BMJ Open

LAMP4yaws: *Treponema pallidum, Haemophilus ducreyi* loop mediated isothermal amplification – protocol for a cross-sectional, observational, diagnostic accuracy study

Becca Louise Handley ,[1] Camila González-Beiras,[2] Serges Tchatchouang,[3] Laud Antony Basing,[4] Kouadio Aboh Hugues,[5,6] Mohammed Bakheit,[7] Lisa Becherer,[8] Christina Ries,[9] Earnest Njih Tabah,[10,11] Tania Crucitti,[12] Nadine Borst,[8] Simone Lüert,[9] Sieghard Frischmann,[7] Tamara Haerpfer,[8] Emelie Landmann,[7] Ivy Amanor,[4] Aboubacar Sylla,[6] Mireille S Kouamé-Sina,[6] Jean P Ndzomo-Ngono,[3] Adingra Tano,[6] Daniel Arhinful,[4] Patrick Awondo,[3] Solange Ngazoa Kakou,[6] Sara Eyangoh,[3] Kennedy Kwasi Addo,[13] Emma Michele Harding-Esch ,[1] Sascha Knauf,[9] Oriol Mitjà,[2] Michael Marks [1,14]

To cite: Handley BL, González-Beiras C, Tchatchouang S, *et al*. LAMP4yaws: *Treponema pallidum*, *Haemophilus ducreyi* loop mediated isothermal amplification – protocol for a cross-sectional, observational, diagnostic accuracy study. *BMJ Open* 2022;**12**:e058605. doi:10.1136/bmjopen-2021-058605

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-058605).

Received 23 October 2021
Accepted 18 February 2022

For numbered affiliations see end of article.

Correspondence to
Ms Becca Louise Handley; rebecca.handley1@lshtm.ac.uk

ABSTRACT

Introduction Yaws, caused by the bacterium *Treponema pallidum* subsp. *pertenue,* is a neglected tropical disease targeted for eradication by 2030. Improved diagnostics will be essential to meet this goal. Diagnosis of yaws has relied heavily on clinical and serological tools. However, the presence of coendemic cutaneous skin ulcer diseases, such as lesions caused by *Haemophilus ducreyi* (*HD*), means these techniques do not provide a reliable diagnosis. Thus, new diagnostic tools are needed. Molecular tools such as PCR are ideal, but often expensive as they require trained technicians and laboratory facilities, which are often not available to national yaws programmes.

Methods and analysis The LAMP4yaws project is a cross-sectional, observational, diagnostic accuracy study of a combined *Treponema pallidum* (*TP*) and *HD* loop mediated isothermal amplification (TPHD-LAMP) test performed under real world conditions in three endemic countries in West Africa. Individuals with serologically confirmed yaws will be recruited in Cameroon, Côte d'Ivoire and Ghana. Each participant will provide paired swabs, one of which will be sent to the respective national reference laboratory for yaws quantitative PCR and the other will be tested for both *TP* and *HD* using the TPHD-LAMP test at local district laboratories. Sensitivity and specificity of the TPHD-LAMP test will be calculated against the reference standard qPCR. We will also assess the acceptability, feasibility and cost-effectiveness of the test. We anticipate that results from this study will support the adoption of the TPHD-LAMP test for use in global yaws eradication efforts.

Ethics and dissemination We have received ethical approval from all relevant institutional and national ethical committees. All participants, or their parents or guardians, must provide written informed consent prior to study enrolment. Study results will be published in an open access journal and disseminated with partners and the World Health Organization.

Trial registration number NCT04753788.

Strengths and limitations of this study

- A multicountry evaluation of the new *Treponema pallidum* and *Haemophilus ducreyi* loop mediated isothermal amplification (TPHD-LAMP) test performed in a large 'real-world' programmatic setting.
- Capacity building and strengthening of in-country teams to aid with future yaws eradication efforts.
- Extensive social science analysis will allow us to highlight barriers to achieving yaws eradication and enable us to determine if the new LAMP test is acceptable, feasible and costeffective for use in national yaws programmes.
- The TPHD-LAMP is not a true point of care test and currently requires laboratory facilities to carry out the test.
- The study is only being carried out in three of 15 yaws endemic countries. With a large burden of yaws in Asia and the Western Pacific, an evaluation in these populations may be required to ensure local context, including genetic variation, does not affect the performance of the TPHD-LAMP test.

INTRODUCTION

Yaws is a neglected tropical disease (NTD) caused by the spirochete bacterium *Treponema pallidum* subsp. *pertenue* (*TPE*), and is closely

related to the causative agent of syphilis, *Treponema pallidum* subsp. *pallidum*.[1 2] Yaws mainly affects children aged 5–15 years[1] living in endemic areas. It is mostly found in rural, remote communities in the tropics with scarce access to health facilities and sanitation.[3] The disease is spread through direct skin-to-skin contact.[4] Yaws presents in stages: primary yaws appears as a single papilloma or ulcer, and is highly infective. After the initial active infection, a period of latency ensues where the patient appears clinically healed but is still seroreactive for the disease. If untreated, the patient may develop secondary yaws, affecting the lymph nodes and bones. This may be followed by further periods of latency and eventually if left untreated, up to 10% of cases may develop tertiary disease which can result in disfiguring and destructive lesions, which can lead to life-long deformity and disability.[4]

Under the new NTD roadmap 2021–2030, yaws is targeted for eradication by 2030.[5] It is anticipated that this goal will be achieved through mass drug administration (MDA) with azithromycin, an antibiotic shown to be effective against yaws infection as a single oral dose treatment. Azithromycin is distributed to entire endemic communities, where there is at least one single case of serologically or molecularly confirmed yaws, known as Total Community Treatment (TCT). After an initial round of TCT, eradication efforts move to targeted treatment of remaining cases and their close contacts (Total Targeted Treatment). Clinical diagnosis of yaws can be challenging as other pathogens can cause skin ulcers with similar characteristics to yaws. The most common of these is *Haemophilus ducreyi* (*HD*), which can be responsible for over 50% of the ulcers clinically diagnosed as yaws.[6] Currently, molecular confirmation through PCR is the gold-standard diagnosis for yaws but is often unavailable at the district level in yaws-endemic countries.

