Guidance on sampling techniques for laboratory-confirmation of *Mycobacterium ulcerans* infection (Buruli ulcer disease)

World Health Organization

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Background

Advances in the clinical management of *Mycobacterium ulcerans* infection (Buruli ulcer disease) have shifted options for treatment from surgical management to combination antibiotic therapy. Antibiotic treatment with rifampicin and streptomycin or rifampicin and other oral regimens has made possible decentralized treatment where previously hospital-based surgical treatment was the only option. As a result of these advances, the number of surgical interventions has reduced (today, about 40% of patients are treated without the need for surgery) and recurrences of the infection have fallen to almost zero.

Confirmation of cases using laboratory methods – polymerase chain reaction and direct smear examination – has become a central issue in the overall management of the disease. Although cultures are not essential for diagnosis of the infection and the immediate clinical management of patients, identifying cases of treatment failures and recurrences of infection may require the detection of viable bacilli. Cultures may also be necessary if drug-resistant strains of *M. ulcerans* emerge.

In many countries where Buruli ulcer is endemic, 70–100% of patients present with ulcerative lesions and 0–30% present with non-ulcerative lesions. Since 2007, excellent progress has been made in using the fine-needle aspiration technique to collect samples from clinically diagnosed cases with non-ulcerative lesions. Until then, punch biopsy was the preferred technique to the more invasive surgical biopsy for obtaining samples from non-ulcerative lesions. Punch biopsy is used in a few countries mainly for research purposes. Today, fine-needle aspiration is used in a number of countries to obtain specimens for laboratory confirmation of infection. Punch biopsy is a less preferred choice, although its use may be limited to the special circumstances indicated below. Although surgical treatment may be performed less often today, cases that are surgically treated at any point in time should be an opportunity to provide samples for laboratory analysis.

Methods used for diagnosis

Four methods are commonly used for laboratory confirmatory of *M. ulcerans* infection: direct smear examination, polymerase chain reaction, culture and histopathology. The pros and cons of these techniques are summarized below.

Method	Pros	Cons
Direct smear examination	• Easy to perform at local level • Does not require expensive materials and equipment • Rapid results • Uses swabs, fine-needle aspiration and biopsy samples	• Low sensitivity (<60%) Needs trained personnel • Needs external quality assurance
Polymerase chain reaction	• Results fairly rapid • Uses swabs, fine-needle aspiration and biopsy samples • High sensitivity (>95%)	• Requires a sophisticated laboratory • Expensive to perform • Needs trained personnel • Requires strict quality control
Culture of *M. ulcerans*	• Uses swabs, fine-needle aspiration and biopsy samples	• Requires a sophisticated laboratory • Needs trained personnel • Results take >8 weeks • Low sensitivity (20–60%) • Not useful for immediate patient management
Histopathology	• Sensitivity is about 90% • Results fairly rapid (if services are available) • Useful in establishing differential diagnosis and monitoring unexpected response to treatment	• Requires a sophisticated laboratory • Expensive to perform • Needs trained personnel • Requires invasive procedure (i.e. biopsy)

Sampling techniques

Three techniques are used to collect specimens: swabs, fine-needle aspiration and biopsy (punch or surgical). Specimens may be used for routine diagnosis and clinical management of patients, and research.

1. Routine clinical management and case-finding

Swabs and fine needle aspiration are simple procedures that an be undertaken at any level (community, health centers, hospitals) during routine management or case-finding in communities.

1.1 Swabs

Specimens obtained by swabs should be taken from the undermined edges of a clinically diagnosed Buruli ulcer. Physicians or experienced health workers can perform this technique. In general, most patients present with ulcers so this technique is widely applicable in every setting. However, every effort should be made to minimize pain and bleeding, and proper training provided to health workers to perform this technique.

1.2 Fine needle aspiration

Fine-needle aspiration (FNA) is mainly used to obtain samples from clinically-diagnosed non-ulcerative lesions (nodule, plaque and oedema). This technique is necessary in up to 30% of patients (depending on the setting) and is simple enough to be applied more widely in the field. FNA may also be used in some ulcerative lesions where it is difficult to take swabs because of healing edges. Only physicians or experienced health workers should perform this technique; ongoing training and regular supervision should be provided to health workers to improve their skills.

Extreme care should be exercised when performing fine-needle aspiration around the head and neck area (especially around the eyes) and the genitalia. Where necessary, an expert clinician should perform this technique in order to minimize any unintended damage to important organs or structures.

WHO recommends that a maximum of two swabs or two fine-needle aspirations be taken for each lesion depending on the experience of the person performing the technique.