PART 15 Troubleshooting Issues with Coagulation laboratory tests

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TOPICS COVERED

- ✓ Unexpected Results on an Individual Test Sample
- ✓ Out of Range IQC Results
- ✓ How to Investigate Out-of-Consensus Results in External Quality Assessment Surveys

Unexpected Results on an Individual Test Sample: Problems related to coagulation testing occur in all coagulation laboratories irrespective of which methods reagents and equipment are in use. Inaccurate results can occur as a consequence of issues with a particular sample due to issues with the sample itself. This can be a consequence of inadequate sample collection processing or storage prior to analysis. These issues are discussed in Part 3 of this manual. Recommendations on controlling preanalytical variables are also available, specifically related to hemophilia and allied disorders (Kitchen et al, 2020), and in relation to sample collection (Kitchen et al, 2021a) and processing (Kitchen et al, 2021b) in all aspects of coagulation laboratory testing, and are not discussed further here. Specific issues can occur during the analysis of a sample which relate to the reagent or sample handling during that specific test, despite successful analysis of adjacent samples just before or just after the sample with a questionable result. Many analyzers use a probe to automatically aspirate samples and such probes sometimes descend until detection of a liquid and then aspirate a suitable test sample volume to complete the testing. If that test sample has bubbles on the surface, this may lead to mis-sampling with inadequate volume, following be falsely abnormal results such as falsely prolonged screening tests results or falsely low activity in calibrated assays. Inaccurate pipetting of reagents during analysis can also lead to inaccurate results, for example, if a probe used for reagent movement in an analyzer has moved out of alignment. This would likely affect multiple sample results. It may be useful to run 10 replicates of the same sample to assess the precision of results, which is normally compromised if probe misalignment has occurred. Reagent areas on analyzers are usually maintained at a constant temperature, often chilled below ambient temperature but with reaction mixtures warmed to 37°C during analysis. Most coagulation test results are highly temperature dependent, therefore any drift outside a narrow acceptable range in the cooling of regents or in particular heating of reaction mixtures, can cause inaccurate results which are generally falsely abnormal. False normal results are much rarer than false abnormal ones, and are only regularly seen in relation to the false shortening of APTT as a consequence of pre-analytical issues such as in vitro hemolysis. Fully automated coagulometers often utilize wash/cleaning solutions that rinse through probes in between successive pipetting operations. This process normally avoids carryover of sample or reagent from one test into the following reaction mix, but such events have occurred. For example, partial sample carryover into a following sample reaction mixture has occurred in the past in relation to pathological components in the sample such as anti-phospholipid antibodies or paraproteins, or related to therapeutic drugs, including emicizumab, which can then cause false shortening of APTT in the following sample. Reagent components such as heparin neutralizer in reagents have carried over into following samples, which could lead to loss of heparin activity if present in the following sample. Reagent carryover effects have been largely eradicated when reagents and instruments from the same manufacturer are combined. This is more likely to be an issue if a reagent from one manufacturer is used on an analyzer from a different manufacturer, if that combination has not been validated for use. It is critical that laboratories follow diagnostic company guidance on permitted intervals between preventative maintenance visits to minimize risks of generating patient sample results that cannot be safely released for patient management decisions. It is important that laboratories receive sufficient patient/clinical information as is needed for experienced laboratory

staff to identify unexpected or unusual test results wherever possible. Any unexpected test result should be reanalyzed to exclude the possibility of analytical error. Where analytical error is excluded as an explanation for an unexpected result that does not seem to fit the patient's clinical picture, a repeat sample should be obtained for confirmation of the result.