In the campaign for yaws eradication, it is vital that all endemic communities are identified. In post-MDA surveillance, it is key that no yaws re-emergence is missed or misdiagnosed. While PCR is a highly sensitive and specific tool for yaws diagnosis and, therefore, recommended by WHO to aid with eradication, it depends on trained laboratory staff and requires well-equipped laboratory facilities. This is often not available in remote yaws endemic settings with little infrastructure. A combined *Treponema pallidum (TP*) and *HD* loop mediated isothermal amplification test (TPHD-LAMP)[7 8] has been developed for simultaneous detection of these two primary causes of yaws-like lesions. This test is user-friendly and does not require costly thermocyclers. Therefore, it is suitable for use in local hospital laboratories close to the patient, to test for yaws and *HD*. The TPHD-LAMP test has been previously evaluated in a laboratory setting[8] using swab samples collected during yaws studies in Papua New Guinea and Ghana with promising results: sensitivities of 84.7% (95% CI 72.5% to 92.4%) for *TP* and 91.6% (95% CI 85.8% to 95.3%) for *HD* were reported. The respective specificities were 95.7% (95% CI 92.0% to 97.8%) and 84.7% (95% CI 77.4% to 90.1%). The sensitivities for both targets were reduced in mixed infection samples: 68.4% (95% CI 43.5% to 86.4%) for *TP* and 73.7% (95% CI 48.6% to 89.9%) for *HD*. However, the test is yet to be evaluated in a real-world setting. The TPHD-LAMP test can also potentially be adapted to include *TP* macrolide resistance detection, a feature which is hugely important in light of the recent detection of macrolide-resistant *TPE* strains, which emerged after MDA campaigns in Papua New Guinea.[9 10]

METHODS

Study design

Study sites, population and participant eligibility

The study will take place in three countries in West Africa: Cameroon, Côte d'Ivoire and Ghana. These three countries have been selected as they contribute to the highest recorded prevalence of yaws in Africa.[11 12] In each country, national disease reporting data have been used to select districts that are suspected or known to be highly endemic for yaws, facilitating rapid fulfilment of the required number of yaws cases for the study. The number of selected villages/communities will depend on the prevalence of the disease and the length of time needed to reach our target sample size. Recruitment will cease whenever the desired sample size is reached.

Aims

The primary aim of the project is to perform a cross-sectional, observational, diagnostic accuracy evaluation of the novel TPHD- LAMP test to assess its value as a diagnostic tool for use in yaws eradication and surveillance programmes. This will include testing the TPHD-LAMP test's diagnostic accuracy against the reference standard real-time qPCR tests performed at national reference laboratories, and performing social science and health economic studies to assess its feasibility for deployment in yaws-endemic areas.

Objectives

Primary objective

(1) Assess the diagnostic accuracy (sensitivity and specificity) of the TPHD-LAMP test for *TP* and *HD* diagnosis under programmatic conditions in yaws-endemic areas in Ghana, Côte d'Ivoire and Cameroon in order to determine its suitability as a diagnostic tool in yaws eradication programmes.

Secondary objectives

1. Perform acceptability and feasibility and health economic studies to see if it is realistic to use the TPHD-LAMP test to aid with eradication efforts and/or surveillance.
2. Develop an external quality assurance (EQA) scheme for the molecular diagnosis of yaws and *HD*.

Data and sample collection for objective 1

Participant enrolment will take place between April 2021 and September 2022. Recruitment will cease prior to this

