Article published online: 2023-11-15

International Council for Standardization in Haematology Guidance for New Lot Verification of Coagulation Reagents, Calibrators, and Controls

Robert C. Gosselin, CLS[1] Donna Castellone, MS, MASCP, MT (ASCP) SH[2] Akbar Dorgalaleh, PhD[3] Kieron Hickey[4] Giuseppe Lippi, MD[5] Karen Moffat, BEd, MSc, ART, FCSMLS(D)[6] Rebecca O'Toole, MSc[7] Joe Rigano, BAppSci (MLS) (Dis), FAIMS[8]

[1] CLS Hemostasis and Thrombosis Center, University of California, Davis Health System, Sacramento, California
[2] New York Presbyterian Hospital—Columbia, New York, New York
[3] Hamin Pazhuhan Tis Institute, Tehran, Iran
[4] Sheffield Laboratory Medicine, Royal Hallamshire Hospital, Sheffield, United Kingdom
[5] Section of Clinical Biochemistry and School of Medicine, University of Verona, Verona, Italy
[6] Department of Pathology and Molecular Medicine, McMaster University, Hamilton Ontario, Canada
[7] Hematology Department, Wellington Hospital and Health Care, Wellington, New Zealand
[8] CCS Northern Pathology Victoria, Melbourne, Australia

Address for correspondence Robert C. Gosselin, CLS, 1201 8th Avenue, Sacramento, CA 95818 (e-mail: rcgosselin@outlook.com).

Semin Thromb Hemost

Abstract

The clinical laboratory uses commercial products with limited shelf life or certain expiry dates requiring frequent lot changes. Prior to implementation for clinical use, laboratories should determine the performance of the new reagent lot to ensure that there is no significant shift in reagent performance or reporting of patient data. This guideline has been written on behalf of the International Council for Standardization in Haematology (ICSH) to provide the framework and provisional guidance for clinical laboratories for evaluating and verifying the performance of new lot reagents used for coagulation testing. These ICSH Working Party consensus recommendations are based on good laboratory practice, regulatory recommendations, evidence emerged from scientific publications, and expert opinion and are meant to supplement regional standards, regulations, or requirements.

Keywords
- new lot reagent verification
- coagulation testing
- laboratory

The clinical laboratory uses commercial products with limited shelf life or certain expiry dates. As such, laboratories constantly need to change reagents, a process which is usually transparent to health care providers. Reagent lot-to-lot variation can cause a significant analytical error, with changes that may generate a significant shift in patient data. The Clinical and Laboratory Standards Institute (CLSI) document for reagent lot verification of performance is primarily targeted for clinical chemistry tests, which have more robust and standardized performance characteristics than most hemostasis-based assays.[1] Other guidance for laboratories includes documents provided by international regulatory organizations such as the International Organization for Standardization (ISO) 15189 document[2,3] or regional regulatory agencies such as the Food

Issue Theme Laboratory diagnostics for thrombosis and hemostasis testing – Part III; Guest Editors: Kristi J. Smock, MD, Karen A. Moffat, BEd, MSc, ART, FCSMLS(D)

DOI https://doi.org/10.1055/s-0043-1776405.
ISSN 0094-6176.

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

and Drug Administration (FDA)[4] and the Centers for Medicare and Medicaid Services[5] in the United States. However, these regulatory agencies provide little details or recommendations about how to perform new lot verification of reagents. ISO 15189 (section 5.3.2.3 Reagents and consumables) states that "Each new formulation of examination kits with changes in reagents or procedure, or a new lot or shipment, shall be verified for performance before use, or before release of results, as appropriate. Consumables that can affect the quality of examinations shall be verified for performance before placing into use.."[2] In 2018, the FDA updated their guidance document for industry, noting that "partial validation" can be used to "evaluate modifications of already validated bioanalytical methods" and can be limited to assessing a single intra-assay accuracy and precision evaluation although recognizing "nearly full validation" may also be suitable.[4] The Clinical and Laboratory Improvement Amendments (CLIA, section §493.1255 Standard: Calibration and calibration verification procedures) specifies calibration verification procedures if a "complete change of reagents for a procedure is introduced, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes." but also indicates (section §493.1256: Standard: Control procedures) to "Perform control material testing as specified in this paragraph before resuming patient testing when a complete change of reagents is introduced…."[5] No other laboratory guidance related to reagent changes made in a coagulation laboratory is addressed, including reagents or test platforms that are not calibrated. The laboratory accrediting agency College of American Pathologists (CAP) require "New reagent lots and shipments are checked against old reagent lots or with suitable reference material before or concurrently with being placed in service."[6] providing additional guidance for acceptable samples or material used for quantitative and qualitative tests. However, this guidance is limited to recommendations for a number of samples to be tested, statistical acceptability, or other requirements. CAP has additional requirements for the confirmation of specific methodology calibrations for verifying the analytical measurement range (AMR), which will be described later.

In the clinical coagulation laboratory, the commonly used testing principles are either chronometric (clot based), chromogenic, immunologic, or latex particle agglutination/aggregation.[7] Coagulation test reagents may be either (1) reagents for assays that do not require calibration (e.g., activated partial thromboplastin time [aPTT] or lupus anticoagulant [LAC] screen), with results reported in raw units (e.g., seconds); (2) reagents for tests that require calibration (e.g., D-dimer or coagulation factor assays), with results reported as quantitative values usually determined from a calibration curve (e.g., IU/dL); (3) reagents for assays where a reported result is either qualitative (i.e., fibrin monomer), semiquantitative (i.e., some heparin-induced thrombocytopenia assays), or used as an ancillary reagent (normal pooled plasma, NPP); (4) internal quality control (IQC) that may have assigned test values (and acceptable result ranges) or require local determination of target mean and ranges, and (5) calibrator materials with assigned values. Molecular diagnostics or flow cytometry are beyond the scope of this document and will not be addressed.

A particular challenge for hemostasis laboratories is the verification of performance for new lots of prothrombin time (PT) or aPTT reagents, as these tests are often the first-line investigations performed on patients with query hemostatic challenges. They have multiple indications for use, including screening for clotting factor deficiencies and measuring the efficacy of treatment, which may be either anticoagulation or replacement therapy. Presumably, tests that require calibration, such as factor assays, may mitigate any biases associated with the variability of new reagent lots, especially if the same calibrator material is used for multiple lots of reagents. However, even with calibrated tests, the instrument limitations for thresholds (e.g., lower limit of quantitation) may fluctuate between reagent lots. Other potential variables that may introduce the risk of introducing testing bias, such as poor instrument precision, are beyond the scope of this document.

