thers.com, for detailed information on biochemistry books, visit our website www.jaypeebrothers.com, for detailed information on biochemistry books

# Practical Clinical Biochemistry Methods & Interpretations

As per the Competency-based Medical Education Curriculum (NMC)

# Ranjna Chawla







# Contents

1.	HAZARDS IN THE CLINICAL BIOCHEMISTRY LABORATORY	1
	<ul> <li>Hazards from Dangerous Chemicals</li> <li>Infection Hazards</li> </ul>	1
	<ul> <li>Management of Laboratory Waste</li> <li>First Aid and Emergency Treatment in the Laboratory</li> </ul>	4 5
2.	SPECIMEN COLLECTION AND PRESERVATION	9
	<ul> <li>Taking Care of the Intra- and Extra-laboratory Factors for Reliable Results</li> <li>Collection and Preservation of Biological Fluids</li> <li>Method of Taking Blood Specimen and Separating the Serum Aseptically</li> <li>Precautions for Taking Blood Sample</li> <li>Anticoagulants Used in Biochemical Analysis</li> <li>Special Blood Containers (Vacutainers)</li> <li>Specimen Type</li> <li>Preservation, Storage, and Transport of Blood Sample</li> <li>Common Changmat that Oppuring Blood Sample</li> </ul>	9 11 11 12 13 14 15
_	Common Changes that Occur in Blood Samples	16
3.	QUALITY CONTROL IN CLINICAL LABORATORY	21
4.	<ul> <li>To Assess the Validity of Results on Patient Specimens</li> <li>Type of Material Used for Quality Control</li> <li>Evaluation of Quality Control Results</li> <li>Target Average Value of Quality Control Pool</li> <li>Temporary Target Average and Final Target Average</li> <li>Frequency of Analysis of Quality Control Sample</li> <li>Monitoring Quality Control Data</li> <li>Calibration and Calibration Materials</li> <li>Evaluation of Daily, Weekly, and Monthly Results to Maintain Accuracy and Precision</li> <li>Internal Quality Control Program</li> <li>External Quality Control Program</li> <li>Water Quality for Accuracy of Laboratory Results</li> </ul>	21 21 22 23 23 24 26 26 26 27 28 35 <b>40</b>
5.	REVIEW OF ANALYTICAL CHEMISTRY	48
	<ul> <li>Concentration</li> <li>Dilution Problems</li> <li>Plasma Osmolality</li> <li>Urine Osmolality</li> </ul>	48 49 49 51
6.	DETERMINATION OF PH	52
	<ul> <li>pH and its Significance</li> <li>Buffers</li> <li>Methods for Determination of pH</li> <li>Operation of pH Meter</li> </ul>	52 52 52 53

Contents
----------

7.	TESTS FOR CARBOHYDRATES	56
4	Tests for Carbohydrates	56
4	Reactions of Disaccharides	60
4	Reactions of Polysaccharides	60
4	<ul> <li>Scheme for Identification of Unknown Carbohydrates</li> </ul>	62
8.	PRECIPITATION REACTIONS FOR PROTEINS	63
4	Precipitation by Salts	63
4	Isoelectric Precipitation	63
•	Precipitation by Organic Solvents	64
	Precipitation by Actor Agents	64
	Precipitation by Heavy Metahons     Precipitation by Heavy Metahons	64
9.	COLOR REACTIONS FOR PROTEINS	66
•	General Reactions of Proteins	67
•	<ul> <li>Scheme for Identification of an Unknown Protein</li> </ul>	71
10. <sup>-</sup>	TESTS FOR LIPIDS	72
	Qualitative Tests for Linids	
	Test for Unsaturation (Bromination Test)	72
	Oualitative Tests for Glycerol	73
4	Qualitative Tests for Cholesterol	74
11. (	GASTRIC JUICE ANALYSIS	75
4	Qualitative Analysis of Gastric Juice	75
4	<ul> <li>Examination of the Specimen Volume and Appearance</li> </ul>	75
•	<ul> <li>Determination of Free and Total Acidity</li> <li>Interpretation</li> </ul>	75 77
<b>12.</b>	PHYSICAL EXAMINATION OF URINE	79
•	Specimen Collection and Preservation	79
•	Composition of Normal Urine	80
4	Physical Examination of Urine Color	80
13.	CHEMISTRY OF NORMAL URINE	84
4	Tests for Inorganic Constituents	84
4	Tests for Organic Constituents	84
14.	ABNORMAL CONSTITUENTS OF URINE	86
4	Abnormal Constituents of Urine	86
4	Dipstick Test	91
4	Diseases Identified	92
15.	URINARY ASCORBIC ACID	96
4	Ascorbic Acid Saturation Test	96
4	Determination of Ascorbate by Titration with 2,6-Dichlorophenolindophenol	97
16.	REDUCING SUGARS IN URINE	99
4	Benedict's Method for Estimation of Reducing Sugar in Urine	99
4	<ul> <li>Other Reducing Substances in Urine</li> </ul>	100

### xii

Contents	xiii
17. HEMOGLOBIN AND ITS DERIVATIVES	103
<ul> <li>Detection of Hemoglobin and its Derivatives</li> </ul>	104
<ul> <li>Spectroscopic Study of the Pigments</li> </ul>	104
<ul> <li>Preparation of Hemin Crystals</li> </ul>	106
Ouantitative Determination of Hemoglobin	106
Hemoglobin and Related Chromoprotein	107
Hemoglobin that related enronoprotein     Hemoglobin Electrophoresis	108
Normal Hemoglobin Values	109
18. SPECTRAL TECHNIQUES	111
Absorption Spectroscopy	111
<ul> <li>Standard Curve (Calibration Curve)</li> </ul>	113
<ul> <li>Type of Instruments</li> </ul>	113
<ul> <li>Essential Parts of Photocolorimeter</li> </ul>	114
19. AUTOMATION	119
<ul> <li>Classification of Analyzers</li> </ul>	119
<ul> <li>Profiles of Analyzers</li> </ul>	120
<ul> <li>Benefits of Analyzers</li> </ul>	123
Total Lab Automation	123
<ul> <li>Integrated Modular Preanalytical System Components</li> </ul>	124
Autoverification	125
20. LIVER FUNCTION TESTS	127
♦ Liver Function Tests	127
<ul> <li>Serum Enzymes in Liver Diseases and Jaundice</li> </ul>	127
Synthesis	128
Detoxification	128
Bromsulfthalein (BSP) Excretion Test	128
Henatitis Markers	130
<ul> <li>Hepatitis A Virus (HAV)</li> </ul>	130
<ul> <li>Hepatitis R Virus (HRV)</li> </ul>	130
<ul> <li>Hepatitis B Virus-DNA (HBV-DNA)</li> </ul>	130
<ul> <li>Hepatitis C Virus (HCV)</li> </ul>	137
<ul> <li>Hepatitis D Virus (HDV)</li> </ul>	132
<ul> <li>Hepatitis E Virus (HEV)</li> </ul>	132
21. CARDIAC FUNCTION TESTS	137
<ul> <li>Test for Coronary Heart Disease Risk Evaluation</li> </ul>	137
<ul> <li>Diagnostic Indicators of Myocardial Infarction</li> </ul>	138
22. KIDNEY FUNCTION TESTS	155
Tests of Glomerular Function	155
Tests of Tubular Function	156
23. PANCREATIC FUNCTION TESTS	163
Functions of the Pancreas	163
Pancreatic Juice	163
External Secretion of Pancreas	164
<ul> <li>Tests for Pancreatic Diseases</li> </ul>	164

