

# SEED Coagulation

Sysmex Educational Enhancement and Development  
July 2012



## Quality control in coagulation testing

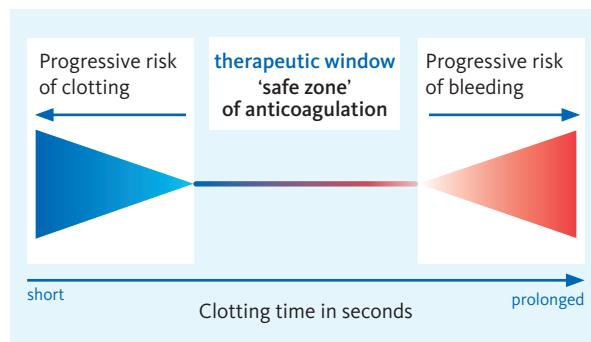
### The prothrombin time and activated partial thromboplastin time

As discussed in previous editions, the main indication for prothrombin time (PT) (expressed as an international normalized ratio or INR) and activated partial thromboplastin time (APTT) testing is to monitor the anticoagulant effects of warfarin and heparin respectively. Both drugs have a very narrow therapeutic window. This means that the patient will be adversely affected if the test results fall outside of this range – if too high they may bleed or if too low they are at risk of developing a blood clot (Fig. 1). Millions of patients are on chronic anticoagulant therapy worldwide so the consequences of issuing erroneous test results are far reaching.

In this regard, it is of paramount importance that the PT and APTT results issued by the laboratory are accurate. The only way to ensure this is to apply stringent internal quality control procedures.

### Prothrombin time reagent

Many of the coagulation reagents and controls that are required for coagulation testing come in a lyophilised formulation. Lyophilisation refers to the process of freeze drying the liquid reagent which removes the liquid component and leaves a dry powder behind. As clot based coagulation testing requires the key components (e.g. tissue factor in the thromboplastin reagent Innovin<sup>®</sup>) to be biologically active lyophilisation is necessary to preserve this function. In the lyophilised format, the tissue factor is preserved thereby giving the PT reagent a very long shelf life. Before use, the PT reagent needs to be reconstituted with the exact amount of diluent (liquid) as prescribed in the package insert. This reconstitution effectively brings the reagent back to life and ready to use in testing. However, it must be noted that once converted back into a liquid, the reagent can only be used for a very limited period. This is referred to as the 'open vial stability'. Whilst the expiry times are very long (measured in months or years), the open vial stability of reagents and controls is measured in hours or days. It is therefore essential to ensure that reagents are correctly labeled with the date and time when reconstitution took place and to ensure that the items are stored appropriately. The individual package inserts will provide details of the open vial stability under different storage conditions (Fig. 2).



**Fig. 1** Diagrammatic representation of the 'therapeutic window' of clotting times for patients on anticoagulant therapy

The lack of adherence to the storage conditions and times is a major source of error in the coagulation laboratory.

### Storage and Stability

Store at +2 to +8 °C. At this temperature, the unopened reagent can be used until its expiry date (see vial label).

Stability after reconstitution:	+2 to +8 °C	10 days (closed vial)
	+15 to +25 °C	5 days (closed vial)
	+37 °C	24 hours (closed vial)

Information about on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

#### Do not freeze!

**Signs of expiry:** Absence of vacuum when opening the vial; reagent is difficult to reconstitute; results are not reproducible.

**Fig. 2** Extract of Innovin package insert highlighting storage and stability conditions after reagent reconstitution

### Activated partial thromboplastin time reagent

In contrast to the thromboplastin reagents used for PT testing, the APTT reagent Actin FS® comes in liquid formulation. This is because the activator which initiates the clotting reaction is an inert substance that has no inherent biological function. It is the charged nature of this substance (ellagic acid) that reacts with clotting factor XII (the starting point of the APTT) rather than enzymatic activity. Consequently the reagent is stable as a liquid although the shelf life is somewhat shorter than the lyophilized reagents. Once opened, the same open vial stability issues apply as exposure to air initiates a slow decline in the stability of the product. The performance of the product is only guaranteed by the manufacturer if the storage times and conditions are strictly adhered to.

