**Institution Name:** Directorate General of Specialized Medical Care, MoH

**Document Title:** Activated Partial Thromboplastin Time (APTT) Guideline

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<tr>
<th>Approval Process</th>
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<th>Signature</th>
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<tbody>
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<td></td>
</tr>
</tbody>
</table>
Contents Table:

Acknowledgement........................................................................................................3
Acronyms..........................................................................................................................4
1. Introduction......................................................................................................................5
2. Scope..............................................................................................................................5
3. Purpose...........................................................................................................................5
4. Definitions......................................................................................................................5
5. Recommendations.........................................................................................................6
6. Procedure......................................................................................................................6
7. Responsibilities.............................................................................................................12
8. Document History and Version Control......................................................................13
9. Related Documents......................................................................................................13
10. References..................................................................................................................14
Acknowledgement

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<table>
<thead>
<tr>
<th>Name</th>
<th>Hospital/Center</th>
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<tbody>
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**Acronyms:**

<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>APTT</td>
<td>Activated Partial Thromboplastin Time</td>
</tr>
<tr>
<td>UFH</td>
<td>Unfractionated Heparin</td>
</tr>
<tr>
<td>TAT</td>
<td>Turnaround time</td>
</tr>
<tr>
<td>RI</td>
<td>Reference interval</td>
</tr>
<tr>
<td>Het</td>
<td>Haematocrit</td>
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<tr>
<td>PPP</td>
<td>Platelet Poor Plasma</td>
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<tr>
<td>PNP</td>
<td>Pooled Normal Plasma</td>
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<tr>
<td>PT</td>
<td>Prothrombin Time</td>
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Activated Partial Thromboplastin Time (APTT) Test Guideline

1. Introduction
Partial Thromboplastin Time (APTT) test is used to detect abnormalities in the intrinsic pathway of the coagulation cascade. It is sensitive for abnormalities in coagulation factors XII, XI, IX, VIII, V, II, X and fibrinogen. This test is used mainly to screen for a bleeding disorder, Lupus anticoagulant and monitor unfractionated heparin (UFH) therapy.

2. Scope
This document is applicable to all scientists / pathologists working in hemostasis/coagulation laboratories.

3. Purpose
3.1 To understand the principle of the APTT test.
3.2 To aid in selection and validation of APTT reagents.
3.3 To aid in establishing APTT testing in all laboratories.

4. Definitions
4.1 Turn around Time (TAT): The time is taking to process a sample until release of test’s results.
4.2 Internal quality control: testing of commercial reagents with known values to ensure stability of reagents and reproducibility of results.
4.3 Reference Intervals (RI): range of normal results in healthy population.
4.4 External Quality Testing Scheme: A testing program that distributes identical samples for testing to different laboratories for comparison of performance of reagents and technologies and reproducibility of results.
4.5 Westgard rules: Statistical rules used to define specific performance limits for a particular assay and can be used to detect both random and systematic errors.
4.6 Sensitivity: is the ability of a test to correctly identify those with the disease (true positive rate).
4.7 Specificity: is the ability of the test to correctly identify those without the disease (true negative rate).

5. **Recommendations**

5.1 The sample on receipt fulfils patient identification and labeling criteria.
5.2 Phlebotomists trained on how to collect blood samples for coagulation assays.
5.3 All samples deemed unacceptable due to pre-analytic handling and unfulfilled transport requirements should be rejected.
5.4 Improper labelling of aliquots as citrated plasma should be rejected.
5.5 Turnaround time (TAT) shall be defined in each laboratory for both routine and urgent samples.
5.6 Each laboratory establishes and validate reference intervals (RI) for their instrument reagent combination for APTT. The RI shall be verified with any change in reagent, lot number, instrument, collection system, or once per year.
5.7 Internal quality control testing is performed to ensure accurate results.
5.8 Equipment’s is maintained as per the manufacturer recommendations and maintenance logs are recorded.

