

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات



Hematology Department

STANDARD OPERATING PROCEDURES (SOPs)

April 2016

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

أهلاً:

❖ إلى أرواح شهدائنا الأبرار وإلى أرواح شهدائنا خاصة في مهنة التحاليل الطبية

الشهيد/رامي السلوت

والشهيد/حسام راضي

❖ وكذلك إلى روح الزميل/حسام أبو شمالة

وروح الزميل/اديب علوان

❖ وإلى مرضانا الزملاء/مهند الشنطي

والزميل محمود الجرو (وهو عضو فريق اعداد SOPs الميكروبيولوجي)

نسأل الله لهم الشفاء العاجل وللشهداء الفردوس الأعلى .

❖ إلى كل من ينتمي إلى هذه المهنة الإنسانية من زميلات وزملاء

نهدي هذا العمل رمزاً للوفاء

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شكر وتقدير

يتقدم فريق عمل مختبرات وبنوك دم المستشفيات بجزيل الشكر والتقدير إلى كل من الأخ الدكتور/ يوسف ابو الريش "وكيل وزارة الصحة"، وإلى الأخ الدكتور/ عبد اللطيف الحاج" مدير عام المستشفيات" لما قدموه من دعم وتسهيل من اجل انجاز هذا العمل وكذلك إلى الاستاذ/ شاكر ابو شعبان" مدير دائرة مختبرات وبنوك دم المستشفيات سابقا" لما قدمه من دعم ومشورة في جميع مراحل انجاز هذا العمل . كما نتقدم بجزيل الشكر والتقدير الى كل من فريق الاعداد وفريق المراجعة لما بذلوه من جهد من اجل اتمام هذا العمل وكذلك الى جميع الاخوة العاملين في مختبرات وبنوك دم المستشفيات اللذين سيكون لهم الدور الرائد في تطبيق هذه الإجراءات. وكذلك نتقدم بجزيل الشكر والعرفان إلى مؤسسة العون الطبي للفلسطينيين لرعايتهم طباعة هذا الدليل.

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بسم الله الرحمن الرحيم

قال تعالى: (و قل اعملوا فسيرى الله عملكم ورسوله و المؤمنين) صدق الله العظيم

الأخوة والأخوات الزملاء الأكارم...

السلام عليكم ورحمة الله وبركاته..... أما بعد

انه لمن دواعي سروري أن أضع بين أيديكم (دليل طرق الفحوصات المخبرية الموحد)
(Standard Operating Procedure SOPs) والذي يتضمن توثيقاً وتفصيلاً لطرق عمل
الفحوصات التي تقدمها مختبرات وبنوك دم المستشفيات، وذلك من أجل تنظيم العمل وضمان جودته
في جميع جوانب خدمات المختبرات وبنوك الدم.

تأتي الحاجة إلى هذا العمل تأكيداً وترسيخاً لمبدأ العمل بروح الفريق الواحد وتوثيق وتوحيد طرق
العمل المبني على أسس علمية والذي تنتهجه وزارة الصحة والإدارة العامة للمستشفيات في كافة
مناحي العمل وتطلعاً للراقي بمهنة التحاليل الطبية خاصة، و بجودة الخدمات الطبية المقدمة في الوزارة
بشكل عام.

كما يعتبر هذا الدليل الأول والموحد لجميع مختبرات وبنوك دم المستشفيات و الذي سيكون له بمشيئة
الله الأثر الايجابي على جودة الخدمات المخبرية ومجمل الأداء الفني من أجل الارتقاء بالخدمات
المقدمة للمواطن.

في النهاية لا يسعني إلا أن أتقدم بخالص الشكر و التقدير لفريق عمل دائرة مختبرات وبنوك دم
المستشفيات وجميع من ساهم في انجاز هذا العمل سواء في الإعداد أو المراجعة أو الطباعة والإخراج.

د. عبد اللطيف الحاج

مدير عام المستشفيات

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فريق الإعداد

الاسم	مكان العمل
فريد حسن ابو العمرين	مجمع الشفاء الطبي
أمني سهيل الهندي	مجمع الشفاء الطبي
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فريق المراجعة

الاسم	مكان العمل
عميد مشتهى	الإدارة العامة للمستشفيات
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Contents

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2	Sysmex KX-21 Hematology Analyzer	SP 02
3	Cell-Dyn 1800 Hematology Analyzer	SP 03
4	CELL DYN 3500-3700 Hematology Analyzer	SP 04
5	ACL Automated Coagulation Analyzer (Factor Deficient Plasma VIII, IX, XI, XII Tests- HemosIL®)	SP 05
6	ACL Automated Coagulation Analyzer (PT-FIB/APTT Tests- HemosIL®)	SP 06
7	CoaLAB 1000 Automated Coagulation Analyzer	SP 07
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ABX Micros ES60 Hematology Analyzer

SOPs\ HGA \.....H/ Haem /01

Version: ...1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

The ABX Micros ES60 performs automated blood counts and requires no manual operations for aspirating blood, dilutions, measuring, calculations, print-outs and computer transfer of data. It measure the following 18 hematologic parameters: WBC , LYM% , LYM# , MON% , MON# , GRA% , GRA# , PDW* , PCT* , RBC , HGB , HCT , MCV , MCH , MCHC , RDW , PLT , MPV.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

About 2-3 ml of venous blood collected into EDTA tubes.

Specimens should be transported at room temperature 18 - 26°C and can be store in the refrigerator of 2 - 8°C up to 6 hours. If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis.

Specimen reception:

Reception of request and sample should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Criteria for rejection haematology specimens

1. When the identification is missing /inadequate.
2. Insufficient quantity.
3. Inappropriate container.

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4. Inappropriate transport/storage.
5. Unknown duration of delay.
6. Clotted sample.

Equipment & Items required:

Diluent: ABX Minidil LMG (10L).

Cleaner: ABX Miniclean (1L).

Lyse: ABX Minilyse LMG (1L).

Abbreviations:

CBC: Complete blood count.

EDTA: Ethylene diamine tetra acetic acid

WBC: White Blood Cells

LYM%: Lymphocyte percentage

LYM#: Lymphocyte absolute value

MON%: Monocyte percentage

MON#: Monocyte absolute value

GRA%: Granulocyte percentage

GRA#: Granulocyte absolute value

RBC: Red Blood Cells

HGB: Hemoglobin

HCT: Hematocrit

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

RDW: Red cell Distribution Width

PLT: Platelets

MPV: Mean Platelet Volume

PDW*: Platelet Distribution Width

PCT*: Plateletcrit

NRBCs: Nucleated Red Blood Cells

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Procedure:

1. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
2. Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
3. The instrument performs an initialization phase for the internal electronics. Please wait.
4. Once the initialization phase is complete, the ABX Micros ES60 OT/CT will automatically run a startup cycle.
5. If the ABX does not automatically run a startup cycle after the initialization phase is completed, press "Startup" button in the "Status" area to initiate a startup cycle.
6. Then, the instrument will perform a blank cycle for a reference blank count (an analysis cycle based on reagents without any blood sample).
7. Check and verify that the reference blank counts do not exceed the following parameter limits: WBC < 0.3, RBC < 0.02, HGB < 0.3, PLT < 10 then: Press "OK" button to validate blank results.
8. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
9. Entering patient ID, sample ID, Patient name, etc
10. Follow the indications displayed in the "Sample analysis" dialog box to run the analysis.
 - a. Mix the sample gently and thoroughly.
 - b. Remove the cap from the sample tube.
 - c. Place the sample beneath the sampling needle.
 - d. Raise up the tube so that the sampling needle lowers into the blood and press the manual sample bar.
 - e. The analysis cycle will begin.
11. When the analysis is completed, the "Sample analysis" dialog box is closed and results are displayed in the "Result display" menu for print out.
12. Dilute the sample if White blood cell counts $\geq 100,000$ /mm³ and platelet counts $\geq 1,000,000$ /mm³ are outside the linearity specifications of the instrument.

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Quality control procedures:

1. At the beginning of each work shift all parameters are tested with blood control.
2. The 3 levels include: Abnormal Low, Normal, Abnormal High
3. Controls are stored at 2-8°C and brought to room temperature on a roller mixer before use .
4. Controls are gently inverted many times according to the manufacturer's instruction before use.
5. From the RUN screen, press [SPECIMEN TYPE].
6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key.
7. Control values must be within three standard deviation, otherwise the measurement has to be repeated, if the control still out of range:
 - a. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
 - b. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable. If this does not solve the problem:
 - ✓ Prepare new control(s) and try again.
 - ✓ If the controls are still out, inform your supervisor to check the operator's manual, or recalibrate instrument and If controls are still out,. Contact Medical Maintenance where applicable, or servicing engineer.
8. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).

Linearity:

PARAMETER	LINEAR RANGE
WBC ($10^3/\text{mm}^3$)	0.4 – 106.6
RBC ($10^6/\text{mm}^3$)	0.2 – 8.1
HGB (g/dl)	0.68 - 26
PLT ($10^3/\text{mm}^3$) (A)	13 – 2777
PLT ($10^3/\text{mm}^3$) (B)	13 - 4856
HCT (%)	2.0 - 80

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Limitations/ Interfering substance:

Verification of any "Abnormal" test result (including flagged results or results outside their normal range) is due to the following listed:

- **WBC White Blood Cells (Leukocytes):**

NRBC, Non-lysed Red Cells, Multiple myeloma, Hemolysis, Leukemia, Increased turbidity, Chemotherapy, Cryoglobulins.

- **RBC Red Blood Cells (Erythrocytes):**

High WBCs, Agglutinated red blood cells, Cold agglutinins.

- **HGB (Hemoglobin):**

Turbidity of the blood sample which may be due to Elevated WBC or Elevated Lipids, Fetal bloods mixed with maternal bloods may produce a falsely elevated hemoglobin value.

- **PLT (Platelets):**

Very small erythrocytes, Agglutinated red blood cells, Giant platelets in excessive numbers, Chemotherapy, Hemolysis, RBC inclusions, Platelet agglutination.

Expected values:

Age	HGB (g/dL)	HCT (%)	RBC (x10 ⁶ /μL)	MCV (fl)	MCH (pg)	MCHC (%)	RDW (%)	PLT (x10 ³ /μL)	WBC (X10 ³ /μL)	Neutro %	Lympho %	Eosino %	Baso %	Mono %
0 - 3 Day	14.5 - 22.5	45 - 67	4.00 - 6.60	95 - 121	31 - 37	29 - 37	12.0 - 18.0	150 - 450	9.0 - 35.0	32 - 62	19 - 29	0 - 2	0 - 1	5 - 7
4 - 9 Day	13.5 - 19.5	42 - 66	3.90 - 6.30	88 - 126	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 21.0	19 - 49	26 - 36	0 - 2	0 - 1	5 - 7
10 - 14 Day	12.5 - 20.5	39 - 63	3.60 - 6.20	86 - 124	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 20.0	14 - 34	36 - 45	0 - 2	0 - 1	6 - 10
15 - 30 Day	10.0 - 18.0	31 - 55	3.00 - 5.40	85 - 123	28 - 40	29 - 37	11.5 - 16.0	150 - 450	5.0 - 19.5	15 - 35	43 - 53	0 - 2	0 - 1	7 - 11
2 - 6 Month	9.5 - 13.5	29 - 41	3.10 - 4.50	74 - 108	25 - 35	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.5	13 - 33	41 - 71	0 - 3	0 - 1	4 - 7
7-24 month	10.5 - 13.5	33 - 49	3.70 - 5.30	70 - 86	23 - 31	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.0	15 - 35	45 - 76	0 - 3	0 - 1	3 - 6
2 - 6 Years	11.5 - 15.5	34 - 40	3.90 - 5.30	75 - 87	24 - 30	32 - 36	11.5 - 15.0	150 - 450	5.5 - 15.5	23 - 45	35 - 65	0 - 3	0 - 1	3 - 6
6 - 12 Years	11.5 - 15.5	35 - 45	4.00 - 5.20	77 - 95	25 - 33	32 - 36	11.5 - 15.0	150 - 450	4.5 - 13.5	33 - 61	28 - 48	0 - 3	0 - 1	3 - 6
12 - 18 Years (Male)	13.0 - 16.0	36 - 51	4.50 - 5.30	78 - 98	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
12 - 18 Years (Female)	12.0 - 16.0	33 - 51	4.10 - 5.10	78 - 102	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
>18 Years (Male)	13.5 - 17.5	37 - 53	4.50 - 5.90	80 - 100	26 - 34	32 - 36	11.5 = 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6
>18 Years (Female)	12.0 - 16.0	33 - 51	4.00 - 5.20	80 - 100	26 - 34	32 - 36	11.5 - 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6

Interpretation of the results:

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

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Reporting result:

According to lab policy.(automated printing or computerized)

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Sysmex KX-21 Hematology Analyzer

SOPs\ HGA \.....H/ Haem / 02

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose/Definition:

The KX-21 performs speedy and accurate analysis of 18 parameters in blood (Whole WBC, LYM%, MXD%, NEUT% , LYM# , MXD#, NEUT# , RBC count, Hemoglobin, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, PDW, MPV, P-LCR)

The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the noncyanide hemoglobin method.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
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Specimen requirements:

About 2-3 ml of venous blood collected into EDTA tubes.

Specimens should be transported at room temperature 18 - 26°C and can be store in the refrigerator of 2 - 8°C up to 6 hours.. If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis.

Specimen reception:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Criteria for rejection haematology specimens

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1. When the identification is missing /inadequate.
2. Insufficient quantity
3. Inappropriate container
4. Inappropriate transport/storage
5. Unknown duration of delay
6. Clotted sample

Equipment & Items required:

Sysmex KX -21N analyzer

Reagent	Storage Conditions
Sysmex KX-21 diluent	5 - 30°C
Stromatolyser- KX 21	5 - 30°C
CellClean (detergent)	1 - 30°C

Abbreviations:

CBC: Complete blood count.

EDTA: Ethylene diamine tetra acetic acid

WBC: White Blood Cells

LYM%: Lymphocyte percentage

LYM#: Lymphocyte absolute value

MON%: Monocyte percentage

MON#: Monocyte absolute value

GRA%: Granulocyte percentage

GRA#: Granulocyte absolute value

RBC: Red Blood Cells

HGB: Hemoglobin

HCT: Hematocrit

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

RDW: Red cell Distribution Width

PLT: Platelets

MPV: Mean Platelet Volume

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PDW*: Platelet Distribution Width

PCT*: Plateletcrit

P-LCR: Platelet larger cell ratio

Procedures:

1. Check to see that the reagents needed for the number of the samples to be processed for the day are available.
2. Turn ON the power switch on the right side of the unit. Self-check, auto rinse, and background check will be automatically performed, and the "Ready" (ready for analysis) will appear.

Permissible background counts

WBC	0.3 [$\times 10^3/\mu\text{L}$] or less
RBC	0.02 [$\times 10^6/\mu\text{L}$] or less
HGB	0.1 [g/dL] or less
PLT	10 [$\times 10^3/\mu\text{L}$] or less

3. When auto rinse and background check are normally completed, "Ready" is displayed.
4. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
5. If the result of quality control in acceptable range input your blood samples.
6. Input from the panel keyboard.
7. Press [SAMPLE No.] key in the Ready status.
8. Entering patient ID, sample ID, Patient name, etc
9. Press [ENTER] key, This will fix the sample No. and the status becomes ready for analysis.
10. Mix the sample sufficiently before analysis.
11. Remove the plug while taking care not to allow blood scatter.
12. Set the tube to the sample probe, and in that condition, press the start switch.
13. when the LCD screen displays "Analyzing," remove the tube.
14. After that, the unit executes automatic analysis and displays the result on the LCD screen.

