# Purpose

Prothrombin Time is a coagulation screening test used for the evaluation of the extrinsic coagulation pathway and the monitoring of oral vitamin K antagonist therapy (Warfarin) using the International Normalised Ratio (INR).

# Method Principle

The principle of the test consists of the use of calcium thromboplastin to activate factor VII in the extrinsic pathway and measure the clotting time of the patient plasma sample (Prothrombin time). The prothrombin time is used in the calculation of the INR.

* 1. **STAR Methodology**
* 50 µL of patient plasma is warmed for 240 seconds at 37°C
* 100 µL of NeoPTimal is added
* Clot formation is measured between 4 and 200 seconds
* INR is calculated

# Work Health & Safety

PPE must be used when handling patient samples and plasma reagents.

# Definitions

PPE Personal Protective Equipment

PT Prothrombin Time

INR International normalised ratio

MNPT Mean normal prothrombin time

ISI International sensitivity index

RBT Rabbit Brain Thromboplastin

WHO World Health Orgainsation

QC Quality Control

STA-R MAX Stago STA-R Max analyser (Alfred)

STA-R MAXINE Stago STA-R Max analyser (Alfred)

STA-R Compact Stago STA-R Compact Max analyser (Sandringham)

# Sample Preparation

For sample requirements and pre-analytical processing refer to:

* CD\_HA\_0024\_Specimens for Coagulation tests

Samples for PT/INR May be stored up to 24 hours at 20+/-5°C before testing. For longer storage, freeze aliquots of plasma at -80°C in false bottom screw capped tubes.

Thaw for 10 minutes at 37°C just before testing.

**Samples should not be run if stored 2-8°C.**

# Equipment & Reagents

* 1. **Equipment**
* STA-R MAX and STA-R COMPACT MAX coagulation analysers
  1. **Reagents**
* Diagnostica Stago NeoPTimal reagent is stored in the coagulation refrigerator. Surplus stock is stored in the Pathology walk in cool room.
  + 1. **STA NeoPTimal**
* Each pack of reagent contains paired vials of lyophilised thromboplastin reagent and diluent containing CaCl2.
* Carefully pour the bottle diluent into a bottle of reagent (brown).
* Date, time and initial the bottle of NeoPTimal.
* Stand at room temperature (18-25°C) for 30 minutes. Mix vigorously and add a white stirring bar to the bottle before use.
* Once reconstituted, NeoPTimal is stable with the perforated plastic lid in place for 48 hours on board the STA-R MAX analysers.
* Add stir-bar before use. Load using bar-code on the bottle into section R2 of the reagent drawer of the STA-R MAX.
* At the Alfred site multiple vials of NeoPTimal are reconstituted each morning but there should always be a minimum of 1 vial of reconstituted NeoPTimal in the white rack in the coagulation refrigerator. Before use, mix vigorously and then bring to room temperature.

