

ANTIBIOTICS MONOGRAPHS | NO. 2

ASSAY METHODS OF ANTIBIOTICS

A LABORATORY MANUAL

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Under the Editorial Direction of

Henry Welch, PH.D., and Félix Martí-Ibáñez, M.D.

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A Laboratory Manual

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U. S. Department of Health, Education, and Welfare*

FOREWORD

By Henry Welch, PH.D., and Félix Martí-Ibáñez, M.D.

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FOREWORD

Ever since Pasteur introduced the microbial doctrine and Ehrlich's Chromotherapy ushered in the era of modern chemotherapy, the laboratory has been the main battleground of Medicine. Gone are the days of philosophical investigation in the "pristine and serene" atmosphere of the laboratory. In today's laboratory the most important branch of Therapeutics was born and clinical diagnosis is each day brought to completion. If the clinician orients diagnosis on the basis of the exploration of the patient, in the laboratory the diagnosis is pinned to a basis so precise as to be sometimes almost mathematical, and it is confirmed or rejected. This is also the place where an infinite number of substances endowed with chemotherapeutic powers have been tested or discovered. If the hospital is art, the laboratory is science, and there the research worker, aided by his test tubes, often announces whether the patient will live or die.

In the hospital the physician struggles with symptoms and signs; in the laboratory the research worker zealously pursues the microbial or biochemical etiology of disease until he corners it like an animal at bay and then turns it over to the relentless pack of dogs of modern chemotherapeutic agents.

It was the physician's daily chores that gave historical rise to early clinical medicine. The laboratory came much later and was the fruit of the research man's wish to carry his understanding of the patient one step beyond what his senses could reveal. If the clinic is the expression of man seeking the truth about disease with his senses, the laboratory represents the historical yearn to plumb the abyssal mysteries of organic cells and humors; it is an attempt to extend, with the help of the clear hard pupil of the microscope, the search for the invisible cause of disease beyond the limits of what can be seen with the naked eye.

Antibiotics, perhaps the greatest accomplishment of modern research, have, since their inception, imparted to the laboratory a tremendous importance in Medicine. No longer is the mission of the laboratory to investigate

some simple diagnostic tests. The laboratory must now produce constantly new curative weapons, and it must identify, isolate, measure, and stabilize them, determine their purity, and also act as wise adviser and mentor to the clinic. Without the laboratory, there would be no Antibiotic Era, and Therapeutics could not aspire to anything loftier than the empirical application of remedies extracted from Nature. In the laboratory, man, with the help of methods, instruments, and reactives, discovers and imprisons in his brain the immutable laws that rule the world of chemotherapeutics.

Since 1942 literally thousands of antibiotic substances have been isolated in laboratories. Many, in fact the great majority, have been discarded because of poor antimicrobial activity or high toxicity. There are now available on the American pharmaceutical market 17 useful antibiotics and hundreds of preparations of penicillin, tyrothricin, streptomycin, dihydrostreptomycin, bacitracin, chloramphenicol, chlortetracycline, oxytetracycline, tetracycline, polymyxin, viomycin, neomycin, erythromycin, carbomycin, fumagillin, nystatin, and anisomycin.

The useful methods for analysis of all these antibiotic preparations are included in this monograph, the second in a series dedicated to present a concise and accurate picture of each of the principal antibiotics and its application in clinical medicine.

This monograph is the first and only source in which useful and practical methods for the assay of all these antibiotic preparations are described. Many of the methods are "official" in that they are the ones used by the Food and Drug Administration in determining the identity, strength, quality, and purity of the so-called "certifiable" antibiotics. However, the others are not neglected and the most useful and acceptable methods for those antibiotics not certified by the Government are also included.

The first 15 chapters of this book describe the methods used for determining the potency of the salts of the 17 basic antibiotics. The various methods for preparations, such as tablets, troches, and ointments, are also given. In addition, where applicable, methods of analysis of body fluids and tissue are described. Assay of mixtures of antibiotics in pharmaceutical dosage forms are detailed in Chapter 16, while Chapters 17 and 18 describe methods of identification and the tests for toxicity, pyrogens, histamine, and sterility. Methods of determining bacterial sensitivity to the antibiotics are given in Chapter 19, and special methods are described in Chapter 20. All media, solutions, reagents, and apparatus are grouped in the final chapter of this comprehensive volume.

