



## Contents

50 aPTT test cards, individually sealed in foil pouches.

## INTENDED USE

The Cascade POC aPTT test cards are to be used with the Cascade POC analyzer and are intended for the determination of the activated Partial Thromboplastin Time (aPTT) of citrated whole blood or plasma.

The aPTT test cards, together with the analyzer, are especially suited for professional use in decentralized areas of testing near the site of patient care, as well as for use in the more traditional clinical laboratory.

## SUMMARY

The aPTT test is sensitive to deficiencies in the intrinsic and common pathways of the coagulation process.<sup>1,2</sup> Although the aPTT test is sensitive to severe deficiencies of all clotting factors except Factor VII, its primary use is in screening for deficiencies in Factors VIII, IX, XI, XII, prekallikrein (Fletcher factor), and high molecular weight kininogen (Fitzgerald factor). The aPTT test is also useful for monitoring the effectiveness of heparin therapy since the clotting time increases in proportion to the heparin level in the blood.<sup>3,5</sup>

The aPTT test was first reported in 1953 by Langdell, et al.<sup>1</sup> The authors described the effect of crude cephalin on hemophilic plasma. Later modifications included the addition of an activator to provide optimal contact activation, thereby minimizing the influence of other surfaces.<sup>2</sup>

While the aPTT test card is similar to other aPTT tests, the Cascade POC system is designed to eliminate many of the variables encountered with other coagulation methods. Precise pipetting of reagent or sample and manual timing skills are not a factor with the aPTT test card. Many of the variables encountered with sample transport and handling are avoided.

## PRINCIPLE

The Cascade POC aPTT test consists of the recalcification of plasma in the presence of phospholipid (platelet substitute) and an activator, with the subsequent measurement of the time necessary for clot formation. The test is sensitive to deficiencies of the intrinsic and common pathways, including Factors II, V, VIII, IX, X, XI, XII, prekallikrein, and high molecular weight kininogen. It is especially sensitive to the presence of heparin. The aPTT test should not be used to detect deficiencies of fibrinogen or Factor VII.

## REAGENT

For in vitro diagnostic use only.

Components	Storage	Stability
Phospholipid, calcium chloride, magnesium aluminum silicate, buffers, stabilizers, and paramagnetic iron oxide particles	2–8°C (36–46°F)	Unopened – until the expiration date on the pouch label or Unopened – 2 weeks

**CAUTION:** Exposure of the test cards at any time to magnetic objects or fields (for example, an MRI instrument) can corrupt the encoded information and prevent the analyzer from performing the test.

**CAUTION:** Any pouches not kept refrigerated should be dated and should not be used beyond this 2-week period.

## SPECIMEN COLLECTION AND PREPARATION

The aPTT test cards may be used with citrated whole blood or plasma collected and processed according to recognized standards for the handling of blood specimens for blood coagulation studies.<sup>6</sup> Add whole blood to 109 mM (3.2%) of the dihydrate form of sodium citrate, in a proportion of nine parts whole blood to one part anticoagulant. Mix the blood by gentle inversion with the anticoagulant immediately after collection.

Test whole blood specimens within 15 minutes of collection, especially if the patient is being monitored for heparin therapy.

To obtain plasma, centrifuge the blood immediately at a minimum RCF of 1000 x g at 4°C (39°F) for 10 minutes. After centrifugation, separate the plasma from cellular elements, especially platelets, and transfer the plasma to a clean plastic test tube using a pipette with a non-wettable surface, store tightly capped at 2 to 8°C (36–46°F) for up to 4 hours. Allow refrigerated specimens to warm to room temperature before testing.

## PROCEDURE

### Materials Required But Not Provided:

- Cascade POC analyzer
- Cascade POC Operator's Manual
- Blood sampling materials such as venipuncture needles, syringes, alcohol swabs, vacuum tubes containing sodium citrate
- Sample transfer devices (pipettes or droppers) capable of delivering approximately 30 to 35 µL
- Quality control material

## STEP-BY-STEP

1. Equilibrate test cards at room temperature (20 to 25°C or 68 to 77°F) before removing from the foil pouch.

**CAUTION:** The test card must be used within 15 minutes after the pouch is opened. Pouches of cards should not be repeatedly warmed and returned to the refrigerator.

