

Textbook of **CLINICAL CHEMISTRY**

Editor:

NORBERT W. TIETZ, Ph.D.

Professor, Department of Pathology,
Director of Clinical Chemistry,
College of Medicine,
University of Kentucky, Lexington, KY

Editorial Committee:

N. V. BHAGAVAN, Ph.D.

WENDELL T. CARAWAY, Ph.D.

REX B. CONN, M.D.

JOHN F. KACHMAR, Ph.D.

ELIZABETH L. PRUDEN, Ph.D., M.T. (ASCP)

RONALD J. WHITLEY, Ph.D.

1986

W. B. SAUNDERS COMPANY

W. B. Saunders Company: West Washington Square
Philadelphia, PA 19105

Library of Congress Cataloging in Publication Data

Main entry under title:

Textbook of clinical chemistry.

1. Chemistry, Clinical. I. Tietz, Norbert W.,
1926- [DNLM: 1. Chemistry, Clinical. QY 90 T355]
RB40.T44 1986 616.07'56 83-20410
ISBN 0-7216-8886-1

Editor: Dudley Kay

Developmental Editor: Dave Kilmer

Designer: Terri Siegel

Production Manager: Frank Polizzano

Textbook of Clinical Chemistry

ISBN 0-7216-8886-1

© 1986 by W. B. Saunders Company. Copyright under the Uniform Copyright Convention. Simultaneously published in Canada. All rights reserved. This book is protected by copyright. No part of it may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without written permission from the publisher. Made in the United States of America. Press of W. B. Saunders Company. Library of Congress catalog card number 83-20410

Last digit is the print number: 9 8 7 6 5 4 3 2 1

CONTENTS

CHAPTER 1	
LABORATORY PRINCIPLES AND INSTRUMENTATION	1
CHAPTER 1A. GENERAL LABORATORY TECHNIQUES AND PROCEDURES	3
<i>by Edward W. Bernes, Jr., Ph.D., and Donald S. Young, M.B., Ph.D.</i>	
Chemicals and Related Substances	3
Reagent Grade Water 3; Reagent Grade or Analytical Reagent Grade (AR) Reagents 6; Desiccants and Use of Desiccators 8	
General Laboratory Supplies.....	10
Glassware 10; Plasticware 11; Synthetic and Rubber-Base Tubing 15	
Volumetric Equipment and Its Calibration	15
Pipets 15; Burets 21; Volumetric Flasks 22	
Centrifuges 23; Procedures for Concentrating Solutions 27; Separatory Funnels 27; Laboratory Mixers and Homogenizers 28; Filtration 28; Balances and Weighing 30; Concept of Solute and Solvent 32; Physical and Chemical Units 34; Buffer Solutions and Their Action 35	
Units of Measurement.....	37
International System of Units 37; Reporting 42; Conversion of Units 44	
Safety.....	45
CHAPTER 1B. ANALYTICAL PROCEDURES AND INSTRUMENTATION.....	55
<i>Coordinated by Carl A. Burtis, Ph.D.</i>	
Section One. Photometry	55
<i>by Wendell T. Caraway, Ph.D.</i>	
Nature of Light 55; Beer's Law 56; Components of Spectrophotometers 61; Standardization of Spectrophotometers 69	
Flame Photometry 72; Atomic Absorption Spectrophotometry 75	
Section Two. Fluorometry, Nephelometry, and Turbidimetry.....	78
<i>by Thomas O. Tiffany, Ph.D.</i>	
Fluorescence and Light Scattering Measurements	78
Approaches to Fluorescence Measurements.....	79
Time Relationships of Fluorescence Emission 80; Fluorescence Polarization 81; Limitations of Fluorescence Measurements 83; Instrumentation for Measurement of Fluorescence 85; Fluorometers for Special Applications 90	
Light Scattering Measurements 91; Nephelometric and Turbidimetric Measurements of Scat- tered Light 94	
Section Three. Electrophoresis.....	98
<i>by Emanuel Epstein, Ph.D.</i>	
Theory of Electrophoresis 98; Effect of Buffer on Electrophoretic Migration 100; Electro- endosmosis or Endosmosis 100	
A General Method for Electrophoresis	101
Paper Electrophoresis (PE) 104; Agarose Gel Electrophoresis (AGE) 104; Cellulose Acetate Electrophoresis (CAE) 105; Polyacrylamide Gel Electrophoresis (PAGE) 105; Starch Gel Electrophoresis 106; Isoelectric Focusing (IEF) 106; Limitation and Errors 108; Problem Solving 109	
Section Four. Electrochemistry	110
<i>by Ole Sjoaard-Andersen, M.D., Ph.D.</i>	