6. **Procedure**

6.1 Principle of APTT Test:

6.1.1 One volume of Platelet Poor Plasma (PPP) is mixed with one volume of APTT reagent and incubated for 3-5 minutes at 37°C then one volume of Calcium Chloride is added (all pre-warmed to 37°C).

6.1.2 The end point in the manual technique is detected by starting the incubation of the PPP with the APTT reagent until the calcium is added and stopped when the clot forms.

6.1.3 The time taken from the addition of calcium to the formation of the fibrin clot is known as APTT.

6.1.4 The APTT test is measured in seconds.
6.1.5 In automated method for the APTT, the clot formation is detected when the optical
density of the mixture has exceeded a certain threshold (clot formation makes the
mixture opaquer and less light passes through).

6.1.6 In an automated coagulometer, the end point (i.e. formation of the clot) is detected
electronically by photo-optical or electro-mechanical techniques.

6.1.7 Some photo-optical coagulometers allow the detection of biphasic APTT waveform
which is seen in disseminated intravascular coagulation (DIC) and it appears that
this wave is sensitive and specific for DIC.

6.2 APTT Reagent:

6.2.1 It is composed of a standard amount phospholipid (cephalin) and a contact activator
(e.g. Kaolin, micronized silica or ellagic acid).

6.2.2 Different APTT reagents will have different sensitivities to coagulation factor
deficiencies, detection of Lupus anticoagulant and UFH.

6.2.3 The choice of APTT reagent will depend on the sensitivity priority of each
laboratory. Sometimes more than one reagent may be required in the same
laboratory.

6.2.4 The selection of an APTT reagent should take into account the following
specifications:
6.2.4.1 The selected reagent must be compatible with the automated coagulometer.
6.2.4.2 Stability of the reagent once in use.
6.2.4.3 Lot to lot consistency, especially APTT sensitivity to heparin.
6.2.4.4 Availability of 5-6 months’ supply of a single lot number.
6.2.4.5 Performance of the reagent and number of its users in an external quality
testing scheme.

6.2.5 APTT reagent sensitivity to deficiencies of coagulation factors:
6.2.5.1 This depends on the type of activator used in the reagent e.g. silica is
sensitive to mild deficiencies of factors VIII, IX and XI at levels of 0.35-0.4
IU/ml which are clinically significant.
6.2.5.2 APTT reagent that is too sensitive for very mild deficiencies e.g. greater than
0.4-0.5IU/ml, is generally not necessary.
6.2.5.3 A normal APTT does not exclude these mild factor deficiencies.

6.2.5.4 The APTT can be made more or less sensitive to specific coagulation factors by varying the incubation time.

6.2.5.5 A short incubation time e.g. 2 minutes makes the test very sensitive to the level of contact factors whereas a long incubation period makes it very insensitive to these factors. Many laboratories employ a 5 minutes incubation period. If a contact factor deficiency is suspected, then comparing a short and long incubation time may be useful.

6.2.5.6 It is useful to note that the degree of prolongation of the APTT can give an idea of the underlying abnormality. For example, an APTT that is grossly prolonged e.g. >120s is more likely to be due to a contact factor deficiency than to a deficiency of factor VIII or IX. Conversely an APTT in the region of 70-80s is more in keeping with a diagnosis of severe haemophilia A or B rather than a contact factor deficiency.

6.2.5.7 Verification of the APTT reagent’s sensitivity to coagulation factor deficiency need to be carried out as follows:

6.2.5.7.1 The old reagent needs to be compared against the new reagent

6.2.5.7.2 Factor deficiency sensitivity for factor VIII (FVIII), factor IX (FIX) and factor XI (FXI) need to be tested.

6.2.5.7.3 This is not required for factor XII (FXII) as FXII deficiency is not associated with increased bleeding risk.

6.2.5.7.4 This can be performed by testing pooled normal plasma (PNP) diluted with factor deficiency plasma at different levels e.g. 75, 50, 40, 20 and 10% to cover severe, mild and moderate deficiency.