Regional and international regulatory agencies identify that the verification of new reagents should be performed, but little guidance is provided for laboratories.[2,3,5] The purpose of this document, from the International Council for Standardization in Haematology (ICSH), is to provide laboratory guidance for the verification of the performance of new reagent lots used for hemostasis testing. These ICSH Working Party consensus recommendations are based on good laboratory practice, regulatory recommendations, evidence emerged from scientific publications, and expert opinion. The resources (e.g., laboratory staffing, sample availability, and financial constraints) required for new lot reagent verification of performance will vary, and thus, we will provide tiered recommendations (minimal and optimal).

This document is not intended to supersede any national, regional, local, or institutional requirements. Laboratories that modify regulatory-approved reagents or reagents that are used outside their intended use may constitute a laboratory-developed test (LDT), which may have more stringent requirements for performance verification. The verification of performance for new reagents or instrument platforms has already been addressed in previous ICSH documents and, therefore, will not be addressed here.[8,9] If a new reagent supplied by the same manufacturer is introduced, this should be treated as a new method, needing more robust evaluation and performance verification.

New Lot Reagent Selection—General Guidance

The selection of new lot reagent material should be predicated on both laboratory needs and manufacturer capacity. Manufacturers generally follow their own described quality control procedures to ensure reagent performance. Nevertheless, the laboratory should have the option of choosing which lot may give optimal performance for their given patient population. With each new reagent batch, the instructions for use (IFU) should be reviewed for any changes to the performance claims

of reagents. Any such changes require evaluation by the laboratory and may impact lot-to-lot variation. Considerations for laboratories should include the expiry date and manufacturer inventory for providing the longest dating possible for clinical use before another new lot of reagents is required. For some automated analyzers, creating duplicate or shadow test protocols would allow laboratory professionals to program both existing lot reagents and new lot reagent tests concurrently on patient samples that would expedite method comparison and avoid potential bias associated with delays in new lot reagent testing. For laboratories with multiple analyzers, designating a single analyzer could be considered, with results from each analyzer correlated to a predicate device. Such an approach may ensure consistency across all analyzers and institutions. Given the variable ex-vivo stability of coagulation factors, treatments, etc., the comparison of new lot reagents to existing lot reagents should occur concurrently (within 1 hour).

Lastly, each laboratory should develop a written protocol, process, or plan for performance verification of new reagent lots. Testing of new lot reagents should be performed to mimic laboratory practice, preferentially encompassing different shifts and different testing personnel and laboratory environment, although this process may be unwieldy in networks with a vast number of testing sites and/or instruments. Creating a biorepository of normal and abnormal samples may expedite the evaluation process, although surrogate (contrived) samples and external quality assurance material can also be used. For hemostasis laboratories that provide an aPTT-based heparin therapeutic range (HTR), it may be prudent to coordinate with reagent manufacturer to evaluate two different reagent lots to avoid potential HTR changes, which will likely have downstream effects (e.g., heparin dose changing algorithms). This approach would provide a choice for selecting which lot to be selected based on recommended performance criteria for HTR determination.[10] The use of statistical methods (i.e., correlation, regression, and slope) is certainly appropriate, but whether differences between reagent lots are "clinically significant" may be a more important measure for patient care.[7] There is conflicting guidance as to the acceptability of simultaneous changing of IQC material with new lot reagents.[1,2] Whether to change IQC material concurrently with new lot reagents or modifying existing IQC thresholds with new lot reagents may be restricted by regional regulatory authorities. However, IQC testing (precision assessment) should be part of the new lot verification of performance, whether changed concurrently or not.

The local measurement of uncertainty is a valuable tool to use as a threshold for comparing differences between results on new and existing lot numbers. Results falling outside the acceptable limits may be significant as they exceed the normal day-to-day biological and analytical variation. These should be assessed on an individual basis, with regard to reference range cut-offs and clinical decision points.

General Recommendations

- New reagent lots based on the longest expiry date, especially for screening tests (PT, aPTT, LAC, and thrombin time [TT]), which may require more robust verification of performance should be selected.
- Appropriate PT and aPTT reagents with the consideration of PT ISI (International Sensitivity Index), factor sensitivity, LAC sensitivity or insensitivity, as well as heparin sensitivity should be determined.
- For automated analyzers, duplicate test protocol(s) should be generated to allow for concurrent analysis of new lots of reagents with the existing lot when testing patient samples.
- When more than one analyzer is regularly used in a single diagnostic laboratory for patient testing, verification should be performed across all instruments.
- For laboratories with multiple analyzers across multiple institutions, new reagent lots should preferably be verified on each analyzer.
  - A written plan for alternative strategies is recommended for larger networks where this recommendation for new reagent lot comparison between sites and instruments may not be feasible.
- The testing of new and existing lots of reagents should be performed concurrently (optimally, within 1 hour).
- Reagent verification of performance should be performed by at least two different testing personnel over at least 5 working days.
- Precision assessment should be part of the new lot verification of performance, whether IQC is changed concurrently or not.
- Given the fluctuations in throughput and temperature over the course of a day, patient comparison and internal QC testing should not be limited to specific times in the day; rather, it should preferably be representative of the operating times of a laboratory, standardizing any environmental influence.
- Each laboratory should develop a protocol, process, or plan for the performance of verification of new reagent lots that mimics laboratory practice and defines desired statistical outcomes.
  - The plan should identify the intended use of each test to ensure identification of the appropriate patient population and samples, reflective of a wide spectrum of conditions (e.g., mild-severe disease, anticoagulant status, etc.) that shall be used for comparison testing, also reflecting the performance characteristics of the reagents.
  - Samples should represent results across the reportable range, as well as samples from patients under specific conditions (LAC, factor deficient, heparin, liver disease, low fibrinogen, high fibrinogen, etc.).
  - Surrogate samples, including sample pools or contrived, can be used for new lot reagent comparisons. If testing is to be delayed, or cannot be performed within optimal time limits, frozen storage of plasma aliquots should be considered.
- New lots of more stable reagents such as buffers, diluents or calcium chloride, saline and water, and other consumables

that can affect the quality of examinations should be verified for performance before placing into use.2 Readership should consult regulatory authorities for clarification and local regulatory requirements.
- Testing of residual or banked external quality assurance (EQA) samples would be recommended if available and can be used to demonstrate new lot accuracy.
- Each laboratory must have a documentation system in place to address performance verification of new reagent lots. This system must have director or designate approval prior to use and shall be maintained in accordance with local accreditation or regulatory requirements. This system must include a general performance policy for reagent lot verification, including specific requirements for each measurand (or groups of assays by method) and new lot acceptability requirements.

