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Establishment of New Coagulation Tests-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

A. This applies to tests classified as moderate or high complexity under the Clinical Laboratory Improvement Amendments (CLIA) '88 guidelines and includes the following test categories:

1. Individual procedures on multi-channel instruments
2. Individual procedures on single-channel instruments
3. Manual procedures

II. ACRONYMS:

A. Activated Partial Thromboplastin Time (PTT)
B. Clinical Laboratory Improvement Amendments (CLIA)
C. Coefficient of Variation (CV)
D. Food And Drug Administration (FDA)
E. International Normalized Ratio (INR)
F. Prothrombin Time (PT)
G. Standard Deviation (SD)
H. Thrombin Time (TT)
I. World Health Organization (WHO)

III. PROCEDURE:

A. **FDA Approved Method:**

1. Prior to reporting patient results using a new laboratory test, data must be obtained and retained on file, which verifies that the laboratory is achieving manufacturer's performance specifications for any test, which is FDA, approved. These performance specifications include:
   a. Accuracy
   b. Correlation Study

c. Precision

d. Reportable Range

e. Reference Range

2. In addition, information documenting the likelihood of interfering substances, which are likely to be present in the sample, should be evaluated. For FDA approved methods, data provided by the manufacturer may be used for this, and it should be included in the procedure description.

3. **Evaluation Requirements for FDA Approved Methods:**

   a. **Accuracy:** Accuracy is defined as closeness of the agreement between the result of a measurement and a true value. The concept of a true value is frequently not applicable to coagulation testing. This is especially true of the global screening tests. While a degree of standardization has been achieved for the International Normalized Ratio (INR), there is no standard for the PT, APTT and TT. Verification of the accuracy of new quantitative procedures is a requirement prior to reporting patient results. Accuracy and Precision verification can be combined into one procedure for Fibrinogen and Factor Assays. If precision verification has already been performed the values generated during the precision testing can be used for accuracy verification. A valid standard curve must be established prior to performance of a test is required. A control/standard plasma calibrated against the WHO standard should be used to verify results.

      i. **Accuracy Procedure for Fibrinogen and Factor Assays:**

         a. Reconstitute appropriate standard or control materials and reagents as recommended on the package insert.

         b. Prepare dilutions as appropriate (factor assays). Store refrigerated or on ice until ready.

         c. Run test(s) in replicates of 10.

         d. Compare the results with the assigned values for controls for fibrinogen.

         e. Calculate the Mean, SD and %CV for controls.

         f. Accuracy is considered acceptable if the results are within ± 20% of the assayed or calculated value.

      ii. **Accuracy Procedure for special coagulation assay**s: Accuracy may be assessed by assaying any of the following:

         a. Standard solutions prepared to a known concentration

         b. Calibrators of a lot number different from that used to calibrate the method

         c. Quality control materials with assigned values

         d. Proficiency testing specimens for which consensus values are known At least three such materials with different levels of the analyte should be used. Results should fall within + 20 % of the assigned values to be considered acceptable.

   b. **Correlation Study:**

      i. For most coagulation assays, the concept of a true value is not applicable. In these circumstances comparability may serve as a substitute for accuracy determination. For the comparison of the coagulometer/reagent system, use the laboratory's current

coagulometer/reagent system or a recognized reference method. The selection of samples for method verification studies is critical. They must represent a range of values from normal to abnormal. This is an absolute requirement for regression analysis. There must be a spread of values in order for the regression analysis to work effectively.

a. Select 40 patient samples (20 normal and 20 abnormal). Perform specified testing over several days.
b. Be certain not to exceed the linear range of either method.
c. Test samples on the current system within one hour after performing test on new system. If testing cannot be done immediately samples can be stored at 2 - 8ºC for up to 2 hours.
d. Assay the samples by both methods as you would test a patient sample.
e. Repeat discrepant results by both methods, if possible.
f. Subject results to statistical analysis using linear regression analysis and the paired t test.
g. Graph the regression plot with the evaluation method as the y-axis and the reference method as the x-axis.
h. Obtain the slope, intercept and standard error of the estimate for the regression line, as well as the correlation coefficient and t value for the two sets of data. Proportional bias may be estimated from the slope of the line and constant bias from the intercept. The t test indicates whether there is a significant difference between the means of the two sets of results and is very sensitive to bias, whether constant or proportional. It is dependent, however, on the amount of random variability in the data. Neither the regression data nor the t test is useful in identifying which method is contributing to the bias.

ii. **Correlation Coefficient**:The correlation coefficient and the standard error of the estimate give a measure of the random variability in the data and also do not indicate which method may be at fault. A general guide for acceptability of the correlation coefficient (R) is that R^2 should not be less than 0.85 (R=0.92).

iii. **Standard Error of the Estimate:** The standard error of the estimate (SEE) measures the spread of the x-y data around the linear regression line. If both methods have the same constant precision SD across the full analytical range, SEE should be about 1.4 times the precision SD.

c. **Precision:** Precision is the relationship of the results of repeated analyses performed on the same material and is expressed as (SD) and (CV). Both within-run and day-to-day precision should be assessed.

i. Perform at least 20 replications of the new test method. Perform 10 replicates of a normal control and 10 replicates of an abnormal control.

