

# Abbott i-STAT™ PT/INR METHOD AND SAMPLE COLLECTION

## 1. PURPOSE AND SCOPE

The purpose of this document is to describe the procedure for performing an INR test using the Abbot i-STAT analyser. The i-STAT meter can be used by healthcare professional for measuring INR. This method is to be used for the determination of prothrombin time (PT) and INR in capillary and venous whole blood. This test is used for monitoring oral anticoagulant therapy and long-term therapy with Coumarin derivatives.

## 2. HAZARDS

### Patient Samples

All patient samples should be treated as potentially infectious and handled appropriately. Some quality control solutions contain human source material. Standard precautions should be employed. Personal Protective Equipment should be worn when processing samples, quality control testing and maintenance procedures.

## 3. CLINICAL SIGNIFICANCE

Oral anticoagulation therapy is the established treatment for patients suffering from a range of conditions in which it is necessary to inhibit the formation of blood clots within the circulation.

The major indications for anticoagulation include:

- Prevention of thrombosis in patients with prosthetic heart valves/stents
- Treatment and secondary prevention of venous thromboembolism.
- Primary prevention of venous thromboembolism in high risk patients
- Primary prevention of stroke in patients with atrial fibrillation.

Oral anticoagulant drugs work by antagonizing the effects of Vitamin K and this reduces the blood's ability to form a clot. This effect can be measured by determining the prothrombin time (PT) in a patient's blood and comparing it with a standard figure. The resulting ratio is called the Internal Normalised Ratio (INR).

INR is a good indicator of effectiveness and risk of bleeding during Warfarin therapy. Regular INR testing is required to adjust Warfarin dose in patients to maintain their INR to as near the appropriate target INR as possible. Optimum target INR figures have been established for different diagnoses.

Warfarin is a potentially hazardous drug causing major bleeding in 1-2% of people treated, and intracranial bleeding in about 0.1-0.5% during each year of therapy (Gallus A et al MJA 2000; 172: 600-605). Patients on therapy should be monitored closely.

## 4. TEST PRINCIPLE

The i-STAT PT/INR test is a whole blood determination of the prothrombin time used for monitoring oral anticoagulant (Warfarin) therapy. The test determines the time required for complete activation of the extrinsic pathway of the coagulation cascade when initiated (activated) with a thromboplastin.

In a prothrombin time test, coagulation is initiated by mixing the sample with tissue thromboplastin. In traditional prothrombin time tests, complete activation is indicated when activated thrombin converts fibrinogen to fibrin and extensive or localized clots are detected mechanically or optically. The i-STAT PT/INR test is similar except that the endpoint is indicated by the conversion of a thrombin substrate other than fibrinogen. An electrochemical sensor is used to detect this conversion.

The PT/INR test result is reported as an International Normalised Ratio (INR) and, optionally, in seconds. The INR is the recommended method of result reporting for monitoring of oral anticoagulant therapy.

$$\text{INR} = \left[ \frac{\text{Patient Plasma prothrombin time (sec)}}{\text{[Mean Normal Plasma prothrombin time (sec)]}} \right]^{\text{ISI}}$$

If results appear inconsistent with the clinical assessment, the patient sample should be recollected and retested using another cartridge.

### 4.1 Interference

- **Warning:** Use in any other scenarios, particularly snakebite, constitutes “off label use” and may result in misleading or incorrect results. A patient may be suffering from significant undiagnosed envenomation with a potential for serious complications.
- The presence of exogenously added heparin, citrate, oxalate, or EDTA from blood collection devices will interfere with test results.
- Glass syringes or tubes may prematurely activate coagulation, resulting in accelerated clotting times and lower INR's. Venous samples must be collected into plastic syringes or tubes.
- PT/INR results may be affected by commonly administered drugs.
- The i-STAT PT/INR test has not been characterised with patients that have lupus anticoagulant antibodies. If the presence of lupus anticoagulant antibodies is known or suspected, consider using a prothrombin time laboratory assay using reagent that is known to be insensitive to lupus anticoagulant antibodies or an alternative method.

### 4.2 Limitations of the test

- The i-STAT PT/INR test is not affected by fibrinogen concentrations between 2.06  $\mu\text{mol/L}$  to 15.91  $\mu\text{mol/L}$ .
- The i-STAT PT/INR test is not affected by unfractionated heparin concentrations up to 1.0 U/mL.
- Haematocrit in the range of 24 – 54 % PCV have been demonstrated not to affect results.

