Evaluation of an IS2404 LAMP protocol, a simple and rapid test for diagnosis of Buruli Ulcer in low-resource settings

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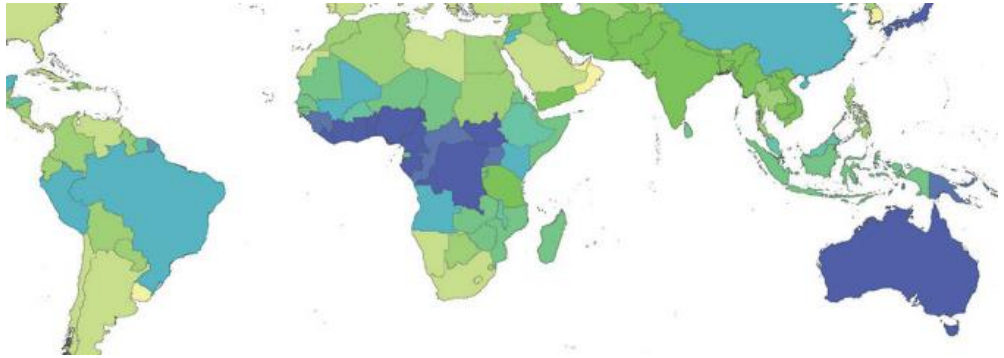
Conflict of Interest:
The author has declared no conflict of interest.

Buruli ulcer (*Mycobacterium ulcerans* infection)

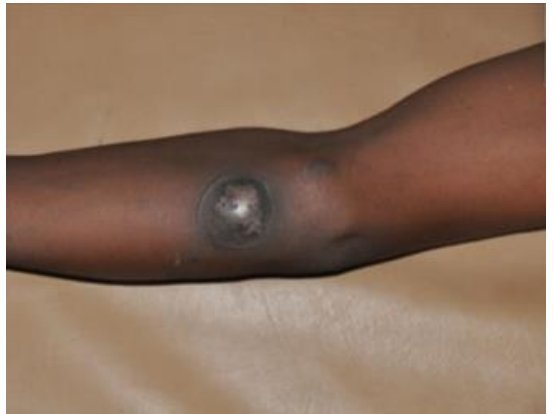
- **Destructive disease of the skin and cutaneous tissues**
  - Infectious disease of poverty and Neglected Tropical Disease (NTD) of the skin
  - Reported in 33 countries worldwide, mostly in West Africa and Australia
    - 73% of cases in Côte d'Ivoire, Ghana, and Benin (Yotsu et al., 2018)
    - 50% of patients children < 15 years of age (Yotsu et al., 2018)
  - Unclear mode of transmission
    - Environmental transmission, associated with freshwater ecosystems (Muleta et al., 2021)
    - Mammalian reservoirs and insect vectors, e.g., possums, mosquitoes (Mee et al., 2024)



Simpson et al., 2019

Buruli ulcer (*Mycobacterium ulcerans* infection)

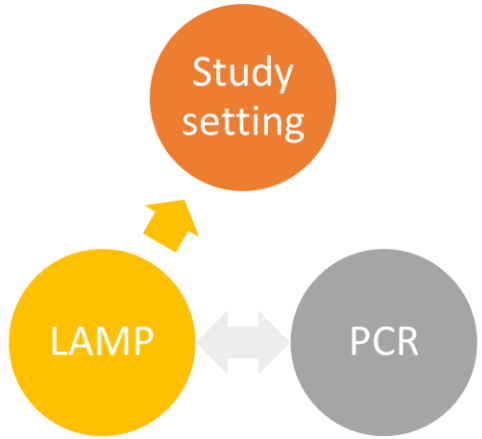
- Early detection crucial
  - Late-stage complications with bone involvement, disseminated disease
  - Surgery, long-term disability
- Treatment successful with antibiotics:
  - oral rifampicin + injectable streptomycin once daily - nephro- and ototoxicity, daily visits
  - all-oral rifampicin and clarithromycin twice daily, 8 weeks (Philipps et al., 2020)
- Current diagnostic methods can't meet point-of-care (PoC) needs
  - WHO recommendation: Molecular confirmation with qPCR before treatment
    - Requires laboratories with appropriate equipment
    - Conducted in only 30% of patients (2018) (Popa et al., 2023)
    - Reference laboratory network BU-LABNET (Marion et al., 2022, www.africabulabnet.org)
- Socioeconomically deprived patients
  - Delays, complications with diagnosis, treatment
  - Stigma