Noncalibrated Coagulation Tests

There are several tests in the coagulation laboratory that are not calibrated, including, but not limited to, the PT with international normalized ratio (INR), aPTT, TT, reptilase time, dilute Russell's viper venom time, other clotting tests that are reported in seconds, platelet aggregation or function studies, or point-of-care devices such as the activated clotting time (ACT) or thromboelastography or rotational thromboelastometry. Tests that are not calibrated should be scrutinized for both the intended use and population at each testing site, to assure that proper patient populations are considered throughout the verification process. Tests such as the PT and aPTT have multiple clinical uses (clotting factor deficiency assessment, anticoagulant monitoring, or replacement therapy monitoring) and likely the complexity of the verification process will be secondary to the local intended use. Laboratories should incorporate into their evaluation a mix of abnormal samples (including surrogate samples when patient samples are unavailable) that reflect the expected patient population to be tested. Tests with limited or specific applications, such as LAC, may require specific patient populations for method comparison, and each laboratory should consider obtaining formal approval by accredited bodies (i.e., local ethics committee) for storing abnormal samples during the course of routine testing aimed at creating a sufficient bank of abnormal plasmas to expedite performance verification of new reagent lots. If multiple sites need to be correlated, plasma pools may be created combining patient samples with similar results, conditions, or therapies.

Prothrombin Time Reagents

The PT is used for evaluating factor abnormalities (deficiencies or dysfunctional proteins) in the extrinsic and common pathway (factors II, V, VII, X, and fibrinogen), monitor vitamin K antagonists (VKA) therapy, such as warfarin, or monitor efficaciousness of reversal (e.g., prothrombin complex concentrates) or factor replacement therapy. The INR was designed to standardize the monitoring of VKA anticoagulation[11] and may not be suitable for other indications, such as liver disease,[12] although sometimes the laboratory may report both the PT and INR values for any given patient. With new reagent lots, the mean normal prothrombin time (MNPT) should also be determined for the new lot, using 20 fresh samples from healthy individuals,[13] although the World Health Organization publications for INR guidance have indicated the use of frozen citrated normal plasma for MNPT determination[14] and other methods have been described and may be considered after local validation.[15–18] After the calculation of MNPT, it may be prudent to have at least two experienced laboratory staff review the INR calculations and instrument test protocol modifications to assure manual entry accuracy. Afterward, INR performed over a range of INR targets is necessary to ensure no significant change in INR between reagent lots. Prior to initiating the verification of the reagent lot, verify the manufacturer's correct ISI using certified plasma or other described methods as appropriate.[15–18] Commercially prepared frozen normal donor citrated plasma or lyophilized plasma material may also be suitable for MNPT if the citrate concentration used for donor collection is the same concentration used locally. Patient samples are preferred when comparing different reagent lots to avoid issues with the commutability of IQC materials.

To evaluate the INR performance of the new thromboplastin lot, a minimum of 20 samples of patients on stable VKA therapy should be tested in parallel and evaluated with new and existing reagent lots, unless the laboratory uses an alternate method that has been locally validated.[15–18] To evaluate the PT performance of a new thromboplastin lot, samples should be selected to include normal subjects, patients on oral VKA therapy representing both the therapeutic and nontherapeutic ranges, and if possible, at least samples of 20 patients with conditions affecting the extrinsic and common pathway should be included (e.g., patients with history of liver disease, consumptive coagulopathies [e.g., disseminated intravascular coagulation; DIC], low fibrinogen (hypofibrinogenemia), or dysfunctional fibrinogen (dysfibrinogenemia or hypodysfibrinogenemia) and tested with new and existing reagents. If possible, samples from neonates or newborns may also provide sources for an abnormal PT. If new lot PT reagent comparison statistical thresholds are not met, then assessing factor sensitivity may be useful, where samples of known factor concentration, optimally a commercial calibrator, are diluted in respective factor-deficient plasma and PT testing is then performed. Alternatively, and if available, characterized patient samples could be used. The factor sensitivity determination should be performed using surrogate or patient samples having approximately 30 to 60% factor activity to help determine at what factor level the PT becomes prolonged. Evaluation of new lot PT reagents using samples from patients taking direct oral anticoagulants (DOACs) are not recommended, given their variability for assessing this class of oral anticoagulants.[19] The use of INR calibrants or material that can verify the local INR/ISI used for testing should be considered if available and applicable to the reagent and instrument platform being used.

Recommendations for verification of performance of new lot PT testing:

- Manufacturer ISI results should be verified using appropriate INR calibrators or equivalent material or an alternative method that has been locally validated. If ISI verification fails,
  - Ensure that correct ISI and MNPT have been properly recorded.
  - If verification still fails, consult with the reagent manufacturer for resolution or seek a new reagent lot.
- The MNPT for the new lot number using either 20 fresh or frozen samples from healthy adult individuals should be determined. The use of alternative plasma sources or an alternative method can be considered if locally validated.
- The INR/PT reference interval (RI) should be verified using 20 samples collected from nonanticoagulated patients using the CLSI-approved transference method[20] or an alternative method that has been locally validated. Failure to achieve statistical limits may require a new RI determination.
- At least 20, but ideally at least 40, normal and described abnormal samples should be tested concurrently with existing and new lot reagents for both PT (in seconds) and INR (for VKA therapy samples if reported) testing. Surrogate samples (i.e., pooling or modified) are acceptable when patient samples are unavailable, but commutability should be considered in this situation.
- For patient comparisons, the testing should be performed within stability limits described for PT[21] or longer if locally validated.
- Verification should preferably be performed across all analyzers used for diagnostic testing, unless there are alternate procedures in place to assess comparability of performance.
- Regression acceptability should be determined locally; however, linear correlation (e.g., Spearman's) and regression (e.g., Passing and Bablok) are recommended, with acceptable criteria of >0.95 (coefficient of correlation) and slope between 0.90 and 1.10, respectively.[22] Bias estimation may be a useful determination, with expected bias similar as observed during the method verification of performance. For normal samples, at least 90% should be within current or manufacturer-defined RI to verify the RI.
  - MNPT may also require changing for those sites reporting INRs.[17]
    Note: It is good laboratory practice to always establish a new MNPT with a new lot of PT reagent when reporting INR. For laboratories reporting the prothrombin index, verification of the reporting method with each new reagent lot is required.
- If statistical thresholds fail, then factor sensitivity testing may be useful to identify factor sensitivity differences between reagent lots.
- Direct INR IQC values (targets and limits) should be assessed for the new lot number using appropriate quality control material.

Activated Partial Thromboplastin Time Reagents

Similar to the PT, the aPTT is used for evaluating factor abnormalities in the intrinsic and common pathway (factors II, V, VIII, IX, X, XI, XII, other contact factors and to a lesser degree, fibrinogen), monitoring unfractionated heparin (UFH) therapy, monitoring direct thrombin inhibitor (DTI) therapy (e.g., argatroban), or monitoring the efficaciousness of some therapies (e.g., factor VIII replacement therapy or reversal agents such as protamine sulfate).