- Cubicin (daptomycin for injection) has been found to cause a concentration-dependent false prolongation of prothrombin time (PT) and elevation of INR when using the i-STAT PT/INR test.
- The i-STAT PT/INR test may report a false prolongation of the prothrombin time (PT) and an elevation of the INR on samples contaminated with Chlorhexidine Gluconate.
- The i-STAT PT/INR test is not intended for evaluating individual factor deficiencies.

### 4.3 Accuracy

Method comparison data were collected at three clinical sites using a protocol in accordance with the CLSI Guideline EP9-A. Venous samples from outpatients undergoing routine oral anticoagulation therapy were collected in plastic tubes and analyzed in duplicate on 3 lots of cartridges on the i-STAT System; plasma from tubes containing a citrate anticoagulant were analyzed in duplicate on the comparative instruments using Dade Innovin reagent. Deming regression analysis was performed on the first replicate of each sample. In the method comparison table below, n is the number of specimens in the data set,  $S_{y.x}$  is the standard error of the estimate, and r is the correlation coefficient.

Statistic	i-STAT vs. Behring BCS <sup>®</sup> and Dade <sup>®</sup> Innovin <sup>®</sup> reagent	i-STAT vs. STA Compact <sup>®</sup> and Dade <sup>®</sup> Innovin <sup>®</sup> reagent	i-STAT vs. Electra <sup>®</sup> 700 and Dade <sup>®</sup> Innovin <sup>®</sup> reagent
n	183	180	177
Mean (INR)	2.3	2.3	2.5
Range (INR)	1.0 – 3.9	1.0 – 4.3	1.0 – 4.6
S <sub>x</sub> (INR)	0.729	0.777	0.779
Slope	0.922	1.013	0.914
Intercept (INR)	0.402	0.012	0.054
r	0.898	0.943	0.948
S <sub>y.x</sub>	0.322	0.272	0.191

### 4.4 Precision

Typical imprecision data for venous whole blood samples are presented in the table below for sample duplicates collected at two clinical sites. Typical imprecision data for capillary whole blood samples are presented for sample duplicates collected at one clinical site using a single capillary stick.

Statistic	Site 1 (venous)	Site 2 (venous)	Site 3 (capillary)
n	181	102	33
Mean (INR)	2.6	2.4	2.5
%CV	4.7%	4.0%	4.6%

Typical imprecision data for lyophilized plasma material are presented below for studies performed at an Abbott Point of Care Inc. facility and during clinical trials.

Plasma Control	Mean	SD	%CV
Level 1	1.1 (INR)	0.05	4.5%
Level 2	2.5 (INR)	0.17	6.9%

## 5. INSTRUMENT



### Product specifications

#### 5.1 Operating Conditions and Technical Data

Temperature range	16° – 30°C
Relative humidity	Up to 90 % (non-condensing)
Maximum altitude	Not stated
Position	Place the meter on a level, vibration-free surface with display facing up including when it is in downloader. Place the meter away from devices that gives off heat and away from direct sunlight
Reportable Range	0.9 – 8.0 Performance characteristics have not been established for INR's above 6.0.
Sample size	Between 20 – 45 µL. Overfilling may result in cartridge damage
Test time	5 minutes
Memory	4000 patient results and 1000 control results stored on analyser
Barcode scanner	Yes - Laser Diode 650 nm Maximum Output 1.0 mW.
Connectivity	Yes
Scanner	Laser Diode 650 nm Maximum Output 1.0 mW.
Battery operation	2 x 9V lithium batteries or Rechargeable power pack
Mains connection	Yes via downloader only
Number of tests with fully charged battery	Not stated

Safely class	Not stated
Automatic power-off	Yes
Dimensions	230 x 80 x 70 mm
Weight	650g

## 5.2 Storage and transport conditions

Temperature range Meter (In original container)	-10° to +46°C
Relative humidity	Up to 90% (non-condensing)

## 6. SPECIMEN REQUIREMENTS

### 6.1 Sample Material

Fresh whole blood without anticoagulants collected in a plastic syringe or plastic evacuated tube without clot activators or serum separators. Device used to transfer sample to cartridge must be plastic. Fresh capillary whole blood dispensed directly into the cartridge from the finger.

