

Evaluating the Unfractionated Heparin Sensitivity of new aPTT Reagents

When changing the activated partial thromboplastin time (aPTT) method, a laboratory must determine the responsiveness of the method (reagent/instrument) to unfractionated heparin if the aPTT is to be used to monitor heparin anticoagulation with the aPTT. In order to determine the heparin sensitivity, the laboratory has several options from which to choose; three are listed below. The first is to use a heparin assay to correlate heparin concentration with the new aPTT reagent. The second is to use a cumulative summation method to select a new reagent that has the same heparin responsiveness as the old reagent. The third is to recalibrate the instrument so that the heparin responsiveness of the new method remains the same (or nearly the same) even when the reagents may be different. A summary of the methods follows:

1. Validation of heparin sensitivity of the aPTT using an assay of heparin:

This can be done by simultaneously determining the aPTT (seconds) and heparin concentration (U/mL) using specimens from patients receiving unfractionated heparin for the treatment of thromboembolism. A dose-response curve can be calculated from the data using regression analysis and the aPTT range corresponding to a heparin concentration of 0.3 - 0.7 U/mL (by a factor Xa inhibition assay) can be derived.

In contrast to the high degree of correlation between heparin added in vitro and aPTT prolongation, several published comparisons have shown more variability for paired data points comparing the aPTT to heparin concentration in ex vivo specimens, yielding r2 values of < 0.50 in many cases. Less than 50% of the variation in aPTTs in heparinized plasmas is explained by differences in the heparin concentrations in those plasmas. In one study, the correlation was better for aPTT/anti-Xa comparisons than for aPTT/protamine titration comparisons suggesting that the functional Factor Xa inhibitory assay may be preferable to the protamine titration assay for establishing the therapeutic interval.

Although the use of the heparin assay may be preferred, the potential burden of such a recommendation should not be overlooked. For example, laboratory validation of the therapeutic range by the performance of simultaneous aPTT and heparin assays may be overwhelming, if not technically infeasible, in small hospitals. An alternative approach is described in section 2 below.

This factor Xa inhibitory comparison method implies that all clinicians will change their behavior, that is, adjust their therapeutic interval for the aPTT, with each change of reagent in the laboratory. Changing the behavior of a diverse group of clinicians can be difficult.

2. Validation of heparin sensitivity of the aPTT using ex vivo heparin specimens: Comparison with an existing, validated aPTT reagent:

The goal of this method is for the laboratory to select a reagent that has the same (or nearly the same) heparin responsiveness as the one currently in use. By doing so, the clinician behavior need not change. Of importance is to control for, and prevention of, drift with multiple changes over time.

Below is described a method for reagent evaluation that will identify reagents that are sufficiently similar for clinical purposes. It uses the cumulative summation method to control for drift. The method is as follows:

 Accumulating patient specimens. There are no data to dictate the number of patient specimens that should be used to perform comparative testing of two aPTT reagents on ex vivo heparin specimens. In the examples provided in the table, more than 30 specimens were used. No more than two specimens from a single patient were included in this study. The specimens can be collected prior to the time that the new reagent will be evaluated in the laboratory. They are carefully centrifuged to remove all platelets (< 10 x 10³/µL), and frozen (-20°C to -70°C) in aliquots for future aPTT reagent comparisons. Doing so should not be a particular burden, even for the smallest laboratory.

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- Selecting a new aPTT reagent to test. Laboratories can obtain reagents from suppliers, with responsiveness
 to heparin that would be predicted to be similar to the reagent currently being used. This depends on the
 manufacturers knowing the heparin sensitivities of their aPTT reagents so they can supply a reagent with similar
 sensitivity to heparin when requested.
- **Comparison testing.** Once a potential replacement reagent has been selected for testing, split specimens can be used to perform the aPTT using each of the two reagents on the instrument(s) used in production. The comparison data are plotted with the old reagent on the x-axis and new on the y-axis. Visual or regression analysis can be used to judge the acceptability of the comparison data and to identify discrepant and outlier results. The data for each aPTT reagent are summed and the mean and standard deviation determined. The difference between the means of the new and old aPTT reagents are then recorded for future reference.
- A cumulative summation of differences. Each time there is a change in reagents or instrument, comparison testing should be performed. In addition to recording the difference in the mean, the laboratory should prepare a cumulative summation of the differences that have occurred in the past. In doing so, the cumulative shift in the reagent performance in the presence of heparin can be determined. A difference between reagent means or a cumulative change of more than seven seconds is reason for concern and requires action. Suitable actions might include evaluating a different reagent to find one with an acceptable level of variation, informing all clinicians using heparin of the change in the therapeutic interval and recommending that they change their thresholds, or reverifying the aPTT using comparison with heparin concentration.

As one begins this process it is extremely important to keep in mind that different lots of reagent of the same "name" from the same manufacturer usually have sensitivities to heparin that differ from those of prior lot numbers. Sometimes these differences are substantial and can be either intentional or unintentional on the part of the manufacturer.

Number	Mean Old Lot (Sec.)	Mean New Lot (Sec.)	Difference New-Old (Sec.)	CumSum (Sec.)	Action
1 (2001)	78.6 (1)	73.9 (2)	-4.7	-4.7	Accept
2a (2002)	48.3	41.7	-6.6	-11.3	Reject
2b (2002)	47.6 (2)	53.6 (3)	+6.0	+1.3	Accept
3 (2003)	71.9 (3)	72.3 (4)	+0.4	+1.7	Accept
4a (2004)	62.0	71.2	+9.2	+10.9	Reject
4b (2004)	62.8 (4)	60.3 (5)	-2.5	-0.8	Accept

Cumulative summation of reagent mean differences

Patient data set (new reagent evaluations in sequence)

Comparisons are made within patient data sets, new and old reagents, not between data sets.

In the example shown in the table, there were four changes involving the evaluation of six reagents. The protocol described above was used to generate all of the data points. Different sets of patient specimens were analyzed, with each reagent change explaining the variation of mean values for each patient data set. The reagents tested in numbers 2 and 4 are examples of reagents that have unacceptable variability (reagent 2a has a CumSum that is above seven, which is too high despite the differences in the mean being less than seven seconds; reagent 4a has

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a difference in the mean that is greater than seven seconds). As noted in the column to the far right of the table, both of these reagents were rejected, and in both cases different reagents (2b and 4b respectively) were tested and the problem resolved.

It is of interest that the cumulative summation has remained within a range that has not made it necessary to inform clinicians of the need to change the therapeutic range for the aPTT. Using aPTT reagents from a single manufacturer facilitates the simplicity of the method. In addition, this method can be applied even if there is an instrument change.

3. Validation of heparin sensitivity of the aPTT using instrument recalibration:

The principle of this method is that some instruments can be adjusted (calibrated) to make the response of a new reagent match the response of the prior reagent. Using this method, the variability of the reagents to heparin can be compensated for by adjusting the response of the instrument itself. Most instruments do not have this capability, but for those that do, this method can work well.

Laboratories that recommend that the aPTT be used to monitor unfractionated heparin therapy must determine the sensitivity of the method to heparin, inform the clinicians of the sensitivity, and recommend a therapeutic interval. This must be done each time there is a change in the method.

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