In contrast calcium chloride is a completely inert chemical hence it has a very long shelf life included a long stability even after opening.

### Control plasmas

As for any laboratory investigation, the accuracy of PT and APTT results must be monitored on a regular basis using controls. As the PT and APTT essentially measure the reactivity of clotting factors, culminating in the conversion of fibrinogen to fibrin, the control material has to contain functional clotting factors. However, we know that blood taken for coagulation testing should ideally be tested within four hours as the clotting factors start to deteriorate quite rapidly. It therefore follows that controls for clot-based coagulation testing must be comprised of plasma. As for the PT reagent, the function of the clotting factors is preserved through lyophilisation. Likewise, once reconstituted, control plasmas have the same short viability as do patient samples.

If the test method is working well, then the result obtained for the control sample should be within the specified range of acceptable results – the so-called target values. It is important to always use a control with a target value that is aligned with what is clinically relevant. In the context of using the APTT for heparin monitoring, it would be important to have a control with a target value that is within the therapeutic range. In line with this general principle Sysmex, with its reagent partner Siemens, has three levels of controls for PT (INR) and APTT testing (Tab. 1).

Citrol® Level 1	Normal range
Citrol® Level 2	Low therapeutic range
Citrol® Level 3	High therapeutic range

**Tab. 1** Control plasmas used for PT (INR) and APTT testing

### Control plasma target values

Control plasmas for PT and APTT testing may be assayed or unassayed. A control plasma that is deemed to be 'assayed' is supplied with target values. The target values assigned to a control plasma are specific to the reagent and analyser used to generate the test result. It is therefore important to ensure the correct target range is used. Unassayed control plasmas do not have target values assigned. If a laboratory chooses to use unassayed control plasmas, they must be aware that they will have to generate their own target range by processing the new lot of control plasma on an analyser five times per day for at least five consecutive days.

LOT	528104	EXP	2014-01-30			
Reagents/Reagenzien/Réactifs Reagenti/Reactivos/Reagentes Reagens/Reagenser/Αντιδραστήρια	Assigned value Sollwert valeur-cible valor teórico valor nominal analyseværdi analytiska värde Τιμή αναφοράς	Confidence interval Vertrauensbereich domaine de confiance Intervallo di accettabilità intervalo de confianza intervalo de confiança konfidensinterval konfidentsinterval Τιμή αναφοράς	Assigned value Sollwert valeur-cible valor teórico valor nominal analyseværdi analytiska värde Τιμή αναφοράς	Confidence interval Vertrauensbereich domaine de confiance Intervallo di accettabilità rango de confianza intervalo de confiança konfidensinterval konfidentsinterval Τιμή αναφοράς	Assigned value Sollwert valeur-cible valor teórico valor nominal analyseværdi analytiska värde Τιμή αναφοράς	Confidence interval Vertrauensbereich domaine de confiance Intervallo di accettabilità rango de confianza intervalo de confiança konfidensinterval konfidentsinterval Τιμή αναφοράς
<b>Mechanical instruments/ Mechanische Instrumente/ Instruments mécanique/ Strumentazione meccanica/ Instrumentos mecánicos/ Instrumentos mecânicos/ Mekaniske instrumenter/ Tekniska instrument/ Μηχανικά όργανα</b>						
Prothrombin time/Thromboplastinzeit/ Temps de Quick/Tempo di protrombina/ Tiempo/Tempo de tromboplastina/ Protrombintid/χρόνο προθρομβίνης				PT/TQ		
%				INR		
Thromborel® S	80	70 - 90	1.11	0.98 - 1.24	12.4	10.9 - 13.9
Innovin®	94	83 - 105	1.02	0.90 - 1.14	9.5	8.4 - 10.6

**Fig. 3** An extract from the assay data sheet of a normal level of control plasma

The results obtained are then used to calculate the target range using the formula of 'mean  $\pm$  2SD'. During this time of establishing such a target range, the daily testing must continue to be controlled using the previous lot number of control plasma. In other words, during the days that a new target range is being established, two lots of control plasmas must be used. An example of an assay data sheet for a control plasma is shown in Fig. 3.

