Dedication

We dedicate the third edition of the *Clinical Microbiology Procedures Handbook* to Henry D. Isenberg. Henry was a pioneer in clinical microbiology who spearheaded the field of microbial diagnosis for more than a half century. He was a gifted mentor, scholar, and scientist who inspired several generations of clinical microbiologists. We are very fortunate to have worked with such an outstanding microbiologist and colleague.
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How To Use This Handbook

General Format
The third edition of this handbook has been divided into three volumes containing the front matter, 16 sections (composed of “procedures”), and an index. Volume 1 contains the front matter of the handbook plus sections 1 through 4. Volume 2 contains sections 5 through 9. Volume 3 contains sections 9 through 16 and the index.

Included at the front of each volume is a short table of contents listing the items contained in the front and back matter and the 16 sections of the handbook. In addition to the table of contents for the entire handbook, each section is immediately preceded by a detailed table of contents for that section, giving the section editors’ names, the procedure titles included in that section, and the authors’ names for each procedure.

Sections
The content of the handbook has been organized into 16 sections as follows:

- Section 1: Procedure Coding, Reimbursement, and Billing Compliance
- Section 2: Specimen Collection, Transport, and Acceptability
- Section 3: Aerobic Bacteriology
- Section 4: Anaerobic Bacteriology
- Section 5: Antimicrobial Susceptibility Testing
- Section 6: Aerobic Actinomycetes
- Section 7: Mycobacteriology and Antimycobacterial Susceptibility Testing
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- Section 13: Epidemiologic and Infection Control Microbiology
- Section 14: Quality Assurance, Quality Control, Laboratory Records, and Water Quality
- Section 15: Biohazards and Safety
- Section 16: Bioterrorism

Procedures
Each section listed above consists of procedures. The procedures have been numbered and are referred to by number in cross-references in the text. The procedure number consists of the section number plus the number of the procedure (plus the number of a subprocedure if applicable). For example, “procedure 5.6” is the sixth procedure in section 5; “procedure 7.4.2” is the second subprocedure of the fourth procedure in section 7.
Page Numbers
The page number within a procedure is the procedure number followed by the number of the page within the procedure. Thus, from the examples given above, “page 5.6.10” is the 10th page within procedure 5.6, and “page 7.4.2.3” is the 3rd page within procedure 7.4.2. In all cases, the last number is the page number within a procedure.

The index is numbered beginning with an “I” followed by the number of the page within the index. For example, “page I.3” is the third page in the index.
Abbreviations

In this handbook, most abbreviations have been introduced in parentheses after the terms they abbreviate on their first occurrence, e.g., “a central nervous system (CNS) specimen.” Some exceptions to this rule are explained below and given in Tables 1 to 4.

Because of their frequent use in this handbook and/or their familiarity to readers, the terms listed in Table 1 have been abbreviated in the procedures; i.e., they have not been spelled out or introduced. Based on the editorial style for books and journals published by the American Society for Microbiology (ASM), the abbreviations listed in Table 2 have also been used without introduction in this handbook. Table 3 lists abbreviations that have been used without introduction in the bodies of tables. Abbreviations for commonly accepted units of measurement have been used without definition if they appeared with numerical values. Table 4 lists some common units of measurement appearing in this handbook. These last two items are also based on ASM style.

As readers use the various procedures in this handbook and see unfamiliar abbreviations that are not defined in the procedures themselves, they should refer to these tables for definitions.

Table 1 Common abbreviations used without introduction in this handbook

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BAP (not SBA)</td>
<td>5% Sheep blood agar plate</td>
</tr>
<tr>
<td>BHI</td>
<td>Brain heart infusion</td>
</tr>
<tr>
<td>BSL</td>
<td>Biosafety level</td>
</tr>
<tr>
<td>CAP</td>
<td>College of American Pathologists</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CHOC</td>
<td>Chocolate agar</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute (formerly NCCLS)</td>
</tr>
<tr>
<td>CMPH</td>
<td><em>Clinical Microbiology Procedures Handbook</em> (first edition)</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMB</td>
<td>Eosin-methylene blue</td>
</tr>
<tr>
<td>EPCM</td>
<td><em>Essential Procedures for Clinical Microbiology</em></td>
</tr>
<tr>
<td>GLC</td>
<td>Gas-liquid chromatography</td>
</tr>
<tr>
<td>JCAHO</td>
<td>Joint Commission on Accreditation of Healthcare Organizations</td>
</tr>
<tr>
<td>MAC</td>
<td>MacConkey agar</td>
</tr>
<tr>
<td>MSDS</td>
<td>Material safety data sheet</td>
</tr>
</tbody>
</table>