The authors of this monograph have had wide experience in the field of antibiotics. Doctor Grove as assistant director of the Division of Antibiotics of the Food and Drug Administration has kept close contact with the development of antibiotic methods through supervision of a large group of bacteri-

ologists and chemists who analyze daily hundreds of antibiotic dosage forms. Doctor Randall as director of the research group in the same Division has been responsible for the development or modification and improvement of many of the methods included in this monograph. It is an interesting historical point that the first official assays of crude penicillin were carried out in 1943 in his laboratory.

The correlation of this large group of methods has been a monumental task and is so extremely well done that this book will be of great value to laboratory technicians and research workers in the antibiotic field. Industrial laboratories, where thousands of analyses of these drugs are made daily, and hospital, state, and municipal agencies will find this book a rich and rewarding source of useful information. This Manual is written in the clear, terse style that has become the characteristic idiom—chary of word, rich in meaning—of laboratory investigators. For the research man has beaten out for himself a sort of scientific shorthand. Methods, instruments, and systems are grouped on the pages of this book with the precision of soldiers lined up for the most exacting inspection. Indeed, since a laboratory manual is always the most eloquent of scientific books, it teaches us the art of saying a maximum of useful things with a minimum of verbiage, thus providing a supreme vehicle for the transmission of scientific knowledge. The clinician and the research man welcomes books like this Manual, on the pages of which the laboratory holds high its historical mission of seeker of scientific truth.

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P R E F A C E

The value of antibiotics in the treatment of infectious diseases of man and the impact they have had on public health is well known by everyone today. The use of antibiotics in the treatment of animal diseases and in animal feeds for growth development has also become commonplace. The more recent use of these drugs for plant diseases further demonstrates their ubiquity. It is exceedingly important therefore to have accurate means of testing and controlling this outstanding class of drugs. Many methods of assay have been described in the scientific literature and there are many unpublished methods or modifications of methods. However, there is not available a compilation of tested and proved procedures for all of the antibiotics commonly distributed today. It is believed therefore that a real need for such a book exists.

The official regulations, promulgated under section 507 of the Federal Food, Drug, and Cosmetic Act, contain tests and methods of assay for penicillin, streptomycin, dihydrostreptomycin, chlortetracycline, tetracycline, chloramphenicol, bacitracin, and also a few of these antibiotics combined with polymyxin or neomycin. However, these regulations do not contain methods for various other antibiotics nor methods for determining antibiotics in body fluids, tissues, animal feeds, or milk. The regulations also do not contain methods for determining the sensitivity of an organism to an antibiotic or identification tests.

The present book gives practical tests and methods of assay for all of the antibiotics that are being distributed commercially in the United States today and for the various preparations and substances in which they may occur. Because of the tremendous amount of research being conducted to find new antibiotics, this book will probably not be published very long before some new ones will be introduced for clinical or other use, further adding to the list of these important drugs. It is believed, however, that such a wide variety of methods are presented that it will be a relatively simple matter to adapt them to any new antibiotics that may come along. It is hoped that this book will be of value not only to those who are engaged in the testing of antibiotics, but to students and teachers whose field of interest embraces this important and useful class of drugs.

The authors wish to express their sincere appreciation to Dr. Henry Welch, Dr. William Wright, and Mr. Amiel Kirshbaum for their helpful suggestions and review of the manuscript. We also wish to thank Dr. Joseph DiLorenzo for preparing the various charts and figures in the book.

