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THIRD EDITION

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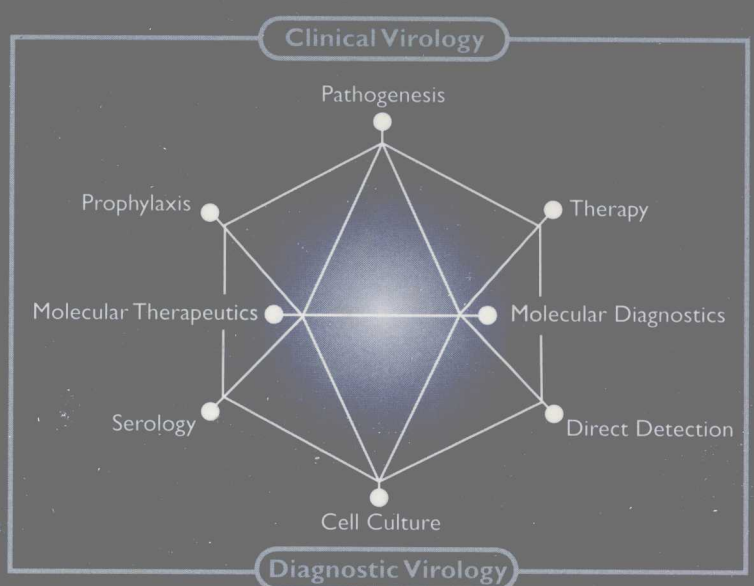

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ASM PRESS

WASHINGTON, D.C.

Address editorial correspondence to ASM Press, 1752 N St., NW, Washington, DC 20036-2904, USA

Send orders to ASM Press, P.O. Box 605, Herndon, VA 20172, USA
Phone: 800-546-2416; 703-661-1593
Fax: 703-661-1501
E-mail: books@asmusa.org
Online: www.asmpress.org

American Society for Microbiology
1752 N St. NW
Washington, DC 20036-2904

Library of Congress Cataloging-in-Publication Data
Clinical virology manual/edited by Steven Specter, Richard L. Hodinka, and Stephen A. Young—3rd ed.
p. cm.
Includes bibliographical references and index.
ISBN 1-55581-173-6
1. Diagnostic virology—Handbooks, manuals, etc. I. Specter, Steven. II. Hodinka, Richard L. III. Young, Stephen A.
QR387.C48 2000
616'.0194—dc21 00-040163

10 9 8 7 6 5 4 3 2 1

We dedicate this book to our colleague and friend Jerry Lancz, who served as an editor of the first two editions but has since retired. His foresight and insights created a publication that has brought greater understanding and unity to a field that continues to expand. We also dedicate it to our wives, Randie, Kitty, and Linda, and to our children, Ross, Rachel, Ryan, Tyler, Brett, and Jesse, whose patience and support sustain us through all our endeavors.

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Preface to the Third Edition

The aims of the *Clinical Virology Manual* remain the same as those of the first edition; thus, the original preface is included to describe those goals. The third edition is updated and expanded from the second edition. It has been expanded from 36 chapters to 39 chapters and 3 appendixes. The original section on reference laboratories now comprises the appendixes. Many of the chapters have been updated and expanded, while some of the more standard virology techniques of the past have been retained from the second edition (chapters 6, 8, 11, and 12). Four new chapters have been added to the Laboratory Procedures section; these include replacing the chapter on PCR with one chapter on molecular diagnostics and one on quantitative molecular technologies, as well as chapters on the use of flow cytometry in viral diagnostics and automation in the virology laboratory. These are intended to address much of the modernization that has occurred in the past several years. In the Viral Pathogens section, we have separated the coverage of viral hepatitis into two chapters along the lines of route(s) of infection; separated cytomegalovirus, Epstein-Barr virus, and varicella-zoster virus from human herpesvirus 6; added human herpesviruses 7 and 8 to the latter chapter; and added a chapter on rodent-borne viruses. The information in the appendixes has been updated.

This edition also brings several major changes, including a new publisher, the retirement of one of the original editors, and the addition of two new editors. We are pleased that ASM Press is now publishing this edition and hope that ASM members as well as nonmembers will find this manual a useful adjunct to the *Manual of Clinical Microbiology* and *Manual of Clinical Laboratory Immunology*. There are a number of chapters for which the authors have changed as a result of change of professional focus, retirement, or death. We hope that this edition is a credit to those who preceded this effort, especially Jerry Lancz, to whom this edition is dedicated.

