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Validation of New Reagent Lot Numbers
Stago Compact Max Analyzers

Principle
Lot to lot variance must be checked to ensure valid patient results.

Specimen
Sodium Citrated Plasma

Instrumentation/Equipment
Data is entered into templates and sent to Stago Technical Support Specialist for evaluation. Upon completion, Stago TSS will send results to review.

Procedure:
Validation of New lot numbers of reagents**.**

A. Loading New Lot of Reagents

1. From Main Menu select Methodologies and print out the test set up for the "NEW" assays.
2. Open the product drawer and barcode and load the current lot of reagents you are using.
3. Next barcode and load the new lot of reagents. You will be prompted to scan the bar code sheet for each new lot of reagent.
4. Pass the reagent bar code sheet in front of the bar code reader and complete loading.
5. Do not scan the bottle of the new lots of controls. Instead type the ID and name exactly as it is listed on page 3 of the test set up for the "NEW" assays.
    a. Type the vial size, stability and lot number and load vial into the drawer.
    b. You will get a pop up box alerting you that changes are detected.
    c. Type "YES" and confirm.
    d. Do the same for the abnormal control.
6. Close the product drawer and continue with each new assay you are running.

QC Ranges

1. If you are not changing lots of controls along with your "NEW" assay reagent lot, you must still QC the "NEW" assays.
2. Current lot of Stago Coag Plus controls are scanned on as you normally would and the ranges are automatically programmed with the "NEW" assays.
3. BioRad D-dimer controls are user defined and must have the QC range entered before controls can be run.
    a. Click on the QC icon to access the Quality Control Screen.
    b. Double click on NEW DDI and then click on Modify threshold at the bottom

c. of the page. When the pop up box comes up type in the QC minimum and maximum ranges from the BioRad D-dimer package insert.
d. Click confirm.
e. Enter the access code and confirm.
f. Exit out of QC file

Calibration

1. From the Main Menu select Calibration and select NEW PT by double clicking.
2. Click on Modify parameters at the bottom of the page.
3. Enter your access code and confirm.
4. You must type in a reference time (geometric mean). Type a normal PT such as 13.2. Note: This will not be used to report patient results.
5. Click on the drop down button in the lot column under the Reagents section of the screen.
6. Choose the correct lot number for the "NEW PT" reagent from the drop down list.
7. Click the confirm button at the bottom of the page and the correct ISI will populate on the screen for this lot number. Check the information with your barcode sheet.
8. Exit this screen by clicking the door icon at the bottom right of the screen.
9. Proceed in the same manner to choose the new lot numbers under Calibration for NEW PTT, NEW FIB, and NEW DDI if needed.
10. Note: for NEW FIB and NEW DDI you will need to click the Run Controls button before choosing the lot number for the reagent.
11. Exit the Calibration menu.

Running QC

1. Click on the QC icon or go to the Quality control menu.
2. Check the boxes beside the assays you wish to order QC. For example, PT, PTT, Fib, NEW PT, NEW PTT and NEW Fib and click on the "Go" icon at the top right of the screen.

B. Running Lot Conversion Samples

Note: You may give the samples prefixes according to the types of samples you are running. For example: Norm-1, Norm-2 etc. for normal samples.

1. Open the sample drawer and click on the Manual Mode button if you are not already in that mode.
2. Click on Change Identity to input a prefix.
3. The Default Identity window box appears.
4. Type the prefix you want and click Confirm. The prefix will now be in the ID window and you can just add a number. To erase it click on Change identity again and backspace the prefix to remove it.
5. Select the tests you need by double clicking them and Confirm your selection.
6. Continue in the same manner until all samples are ordered.

7. Exit the sample loading menu.

PT Reagents

Required Specimens: 20 specimens from normal patients not on anticoagulation therapy
20 abnormal samples from patients on oral anticoagulation therapy

1. Parallel testing of a new lot of PT reagent should be completed well in advance of the expiration date of the old lot. Parallel testing of new lots of PT reagents also includes verifying the reference range, geometric mean and scanning the correct ISI (international Sensitivity Index) into the coagulation analyzer.

2. To verify the reference range and geometric mean it is necessary to collect specimens from 20 "normal" patients and to run a PT with the new lot of reagent. 90% of the samples must fall within the current range (in seconds) in order to verify the range and geometric mean. If the sample values do not fall within the current range, a new reference range study must be conducted. The Stago TSS will calculate the new range and geometric mean.

3. Validate the PT reference range with 20 specimens. If the reference range does not validate, perform a new reference range study using at least 40 specimens.

4. Perform comparison studies using the INR between the old and new lot number to verify the consistency of patient results and controls. Use the 20 normal samples along with 20 abnormal samples. The R value for the correlation study should be >0.95.

5. Perform a manual check of the INR and compare with the instrument generated INR result.

6. Protime Controls should be run on all three shifts. After a minimum of 30 runs, the data will be evaluated to establish the mean and SD's. The mean should be within the established ranges for that particular lot.

APTT Reagents

Required Specimens: 20 specimens from normal patients not on anticoagulation therapy
20 specimens from patients receiving unfractionated heparin therapy

1. Parallel testing of PTT reagents should be conducted well in advance of the expiration of the old reagent.

2. Perform comparison studies between the old and new lot number using patient samples. The R value for the correlation study should be R >0.95.