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15. Then the unit turns to the ready status, becoming ready for analysis of the next samples.

Quality control procedures:

1. At the beginning of each work shift, all parameters are tested with blood control.
2. The 3 levels include: Abnormal Low, Normal, Abnormal High
3. Controls are stored at 2-8°C and brought to room temperature on a roller mixer before use .
4. Controls are gently inverted eight times according to the manufacturer's instruction before use.
5. From the RUN screen, press [SPECIMEN TYPE].
6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key.
9. Control values must be within three standard deviations, otherwise the measurement has to be repeated, if the control still out of range:
 - a. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
 - b. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable, If this does not solve the problem:
 - ✓ Prepare new control(s) and try again.
 - ✓ If the controls are still out, inform your supervisor to check the operator's manual, or recalibrate instrument and If controls are still out,. Contact Medical Maintenance where applicable, or servicing engineer.
7. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).
8. Dilute the sample if White blood cell counts $\geq 100,000$ /mm³ and platelet counts $\geq 1,000,000$ /mm³ are outside the linearity specifications of the instrument.

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Linearity:

1) Whole blood mode	
WBC:	1.0 - 9.9 ($\times 10^3/\mu\text{L}$) ± 0.3 ($\times 10^3/\mu\text{L}$) or less
	10.0 - 99.9 ($\times 10^3/\mu\text{L}$) $\pm 3\%$ or less
RBC:	0.30 - 0.99 ($\times 10^6/\mu\text{L}$) ± 0.03 ($\times 10^6/\mu\text{L}$) or less
	1.00 - 7.00 ($\times 10^6/\mu\text{L}$) $\pm 3\%$ or less
HGB:	0.1 - 10.0 (g/dL) ± 0.2 (g/dL) or less
	10.0 - 25.0 (g/dL) $\pm 2\%$ or less
HCT:	10.0 - 33.3 (HCT%) ± 1.0 (HCT%) or less
	33.4 - 60.0 (HCT%) $\pm 3\%$ or less
PLT:	10 - 199 ($\times 10^3/\mu\text{L}$) ± 10 ($\times 10^3/\mu\text{L}$) or less
	200 - 999 ($\times 10^3/\mu\text{L}$) $\pm 5\%$ or less
	(However, RBC $< 7.00 \times 10^6/\mu\text{L}$)

Limitations/ Interfering substance:

The following is a list of possible substances that may interfere with the listed parameters.

1. WBC: platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyse-resistant RBCs in patients with haemoglobinopathies, severe liver disease or neonates.
2. RBC: Cold agglutinins, severe microcytosis, fragmented RBCs, large numbers of giant platelets, in vitro haemolysis.
3. HGB: Lipemia, abnormal proteins in blood plasma, severe leukocytes (above 100,000/ μL). The effect of abnormal proteins and Lipemia may be removed by plasma replacement or plasma blank procedures.
4. HCT: Cold agglutinins, leukocytosis (above 100,000/ μL), abnormal red cell fragility.
5. PLT: Pseudothrombocytopenia, platelet aggregation, increased microcytosis, megalocytic platelets
6. Low sample volume of <1 mL may dilute patient samples with EDTA in the collection tube giving falsely low results. If a low sample volume is expected, use a pediatric EDTA tube; fill to the second line and mix well.
7. Dilute the sample if White blood cell counts $\geq 100,000$ / mm^3 and platelet counts $\geq 1,000,000$ / mm^3 are outside the linearity specifications of the instrument

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Expected values:

Age	HGB (g/dL)	HCT (%)	RBC (x10 ⁶ /μL)	MCV (fl)	MCH (pg)	MCHC (%)	RDW (%)	PLT (x10 ³ /μL)	WBC (X10 ³ /μL)	Neutro %	Lympho%	Eosino %	Baso %	Mono %
0 - 3 Day	14.5 - 22.5	45 - 67	4.00 - 6.60	95 - 121	31 - 37	29 - 37	12.0 - 18.0	150 - 450	9.0 - 35.0	32 - 62	19 - 29	0 - 2	0 - 1	5 - 7
4 - 9 Day	13.5 - 19.5	42 - 66	3.90 - 6.30	88 - 126	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 21.0	19 - 49	26 - 36	0 - 2	0 - 1	5 - 7
10 - 14 Day	12.5 - 20.5	39 - 63	3.60 - 6.20	86 - 124	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 20.0	14 - 34	36 - 45	0 - 2	0 - 1	6 - 10
15 - 30 Day	10.0 - 18.0	31 - 55	3.00 - 5.40	85 - 123	28 - 40	29 - 37	11.5 - 16.0	150 - 450	5.0 - 19.5	15 - 35	43 - 53	0 - 2	0 - 1	7 - 11
2 - 6 Month	9.5 - 13.5	29 - 41	3.10 - 4.50	74 - 108	25 - 35	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.5	13 - 33	41 - 71	0 - 3	0 - 1	4 - 7
7-24 month	10.5 - 13.5	33 - 49	3.70 - 5.30	70 - 86	23 - 31	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.0	15 - 35	45 - 76	0 - 3	0 - 1	3 - 6
2 - 6 Years	11.5 - 15.5	34 - 40	3.90 - 5.30	75 - 87	24 - 30	32 - 36	11.5 - 15.0	150 - 450	5.5 - 15.5	23 - 45	35 - 65	0 - 3	0 - 1	3 - 6
6 - 12 Years	11.5 - 15.5	35 - 45	4.00 - 5.20	77 - 95	25 - 33	32 - 36	11.5 - 15.0	150 - 450	4.5 - 13.5	33 - 61	28 - 48	0 - 3	0 - 1	3 - 6
12 - 18 Years (Male)	13.0 - 16.0	36 - 51	4.50 - 5.30	78 - 98	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
12 - 18 Years (Female)	12.0 - 16.0	33 - 51	4.10 - 5.10	78 - 102	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
>18 Years (Male)	13.5 - 17.5	37 - 53	4.50 - 5.90	80 - 100	26 - 34	32 - 36	11.5 = 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6
>18 Years (Female)	12.0 - 16.0	33 - 51	4.00 - 5.20	80 - 100	26 - 34	32 - 36	11.5 - 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6

Interpretation of the results:

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

Reporting result:

According to lab policy.(automated printing or computerized)

Hematology Standard Operating Procedures (SOPs)

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Cell-Dyn 1800 Hematology Analyzer

SOPs\ HGA \.....H\ Haem \ 03

Version: 1	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

The Cell-Dyn 1800 Hematology Analyzer performs a Complete Blood Count (CBC), Platelet Count, and a Three-Part Differential. Whole blood is aspirated, diluted, and then divided into two samples. One sample is used to analyze the red blood cells and platelets while the second sample is used to analyze the white blood cells and hemoglobin.

Electrical impedance is used to count the white blood cells, red blood cells, and platelets as they pass through an aperture. As each cell is drawn through the aperture, a change in electrical resistance occurs generating a voltage pulse. The number of pulses during a cycle corresponds to the number of cells counted. The amplitude of each pulse is directly proportional to the cell volume.

Responsibilities:

- All hematology department personal are required to be knowledgeable of this procedure.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.
- New employees are trained and assessed for competence before they can handle patient sample.

Specimen requirements:

Whole blood collected in an EDTA tube.

The instrument aspirates 30 µl of patient sample.

Specimen receptions:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Criteria for rejection haematology specimens

1. When the identification is missing /inadequate.

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2. Insufficient quantity
3. Inappropriate container
4. Inappropriate transport/storage
5. Unknown duration of delay
6. Clotted sample

Equipment & Items Required:

1. Cell-Dyn Diluent, Cell-Dyn Lytic Agent, Cell-Dyn Detergent:
 - a. Stable at room temperature until the expiration date on the container.
 - b. Protect from direct sunlight, extreme heat, and freezing during storage.
 - c. Do not use if reagent has been frozen.
2. Enzymatic Cleaner:
 - a. Stable at 2-8°C until the expiration date on the container.
 - b. Do not use if reagent has been frozen.

Abbreviations:

CBC: Complete Blood Count

EDTA: Ethylene diamine tetra acetic acid

AMR: Analytical Measurement Range

Procedures:

1. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
2. Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
3. Press MAIN to return to the MAIN MENU. At the MAIN MENU, enter in the operator ID and press RUN, next press SPECIMEN TYPE.

The results shall be within the following specifications.

Parameter	Background Limit
WBC (K/mm ³)	≤ 0.3
RBC (M/mm ³)	≤ 0.05
HGB (g/dL)	≤ 0.1
PLT (K/mm ³)	≤ 5

4. If the Open Mode Background count results are acceptable, proceed to Step 4.

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5. If the Open Mode Background count results fail, press CLEAR ORIFICE to clear the orifice. Press MAIN then SPECIAL PROTOCOL then AUTO CLEAN and put enzymatic cleaner in tube and place the sample probe in the tube and press RUN. When cleaning is complete, press NORMAL BACKGROUND and press the Plate.

6. To perform patient testing:

- A. Press MAIN to return to the MAIN MENU screen. Enter in the Operator ID and press RUN. Press SPECIMEN TYPE then press PATIENT SPECIMEN. Verify that RUN Ready is displayed in the Status Box.
- B. Mix the patient sample well and remove the cap.
- C. Place the sample probe in the tube so that the end is immersed in the sample but not resting on the bottom of the tube.
- D. Press the Touch Plate to start the run. The Status Box on the RUN menu indicates the stage of the run.
- E. When Remove Specimen is displayed in the Status Box and the probe has moved up through the wash block remove the sample tube and replace the tube cap. A beep will indicate that the probe cleaning cycle has begun.
- F. After the probe cleaning cycle is complete, the probe will move down into position for the next sample and the results will be displayed on the screen.
- G. If needed, press PRINT REPORT for a hardcopy of the report.
- H. Dilute the sample if White blood cell counts $\geq 100,000$ /mm³ and platelet counts $\geq 1,000,000$ /mm³ are outside the linearity specifications of the instrument.

Quality control procedure:

1. At the beginning of each work shift, all parameters are tested with blood control.
2. The 3 levels include: Abnormal Low, Normal, Abnormal High
3. Controls are stored at 2-8°C and brought to room temperature on a roller mixer before use .
4. Controls are gently inverted many times according to the manufacturer's instruction before use.
5. From the RUN screen, press [SPECIMEN TYPE].

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6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key.
7. Control values must be within three standard deviations, otherwise the measurement has to be repeated. if the control still out of range:
 - a. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
 - b. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable. If this does not solve the problem:
 - ✓ Prepare new control(s) and try again.
 - ✓ If the controls are still out, inform your supervisor to check the operator's manual, or recalibrate instrument and If controls are still out,. Contact Medical Maintenance where applicable, or servicing engineer.
8. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).

Linearity:

Analytical Measurement Range (Linearity)

Analyte	AMR
WBC (K/mm ³)	0.5 – 99.9
RBC (M/mm ³)	1.0 – 7.00
HGB (g/dL)	2.5 – 24.0
MCV (fL)	50 – 200
PLT (K/mm ³)	10 – 999
MPV (fL)	5.0 – 20.0

Limitations/ Interfering substance:

The following is a list of possible substances that may interfere with the listed parameters.

1. WBC: platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyse-resistant RBCs in patients with haemoglobinopathies, severe liver disease or neonates.
2. RBC: Cold agglutinins, severe micryocytosis, fragmented RBCs, large numbers of giant platelets, in vitro haemolysis.
3. Hgb: Lipemia, abnormal proteins in blood plasma, severe leukocytes (above 100,000/ μ l). The effect of abnormal proteins and Lipemia may be removed by plasma replacement or plasma blank procedures.

Hematology Standard Operating Procedures (SOPs)

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4. Hct: Cold agglutinins, leukocytosis (above 100,000/ μ L), abnormal red cell fragility.
5. PLT: Pseudothrombocytopenia, platelet aggregation, increased microcytosis, megalocytic platelets.
6. Low sample volume of <1 mL may dilute patient samples with EDTA in the collection tube giving falsely low results. If a low sample volume is expected, use a pediatric EDTA tube; fill to the second line and mix well.

Expected values:

Age	HGB (g/dL)	HCT (%)	RBC (x10 ⁶ / μ L)	MCV (fl)	MCH (pg)	MCHC (%)	RDW (%)	PLT (x10 ³ / μ L)	WBC (X10 ³ / μ L)	Neutro %	Lympho%	Eosino %	Baso %	Mono %
0 - 3 Day	14.5 - 22.5	45 - 67	4.00 - 6.60	95 - 121	31 - 37	29 - 37	12.0 - 18.0	150 - 450	9.0 - 35.0	32 - 62	19 - 29	0 - 2	0 - 1	5 - 7
4 - 9 Day	13.5 - 19.5	42 - 66	3.90 - 6.30	88 - 126	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 21.0	19 - 49	26 - 36	0 - 2	0 - 1	5 - 7
10 - 14 Day	12.5 - 20.5	39 - 63	3.60 - 6.20	86 - 124	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 20.0	14 - 34	36 - 45	0 - 2	0 - 1	6 - 10
15 - 30 Day	10.0 - 18.0	31 - 55	3.00 - 5.40	85 - 123	28 - 40	29 - 37	11.5 - 16.0	150 - 450	5.0 - 19.5	15 - 35	43 - 53	0 - 2	0 - 1	7 - 11
2 - 6 Month	9.5 - 13.5	29 - 41	3.10 - 4.50	74 - 108	25 - 35	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.5	13 - 33	41 - 71	0 - 3	0 - 1	4 - 7
7-24 month	10.5 - 13.5	33 - 49	3.70 - 5.30	70 - 86	23 - 31	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.0	15 - 35	45 - 76	0 - 3	0 - 1	3 - 6
2 - 6 Years	11.5 - 15.5	34 - 40	3.90 - 5.30	75 - 87	24 - 30	32 - 36	11.5 - 15.0	150 - 450	5.5 - 15.5	23 - 45	35 - 65	0 - 3	0 - 1	3 - 6
6 - 12 Years	11.5 - 15.5	35 - 45	4.00 - 5.20	77 - 95	25 - 33	32 - 36	11.5 - 15.0	150 - 450	4.5 - 13.5	33 - 61	28 - 48	0 - 3	0 - 1	3 - 6
12 - 18 Years (Male)	13.0 - 16.0	36 - 51	4.50 - 5.30	78 - 98	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
12 - 18 Years (Female)	12.0 - 16.0	33 - 51	4.10 - 5.10	78 - 102	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
>18 Years (Male)	13.5 - 17.5	37 - 53	4.50 - 5.90	80 - 100	26 - 34	32 - 36	11.5 = 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6
>18 Years (Female)	12.0 - 16.0	33 - 51	4.00 - 5.20	80 - 100	26 - 34	32 - 36	11.5 - 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6

Interpretation of the result:

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

Reporting result:

According to lab policy.(automated printing or computerized)

Hematology Standard Operating Procedures (SOPs)

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CELL DYN 3500-3700 Hematology Analyzer

SOPs\ HGA \.....H\ Haem \04

Version: 1	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

The CELL-DYN 3500 system is an automated hematology analyzer that uses electrical impedance, flow cytometry, laser light scatter, and spectrophotometric technologies to measure the following 22 hematologic parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW and PCT. The instrument also determines the percent WBC values and calculates the absolutes for NEU, LYMPH, MONO, EOS and BASO.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

About 2-3 ml of venous blood collected into EDTA tubes.