STEVEN SPECTER
RICHARD L. HODINKA
STEPHEN A. YOUNG

Preface to the First Edition

Clinical virology is an area that is undergoing rapid expansion. As a service for patient care, the utility of the clinical virology laboratory has increased significantly in the past decade. Due to the availability of commercial test kits, sophisticated yet simple diagnostic reagents, and the standardization of laboratory assays, accurate, reliable and, in many instances, rapid protocols are currently available for the diagnosis of a variety of viral agents producing human infections. Thus, the demands (on both the physician and the clinical laboratory virologist) for the diagnosis of viral infections will continue to increase. With this in mind, this volume is written as both an aid to the clinician and as a guide for the clinical laboratory.

This manual has three sections. The first describes laboratory procedures to detect viruses. The initial chapters deal with quality control in the laboratory and specimen handling, areas that are critical for an effective diagnostic laboratory. This is followed by individual chapters that provide information or a detailed protocol on how to set up and test samples for viral diagnosis using this technique. Both classical and the newer, more experimental techniques are described in detail.

The second section focuses on the viral agents. Viruses are grouped into chapters based on a target organ-system categorization. In this way, viruses producing infection in a particular organ or tissue are discussed and compared in a single chapter. This approach more accurately reflects the problems and choices faced by the attending physician and clinical technician for the diagnosis of a viral infection. Each chapter includes information relating basic, pathogenic, immunologic, and protective measures concerning each virus group, as well as information on its isolation, propagation, and diagnosis. This section also includes a chapter on *Chlamydia*. There are two reasons for including this family: the clinical laboratory often isolates and diagnoses *Chlamydia*, and the techniques used in its isolation and diagnosis are used in other instances.

The third section is designed to be used for reference. Here we supply information about Federal Reference Laboratories at the Centers for Disease Control and their role in the diagnosis of viral infection. The diagnostic and regulatory activities of state health laboratories and services available at individual hospital laboratories are provided in survey form. This listing is somewhat incomplete in that it contains information provided in response to an initial questionnaire and follow-up.

The aim and scope of this volume are service: to the physician, as a source of basic and clinical information regarding viruses and viral diseases, and to the laboratories, as a reference source to aid in the diagnosis of virus infection by providing detailed information on individual techniques and the impetus to expand services offered.

STEVEN SPECTER
GERALD LANCZ

Contents

LABORATORY PROCEDURES FOR DETECTING VIRUSES

I

Quality Assurance in Clinical Virology

ANN WARFORD

1

Quality assurance in clinical virology requires a comprehensive program for surveillance and improvement of all aspects of laboratory services. Laboratory testing for health assessment, disease diagnosis, or treatment begins with patient preparation and sampling and continues through testing, reporting of results to patient care providers, and appropriate notification of results and test interpretation. In a 1996 report of a prospective study of the type and frequency of laboratory testing problems in primary care physicians' offices during a 6-month period, a rate of 1.1 problems per 1,000 visits was found (Nutting et al., 1996). Twenty-seven percent of these test problems had an impact on patient care, including serious effects such as unnecessary hospitalization, prolonged hospital stay, more invasive diagnostic procedures, and delays in treatment. However, only 25% of the laboratory problems involved test analysis or inconsistent results; 75% of errors occurred in specimen collection and transport (43%) or timely provider notification of results (32%). This and other studies (Boone et al., 1982; Bartlett et al., 1994) confirm the need for laboratory involvement in improving the total testing process, including preanalytical and postanalytical steps, if laboratory services are to be meaningful and beneficial in patient health care.

