Activated Partial Thromboplastin Time (aPTT)

Intended Use
The aPTT is a unitized coagulation test intended for in vitro use in performing a quantitative, one-stage Activated Partial Thromboplastin Time (aPTT). The test is performed using fresh canine or feline whole blood. This test is to be used in the Coag Dx™ Analyzer and is also compatible with the SCA2000™ Coagulation Analyzer.

This test is for veterinary use only and not for human diagnostic use.

Summary and Explanation
The aPTT is a measure of the intrinsic and common coagulation pathways, which involves all coagulation factors except Factors VII and III (tissue factor). The aPTT is a modification of the Partial Thromboplastin Time (PTT). The PTT uses a phospholipid derived from either brain or lung tissue to mimic the role of platelets in the coagulation process. The aPTT contains a contact activating substance to standardize the activation of Factor XII, thereby providing a more precise and sensitive assay. The addition of a contact activator such as glass, kaolin or diatomaceous earth distinguishes the aPTT from the PTT.

The aPTT assay resolution is achieved through the use of a platelet factor 3 substitute and a kaolin activator, and does not require an incubation step.

Principle of Operation
The coagulation analyzer utilizes a mechanical end point clotting mechanism in which clot formation occurs within the disposable aPTT cartridge. Following whole blood sample introduction, the analyzer precisely measures 15 microliters of blood and automatically moves it into the test channel within the aPTT cartridge. The remainder of the blood sample not needed for testing is automatically

Limitations
Test results under 12 seconds and over 350 seconds are not reported. Instead, either “Out of range - Lo” or “Out of range - Hi” is displayed. aPTT values less than 12 seconds may indicate excessive blood coagulation activation, possibly due to specimen contamination upon collection or processing, and should be repeated.

References

Suggested Readings


drawn into the waste channel of the cartridge. Sample/reagent mixing and test initiation are also performed automatically, requiring no operator interaction. After mixing with the reagent, the sample is then moved back and forth within the test channel and observed for clot formation.

The clot detection mechanism consists of a series of LED optical detectors aligned with the test channel of the cartridge. The speed at which the blood sample moves back and forth between the two detectors is measured. As clot formation begins, blood flow is impeded and the movement slows. The analyzer recognizes that a clot end point has been achieved when the movement decreases below a predetermined rate. The whole blood aPTT test result is reported in whole seconds.

Reagents
Each box of aPTT test cartridges contains:

- 10 pouches, each containing one aPTT test cartridge and one dessicant

The aPTT test cartridge is a self-contained disposable test chamber preloaded with a dried preparation of kaolin, phospholipid, stabilizers and buffers. Each cartridge is individually packaged in a pouch. Cartridge pouches are stamped with a lot-specific expiration date.

Caution: All used test cartridges should be considered as potentially infectious, handled with care and disposed of properly.

Storage and Stability
When refrigerated (2°–8°C), the foil-pouched aPTT cartridges are stable until the marked expiration date. Room temperature storage (15°–30°C) is optional for unopened, pouched cartridges. aPTT cartridges should not be exposed to temperatures in excess of 37°C.

Note: Room temperature redating is to a maximum of 4 weeks, but must never exceed the marked expiration date. Redating is necessary if stored at room temperature.

Mark the outer box with the new expiration date when cartridges are stored at room temperature.

Operating Instructions
Before performing any assay, refer to the IDEXX Coag Dx™ Analyzer Operator’s Guide for detailed operating instructions.

Material provided
- aPTT test cartridges

Material required (not provided)
- Coagulation analyzer
- Plastic syringes
- 23-gauge needle or larger (for syringe sampling)

Note: aPTT test cartridges must be at room temperature prior to use. Once removed from the refrigerator, this may take up to 60 minutes. For best results, the pouch should be opened immediately prior to testing.

Test procedure
Refer to the IDEXX Coag Dx™ Analyzer Operator’s Guide if any fault message should appear during this procedure.

1. Insert a test cartridge into the cartridge opening of the analyzer. The cartridge must be inserted with the blood reservoir facing up. The analyzer automatically identifies the test cartridge and displays the test type.
2. During the warming stage, observe the display for fault messages.
   - The analyzer emits an audible tone when it is ready and alternately displays the “Add Sample” and “Press Start” messages. The analyzer remains in the “Ready” mode for 5 minutes before a “START... timeout” message displays. If this occurs, a new test cartridge must be placed in the analyzer.
3. Obtain the blood sample. (See the Sample Collection section for more information.)
4. Immediately dispense one drop of blood into the center sample well of the test cartridge, fill from the bottom of the well up. This may be done either with or without a needle. A sufficient quantity of blood must be added directly to the center sample well to fill it flush to the top. Should a large drop of blood extend above the center sample well, push it over into the outer sample well.
5. Press the Start key. A single beep signals the start of the test. The analyzer automatically mixes the sample with the reagent and detects clot.
6. The analyzer emits a single beep when the test is complete.
   - The test result, in seconds for whole blood, remains on the screen until the test cartridge is removed from the analyzer and for 120 seconds following its removal.

Sample Collection
Blood samples to be used for coagulation testing must be collected in the following manner to prevent contamination with tissue thromboplastin, or indwelling intravenous (I.V.) solutions that interfere with the coagulation assays. Poorly collected blood samples with visible clotting or debris accumulation must be discarded and a fresh sample collected.

Patient excitement should be minimized as this can increase platelet count, platelet aggregation, and the levels of von Willebrand Factor (vWF), fibrinogen, and Factors V and VIII. Prolonged venous stasis and excessive probing for the vessel should be avoided. Use of the cephalic or saphenous veins are advised as bleeding is easier to control from these sites (Green, et al, 1995). If a syringe is used, it should have a 23-gauge needle or larger. Use of excessive force when expelling the blood specimen through the needle may cause hemolysis.

Note: Samples must not be collected until the analyzer indicates “Add Sample” and “Press Start.”

Syringe sample, from a venipuncture
1. Prepare the venipuncture site by cleansing with alcohol and allowing to air-dry completely.
2. Obtain a minimum of 0.2 cc of blood.

Operating Precautions
Do NOT use cartridges that are past their marked expiration date, or which have been improperly stored.

Do NOT force a cartridge into the analyzer. If resistance to insertion is encountered, gently remove the cartridge and examine the cartridge slot. Remove any obstruction before attempting further use of the analyzer.

Specimen collection and handling for all coagulation testing requires careful adherence to guidelines. As with all diagnostic tests, test results should be scrutinized in light of a specific patient’s condition and anticoagulant therapy. Any results exhibiting inconsistency with the patient’s clinical status should be repeated or supplemented with additional test data.

The aPTT is affected by poor technique including blood collection and the transfer of blood to the sample well. The accuracy of the test is largely dependent upon the quality of the blood specimen, which may be affected by:

- Foaming of the sample
- Hemolysis of the sample
- Clotted or partially clotted blood

Test results greater than 350 seconds should be considered abnormally high and the test should be repeated or reported as >350 seconds.

Performance Characteristics
Reference range
Whole blood samples were obtained from normal healthy animals and tested with the aPTT assay. Reference ranges were developed as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Canine (sec)</th>
<th>Feline (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Range</td>
<td>60–93</td>
<td>60–115</td>
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