Technical Note

Development and Implementation of a Coagulation Factor Testing Method Utilizing Autoverification in a High-volume Clinical Reference Laboratory Environment

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Received: 12 November 2016 Accepted: 28 March 2017 Published: 19 June 2017

Abstract

Background: Testing coagulation factor activities requires that multiple dilutions be assayed and analyzed to produce a single result. The slope of the line created by plotting measured factor concentration against sample dilution is evaluated to discern the presence of inhibitors giving rise to nonparallelism. Moreover, samples producing results on initial dilution falling outside the analytic measurement range of the assay must be tested at additional dilutions to produce reportable results. **Methods:** The complexity of this process has motivated a large clinical reference laboratory to develop advanced computer algorithms with automated reflex testing rules to complete coagulation factor analysis. A method was developed for autoverification of coagulation factor activity using expert rules developed with on an off the shelf commercially available data manager system integrated into an automated coagulation platform. **Results:** Here, we present an approach allowing for the autoverification and reporting of factor activity results with greatly diminished technologist effort. **Conclusions:** To the best of our knowledge, this is the first report of its kind providing a detailed procedure for implementation of autoverification expert rules as applied to coagulation factor activity testing. Advantages of this system include ease of training for new operators, minimization of technologist time spent, reduction of staff fatigue, minimization of unnecessary reflex tests, optimization of turnaround time, and assurance of the consistency of the testing and reporting process.

Keywords: Anticoagulants, autoverification, coagulation factor inhibitors, coagulation factors, coagulation testing, compliance, data management, expert rules, factor parallelism, hemostasis, laboratory automation, lupus anticoagulants, middleware, preanalytics, thrombosis

Introduction

Coagulation factor activity testing is performed as an aid in the investigation of various clinical disease states related to bleeding and hypercoagulability.[1,2] In particular, this testing supports the diagnosis of factor deficiencies present in hereditary and acquired hemophilia as well as aiding in the diagnosis of hepatic disorders, nutritional deficiencies of Vitamin K, warfarin treatment, consumptive coagulopathies, and von Willebrand Disease. In addition, factor activity testing provides information about elevations of factors as may be present in thromboembolic complications, coronary atherosclerosis, renal failure, diabetes, and inflammatory syndromes.

There are eight factor activity assays commonly performed using one-stage methodology: prothrombin (factor II, [FII]), FV, FVII, FVIII, FIX, FX, FXI, and FXII. The underlying principle of all functional clotting factor assays involves measuring the clotting time when the test plasma is added to a clotting system deficient in the specific clotting factor to be measured.[1,2] The degree of correction of the clotting time correlates to the factor activity in the test plasma. These assays are calibrated against dilutions of reference plasmas.[1,2]

Good laboratory practice, Clinical Laboratory Standards Institute (CLSI), and the College of American Pathologists (CAP)

Access this article online

Quick Response Code:

Website: www.jpathinformatics.org

DOI: 10.4103/jpi.jpi_95_16

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How to cite this article: Riley PW, Gallea B, Valcour A. Development and implementation of a coagulation factor testing method utilizing autoverification in a high-volume clinical reference laboratory environment. J Pathol Inform 2017;8:25.

Available FREE in open access from: http://www.jpathinformatics.org/text.asp?2017/8/1/25/208471

Hematology checklist stipulate that at least three dilutions of test plasma in buffer or factor deficient plasma should be tested.[3,4] Further, dilutions are measured when factor levels fall outside of the analytical measurement range of the assay, defined as the limited calibration range tested. Reporting the average of three dilutions improves accuracy and allows for detection of inhibitors. A graphical representation of a typical calibration curve and multiple dilutions of a patient sample are displayed in Figure 1. In this graph, the concentration of undiluted calibrator is defined as 100%. A straight, horizontal line with a y-axis value of 100% reflects the linear regression of the dilution-corrected calibrator activities versus their corresponding dilution. A second line, parallel to the first one, represents the linear regression of the dilution-corrected patient activities versus their corresponding dilutions.

