

**Therapeutic Drug Monitoring
and Toxicology
by Liquid Chromatography**

CHROMATOGRAPHIC SCIENCE

A Series of Monographs

Editor: JACK CAZES
Fairfield, Connecticut

- Volume 1: Dynamics of Chromatography (out of print)
J. Calvin Giddings
- Volume 2: Gas Chromatographic Analysis of Drugs and Pesticides
Benjamin J. Gudzinowicz
- Volume 3: Principles of Adsorption Chromatography: The Separation of Nonionic Organic Compounds (out of print)
Lloyd R. Snyder
- Volume 4: Multicomponent Chromatography: Theory of Interference (out of print)
Friedrich Helfferich and Gerhard Klein
- Volume 5: Quantitative Analysis by Gas Chromatography
Joseph Novák
- Volume 6: High-Speed Liquid Chromatography
Peter M. Rajcsanyi and Elisabeth Rajcsanyi
- Volume 7: Fundamentals of Integrated GC-MS (in three parts)
Benjamin J. Gudzinowicz, Michael J. Gudzinowicz, and Horace F. Martin
- Volume 8: Liquid Chromatography of Polymers and Related Materials
Jack Cazes
- Volume 9: GLC and HPLC Determination of Therapeutic Agents (in three parts)
Part 1 edited by Kiyoshi Tsuji and Walter Morozowich
Part 2 and 3 edited by Kiyoshi Tsuji
- Volume 10: Biological/Biomedical Applications of Liquid Chromatography
Edited by Gerald L. Hawk
- Volume 11: Chromatography in Petroleum Analysis
Edited by Klaus H. Altgelt and T. H. Gouw
- Volume 12: Biological/Biomedical Applications of Liquid Chromatography II
Edited by Gerald L. Hawk
- Volume 13: Liquid Chromatography of Polymers and Related Materials II
Edited by Jack Cazes and Xavier Delamare
- Volume 14: Introduction to Analytical Gas Chromatography: History, Principles, and Practice
John A. Perry

- Volume 15: Applications of Glass Capillary Gas Chromatography
Edited by Walter G. Jennings
- Volume 16: Steroid Analysis by HPLC: Recent Applications
Edited by Marie P. Kautsky
- Volume 17: Thin-Layer Chromatography: Techniques and Applications
Bernard Fried and Joseph Sherma
- Volume 18: Biological/Biomedical Applications of Liquid Chromatography III
Edited by Gerald L. Hawk
- Volume 19: Liquid Chromatography of Polymers and Related Materials III
Edited by Jack Cazes
- Volume 20: Biological/Biomedical Applications of Liquid Chromatography IV
Edited by Gerald L. Hawk
- Volume 21: Chromatographic Separation and Extraction with Foamed Plastics and Rubbers
G. J. Moody and J. D. R. Thomas
- Volume 22: Analytical Pyrolysis: A Comprehensive Guide
William J. Irwin
- Volume 23: Liquid Chromatography Detectors
Edited by Thomas M. Vickrey
- Volume 24: High-Performance Liquid Chromatography in Forensic Chemistry
Edited by Ira S. Lurie and John D. Wittwer, Jr.
- Volume 25: Steric Exclusion Liquid Chromatography of Polymers
Edited by Josef Janča
- Volume 26: HPLC Analysis of Biological Compounds: A Laboratory Guide
William S. Hancock and James T. Sparrow
- Volume 27: Affinity Chromatography: Template Chromatography of Nucleic Acids and Proteins
Herbert Schott
- Volume 28: HPLC in Nucleic Acid Research: Methods and Applications
Edited by Phyllis R. Brown
- Volume 29: Pyrolysis and GC in Polymer Analysis
Edited by S. A. Liebman and E. J. Levy
- Volume 30: Modern Chromatographic Analysis of the Vitamins
Edited by Andre P. De Leenheer, Willy E. Lambert, and Marcel G. M. De Ruyter
- Volume 31: Ion-Pair Chromatography: Theory and Biological and Pharmaceutical Applications
Edited by Milton T. W. Hearn
- Volume 32: Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography
Edited by Steven H. Y. Wong

Other Volumes in Preparation

Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography

Edited by

Steven H. Y. Wong

*Assistant Professor of Laboratory Medicine
and Director, Drug Analysis Division
Department of Laboratory Medicine
University of Connecticut School of Medicine
Farmington, Connecticut*

MARCEL DEKKER, INC.