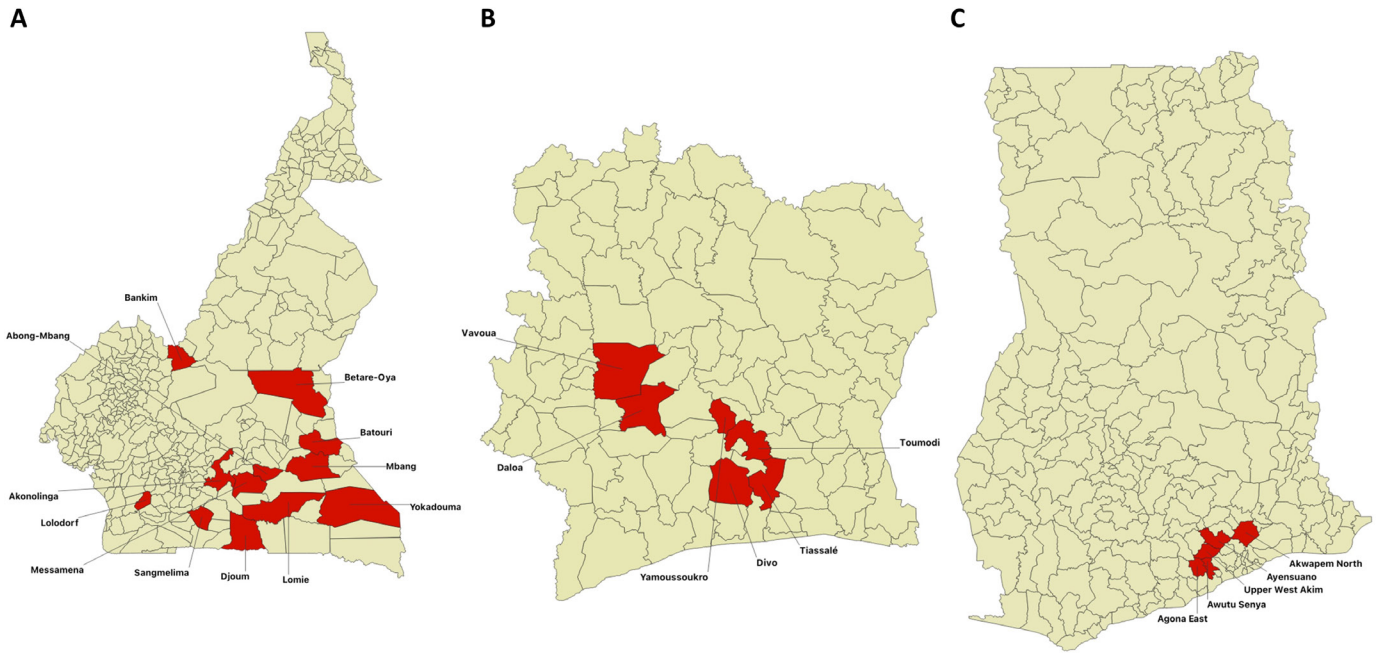

Figure 1 Highlighted districts indicate where active case searching will take place in Cameroon (A),Côte d'Ivoire (B) and Ghana (C)

if the desired sample size is met. Laboratory procedures will be conducted concurrently with the aim to complete all assays by November 2022. In each district (figure 1), we will use active case finding to identify eligible participants for the study. Primarily, we will include those seropositive for treponemal and non-treponemal antibodies as this is the best indicator of an active yaws infection. Recruitment will consist of rapidly screening participants living in communities with reported cases of suspected or confirmed yaws. Individuals will be invited to participate, based on the following inclusion and exclusion criteria.

Inclusion criteria

- Able to give written informed consent if over the age of consent (≥18 years in Côte d'Ivoire and Ghana, ≥21 years in Cameroon), or parent/guardian provides written consent.
- Verbal assent if under the age of consent.
- Presenting with a yaws-like lesion.
- Resident in yaws-endemic district.
- Participants aged 6 months and older.

Exclusion criteria

- Does not consent/unwilling to participate.
- Refusal of village chief (for village inclusion), or refusal of individual and/or guardian (for individual inclusion).
- Permanent disability that prevents or impedes study participation and/or comprehension.
- Participants under 6 months of age.

Screening for the primary objective will consist of a visual examination of a participant's arms, legs, head and torso to look for the presence of lesions consistent with yaws, such as papilloma which tend to have a yellow crust, or yaws ulcers which are typically round, have raised edges and are not painful. All screening will be performed by healthcare workers trained to recognise yaws and perform serological tests. Following written informed consent, participants presenting with yaws-like lesions will undergo an SD Bioline (Abbott, USA) test which detects long-lived anti-treponemal antibodies; these markers generally remain positive for life after exposure to a treponemal infection. If reactive, a Chembio Dual Path Platform test (Chembio Diagnostics, New York, USA) will be performed which will allow for simultaneous detection of both treponemal and non-treponemal antibodies the latter of which are a more reliable indicator of current infection. Results of all serological tests will be recorded in Open Data Kit (ODK) data collection forms. Participants reactive for both treponemal and non-treponemal antibodies will be invited to enrol in the study (figure 2). In addition, up to 10% of participants with negative serology for either test will be randomly selected using the Open Data Kit (ODK) data collection forms and invited to enrol, allowing us to sample from children with negative serology that may not have seroconverted yet, for example, if they are early in an infection (in an early infection, there may be detectable bacteria using molecular diagnostic tests but individuals may not have yet mounted a serological response).

For each participant, we will collect two swabs from their largest yaws-like lesion. If presenting with a yaws papilloma, a curette will be used to lift the scab first. If needed, saline solution will be used to moisten the wound. The two swabs will be simultaneously rolled over a 1 cm^2 area of the lesion for five seconds. Both swabs will then be stored separately in 500 µL of lysis buffer (10 mM

Tris-HCL, pH 8.0; 100 mM EDTA, pH 8.0; 0.5% SDS)[13] within cool boxes, until transport to a refrigerator at the local district laboratory, within 48 hours of collection. A lesion assessment will be carried out, including recording the size, shape and other physical features of the lesion. We will ask if the participant has suffered with these yaws-like lesions before and if they have received treatment for the current presentation.

All participants with yaws-like lesions will be offered treatment with 30 mg of azithromycin/kg (max 2 g),[14] regardless of whether they choose to enrol in the study or their serology results. As per WHO guidelines,[14] after 2–4 weeks, all participants with serologically-confirmed yaws will be followed up to ensure clinical resolution. Any participants with suspected treatment failure (those presenting with lesions that have not healed, or are worse), will be treated with injectable benzathine penicillin as per WHO guidelines[14] and two additional swabs will be collected to test for mutations conferring macrolide resistance in *TPE*.

Sample size considerations

To measure a 95% sensitivity of the TPHD-LAMP test (±3%), a total of 210 qPCR positive *TP* cases are needed.[15] A similar number of cases are required to assess sensitivity and specificity for *HD*. We calculated the number of participants we would need to screen and enrol to achieve our target of 210q PCR-positive cases based on previously available data and assumptions: (1) 10% of suspected cases have positive rapid-diagnostic-test (RDT) serology results[16] and (2) approximately 35% of RDT-positive cases have qPCR-detectable *TP*,[17] with a similar number positive for *HD*.[18 19] Even in endemic districts of West Africa, previous case searches report suspected childhood yaws prevalence of ≤10%. Our active case searches, therefore, need to screen approximately 60 000 individuals to identify an average 600 serology-positive and 210 qPCR-positive cases for primary analysis (figure 2).