(continued)
### Abbreviations

**Table 1** Common abbreviations used without introduction in this handbook *(continued)*

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.A.</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>NBS</td>
<td>National Bureau of Standards (pertaining to a special calibrated thermometer)</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>OSHA</td>
<td>Occupational Safety and Health Administration</td>
</tr>
<tr>
<td>PMNs</td>
<td>Polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PPE</td>
<td>Personal protective equipment</td>
</tr>
<tr>
<td>QA</td>
<td>Quality assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red blood cells or erythrocytes</td>
</tr>
<tr>
<td>TCBS</td>
<td>Thiosulfate citrate bile salt sucrose agar</td>
</tr>
<tr>
<td>THIO</td>
<td>Thioglycolate broth</td>
</tr>
<tr>
<td>TSA</td>
<td>Trypticase soy agar or tryptic soy agar</td>
</tr>
<tr>
<td>TSB</td>
<td>Trypticase soy broth or tryptic soy broth</td>
</tr>
<tr>
<td>WBCs</td>
<td>White blood cells or leukocytes</td>
</tr>
</tbody>
</table>

**Table 2** Additional abbreviations used without introduction (according to ASM style)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>AMP, ADP, ATP, GTP, dCMP, ddGTP, etc.</td>
<td>Adenosine 5′-monophosphate, adenosine 5′-diphosphate, adenosine 5′-triphosphate, guanosine 5′-triphosphate, deoxyctydine 5′-monophosphate, dideoxyguanosine triphosphate, etc.</td>
</tr>
<tr>
<td>ATPase, dGTPase, etc.</td>
<td>Adenosine triphosphatase, deoxyguanosine triphosphatase, etc.</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming unit(s)</td>
</tr>
<tr>
<td>cRNA</td>
<td>Complementary ribonucleic acid</td>
</tr>
<tr>
<td>DEAE</td>
<td>Diethylaminoethyl</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNsae</td>
<td>Deoxyribonuclease</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetate, ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGTA</td>
<td>Ethylene glycol-bis(β-aminoethyl ethyl)-N,N′,N′,N′-tetraacetic acid</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal inhibitory concentration</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NAD+</td>
<td>Oxidized nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Reduced nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NADPH</td>
<td>Reduced nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>oligo(dT), etc.</td>
<td>Oligodeoxythymidyl acid, etc.</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PFU</td>
<td>Plaque-forming unit(s)</td>
</tr>
<tr>
<td>poly(A), poly(dT), etc.</td>
<td>Polyadenylic acid, polydeoxythymidyl acid, etc.</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer ribonucleic acid</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
</tbody>
</table>
Table 3 Abbreviations used without introduction in the bodies of tables

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>amt</td>
<td>Amount</td>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>approx</td>
<td>Approximately</td>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>avg</td>
<td>Average</td>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>concn</td>
<td>Concentration</td>
<td>sp act</td>
<td>Specific activity</td>
</tr>
<tr>
<td>diam</td>
<td>Diameter</td>
<td>sp gr</td>
<td>Specific gravity</td>
</tr>
<tr>
<td>expt</td>
<td>Experiment</td>
<td>temp</td>
<td>Temperature</td>
</tr>
<tr>
<td>exptl</td>
<td>Experimental</td>
<td>tr</td>
<td>Trace</td>
</tr>
<tr>
<td>ht</td>
<td>Height</td>
<td>vol</td>
<td>Volume</td>
</tr>
<tr>
<td>mo</td>
<td>Month</td>
<td>vs</td>
<td>Versus</td>
</tr>
<tr>
<td>mol wt</td>
<td>Molecular weight</td>
<td>wk</td>
<td>Week</td>
</tr>
<tr>
<td>no.</td>
<td>Number(s)</td>
<td>wt</td>
<td>Weight</td>
</tr>
<tr>
<td>prepn</td>
<td>Preparation</td>
<td>yr</td>
<td>Year</td>
</tr>
</tbody>
</table>

Table 4 Some common units of measurement used in this handbook

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
</tbody>
</table>

Icons

The three icons listed below are used throughout this handbook. The icons direct the readers to follow important directions as they carry out the procedures. As a reminder, an explanation of the icon appears next to it at each appearance in the text.