### Why the use of third party reagents and controls is problematic

The use of third party reagents and controls is problematic for several reasons. Firstly, the target ranges on the assay data sheet that are supplied with assayed control plasmas are generally only available for reagents and analysers belonging to the same manufacturer. If the exact reagent and analyser (make and model) combination is not listed, then the control plasma is in essence 'unassayed' for that combination or analytical system. The laboratory will then need to establish its own target range. This approach is not recommended unless the laboratory personnel are highly experienced in coagulation testing. Results obtained using the same reagent but different models of analyser from the same manufacturer are not going to be identical. Differences are to be expected as the clotting time generated is the product of the entire analytical system i.e. the analyser, the protocol set up and the reagents. As the therapeutic range for anticoagulation is narrow, any small shifts in clotting times may result in a change in clinical management. In this regard it is imperative that the control target values are specific for the reagent and analyser model in use.

A second major problem with the use of third party reagents is that the protocols on automated analysers are fine-tuned by the manufacturer for specific reagents which are not interchangeable. If a laboratory chooses to use reagents other than those recommended by the manufacturer then the responsibility of ensuring that the analyser is correctly set-up is entirely their own.

### Are all variables that have an influence on test results detectable through the use of control plasmas?

It is important to be aware of the fact that there are several pre-analytical factors that can give rise to erroneous test results as these factors are sample specific and will not be detected by the standard internal quality control procedures. Control plasmas will only assess the analytical component of the testing process. Any error introduced into patient sample testing due to sample processing pre-analysis will not be identified through the use of control plasmas.

### How frequently should control plasmas be processed?

As a general rule of thumb, control plasmas should be processed at least once per 8 hour shift. Both a normal and at least one abnormal control should be included.

### How does one determine whether the quality control results are acceptable or not?

One must remember that the purpose of performing internal quality control is to ensure the reliability of patient results. The values obtained for each level of control plasma must be compared with the acceptable target range as per the data assay sheet that is specific for the lot number of the

control and reagent and analyser combination. The value must be within the specified range. However, the target range provided is generally rather wide and in practice the range of values obtained on a single specific analyser in a laboratory from day to day will be much tighter. The degree of inter-day variation that is considered acceptable will be guided by the CV% of the assay. The CV% is a measure of the precision of the test. If a single value shows a significant shift from what was obtained on previous days, or a continuous trend deviating in the same direction, a possible source of error should be sought. Patient results should be withheld until the problem has been rectified.

### **How would one go about troubleshooting failed QC results?**

A number of baseline factors need to be considered when evaluating the possible sources of error:

- Is an isolated parameter affected or are all parameters affected?
- If more than one, do these parameters have a common testing principle or not; e.g. clot-based tests only or are chromogenic parameters (e.g. antithrombin) also affected?
- Is only one level of control affected or are all out of range?
- Is the direction of deviation the same for all levels and all parameters i.e. do all have values that are too high or all are too low?
- Is this a once off event or has the same problem been observed before?
- Has the open vial stability of all reagents been adhered to?
- Has the expiry date of all reagents been adhered to?
- Does the lot number of the control material used match that of QC target range?
- Have the reagents and controls been correctly reconstituted?
- Was the diluent used for reconstitution the correct one?
- Was the diluent of good quality?
- Was there a problem with pipetting?
- Was the reagent adequately mixed and allowed to stand for the appropriate amount of time before use?
- Was the analyser correctly calibrated (if applicable)?
- Has analyser maintenance been carried out as prescribed?

The first step to be undertaken when troubleshooting internal quality failures is to judge the anticipated time delay before the problem is likely to be resolved. This will determine whether alternate plans need to be made for patient sample analysis as no patient can be released until the quality control results are once again shown to be within acceptable performance limits.

