Analytical Aspects of Drug Testing

Edited by Dale G. Deutsch

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with foreword by

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VOLUME 100

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FOREWORD

Analytical toxicologists are faced with ever-increasing demands for more sensitive and specific methods to detect the increasing number of clinical agents in common use. To help satisfy this demand, the editor of this volume has assembled a series of monographs describing the cutting edge of recent advances in analytical techniques. By adapting these techniques to the analysis of biological specimens, toxicologists will be able to provide data to those concerned that may help resolve problems related to proper use, misuse, or abuse of drugs. These problems include the ongoing search for: (a) causes of poisonings, (b) improved therapeutic approaches using currently available drugs, (c) the development of new drugs and their pharmacology, (d) means to insure a community, military, and industrial environment free of drug abuse, and (e) ascertaining the implications of substances detected in biological specimens during medicolegal investigations.

Analysts have responded to these demands in an exemplary fashion. They have developed techniques and procedures whose precision, accuracy, specificity, and sensitivity have met initial goals. As these goals were met, other more sensitive and specific demands were made because more knowledge and insights were acquired. This continuing process creates the need to modify the current procedures, a need that can be satisfied only by utilizing the latest modern technology.

The authors of the chapters in *Analytical Aspects of Drug Testing* provide significant information on these technological advances and suggest how they can be applied to drug analyses. Rightfully, the volume begins with the topic of quality assurance and ends with material designed to help interpret the results obtained by any of the innovative techniques described in between these two chapters. Also included in the volume are discussions on separation techniques, immunoassays, and the many chromatographic techniques that have become essential to adequate laboratory performance. In many instances these techniques involve relatively expensive equipment and specially trained personnel. The rising costs of health care will determine the extent to which these improved techniques will be used. The cost/benefit ratio will be a deciding factor in this decision.

Refined and improved laboratory data are achievable. Reliable as they may be, they may have little or no value in patient care if personnel beyond the laboratory fail in their responsibilities. *Cooperation* is the key word. The attending physician concerned with monitoring proper use of a therapeutic drug (a) must initiate an explicit request for a laboratory result; (b) must insure that the proper procedures are in place for obtaining the designated specimen at a proper time (it is essential that the exact time of sampling be noted as well as the time the last dose of medicine was given); (c) must provide for prompt delivery of the specimen to the laboratory; (d) should inform the laboratory of all relevant clinical information; and (e) should be available when the laboratory result is reported.

If a suspected poisoning is being investigated, both blood and urine specimens are required. A history of medications taken by the patient prior to the incident that led to the need for treatment, a list of medications administered to the patient by the attending staff, and the patient's presenting symptoms and current status should be reported to the toxicologist. In medico-legal investigations (and these include testing urines for drugs subject to abuse), a chain of custody must be established and maintained. This requires a written document listing all those who handle the specimens as they proceed from obtaining the specimen from the person involved in an incident to all the laboratory staff members doing the analyses. Laboratory security must be assured and written records must be kept of each person who handles each specimen, including the times of analyses, the storage and ultimate disposition of the specimens, and all original data on standards, quality control, and positive results. Criteria for maintenance, quality control and analytical procedures must be available in a suitable volume. Evidence of supervisory inspections of all results must be documented. Failure to observe these essential steps could vitiate the legal value of the most adequate laboratory result.

The laboratory, too, has responsibilities beyond that of providing an adequate method for a particular need. Quality assurance is essential to quality analyses. However accurate and reliable the analysis, the result will be undesirable and valueless if the sample is mislabeled, if it is confused with another, if proper records (and in medico-legal instances the related analytical data and the specimen) are not kept, if reports are not checked prior to release and then not reported properly. Quality assurance provides for continuous monitoring of all steps in the laboratory; it is essential. Too often, most laboratories consider quality control procedures (essential as they are) to be sufficient and equivalent to quality assurance. There is no substitute for quality assurance and it is obligatory in every laboratory.

Notwithstanding optimal compliance with all of the abovementioned details, a laboratory result is only of value if it is interpreted properly and put to use in patient care. Improper or inadequate interpretation of an excellent laboratory datum can negate all previous well-accepted procedures. Many clinical laboratory scientists feel their role is satisfied when they submit a proper report. This is debatable. They should put emphasis on the clinical aspect of the "clinical" laboratory scientist (or clinical chemist or clinical toxicologist) and collaborate with the clinician, when appropriate, to insure proper interpretation of a laboratory result. Clinical decisions should never be made solely on the basis of a laboratory result. This is all the more true in urinalysis for drugs subject to abuse, where misinterpretation of the laboratory data can result in an unwarranted discharge of employees.

