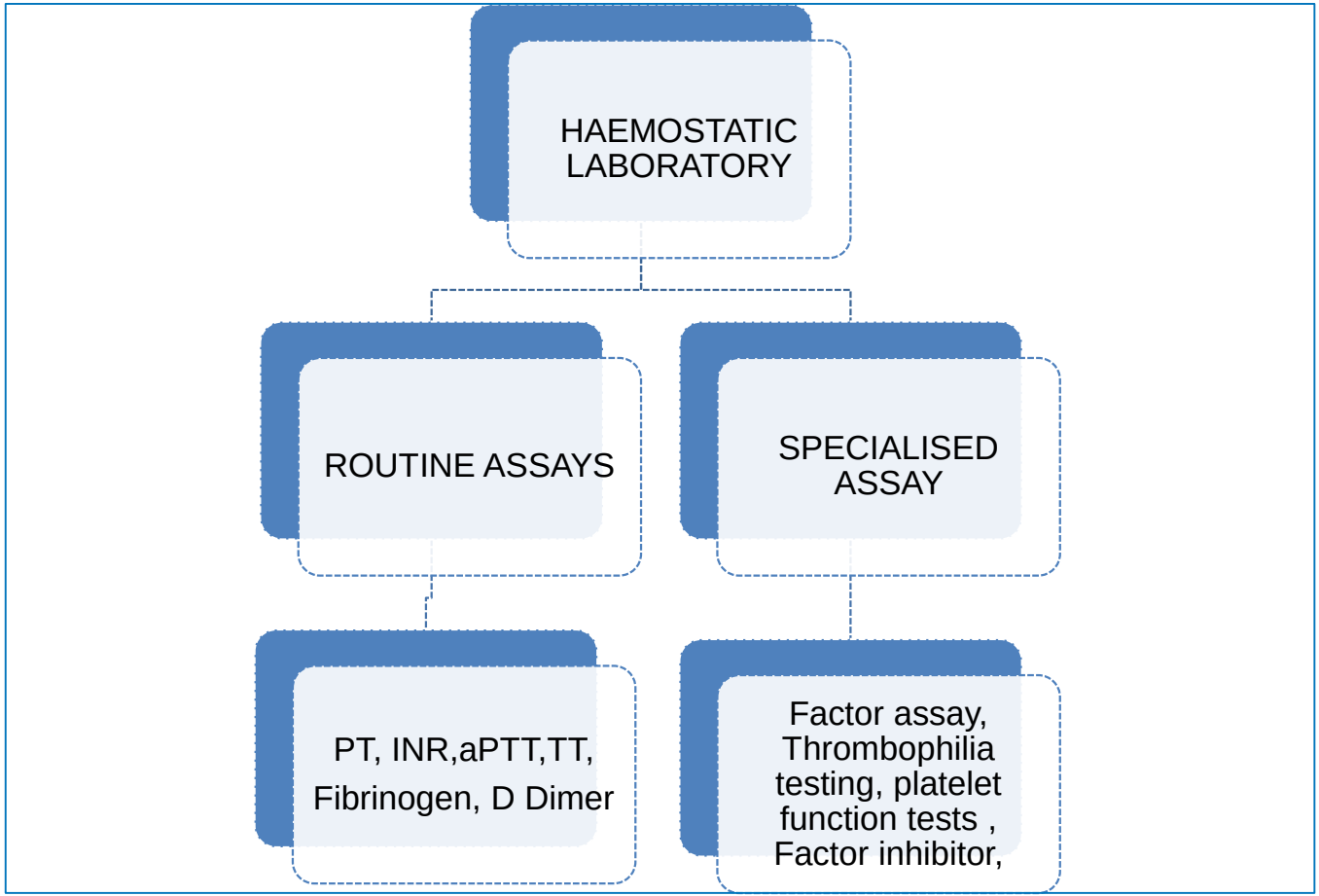
QUALITY PERSPECTIVES IN A COAGULATION LABORATORY

– FROM THEORY TO THE BENCH

DR . ARPITA ROY DAM
NATIONAL HEAD QUALITY ASSURANCE &
HEAD HEMATOLOGY SERVICES
AGILUS DIAGNOSTICS LTD



Lack of assay standardization together with nonharmonized test results between different measurement methods, can potentially lead to incorrect decision in patient's treatment.