24. THYROID FUNCTION TESTS	171
<ul> <li>Current Methods for Measuring Thyroid-related Hormones</li> <li>Enzyme Immunoassay for the Quantitative Determination of</li> </ul>	171
<ul> <li>Thyrotropin (TSH) and Total T4 and T3</li> <li>Assessment of Thyroid Function and Interpretation</li> </ul>	172 172
25. TUMOR MARKERS	178
<ul> <li>Overview of Role of Laboratory Tests</li> </ul>	178
<ul> <li>Classes of Biochemicals Used as Tumor Markers</li> </ul>	179
<ul> <li>Types of Analytes Used</li> </ul>	179
Other Proteins	181
<ul> <li>Enzymes</li> <li>Matheodology Llood for Turner Merkers</li> </ul>	182
Methodology used for fumor markers	183
26. SERUM TOTAL PROTEINS AND ALBUMIN-GLOBULIN RATIO	187
Serum Proteins Investigation	187
<ul> <li>Serum Protein Estimation by Biuret Method</li> </ul>	187
<ul> <li>Calculation of Albumin–Globulin Katio</li> <li>Quantitative Estimation of Albumin by RCC Mathed</li> </ul>	188
	189
27. SERUM ALKALINE AND ACID PHOSPHATASE	196
<ul> <li>Alkaline Phosphatase</li> </ul>	196
<ul> <li>Serum Alkaline Phosphatase Estimation</li> </ul>	196
<ul> <li>Photometric Determination of Alkaline Phosphatase</li> <li>Asid Phosphatase</li> </ul>	197
Acid Phosphatase	199
28. SERUM AMINOTRANSFERASES (TRANSAMINASES)	202
<ul> <li>Determination of Aminotransferases</li> </ul>	202
<ul> <li>Levels of Aminotransferases in Different Tissues</li> </ul>	204
29. SERUM AMYLASE	206
<ul> <li>α-Amylase</li> </ul>	206
<ul> <li>Serum Amylase Estimation</li> </ul>	206
<ul> <li>Amylase (CNPG3 Method)</li> </ul>	207
<ul> <li>Increased Plasma Amylase</li> </ul>	207
<ul> <li>Decreased Plasma Amylase</li> </ul>	208
30. SERUM BILIRUBIN	209
Determination of Serum Bilirubin	209
<ul> <li>Determination of Total and Direct Bilirubin</li> </ul>	210
<ul> <li>Determination of Serum Bilirubin by the Method of Jendrassik and Grof</li> </ul>	210
<ul> <li>Retention Jaundice (Unconjugated Hyperbilirubinemia)</li> </ul>	211
Regurgitation Jaundice     Missed Line activities are in	212
Mixed Hyperbillrubinemia     Livinary Einding In Jaundico	212
	212
31. BLOOD UREA AND UREA CLEARANCE	218
<ul> <li>Choice of the Sample</li> </ul>	218
Determination of Blood Urea	218
<ul> <li>Urinary Urea</li> <li>Interpretation</li> </ul>	221
▼ interpretation	222

### xiv

	Contents	xv
32.	SERUM CREATININE AND CREATININE CLEARANCE	224
	<ul> <li>Determination of Serum Creatinine (Jaffe's Alkaline Picrate Method)</li> </ul>	224
	<ul> <li>Estimation of Creatinine (Jaffe's Reaction without Deproteinization, Kinetic Method)</li> </ul>	225
	Urinary Creatinine	226
	Creatinine Clearance	226
33.	SERUM URIC ACID	230
	<ul> <li>Determination of Serum Urate (Caraway's Method)</li> </ul>	230
	♦ Other Methods of Estimation	231
	♦ Hyperuricemia	232
	◆ Hypouricemia	233
	Determination of Urate in Urine	233
34.	BLOOD SUGAR	235
	Choice of Blood Specimen	235
	♦ Glucose Oxidase Peroxidase Method	236
	♦ Glucose Hexokinase Method	236
	<ul> <li>Alkaline Copper Reduction Method</li> </ul>	236
	Ferricyanide Method	238
	♦ Hyperglycemia	239
	♦ Causes of Insulin Resistance	240
	<ul> <li>Capillary Blood Glucose (CBG) Test</li> </ul>	241
35.	GLUCOSE TOLERANCE TEST	249
	Oral Glucose Tolerance Test	249
	<ul> <li>Extended Oral Glucose Tolerance Test</li> </ul>	252
	<ul> <li>Cortisone Stressed Glucose Tolerance Test</li> </ul>	252
	<ul> <li>Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT)</li> </ul>	252
	◆ Factors Affecting Glucose Tolerance Test	252
	<ul> <li>Some Special Tests for Diagnosis of Diabetes</li> </ul>	253
36.	BLOOD LIPIDS	258
	Ultracentrifugation and Electrophoresis	258
	<ul> <li>Precautions for Taking Blood Samples for Lipid Analysis</li> </ul>	258
	♦ Cholesterol	259
	<ul> <li>Determination of HDL Cholesterol</li> </ul>	262
	<ul> <li>Determination of LDL Cholesterol</li> </ul>	263
	<ul> <li>Trialycerides (TG)</li> </ul>	265
	<ul> <li>Risk Factors of Coronary Heart Disease</li> </ul>	266
37.	SERUM INORGANIC PHOSPHORUS	281
	Phosphorus in Blood	281
	<ul> <li>Determination of Inorganic Phosphate in Serum</li> </ul>	281
	♦ Hyperphosphatemia	283
	<ul> <li>Hypophosphatemia</li> </ul>	283
	<ul> <li>Phosphate Excretion</li> </ul>	283
38.	SERUM CALCIUM	285
	Determination of Total Serum Calcium	285
	<ul> <li>Estimation of Total Serum Calcium by O-Cresolphthalein Complexone</li> </ul>	
	(Endpoint Colorimetric Method)	285

