



## Contents

50 PT-C test cards, individually sealed in foil pouches.

## INTENDED USE

The Cascade POC PT-C test cards are to be used with the Cascade POC analyzer and are intended for the determination of the Prothrombin Time (PT) of citrated whole blood or plasma.

The PT-C 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 PT test was first reported by Quick<sup>1</sup> in 1935. It has become one of the most useful tests for evaluating the extrinsic and common pathways of the coagulation process. There are two major applications for this test: (1) as a screening tool for inherited or acquired disorders of factors II, V, VII, and X; and (2) as a method for monitoring oral anticoagulant drug therapy. The warfarin type drugs reduce the activity of Factors II (prothrombin), VII, IX, and X.<sup>2</sup>

The PT-C test card is based on Quick's method; however, 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 PT-C test card. Many of the variables encountered with sample transport and handling are avoided.

## PRINCIPLE

The Cascade POC PT-C test card provides a one-stage method that measures the clotting time of the sample after combining it with tissue thromboplastin and calcium in a prewarmed card. By providing tissue thromboplastin, the intrinsic pathway factors (Factors VIII, IX, XI, and XII) are not detected, and a deficiency in one or more of them will not be reflected in the result. The PT-C test card screens for deficiencies in Factors II (prothrombin), V, VII, and X, as well as monitoring the effects of warfarin therapy.

## REAGENT

For *in vitro* diagnostic use only.

Components	Storage	Stability
Thromboplastin extracted from human placenta, buffers, stabilizers, and paramagnetic iron oxide particles	2–8°C (36–46°F)	Unopened – until the expiration date on the pouch label or Unopened – 1 week
	20–25°C (68–77°F)	

**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 1 week period. It should be noted that stability and sensitivity of thromboplastins are susceptible to elevated temperatures. For optimal test card performance, remove PT-C test cards from refrigerated storage (2 to 8°C, or 36 to 46°F) and warm to room temperature (20 to 25°C, or 68 to 77°F) immediately prior to use. PT-C cards are stable at room temperature (20 to 25°C, or 68 to 77°F) for 7 days. Performance of the test cards stored at temperatures above 25°C or 77°F may be affected.

**POTENTIAL BIOHAZARD:** Human source material. Treat as potentially infectious. Thromboplastin batches prepared from human placentas have been examined for hepatitis B surface antigen (HBsAg) and for antibodies to HCV and HIV by FDA-required tests and found to be nonreactive. Because no known test method can offer complete assurance that hepatitis B virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent, all human-based products should be handled in accordance with good laboratory practices, using appropriate precautions.<sup>5-8</sup>

## SPECIMEN COLLECTION AND PREPARATION

The PT-C 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>3</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 on collection. Collection in 109 mM (3.2%) sodium citrate is recommended by the International Society for Thrombosis and Hemostasis and is used for international reference material International Sensitivity Index (ISI) assignments.<sup>4</sup>

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 1500 g for 15 minutes. After centrifugation, separate the plasma from the cellular elements, especially platelets, and then 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 to 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.
8. When prompted, press the **YES** key if you want the analyzer to compute the INR. If the sample was collected with 109 mM (3.2%) citrate, you can use the analyzer to compute the INR. The PT-C result is displayed along with the INR.  
**CAUTION:** Press the **NO** key if you do not want the analyzer to compute the INR.
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 calibrations 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 (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 available for use 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

Samples from 121 normal individuals were tested with the PT-C test card. The 95% confidence interval for citrated whole blood was 8.1 to 13.3 seconds and for citrated plasma was 9.6 to 13.1 seconds. 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.

## RESULTS

The analyzer reporting units are in seconds. The results are displayed at the end of the test procedure. The analyzer automatically calculates the ratio of the patient's result to the mean of the normal range. A mean value is encoded on each card and can be modified through the supervisory menu. Refer to the operator's manual for instructions. The PT-C test is capable of reporting results up to 150 seconds. Verify results > 150 seconds by repeat testing.

