# TPP for a Rapid Test for Diagnosis of Buruli Ulcer at the Primary Health-Care Level

## Buruli ulcer

Buruli ulcer (BU) is a chronic debilitating skin disease caused by *Mycobacterium ulcerans*. It has been reported in 33 countries in Africa, the Americas, Asia and the Western Pacific. Most cases of BU occur in tropical and subtropical regions except in Australia, China and Japan. Out of the 33 countries, 14 regularly report data to the World Health Organization (WHO). The highest burden is in sub-Saharan Africa, where the majority of people affected are children below 15 years of age. The annual number of suspected BU cases reported globally was around 5000 cases up until 2010 when it started to decrease until 2016, reaching its minimum with 1961 cases reported. Since then, the number of cases has started to rise again every year, up to 2713 cases in 2018. The reasons for the decline and subsequent increase are not clear. *Mycobacterium ulcerans* is an environmental bacterium and produces a unique toxin – mycolactone, which is responsible for the pathogenesis of disease. BU often starts as a painless swelling (nodule), a large painless area of induration (plaque) or a diffuse painless swelling of the legs, arms or face (oedema). The disease may progress with no pain or fever. Without treatment, or sometimes during antibiotic treatment, the nodule, plaque or oedema will ulcerate within 4 weeks. Bone is occasionally affected, causing deformities. Although mortality from BU is low, the main problem is long-term disability in an estimated 25% of those affected. The mode of transmission to humans remains unknown. Therefore, the objective of BU control is to minimize the suffering, disabilities and socioeconomic burden. Early detection and antibiotic treatment are the cornerstones of the control strategy [1].

## Public Health Response

In 2004, the Fifty-seventh World Health Assembly adopted resolution WHA57.1 on surveillance and control of Buruli ulcer, urging the Member States in which the disease is or threatens to become endemic to support enhanced surveillance of the disease and accelerate the development of tools for diagnosis, treatment and prevention. The Cotonou Declaration on BU, adopted by the Heads of States of affected countries in Benin in 2009, called on countries to ensure that cases are detected at an early stage to reduce the frequency of disabilities. Confirmation of cases is essential to ensure that patients treated with antibiotics for 8 weeks are true cases of BU, and WHO thus requires all endemic countries to ensure that at least 70% of cases reported are laboratory-confirmed [2].

## Available Diagnostic Tools

Some progress has been made on diagnostic tools. The current diagnostics tests for BU are microscopy, bacterial culture, histology and polymerase chain reaction (PCR) for insertion sequence (IS) 2404. Microscopy is the most available method in endemic countries but has challenges with sensitivity. Out of the four traditional methods used to diagnose BU, PCR is regarded as the gold standard [3-5]. Although this method is accurate, reference laboratories tend to be far from affected areas making it a challenge to obtain immediate results for patient management.

Another indirect gap in BU diagnostics is a lack of sustained capacity building for all peripheral health facility laboratories and health workers in endemic areas. Although often remote from locations where

BU is endemic, there is a need to provide continuous training for laboratory staff tasked with providing routine diagnostic services for clinics to which patients with BU present. This will help to cut down on turnaround time compared to transporting samples to reference laboratories, which are usually located in the cities [6]. In addition, training of health workers to enhance their awareness of BU case identification and management is a key need identified in the 2021-2030 road map for NTDs. For instance, this could be a part of a health ministry training module. Country ownership through domestic funding of these interventions needs to be encouraged to ensure that it becomes part of the normal roles in peripheral health facilities [4]. Operational and implementation research is required to address programmatic bottlenecks in local health systems. New diagnostic tools can be fully tested in the peripheral health facilities to help provide tailor-made innovative approaches to synergizing regular operations of district health facilities with NTD diagnostics provision [7].

**The WHO Diagnostic Technical Advisory Group for Neglected Tropical Diseases**

The WHO Department of Control of Neglected Tropical Diseases (NTD) set up the Diagnostic Technical Advisory Group (DTAG) to be the principal advisory group to WHO on NTD diagnostics. This group works to ensure a unified method will be used to solve NTD diagnostic needs and to direct WHO strategies to develop efficient diagnostic tools. The first meeting of the group occurred in Geneva, Switzerland in 2019 [3]. The DTAG noted the following diagnostic needs for Buruli ulcer:

- Rapid point-of-care tests targeting mycolactone – for individual diagnosis at PHC/community level.
- Loop-mediated isothermal amplification (LAMP) and/or Recombinase Polymerase Amplification (RPA) – design locked tests could replace home-brewed PCR methods – for individual diagnosis.