Contents	
----------	--

<ul> <li>♦ Нуро</li> <li>♦ Нуре</li> </ul>	calcemia rcalcemia	287 287
39. SERUM	AND URINE CHLORIDE	290
<ul><li>Choic</li><li>Deter</li><li>Urine</li></ul>	e of Specimen mination of Serum Chloride Chloride	290 290 292
40. CEREBR	OSPINAL FLUID	293
<ul> <li>Exam</li> <li>Deter</li> <li>Deter</li> <li>Deter</li> <li>Deter</li> </ul>	ination of CSF mination of Total Protein mination of Globulins mination of Glucose mination of Chloride	293 295 295 296 297
41. ANALYS	IS OF FOODSTUFFS	299
<ul> <li>Anim</li> <li>Milk /</li> <li>Egg /</li> <li>Veget</li> <li>Breact</li> <li>Potat</li> </ul>	al Products Analysis Analysis table Products I 0	299 299 301 302 302 303
42. CHROM	ATOGRAPHY	304
<ul> <li>Adso</li> <li>Ion E:</li> <li>Partit</li> <li>Exclu</li> <li>Relati</li> <li>Separ</li> <li>Prepa</li> <li>Loadi</li> </ul>	rption Chromatography xchange Chromatography ion Chromatography sion Chromatography ive Advantages and Disadvantages of Different Chromatographic Methods ration of Mixture of Amino Acids by Paper Chromatography aration of Samples ing of Chromatogram	304 304 305 305 305 306 306
43. ELECTR	OPHORESIS	309
<ul> <li>Types</li> <li>Types</li> </ul>	s of Electrophoresis s of Electrophoresis Used for Separation of Serum Proteins	309 310
		215
<ul> <li>Principal</li> <li>Types</li> <li>Vario</li> <li>Samp</li> <li>Care a</li> <li>Atom</li> <li>Interp</li> <li>Thera</li> <li>Lead</li> </ul>	ipie s of Flame Photometers us Parts of Flame Photometer ole Dilution and Maintenance of Flame Photometers nic Absorption Spectroscopy (AAS) oretation of Changes in Sodium and Potassium upeutic Monitoring of Lithium Poisoning	315 315 316 317 317 317 318 320 321
45. ARTERIA	AL BLOOD GAS ANALYZER	322
<ul><li>Calib</li><li>Preca</li><li>Types</li></ul>	ration utions s of Possible Acid-base Disturbances	322 323 323

### xvi

	Contents	xvii
46.	BODY FLUIDS AND THEIR COMPOSITION	330
	◆ Anion Gap	330
47.	IMMUNOLOGY	335
	<ul> <li>First Level of Detecting Interaction of Antibodies and Antigens</li> <li>Precipitation</li> <li>Agglutination</li> <li>Light Scattering Technique for the Measurement of Antigen–Antibody Reaction</li> <li>Second Level of Detecting Interaction of Antibodies and Antigens</li> <li>Antigen–Antibody Reactions Using Fluorescent Labels</li> <li>Radioimmunoassay Methods</li> <li>Immunoassays Using Enzymes-linked Antibody or Antigen, Enzyme-linked Immunosorbent Assay</li> </ul>	335 335 338 340 341 341 342 343
48.	ISOTOPES IN CLINICAL CHEMISTRY	347
	<ul> <li>Basic Structure of an Atom</li> <li>Units of Radioactivity Measurements</li> <li>Principles of Radiation and Radioactivity</li> <li>Measurement of Radioactivity</li> <li>Applications of Radioisotopes</li> <li>Safety Rules for Handling Radioactive Material</li> </ul>	347 347 348 349 351 352
49.	HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME	353
	<ul> <li>Structure and Function of HIV Gene</li> <li>Specific Tests</li> <li>Collection, Transport, and Storage of Samples</li> <li>Tests to be Conducted for Laboratory Diagnosis of HIV-infected Patient</li> <li>Interpretation</li> </ul>	353 355 359 359 360
50.	THERAPEUTIC DRUG MONITORING	365
	<ul> <li>Purpose for Therapeutic Drug Monitoring</li> <li>Commonly Monitored Drugs</li> <li>Normal Therapeutic Range of Antiepileptics</li> <li>Therapeutic Drug Monitoring Technologies</li> </ul>	365 365 365 366
51.	ANEMIA PROFILE	370
	<ul> <li>Forms of Anemia</li> <li>Methods to Diagnose Anemia</li> <li>Other Related Assays for Finding Causes of Anemia</li> </ul>	370 370 374
52.	BONE MARKERS	377
	<ul> <li>Laboratory Investigations for Osteoporosis</li> <li>Bone Turnover Markers</li> <li>Procollagen Type 1 N-Terminal Propeptide and Procollagen Type 1 C-Terminal Propeptide (P1NP and P1CP/C1CP)</li> <li>Bone Resorption Markers</li> </ul>	377 379 380 381
53.	COMPONENTS ASSOCIATED WITH RESEARCH WORK AND ITS PUBLICATION	386
	<ul> <li>Part I: Research Question, Hypothesis, and Objectives</li> <li>Part II: Study Designs</li> <li>Part III: Sampling</li> </ul>	386 387 391

<ul> <li>Part IV: Sample Size Calculation</li> <li>Part V: Guidelines for Writing Research Manuscripts</li> <li>Part VI: Where to Publish</li> <li>Part VII: Bibliometric Indices to Assess Academic Contribution of a Researcher</li> <li>Part VIII: Where not to Publish</li> <li>Part IX: Plagiarism</li> <li>Part X: Different Types of Authors</li> </ul> 54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES <ul> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	
<ul> <li>Part V: Guidelines for Writing Research Manuscripts</li> <li>Part VI: Where to Publish</li> <li>Part VII: Bibliometric Indices to Assess Academic Contribution of a Researcher</li> <li>Part VIII: Where not to Publish</li> <li>Part IX: Plagiarism</li> <li>Part X: Different Types of Authors</li> </ul> 54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES <ul> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	393
<ul> <li>Part VI: Where to Publish</li> <li>Part VII: Bibliometric Indices to Assess Academic Contribution of a Researcher</li> <li>Part VIII: Where not to Publish</li> <li>Part IX: Plagiarism</li> <li>Part X: Different Types of Authors</li> </ul> 54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES <ul> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	395
<ul> <li>Part VII: Bibliometric Indices to Assess Academic Contribution of a Researcher</li> <li>Part VIII: Where not to Publish</li> <li>Part IX: Plagiarism</li> <li>Part X: Different Types of Authors</li> </ul> 54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES <ul> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	398
<ul> <li>Part VIII: Where not to Publish</li> <li>Part IX: Plagiarism</li> <li>Part X: Different Types of Authors</li> </ul> 54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES <ul> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	403
<ul> <li>Part IX: Plagiarism</li> <li>Part X: Different Types of Authors</li> <li>54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES</li> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	403
<ul> <li>Part X: Different Types of Authors</li> <li>54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES</li> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	404
<ul> <li>54. LEAN SIX SIGMA (6α) APPROACH FOR CLINICAL LABORATORIES</li> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	405
<ul> <li>Aim of Lean</li> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (60)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	406
<ul> <li>Traditional Versus Lean Laboratory</li> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	406
<ul> <li>Aim of Six Sigma (6σ)</li> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	407
<ul> <li>Why the Name Six Sigma</li> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	407
<ul> <li>Phases of Laboratory Testing where Defects (Delays) can Occur</li> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	408
<ul> <li>Calculation of Six Sigma</li> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	408
<ul> <li>Functioning at Different Sigma Levels</li> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	408
<ul> <li>Visualizing World Class Quality Beyond Calculations</li> </ul>	412
	414
<ul> <li>Six Sigma DMAIC Methodology</li> </ul>	417
<ul> <li>Benefits, Limitations, and Challenges of Six Sigma</li> </ul>	417
<ul> <li>Lean and Six Sigma are Combined to Form Lean Six Sigma (LSS)</li> </ul>	418
55. ADDITIONAL TOPICS AS PER REVISED NMC GUIDELINES FOR UNDERGRADUATE CURRICULUM	423
	422
<ul> <li>Immunoairrusion</li> <li>Pasis and Patienals of Biachemical Tests Dans in Edoma</li> </ul>	423
<ul> <li>Basis and Rationale of Biochemical Tests Done in Edema</li> <li>DNA labelation from Tigue and Blood</li> </ul>	424
<ul> <li>DINA Isolation from Tissue and Blood</li> <li>Electrolyte Analysis by ISE</li> </ul>	424
<ul> <li>Electrolyte Analysis by ISE</li> <li>Advantages and/or Disadvantages of Use of Unsaturated Saturated and Trans Eats in Eeed</li> </ul>	420
<ul> <li>Advantages and/or Disadvantages of ose of onsaturated, saturated and mans rats in rood</li> <li>Screeping of Uring for Inhorn Errors</li> </ul>	427
<ul> <li>Ecod Itoms with High and Low Chromic Index and their Importance in Diet</li> </ul>	420
<ul> <li>Baseline Diagnostic, Prognostic, and Discharge Investigations in Clinical Biochemistry</li> </ul>	429
56. UNCERTAINTY OF MEASUREMENT IN LABORATORY MEDICINE	431
What is Uncertainty of Measurement?	/131
<ul> <li>Sources of Uncertainty</li> </ul>	/122
Guidelines of Measurement Uncertainty	432
Ways to Express Uncertainty	433
<ul> <li>Different Approaches to Calculate the Uncertainty in Laboratory Medicine</li> </ul>	433
Allowable Total Error Concent for Measuring Uncertainty	437
<ul> <li>Use of TEa for Proficiency Testing and Use of MU for Patients Test Results</li> </ul>	+37
Index	437

