Report of a WHO–FIND meeting on diagnostics for Buruli ulcer

Geneva, 26–27 March 2018

World Health Organization

FIND
Because diagnosis matters

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Contents

Abbreviations

BU	Buruli ulcer
ELISA	enzyme-linked immunosorbent assay
EQA	external quality assurance
FIND	Foundation for Innovative New Diagnostics
fTLC	fluorescent thin-layer chromatography
LAMP	loop-mediated isothermal amplification
mAbs	monoclonal antibodies
PCR	polymerase chain reaction
RDT	rapid diagnostic test
RPA	recombinase polymerase amplification
TPP	target product profile
WHO	World Health Organization

1. Background

The target of the World Health Organization (WHO) roadmap on neglected tropical diseases[1] for Buruli ulcer (BU) is that by 2020, 70% of all cases are detected at an early stage and cured with antibiotics in all countries where the disease is endemic. The Foundation for Innovative New Diagnostics (FIND) is collaborating with WHO to achieve this target for control of the disease. FIND's main focus is on promoting and supporting the development of new diagnostic tools to improve early detection of BU. The current FIND strategy on BU diagnostics was developed after a meeting of experts convened by WHO and FIND in 2013. Since then, FIND has been working with partners in academia and industry to develop a rapid test for screening and diagnosis at the community level, and to develop a molecular test for confirmatory diagnosis at the microscopy laboratory or district hospital level; and is supporting WHO in the evaluation and implementation of fluorescent thin-layer chromatography (fTLC) to detect mycolactone in lesions from BU suspected cases.

During a meeting of the WHO Technical Advisory Group (TAG) on Buruli ulcer (Geneva, 21 March 2017), a number of problems with laboratory confirmation of BU were identified: (i) low rate of polymerase chain reaction (PCR) confirmation in a number of endemic countries; (ii) long delays in getting results from laboratories; (iii) low participation in external quality assurance (EQA) programme by national reference laboratories; and (iv) lack of funding for sustaining the EQA programme. The TAG noted with satisfaction the progress made to develop diagnostic tests for BU by many research groups; however, considerable time is still needed to optimize methods and to progress them to field testing.

To accelerate progress, WHO and FIND convened a second global meeting at WHO headquarters in Geneva, Switzerland with the aim of establishing an action plan to develop new diagnostic solutions for BU and to create a framework of collaboration to address unmet needs in BU diagnostics.

[1] Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation. Geneva: World Health Organization; 2012 (http://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf).

2. Meeting summary

The meeting was held from 26 to 27 March 2018 to review and discuss the following topics:

- Advances and challenges in the use of fTLC, and new approaches to detecting mycolactone using monoclonal antibodies (mAbs).
- The status of development of rapid diagnostic tests (RDTs) targeting the MUL_3720 protein.
- The role of PCR as a reference test, and hurdles in providing a confirmatory diagnosis and in establishing a quality assurance programme.
- New molecular tools with potential for implementation at a level lower than in the national or regional reference laboratory, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA).
- The need to harmonize and standardize methods for collection and preparation of specimens, so samples can be referred for diagnosis and stored for evaluation of new diagnostic tests in optimal conditions.
- Barriers to accessing early diagnosis and treatment, including coordination at the programme level, and lack of adequate diagnostic tools.
- Defining target product profiles (TPPs) to guide the development of new diagnostic tools that can be applied at different levels of the health system. Participants agreed that two TPPs would be developed to address the current gaps: (i) a rapid test for BU diagnosis at the primary health-care level; and (ii) a test for diagnosis of BU that can also assist in treatment monitoring and differential diagnosis at the district hospital or reference centre.

3. Key discussion points

3.1. Targeting mycolactone for the diagnosis of Buruli ulcer

- Preliminary results show that mycolactone or its metabolites may be present in the urine of mice infected with *Mycobacterium ulcerans* and in cases of BU, but further research is needed. Levels of mycolactone in ulcerative lesions decrease with treatment, highlighting its potential as a test of cure. Studies by Johns Hopkins University on liquid chromatography-mass spectrometry using experimental infections in mice and guinea-pigs show that the concentration of mycolactone is highest in the centre of the lesion, which may have implications for collection of samples for mycolactone detection tests.