Yotsu et al. (2018)

Study aims

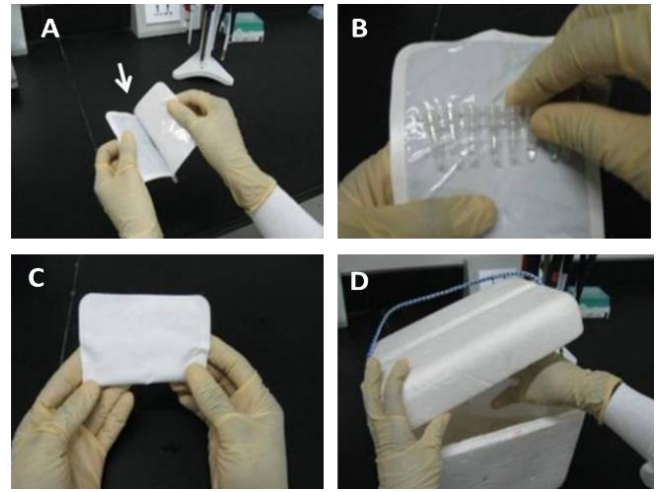
- 1. Diagnostic test accuracy (DTA): analysis of the performance of new molecular diagnostic test as compared to PCR, protocol suitable for low-resource/PoC-setting
  - Loop-mediated isothermal amplification (LAMP) method (Notomi et al., 2000)
    - DNA detection, constant temperature of 60°C - simple heat sources
    - Sensitive and robust, naked-eye or simple UV detection of results
    - Potential to be implemented at PoC?
- 2. Implementability of LAMP at PoC

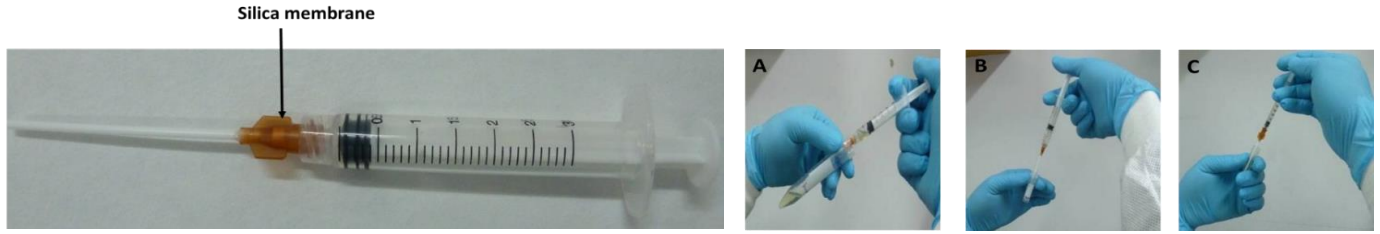


- Study (= target) setting: Pakro Health Centre (PHC) as PoC
  - Eastern Region, est. 1991 by Ghana Health Service
  - Inhabitants of village, surrounding farming hamlets (9000 inh)
  - Public electricity grid without generator
- Reference laboratory
  - NMIMR (Noguchi Memorial Institute for Medical Research, Accra)

Methods

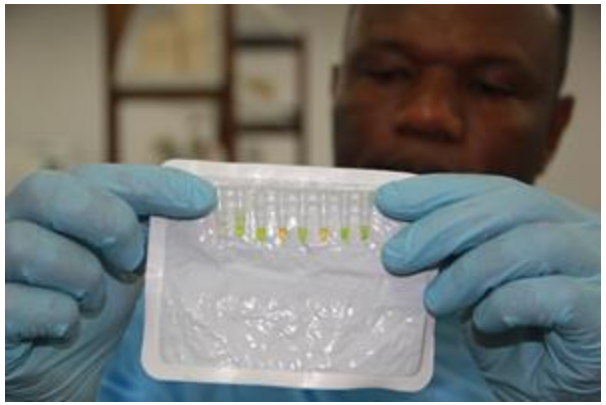
- **Study at NMIMR:** N=64 samples (fine needle aspirates, swabs), comparison of LAMP vs. PCR
  - LAMP protocol, requiring no electricity:
    - DNA extraction: Syringe-based method (EasyNAT, Ustar Biotechnologies) (LAMP^{sm})
    - Amplification: Heat source commercial 'pocket warmer' (pwLAMP)
- **Focus group discussions, individual interviews**
  - Researchers, health care professionals (doctor, nurse), community-based surveillance (CBS) volunteers
    - Diagnostic workflow and timelines
    - Conditions at the PoC
    - Challenges/barriers for patients and CBS volunteers
- **Study approved** by ethics committees of NMIMR and University of Oxford