**Wash hand with warm soapy water and dry thoroughly prior to collecting samples**

#### Capillary blood (from finger prick)

- Before performing capillary puncture, stimulate blood flow in the fingertip by washing the hands or soaking the fingertip in warm water. Dry fingertips before proceeding
- Puncture a non-callused area on the side of the fingertip.
- Do not perform a test with a drop of blood from a previous puncture.
- Use a lancing device that provides a deep puncture so that blood flows freely.
- Immediately after lancing, massage gently along the side of your finger to obtain a large drop without pressing or squeezing too hard.
- Immediately (within 15 seconds) apply a large drop well-rounded drop without air bubbles to the application area. The first drop of blood must be used.
- Do not use glass capillary tubes or capillary tubes that contain anticoagulants

#### Venepuncture

- Blood must be collected in a plain plastic syringe without using anticoagulants using a 23-gauge needle or larger.

## 7. CARTRIDGES / REAGENTS

A single-use disposable cartridge contains micro fabricated sensors, a calibrant solution, fluidics system, and a waste chamber. A whole blood sample of approximately 1 to 3 drops is dispensed into the cartridge sample well, and the sample well is sealed before inserting it into the analyser.

## 7.1 Storage and handling

- Cartridges are sealed in individual pouches or portion packs.
- Store the main supply of cartridges in the refrigerator at a temperature between 2° - 8°C till the expiry date. Do not allow cartridges to freeze.
- Cartridges may be stored at room temperature (18° - 30°C) for up to 2 weeks (the time frame indicated on the cartridge box).
- Cartridges should not be returned to the refrigerator once they have been at room temperature, and should not be exposed to temperatures above 30°C.
- If the pouch has been punctured, the cartridge should not be used. Write the date on the cartridge box or individual cartridge pouches to indicate the room temperature expiration date.
- Cartridges should remain in pouches until time of use.
- Do not use after the labeled expiration date.
- The cartridge must sit at room temperature for 5 minutes before use.
- Handle the cartridge from its side edges and avoid touching the contact pads or exerting pressure over the centre of the cartridge.

## 8. CALIBRATION

Calibration is set at the factory during manufacturing and may be updated as necessary as part of regular software upgrades.

## 9. QUALITY CONTROL

### **Quality control material (perform as per your organisation protocol)**

Accurately testing known levels of prothrombin time ensures that the system and your technique used in testing give accurate results on patient tests. The control solutions have defined (known) values. The results for these solutions must first fall within a certain acceptable range in order to allow valid patient testing.

A quality control test should be performed every time a new shipment of test cartridges are received, when a new lot number of cartridges are used, if the clinical picture does not correlate with the patient test results, after major maintenance, and at a minimum of once a month.

Enrolling in an External Quality Assurance Program is encouraged to objectively compare results with other users using the same method of testing. If an External Quality Assurance Program is not available, monthly lab comparison is encouraged.

The Abbott i-STAT Analyser uses the following methods for quality:

- Electronic Stimulator Check (Electronic QC)
- Control solutions

### **9.1 Electronic Control**

Perform electronic simulator check on analyser once per day. The Electronic Simulator is inserted into the cartridge port of the analyser to verify electrical measurement. A PASS/FAIL message indicates whether the analyser's

measurements are within specification. If a FAIL message appears in the display window on the analyser, repeat the test. If the instrument still fails, document the problem on the Instrument QC/Event Log and contact the Manufacturer or APPN for assistance. Do not use the device.

The Electronic Simulator should be stored at room temperature and protect contact pads from contamination by replacing the plastic cap and placing the Electronic Simulator in its protective case after use. To perform an electronic simulator check:

- Turn the analyser on.
- Press the Menu key to access the Administration Menu.
- Press the 3 key for Quality Tests
- Press the 4 key for Simulator.
- Scan or enter Operator ID.
- Enter the Simulator ID (serial number).
- Insert the simulator into the cartridge port.
- View results on analyser's screen.
- If PASS is displayed, continue to use the analyser.
- If FAIL is displayed for the external simulator, reinsert the simulator.
- If FAIL is displayed a second time, do not use the analyser and contact your Support Services representative.

## 9.2 Running control solutions

The control solutions has two level:

- i-STAT PT quality control, level 1
- i-STAT PT quality control, level 2

The control solutions should be stored in the refrigerator at 2° - 8°C. Do not use after expiration date on the box and vials. Controls should be used immediately after reconstitution.

