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Establishment of Quantitative PCR (qPCR) and Culture Laboratory Facilities in a Field Hospital in Benin: 1-Year Results

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No simple diagnostic tool is available to confirm Mycobacterium ulcerans infection, which is an emerging disease reported in many rural areas of Africa. Here, we report the 1-year results of a hospital laboratory that was created in an area of endemicity of Benin to facilitate the diagnosis of M. ulcerans infection.

Buruli ulcer is a neglected tropical disease occurring mainly in poor, rural communities in West Africa and is caused by the environmental pathogen Mycobacterium ulcerans. This bacillus produces a unique toxin called mycolactone, which has cytotoxic and immunomodulatory activity and often causes severe skin ulcerations, disfigurement, and disability, with children the most affected. A combination of rifampin and streptomycin or rifampin and clarithromycin has been recommended by the World Health Organization (WHO) since 2004 as the first-line treatment for all stages of the disease. This combination results in better cure rates when compared to the previous one-drug (rifampin) regimen (66). The need for a new approach led to the establishment of the laboratory at the Buruli ulcer treatment center in Pobé, Benin, in 2011. This laboratory was recently upgraded to provide quantitative PCR (qPCR) and culture facilities for the management of patients. Since November 2012, the field laboratory established at the Buruli ulcer treatment center in Pobé, Benin, has been fully equipped to carry out M. ulcerans diagnosis and has three separate rooms to avoid cross-contamination. Technicians have been trained to perform quantitative PCR (qPCR) and culture in addition to smear examination. Here, we evaluated the 1-year results of this laboratory, which is the first one associated with a specialized treatment center located in a rural area of endemicity and able to carry out qPCR, culture, and direct smear examination (DSE) of a given sample. Detection and quantification of M. ulcerans by qPCR were compared with culture and Ziehl-Neelsen staining results, and the beneficial effects of this laboratory approach on patient management were evaluated.

Among 394 specimens analyzed, 203 (51%) were taken by swab, 101 (26%) by FNA, and 90 (23%) by biopsy. The samples were analyzed by molecular tests (qPCR) and microscopic tests (culture and Ziehl-Neelsen staining). Briefly, swabs were rehydrated and biopsy specimens were minced in 2 ml of sterile water. The Kubiaca method was used to decontaminate 400 μl of swab and skin biopsy specimens to isolate M. ulcerans strains (6). FNA specimens were not decontaminated, because the sampling procedure is considered sterile. After inoculation onto Lowenstein-Jensen medium, growth was monitored weekly for 5 months. Ziehl-Neelsen staining was performed as described previously (3). To prepare DNA, 400 μl of suspension was centrifuged, resuspended in 50 mM NaOH solution, and heated at 95°C for 10 min. DNA was then purified with a QIAquick PCR purification kit (Qiagen), according to the manufacturer’s instructions. Negative and positive controls were systematically included. IS2404 primers and probe were selected from Rondini et al., and amplification and analysis were performed with a Step One real-time PCR system (Applied Biosystems) (4, 7). Sample DNA was quantified from an external standard curve made with a series of six 10-fold serial dilutions of M. ulcerans (strain 1G897) DNA (Fig. 1A).

Of the 394 samples, 163 (41%) were qPCR positive, confirming the diagnosis of Buruli ulcer. The proportions of samples identified as positive for M. ulcerans by PCR were similar for FNA specimens, swabs, and skin biopsy specimens (P = 0.68). Indeed, M. ulcerans DNA was detected in 78 swabs (38%), 47 FNA specimens (47%), and 38 skin biopsy specimens (42%). The bacterial loads estimated by qPCR differed according to the type of sampling (with median loads of 24 × 10^3, 21 × 10^3, and 25 × 10^4 bacteria/ml in swabs, FNA specimens, and skin biopsy specimens, respectively) (Table 1). Skin biopsy specimens contained the largest amount of M. ulcerans DNA (P < 0.0001), followed by swabs (P < 0.01) (Fig. 1B). The amount of bacterial DNA detected did not depend on the WHO category of the lesion (1), and this was true for all types of sampling methods (data not shown).

