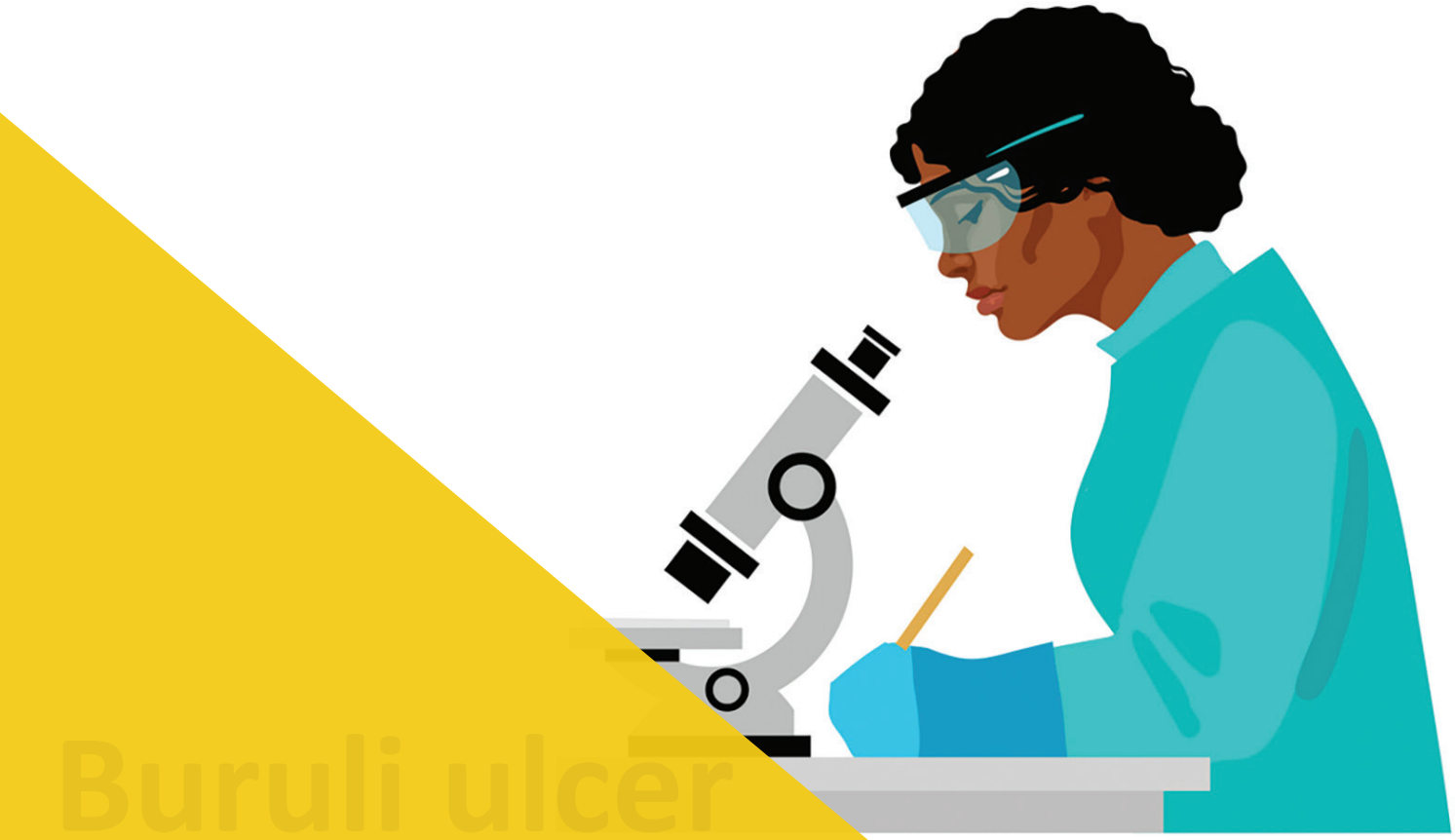
First meeting of the network on Buruli ulcer PCR laboratories in the WHO African Region