Potentiometry	110
Redox Electrodes 113; Ion-Selective Membrane Electrodes 116; Gas Electrodes 121; Enzyme Electrodes 122; Liquid-Liquid Junction Potentials 123	
Polarography 124; Amperometry 125; Coulometry 127; Conductometry 128	
Section Five. Osmometry	129
<i>by Esther F. Freier, M.S. M.T.(ASCP)</i>	
Osmosis 129; Colligative Properties 130	
The Freezing Point Depression Osmometer 131; The Vapor Pressure Osmometer 132; The Colloid Osmotic Pressure Osmometer 133	
Section Six. Chromatography	135
<i>by Larry D. Bowers, Ph.D.</i>	
Forms of Chromatography 136; Fundamental Properties of Chromatography 139	
Separation Mechanisms	144
Ion-Exchange Chromatography 144; Steric Exclusion Chromatography 145; Adsorption Chromatography 146; Partition Chromatography 147; Affinity Chromatography 148	
Section Seven. Gas Chromatography.....	149
<i>by Sati C. Chatteraj, Ph.D.</i>	
Basic Principles of Technique 150; The Gas Chromatograph 151	
Section Eight. High Performance Liquid Chromatography	159
<i>by M. David Ullman, Ph.D., and Carl A. Burtis, Ph.D.</i>	
Instrumentation	159
Practical Operation	170
Sample Preparation 170; Tubing Assembly 171; Mobile Phase 171; Pumps 171; Injector 172; Column 172; Detector 172; Safety 172	
Section Nine. Basic Principles of Radioactivity and Its Measurement	173
<i>by Edward R. Powsner, M.D.</i>	
Atomic Structure, Radiation, and Radioactivity.....	173
Interaction of Radiation with Matter.....	183
Excitation and Ionization 183; Particulate Radiation 184; Electromagnetic Radiation 184; Units of Radiation Exposure and Radiation Dose 186	
Detection and Measurement of Radioactivity	186
Autoradiography 186; Gas-Filled Detectors 186; Scintillation Detectors 187; Electronics of Scintillation Counting 190; Efficiency of Scintillation Counting 191; Counting Statistics 193	
Radiation Safety	194
Section Ten. Mass Spectrometry	197
<i>by Brian D. Andresen, Ph.D., and Bethany L. Wise, Ph.D., M.T.(ASCP)</i>	
The Instrument 198; Gas Chromatography-Mass Spectrometry 204; Isotope Dilution Mass Spectrometry (IDMS) 206	
CHAPTER 1C. PRINCIPLES OF IMMUNOCHEMICAL TECHNIQUES.....	209
<i>by Gregory J. Buffone, Ph.D.</i>	
Antigen-Antibody Binding—Theory of Interaction	211
Qualitative Methods for Detecting Proteins	215
Passive Gel Diffusion 215; Immunoelectrophoresis 217	
Quantitative Methods for Measuring Proteins.....	222
Radial Immunodiffusion (RID) and Electroimmunoassay (EIA) 222; Turbidimetric and Nephelometric Assays 223; Techniques for Detecting Primary Association Between Antigen and Antibody 224; Immunocytochemistry 229; Agglutination Assays 230	
Monoclonal Antibodies.....	231
CHAPTER 1D. AUTOMATION IN THE CLINICAL LABORATORY	236
<i>by Ernest MacLin, P.E. and Donald S. Young, M.B., Ph.D.</i>	

