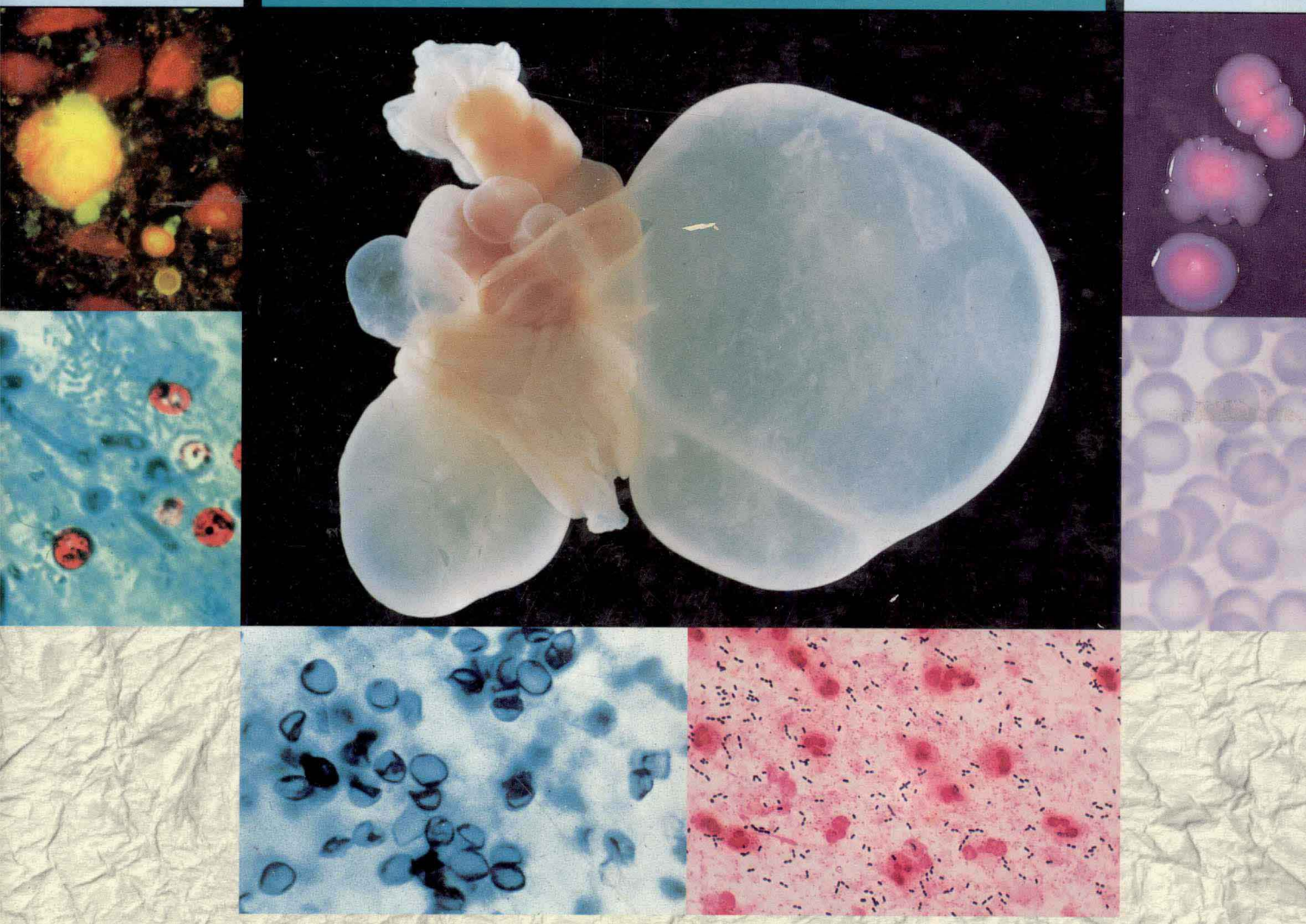
CLINICAL AND PATHOGENIC

MICROBIOLOGY

SECOND EDITION



Barbara J. Howard

John F. Keiser
Thomas F. Smith
Alice S. Weissfeld
Richard C. Tilton

CLINICAL AND PATHOGENIC MICROBIOLOGY

Second Edition

BARBARA J. HOWARD, D.A., M.T.(ASCP)
Associate Professor, Department of Biology
Director, Programs in Clinical Laboratory Science
The Catholic University of America
Washington, D.C.
Editor-in-Chief

JOHN F. KEISER, M.D., Ph.D., Diplomate, ABMM
Chief, Microbiology Laboratory
Assistant Professor
Department of Pathology
George Washington University Medical Center
Washington, D.C.

ALICE S. WEISSFELD, Ph.D., Diplomate, ABMM
Director, Microbiology Specialists Inc.
Adjunct Assistant Professor
Department of Microbiology and Immunology
Baylor College of Medicine
Houston, Texas

THOMAS F. SMITH, Ph.D., Diplomate, ABMM
Professor of Microbiology and Laboratory Medicine
Chair, Division of Clinical Microbiology
Director, Virus Laboratory
Mayo Clinic
Rochester, Minnesota

RICHARD C. TILTON, Ph.D., Diplomate, ABBM
Senior Vice President and Chief Scientific Officer
North American Laboratory Group
New Britain, Connecticut

Editorial Assistant.
Joanne Comerford, M.C.M., M.T. (ASCP)
Supervisor, Genotyping Laboratory
George Washington University Medical Center
Washington, D.C.

Mosby

St. Louis Baltimore Boston Chicago London Philadelphia Sydney Toronto

Mosby
Dedicated to Publishing Excellence

Sponsoring Editor: Jim Shanahan
Project Manager: Nancy C. Baker
Project Supervisor: George Mary Gardner
Proofroom Manager: Barbara M. Kelly

A C.V. Mosby imprint of Mosby-Year Book, Inc.

Mosby-Year Book, Inc.
11830 Westline Industrial Drive
St. Louis, MO 63146

Cover illustrations: *Center,* Racemose form of *Cysticercus* removed from fourth ventricle of brain in young woman from Central America. (Photograph by Barbara Neuburger, Medical Photography, Biomedical Communications, George Washington University Medical Center, Washington, D.C.) *Counterclockwise,* Direct fluorescent antibody stain of herpes simplex II in genital ulcer. (Courtesy Peter McEvoy, Walter Reed Army Medical Center, Washington, D.C.). *Cryptosporidium.* In acid-fast stain of stool sample, *Cryptosporidium* oocysts stain red, whereas everything else stains blue. Cysts of *Pneumocystis carinii* stained with Grocott-Gomori methenamine–silver nitrate stain. Gram stain of sputum showing gram-positive, lancet-shaped diplococci of *Streptococcus pneumoniae* and gram-negative short, slender rods of *Haemophilus influenzae.* (Courtesy Carol Ormes, Washington Hospital Center, Washington, D.C.). Trypomastigote of *Trypanosoma cruzi* (Giemsa stain). Pink, mucoid colonies of *Klebsiella pneumoniae* on MacConkey agar. (Courtesy Carol Ormes, Washington Hospital Center, Washington, D.C.).