New York and Basel

Library of Congress Cataloging in Publication Data

Main entry under title:

Therapeutic drug monitoring and toxicology by liquid chromatography

(Chromatographic science ; v. 32)

Includes index.

1. Drugs--Analysis.
2. Liquid chromatography.
3. Patient monitoring.
4. Chromatographic analysis.

I. Wong, Steven H. Y. (Steven How-Yan), [date].

II. Series.

RB56.T443 1985 615'.7 84-23061

ISBN 0-8247-7246-6

COPYRIGHT © 1985 by MARCEL DEKKER, INC. ALL RIGHTS RESERVED

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

MARCEL DEKKER, INC.

270 Madison Avenue, New York, New York 10016

Current printing (last digit):

10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

Dedicated to
my wife, Gretta,
my daughters, Heather and Amanda,
and my parents

FOREWORD

It has been just over 10 years since the technique of high-performance liquid chromatography took its first hesitant steps into the clinical laboratory. Since that time, it has deservedly become the technique of choice for many applications in therapeutic drug monitoring and toxicology. Modern liquid chromatography (LC) has made it possible for laboratorians to routinely measure many previously elusive analytes. Just as importantly, it has allowed the determination of highly polar, conjugated metabolites of therapeutic agents, the concentrations of which may be predictive of certain metabolic diseases or drug overdose. This latter capability of LC is not shared by currently available immunoassays. There is promise for the future: improved sensitivity, more specific detectors, and partial or full automation are now becoming available or are on the horizon.

This volume is an excellent representation of the many areas of active investigation in the clinical application of LC to drug analysis. Its editor and contributors are to be congratulated for their straightforward and pragmatic approach to this instrumental technique. I believe that their effort will promote and encourage the adoption of LC as a practical analytical technique by numerous clinical laboratories throughout the world.

Randall C. Baselt, Ph.D.
Professor and Director of Toxicology
University of California School of Medicine
Davis, California

PREFACE

Therapeutic drug monitoring (TDM), since its emergence in the 70s, has been established as a well-accepted subspecialty of clinical medicine. Undoubtedly, laboratory measurements of TDM and toxicology have been greatly enhanced by the advances in instrumentations such as immunoassay and chromatography. Liquid chromatography (LC), in particular, has played a very active and vital role, by providing fast, specific, cost-effective, and in selected cases (such as antidepressants, antihypertensives, and immunosuppressives), the preferred or only mode of analysis for drugs and metabolites, often before the immunoassay. Indeed, for comparison studies, the LC assay is often chosen as the reference method. In spite of numerous books, articles, and workshops dealing with various aspects of TDM and toxicology, a comprehensive and dedicated treatise of LC for these areas is still lacking. Furthermore, with the present emphasis on the new health-care economics, such as the Diagnostic Related Group (DRG) reimbursement policy, laboratories might want to reexamine the choice of instrumentation and methods. It is in light of these considerations that this book was conceived and finalized. The approach is to present a current account of the various LC instrumentations for TDM and toxicology drug assays, and to review the clinical pharmacology of major classes of drugs and their LC analyses. Each chapter is based upon its contributor's personal experience. The book should be useful to practitioners such as laboratory directors, toxicologists, and clinicians, and to interested nonpractitioners such as analytical chemists, immunologists, and instrument manufacturers.

The first part includes chapters on the principles of TDM, sampling techniques, and various instrumentation topics, such as the computer,

mass spectrometry, fluorescence, and electrochemical detection. The second part consists of chapters on six major classes of drugs, beginning with a review of their clinical pharmacology and LC analyses, followed by recommended LC procedures. Emphasis is placed on some of the recently introduced drugs and their LC analyses. The third part consists of chapters on medicolegal guidelines, LC analyses of miscellaneous groups of drugs, and various laboratory management considerations. It is hoped that through this updated and coordinated treatment, the roles of LC might be better defined to meet the present needs of the laboratory for TDM and toxicology drug measurements.