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CONTENTS

FOREWORD by Henry Welch, PH.D., and Félix Martí-Ibáñez, M.D.	xxi
-----------------------------------------------------------------	-----

PREFACE	xxv
---------	-----

1 INTRODUCTION	1
----------------	---

2 PENICILLIN	7
--------------	---

Section A. Microbiologic assay methods for penicillin.	7
--------------------------------------------------------	---

METHOD 1. Cylinder-plate method using <i>Micrococcus py-</i> <i>ogenes</i> var. <i>aureus</i> as the test organism.	7
------------------------------------------------------------------------------------------------------------------------	---

METHOD 2. Cylinder-plate method using <i>Sarcina lutea</i> as the test organism.	14
-------------------------------------------------------------------------------------	----

Section B. Chemical method for penicillin.	16
--------------------------------------------	----

The iodometric titration method.	17
----------------------------------	----

Section C. Assay of penicillin salts and pharmaceutical dosage forms.	17
--------------------------------------------------------------------------	----

Sodium, potassium, and calcium penicillins.	17
---------------------------------------------	----

Aluminum penicillin.	18
----------------------	----

Procaine penicillin G.	18
------------------------	----

Ephedrine penicillin G.	19
-------------------------	----

1-Ephenamine penicillin G.	19
----------------------------	----

Benzathine penicillin G.	19
--------------------------	----

Penethamate.	20
--------------	----

Sodium and potassium penicillin O.	20
------------------------------------	----

2-Chloro-procaine penicillin O.	21
---------------------------------	----

Dibenzylamine penicillin.	21
---------------------------	----

Hydrabamine dipenicillin G.	21
-----------------------------	----

Penicillin ointment.	22
Penicillin tablets.	23
Penicillin troches (pastilles, lozenges).	23
Penicillin dental cones.	24
Penicillin with vasoconstrictor (nose drops).	24
Penicillin tablets containing aluminum penicillin.	24
Penicillin sulfonamide powder.	24
Penicillin vaginal suppositories.	24
Penicillin bougies.	24
Procaine penicillin in oil.	24
Penicillin for inhalation therapy.	25
Procaine penicillin for aqueous injection.	25
Procaine penicillin and buffered crystalline sodium or potassium penicillin for aqueous injection.	25
Procaine penicillin and crystalline sodium or potassium penicillin in oil.	27
1-Ephenamine penicillin G for aqueous injection.	28
1-Ephenamine penicillin G in oil.	28
Benzathine penicillin G oral suspension.	28
Benzathine penicillin G for aqueous injection.	28
Benzathine penicillin G and buffered crystalline sodium or potassium penicillin for aqueous injection.	28
Benzathine penicillin G, procaine penicillin, and buffered crystalline sodium or potassium penicillin for aqueous injection.	29
Ephedrine penicillin tablets.	29
Buffered penicillin powder.	30
Penicillin tooth powder.	30
Penethamate (diethylaminoethyl ester penicillin G hydriodide) for aqueous injection.	30
Chloroprocaine penicillin O for aqueous injection.	30
Hydrabamine penicillin oral suspension.	30
Penicillin capsules.	31
Penicillin oral suspension.	31

Section D. Determination of penicillin in body fluids and other substances.

Blood serum.	31
Urine.	32
Tissue.	32
Milk.	32
Animal feed supplement and (mixed) final feeds.	32

3 STREPTOMYCIN AND DIHYDROSTREPTOMYCIN 34

Section A. Microbiologic assay methods for streptomycin and dihydrostreptomycin. 34

- METHOD 1. Cylinder-plate method using *Bacillus subtilis* as the test organism. 34
- METHOD 2. Turbidimetric method using *Klebsiella pneumoniae* as the test organism. 36
- METHOD 3. Modified cylinder-plate assay method using *Bacillus subtilis*. 38

Section B. Chemical methods for streptomycin and dihydrostreptomycin. 38

- METHOD 1. Maltol method for streptomycin. 40
- METHOD 2. Ultraviolet spectrophotometric method for dihydrostreptomycin. 41
- METHOD 3. Oxidized nitroprusside method for streptomycin or dihydrostreptomycin. 42

Section C. Assay of streptomycin and dihydrostreptomycin salts and pharmaceutical dosage forms. 42