REGULATORY REQUIREMENTS

Effective September 1992, with the implementation of the federal Clinical Laboratory Improvement Amendments of 1998 (CLIA-88), all clinical laboratories in the United States are regulated by the Health Care Financing Administration (HCFA) unless state health department regulations exceed and are approved by HCFA (Centers for Disease Control and Prevention [CDC], 1992; HCFA, 1992). The provisions of CLIA-88 include licensure, inspections conducted by HCFA or HCFA-approved organizations such as the College of American Pathologists (CAP) and the Joint Commission for Accreditation of Healthcare Organizations (JCAHO), and sanctions for failure to meet mandated standards. The stated purpose of CLIA-88 regulation of laboratories is to improve laboratory quality and achieve accurate and reliable laboratory results. The main quality standards of the regulatory and accrediting organizations can be categorized as personnel qualifications, responsibilities and competency assessment, proficiency testing for all analytes and staff, written and approved procedures, method verification and validation, test reagent and equipment quality control and preventive maintenance and, lastly, patient test management, which includes ongoing assessment and improvement of all laboratory services. In the references, those references that are marked with an asterisk provide in-depth information regarding U.S. clinical laboratory regulations and accreditation requirements as well as useful quality assurance resources.

PROCEDURE MANUAL

An essential tool for the laboratory staff is a complete and current procedure manual available at the bench. The manual should contain a detailed, stepwise procedure for all tasks performed in the laboratory written according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) (1996). The required elements of the procedure manual are title, test principle, patient preparation, specimen collection, transport and storage, reagents, standards and controls, supplies, instrumentation (including calibration and maintenance), quality control frequency and acceptable limits, corrective action for unacceptable quality control, test steps, calculations, expected values, reference range, critical values, linearity and detection limits, method limitations and interfering substances, method validation, references, implementation and review dates, and author(s).

A copy of a manufacturer's kit package insert does not meet the requirements for the laboratory's written procedures. In addition to formal procedures for each type of patient test performed, written procedures are required for care provider sample collection and handling and must specify specimen rejection criteria, such as shown in Table 1. Specimen collection information must also be provided to medical and nursing staffs and as part of periodic laboratory hospital inservice education programs to be effective. No procedure in the laboratory can compensate for erroneous specimen collection and handling. Written protocols are also required for proficiency testing, safety, and the quality assurance and improvement program. Each written procedure must be reviewed and approved by the laboratory director and updated when method improvements are implemented.

TABLE 1 Examples of specimen rejection criteria[a]

Problem	Specimen	Test	Action	
			Reject (phone for new sample)	Process and test with disclaimer
Delay in transit	Clotted blood	Serology		>24 h
	Whole blood (unspun)	Culture/PCR	>12 h (whole blood)	6–12 h (whole blood)
	Serum or plasma (RT)	PCR	>72 h (RT)	25–72 h (RT)
	Serum or plasma (cold)	PCR		>72 h (cold pack, refrigerated)
	PPT tube (unspun)	PCR	>12 h (unspun/whole blood)	6–12 h (unspun/whole blood)
	PPT tube (spun)	PCR	>72 h (RT)	25–72 h (RT)
	PPT tube (spun)	PCR		>72 h (cold pack, refrigerated)
	Nonblood	Clostridium difficile toxin		>4 h (*C. difficile* toxin in stool)
		Viral culture		>48 h (refrigerated) for viral cultures
Heparin (green top)	Whole blood	PCR	Any (cannot use for PCR)	
Hemolysis	Serum	Serology	Looks like whole blood	Mild/moderate hemolysis (note serum appearance in computer)
Lipemia/icterus	Serum	Serology		Note appearance in computer
Mislabeled or unidentified	Any (except surgery)	Any	Reject/recollect	Tissue/CSF (have physician identify and sign, add disclaimer)
Dry swab, wood, calcium alginate, or charcoal swab	Swab	Culture		Note unsatisfactory swabs in computer with disclaimer
Container gross external contamination	Any (except surgery)	Any	Reject and recollect	Tissue/CSF (have submitter or supervisor disinfect with bleach)
Duplicate (<24 h)	Any except surgery (BAL, biopsy, CSF)	Any	Reject duplicate blood, urine, or stool	Process if requested by physician
Fixative (Formalin)	Any	Any	Reject and recollect	
Non-VTM (Bacti culturette)	Swab	Culture/DFA	Cannot use culturette for DFA/EIA or *Chlamydia*	Can use culturette for viral culture (transfer to VTM as soon as possible)
Nonstandard source or collection method	Sputum or stool for respiratory viruses	Culture/DFA	Reject, recollect NP/Tht/BAL	Add disclaimer
QNS	Any	Any		Call for physician's test priority list
Inadequate cellular material	Lesions, swabs	DFA	Call for recollection	

[a]Abbreviations: RT, room temperature; PPT, plasma processing tube (BD); BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; VTM, viral transport medium (SP buffer); DFA, direct fluorescent-antibody assay; EIA, enzyme immunoassay; NP, nasopharyngeal swab; Tht, throat swab; QNS, quantity not sufficient.