1 2 3 4 5 6 7 8 9 0 GW/MV/SE 98 97 96 95 94

Library of Congress Cataloging-in-Publication Data

Clinical and pathogenic microbiology / [edited by] Barbara Howard . . . [et al.]0–2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-8016-6426-8
1. Medical microbiology. 2. Diagnostic microbiology.
I. Howard, Barbara J.
[DNLM: 1. Mycology—methods. 2. Parasitology—methods.
3. Virology—methods. QY 110 C641 1993]
QR46.C57 1993
616′.01—dc20
DNLM/DLC 92-48369
for Library of Congress CIP

PREFACE

The opening comments in the preface to the first edition of this book remain applicable: "*Clinical and Pathogenic Microbiology* combines in one volume the study of the materials and methods used for identification of pathogenic organisms and the study of these organisms in relation to their disease processes in humans."

As with the first edition, the authors have sought to strike a balance between theory and practice and between identification procedures and clinical significance of organisms. As before, we have attempted to present microbiology within an epidemiologic, diagnostic, and clinical framework.

Just as microbiology has changed since the first edition, so has this book. The chapter on safety reflects the heightened and vital concern with safety in the laboratory. The continued adaptation of molecular biologic techniques to clinical microbiology testing has resulted in a new chapter on molecular techniques as well as a separate subheading, "Identification by Molecular Methods," in the chapters on various bacteria. New chapters on mycoplasmas and retroviruses and expanded sections on organisms such as *Rochalimaea* underscore the increasing importance of these microorganisms in the laboratory.

The countless references from the last 3 years reflect the authors' concern that the book be as up-to-date as possible. In addition to updating material, the second edition seeks to be more focused on clinically relevant information. The chapters on specimen collection and processing and antimicrobial susceptibility testing have been expanded considerably as a reflection of the paramount importance of these topics in clinical microbiology.

Most important, this book is primarily written for instructional purposes, and as such it seeks to present the most pertinent and vital information in a clear and concise manner without overwhelming the reader.

Among the numerous people I would like to thank for their assistance are Pat Loughlin, and Diane Haddick, for their endless typing and retyping and Joanne Comerford for her superb editorial assistance and dogged commitment to this project. I thank many people for making helpful comments about portions of the text, including Dr. Robert Weaver for his careful review of Chapter 17. Perhaps the most important people to thank are the many readers who not only made the first edition a success but also provided many insights and helpful suggestions that have provided the basis for many of the revisions.

Barbara J. Howard

PREFACE TO FIRST EDITION

Although separate treatments of clinical and pathogenic microbiology abound, no previous work has satisfactorily integrated these two subject areas. *Clinical and Pathogenic Microbiology* combines in one volume the study of the materials and methods used for the identification of pathogenic organisms and the study of these organisms in relation to their disease processes in humans. The authors have striven to create a comprehensive book that strikes a balance between theory and practice.

Clinical and Pathogenic Microbiology is intended as a text for medical technology students and students in pathogenic and diagnostic microbiology courses at advanced undergraduate and graduate levels. In addition, clinical laboratories will find the book to be a well-documented reference tool.

The reader will find comprehensive coverage of mycology, parasitology, and virology, which should obviate the need for supplementary texts in these areas. The use of consistent subheadings throughout the text makes all material readily accessible.

The student can easily assimilate complex material in *Clinical and Pathogenic Microbiology* because the text has not neglected extensive explanatory information and coverage of basic science. The reader is led through difficult material in a logical fashion. Basic concepts that are presented early in the text are carefully followed and given enhanced treatment through the remainder of the text.

I acknowledge gratefully the assistance of many individuals in the preparation of this book. Invaluable suggestions and reviews of chapters were made by Dean (Ike) Armstrong, Marilyn S. Bartlett, Don Brenner, William J. Brown, Elizabeth P. Cato, Patricia Charache, Marie B. Coyle, Cecil S. Cummins, Madeline Ducate, Richard R. Facklam, James C. Feely, Lynn S. Garcia, G.L. Gilardi, Patricia Greenup, Dieter H.M. Groschel, George R. Healy, Dannie G. Hollis, Marguerite M. Jackson, J. Michael Janda, William M. Janda, Samuel W. Joseph, Raymond L. Kaplan, John F. Keiser, Michael T. Kelly, Mogens Kilian, Wesley E. Kloos, James T. Kvach, Jean F. MacFaddin, Abe Macher, J. Kenneth McClatchy, Michael R. McGinnis, Cedric A. Mims, Linda Minnich, Josephine A. Morello, David Power, Eileen L. Randall, Alan M. Rauch, Arthur L. Reingold, Morrison Rogosa, Louis D.S. Smith, Walter E. Stamm, Thomas F. Smith, A. von Graevenitz, and Robert E. Weaver. I am also most appreciative of the skill of Jack P. Tandy in preparing our illustrations and for the photographs supplied by Leon J. LeBeau, Carol A. Ormes, and many others cited in the text. The work would never have been realized without the expert typing and retyping of the manuscript by numerous individuals including Diane Haddick, Grace Cannata, Judy Smith, Pansy Palmer, Adrianne Lucke, and Gloria Condit. A very special thanks goes to Thu-Thao Trinh, Maria Finelli, Joanne Comerford, and Eugene R. Kennedy, who assisted in checking references, renumbering references, proofreading galleys, and perhaps most important, keeping up my spirits. And finally I extend my deepest gratitude to my family for their unending patience and support.

Barbara J. Howard

CONTRIBUTORS

Rebecca D. Almazan, B.S., M.T.(ASCP) S.M.
Formerly Supervisor, Clinical Microbiology Laboratory
Walter Reed Army Medical Center
Washington, D.C.

Martin Altwegg, Ph.D.
Institute of Medical Microbiology
University of Zurich
Zurich, Switzerland

Raymond C. Bartlett, M.D.
Professor, Laboratory Medicine
University of Connecticut
School of Medicine
Formerly Director, Division of Microbiology
Department of Pathology and Laboratory Medicine
Hartford Hospital
Hartford, Connecticut

Alain Blanchard, D.Sc.
Director of Research
Institut Pasteur
Paris, France
Department of Microbiology
University of Alabama at Birmingham
Schools of Medicine and Dentistry
Birmingham, Alabama

Edward J. Bottone, Ph.D., Diplomate, ABMM
Professor of Microbiology
Professor of Pathology
Mount Sinai School of Medicine
Director, Clinical Microbiology Laboratories
The Mount Sinai Hospital
New York, New York

Philippe Brouqui, M.D., Ph.D.
Assistant Professor
Unité des Rickettsies
Marseille, France

James R. Campbell, Ph.D.
Program Manager for Molecular Biology
Center for Biomolecular Science
Naval Research Laboratory
Washington, D.C.

Joseph M. Campos, Ph.D.
Director, Microbiology/Virology Laboratory
Children's National Medical Center
Professor
Departments of Pediatrics, Pathology, Microbiology/Immunology
George Washington University Medical Center
Washington, D.C.

Gail H. Cassell, M.S., Ph.D.
Professor and Chairman, Department of Microbiology
University of Alabama at Birmingham
Schools of Medicine and Dentistry
Birmingham, Alabama

Jill E. Clarridge, Ph.D.
Chief, Microbiology Section
Veterans Administration Medical Center
Assistant Professor
Departments of Pathology and Microbiology
Baylor College of Medicine
Houston, Texas

Donna M. Crabb, B.S., M.T.(ASCP)
Medical Technologist
Diagnostic Mycoplasma Laboratory
Department of Microbiology
University of Alabama at Birmingham
Schools of Medicine and Dentistry
Birmingham, Alabama

Carolyn D. Deal, Ph.D.
Research Microbiologist
Department of Bacterial Diseases
Walter Reed Army Institute of Research
Washington, D.C.

