Essentials for the Small Laboratory and Physician's Office

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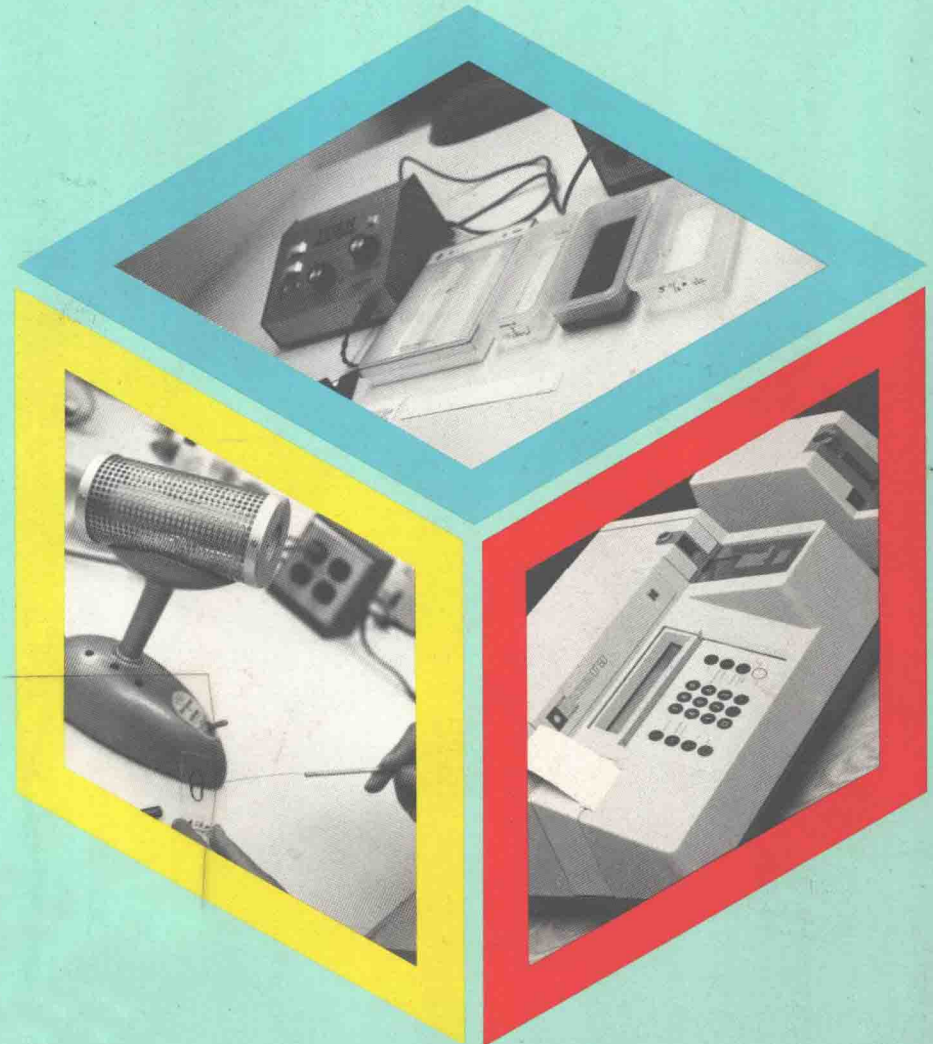

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ESSENTIALS FOR THE SMALL LABORATORY AND PHYSICIAN'S OFFICE

To my husband, Mark; sons, Patrick and Jonathan; parents; and parents-in-law for their support and devotion.

K.B.-McB.

To Wilson G. Brown, M.D., who encouraged my professional development early in my career.

D.L.R.

PREFACE

The area of medical laboratory sciences is undergoing significant changes. A shift of laboratory testing is occurring from larger health care institutions to smaller laboratories and physicians' offices and clinics. The personnel performing the laboratory tests include: (1) medical technologists (MTs), (2) medical laboratory technicians (MLTs), (3) registered nurses (RNs), (4) licensed vocational nurses (LVNs), (5) medical assistants, and (6) physicians. In addition, changing technology that employs dry reagent systems is leading to the development of many decentralized laboratories in wards, operating rooms, and emergency rooms.