3. To verify the PTT reference range it is necessary to collect specimens from 20 "normal" patients and to run a PTT with the new lot of reagent. 90% of the samples must fall within the current range in order to verify the range. If the sample values do not fall within the current range, a new reference range study must be conducted.

4. Data is entered into the template and sent to Stago TSS to evaluate the acceptability of old normal and therapeutic ranges. If a new therapeutic range is needed, it should be established using the factor Xa inhibition assay to determine heparin sensitivity.

5. APTT Controls should be run on all three shifts. After a minimum of 30 runs, the data will be evaluated to establish the mean and SD's. The mean should be within the established ranges for that particular lot.

Determination of the APTT values corresponding to the Heparin Therapeutic Range

Heparin Anti-Xa Assays

The recommendations presented by the Collage of American Pathologists during Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy state that it is now apparent that laboratories must determine the appropriate therapeutic range for their own APTT system used to monitor heparin therapy. Ideally this should be done by **simultaneously** determining the APTT and heparin concentration for samples from patients receiving heparin for the treatment of thromboembolism. A dose-response curve can be calculated from the data using regression analysis and the APTT values corresponding to a heparin concentration of 0.3 to 0.7 U/mL (by factor Xa inhibition assay) can be derived. The anti-Xa based assays have become the method of choice to determine heparin concentration. These assays have excellent sensitivity and specificity and are least affected by the variables listed above.

Procedure to determine heparin therapeutic range by Anti-Xa assay:

1. Obtain the heparin used in patient therapy from the pharmacy in order to calibrate the assay.

    a. Using normal saline, make appropriate dilutions until the Heparin concentration is 10 U/ml.

    b. Using Standard Human Plasma, make a 1.0 U/ml Heparin Stock Standard. This is used in place of the calibrator with the assay value being 1.0 U/ml.

Dilutions are as follows:

	Dilution	UFH	Saline	Concentration
Pharmaceutical Heparin Concentration: 10,000 U/mL (3 step dilution)	(1:10)	1.0 ml	9.0 ml	1000 U/mL
	(1:10)	1.0 ml from tube #1	9.0 ml	100 U/mL
	(1:10)	1.0 ml from tube #2	9.0 ml	10 U/mL
Pharmaceutical Heparin Concentration: 5,000 U/mL (3 step dilution)	(1:5)	1.0 ml	4.0 ml	1000 U/mL
	(1:10)	1.0 ml from tube #1	9.0 ml	100 U/mL
	(1:10)	1.0 ml from tube #2	9.0 ml	10 U/mL
Pharmaceutical Heparin Concentration: 1,000 U/mL (2 step OR 1 step dilution)	(1:10)	1.0 ml	9.0 ml	100 U/mL
	(1:10)	1.0 ml from tube #1	9.0 ml	10 U/mL
	OR	0.1 ml	9.9 ml	10 U/mL
Pharmaceutical Heparin Concentration: 100 U/mL				
	(1:10)	1.0 ml	9.0 ml	10 U/mL

Note: The stock heparin solution (10 U/mL in isotonic saline) is stable for 1 week at 2-8^{o} C.

2. Set up the anti-Xa assay on the Stago Compact Max analyzers

3. Collect citrated blood from 30-40 patients receiving unfractionated heparin therapy and also 5-10 normal patients.

4. Measure the PT/INR and APTT. If the INR is <1.3 this sample can be included in the study.

5. Measure the heparin concentration with the anti-Xa assay.

6. Perform a linear regression analysis with the trend line of the data with the heparin concentration on the x axis and the APTT result on the Y axis.

7. Using the corresponding regression equation determine the APTT values corresponding to the ex-vivo heparin results at 0.3 U/mL and 0.7 U/mL.

   Example:
   Regression equation Y=50 x + 60
   - APTT corresponding to 0.3 U/mL: 75 seconds
   - APTT corresponding to 0.7 U/mL: 95 seconds

   APTT values corresponding to the heparin therapeutic range are 75 – 95 seconds.

Note: All APTT testing should be performed on fresh samples. The heparin anti-Xa testing may be performed on frozen samples if the sample was double spun as described in the preparation of the fresh normal plasma pool. To thaw the frozen samples place them into a 37 degree centigrade water bath for 5-10 minutes.

D. Fibrinogen and D-Dimer reagents (Pre-calibrated tests)

1. Parallel testing of Fibrinogen and D-Dimer reagents should be conducted well in advance of the expiration of the old reagent.

2. Perform comparison studies between the old and new lot number using patient samples. Test at least 10 samples across the analytical measurement range of the analyzer. The R value for the correlation study should be R=>0.95.

3. Results are entered into templates provided by Stago and sent to Stago TSS to evaluate.

4. Fibrinogen Controls and D-Dimer controls should be run on all 3 shifts accumulating 40-50 runs. Assayed values have been established for each control and should not exceed +/- 3 SD's.

Note: Instrument comparison must be performed after any reagent lot change. See Protime, PTT, Fibrinogen, D-Dimer procedure in the Coagulation manual.

Policy Created by: _____ Date: _____

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REVISION HISTORY			
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Lead	Date	Coordinator/ Manager	Date	Medical Director	Date