To reiterate, there is more to analysis than the result of an executed analytical procedure. A significant amount of ancillary efforts are required, which involve laboratory personnel, clinicians, and administrators. It is essential that these groups be aware of their respective obligations and that they fulfill them in an exemplary way. This can only be achieved if communication pathways are patent and used as necessary. When this is accomplished, then laboratory data can be put to effective use in improved patient care.

IRVING SUNSHINE

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PREFACE

Drug testing affects many areas of our society. Among the most publicized are the workplace and employee programs, but it has also proliferated in athletic programs, the military, correctional facilities, and rehabilitation programs. Drug testing also plays a very crucial role in the medical setting. In the clinical laboratory the toxicologist performs therapeutic drug monitoring, emergency room toxicology for the overdosed or poisoned patient, and testing for substance abuse in patients from psychiatry, neurology, obstetrics and gynecology, pediatrics, sleep clinics, and other areas.

Due to the continued proliferation of new technology, the field of analytical toxicology is expanding vigorously, particularly in the area of drug testing. This book is designed to update readers on previously established technology, outline new techniques that are now being used in the more advanced laboratories and explore a number of techniques which are still in developmental stages. Many of the chapters in this volume contain data presented for the first time.

The first chapter is presented in recognition of the fact that sophisticated analytical technology does not, in itself, insure quality results. Chapter 1, written by Dr. Jenny, a scientist in charge of the New York State proficiency testing program, is a primer on quality assurance for laboratories performing clinical and workplace drug testing. It contains explicit procedures which, if followed, insure that the integrity of good analytical procedures is not compromised by any weakness in the steps in the process, from collection of the sample to the reporting of results. Chapter 1 includes such important topics as: specimen collection, documentation, the standard operating procedure manual (SOPM), laboratory security, quality control, and analytical method performance characteristics.

One of the simplest and most widely used methods for drug testing in the toxicology laboratory is the immunoassay. Assays using Syva EMIT reagents for each of ten drug classes are discussed in detail in Chapter 2. Comprehensive tables listing those drugs that yield a positive response as well as those which cross-react to produce a false positive are pre-

sented. Chapter 2 also includes the principles of the enzyme immunoassay, methods for specimen collection, assay conditions, and types of commercial instruments used with the reagents.

As described in Chapter 3, solid phase extraction (SPE) procedures are becoming popular in analytical toxicology because they are more rapid, selective, and require smaller volumes of organic solvents than do liquid–liquid extraction procedures. By applying the principles described by the author of Chapter 3, Martha Harkey, one can develop an extraction protocol for any drug. An appendix to Chapter 3 contains SPE methods for many drugs, including delta-9-carboxy tetrahydrocannabinol, from urine.

With the availability of less expensive, more compact, and easier-to-use models of gas chromatograph/mass spectrometers (GC/MS), they are becoming standard equipment for toxicology laboratories. In Chapter 4, GC/MS for drug detection using library search routines is illustrated for the identification of unknown substances as well as for the confirmation of specific drugs. In Chapter 9, procedures for GC/MS screening and analysis of anabolic steroids are described in detail. These state-of-the-art methods were used in the anabolic steroid screening program during the 10th Pan American Games in 1987.

The emergence of high performance liquid chromatography (HPLC) for drug analysis is reflected by three novel chapters in this volume. Chapter 5 details methods of screening for drugs using HPLC with an ultraviolet photodiode array detector. Drugs are identified by a combination of retention indices and ultraviolet spectra, using a computerized spectral library search program written by authors Hill and Langner. Two evolving techniques for toxicology and therapeutic drug monitoring are microbore liquid chromatography (MBLC) and direct-sample-analysis, both of which are presented in Chapter 6. These methods fulfill the need to analyze smaller volumes, such as those encountered in neonatal and pediatric patient samples, with accelerated turnaround times. Examples are given for the clinical analysis of theophylline, caffeine, procainamide, and chloramphenicol. In Chapter 7, a technique destined to become a routine tool in toxicology combines the convenience of high performance liquid chromatography with the specificity of mass spectroscopy (HPLC/MS). Author Robert Voyksner describes applications of HPLC/MS for the analysis of steroids, alkaloids, mycotoxins, drugs, and pesticides.

In some instances it would be desirable to perform toxicology tests with dipsticks and observe color development visually or with a simple machine—as is now done for glucose. Chapter 8 reviews the dry reagent chemistries that have been developed for clinical toxicology and therapeutic drug monitoring. The principles of these various techniques are

described, with examples of theophylline, phenytoin, primidone, carbamazepine, gentamicin, tobramycin, phenobarbital, and amikacin.

No volume on drug testing would be complete if it did not, in addition to describing the analytical methodology, profile a drug of abuse. In Chapter 9 this is done for cocaine, a drug of abuse very much in the public eye. The clinical properties and toxicity, routes of administration, metabolic disposition and kinetics, neurochemical mechanism, and methods of analysis are presented. Furthermore, any volume in this area would be amiss if it did not, in addition to describing the analytical techniques, describe the problems and pitfalls of drug testing. The final chapter, Chapter 11, addresses these issues in terms of screening and confirmation tests such as the immunoassay and chromatographic methods, as well as discussing quality assurance issues.