STAFF

The key to a quality viral diagnostic service is the laboratory staff. Staff qualifications for education, experience, training, and licensure or certification vary greatly among regulatory and accrediting agencies, with CLIA-88 having the minimum requirements (August et al., 1990; HCFA, 1992). Virology testing is categorized in CLIA-88 as moderate- and high-complexity testing, with only a few infectious mononucleosis serology kits listed as "waived," i.e., exempt from many CLIA-88 regulations. Virology laboratories can offer level-one testing, which consists of immunoassays for antigen detection without microscopy, or level-two high-complexity testing for viral isolation and identification and all other viral diagnostics. Because most virology methods are complex and subjective, requiring independent analysis and decisions, adequate education and training in theory

and methods are essential for quality results. Several studies have correlated the level of education, training, and certification or licensure with laboratory performance quality as measured in proficiency surveys (Gerber et al., 1991; Hancock et al, 1993; Woods and Bryan, 1994; CDC, 1996; Shahangian, 1998). Continuing education is certainly desirable for all virologists in this rapidly changing field and is required in some states, particularly those with licensure requirements for laboratory personnel. Among the laboratory director's responsibilities are written qualifications, duties, and responsibilities for all staff and assurance that staffing levels are adequate for the type and volume of testing performed. Excessive workloads are not consistent with quality, particularly with subjective tasks requiring judgment, such as microscopy.

PROFICIENCY TESTING

CLIA-88 has adopted an external, graded proficiency test program(s) (PT) as the main indicator of the quality of laboratory testing performance. All laboratories must participate in PT for each analyte or test for which patient testing is performed; laboratories that fail consecutive challenges or two of the three annual testing events are subject to severe sanctions. Proficiency testing must be performed in the same manner and with the same staff as are routine patient samples. Known proficiency samples are an imperfect measure of a laboratory's performance accuracy and reliability because (i) they are recognized challenges which have penalties for failure and are prone to special attention; (ii) they test only the analytical phase of testing, not specimen collection, transport, or usual result reporting; (iii) they consist of a laboratory adapted virus(es) or pooled, processed body fluids spiked with analyte, which may have a matrix effect which renders them inaccurate with certain methods; and (iv) they cannot test analyte concentrations near the assay cutoff due to nonconsensus results with borderline levels. However, PT samples do still detect staff human errors and some poorly performing methods. PT unknown sample testing and analysis of results provided by programs such as CAP PT surveys also provide an educational resource for the laboratory. If no graded proficiency samples are available for tests performed, the laboratory must validate these methods for accuracy and reliability twice annually by other means, such as samples split with a reference laboratory, known samples, and patient clinical correlations including chart review. Blind quality control has been reported to offer the best measure of routine laboratory performance and can be accomplished with samples split and relabeled prior to receipt in the laboratory to assess reproducibility (Boone et al., 1982; Farrington, et al., 1995; Gray et al., 1995a, 1995b; Shahangian, 1998). Inadequate PT performance is the most common post-CLIA-88 inspection citation (Table 2) (Chapin and Baron, 1996; Belanger, 1998). Any type of PT assessment is useless without investigation and efforts to improve system problems. PT failures provide an opportunity for evaluation of factors contributing to test performance problems (Table 3), and use of total quality management methods with staff input from all sections and levels is recommended and outlined by NCCLS (1997) and others (Engebretson and Cembrowski, 1992). Investigations by CDC and CAP showed that approximately 20% of repeated PT failures had no cause identified by the laboratory and that on-site technical consultation was required for performance improvement (Boone et al., 1982; Hoeltge and Duckworth, 1987).

TABLE 2 Top HCFA inspection CLIA-88 deficiencies cited 1996 to 1998[a]

Proficiency testing program for each specialty and subspecialty inadequate
Quality assurance plan; lack of comprehensive written plan for maintaining quality of overall testing process, identifying problems, and implementing corrective action
Quality control not documented with at least two levels of controls for each day of testing
Preventive maintenance and function checks of instrumentation inadequate
Competency assessment program of staff performance inadequate
Daily supervisory review of quality control, preventive maintenance, and patient test results not performed
Procedure manual and job descriptions without lab director's written designation of responsibilities and duties of staff
Correlation of multiple test methods for same analytes not documented

[a]Sources: Chapin and Baron, 1996; Belanger, 1998.