Centre Pasteur du Cameroon,
Yaoundé, 21–24 October 2019

World Health Organization

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Contents

Abbreviations and acronyms

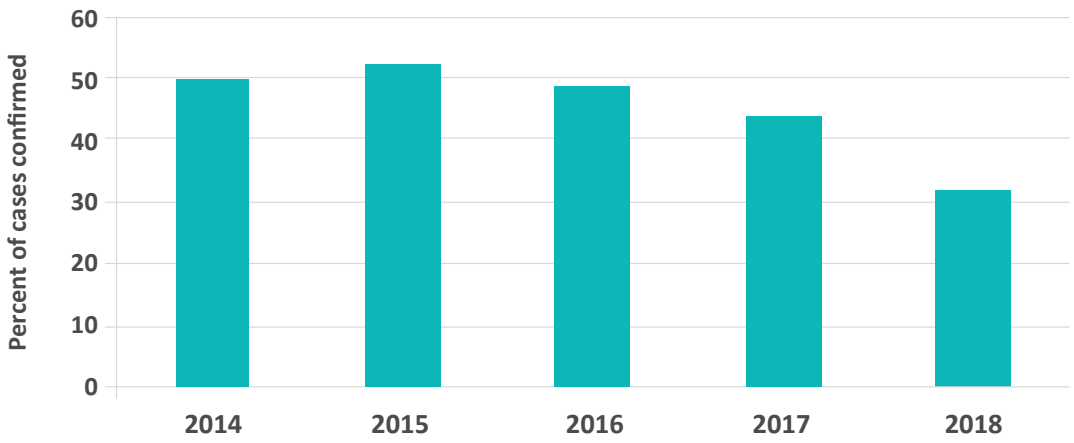
CPC	Pasteur Center of Cameroon
EQA	external quality assessment
ITM	Institute of Tropical Medicine
NTD	neglected tropical disease
PCR	polymerase chain reaction
SOP	standard operating procedure
WHO	World Health Organization

1. Background

Buruli ulcer is caused by infection with *Mycobacterium ulcerans*. The disease is reported in more than 33 countries worldwide, but only about half of these countries regularly report data to WHO; most cases are reported from subregions of West and Central Africa. The mode of transmission is not known. About half of those affected are children aged under 15 years; there is no gender difference. Diagnosis is based mainly on clinical and epidemiological characteristics. Of the four methods used for laboratory confirmation (microscopy, polymerase chain reaction (PCR), histopathology and culture), PCR is the most rapid and widely used. Other rapid methods for detection of mycolactone in lesions from suspected cases, such as fluorescent thin-layer chromatography, are under evaluation in four countries in Africa. Research to develop point-of-care tests is in progress. Treatment of Buruli ulcer comprises 8 weeks of combined antibiotics (rifampicin and clarithromycin). Complementary therapies such as wound care, skin graft and prevention of disability are needed in some cases to ensure full recovery.

The target set by the World Health Organization (WHO) for control of Buruli ulcer is for countries to achieve a rate of case confirmation by PCR of at least 70%. All endemic countries have at least one PCR facility to support confirmation of cases. However, most countries in the WHO African Region have not been able to reach the target, and the rate of case confirmation has been declining (see Fig. 1).

Fig. 1 Average percentage of suspected Buruli ulcer cases confirmed in the WHO African Region, 2014–2018



During the 12th meeting of the WHO Technical Advisory Group on Buruli ulcer (Geneva, 27 March 2019), the Institute of Tropical Medicine (ITM) announced that it was discontinuing the external quality assurance (EQA) programme. In response, the Group recommended that EQA be assumed by an African country in which Buruli ulcer is endemic, for sustainability purposes.

At its previous meeting (Geneva, 21 March 2017), the Group identified a number of problems with laboratory confirmation of cases, namely: (i) the low rate of PCR confirmation in a number of endemic countries; (ii) the long delays in receiving results from the laboratories; (iii) the low rate of participation in the EQA programme by national reference laboratories; and (iv) the lack of funding to sustain the EQA programme.

As ITM has discontinued the external evaluation process, it is imperative that a new model of EQA is rapidly proposed that will improve the performance of laboratories involved in the molecular diagnosis of Buruli ulcer in endemic countries in Africa to ensure that patients receive correct diagnostic results and that the data recorded by WHO are accurate, reliable and comparable with those of other continents such as Australia.