# Calibration & Quality Control

* 1. **Controls**
* Refer document CD\_HA\_0789\_Coagulation Controls Calibrators.
* Controls are stored in the coagulation refrigerator, surplus in the pathology walk in cool room.
  + 1. **STA Routine QC 2mL N + P**
* Load bottles using the label barcode into section R0 of the STA-R MAX.
* Controls are manually programmed by selecting **PT INR** or **QC INR** QC from the STA-R MAX QC screen, according to the schedule in CD\_HA\_0961\_STA-R Max Coagulation Analyser Routine Operation.
* Controls for **PT INR** are automatically run when the reagent is changed.
* Controls for **PT INR** are run automatically every 4 hours if a sample is run.
* Controls for **QC INR** are run manually every 24hrs.
  1. **Calibration**
* Stago supplies a single lot of NeoPTimal reagent that usually lasts for approximately 12 months.
* Reagent size options are 5ml, 10ml and 20ml
* Stago will alert the laboratory when a new lot number is available. Reagent can then be purchased to perform the validation and calibration. During the calibration QC testing must be performed on the new lot of NeoPTimal as with the current lot i.e. every new bottle and every 8 hours.
* External quality assurance programmes such as RCPA assess the overall accuracy of the ISI and calculated MNPT.
* Calibration involves the determination of the MNPT and the assigning of the ISI.
* **NOTE: Calibration of factors II, V, VII and X on STA-R MAX and STA-R MAXINE with the new lot number of NeoPTimal is also required prior to implementation of the new lot.** 
  + 1. **Mean Normal Prothrombin Time**
* Each new lot number of NeoPTimal reagent requires a MNPT to be calculated for each analyser.
* Set up “**NB INR10**” assay with the stated ISI in the pack insert of the new lot number of NeoPTimal (barcoded).
* Enter the MNPT from the current NeoPTimal lot in the reference time field for the “**NB INR10**”.
* Ensure that the **PT INR** assay has only got the current lot number of reagent calibrated.
* Ensure that the “**NB INR10**” only has the new lot number of reagent calibrated.
* Test at least 100 fresh normal patient plasmas
  + with INRs of 0.90–1.10 and APTTs of 28.0–36.0 s
  + over 5–7 days using the new lot of reagent.
* Manage the new lot of reagent as you would the current lot of reagent i.e. leaving the reagent on the analyser until it is empty or the on-board stability has expired.
* Once sufficient patient samples have been tested, export the data to USB.
* For STA-R MAX and MAXINE select:
  + - System
    - Archiving
    - Archiving Export
    - Export Archives
    - Select Date Range
    - Update
    - PT INR +/- NR INR, NB INR05, NBINR10, NBINR20
    - Export
    - Removable Disk (D:)
    - Open
    - Save.
* For STA-R Compact select:
  + - User Maintenance
    - Utilities
    - Export Patient Results
    - File Name (SDMH, Date, PT Correlation)
    - Transfer
    - Browse
    - USB Drive (D:)
    - OK.
  + Enter data into the “MNPT STA-R MAX NeoPTimal Lot \_\_\_ Expiry \_\_\_ Master Copy Template” Excel file corresponding to the analyser located in:
    - ***H:\AAA\_Quality\UNIT\_HAem\_q\Validation Data\Coagulation\NeoPTimal.***
    - Save the file in a new folder identified with the new lot number and expiry date of NeoPTimal and include the new lot number and expiry date of NeoPTimal in the file name.
    - **DO NOT save data in the Master Copy Template.**
* Follow the instructions in the Excel spreadsheet to calculate the geometric mean for each day of “PT INR” +/- NR INR, NB INR05, NB INR10 or NB INR20 . This is the daily MNPT.
* The daily MNPT calculated for the current lot NeoPTimal (PT INR) should be consistent between each day of testing.
* The daily MNPT for the current lot should correspond to the original MNPT calculated when the reagent was accepted for use. This MNPT can be viewed in the calibration screen for PT OPT under reference time.
* The daily MNPT calculated for “NB INR05, NB INR10 or NB INR20” should be consistent for each day of testing.
  + The daily MNPT should be within +/- 0.5 seconds of the average GEOMEAN.
  + If results fall outside of these limits review data for outliers or incorrect result entry and perform further correlations to achieve a daily MNPT within the acceptable limits.
* Once all the data has been reviewed, the average geometric mean daily MNPT has been determined for the new lot and analyser.
* Repeat the above process for STA-R MAXINE and STA-R Compact.
  + 1. **The ISI**
* The ISI on the kit insert for the lot number of NeoPTimal should be used in the analyser calculation of INR.
* This is established for each reagent lot by Diagnostic STAGO using the method recommended by the WHO (world health organisation) using a secondary standard RBT (rabbit brain thromboplastin).
  + 1. **Correlation Between Current Lot Number and New lot Number**
* The data collected for the MNPT can be used for normal patient correlations.
* On the STA-R MAX on every patient sample that has a PT INR requested perform a “NB INR05, NB INR10 or NB INR20” assay within 15 mins of the original result.
* Test at least 100 normal patients with INRs from 0.9-1.1
* Test at least 50 patients on warfarin therapy with INR results ranging from 2.0–5.0
* Repeat the above process for STA-R MAXINE
* For the STA-R Compact test 50 normals and 25 patients on warfarin as above
* Once sufficient patient samples have been tested, export the data via USB and enter into the “PT Correlation STA-R MAX NeoPTimal lot\_\_\_ expiry\_\_\_ Master Copy Template” Excel file corresponding to the analyser located in:
  + ***H:\AAA\_Quality\UNIT\_HAem\_q\Validation Data\Coagulation\NeoPTimal.***
  + Save the file in the folder identified with the new lot number and expiry date of NeoPTimal and include the new lot number and expiry date of NeoPTimal in the file name.
  + **DO NOT save data in the Master Copy Template.**
  + Follow the instructions in each Excel file to analyse the data.