There is no fixed troubleshooting procedure. Each problem needs to be assessed on a case by case basis. A step by step approach should be adopted which follows a logical sequence of checks. The obvious should be checked first. Judgment should be prudent taking both cost and time factors into consideration. One should try to gauge whether the problem is likely to be due to faulty control material, faulty reagents or a faulty analyser. As a rule of thumb, the problem is most likely to lie with the control material as this is the most labile, followed by the reagents and lastly the analyser which is the most stable component of the analytical system.

### **What kind of problems are commonly associated with the control plasma?**

Because the reconstituted control plasma is very labile with a short open vial stability (maximum 16 hours for Citrol), the use of 'old' control material is the commonest cause of failed internal quality control. This is readily preventable if the laboratory adheres to the practice of labelling each reconstituted vial with the date, time and signature of the person who opened it. All parameters are likely to be affected and usually all levels as in general the person reconstituting the controls for the day (or particular shift) will make up a vial for each level to be used at that time. Another consideration is the accuracy of pipetting the diluent. An excess of diluent would result in a dilution of clotting factors with prolonged times and too little would give rise to a concentrated sample with shorter than expected clotting times. The diluent for control plasma is distilled water. It is strongly advised that laboratories use sterile 'water for injection' as this comes in a small sized single use sterile packaging. It is however common practice for laboratories to source the distilled water for control plasma and reagent reconstitution from a local purification system where sterility, especially during the collection process is not always maintained. The growth of fungal elements is relatively common which can interfere with analyser optics and consequently test results. A simple check would be to make up a fresh vial of control

using sterile water and retesting the sample. Power failures which lead to intermittent loss of cold chain maintenance must also be considered as possible sources of error as the open vial stability of Citrol control plasma is reduced from 16 hours if kept stoppered and in the fridge at 2 – 8°C to only 8 hours if the temperature rises to 15°C.

### **What are the commonest problems associated with reagents?**

The commonest problem, as for control plasmas, is the lack of adherence to the maximum open vial stability period and the correct reconstitution where appropriate. If the reagent is the problem then all levels of control plasma would be affected but only those parameters that have a reagent in common. For example, if the suspect reagent is Innovin, then the PT (INR) results and extrinsic factors will be affected, but the APTT and intrinsic factors will be within limits. Another common source of error with INR results is the use of a new lot number or reagent without entering the new ISI value and newly determined mean control value into the method protocol on the analyser. The INR values that are produced by the analyser will be based on the clotting time of the plasma sample measured with a new lot of reagents but using the mean control value and ISI that belong to an earlier lot numbers. Although such differences tend to be small, they can be significant enough to cause quality control failure. It is an extremely rare occurrence for reagents for which cold chain has been maintained at all times, accurately reconstitution and open vial stability limits adhered to be faulty.

### **What kind of problems may be due to the analyser?**

The analyser is least likely to be the cause of failed quality control. However there are some simple things that may give rise to faulty results. Any spillage of plasma into the detection wells can interfere with optics. Likewise if the intensity of the LED lamps that are the core component of the optical clot detection system needs to be checked from time to time as part of regular analyser maintenance. If this is not done then a drift in results is likely to occur. The temperature of the detection wells needs to be at  $37 \pm 1^\circ\text{C}$ . There is an easy self-check on the analysers to confirm that the temperature is correct. As clot formation depends on the biological activity of clotting factors, which function optimally at body temperature, any small deviation up or down can have a significant effect on test results. As each of the detection wells has its own LED lamp and temperature control, faulty test results are generally restricted to a single detection well and is thus relatively easily identified.

### **Take home message**

Internal quality control is an integral process of laboratory testing which is no different for coagulation testing. Always ensure that the target values used are appropriate for the reagent and analyser combination. In the event that the quality control samples fail, patient results must be withheld, and possibly retested once the problem has been resolved. There can be multiple sources of error and hence a logical troubleshooting approach must be adopted. In general however, the control plasma is most likely to be the culprit, followed by the reagents and lastly the analyser.

*Compiled by*  
**Dr. Marion Münster**