Variability between laboratories performing coagulation tests with identical platforms: a nationwide evaluation study; Nagler et al. Thrombosis Journal 2013, 11:6

- ✓ Data from eight laboratories measuring fibrinogen twice in twenty healthy subjects with one out of 3 different platforms and single measurements of prothrombin time (PT), and coagulation factors II, V, VII, VIII, IX, X, XI and XIII were analysed.
- ✓ The variability for fibrinogen measurements within a laboratory ranged from 0.02 to 0.04, the variability between laboratories ranged from 0.006 to 0.097.
- ✓ Variance components that could be attributed to technicians or laboratory procedures were substantial, led to disappointingly low intraclass correlation coefficients for several factors and were pronounced for some of the platforms.
- ✓ The findings call for sustained efforts to raise the level of standardization of structures and procedures involved in the quantification of coagulation factors.

agilus diagnostics

ACLA American Clinical Laboratory Association

Home | Contact Us | Annual Meeting | Enter Your Search Terms...

ABOUT US | VALUE OF LAB TESTING | ISSUES | TAKE ACTION | NEWS | RESOURCES | JOIN US

ACLA NEWS

Like 5 Tweet 11

Importance of Clinical Lab Testing Highlighted During Medical Lab Professionals Week

April 17, 2014 *Categories: ACLA News, ACLA Press Releases, Featured News, News, Value of Labs*

Washington, D.C. – In recognition of the 300,000 medical laboratory professionals across the country who perform and interpret more than 10 billion laboratory tests annually, the American Clinical Laboratory Association (ACLA) today joined the American Society for Clinical Pathology in celebrating Medical Lab Professionals Week, held April 20-26, 2014.

"At a time when lab tests guide more than 70 percent of medical decisions and personalized medicine opens new windows to wellness, clinical lab professionals play an increasingly important role in today's healthcare system," said ACLA President Alan Mertz. "From early detection and diagnosis of disease to individualized treatment plans based on a person's unique genetic makeup, clinical lab testing is key to improving healthcare quality and containing long-term health costs."

NEWS BY TOPIC

- Reimbursement and Coverage
- Laboratory Developed Tests
- Patient Access to Lab Services
- Value of Labs
- IOAS Exception and Self-Referral
- Regulatory Issues

"lab tests guide more than 70 percent of medical decisions"

QUALITY IN COAGULATION LABORATORY

There are 2 parts to QA:

- Internal Quality Control [QC] - 'Are my results today the same as yesterday?'
- External Quality Assurance [EQA] - 'Are my results the same as other labs performing the same test?'

Internal QC is the monitoring of any haemostatic test performed in the laboratory to ensure that there is no day-to-day or within the day variation. Commonly this involves includes a statistical analysis of the tests that have been performed and the use of control materials with an assigned value e.g. a Factor VIII standard.

External QA involves the evaluation of the performance of the laboratory in a particular test or tests by an external agency e.g. UK NEQAS, CAP , CMC Vellore etc. Such schemes are usually organised on a national or international bodies and the analysis is retrospective with a comparison of labs within the peer group.

QUALITY BEGINS AND ENDS WITH THE PATIENT

PRE-ANALYTICAL
ANALYTICAL
POST-ANALYTICAL

LABORATORY QUALITY ASSURANCE PROGRAM

➢ PRE EXAMINATION PHASE

- Standardization of collection procedure
- Specimen collection
- Specimen mixing
- Sample integrity
- Operator training/ competency

➢ EXAMINATION PHASE

- Calibrations of equipment
- Daily Quality Control
- Linearity & dilutions (where applicable)
- Test limitations & instrument flaggings

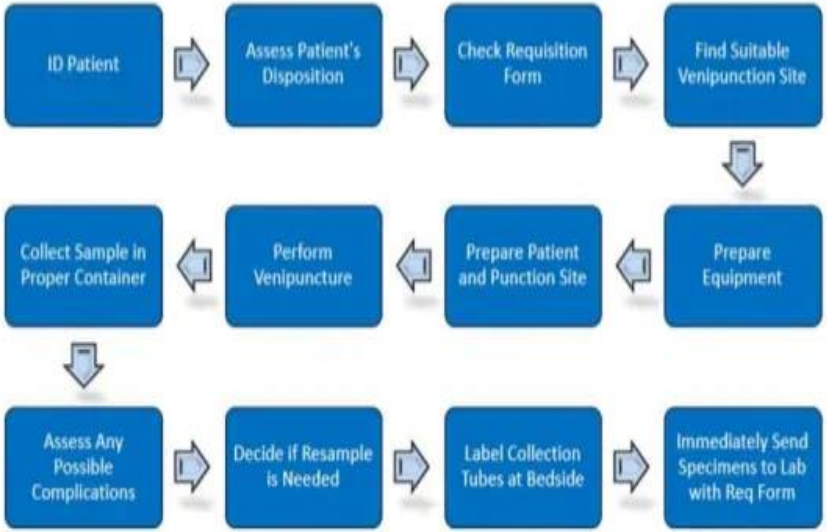
➢ POST EXAMINATION PHASE

- Delta checks
- EQA / proficiency testing
- RCA and CAPA of nonconformities

QUALITY MANAGEMENT IN PRE-ANALYTICAL PHASE

RIGHT QUALITY STARTS WITH THE RIGHT SAMPLE !!

