PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (http://bmjopen.bmj.com/site/about/resources/checklist.pdf) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

| TITLE (PROVISIONAL) | LAMP4YAWS - TREPONEMA PALLIDUM, HAEMOPHILUS DUCREYI LOOP MEDIATED ISOTHERMAL AMPLIFICATION: PROTOCOL FOR A CROSS-SECTIONAL, OBSERVATIONAL, DIAGNOSTIC ACCURACY STUDY |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AUTHORS             | Handley, Becca; González-Beiras, Camila; Tchatchouang, Serges; Basing, Laud; Hugues, Kouadio; Bakheit, Mohammed; Becherer, Lisa; Ries, Christina; Njih Tabah, Earnest; Crucitti, Tania; Borst, Nadine; Lüert, Simone; Frischmann, Sieghard; Haerpfer, Tamara; Landmann, Emelie; Amanor, Ivy; Sylva, Aboubacar; Kouamé-Sina, Mireille S.; Ndzomo-Ngono, Jean P.; Tano, Adingra; Arhinful, Daniel; Awondo, Patrick; Ngazoa Kakou, Solange; Eyangoh, Sara; Addo, Kennedy Kwasi; Harding-Esch, Emma; Knauf, Sascha; Mitjà, Oriol; Marks, Michael |

GENERAL COMMENTS

In this study, the authors described a protocol for a clinical evaluation of a new combined diagnostic test - Treponema pallidum and Haemophilus ducreyi loop mediated isothermal amplification. In recent years, isothermal amplification detection has been widely used in the detection of various pathogenic microorganisms. In recent years, isothermal amplification detection has been widely used in the detection of various pathogenic microorganisms. But there are still some comments need to consider.

1. The authors intend to screen approximately 60,000 individuals to identify an average 600 serology-positive and 210 qPCR-positive cases for primary analysis. How long is the project duration and whether the sample size can be guaranteed? It would be better if samples in the incubation period and after treatment, at high concentration, could be collected.

2. In particular, it is very easy to cause contamination of LAMP assay. How did the protocol avoid the occurrence of contamination by aerosol (especially at high concentration template)?

3. The extracted DNA can be divided into two parts for amplification in two different laboratories, and then the test results were compared the consistency.

REVIEWER | Nzelu, Chukwunonso  
University of Calgary, Microbiology, Immunology, and Infectious Diseases |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| REVIEW RETURNED | 24-Dec-2021 |
GENERAL COMMENTS

The protocol manuscript by Handley et al. provides an elaborative overview of LAMP4yaws project for evaluation of the novel TPHD-LAMP test in yaws-endemic areas of Ghana, Cote d’Ivoire and Cameroon. The manuscript reads well, all sections are well delineated and concise. The authors also outlined the study objectives, testable hypotheses and the study limitations. To my knowledge, the authors have covered a decent range of detailed study plan.

I have the following comments:
- The authors should specify the age of consent in the methods section.
- Under Data and sample collection for Objective 1: State, if any, the distinct features of yaw lesion to look out for.
- Indicate clearly if the sample collection will be done by clinicians only?
- Line 40-46: “……….for example, if they are early in an infection” The authors should explain the purpose for this negative serology sample collection. Is it to assess the sensitivity of the test in early infection detection?
- Laboratory analysis section: indicate the concentration/volume of DNA template to be used.
- The authors should provide reasons for the choice of the 16S rRNA genes targets for TP and HP.
- Under External Quality Assurance: Using the word “on-site” monitoring can be confused to mean field site monitoring or surveillance. It is important to note that district laboratory test can not be referred to on/field-site test. Therefore, the authors should change the word “on-site” to “district laboratory monitoring” all through the manuscript.
- The authors should provide the proposed dates (months/year) of the study.
- Under the Ethics: Correct the typo; “Children over ten years “of” will also……” Remove “of”

Minor comment: Line numbering, the Continuous option instead of Restart each page option is better for manuscript reviews.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. J. Yuan, Capital Institute of Paediatrics

Comments to the Author:
In this study, the authors described a protocol for a clinical evaluation of a new combined diagnostic test - Treponema pallidum and Haemophilus ducreyi loop mediated isothermal amplification. In recent years, isothermal amplification detection has been widely used in the detection of various pathogenic microorganisms. But there are still some comments need to consider.

1. The authors intend to screen approximately 60,000 individuals to identify an average 600 serology-positive and 210 qPCR-positive cases for primary analysis. How long is the project duration and whether the sample size can be guaranteed? It would be better if samples in the incubation period and after treatment, at high concentration, could be collected.

Cameroon, Cote d’Ivoire and Ghana were selected for the study as they have the highest prevalence of yaws in West Africa. Based on national reporting data within each country and with participant recruitment allocated for 18 months (April 2021 – September 2022) we expect to reach the desired...
sample size in this time. We have included the sample recruitment timeframe on page 5, lines 139-142. We are focusing on collecting only patients with active yaws presenting with lesions as we require lesion swabs, harbouring bacteria, for the evaluation of the TPHD LAMP.