Roger Y. Dodd, Ph.D.
Head of Transmissible Diseases Department
American Red Cross
Holland Laboratory
Rockville, Maryland

Lynn B. Duffy, B.S., M.T.(ASCP)
Technical Director
Diagnostic Mycoplasma Laboratory
Department of Microbiology
University of Alabama at Birmingham
Schools of Medicine and Dentistry
Birmingham, Alabama

David O. Freedman, M.D.
Director, UAB Travelers Health Clinic
Division of Geographic Medicine
Department of Medicine
University of Alabama at Birmingham
Birmingham, Alabama

Robyn R. M. Gershon, Dr.PH., M.T.(ASCP)
Research Associate
The Johns Hopkins University
School of Public Health
Department of Environmental Sciences
Baltimore, Maryland

Paul A. Granato, Ph.D.
Clinical Microbiologist
Department of Pathology
Crouse Irving Memorial Hospital
Clinical Professor of Pathology
State University of New York Health Science Center
Syracuse, New York

Wayne W. Grody, M.D., Ph.D.
Associate Professor
Divisions of Molecular Pathology and Medical Genetics
Department of Pathology and Laboratory Medicine
UCLA School of Medicine
Director, Diagnostic Molecular Pathology Laboratory
UCLA Medical Center
Los Angeles, California

Bruce A. Gunn, Ph.D., S.M.(AAM)
Microbiology Section
Department of Pathology and Area Laboratory Services
Tripler Army Medical Center
Honolulu, Hawaii

Gerri S. Hall, Ph.D.
Staff Microbiologist
Cleveland Clinic Foundation
Cleveland, Ohio

Jean Handy, Ph.D.
Associate Professor of Microbiology and Immunology and of Pathology
University of North Carolina School of Medicine
Associate Director, Clinical Microbiology/Immunology Laboratories
University of North Carolina Hospitals
Chapel Hill, North Carolina

Janet A. Hindler, M.C.L.S., M.T.(ASCP)
Senior Specialist
Department of Pathology and Laboratory Medicine
Clinical Microbiology Section
University of California at Los Angeles
Los Angeles, California

Barry Holmes, M.Sc., Ph.D., C.Biol, F.I. Biol.
Head, Identification Services Laboratory
National Collection of Type Cultures
Central Public Health Laboratory
London, England

Carrie A. Norton Hughes, Ph.D.
Research Associate
Department of Microbiology
University of Minnesota
Minneapolis, Minnesota

J. Michael Janda, Ph.D.
Research Scientist
Microbial Diseases Laboratory
California Department of Health Services
Berkeley, California

John L. Johnson, Ph.D.
Professor of Microbiology
Department of Anaerobic Microbiology
Virginia Polytechnic Institute and State University
Blacksburg, Virginia

Russell C. Johnson, Ph.D.
Professor, Department of Microbiology
University of Minnesota
Minneapolis, Minnesota

Raymond L. Kaplan, Ph.D.
Technical Manager, Microbiology
SmithKline Beecham Laboratories
Tucker, Georgia

John Klaas II, Ph.D.
Data Administrator
Information Technology and Services Division
Babson College
Babson Park, Massachusetts

Wesley E. Kloos, Ph.D.
Professor of Genetics and Microbiology
North Carolina State University
Raleigh, North Carolina

Mark T. LaRocco, Ph.D.
Director of Clinical Microbiology
St. Luke's Episcopal Hospital
Houston, Texas

Mary Ellen Mangum, Dr.P.H., M.T.(ASCP)
Quality Assurance Technologist
Microbiology/Immunology Laboratories
University of North Carolina Hospitals
Chapel Hill, North Carolina

Michael R. McGinnis, Ph.D.
Professor and Vice Chairman, Department of Pathology
University of Texas Medical Branch
Galveston, Texas

Peter L. McEvoy, Ph.D.
Director, Microbiology Laboratory
Walter Reed Army Medical Center
Washington, D.C.

Ann Marie McNamara, Sc.D., M.T.(ASCP)
Director, Microbiology Division
U.S. Department of Agriculture
Food Safety and Inspection Service
Sciences and Technology
Washington, D.C.

J. Michael Mullins, Ph.D.
Professor and Chair
Department of Biology
The Catholic University of America
Washington, D.C.

A. William Pasculle, Sc.D.
Assistant Director of Microbiology
University of Pittsburgh Medical Center
Associate Professor of Pathology
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

Marie T. Pezzlo, M.A., F(AAM)
Senior Supervisor, Medical Microbiology
University of California
Irvine Medical Center
Orange, California

Didier Raoult, M.D., Ph.D.
Professor and Director
Department of Clinical Microbiology
Unité des Rickettsies
Marseilles, France

John C. Rees, Ph.D., M.T.(ASCP)
Director, Clinical Immunology and Serology
Washington Hospital Center
Washington, D.C.

John H. Richardson, D.V.M., M.P.H.
Formerly Director and Biosafety Officer
Environmental and Occupational Safety
Emory University
Atlanta, Georgia

Barbara M. Soule, R.N., M.P.A., C.I.C.
Director, Epidemiology and Safety
St. Peter Hospital
Olympia, Washington

Vernon L. Tesh, Ph.D.
Assistant Professor
Department of Medical Microbiology and Immunology
College of Medicine
Texas A & M University
College Station, Texas

John H. Thompson, Ph.D.
Emeritus Professor of Laboratory Medicine
Mayo Clinic
Rochester, Minnesota

Ken B. Waites, M.D.
Associate Professor, Department of Pathology
Department of Microbiology
Department of Rehabilitation Medicine
Director of Clinical Microbiology
University of Alabama Hospital
University of Alabama at Birmingham
Schools of Medicine and Dentistry
Birmingham, Alabama