BLOOD COLLECTION PROCEDURE



7

Phlebotomy Technique: Recommended Order of Draw[1]

Closure Colour	Collection Tube	Mix by Inverting
1	Sterile samples (e.g., Blood Cultures)	
2	Citrate tubes*	3 to 4 times
	ACD tubes	8 to 10 times
3	SST™II Gel tubes	8 to 10 times
	Plain Serum tubes (with clot activator)	8 to 10 times
4	PST™II Gel tubes	8 to 10 times
	Heparin tubes	8 to 10 times
5	EDTA tubes	8 to 10 times
6	Fluoride Oxalate and Fluoride EDTA (glucose tubes)	8 to 10 times

TABLE 2. EFFECTS OF ANTICOAGULANTS ON COAGULATION TEST RESULTS

Medication	Mechanism of action	Test result if anticoagulant present	Test to quantify drug (if needed)
Heparin	Enhances antithrombin effect on thrombin and factor Xa	Prolongs APTT	APTT or drug-specific anti-Xa assay
Low-molecular-weight heparin	Enhances antithrombin effect on factor Xa	May prolong APTT but cannot be reliably excluded even with normal PT/APTT	Drug-specific anti-Xa assay
Apixaban	Factor Xa inhibitor	May prolong PT but cannot be reliably excluded even with normal PT/APTT	Drug-specific anti-Xa assay
Rivaroxaban	Factor Xa inhibitor	Prolongs PT (with sensitive reagent)	Drug-specific anti-Xa assay
Dabigatran	Direct thrombin inhibitor	Prolongs TT* and APTT	Dilute TT

Abbreviations: APTT = activated partial thromboplastin time; factor Xa = activated factor X; NOAC = nonvitamin K oral anticoagulant; PT = prothrombin time; TT = thrombin time.
* TT measures the time taken after addition of exogenous thrombin for conversion of fibrinogen to fibrin, the final step in clot formation, common to the intrinsic and extrinsic pathways.

PRE-ANALYTICAL VARIABLES IN COAGULATION SAMPLES

- The current CLSI guidelines favor the use of the lower **citrate (3.2%)** concentration for most coagulation assays, Specimens collected in 3.8% buffered sodium citrate may overestimate the PT and APTT and underestimate fibrinogen.
- Tubes should be **adequately filled** (to the mark noted on the tube if provided) or to no less than 90% of this total volume. Under-filling may cause significant sample dilution and may also provide falsely prolonged clotting times due to the excess calcium-binding citrate present.
- Too high a **hematocrit** will influence the anticoagulant to plasma ratio and thus test results in coagulation samples An adjustment in the ratio of anticoagulant solution/volume of blood at different packed cell volume when hematocrit values are above 55% may be undertaken using CLSI recommendations, although a simplified method is to remove 0.1 mL of sodium citrate from a 5 mL 3.2% sodium citrate evacuated tube prior to collection.
- Coagulation tests (specially aPTT based) to be performed within 4 hrs from collection. If aPTT based testing cannot be performed within these times, platelet-poor plasma should be removed from the cells by double centrifugation and frozen at –20°C for up to 2 weeks or at –70°C for up to 12 months.
- Prior to testing, samples should be completely thawed in a 37 C water bath (for about 5 minutes) and mixed thoroughly prior to testing.
- Platelet-poor plasma should have a residual platelet concentration of less than 10 X 10 9/L.
- *Collection of blood for coagulation testing through intravenous lines that have been previously flushed with heparin should be avoided*
- *All coagulation samples to be checked for clots before assay*

PRE-ANALYTICAL VARIABLES IN COAGULATION SAMPLES

- The pre-analytical phases of testing include specimen collection, transportation of whole blood specimens to the laboratory, specimen processing and storage
- One of the most critical aspects of specimen collection is proper patient and also sample identification
- Samples should be collected in a relatively non- traumatic manner and blood should flow freely in to the specimen container. Closed vacuum vacutainer system is preferable 3.2% Sodium Citrate vials
- The blood and anticoagulant must be promptly and adequately mixed to avoid *in vitro* clot formation.
- The required blood to anti-coagulant ratio is 9:1 and therefore overfilling or under filling of the evacuated tube should be avoided as this can introduce result error.
- EDTA or heparin anticoagulated tubes for haemostasis testing will lead to aberrant results
- When the sample matrix is unknown or in doubt, performance of Na, K and Ca assays on the sample can be undertaken to help determine the sample matrix. Samples in sodium citrate tend to have high sodium and low calcium values while samples in potassium EDTA will have elevated potassium levels with undetectable calcium values
- PT/INR evaluation are stable at room temperature for 24 hrs. Samples can be stored as whole blood or centrifuged.
- Samples for APTT and for special coagulation assays should be maintained at room temperature if testing will be complete within four hrs. Samples that can not be tested in this time frame should be spun and the plasma frozen in a freezer that does not undergo automatic freeze thaw cycles.

