

2018

# **The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex: technical guide**



**World Health  
Organization**

**FIND**

Because diagnosis matters

WHO collaborating centre for the evaluation  
of new diagnostic technologies



# **The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex: technical guide**

WHO/CDS/TB/2018.19

© World Health Organization 2018

Some rights reserved. This work is available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo>).

Under the terms of this licence, you may copy, redistribute and adapt the work for non-commercial purposes, provided the work is appropriately cited, as indicated below. In any use of this work, there should be no suggestion that WHO endorses any specific organisation, products or services. The use of the WHO logo is not permitted. If you adapt the work, then you must license your work under the same or equivalent Creative Commons licence. If you create a translation of this work, you should add the following disclaimer along with the suggested citation: “This translation was not created by the World Health Organization (WHO). WHO is not responsible for the content or accuracy of this translation. The original English edition shall be the binding and authentic edition”.

Any mediation relating to disputes arising under the licence shall be conducted in accordance with the mediation rules of the World Intellectual Property Organization.

**Suggested citation.** The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex: technical guide. Geneva: World Health Organization; 2018 (WHO/CDS/TB/2018.19). Licence: CC BY-NC-SA 3.0 IGO.

**Cataloguing-in-Publication (CIP) data.** CIP data are available at <http://apps.who.int/iris>.

**Sales, rights and licensing.** To purchase WHO publications, see <http://apps.who.int/bookorders>. To submit requests for commercial use and queries on rights and licensing, see <http://www.who.int/about/licensing>.

**Third-party materials.** If you wish to reuse material from this work that is attributed to a third party, such as tables, figures or images, it is your responsibility to determine whether permission is needed for that reuse and to obtain permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

**General disclaimers.** 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 WHO 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 and dashed 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 WHO 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 WHO 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 WHO be liable for damages arising from its use.

Printed in Spain

## Contents

<b>Acknowledgements.....</b>	<b>v</b>
<b>Abbreviations.....</b>	<b>vi</b>
<b>Glossary of terms .....</b>	<b>viii</b>
<b>Executive summary .....</b>	<b>x</b>
<b>Background.....</b>	<b>xi</b>
Scope of the document.....	xii
References .....	xii
<b>1. Review of the current methods available for the sequencing of <i>Mycobacterium tuberculosis</i> complex.....</b>	<b>1</b>
1.0 Introduction.....	1
1.1 Whole genome sequencing versus targeted next-generation sequencing .....	1
1.2 The next-generation sequencing workflow.....	2
1.2.1 DNA extraction and quality control.....	3
1.2.2 DNA library preparation.....	4
1.2.3 Next-generation sequencing: Platforms and considerations .....	5
1.2.4 Next-generation sequencing data analysis .....	10
1.3 References.....	11
<b>2. Principles of using graded resistance-conferring mutations to detect resistance to anti-tuberculosis compounds.....</b>	<b>14</b>
2.0 Introduction.....	14
2.1 Methods.....	14
2.1.1 Systematic review of mutations.....	14
2.1.2 Systematic review of MIC data.....	15
2.1.3 Summary data presentation .....	17
2.2 Results.....	17
2.2.1 Isoniazid .....	17
2.2.2 Rifampicin.....	22
2.2.3 Fluoroquinolones .....	31
2.2.4 Pyrazinamide .....	35
2.2.5 Second-line injectable drugs.....	49
2.3 Conclusions .....	57
2.4 References .....	58

**3. Accuracy of sequencing in a multi-country, population-based study for determining drug resistance in *Mycobacterium tuberculosis* complex ..... 64**

3.0 Introduction ..... 64

3.1 Methods..... 64

3.1.1 Study design and participants..... 64

3.1.2 Phenotypic testing ..... 64

3.1.3 Sequencing methods..... 65

3.1.4 Statistical analysis..... 66

3.2 Results ..... 67

3.2.1 Quality of sequencing results ..... 67

3.2.2 Sequencing results ..... 67

3.3 Conclusions ..... 70

3.4 References ..... 71

**4. Implementation considerations for drug-resistant tuberculosis sequencing in low- and middle-income countries..... 74**

4.0 Introduction ..... 74

4.1 Setting up an NGS workflow ..... 74

4.2 Additional equipment requirements..... 75

4.3 Support and training requirements..... 77

4.4 Bioinformatics and data interpretation ..... 79

4.4.1 Results reporting..... 82

4.5 Quality assurance and control ..... 84

4.6 Data and Information technology requirements..... 85

4.6.1 Data storage..... 85

4.6.2 Data transfer/ upload..... 87

4.7 Ethical considerations for the use of information obtained from human samples..... 87

4.8 References ..... 88

**Appendix 1. Example of an End-to-End Sequencing Workflow for TB DST: The Genoscreen Deeplex®-MycTB Targeted NGS workflow for the Illumina MiSeq ..... 90**

**Appendix 2. Example of an End-to-End Sequencing Workflow for TB DST: The Oxford Nanopore Sequencing Technologies WGS workflow for MinION..... 92**

**Appendix 3. Target Product Profile - Detection of resistance associated mutations in *Mycobacterium tuberculosis* complex utilizing Next Generation Sequencing..... 94**

## Acknowledgements

The development of this document was led by FIND with contributions from Sophia Georghiou, Timothy Rodwell, Rebecca Colman, Paolo Miotto, Arnold Bainomugisa, Andrea Cabibbe, Anita Suresh and Claudia Denking. Technical input and review was provided by Christopher Gilpin, Alexei Korobitsyn, Anna Dean, Karin Weyer and Matteo Zignol (WHO Global TB Programme).