2. Remove the test card from its foil pouch and hold it so that the full test name is right side up and facing you.
3. Pass the test card firmly and steadily through the card reader. The analyzer interprets the encoded information on the test card and displays prompts for each step of the procedure.
4. When prompted, place the test card in the analyzer, and allow to warm.  
**CAUTION:** Do not leave the test card in the analyzer for longer than 15 minutes before applying the sample. Prolonged warming of the card can affect the performance of the test.
5. When prompted, add 30 to 35 µL of sample into the sample well (colored circle) on the test card. Sample placement automatically initiates testing.
6. At the end of the test, confirm that the test was performed with the analyzer set to the appropriate sample type. The sample type is displayed along with the result at the end of the test.
7. When the card is removed from the analyzer at the end of each test, ensure that the entire reaction chamber was filled with sample. If an inadequate amount of sample was added to the card, repeat the test, using a fresh card.
9. Dispose of the test card and other contaminated items in a manner approved for biohazardous material.

## Procedural Notes

- The analyzer is preset to provide a constant temperature of 37 ± 0.3°C (98.6 ± 0.5°F) and will automatically prewarm the test card before prompting the user to apply the sample drop. All other mathematical parameters necessary are magnetically encoded on each test card. Refer to the operator's manual for details.
- To maintain a fully charged battery, leave the unit plugged into its power supply which is, in turn, plugged into an AC outlet. Leave the power switch in the OFF position while storing the analyzer.
- The Operator Identification Code and the Quality Control Lockout are optional features. Refer to the operator's manual if either of these features has been enabled.
- Operate the analyzer only at ambient temperatures between 18 to 32°C or 64 to 90°F.
- Ensure that the sealed pouch containing a test card has reached room temperature and that the analyzer is either plugged into an appropriate AC wall outlet or has a sufficiently charged battery.
- Collect the sample as described in Specimen Collection and Preparation.

## QUALITY CONTROL

**Calibration:** Operator calibration is not required. Calibration of both the analyzer and test cards was performed at the time of manufacture.

Daily quality control (QC) is good laboratory practice and is required by most states in the U.S. and the Clinical Laboratory Improvement Amendment, 1988 (CLIA '88). Quality control procedures are part of an overall quality assurance program to ensure the accuracy and reliability of patient results and reports. Monitoring the results of QC analyses can alert you to possible system performance problems. Healthcare professionals should follow proper local and national guidelines for quality control and check with appropriate licensing/accrediting bodies to ensure that QC programs meet established standards. It is recognized nationally that medical and laboratory instrumentation be enrolled in a quality assurance program. Participation in inter-laboratory QC survey programs allows for the comparison with systems in other laboratories and may help identify possible errors not detected by intra-laboratory QC testing alone.

There are two types of quality control that may be used on the Cascade POC: Electronic Quality Control (EQC Test Card) and commercial plasma controls.

The EQC Test Card ensures that the electronic components of the Cascade POC analyzer are working properly. The purpose of the EQC Test Card is to offer a simple and economic alternative to the daily use of Cascade POC test cards and plasma controls. However, the EQC test card is not intended to permanently replace plasma controls.

At least two levels of EQC quality control must be performed every 8 hours of operation when patient samples are tested. It is imperative that, at a minimum, commercial plasma controls are tested in the following situations:

- With each new box of test cards or at least once per week
- With each new shipment of test cards
- With each new lot number of test cards
- Whenever improper storage or handling of test cards is suspected
- Whenever patient results appear abnormally high or low

This testing is in addition to the daily EQC testing. For more detailed information about quality control for the Cascade POC, refer to the Cascade POC Operator's Manual, the EQC test card package inserts, or contact your local authorized distributor.

## REFERENCE VALUES

A study was conducted at three clinical sites which included 60 normal healthy donors (24 males and 36 females ranging in ages between 21 and 59 years). These results should be used as a guideline only. Operators should establish their own expected values based on their own population of normal individuals. It is suggested that a minimum of 20 individuals be included in the study. Specimens should be collected and handled in the same manner that the operator expects to use for patients.

Sample Type	Mean (sec)	Range (sec)	SD (sec)
Plasma	29.0	21.8 – 36.2	3.6
Blood	29.5	22.5 – 36.5	3.5

## RESULTS

The analyzer reporting units are in seconds. The results are displayed at the end of the test procedure. The aPTT test is capable of reporting results up to 300 seconds. Verify results > 300 seconds by repeat testing.

## LIMITATIONS

The influence of many seemingly insignificant environmental factors can affect aPTT testing.<sup>7</sup> Recommended specimen handling procedures should be strictly followed.