### Lean Six Sigma (6 $\sigma$ ) Approach for Clinical Laboratories

BC14.21 Describe Quality control.

CHAPTER

#### INTRODUCTION

Quality in laboratory medicine needs to be maintained to give correct results for better diagnosis and disease management. Around 70% of the diagnosis of diseases is based on laboratory results. The goal of laboratory medicine is to give correct results to the right patient at the right time. This is to prevent any possibility of error and possible serious or fatal consequences in the proper treatment. So, in the health-care industry, the need to improve the quality of laboratory test results to a very high level has become the need of the hour.

The constant improvement of laboratory performance is very critical in maintaining accurate laboratory results. The automated results produced are closely monitored to make sure that no unreliable reports are dispatched. This is done by running internal control programs and by taking part in external quality assurance schemes.

For management of quality in business and industry, Six Sigma techniques are widely used. Since past decade, these techniques have also been successfully applied in the healthcare sector. Lean and Six Sigma in health-care settings have helped in eliminating nonvalue adding steps (which help in enhancing laboratory efficiencies), in choosing an ideal Westgard rule (which helps in improving the quality to a Six Sigma level) and in drawing charts and monograms (which help in visualizing quality beyond calculations). This chapter covers the practical utility of lean Six Sigma method in improving the processes and quality in a clinical laboratory.

#### **AIM OF LEAN**

Anything that does not add value is considered a waste. The aim of lean is to cut time by eliminating nonvalue adding steps. So lean is a philosophy that helps in enhancing productivity by eliminating waste. In the laboratory setting, waste is often associated with waste of consumables, reagents, blood products, and technician's time.

This lean management theory was developed during 1990s by **Toyota Production System (TPS)**. Guiding principles of the lean manufacturing approach were formulated in 2001. Lean was designed to carry out more with fewer available resources, which helped in adding values and attitudes needed to sustain continuous improvement in the long run.

**Lean applied to industries:** There are seven types of waste that lean attempts to mitigate, i.e., overproduction, inventory, waiting, unnecessary transport, unnecessary processing, unnecessary human motions, and defects.

**Lean applied to clinical laboratories:** In clinical laboratories, the following five principles can help meet the goal:

- 1. *To see what customer values:* This requires a precise understanding of the specific needs of the customer. For clinical laboratories, the customer's perception of value is receiving accurate results in a timely way.
- 2. To understand the value stream: Value stream are those activities that, when done correctly and in the right order, give service that the customer values. For a laboratory, the value stream includes the set of activities needed to collect, transport, and process specimens in such a way that correct test results can be determined and provided back to the clinician.

- 3. *To improve the workflow:* In a lean organization work should flow steadily, without interruption from one value-adding activity to the next. Value-adding activities include those actions needed to get a result. Conversely, nonvalue adding includes those actions that serve no purpose in achieving a result. Such activities include the rework necessary to address problems of incorrectly ordered tests, instrument downtimes, and lost specimens.
- 4. *To pull the work through the system*: The system should react to the customer's demand. In other words, customers pull the work through the system. In nonlean organizations work is pushed through the system at the convenience of the operators, and so produces outputs which are not required. One should pull and not push through the value stream. The use of Kanban cards (visual cards as a signaling system), at the various benches within the laboratories, can help pull value through the stream. For example, specimens can be provided as the need exists, and not being pushed, potentially causing a backlog.
- 5. *Perfection in work:* A perfect process delivers just the right result to the patient. In this, every step is value-adding, producing a good result and desired output every time. In such actions, there is continuous flow and flexibility that does not cause any delay. As the first four principles are implemented, one gets to understand the system even better, and from this understanding, creates ideas for more improvement. A lean system thus becomes leaner and faster, and it becomes easier to find and eliminate waste.

#### TRADITIONAL VERSUS LEAN LABORATORY

In a traditional laboratory, every section is separate having its own staff and space. Daily workload is usually not evenly distributed since it is based on the workload of a particular section. Cross-training, i.e., learning how to do more than one specific job, is also a key to achieving a lean laboratory operation. It is also required to keep in view the shortage of laboratory professionals.

In a lean laboratory workplace, automated analyzers (for routine chemistry, immunoassay, coagulation profile, etc.,) are placed together in a U-shape. These automated analyzers are placed as close to the central processing area as possible to decrease excess motion. Manual testing can be in an area that is further away from specimen processing.

A lean laboratory workplace provides several advantages:

- ✤ Less staff is required
- Less wasted motion
- Enhanced teamwork.

#### AIM OF SIX SIGMA (6)

## Six Sigma Principles in Industrial Sector

Six Sigma methodologies, a manufacturing strategy, were developed in 1986 by Motorola. It was developed with the goal of decreasing the defect rates in production. It has significantly improved production efficiency in various industries. The goal of Six Sigma  $(6\sigma)$  is to bring defects at a rate of 3.4 defects per million opportunities (DPMO), i.e., 3.4 defects in 1,000,000 opportunities.

### Six Sigma Principles in Healthcare Sector

One of the first healthcare organizations to start Six Sigma was Commonwealth Health Corporation in 1998 in US. This was to help meet the high quality and near zero defect rates. Six Sigma improves assay quality by identifying biased and imprecise assays so that quality monitoring strategies can be worked out. It enables the laboratory to objectively and quantitatively assess the performance of the assays and instruments. Six Sigma offers ways to make fewer mistakes in all their activities (ranging from filling in a requisition form to the most complicated analytical process and report delivery) by eliminating errors before they appear. The starting point of 6-sigma is to define what is considered as an error, to develop processes to quantify errors, and to use corrective procedures. Attainment of Six Sigma is envisaged as the gold standard for defining a world class measure of quality in the clinical laboratory. It can help cut costs associated with reagents, supplies, control material, labor, and quality control failure investigations.

