COAGULATION

1. What is coagulation?
   Coagulation is the natural mechanism by which the body protects itself from external and internal bleeding following injury.

2. In general terms, how does coagulation occur?
   The three main elements involved in the coagulation of blood are:
   - the vessels, which contract,
   - the platelets (thrombocytes), which clump together and adhere to the vessel walls,
   - the coagulation factors. Coagulation factors are proteins which are found in blood and which are activated by platelets or by injured tissue. Fibrin, the last link in the chain coagulation factors, is formed from its precursor, fibrinogen. Fibrin is responsible for “sealing” the injured vessel: the blood has coagulated.

3. How do medicines that reduce coagulation of the blood work?
   In patients with certain diseases it is necessary to reduce the excessive tendency of the blood to coagulate. This is done by administering certain medicines, such as Coumadin, which prevent the blood coagulating too quickly. This prevents the formation of blood clots, also known as thrombi or emboli. Natural coagulation is not, however, completely suppressed by these drugs. Anticoagulants such as Marcumar, Falithrom, or Sintrom inhibit coagulation of the blood, i.e. it takes longer for the blood to coagulate.

4. What part of the coagulation process does the administration of anticoagulants influence?
   Coumadin and warfarin (coumarin derivatives) inhibit coagulation of the blood by influencing the coagulation factors. Medicines such as aspirin (ASA) do not influence the coagulation factors, instead they make the deposition and adhesion of the platelets (thrombocytes) more difficult. Medicines of this type include platelet aggregation inhibitors such as ticlopidine.

5. How do oral anticoagulants work?, What role does vitamin K play?
   Vitamin K is required for the formation of important coagulation factors. Oral anticoagulants (so-called coumarins) dose-dependently inhibit the action of vitamin K. Vitamin K promotes the formation of active coagulation factors. Coumarins displace vitamin K from the liver, so formation of active coagulation factors is reduced. Coumarins are antagonists of vitamin K.

6. What drugs contain coumarin derivatives?
   - Coumadin®, active ingredient warfarin (Bristol Myers Squibb) particularly in USA
   - Marcumar®, active ingredient phenprocoumon (Roche Pharma) particularly in D, AU, NL
   - Falithrom®, active ingredient phenprocoumon (SalutasFahlberg) particularly in D, AU, NL
   - Sintrom®, active ingredient acenocoumarol (Novartis) particularly in GB, F, ES
   - Tromexan®, active ingredient ethyl biscoumacetate (Novartis) particularly in B, Croatia
MONITORING

7. How is oral anticoagulant therapy monitored?
The Prothrombin time (PT) test is the standard for monitoring oral anticoagulation therapy. Treatment with anticoagulants needs to be monitored regularly by means of blood tests. This can be done using blood from a fingertip or from a vein. In the laboratory, a substance, thromboplastin, is added to the blood. This triggers coagulation via a certain biological “pathway”, and a blood clot is formed. The “coagulation time” is measured: this is known as the prothrombin time (PT).

8. What is the PT thromboplastin reagent?
Thromboplastin is a reagent, a test substance, used for the determination of the coagulation time (PT). The unit in which the prothrombin time (PT) is usually cited is the INR value (for oral anticoagulation therapy) or seconds (for factor screening PT use). Thromboplastin is also called “tissue factor” (TF).

9. What materials are used to produce the different thromboplastins/reagents?
They may be prepared from biological material such as rabbit or bovine lung or brain. Reagents prepared by genetic engineering techniques, so-called recombinant thromboplastins, are also common today.

10. What material is used to prepare the i-STAT PT reagent?
The i-STAT currently employs a recombinant thromboplastin. It has been assigned an ISI of around 1.05. The associated Mean Normal PT (MNPT) for the i-STAT thromboplastin reagent is 12.0 seconds. The use of the International Sensitivity Index (ISI) permits the comparison of reagent strength against a known standard. Thus, the thromboplastin “strength” can be standardized by using an “indexing” factor called the ISI. Every manufacturer standardizes its reagent against a standard reagent proposed by the WHO, the reference thromboplastin. The reference thromboplastin has an ISI of about 1.0. Every manufacturer determines the ISI value of each reagent batch by comparison with the WHO reference thromboplastin.