Specimens should be transported at room temperature 18 - 26°C and can be store in the refrigerator of 2 - 8°C up to 6 hours.. If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis.

Specimen reception:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Criteria for rejection haematology specimens

1. When the identification is missing /inadequate.
2. Insufficient quantity
3. Inappropriate container

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4. Inappropriate transport/storage
5. Unknown duration of delay
6. Clotted sample

Equipment & Items required:

1. EQUIPMENT:

- 1.1. CELL-DYN 3500 Analyzer
- 1.2. Specimen rotator

2. REAGENTS:

- 2.1. CELL-DYN diluent
- 2.2. CELL-DYN WIC/HGB lyse
- 2.3. CELL-DYN sheath reagent
- 2.4. CELL-DYN detergent
- 2.5. CELL-DYN control material (low, normal & high).
- 2.6. CELL-DYN calibrator
- 2.7. CELL-DYN enzymatic cleaner

NOTE: The diluent, detergent, sheath & lyse reagents are stored at room temperature. The enzymatic cleaner is stored at 2-8 ° C. All reagents are stable until the manufacturer's expiration date. Reagents are packaged ready to use.

Abbreviations:

CBC: Complete blood count.

EDTA: Ethylene diamine tetra acetic acid

WBC — White Blood Cell or Leukocyte count

NEU — Neutrophil absolute count

%N — Neutrophil percent

LYM — Lymphocyte absolute count

%L — Lymphocyte percent

MONO — Monocyte absolute count

%M — Monocyte percent

EOS — Eosinophil absolute count

%E — Eosinophil percent

BASO — Basophil absolute count

%B — Basophil percent

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RBC — Red Blood Cell or Erythrocyte count

HGB — Hemoglobin concentration

HCT — Hematocrit

MCV — Mean Corpuscular Volume

MCH — Mean Corpuscular Hemoglobin

MCHC — Mean Corpuscular Hemoglobin Concentration

RDW — Red Cell Distribution Width

PLT — Platelet or Thrombocyte count

MPV — Mean Platelet Volume

PDW*— Platelet Distribution Width

PCT* — Plateletcrit

RETIC % — Reticulocyte Percent

RETIC ABS — Reticulocyte Absolute

IRF — Immature Reticulocyte Fraction

WIC: WBC Impedance Count

WOC: WBC Optical Count

Procedures:

1. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
2. Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
3. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
4. Entering patient ID, sample ID, Patient name, etc
5. With the analyzer in the ready mode, select [RUN], [SPECIMEN TYPE] and then [PATIENT].
6. Enter the following information in the indicated fields:
 - a. NEXT ID: Manually enter or barcode.
 - b. PATIENT FIELD: Type in the patient's first and last name.
 - c. PARAMETER SET: Enter the number 1.
7. Place the sample under the probe and immerse the probe in the specimen.

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8. Press the touch plate, which is located behind the probe to begin aspiration.
9. Remove the specimen from the probe when the beep sounds. The wash block will move down the probe and clean it.
10. Upon completion, the wash block returns to the starting position and the specimen results are displayed. Do not begin testing the next patient until the current patient results are displayed.
11. Dilute the sample if White blood cell counts $\geq 100,000$ /mm³ and platelet counts $\geq 1,000,000$ /mm³ are outside the linearity specifications of the instrument.
12. Print patient reports.

Quality control procedures:

1. At the beginning of each work shift, all parameters are tested with blood control.
2. The 3 levels include: Abnormal Low, Normal, Abnormal High
3. Controls are stored at 2-8°C and brought to room temperature on a roller mixer before use .
4. Controls are gently inverted eight times according to the manufacturer's instruction before use.
5. From the RUN screen, press [SPECIMEN TYPE].
6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key.
7. Control values must be within three standard deviations, otherwise the measurement has to be repeated if the control still out of range:
 - a. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
 - b. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable. If this does not solve the problem:
 - ✓ Prepare new control(s) and try again.
 - ✓ If the controls are still out, inform your supervisor to check the operator's manual, or recalibrate instrument and If controls are still out,. Contact Medical Maintenance where applicable, or servicing engineer.
8. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).

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9. Limitations/ Interfering substance:

PARAMETER	LINEAR RANGE	ACCEPTABLE LIMITS
WIC	0 - 99.9 K/uL	+ 0.4 or 3.0%
WOC	0 – 250 K/uL	± 0.4 or 4.0%
RBC	0 - 8 M/uL	± 0.1 or 2.5%
HGB	0 – 24 g/dl	± 0.3 or 2.0%
MCV	50 - 200fL	± 3.0 or 3.0%
PLT	0 - 2000Ku/L	± 10.0 or 7%
MPV	5 - 18 fl	± 1.0 or 6.0%

NOTE: If the upper limits of linearity for cell counts or hemoglobin are exceeded, the patient sample must be diluted and retested within 1 hour. Calculate the new values for each parameter according to the dilution factor used. The MCV, MCH, MCHC and MPV are unaffected by dilution and do not require dilution or correction.

Interfering substance: The cell Dyn 3500 has been designed to detect and flag samples that contain interfering substances. The following list indicates the substances that may interfere. Refer to SOP Book 2 Section A.2 Verification of spurious results, Section A.4 Data Flagging or the Cell Dyn operator's manual, chapter 3, for parameter flags and corrective actions.

1. WBC: NRBCs lytic-resistant RBCs, PLT clumps, fragile WBCs.
2. RBC: Elevated WBC count, increased numbers of giant PLTs, auto-agglutination and in vitro hemolysis.
3. HGB: Elevated WBC count, increased plasma proteins, lipemia, icterus and lyse-resistant RBC's . Refer to the Spurious Results SOP Book 2 Section A.2 for additional information.
4. MCV: Elevated WBC count, hyperglycemia, in-vitro hemolysis, increased number of giant PLTs, cryoglobulin and cryofibrinogen. If cold agglutinins are suspected (increased MCV, with spurious HGB, MCH and MCHC results), refer to the Spurious Results SOP Book 2 Section A.2.
5. PLT: WBC fragments, in vitro hemolysis, microcytic RBCs, cryoglobulin PLT clumping and increased numbers of giant PLTs.

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6. Specimens greater than (>) 24 hours old may cause multiple flags. Do not perform a differential count unless the slide was made the day the specimen was collected.

Expected values:

Age	HGB (g/dL)	HCT (%)	RBC (x10 ⁶ /μL)	MCV (fl)	MCH (pg)	MCHC (%)	RDW (%)	PLT (x10 ³ /μL)	WBC (X10 ³ /μL)	Neutro %	Lympho%	Eosino %	Baso %	Mono %
0 - 3 Day	14.5 - 22.5	45 - 67	4.00 - 6.60	95 - 121	31 - 37	29 - 37	12.0 - 18.0	150 - 450	9.0 - 35.0	32 - 62	19 - 29	0 - 2	0 - 1	5 - 7
4 - 9 Day	13.5 - 19.5	42 - 66	3.90 - 6.30	88 - 126	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 21.0	19 - 49	26 - 36	0 - 2	0 - 1	5 - 7
10 - 14 Day	12.5 - 20.5	39 - 63	3.60 - 6.20	86 - 124	28 - 40	28 - 38	13.0 - 18.0	150 - 450	5.0 - 20.0	14 - 34	36 - 45	0 - 2	0 - 1	6 - 10
15 - 30 Day	10.0 - 18.0	31 - 55	3.00 - 5.40	85 - 123	28 - 40	29 - 37	11.5 - 16.0	150 - 450	5.0 - 19.5	15 - 35	43 - 53	0 - 2	0 - 1	7 - 11
2 - 6 Month	9.5 - 13.5	29 - 41	3.10 - 4.50	74 - 108	25 - 35	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.5	13 - 33	41 - 71	0 - 3	0 - 1	4 - 7
7-24 month	10.5 - 13.5	33 - 49	3.70 - 5.30	70 - 86	23 - 31	30 - 36	11.5 - 16.0	150 - 450	6.0 - 17.0	15 - 35	45 - 76	0 - 3	0 - 1	3 - 6
2 - 6 Years	11.5 - 15.5	34 - 40	3.90 - 5.30	75 - 87	24 - 30	32 - 36	11.5 - 15.0	150 - 450	5.5 - 15.5	23 - 45	35 - 65	0 - 3	0 - 1	3 - 6
6 - 12 Years	11.5 - 15.5	35 - 45	4.00 - 5.20	77 - 95	25 - 33	32 - 36	11.5 - 15.0	150 - 450	4.5 - 13.5	33 - 61	28 - 48	0 - 3	0 - 1	3 - 6
12 - 18 Years (Male)	13.0 - 16.0	36 - 51	4.50 - 5.30	78 - 98	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
12 - 18 Years (Female)	12.0 - 16.0	33 - 51	4.10 - 5.10	78 - 102	25 - 35	32 - 36	11.5 - 14.0	150 - 450	4.5 - 13.0	34 - 64	25 - 45	0 - 3	0 - 1	3 - 6
>18 Years (Male)	13.5 - 17.5	37 - 53	4.50 - 5.90	80 - 100	26 - 34	32 - 36	11.5 = 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6
>18 Years (Female)	12.0 - 16.0	33 - 51	4.00 - 5.20	80 - 100	26 - 34	32 - 36	11.5 - 13.1	150 - 450	4.5 - 11.0	35 - 66	24 - 44	0 - 3	0 - 1	3 - 6

Interpretation of the result:

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

Reporting result:

According to lab policy.(automated printing or computerized)

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

ACL Automated Coagulation Analyzer

(Factor Deficient Plasma VIII, IX, XI, XII Tests- HemosIL®)

SOPs\ HGA \.....H\ Haem \05

Version: 1	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose/Definition:

The ACL analyzer is a fully automated, microcomputer-controlled, nephelometric microcentrifugal instrument capable of performing the following tests:

- PT-FIB (Prothrombin Time and Fibrinogen Level).
- APTT (Activated Partial Thromboplastin Time).
- PT-FIB/APTT (all three tests run simultaneously).
- Single Factors (II, V, VII, VIII, IX, X, XI, XII).

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen Requirements:

- Plasma sample, The anticoagulant of choice for coagulation studies is 3.2% Sodium Citrate (Blue Top Tube).
- The standard ratio for citrated specimens is nine (9) parts of blood + one (1) part of anticoagulant, (9:1 ratio) that is critical for valid results.
- Platelet poor plasma required, Set the centrifugation speed to 3,000 rpm and centrifuge a sample for 10 minutes.
- Separate the plasma from the cells within 30 minutes after centrifugation of the sample and place the plasma sample in a plastic tube.

Hematology Standard Operating Procedures (SOPs)

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- If the specimen will be tested within two to four hours after centrifugation, it may be kept at room temperature.
- Freeze the specimen if it cannot be processed within four (4) hours after collection.
- Plasma can be frozen at (-20°C) or lower for at (7) days or at (-80°C) for six (6) months without loss of most factors.
- Thaw rapidly at (37°C) in a water bath. Remove the plasma sample as soon as it is thawed and perform the test immediately. Samples are viable for a maximum of (2) hours at room temperature after they have been thawed .
- Plasma samples cannot be re-frozen.

Specimen reception:

1. Prior to performing coagulation testing on patient samples, verify the patient identification. Any discrepancy must be investigated before processing the specimen.
2. Check the specimen for clots, visually hemolyzed and lipemic samples.
3. Ensure the specimen is labeled and label a sample cup.

Criteria for rejecting hematology specimen:

- When the identification is missing /inadequate.
- Clotted specimens.
- Lipemic, icteric or hemolyzed plasma samples
- Incomplete filling of tube or over-filled samples.
- Inappropriate container.
- Unknown duration of delay.
- Inappropriate transport/storage

Abbreviations:

- APTT: Activated Partial Thromboplastin Time
- SD: Standard Deviation

Equipment & Items required:

- ACL Analyzer
- Centrifuge
- Sample cups (0.5mL capacity)

Hematology Standard Operating Procedures (SOPs)

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- Rotors
- Plastic test tubes
- Reference Solution
- Factor deficient plasma VIII
- Factor deficient plasma IX
- HemosIL APTT reagent
- HemosIL Calcium Chloride reagent
- HemosIL Normal and Abnormal controls
- HemosIL Calibration plasma
- HemosIL Factor Diluent

Reagents Preparation:

Factor deficient plasmas VIII, IX : Reconstitute all deficient plasmas with 1.0ml of deionized water. Swirl gently and let stand for 30 minutes at room temperature. Maintain at 2-8°c for no more than four hours.

APTT Reagent (SynthAFax): APTT reagent comes ready for use. Each vial must be mixed by inversion several times before use to assure homogeneity of the reagent.

Calcium Chloride (0.025M): Calcium Chloride comes ready for use. Mix well by inversion before use.

Calibration Procedure:

1. Dilute the calibration plasma (or pooled plasma) 1+4 with factor diluent according to the program selected.
2. Place diluted calibration plasma in the pool position of the special (factor assay) sample tray.
3. Place factor diluent in the Dil position. Place factor deficient plasmas in appropriate positions of the sample tray.
4. Place the APTT reagent in the reagent reservoir No.2 and the calcium chloride in the reagent reservoir No.3.
5. Select the factor **VIII or IX** program and follow the instructions for calibration displayed on the ACL analyzer video screen.

Hematology Standard Operating Procedures (SOPs)

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Assay Procedure:

1. Dilute patient samples (1+4) with factor diluent (100µl sample with 400µl).
2. According to the sensitivity range required, dilute calibration plasma as follows:
 - a. High curve (1+4) with factor diluent (100µl+400µl factor diluent).
 - b. Low curve (1+79) with factor diluent (100µl+7.9ml factor diluent).
3. Place pre-diluted samples in the appropriate positions of the sample tray.
4. Place pre-diluted calibration plasma in the pool position of the sample tray.
5. Place factor diluent in the Dil position of the sample tray.
6. Place factor deficient plasmas in the appropriate position of the sample tray.
7. Place the APTT reagent in the reagent reservoir No.2 and the calcium chloride in the reagent reservoir No.3.
8. Select the single factor program and follow the instructions displayed on the ACL analyzer video screen.

Quality Control:

- Normal and abnormal control will be run on each tests.
- Control results must be within the specified ($\pm 2SD$) limits of the quality control chart.
- If one or more level of control is outside $+ 3SD$, do not report patient results, Perform troubleshooting action:
 - ✓ check reagent levels and expiration dates.
 - ✓ Repeat the control again, If it is still outside $+ 3SD$.,
 - ✓ reconstitute new controls,
 - ✓ check instrument maintenance/cleaning, and repeat.
 - ✓ If results are still outside limits, notify the Hematology Supervisor
- Immediately, Corrective action must be taken before reporting patient results.
- ✓ Out of range controls will be recorded on the appropriate (normal/abnormal control) Service Troubleshooting Log along with the corrective action taken.

Limitations/ Interfering Factors:

1. Hemolysis can cause clotting factor activation.
2. Lipemia and icterus interfere with the spectrophotometric measurements resulting in falsely decreased end point determinations.