### External Review Group

Heidi Albert (FIND South Africa, Cape Town, South Africa); Heather Alexander (Division of Global HIV and Tuberculosis, US Centers for Disease Control and Prevention, Atlanta, USA); Martina Casenghi (Elizabeth Glaser Pediatric AIDS Foundation, Geneva, Switzerland); Chris Coulter (Queensland Department of Health, Brisbane, Australia); Daniela Cirillo (San Raffaele Scientific Institute, Milan, Italy); Masoud Dara (WHO Regional Office For Europe, Copenhagen, Denmark); Soudeh Ehsani (WHO Regional Office For Europe, Copenhagen, Denmark); Lucilaine Ferrazoli (TB and Mycobacteriology Laboratory Adolfo Lutz Institute, Sao Paulo, Brazil); Bernard Fourie (University of Pretoria, South Africa); Levan Gagnidze (International Organization for Migration, Bangkok, Thailand); Petra de Haas (KNCV Tuberculosis Foundation, The Hague, The Netherlands); Zahra Hasan (The Aga Khan University, Karachi, Pakistan); Rumina Hasan (The Aga Khan University, Karachi, Pakistan); Sven Hoffner (Karolinska Institute, Stockholm, Sweden); Nguyen Van Hung (National TB Reference Laboratory, National Lung Hospital, Hanoi, Viet Nam); Nazir Ismail (National Institute of Communicable Diseases, Johannesburg, South Africa); Connor Meehan (Institute of Tropical Medicine, Antwerp, Belgium); Igor Mokrousov (St Petersburg Pasteur Institute, St. Petersburg, Russian Federation); Moses Joloba (National Reference Laboratory of the National TB and Leprosy Programme, Kampala, Uganda); Lindiwe Mvusi (National Department of Health, South Africa); Alaine Umubyeyi Nyaruhirira (Management Science for Health, Gauteng, South Africa); Daniel Orozco (Partners In Health, Boston, USA); Jamie Posey (Mycobacteriology Laboratory Branch, Centers for Disease Control and Prevention, Atlanta USA); Leen Rigouts (Institute of Tropical Medicine, Antwerp, Belgium); Camilla Rodrigues (P.D. Hinduja Hospital & Medical Research Centre, India); Marco Schito (Critical Path Institute, Tucson, USA); Kaiser Shen (US Agency for International Development (USAID), Washington, USA); Thomas Shinnick (Independent consultant, USA); Alena Skrahina (Republican Scientific and Practical Center for Pulmonology and Tuberculosis, Minsk, Belarus); Khairunisa Suleiman (Médecins Sans Frontières Access Campaign, Geneva, Switzerland); Nairobi, Kenya); Elisa Tagliani (San Raffaele Scientific Institute, Milan, Italy); Sabira Tahseen (National TB Reference Laboratory, National Institute of Health, Islamabad, Pakistan); Maria Alice Telles (Tuberculosis Laboratory Independent Consultant for PAHO, Sao Paulo, Brazil); Francis Varaine (Médecins Sans Frontières, France); William Wells (US Agency for International Development (USAID), Washington, USA).

### Target Product Profile (TPP): Detection of resistance associated mutations in *Mycobacterium tuberculosis* complex utilizing Next Generation Sequencing

The TPP was drafted by FIND (Rebecca Colman, Timothy Rodwell, David Dolinger and Claudia Denking) with comments incorporated from CPTR assay development working group, CPTR surveillance working group, New Diagnostic Working Group, and a web-based survey sent out to GLI listserve and GHDonline. The final consensus TPP is presented in Appendix 3.

### Acknowledgement of financial support

FIND provided funding for performing the systematic review and preparing the draft guide. Funding from the United States Agency for International Development through USAID-WHO Consolidated Grant No. GHA-G-00-09-00003 / US2014-741 is also gratefully acknowledged.

Abbreviations

AMK	amikacin
BDQ	bedaquiline
CAP	capreomycin
CB	clinical breakpoint
CC	critical concentration
CFZ	clofazimine
CPTR	Critical Path to TB Drug Regimens
DCS	D-cycloserine
DLM	delamanid
DR-TB	drug-resistant tuberculosis
DST	drug susceptibility testing
FQs	fluoroquinolones
GFX	gatifloxacin
INH	isoniazid
IT	information technology
KAN	kanamycin
LFX	levofloxacin
LJ	Löwenstein-Jensen
LR	Likelihood ratio
LZD	linezolid
MDR-TB	multidrug-resistant tuberculosis
MXF	moxifloxacin

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\\_25541](https://www.yunbaogao.cn/report/index/report?reportId=5_25541)