Out-of-Range IQC Results: As described in Part 2 of this manual, it is convenient to keep a record of IQC results on each IQC material in the form of a chart. Many analyzers use the Levey-Jennings approach as shown in Figure 23. There are IQC systems available to assist laboratories in troubleshooting issues, such as the Westgard rules (www.westgard.com) which use 5 different control rules. These have few false alarms and give confidence in error detection. On the other hand, the patterns of IQC testing required for effective use of such systems are not well suited to regular use in most coagulation laboratories. The potential clinical consequences of laboratory error in management of patients with hemophilia and allied disorders, means a cautious approach to out-of-range IQC results is safer for patients. For this reason, any out-of-range IQC should be assessed and during investigation testing and reporting of patient results should be suspended. It is useful to identify the case of an out-of-limits IQC to help avoid future delays in processing samples. If a retest on the same vial of QC material generates a result which is clearly in range, then there may be an analyzer issue which can be assessed by performing 10 replicates on the same test sample. An analyzer issue is indicated if there is wide variability amongst the replicates. More often in routine coagulation testing, a repeat test on the same material is again out of range, and replacement of the material with a new vial or aliquot generates an in-range result, confirming that the IQC material itself was the source of the problem. In this case, patient results are safe to be released. If, on the other hand, testing a new vial/aliquot of IQC also generates a similar out-of-range result, there is an issue with the test system that would also impact patient results. In this case, the reagents used for the test should be replaced in sequence with a new IQC after each replacement of a reagent. Once a replacement reagent leads to an in- range IQC result, that reagent is identified as the source of the problem. This should be noted in IQC records so that patterns can be identified and a new assessment of reagent stability and use initiated. If replacement of all relevant reagents is still associated with out-of-range results, then that analyzer should be withdrawn from use pending review by the manufacturer and the laboratory should switch to a backup, ideally an alternative coagulometer giving the same results or, for tests with clotting endpoints, a manual technique (see Part 4 of this manual). Any patient results obtained since the previous in-range IQC result should be reviewed with repeat testing to establish where in the sequence of sample testing the problem may have commenced. If patient results from samples analyzed after the previous in-range IQC have been released, the laboratory should retest and recall any results that are not conformed, and should also review its IQC testing protocols since adequate IQC testing should avoid the necessity to recall any patient results whatsoever. Figure 23 shows the Levey-Jennings plot of APTT IQC results. The dotted red lines show the upper and lower limits of the acceptable range for this material. The first series of results are inside the range other than one on the lower limit. The second section shows a gradual and progressive increase in the clotting times. This trend occurs if one component of the test gradually changes over time. This is unlikely to occur in relation to commercial lyophilized IQC material stored according to manufacturer's instructions, but can occur if the IQC material has been locally prepared as described in Part 2. Alternative causes could be a gradual change in one of the reagents if not stored appropriately or by a gradual deterioration in some aspect of the endpoint detection system used (e.g. a deterioration in light source of a photo optical system). These types of problem are rare with modern automated coagulometers.

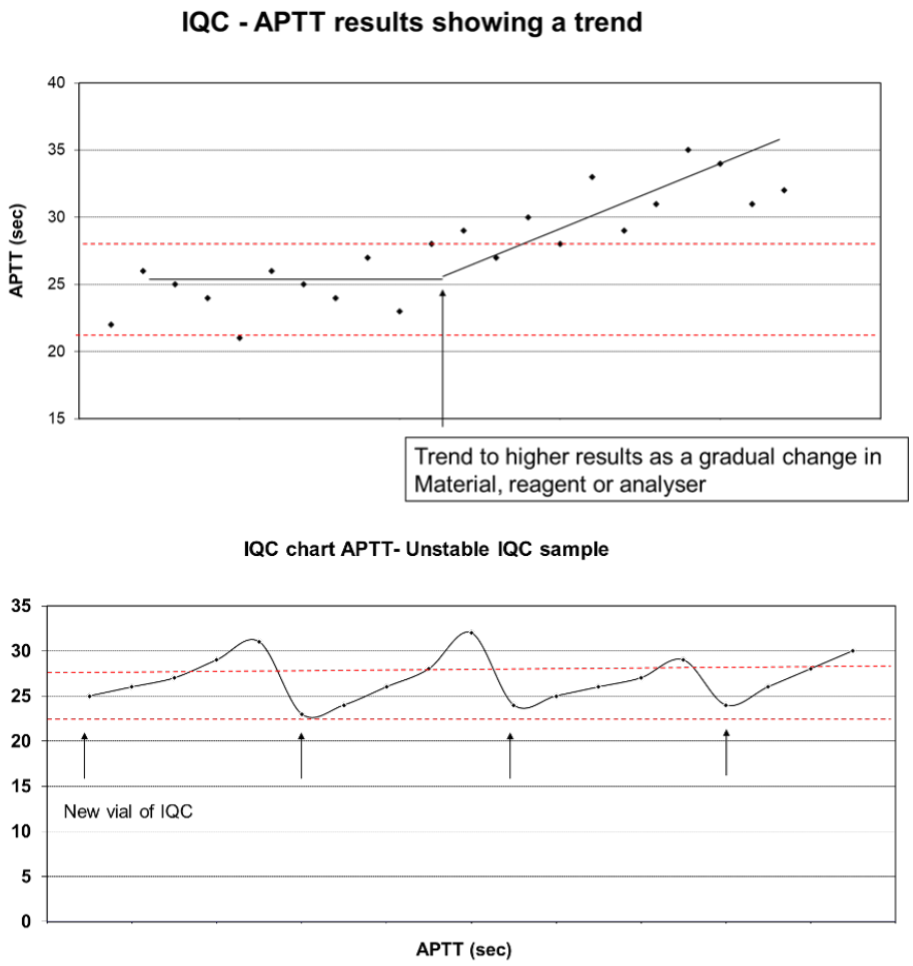

Figure 23. Levey-Jennings plot of APTT results on an unstable IQC material. The dotted red lines show the upper and lower limits of the acceptable range for this material. Each solid black arrow indicates when a new vial of IQC was loaded on to the analyzer. For each new vial there is a gradual increase in APTT over time. This example occurred because of a locally prepared frozen IQC material that was unstable after thawing. This can in principle also occur after reconstitution of lyophilized samples if not properly prepared or if the water used to reconstitute is contaminated.