Training

Prior to participant enrolment, standard operating procedures (SOPs) for all aspects of the study will be developed. All healthcare workers and laboratory staff will receive study-specific training according to the relevant SOPs. This will include an introduction to the protocol and study rationale as well as Good Clinical Research Practice and Good Clinical Laboratory Practice sessions. Trainees will be introduced to the study documentation and data entry forms and will be shown how to correctly perform the informed consent procedure. Healthcare workers will be trained in conducting serological tests and interpreting the results as well as lesion swab collection and sample storage. Laboratory technicians in the district laboratories will be shown how to store and process samples, including DNA extraction and the TPHD-LAMP test. We will also explain the importance of maintaining a clean environment within which to work, as well as teaching about important sources of contamination, how to avoid and detect this (including the use of no template controls and negative extraction controls) and how to respond if any issues with the laboratory techniques arise. In each national reference laboratory, the qPCR tests will be validated prior to use. Training will be provided in-person by the international study coordinator (BLH), the field coordinator (CG-B), work package lead on capacity building (ST) and the study coordinators in each country (LAB, KAH and ST).

Laboratory analysis

TPHD-LAMP conducted at district laboratories

The TPHD-LAMP test will be conducted at selected local district hospitals or health centres (table 1). We will

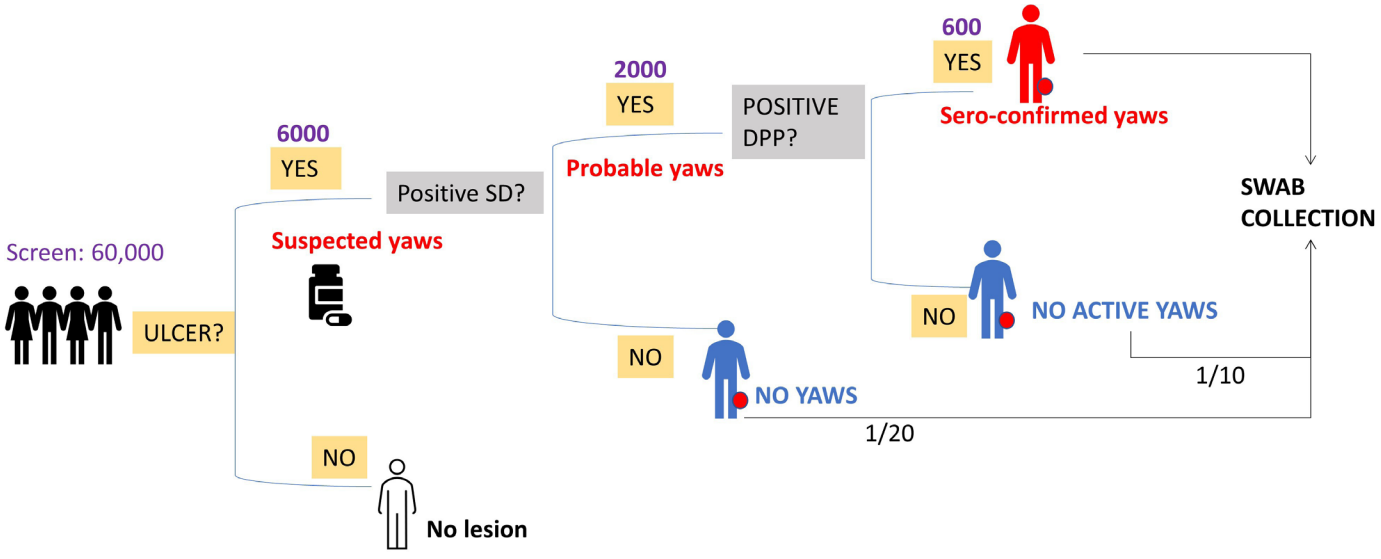

Figure 2 Schematic of the LAMP4yaws recruitment strategy. To find 6000 suspected of ways, we expect to need to screen around 60 000 people. Of these, we expect one-third to be positive for treponemal antibodies and around 10% of individuals to be positive for both treponemal and non-treponemal antibodies.These participants will be invited to enrol in the study.

Table 1 District and reference laboratories involved in sample processing

Country	Cameroon	Côte d'Ivoire	Ghana
Reference laboratories	Centre Pasteur du Cameroun	Institut Pasteur de Côte d'Ivoire	Noguchi Memorial Institute for Medical Research
District laboratories	To be confirmed*	Divo District laboratory Tabo District laboratory Yamoussoukro District Laboratory	Tetteh Quarshie Memorial Hospital Asuboi Health Centre Adeiso Health Centre

*District laboratories in Cameroon will be selected as the study proceeds, dependant on successful case finding.

provide the local teams with all the necessary equipment, reagents and consumables required to perform the test.

When samples reach the district laboratory, DNA will be extracted from a 200 µL sample of swab lysis buffer using the MAST ISOPlex DNA/RNA extraction kit and eluted into 80 µL RNAse free water. This kit has been chosen because it is a magnetic bead-based extraction that forgoes the need for fast centrifugation. After extraction, the DNA eluate is stored at 2°C–8°C until the TPHD-LAMP test is performed, within 1 week.

To perform the TPHD-LAMP test,[8] 5 µL of DNA is mixed with reaction buffer, Bst Polymerase and a dye mix. The test is then incubated at 64°C in a MAST Tubescanner for 60 min. To ensure the test is performing correctly, that samples have been successfully extracted and that contamination has been avoided, a negative extraction control (extracted lysis buffer), a positive control (a plasmid containing both pathogens' target gene sequence) and a no template control (NTC) of double-distilled water (ddH_20) will be included in each run. The TubeScanner machine controls the temperature and reads fluorescent signals of a multiplex mediator probe. The result output for each target is positive or negative and is generated within 1 hour. Tests which fail quality control (one or more of the controls showing discordant results) will be repeated and troubleshooting will occur if the problems persist. Results will be immediately entered into an ODK electronic data entry form. Results will not be relayed to participants as the test has not received regulatory approval and is therefore for research purposes only.