1. For coagulation screening assays laboratories should evaluate acceptable concentration of interfering substances for their own system (reagent/ coagulometer).

2. If intravascular or in vivo haemolysis is suspected, along with the results appropriate notation on the test report (intravascular haemolysis) should be given.

3. Use of mechanical and/or electromechanical clot detection methods is recommended whenever it is possible for samples that contain substances interfering with light transmission (Haemolysis, hyperbilirubinemia and lipemia)

4. Thawing of frozen plasma samples should be performed rapidly, for 10 minutes, at 37 °C in an incubator, dry thermo block or water bath.

5.To ensure sample integrity prior to testing, thawed sample should be thoroughly mixed by the gentle inversion of sample for 180° and return to starting position for 6-times.

QUALITY MANAGEMENT IN ANALYTICAL PROCESS

ANALYTICAL – ACCURACY VS PRECISION

1. **Accuracy**: Accuracy is a measure of how close reported test results are to the “true” value, which is typically a target defined by a “gold standard” test

2. **Precision**: Precision is a measure of how close test results are to each other when they are repeated multiple times on the same sample.

QUALITY ASSURANCE INSIDE LAB

- VALIDATION & VERIFICATION OF EQUIPMENT
- STANDARDIZATION OF METHOD & REAGENTS
- REFERENCE INTERVAL CHECKS
- CALIBRATION OF EQUIPMENT INCLUDING
- CALIBRATION OF NON-CRITICAL LAB EQUIPMENTS LIKE PIPETTES,TIMERS,CENTRIFUGE ETC
- INTERNAL QUALITY CONTROL
- EXTERNAL QUALITY ASSESSMENT (EQA)
- STATISTICAL ANALYSIS OF LAB DATA
- MONITORING DEVIATIONS ,NON-CONFIRMITIES IN LAB
- PERFORMING ELABORATE ROOT CAUSE ANALYSIS AND EFFECTIVE CAPA FOR OUTLIERS

Name of Laboratory : [redacted]
City Gurgaon

Verification Report No : 2324-10

Test/Equipment/Method Under Verification : Coagulation Analyser

Verification Report Issued on : 05.03.2024

3. Test Verification Protocol		
Experiment	**Experiment Design (Protocol)**	**Acceptance Criteria**
1. Precision A. Intra Assay B. Inter Assay	A. 1 Abnormal Sample 10 times B. 1 Abnormal Sample 3 run with 4 Replicates	CV % < 10 both Experiment Interpretation should be Concordant
2. Accuracy	Level 1 and Level 2 Control 10 times	r^2>0.95
3. Biological Reference Interval Verification	20 Normal	90 % population of total data should must be within the manufacturer Biological reference interval.

Sr. No	Parameters	Intra-Assay Variation	Inter-Assay Variation	Accuracy	BRI
		% C.V. < 10%	% C.V. < 10%	r^2> 0.95	95 % Population Acceptance with Manufacturer
	Factor IX	2.87	5.41	r^2 0.9793 SE : 3.19	100%
	Protein C	4.43	2.55	r^2 0.9708 SE : 4.14	100%
	Protein S	8.29	5.65	r^2 0.9628 SE : 3.54	100%
	DRVV S	2.35	2.50	r^2 0.9999 SE : 1.25	100%
	DRVV C	2.47	0.97	r^2 0.9833 SE : 0.61	100%
	ANTI-T III	2.11	2.94	r^2 0.9954 SE : 1.77	100%
	TT	1.18	1.68	NA SE : 0.00	100%

INTERNAL QUALITY CONTROLS & CALIBRATION

- **Commercial IQC material using two different levels are to be used for all coagulation tests –**
  - ✓ each eight hours of patient testing
  - ✓ each time there is a change in reagents
  - ✓ more frequently if specified in manufacturer's instructions, laboratory procedure,

- *Calibration and verification of calibration of methods must be performed following the manufacturer's instructions, at minimum, including the number, type, and concentration of calibration materials, frequency of calibration, and criteria for acceptable performance*

- *Laboratories must either recalibrate or perform calibration verification at least every six months and if any of the following occur:*
  1. *At changes of reagent lots unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client results*
  2. *If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range*
  3. *After major preventive maintenance or change of critical instrument component*
  4. *When recommended by the manufacturer*