#### WHY THE NAME SIX SIGMA

Sigma ( $\sigma$ ) is the Greek mathematical symbol for standard deviation (SD). The sigma for a process refers to the number of SDs from the mean, before it is outside the acceptable limits. In Six Sigma, variation up to 6-sigmas, i.e., 6 standard deviations should fit within the tolerance limits for the process: hence, the name Six Sigma. Any process can be evaluated by determining how many sigmas fit within the tolerance limits, e.g., if sodium has Six Sigma performance, then the mean could shift by six SDs, and still meet the laboratory requirements.

#### PHASES OF LABORATORY TESTING WHERE DEFECTS (DELAYS) CAN OCCUR

Three phases where defects can occur are preanalytical, analytical, and postanalytical phases.

#### **Preanalytical Phase**

Counting defects by measuring preanalytical errors out of the total number of samples received, e.g., hemolyzed, clotted, lipemic, icteric, QNS (quantity not sufficient), wrong collection, etc.

#### **Analytical Phase**

Predicting process performance by measuring variation in all analytical processes.

To understand the analytical method performance on the sigma metrics scale, we do not have an easy comparative value against which we judge the test result. So instead, we control the data we are already collecting imprecision (expressed as coefficient of variation, CV) and trueness (expressed as bias).

#### **Postanalytical Phases**

Stat turnaround-times (TAT), i.e., time from order to completion of emergency department, is an indicator tracked by most laboratories. It is quite simple to know whether a test result is beyond the acceptable TAT. Laboratories have the desired TAT and real TAT and have to simply count the number of times the real TATs exceed that desired TAT. A defect would be any result not reported within the specified turnaround time.

#### **CALCULATION OF SIX SIGMA**

Six Sigma is both a metric and a methodology. Six Sigma as a metric requires defects to be clearly defined and is used as a scale for quality. Six Sigma as a methodology requires measuring variation and is used as a scale for measure.

# Calculation of Sigma by Measuring Defects

This sigma approach is useful for pre- and postanalytical processes. Following steps are carried out:

# Inspecting Outcome and Counting Defects

Calculating defects per million opportunities (DPMO)

DPMO = Number of defects × 1,000,000/ number of opportunities.

Converting DPM to sigma metric— Conversion of DPMO to sigma metric is done by using standard Six Sigma tables available.

If one knows what percent of test results fail the desired target, such as preanalytical errors **(Table 54.1)**, one can convert that into a DPM, which in turn becomes a sigma metric. If Six Sigma is carried out properly, it ensures that internal processes are running at best efficiency.

TABLE 54.1: A hyp and defects.	oothetical calculation	of sigma for preanaly	tical errors	by meası	uring total opportunities
Preanalytical errors	No. of total samples/ opportunities	No. of defects out of total samples	% Error	DPMO	Process sigma from Six Sigma tables/process sigma calculator
Hemolyzed	1,000	20	2%	20,000	3.55
High PCV	1,000	10	1%	10,000	3.83
Wrong collection	1,000	7	0.7%	7,000	3.96
Lipemic	1,000	1	0.1%	1,000	4.6

<b>TABLE 54.2:</b> Error rate, DPM, and corresponding sigma.				
Error rate	DPM	Sigma		
Acceptable				
5%	50,000 DPM 3.15			
1%	10,000 DPM	3.83		
Goal				
0.1%	1,000 DPM	4.6		
0.01%	100 DPM	5.2		
Excellent				
0.001%	10 DPM	5.8		

The goal should be an error rate of 0.1% or 1,000 DPM (4.6-sigma) to 0.01% or 100 DPM (5.2-sigma), and ultimately 0.001% or 10 DPM (5.8-sigma). A defect rate from 1% or 10,000 DPM to 5% or 50,000 DPM are often considered acceptable **(Table 54.2)**.

A Six Sigma is defined as 3.4 defects per million opportunities (DPMO) **(Table 54.3)**. A

<b>TABLE 54.3:</b> Table showing sigma metric, DPMO, percent defects, and percent yield.			
Sigma metrics	DPMO	Percent defects	Percentage yield
1	691,462	69%	31%
2	308,538	31%	69%
3 Minimum	66,807	6.7%	93.3%
4	6,210	0.62%	99.38%
5	233	0.023%	99.977%
6 World class	3.4	0.00034%	99.99966%
7	0.019	0.0000019%	99.9999991%

three-sigma process has about 66,807 defects per million tests.

#### Calculation of Sigma by Measuring Variation and Predicting Process Performance

This sigma approach is useful for analytical processes and is carried out in the following way:

#### To Calculate CV, Bias, and Total Allowable Errors (TEa) for an Analyte

Quality control includes management of internal and external quality. The two components which define the quality specifications of the patient results are: bias and CV. Internal quality helps in maintaining precision and accuracy which helps to calculate CV, whereas external quality control helps in maintaining accuracy which helps to calculate bias.

TEa is calculated by the specification given by CLIA/RCAP/RiliBÄK or other standard regulations as selected by the user.

#### Coefficient of variation (CV)

Closeness of values to each other, i.e., imprecision, is used to describe the variation of a test. The CV expresses the variation as a percentage of the mean. CV is calculated from the calculated laboratory mean and calculated standard deviation procured from the internal quality control data over preceding months.

CV% = (Standard deviation/Laboratory mean) × 100%

A test with high standard deviation means poor precision, greater instability, and high random error in the laboratory.

Generally, for all parameters, CVs of 5% or less generally denote a good method performance, whereas CVs of 10% and higher imply unsatisfactory performance.

So, CV provides a general perception about the performance of a method. The CVs and standard deviations (SDs) can be calculated using the Graph Pad Prism version 5.0.

#### Bias

Bias is deviation from the true value (accuracy). It indicates systematic difference between the result obtained by the laboratory's test method and the one obtained from an accepted reference. The true value with some traceability can be one of the following:

- From proficiency testing (PT)/external QC program
- From mean of peer group, e. g., participation in unity report (interlaboratory program available to clinical diagnostic laboratories for day-to-day performance comparison with peer group with at least 12 participants).
- From a comparative method (new vs old).
- From observed laboratory mean vs target mean.
- Bias from reference material or reference method/manufacturer value.

Bias emphasizes lack of agreement among methods being compared. Systematic error is detected as positive or negative bias for a given analytical method.

Bias =

Designated mean (Dm)–Laboratory mean(LM) Designated mean (Dm)

#### × 100

Designated mean (Dm) is the means of all laboratories using same instrument and method and is also provided by the reagent manufacturer.

#### Total allowable error (TEa)

Refers to allowable difference from the true value, i.e., the degree of change that needs

to be detected in an analyte for a clinically important decision to be made.