Whole blood samples containing heparin that are allowed to stand at room temperature will exhibit a progressive shortening of the aPTT.<sup>7</sup> This is thought to be due to the release of Platelet Factor 4 (PF4) from the platelets, which is known to have a neutralizing effect on heparin.<sup>8</sup> When performing an aPTT test to monitor patients for heparin, avoid handling samples in a manner that could induce the release of PF4 from platelets. This includes traumatic collection, refrigeration, or delay in testing or processing.

The aPTT test cards are not suitable for monitoring oral anticoagulant therapy nor can they detect deficiencies of Factor VII or fibrinogen.

## INTERFERENCES

The presence of oxalate, EDTA, or any additive other than sodium citrate may interfere with the test.<sup>9</sup> Hemolysis should not affect the results; however, it is often an indication of poor specimen quality. Moderate lipemia and a hematocrit of 0% to 55% will not normally interfere with the results obtained with the aPTT test card.

## PERFORMANCE CHARACTERISTICS

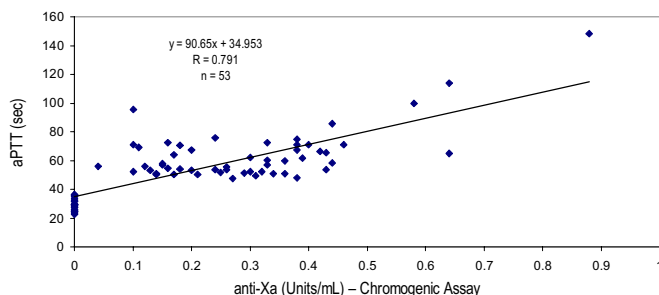
**HEPARIN SENSITIVITY:** The effect of heparin as an anticoagulant can depend on many factors, including the source, type, and manufacturer of heparin used; differences in an individual's response to the drug; and the action of other medications being given. While monitoring a patient on heparin therapy, aPTT results can vary with the amount of heparin administered, the timing of sample collection, the manner in which the sample is handled, and the type of heparin being used.<sup>8</sup>

The aPTT test cards are sensitive to the presence of therapeutic levels of heparin in the sample. The aPTT test card clotting times associated with a given concentration of heparin can be determined by performing in vitro heparin response studies.<sup>4</sup> A typical in vitro heparin curve obtained by adding unfractionated porcine heparin to citrated whole blood is represented in the following table:

Unfractionated Heparin (U/mL)	aPTT (sec)	Unfractionated Heparin (U/mL)	aPTT (sec)
0.0	31.8	0.30	78.9
0.05	36.8	0.40	104.7
0.10	44.5	0.50	128.3
0.20	59.4		

A study was performed at a clinical site comparing the Cascade POC aPTT to a chromogenic anti-Xa assay, using citrated samples from heparinized patients. The following results were obtained:

Correlation of aPTT Results to a Chromogenic Anti-Xa Assay-Heparinized Patients



**SENSITIVITY:** The aPTT has been shown to be sensitive to clotting factors in the intrinsic pathway and may be used to screen for moderate and severe deficiencies of these factors. It is not sensitive to deficiencies of fibrinogen or factor VII and may not detect mild or moderate Factor IX deficiencies.

**PRECISION:** Precision studies performed using the aPTT test cards and two levels of quality control plasma (n = 40 for each) produced the following results. Since val-

ues obtained with controls from other manufacturers may differ, operators should establish their own expected ranges for controls.

Within-run		Normal	Abnormal	
Mean (sec)		36.0	75.1	
SD (sec)		1.0	2.2	
CV (%)		2.9	2.9	
Run-to-run		Normal	Abnormal	
Mean (sec)		35.7	74.2	
SD (sec)		1.1	2.7	
CV (%)		1.2	1.8	
Lot-to-lot		1	2	3
Normal	Mean (sec)	34.9	34.5	34.9
	SD (sec)	0.5	0.6	0.5
	CV (%)	1.5	1.8	1.4
Abnormal	Mean (sec)	69.0	73.8	69.6
	SD (sec)	1.6	2.1	1.8
	CV (%)	2.4	2.8	2.5

**COMPARISON:** Multiple site comparison studies were done on citrated plasma and citrated whole blood with the following results.

Citrated Plasma - n = 257

r = 0.703 to 0.838

Citrated Whole Blood - n = 115

r = 0.758 to 0.835

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