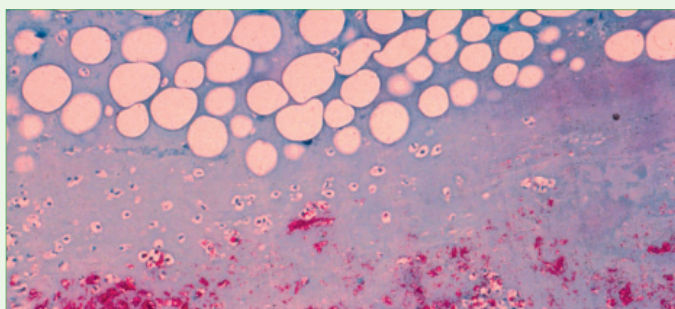


LABORATORY DIAGNOSIS OF BURULI ULCER

A MANUAL
FOR HEALTH CARE
PROVIDERS



Edited by : Françoise Portaels



World Health
Organization

LABORATORY DIAGNOSIS OF BURULI ULCER

A MANUAL FOR HEALTH-CARE PROVIDERS

Edited by: Françoise Portaels



**World Health
Organization**

WHO Library Cataloguing-in-Publication Data

Laboratory diagnosis of buruli ulcer: a manual for health care providers / edited by Françoise Portaels.

1. Buruli ulcer – diagnosis. 2. Buruli ulcer – prevention and control. 3. *Mycobacterium ulcerans*. I. Portaels, Françoise. II. World Health Organization.

ISBN 978 92 4 150570 3

(NLM classification: WC 302)

© World Health Organization 2014

All rights reserved. Publications of the World Health Organization are available on the WHO web site (www.who.int) or can be purchased from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int).

Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press through the WHO web site (http://www.who.int/about/licensing/copyright_form/en/index.html).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

Printed in Italy

WHO/HTM/NTD/IDM/2014.1

CONTENTS

ACKNOWLEDGEMENTS	iv
ILLUSTRATIONS AND TABLES	iv
ABBREVIATIONS	vi
1. INTRODUCTION AND IMPORTANCE OF LABORATORY CONFIRMATION OF BURULI ULCER	1
2. COLLECTING CLINICAL SPECIMENS	2
2.1 TYPES OF CLINICAL SPECIMENS	2
2.1.1 FINE-NEEDLE ASPIRATION	2
2.1.2 SWABS	2
2.1.3 BIOPSIES (PUNCH OR SURGICAL)	3
2.2 COLLECTING SPECIMENS IN THE FIELD	3
2.3 COLLECTING SPECIMENS AT TREATMENT CENTRES	3
3. STORING AND TRANSPORTING CLINICAL SPECIMENS	4
3.1 FOR BACTERIOLOGICAL ANALYSIS	4
3.2 FOR POLYMERASE CHAIN REACTION	5
3.3 FOR HISTOPATHOLOGICAL ANALYSIS	5
4. METHODS OF LABORATORY CONFIRMATION AND THEIR LIMITATIONS	6
4.1 LABORATORY TESTS FOR DIAGNOSIS OF BURULI ULCER	6
4.2 SPECIMEN PREPARATION	7
4.3 DIRECT SMEAR EXAMINATION	7
4.4 IN VITRO CULTURE	8
4.4.1 DECONTAMINATION BEFORE CULTURE	8
4.4.2 CULTURE MEDIA	8
4.4.3 CULTURE CONDITIONS AND INCUBATION TIMES	8
4.4.4 SPECIFIC IDENTIFICATION OF <i>MYCOBACTERIUM ULCERANS</i>	8
4.5 PCR FOR IS2404	9
4.5.1 DNA EXTRACTION PROCEDURES	9
4.5.2 IDENTIFICATION OF <i>MYCOBACTERIUM ULCERANS</i> BY PCR	10
4.5.2.1 GEL-BASED PCR	10
4.5.2.2 REAL-TIME PCR	10
4.6 SPECIFIC IDENTIFICATION OF OTHER MYCOBACTERIA POSITIVE FOR IS2404	11
4.7 HISTOPATHOLOGICAL METHODS	11
4.7.1 SELECTION OF SITE FOR BIOPSY SPECIMEN	11
4.7.1.1 NONULCERATIVE LESIONS	12
4.7.1.2 ULCERATIVE LESIONS	12
4.7.2 FIXATION OF TISSUE	12