To evaluate abnormal samples for aPTT testing, these could be collected from patients on heparin therapy or liver disease, consumptive coagulopathies, known intrinsic clotting factors deficiencies, or neonates, which should then be tested with new and existing reagent platforms. As with the PT, the aPTT has variable sensitivity for assessing DOACs, and thus, the use of DOAC samples for new reagent lot comparisons is usually not recommended.[19] If the aPTT is used for UFH monitoring, then at least 20 samples from patients on active UFH treatment (not prophylaxis) are required for HTR determination.[10] For HTR determination, avoid samples with marked inflammatory conditions, as raised fibrinogen and factor VIII may factitiously repress the aPTT clotting time while on UFH therapy. Similarly, avoid the use of samples showing aPTT prolongation compounded by secondary factors (e.g., factor deficiencies, on concomitant VKA therapy).

Parenteral DTI monitoring is likely to be predicated on baseline aPTT and target ratios after the initiation of infusion and is not the same therapeutic range as determined for UFH.[23] Samples collected from normal subjects or samples that are within normal limits of existing reagent should also be incorporated in the method comparison. aPTT reagents that are used for purposes of LA testing, which may or may not be the primary screening aPTT reagent, would require additional population-defined samples to be evaluated (see LA reagent section below).[24] If the aPTT comparison statistical thresholds are not met, then assessing factor sensitivity may be considered in the same fashion as described for the PT, although alternative strategies have also been described.[25] The aPTT factor sensitivity determination should be performed using multiple surrogate samples ranging from approximately 30 to 60% factor activity. For laboratories that perform testing for diagnosis and monitoring of hemophilia, verification of new lot aPTT reagent sensitivity to factors VIII, IX, and possibly XI should also be considered, regardless of whether statistical thresholds are met.

Recommendations for verification of performance of new lot aPTT testing:

- The aPTT RI should be verified using 20 samples collected from nonanticoagulated patients using the CLSI-approved transference method.[20,26] Failure to achieve statistical limits (at least 90% of the results using new lot reagent on samples collected from nonanticoagulated normal subjects are within the existing normal range) may require a new RI determination.
- At least 20, but ideally at least 40, normal and abnormal samples (as described above) should be tested concurrently (within 1 hour) with existing and new lot reagents for aPTT (in seconds).
  - If the aPTT is used for monitoring UFH infusion, then a minimum of 20 samples from patients on heparin therapy are required for HTR verification.[10]

  - Acceptability and generation of HTR is outside the scope of this document, but additional guidance is available.[10,27]
- HTR verification should preferentially be performed across all analyzers used for diagnostic testing.
- Samples should be used on patients with UFH treatment doses only, with no concomitant additional anticoagulant therapy.
- Normal to near-normal INR should be used, with no more than two samples from the same patient.
- Failure to meet acceptable thresholds would require new HTR determination comparing aPTT times to anti-FXa (activated factor X) measurements.
- HTR changes should be communicated as soon as possible to the appropriate health care units, to allow modifications of UFH dosing protocols. The laboratory must provide:
  - Existing HTR
  - New HTR
  - New reagent implementation date and time
- For patient sample comparisons, the statistics used should be determined locally; however, linear correlation (e.g., Spearman's) and regression (e.g., Passing and Bablok) is recommended with acceptable criteria of >0.95 (coefficient of correlation) and slope between 0.90 and 1.10, respectively.[22] A bias estimation may be a useful adjunct.
  - If statistical thresholds fail, then factor sensitivity testing may be considered.
- IQC values (targets and limits) should be assessed for the new lot reagents using either assayed or unassayed control material.

Thrombin Time Reagents

The TT test is similar to fibrinogen determination, in that an exogenous thrombin reagent is added to a patient plasma sample. For TT, the test sample is typically neat (undiluted) or slightly diluted, and the thrombin reagent has a relatively low concentration (approximately 2–10 NIH U/mL), whereas the thrombin reagent used for fibrinogen testing will have higher concentrations of thrombin (approximately 35–100 NIH U/mL), and additionally use diluted patient plasma.[28] The TT measures the clotting time after thrombin addition, thus evaluating the conversion of fibrinogen to fibrin or clot formation. Abnormal TTs are seen in patients with low or dysfunctional fibrinogen (hypofibrinogenemia and dysfibrinogenemia, respectively, or combination of both) or drugs that will inhibit thrombin such as heparins, and parenteral or oral DTIs. Abnormal TTs can also be seen in patients with DIC, where elevated fibrin(ogen) degradation products can inhibit fibrin polymerization thus preventing clot formation.

Recommendations for verification of performance of new lot TT testing:

- At least 20 normal and 20 abnormal samples (low fibrinogen or on UFH therapy) should be tested concurrently with existing and new lot reagents for TT.
- Correlation (e.g., Spearman's) and regression (e.g., Passing and Bablok) acceptability should be >0.95; slope between 0.90 and 1.10, respectively, and bias estimation.[22]
  - For normal samples, at least 90% should be within current or manufacturer-defined RI.
  - Failure to achieve statistical limits may require a new RI determination.

Normal Pooled Plasma

NPP is commonly used in the coagulation laboratory for the performance of PT or aPTT mixing studies, as well as for normalizing LAC test results. The manufacturers of NPP do not usually provide a certificate of analysis detailing factor activity levels for each lot but rather assure that at least 80% (80 U/dL or 0.80 IU/mL) factor levels are present. NPP used for more targeted applications (i.e., factor VIII Bethesda assay) should be assessed prior to use to determine factor level(s). While an optimal level (near 100%, 100 U/dL or 1.00 IU/mL) is recommended,[29] each laboratory should determine their optimal NPP performance for the given indication.

Recommendations for new lot NPP assessment:

- For laboratories that use NPP for mixing studies, at least three known inhibitor (e.g., samples with LAC or factor inhibitors) and three known factor deficient (e.g., samples from patients on VKA or with specific factor deficiency) samples should be tested.
  - Acceptability would be an agreement with mixing study interpretation locally defined[30] as follows:
    (a) The interpretation of "correction" for factor-deficient samples.
    (b) The interpretation of "noncorrection" for inhibitor samples or samples with inhibitory characteristics (i.e., LAC).