### Preparing the meter

- Turn on the meter by pressing the 'Power' button once
- Press the Menu key to access the Administration Menu
- Press the 3 key for Quality Tests.
- Press the 1 key for Control.
- Press the 1 key for i-STAT Cartridge. Scan or Enter Operator ID. Repeat if prompted.
- Scan the Cartridge Lot number from the cartridge pouch by pressing and holding the "Scan" button.
- To scan a barcode, align the red laser light so it covers the entire barcode. The device will beep when it reads the barcode successfully
- If the cartridge pouch does not have a barcode enter the lot number manually. You may ignore the letters
- The meter will now prompt you to insert the cartridge.

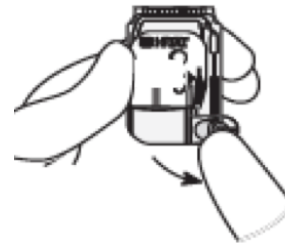
### Preparing the control solution

- Prior to use, allow one vial each of the lyophilized plasma and calcium chloride reconstituting fluid to stand at room temperature for a minimum of 45 minutes.

- Remove the cap and stopper from the vials and pour the entire contents of the calcium chloride vial into the lyophilized plasma vial. Place the stopper back on the reconstituted vial.
- Allow the vial to sit for 1 minute and then mix the contents by swirling gently for 1 minute, then inverting slowly for 30 seconds.

### Applying the control solution

- Allow the individual unopened cartridge pouch to sit at room temperature for 5 minutes before use
- Remove cartridge from the pouch. Handle the cartridge from its edges. Avoid touching the contact pads or exerting pressure over the center of the cartridge.
- Use a plastic pipette, syringe, or capillary tube without anticoagulant to transfer the solution to the cartridge.
- Discard the first drop from the syringe to clear unseen bubbles.
- Touch the next drop to the well allowing cartridge to draw sample in. **DO NOT** use a needle. Fill only to the blue arrow as indicated on the cartridge.
- Immediately seal the cartridge until it clicks into place. This process must be completed within 30 seconds of the complete reconstitution of the control sample.



### Inserting the cartridge into the meter

- To insert the cartridge into the cartridge port, grasp the cartridge by the thumb recess.
- Hold the device by the other hand and gently guide the cartridge into the analyser until fully inserted and it clicks into place.
- The analyser must remain on a level surface with the display facing up during testing.

**NOTE:** Do not remove the cartridge while 'Cartridge Locked' message is displayed on the screen.

### Results

- The countdown will begin as the sample is analysed.
- The results will then be displayed on the screen.
- Compare results to the value assignment sheet ranges. If results are within the expected ranges, use the cartridges as needed. Record your results.
- Remove the cartridge after the 'Cartridge Locked' message disappears. The analyser is ready for the next test immediately.

### Remedial Action

If any results are outside the published expected ranges:

**DO NOT USE** cartridges from the suspect lot. Quarantine the suspect lot. Notify the i-STAT System Coordinator immediately. Record the QC failure in the i-STAT QC Action Log along with the action taken.



### 9.3 Laboratory Comparison

If your policy states you must perform laboratory comparison then perform a venepuncture sample for the laboratory by collecting blood in a Sodium Citrate (blue top) tube filled to the fill line and mixed gently. Perform a fingerprick and run a sample on the Abbott i-STAT analyser. Collection of both the venepuncture and capillary sample should occur at the same time. Record and compare the results ensuring they are in acceptable range for your organisation.

## 10. TEST PROCEDURE

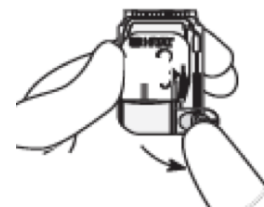
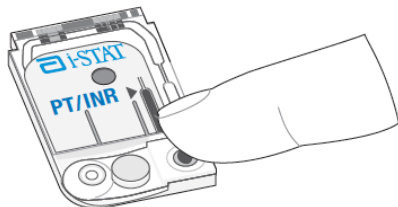
### 10.1 Test Procedure

#### Preparing the meter

- Press ① to turn on the device and the analyser performs a self-check
- Press ② to select 'i-STAT Cartridge'
- Scan or Enter Operator ID. Repeat if prompted.
- Scan or Enter Patient ID. Repeat if prompted.
- Scan the lot number on the cartridge pouch by position barcode 10-15 cm from the scanner window and press and hold 'Scan' to activate the scanner
- Align the red laser light so it covers the entire barcode. The device will beep when it reads the barcode successfully  
**NOTE:** If the cartridge pouch does not have a barcode enter the lot number manually. You may ignore the letters
- The meter will now prompt you to insert the cartridge.