Reference standard qPCRs conducted at national reference laboratories

One of each lesion swab sample will be transported to the national reference laboratory in each country (table 1) and stored at 2°C–8°C until processing. Here, DNA will be extracted using the Qiagen DNA mini Qiamp kit (Qiagen, Germany). Again, the sample will consist of 200 µL of lysis buffer and DNA will be eluted into 100 µL of RNAse free water.

First, 5 µL of DNA will be tested using a qPCR targeting the human endogenous gene RNAse P.[20 21] This will allow for confirmation that the sample contains human tissue and has been extracted successfully. Samples positive for the RNAse P gene will then be tested for *TP* and *HD* using two separate PCR reactions, again 5 µL of DNA will be used in each reaction. The *polA* gene and the V8 region of the 16S ribosomal RNA gene will be targeted for *TP* and *HD,* respectively.[22] These targets have been selected as they have been widely used for the detection of *TPE* and *HD*.[22] All samples will be tested twice within one assay and if results for any sample are discordant, the qPCR will be repeated for that specimen. Sample results will be considered positive or negative for each target and these results will be recorded on an ODK form for real-time analysis. Plasmids containing both gene targets, a negative extraction control (extracted lysis buffer) and an NTC (ddH_20) will be included in each qPCR run and if any discordant results of controls are recorded the assay will be repeated.

Data analysis plan for the primary outcome

The national reference laboratory qPCR results will be considered the reference standard result. We will calculate the sensitivity and specificity of the new test as well as the positive and negative predictive values. These will be calculated separately for both pathogens. A secondary analysis on mixed infection samples will also be conducted. Data analysis will be performed using R V.4.0.2[23] or above.

Social science and health economic methods

We will investigate how cost-effective the TPHD-LAMP test is, as well as the feasibility and ease with which it can be conducted at local district laboratories. The health economic analysis will involve costing all aspects of both diagnostic tests, from sample collection to results generation, including shipping and transport costs, and staff time and salaries. We will perform a knowledge attitudes and practice questionnaire with community members, healthcare workers, traditional healers and other key stakeholders. This will allow us to highlight potential barriers to yaws eradication as well as local beliefs regarding yaws diagnosis and treatment and perceptions of the disease. Separate questionnaires have been designed for healthcare workers, district laboratory technicians and reference laboratory staff to determine how feasible and/or acceptable the TPHD-LAMP test is in its current form. All questionnaires will be completed in ODK. Data will be analysed using a mixture of quantitative and qualitative measures, which will be covered elsewhere.

External quality assurance

A second objective of the project is to develop and use an EQA scheme for *TPE* and *HD* diagnosis. The EQA scheme in the LAMP4Yaws project comprises three components: in-country laboratory monitoring, re-testing of a subset of samples at partner institutions, and proficiency testing (PT). For in-country laboratory monitoring, the Study Coordinator (BLH) and capacity building lead (ST) will be involved in the initial laboratory skills training and assay validation at both the reference and district laboratories and will also participate in monitoring visits to all laboratories involved in the project. For the second component, DNA extracts of a subset of 20% of samples collected during the study will be retested with both the TPHD-LAMP and qPCR tests at a yaws-specialised laboratory at the Friedrich-Loeffler-Institut. In case of substantial variances, an on-site evaluation will be performed.

For the final EQA component we will develop PT panels for the reference laboratories performing the qPCRs and for the district laboratories performing the TPHD-LAMP tests. For this we will create two plasmids (one with the *TP polA* gene target and one with the *HD* 16SrRNA gene target). Following amplification, plasmids will be quantified using digital PCR, tested for long-term stability and the limits of detection on both the TPHD-LAMP and qPCR platforms. Cotton swabs will be spiked with HEK293 cells to simulate the human cell background of a clinical sample. Subsequently, a panel of seven swabs will be prepared, with swabs containing either one, both or none of the plasmids in different concentrations and combinations. The participating laboratories will receive a blinded PT panel transported at ambient temperature. Each laboratory will be bi-annually re-evaluated using a new set of blinded samples. The basic criteria for successful participation will be the correct identification of the NTC and the high positive samples in all triplicates, as well as an appropriate SD within the triplicates. We will implement a 3-grade evaluation system, where the participants can obtain perfect results, fulfil the minimum requirements, or fail. Only laboratories which have fulfilled the minimum requirements will be able to start or proceed with the testing of clinical samples. If a laboratory fails the PT, it will undergo a detailed quality assessment to identify critical points to take appropriate corrective measures before repeating the PT.

Ethics

We have ethical approval from the London School of Hygiene & Tropical Medicine (LSHTM) ethics committee (Reference: 21633, 19 August 2021) as well as local and national committees in each country. We were granted approval to conduct the study from Cameroon's National Ethics committee for Human Research (22 December 2020), the National Research Ethics Committee in Côte d'Ivoire (16 September 2020) and in Ghana by the Noguchi Memorial Institute for Medical Research NMIMR institutional review board (6 November 2020) and Ghana health service National Research Ethics Committee (29 April 2021). Prior to any study procedures, written informed consent will be obtained from participants or from parents or guardians if the participant is under the age of consent. Following national recommendations, informed consent will be conducted in English or French, with the assistance of a community representative translating into the local language, if necessary. If a person is illiterate, the study information and consent procedure will be clearly explained to them by a member of the study team or a local translator. Children over ten years old will also be required to give verbal assent for enrolment. If any participants wish to withdraw from the study at any point post-enrolment, that participant's information will be completely eliminated. Consent forms, approved by all relevant ethical review boards, will be available in English and French, as appropriate (see online supplemental material 1).

Data storage and security

All participants recruited will be randomly assigned a study identification number and only coded data will be analysed. Data on the: (1) number of screened individuals, (2) number of treponemal and non-treponemal antibody rapid tests conducted and (3) number of serologically confirmed cases, will be shared with national programme managers at regular intervals throughout the study. Data collected through ODK will be stored on the LSHTM ODK Central Server. Access to this will be limited to the principal investigator (MM), the international study coordinator (BLH) and the field coordinator (CG-B). Data will be made available to study personnel on reasonable request. Any data downloaded from ODK central will be stored on institutional network drives with firewalls and security measures in place. Hard copies of signed consent forms will be stored in secured offices at the reference laboratory in each country.