### Perform PT and INR correlations between STA-R MAX and STA-R MAXINE using the new lot number of NeoPTimal.

* Test at least 100 normal patients 0.90-1.10
* Test at least 30 patients on warfarin therapy with INR results ranging from 2.0–5.0.
* Once sufficient patient samples have been tested, export the data via USB and enter into the “PT Correlation STA-R MAX NeoPTimal lot\_\_\_ expiry\_\_\_ Master Copy Template” Excel file.

### Finally, perform a correlation between the STA-R MAX and STA-R Compact at Sandringham

Test at least 20 samples including normal patients and patients on warfarin therapy with INR results ranging from 2.0–5.0.

Export the data via USB and enter into the “PT Correlation STA-R MAX NeoPTimal lot\_\_\_ expiry\_\_\_ Master Copy Template” Excel file.

* See CD\_HA\_ 0831 Correlation Studies – FBE and Coagulation
* **Correlations are acceptable when the mean difference is within +/- 5.0%.**
* If the mean difference is outside +/- 5.0%, ensure that the correct MNPT and ISI has been assigned to “PT INR” and “NB INR05, NB INR10 or NB INR20” for each analyser. Perform additional testing as required to achieve a mean difference within +/- 5%.
* When the correlations have been deemed acceptable, complete the “SIGN OFF” tab in the “MNPT STA-R NeoPTimal Lot \_\_\_ Expiry \_\_\_ Master Copy Template” Excel file corresponding to each analyser. The Excel file will be password protected. If access is required, discuss with a senior scientist.
* Prior to using the new lot of NeoPTimal for testing and reporting patient results, calibrate the PT OPT test with the new lot number by scanning the ISI from the package insert and update the reference time using the MNPT that was individually determined for each analyser.