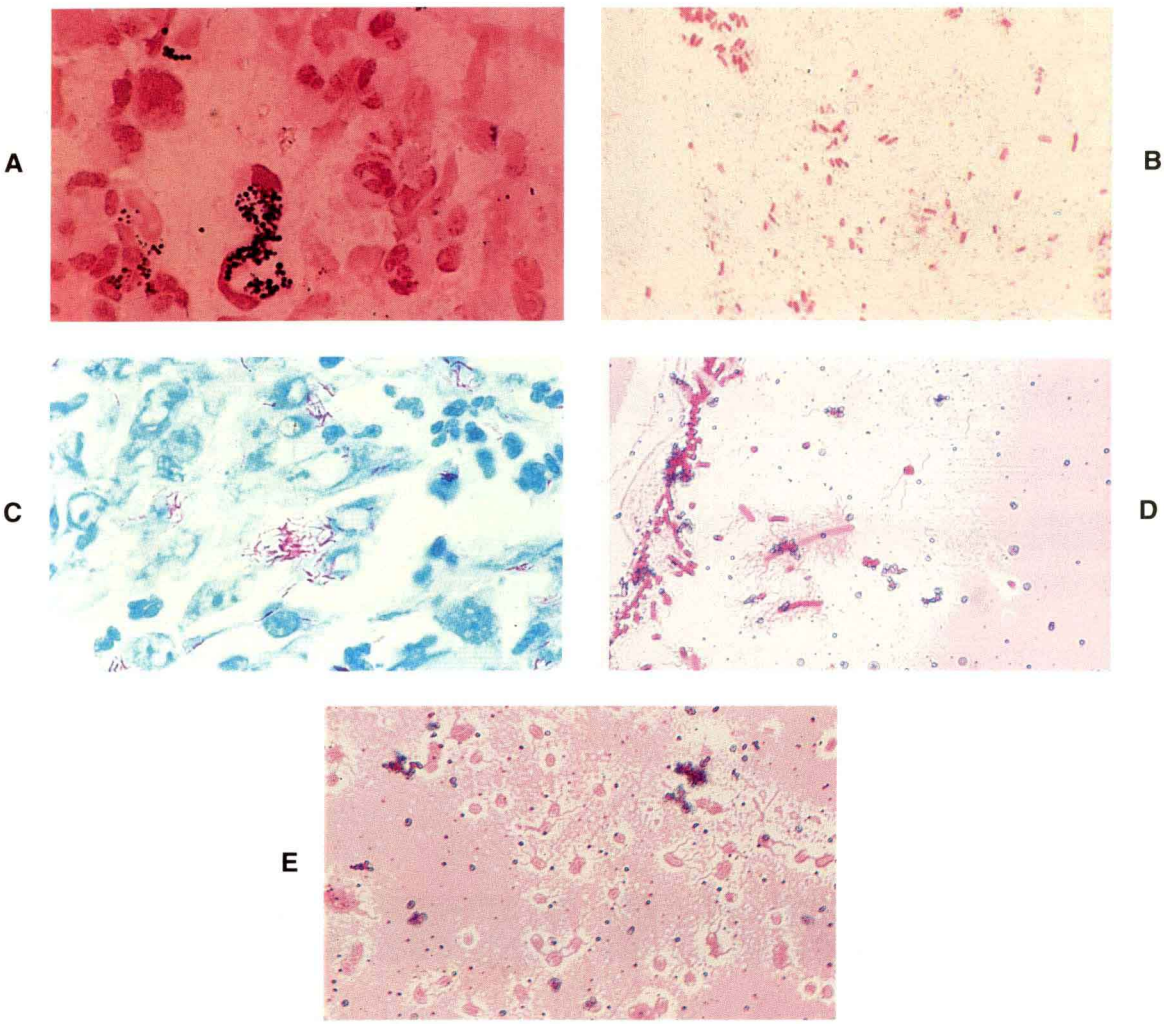


PLATE 1. **A,** Gram stain showing clusters of gram-positive cocci *(Staphylococcus aureus)* in sputum. **B,** Gram stain showing gram-negative rods of *Yersinia enterocolitica* in fluid (Courtesy of Jill Clarridge, VA Medical Center, Houston) **C,** Ziehl-Neelsen stain of tissue. Acid-fast organisms of *Mycobacterium kansasii* stain red against blue background. (Courtesy Carol Ormes, Washington Hospital Center, Washington, D.C.) **D,** flagella stain of *Proteus mirabilis*. Flagella are distributed across entire cell surface; this is called peritrichous flagellation. **E,** flagella stain of *Aeromonas hydrophila*. This organism demonstrates polar flagellation; that is, flagella are present only at poles.

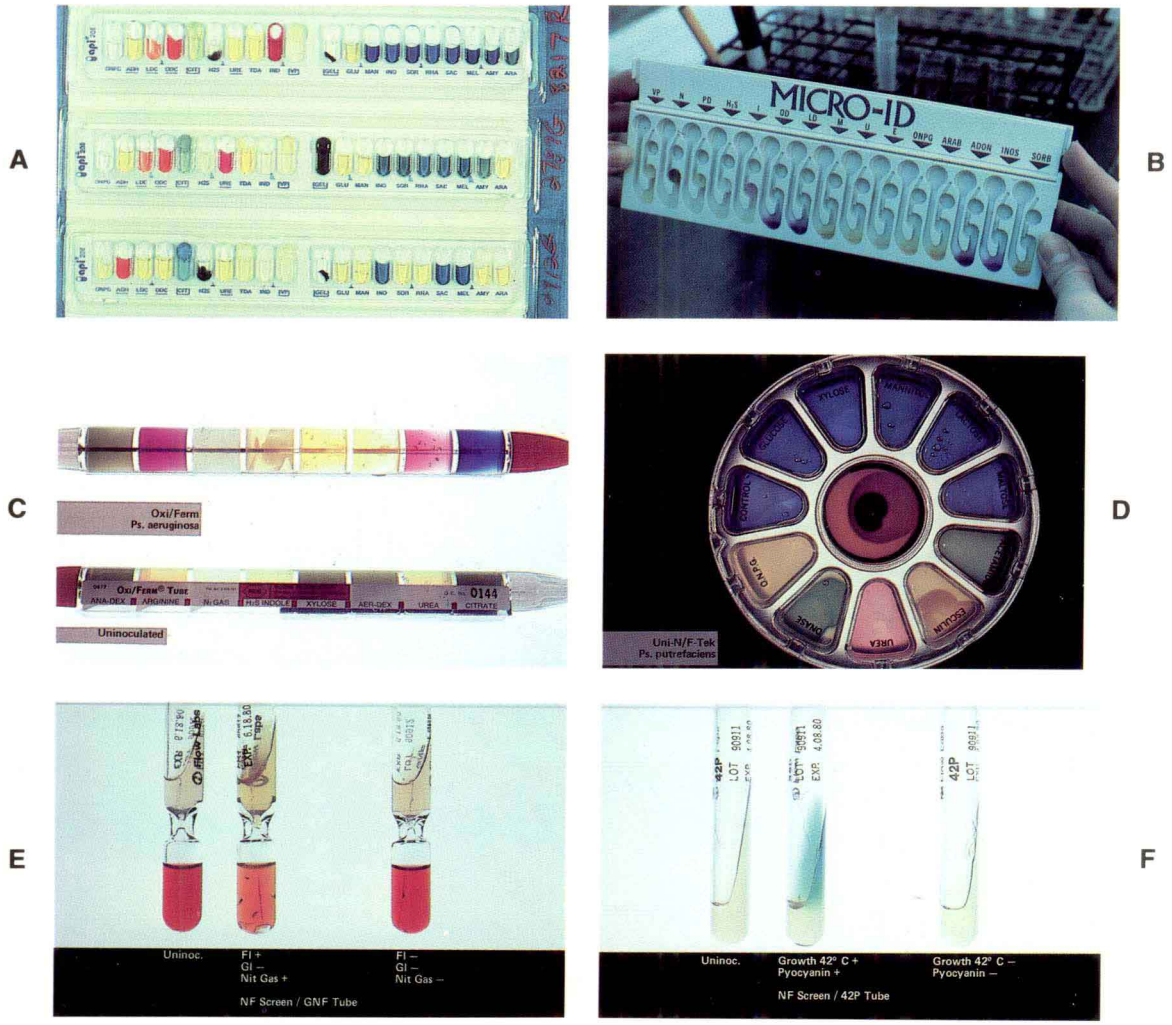


PLATE 2. **A,** API20E system. **B,** Micro-ID system. **C,** Oxi-Ferm system. **D,** Uni-N/F-Tek system. **E,** GNF screening tube; glucose fermentation is detected in the base of the agar and denitrification and fluorescein production in the upper portion. **F,** the 42P screening tube detects growth at 42° C and production of pyocyanin. Blue-green color is due to pyocyanin production by *Pseudomonas aeruginosa*.