**NTD Road Map 2021-2030**

Buruli ulcer is one of the diseases targeted for control in the 2021–2030 NTD road map. The main 2030 target for BU is to reduce the proportion of cases diagnosed in Category III from 30% (baseline) to less than 10%. To achieve this goal, decentralized testing, i.e. testing at the public health centre and/or community level, is key. Therefore, one of the critical actions for BU is to “Develop rapid diagnostic tools for use in public health and community centres to ensure early diagnosis, reduce morbidity and confirm cases”. A rapid test targeting the toxin mycolactone will address a second priority for Buruli ulcer highlighted in the roadmap: “Improve detection of viable *M. ulcerans* in wound samples to distinguish between treatment failure and paradoxical reaction with methods such as mycolactone detection and 16S rRNA”.

**Background and scope for the TPP**

In 2009, WHO's Second International Conference on Buruli Ulcer Control and Research, resolved to strengthen the capacity of national laboratories to confirm cases of the disease but advised that "efforts are still needed to develop simple diagnostic tools usable in the field as well as disability prevention methods" [2].

In 2013, the WHO together with the Foundation for Innovative New Diagnostics (FIND) convened a meeting of BU experts in Geneva [8]. In this meeting two priority unmet needs in BU diagnosis were identified:

- A diagnostic test for the early detection of BU in symptomatic patients with sufficient positive predictive value to put patients on appropriate treatment.
- A screening test at the primary or community level for symptomatic patients with ulcer.

In March 2018, WHO and FIND convened a global meeting 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 [9]. At this meeting, participants agreed to develop a target product profile (TPPs) to address the need for a rapid test for BU diagnosis at the primary health-care level.

**Audiences engaged and external consultations to develop the TPP**

Preliminary work before the WHO-FIND meeting in 2018 [9] included definition and discussion of Use Cases for a BU diagnostic test that could be used at the point-of-care, at community or public health centre level. A team of 21 participants from research organizations, NGOs, industry and product development partnerships, national programmes and the WHO were involved in the preparation and discussion of the TPP, and the draft was published at the WHO website [*list of participants and draft published available in reference* 9].

**References**


1. https://www.who.int/health-topics/buruli-ulcer

2. WHO. Cotonou Declaration on Buruli Ulcer. Geneva, Switzerland: World Health Organization. https://apps.who.int/iris/handle/10665/329410

3. WHO. Report of the first meeting of the WHO Diagnostic Technical Advisory Group for Neglected Tropical Diseases. Geneva, Switzerland: World Health Organization 2019 30-31 October 2019. https://www.who.int/neglected_diseases/resources/9789240003590/en/

4. WHO. Ending the Neglect to Attain the Sustainable Development Goals. A Road Map for Neglected Tropical Diseases 2021–2030. https://www.who.int/neglected_diseases/Ending-the-neglect-to-attain-the-SDGs--NTD-Roadmap.pdf

5. Sakyi SA, Aboagye SY, Darko Otchere I, Yeboah-Manu D. Clinical and Laboratory Diagnosis of Buruli Ulcer Disease: A Systematic Review. Can J Infect Dis Med Microbiol. 2016;2016:5310718. doi: https://10.1155/2016/5310718

6. WHO. Integrating Neglected Tropical Diseases in Global Health and Development Fourth WHO Report on Neglected Tropical Diseases. 2017. https://www.who.int/neglected_diseases/resources/9789241565448/en/

7. WHO. Report of a WHO–FIND meeting on diagnostics for Buruli ulcer Geneva, 26–27 March 2018. https://www.who.int/publications/i/item/WHO-CDS-NTD-IDM-2018.09-eng

8. WHO 2014. Report of a WHO-FIND consultative meeting on diagnostics for Buruli ulcer, Geneva, Switzerland, 21 November 2013. http://apps.who.int/iris/bitstream/10665/112669/1/WHO_HTM_NTD_IDM_2014.2_eng.pdf?ua=19.

9. WHO 2018. Report of a WHO–FIND meeting on diagnostics for Buruli ulcer. Geneva, Switzerland. https://apps.who.int/iris/handle/10665/274607