11. Since the reagent ISI is so integral to the system, will I need to validate the ISI (or performance) for each i-STAT PT lot?
No, you do not need to recalibrate or revalidate the ISI for every i-STAT PT lot. Since lab PT systems usually consist of a reagent and instrument, and are open systems (can use any combination reagent and instrument), it is not uncommon for a lab to do local adjustments to the manufacturer’s ISI assignment for that reagent. This will take into consideration any reagent ISI changes due to using a different (local) analyzer than the one that was used for the original ISI assignment (at the manufacturer). Since the i-STAT PT cartridges are custom built for a sole instrument platform, we can customize the cartridge performance (ISI) for all future production lots. Since the lot performance is so tightly controlled, there is likely NO change in the ISI of our reagent from lot to lot. This helps alleviate any need for lot-to-lot crossover studies or new local ISI verification of an i-STAT PT lot.

12. What units are used to measure the extent to which coagulation is inhibited?
A “unit” is specified for the measurement of coagulation or the inhibition of coagulation, just as grams are used as units of weight. The usual units for the measurement of the inhibition of Vitamin K coagulation factors is the INR.

13. I hear that the traditional PT is based on the Quick principle, what is that?
An American scientist named Dr. Armand James Quick developed this method of measuring the coagulation time in the 1930s. The principle of the Quick measurement is as follows: blood plasma or capillary blood is mixed with a coagulant (= thromboplastin) in a test tube and made to coagulate. The time the sample takes to coagulate is measured. The time taken for it to coagulate is measured at a constant temperature of 37°C. The coagulation process
triggered in this way takes place in the so-called EXTRINSIC pathway, and involves the activation of various coagulation factors.

14. What coagulation factors does the Quick value measure?
The Quick value measures the most important vitamin K-dependent coagulation factors: II, V, VII and X.

15. Why do I get different (Quick) lab plasma PT values when compared to the i-STAT PT test values?
Different results are obtained depending on the laboratory method used and the thromboplastin used to determine the Quick value (in seconds). It is perfectly possible for different results to be obtained even when the same blood sample is measured in different laboratories. To minimize this, several standards have been created.

16. Why is it not possible to directly compare PT results (in Quick seconds) to other PT result performed at different locations?
This is due to the different properties of the thromboplastins used. Different manufacturers use different starting materials and purification procedures, so each thromboplastin has its own unique properties. Each reagent manufacturer specifies the particular normal range of its reagent. This is why there are so many different normal ranges. To minimize this, several standards have been created.

17. What is the INR?
To help standardize PT testing reagents and test results across many differing product and testing locations, the WHO recommended the use of the INR instead of the Quick value as the units for the measurement of coagulation right back in the early 1980s. It is now the global standard and recommendation for PT oral anticoagulation therapy test reporting.

18. How is the INR calculated?
The INR is also calculated from the patient’s coagulation time in seconds. However, the calculation also takes into account the reagent ISI. (the ISI s calculated by each manufacturer by comparison with an international standard thromboplastin. This compensates for the differing sensitivities of the reagents on the market).

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

The patient’s coagulation time is first divided by the coagulation time of a healthy individual (normal value). This ratio shows the increase in the coagulation time of the patient compared with a healthy individual. Since, however, different thromboplastins have different sensitivities (depending on their origin) the calculated sensitivity factor (ISI) needs to be taken into account. The ISI is an exponent, the ratio is raised to the power of the ISI.