- Run quality controls everyday, before running the patient's sample
- Plot Levy Jennings Chart , using Standard Deviation, CV , MU.
- Daily checks and regular reviews of QC data along with trends
- Use Westgard rules to evaluate the QC values as acceptable or not.
- RCA & CAPA on outliers as well as trend analysis for bias estimation
- Define your "warning" and " rejection" westgard rules

POINTERS TO RIGHT ANALYTICS

- For PT, ISI value should be appropriate to the particular PT reagent and instrumentation used.
- The calculation of the INR is adjusted using the appropriate ISI value for every new lot of PT reagent, changes in types of reagent, or change in instrumentation. INR=(PT of patient / PT of geometric mean normal population)*ISI*
- The appropriate **geometric mean of the PT reference interval is used in the INR calculation (MNPT).**
- There are checks of patient reports for correct INR calculations, patient values, and reference intervals under the following circumstances.
  1. Change in lot or type of PT reagent
  2. Change in instrument
  3. Establishment of new PT reference interval
  4. Change in INR calculation
  5. At defined intervals, in the absence of the above changes
- **Reference intervals for PT and aPTT should be current for the reagent or lot number, and are appropriately determined.**

STANDARDIZATION OF ROUTINE COAGULATION TESTS

PROTHROMBIN TIME

- Recombinant thromboplastins with ISI values below 1.2 should be used for PT determination.
- ISI value for a specific combination of thromboplastin/ coagulometer should be used whenever it is possible.
- MNPT calculation with every new lot of reagent
- INR calculation checks on equipment to be done
- Reference ranges to be verified with reagent lot change

FIBRINOGEN

- Fibrinogen functional assay is the most reliable method for routine use in clinical laboratory.
- Fibrinogen immunoassays that measure concentration of fibrinogen rather than its functional activity are recommended in differential diagnosis of dysfibrinogenemia.
- Results of fibrinogen activity test should be expressed as g/L.

D-DIMER

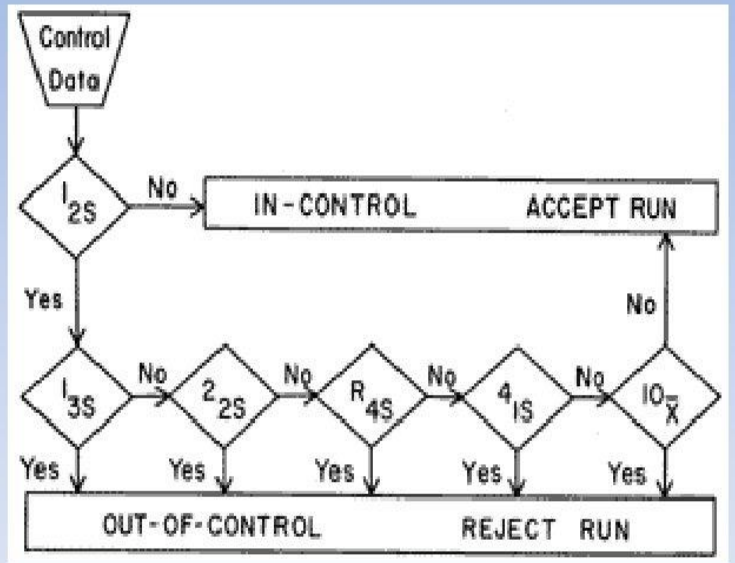
1. D-dimer results should be expressed in mg/L DDU or FEU.
2. D-dimer measurement method in use should be declared on the test report along with the test result.
3. Assay kits that do not provide information about the type of unit used (DDU or FEU) in the assay should not be used.

aPTT

1. To performing aPTT as a screening test, it is recommended to use aPTT reagents sensitive to factor deficiency, but at the same time it does not have to be sensitive to LA.
2. aPTT assay is an appropriate assay for UFH therapy monitoring.
3. If aPTT is used for UFH therapy monitoring, general recommendation for laboratories is to test the sensitivity of aPTT reagents in order to establish appropriate therapeutic interval.
4. aPTT should not be used for LMWH monitoring. For estimating LMWH therapeutic response anti-Xa assay should be used exclusively.
5. The reference ranges should be verified with reagent lot change
6. The aPTT ratio should always be reported together with the result expressed in seconds.