TEa is a model that combines both CV (imprecision) and bias (trueness) of a method to calculate the impact on a test result. The sigma metrics were calculated using PT/ EQA groups, CLIA, RCPA, biological variation database—minimum/optimum/desirable, RICOS Goals, RiliBÄK, etc. Following have been different models of TEa:

- Solution 250 model TEa: Early definition of TEa used a 2SD model (meaning that 95% of the measured results were expected to be within the TEa).
- SSD model for TEa: Present day model (CLIA, CAP, New York state regulatory guidelines) uses a 3SD model (99.7% of your data will be within TEa)

TEa (%) = Bias (%) +  $1.65 \times CV$  (%)

(1.65 implies that 95% of the results fall within the TEa limit given a Gaussian distribution).

#### Selecting the optimum TEa

*If TEa is too large* ability to correctly interpret results is compromised.

**If TEa is too small:** The costs for keeping the process in control become excess.

The just right TEa allows correct interpretation of clinical issues and reasonable control of process costs. There is no consensus on the perfect TEa, but it is an important statistical quality control (SQC) tool. However, selecting the optimum TEa is the prerogative of a laboratory.

Example when TEa for sodium is too big
 ±9 (reference interval for sodium is 136-144 mmol/L)

If target value of QC 140  $\pm$ 3 mmol/L (1SD)  $\pm$ 3 SD becomes 131–149. This TEa completely covers the reference interval so that any discrimination between health and disease is impossible. So, TEa should never be wider than the reference interval.

 When TEa for sodium is too small = ±0.3 If target value of QC 140 ±0.1 mmol (1SD) ±3 SD becomes 139.7-140.3. This TEa fails, because cost to maintain process control is expensive. TEa should never be smaller than the last reportable digit. Typical SD is 1 mmol/L, which makes TEa of  $\pm 3$ having range 137–143, which is well within reference interval range.

According to CLIA, TEa is  $\pm 4$  mmol/L. This prevents setting unrealistic expectations at the low end and avoids unachievable value.

If the difference between the true concentration of an analyte and the reported concentration in a patient's sample exceeds TEa, the result is considered unreliable. **Table 54.4** enlists TEa for some common biochemical parameters as given by Clinical Laboratories Improvement Act (CLIA) guidelines 1988, RCPA-QAP, RiliBÄK, and Ricos Goal desirable TEa.

**TABLE 54.4:** TEa for some of the common biochemical as per CLIA recommendation, RCPA-QAP, RiliBÄK and Ricos Goal desirable TEa.

	CLIA	RCPA-QAP	RiliBÄK	Ricos Goal desirable TEa
Glucose	Target value ±10% or 6 mg/dL (greater)	±8%	±15%	7%
Urea	Target value ±9% or 2 mg% (greater)	±12%	±20%	15.6%
Creatinine	Target value $\pm 15\%$ or $\pm 0.3$ mg/dL (greater)	±8%	±20%	8.9%
T. Bil	Target value $\pm 20\%$ or 0.04 mg/dL (greater)	±12%	±22%	27%
T. Protein	±10%	±5%	±10%	4%
Albumin	±10%	±6%	±20%	4%
Uric acid	±17%	±8%	±13%	12%
Cholesterol	±10%	±6%	±13%	9%
TG	±25%	±12%	±16%	26%
HDL-c	±30%	±12%	-	12%
Na	Target value ±4 mmol/L	±2%	±5%	0.7%
К	Target value ±0.5 mmol/L	±5%	±8%	6%
CI	5%	3 mmol/L <100 mmol/L, 3% >100 mmol/L	8%	1.5%
Ca	0.25 mmol/L	0.1 mmol/L <2.5 mmol/L, 4% >2.5 mmol/L	10%	2.6%
AST	±20%	±12%	±21%	16.7%
ALT	±20%	±12%	±12%	27.5%
ALP	±30%	±12%	±18%	12%
Amylase	±30%	±10%	-	15%
СРК	±30%	±12%	±20%	30%
Iron	±20%	±12%	-	30.7%
Mg	25%	±8%	±15%	5%
Cortisol	25%	±15%	±30%	23%
fT4	Target value ±3SD	±12%	±20%	7%
TSH	Target value ±3SD	±20%	±24%	23%

*CLIA*—Clinical Laboratories Improvement Act.

*RCPA-QAP*: The Royal College of Pathology of Australia—Quality Assurance Performance.

*RiliBÄK*: Guidelines of German Medical Associates for the Quality Assurance of Laboratory Medical Examination.

Ricos Goals: Biological variation database.

#### To Calculate Sigma Using Standard Analytical Sigma Metric Equation

For analytical processes, with known total allowable error, and for which analytical performance can be estimated in the form of accuracy (bias) and precision (CV), sigma is calculated as follows:

$$\sigma \frac{\text{TEa} - \text{Bias}}{\text{CV}}$$

All variables are in their actual units, but the percentage version of the equation is most popular.

#### FUNCTIONING AT DIFFERENT SIGMA LEVELS

Starting from the goal is to cut the number of defects in half, is a more realistic goal than trying to meet 6-sigma for every process.

- Achieving a sigma level equal to or more than 6 must be encouraged in all laboratories. Six Sigma concentrates on regulating a process to 6 SDs. Performance above Six Sigma levels is a world class performance. When the method sigma is greater than or equal to 6, stringent internal QC rules need not to be adopted. In such cases, false rejections can be minimized by relaxing control limits up to 3SD.
- Achieving at sigma metric from 6.0 to 3.0 represents the range from best case to worst case.
- Functioning at the 3-sigma level is regarded as the least acceptable level of quality. A good performance is indicated by a sigma level more than 3 but a sigma level less than 3 is a sign of a poor performance procedure. Methods with sigma performance less than 3 are not considered acceptable for

production and calls for the adoption of a newer and better method as quality of the test cannot be assured even after repeated QC runs.

### Changes in performance in laboratory to meet high sigma:

- To meet 3-sigma—usually only obvious changes and corrections are required.
- To meet the 4-sigma—processes must also be improved.
- To meet 5-sigma—the design of processes must be improved.
- To meet 6-sigma—requires rigorous tools and a design for perfection.

Should sigma value more than 6 be achieved: Sigma value of 14, 20, and 30 are essentially no different from each other. If sigma is increased from 6-sigma to 7-sigma, defects reduce from 340 to 2 defects per 100 million (Table 54.5). Although, real improvement from achieving 5-sigma to 6-sigma is very less from achieving 6-sigma to 7-sigma, but practically improvement from 5-sigma to 6-sigma is more effective. The increase in cost incurred in achieving 6-sigma to 7-sigma may not justify the advantages of increase in sigma metrics from 6 to 7.

Extremely high sigma metrics may show that you have chosen a tighter quality need.