19. What does an INR value of 1.0 or 2.0 mean?
A person who is not taking anticoagulant medication (oral anticoagulant) has an INR of about 1.0. A measured INR of 2.0 means that it takes twice as long for the blood to coagulate. At an INR of 3.0, therefore, it takes three times as long for coagulation to occur. To sum up: The higher the INR number, the greater the inhibition of coagulation.

20. What advantages does use of the INR offer?
The INR is the international result unit recommended worldwide for the prothrombin time (PT) measurements. Reasons for the use of the INR are:
- INR values can be compared, i.e. they permit the comparison of coagulation measurements despite the use of different thromboplastins.
- The INR permits standardization of the degree of inhibition of coagulation for certain indication groups independent of the thromboplastin and the instrument used.
- The INR permits improved monitoring and treatment of patients.
21. What limitations are there on the use of the INR?
   The INR does not produce absolute (perfect) comparability between measurements obtained at different laboratories or using different thromboplastins or different systems. The INR must not be used in coagulation factor screening (preoperative, postoperative) in patients who are not being treated with anticoagulants.

22. What is an indication-related therapeutic range?
   Various clinical specialist societies have issued recommendations about how strongly coagulation should be inhibited in specific diseases. These recommendations offer optimal protection from thrombosis combined with a minimal risk of bleeding in the diseases concerned. Because there are a number of different specialist societies, there are also (slight) differences in the recommendations, and the recommendations are also changed from time to time in the light of the most recent medical knowledge. The commonly used recommendations can be found within the American Heart Association published guidelines. With all of these clinical recommendation available, it is desirable that a customer obtains this information for themselves. These ranges typically include the following:
   - High anticoagulation INR range: 3.0-4.0
   - Moderate anticoagulation INR range: 2.5-3.5
   - Low anticoagulation INR range: 2.0-3.0

RESULTS

23. If INR values can be compared, why are there differences between the competitor’s whole blood PT and the i-STAT PT system?
   Even INR values do not provide absolute foolproof comparability. The reliability of the INR depends on the quality of the prothrombin time measurement. Handling effects are decisive in the quality of the result. This is true both for whole blood and laboratory plasma method. The different ISI values of the thromboplastins used can also influence the result, especially at higher INR values. Differences can also come about because different thromboplastins have differing sensitivities to vitamin K-dependent coagulation factors. Other than reagent, the instrument plays a part in the overall ISI assessment. As such, the ISI is actually a generic assignment in the lab markets. For example, Dade sells Innovin® that has a 0.96 ISI. That 0.96 is established on a Behring BFA instrument. When using the same reagent on an MLA 700, the ISI on this combo more closely resembles a 1.08. In practice, one of the advantages a whole blood PT has over the lab is it is a CLOSED system. So the ISI is very reliable from lot to lot since the analyzer is known and standardized.

24. What level of difference is tolerable in duplicate measurements of the INR with the i-STAT PT test?
   Duplicate PT measurements carried out by experienced whole blood PT operators in our clinical validations witnessed an average 5.6% coefficient of error. This can translate into only a ± 0.1 to 0.25 INR unit difference.

25. The laboratory reports a different INR value than the i-STAT PT on a patient?
   Likely a handling error (collection or test performance), error in the test procedure, patient on heparin (too much for comparative PT), hematocrit value outside the normal range: 32-50%, residual alcohol/soap on the skin from puncture site. If possible, re-perform the PT testing for this patient.

26. Are INR values affected by taking other medicines?
   A number of medicines increase or reduce the effects of anticoagulants. In every case check “Interactions”, “Side effects”, “Indications”, “Contraindications”, and “Special information” on the package insert which comes with the medication for any references to interactions with oral anticoagulants.
27. What medicines increase the effect of coumarins (Coumadin, warfarin)?
An increase in the effect means: risk of bleeding increases – INR increases.
- Action via displacement and/or inhibition of the metabolism of coumarins:
  - Analgesics (e.g. acetylsalicylic acid)
  - Antirheumatics (e.g. phenylbutazone)
  - Antibiotics (e.g. penicillins, ampicillins, tetracyclines)
  - Anticholesteraemics (statins, fibrates)
  - Antiarrhythmic (e.g. amiodarone, quinidine)
  - Antidepressants (tricyclic antidepressants)
  - Diuretics (e.g. ethacrynic acid)
  - Uric acid lowering agents (e.g. allopurinol)
  - Hypoglycaemic medicines (e.g. sulfonylureas)
  - Thyroid hormones