For culture experiments, we analyzed only qPCR-positive specimens that were taken from qPCR-positive patients who had not started antibiotic treatment. Despite the relationship between the bacterial load estimated by qPCR and the type of sample, the positive culture rates were approximately the same for all three sampling methods: 61% (41/67) for swabs, 63% (24/38) for FNA specimens, and 62% (10/16) for skin biopsy specimens (Table 1). Three cultures (2.5%) showed fungal contamination, and the median incubation time required to obtain a positive culture was 45 days (minimum, 30; maximum, 120). Bacterial load estimated by
qPCR was associated with a positive culture rate for swabs and FNA specimens. Indeed, the bacterial load was significantly higher in culture-positive specimens than in culture-negative specimens (Fig. 2A). However, the positive culture rate reached a plateau at around 80%, at which point an increase in bacterial load had no effect on the proportion of positive cultures (Fig. 2B). DSE results after Ziehl-Neelsen staining were positive in 47% of qPCR-positive specimens. The levels of sensitivity of DSE were highly dependent on the type of sampling and were 13% in FNA specimens, 52% in swabs, and 82% in skin biopsy specimens (Table 1), which is consistent with the results of a previous study (4).

The Raoul Follereau hospital in Pobè, Benin, was established in 2004 and is located in the middle of an area of endemicity in southeastern Benin, close to the Nigerian border. This center treats around 150 Buruli ulcer patients per year. The creation of a molecular laboratory inside the hospital has enabled the direct processing of samples. We chose to use a qPCR method rather than a gel-based PCR method because this method minimizes cross-contamination from amplicons, is faster and more sensitive than gel-based PCR, and involves less toxic waste. Here, we describe for the first time the bacterial load of M. ulcerans DNA by qPCR analysis of swabs, FNA samples, and biopsy specimens. (A) Representative external standard curve made from a series of six 10-fold serial dilutions of M. ulcerans DNA. Ct, threshold cycle. (B) Estimation of bacterial loads in swabs, FNA samples, and biopsy specimens (n = 163). **, P < 0.01; ***, P < 0.0001. Bacterial load data show the number of bacteria per ml detected by qPCR.

![Quantification of M. ulcerans DNA by qPCR analysis of swabs, FNA samples, and biopsy specimens.](image)

**Table 1** Comparison of qPCR, DSE, and culture results with specimens obtained by swab, FNA, and biopsy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Swab</th>
<th>FNA specimen</th>
<th>Biopsy specimen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>% qPCR-positive specimens (no. of qPCR-positive specimens/total no. of specimens)</td>
<td>38 (78/203)</td>
<td>47 (47/101)</td>
<td>42 (38/90)</td>
<td>42%</td>
</tr>
<tr>
<td>Median bacterial DNA load estimated by qPCR (no. of bacteria/ml)</td>
<td>23,500</td>
<td>2,100</td>
<td>250,000</td>
<td>91,900</td>
</tr>
<tr>
<td>% positive DSE* (no. of qPCR-positive specimens/total no. of specimens)</td>
<td>52 (41/78)</td>
<td>13 (6/47)</td>
<td>82 (31/38)</td>
<td>47%</td>
</tr>
<tr>
<td>% positive culture* (no. of qPCR-positive specimens/total no. of specimens)</td>
<td>61 (41/67)</td>
<td>63 (24/38)</td>
<td>62 (10/16)</td>
<td>62%</td>
</tr>
</tbody>
</table>

* DSE, direct smear examination.

* Data include only samples taken before the beginning of antibiotic treatment.
gence of resistance to antibiotics and relapses and is essential for research on environmental dissemination and transmission.

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REFERENCES

10. Reference deleted.

FIG 2 Sensitivity of culture in qPCR-positive specimens. (A) Bacterial load detected by qPCR in swabs, FNA samples, or biopsy specimens divided according to whether they gave positive or negative culture results. *, P < 0.1; ***, P < 0.001. (B) Positive culture rate as a function of bacterial load detected by qPCR in specimens (n = 118). Bacterial load data show the number of bacteria per ml determined by qPCR.