Dissemination

The findings of this study will be reported back to all the relevant project stakeholders. The results of the study will be published in an Open Access journal. If this study's findings indicate that the new TPHD-LAMP test could be deployed successfully to aid with yaws eradication, we will engage with WHO and national and international networks to ensure relevant policy makers, public health advisors and researchers are aware of the study and its findings.

Patient and public involvement

There was no patient and public involvement in the design of this study protocol.

DISCUSSION

Under the new NTD road map 2021–2030, yaws and dracunculiasis are the only two diseases targeted for eradication. Currently, PCR is the most sensitive and specific tool for yaws diagnosis. However, it is not always available in endemic countries, especially in the most remote

and rural areas where the disease is more prevalent. To achieve eradication, sensitive diagnostic tools that can detect a singular case of yaws are needed. This will be important both for deciding whether to implement MDA and for surveillance post-MDA. These diagnostic methods will also need to be highly specific so as to not mis-diagnose yaws resulting in unnecessary interventions, and potentially leading to the development of antimicrobial resistance.

The LAMP4yaws study will assess the accuracy of the TPHD-LAMP by conducting an evaluation of the test performed at local rural laboratories compared with the reference standard qPCR conducted at national reference laboratories. The TPHD-LAMP test has shown promising results during a laboratory evaluation but it is also important to assess its performance under programmatic conditions. Isothermal tests, such as LAMP, offer a solution to the problems faced by PCR, as they are cheap, easy to perform and can be operated close to the patient. Having a test that can be conducted locally, allows for prompt detection of yaws cases, which will be particularly important post-MDA to prevent onward transmission of remaining cases. The establishment of the EQA scheme and strengthening of in-country yaws diagnostic capacity will be an invaluable aid for future eradications efforts in our three study countries in West Africa, as well as other yaws-endemic countries globally.

There are some limitations to this study. First, the LAMP4yaws test is not a true point of care test as it must be performed within a basic laboratory facility. In its current form it also requires a cold chain. Power shortages in the district laboratories may not be uncommon, thus conducting the TPHD-LAMP assay could be hindered in these conditions. The TPHD-LAMP test also relies on reagents produced by a single company based in Europe. If the TPHD-LAMP is to be adopted by national programmes, the infrastructure to access these products easily at a low cost will be essential. The acceptability and feasibility and cost-benefit analysis will allow us to highlight which challenges require overcoming before the test can be successfully deployed.

If the TPHD-LAMP is shown to be useful, further work would include evaluating the test in other yaws endemic countries, such as Papua New Guinea and Solomon Islands, where the highest burden of yaws is found. This will allow us to ensure difference in local context and genetic variation of *TPE* will not affect the performance of the TPHD-LAMP. A recombinase polymerase amplification test for *TP* and *HD* has also recently been developed.[24] Similarly, this test relies on isothermal amplification, which can be conducted close to the patient and has performed well in a laboratory evaluation. Pursuing a clinical evaluation in a programmatic setting will also be useful.

Finally, the detection of azithromycin resistant *TPE* strains in a post-MDA setting in Papua New Guinea[9 10] has the potential to derail eradication efforts, which are primarily based on mass treatment with this antibiotic. The LAMP test is currently under development to include multiplex detection of *TP* and the point mutations that confer resistance. If resistance emerges, its prompt detection is vital to prevent transmission of these strains. A successful evaluation of this test when available could be key to allowing prompt detection of these cases.

The need for new point of care diagnostics for yaws has been on the public health agenda for years. However, this need has become more urgent since the documented emergence of azithromycin resistance. Successful evaluation of the TPHD-LAMP test, demonstrating high sensitivity and specificity, could provide an invaluable tool for the future of yaws eradication.

Author affiliations
[1]Clinical Research Department, London School of Hygiene and Tropical Medicine Faculty of Infectious and Tropical Diseases, London, UK
[2]Department of Infectious Diseases, Hospital Universitari Germans Trias i Pujol, Badalona, Spain
[3]Centre Pasteur du Cameroun, Yaounde, Cameroon
[4]University of Ghana Noguchi Memorial Institute for Medical Research, Accra, Greater Accra, Ghana
[5]National Program of African Trypanosomiasis Elimination, Abidjan, Côte d'Ivoire
[6]Institut Pasteur de Cote d'Ivoire, Abidjan, Lagunes, Côte d'Ivoire
[7]Mast Diagnostica GmbH, Reinfeld, Germany
[8]IMTEK - Department of Microsystems Engineering, University of Freiburg, Freiburg im Breisgau, Germany
[9]Institute of International Animal Health/One Health, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany
[10]National Buruli Ulcer, Leprosy, Yaws and Leishmaniasis Control Program, Ministry of Public Health, Yaounde, Centre Region, Cameroon
[11]Public Health & Epidemiology, University of Dschang, Yaounde, West Region, Cameroon
[12]Experimental Bacteriology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar
[13]Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana
[14]Hospital for Tropical Diseases, London, UK

Acknowledgements We are grateful to all the district healthcare workers and laboratory technicians that will be involved in screening and enrolling participants and performing the laboratory tests vital to this study. We are also grateful to Esther Amon for her administrative support during this project.