CAUTION NOTE: This list is not complete. Only the most common representatives of a group are cited. Please refer to the drug package insert for more complete details.

28. What medicines reduce the action of coumarins (Marcumar)
A reduction in the effect means: risk of thrombosis increases – INR decreases.
- Antiepileptics (e.g. carbamazepine, barbiturates)
- Antifungals (e.g. griseofulvin)
- Tuberculosis drugs (e.g. rifampicin)
- Vitamin products (vitamin K)
- Care is required with laxatives, as the effect on the inhibition of coagulation is unpredictable

CAUTION NOTE: This list is not complete. Only the most common representatives of a group are cited. Please refer to the drug package insert for more complete details.

29. What is the range of heparin levels in patients receiving heparin. i-STAT PT is insensitive to heparin up to 1 U/mL. Should we expect trouble in any particular patient population? If so, is heparin a well-known interfering substance for the PT test?
The heparin insensitivity of the PT is simply to help a population of patients known as “crossover” patients. When getting discharged for an angioplasty, it is possible that these patients have some residual heparin anticoagulation from the day before. Since warfarin takes about 72 hours to have effect, many are kept on a low-grade heparin drip or allowed to naturally metabolize the heparin (so there is a low-grade anticoagulation going on while the warfarin starts to kick in. These crossover patients typically have much less than 1.0 units of heparin per mL of blood on board. 1.0 is usually the intraoperative concentration of heparin.

30. Other common sources of error?
- Interference from heparin injections overlapping with the administration of Coumadin? The i-STAT PT is insensitive up to 1.0 unit/mL of heparin
- Was the hematocrit value outside the normal range (> 50%)? The i-STAT PT is not affected by HCT values between (24%-52%)
- Did you press or squeeze too much when obtaining the blood? Squeezing can contaminate the blood sample with thromboplastin from the puncture wound (and shortening the result).
- Did it take more than 15 sec from pricking the finger to application of the blood to the test cartridge? Large delays in applying the sample can allow the blood to begin clotting (and shortening the result).
- Was the fingertip pricked too early? Prick the finger immediately before applying blood to cartridge. *Large delay from puncture to application of the sample can allow the blood to begin clotting (and shortening the result).*
- Was the same fingertip pricked again after an unsuccessful measurement? *Multiple punctures in one location can contaminate the blood sample with blood clot or thromboplastin from a previous puncture wound (and shortening the result).*

31. Is there a calibration verification or linearity procedure for the Prothrombin Time test used to monitor oral anticoagulation therapy?

Since the coumarin class drugs are Vitamin K antagonists, and they affect the synthesis of certain coagulation factors produced in the liver, Coumadin does not really cause any anticoagulation directly. Thus is it impossible for an “in vitro” application of Coumadin to a blood sample to prolong its clotting time. As such, there are no commercial “linearity” kits or procedures for PT/INR testing.

