PROTHROMBIN TIME/ (PT/INR)

The i-STAT® PT/INR test is a whole blood determination of the prothrombin time used for monitoring oral anticoagulant (Coumadin or 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 added thrombin substrate is H-D-phenylalanyl-pipecolyl-arginine-p-amino-p-methoxydiphenylamine, which has the structure:

Phenylalanine - Pipecolic acid - Arginine -- NH - C_6H_4 - NH - C_6H_4 - OCH_3

Thrombin cleaves the amide bond at the carboxy terminus of the arginine residue (denoted by the two dashes) because the bond structurally resembles the thrombin-cleaved amide linkage in fibrinogen. The product of the thrombin-substrate reaction is the electrochemically inert tripeptide Phenylalanyl - Pipecolyl - Arginine and the electroactive compound NH_3^+ - C_6H_4 - NH - C_6H_4 - OCH_3. A formation of the electroactive compound is detected amperometrically and the time of detection is measured.

The PT/INR test result is reported as an International Normalized Ratio (INR) and, optionally, in seconds. The INR is the recommended method of result reporting for monitoring of oral anticoagulant therapy.[1] A Mean Normal i-STAT prothrombin time (sec) and an ISI are determined following the WHO recommendations at a CAP-accredited facility. INR results are calculated using the following equation:

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

The optionally displayed units of seconds reflect traditional plasma PT times. The reported time is derived from the PT/INR result and the equation below using an ISI of 1.05 and a typical Mean Normal Plasma PT time of 12.0 seconds.

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

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

Intended Use

The i-STAT PT, a prothrombin time test, is useful for monitoring patients receiving oral anticoagulation therapy such as Coumadin or warfarin.

Contents

Each i-STAT PT/INR cartridge provides a sample collection chamber, sensors to detect the coagulation endpoint and dry reagents necessary to initiate and allow coagulation. Inert matrix components and reagents are coated on a section of the sensor channel and include the following reactive ingredients:

Reactive Ingredient	Biological Source	Minimum Quantity
Recombinant Tissue Thromboplastin	Human	0.18 mg
Heparinase I	*Flavobacterium heparinum*	0.018 IU
Thrombin Substrate	Not Applicable	0.4 µg

Metrological Traceability

The i-STAT System test for Prothrombin Time (PT/INR) measures the International Normalized Ratio (dimensionless) expressing the relative time interval required for complete activation, by thromboplastin, of the coagulation cascade in capillary or venous whole blood for *in vitro* monitoring of oral anticoagulant (Coumadin or warfarin) therapy. PT/INR values assigned to i-STAT's controls are traceable to the World Health Organization (WHO) international reference measurement procedures and the International Reference Preparation recommended by the WHO.[2] i-STAT System controls are validated for use only with the i-STAT System and assigned values may not be commutable with other methods. Further information regarding metrological traceability is available from Abbott Point of Care Inc.

Expected Values

Test/Abbreviation	Units	Verified Clinical Range
Prothrombin Time/ (PT/INR)	INR	0.9 - 6.0*

*The performance characteristics of the i-STAT PT/INR measurement have not been established at INRs above 6.0.

Performance Characteristics

The typical performance data summarized below were collected in healthcare facilities by healthcare professionals trained in the use of the i-STAT System and comparative methods.

Imprecision

Initial studies were conducted to collect imprecision data for venous and capillary whole blood samples. Imprecision data for venous whole blood samples were collected in duplicate at two clinical sites. Imprecision data for capillary whole blood samples were collected in duplicate at one clinical site using a single capillary stick. The table below summarizes this data.

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%

The below imprecision data for lyophilized plasma control material were collected during studies at an Abbott Point of Care facility and during clinical trials. SD and %CV are typical of current performance. Current Value Assignment Sheets should be referenced for applicable plasma control mean data.

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

Reference Interval

In a study to determine a reference interval for PT/INR, venous samples from healthy volunteers were collected in plastic tubes, and whole blood was analyzed with one lot of cartridges on the i-STAT System. Capillary samples were obtained from the same volunteers using Softclick Pro (setting of 3) and analyzed on the same cartridge lot. Reference intervals for INR in venous and capillary samples were determined according to the CLSI Guideline C28-A2.[3] The data are summarized in the table below:

Statistic	Venous whole blood	Capillary whole blood
n	120	119
Mean (INR)	1.0	1.0
SD	0.1	0.1
Reference Range (INR)	0.8 - 1.2	0.8 - 1.2

Due to the many variables that may affect PT/INR results, each laboratory should establish its own reference interval.