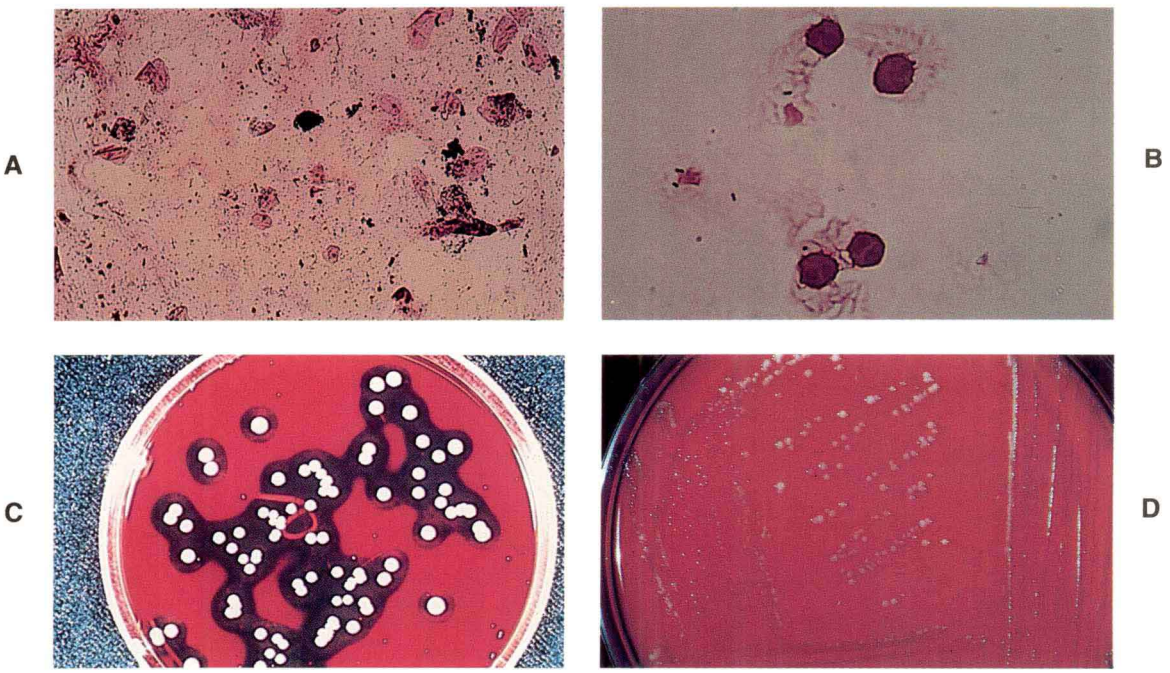


PLATE 3. **A,** Gram-stained sputum smear with greater than 25 squamous epithelial cells per low power field. **B,** Gram stain of cerebrospinal fluid with *Streptococcus pneumoniae*. **C,** β-hemolytic colonies of *Staphylococcus aureus* on sheep blood agar. **D,** colonies of *Staphylococcus epidermidis* on sheep blood agar (1:4.5). (Courtesy of Dr. Leon J. LeBeau, Department of Biocommunication Arts, Medical Center, University of Illinois at Chicago.)

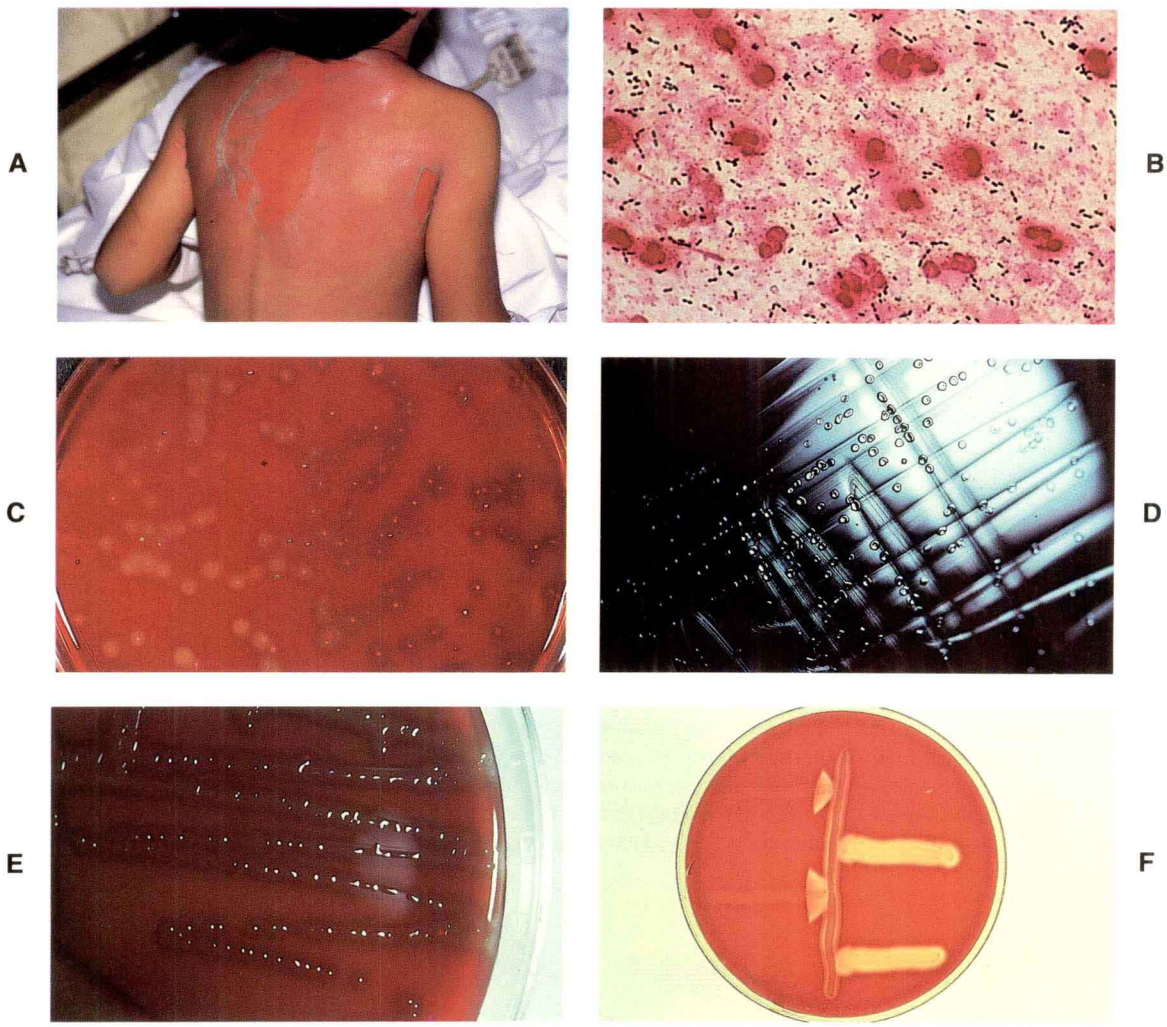


PLATE 4. **A,** scalded skin syndrome. (Courtesy of Dr. Marian Melish, Kapiolami Children's Medical Center, Honolulu.) **B,** Gram stain of sputum showing gram-positive, lancet-shaped diplococci of *Streptococcus pneumoniae* and gram-negative, short, slender rods of *Haemophilus influenzae*. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **C,** group A β-streptococcus on sheep blood agar (1:4.5). Note large zone of hemolysis in relation to size of colony. **D,** umbilicated colonies of *S. pneumoniae*. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **E,** watery-appearing mucoid colonies of *S. pneumoniae*. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **F,** CAMP test. Arrowhead hemolysis demonstrated by two strains on *left* is a positive reaction and is presumptive identification of group B β-streptococcus. Isolates on *right* are negative for CAMP test. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.)