2. In particular, it is very easy to cause contamination of LAMP assay. How did the protocol avoid the occurrence of contamination by aerosol (especially at high concentration template)?

All lab technicians involved in the study will undergo intense training to ensure that they were confident at performing the techniques and in taking appropriate precautions needed to avoid contamination. As contamination cannot always be avoided, appropriate controls are included in each run to detect potential contamination. These include no template controls (ddH2O) and negative extraction controls (extracted lysis buffer). If discordant results are detected for the controls, the assay will be run again. If discordant results are repeated, the laboratory technicians are instructed to contact the study coordinator so an investigation into the source of potential contamination can be carried out. We have made this clearer in the training subsection (Page 8, lines 216-219) and the laboratory analysis section (Page 9, lines 235-236).

3. The extracted DNA can be divided into two parts for amplification in two different laboratories, and then the test results were compared the consistency.

There has previously been a laboratory evaluation of the TPHD LAMP performed as the reviewer suggests (ref 8 in paper). The LAMP4yaws study is a real-world clinical evaluation of all stages of test performance including DNA extraction, sample processing and testing. Therefore, this requires both the district and reference laboratory to perform each step independently.

Reviewer: 2
Dr. Chukwunonso Nzelu, University of Calgary

Comments to the Author:

The protocol manuscript by Handley et al. provides an elaborative overview of LAMP4yaws project for evaluation of the novel TPHD-LAMP test in yaws-endemic areas of Ghana, Cote d’ Ivoire and Cameroon. The manuscript reads well, all sections are well delineated and concise. The authors also outlined the study objectives, testable hypotheses and the study limitations. To my knowledge, the authors have covered a decent range of detailed study plan.

1. The authors should specify the age of consent in the methods section.
We have now included this in the manuscript (Page 6, lines 148-149)

2. Under Data and sample collection for Objective 1: State, if any, the distinct features of yaw lesion to look out for.
We have included a brief description of the main clinical features of active yaws (Page 6, lines 161-162)

3. Indicate clearly if the sample collection will be done by clinicians only?
Trained healthcare workers will be responsible for the screening and enrolment of all participants. We have included this on page 6 lines 163-164.

4. Line 40-46: “……….for example, if they are early in an infection” The authors should explain the purpose for this negative serology sample collection. Is it to assess the sensitivity of the test in early infection detection?

It is recognised that in the first few weeks of infection people can have detectable levels of bacteria by molecular tests but not by serology. This has previously been demonstrated by PCR but not yet using LAMP, therefore we will collect samples from a subset of these people to see if we can demonstrate this. We have indicated this in the document (page 6, lines 175-176)
5. Laboratory analysis section: indicate the concentration/volume of DNA template to be used. We have included this in both the district lab analysis and reference lab analysis sections (page 9, line 233 and page 10, line 253).

6. The authors should provide reasons for the choice of the 16S rRNA genes targets for TP and HP. The selected targets are based on previously published and validated assays. Previous works validating these targets are provided in the paper. For both pathogens this is reference 22 in the paper. We have clarified this on page 9, line 258.

7. Under External Quality Assurance: Using the word “on-site” monitoring can be confused to mean field site monitoring or surveillance. It is important to note that district laboratory test can not be referred to on/field-site test. Therefore, the authors should change the word “on-site” to “district laboratory monitoring” all through the manuscript. We have changed “on-site monitoring” to “in-country laboratory monitoring” as this involved both training and monitoring of the reference laboratories as well as the districts labs in each country. (Page 11, lines 286, 287 and 289)

8. The authors should provide the proposed dates (months/year) of the study. We have included the study time frame in the methods section (page 5, line 139-142).

9. Under the Ethics: Correct the typo; “Children over ten years “of” will also……” Remove “of” We have corrected this typo (page 11, line 322) and the document has been thoroughly proofread to look for any other grammatical errors and typos, which have been corrected if detected.

10. Minor comment: Line numbering, the Continuous option instead of Restart each page option is better for manuscript reviews. We have included continuous line numbers in the document.

**VERSION 2 – REVIEW**

| REVIEWER                  | Yuan, J.  
|                          | Capital Institute of Paediatrics |
| REVIEW RETURNED          | 18-Feb-2022 |

| GENERAL COMMENTS        | No further comments. |

| REVIEWER                  | Nzelu, Chukwunonso  
|                          | University of Calgary, Microbiology, Immunology, and Infectious Diseases |
| REVIEW RETURNED          | 01-Feb-2022 |

| GENERAL COMMENTS        | In the revised version of their manuscript, the authors have addressed the deficiencies and performed the required amendments. Authors responded to all reviewers comments. Acceptance of the manuscript for publication is recommended. |