**TPP for a Rapid Test for Diagnosis of Buruli Ulcer at the Primary Health-Care Level**

| **1. Scope** | **Minimum** | **Ideal** | **Annotations** |
|---|---|---|---|
| 1.1 Goal of the test. Intended Use | Confirmation of Buruli ulcer | | Used in patients who self-present at health centre, or in active case finding activities. The test is done after clinical assessment. |
| 1.2 Target population | Suspected cases, ulcerated lesions (advanced stages) | Suspected cases early and late stages | |
| 1.3 Target operator of the test | Nurse, laboratory technician | Nurse, laboratory technician, Community Health worker | |
| 1.4 Lowest setting for implementation | Health centre | Community, as part of active case finding campaigns | |
| 1.5 Target analyte to be detected | Mycolactone, bacterial protein or DNA | | Antibody response is not a good marker of disease in BU-endemic areas. DNA and proteins are usually an integral part of bacteria, which may not distributed homogeneously in the lesion. However, DNA detection (by PCR) is the recommended test for confirmation. Mycolactone is secreted and could be detected throughout the lesion. |
| **2. Performance characteristics** | **Minimum** | **Ideal** | **Annotations** |
| 2.1 Clinical sensitivity (assessed in a latent class analysis) | Non-inferior than Ziehl-Neelsen microscopy | Non-inferior than Polymerase Chain Reaction | Usual diagnostic tests are AFB microscopy (low sensitivity and specificity) and PCR (at reference centre level). At community level diagnosis is based on clinical signs. |
| 2.2 Clinical specificity (assessed in a latent class analysis) | Non-inferior than Ziehl-Neelsen microscopy | Non-inferior than Polymerase Chain Reaction | |
| 2.3 strain specificity | African strains | Global | Extremely low level of genetic diversity in M. ulcerans would avoid the need to pay extra effort in identifying targets that are common across M. ulcerans isolates from different regions. |
| 2.4 Type of analysis (quantitation) | Qualitative | | |

| 3. Test procedure | Minimum | Ideal | Annotations |
|---|---|---|---|
| 3.1 Training needs. Time dedicated to training session for end users, including sample collection | 2 days | 1 day | Minimally invasive sampling procedures applied to skin lesion (nodule or ulcer). Fine Needle Aspirate, swab. This may require training in the case of Community Health Worker. |
| 3.2 Sample type | Lesion swab | Lesion swab, fine needle aspirate (FNA) | Early stage lesions are not ulcerated and a swab cannot be taken, an FNA is needed in these cases. |
| 3.3 Sample preparation. Total steps | 3-5 steps | Direct testing on sample | Sample might need to be eluted/added to specific buffer. |
| 3.4 Number of steps to be performed by operator | < 10; 1 timed steps | < 3; 1 timed steps | |
| 3.5 Need for operator to transfer a precise volume of sample | Acceptable with a disposable transfer device provided | No | Sample may need to be eluted in specific buffer (included in the kit) |
| 3.6 Time to result | Same day | <20 min | |
| 3.7 Internal control | Included | | Positive control to confirm validity of the test |
| 3.8 Reading system. Interpretation of results | Visual (naked eye) or simple reading device | Visual (naked eye) | See 3.9 |
| 3.9 Auxiliary equipment | Test reader (for lateral flow assay, dual path platform, or similar) | None, instrument free (required materials are included in the kit) | There are RDTs that generate a fluorescent signal that increases sensitivity, a reader is needed to detect this signal. In these cases a connectivity option could be desirable, enabling sending results to a reference lab, coordinator, reporting system, etc. thresholds |
| 3.10 Power Requirements | Battery operated | None required | If a reading device is needed it should be small, portable or hand-held instrument (<1 kg) that can operate on rechargeable battery or solar power lasting at least 4 h (8 h preferred) |
| 3.11 Need for maintenance/spare parts | None | | |
| **4. Operational characteristics** | **Minimum** | **Ideal** | **Annotations** |
| 4.1 Operating conditions | 5-40°C, 80% relative humidity | 5-50°C, 90% relative humidity | High environmental temperatures and high humidity are often a problem in countries where BU is endemic. |
| 4.2 Reagent kit transport | No cold chain required. Tolerance of transport stress for a minimum of 72 hours at -15°C to +50°C | No cold chain required. Tolerance of transport stress for a minimum of 1 week at -15°C to +50°C | Refrigerated transport is costly and often cannot be guaranteed during the entire transportation process. Frequent delays in transport are common |

| | | | |
|---|---|---|---|
| 4.3 Reagent kit storage / stability | No cold chain required. >12 months at 40°C, 70% relative humidity | No cold chain required. 24 months at 50°C, 90% relative humidity | Should be able to tolerate transport stress (48 h at 50ºC). To include test quality detector (for surpassed temperature or humidity) |
| 4.4 Reagents reconstitution. Need to prepare the reagents prior to utilization | Few simple steps | All reagents ready-to-use | Simple steps like resuspension of lyophilized reagent |
| 4.5 In use stability | >1h for a single use test after opening the pouch | >2h for a single use test after opening the pouch | |