Lupus Anticoagulant Reagents

LAC are part of a heterogenous group of autoantibodies directed against anionic phospholipids (PL).[31] First described in a patient with systemic lupus erythematosus (SLE), these autoantibodies are associated with thrombotic risk and pregnancy complications.[32] LAC screening is part of a panel of antiphospholipid antibody measurements used to identify patients suspected of having the antiphospholipid antibody syndrome (APS)[32] and also used for SLE diagnosis.[33] LAC is a common cause for a prolonged aPTT (and sometimes PT) that often demonstrates as an inhibitor (noncorrection of mixing studies). There are several types of LAC assays that could be used, including those based on snake venoms (i.e., Russell viper, ecarin, and taipan), hexagonal PL configurations, and neutralization of antibodies using excess PL, platelets, or platelet derivatives.[31,32] Given the heterogeneity of these antibodies, combined with the lack of test standardization, the methods used for screening the presence of LAC are PL type and concentration dependent, which requires the prolongation of a screening method to proceed with establishing LAC characteristics of inhibitor and PL

dependence.[31,32] The use of integrated systems (screening and confirmation from the same method or source) and calculating normalized ratios for results reporting may aid in mitigating new reagent lot differences. Consult the manufacturer's IFU for guidance as some IFUs indicate reference ranges, cutoffs, or both, but local verification of performance for these assays has already been described.[9] Appropriate determination or confirmation (transference) of manufacturer cutoff value (seconds or ratios) for any LAC method is critical for diagnostic accuracy. Comparison studies should include samples near or at decision or cutoff values. Although samples with LAC are recommended to be reported as positive (not semiquantitative such mild or strong),[32] assessing a breadth of abnormal samples should be considered. Laboratories that normalize LAC test results must incorporate the material used (i.e., NPP, normal mean, and mixed normal mean) concurrently during new lot reagent verification of performance. It may be beneficial to concurrently evaluate new lots of NPP during new lot verification of LAC reagents.

Given the heterogeneity of these antibodies and the lack of international standards for this assay, the statistical thresholds (as described below) may not be met. As such, the use of percentage agreement (total, negative, and positive) could be considered, with an optimal goal of 100% agreement. Failure to achieve 100% may suggest a required change in the cutoff value. New lot numbers for LAC confirmatory reagents should be tested to confirm that the new lot number corrects the abnormal control, thereby demonstrating the PL-dependent nature of LAC. However, if the laboratory has the capacity to do so, it might be preferred to apply similar recommendations to those of the screen test reagent described below.

Recommendations for verification of performance of new lot LA assays:

- At least 20, but ideally at least 40 patient, samples being investigated for possible APS or SLE should be tested concurrently with existing and new lot reagents for the LAC screening test (in seconds). For patient comparisons, the statistics used should be determined locally; however, liner regression is recommended, with the acceptable criterion of >0.95 (coefficient of correlation) and 0.90 to 1.10 (slope).[22] A bias estimation may be a useful adjunct.
  - At least a minimum of 20%, but ideally 40%, of the patient comparison samples should be LAC positive and the full lupus LAC panel should be performed (e.g., screen, mix, and confirm).
- IQC values (targets and limits) for both screening and confirmatory reagents should be assessed for the new lot number using either assayed or unassayed control material.
- For laboratories that provide normalized ratio reporting using the mean of the normal RI, at least 20 samples collected from normal donors should be used to help establish appropriate normalized cutoffs.
  - Frozen normal donor samples may be suitable if citrate concentration is the same as used for patient testing.
  - Alternative strategies such as the use of NPP for normalizing results can be used once locally validated.

Qualitative (Screening) Assays

There are several coagulation assays that provide qualitative (positive or negative) results (e.g., some heparin induced thrombocytopenia tests) or results that are associated with a given diagnostic threshold (e.g., some activated protein C resistance, APCR assays). These assays may or may not have kit-provided quality control material.

Recommendations for qualitative (screening) assays:

- At a minimum, testing of a positive and negative sample that is independent of the control material provided (if applicable)
  Note: Regulatory agencies may not accept kit-provided control material as acceptable performance verification of new reagent lots. For these laboratories, verification of performance can be achieved by:
  - Testing of previously analyzed negative and positive patient samples.
  - Additional testing of sample(s) near or at the diagnostic threshold or cut-off is optimal.
- Refer to the manufacturer's IFU for additional testing recommendations.

Slide Agglutination Assays

Slide agglutination assays are immunologic methods that use either antibody-complexed latex bead (latex immunoassays) or coated red blood cells (hemagglutination assays) that are performed on reusable glass/ceramic (reusable) or plastic/treated paper (disposable) plates. These tests may be semiquantitative or purely qualitative. Controls for these tests are usually qualitative (positive and negative controls).

Recommendations for verification of performance of new lot slide agglutination assays:

- At a minimum, testing of new reagents with existing control material. Acceptability of the new lot would be achieving the expected results for both positive and negative controls.
- Stored plasma from previously tested using semiquantitative methods should be additionally considered to assure longitudinal continuity of result reporting, with the acceptability of new lot demonstrating equivalence (locally determined).

Platelet Aggregation or Platelet Testing Reagents

There are several platforms that assess platelet function, including rapid whole blood methods, impedance methods, flow cytometry methods, and traditional platelet-rich plasma methods. The veracity of these methods for assessing platelet function is outside the scope of this document. Viscoelastic measurements used for global coagulation assessment, including platelet function, will be discussed below.

Reagents for platelet function assessment may be single-use cartridges or separate reagents (agonists) that are used as

part of a profile for assessing platelet function or determine the efficacy of antithrombotic therapy.[34–38] In general, the platelets present in patient whole blood or plasma are exposed to an agonist(s) that, in normally functioning platelets, cause the platelets to undergo activation, shape change, agglutination, degranulation, and aggregation. The testing conditions may include shear rates (closed vacuum or cone/plate system) or more static platforms such as magnetic stirring within a glass or plastic container. The difficulty associated with quality assurance for platelet function testing is the ability to assess abnormal samples, given the limited stability of platelets in citrated blood or plasma (maximum 4 hours). Quality control for platelet function testing may be limited to either (1) electronic assessment of instrumentation (primarily limited to close system methods) or (2) normal donor samples tested concurrently for each day of use. While normal donor testing may assure that there are no factitious abnormal results, testing a normal donor does not assure the suitability of reagents for detecting platelet function defects. This may be of particular concern when reagents are prepared locally, in lieu of commercial sources. Contrived samples can be considered, using normal donor blood enriched with aspirin or specific antithrombotic drugs that do not require in-vivo metabolizing targeting adenosine diphosphate or platelet glycoprotein IIb-IIIa receptors, to create abnormal aggregation responses or profiles.

Recommendations for performance verification of new lot of reagents or cartridges for platelet testing.

- For platelet testing using reagent cartridges, at a minimum one normal and abnormal donor should be tested and compared with the existing lot of reagent(s).
- For platelet aggregation reagents, at a minimum one, but optimally at least three normal donors should be tested and compared with existing lot of reagent(s).
  - Optimally, in addition to normal samples, testing three (3) abnormal patient samples or surrogate (contrived) samples specimens should be considered, especially when local reagent preparation is used for testing to assure longitudinal continuity of result reporting.
- Laboratories should collate historical data from normal donors to aid in the reassessment of new batches of reagents.
- Due to limitations in QC and difficulty in evaluating batch-to-batch variation, consideration should be made on how to limit batch change (use of a large batch of frozen reagents, etc.)
- As there are no commercial manufacturer control materials for platelet-rich plasma aggregation, an individualized quality control plan (IQCP), or as regional regulations required, should be considered for platelet function testing.