Based on the Group's recommendation, WHO intends to transfer the programme to a volunteer laboratory in an endemic country in Africa that demonstrated good performance during the previous rounds of EQA. The Mycobacteriology service of the Centre Pasteur du Cameroun (CPC) was judged to be one of the best performing laboratories and was asked to propose a new model to improve the performance of laboratories involved in the molecular diagnosis of Buruli ulcer in the endemic countries in Africa.

In response, WHO sent two consultants to visit CPC on 23–25 April 2019 in order to:

- assess the capacity and needs of CPC for potential designation as the reference laboratory for Buruli ulcer in Africa and to conduct the EQA programme; and
- plan a meeting of all the laboratories involved in confirmation of cases of Buruli ulcer during the second half of 2019.

From the laboratory visit and the discussions about the laboratory network, the consultants recommended that, given the experience and technical expertise of the Mycobacteriology unit of CPC, the CPC should be designated as the Coordinating Centre for the new EQA programme.

The first meeting of the Buruli ulcer laboratory network (BU-LABNET) ulcer was held at CPC in Yaoundé, Cameroon, on 21–24 October 2019. The agenda is given in Annex 1. The meeting was attended by representatives from 11 laboratories from nine endemic countries and external experts (see Annex 2 for the list of participants), with simultaneous interpretation in English and French.

2. Meeting summary

The following topics were reviewed and discussed:

- terms of reference of BU-LABNET: membership, functioning and support;
- harmonization of standard operating procedures (SOPs) for PCR-based diagnosis of Buruli ulcer;
- organization of the new EQA programme;
- harmonization of data collection;
- integration of PCR-based case confirmation with other neglected tropical disease (NTDs) amenable to case management, including yaws, leprosy and leishmaniasis;
- research activities to be conducted within the network; and
- establishment of a BU-LABNET WhatsApp group to facilitate communication.

3. Main discussion points

3.1 Terms of reference and functioning of the network

3.1.1 *Membership*

Membership of the laboratory network includes 11 laboratories in endemic countries performing quantitative PCR-based (qPCR) diagnosis of Buruli ulcer.

- **Benin**
  - Laboratoire de Référence de Mycobactéries de Cotonou
  - Centre de Dépistage et de traitement de l'ulcère de Buruli, Pobé
- **Cameroon**: Centre Pasteur du Cameroun
- **Côte d'Ivoire**: Institut Pasteur de Côte d'Ivoire
- **Democratic Republic of the Congo**: Institut National de Recherche Biomédicale
- **Gabon**: Centre International de Recherches Médicales de Franceville
- **Ghana**
  - Kumasi Centre for Collaborative Research in Tropical Medicine
  - Noguchi Memorial Institute for Medical Research
- **Liberia**: National Public Health Reference Laboratory
- **Nigeria**: Nigerian Institute of Medical Research
- **Togo**: National Institute of Hygiene

3.1.2 *Coordination*

- The network will be coordinated by the Pasteur Centre of Cameroon with the support of an advisory group and a panel of experts.
- To facilitate communication, a BU-LABNET WhatsApp group has been established.

The advisory group will comprise the following representatives:

- one member from the Coordinating Centre (Sara Eyangoh);
- two members from WHO (Sheick O. Coulibaly from the Regional Office for Africa and Kingsley Asiedu from headquarters);
- two members from the laboratory network: one from an English-speaking country (Michael Frimpong from the Kumasi Centre for Collaborative Research in Tropical Medicine, Ghana) and one from a French-speaking country (Wemboo Afiwa Halatoko from the Institut National d'Hygiene, Togo); and
- three experts (Sundeep Chaitanya, Gisela Bretzel and Estelle Marion).

The panel of experts will comprise a group of volunteer experts to support laboratory assessment, capacity-building and evaluation of needs. Sundeep Chaintanya will chair the panel. Sundeep Chaitanya, Gisela Bretzel, Estelle Marion, Marcus Beissner, Michael Frimpong, Solange Kakou Ngazoa and Sara Eyangoh will be volunteers.

3.2 Harmonization of standard operating procedures

The SOPs currently used by laboratories in the network were compared using four criteria:

- feasibility,
- cost,
- robustness and
- repeatability.