4.7.3 PREPARING HISTOPATHOLOGICAL SECTIONS	12
4.7.4 GROSS CHANGES	12
4.7.5 HISTOPATHOLOGICAL CHANGES BEFORE ANTIBIOTIC TREATMENT	13
4.7.5.1 SKIN CHANGES	13
4.7.5.2 LYMPH NODES	18
4.7.5.3 BONE CHANGES	19
4.7.5.4 PATIENTS WITH EXTENSIVE DISEASE	21
4.7.6 IMMUNOCHEMISTRY AND ANALYSIS OF CHEMOTHERAPY- ASSOCIATED LOCAL IMMUNE RESPONSES	21
4.7.7 PARADOXICAL REACTIONS	25
5. QUALITY ASSURANCE	26
6. ROLES OF NATIONAL PROGRAMMES, HEALTH FACILITIES AND LABORATORIES	27
6.1 NATIONAL BURULI ULCER CONTROL PROGRAMMES	27
6.2 HEALTH FACILITIES	27
6.3 LABORATORIES	27
6.3.1 PERIPHERAL LABORATORIES	27
6.3.2 NATIONAL REFERENCE LABORATORY	27
6.3.3 INTERNATIONAL (SUPRANATIONAL) REFERENCE LABORATORY	28
6.3.4 GLOBAL NETWORK OF LABORATORIES FOR CONFIRMING <i>MYCOBACTERIUM ULCERANS</i> DISEASE (BURULI ULCER)	28
7. PROPOSED ALGORITHM FOR DIAGNOSING BURULI ULCER	30
REFERENCES	31
ANNEXES	35
ANNEX 1. COLLECTING CLINICAL SPECIMENS	37
ANNEX 2. TRANSPORT MEDIA FOR BACTERIOLOGICAL ANALYSES	41
ANNEX 3. PREPARING SPECIMENS FOR BACTERIOLOGICAL ANALYSES	43
ANNEX 4. DIRECT SMEAR EXAMINATION	44
ANNEX 5. IN VITRO CULTURE OF <i>MYCOBACTERIUM ULCERANS</i>	51
ANNEX 6. POLYMERASE CHAIN REACTION PROTOCOLS	62
ANNEX 7. HISTOPATHOLOGICAL STAINING TECHNIQUES	76
ANNEX 8. QUALITY ASSURANCE	94
ANNEX 9. RELEVANT FORMS (BU 01 AND BU 03)	98
ANNEX 10. GUIDANCE ON SAMPLING TECHNIQUES FOR LABORATORY CONFIRMATION OF <i>MYCOBACTERIUM</i> <i>ULCERANS</i> DISEASE (BURULI ULCER)	100

ACKNOWLEDGEMENTS

Written by:

Professor Françoise Portaels, Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Dr Miriam Eddyani, Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Ms Caroline Lavender, Mycobacterium Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Victoria, Australia

Dr Richard Phillips, Department of Medicine, Komfo Anokye Teaching Hospital, Kumasi, Ghana

Dr Gisela Bretzel, Department of Infectious Diseases & Tropical Medicine, University Hospital, Ludwig-Maximilians University, Munich, Germany

Dr Marcus Beissner, Department of Infectious Diseases & Tropical Medicine, University Hospital, Ludwig-Maximilians University, Munich, Germany

Dr Dissou Affolabi, Mycobacteria Reference Laboratory, Cotonou, Benin

With contributions from:

Dr Kingsley Asiedu, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Dr Luc Brun, Department of Pathology, University of Parakou, Parakou, Benin

Professor Bouke de Jong, Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Dr Sara Eyangoh, Mycobacteria Laboratory, Pasteur Center of Cameroon, Yaoundé, Cameroon

Dr Janet Fyfe, Mycobacterium Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Victoria, Australia

Dr Solange Kakou-Ngazoa, Microbiology, Pasteur Institute of Côte d'Ivoire, Abidjan, Côte d'Ivoire

Professor Anatole Kibadi Kapay, Plastic Surgery Unit, University Hospital of Kinshasa, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Dr Wayne M. Meyers, Armed Forces Institute of Pathology, Washington D.C., United States of America

Dr Kazue Nakanaga, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Associate Professor Daniel O'Brien, Department of Infectious Diseases, The Geelong Hospital, Barwon Health, Geelong, Australia

Professor Gerd Pluschke, Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland

Dr Jean-Jacques Roux, Anatomy and Pathological Cytology unit, Hospital of Chambéry, Chambéry, France

Dr Marie-Therese Ruf, Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland

Dr Armand Van Deun, Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Mr Koen Vandelannoote, Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Professor Dorothy Yeboah-Manu, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana, and

The WHO Buruli Ulcer Laboratory Network Working Group.

This document was produced with the support of Anesvad, Spain (<http://www.anesvad.org>).

预览已结束，完整报告链接和二维码如下：

https://www.yunbaogao.cn/report/index/report?reportId=5_27910