#### Applying the sample

- Handle the cartridge from its edges and avoid touching the contact pads or exerting pressure over the center of the cartridge.
- Clean and prepare the finger to be sampled. Allow finger to dry thoroughly before sampling.
- Perform a fingerstick and immediately (within 15 seconds) apply the first drop of blood to the sample well. Once in contact with the sample well, the blood will be drawn into the cartridge. Fill only to the blue arrow indicated on the cartridge.
- Fold the sample closure over the sample well and press the rounded end of the closure until it snaps into place.  
**NOTE:** It is possible to bring the cartridge to the finger for easier application.



#### Inserting the cartridge

- To insert the cartridge into the cartridge port, grasp the cartridge by the thumb recess. Hold the device by the other hand and gently guide the cartridge into the analyser until fully inserted and clicks into place.
- The analyser must remain on a level surface with the display facing up during testing.

**NOTE:** Do not remove the cartridge while 'Cartridge Locked' message is displayed on the screen.



### Results

- The countdown will begin as the sample is analysed.
- The results will then be displayed on the screen.
- Record your results.
- Remove the cartridge after the 'Cartridge Locked' message disappears. The analyser is ready for the next test immediately.

### 10.2 Venepuncture

- Immediately after venipuncture, discard the first 4 drops of blood and apply 5th drop to the sample well (a large well-rounded drop without air bubbles). Once in contact with the sample well, the blood will be drawn into the cartridge.

## 11. RESULTS

- The i-STAT analyser contains a microprocessor that performs all calculations required for reporting results.
- Results can be printed to the HP Portable Printer or the Martel Portable Printer with or without Downloader / Recharger. See printer manufacturers' instructions
- **NOTE:** Results printed on thermal paper will fade with time and are therefore not acceptable as a permanent chartable record.
- Results can be transmitted from the i-STAT Portable Clinical Analyser to the Central Data Station/Data Manager. See manufacturer's instructions

### 11.1 Non-anticoagulated Range

INR: 0.8 – 1.2

### 11.2 Therapeutic Range

The anticoagulant effect of Warfarin should be kept at an International Normalised Ratio (INR) of about 2.5 (desirable range, 2.0 –3.0), although a higher level may be needed in certain clinical conditions. The risk of bleeding increases exponentially with the INR result and becomes clinically unacceptable once the INR exceeds 5.0.

### 11.3 Unusual Results

An unexpected result may include any result that falls outside the therapeutic range, or a result that falls inside the therapeutic range but is not consistent with the clinical symptoms (e.g. bleeding or bruising).

### **Causes of unexpected results**

- Changes in diet, lifestyle or taking nutritional supplements.
- Certain prescription drugs and over the counter medicines (e.g. antibiotics).
- Anti-phospholipid antibodies (APA) such anti-cardiolipin antibodies or lupus antibodies may falsely prolong coagulation times using the system. Where APA are known to be present, it is imperative that a result be obtained from a laboratory using an APA insensitive method.
- Liver disease, congestive heart failure, thyroid dysfunction, and other diseases or conditions can affect the action of oral anticoagulants and the INR value.

### **What to do when you get an unexpected result**

- If the result is outside the therapeutic range, follow your clinic's steps for re-testing.
- If, after re-testing, the result is still outside the therapeutic range, consider the above causes.
- An INR  $\geq 4$  should be confirmed with the laboratory or if not possible then Point of Care INR.
- If the result falls within the therapeutic range, but there is reason to believe the INR could be significantly different (e.g. bleeding or bruising), testing by an alternative method should be arranged immediately.

## **12. MAINTENANCE**

- Clean the display and case with a gauze pad moistened with a mild non-abrasive cleaner, detergent, soap and water, alcohol or 10% bleach solution.
- Wipe over with another pad moistened with water and dry.
- Use the electronic simulator daily.

## **13. REFERENCES**

This method has been adapted from the i-STAT System – Abbott Point of Care Procedure Manual, PT/INR test cartridge and control solution package inserts.