- ![Biohazard icon] It is imperative that these cultures be handled in a biological safety cabinet.
- ![Reagent container icon] Include QC information on reagent container and in QC records.
- ![Standard precautions icon] Observe standard precautions.
Preface

The third edition of the *Clinical Microbiology Procedures Handbook* (CMPH) is based on the value and user requirements following the first and second editions of CMPH, the companion volume *Essential Procedures for Clinical Microbiology*, and the 2007 update of the second edition.

Almost all of the sixteen sections of the second edition of CMPH have been revised and updated; sections that did not require extensive revision will be updated during the next cycle of changes.

The purpose of the third edition of CMPH remains constant. That is to provide everyone engaged in the microbiological analysis of clinical specimens with procedures that will enable them to correctly perform the appropriate tasks. CMPH remains a cookbook that provides step-by-step descriptions of the numerous procedures used by workers at the bench.

As with the second edition of CMPH and the 2007 update of the second edition, there is increased emphasis on molecular approaches, bioterrorism, infection control in medical facilities, and the host’s immunological responses to microbial challenges. Also, continued emphasis is placed on the need to respond to governmental regulations and fiscal constraints. Highly experienced workers with many years of bench experience have written these procedures, and the format adheres to CLSI document GP02-5A (5th ed., 2006).

All procedures have been reviewed extensively by section editors, the editor in chief, and the ASM Press editors. We continue to encourage the users of these documents to bring new methods of universal relevance to our attention so they can be incorporated into the next update and shared with the clinical microbiology community.

Readers are reminded that naming any specific product in CMPH is not intended as an endorsement of that specific product or a suggestion to exclude other equally acceptable products. CMPH is for laboratory use only by qualified, experienced individuals or by personnel under the direct supervision of qualified, experienced individuals. Every effort has been made to ensure that the contents of this update are comprehensive, accurate, reliable, and reproducible.

Not all existing microbiological protocols are included; however, the editors and authors are familiar with all commonly used protocols. As newer protocols become more widely accepted and used, they will be incorporated into future CMPH revisions.

The third edition of CMPH is available in print, on CD, and as a downloadable PDF.

*Lynne S. Garcia*
Acknowledgments

I thank each of the section editors and authors for their tremendous efforts in planning and completing the third edition of CMPH. Special thanks go to everyone who participated in the original version, the second edition, and the 2007 update of the second edition for their comprehensive contributions in developing and updating these diagnostic procedures for all microbiologists. Each new edition and update builds on the expertise of the previous editors and authors, all of whom provided outstanding contributions to CMPH. We continue to acknowledge and thank the late Dr. Henry D. Isenberg for his outstanding guidance and leadership during the development and updating of CMPH. All microbiologists owe him a tremendous debt of gratitude; he will always be known as the “father of CMPH” and its greatest supporter.

Our editors and authors join me in thanking the officers of ASM, the Publications Board, and, especially ASM Press. As editor in chief, I particularly want to acknowledge John Bell, Cathy Balogh, Susan Birch, and Jeff Holtmeier of ASM Press for their efforts in supporting this and former editions of CMPH.

It has been a great privilege to work with this current group of editors and authors on the third edition, and we all continue to support CMPH in memory of Dr. Isenberg.

Lynne S. Garcia
Reader Response Form

Dear Reader:
We solicit your help in improving the *Clinical Microbiology Procedures Handbook* (CMPH). Updates will be published periodically to keep CMPH current, accurate, and reliable. Your guidance will play an important role in achieving our objective of making CMPH the most useful laboratory procedures guide available. *Please copy this page for your continued use.*

1. Have you found any errors? Please list the section, procedure, and page number; describe the error.

2. Please list procedures that you deem to be outdated, confusing, or inadequately presented. List the section, procedure, and page number; explain.

3. Indicate the topics that you would like to see added. Please list your reasons for the selection.

4. Additional comments.

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Microbiological analysis of clinical specimens is a constantly changing discipline; new methods and technologies emerge. The contributors to the third edition of CMPH believe that the procedures and guidelines suggested here are from reliable sources and in line with the practices accepted at the time of publishing. Readers are reminded that the naming of any specific product is not intended as an endorsement of that specific product by ASM Press or any other agency, nor is it a suggestion to exclude other equally acceptable products. CMPH is for laboratory use only by qualified, experienced individuals or by personnel under the direct supervision of qualified, experienced individuals. Every effort has been made to ensure that the contents of this update are comprehensive, accurate, reliable, and reproducible.
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