WESTGARD RULES FOR QC

Westgard Multirule QC

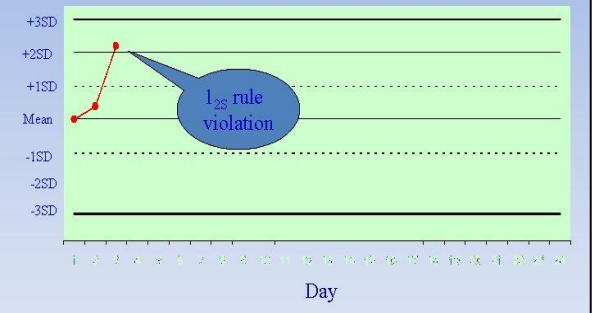


Westgard Rules

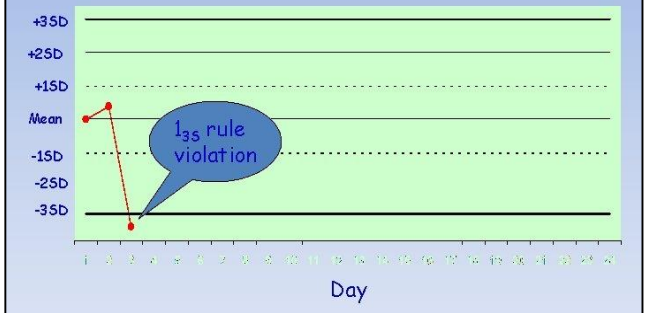
(Generally used where 2 levels of control material are analyzed per run)

- 1_{2S} rule
- 1_{3S} rule
- 2_{2S} rule
- R_{4S} rule
- 4_{1S} rule
- $10_{\bar{X}}$ rule

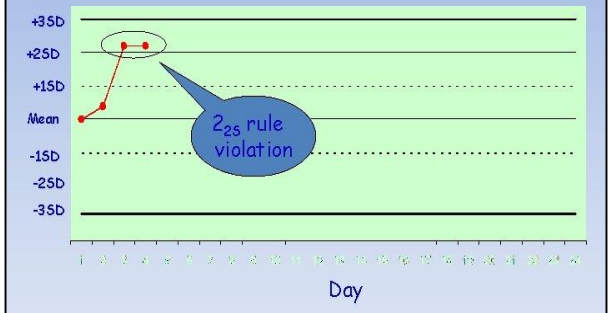
1_{2S} Rule = A warning to trigger careful inspection of the control data



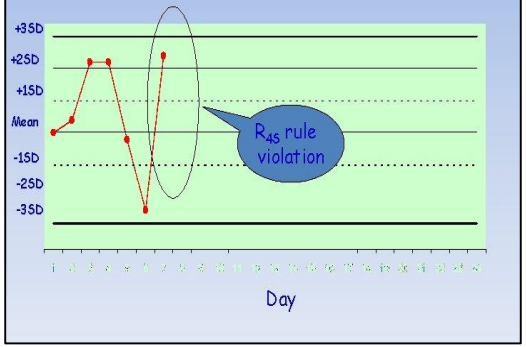
1_{3S} Rule = Reject the run when a single control measurement exceeds the +3SD or -3SD control limit



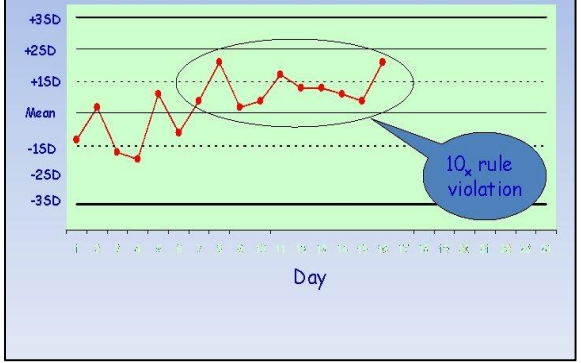
2_{2S} Rule = Reject the run when 2 consecutive control measurements exceed the same +2SD or -2SD control limit