Results 1 (Ahortor et al., under review PLOS NTDs)

- Sensitivity = 0.836, specificity = 1 as compared to PCR
- Limit of detection = 30 copies of multi-copy target *IS2404*
- **Meta-analysis** on performance of LAMP for diagnosis of BU (Erber et al., in prep.)
  - 9 separate experiments included (665 clinical samples, 7 studies 2012-2023)
  - Cumulative sensitivity = 0.84 (0.80,0.87), specificity = 0.98 (0.9,1)

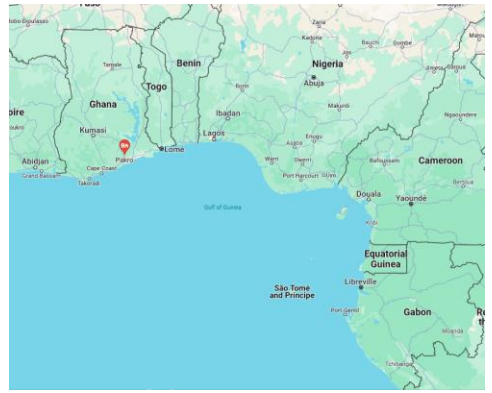


	hbLAMP^{CM}			IS*2404*PCR^{SM}			hbLAMP^{SM}			pwLAMP^{SM}		
	(+)	(-)	Total	(+)	(-)	Total	(+)	(-)	Total	(+)	(-)	Total
IS2404 PCR (+)	54	2	56	49	6	55	46	9	55	46	9	55
IS2404 PCR (-)	0	8	8	0	9	9	0	9	9	0	9	9
Total	54	10	64	49	15	64	46	18	64	46	18	64
% Positivity	84.4			76.6						71.9		
% Sensitivity	96.4			89.1						83.6		

IS*2404* PCR^{CM} = IS*2404* PCR performed using DNA recovered by the CM
hbLAMP^{CM} = conventional LAMP assay performed using DNA recovered by the CM
IS*2404* PCR^{SM} = IS*2404* PCR performed using DNA recovered by the SM
hbLAMP^{SM} = conventional LAMP assay performed using DNA recovered by the SM
pwLAMP^{SM} = pocket warmer assay performed using DNA recovered by the SM

Results 2 (Ahortor et al., under review PLOS NTDs)

- Buruli ulcer perceived as **non-medical condition**
  - Often traditional (herbal/spiritual) treatment sought first, late-stage presentations
- **Diagnosis of Buruli ulcer in Pakro**
  - High accuracy of screening based on clinical picture (>90% later confirmed)
  - Samples sent to NMIMR for PCR confirmation by 'public' transport (55 km)
  - Time delay around 1 week
- **Challenges,** barriers for patients
  - Costs
  - Availability of public transport
- **Challenges in implementation of LAMP** at PoC
  - Infrastructure
  - Elevated temperature
  - Training needs of staff



Summary and discussion 1

- LAMP has the potential for PoC-suitable diagnosis of Buruli Ulcer
- Target Product Profile (TPP) for BU diagnosis (WHO DTAG, 2022)
  - Molecular method (DNA-based), results available the same day
  - Minimal auxiliary equipment, low costs, independence of constant electricity supply
  - Sensitivity and specificity non-inferior to staining/microscopy

- Limitations
  - Transfer to PoC, implementation studies in endemic regions
  - Training needs of staff
    - Contamination risk
  - Little interest and support at present

Summary and discussion 2

- Diagnosis of Buruli Ulcer in low-resource settings
  - Importance of diagnosis based on clinical criteria
    - Clinical score (WHO 2020 BU reporting forms; Mueller et al., 2016/MSF)
  - Integrated PCR systems, e.g., Biomeme PCR (Frimpong et al., 2023)
  - Risks associated with false positive diagnosis (Olliaro and Torreele, 2021)
    - Superior safety profile of all-oral rifampicin and clarithromycin (Philipps et al., 2020)

- Clear benefits of Studies-Within-Trials (SWATs) (www.trialforge.org)
  - Small, often qualitative studies embedded in studies or trials, addressing specific aspects
    - Recruitment and informed consent procedure, retention (Boxall et al., 2022; Negussie et al. 2016)
    - Study initiation and –process, workflow and context (Erber et al., 2021)
    - Insights into perspectives of HCPs, patients
  - Interviews and focus group discussions

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