With the expansion of laboratory services into these ambulatory areas, which have many health professionals who have not had extensive education and/or experience in clinical laboratory techniques and quality assurance measures, a book was needed containing the "essentials" of medical laboratory technology. The book was designed to benefit both student and practicing professionals who perform these laboratory assays. It includes laboratory procedures commonly performed in ambulatory and physicians' offices; proper collection procedures for blood and other body fluids to be analyzed through laboratory techniques; many lists, illustrations, and tables that detail the laboratory tests for the working professional to quickly consult; current usefulness of these procedures and implications of the results; cost-effective strategies and instrument selection criteria for implementation of laboratory testing; reference intervals (ranges) of normal variation in laboratory tests' results according to sex and age groups (e.g., newborn, pediatric, adult, senior adult); and quality assurance, quality control, and safety procedures that should be followed in the performance of these laboratory tests.

The availability of a large menu of sophisticated assays for the smaller laboratory and/or physician's office brings the professional responsibility of determining the reliability and accuracy of each of these laboratory results through quality assurance and control concepts. The practicing physician

and/or laboratory director must understand these quality assurance principles and make certain that the personnel performing the laboratory assays can perform the necessary quality control procedures.

This book is unique in that for the practicing physician and/or laboratory director, it serves as a "reference" for quality assurance, quality control, and safety standards; and for the laboratory personnel, it serves as a "handbook" for quality assurance in blood collection and laboratory procedures. The awareness of quality control techniques and quality assurance by the health professional involved in laboratory testing leads to increased laboratory accuracy and precision of results and decreases the likelihood of legal problems from poorly derived laboratory results.

The overall intent of this book is to provide greater insight into medical laboratory technology and procedures for health care professionals, with a resultant product of "quality patient care."

We are indebted to many individuals for assistance in preparing this textbook. We would like to express appreciation to Barbara Smith Michael, Gordon Briggs, the College of American Pathologists; Mark Irish with Sarsedt, Inc.; Ted Turaschek with Clay Adams; Allison Bausback with International Technidyne Corp.; Kenneth E. Ballinger, Jr., with E.I. DuPont DeNemours and Co.; O.Q. Hansen with Buffalo Medical Specialties Mfg., Inc.; and Karen Kaplan, who provided the computer illustration of the blood cells.

We would like to thank Sheryl Stout Parker, a postbaccalaureate student in The University of Texas Biomedical Communications Program, who was the photographer for the book. Also, we wish to thank Connie Rogers, a postbaccalaureate student in The University of Texas Biomedical Communications Program for her illustrations for the book. We would like to express appreciation to John Kuykendall at The University of Texas Cancer Center, M. D. Anderson Hospital and Tumor Institute, for the photographs of urinary sediment in chapter 4.

We are indebted to our families for enduring the many hours spent developing this book.

We hope that this book will create a better understanding and practice in medical laboratory science.

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CONTENTS

1 Principles of Laboratory Work

CLINICAL LABORATORY ANALYSES

Laboratory procedures are performed to obtain results that can assist the physician in screening, diagnosing, or monitoring the patient's disease or disability. Table 1–1 gives the steps that are involved in requesting, performing, and evaluating a measured quantity (analyte) in blood, urine, cerebrospinal fluid (CSF), or other body fluid. The first step in the performance of clinical laboratory procedures is properly to prepare the patient for the particular assay to be performed (e.g., having the patient abstain from eating for 8–12 hours prior to certain laboratory procedures), which is followed by the collection of biologic specimen (e.g., blood, urine), analysis, and production of laboratory results and a report.

The laboratory results are expressed in some type of unit (e.g., plasma potassium equals 4.1 mEq/L, where 4.1 is the number for the substance concentration and mEq/L is the symbolized unit). The following three segments of information should be presented when a laboratory result is given in a report: (1) "system" of which a component or function is measured (e.g., plasma); (2) "component" (e.g., potassium); and (3) "kind of quantity" (e.g., substance concentration). Two types of units are now being used for the reporting of laboratory results: (1) conventional units and (2) SI units (Systeme Internationale d'Unites). Table 1–2 provides examples of clinical chemistry and hematology tests as related to their conventional and SI

TABLE 1–1.