- Streptomycin sulfate, streptomycin hydrochloride, streptomycin trihydrochloride calcium chloride, dihydrostreptomycin sulfate, and dihydrostreptomycin hydrochloride. 42
- Streptomycin or dihydrostreptomycin ointments. 43
- Streptomycin or dihydrostreptomycin tablets. 44
- Streptomycin sulfate solution or dihydrostreptomycin sulfate solution. 44
- Streptomycin or dihydrostreptomycin syrup. 44
- Streptomycin, pectin, and kaolin in aluminum hydroxide gel. 44
- Streptomycin or dihydrostreptomycin otic (ear drops). 44
- Streptomycin, or dihydrostreptomycin, kaolin, pectin, and aluminum hydroxide gel powder (veterinary). 44
- Streptomycin or dihydrostreptomycin for inhalation therapy. 45
- Streptomycin-dihydrostreptomycin sulfate. 45
- Solution of streptomycin-dihydrostreptomycin sulfate. 45
- Streptomycylidene isonicotinyl hydrazine sulfate. 45

Streptomycin-dihydrostreptomycin sulfate and isonicotinic acid hydrazide for aqueous injection.	45
-------------------------------------------------------------------------------------------------	----

Section D. Determination of streptomycin or dihydrostreptomycin in body fluids and other substances. 46

Blood serum.	46
Urine.	46
Tissue.	46
Milk.	46

4 THE TETRACYCLINES (Chlortetracycline, Oxytetracycline, and Tetracycline) 48

Section A. Microbiologic assay methods for the tetracyclines. 48

METHOD 1. Turbidimetric assay using <i>Micrococcus pyogenes</i> var. <i>aureus</i> as the test organism.	48
METHOD 2. Cylinder-plate method using <i>Bacillus cereus</i> var. <i>mycoides</i> as the test organism.	50

Section B. Chemical methods for chlortetracycline, oxytetracycline, and tetracycline. 52

METHOD 1. Ferric chloride colorimetric method for chlortetracycline, oxytetracycline, and tetracycline.	53
METHOD 2. Acid colorimetric method for chlortetracycline or tetracycline.	54
METHOD 3. Ultraviolet spectrophotometric method for oxytetracycline or tetracycline.	55
METHOD 4. Fluorometric method for chlortetracycline.	56

Section C. Assay of the tetracyclines and their pharmaceutical dosage forms. 56

Chlortetracycline, chlortetracycline hydrochloride, oxytetracycline, oxytetracycline hydrochloride,

tetracycline, and tetracycline hydrochloride.	56
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride for intravenous injection.	58
Oxytetracycline hydrochloride or tetracycline hydrochloride for intramuscular injection.	59
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride ointment.	59
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride capsules.	59
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride tablets.	59
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride troches.	60
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride powder (for oral use).	60
Oxytetracycline or tetracycline powder.	60
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride ophthalmic (eye drops).	60
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride otic (ear drops).	60
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride dental cones.	61
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride dental paste.	61
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride surgical powder.	61
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride suppositories.	61
Chlortetracycline hydrochloride gauze packing.	61
Oxytetracycline hydrochloride gauze pads.	61
Chlortetracycline hydrochloride dressing.	62
Chlortetracycline hydrochloride, oxytetracycline hydrochloride, or tetracycline hydrochloride with vasoconstrictor (nose drops).	62
Calcium chlortetracycline oral drops.	62
Calcium chlortetracycline syrup.	62
Calcium chlortetracycline oral suspension with sulfonamides.	62
Calcium chlortetracycline cream.	62
Oxytetracycline hydrochloride in oil.	63
Tetracycline oral suspension.	63

Section D. Determination of the tetracyclines in body fluids and other substances.	63
Blood serum.	63
Urine.	64
Tissue.	64
Milk.	64
Animal feed supplements and mixed feeds.	64
 5 CHLORAMPHENICOL	 66
Section A. Microbiologic assay methods for chloramphenicol.	66
METHOD 1. Cylinder-plate method using <i>Sarcina lutea</i> as the test organism.	66
METHOD 2. Modification of method 1 for the assay of chloramphenicol in blood serum and other substances.	68
Section B. Chemical methods for chloramphenicol.	69
METHOD 1. Ultraviolet spectrophotometric method.	69
METHOD 2. Colorimetric method.	70
METHOD 3. Modified method 2 for body fluids, tissue, and other substances.	71
Section C. Assay of chloramphenicol and its pharmaceutical dosage forms.	71
Chloramphenicol.	71
Chloramphenicol palmitate.	72
Chloramphenicol capsules.	72
Chloramphenicol ointment.	72
Chloramphenicol ophthalmic.	73
Chloramphenicol otic (ear drops).	73
Chloramphenicol solution.	73
Chloramphenicol for aqueous injection (suspension).	73
Chloramphenicol palmitate oral suspension.	73
Section D. Determination of chloramphenicol in body fluids and other substances.	74
Blood serum.	74