Viscoelastic Measurements

Viscoelastic measurements are tests that measure the physical properties of clotting blood.[39,40] The measurements of clotting blood encompass either resistance (torque), sound, or other physical properties that are measured continuously in real time, ultimately resulting in graphical representation of clot initiation, fibrin polymerization, and platelet aggregation, with thrombin generation, and potentially assessment of clot lysis 30 to 60 minutes after maximal clot formation. Reagents are typically instrument-specific, may be single-use cups or cartridges, with some systems allowing local modifications or enhancements using different agonists than provided by the manufacturer. Most commercial sources for viscoelastic reagents provide suitable control material (two levels, low and high), although these control materials may not address each available reagent or address each reportable parameter.

Recommendations for the verification of performance of new lot viscoelastic measurement reagents:

- At a minimum, testing of new reagents with commercially available control material, prior to clinical use. Acceptability of new lot would be recovering expected results (provided by the manufacturer) for each parameter.
- Ideally, for reagents or kits that do not have quality control or do not provide any abnormal results for reported parameters, concurrent testing (with existing lot reagents) with at least one normal and one abnormal patient (or surrogate sample) should be considered to assure longitudinal continuity of result reporting. Refer to regional regulatory authorities for further guidance if an individualized quality control plan is required.[41]

Other Point-of-Care Tests

Point-of-care tests (POCT) methods using citrate or native whole blood for hemostasis testing have been available for decades, which are mostly targeted for use by nonlaboratory professionals or patient self-assessment (after appropriate training). The most common coagulation test menu using POCT platforms include the ACT, PT (and/or INR), aPTT, and D-dimer.[42,43] POCT methods are single-use cartridges that are instrument-specific, and some cartridges (primarily PT/INR or aPTT) have internal features or controlling mechanisms to aid in determining the veracity of the sample collection and test result. POCT cartridges are lot-specific and typically packaged to contain multiple test cartridges (e.g., 25 per box).

Recommendations for the verification of performance of new lot POCT reagents (not POCT for patient self-assessment):

- At a minimum, testing of new lot POCT reagents with the specific quality control material available from cartridge manufacturer if required prior to clinical use. Acceptability of a new lot would be recovering expected results (provided by the manufacturer).
  - This recommendation is harmonized with the ICSH guidance document for point-of-care INR and D-dimer testing.[44]
- Verification of new lot performance using EQA material would be an additional consideration, especially if the quality control material fails to meet expectations.

Acceptability of a new lot would be defined by the respective EQA summary.
- Unexpected INR result(s) from a new lot POCT INR cartridge should be confirmed with a laboratory INR determination prior to dose adjustment.

Calibrated Coagulation Tests

With the exception of some POCTs, the aforementioned coagulation tests are mostly assays that report measurements of time (chronometric) or some coagulation function over time. Tests that are reported in units per volume (e.g., mg/dL and IU/mL) are calibrated and mitigate to some degree the need for new RI assessment but still require accuracy assessment between reagent lots to assure transparency to clinicians. The frequency of calibration may be regionally defined by regulatory agencies or by the reagent manufacturer (e.g., every 6 months), but all new reagent lots require calibration prior to clinical use. Acceptability of calibration is beyond the scope of this document but is likely defined by the instrument manufacturer IFU. Consider performing precision checks regularly (e.g., monthly) to reduce the risk of introducing bias by calibrating a test on an imprecise instrument. Where appropriate, using calibrator(s) and controls from different commercial sources may provide additional assurance of testing accuracy. However, this may be considered an LDT by local regulatory authorities, which may require additional validation.

Calibrated coagulation tests include chronometric, chromogenic, immunoturbidimetric, immunochemiluminescent, immunofluorometric, enzyme-linked immunosorbent assay, and others. Commercially available calibrated tests are commonly provided as kits containing the required reagents, which may or may not also contain the required calibrator(s) or controls. Factor assays are commonly performed using one-stage clot-based methods, where each required reagent is obtained separately and changing all reagent lots concurrently may be considered. Evaluation of new lots of more stable reagents such as buffers, diluents or calcium chloride, saline, and water may not be required prior to clinical use.[2] For laboratories with multiple analyzers (within institution or institution network), it may be of value to check clotting times of factor curves between analyzers as well as checking optical density readings of chromogenic and immunoturbidimetric curves between analyzers (and between calibration runs), especially when there is a deviation in final results between testing sites. Solid phase assays often utilizing chemiluminescent technology have calibration curves preestablished by reagent manufacturers. For some tests, international consensus RIs are available (e.g., anticardiolipin or anti-β2 glycoprotein I antibodies) so that batch-to-batch performance is partially relevant where reassessment of RI is unfeasible.

Recommendations for verification of performance of new lot reagents that require calibration.

- After calibration, at a minimum, each laboratory should assess new reagents with commercially available (ideally assayed) control materials or in-house QC prior to clinical use. Acceptability of a new lot would be recovering expected results (provided by the product manufacturer) for each parameter.
  Note: Regulatory agencies may not accept kit-provided control material as acceptable new lot reagent verification of performance. For these laboratories, the verification of calibration and new lot reagent performance can be achieved by:
  - Alternative (third party) sources of control or calibrator material that cover the AMR can be used if the commutability of the controls or calibrators is demonstrated.
  - A more robust new lot evaluation would include an imprecision and accuracy assessment by testing three replicates of three different levels covering the AMR.[4]
  - A minimum of 3 to 5 patient or commercial samples at medical decision levels, but ideally 10 to 20 patient samples tested using previously verified reagents spanning the reportable range.[45]
  - Surrogate and stored frozen plasma samples may be used. Frozen plasma samples should be tested within 2 hours of thaw.
  - Calibrator or control material from another reagent or kit lot, if allowed by manufacturer IFUs (no target value specificity to a given lot indicated).
  - Testing of certified reference materials.
    Note: Excluding clot-based assays, some regulatory agencies may require the AMR range to be verified prior to clinical use, especially when a single calibrator is used to create AMR. AMR verification can be achieved using previously tested patient samples or commercially available assayed material.
- IQC values (targets and limits) should be assessed for the new lot reagents using assayed (optimal) control material.
- Factor-deficient plasma used for one-stage factor assay testing should be evaluated to ensure the deficient material contains <0.01 IU/dL ($<1\%$) of factor.
- Laboratories servicing specialized patient populations (e.g., emergency departments and hemophilia treatment centers) may consider assessing/verifying lower limit of quantitation, especially if calibrated tests are used for screening purposes.[29,46,47]
- For methods using preestablished calibration curves, a local calibration is performed and the acceptable limits are set by the manufacture.
  - Quality control material should be tested with acceptable performance prior to use.