- The stability of mycolactone for testing requires collection of samples in absolute ethanol and protection from light; the use of plastic tubes is not advised as mycolactone adheres to this material. The use of siliconized or glass tubes is encouraged.
- Data from the *mycostudy*, presented by the University of Ghana, show variable sensitivity (25–80%) and specificity (35–75%) of fTLC across sites. The method is standardized and appears straightforward, but the interpretation of results can be challenging, especially when swab samples are analysed. The accuracy of the PCR methods from the different national laboratories (with different protocols) used as a reference test in this study might not be ideal and could compromise the results of the evaluation.
- Different mAbs against mycolactone or mycolactone analogues have been developed using a library of either recombinant mAbs and selection with phage and yeast display (Specifica) or mouse hybridoma cells (Swiss Tropical and Public Health Institute). Assemblies of clones producing scFv and full antibodies have been generated by Specifica and the Swiss Tropical and Public Health Institute respectively. These present high affinity, in the range of the mycolactone concentration found in lesions from infected mice and BU cases (1–1000 nM). Preliminary testing has been conducted using competition enzyme-linked immunosorbent assays (ELISAs). With the availability of more synthetic mycolactone, it was proposed that open Fv ELISA and open sandwich assays could be developed, in which antibodies recognizing antibody-mycolactone complexes can be used. Both groups would join forces to work on the development of an RDT using mAbs to detect mycolactone in clinical samples. mAbs for use in the development of a prototype RDT may be ready in less than one year. Studies to assess the stability of mycolactone in stored samples will be needed.
- Access to synthetic mycolactone is an important aspect in the development of mycolactone detecting tests in order to conduct feasibility studies and as a control in the fTLC test. Professor Kishi (Harvard University) has produced large quantities of synthetic mycolactone; some are stored in his laboratory and some at WHO.

3.2. Protein (MUL_3720) capture assay in the diagnosis of Buruli ulcer

- The Swiss Tropical and Public Health Institute has developed 19 mAbs against the *M. ulcerans* surface protein MUL_3720. With PCR as reference, a pair of mAbs used in a capture ELISA shows very high specificity, but moderate sensitivity (*c*60%).

- Abbott/Standard Diagnostics has produced two prototype RDTs based on selected anti-MUL_3720 mAbs and an avidin-biotin system, which have a sensitivity in the range of 3–6 ng/mL when *M. ulcerans* protein lysates are tested. Prototypes are being produced to test clinical samples.

3.3. Molecular diagnosis of Buruli ulcer

- Evaluation of the performance of PCR/quantitative PCR (qPCR) in multiple centres by an EQA programme led by the Institute of Tropical Medicine, Antwerp has shown improvement by the participating laboratories (during 2009–2014), but some limitations remain: approximately 20% of the laboratories reported false–positive results and 30% were unable to detect weak positive samples; and the participation rate is decreasing. Participation in EQA may depend on the availability of funds for PCR/qPCR reagents, which may also affect confirmation of referred samples. Around 50% of the laboratories use home-brewed DNA extraction methods and the PCR/qPCR methodology used varies among laboratories; thus a quality assurance programme targeting harmonization rather than standardization is preferred.
- A recent study conducted by the Institute of Tropical Medicine, Antwerp and partners in Benin revealed that although clinical diagnosis has higher sensitivity than laboratory tests, it may miss BU cases, especially in the early stages (nodular forms). With declining BU incidence, the accuracy of clinical diagnosis will also decrease. Awareness of BU must therefore be sustained while rapid and cost–effective diagnostic tests are developed, as PCR should be reserved for microscopy-negative BU suspects. However, microscopy for BU is not done in many hospitals.
- Studies on the evaluation of simpler approaches to the molecular diagnosis of BU using stored clinical samples were presented, showing promising results. An evaluation of RPA using stored samples from Ghana, presented by the Kumasi Centre for Collaborative Research in Tropical Medicine, showed 86% sensitivity