Urine.	74
Tissues and other substances.	75

6 BACITRACIN 76

Section A. Microbiologic assay methods for bacitracin. 76

METHOD 1. Cylinder-plate method using <i>Micrococcus flavus</i> as the test organism.	76
---------------------------------------------------------------------------------------	----

METHOD 2. Modified cylinder-plate method for the determination of bacitracin in body fluids, tissues, and other substances.	78
-----------------------------------------------------------------------------------------------------------------------------	----

Section B. Assay of bacitracin and its pharmaceutical dosage forms. 78

Bacitracin.	78
Bacitracin methylene disalicylate.	78
Zinc bacitracin.	79
Bacitracin ointment.	79
Bacitracin tablets.	79
Bacitracin troches.	79
Bacitracin with vasoconstrictor.	79
Bacitracin ophthalmic.	80

Section C. Determination of bacitracin in body fluids and other substances. 80

Blood serum.	80
Urine.	80
Tissue.	80
Milk.	80
Animal feed supplements and mixed feed.	80

7 TYROTHRINIC 82

Section A. Microbiologic assay method for tyrothricin. 82

METHOD. Turbidimetric assay method using <i>Streptococcus faecalis</i> (Group D) strain M 19 as the test organism.	82
--------------------------------------------------------------------------------------------------------------------	----

Section B. Assay of tyrothricin and its pharmaceutical dosage forms.

Tyrothricin powder.	83
Alcohol or propylene glycol solutions of tyrothricin.	84
Tyrothricin troches.	84
Tyrothricin ointments or creams.	84
Tyrothricin in mineral oil emulsions.	85
Tyrothricin nose drops.	85
Tyrothricin otic (ear drops).	85
Tyrothricin mouth wash.	85
Tyrothricin adhesive gauze pads.	85

8 POLYMYXIN

Section A. Microbiologic assay methods for polymyxin.

METHOD 1. Cylinder-plate assay using <i>Brucella bronchiseptica</i> as the test organism.	86
METHOD 2. Modification of method 1.	88
METHOD 3. Modification of method 2 for the assay of polymyxin in blood serum and other substances.	88

Section B. Assay of polymyxin and its pharmaceutical dosage forms.

Polymyxin sulfate.	88
Polymyxin sulfate ointment.	89
Polymyxin sulfate tablets soluble.	89
Polymyxin sulfate otic solution.	89

Section C. Determination of polymyxin in body fluids and other substances.

Blood serum.	90
Urine.	90
Tissue.	90
Milk.	90
Animal feed supplements and mixed feeds.	90

9 NEOMYCIN

91

Section A. Microbiologic assay methods for neomycin. 91

METHOD 1. Cylinder-plate assay using *Micrococcus pyogenes* var. *aureus* as the test organism. 91

METHOD 2. Modification of method 1 for the assay of neomycin in blood serum and other substances. 93

Section B. Assay of neomycin and its pharmaceutical dosage forms. 93

Neomycin sulfate. 93

Neomycin sulfate ointment. 94

Neomycin sulfate tablets. 94

Neomycin sulfate ophthalmic. 95

Neomycin sulfate nasal spray. 95

Section C. Determination of neomycin in body fluids and other substances. 95

Blood serum. 95

Urine. 95

Tissue. 95

10 ERYTHROMYCIN

96

Section A. Microbiologic assay methods for erythromycin. 96

METHOD 1. Cylinder-plate assay using *Sarcina lutea* as the test organism. 96

METHOD 2. Modification of method 1 for the assay of erythromycin in blood serum and other substances. 98

Section B. Physicochemical method for erythromycin. 98

Spectrophotometric method. 99

Section C. Assay of erythromycin and its pharmaceutical dosage forms. 100

Erythromycin base. 100

Erythromycin dihydrate. 100

Erythromycin stearate. 100