TABLE 3 Troubleshooting unacceptable patient or proficiency test results

Procedure or method
• Equipment, reagents, standards, quality control materials
• Limitations of methodology: sensitivity, specificity, precision, linear range
• Written procedure erroneous
Technical factors
• Incubation time, temperature, humidity, carbon dioxide
• Pipetting, dilutions, calculations
• Misinterpretation, not following written protocol
Staff or staffing
• Training, experience, continuing education
• Use of overtime, per diem, rotating staff
• Workload-to-staff ratio
Clerical error(s)
• Mislabeling, transcription, units, computer entry
Sample or sampling
• Transport time and/or temperature
• Interfering substances, contamination
• Organism or analyte not present or not viable on receipt
Obtain input on preventive measures from lab staff and others

STAFF COMPETENCY ASSESSMENT PROGRAM

Annual competency assessment and training verification of laboratory staff is also mandated by CLIA-88 and is another of the main HCFA inspection deficiencies cited (Table 2). Competency assessment is even more critical to the quality of laboratory testing since it requires evaluation of testing personnel in all of the routine patient testing procedures, including preanalytical and postanalytical steps, quality control, and instrument methods, as well as analysis. The mandated competency assessment procedures (Table 4) are (i) direct observation of test performance, instrument main-

TABLE 4 Staff training verification and competency assessment documentation

Technical supervisor must assess and verify staff performance of procedures promptly, accurately, and proficiently at least annually by use of the following:

- Direct observation of routine test performance, instrument maintenance and function checks, and microscopy and interpretation
- Monitoring worksheets, result recording, and reporting
- Testing proficiency samples, previously analyzed specimens, blind controls, and/or reference samples
- Daily review of quality control records and preventive maintenance records
- Additional procedures such as written or verbal tests, continuing education, problem solving of test failures, and evaluation of critical incidents, error reports, or complaints
- Reevaluation required with each change in methods

tenance and function checks, and microscopy and interpretation; (ii) monitoring worksheets, result recording, and reporting; (iii) testing of proficiency samples, previously analyzed samples, and blind controls or reference samples; and (iv) daily review of quality control and preventive maintenance records. Competency assessment can also consist of continuing education, written or oral tests, and evaluation of critical incidents, error reports, or complaints and should include evaluation of problem-solving ability, particularly concerning test failures. Evaluation of testing staff for troubleshooting ability can be aided by use of the form shown in Fig. 1, which is recommended for documenting reports of laboratory problems and complaints. By incorporating many of the documents normally used in laboratory operations, competency verification does not have to be an onerous process. Some assessment documentation items might include a training checklist created from the major and critical steps of the procedure manual, daily worksheet and results review checks, repeat testing of positive and equivocal results that are normally performed, confirmatory test results, and review of microscopy, quality control, and preventive maintenance results. As with proficiency testing, poor staff competency indicates the need for evaluation of laboratory systems for recruiting, staffing patterns, training, continuing education, and retention of a qualified staff.

METHOD PERFORMANCE VERIFICATION AND VALIDATION

Method performance verification is required for all U.S. Food and Drug Administration (FDA)-approved instruments, kits, and test systems to demonstrate that accuracy, precision, and reportable range are comparable to those established by the manufacturers. This verification usually consists of parallel testing of the new product with a standard method of known performance characteristics. A minimum of 20 known positive specimens and 50 negative samples has been recommended for this evaluation by McCurdy and colleagues (Elder et al., 1997). For non-FDA-approved methods, establishment of the performance characteristics of accuracy, precision, analytical sensitivity, specificity, interfering substances, and reportable and references ranges is CLIA-88 mandated. Recommendations for in-house developed molecular assay validation are specified by

Problem Description (include test, date, sample ID)
Steps taken to evaluate and solve problem:
Problem reported to: Date:
Corrective Action:
Further Preventive Measures:
Comments:
Prepared by: Date:
Reviewed by: Date:

FIGURE 1 Report of laboratory problem, complaint, or error (adapted from August et al., 1990).