# Process

* 1. **Ordering tests**
* Test is ordered in Cerner as INR or PT
* INR and PT are a part of test groups INAP and COAG
  1. **Running assay on analyser**
* Ensure controls are within acceptable limits
* For sample acceptance, loading and result processing refer to:
  + CD\_HA\_0961\_STA-R MAX Coagulation Analyser Routine Operation
  1. **Result Processing**
* Ensure that the values obtained for the controls are within the ranges stated in the Assay Value insert provided in the box. If the control values are outside the stated ranges, check all components of the test system to ensure that all are functioning correctly, i.e. assay conditions, reagents, integrity of the plasmas being tested, etc. If necessary repeat the patient tests, once the controls are within range.
* Should the results be unexpectedly high or low,
  + Check clinical details for therapy eg warfarin, doacs or NOVO7
  + Re-run the sample in the first instance
  + Remove the sample from the analyser and check the blood sample for over filling, under filling, high haematocrit or gross haemolysis.
    - ????????????
  + Remove the plasma into a false bottom tube and check the red cell button for clots.
    - If clotted ???????????
  + If the sample is suitable, reload the plasma in false bottom tube and re-run the test.
  + Check for the presence of excess heparin (APTT > 300 seconds and TCT > 300 seconds). Report PT and INR as “Unsuitable” if APTT > 300 seconds due to heparin.
* If “M>Mmax” appears as a PT result, and the specimen has been checked, report as >200 secs and INR as >20.0.
* Should the INR be <0.8 and the patient **NOT** on Novo 7, report as “Unsuitable” and request repeat samples and add result comment of “Query Activated Specimen”.
* NOVO7 Patients will have an extremely short PT/INR because of the activated factor VII in this product. – where;’s the comment?
* Warfarin therapy, liver disease and DIC are the most common causes of raised INR.
* Always contact the Pathologist and Doctor when the INR is >4.5 due to Warfarin.
* DOACs (Rivaroxaban, Apixaban and Dabigatran) above their respective expected peak levels can cause an elevated INR.
* Always contact the Pathologist and Doctor when the INR is >2.5 due to a DOAC.
* For other abnormal results refer to *COA\_POL\_002\_ Abnormal Result Investigation and Critical Result Notification*
  1. **PT Correction Test**
* Select a fresh normal plasma collected within the last 4 hours with an INR between 0.95–1.05, an APTT between 31–33 secs and Fibrinogen between 2.0–4.0g/L.
* Transfer a minimum of 1.0 mL of the normal plasma into a labelled microcup and insert into a brass adapter.
* Using the STA-R Max Analyser Reagent Manual Barcodes sheet scan the reagent ID: POOL then manually enter the INR as the lot number and select microvolume.
* Load the normal plasma into the R0 section of the reagent drawer on the STA-R MAX analyser.
* Select the “INRCOR” test. The STA-R MAX analyser will perform a PT/INR on a 1:1 mixture of patient plasma and normal plasma.
* While the INRCOR is running, branch to DOE from the patients ARE and register “PTC”.
* Once testing is complete, enter the INR of the normal plasma into the “INR Normal” field. This will allow automatic interpretation and reporting of the INR Mix.
* The INR **corrects** with normal plasma if the INRCOR is <1.3 and the normal plasma is between 0.95–1.05. Automatic reporting comment “Prolonged INR which corrects with 1:1 mix of normal plasma”.
* The INR **does not correct** with normal plasma if the INRCOR is >1.3 and the normal plasma is between 0.95–1.05. Automatic reporting comment “Prolonged INR which fails to correct with 1:1 mix of normal plasma.”
* Print and refer results to the Haematologist to assess whether further testing is required.

# Interferences

NeoPTimal is insensitive to unfractionated heparin up to 1.0 IU/mL and LMWH up to 1.5 IU/mL.

Hirudin (Lepirudin), Argatroban, Dabigatran and other direct thrombin inhibitors may lead to a prolongation of the Prothrombin Time.

Direct Xa inhibitors (Rivaroxaban and Apixaban) may also prolong the prothrombin time.

# Calculations

PT is the raw data obtained from the assay measured in seconds

MNPT is calculated using normal patients and is specific to each analyser

INR is the International Normalised Ratio calculated by the analyser and transmitted to Cerner

ISI

INR = Patient sample PT (sec)

Mean Normal PT (sec)

# Biological Reference Intervals

**Normal Range:**

INR 0.9 – 1.3

**Therapeutic Range With Warfarin:**

INR 2.0 – 3.5

# Related Documents

# References

STA NeoPTimal package insert

|  |  |  |
| --- | --- | --- |
|  | MAX | MAXINE |
| INR 0.9-1.1  APTT 28 – 36 sec  **100 patients**  Add profile 5 |  |  |
| INR 2.0 – 5.0  Patient on warfarin  **30 patients**  Add profile 5 |  |  |

STOP !!

LIS DOWNLOADING

IS

TURNED OFF

INR CORRELATION UNDERWAY