Acknowledgment

I thank Steven H. Y. Wong for recommending me to Wiley to edit this volume when he was the chairman of the Therapeutic Drug Monitoring and Clinical Toxicology Division of the American Association for Clinical Chemistry. Lou C. Deutsch, my wife, generously provided me with grammar, punctuation, and emotional support throughout this undertaking.

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Stony Brook, New York
December, 1988

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CHAPTER

1

QUALITY ASSURANCE

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Analytical toxicology, as performed in the clinical laboratory, is generally applied to two principal areas: (1) diagnosis and treatment of patients with acute or chronic exposure to toxic substances, and (2) substance abuse testing for treatment programs and the workplace. Laboratory activities required to conduct toxicological investigations include sample collection, transport, accessioning, analysis, and reporting of analytical results. Associated with each of these major activities are additional tasks that must be performed to ensure the integrity of the testing process.

Paramount among all laboratory activities, however, is the quality assurance program. Regardless of how meticulously an analytical procedure is performed, the usefulness of the testing result is compromised, perhaps negated, by any weakness in the test process chain. It is the laboratory's obligation to the clinician and the clinician's patients to establish guidelines for the proper execution of each testing component. The quality of each testing result cannot be "assured"; however, a quality assurance program, properly designed and implemented, minimizes the frequency and severity of testing errors.

The design of a quality assurance program must be a collaborative effort between the physician and the laboratory director. The physician's role in this process is to describe the laboratory service required for good patient care. The description must include the scope of toxicology service, desired assay performance characteristics (sensitivity, specificity, accuracy, precision), the anticipated impact on patient care, and the expected turnaround time for reports. With this information, the laboratory director can outline laboratory requirements for specimen type and volume, sample preservation and transportation, and documentation of pertinent clinical data that might guide the course of laboratory investigation and also permit interpretive reporting.

With the ground rules in place, the laboratory has the distinct advantage of making informed decisions in the development of a viable toxicology service. Objective guidelines for quality assurance can then be established to include the specific demands imposed by the health-care practitioner.

In this chapter, I first focus on the various applications of analytical toxicology services, describing quality assurance guidelines that are necessary if the laboratory is to produce a quality product that is consistent with medical requirements for good patient care. I then provide a more detailed discussion of the preparation and use of reference and quality-control materials and of the general elements of a quality assurance program.

1. CLINICAL TOXICOLOGY

Drug overdose and exposure to nondrug toxic agents are responsible for a sizable percentage of the total cases treated in our nation's emergency rooms (1). The symptoms produced by these agents mimic those that accompany many medical conditions; the urgency of the situation and the use of notoriously inaccurate clinical histories further complicate differential diagnosis (2). The toxicological analysis of body fluids is used to establish or exclude poisoning as the cause of the patient's acute illness.

The confirmation of suspected drug overdose or exposure to toxic substances permits discontinuation of further diagnostic steps and the application of specific therapeutic measures, if available.

1.1. Medical Requirements

Management of the poisoned patient generally involves support of vital functions until the poison is cleared from the system (3). Specific antidotal therapies, however, are available for a few intoxicants, for example, acetaminophen, salicylate, antidepressants, barbiturates, ethylene glycol, methanol, mercury, iron, and lead (3, 4). The antidotes are also potentially harmful to the patient and must be used judiciously. The intoxicant blood concentration is often of prognostic value and is used to assess the necessity for specific therapeutic intervention.

The clinical staff therefore requires a level of support that includes the following (5):

1. A broad-spectrum urine drug screen capable of detecting drugs commonly abused in the community it serves.
2. Quantitation or semiquantitation of the amount of drug in blood, when necessary and appropriate, to document the correlation of concentration and effect. This is done to rule out other underlying causes of the medical condition.
3. Reliable serial determinations of blood concentration of those drugs for which there is a specific antidote for assessment of prognosis and course of therapy.
4. Expeditious reporting of toxicological analyses to ensure prompt, optimal patient care.
5. Twenty-four-hour service.

1.2. Laboratory Requirements

Perhaps more than in any other area in laboratory medicine, the toxicologist requires the active participation of clinical staff during the toxicological investigation of any disease state of unknown origin. The toxicologist must rely upon the astute diagnostician to limit the universe of drugs to a drug or class of drugs that might produce the symptoms expressed by the patient. The laboratory's productivity and quality assurance in terms of patient care depend critically on the ongoing communication between clinical staff and the toxicologist.

Access to the patient during the acute crisis is generally limited to the