The editor gratefully acknowledges the effort of the contributors. He is indebted to his chairman, F. William Sunderman, Jr., M.D., for his encouragement and support; and his former mentor, Dr. Hans J. Ache, for introducing liquid chromatography to his graduate research. During the initial planning of this book, valuable suggestions were solicited from the following persons: Drs. Richard P. Spencer, Jack Cazes, Charles Pippenger, R. P. W. Scott, B. L. Karger, L. R. Snyder, and John Dolan. He also wishes to thank Drs. Dennis Hill, Carlos Santiago, Ralph Rodriguez, Mitchell Gandleman, and Nemat Marzouk for reviewing the manuscripts; and Mary Gagnon who undertook the laborious and important tasks of typing the manuscripts and correspondence. His family's understanding and support has inspired and enhanced the undertaking and completion of this project.

Steven H. Y. Wong

CONTRIBUTORS

Vijay Aggarwal, Ph.D. Department Head, Department of Clinical and Industrial Toxicology, American Bio-Science Laboratories, Van Nuys, California

H. Dix Christensen, Ph.D.* Visiting Professor, Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, Florida

Christine Eckers, Ph.D.† Research Associate, Equine Drug Testing Program, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York

Jack Henion, Ph.D. Associate Professor of Toxicology, Equine Drug Testing Program, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York

Zafar H. Israili, Ph.D. Associate Professor of Medicine, Emory University School of Medicine, and Atlanta Veterans Administration Medical Center, Atlanta, Georgia

Pokar M. Kabra, Ph.D. Associate Professor, Department of Laboratory Medicine, University of California, San Francisco, San Francisco, California

Present affiliations:

*Associate Professor, Department of Pharmacology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

†GC/MS Specialist, Analytical Instrumentation Group, Hewlett-Packard Ltd., Winnersh, Berkshire, England

Peter T. Kissinger, Ph.D. Professor of Chemistry, Department of Chemistry, Purdue University, and President, Bioanalytical Systems, Inc., West Lafayette, Indiana

Carol Lavrich, B.S.* Technical Representative, Sales Department, Bioanalytical Systems, Inc., West Lafayette, Indiana

Fu-Chung Lin, Ph.D. Manager, Research and Development Laboratories, Becton Dickinson Vacutainer Systems Division, Rutherford, New Jersey

David J. Miner, Ph.D. Analytical Development Division, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Indiana

Sheshadri Narayanan, Ph.D. Clinical Professor of Pathology, Department of Pathology, New York Medical College Metropolitan Hospital Center, New York, New York, and Director, Research and Development Laboratories, Becton Dickinson Vacutainer Systems Division, Rutherford, New Jersey

Allen H. Neims, M.D., Ph.D. Professor and Chairman, Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, Florida

C. E. Pippenger, Ph.D. Head, Section of Applied Clinical Pharmacology, Department of Biochemistry, Cleveland Clinical Foundation, Cleveland, Ohio

Kevin F. Scott, Ph.D. Inorganic Chemistry Laboratory, Oxford University, Oxford, England

Steven J. Soldin, Ph.D., F.A.C.B. Associate Biochemist and Director, Therapeutic Drug Monitoring, University of Toronto, and Associate Professor, Department of Clinical Biochemistry and Pharmacology, Hospital for Sick Children, Toronto, Ontario, Canada

Robert Weinberger, Ph.D. Senior Applications Scientist, Chromatography Systems, Kratos Analytical Instruments, Ramsey, New Jersey

Steven H. Y. Wong, Ph.D. Assistant Professor of Laboratory Medicine, and Director, Drug Analysis Division, Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, Connecticut

Present affiliation:

*Technical Representative, Sales Department, Waters Associates, Inc., Milford, Massachusetts

CONTENTS

Foreword (<i>Randall C. Baselt</i>)	v
Preface	vii
Contributors	ix
1. Introduction	1
<i>Steven H. Y. Wong</i>	
1. FUNDAMENTALS AND CURRENT CLINICAL INSTRUMENTATION	
2. Principles of Therapeutic Drug Monitoring	11
<i>C. E. Pippenger</i>	
I. Introduction	11
II. Pharmacodynamics: Site and Mechanism of Drug Action	16
III. Pharmacokinetics	19
IV. Guidelines for Routine Therapeutic Drug Monitoring	28
V. Conclusion	36
References	36
3. Liquid Chromatography and Other Methodologies for Therapeutic Drug Monitoring and Toxicology	39
<i>Steven H. Y. Wong</i>	
I. Introduction	39
II. Liquid Chromatography	40
III. Gas Chromatography	53
	<i>xi</i>