#### Graphic Presentation of Six Sigma using Total Allowable Error (TEa) for Given Analyte, Systematic Error (Bias %) and Imprecision (CV %)

If we measure some analyte in the patient's sample, then the true value that represents the patient's real clinical status is in the center **(Fig. 54.1)**. As we run tests on this patient again, we never get all the results to

<b>TABLE 54.5:</b> Quantification of sigma beyond six.		
Sigma	DPMO	Defects in whole no
бѕ	3.400 DPMO	3.4 defects per MO
7s	0.020 DPMO	2 defects per 100 MO
7.5s	0.001 DPMO	1 defect per 1,000 MO
7.86s	0.0001 DPMO	1 defect per 10,000 MO
8.53s	0.000001 DPMO	1 defect per 1,000,000 MO



Fig. 54.1: Graphic presentation of Six Sigma metrics.

be exactly the same, and the values take on the form of a normalized distribution around that value instead. The TEa is the tolerance limits on either side. If we can squeeze six standard deviations of our analytical method distribution within that TEa, in the absence of any bias, we will meet that Six Sigma goal and expect to generate fewer than four clinical defective results. When bias exists, it shifts our distribution away from the patient's true value. On the other hand, when we have a larger and larger imprecision, it spreads our distribution wider and wider. The combined impact of imprecision and bias may cause the thicker parts of the tails of our distribution to exceed the TEa, which means generating more defective test results.

To implement suitable Westgard QC rules for each analyte: Quality control in healthcare system is still less understood because of the relative complexity in choosing an appropriate Westgard rule. The aim of Six Sigma is to simplify the process of choosing Westgard rule and thus to improve the reliability of the results of diagnostic tests. Selecting an appropriate Westgard rule is done using the Six Sigma principle, along with total allowable error, method imprecision, and bias for that particular analyte. The goal is to meet the highest possible sigma scale within the acceptable limits of total allowable error. Selection of several OC materials and repetitions (measurements) needed is also

done as per sigma values achieved as per the following Westgard rules:

 $\geq 6\sigma - 2$  levels of QC per day with a  $1_{3.5s}$  greater rule

 $5\sigma$  – 2 or 3 levels of QC per day with a  $1_{_{2.5s}}$  or  $1_{_{3s}}$  rule

 $4\sigma$  – 3 or 4 levels of QC per day with a  $1_{3/2_{2s}}/R_{4s}/4_{1s}$  rule

 $3.5\sigma$  – 6 QC per day with a  $1_{_{3s}}\!/2_{_{2s}}\!/R_{_{4s}}\!/4_{_{1s}}$  rule

Less than  $3.5\sigma$ —most affordable levels of QC per day with a  $1_{3s}/2_{2s}/R_{4s}/4_{1s}$  rule as the sigma value decreases, the chance of missing an error increases. Therefore, the best Westgard rule is the one with a sigma value closest to, but smaller than, the sigma value of the test. For low sigma values (<3.5-sigma) reducing analytical bias and imprecision is a key to improve the quality. For lower sigma values, more QC samples and following more Westgard rules are recommended. Example:

a. For total protein:

TEa = 10%

If bias of a laboratory for it is 3% and CV% at 2% for Protein Sigma = (TEa-Bias)/CV% = (10% - 3%)/2% = 3.5 S

So, Westgard rule corresponding to sigma of 3.4 is chosen to check the performance of cholesterol assay, i.e., a multirule of 13s/2 22s/R 4s/41s is applied with two levels of control.

b. For TG: TEa = 25%

If bias of a laboratory is 13% and CV% at 2% for TG  $\,$ 

Sigma = (TEa - Bias)/CV%=(25% - 13%)/2% = 6SSo, a Westgard rule of 13.5s two levels of

control is enough to check its performance. The advantage of adopting a performance goal of Six Sigma is that small shifts in mean (classically described as a shift of 1.5 SD from the mean) will still be acceptable within the tolerance limits, without increasing the defect rate.

#### VISUALIZING WORLD CLASS **QUALITY BEYOND CALCULATIONS**

#### a. Method Decision Chart

Calculation of sigma metrics is, although taxing, but at the same time it can be accomplished. But, with so much sample load, laboratories want simplicity and not so many calculations to be added to their files. Method decision chart often also called a sigma bull's-eye graph (Fig. 54.2) is a visual tool that takes all the information from the equation and turns it into a graphic format.

This chart arranges the imprecision along the x-axis and bias along the y-axis, thus the performance data from an analytical method transforms into graph coordinates. The scale of Y-axis is 0 to TEa and scale of X-axis is 0 to TEa/2. Sigma metrics lines are drawn as per Table 54.6.

TABLE 54.6:	Calculation of	X-axis and Y-a	kis points
for drawing S	Sigma metrics	lines.	

Criterion	Y-axis	X-axis	Sigma
Bias +2S	TEa	TEa/2	2
Bias +3S	TEa	TEa/3	3
Bias +4S	TEa	TEa/4	4
Bias +5S	TEa	TEa/5	5
Bias +6S	TEa	TEa/6	6

Sigma metrics zones superimposed on the graph (Fig. 54.2) are the following:

- The zone closest to the graph's origin is \$ with world class quality, i.e., Six Sigma.
- Followed by Six Sigma is five sigma zone which is excellent.
- Then is four sigma zone considered as good.
- \* After four sigma is three sigma zone, i.e., marginal and then is two sigma zone, i.e., poor.

The rest of the graph, for sigma metrics performance below two sigma, is labeled as unacceptable. As methods get closer to the bull's eye, it means the sigma metrics are higher and fewer defects are being generated.

A normalized method decision chart means multiple analytes with different TEa's can be adjusted to be displayed on a single chart. So, on this visual tool we can know sigma as



Normalized method decision chart

Fig. 54.2: Normalized sigma metrics bull's-eye chart.

per the intersect point that we get from the graphic format (CV is plotted on X-axis and Bias on Y-axis), which we had calculated in the preceding chapter from standard analytical sigma metric equation.

$$\sigma \frac{\text{TEa} - \text{Bias}}{\text{CV}}$$

#### b. Operational Process Specifications (OPSpecs) Charts

An OPSpecs chart plots the allowable bias versus the allowable imprecision, and provides the information needed to select appropriate quality control rules, and number of runs required to assure a defined quality need. It can be used for the following:

- Using the TEa, precision and accuracy of an analyte, ideal Westgard rule can be selected using OPSpecs charts.
- Is a tool that details how many rules and controls are needed to give the necessary error detection (with a minimum of false rejection) for the method.
- Consider how improving a test's precision and accuracy will change the operating point of the method, and therefore, result in simpler and less expensive quality control.
- Estimate the largest allowable imprecision for a test from the X-intercepts of the

operating lines for the QC rule being implemented.

It is a graph very similar to the Six Sigma method decision chart, in that the imprecision CV forms the X-axis, and the bias forms the Y-axis (Fig. 54.3). Where the OPSpecs chart differs from method decision chart is that the lines displayed on the chart are no longer sigma metrics zones, they represent the performance of different QC rule.

