/dL	≤0.1 g/dL
PLT	20×10^3/µL	≤5×10^3/µL

B. **Analytical Specificity (Interference and Cross-Reactivity)**

The following conditions are explicitly cited in the manual as interfering factors that can compromise the analytical specificity and accuracy of results, requiring technician intervention.

Parameter	Interfering Factor/Condition	Observed Effect on Result	Indicated Action (Limitation)
WBC	**Nucleated Red Blood Cells (NRBCs)**	Falsely elevated WBC count.	WBC count must be corrected manually using peripheral blood smear findings.
	Lysis-Resistant RBCs	Falsely elevated WBC count.	Indicated by the "I" Flag. Rerun sample; if flag persists, examine smear and correct.
RBC / Indices (MCV, MCH)	**Cold Agglutinins**	Falsely decreased RBC count; Falsely increased MCV and MCH.	Indicated by the "Q" Flag. Requires warming of the sample to 37°C and rerun.
HGB	**Lipemia (Severe Turbidity)**	Falsely elevated HGB	Indicated by the "G" Flag. Requires confirmation and

Parameter	Interfering Factor/Condition	Observed Effect on Result	Indicated Action (Limitation)
		concentration due to turbidity.	appropriate correction technique (e.g., plasma blank).
PLT	**Platelet Aggregates, Clots, Fibrin**	Falsely decreased Platelet count.	Indicated by the "P" Flag. Requires smear review and may necessitate collection of a new sample in an alternate anticoagulant (e.g., Sodium Citrate) for verification.

C. **Reportable Scope (Linearity and Dilution)**

The measurable and reportable scope is defined by the linearity limits of the instrument.

Parameter	Linear Measuring Range (Reportable Scope)	Dilution/Reporting Instructions (When Range is Exceeded)
WBC	1.0 - 99.9 * 10^3/µL	If a result is flagged with a **"*" (Reject)** and exceed the linear upper limit, the sample **must be diluted** with diluent and rerun. The final result must be calculated by multiplying the displayed value by the dilution factor.
RBC	0.2 - 8.0 * 10^6/µL	
HGB	1 - 25.0 g/dL	
HCT	5 - 70 vol%	

Parameter	Linear Measuring Range (Reportable Scope)	Dilution/Reporting Instructions (When Range is Exceeded)
PLT	20 - 999 * 10^3/µL	

D. **General Method Limitations**

- The instrument's 3-part differential categorizes WBCs based solely on **volume (size)**. It **cannot replace a manual microscopic differential**, which relies on visual morphology and staining characteristics.
- Results outside the established measuring range (linear scope) are **rejected with a "*"** and **cannot be validated** without proper dilution and calculation.
- All samples must be processed within the validated time frame using the specified **EDTA K3** or **EDTA K2** anticoagulant to prevent cellular degradation or morphological changes that compromise accuracy.

5. Responsibilities

1. Laboratory Program committee:
    - Ensure that document is approved by ministry of Health and distributed to all institutions
2. Task force creating the document:
    - Ensure that the document is in accordance with Ministry of Health guidelines and is evidence based
    - Ensure that the document is sent to the relevant individuals in different health institutions for review
    - Ensure that the document is modified after receiving relevant feedback from reviewers
    - Ensure that the document is updated where appropriate
3. Laboratory Head/ supervisor
    - Review the document and provide feedback where appropriate
    - Ensure that local standard operating procedures are in line with document
    - Ensure that all staff are trained and adhere to the document
4. Technical staff
    - Be familiar with this document and adhere to it where applicable

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	August 2031

7. References

- International Council for Standardization in Haematology (ICSH). *ICSH recommendations for the standardization of complete blood count (CBC) and related parameters*. Int J Lab Hematol. 2014;36(6):598–606.
- HORIBA ABX SAS (HORIBA Medical). *ABX Micros ES 60 Hematology Analyzer User Manual*. 2019. Entire document, specifically Sections 4, 6, and Maintenance Procedures.

8. Annexes

Table.1: Specific Parameter Flags

The following flags indicate that the result for a specific parameter may be inaccurate or that the analyzer has detected a morphological abnormality in the sample, requiring further investigation.

Flag	Parameter(s)	Detail/Interpretation	Indicated Action
P	PLT, MPV, PCT, PDW	Platelet Problem: Possible aggregation, clot, or fibrin.	Check sample quality. Rerun the sample. If the flag persists, check for aggregates on the smear, and perform a manual count or alternative method.
Q	RBC, HGB, HCT, MCV, MCH, MCHC, RDW	RBC Problem: Sample abnormality (e.g., cold agglutinin, abnormal HGB, hemolysis, lipemia).	Check sample quality and appearance. If the flag persists, perform a manual count and smear review.
G	HGB, HCT, MCH, MCHC	Hemoglobin Problem: High or low HGB results with discrepancy in other RBC parameters.	Check samples for hemolysis or lipemia.
L	HCT, MCV, MCH, MCHC, RDW	Erythrocyte Abnormalities: High background noise in the RBC channel or presence of interfering cells (e.g., giant platelets).	Check the sample and the reagent quality.

Table.2: Differential White Blood Cell (DIFF) Flags

These flags relate to the 3-part differential (Lymphocytes, Monocytes, Granulocytes) and suggest an abnormal cell population or an interference that affects the white blood cell (WBC) count and/or differential.

Flag	Parameter(s)	Detail/Interpretation	Indicated Action
O	LYM, MON, GRA	Incomplete Separation: The separation line between two populations (LYM/MON or MON/GRA) is not complete.	The sample must be checked. Rerun the sample. If the flag persists, perform a blood smear examination and manual differential count.
N	WBC	Abnormal Granulocyte Population: May indicate the presence of abnormal granulocytes (e.g., left shift, immature cells).	Rerun the sample. If the flag persists, perform a blood smear examination and manual differential count.
T	LYM	Abnormal Lymphocyte Population: Suggests the presence of atypical or reactive lymphocytes.	Rerun the sample. If the flag persists, perform a blood smear examination and manual differential count.
I	WBC	WBC Interference/Suspect: Presence of nucleated red blood cells (NRBCs) or unlysed red blood cells (RBCs) interfering with the WBC count.	Rerun the sample. If the flag persists, examine a peripheral blood smear to confirm NRBCs and correct the WBC count if necessary.

Table.3: General Flags

Flag	Parameter(s)	Detail/Interpretation	Indicated Action
*	All parameters	Parameter Reject: Result is not consistent or out of the measurement range.	Check the sample for clots or bubbles. Check instrument maintenance and reagents. The sample must be re-analyzed.
!	All parameters	Suspicion: Result is potentially questionable.	The sample must be re-analyzed.