Procedural Steps in Requesting, Collecting, Performing, and Evaluating a Measured Quantity From a Body Fluid

I. A quantitative measurement of an analyte in a patient's body fluid (e.g., blood) is requested by a physician
II. The laboratory assay for that analyte is performed by personnel and involves the following
  A. Preassay
    1. Preparation of the patient
    2. Collecting and processing the specimen
    3. Storing the specimen prior to the measurement phase
  B. Assay (manually or by instrumentation):
    1. Taking an aliquot of the specimen for the assay
    2. Dispensing the aliquot into a reaction vessel
    3. Combining the aliquot with one or more reagents
    4. Recording some physical-chemical consequence of the reaction.
    5. Computing the value of the quantity measured
  C. Postassay
    1. Acceptance or rejection of the value dependent on quality control checks
    2. If the value (result of the measurement) is of quality, a report of the result is sent to the requesting physician
III. The laboratory result is evaluated by the physician
  A. The laboratory result is compared by the physician with other known patient diagnostic information
  B. A clinical decision (at least partially) is made by the physician based on the laboratory result

units and a list of conversion factors necessary to use when transferring from conventional units to SI units.

To derive a laboratory result on a patient's specimen, an analytical method must be used. The method includes a procedure, supplies, reagents, and/or instruments which are necessary for the analyst to obtain a result on the "analyte." Each analytical method employed to obtain laboratory results should have a set of written instructions which contain the following information (see example in Table 1–3):

1. Outline of the principle of the laboratory assay with appropriate references to scientific journals.

2. Specification of instruments, reagents, controls, and supplies necessary in the performance of the assay.

3. Explicit instructions on how to make the reagents and calibration standards (calibrators).

4. Specimen requirements with the necessary minimum volume, an-

ticoagulants, or preservatives required and recommended storage conditions.

5. Full description of the analytical procedural steps including the calibration procedure and necessary calculations.

6. The range of the concentrations over which the analytical method is applicable (analytical range).

7. Special safety precautions in handling specimens, preparing reagents, disposing of waste (e.g., radioactive materials), and appropriate methods of decontamination.

TABLE 1–2.

Conventional and SI Units for Clinical Chemistry and Hematology Tests

LABORATORY TEST—CLINICAL CHEMISTRY	CONVENTIONAL UNIT	FACTOR*	SI UNIT
Acetominophen	µg/ml	6.61	µmol/L
Albumin	gm/dl (gm/100 ml)	144.9	µmol/L
Aldolase	mU/ml	7.4	nmol.s^{-1}/L
Alkaline phosphatase	IU/L	—	µmol.s^{-1}/L or Katal/L
Ammonia	µg/dl (µg/100 ml)	0.59	µmol/L
Amylase	U/ml	—	arb. unit
Barbiturate	µg/ml	—	µmol/L
Bilirubin	mg/dl (mg/100 ml)	17.1	µmol/L
Bromide	µg/ml	0.125	mmol/L
Calcium	mg/dl (mg/100 ml)	0.25	mmol/L
Chloride	mEq/L	1.0	mmol/L
Cholesterol	mg/dl (mg/100 ml)	0.026	mmol/L
Cortisol	µg/dl (µg/100 ml)	0.0276	µmol/L
Creatine phosphokinase (CK or CPK)	mU/ml	1.0	µmol.s^{-1}/L or Katal/L
Creatinine	mg/dl (mg/100 ml)	88.4	µmol/L
Digoxin	ng/ml	1.28	nmol/L
Estriol	µg/L	3.47	nmol/L
Ferritin	µg/L	2.2	pmol/L
Folic acid	µg/dl (ug/100 ml)	22.7	nmol/L
Glucose	mg/dl (mg/100 ml)	0.055	mmol/L
Haptoglobin	mg/dl (mg/100 ml)	0.118	µmol/L
HDL cholesterol	mg/dl (mg/100 ml)	0.026	mmol/L
Iron	µg/dl (µg/100 ml)	0.179	µmol/L
Iron-binding capacity (IBC)	µg/dl (µg/100 ml)	0.179	µmol/L
Lead	µg/L	0.207	nmol/L
Lipase	U/ml	—	arb. unit
Lithium	mEq/L	1.0	mmol/L
LDL cholesterol	mg/dl (mg/100 ml)	0.026	mmol/L
Magnesium	mg/dl (mg/100 ml)	0.41	mmol/L
Phosphorus	mg/dl (mg/100 ml)	0.323	mmol/L
Potassium	mEg/L	1.0	mmol/L
Protein: total	gm/dl (g/100 ml)	10.0	gm/L
Quinidine	µg/ml	3.09	µmol/L
Salicylate	mg/dl (mg/100 ml)	0.072	mmol/L
Sodium	mEq/L	1.0	mmol/L
Theophylline	µg/ml	5.55	µmol/L
Thyroxine	µg/dl (ug/100 ml)	12.9	nmol/L
Triglycerides	mg/dl (mg/100 ml)	0.011	mmol/L
Urea nitrogen (BUN)	mg/dl (mg/100 ml)	0.356	mmol/L
Uric acid	mg/dl (mg/100 ml)	59.5	µmol/L