Hematology Standard Operating Procedures (SOPs)

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Expected values:

- Reference ranges: 50-150%.

Interpretations of results:

- A Factor VIII deficiency indicates the possible presence of Hemophilia A or von Willebrand's disease. Hemophilia A is a sex-linked recessive trait. Hemophilia patients are classified by the amount of Factor VIII activity measured in their plasma, severe (<1%), moderate (1-5%) and mild (5-30%). Von Willebrand's disease is an autosomal dominant trait exhibiting decreased levels of Factor VIII coagulant activity, affecting both sexes equally. A differential diagnosis is made based on the results of other specialized coagulation tests, in conjunction with Factor VIII coagulant activity level.
- Factor IX has a decreased activity in a congenital condition known as hemophilia B or Christmas Disease, which is sex-linked recessive. The most common cause of an acquired deficiency of blood clotting factors is hepatic dysfunction due to liver cell damage or non-availability of vitamin K to the liver. Fibrinogen, factors II, VII, IX, X and possibly factor V are produced in the liver and all except fibrinogen and factor V require vitamin K for normal synthesis.

Reporting Results:

- **Factor VIII or IX (Activity)%**

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

ACL Automated Coagulation Analyzer

(PT-FIB/APTT Tests- HemosIL[®])

SOPs\ HGA \.....H\ Haem \06

Version: 1	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose/Definition:

The ACL analyzer is a fully automated, microcomputer-controlled, nephelometric microcentrifugal instrument capable of performing the following tests:

- PT-FIB (Prothrombin Time and Fibrinogen Level).
- APTT (Activated Partial Thromboplastin Time).
- PT-FIB/APTT (all three tests run simultaneously).
- Single Factors (II, V, VII, VIII, IX, X, XI, XII).

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen Requirements:

- Plasma sample, The anticoagulant of choice for coagulation studies is 3.2% Sodium Citrate (Blue Top Tube).
- The standard ratio for citrated specimens is nine (9) parts of blood + one (1) part of anticoagulant, (9:1 ratio) that is critical for valid results.
- Platelet poor plasma required, Set the centrifugation speed to 3,000 rpm and centrifuge a sample for 10 minutes.
- Separate the plasma from the cells within 30 minutes after centrifugation of the sample and place the plasma sample in a plastic tube.

Hematology Standard Operating Procedures (SOPs)

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- If the specimen will be tested within two to four hours after centrifugation, it may be kept at room temperature.

Specimen reception:

1. Prior to performing coagulation testing on patient samples, verify the patient identification. Any discrepancy must be investigated before processing the specimen.
2. Check the specimen for clots, visually hemolyzed and lipemic samples.
3. Ensure the specimen is labeled and label a sample cup.

Criteria for rejecting hematology specimen:

- When the identification is missing /inadequate.
- Clotted specimens.
- Lipemic, icteric or hemolyzed plasma samples
- Incomplete filling of tube or over-filled samples.
- Inappropriate container.
- Unknown duration of delay.
- Inappropriate transport/storage

Abbreviations:

- PT-FIB: Prothrombin Time and Fibrinogen Level
- APTT: Activated Partial Thromboplastin Time
- INR: International Normalized Ratio
- ISI: International Sensitivity Index
- DIC: disseminated intravascular coagulation
- VWF: Von Willebrand factor
- SD: Standard Deviation

Equipment & Items required:

- ACL Analyzer
- Centrifuge
- Sample cups (0.5mL capacity)
- Rotors
- Pipettes (1mL capacity)
- Plastic test tubes

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

- Reference Solution
- HemosIL PT-FIB reagent
- HemosIL APTT reagent
- HemosIL Calcium Chloride reagent
- HemosIL Normal and Abnormal controls
- HemosIL Calibration plasma
- HemosIL Sample Diluent

Calibration:

Calibration is necessary for every new reagent lot (thromboplastin), new lot of reference emulsion, when indicated by QC information, after major maintenance or service.

Calibration Procedure:

1. Reconstitute 2 vials of lyophilized Calibration Plasma with one (1) mL of Reagent Grade Water each, Invert gently to mix. Do not shake. Maintain the calibration plasma at room temperature for 30 minutes before use, stable for two hours from reconstitution.
2. Fill one 1 mL sample cup with HemosIL Sample Diluent and place it in the "DILUENT" position in the sample tray. Fill another 1 mL sample cup with both vials of reconstituted Calibration Plasma and place it in the "POOL" position on the sample tray.
3. Put the Reference Solution, Thromboplastin reagent into the appropriate positions on the instrument.
4. Load a new rotor into the rotor holder.
5. Press PROG to return to the READY menu. Select PT-FIB using the "↑" or "↓" and press ENTER.
6. The "Check" frame is displayed. Press "↑" to start the calibration cycle.
7. Enter the reference values of all parameter then press ENTER. Press "↓" key to start the Calibration.
8. At the end of the analysis, the calibration data, to include results and graphics, will be printed and stored in the memory of the instrument.

Reagents Preparation:

PT-FIB HS reagent:

Hematology Standard Operating Procedures (SOPs)

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- Reconstitute each vial of thromboplastin reagent with one vial of buffer. Mix by gentle inversion to ensure complete resuspension (**DO NOT SHAKE**).
- Label the reconstituted reagent vial with the date and initials of tech placing reagent in use. Maintain at room temperature for (30) minutes before using. A teflon coated magnetic stir bar must be inserted into the reagent reservoir for continuous mixing action.

Stability after reconstitution:

- 8 Hours at 15°C (on the ACL with continuous stirring), 3 days at 2 to 8°C (in the original bottle).
- Do not freeze, do not use reagents after the expiration date.

APTT Reagent:

APTT reagent comes ready for use. Each vial must be mixed by inversion several times before use to assure homogeneity of the reagent.

Calcium Chloride:

Calcium Chloride (**0.025 M**) comes ready for use. Mix well by inversion before use.

Procedure:

1. From the READY status of the instrument, select the desired test (i.e., PT-FIB, PT-FIB/APTT or APTT) by using “↑” or “↓” keys and press ENTER.
2. The "Check" frame is displayed.
3. Empty the PT-FIB HS (thromboplastin) vial content into reservoir number 1 on the instrument. Empty the APTT vial content into reservoir number 2 (APTT) on the instrument, Empty the Calcium Chloride vial content into reservoir number 3 (CaCl₂) on the instrument. Ensure that the Reference Solution volume is sufficient to perform testing.
4. Press “↓” to continue.
5. Load the sample tray with patient plasma. For PT-FIB/APTT testing, only eight (8) samples can be programmed to run at one time. For PT-FIB testing, 18 samples can be programmed and loaded on the sample tray. Calibration plasma loaded in the pool position of the sample tray.
6. Load a new rotor on the instrument.
7. Press the commands key to start analysis.

Hematology Standard Operating Procedures (SOPs)

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8. At the end of analysis the "Results" frame is displayed and results will be printed out for patient samples. Review the results that are to be filed and certified.

Quality Control:

- Normal and abnormal control will be run on each tests.
- Control results must be within the specified ($\pm 2SD$) limits of the quality control chart.
- If one or more level of control is outside $+ 3SD$, do not report patient results, Perform troubleshooting action:
 - ✓ check reagent levels and expiration dates.
 - ✓ Repeat the control again, If it is still outside $+ 3SD$.,
 - ✓ reconstitute new controls,
 - ✓ check instrument maintenance/cleaning, and repeat.
 - ✓ If results are still outside limits, notify the Hematology SupervisorImmediately, Corrective action must be taken before reporting patient results.
- ✓ Out of range controls will be recorded on the appropriate (normal/abnormal control) Service Troubleshooting Log along with the corrective action taken.

Limitations/ Interfering Factors:

1. Hemolysis can cause clotting factor activation and falsely decrease end point measurements.
2. Lipemia and icterus interfere with the spectrophotometric measurements resulting in falsely decreased end point determinations.
3. Contaminated reagents will give inaccurate results.
4. Samples with a fibrinogen levels < 25 mg/dl should be suspected of being a serum sample and rejected.
5. APTT assay results may be affected by many commonly administrated drugs.

Expected values:

PT seconds: **11.5-15.0**

PT activity: **70-120%**

INR: **0.90-1.15**

Therapeutic levels of INR **2.0 – 3.0** target range **2.5**

Fibrinogen: **(200-400)mg/dl (2.00-4.00) g/l**

Hematology Standard Operating Procedures (SOPs)

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PTT seconds: lies between (27-35) seconds.

Note: recommendation that each laboratory determines its own normal range according to the type of reagents.

Interpretation of results:

Interpretation of PT and PTT in patients with a Bleeding or Clotting Syndrome:

Prolonged PT:

Inherited: Factor VII deficiency

Acquired: Vitamin K deficiency, Liver disease, Warfarin use, Factor VII inhibitor

Prolonged APTT:

Inherited: vWF, factor VIII, IX, XI, XII deficiency

Acquired: Heparin use, Inhibitor of vWF, factor VIII, IX, XI, XII, Antiphospholipid antibodies

Prolonged both PT and APTT:

Inherited: Prothrombin, fibrinogen, factor V, X or combined factor deficiency

Acquired: Severe Liver disease, Disseminated intravascular coagulation (DIC).

Supratherapeutic heparin or warfarin, Combined heparin or warfarin use, Inhibitor of prothrombin, fibrinogen, factor V, X, Direct thrombin inhibitor.

Reporting Results:

- The PT and PTT tests are reported in seconds along with the reference ranges.
- PT test results reported in:
 - ✓ Time (Seconds)
 - ✓ Ratio ($PT_{\text{patient}}/PT_{\text{control}}$)
 - ✓ Activity Percentage
 - ✓ INR
- The ACL Analyzer will automatically calculate the INR value when the ISI value is entered in the ACL. This value will appear beside the PT result.

$$INR = \left[\frac{PT_{\text{Patient}}}{PT_{\text{Reference Plasma}}} \right]^{ISI}$$

Note: The International Sensitivity Index (ISI) is an experimentally derived measurement, usually provided by the thromboplastin manufacturer "according to package insert".

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

CoaLAB 1000 Automated Coagulation Analyzer

(PT-FIB/APTT Tests- LABiTec)

SOPs\ HGA \.....H\ Haem \07

Version: 1	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose/Definition:

The CoaLAB 1000 analyzer, is a fully automated photooptical blood plasma hemostasis instrument capable for performing a wide range of coagulometric, chromogenic and immunologic coagulation tests such as Prothrombin time, activated partial Thromboplastin time, Fibrinogen, special tests such as single factor assays, Anti-Thrombin III, Protein C, Protein S, C-Reactive Protein, D-Dimer and others based on the wavelength available in different analyzer types.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen Requirements:

- Plasma sample, The anticoagulant of choice for coagulation studies is 3.2% Sodium Citrate (Blue Top Tube).
- The standard ratio for citrated specimens is nine (9) parts of blood + one (1) part of anticoagulant, (9:1 ratio) that is critical for valid results.
- Platelet poor plasma required, Set the centrifugation speed to 3,000 rpm and centrifuge a sample for 10 minutes.
- Separate the plasma from the cells within 30 minutes after centrifugation of the sample and place the plasma sample in a plastic tube.
- If the specimen will be tested within two to four hours after centrifugation, it may be kept at room temperature.

Hematology Standard Operating Procedures (SOPs)

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Specimen reception:

1. Prior to performing coagulation testing on patient samples, verify the patient identification. Any discrepancy must be investigated before processing the specimen.
2. Check the specimen for clots, visually hemolyzed and lipemic samples.
3. Ensure the specimen is labeled and label a sample cup.

Criteria for rejecting hematology specimen:

- When the identification is missing /inadequate.
- Clotted specimens.
- Lipemic, icteric or hemolyzed plasma samples
- Incomplete filling of tube or over-filled samples.
- Inappropriate container.
- Unknown duration of delay.
- Inappropriate transport/storage

Abbreviations:

- PT-FIB: Prothrombin Time and Fibrinogen Level
- APTT: Activated Partial Thromboplastin Time
- INR: International Normalized Ratio
- ISI: International Sensitivity Index
- DIC: disseminated intravascular coagulation
- VWF: Von Willebrand factor

Equipment & Items required:

- CoaLAB 1000 analyzer.
- Centrifuge
- Sample-cups (0.5mL capacity)
- Cuvette ring contains a 1 x 4 mm stir bar
- Pipettes (1mL capacity)
- Plastic test tubes
- Two containers for rinsing system " Distilled water and waste containers"
- Washing solution

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

- Cleaning solution
- LABiTec PT reagent
- LABiTec APTT reagent
- LABiTec Calcium Chloride reagent
- LABiTec Fibrinogen reagent
- LABiTec Normal and Abnormal controls
- Calibration plasma

Calibration:

- Before starting a routine run operation:
 - ✓ Check the level of the distilled water container,
 - ✓ Empty the waste water container.
 - ✓ Fill liquid system and flush system.
- System Start-up:
 - ✓ Set the power switch on.
 - ✓ The analyzer now runs through a full initialization procedure checking all internal modules automatically don't replace reagents or washing solution or c-ringing during initialization.
 - ✓ Once the initialization process is finished the MAIN MENU appears in the display.

Calibration is necessary for every new reagent lot, when indicated by QC information, after major maintenance or service.

Use this menu to calibrate a test manually or by automated calibration, to define or change the calibration parameters for a test.

A calibration is required for test to which the raw values need to be converted into concentration units/activities.

1. From the Main Menu, press the button Setup to access the Setup Menu.
2. From the Setup Menu, press the button Calibration to access the Calibration Menu.
3. Select a test for calibration by using the arrow keys first to select the test and then press the OK button to continue. The calibration for test screen appears.

Hematology Standard Operating Procedures (SOPs)

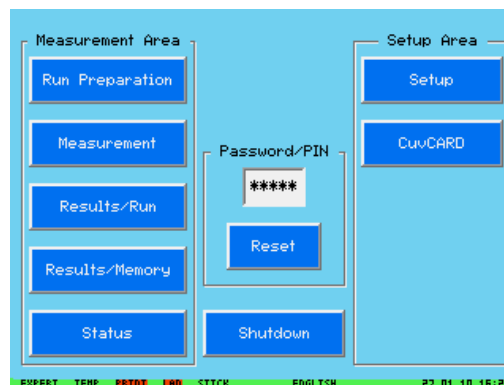
وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

The analyzer offers two different ways to calibrate a test.

- Under Edit Calibration a test can be calibrated manually by editing the calibration data previously measured. Enter manually the evaluated results achieved by a normal measurement with dilutions or if available with standard plasmas or those which have been provided in the package inserts of the reagent supplier used.
- Under Auto Calibration a test will be automatically diluted from a standard and the measured calibration data will be automatically added into a calibration curve and memorized for the test.

Procedure:

- From the Main Menu, press on the **Run Preparation** the following display appears.



- Run preparation:
 - ❖ Load C-ring: load a new cuvette ring.
 - ❖ Load reagents: load tests, set reagent vials, re-new reagents, define positions.
 - ❖ Load samples: load patient samples (Routine & STAT), edit patient ID.
 - ❖ Rinsing modes: flush, intensive wash, cleaning, maintenance.
- From the Main Menu, press on the **Measurement** to access the Measurement Menu.
 - ❖ Start the run
 - ❖ During run: add STAT runs, show status, Immediate STOP, show results.
 - ❖ Show results (all, by sample, by test), display curve, print results/graphs.
- From the Main Menu, press the button CuvCARD to load C-ring balance.

