Instrumented Systems for Microbiological Analysis of Body Fluids

Authors

Lorraine S. Gall, Ph.D.

William A. Curby, M.S.

Instrumented Systems for Microbiological Analysis of Body Fluids

Authors

Lorraine S. Gall, Ph.D.
Associate Professor of Epidemiology
Department of Virology and Epidemiology
Baylor College of Medicine
Houston, Texas

William A. Curby, M.S.
Head, Sias Biophysics Research Unit
Lahey Clinic Foundation
Boston, Massachusetts

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data

Gall, Lorraine S
Instrumented systems for microbiological analysis of body fluids.

Includes bibliographies and index.
1. Medical microbiology- -Instruments. 2. Body fluids- -Microbiology. 3. Body fluids- -Examination. 4. Medical microbiology- -Automation. I. Curby, William A., joint author. II. Title.
QR67.G34 616'.01'028 79-706
ISBN 0-8493-5681-4

Direct all inquiries to CRC Press, Inc., 2000 N.W. 24th Street, Boca Raton, Florida 33431.

International Standard Book Number 0-8493-5681-4

Library of Congress Card Number 79-706
Printed in the United States

PREFACE

The need for improved microbiological techniques for analyzing body fluids, which combine speed and accuracy at a reasonable cost, is evident to serve as a guide to the doctor in treating his patients adequately and more promptly. The first step in speeding up microbial analysis was a series of kits, mainly intended for identifying enteric bacteria that did not completely meet the needs for improving microbial analysis. In about 1965, instrumented systems for microbiological analyses of varying degrees of sophistication began to appear, but these did not make a successful market penetration. Beginning in 1970 several sophisticated decision-making instrumented systems were displayed at scientific meetings and were the subject of numerous scientific papers. Six of these systems capable of detection, enumeration, antibiotic susceptibility testing or identification are on or approaching the market. As a result of these activities, microbiologists are faced with evaluating these instruments for possible use in their laboratories and bioengineers are increasingly being called upon to design these systems. To intelligently design or evaluate these instrumented systems, information on the theoretical and practical aspects of instrumented microbiological analytical systems is necessary. It is the purpose of this monograph to set forth the theoretical rules and practical considerations as a basis for decisions, and to illustrate the application of these aspects to the design and evaluation of instrumented microbiological analysis of body fluids.

The first two sections of the monograph discuss the theoretical rules and practical aspects involved in instrumentation of a microbiological assay of body fluids. Following a brief discussion of several early systems that did not receive market acceptance, there is an in depth illustration of the pulse height analyzer as an instrumented system designed especially to apply these theoretical and practical considerations. The next six chapters describe various instrumented systems illustrating several different approaches to microbiological analysis of body fluids, followed by an evaluation of how well these instrumented systems reach the goals for yielding ideal analytical results. The last two sections deal with some less sophisticated instruments, and with the possibilities for the future in microbiological assays using sophisticated decision-making instrumented systems.

Lorraine S. Gall
William A. Curby

THE AUTHORS

Lorraine Sibley Gall, Ph.D., was born in 1915 in Binghamton, New York, where she graduated from high school as valedictorian, and then enrolled in the bacteriology course at Cornell University, receiving a B.S. degree in 1938. For the following six years she conducted research in nutritional microbiology, and then returned to Cornell to study for a Ph.D. in that subject, submitting a thesis on rumen microbiology. In 1947 she continued her studies with a post-doctorate fellowship at Yale University.

The next ten years were spent in productive research on the isolation and basic function of anaerobic rumen bacteria, first at Ohio Agricultural Experiment Station, and continuing at National Dairy Research Laboratory on Long Island, where her fistulated cattle, Christopher and Isabella, were curiosities and inspired front page coverage of her work in the New York Herald Tribune. Her recognition as an authority on rumen bactera led to the award in 1954 of a Senior Research A Fulbright grant to New Zealand and Australia, where she studied the influence of pasture on rumen flora and lectured on her techniques for isolating rumen anaerobes.

Returning to the United States, she found her strong convictions, that high-roughage low-concentrate feeding produced the most economical, efficient rumen function were not welcomed or accepted by the grain-oriented economy of the U.S. Dr. Gall then turned her attention to human microbiology. Intrigued by the trip to New Zealand, she combined business with travel and trained detailmen throughout the world for American Cyanamid, Lederle International Division; but after five years living out of a suitcase and several unpleasant incidents in an increasingly hostile world, she returned to research.

During the next ten years, while working for Republic Aviation Corporation and IBM, she participated in research programs related to the man-in-space project, monitoring the effect of various simulated space environments on the bacteria living in or on the body of man, with emphasis on the influence of diet on the intestinal anaerobes. Manually monitoring the microbiological status of the space capsule during flight is complicated by the difficulty of performing microbiological procedures in a weightless environment. This led Dr. Gall to investigate the automation of the microbiological analyses, and this has been her chief interest for the past ten years.

Her present assignment at Ames Division of Miles Laboratories is to continue the work started at Grumman Health Systems on an automated microbiological instrument that will perform a complete analysis in a matter of hours rather than days.

At present she is working on development of biomedical instrumentation as an Associate Professor of Epidemiology at Baylor College of Medicine in Houston, Texas.

Dr. Gall has published about 100 scientific papers and many popular articles. She has been recognized by inclusion in *American Men of Science* and several *Who's Who,* such as *Who's Who of Women, Education, Commerce and Industry,* and *Dictionary of International Biography.* She was elected to Sigma Xi, Phi Kappa Phi, Sigma Delta Epsilon, and International Platform Association, and is a member of American Society for Microbiology and Society for Industrial Microbiology. Her hobbies are duplicate bridge and nature study.