(Continued.)

TABLE 1–2 (cont.).

LABORATORY TEST—CLINICAL CHEMISTRY	CONVENTIONAL UNIT	FACTOR*	SI UNIT
Hematology			
Complete blood count			
Hematocrit	%	0.01	No unit
Hemoglobin	gm/dl (gm/100 mL)	0.155	mmol/L
Leukocyte count	/mm^3	1,000,000	10^9/L
Erythrocyte count	/mm^3	1,000,000,000	10^{12}/L
Mean corpuscular volume (MCV)	μm^3	1.0	fl
Mean corpuscular hemoglobin (MCH)	pg		fmol
Mean corpuscular hemoglobin concentration (MCHC)	%		mmol/L
Erythrocyte sedimentation rate (ESR)	mm/hr	1.0	mm/hr
Erythrocyte enzyme: Glucose-6-phosphate dehydrogenase (G6PD)	U/gm Hb	1.0	U/gm
Platelet count	/mm^3	1,000,000	10^9/L
Platelet function tests:			
Clot retraction	%/2 hr		/2 hr
Coagulation tests:			
Bleeding time	min	60	sec
Prothrombin time (PT)	sec	1.0	sec
Partial thromboplastin time (PTT)	sec		sec

*Factor: conventional unit multiplied by factor to obtain SI unit.

QUALITY CONTROL OF ANALYTICAL METHODS

The performance of any analytical method must be judged according to the accuracy of laboratory results obtained from the procedure. Quality control monitoring is a means of assuring that the analytical method is as accurate as possible. Included in the monitoring process are patients' specimens, instruments, reagents, supplies, glassware and personnel. The quality of the specimen is critical (see Chapter 2 on Specimen Collection). Also, specimen processing and proper technique during performance of the method are essential in obtaining accurate and correct laboratory results. The quality of an analysis is dependent upon the following terms:

1. *Accuracy:* the closeness of a laboratory result to the true value; correctness. The true or correct value is determined by comparison to a standard or calibrator (substance or chemical of known composition).
2. *Precision:* the closeness of laboratory results to one another when

TABLE 1–3.

Microbilirubin Analytical Method for Quantitative Determination of Serum Direct and Total Bilirubin by a Modified Evelyn-Malloy Procedure

PRINCIPLE

In 1883, Ehrlich[5] developed a total bilirubin chemistry based on the diazotization and coupling reactions of sulfanilic acid with bilirubin. In 1937 Malloy and Evelyn[6] improved the method of Ehrlich by developing a modified reagent that stabilized the pH-sensitive color of the azobilirubin. The EM Diagnostic Systems, Inc., Direct and Total Bilirubin chemistry is based on the Ehrlich concept with the modifications of Malloy and Evelyn.[6]

Total Bilirubin: bilirubin in serum is coupled with diazotized sulfanilic acid to form azobilirubin. The absorbance at 560 nm is proportional to the bilirubin concentration. Protein-bound (indirect) bilirubin is liberated by methanol, allowing the rapid measurement of total bilirubin.