Quality Control:

- Normal and abnormal control will be run on each tests.

Hematology Standard Operating Procedures (SOPs)

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- Control results must be within the specified ($\pm 2SD$) limits of the quality control chart.
- If one or more level of control is outside $+ 3SD$, do not report patient results, Perform troubleshooting action:
 - ✓ check reagent levels and expiration dates.
 - ✓ Repeat the control again, If it is still outside $+ 3SD$;
 - ✓ reconstitute new controls,
 - ✓ check instrument maintenance/cleaning, and repeat.
 - ✓ If results are still outside limits, notify the Hematology SupervisorImmediately, Corrective action must be taken before reporting patient results.
- ✓ Out of range controls will be recorded on the appropriate (normal/abnormal control) Service Troubleshooting Log along with the corrective action taken.

Limitations/ Interfering Factors:

1. Hemolysis can cause clotting factor activation and falsely decrease end point measurements.
2. Lipemia and icterus interfere with the spectrophotometric measurements resulting in falsely decreased end point determinations.
3. Contaminated reagents will give inaccurate results.
4. Samples with a fibrinogen levels < 25 mg/dl should be suspected of being a serum sample and rejected.
5. APTT assay results may be affected by many commonly administered drugs.

Expected values:

PT seconds: (11.5-15.0)

PT activity: (70-120)%

INR: (0.90-1.15)

Therapeutic levels of INR (2.0 – 3.0) , target range (2.5)

Fibrinogen: (200-400)mg/dl (2.00-4.00) g/l

PTT seconds: lies between (27-35) seconds.

Hematology Standard Operating Procedures (SOPs)

وزارة الصحة - الإدارة العامة للمستشفيات - دائرة مختبرات وبنوك دم المستشفيات

Interpretation of results:

Interpretation of PT and PTT in patients with a Bleeding or Clotting Syndrome:

▪ Prolonged PT:

Inherited: Factor VII deficiency

Acquired: Vitamin K deficiency, Liver disease, Warfarin use, Factor VII inhibitor

▪ Prolonged APTT:

Inherited: vWF, factor VIII, IX, XI, XII deficiency

Acquired: Heparin use, Inhibitor of vWF, factor VIII, IX, XI, XII, Antiphospholipid antibodies

▪ Prolonged both PT and APTT:

Inherited: Prothrombin, fibrinogen, factor V, X or combined factor deficiency

Acquired: Severe Liver disease, Disseminated intravascular coagulation (DIC).

Supratherapeutic heparin or warfarin, Combined heparin or warfarin use, Inhibitor of prothrombin, fibrinogen, factor V, X, Direct thrombin inhibitor.

Reporting Results:

- The PT and PTT tests are reported in seconds along with the reference ranges.
- PT test results reported in:
 - ✓ Time (Seconds)
 - ✓ Ratio ($PT_{\text{patient}}/PT_{\text{control}}$)
 - ✓ Activity Percentage
 - ✓ INR
- The ACL Analyzer will automatically calculate the INR value when the ISI value is entered in the ACL. This value will appear beside the PT result.

$$INR = \left[\frac{PT \text{ Patient}}{PT \text{ Reference Plasma}} \right]^{ISI}$$

Note: The International Sensitivity Index (ISI) is an experimentally derived measurement, usually provided by the thromboplastin manufacturer "according to package insert".

Hematology Standard Operating Procedures (SOPs)

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Thrombolyzer compact X

SOPs\ HGA \.....H\ Haem \08

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition

Thrombolyzer compact x is a classic, fast and reliable coagulation analyzer, it performs up to 160 tests/ hour, capable of performing the following tests PT,PTT,FIBRONGEN , ATIII, TT.

Responsibilities:

- Hematology department personal are required to be knowledgeable of this procedure
- The head of the department must resolve any problem with the process and difficulties in using this sop
- New employees are trained and assessed for competence before they can handle patient sample

Specimen requirements:

- Plasma sample, The anticoagulant of choice for coagulation studies is (3.2%) Sodium Citrate (Blue Top Tube).
- The standard ratio for citrated specimens is nine (9) parts of blood + one (1) part of anticoagulant, (9:1 ratio) that is critical for valid results.
- Platelet poor plasma required, Set the centrifugation speed to (3,000) rpm and centrifuge a sample for (10) minutes.
- Separate the plasma from the cells within 30 minutes after centrifugation of the sample and place the plasma sample in a plastic tube.
- If the specimen will be tested within two to four hours after centrifugation, it may be kept at room temperature.

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Specimen reception:

- Prior to performing coagulation testing on patient samples, verify the patient identification. Any discrepancy must be investigated before processing the specimen.
- Check the specimen for clots, visually hemolyzed and lipemic samples.
- Ensure the specimen is labeled and label a sample cup.

Criteria for rejecting hematology specimen:

- When the identification is missing /inadequate.
- Clotted specimens.
- Lipemic, icteric or hemolyzed plasma samples
- Incomplete filling of tube or over-filled samples.
- Inappropriate container.
- Unknown duration of delay.
- Inappropriate transport/storage

Abbreviations:

PT : Prothrombin Time.

APTT: activated thromboplastin time.

TT: thrombin time.

ATIII : anti thrombin III.

INR: International Normalized Ratio

ISI: International Sensitivity Index

Equipment & Items required:

- PT reagent .
- PTT reagent.
- CaCl_2 reagent .
- Sample cup.
- Cuvette rack segment
- Dropper .
- Centrifuge.
- Probe cleaner solution

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Calibration :

1. Form the "calibration" menu's test window choose the test you want to used, then press (enter).
2. Move the cursor to the "Manual" box, using the (enter) keys, confirming this box by pressing (enter) the values can be entered.
3. Should you want to use less calibration points for the calibration, change the values of the last positions to 0 (zero).
4. Having completed the changes to the table, change the Normal, ISI, Min, and Max values, if necessary.
5. With all changes done, press (Esc). The cursor moves to the "Curve" box (enter). You can now view the new curve representation. Press (esc) to exit the graph.
6. The cursor moves to the "Valid" box (enter). If you wish to keep the old values, change the "Valid: Yes" box using the (space) key to "No" before exiting the menu with (enter). The cursor moves to the "Manual" box (esc).

Procedure :

1. System start-up: set the power switch on.
2. Before starting a routine run operation:
 - ✓ check the level of the distilled water container,
 - ✓ empty the waste water container.
3. The analyzer now runs through a full initialization procedure checking all internal modules automatically.
4. From the main menu, select patient preparation enter the data on keyboard and select the desired test (a, b, c, or d) by using “↑” or “↓” keys and press enter.
5. Add enough volume of plasma in sample cup .
6. Put sample cup in the rack sample of thrombolyzer according to their position
7. Press ESC then press F₂ to start working the test.
8. Wait for result.
9. Record the result in the result book

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Quality Control:

- Normal and abnormal control will be run on each tests.
- Control results must be within the specified ($\pm 2SD$) limits of the quality control chart.
- If one or more level of control is outside $+ 3SD$, do not report patient results,
Perform troubleshooting action:
 - ✓ check reagent levels and expiration dates.
 - ✓ Repeat the control again, If it is still outside $+ 3SD$;
 - ✓ reconstitute new controls,
 - ✓ check instrument maintenance/cleaning, and repeat.
 - ✓ If results are still outside limits, notify the Hematology Supervisor
Immediately, Corrective action must be taken before reporting patient results.
 - ✓ Out of range controls will be recorded on the appropriate
(normal/abnormal control) Service Troubleshooting Log along with the corrective action taken.

Limitations/ Interfering Factors:

1. Hemolysis can cause clotting factor activation and falsely decrease end point measurements.
2. Lipemia and icterus interfere with the spectrophotometric measurements resulting in falsely decreased end point determinations.
3. Contaminated reagents will give inaccurate results.
4. Samples with a fibrinogen levels < 25 mg/dl should be suspected of being a serum sample and rejected.
5. APTT assay results may be affected by many commonly administrated drugs.

Expected values:

PT seconds: **(11.5-15.0)**

INR: **(0.90-1.15)**

Therapeutic levels of INR **(2.0 – 3.0)** , target range **(2.5)**

PTT seconds: lies between **(27-35) seconds.**

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However, this varies widely between laboratories and is dependent upon a number of variables including whether the test is automated or manual, the type of activator and the incubation times employed in the test.

Note: recommendation that each laboratory determines its own normal range

Interpretation of the results:

Interpretation of PT and PTT in patients with a Bleeding or Clotting Syndrome

Prolonged PT:

Inherited: Factor VII deficiency

Acquired: Vitamin K deficiency, Liver disease, Warfarin use, Factor VII inhibitor

Prolonged APTT:

Inherited: vWF, factor VIII, IX, XI, XII deficiency

Acquired: Heparin use, Inhibitor of vWF, factor VIII, IX, XI, XII, Antiphospholipid antibodies

Prolonged both PT and APTT:

Inherited: Prothrombin, fibrinogen, factor V, X or combined factor deficiency

Acquired: Severe Liver disease, Disseminated intravascular coagulation (DIC).

Supratherapeutic heparin or warfarin, Combined heparin or warfarin use, Inhibitor of prothrombin, fibrinogen, factor V, X, Direct thrombin inhibitor.

Reporting result:

- The PT and PTT tests are reported in seconds along with the reference ranges.
- PT test results reported in:
 - ✓ Time (Seconds)
 - ✓ INR
- The thrombolyzer Analyzer will automatically calculate the INR value when the ISI value is entered in the thrombolyzer This value will appear beside the PT result.

$$INR = \left[\frac{PT \text{ Patient}}{PT \text{ Reference Plasma}} \right]^{ISI}$$

Note: The International Sensitivity Index (ISI) is an experimentally derived measurement, usually provided by the thromboplastin manufacturer "according to package insert"

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PROTHROMBIN TIME (MANUAL TEST)

SOPs\ HGA \.....H\ Haem \09

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Definition & Purpose :

- The prothrombin time is the time required for the plasma to clot after an excess of thromboplastin and an optimal concentration of calcium have been added.
- The PT measures functional activity of the extrinsic and common pathways (VII, V, and X, prothrombin, and fibrinogen).
- PT is the most widely used method for monitoring patients receiving oral anticoagulant as warfarin therapy.

Responsibilities:

- Hematology department personal are required to be knowledgeable of this procedure
- The head of the department must resolve any problem with the process and difficulties in using this sop
- New employees are trained and assessed for competence before they can handle patient sample

Specimen Requirements:

- Plasma sample, The anticoagulant of choice for coagulation studies is 3.2% Sodium Citrate (Blue Top Tube).
- The standard ratio for citrated specimens is nine (9) parts of blood + one (1) part of anticoagulant, (9:1 ratio) that is critical for valid results.
- Platelet poor plasma required, Set the centrifugation speed to 3,000 rpm and centrifuge a sample for 10 minutes.
- Separate the plasma from the cells within 30 minutes after centrifugation of the sample and place the plasma sample in a plastic tube.
- If the specimen will be tested within two to four hours after centrifugation, it may be kept at room temperature.

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Specimen reception:

1. Prior to performing coagulation testing on patient samples, verify the patient identification. Any discrepancy must be investigated before processing the specimen.
2. Check the specimen for clots, visually hemolyzed and lipemic samples.
3. Ensure the specimen is labeled and label a sample cup.

Criteria for rejecting hematology specimen:

- When the identification is missing /inadequate.
- Clotted specimens.
- Hemolyzed plasma samples
- Incomplete filling of tube or over-filled samples.
- Inappropriate container.
- Unknown duration of delay.
- Inappropriate transport/storage

Abbreviations:

- **PT** : prothrombin time.
- **I.N.R** : international normalized ratio
- **ISI** : international sensitivity index
- **DIC** : disseminated intravascular coagulation
- **VWF** : Von Willebrand factor
- **SD** : Standard Deviation

Equipment & Items required:

Calcified thromboplastin reagent, Fresh pooled plasma normal control, abnormal control, Water bath at 37°C, Tissue paper, Stop watch, Glass tube, Yellow tips , Micro pipette 50-200 µl.

Procedure:

1. Reconstitute tissue thromboplastin reagent according to instructions. Label the thromboplastin with the time, date and initials.
2. Pipet **100 µL** of plasma into test tubes.
3. Allow at least 1-3 minute for the control and sample to reach 37° C in water bath.

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4. Pipet 200 μ L of PT reagent into the tube containing the plasma. Start the stop watch simultaneously.
5. Mix the tube and leave in the water bath for a minimum of 7-8 seconds. Then remove, wipe the exterior, tilt back and forth gently until a visible clot is formed. As the clot forms, the mixture will gelatinize and may turn cloudy.
6. Stop the stop watch immediately when the clot begins to form and record the time in seconds.
7. Repeat the procedure for the second run of plasma to confirm result and Record the time.
8. If results are not within required limits, a third run should be performed and average the two that match within acceptable limits.

Quality control procedures:

The normal control and the abnormal control will be run on each tests. The control results must be within the specified ($\pm 2SD$) limits of the quality control chart.

- If one or more level of control is outside + 3SD, do not report patient results.

Perform troubleshooting action:

- check reagent levels and expiration dates.
- Repeat the control again. If it is still outside + 3SD,
 - ✓ reconstitute new controls and reagents,
 - ✓ check water bath temperature, and repeat.
- If results are still outside limits, notify the Hematology Supervisor immediately. Corrective action must taken before reporting patient results.
- Out of range controls will be recorded on the appropriate (normal/abnormal control) Service Troubleshooting Log along with the corrective action taken.

Expected values:

The normal values for the prothrombin time range from 11.5 to 15.0 seconds and it is different from lab to lab depend on the source of thromboplastin used.

PT seconds: **11.5-15.0**.

PT activity: **70-120%**

INR: **0.90-1.15**

Therapeutic levels of INR **2.0 – 3.0** target range **2.5**

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Note: recommendation that each laboratory determines its own normal range.

Limitations/ Interfering substance:

1. Associated with specimen (Pre-analytical)

- Inappropriate ratio of anticoagulant to blood
- Delay in testing or processing
- Inappropriate storage

2. Associated with Reagent (Analytical)

- Incorrect preparation of reagents
- Use of reagents beyond reconstituted stability time or expiration date
- Contaminated reagent.

3. Associated with procedure

- Incorrect temperature
- Incorrect incubation times.
- Incorrect volumes of sample, reagents or both

Also test may be affected by :

- Severe diarrhea or vomiting that causes fluid loss and dehydration, this may increase the INR.
- Getting a lot of vitamin k may decrease the INR.
- Medication: antibiotics, aspirin, cimetidine.

Interpretation of the results:

Interpretation of PT and PTT in patients with a Bleeding or Clotting Syndrome

Prolonged PT:

Inherited: Factor VII deficiency

Acquired: Vitamin K deficiency, Liver disease, Warfarin use, Factor VII inhibitor

Prolonged APTT:

Inherited: vWF, factor VIII, IX, XI, XII deficiency

Acquired: Heparin use, Inhibitor of vWF, factor VIII, IX, XI, XII, Antiphospholipid antibodies

Prolonged both PT and APTT:

Inherited: Prothrombin, fibrinogen, factor V, X or combined factor deficiency

Acquired: Severe Liver disease, Disseminated intravascular coagulation (DIC).

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Suprathapeutic heparin or warfarin, Combined heparin or warfarin use, Inhibitor of prothrombin, fibrinogen, factor V, X, Direct thrombin inhibitor.