Instrumental Concepts.....	236
Definitions 237; Specimen Identification 238; Specimen Preparation 240; Specimen Handling, Presentation, and Transport 241; Removal of Protein and Other Interferents 243; Sample Transport and Delivery 244; Reagent Handling 246; Reagent Delivery 249	
Chemical Reaction Phase.....	249
Mixing and Incubation in Centrifugal Analyzers 251; Reaction Vessels and Cuvets in Discrete Systems 252	
Measurement Approaches.....	255
Systems Using Absorbance/Transmittance Photometry 255; Other Forms of Photometry 257; Systems Using Electrochemical Measurements 258; Signal Processing, Data Handling, and Microprocessors 259	
Examples of Automated Clinical Chemistry Systems.....	260
ASTRA 261; COBAS-BIO 263; DEMAND 265; Ektachem 700 267; Parallel 269; SMAC II 272	
Selection of Instruments 274; Trends in Instrumentation 276	
CHAPTER 2	
ACQUISITION, MANAGEMENT, AND APPLICATION OF LABORATORY DATA .	287
CHAPTER 2A. STATISTICAL PROCEDURES.....	287
<i>by Robert O. Kringle, M.S., and George F. Johnson, Ph.D.</i>	
Sources of Variation in Analytical Data 287; The Role of Statistics in Analytical Work 288	
Fundamental Statistical Concepts.....	289
Basic Concepts 289; The Gaussian Probability Distribution 293; Student's <i>t</i> Probability Distribution 296	
Sampling Distributions.....	297
Independence and Random Sampling 298; Sampling Distributions 298; Estimation of Population Parameters 300; Significance Testing 300	
Inference About Population Means.....	305
The Randomized Comparison 305; The Randomized Paired Comparison 307	
Designing Experiments in Analytical Work.....	309
Confidence Intervals for Population Means 310; The Poisson Distribution 314	
Inferences About Population Variances.....	315
Inferences About a Single Population Variance 316; Inference About the Ratio σ_A^2/σ_B^2 of Two Population Variances 319	
The Study of Variation in Analytical Work.....	321
The Expected Value and Variance of a Linear Combination of Random Variables 321; Transmission of Error 323; Estimating Variance Components by Nested ANOVA 324; Inferences Concerning Variances Estimated in One-Stage and Two-Stage Nested ANOVA 328	
Regression Analysis 332; The Total Analytical Error of an Analyzer System 343; Statistical Quality Control 350; Outliers 352; Simulation of Random Events 354	
CHAPTER 2B. ESTABLISHMENT AND USE OF REFERENCE VALUES.....	356
<i>by Helge Erik Solberg, M.D., Ph.D.</i>	
The Concept of Reference Values 356; Selection of Reference Individuals 359; Specimen Collection 364	
Importance of Analytical Procedures and Quality Control.....	366
Statistical Procedures Used in Establishing Reference Values.....	366
Determination of Reference Limits: General Considerations 371; Presentation of an Observed Value in Relation to Reference Values 379; Multivariate, Population-Based Reference Regions 380; Subject-Based Reference Values 382; Transferability of Reference Values 383	
Dynamic vs. Static Interpretation of Clinical Chemistry Data.....	384
CHAPTER 2C. ANALYTICAL GOALS AND CLINICAL RELEVANCE OF LABORATORY PROCEDURES.....	387
<i>by Robert S. Galen, M.D., M.P.H., and Theodore Peters, Jr., Ph.D.</i>	
Analytical Goals 388; Analytical Performance Compared with Analytical Goals 393	

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.