The presence of a factor inhibitor (either specific or nonspecific) in patient samples results in underrecovery of factor levels in clot-based assays. The extent to which inhibitors diminish the measured activity is dependent on inhibitor strength and on the sensitivity of the reagent/instrument combination used. Dilution of the patient sample in the presence of a nonspecific inhibitor tends to diminish the extent of inhibition. This phenomenon (referred to as nonparallelism) causes the slope of the line defined by the dilution-corrected patient activities versus dilution to be different from the slope of a similar line produced the calibrator which is devoid of inhibitor.[1,2,5,6] This phenomenon is displayed graphically in Figure 2. Specific factor inhibitors do not demonstrate nonparallelism in the factor activity assay to which the inhibitor is directed.

The definition of nonparallelism may vary between laboratories and reagent/instrument systems. For example, a laboratory may stipulate that the dilution-corrected results must agree within a specified tolerance for the average of all three results to be reported. When nonparallelism is observed, higher dilutions may be tested in an effort to dilute out the inhibitor effect. If further consecutive dilutions produce a correction of the nonparallelism (results matching within the specified threshold) the highest activity value obtained should be reported with a comment about the presence of a nonspecific inhibitor made in the laboratory report.

The results produced by the laboratory must be transmitted to the laboratory information system (LIS) for delivery to the physician. Modern laboratories employ data manager software systems (also known as middleware) to expedite this process. The middleware can provide additional functionality such as insertion of interpretive comments, ordering of additional tests, and display and storage of quality control (QC) results. Good laboratory practice requires that all test results be reviewed before release to the physician. Reportable results must be associated with acceptable QCs and an absence or review of instrument performance flags. Middleware can enable the laboratory to employ Boolean logic statements to review test results before release. This software-mediated review and release of results, also referred to as autoverification, provides an efficient process that serves to reduce technician time and standardize result reporting.

Technical Background

Prospective validation of the expert rules was accomplished using 552 different anonymized plasma samples, with each sample taken through all pathways of the expert rules system and no failures of parallel versus nonparallel differentiation found. The samples were from patients diagnosed with moderate or severe FVIII deficiencies, including seven with nonspecific inhibitors and 25 with preanalytical or other sample integrity issues. These samples were each tested at 1:10, 1:20, and 1:40 dilutions giving a total of 1656 individual results on an STA-R Evolution automated coagulation analyzer using the using the STA Coag Expert data manager system. All dilutions were performed in an automated fashion on the

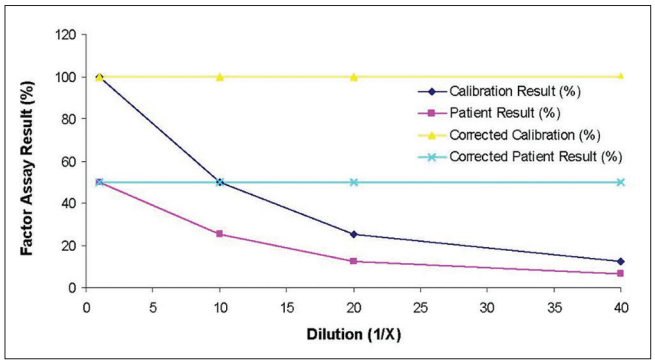

Figure 1: Calibration and hemophilia patient curves before and after correction by multidilution management criteria. Blue lines with filled diamond symbols: Uncorrected results of calibrator plasma samples. Pink lines with filled square symbols: Uncorrected results of hemophilia patient plasma samples. Yellow lines with filled triangle symbols: Results of calibrator plasma samples after dilution factor correction. Light blue lines with X symbols: Results of patient plasma samples after dilution factor correction

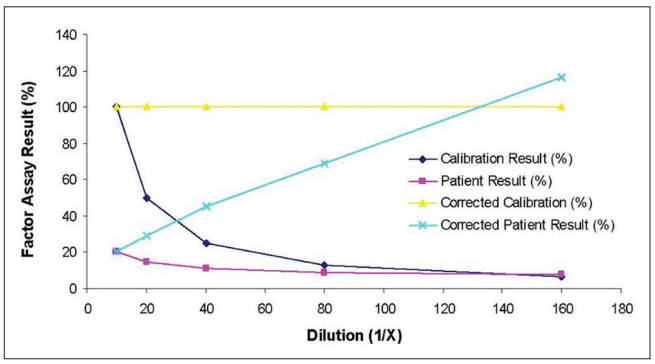

Figure 2: Calibration and nonspecific inhibitor patient curves before and after correction by multidilution management criteria. Blue lines with filled diamond symbols: Uncorrected results of calibrator plasma samples. Pink lines with filled square symbols: Uncorrected results of hemophilia patient plasma samples. Yellow lines with filled triangle symbols: Results of calibrator plasma samples after dilution factor correction. Light blue lines with X symbols: Results of patient plasma samples after dilution factor correction