The following SOPs were adopted by consensus:

SOP1: Standard operating procedure for collection, transport and storage of samples for diagnosis of Buruli ulcer

SOP2: Standard operating procedure for registration and treatment of samples before PCR analysis of Buruli ulcer

SOP3A: Standard operating procedure for extraction and purification of DNA from *Mycobacterium ulcerans* with internal positive control

SOP3B: Standard operating procedure for extraction and purification of DNA from *Mycobacterium ulcerans* without internal positive control

SOP4A: Standard operating procedure for preparation of real-time PCR with internal positive control

SOP4B: Standard operating procedure for preparation of real-time PCR without internal positive control

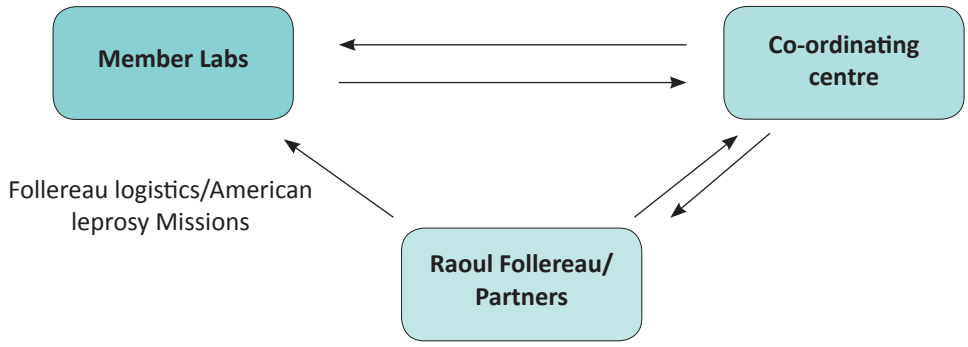
A corresponding list of reference materials and reagents needed for case confirmation by PCR will be appended to the SOPs.

3.3 Support to national laboratories in the network

3.3.1 *Assessment of all laboratories*

This activity will be carried out by members of the expert panel from January to March 2020. Priority laboratories (Gabon and Liberia) will be visited in January 2020, and the others in February and March 2020 (Fig. 2).

Fig. 2 Reagent/consumable procurement plan



3.4 Implementation of the new external quality assurance programme

This activity will be coordinated by CPC, starting in July 2020. The procedures for EQA panel sample preparation, transport and testing will be developed by the Coordinating Centre and distributed to each Member laboratory. Samples will be dispatched **annually** and will constitute blinded pre-tested sample panels. This activity could be done more than once for laboratories that failed the initial assessment in order to monitor improvement.

4. Presentation of laboratory activities

Representatives from 11 laboratories presented their respective laboratory activities based on the reporting template shared. Discussion focused on collection and transportation of samples to the laboratory, as well as coordination with national Buruli ulcer control programmes, to ensure that patients benefit from the quality of testing implemented through the network.

4.1 Group 1: Integration of other skin NTDs

The so- called skin NTDs, which include yaws, leprosy and cutaneous leishmaniasis, could be integrated into the Buruli ulcer platform, with procedures harmonized for each disease. Some laboratories have already begun to integrate this type of platform.

4.2 Group 2: Research

This network offers an important opportunity to conduct or participate in multicentric research activities such as clinical trials evaluating new drug treatments, diagnostic tools, molecular epidemiology and mapping studies and surveillance of antimicrobial resistance.

First, the group will systematically review publications highlighting common activities conducted by the network.

5. Next steps

5.1 Activate the Coordinating Centre

- Recruit project manager (by January 2020): to be recruited by CPC (based in CPC) and funded by American Leprosy Missions.