ii. Perform day-to-day measurements (20 to 40 total points). Each run should include one normal and one high abnormal for PT, APTT, and TT. For special coag tests and Clauss Fibrinogen assay the abnormal should be close to clinically significant decision points (e.g., the lower limits of normal reference interval for antithrombin activity).

iii. For each of five (5) days, run 2 levels of the test in triplicate.

iv. Compute the mean, standard deviation and coefficient of variation (CV) for the within-run and day-to-day precisions.

v. Acceptable performance will be judged by a pathologist on an individual basis, but generally, the within-run CV should not exceed 5 % and the day-to-day CV should not exceed 7 %. For clotting tests, 10-15% CV is allowable.

d. **Reportable Range:** The reportable range of the procedure is established by the range of linearity of the method. This range should be available from the manufacturer, but may be further modified as needed by performing linear limits studies using a quality control material or high/low patient sample, which has been diluted. Since Prothrombin Time and Activated Partial Prothrombin Time are reported in seconds, verification of linearity is not required for PT and aPTT assays.

i. To determine the Linear Range:

a. Use at least 5 different dilutions to obtain 5 points for the curve.

b. Plot the results of these 5 points as observed (y-axis) versus expected (x-axis) on linear graph paper.

c. Points which deviate by more than 10 % (20% for fibrinogen) from the expected value should be considered unacceptable and the point (value) at which this first occurs should be used as the upper limit of linearity. Points scattering about the line by more than 10 % (20% for fibrinogen) indicate a precision problem and should be investigated as such.

e. **Reference Range:**
Before using a manufacturer's recommended reference range, results from the patient correlation study should be assessed for significant differences between methods. If there is no clinically significant difference, the existing reference range may be used. If a clinically significant difference exists, results from the new method may be factored to correspond to the old reference range. If a new range is to be adopted, either due to a significant difference or because a new method not previously performed in the laboratory is introduced, a series of specimens from individuals presumed normal should be assayed.

i. To determine a new Reference Range:

a. Use 20-40 "normal" patient samples; these may be samples from "normal" individuals in the laboratory. The effect of sex, drugs, hormonal state, diet/dietary supplements, etc. may need to be taken into account depending on the assay (e.g., Protein S, APC resistance).

b. Samples should be tested fresh (within four hours at room temperature) and/or after freezing at -70ºC. Frozen plasma samples should be rapidly thawed at 37ºC in a water bath, gently mixed, and tested immediately.

c. If the data appears approximately gaussian in distribution, compute the mean, standard deviation and 95 % confidence interval. Consult with a pathologist to determine the new reference range to be used.

d. Data calculations are performed with the aid of computer excel application and or the EP evaluator.

B. **Non-FDA Approved Methods**

1. For tests which are not FDA approved, i.e., modifications of a manufacturer's procedure, methods adopted from literature descriptions of procedures or methods developed in-house, the following additional performance specifications must also be evaluated and documented:
    a. Appropriate Calibration Procedures
    b. Appropriate Quality Control Procedures
2. The following sections describe in detail, the specific data to be gathered to satisfy each of the above requirements. Once evaluation studies are complete, the data should be reviewed by a pathologist and the procedure approved for clinical use. All data supporting the performance of the new test method must be retained for as long as the method is in use.
3. On approval, the procedure should be dated for the time it is put into use and signed by a pathologist. It should be reviewed every 2 years thereafter as part of the general procedure manual review. Any changes made to the procedure must be approved by a pathologist, have supporting data to document that the change does not affect test results and be signed by a supervisor and pathologist. Effort should be made to have the procedure retyped as soon as possible. Data documenting the acceptability of the change should be filed with the original evaluation data for the procedure.
4. **Evaluation Requirements for non-FDA Approved Methods:**
    a. **Calibration Procedure:**
        i. In cases where there is no manufacturer's recommendation for calibration, the laboratory must establish the frequency and materials to be used. Generally for manual procedures, calibration must be done each run.
        ii. If an in-house calibration procedure is set up on an automated instrument, the method should be assessed by calibrating, using selected standard material and running quality control to determine the calibration viability.
        iii. Based on results from this study, establish the calibrators to be used and the frequency of recalibration. Enter this information into the procedure for this assay.
    b. **Quality Control Procedure:**
        i. Quality control must be checked with at least 2 levels each time calibration is performed. For methods which may not require calibration each run, such as those on automated analyzers, the frequency of quality control checking should be established by performing a stability study.
        ii. To perform a quality control stability study, control material should be run at periodic intervals to determine the stability of the quality control material and reagent system. In any case, quality control must be performed at least once every 8 hours.

IV. REFERENCES:

A. Clinical Laboratory Improvement Amendments of 1988. Final Rule. 42CFR Part 405, et al
B. Protocol for the Evaluation, Validation, and Implementation of Coagulometers; Approved Guideline. H57-A Vol. 28 No.4.
C. Dade Behring Hemostasis CA 7000 Installation Package, Accuracy Verification, Rev 11/1/03.
D. EP Evaluator, Release 7, Copyright 1995 – 2005.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	6/24/2021
Coagulation Medical Director Designee	Marc Smith: System Med Dir, Coagulation	6/4/2021
Policy and Forms Steering Committee Approval (if needed)	Tamara Sabih: Medical Technologist Lead	6/2/2021
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	6/2/2021
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Applicability

Royal Oak