STA-R Evolution. Reagents used for testing included the STA-PTT A as the activated partial thromboplastin time (aPTT) reagent along with the STA-Deficient VIII immunodepleted plasma following automated programming of standard FVIII testing procedures as supplied by the manufacturer into expert rules format (Diagnostica Stago Inc., Parsippany, NJ, USA). All expert rules programming and validation were performed by the manufacturer, with all rules implemented directly in the STA Coag Expert data manager. The calibrator used was the STA – Unicalibrator and the QC material used was the STA-System Control N + P. STA-$CaCl_2$ 0.025 M reagent was used to recalcify the samples and STA – Owren-Koller was used as a diluent for the reagents and plasma samples.

The results of FVIII testing were classified into four ranges requiring different expert rule strategies: very low result (<5%), low result (5%–15%), "standard" results (15%–100%), and high results (>100%). Classification of the results in this manner allowed for the determination of five relevant ratio-based calculations to reproducibly analyze the factor assay results. The results determined by the algorithm were checked against the results as determined from the previously used standard operating procedures not incorporating the expert rules. The procedure incorporating the expert rules was able to classify the patient results in the same manner as the methods previously used but in a more standardized and automated fashion. Thus, the development of the coagulation factor testing expert rules followed a very robust, prospective path using patient samples as described above, reflecting the real-world conditions typical of the daily reference laboratory workload.

Factor Expert Rules

The testing cascade and rules for factor activity assays that were programmed into the STA Coag Expert data manager are displayed in Figure 3. Starting from the top left point of the flow chart, where the test is ordered through the LIS (factor assay ordered); initial testing at three different dilutions (1:10, 1:20, and 1:40) is performed. The mean of these three dilutions is used to determine the next step. If the mean is less than 5%, the sample is retested with a low curve, and the result reported from the low curve, and results with a mean below 5% are not tested for nonparallelism [top branch of Figure 3]. If the mean is between 5% and 15%, the 1:40 dilution is ignored, and the 1:10 and 1:20 dilutions are compared for parallelism. Nonparallelism is ruled out by comparing the 1:10 and 1:20 results to the mean of the two dilutions [second branch from the top of Figure 3]. If an individual dilution result is beyond 15% of the mean, a reflex test is run using a 1:5 dilution. If the results meet the parallelism criteria (defined as the results from each dilution falling within 15% of the mean of all two or three results), the reportable result is calculated as the average of results generated from the 1:10 and 1:20 dilutions. The CAP Hematology checklist recommends that individual results of each dilution performed for a coagulation factor assay agree within 20% agreement to be considered parallel.[3] Thus, although a 20% threshold could have been used, a more discriminative 15% threshold is arbitrarily utilized based on the expected reproducibility of duplicate testing observed empirically by the Center for Esoteric Testing (CET) staff before the expert rules had been implemented. If the results fail to meet the parallelism criteria, as described by the multidilution management criteria above, a 1:5 dilution is tested. The 1:20 dilution is ignored and the 1:10 and 1:5 dilutions are tested for nonparallelism by comparing the 1:5 and 1:10 results to the mean of the two dilutions. If the individual results are beyond 15% of the mean, the results are considered nonparallel. If the results meet the parallelism criteria, the result of the 1:5 dilution is reported.