William A. Curby, M.S., is Head of the Sias Biophysics Research Unit of the Lahey Clinic Foundation and Director of the Alice Sias Memorial Laboratory in Brookline, Massachusetts.

He received his B.S. from Tufts University, Medford, Massachusetts, in 1950 and his M.S. from Tufts University in 1953. From 1953 to 1958, while a Research Assistant Professor at Tufts, he was sponsored by Tufts School of Dental Medicine to study a program involving course work at Massachusetts Institute of Technology, Tufts Uni-

versity School of Medicine, and Boston University School of Medicine for the purpose of putting physics, biology, and medical science into a combined discipline. From 1948 to 1960 Mr. Curby was a U.S. Public Health Fellow at Brandeis University, Waltham, Massachusetts, in a biophysics-biochemistry combination study program.

William Curby has been Principal Investigator for the Department of Defense, National Institute of Health, and Environmental Protection Agency research grants and contracts. For the past several years he has been a lecturer at Northeastern University, Boston, Massachusetts, at the Center for Continuing Education, teaching current state-of-the-art courses related to light energy measurement, photophysics, and advanced optics. He has been a consultant to industrial and federal groups in instrumentation and systems development.

Mr. Curby's research interests have been directed toward the study of real time analyses of biology and medical-physical phenomena. He is the holder of U.S. and foreign patents related to ultrafast-acting sensors for forces and for temperature, for microparticle capture and analysis, and for sensoring and processing methods related to automated monitoring and evaluating of real time changes in the physical and chemical characteristics of living cells. In addition to patents, Mr. Curby has published many papers, scientific articles, and reports on instrumentation and application of changes of cellular characteristics in response to applied physical and chemical stresses.

ACKNOWLEDGMENT

The considerable support of the Ames Division of Miles Laboratories, Elkhart, Indiana, during the preparation of this manuscript is deeply appreciated.

The courtesy and helpfulness of the personnel of several commercial firms who participated in the preparation of and reviewed the chapters related to their product is gratefully acknowledged, especially Dr. J. E. Ahnell of Johnston Laboratories, Inc., Dr. Phillip Wyatt of Science Spectrum, Inc., Mr. Robert C. Matteline of Vitek Systems, Inc., Dr. David C. Mayfield of Abbott Diagnostics Division, Dr. Paxton Cady of Bactomatic, Inc., and Dr. P. Blume of Good Samaritan Hospital, Portland, Oregon.

The author wishes to acknowledge the support and assistance of Sandy Pearlman of CRC Press in the preparation of this volume. The help of many in typing the manuscript is appreciated, especially Vicki Bilenker, Vicki Carpenter, Joan Chapman, Jann Charlston, Mary Drummond, and Gloria Froelich.

TABLE OF CONTENTS

Chapter 1

INTRODUCTION

I. NEED FOR INSTRUMENTED SYSTEMS FOR MICROBIOLOGICAL ANALYSIS OF BODY FLUIDS

Remarks like, "You bug-hunters have progressed about three years beyond Pasteur!" have spurred microbiologists to think increasingly about improving the methods for microbiological analysis of body fluids. Their commitment has been strengthened when physicians explain that they do not send clinical specimens to the bacti lab because "the patients are either dead or gone home by the time the results are available." Clearly, better, speedier, microbiological analysis is needed.

II. GOALS FOR INSTRUMENTED SYSTEMS

What are the methods that require improvement? Briefly, a complete microbiological analysis using traditional methods usually consists of three major interrelated phases: (1) detection of pathogenic bacteria, sometimes coupled with enumeration, which takes a 16- to 24-hr incubation period to isolate bacterial colonies on agar media for enumeration and further testing, (2) antibiotic-susceptibility testing on the pure isolate by the Kirby-Bauer disc method or for Minimum Inhibitory Concentration (MIC), which takes another 24 hr, and (3) identification of the pure culture of predominating pathogens by differential selective media or antisera which may require 2 to 3 days after isolation. Thus, a complete analysis may take 3 to 4 days. Since the procedures are usually performed individually and manually, the results are often reported slowly and are sometimes inaccurate, as well as costly.

The problems have been stated. There is a need for more speed, accuracy, and lower cost in microbiological analysis. What has been done to achieve these goals?

In the late 1960's and continuing into the 1970's, simple instruments aimed at assisting one discrete part of the analysis were devised, mainly as labor-saving devices. These included media dispensers, plate streakers, colony counters, gram stainers, antibiotic-sensitivity readers, and microdilutors. These helped to relieve the tedium of some processes, speeded up the analysis to a limited extent, and by reducing the manual work load, may have cut costs. However, most of these devices have had little impact on the average microbiological lab. Perhaps the exception may be the microdilutors aimed at performing MIC tests. They seem to have a chance of challenging Kirby-Bauer's dominance in the field of antibiotic-susceptibility testing. These devices will be covered briefly in a later chapter.

III. HISTORY OF RAPID PROCEDURES USING KITS

Starting in the late 1960's and early 1970's, attempts to improve microbiological analysis got into full swing with the appearance of many products termed, "rapid methods". Many of these "systems" centered around kits which employed conventional metabolic tests to identify previously isolated bacterial cultures using paper strips, or miniature "test tubes", or containers with a preset battery of tests. Among these were Pathotec® (Warner-Lambert), R/B® (Corning), Enterotubes® and Oxiferm® (Roche), API® (Analytab Products, Inc.), and Minitek® (BBL). Most were