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.

Repeat sampling may be indicated if the results of polymerase chain reaction of the initial samples are negative despite a strong clinical diagnosis.

1.3 Biopsy (punch or surgical)

Samples obtained from swabs and fine-needle aspiration are sufficient in most cases. Punch biopsy or surgical biopsy may be used under the following circumstances or when the diagnosis is in the direct interests of the patient (for example, when swabs and fine-needle aspiration have been tried or abandoned). Surgical biopsy may be preferable when larger diagnostic sample specimens are required for histopathological analyses.

Indications and suggested sampling techniques

Indication	Suggested sampling technique
1. To establish the differential diagnosis of Buruli ulcer	Surgical or punch biopsy
2. To investigate the cause of a "paradoxical reaction"	Punch or surgical biopsy
3. To determine treatment failure following successful administration of high-quality antibiotics	Punch or surgical biopsy
4. To establish a possible recurrence of infection (albeit hard to define today)[a]	Punch or surgical biopsy
5. To establish the possible development of cancerous changes	Surgical or punch biopsy
6. To reconfirm in clinical trials the clinical diagnosis by at least two laboratory methods [a] and to evaluate disease process and therapeutic efficacy	Punch or surgical biopsy

[a] For microbiological investigations, a swab may be preferred if the lesion has ulcerated.

Conditions

- Biopsies should be carried out by a trained physician (or an experienced health worker) who has examined the patient and come to the decision that a biopsy is the only option at his or her disposal.
- Samples for histopathological analysis (or microbiological analysis) should be taken from a single biopsy rather than multiple punch biopsies.
- Biopsies should not be performed on lesions of the face (for cosmetic reasons) and other critical sites (for example the head and neck area and the genitalia).
- Punch biopsy should be performed in a setting where the risk of infection can be minimized and where facilities are available to manage any profuse bleeding.
- All necessary steps should be taken to minimize pain and discomfort to patients.

2. Research

Many of the techniques described above and their conditions for use also apply for research purposes. In exceptional cases or where ethically sound justifications have been made (such as why routine samples or procedures cannot be used or where there is a need to answer critical research questions), explanations should be detailed in the research protocol. Information should also be provided to patients (under the section on information for patients) and included in the consent form. Single biopsies should be taken by experts only. Certain anatomical sites (the face, neck and genitalia) are exempt from punch biopsies.

The protection of patients in research is an international standard that should not differ from country to country. It is therefore essential that the Buruli ulcer community develops its own code of ethics to uphold such standards of medical practice within its research work.

Samples for research are still possible!

Researchers should be assured that opportunities for obtaining samples from patients to enhance research are available and should be explored.

Histopathology (and microbiology)

Debridement or limited excision of tissue from necrotic ulcers or lesions during or after antibiotic treatment provides an opportunity to obtain samples for research.

Cultures

Adequate cultures are possible even if taken from the swabs of 70–100% of patients with ulcerative lesion. Further work is needed to determine how many cultures can be obtained from samples of fine-needle aspiration. It is not necessary to obtain cultures from every patient or lesion as long as there is no evidence for the emergence of antibiotic resistant *M. ulcerans* strains.

Mycolactone

The results of new methods for detecting mycolactone from tissues are encouraging. Samples taken from the necrotic part of ulcerative lesions (in 70–100% of cases) may provide mycolactone for analyses. In particular for non-ulcerative lesions, further studies are needed to determine the feasibility of detecting mycolactone from samples obtained through routine simple sampling techniques.