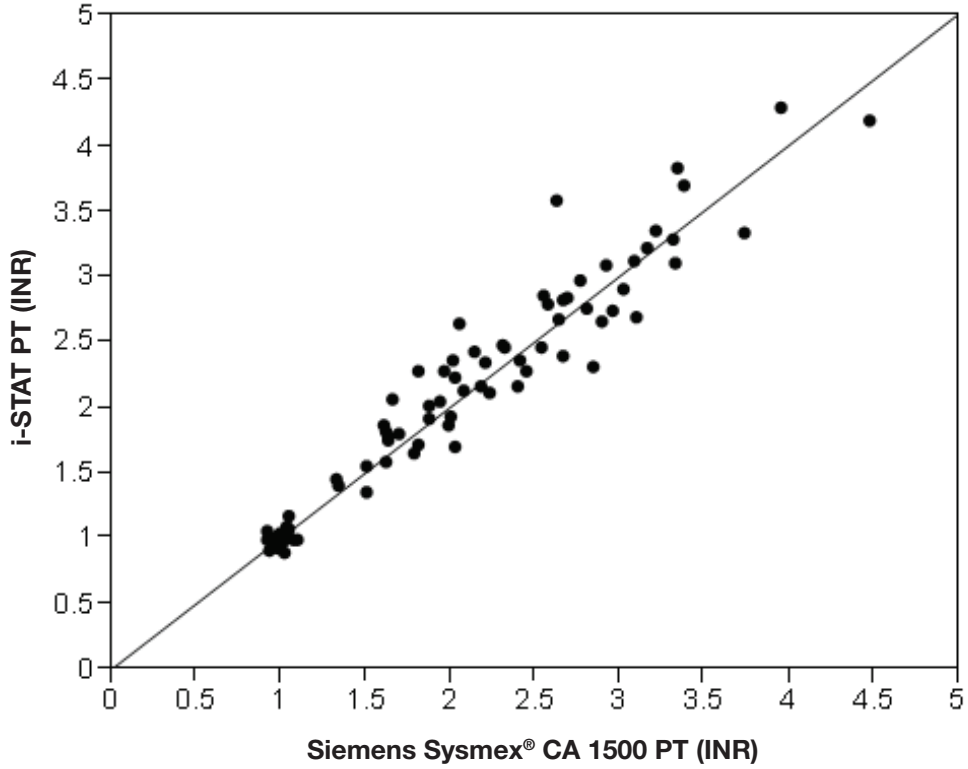
Method Comparison

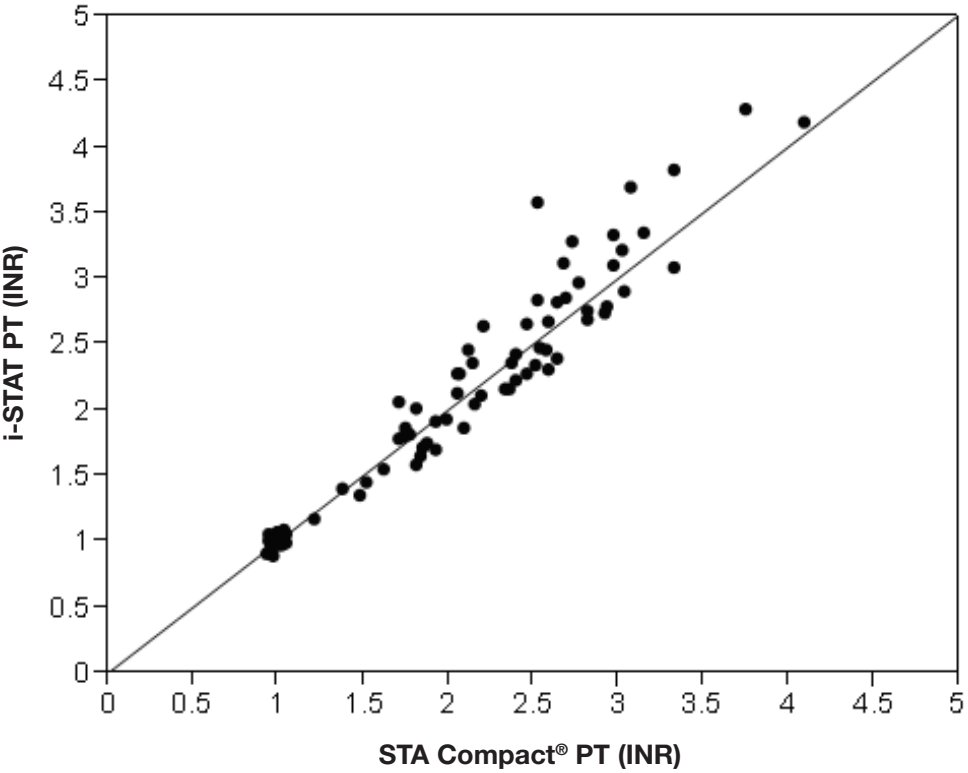
Method comparison data were collected at the Hemostasis Reference Laboratory (Hamilton, Ontario, Canada). Venous samples from outpatients undergoing routine oral anticoagulation therapy were collected in plastic tubes and analyzed in duplicate on multiple lots of cartridges on the i-STAT System; plasma from tubes containing a citrate anticoagulant were analyzed in duplicate on the comparative instruments using Dade® Innovin®, STA Neoplastine® CI Plus, and the HemosIL® RecombiPlasTin 2G® reagents.