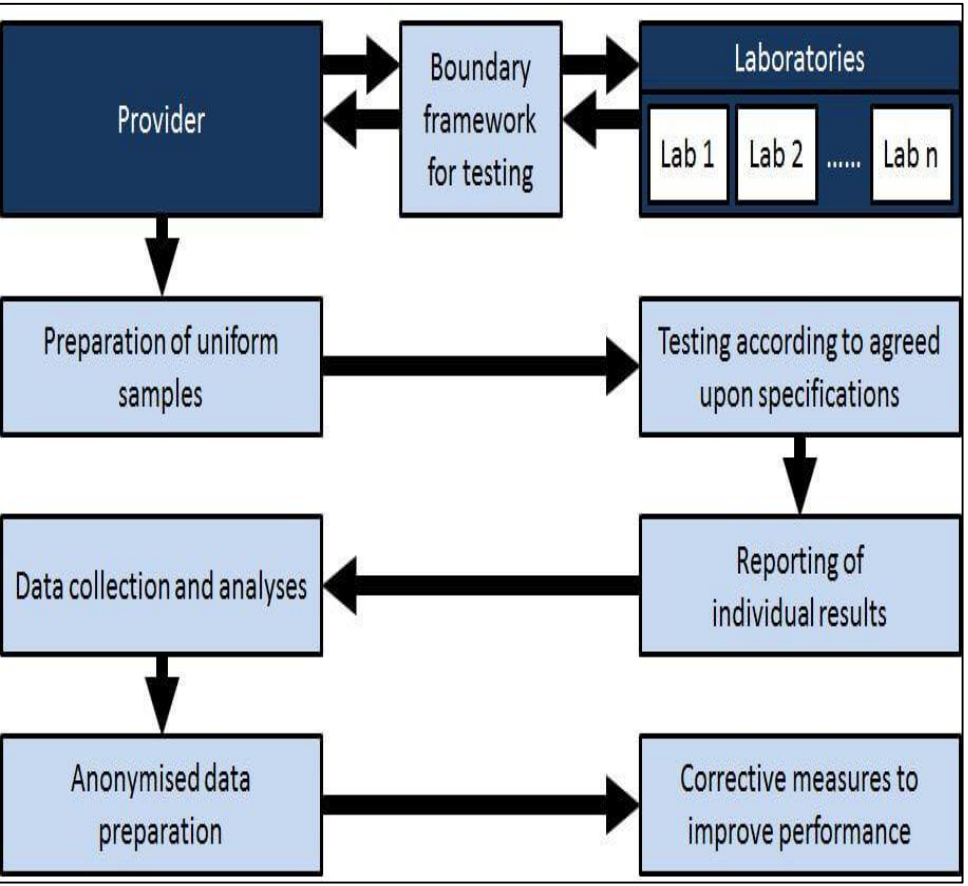
R_{4S} Rule = Reject the run when 1 control measurement exceed the +2SD and the other exceeds the -2SD control limit



10_x Rule = Reject the run when 10 consecutive control measurements fall on one side of the mean



EXTERNAL QUALITY ASSESSMENT PROGRAM / PROFICIENCY TESTING



- The primary aim of a PT is to provide an objective evidence of competence and analytical performance of a participating lab.
- Provides quantitative data covering all the aspects of a lab functioning – reception of sample , storage conditions, processing procedures, calibrations and maintainence of equipment, data generation, result reporting etc.
- Can be a measure of accuracy and precision too
- Report data received from PT provider may act as a effective educational and training tool
- Provides lab a tool to check for BIAS against other peer group labs and do RCA & CAPA

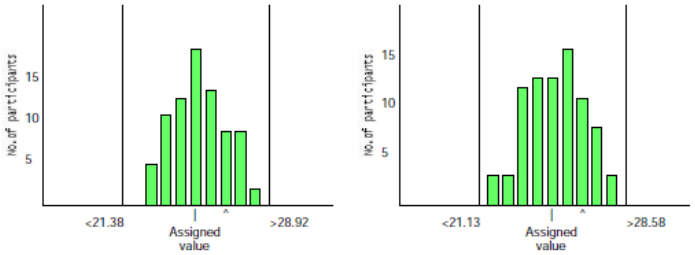
EQA data analysis

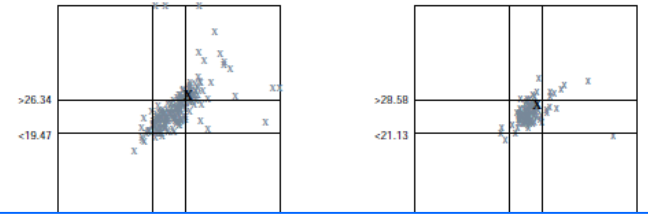
ISHBT-CMC EQAS — HAEMOSTASIS MODULE REPORT

Participant # [redacted] **Survey # 602024**

Parameter **Prothrombin Time**

Group	Prothrombin Time Regent Stago Neoplastin CI Plus	
Sample #	602024 C1	602024 C2
Your result (secs)	26.70	26.50
Your peer group Assigned Value & LAP	25.15(21.38-28.92)	24.86(21.13-28.58)
Limits of acceptable performance	Assigned value ±15%	Assigned value ±15%
Number of Participants	96	96
Coefficient of Variation CV (%)	7.14	7.84
Your performance	Within consensus	Within consensus





Important: 2025 Program-specific CLIA updates enclosed

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

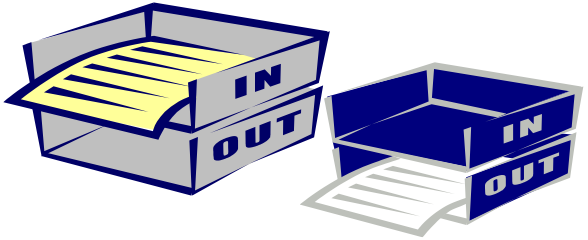
Surveys and Anatomic Pathology Education Programs

Coagulation, Limited
CGL-A 2024
Participant Summary
1.0 Credit of Continuing Education Available

Analyte	Target Value	Evaluation Criteria
Activated Partial Thromboplastin Time	Peer Group	± 15%
D-dimer, Quant	Peer Group	± 3 SD
Fibrinogen	Peer Group	± 20%
International Normalized Ratio	Peer Group	± 20%
Prothrombin Time	Peer Group	± 15%
Special INR Evaluation	If at least 80% of the reported INRs are within ± 0.2 of the calculated INR, the result is Acceptable Performance.	