Reporting Results:

- The PT is reported in seconds along with the reference ranges.
- PT test results reported in:
 - ✓ Time (Seconds)
 - ✓ Ratio (PT_{patient}/PT_{control})
 - ✓ Activity Percentage
 - ✓ INR

$$\text{INR} = \left[\frac{\text{PT Patient}}{\text{PT Reference Plasma}} \right]^{\text{ISI}}$$

Note: The International Sensitivity Index (ISI) is an experimentally derived measurement, usually provided by the thromboplastin manufacturer "according to package insert".

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PARTIAL THROMBOPLASTIN TIME (MANUAL TEST)

SOPs\ HGA \.....H\ Haem \10

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Definition & Purpose :

The activated partial thromboplastin time is the time required for plasma to clot when maximal surface contact activation, optimal phospholipid, and calcium concentration are provided. The PTT measures functional activity of the intrinsic and common pathway as well as screen for coagulation inhibitors such as the lupus-like anticoagulant. APTT is the most widely used method for monitoring intravenous heparin anticoagulant therapy.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen Requirements:

- Plasma sample, The anticoagulant of choice for coagulation studies is 3.2% Sodium Citrate (Blue Top Tube).
- The standard ratio for citrated specimens is nine (9) parts of blood + one (1) part of anticoagulant, (9:1 ratio) that is critical for valid results.
- Platelet poor plasma required, Set the centrifugation speed to 3,000 rpm and centrifuge a sample for 10 minutes.
- Separate the plasma from the cells within 30 minutes after centrifugation of the sample and place the plasma sample in a plastic tube.
- If the specimen will be tested within two to four hours after centrifugation, it may be kept at room temperature.

Hematology Standard Operating Procedures (SOPs)

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Specimen reception:

1. Prior to performing coagulation testing on patient samples, verify the patient identification. Any discrepancy must be investigated before processing the specimen.
2. Check the specimen for clots, visually hemolyzed and lipemic samples.
3. Ensure the specimen is labeled and label a sample cup.

Criteria for rejecting hematology specimen:

- When the identification is missing /inadequate.
- Clotted specimens.
- Hemolyzed plasma samples
- Incomplete filling of tube or over-filled samples.
- Inappropriate container.
- Unknown duration of delay.
- Inappropriate transport/storage

Abbreviations:

- APTT (activated partial thromboplastin time).
- CaCl_2 (calcium chloride)
- DIC (disseminated intravascular coagulation).
- VWF (Von Willebrand factor)

Equipment & Items required:

Partial thromboplastin reagent: consists of phospholipids and a contact activator, CaCl_2 (0.025M), Fresh pooled plasma normal control, abnormal control, Water bath at 37°C, Tissue paper, Stop watch, Glass tube, Yellow tips , Micro pipette 50-200 µl.

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Procedure:

1. Pre-warm a sufficient quantity of 0.025M calcium chloride reagent to 37°C.
2. Pipette 100 µL of patient plasma into a labeled test tube.
3. Into test tube, add 100 µL of partial thromboplastin reagent.
4. Incubate the patient plasma/partial thromboplastin mixture at 37°C for a minimum of three (3) minutes (optimum activation of contact factors).
5. Add 100 µL of calcium chloride into the patient plasma / partial thromboplastin mixture and start the stop watch immediately.
6. After 20 seconds, remove the tube from the water bath. Wipe off the outside of the tube. Gently tilt the tube back and forth until a visible clot forms.
7. Immediately record the time in seconds.
8. The result must be run in duplicate.

Quality control procedures:

The normal control and the abnormal control will be run on each tests. The control results must be within the specified ($\pm 2SD$) limits of the quality control chart.

- If one or more level of control is outside + 3SD, do not report patient results.
Perform troubleshooting action:
 - check reagent levels and expiration dates.
 - Repeat the control again. If it is still outside + 3SD,:
 - ✓ reconstitute new controls and reagents,
 - ✓ check water bath temperature, and repeat.
 - If results are still outside limits, notify the Hematology Supervisor immediately.
Corrective action must taken before reporting patient results.
- Out of range controls will be recorded on the appropriate (normal/abnormal control) Service Troubleshooting Log along with the corrective action taken.

Expected values:

PTT reported in seconds: lies between **27-35 seconds**. However, this varies widely between laboratories and is dependent upon a number of variables including whether the test is automated or manual, the type of activator and the incubation times employed in the test.

Note: recommendation that each laboratory determines its own normal range.

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Limitations/ Interfering Factors:

1. Associated with specimen (Pre-analytical)

- Inappropriate ratio of anticoagulant to blood
- Delay in testing or processing
- Inappropriate storage

2. Associated with Reagent (Analytical)

- Incorrect preparation of reagents
- Use of reagents beyond reconstituted stability time or expiration date
- Contaminated reagent.

3. Associated with procedure

- Incorrect temperature
- Incorrect incubation times (\uparrow incubation time= \downarrow PTT due to contact activation and $> 5\text{min}$ heating will result in loss of heat-labile factor V)
- Incorrect volumes of sample, reagents or both

4. Some drugs such as heparin, antihistamine ascorbic acid ,chlorpromazine and salicylates.

Interpretation of the results:

Interpretation of PT and PTT in patients with a Bleeding or Clotting Syndrome

Prolonged PT

Inherited: Factor VII deficiency

Acquired: Vitamin K deficiency, Liver disease, Warfarin use, Factor VII inhibitor

Prolonged APTT

Inherited: vWF, factor VIII, IX, XI, XII deficiency

Acquired: Heparin use, Inhibitor of vWF, factor VIII, IX, XI, XII, Antiphospholipid antibodies

Prolonged both PT and APTT

Inherited: Prothrombin, fibrinogen, factor V, X or combined factor deficiency

Acquired: Severe Liver disease, Disseminated intravascular coagulation (DIC).

Supratherapeutic heparin or warfarin, Combined heparin or warfarin use, Inhibitor of prothrombin, fibrinogen, factor V, X, Direct thrombin inhibitor.

Reporting Results: PTT reported in seconds.

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Bleeding time

SOPs\ HGA \.....H\ Haem \11

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

- Bleeding time is defined as the time taken for a standard skin wound to stop bleeding upon vessel injury, platelets adhere and form a haemostatic platelet plug. bleeding time measures the ability of these platelets to arrest bleeding and therefore measures platelets function as well as the integrity of the vessel wall.
- Bleeding time: it is a test for :
 - Capillary response to injury.
 - Platelet function: stick to each other and form plug (aggregate), and break and release thromboplastin.
- There are several methods of performing the bleeding time:
 - Duke method
 - IVY method.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure
- The head of the department must resolve any problem with the process and difficulties in using this sop
- New employees are trained and assessed for competence before they can handle patient sample

Specimen requirements:

Patient Preparation: Check the patient's history for recent use of drugs that prolong bleeding time. If the test is being used to identify a suspected bleeding disorder, it should be postponed and the drugs discontinued. If the test is being used preoperatively to assess hemostatic function, it should proceed as scheduled.

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Specimen reception:

1. Explain to the patient this test is used to measure the time required to form a clot and stop bleeding.
2. Reassure the patient that, although he may feel some discomfort from the incisions, the antiseptic, advise the patient that the incisions will leave two small, hairline scars that should be barely visible when healed.

Equipment & Items required:

Blood pressure cuff, disposable lancet, 70% alcohol, filter paper, bandage, stopwatch.

Abbreviations:

B.T: Bleeding time

Procedures:

Duke method

1. Gently clean the lobe of the ear with cotton wool and alcohol, do not rub. Allow to dry.
2. Puncture the ear lobe, with the lancet, making the incision 2-4mm deep the blood should flow freely, without any need to squeeze the ear lobe. Start the stopwatch.
3. After 30 seconds collect the first drop of blood on a corner of the filter-paper
4. Do not touch the skin with the paper.
5. Wait 30 seconds more. Collect the second drop of blood in the same way, a little further along the strip of paper.
6. Continue to collect one more drop of blood every 30 seconds. The drops become progressively smaller.
7. When no more blood appears, stop the timer and record the time.

Ivy method

1. Blood pressure cuff is placed on arm and inflated to 40mm Hg
2. With pressure maintained, the arm is cleaned and a lancet is again used to make a 2-4 mm cut on the volar aspect of the forearm, being careful NOT to cut any visible blood vessels.
3. Filter paper is used to blot blood every 30 seconds until it stops flowing.
4. If bleeding continues for more than 15 minutes, the procedure should be discontinued.

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Limitations/ Interfering substance:

1. If the patient has taken aspirin or aspirin-containing compounds 7 to 10 days prior to the procedure, the bleeding time may be prolonged.
2. Other medications such as dextran, streptokinase, and streptodornase may also affect the bleeding time.
3. Results may be affected by an improperly performed puncture. A puncture that is too shallow, too deep, or in an inappropriate location will adversely affect test results.
4. The alcohol must be completely dried before making the puncture. If residual alcohol is on a puncture site, the bleeding time will be erroneously prolonged.
5. If the phlebotomist allows the filter paper to touch the wound, the platelet clot may be dislodged, causing falsely elevated results.
6. Blood pressure cuff should be maintained exactly at (40mm Hg).

Expected values:

Duke method: 1–3 minutes

Ivy method: 3 to 6 minutes

Interpretation of the results:

A. Abnormal increased results could be due to:

1. Thrombocytopenia, with failure to produce a platelet plug.
2. Poor platelet function
3. Failure of vessel constriction on injury
4. Puncture of larger blood vessel (technical error)
5. Disturbing the clot (technical error)

B. **Prolonged bleeding time** may indicate the presence of disorders associated with such as Hodgkin's disease, acute leukemia, disseminated intra vascular coagulation, hemolytic disease of the newborn, liver disease- end stage and in some cases of Von Willebrand Disease, Hypofibrinogenaemia, Bernard-Soulier disease and Glanzmann's thrombasthenia.

C. **Decreased BT**: Not clinically significant.

Reporting result:

Bleeding time:minute :.....sec

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Clotting time

SOPs\ HGA \.....H\ Haem \12

Version: 1	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

It is the time required for blood to clot without the presence of any substance .

The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors. Variations are wide and the test sensitivity is limited. Whole blood, when removed from the vascular system and exposed to a foreign surface, will form a solid clot. Within limits, the time required for the formation of the solid clot is a measure of the coagulation system.

There are various methods for determining the clotting time, the most common being the capillary tube method and slide method.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure
- The head of the department must resolve any problem with the process and difficulties in using this sop
- New employees are trained and assessed for competence before they can handle patient sample

Specimen requirements:

- 1- About 3 drops of blood on a glass slide and examining it for clot formation.
- 2- About 200 ul blood in capillary tube.

Specimen reception:

Explain to the patient this test is used to measure the time required to form a clot and explain how the test will be performed.

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Equipment & Items required:

Alcohol swab, lancet, glass slide, stopwatch and capillary tubes.

Abbreviations:

C.T: Clotting time

Procedures:

Capillary tube method.

1. Clean the patient finger with alcohol swab and allow to dry.
2. Pricked the finger by lancet, remove the first drop of blood.
3. Squeeze the finger to obtain a larger drop of blood and fill two capillary tubes with blood.
4. After one minute start breaking small pieces of the capillary tube every 30 second until a fibrin thread is seen between the two broken ends.

Slide method

1. Clean one finger with the alcohol swab then puncture it with the lancet.
2. Place a large drop of blood on the glass slide. Start the stopwatch.
3. After one minute, dip the lancet all the way into the blood drop then slowly lift the lancet out.
4. If you see a gel-like thread of fibrin (blood clot protein) the blood has clotted.
5. Record the clotting time.
6. If no piece of fibrin is observed, keep repeating the dipping procedure until the thread is observed.

Limitations/ Interfering substance:

- The test sensitive only in extreme factor deficiencies, and is insensitive to high doses of heparin.
- The following variables tend to decrease the clotting time: rough handling of the blood specimen, presence of tissue fluids (traumatic venipuncture).

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Expected values:

Slide method (2 to 7) minutes

Capillary tube method (2 to 8) minutes

Interpretation of the results:

- In coagulation disorders like hemophilia, clotting time is prolonged but bleeding time remains normal.
- Clotting time is also prolonged in conditions like vitamin K deficiency, liver diseases, disseminated intravascular coagulation, overdosage of anticoagulants etc.

Reporting result:

Clotting timeminutes : seconds.

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Blood film (leishman's stain)

SOPs\ HGA \.....H\ Haem \ 13

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

- Describe the procedure for preparing thin blood smear and staining with leishman's stain for the purpose of diagnosis of different anemia's, leukemia's, viral and parasitic infections, in addition associated with inclusion bodies, allergic reactions, hemoglobinopathies, and platelets disorders
- It is based on a methanolic mixture of "polychromed" methylene blue and eosin.
- The methanolic stock solution is stable and also serves the purpose of directly fixing the smear eliminating a prefixing step.

Responsibilities:

- Hematology department personal are required to be knowledgeable of this procedure
- The head of the department must resolve any problem with the process and difficulties in using this sop
- New employees are trained and assessed for competence before they can handle patient sample

Specimen requirements:

Freshly collected venous blood in an EDTA container is recommended.

Specimen reception:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Smears of peripheral blood must be made immediately

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Criteria for rejection hematology specimens:

1. When The Identification Is Missing /Inadequate.
2. Insufficient Quantity
3. Inappropriate Container
4. Inappropriate transport/storage
5. Unknown duration of delay
6. Haemolysed and/or clotted sample reject

Equipment & Items required:

- Leshimen's stain
- Microscope.
- Microscopic slides.
- Distilled water.
- Methanol 96%.
- Droppers.
- Immersion oil.

Abbreviations:

B. film : Blood film.

RBC : red blood cell.

WBC : white blood cell.

CBC : complete blood count.

EDTA : ethylene diamine tetra acetic acid

RNA : Ribonucleic acid

Procedures:

First preparation of leishman's stock stain :

1. Dissolve 1.5 g of leishman's powder in 1000 ml of methanol.
2. Mix well until the powder dissolve completely & let it to stand for 24 hr.
3. filter the stock stain with filter paper.

Second blood film preparation :

1. Small drop of blood placed at the end of the slide.
2. Using another slide, the blood can be spread to make the smear.
3. Let's to dry

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4. Add one volume of leishman's stain on blood film smear for 1 min
5. Add two volume of distilled water .
6. Mix then wait for 15 min.
7. Wash with tape water.
8. let's to dry and observe under microscope.

Quality control procedures:

- A well-made slide will have three distinct regions: a head, body, and tail.
- Repeat counts of differential WBC's and RBC's morphology on selected slides on subsequent days will give an indication of the range in the variation of the results. (include note on reagent quality).
- When a new batch of stain is prepared, decide the best staining time to use, e.g. stain films made from the same blood at different times, e.g. 5, 7, 10, 12, 15 minutes.

Limitations/ Interfering substance:

Causes of incorrectly stained blood smear (too blue):

1. Buffered water or stain is too alkali.
2. Excessive thickness of the smear.
3. Alkaline residue on the slide.
4. Insufficient washing.
5. Prolonged staining.

Causes of incorrectly stained blood smear (too red):

1. Buffered water or stain is too acid.
2. Acid residue on slide.

Entire smear has a pale stain:

1. Under staining
2. Weak stain.
3. Excessive washing on allowing buffered distilled water to stand on slide.
4. Using warm or hot buffered distilled water for washing slide.