Internal Quality Control (IQC) Material

IQC materials are commercially available control materials that may be "unassayed," indicating that the local determination of acceptable ranges is required or "assayed" indicating that the manufacturer has provided a mean or range of expected results. Control material may be available separately or as part of a reagent kit or system. Each laboratory

must conform to the local regulatory requirements of the manufacturer IFU for IQC acceptability. If the local assessment of assayed material does not perform as expected, testing EQA or other traceable assayed material could be considered for troubleshooting purposes.

Recommendations for verification of performance of new lot control material

- For unassayed control material:
  - 20 measurements for each control, for each measurand over at least 20 days or,
  - 5 consecutive days, with two runs per day (at least 4 hours apart) in triplicate for each control material for each assay.
  - Calculation of mean and standard deviation (SD) required, with a range of acceptability: mean $\pm$ 2 SD or,
  - Calculation of mean and with the range of acceptability being mean $\pm 2 \times$ previously determined SD from new method/instrument evaluation.
- For assayed control material:
  - If using as part of a reagent kit or dedicated measurand system, then no assessment is required prior to clinical use. However, material must be within acceptable limits prior to reporting patient results.
    (a) Acceptability and control ranges are defined by the manufacturer's IFU.
    (b) Locally established control ranges should be calculated for each parameter after ensuring that results are within the manufacture's IFU.
  - If the control material is from a different (third party) commercial source than the material used for calibrations, then consider measuring at least once using existing controls and reagents lot prior to clinical use.
    (c) Control range acceptability should be described by designated laboratory personnel, but similar strategies should be considered as described under unassayed control material.
    (d) Ideally, alternative commercial sources for control material should be assayed and traceable to recognized international standards, when available.

Calibrator Material

Calibrator material should be traceable to international standards, when appropriate and available.[47,48] Calibrator (s) may be available as separate material or included within a kit or test system and may consist of a single material that is diluted or provided as separate calibrators to create the AMR.

Recommendations for verification of performance of new lot calibrator material

- For kit-provided calibration material, the control material must be within acceptability limits prior to patient result reporting.
- For calibration material that is not provided or included as part of a reagent kit or system:
  - Acceptability of a new lot calibrator is verified using the control material that covers the AMR.
- For laboratories that use a calibrator material from a different manufacturer than the assayed control material:
  - Consider running the new lot calibrator (as a patient sample) for each measurand in which the calibrator is used.
  - Acceptability of calibrator should be recovery within $\pm$ 10% of the stated measurand value.

Conclusion

Implementing new lots of coagulation testing reagents, calibrators, and controls is a necessary process in the clinical laboratory to assure equivalence or acceptable performance prior to clinical use. It is strongly recommended that each laboratory have a written policy, with acceptability criteria defined for evaluating new lots of coagulation reagents, calibrators, and controls. This document provides the framework and provisional guidance for clinical laboratories in assessing new reagent lot performance, but adherence to regional accrediting or regulatory requirements is also required.

Conflict of Interest
D.C. received honorarium from Whitehouse Webinar Speaker, Stago Webinar Speaker, Coagulation BLOG for Aniara. She served as an Executive Secretary, NASCOLA and is currently serving as a Chairperson, CLSI HILA Committee.
K.M. received support from International Society of Laboratory Hematology (ISLH) and Canadian Society for Medical Laboratory Science (CSMLS) for attending meetings. She is also serving as a Vice President, Membership Engagement in ISLH and as a Board of Directors, Director, Ontario in CSMLS.
R.C.G. received consultation fees from Sysmex America Inc.; University of California, Santa Cruz; University of California, Berkeley; and Diagnostica Grifols. He received honorarium from Diagnostica Stago; Pharmacy Innovations Conference on Antithrombotic Therapy; and Mindray Scientific Forum. He also participated on a Data Safety Monitoring Board or Advisory Board in University of California, Davis Health System, Thrombosis and Hemostasis Subcommittee of Pharmacy and Therapeutics Committee. He received reimbursement for expenses for leadership role from International Council for Standardization in Haematology.

References

1 CLSI. User Evaluation of Acceptability of a Reagent Lot Change. 2nd ed. CLSI Guideline EP26 Clinical and Laboratory Standards Institute; 2022
2 International Organization for Standardization. Medical Laboratories—Requirements for Quality and Competence (ISO Standard Number 15189:2022). Geneva, Switzerland
3 International Organization for Standardization. In Vitro Diagnostic Medical Devices—Measurement Of Quantities in Samples of