QUALITY MANAGEMENT POST –ANALYTICAL PHASE

Information Management System - LMS



MANUAL
TRANSCRIPTION

PATIENT INFORMATION

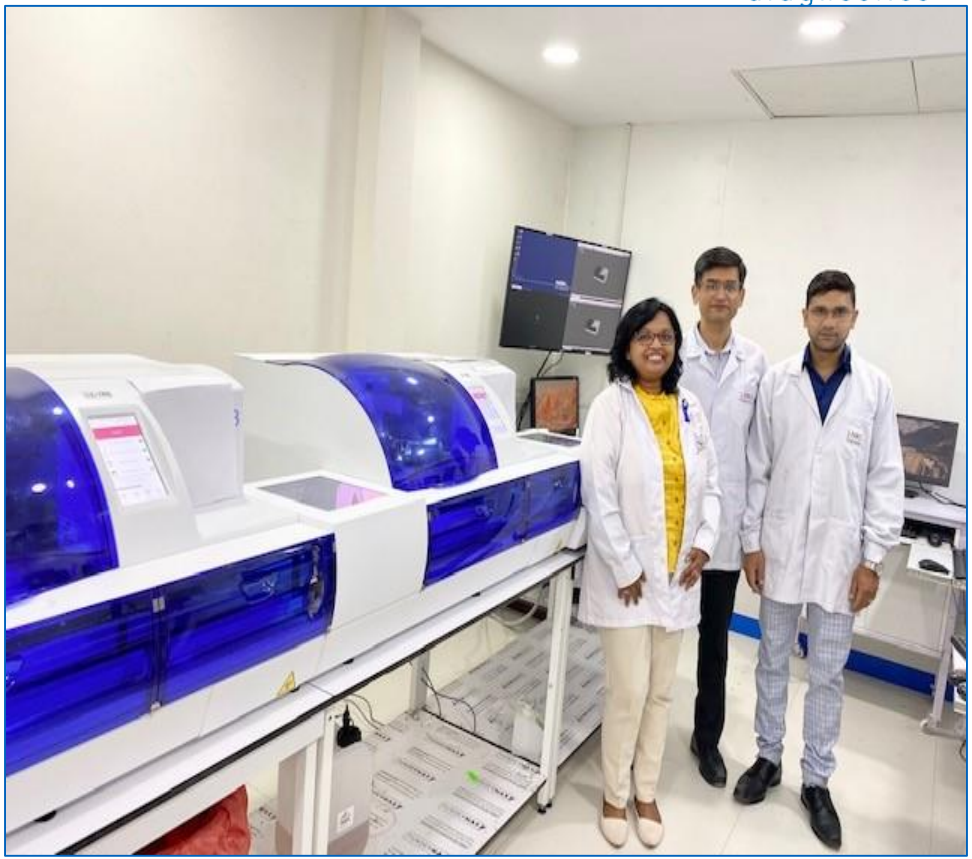
Quality Lab Report

- **accessible**
- **accurate**
- **timely**
- **secure**
- **confidential**
- **private**

FEW POST-ANALYTICAL CHECKS

- If reference intervals are adopted from the literature or manufacturer, it is recommended to verify if such intervals are appropriate for a local population.
- The use of age-adjusted reference intervals is critical for ensuring proper management of children with thrombosis or bleeding disorders.
- LIS interfacing verification to be done at a laboratory defined interval
- Interpretative comments, related to the both, preanalytical and analytical phases of testing,are strongly recommended and should be an integral part of the test report
- Coagulation laboratories are encouraged to define locally clinical relevant critical limits and/ or expand the existing list of critical values in conjunction with local clinical opinion.
- The first critical result should be reported immediately to the physician, and depending on the agreement with the clinical staff, reporting of the each following critical results for the same patient should be managed.

THE HEMOGLOBIN TEAM- AGILUS REFERENCE LABORATORY , GURGAON



REFERENCES

- Bonar et.al.Quality in coagulation and haemostasis testing; *Biochemia Medica 2010;20(2):184–99*
- *CAP guidelines Hematology & Coagulation Checklist*
- Nagler et al. Variability between laboratories performing coagulation tests with identical platforms: a nationwide evaluation studyThrombosis Journal 2013, 11:6
- *Bronić A. et al.,Recommendations for coagulation screening assays and D-dimer : Biochem Med (Zagreb) 2019;29(2):**020503***

agilus diagnostics

Thank You!