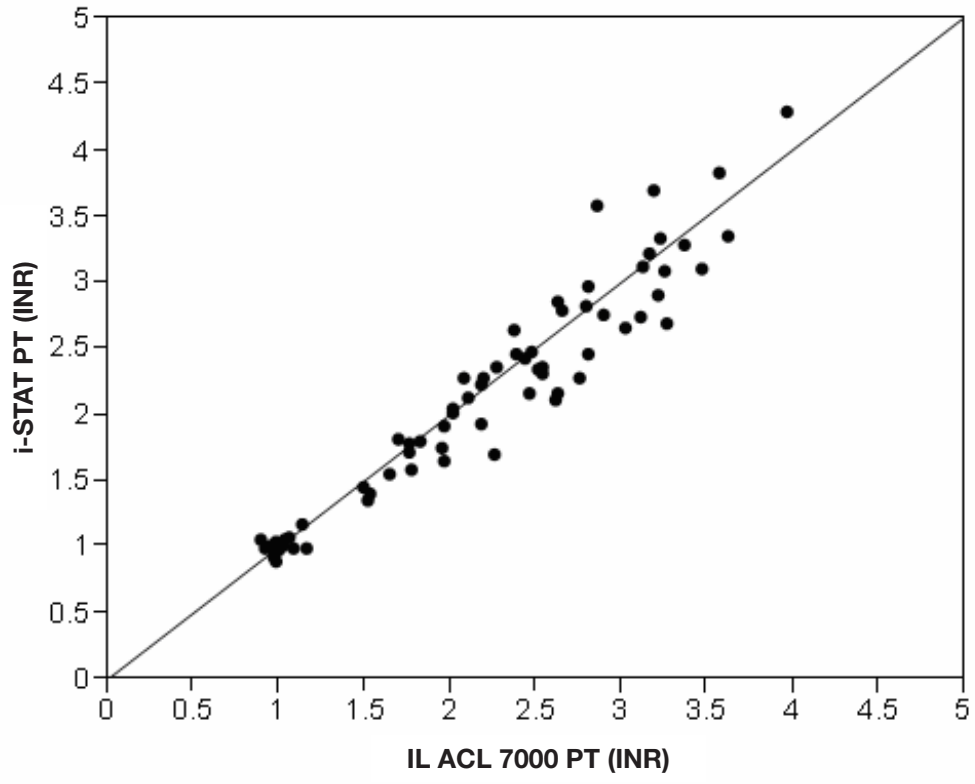
Deming regression analysis[4] was performed on the first replicate of each sample. In the method comparison table below, *n* is the number of specimens in the data set, *Sy.x* is the standard error of the estimate, and *r* is the correlation coefficient.

Method comparisons will vary from site to site due to differences in the sample handling, reagent and instrument systems in use, and other site-specific variables. A correlation study should be performed to establish the differences between the i-STAT PT/INR measurement and other methods used.