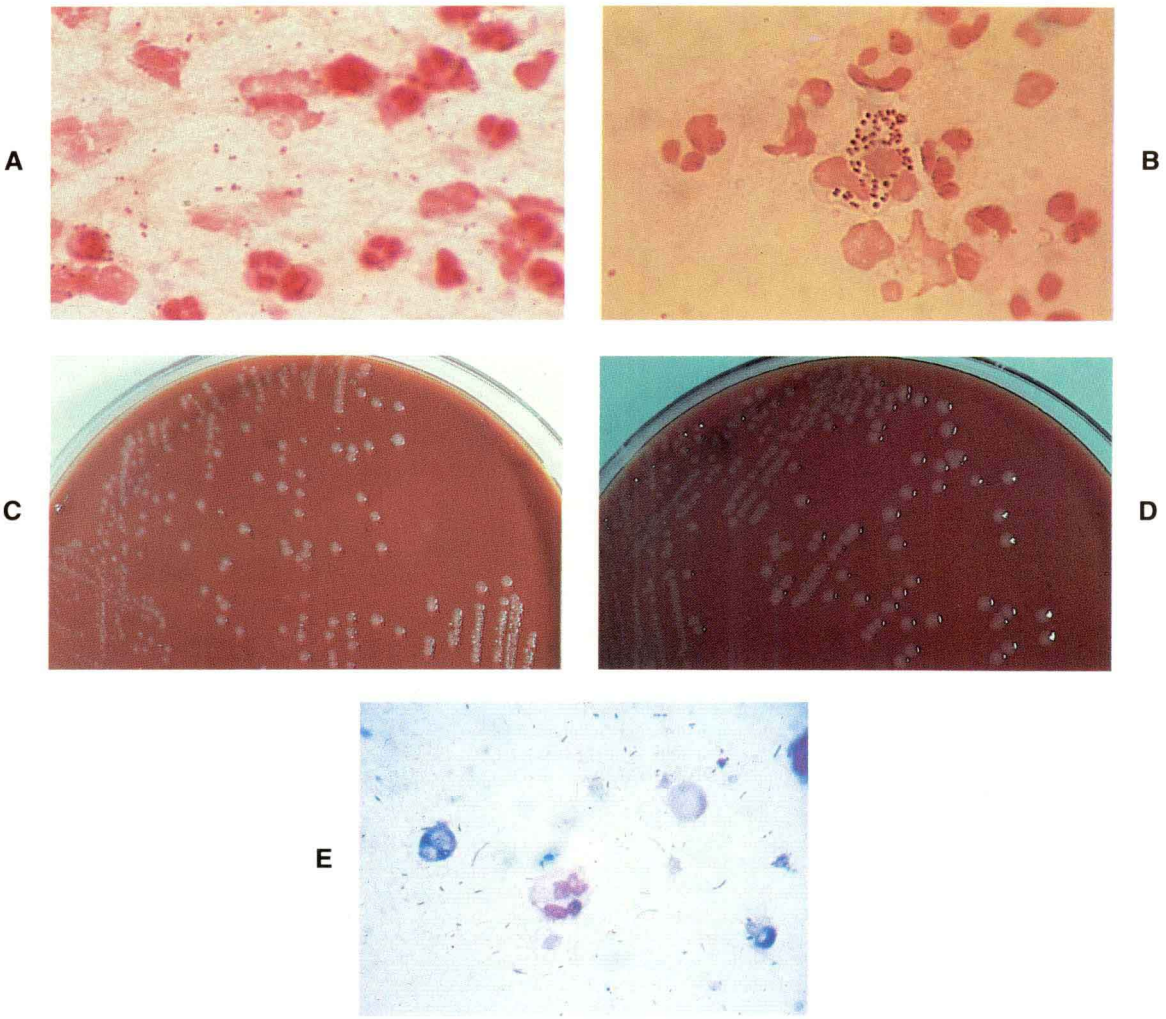


PLATE 5. **A,** Gram stain of sputum with *Moraxella catarrhalis*. This organism typically appears as gram-negative diplococci with adjacent sides flattened. **B,** typical intracellular, gram-negative, kidney bean–shaped diplococci of *Neisseria gonorrhoeae* in exudate from male. **C,** colonies of *N. gonorrhoeae* on chocolate agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **D,** colonies of *Neisseria meningitidis* on chocolate agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **E,** Gram stain of cerebrospinal fluid showing typical short and slender gram-negative rods of *Haemophilus influenzae*. Organisms stain very lightly and may be mistaken for debris.