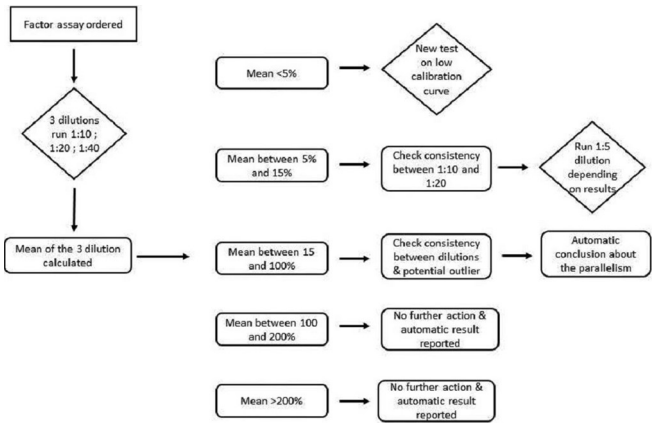

Figure 3: Factor expert rules flow chart. Solid diamonds: Starting point when factor assay is ordered. Solid rectangles: Automated factor assays run as a result of decision points. Rounded rectangles: End-point with assay result reported automatically

If the mean is between 15% and 100%, and the results meet the parallelism criteria as described by the multidilution management criteria described above, the mean of the three dilutions is reported [middle branch of Figure 3]. If the mean is between 100% and 200%, the mean of the 1:20 and 1:40 dilutions is reported [second branch from bottom of Figure 3]. If the mean is greater than 200%, the result of the 1:40 dilution is reported [bottom branch of Figure 3].

Conclusion

Expert rules utilizing autoverification procedures can be applied on the instrument, in the data manager software, or in the LIS. When properly implemented, these expert rules can (1) handle the mundane and error prone task of result verification, allowing medical technologists to focus on the true problem samples, (2) improve the consistency in the quality of test results, and (3) reduce staff fatigue, improving the work environment in the process. Thus, autoverification can produce great efficiencies in laboratory operations and also improve the consistency of test reporting.

Although other studies have been reported on autoverification practices with respect to other commonly run tests in the clinical laboratory, including hepatitis B virus ELISAs,[7] thyroid function profiles,[8] as well as for clinical chemistry

panels,[9,10] we are only aware of two other papers discussing autoverification for coagulation applications.[11,12] In the papers focusing on coagulation applications, autoverification practices are featured with regard to routine coagulation parameters, including prothrombin time (PT), aPTT, and fibrinogen[12] and PT, aPTT, fibrinogen, D-dimer, and AT.[11] However, the work reported here constitutes a novel contribution as it is the first study reporting expert rule and autoverification methods for coagulation factor testing developed in compliance with the CLSI AUTO-10A guideline.[13]

By utilization of the coagulation factor testing expert rules described here, the sample results easily obtained by automated implementation of the rules can allow users to concentrate on inconclusive specimens and results requiring greater levels of attention. Test results utilizing the rules are approved and released automatically in a consistent, standardized manner, allowing for significant decreases in overall turn-around-time compared to standard factor testing processes not employing autoverification procedures. The expert rules were based on the manual practice of the laboratory before implementation of the program. Creating expert rules within the STA Coag Expert data manager system improved the efficiency and consistency of application of the rules. In terms of quantifiable labor savings, the CET staff estimated that implementation of the expert rules has reduced overall labor expenditure by 2%–4%. Given the varied test menu and high workload in the CET, saving labor expenditures by implementation of expert rules for the coagulation factor testing represents a key institutional win.

The expert rules allow for reduced user intervention and nonstandardized judgments, resulting in higher efficiencies, and improved quality of results in a variety of laboratory settings and within a range of institutions. Thus, even though the expert rules described here have been developed for a high volume setting, they still will have a significant benefit in a lower volume setting where staffing levels and specialization challenges are even more prevalent.

In order for readers to be able to implement the complete expert rules algorithms in a local laboratory setting, please contact the corresponding author for a copy of the expert rules.

Acknowledgments

We wish to acknowledge current and former members of the LabCorp CET laboratory who helped to validate and implement the reported method, especially the late Marshall Thompson, who worked diligently to develop the expert rules. These rules are a testament to his legacy as a clinical laboratory scientist. In addition, we acknowledge Magalie Lubineau from Diagnostica Stago, who assisted with information and figures. Last, we acknowledge Dorothy M. (Adcock) Funk who greatly assisted with proofreading and editing.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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