6.2.5.7.5 Then measure the APTT for all dilutions.

6.2.5.7.6 It is preferable to include known patients of FVIII, FIX and FXI at the different severity levels if possible. A minimum of 10 Patients with Haemophilia A and less number of patients with the rarer factor deficiency (FIX and FXI) are tested.
6.2.5.7.7 Sensitivity of the reagent to the factor deficiency is the level of factor at which the APTT is prolonged when compared to the normal RI.

6.2.6 APTT reagent sensitivity to Heparin (UFH) Therapy:

6.2.6.1 The new APTT reagent must be compared against the old APTT reagent to ensure that the new reagent has same sensitivity.

6.2.6.2 It is important to establish that the reagent in use has a linear relationship between clotting times and heparin concentration in the therapeutic range (0.3-0.7 IU/ml).

6.2.6.3 Less sensitive reagent to heparin may lead to overdosing of patient with minimal APTT change while highly sensitive reagent may lead to under-dosing of patients due to the marked changes in the APTT.

6.2.6.4 Heparin sensitivity is determined by preferably using known patients on heparin therapy (20-30 samples) or testing pooled normal plasma which is spiked with heparin at concentration that span therapeutic range, below and above (0.3-0.7 IU/ml).

6.2.6.5 Then measure the APTT on each plasma and determine the APTT ratio (APTT of heparinized plasma/ APTT of plasma without heparin).

6.2.6.6 Measure anti-Xa level of each plasma.

6.2.6.7 Finally plot the APTT ratio against heparin concentration to determine the sensitivity using regression analysis.

6.2.6.8 Anti-Xa level above 0.3 IU/ml should be equivalent to 2.5 APTT ratio (APTT of heparinized plasma/ APTT of plasma without heparin ratio)

6.2.7 APTT reagent sensitivity to Lupus Anticoagulant (LA):

6.2.7.1 It is advisable to use samples of known patients with positive LA test and measure the APTT. A minimum of 10 samples usually required.

6.2.7.2 The performance of this reagent also can be investigated by reviewing the reports of the external quality testing scheme.
6.3 Reference intervals (RIs) and detection limits:
   6.3.1 RIs can be validated by testing a total of 100 healthy subjects with equal distribution of both male and female’s gender for APTT.
   6.3.2 The reference interval can be calculated as the mean ± 2SD or 95%CI (2.5-97.5%).
   6.3.3 Calculate the intra and inter-assay coefficient of variation (CV) by testing a sample by 5x5 or 10x10 modules i.e. 5x5 is 5 duplicates of the same sample run within one analyser cycle and 5 duplicates of the same sample should be run in five-time points (e.g. 5 consecutive days)
   6.3.4 Verification of the RIs for APTT will require testing healthy individuals (n=20) and the mean is calculated as a geometric mean. This need to be done with any change in reagent, lot number, instrument, collection system, or once per year
   6.3.5 The detection limit of the reagent instrument combination must be determined and known

6.4 Internal Quality Controls:
   6.4.1 Daily run of both normal and abnormal quality control materials is necessary to ensure accuracy of results.
   6.4.2 The frequency at which such quality controls are run depends on the workload of the laboratory and stability of the reagents for that particular reagent instrument combination.
   6.4.3 Failure of controls must be determined as per the Westgard rules, documented and corrective actions are taken before resuming patient’s sample testing.