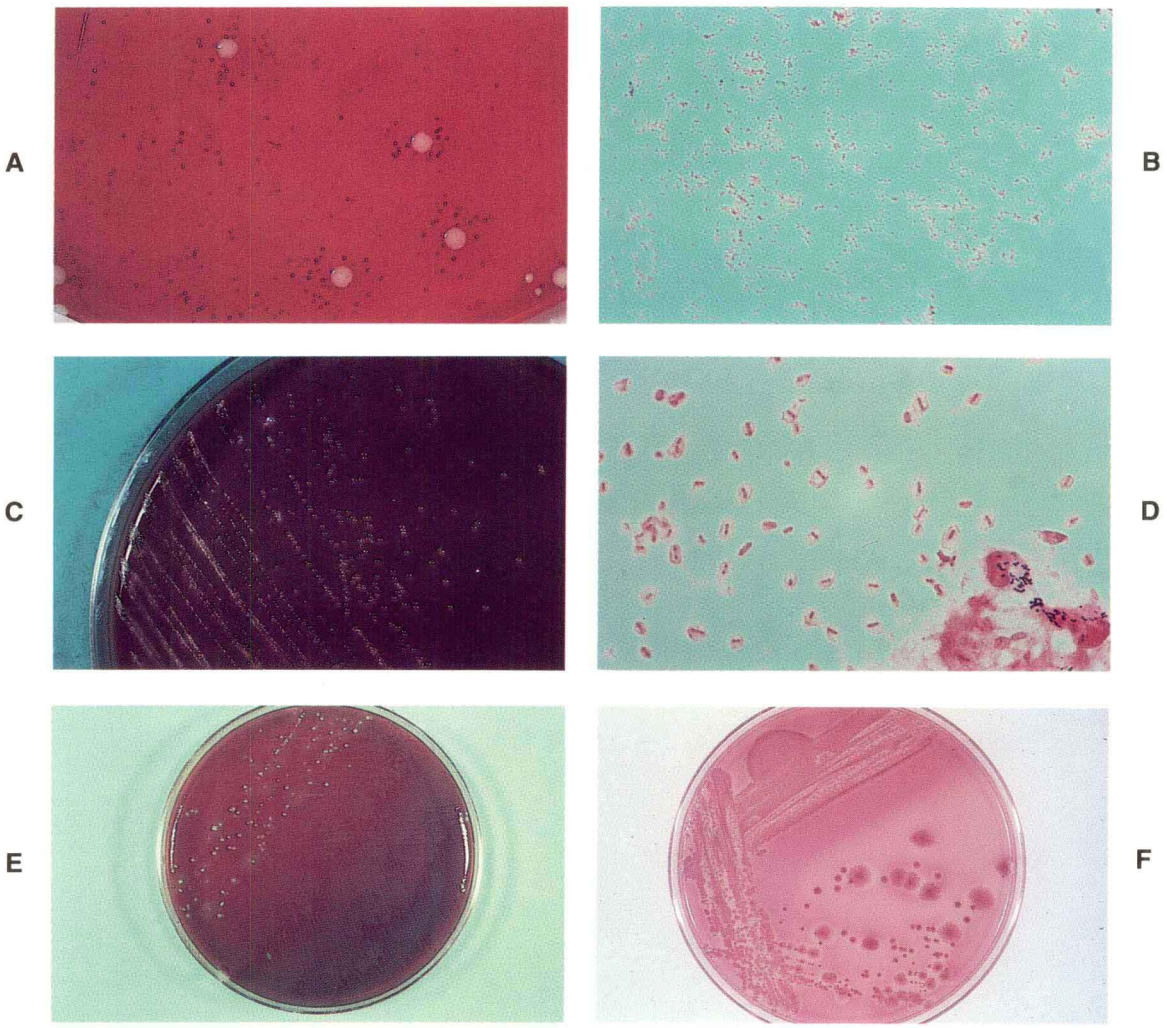


PLATE 6. **A,** *Haemophilus influenzae* satelliting staphylococci on blood agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **B,** Gram stain of *H. influenzae* from chocolate agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **C,** small, smooth, translucent, gray colonies of *H. influenzae* on chocolate agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **D,** direct smear of sputum showing encapsulated *Klebsiella pneumoniae*. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **E,** *Proteus mirabilis* swarming on blood agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **F,** two types of *Escherichia coli* on MacConkey agar.

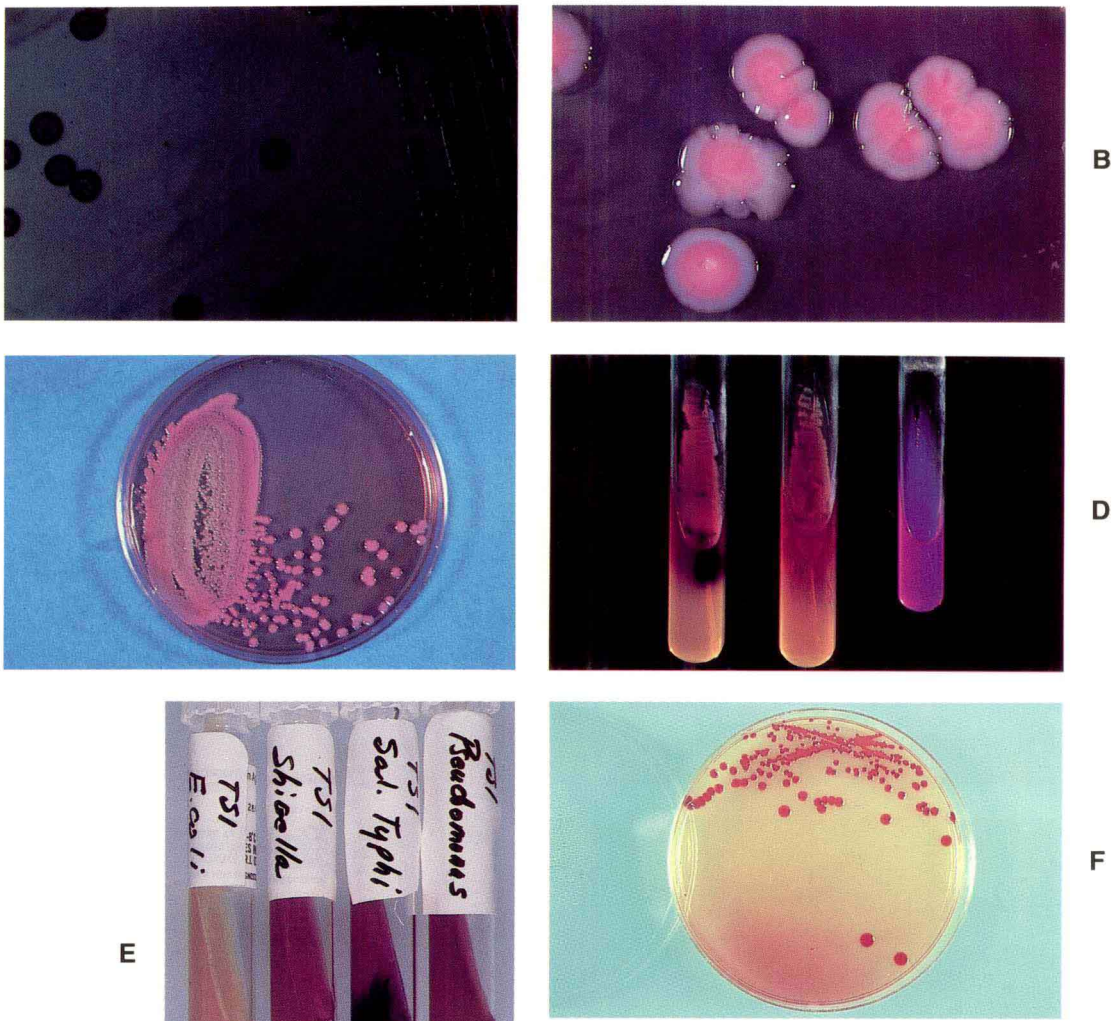


PLATE 7. **A,** metallic sheen of *Escherichia coli* on eosin–methylene blue agar (1:2.5). (Courtesy of Dr. Leon J. LeBeau, Department of Biocommunication Arts, Medical Center, University of Illinois at Chicago.) **B,** pink, mucoid colonies of *Klebsiella pneumoniae* on MacConkey agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **C,** pink colonies of *Enterobacter* on MacConkey agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **D,** triple sugar iron, lysine iron agar, and urea screen of *Proteus*. (Courtesy of Dr. Leon J. LeBeau, Department of Biocommunication Arts, Medical Center, University of Illinois at Chicago.) **E,** Triple sugar iron reactions *(left to right): E. coli,* A/A+/−; *Shigella,* K/A −/−; *Salmonella typhi,* K/A −/+; *Pseudomonas,* K/NC. **F,** pigmented *Serratia marcescens* on MacConkey agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.)