Example: Patient Time = 16.5 sec  
Mean Normal PT = 9.7 sec  
Ratio = 1.7

**International Normalized Ratio (INR):** The analyzer will calculate the INR if requested. The International Sensitivity Index (ISI) is encoded on the magnetic stripe along with the other calibration information and is passed to the analyzer when a test is initiated. At the end of the test, the analyzer will ask the operator if the INR should be calculated. The operator must press the YES key to allow the analyzer to calculate and report this value.

Authorities recommend that the INR should be reported only for those patients who have been stabilized on warfarin therapy.<sup>2</sup> Therefore, it is left to the operator to make this decision.

## LIMITATIONS

The concentration of trisodium citrate used to anticoagulate blood samples is an important variable in the calculation of the INR. Studies indicate that the concentration of citrate affects the ISI assignment for PT-C test cards. The ISI encoded on PT-C test cards is based on determinations with samples collected in 109 mM (3.2%) citrate as recommended by the International Society for Thrombosis and Hemostasis.<sup>4</sup>

Many commonly administered drugs, diseases, and other factors can affect the results obtained in PT testing.<sup>2</sup> If unexpected results are found, the test should be verified by repeat testing. If the results are confirmed, more in-depth testing may reveal a deficiency of one or more factors. INR results inconsistent with the patient's clinical presentation may be an indication of improper test card storage. Since normal values vary from laboratory to laboratory, depending on the technique used, each laboratory should establish its own reference interval. For INR reporting, the geometric mean of the normal reference interval should be used.<sup>9</sup> Since desired ratios may vary depending upon clinical practice and test methodologies, the optimum therapeutic range for this method should be established by each user.<sup>2</sup>

## INTERFERENCES

The presence of oxalate, EDTA, or any additive other than sodium citrate may interfere with the test. Hemolysis should not affect the results; however, it is often an indication of poor specimen quality.<sup>3</sup> Moderate lipemia and a hematocrit of 0% to 55% do not normally interfere with the results obtained with the PT-C test card. Therapeutic levels of heparin may give prolonged PT-C results.

## PERFORMANCE CHARACTERISTICS

Clinical studies indicate the concentration of citrate affects the ISI for PT-C test cards.

**PRECISION:** Precision studies performed using PT-C test cards to test two levels of quality control plasma produced the following results. Since values obtained with controls from other manufacturers may differ, operators should establish their own expected ranges for controls.

### Within-run Precision (30 tests per level)

	Normal	Abnormal
Mean (sec)	12.1	60.9
SD (sec)	0.5	2.6
CV (%)	4.1	4.3

### Run-to-run Precision (20 days/10 tests per day)

	Normal	Abnormal
Mean Range (sec)	10.7 – 12.2	57.4 – 69.7
SD (sec)	0.2 – 0.8	0.8 – 6.1
CV (%)	1.9 – 7.3	1.4 – 9.1

### Lot-to-lot precision (n = 30 each)

	1	2	3
Normal Mean (sec)	12.4	11.9	12.7
SD (sec)	0.7	0.7	0.4
CV (%)	5.8	5.5	3.5
Abnormal Mean (sec)	66.2	60.6	64.4
SD (sec)	4.4	4.2	4.9
CV (%)	6.7	7.0	7.6

**SPECIFICITY:** Studies show that the Helena Cascade POC PT-C test card is sensitive to deficiencies of Factors II, V, VII and X.

**COMPARISON:** Clinical studies with over 300 citrated plasma and citrated whole blood samples from normal donors and from patients on warfarin were done comparing POC PT-C cards with another thromboplastin reagent. Comparisons of both the citrated plasma samples and the citrated whole blood samples gave correlation coefficients of greater than 0.92.

## REFERENCES

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US Pats 4,849,340; 5,110,727; 5,350,676

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