IV.	Radioimmunoassay (RIA)	54
V.	Enzyme Multiplied Immunoassay Techniques (EMIT)	56
VI.	Fluorescence Polarization Immunoassay (FPIA) Using TDX	59
VII.	Substrate-Labeled Fluorescence Immunoassay (SLFIA)	66
VIII.	Nephelometric Inhibition Immunoassay (NIIA)	68
IX.	Latex Agglutination Inhibition Test for Gentamicin and Tobramycin	68
X.	Enzyme-Linked Immunosorbent Assay: ELISA Enzymune-Test TM	69
XI.	Fluorescent Immunoassay: Amerifluor TM	70
XII.	Prosthetic Group Label Immunoassay (PGLIA)	71
XIII.	Automated Turbidimetric Inhibition Assay for Theophylline	71
XIV.	Conclusion	73
	References	73
4.	Sampling Technique	79
	<i>Sheshadri Narayanan and Fu-Chung Lin</i>	
I.	Introduction	79
II.	Devices	79
III.	Components of Materials Used in Specimen Collection Devices	81
IV.	Influence of Specimen Collection in Toxicology	83
V.	Conclusion	85
	References	86
5.	Computer Control of Liquid Chromatographic Analyses	89
	<i>Kevin F. Scott</i>	
I.	Introduction	89
II.	The Anatomy of a Computer-Controlled Liquid Chromatographic System	90
III.	Computer-Controlled Collection of Liquid Chromatographic Data	99
IV.	Data Handling	106
V.	The Selection of an Automatic Liquid Chromatographic System	110
	References	113
6.	Combined Liquid Chromatography-Mass Spectrometry of Drugs	115
	<i>Christine Eckers and Jack Henion</i>	
I.	Introduction	115
II.	Off-Line LC-MS	116
III.	On-Line LC-MS	117

<i>Contents</i>	<i>xiii</i>
IV. LC-MS, MS-MS, and LC-MS-MS	134
V. Discussion	135
VI. Conclusion	138
References	138
7. Drug Determination in Biological Fluids by Liquid Chromatography-Fluorescence	151
<i>Robert Weinberger</i>	
I. Introduction	151
II. Basic Concepts	152
III. Instrumentation	157
IV. Applications of LC-Native Fluorescence	161
V. Precolumn Derivatization	164
VI. Postcolumn Derivatization	170
VII. Phosphorescence	180
References	184
8. Liquid Chromatography-Electrochemistry: Potential Utility for Therapeutic Drug Monitoring	191
<i>Carol Lavrich and Peter T. Kissinger</i>	
I. Introduction to Liquid Chromatography-Electrochemistry (LC-EC)	191
II. Electroactive Drugs	196
III. Review of Applications	202
IV. Future Directions	225
V. Conclusion	229
References	229
II. MAJOR CLASSES OF DRUGS	
9. Antiasthmatics	237
<i>H. Dix Christensen and Allen H. Neims</i>	
I. Introduction	237
II. Rationale for Xanthine Monitoring	238
III. Liquid Chromatography	239
IV. Interferences	253
V. Reliability of Analytical Procedures	256
VI. Conclusion	257
References	258
10. Antibiotics	269
<i>David J. Miner</i>	
I. Introduction	269
II. General Considerations	269

III. Review of Liquid Chromatographic Methods	273
IV. Conclusion	300
References	302
11. Antidepressants	309
<i>Steven H. Y. Wong</i>	
I. Introduction	309
II. Historical Perspectives	310
III. Hypotheses for Depression	311
IV. Clinical Pharmacology	313
V. Rationale for Antidepressant Monitoring and the Role of the Laboratory	324
VI. Sampling Considerations	325
VII. Antidepressants Assays	326
References	340
12. Anticonvulsants	351
<i>Steven J. Soldin</i>	
I. Introduction	351
II. The Rationale for Therapeutic Drug Monitoring	352
III. Clinical Pharmacology	355
IV. Measurement of Anticonvulsant Drugs by HPLC	358
References	363
13. Antihypertensives	367
<i>Zafar H. Israili</i>	
I. Introduction	367
II. Drugs with Central Modes of Action	368
III. Drugs Acting at the α -Adrenoceptor Sites	371
IV. β -Adrenoreceptor Antagonists	372
V. Diuretics	381
VI. Inhibitors of the Renin-Angiotensin System	387
VII. Vasodilators	389
VIII. Drugs with Miscellaneous Modes and Sites of Action	392
IX. Conclusion	394
References	395
14. Antiarrhythmics	405
<i>Pokar M. Kabra</i>	
I. Introduction	405
II. Preparation of Samples	410
III. Chromatography	412
IV. Detection and Quantitation	420
V. Analysis of Metabolites	424