Contributors EMH-E, SK, OM, MM conceived the idea for the study. MB, EL, LB, TH, SF, NB designed the LAMP assay. TC and ST accompanied the development of the training plan and validation of the reference laboratory qPCR methods. SK, CR and SL developed the EQA plan for the study. CG-B, BLH, ST, LAB, KAH, SNK, SE, KKA, EMH-E and EMH-E contributed expertise for the participant enrolment and field implementation. ST, IA, JPN-N, AS, MSK-S, SNK, SE, KKA and BLH contributed expertise on the laboratory aspects of the project. DA, AT, PA contributed to the design of the social science studies for the project. BLH drafted this protocol manuscript. CG-B, ST, LAB, KAH, MB, EL, LB, TH, SF, NB, CR, ST, TC, IA, JPN-N, AS, MSK-S, EMH-E, SL, AT, DA, PA, SNK, SE, KKA, EMH-E, SK, OM and MM read and approved the final draft of this manuscript.

Funding The LAMP4yaws study is fully funded by the European and Developing Countries Clinical Trial Partnership. Grant number: RIA2018D-2495

Competing interests BLH, GCB, ST, LAB, KOH, LB, NB, TC, TH, SL, CR, IA, AS, MSKS, JPNN, PA, AT, ENT, DKA, SNK, SE, KWA, EMHE, SK, OM and MM declare no competing interests. SF, MB and EL are employees of Mast Diagnostics GmbH, which produce and sell the TPHD LAMP kits and products. A patent covering the

mediator displacement probe technique a component of the TPHD-LAMP assay described in the paper has been applied for by the University of Freiburg and Hahn-Schickard.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Becca Louise Handley http://orcid.org/0000-0003-2208-8229
Emma Michele Harding-Esch http://orcid.org/0000-0002-1432-8109
Michael Marks http://orcid.org/0000-0002-7585-4743

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Lamp4yaws: Participant information sheet

Project PI: Michael Marks, London School of Hygiene and Tropical Medicine

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Why are we completing this study?

Yaws is a highly contagious infection and people can get it through direct contact with infected individuals. The condition starts with an ulcer on the skin. If left untreated, the ulcer can develop into deformities on the bone that can last forever. There is a global effort to eliminate yaws from communities by 2030. Sometimes it is not easy to tell if a person has yaws as there are many different germs/bacteria that cause skin ulcers. A new test has been made to see if a person has yaws. We want to know if this test is good enough to detect yaws, and for this, we need to collect samples of people with skin ulcers. We will be recruiting participants in three countries: Cameroon, Cote D'Ivoire and Ghana between 2020-2022.

Why have I been chosen?

Today, we are in your village because yaws occurs here. Our aim is to find 200 people with ulcers and positive blood tests in each country.

What will taking part involve?

We will perform a quick skin examination of the arms, legs and torso to see if you have any signs of yaws on your body and we may perform a finger prick to collect up to six drops of blood from you. If you do have signs of yaws we will also use your blood sample to test to see if you have yaws in your blood. If this test is positive we will also collect some cotton swabs by rubbing the lesion. If you have lesions we will offer you azithromycin antibiotic or refer you to services for free treatment. This drug is safe and is used to treat many other of illnesses. The samples you provide will be tested for yaws within the country. We may send some of your samples to our European partners to improve the test and find out what other germs may be causing lesions or may be common in your community.

After taking your sample, we will come back within 4 weeks to check whether the skin lesion has healed up or not. If the lesion has not healed we may collect more swabs and we will offer you a different drug to treat the lesion.

Do I have to take part?

It is entirely up to you to decide to join the study. We will describe the study and go through the information sheet. If you agree for you or your dependent to take part, we will then ask you to sign a consent form. You or your dependent is free to withdraw at any time, without giving a reason. Even if you refuse to take part in the study we will refer you for free treatment for your ulcer.

What are the possible disadvantages and risks of taking part?

There are very few risks to taking part in this survey. The finger pricks and the ulcer swab can be uncomfortable. We have used these procedures in many surveys and no serious risks have emerged.

What are the possible benefits of taking part
If you are found to have ulcers we will offer a free treatment, or refer you to services for free treatment. This treatment should get rid of these ulcers. The information we get will help improve the diagnosis of people with yaws in all around the world.

What happens when the research study stops?
After the research study stops, the findings of the survey will be published in an easily accessible format. We may store the blood (up to 6 drops) and swabs indefinitely in case anyone should question our results. We may also anonymously test those samples in other related studies.

Photographs may be used for teaching and research purposes, and may be published in open access format meaning they may become available on the internet. However, if photographs are published, we would ensure that the photographs would not be linkable to you as an individual.

Will my taking part in the study be kept confidential?
Yes. All information collected about you or your dependent during the course of the research will be kept strictly confidential.

If you or your dependent joins the study, some parts of your medical records and the data collected for the study may be looked at by authorised persons from the London School of Hygiene & Tropical Medicine. They may also be looked at by representatives of regulatory authorities and by authorised people from the Ministry of Health to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time. If you withdraw from the study, we will destroy all identifiable samples, but we will need to use the data collected up to your withdrawal.

What will happen to the results of the research study?
The results of the study will be published, and shared with healthcare bodies in Ghana, Cote d'Ivoire and Cameroon and the wider international community for the purposes of making policy decisions with the Ministry of Health.

Who is organising and funding the research?
The European and Developing Countries Clinical Trials partnership are paying for the study. The study is being designed and coordinated by, the London School of Hygiene & Tropical Medicine.

Who has reviewed the study?
This study was given a favourable ethical opinion by the London School of Hygiene & Tropical Medicine Research Ethics Committee and the National Health and Research Ethics Committee (Reference: 21633)

Contact Details
Should you have any questions or worries about the project, please feel free to contact any one of the study investigators:
Rebecca Handley (Study coordinator) Rebecca.handley1@lshtm.ac.uk +44207 927 2866

Michael Marks (Principle investigator) Michael.marks@lshtm.ac.uk +44207 927 2457

CONSENT SIGNATURE FORM

Stick ID barcode label here

Title of Research:

Loop mediated isothermal amplification test development, implementation and evaluation for yaws eradication

Statement

☐ I have read the information on this study/research or have had it translated into a language I understand. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.