Variations on staining on different areas of the smear:

1. Buffered water was unevenly applied.
2. Acid or alkali residue on slide.
3. Water wasn't properly drained from slide after washing.

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Precipitated stain:

1. Lack of through washing.
2. Stain hasn't properly filtered.

Evaporation of alcoholic stain may be due to excessive staining time, slide tilted so that stain runs to one end or to titling stain of slide before washing.

Expected Result:

- Erythrocytes stain buff pink to pale bluish-gray.
- Leukocytes: neutrophils, monocytes and lymphocytes have a pale blue-gray cytoplasm and purple nucleus.
- Eosinophil have coarse, pink granules in the cytoplasm.
- Basophils have coarse, deep blue granules in the cytoplasm.
- Platelets have pale blue cytoplasm and diffuse red nuclear material.
- Normal RBC's shape are normocytic, normochromic, no variation in size, no shape variation , and no inclusion bodies.
- Normal WBC's morphology and differential.
- Normal platelets size, shape and distribution.

Interpretation of the results:

- Red cell according to hemoglobin content : normochromic, hypochromic, hyperchromic
- Variation of red cell size (anisocytosis): normocytic, macrocytic, and microcytic
- Variation of red cell shape (poikilocytosis): normal or abnormal; report the presence of sickle cells, target cells, spherocytes, etc.
- Red cell inclusion: RNA, polychromasia, Howell-Jolly bodies, etc.
- Nucleated red blood cells and all types of erythroblasts are abnormal.
- Elevated white blood cell count may mean infection.
- Decreases in white blood cell count may occur with disease progression or may indicate bone marrow suppression.
- Total lymphocyte count: decrease in absolute lymphocyte count may reflect bone marrow suppression.

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- An increase in neutrophils may be due to an acute bacterial infection or hematological malignancies such as myeloid leukemia.
- An increase in eosinophils may be due to a parasitic infection or an allergic reaction.
- An increase in lymphocytes may be due to viral infections or chronic infections such as tuberculosis or lymphocytic leukemia.
- An increase in monocytes is found in hematological malignancies such as chronic myelomonocytic leukemia and certain bacterial and parasitic infections (e.g., typhoid fever, malaria).

Reporting result:

RBC's Morphology:.....

WBC's differential:

Neutrophils:%.

Lymphocytes:.....%.

Eosinophil's:..... %.

Basophils:.....%.

Blast cells:.....%.

Platelets morphology & distribution:.....

Hematology Standard Operating Procedures (SOPs)

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Blood Group \ (Slide Method) BIOTEC LABORATORIES

SOPs\ HGA \.....H\ Haem \14

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

Determine the ABO and Rh group in human blood. ABO and Rh group are determined by detection of the presence or absence of A & B antigen in ABO system and presence or absence of D antigen in Rh system. Because of its low sensitivity, slide method is used only for preliminary grouping.

Responsibilities:

- Hematology department personnel are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The Head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Blood collected in an EDTA container is recommended.

Specimen reception and rejection:

Reception of samples should be recorded, and record time of reception. It is essential to pay strict attention to sample identification and labeling of tubes.

Clotted samples, hemolysed samples and samples collected in other anticoagulants than EDTA are rejected. Samples should be stored at 2-8 °C if not tested immediately.

Equipment & Items required:

- Glass slides, Wooden applicator.
- Anti-A(blue), Anti-B(yellow) and Anti-D reagents.

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Abbreviations:

- ABO : blood group system. Rh : rhesus factor.

Procedures:

1. Bring the reagents at room temperature.
2. Mix blood tube gently.
3. Transfer one drop of whole blood for each test to a glass slide.
4. Transfer one drop of each reagent to each blood drop.
5. Mix whole blood & reagent by using wooden applicator.

Quality control procedures:

The reactivity of blood grouping reagents should be confirmed by testing with known blood group blood samples on each day of use.

Limitations/ Interfering substance:

- Drying up of reaction mixture can cause aggregation of cells, giving false positive results.
- Abnormal plasma proteins, cold auto agglutinins, positive direct anti globulin test and in some cases bacteremia may interfere.

Expected result:

Agglutination indicates a positive result to the corresponding antigen, no agglutination indicates a negative result to corresponding antigen.

Interpretation of the results:

Anti- A	Anti-B	Anti-D	Blood group
-	-	+	O ⁺
+	-	+	A ⁺
-	+	+	B ⁺
+	+	+	AB ⁺
-	-	-	O ⁻
+	-	-	A ⁻
-	+	-	B ⁻
+	+	-	AB ⁻

Reporting result:

Blood group: Rh:.....

Hematology Standard Operating Procedures (SOPs)

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Erythrocyte Sedimentation Rate (Westergren technique)

SOPs\ HGA \.....H\ Haem \15

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose/Definition:

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and CRP.

When well-mixed venous blood is placed in a vertical tube, erythrocytes will tend to fall toward the bottom. The length of fall of the top of the column of erythrocytes in a given interval of time.

Responsibilities:

- Hematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Either venous blood collected directly into sodium citrate and tested within 2 hours, or EDTA anticoagulated blood diluted in sodium citrate can be used.

If EDTA blood is used and kept refrigerated at 4-8°C, citrate dilution of the blood and testing can be delayed for up to 6 hours.

Specimen reception:

Samples must be transported as soon as possible after collection at 18–22°C, and the tests samples should be analyzed within 2 hours after collection.

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Criteria for Rejecting Hematology Specimens:

1. When the identification is missing /Inadequate.
2. Insufficient quantity.
3. Inappropriate container.
4. Inappropriate transport /storage.
5. Unknown duration of delay.

Equipment & Items required:

1. Tri-Sodium citrate, 32 g/l (3.2 % w/v) anticoagulant.
2. Glass Westergren pipettes or when available, disposable plastic Westergren pipettes can be used.
3. Westergren pipettes measures 300mm in length (plastic pipettes are often shorter) and are graduated from 0-200mm. The diameter should not be less than 2.55mm.
4. Timer.

Abbreviations:

ESR: Erythrocyte sedimentation rate

CRP: C-reactive protein.

EDTA: Ethylene diamine tetra acetic acid

Procedures:

1. Pipette 0.4 ml of sodium citrate anticoagulant into a small container.
2. Add 1.6 ml of venous blood or EDTA anticoagulated blood and mix well.
3. Remove the cap of the container and place the sample in the ESR stand (make a note of the number in the patient's notes).
4. Insert a Westergren pipette and ensure it is positioned vertically.
5. Using a safe suction method, draw the blood to the 0 mark of the Westergren pipette, avoiding air bubbles.
6. Check that the ESR stand is level by making sure that the bubble in the spirit level is central. If required, adjust the screws on the bottom of the stand. Re-check that the pipette is vertical.
7. Set the timer for 1 hour. Ensure the ESR stand and pipette will not be exposed to direct sunlight.

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8. At the end of the hour read the height of clear plasma above the upper margin of the column of sedimenting cells to the nearest millimeter.

Quality control procedures:

The most practical way of controlling ESR tests is to follow the test method exactly.

Limitations/ Interfering substance:

1. Using the wrong volume of blood to anticoagulant.
2. Blood not sufficiently mixed with anticoagulant.
3. Clots in the blood.
4. Air bubbles at the top of the column.
5. Testing blood samples at the hottest time of the day, or leaving tests in direct sunlight. Temperatures over 25°C increase sedimentation.
6. Using a pipette, which is, not clean or not dry.
7. Pipette not positioned vertically. Even slight variations from the upright increase sedimentation.
8. Not checking whether the ESR stand is level on the bench.
9. Placing an ESR stand on the same bench as a centrifuge where vibration will interfere with sedimentation.
10. Measuring the ESR when a patient is dehydrated.

Expected values:

- Men...Up to 15 mm/hour
- Women...Up to 20 mm/hour
- Elderly...Up to 20 mm/hour
- Children...Up to 15 mm/hour

Interpretation of the results:

An elevated ESR may be found in:

- Pregnancy (after the third month).
- Acute and chronic infections.
- Rheumatic fever.
- Rheumatoid arthritis.
- Myocardial infection.

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- Nephrosis.
- Acute hepatitis.
- Menstruation.
- Tuberculosis.
- Hypothyroidism.
- Hyperthyroidism.

Adults over 60 years of age frequently have a slightly higher ESR value due primarily to decreased concentrations of plasma albumin.

A decreased ESR will be present in:

- Polycythemia.
- Congestive heart failure.
- Hypofibrinogenemia.
- The presence of red blood cell abnormalities (poikilocytosis, spherocytes, and sickle cells).

Reporting result:

ESR:mm/1st Hour

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Osmotic Fragility Test

SOPs\ HGA \.....H\ Haem \16

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Definition & Purpose:

Osmotic fragility test is a test to measure red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions "hypotonic solutions" .

The test is done to evaluate hemolytic anemia especially hereditary spherocytosis and hemolytic states.

This procedure is employed to help diagnose different types of anemia's, in which the physical properties of the red blood cell are altered.

Responsibilities:

- Hematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample
- The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

Heparinized venous blood sample (2-3 ml) . The test should be carried out within 2 hours of collection or 6 hours if kept at 4°C.

Specimen reception:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Samples must be transported as soon as possible, and the tests samples should be analyzed within 2 hours after collection.

Criteria for rejection haematology specimens

1. When the identification is missing /inadequate.
2. Insufficient Quantity
3. Inappropriate Container

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4. Inappropriate transport/storage
5. Unknown duration of delay
6. Haemolysed and/or clotted sample reject

Equipment & Items required:

Plastic centrifuge tube, Racks, Stopwatch, Parafilm, Automatic pipette 1ml, Automatic pipette 10 to 50 μ L, Stock solutions consist of buffered NaCl 1%, Distilled water, Centrifuge, Calculator, Yellow tips, Blue tip, Cuvette, Spectrophotometer, Vortex.

Abbreviations:

EDTA: Ethylene diamine tetra acetic acid.

O,D: Optical Density.

HS: (hereditary spherocytosis).

NaCl: (sodium chloride).

Procedures:

Prepare dilutions of buffered NaCl and place in appropriately labeled test tube

Test tube	1.0%NaCl (ml)	D.W. (ml)	Final conc. (%)
1	10.0	0.0	1.00
2	8.5	1.5	0.85
3	7.5	2.5	0.75
4	6.5	3.5	0.65
5	6.0	4.0	0.60
6	5.5	4.5	0.55
7	5.0	5.0	0.50
8	4.5	5.50	0.45
9	4.0	6.0	0.40
10	3.5	6.5	0.35
11	3.0	7.0	0.30
12	2.0	8.0	0.20
13	1.0	9.0	0.10
14	0.0	10.0	0.00

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1. Mix the preceding dilutions well, using Parafilm to cover each test tube while mixing.
2. Add 0.050 ml of the patients heparinized blood to each of 14 test tubes.
3. Mix each test tube immediately by gentle inversion in vortex.
4. Allow the test tubes to stand at room temperature for 30 minutes.
5. Remix the test tubes gently and centrifuge at 2500 rpm for 5 minutes.
6. Carefully transfer the supernatants to cuvettes and read on Spectrophotometer at wavelength of 540 nm. Set the optical density at 0.0% hemolysis using the supernatant in test tube 1, which represent the blank, or 0.0% hemolysis. Test tube 14 represent 100% hemolysis.
7. Calculate the percent of hemolysis for each supernatant and will be reported.
8. The result of the test may be graphed.

Quality control procedures:

Freshly collected normal heparinized sample obtained in the same way as that the patient.

Limitations/ Interfering substance:

- This test should be performed immediately because shape change and osmotic conditions change with time.
- Presence of hemolytic organisms in the sample gives invalid results "fragility increased"
- Severe anemia or other conditions with fewer RBCS available for testing.
- Recent blood transfusion .
- If anticoagulants blood is used for test use only heparin as anticoagulant , in order to avoid adding more salts to blood such as oxalate , EDTA.
- Arise in the temperature at which the tests are carried out "decreased osmotic fragility".
- The fragility of the red cells is increased by a fall in pH.
- If hemolysis is present in the 0.85% sodium chloride tube or in the normal control, the buffered sodium chloride stock and working solutions should be discarded and re-prepared.

Expected values:

Hemolysis begins 0.45% and complete 0.35%.

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Interpretation of the results:

Osmotic fragility decreased in:

- ✓ Thalassemia.
- ✓ Iron deficiency anemia.
- ✓ Sickle cell anemia
- ✓ After splenectomy, chronic liver disease
- ✓ Hyponatremia.
- ✓ Polycythemia Vera.

Osmotic fragility increased in :

- ✓ Hemolytic anemia
- ✓ Hereditary spherocytosis.
- ✓ Acquired spherocytosis
- ✓ Hypernatremia
- ✓ And whenever spherocytes are found.
- ✓ The older red cells are also more fragile.

Reporting results:

- Calculate the percent of hemolysis , the percent of hemolysis is calculated as

$$\text{Percent of hemolysis} = \frac{\text{O.D. of supernatant}}{\text{O.D. supernatant tube \#14}} \times 100$$

- The results of the test may then be graphed, with the percent hemolysis plotted on the ordinate (vertical axis) and the sodium chloride concentration on the (horizontal axis).

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Cerebrospinal Fluid Cellular Examination

SOPs\ HGA \.....H\ Haem \17

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & definition :

Cerebrospinal Fluid (CSF) is the product of the secretory activity of the choroid plexus. It is the third major fluid of the body and supplies nutrients to the nervous tissue, removes metabolic waste, and protects the brain and spinal cord from trauma. The purpose of a CSF analysis is to diagnose medical disorders that affect the central nervous system.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.
- New employees are trained and assessed for competence before they can handle patient sample.

Specimen requirements

- A physician collects CSF by lumbar puncture and always under aseptic conditions.
- It is a routine practice to collect three (3) sterile tubes of CSF (1-5 ml per tube) for analysis.
- Tubes should be collected and labeled sequentially by the physician at the time of collection.
- All tubes must be labeled properly and delivered immediately to the following sections:
Tube #1: To Chemistry for protein and glucose or serology study.
Tube #2: To Microbiology for culture and gram stain.
Tube #3: To Hematology for cell count and differential

If only one tube is collected, perform testing in the following order to preserve specimen and avoid contamination:

1. Microbiology - culture and gram stain.
2. Hematology - cell count and differential.
3. Chemistry

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Specimen reception:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Samples must be transported as soon as possible, and the tests samples should be analyzed within 1 hours after collection.

Criteria for rejection haematology specimens

1. when the identification is missing /inadequate.
2. insufficient quantity
3. inappropriate container
4. inappropriate transport/storage
5. unknown duration of delay
6. Clotted bloody sample.

Equipment & Items required:

1. Microscope
2. Counting chamber
3. Automatic pipet
4. Yellow tips
5. Coverslips (supplied with the counting chamber)
6. Tubes 2–5ml
7. Turks solution
8. Glass slides
9. Giemsa stain
10. Methanol
11. Normal saline

Abbreviations:

CSF: cerebrospinal fluid

Hematology Standard Operating Procedures (SOPs)

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Procedures:

For turbid CSF sample

1. Make a 1: 20 dilution using 0.05 ml of the CSF and 0.95 ml of Turks solution.
2. Pipette into a small tube and mix.
3. put one drop from the diluted sample on the counting chamber.
4. Leave the counting chamber on the bench for 5 minutes to allow the cells to settle.
5. Place the chamber on the microscope stage.
6. Count the cells in the 4 WBC squares' using the 10 x objective.
7. the counted WBC in the 4 squares' multiply with 50.