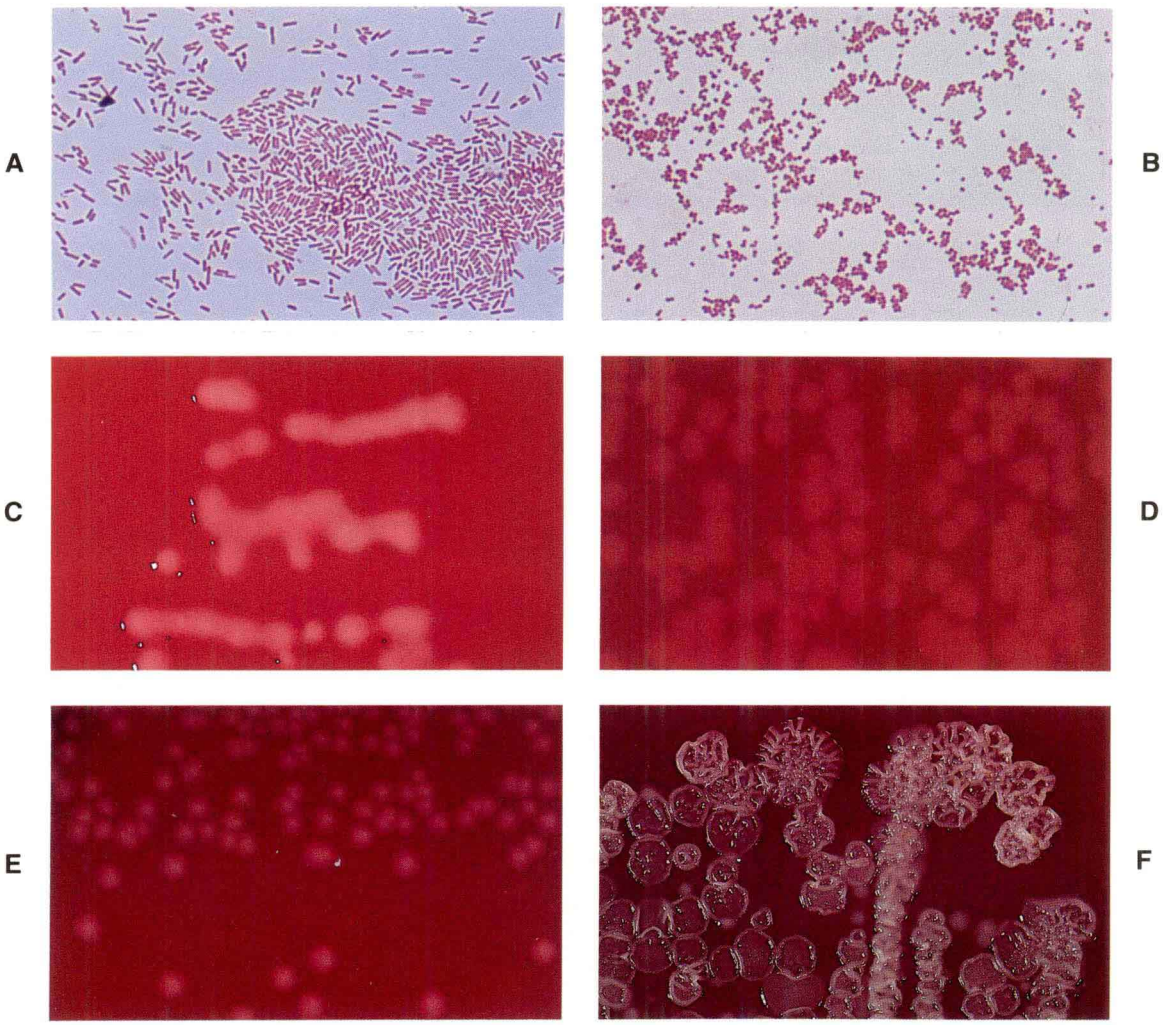


PLATE 8. **A,** long, thin, gram-negative rods of *Pseudomonas aeruginosa*. **B,** gram-negative coccobacilli of *Acinetobacter baumannii*. **C,** mucoid forms of *P. aruginosa* on blood agar after 24 hours incubation. **D,** typical colonies of *P. aeruginosa* with ground-glass appearance. Blood agar after 24 hours incubation. **E,** smooth colonies of *P. aeruginosa* on blood agar after 24 hours incubation. **F,** mixed smooth and wrinkled colonies of *Pseudomonas stutzeri* after 48 hours incubation.

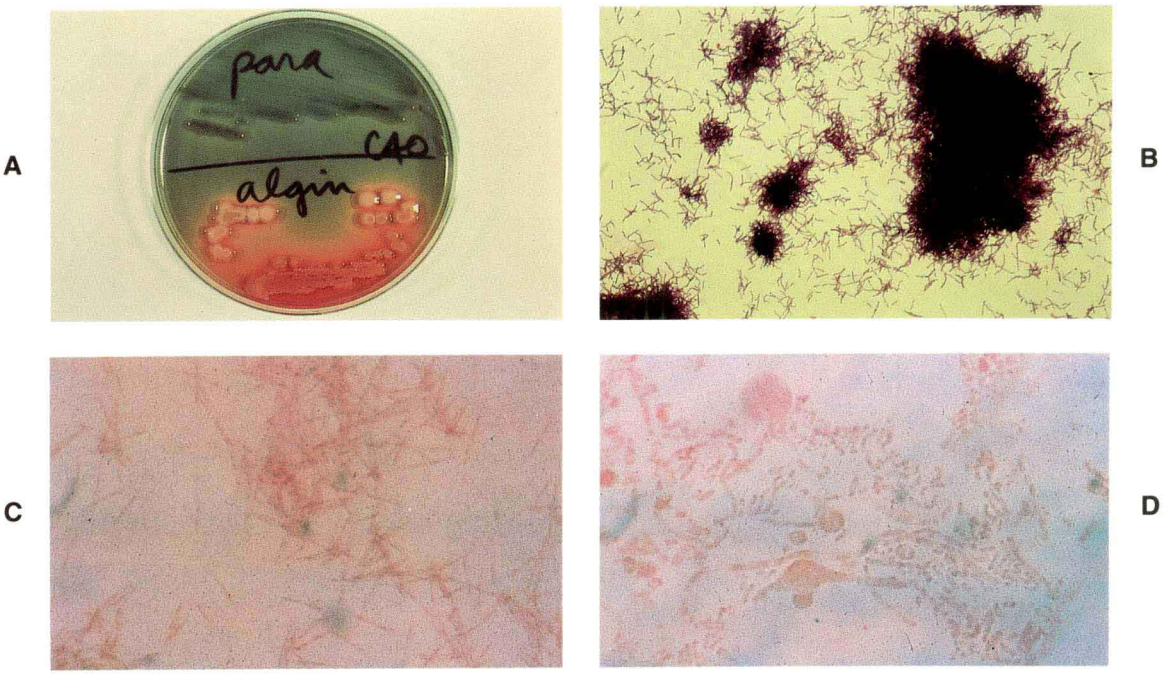


PLATE 9. **A,** colonies of *Vibrio parahaemolyticus (top),* and *Vibrio alginolyticus* on thiosulfate-citrate-bile salts sucrose (TCBS) agar. (Courtesy of Carol Ormes, Washington Hospital Center, Washington, D.C.) **B,** Gram stain of *Actinomyces israelii*. **C,** Gram stain of *Fusobacterium nucleatum* showing long, spindle-shaped bacilli. **D,** Gram stain of *Fusobacterium necrophorum*. Note pleomorphic gram-negative bacilli with swollen areas, filaments, and large round bodies.