Statistic	i-STAT vs. Siemens Sysmex® CA-1500 and Dade® Innovin® reagent	i-STAT vs. STA Compact® and Neoplastine® CI Plus reagent	i-STAT vs. IL ACL 7000 and HemosIL® RecombiPlasTin 2G® reagent
n	78	78	69
Mean (INR)	2.1	2.1	2.2
Range (INR)	0.9 - 4.5	0.9 - 4.1	0.9 – 4.0
Sx (INR)	0.843	0.772	0.840
Slope	0.981	1.074	0.972
Intercept (INR)	0.084	-0.100	0.003
r	0.963	0.964	0.962
Sy.x	0.233	0.229	0.233

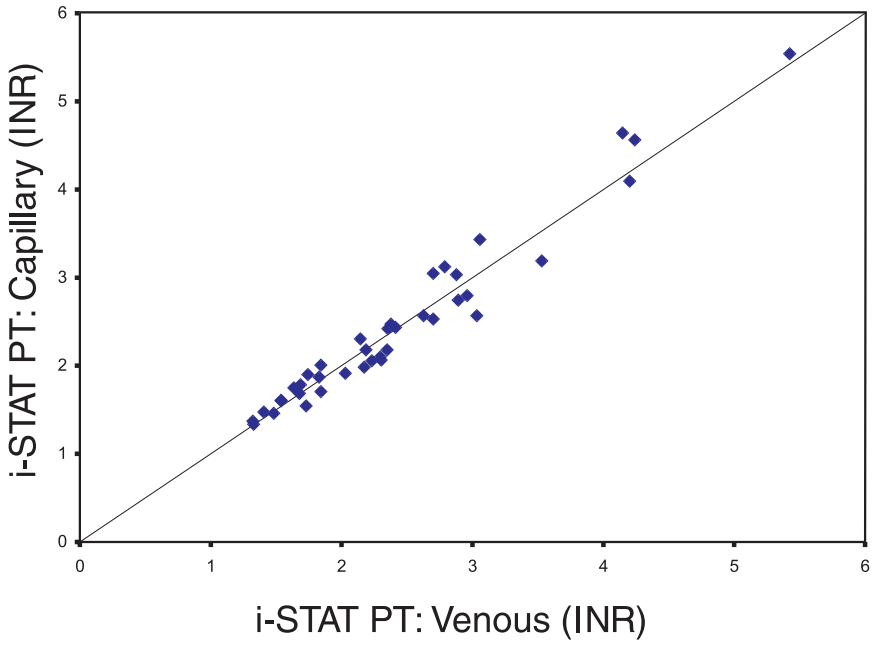

i-STAT PT (INR)
Siemens Sysmex® CA 1500 PT (INR)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5


i-STAT PT (INR)
STA Compact® PT (INR)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5



Data is presented below from one clinical site comparing data from capillary samples to data from venous samples analyzed on the i-STAT System.

Statistic	Capillary vs. Venous
n	39
Mean (INR)	2.4
Range (INR)	1.3 – 5.4
Sx (INR)	0.960
Slope	1.049
Intercept (INR)	-0.098
Sy.x	0.128
r	0.978



Factors Affecting Results

- The presence of exogenously added heparin, citrate, oxalate, or EDTA from blood collection devices will interfere with test results.
- Poor technique in sample collection may compromise the results. (See Specimen Collection and Preparation below.)
- Glass syringes or tubes may prematurely activate coagulation, resulting in accelerated clotting times and lower INRs. Venous samples must be collected into plastic syringes or tubes.
- PT/INR results may be affected by commonly administered drugs.
- Abbott Point of Care has not characterized the i-STAT PT/INR test 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 a reagent that is known to be insensitive to lupus anticoagulant antibodies or an alternate laboratory method.

Limitations of the i-STAT PT/INR Test

- The analyzer must remain on a level, vibration-free surface with the display facing up during testing. A level surface includes running the handheld in the downloader/recharger.
- The i-STAT PT/INR test is not affected by fibrinogen concentrations between 70 and 541 mg/dL. The i-STAT PT/INR electrogenic test methodology does not measure the physical clot and is not dependent on whether or not fibrinogen forms into an actual physical fibrin clot. As such, the i-STAT PT/INR test will not reflect the extension of coagulation time associated with the depletion of fibrinogen (e.g., consumptive coagulopathy), disseminated intravascular coagulation, or defibrination syndrome.
- The i-STAT PT/INR test is not affected by unfractionated heparin concentrations up to 1.0 U/mL.
- Hematocrits 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. It is recommended that for patients being treated with this antibiotic, an alternate method be used to evaluate PT/INR.
- 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.