5.2 Harmonize standard operating procedures

- Compare extraction methods (November 2019)
- Validate master-mix (November 2019)
- Validate SOPs – Advisory Board (December 2019)
- Convene meeting in Angers (November 2019)
- Disseminate SOPs (January 2020)

5.3 Organize activities

- Plan purchases
  - estimate available reagents and duration of use (November 2019)
  - draft new procurement plan (by March 2020)
- Prepare a checklist for expert visits and a template for reports (December 2019?)
- Conduct planning visits to selected laboratories (January to March 2020)
- Compile reports from the laboratories based on the standard template (quarterly from second or third quarter 2020)

5.4 Implement external quality assurance

- Draft SOPs for preparation of high-quality samples
- Prepare shipment of first batch (July 2020)

6. Priority activities and timeline for implementation, 2019–2023

Activity	2019	2020	2021	2022	2023
Finalize BU-LABNET adopted procedures					
Finalize reference list of laboratory materials and reagents needed for BU confirmation by PCR (SOP1,2,3 and 4)					
develop procedures for the external quality assessment program (sample preparation, shipment, reconstitution)					
Ship EQA panel samples to member labs					
Hold laboratory network meeting					
Monitoring and evaluation for continuous improvement					
Onsite refresher training as required					
Procurement of BU PCR reagents and materials					

7. Recommendations

7.1 For the Coordinating Centre

- Disseminate all the harmonized SOPs that were adopted by consensus at this meeting.
- Ensure proper functioning of the advisory board and facilitate interaction with the panel of experts.

7.2 For BU-LABNET

- Ensure that the outcomes of the meeting are implemented by all participating laboratories.

7.3 For national Buruli ulcer control programmes

- Train health workers in clinical diagnosis of Buruli ulcer, collection of high-quality samples, storage and timely transportation to the laboratory.
- Work closely with laboratories to address any problems and delays in laboratory confirmation.

7.4 For WHO

- Officially designate the Centre Pasteur du Cameroon as the Coordinating Centre for BU-LABNET in the African Region.
- Engage national Buruli ulcer control programmes in the quality of sample collection, storage and transport to laboratories.
- Assist in advocacy, communication and fundraising for the implementation of BU-LABNET and the outcomes of the meeting.
- Work with partners in securing high-quality PCR supplies and reagents for BU-LABNET.

7.5 For nongovernmental organizations and donors

- Provide resources to support BU-LABNET to implement confirmation of cases in all countries.
- Support national programmes to implement control activities and collect, store and transport samples to the laboratory.

Annexes

Annex 1. Agenda

Day	Topic	Speakers (bold type) / Facilitators
Monday, 21 October 2019 (day 1)		
08:00–08:30	Arrival of participants	Secretary CPC and WHO (Félicité and Lucie Pascale)
08:30–09:00	Opening ceremony: welcome speech of the Centre Pasteur and WHO	Professor Elisabeth Carniel
09:00–09:30	Opening lecture: "Overview of Buruli ulcer and efforts to improve diagnosis over the past 10 years"	Dr Asiedu Kingsley WHO
09:30–10:00	*Family photograph and coffee break*	
10:00–10:45	Presentation of BU LAB Network	Dr Sara Eyangoh
10:45–12:15	Discussion and adoption of network functioning	All
12:30–13:30	*Lunch*	
13:30–17:30	Laboratory presentations (11 laboratories): activities, identification of strengths and weaknesses, challenges. Presentation: 10min, discussion: 5 min	All
	End of the day	
Tuesday, 22 October 2019 (day 2)		
09:00–10:00	Presentation of SOPs. Synthesis of material and methods used by the 11 laboratories	Dr Sara Eyangoh
10:00–12:00	Discussion of SOPs: DNA extraction, mix preparation, DNA standard, primers and probe, PCR reagents	All
12:00–13:00	*Lunch*	
13:00–15:00	Harmonization and adoption of the SOP	All
15:00–17:00	Discussion of a new model EQA programme; country visit planning	All
	End of the day	
Wednesday, 23 October 2019 (day 3)		
09:00–10:30	Challenges for each lab to implement the harmonized SOPs (5mn presentation of each lab)	All
10:30–12:30	Group1: discussion on Integrated laboratory (NTDs) and role of national programmes	Experts and five laboratories
	Group 2: Multicentric studies using the network	Experts and five laboratories
12:30–13:30	*Lunch*	
13:30–15:30	Restitution of each group and discussion	
15:30–17:00	Recommendations and closing remarks	
	End of the day	
Thursday, 24 October 2019 (day 4): Field visit to Akonolinga District Hospital		

Annex 2. List of participants

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