How to Investigate Out-of-Consensus Results in External Quality Assessment Surveys: Participation in proficiency testing or EQA is an essential requirement for a laboratory to ensure it produces accurate results. Accreditation bodies who assess against ISO standards such as ISO 15189 (2022) require this for any tests where EQA is available. There is an IEQAS focused on hemophilia and allied disorders overseen by the WFH (see Part 2 of this manual). Results obtained in EQA exercises can be used to identify important issues related to the precision and accuracy of coagulation laboratory tests, provided the test material in the EQA program is commutable with patient samples (i.e. behaves in the same way in a particular method as patient samples would). Effective troubleshooting of EQA results that are not within the consensus of results in other centers, is important to ensure safe patient management. When considering outlying EQA results, there are a number of things that should be considered. A local result outside the target derived from the results in other centers, is less of a concern if the difference is not large enough to impact patient management. A clinically significant difference is much more of a problem than a statistical difference that would not be predicted to alter diagnosis or management of a patient.

A single result that is markedly different from the mean or median of results in other centers, to the extent that patient management would be affected had the sample been from a patient the laboratory, should be investigated further. This could include the following:

i) Checking that the sample was stored correctly on receipt, reconstituted correctly, and the test performed according to the written procedure for that method. If the problem was thought to be restricted to analysis of the EQA sample, then patient results would be unaffected.

ii) Checking that the internal QC at the time the EQA test was performed was satisfactory. If not, there was likely an issue with testing that could have affected patient results and patients results should be reviewed.

iii) Consideration of the particular details of the test sample. If abnormal, then the results obtained could be a consequence of the particular defect in the EQA sample.

When a laboratory has outlying results in consecutive surveys that occur over a number of surveys, investigation is needed and should take account of the pattern in the relationship between local results and the mean or median of results in other centers using similar methodology. In addition to the investigations after a single outlying result mentioned above, the following should be considered:

i) The clinical impact of the results should be evaluated. It is possible that, for an assay with very good precision, a laboratory can be persistently out of consensus, but still record results relatively close to the target, with no clinical consequences. It is also possible that if a locally determined reference range is employed, any bias in patient results is compensated by an appropriate reference range.

ii) Results which are consistently reading high or consistently reading low compared to the mean or median result in other centers, is often related to calibration. Typically, labs with such problems have a calibration curve established sometime in the past which is not appropriate for current testing, either because of a change in lot number of an assay component or because day to day variability in test results requires a fresh calibration alongside analysis of test samples. Recalibration typically resolves this issue in centers that are using a historical calibration curve. The possibility that an inappropriate potency has been assigned to the calibrator, although rare, should be considered. When investigating the possibility of a problem related to calibration, it can be useful to analyze a sample with an independently assigned value as a test sample to check how much the results of test samples are being over- or under-estimated. Such a material can also be used to perform a new calibration. The WFH IEQAS program has permission to supply a vial of the ISTH SSC plasma standard for this type of troubleshooting investigation. This has assigned values for a number of different coagulation parameters. The effect of a new calibration can be assessed by analyzing a small group of test samples before and after the new calibration. The SSC plasma standard is not available for routine use in calibration of local assay methods.

iii) Results above the mean or median in some surveys, and below the mean or median in others, suggest imprecision of the assay. This can occur as a consequence of poorly maintained instruments, inadequate reagent handling (i.e. reconstitution or storage), reagent instability, or issues related to staff training or competence.

Where possible, analysis of repeat samples after completion of investigation and after making any necessary improvements, is useful to confirming the success or otherwise of interventions. Retrospective review of past EQA survey results, prior to the problem of outlying results occurring, should be done alongside laboratory records of lot changes, calibrations, instrument service, and changes in methodology, to assess if the change in performance corresponds to any relevant internal changes. EQA data analysis and performance reports are by nature retrospective since analysis and reporting normally take place some time

after tests were performed in the laboratory. Therefore, any problem identified may have affected patient results over the same period of time. The laboratory should review with clinicians the past patient results for any tests where EQA indicates a clinically relevant inaccuracy could have been present. The review should consider whether any patient diagnosis or management could have been adversely affected. Retesting may be needed if the pattern of outlying results could have impacted patients adversely.

References

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