For drawing the chart define the analytical quality need for the test in the form of TEa. Calculate the SD or CV for the method. Calculate the bias. Calculate normalized operating point, which is observed imprecision and inaccuracy expressed as a percentage of the allowable total error. Plot normalized operating point by calculating CV% and bias as follows and selects the relevant QC rule. A normalized method decision chart/OPSpecs chart means multiple analytes with different TEa's can be adjusted to be displayed on a single chart. For example:

- ✤ For 10% TEa, a 2% observed CV would be 20%; a 4% observed bias would be 40%.
- For 20% TEa, a 2% observed CV would be 10%; a 4% observed bias would be 20%.
- For 30% TEa, a 2% observed CV would be 6.7%; a 1% observed bias would be 13.3%. The diagonal lines displayed in the chart





**Fig. 54.3:** OPSpecs chart. (Pr: probability of false rejections; N: number of measurements; R: runs)

represent (in order from right to left) different quality control (QC) rules listed in the key at right (from top to bottom). As performance is closer to the bull's eye, the plotted point is within the ring of that QC rule, which means that a particular rule combination will give adequate error detection and proper analytical quality assurance.

#### c. Deciding QC Frequency and Run Length Using Nomogram

A sigma-metric SQC run size nomogram is for estimating the number of patient samples between QC events for bracketed operation of a continuous analytical testing process.

Up until about a decade ago, QC frequency was entirely determined by rule of thumb. Sometimes, it was planet-based QC frequency: once a day. At other times, it was labor-based QC frequency: once a shift. But it was surely not patient-based QC frequency, driven by the performance of the method and the quality required for the proper use and interpretation of the test results.

Until recently, the decision on QC frequency no longer has to be calculated and understood mathematically but could instead be simply interpreted graphically. Through a series of publications in 2017-18, Westgard extended these graphic simplifications to create more practical QC frequency nomograms. Now laboratories can simply observe a graph that compares their sigma metrics on the X-axis with the number of patient samples that can be run between controls on the Y-axis (Fig. 54.4). The different lines in the graph represent the QC procedures that may be chosen by the laboratory. In order to decide QC frequency, a laboratory should do the following:

- \* Decide the sigma metric of a method
- Find the appropriate QC design
- Find the intersection of the sigma metric and QC procedure line on the nomogram.

If a laboratory has a Six Sigma method, then any QC procedure will do and at least 500 patients or possibly many more can be run in between QC events without the risk of reporting any wrong result.



Fig. 54.4: Sigma metric run size nomogram.

For Six Sigma methods, the QC frequency can be reduced to 1 control per 1,000, 2,000, or even higher numbers of patient samples, or they can use extended QC limits such as 4s or wider.

If a laboratory has a sigma of three or less, then QC frequency must be greatly increased, to something closer to one control per 100 or per 50 patient samples. Methods below 3 and 2-sigma would theoretically require almost constant running of controls, a frequency that may be impractical.

MR6 represents a full implementation of "Westgard **Rules**" using 6 control measurements (either 6 controls run at once or 3 controls run with 2 measurements being made on each control).

MR N4 represents the  $1_{3s}/2_{2s}/R_{4s}/4_{1s}$  multirule procedure with N = 4 control measurements.

MR N2 represents the  $1_{3s}/2_{2s}/R_{4s}$  control rules with N = 2 control measurements.

SRN2. Single-rule procedures using the  $1_{3s}$  control rule and N = 2 control measurements.

SRN4 Single-rule procedures using the  $1_{3s}$  control rule and N = 4 control measurements.

#### SIX SIGMA DMAIC METHODOLOGY

To eliminate defects and reduce variation Six Sigma uses DMAIC methodology. This is a stepwise approach for enhancing quality and producing the desired goals. DMAIC is the following five-stage Six Sigma system to find and solve problems.

**Define**—the problem or opportunity and the expected outcome of laboratory processes.

*Measure*—the current performance and ability. Establish and measure QC systems as per real need. Collect relevant data and document the same.

*Analyze*—to find the cause of the defect under investigation. Periodical analysis of data to investigate and find the deficiency.

*Improve*—by implementing potential solutions. Assess the effectiveness and

relevant improvement. Do pilot runs to prove a new process ability.

*Control*—by standardizing solution and monitoring performance. As per assessment, implement and control the process to make sure that any deviations from target are corrected before they result in defects.

In this, the laboratory turnaround time for each test, total delay time in the sample reception area, and percentage of steps involving risks of medical errors and biological hazards in the overall process are measured.

#### **DMADV** Approach

Apart from the "DMAIC" methodology, which is essentially used for a pre-existing process that is defective, the "DMADV" (Define, Measure, Analyze, Design, and Verify) methodology is used when a new process is being developed or a pre-existing defective process has failed, even the "DMAIC" correction. The difference lies only in the last two steps, "Design"—detailed design of the process to meet the customer needs and "Verify"—the design performance and its ability in meeting the customer needs.

#### BENEFITS, LIMITATIONS, AND CHALLENGES OF SIX SIGMA

#### **Six Sigma Benefits**

Clinical laboratories remain in a constant effort to increase their workload, decrease error, improve the quality, and decrease the cost. To meet these goals, sigma metrics have become a useful tool for all parts of the quality control (QC) design process. Six Sigma not only allows benchmarking the performance of methods and instruments on a universal scale, it allows laboratories to easily visualize performance (using tools like method decision chart, OPSpecs chart, and QC frequency nomogram), optimize the QC rules, and numbers of control measurements they carry out, and now even schedule the frequency of running those controls. The most popular outcomes from Six Sigma are as follows:

### Practical CLINICAL BIOCHEMISTRY Methods & Interpretations

#### Salient Features

- Topics are revised and updated as per the new competency-based curriculum (National Medical Council guidelines) for undergraduate students.
- More than 450 case reports/viva questions with detailed explanation are included.
- Meticulously revised in view of the latest developments in the field of biochemistry.
- A chapter on Uncertainty of Measurement for traceability of test results, encompassing its sources, different approaches for calculation, along with a solved example is added.
- A few other topics such as total lab automation, including integrated pre- and postanalytical automation systems, biomedical waste management as per Central Pollution Control Board guidelines, screening of urine for inborn errors of metabolism, glycemic index and its importance, and hemoglobin electrophoresis are added.
- In addition to routine biochemistry tests, important topics covered include quality control and Six Sigma approaches for clinical laboratories, therapeutic drug monitoring, anemia profile, and bone markers.
- For publishing research work, students can refer to the chapter on *Components Associated with Research Work and Its Publication.*
- Topics are selected and covered considering the requirements of both undergraduate and postgraduate students.
- A useful book for bridging the gap between clinicians and clinical laboratories.

**Ranjna Chawla** PhD is a Senior Biochemist (Scientist IV), Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research (GIPMER), Government of NCT of New Delhi, India. She has around 40 years of experience in various capacities.

She completed her Doctorate from the Postgraduate Institute of Medical Education and Research (PGIMER) in Chandigarh. She has been associated with prestigious institutions such as PGIMER, Chandigarh; Lady Hardinge Medical College (LHMC), Delhi; Government Medical College (GMC), Chandigarh; Guru Nanak Eye Center (GNEC), Delhi, and Nehru Homoeopathic Medical College and Hospital (NHMC), Delhi. She has investigated various projects of UGC, ICMR, and AYUSH and has also published numerous research papers in national and international journals. Dr Chawla has utilized her teaching and research experience to understand the problems of students and incorporate the latest developments in the field of biochemistry.

#### Printed in India



Join us on facebook.com/JaypeeMedicalPublishers Follow us on istagram.com/JaypeeMedicalPublishers