32. What is involved with the standard “evaluation process”?

Two things to remember first; 1) we are in a “replacement strategy” sales mode, so there is a competing whole blood PT device in the account. 2) since none of these other whole blood PT technologies do a fingerstick as well as us, we do not want to use them as the reference PT. Instead, the lab plasma PT is the GOLD STANDARD. As such, that should be the reference PT for our evaluation process. With the lab as the reference PT, there are two protocols in creation. One for a venipuncture, and the other for finger puncture evaluation (versus the lab plasma PT). Each protocol asks for a normal range population and a range of anticoagulated patients throughout the INR range (1.5-4.5)

33. Can the i-STAT PT be used with citrated blood samples?

It is not possible to use citrated (then calcified) blood samples on the i-STAT PT test. In coagulation, ionics are very important proper coagulation. As such, fresh whole blood coagulation tests (i-STAT PT included) are not designed for use with citrated whole blood. The addition of sodium citrate or calcium chloride will likely affect the coagulation chemistry that has been designed to work on native fresh whole blood.
**TERMINOLOGY**

**AMI:**
Acute Myocardial Infarction (AMI) is the rapid development of myocardial necrosis caused by a critical imbalance between the oxygen supply and demand of the myocardium. This usually results from plaque rupture with thrombus formation in a coronary vessel, resulting in an acute reduction of blood supply to a portion of the myocardium

**Atrial Fibrillation:**
Most common arrhythmia, affecting more than 2 million people annually. Research has yet to uncover the definitive cause of AF. In the broadest sense, AF represents the loss of synchrony between the atria and the ventricles.

**Coumarin:**
Coumarin (and coumarin-class drugs) is the most frequently prescribed oral anticoagulant; the fourth most prescribed cardiovascular agent and the overall ninth most prescribed drug in the world.

**EXAMPLES OF COUMARIN-CLASS DRUGS:**
- Coumadin®, active ingredient warfarin (Bristol Myers Squibb) particularly in USA
- Marcumar®, active ingredient phenprocoumon (Roche Pharma) particularly in D, AU, NL
- Falithrom®, active ingredient phenprocoumon (SalutasFahlberg) particularly in D, AU, NL
- Sintrom®, active ingredient acenocoumarol (Novartis) particularly in GB, F, ES
- Tromexan®, active ingredient ethyl biscoumacetate (Novartis) particularly in B, Croatia

**DVT/PE:**
Deep Vein Thrombosis is a blood clot in a deep vein. These may form on the valves within the vein, and may subsequently increase in size to totally occlude the vein. Sometimes parts of the clot may break off and travel in the bloodstream to the lungs and cause serious health problems. (PULMONARY EMBOLISM). Although some clots may never go away, they can be treated both in the hospital and at home to prevent the clot from growing.

**INR:**
The INR is derived from calibrations of commercial PT reagents against the International Reference Preparation (IRP). The manufacturer provides the ISI value for each lot of thromboplastin reagent. The INR is calculated by the formula below:

\[
\text{INR} = \frac{\text{Patient I-STAT PT time (sec)}}{\text{Mean Normal I-STAT PT time (sec)}}^{\text{ISI}}
\]

**ISI:**
This standardization system was introduced by the World Health Organization (WHO) as a common basis for the interpretation of the PT results independent of the sensitivity of PT reagents (which tends to vary). The lower the ISI the more sensitive is the PT reagent (thromboplastin) preparation

**Mean Normal PT (MNPT):**
Normal range establishment of local warfarin population using a fixed ISI reagent/instrument combination. The mean normal PT is essential due to its involvement in the International Normalized Ratio equation.

**Narrow Therapeutic Index (NTI):**
Due to their efficacy nature, drugs like Coumadin are called Narrow Therapeutic Index or NTI drugs; meaning dosage is critical to effective treatment
OAT:
The goal of Oral Anticoagulant Therapy is to administer the lowest possible dose of anticoagulant to prevent clot formation or expansion. The required degree/dosage of anticoagulation varies per patient due to many uncontrollable factors/conditions. Thus frequent monitoring is needed.

PAT:
Pre-Admission Testing is usually performed before any elective surgical procedure. It usually includes a coagulation screen to identify any coagulopathies or coumadin anticoagulation presence. Both of these conditions can contribute to bleeding during and after the surgery.

TITR:
Time in Therapeutic Range indicates the amount of time that the patient's PT INR result is within the desired target range.