Direct Bilirubin: conjugated (direct) bilirubin in serum is coupled with diazotized sulfanilic acid to form azobilirubin. The absorbance at 560 nm is proportional to direct bilirubin concentration. Acid solubilizes only conjugated bilirubin, allowing the measurement of direct bilirubin.

Reagents	*For In Vitro Diagnostic Use*
64985A, T-BILI ACCELERATOR, 2 x 237 ml	
Ingredients:	*Concentration:*
Sulfanilic acid	5.0 gm/L
Hydrochloric acid (conc.)	8.08 ml/L
Methanol	440 ml/L

DANGER! MAY BE FATAL OR CAUSE BLINDNESS IF SWALLOWED. POISON. VAPOR HARMFUL. COMBUSTIBLE. Do not breathe vapor. Keep container closed. Use with adequate ventilation. Wash thoroughly after handling. Keep away from heat and open flame. CANNOT BE MADE NONPOISONOUS. FIRST AID: If swallowed, call a physician immediately.

64985B, T-BILI DIAZO SOLUTION, 1 x 20 ml

Ingredients:	*Concentration:*
Sodium nitrate	20.0 gm/L

WARNING! HARMFUL IF SWALLOWED. FIRST AID: If swallowed call a physician immediately.

64985C, D-BILI ACID REAGENT, 2 x 237 ml

Ingredients:	*Concentration:*
Sulfanilic acid	0.5 gm/L
Hydrochloric acid (conc.)	7.84 ml/L
Preservative	

64985D, D-BILI DIAZO SOLUTION 1 x 20 ml

Ingredients:	*Concentration:*
Sodium nitrite	1.5 gm/L

WARNING! HARMFUL IF SWALLOWED. FIRST AID: If swallowed, call a physician immediately.

64985E, BILIRUBIN CALIBRATOR, 2 x 3 ml

Ingredients:	*Concentration:*
Bilirubin	(see bottle label*)
Human albumin	approx 5.5 gm/dl

(Continued.)

TABLE 1–3 (cont.).

Reconstitute 1 vial of Bilirubin Calibrator Reagent with 3.0 ml of distilled or deionized water. Swirl gently. Allow 5–10 minutes to ensure complete reconstitution. Reconstituted Calibrator is stable refrigerated at 2°–8°C for 1 week (7 days) or for 3 months at ordinary freezer temperatures (– 20°C or colder). Upon thawing, mix thoroughly before use. Do not use if the serum becomes contaminated with bacteria.
*NOTE: Concentration of bilirubin will vary from lot to lot.
WARNING! Plasma and serum donors for the Bilirubin Calibrator are routinely tested and found negative for Hepatitis B Surface Antigen (HBsAg) using test methods with third-generation sensitivity and reagents licensed by the Bureau of Biologics of the Food and Drug Administration. However, all serum-based material may be a potential carrier of disease-causing organisms. Handle this material with the same caution used when working with patient samples.

Storage instructions: Micro Bilirubin Reagent Set must be stored at controlled room temperature (15°–30°C). Storage must not exceed expiration date on box label.

Indications of deterioration: Signs of microbial growth in any of the reagents warrant discontinuance of their use. A slight crystalline precipitate may form in the sodium nitrite solution. This will not effect the performance of the reagent.
Specimen collection and handling

Use clear, unhemolyzed serum which has been separated from the blood cells as soon after collection as possible. At all times, care must be taken to protect the samples from exposure to light.

The well-known light sensitivity of bilirubin dictates that the determination be performed as soon after collection as possible. Overexposure of the samples to light may result in false depression of both total and direct bilirubin results.