☐ I understand that my (my child's) participation is voluntary. I understand I may withdraw from the study at any time without giving a reason and that this will not affect my (my child's) normal care.

☐ I understand that the information or tissue sample collected about/from me(my child) will be stored indefinitely and may be used to support other research in the future, and may be shared anonymously with other researchers, for their ethically-approved projects.

☐ I give consent for lesion photographs taken of me or my child to be taken and used for the purposes of this study, which may involve dissemination to relevant external parties and potential publication online. These will not show any identifiable features.

☐ I know enough about the above named study and agree to take part.

To check appropriate one:

☐ *(A) Participant:*

NAME OF PARTICIPANT_____

SIGNATURE/THUMB PRINT OF PARTICIPANT: _____

☐ (B) *If participant is under the age of consent)*

GUARDIAN'S NAME:_____

GUARDIAN'SSIGNATURE/*THUMBPRINT*:_____

RELATIONSHIP TO THE CHILD: FATHER [ ] MOTHER [ ] OTHER [ ]

Translator (if applicable): I have read this form and the information sheet to the above person and am sure that he/she has understood what is required of someone enrolling in this study and that they agree to enrol in the study.

Signed:…… Date:

Participant information sheet
Lamp4yaws – Social Science studies

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to talk to others about the study, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Why are we completing this study?
Yaws is a highly contagious infection and people can get it through direct contact with infected individuals. The condition starts with an ulcer on the skin. If left untreated, the ulcer can develop into deformities on the bone that can last forever. There is a global effort to eliminate yaws from communities by 2030. We are currently conducting a large study to assess the reliability of a new diagnostic test, alongside this we also want to learn more about peoples understanding of yaws and other skin conditions. We also want to find out if and where people may seek treatments and opinions about yaws diagnostics tests and treatments.

Why have I been chosen?
You have been chosen because you live in a yaws endemic community, or you are a healthcare worker, laboratory scientist of key stakeholder that may be involved in yaws control programs or yaws surveillance.

What will taking part involve?
We are inviting you (or your dependent) to take part in either focus groups discussions or small group/individual interviews about yaws. If you take part these sessions will be recorded with a digital recorder. We estimate that each interview/focus group discussion will last around 60-90 minutes to complete. In the interview/focus group discussion we will ask you (or your dependent) about your (their) experience with yaws in the community. We will also ask you (or your dependent) about your (their) personal experiences relating to care and treatment of yaws and other skin diseases.

Do I have to take part?
It is entirely up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree for you or your dependent to take part, we will then ask you to sign a consent form. You or your dependent are free to withdraw at any time, without giving a reason.

What are the possible disadvantages and risks of taking part?
There are very few risks to taking part in this survey. We will be asking you (your dependant) questions about your (their) daily experience. You (they) don't have to answer questions you (they) don't want to answer. If you feel the need to talk or confide in someone after the group discussion, we can refer you to the appropriate services.

What are the possible benefits of taking part?
There are no direct benefits to you taking part in this study but the information we get will help improve the diagnosis of people with yaws in all around the world.

Will my taking part in the study be kept confidential?

Yes. All information collected about you or your dependent during the course of the research will be kept strictly confidential. All researchers will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site. Your name will not be linked to any reports or articles. The data from this interview will remain confidential and protected. After the interview, we will transfer the information to a password-protected computer and destroy the contents of the recording device.

What will happen if I don't want to carry on with the study?

You can decide whether or not to participate in the group discussion and you are free to withdraw from the study at any time. This will have no influence on your participation in any other research, or any other services you are currently receiving. You can stop the study at any time without any sanction.

What happens when the research study stops?

After the research study stops, the results of the study will be published, and shared with healthcare bodies in the Ghana, Cote d'Ivoire and Cameroon and the wider international community for the purposes of making policy decisions with the Ministry of Health. We may store the information you provided indefinitely, and this may be used by other researchers for future studies. None of the information used by other researchers will be identifiable.

Who is organising and funding the research?

The European and Developing Countries Clinical Trials partnership are paying for the study. The study is being designed and coordinated by, the London School of Hygiene & Tropical Medicine.

Who has reviewed the study?

[**ONCE APPROVED:** This study was given a favourable ethical opinion by the London School of Hygiene & Tropical Medicine Research Ethics Committee and the National Health and Research Ethics Committee.]

Contact Details

Should you have any questions or worries about the project, please feel free to contact any one of the study investigators:

Rebecca Handley (Study coordinator) Rebecca.handley1@lshtm.ac.uk +44207 927 2866
Michael Marks (Principle investigator) Michael.marks@lshtm.ac.uk +44207 927 2457

CONSENT SIGNATURE FORM – SUB-STUDY 4

Stick ID barcode label here

Title of Research:

Loop mediated isothermal amplification test development, implementation and evaluation for yaws eradication

Statement

- ☐ I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction and my questions concerning this study have been answered.
- ☐ I understand that my (my child's) participation is voluntary. I understand I may withdraw from the study at any time without giving a reason and that this will not affect my (my child's) normal care.
- ☐ I understand that the information collected about me (my child) will be used to support other research in the future, and may be shared anonymously with other researchers, for their ethically-approved projects.
- ☐ I give consent to be recorded for this study
- ☐ I know enough about the above named study and agree to take part.

To check appropriate one:

☐ *(A) Participant:*

NAME OF PARTICIPANT_____

SIGNATURE/THUMB PRINT OF PARTICIPANT: _____

☐ (B) *If participant is under 18 years)*

GUARDIAN'S NAME:_____

GUARDIAN'SSIGNATURE/*THUMBPRINT*: _____

RELATIONSHIP TO THE CHILD: [ ] FATHER [ ] MOTHER [ ] OTHER

Witness: I have read this form and the information form to the above person and am sure that he/she has understood what is required of someone enrolling in this study and they agree to enroll in the study.

Signed:…… Date:……