Biological Origin—Requirements for Content and Presentation of Reference Measurement Procedures (ISO standard number 15193:2009). Geneva, Switzerland
4 United States Department of Health and Human Services. Food and Drug Administration. Bioanalytical Method Validation; Guidance for Industry. May 2018. Accessed September 18, 2021 at: https://www.fda.gov/media/70858/download
5 United States Code of Federal Regulations (CFR) Title 42, Chapter IV, Subchapter G, part 493 Laboratory Requirements. Accessed September 18, 2021 at: https://www.ecfr.gov/current/title-42/chapter-IV/subchapter-G/part-493
6 College of American Pathologists (CAP) All Common Checklist. COM.30450: New Reagent Lot Confirmation of Acceptability that New Lot Reagents, 2020. Northfield, IL
7 Favaloro EJ, Gosselin RC, Pasalic L, Lippi G. Hemostasis and thrombosis: an overview focusing on associated laboratory testing to diagnose and help manage related disorders. Methods Mol Biol 2023;2663:3–38
8 Gardiner C, Coleman R, de Maat MPM, et al. International Council for Standardization in Haematology (ICSH) laboratory guidance for the evaluation of haemostasis analyser-reagent test systems. Part 1: instrument-specific issues and commonly used coagulation screening tests. Int J Lab Hematol 2021;43(02):169–183
9 Gardiner C, Coleman R, de Maat MPM, et al. International Council for Standardization in Haematology (ICSH) laboratory guidance for the verification of haemostasis analyser-reagent test systems. Part 2: specialist tests and calibrated assays. Int J Lab Hematol 2021;43(05):907–916
10 Marlar RA, Clement B, Gausman J. Activated partial thromboplastin time monitoring of unfractionated heparin therapy: issues and recommendations. Semin Thromb Hemost 2017;43(03):253–260
11 Kirkwood TBL. Calibration of reference thromboplastins and standardisation of the prothrombin time ratio. Thromb Haemost 1983;49(03):238–244
12 Tripodi A, Chantarangkul V, Mannucci PM. The international normalized ratio to prioritize patients for liver transplantation: problems and possible solutions. J Thromb Haemost 2008;6(02):243–248
13 Adcock D, et al. Validation of INR and local calibration of PT INR systems. Approved guideline. CLSI document H54-A Clinical and Laboratory Standards Institute; 2005
14 Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with vitamin K antagonists Replacement of Annex 3 of WHO Technical Report Series, No. 889 Annex 6. World Health Organization (WHO); 2013. Accessed May 26, 2023 at: https://cdn.who.int/media/docs/default-source/biologicals/blood-products/document-migration/trs_979_annex_6.pdf?sfvrsn=5c9-60b91_3&download=true
15 Favaloro EJ, Hamdam S, McDonald J, McVicker W, Ule V. Time to think outside the box? Prothrombin time, international normalised ratio, International Sensitivity Index, mean normal prothrombin time and measurement of uncertainty: a novel approach to standardisation. Pathology 2008;40(03):277–287
16 Favaloro EJ, McVicker W, Zhang Y, et al. Improving the inter-laboratory harmonization of the international normalized ratio (INR): utilizing the concept of transference to estimate and/or validate International Sensitivity Index (ISI) and mean normal prothrombin time (MNPT) values and/or to eliminate measurement bias. Clin Lab Sci 2012;25(01):13–25
17 Favaloro EJ. Optimizing the verification of mean normal prothrombin time (MNPT) and International Sensitivity Index (ISI) for accurate conversion of prothrombin time (PT) to international normalized ratio (INR). Methods Mol Biol 2017;1646:59–74
18 Favaloro EJ. How to generate a more accurate laboratory-based international normalized ratio: solutions to obtaining or verifying the mean normal prothrombin time and international sensitivity index. Semin Thromb Hemost 2019;45(01):10–21
19 Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants. Thromb Haemost 2018;118(03):437–450
20 CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory. Approved guideline. CLSI document C29–A3c Clinical and Laboratory Standards Institute; 2005
21 CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays—Sixth Edition. CLSI document H21 Clinical and Laboratory Standards Institute; 2022
22 Bilić-Zulle L. Comparison of methods: Passing and Bablok regression. Biochem Med (Zagreb) 2011;21(01):49–52
23 Van Cott EM, Roberts AJ, Dager WE. Laboratory monitoring of parenteral direct thrombin inhibitors. Semin Thromb Hemost 2017;43(03):270–276
24 Favaloro EJ, Kershaw G, Mohammed S, Lippi G. How to optimize activated partial thromboplastin time (APTT) testing: solutions to establishing and verifying normal reference intervals and assessing APTT reagents for sensitivity to heparin, lupus anticoagulant, and clotting factors. Semin Thromb Hemost 2019;45(01):22–35
25 Do L, Favaloro E, Pasalic L. An analysis of the sensitivity of the activated partial thromboplastin time (APTT) assay, as used in a large laboratory network, to coagulation factor deficiencies. Am J Clin Pathol 2022;158(01):132–141
26 CLSI. One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline—Second Edition. CLSI document H47-A2 Wayne, PA: Clinical and Laboratory Standards Institute; 2008
27 Brill-Edwards P, Ginsberg JS, Johnston M, Hirsh J. Establishing a therapeutic range for heparin therapy. Ann Intern Med 1993;119(02):104–109
28 Undas A. Determination of fibrinogen and thrombin time (TT). Methods Mol Biol 2017;1646:105–110
29 CLSI. Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay CLSI document H48 2nd Edition. Clinical and Laboratory Standards Institute; 2016
30 Adcock DM, Moore GW, Montalvão SL, Kershaw G, Gosselin RC. Activated partial thromboplastin time and prothrombin time mixing studies: current state of the art. Semin Thromb Hemost 2023;49(06):571–579
31 CLSI. Laboratory testing for the lupus anticoagulant. Approved guideline H60-A Wayne, PA: Clinical and Laboratory Standards Institute; 2014
32 Devreese KMJ, de Groot PG, de Laat B, et al. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis: update of the guidelines for lupus anticoagulant detection and interpretation. J Thromb Haemost 2020;18(11):2828–2839
33 Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol 2019;71(09):1400–1412
34 Hayward CP, Moffat KA, Raby A, et al. Development of North American consensus guidelines for medical laboratories that perform and interpret platelet function testing using light transmission aggregometry. Am J Clin Pathol 2010;134(06):955–963
35 Mammen EF, Comp PC, Gosselin R, et al. PFA-100 system: a new method for assessment of platelet dysfunction. Semin Thromb Hemost 1998;24(02):195–202
36 Paniccia R, Priora R, Liotta AA, Abbate R. Platelet function tests: a comparative review. Vasc Health Risk Manag 2015;11:133–148
37 CLSI. Platelet Function Testing by Aggregometry. Approved guideline. CLSI document H58-A Wayne, PA: Clinical and Laboratory Standards Institute; 2008
38 Platton S, McCormick Á, Bukht M, Gurney D, Holding I, Moore GW. A multicenter study to evaluate automated platelet aggregometry

on Sysmex CS-series coagulation analyzers-preliminary findings. Res Pract Thromb Haemost 2018;2(04):778–789
39 Carll T, Wool GD. Basic principles of viscoelastic testing. Transfusion 2020;60(Suppl 6):S1–S9
40 Volod O, Runge A. Measurement of blood viscoelasticity using thromboelastography. Methods Mol Biol 2023;2663:709–724
41 Hoeltge GA. Accreditation of individualized quality control plans by the College of American Pathologists. Clin Lab Med 2017;37 (01):151–162
42 Perry DJ, Fitzmaurice DA, Kitchen S, Mackie IJ, Mallett S. Point-of-care testing in haemostasis. Br J Haematol 2010;150(05):501–514
43 Riley RS, Gilbert AR, Dalton JB, Pai S, McPherson RA. Widely used types and clinical applications of D-Dimer assay. Lab Med 2016; 47(02):90–102
44 Fitzmaurice DA, Geersing GJ, Armoiry X, Machin S, Kitchen S, Mackie I. ICSH guidance for INR and D-dimer testing using point of care testing in primary care. Int J Lab Hematol 2023;45(03):276–281
45 Miller WG, Erek A, Cunningham TD, Oladipo O, Scott MG, Johnson RE. Commutability limitations influence quality control results with different reagent lots. Clin Chem 2011;57(01):76–83
46 Rimsans J, Douxfils J, Smythe MA, et al. Overview and practical application of coagulation assays in managing anticoagulation with direct oral anticoagulants (DOACs). Curr Pharmacol Rep 2020;6:241–259
47 Gray E, Kitchen S, Bowyer A, et al. Laboratory measurement of factor replacement therapies in the treatment of congenital haemophilia: a United Kingdom Haemophilia Centre Doctors' Organisation guideline. Haemophilia 2020;26(01): 6–16
48 National Institute of Biological Standards and Controls (NIBSC) Biological Reference Materials, Haemostasis. London, United Kingdom. Accessed September 18, 2021 at: https://www.nibsc.org/products/brm_product_catalogue/sub_category_listing.aspx?category=Biotherapeutics&subcategory=Haemostasis