Specimen Collection and Preparation

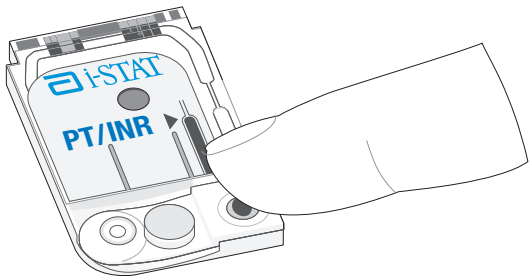
Caution: The i-STAT PT/INR cartridge is designed to accept a sample between 20 and 45 microliters. A single drop of blood from either a finger puncture or as formed at the tip of a syringe will typically be within this range. If a larger volume is delivered to the sample well, use caution when closing the cartridge as excess blood may be expelled from the cartridge.

The i-STAT PT/INR test can be performed using capillary or venous samples.

Skin Punctures

1. Remove cartridge from foil pouch and place the cartridge on a flat surface.
2. Prepare lancet device and set aside until needed.
3. Clean and prepare the finger to be sampled using a 70% aqueous solution of isopropanol (70% v/v).[5] Allow the finger to dry thoroughly before sampling. When disinfecting fingerstick skin puncture sites, swabs or solutions containing substances other than isopropanol (e.g., chlorhexidine gluconate) are not recommended. Refer to the "Limitations of the i-STAT PT/INR Test" section above for more information.
4. Prick the bottom side of the fingertip with the lancet device.
5. Gently squeeze the finger, developing a hanging drop of blood and perform the test with the first sample of blood. *Avoid strong repetitive pressure ("milking") as it may cause hemolysis or tissue fluid contamination of the specimen.*
6. Touch the drop of blood against the bottom of the sample well. Once in contact with the sample well, the blood will be drawn into the cartridge.
7. Apply sample until it reaches the fill mark indicated on the cartridge.
8. Fold the sample closure over the sample well.
9. Press the rounded end of the closure until it snaps into place.

Note: To further simplify the sample application into the test cartridge, it is possible to bring the cartridge to the finger for easier application. Do ensure that the instrument remains on a flat, vibration-free surface for testing.



Venipunctures

- Collection technique resulting in good blood flow must be used.
- The sample for testing should be drawn into a **plastic collection device** (either a plastic syringe or plastic evacuated tube).
- The collection device **cannot contain anticoagulants** such as heparin, EDTA, oxalate, or citrate.
- The collection device **cannot contain clot activators or serum separators.**
- The sample should be immediately dispensed into the sample well of a cartridge. A drop of blood should be touched against the bottom of the sample well. Once in contact with the sample well, the blood will be drawn into the cartridge.
- If a second measurement is required, a fresh sample should be obtained.

Note: Some experts recommend drawing and discarding a (venous) sample of at least 1.0 mL prior to drawing sample for coagulation testing.[6]

References

1. Kirkwood TBL. Calibration of Reference Thromboplastins and Standardisation of the Prothrombin Time Ratio. Thrombosis Haemostasis, 49 (3) 238-244, 1983.
2. L. Poller, The Prothrombin Time (Synonymous with thromboplastin time or Quick test), World Health Organization, Geneva, WHO/LAB/98.3, 1998.
3. CLSI. How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline---Second Edition. CLSI document C28-A2 (ISBN 1-56238-406-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 2000.
4. P.J. Cornbleet and N. Gochman, "Incorrect Least-Squares Regression Coefficients in Method Comparison Analysis," Clinical Chemistry 25:3, 432 (1979).
5. CLSI. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition. CLSI document H4-A6 [ISBN 1-56238-677-8]. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 2008.
6. Corriveau, Donna: Fritsma, George (ed.): Hemostasis and Thrombosis in the Clinical Laboratory. Ed, J.B. Lippinncott Company, Philadelphia, 1988, pp 70-71.

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