<i>Contents</i>	xv
VI. Very-High-Speed Liquid Chromatography	427
References	429
III. LABORATORY MANAGEMENT AND MISCELLANEOUS TOPICS	
15. Medicolegal Guidelines for the Clinical Toxicology Laboratory	437
<i>Vijay Aggarwal</i>	
I. Introduction	437
II. The Medicolegal Environment	438
III. Minimizing Liability	442
IV. The Medicolegal Sample	445
V. Medicolegal Testimony	448
VI. Conclusion	450
Bibliography	451
16. Drugs and Laboratory Management: Clinical and Technical Considerations	453
<i>Steven H. Y. Wong</i>	
I. Introduction	453
II. LC Drug Analysis	453
III. Laboratory Management	453
References	474
17. Conclusion: Current Status and Future Developments	485
<i>Steven H. Y. Wong</i>	
Index	489

INTRODUCTION

STEVEN H. Y. WONG / *University of Connecticut School of Medicine, Farmington, Connecticut*

Therapeutic drug monitoring (TDM) has developed into a well-established subspecialty within the clinical laboratory. It deals with the optimization of the clinical response, as guided by the drug concentration in biological fluid such as plasma or serum. The rationale and the principles for TDM have been extensively reviewed [1-5] and updated in Chap. 2 by Pippenger. Toxicology deals with the identification/quantitation of known or unknown drugs and chemicals [6-9]. Traditionally, some of the modern TDM functions have been regarded as toxicology. Depending on the drug concentration and patient history (such as overdose), a TDM or toxicology measurement may be performed by the same procedure.

The evolution of TDM resulted from the increasing abundance of pharmacokinetic data [10], and from the introduction and/or improvement of instrumentations such as chromatography and immunoassay. With these instrumentations, laboratory personnel can measure the plasma concentration of drugs and metabolites within a reasonable amount of time (usually 1-4 hr), with predictable and acceptable precision (relative standard deviations are usually less than 10%) [11]. Among the various modes of chromatography used for TDM and toxicology, both gas and liquid chromatography have played vital roles. Applications of LC for drug measurement dated back to the early seventies with the advent of LC for drug identification and for pharmacokinetic studies [12]. Liquid chromatography, herein encompassing the following terms, "high-performance liquid chromatography (HPLC)," "high-pressure liquid chromatography (HPLC)," and "high-speed liquid chromatography," has already surpassed gas chromatographic applications for certain areas of TDM such as antidepressant monitoring,

according to a recent survey result of the College of American Pathologists, as shown by Table 1 for the measurement of nortriptyline [13]. Advantages of LC include its selectivity and sensitivity as a result of variety of separation and detection modes. Thus LC has enhanced TDM and toxicology as much as it has other areas of the biomedical and biochemical sciences. But perhaps the difference in regard to LC is the high frequency of daily routine LC clinical analysis—a testimonial to the remarkable state of the instrumentation and column technology.

In carrying out TDM/toxicology measurements, there are two primary considerations—personnel and instrumentation. A dedicated number of personnel (four to five technologists) would enhance the operation with the objective of becoming an active and integral part in the patient health care system. In view of the vast number of drugs and the increasing number of newly introduced drugs on the market, the technologists would have to gain a working knowledge of clinical pharmacology through meetings, discussions, and workshops. Then the personnel would be more proficient towards interpreting drug levels as well as detecting abnormal results or laboratory errors. The dosage regime and the clinical status of the patients would guide the laboratory personnel as to what to anticipate, and therefore to recommend the appropriate drug measurement/toxicology test. The recommendations would include sampling time, types of specimens, and others. Based on the above considerations, it is inherently difficult to train a large laboratory staff to be proficient in various aspects of clinical pharmacology and measurement.