Interfering substances: The results of data gathered in EM Diagnostic Systems, Inc. laboratories show that the following substances interfere with direct and total bilirubin values:

Total bilirubin:
- Hemolysis (500 mg/dl hemoglobin)
- Vitamin A (750 units/ml)
- Turbidity (moderate to gross)
- Tripotassium EDTA
- Sodium fluoride with potassium oxalate
- Sodium oxalate
- Sodium heparin

Direct bilirubin
- Hemolysis (30 mg/dl hemoglobin)
- Vitamin A (750 units/ml)
- Turbidity (moderate to gross)
- Protein (11.5 gm/dl protein)
- Tripotassium EDTA (ethylenediaminetetracetic acid)
- Sodium fluoride with potassium oxalate
- Sodium oxalate
- Sodium heparin

PROCEDURE

Materials Provided
- 64985A, T-BILI Accelerator Reagent
- 64985B, T-BILI Diazo Reagent
- 64985C, D-BILI Acid Reagent
- 64985D, D-BILI Diazo Reagent
- 64985E, Bilirubin Calibrator Reagent

Materials Required But Not Provided

Spectrophotometer capable of reading absorbance at 560 nm 12 x 75 mm (matched to 1% T) cuvet—2 per determination.

Timer accurate to one sec/min

Polyethylene Film

Pipets calibrated "to deliver:"

50 microliters, 3 per determination

2.0 ml, 1 per determination

PROCEDURE OUTLINE

Total Bilirubin (Adult and Pediatric)

1. Pipet 2.0 ml of Reagent A into each of two 12 x 75 mm "matched" spectrophotometer tubes. Label one tube BLANK and the TEST.
2. Add 0.05 ml of Reagent B to the TEST tube. MIX WELL. Vortex or invert against parafilm for at least five (5) seconds. Following completion of Step 2, proceed to sample addition (see Steps 3 and 4) within ten (10) minutes.
3. Add 0.05 ml of sample to the BLANK tube. Mix well.
4. Add 0.05 ml of sample to the TEST tube. Mix well.
5. Allow both tubes to stand at room temperature.
6. After exactly 5 minutes, read both tubes against distilled water at 560 nm on a suitable spectrophotometer.
7. Record the BLANK and TEST readings obtained and calculate the A for each sample (A = A TEST − A BLANK). This absorbance is used in calculating the total Bilirubin result.

Direct Bilirubin

1. Pipet 2.0 ml of Reagent C into each of two 12 x 75 mm "matched" spectrophotometer tubes.
2. Add 0.05 ml of Reagent D to the TEST tube. MIX WELL. Vortex or invert against parafilm for at least five (5) seconds. Following completion of Step 2, proceed to sample addition (see Steps 3 and 4) within ten (10) minutes.
3. Add 0.05 ml of sample to the BLANK tube. Mix well.
4. Add 0.05 ml of sample to the TEST tube. Mix well.
5. Allow both tubes to stand at room temperature.
6. After exactly 2.0 minutes, read both tubes against a distilled water blank at 560 nm on a suitable spectrophotometer.
7. Record the BLANK and TEST readings obtained and calculate the A for each sample (A = A TEST − A BLANK). This absorbance is used in calculating the Direct Bilirubin result.

Stability of Reaction Mixture

The final color developed in the Total Bilirubin procedure is stable for at least 15 minutes. The color developed in the Direct Bilirubin procedure must be read at the exact time indicated. It is recommended that all readings be taken exactly as indicated in the Procedure Outline.

CALIBRATION

1. Reconstitue one vial of Reagent E (Bilirubin Calibrator) with 3.0 ml of distilled or deionized water. Swirl gently.
2. After complete reconstitution (5–10 minutes), substitute the Bilirubin Calibrator in varying sample volumes for the unknown sample in the test procedure for Total bilirubin.
3. The suggested sample volumes to be used are: 5 µl, 10 µl, 25 µl, and 50 µl. Duplicate determinations should be performed at each level.
4. Plot the corrected absorbance values vs the mg/dl value for each sample volume (see below).

(Continued.)