For clear CSF sample:

1. If undiluted CSF is used, no calculation is necessary; count the 9 WBC squares' and another random square'.
2. Gives the number per mm^3 of CSF.
3. If erythrocytes are present: Make a dilution 1: 2 using 0.1 ml of the CSF and 0.1 ml of Turks solution.
4. Count the cells in the 10 WBC squares' using the 10 x objective.
5. The counted WBC in the 10 squares' multiply with 2.

Erythrocytes counting:

1. Make a dilution 1: 200 using 0.01 ml of the CSF and 1.99 ml of saline solution.
2. Count the cells in the 5 RBC squares' using the 40 x objective.
3. The counted RBC in the 5 squares' multiply with 10.000.

N.b: to use other dilution the equation can be used:

Number of cells counted x dilution = cells/ μl

Number of squares counted x vol. of 1 square (0.1)

For bloody sample use the equation as follow:

$$\text{WBCs (added)} = \frac{\text{WBC(blood)} \times \text{RBC (CSF)}}{\text{RBC (blood)}}$$

$$\text{True CSF WBCs} = \text{WBC CSF} - \text{WBC added}$$

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Determination of the leukocyte differential leukocyte count:

Clear CSF sample:

1. Centrifuge the CSF at 3000 g for 10 minutes. Pour off the supernatant fluid into another tube (to be used for other tests).
2. Mix the deposit by tapping the end of the tube, spread on a clean slide and leave to dry.
3. Fix with methanol and stain with a Giemsa stain as described in blood film.

Turbid CSF sample:

1. Pipette one drop of uncentrifuged, mixed CSF on to a slide.
2. Make a thin smear and leave to dry.
3. Fix with methanol and stain with a Giemsa stain as described in blood film section.

Limitations/ Interfering substance

1. Specimens must be well mixed. Failure to mix the specimen can cause invalid results.
2. Leukocytes may begin to lyse within one (1) hour after collection. Cell counts must be performed promptly.

Interpretation of the results:

An increased number of leukocytes can be found in:

Bacterial meningitis mostly neutrophils.

Viral meningitis: mostly lymphocytes.

Other microbial infection.

Expected values:

Normal RBC count : (0) cell/ μ l

Normal WBC count (0-5) cell/ μ l

Hematology Standard Operating Procedures (SOPs)

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Reticulocytes Count \ (Brilliant cresyl blue) RAL. Diagnostics SOPs\ HGA \.....H\ Haem \18

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose & Definition:

Describe the procedure for preparing thin blood smear stained with supra-vital dye in order to visualize reticulocytes.

Reticulocytes are the final immature cells in the red blood cells maturation process, and the only immature red cells seen normally in peripheral blood, they are released from the bone marrow and circulate in the peripheral blood to be fully differentiated within 24 hours.

The reticulocyte count is an important diagnostic tool. It is a reflection of the amount of effective red blood cell production taking place in the bone marrow.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.
- New employees are trained and assessed for competence before they can handle patient sample.

Specimen requirements:

Freshly collected venous blood in an EDTA container is recommended.

Specimen reception & rejection :

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Criteria for rejection haematology specimens

1. When the identification is missing /inadequate.
2. Insufficient Quantity
3. Inappropriate Container

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4. Inappropriate transport/storage
5. Unknown duration of delay
6. Haemolysed and/or clotted sample reject.

Equipment & Items required:

- Microscope.
- Microscope slides.
- Plastic tube.
- Commercially prepared liquid Brilliant cresyl blue .
- Immersion oil.
- Micropipette .

Abbreviations:

- Retics count : Reticulocyte count.
- RBCs: Red blood cells.
- µl: Microliter.

Procedures:

1. Deliver about 100 µl of stain into tube, add equal volume of patient blood.
2. Mix well & incubate 15 min at 37°C.
3. At the end of the time, resuspend the red cells by gentle mixing.
4. Dispense one drop of the mixture on slide.
5. Spread the mixture as the blood film, lets dry.
6. Choose the area of field where cells are undistorted and stain is good, using 100X immersion lens count retics seen per 1000 red cells.
7. Report the result in percentage in the result book.

Result and calculation:

Reticulocyte appears as cell containing dark blue granules or blue network.

Count 1000 RBCs including reticulocytes, the number of reticulocyte is reported as percentage of the total RBCs .

$$\text{Retics \%} = \frac{\text{Retics count}}{1000 \text{ RBCs}} \times 100$$

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Quality control procedures:

Two slides should be done for each retics count, final calculated retics % from the two slides should agree with 15%, if this agreement is not reached, prepare a third slide and count.

Limitations/ Interfering substance:

1. High glucose levels and the use of heparin can cause reticulocytes to stain poorly.
2. Falsely decreased reticulocyte counts can result from under staining the blood with new methylene blue. Be sure the stain/blood mixture incubates the full 15 minutes.
3. Thick and thin spreading are improper for counting.
4. Filtration of the stain is necessary when precipitated material is present which can resemble a reticulocyte.
5. A retractile appearance of erythrocytes should not be confused with reticulocytes. Retractable bodies are due to poor drying owing to moisture in the air.
6. If no reticulocytes are observed after scanning at least two slides, report "none seen".

Expected value:

- Up to 1.5% (15/1000) of total red blood cells in all ages.
- Up to 6% (60/1000) of total red blood cells in neonates but return to adult levels in 1-2 weeks.

Interpretation of the results:

• The reticulocyte count is elevated:

1. In patients with hemolytic anemia including G6PD deficiency attacks and hemolytic diseases of newborn.
2. In those with hemorrhage (acute and chronic).
3. Following treatment of iron-deficiency anemia and the megaloblastic anemia.
4. In patients with uremia.
5. thalassemia, sideroblastic anemia.
6. Pregnancy.
7. Medications such as levodopa, malarial medications, corticotrophin, and fever-reducing medications.

• The reticulocyte count is decreased in cases of:

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- 1) Aplastic anemia.
 - 2) Aplastic crises of hemolytic anemia .
 - 3) Ineffective erythropoiesis pernicious anemia.
 - 4) Untreated pernicious anemia, megaloblastic anemia and iron deficiency anemia.
 - 5) Exposure to radiation or radiation therapy.
 - 6) Medications such as azathioprine, chloramphenicol, dactinomycin, methotrexate, and other chemotherapy medications.
- Reticulocytopenia in the presence of a suggested hemolytic anemia may often make diagnosis difficult. The diagnosis of a hemolytic anemia can be made because the combination of both hemolysis and reticulocytopenia results in a rapidly falling hemoglobin and hematocrit.

Reporting result:

Reticulocytes count : % .

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Blood Film \ (Giemsa Stain) Coral Clinical system

SOPs\ HGA \.....H\ Haem \19

Version:1.....	Head of department:
Date effective: 01/02/2015.....	Quality Officer:
Copy number:1.....	Director of :

Purpose & Definition:

Describe the procedure for preparing thin blood smear and staining with Giemsa stain for the purpose of diagnosis of different anemia's, leukemia's, viral and parasitic infections, in addition associated with inclusion bodies, allergic reactions, hemoglobinopathies, and platelets disorders. Blood film is a visible blood picture that confirms CBC reports and explains them in order to detect and identify abnormal cells.

Responsibilities:

- Haematology department personal are required to be knowledgeable of this procedure.
- The head of the department must resolve any problem with the process and difficulties in using this SOP.
- New employees are trained and assessed for competence before they can handle patient sample.

Specimen requirements:

Freshly collected venous blood in an EDTA container is recommended.

Specimen reception and rejection:

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Smears of peripheral blood must be made immediately

Criteria for rejection haematology specimens

1. When the identification is missing /inadequate.
2. Insufficient Quantity
3. Inappropriate Container
4. Inappropriate transport/storage
5. Unknown duration of delay

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6. Haemolysed and/or clotted sample reject.

Equipment & Items required:

- Microscope.
- Microscopic slides.
- Distilled water.
- Methanol 96%.
- Droppers.
- Immersion oil.
- Giemsa stain.

Abbreviations:

B. film : Blood film.
RBC : Red blood cell.
WBC : White blood cell.
CBC : Complete blood count.
Hb : Hemoglobin.
EDTA : Ethylene diamine tetra acetic acid.

Procedures:

1. Small drop of blood placed at the end of the slide.
2. Using another slide, the blood can be spread to make the smear.
3. Let's dry.
4. Fix blood film with methanol for 2 to 3 min.
5. Wash the slide with distilled water & let the blood film to dry.
6. Dissolve one volume of commercially prepared Giemsa stain + nine volume of distilled water.
7. Cover the slide with working stain for 15 min. then wash with water.
8. Observe the normal & abnormal morphology of WBC & RBC & record the result in the result book.

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Quality control procedures:

A well-made slide will have three distinct regions: a head, body, and tail.

Repeat counts of differential WBCs and RBCs morphology on selected slides on subsequent days will give an indication of the range in the variation of the results. (include note on reagent quality).

Limitations/ Interfering substance:

- A ragged spreader or one that is not smooth can ruin a slide.
- Make sure the drop of blood is not too big or too small.
- Hold the spreader at the correct angle .
- Do not use a glass slide that is dirty or oily.
- Films made from blood that had been standing for more than 6 hours affects the quality of staining.
- Unfiltered stain gives bad staining.
- Long fixation time may give a wrong view of aberrant RBC's.

Expected Result:

- Erythrocytes stain buff pink to pale bluish-gray.
- Leukocytes : neutrophils, monocytes and lymphocytes have a pale blue-gray cytoplasm and purple nucleus. Eosinophil have coarse, pink granules in the cytoplasm. Basophils have coarse, deep blue granules in the cytoplasm.
- Platelets have pale blue cytoplasm and diffuse red nuclear material.
- Normal RBC's shape are normocytic, normochromic, no variation in size, no shape variation , and no inclusion bodies.
- Normal WBCs morphology.
- Normal WBCs differential:
 - Neutrophils: 45% - 65%.
 - Lymphocytes: 35 – 55%.
 - Eosinophil's: up to 3%.
 - Basophils: 0 – 1%.
- Normal platelets size, shape and distribution.

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Interpretation of the results:

- Hb content (red cells color): normochromic, hypochromic, hyperchromic.
- Red cell size: normocytic, macrocytic, and microcytic.
- Anisocytosis: variation in RBCs size.
- Red cell shape: normal or abnormal; report the presence of sickle cells, target cells, spherocytes, etc.
- Poikilocytosis: variation in RBCs shape.
- Red cell inclusion: RNA, polychromasia, punctate basophilia, Howell-Jolly bodies, etc.
- Report the presence of parasites such as sporozoa, nematodes, and trypanosomes as well as bacteria such as spirochetes. .
- Nucleated red blood cells and all types of erythroblasts are abnormal.
- Elevated white blood cell count may mean infection.
- Decreases in white blood cell count may occur with disease progression or may indicate bone marrow suppression.
- Total lymphocyte count: a decrease in absolute lymphocyte count may reflect bone marrow suppression.
- An increase in neutrophils may be due to an acute bacterial infection or hematological malignancies such as myeloid leukemia.
- An increase in eosinophil's may be due to a parasitic infection or an allergic reaction.
- An increase in lymphocytes may be due to viral infections or chronic infections such as tuberculosis or lymphocytic leukemia.
- An increase in monocytes is found in hematological malignancies such as chronic myelomonocytic leukemia and certain bacterial and parasitic infections (e.g., typhoid fever, malaria).

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Reporting result:

RBCs Morphology:.....
WBCs differential:
Neutrophils:%.
Lymphocytes:.....%.
Eosinophil's:.....%.
Basophils:.....%.
Blast cells:.....%.
Platelets morphology & distribution:.....

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Sickling test (Na-meta-bisulfate method)

SOPs\ HGA \.....H\ Haem \20

Version: 1.....	Head of department:
Date effective:	Quality Officer:
Copy number:	Director of :

Purpose& definition :

The purpose of this test is to detect sickle cell disorder (Anemia or Trait). Sickle cell anemia is caused by an abnormal form of hemoglobin known as hemoglobin-S, which tends to precipitate in a way that the red cell takes the sickling shape.

Whole blood is mixed with a sodium metabisulfite reagent on a slide. If the red cells contain an abnormal hemoglobin, they will become sickle-shaped (or half-moon shape). The reagent sodium metabisulfite removes oxygen from the cells, allowing sickling to take place.

Responsibilities:

- Hematology department personal are required to be knowledgeable of this procedure
- The head of the department must resolve any problem with the process and difficulties in using this SOP
- New employees are trained and assessed for competence before they can handle patient sample

Specimen requirements:

Whole blood using EDTA or heparin as anticoagulant sample.

Specimen reception

Reception of samples should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Samples must be transported as soon as possible, and the tests samples should be analyzed within 2 hours after collection.

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Criteria for rejection hematology specimens

1. When The Identification Is Missing /Inadequate.
2. Insufficient Quantity
3. Inappropriate Container
4. Inappropriate transport/storage
5. Unknown duration of delay
6. Haemolysed and/or clotted sample reject

Equipment & Items required:

- Distilled water & plastic tubes
- Na-meta-bisulfate powder
- slide and cover slide
- petroleum jelly
- microscope

Abbreviations:

Na: sodium

D.W: distill water

Procedures:

First preparation of Na-meta-bisulfate

1. prepare 2% of Na-meta-bisulfate solution
2. dissolve 2 gram of Na-meta-bisulfate in 100ml D.W
3. This solution, if stored at 3° or 4°C remains effective for about one week.
4. Stable for 8 hr at room temperature

Second sickle cell test

1. Place a small drop of capillary blood in the centre of a slide.
2. Add an equal drop of the fresh sodium metabisulfite solution.
3. Mix carefully and cover with a cover slide with petroleum jelly , make sure that no air bubbles form.
4. Place the slide in a wet chamber.
5. Examine under the microscope after 15 minutes using the 40 x objective.
6. The test is negative if the red cells remain round.
7. The test is positive if the cells become sickle-shaped, or banana-shaped.

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Quality control procedures:

Its advantage to run positive control each time this test is performed

Limitations/ Interfering substance

1. This test should not performed on infant less than six months old
2. Sickling of red blood cells is maximum at 37°C and decrease as the temperature lowers
3. Sever anemia will cause false negative result
4. Low Hb cause false negative : the use of packed cells will overcome this problem
5. Specimen with 25% Hb F present may cause false negative.
6. Blood from patients with polycythemia ,multiple myeloma ,dysglobulinemia may cause false positives
7. False-negative results may occur if:
 - outdated reagents are used.
 - concentrations of hemoglobin S are low.
 - patients have moderate or severe anemia.
8. In all cases where abnormalities are indicated or suspected electrophoretic confirmation is recommended

Interpretation of the results:

Negative result

- The erythrocytes remain round

If the test is negative, re-examine the slide after a further 30 minutes, then after 2 hours and after 24 hours.

Positive result

- The erythrocytes become sickle-shaped or banana-shaped often with spikes

It is important to examine several parts of the preparation, as sickling can occur more quickly in one part than in another.

- Do not mistake normal erythrocytes lying on their side created cells for sickle cells.

Reporting result:

Positive for sickle cell test.

Negative for sickle cell test.

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