For the measurement or identification of drugs and chemicals, the instrumentation ranges from the simple flame photometer used to measure lithium, to automated equipment such as the Automated Clinical Analyzer (Dupont, Wilmington, Delaware) used for Enzyme Multiplied Immunoassay Technique (EMIT) (Syva, Palo Alto, California), and TDX, for Fluorescence Polarization Immunoassay (FPIA) (Abbott, North Chicago, Illinois), to the more demanding chromatography. The immunoassay technology and its instrumentation are much more adaptable to automation and thus require less involvement of personnel. However, the chromatographic procedures usually require extraction and analysis performed with close personal attention. Depending upon the ability of the personnel, TDM/toxicology chromatographic equipment may range from simple TLC and basic gas or liquid chromatographs, to the more sophisticated chromatographs with various data handling capability such as GC-MS and its associated data bank. In order to utilize these instruments, it will be more efficient to train a dedicated group of technologists.

Among the whole range of instrumentation used by clinical laboratories for drug measurement, chromatography has always played a vital role. From the survey results of the College of American Pathologists [14], phenobarbital measurement (Table 2) is still performed by

Table 1 CAP Survey Results for the Measurement of Nortriptyline (ng/ml)

Specimen/method	No. labs	Mean	S.D.	C.V.	Median	Low value	High value
Specimen Z-5	Target value 100.0						
Gas-liquid chromatography Underivatized	18	—	—	—	90	49	151
High-performance liquid chrom. Solvent extraction	42	98.7	37.2	37.7	—	—	—
All HPLC results	49	97.8	35.5	36.3	—	—	—
All methods/all results	74	102.6	43.0	42.0	—	—	—
Specimen Z-6	Target value 1200.0						
Gas-liquid chromatography Underivatized	18	—	—	—	1243	207	1094
High-performance liquid chrom. Solvent extraction	41	1199.8	201.5	16.8	—	—	—
All HPLC results	48	1175.4	170.3	14.5	—	—	—
All methods/all results	72	1230.9	237.2	19.3	—	—	—

Source: Ref. 13.

Table 2 CAP Survey Results for the Measurement of Phenobarbital (mcg/ml)

Specimen/method	No. labs	Mean	S.D.	C.V.	Median	Low value	High value	
Specimen Z-2		Target value 2.0						
The results listed below were reported as "Equal To" values.								
Photometry								
Spectrophotometry/colorimetry	11	—	—	—	2.0	1.3	6.0	
All photometry results	12	—	—	—	2.0	1.3	6.0	
Gas-liquid chromatography								
Derivatized	11	—	—	—	2.0	0.0	2.4	
Underivatized	14	—	—	—	2.3	1.2	6.1	
All GLC results	24	2.12	0.95	44.7	—	—	—	
High-performance liquid chrom.								
Protein precipitation	21	2.45	0.76	31.2	—	—	—	
Solvent extraction	21	2.30	0.63	27.6	—	—	—	
All HPLC results	45	2.38	0.69	28.8	—	—	—	
Enzyme immunoassay								
Syva	105	2.40	0.88	26.6	—	—	—	
Other	19	—	—	—	2.2	1.0	19.5	

	No. labs	Mode	Low value	High value
Fluorescent immunoassay				
Ames	18	—	0.7	2.5
Fluorescence polarization imm.				
Abbott	84	1.44	—	—
Immunonephelometry/immunoturbid.				
Beckman	65	3.88	—	—
Beckman		1.22	—	—
Beckman		31.4	—	—
Radioimmunoassay				
Clinical assays	13	—	1.7	3.3
All immunoassay results	307	2.34	—	—
All methods/all results	401	2.34	—	—
		1.02	—	—
		43.6	—	—
		1.01	—	—
		43.1	—	—
The results listed below were reported as "Less Than" values.				
Photometry				
Spectrophotometry/colorimetry	36	—	2.0	5.0
Enzyme immunoassay				
Syva	418	—	1.5	5.0
Other	118	—	3.0	5.0
Fluorescence polarization imm.				
Abbott	31	—	0.5	5.0
Immunonephelometry/immunoturbid.				
Beckman	21	—	3.8	8.0

Source: Ref. 14.