6.5 Mixing Study for prolonged APTT test:
   6.5.1 Mixing study is when patient plasma is mixed with normal plasma [ratio 1:1] then the APTT is determined.
   6.5.2 It is performed to distinguish between a clotting factor deficiency or an inhibitor as the cause for the prolonged APTT.
   6.5.3 The testing is done immediately and after 2 hours of incubation at 37°C.
   6.5.4 If the mixture fails to correct the APTT to normal value then this is strongly suggestive of a coagulation factor inhibitor e.g. an acquired FVIII antibody or an antiphospholipid antibody i.e. a lupus anticoagulant.
6.5.5 If the mixture corrects fully then this is in keeping with coagulation factor deficiency
6.5.6 It is prudent to confirm both the presence of inhibitors or coagulation factor deficiency by performing LA testing and coagulation factors assays respectively

6.6 Interpretation of abnormal APTT results:

<table>
<thead>
<tr>
<th>Isolated Prolonged APTT</th>
<th>Deficiencies of coagulation factors: XII, XI, IX &amp; VIII.</th>
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<tr>
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<td>Contact factor deficiency e.g. pre-kallikrein deficiency</td>
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<td></td>
<td>Von Willebrand Disease</td>
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<td></td>
<td>Mild deficiencies of coagulation factors: II, V, X</td>
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<td></td>
<td>Acquired clotting factor inhibitors</td>
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<td></td>
<td>Lupus anticoagulant [LA]</td>
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<tr>
<td>Prolonged APTT with prolonged TT</td>
<td>Unfractionated heparin Therapy</td>
</tr>
<tr>
<td>Prolonged APTT + Prolonged PT</td>
<td>Vitamin K deficiency</td>
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<tr>
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<td>Liver disease due to:</td>
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<tr>
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<td>Direct thrombin inhibitors including Hirudin, Argatroban and Dabigatran.</td>
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<tr>
<td></td>
<td>DIC</td>
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<td></td>
<td>Massive blood transfusion leading to a dilutional coagulopathy</td>
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<td></td>
<td>In patients receiving thrombolytic therapy</td>
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<tr>
<td></td>
<td>In multiple clotting factor deficiencies the APTT becomes prolonged with less severe reductions in factor levels.</td>
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<td></td>
<td>Combined deficiency of clotting factors e.g. Factors V and VIII 'Common Pathway' clotting factor deficiencies - FV, FX, FII and Fibrinogen.</td>
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<tr>
<td>Short APTT</td>
<td>An acute phase response leading to high FVIII levels</td>
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<td></td>
<td>Difficulties in the collection of samples leading to activation of coagulation within the collection tube.</td>
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7. Responsibilities

7.1 Supervisor of the Haemostasis/coagulation laboratory Shall:
   7.1.1 Ensure that a Standard operating procedure is established for the test
   7.1.2 Ensure that an internal quality control program is established for the test.
   7.1.3 Ensure record keeping and control of all documents pertaining to test.
   7.1.4 Ensure that all necessary steps are taken to validate the test performance.

7.2 All Technical Scientists in Haemostasis/coagulation laboratory Shall:
   7.2.1 Follow the standard operating procedure of the test.
   7.2.2 Comply with policies pertaining to performing the test.
   7.2.3 Performing the necessary tests to ensure accurate and reproducible results.
8. Document History and Version Control

<table>
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<th>Version</th>
<th>Description of Amendment</th>
<th>Author</th>
<th>Review Date</th>
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<tr>
<td>01</td>
<td>Initial Release</td>
<td>Dr Sabria Al-Hashami</td>
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**Approved by**: Dr.Kadhim Jaffar Sulaiman

9. Related Documents:

9.1 Sample integrity and Preanalytical variables in Haemostasis tests policy and procedure (MoH/DGSMC/GUD/018/Vers.01).

9.2 Quality control in Haemostasis laboratory and reference intervals policy and procedure (MoH/DGSMC/GUD/017/Vers.01).

9.3 Hemostasis test validation and performance policy and procedure (MoH/DGSMC/GUD/009/Vers.01).

9.4 Monitoring of anticoagulant therapy policy and procedure (MoH/DGSMC/GUD/012/Vers.01).
10. References:

<table>
<thead>
<tr>
<th>Title of book/ journal/ articles/ Website</th>
<th>Author</th>
<th>Year of publication</th>
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</thead>
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