Detection of *Treponema pallidum* DNA from whole blood and earlobe specimens in patients from two STI clinics in Lima, Peru

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BACKGROUND

Epidemiology

- *Treponema pallidum* causes >10 million new syphilis infections worldwide each year[1].
- Within Latin America, syphilis is **concentrated in high-risk populations** including sex workers, men who have sex with men (MSM) and transgender women (TW)[2].
- In Peru, prevalence is **>20% in MSM/TW**[3].

Diagnosis

- Diagnosis of syphilis in Peru relies on a **syndromic approach**.
- **Disadvantage:** many are asymptomatic and those who are symptomatic may remain sexually active without seeking treatment[4]. There are also high reinfection rates, especially in high risk groups[5].
- **Laboratory diagnosis** relies on treponemal (TP-PA) and non-treponemal (RPR or VDRL) tests. However, it can be difficult to distinguish between patients who are truly cured after treatment and those who may continue to be infected, especially those at high risk of reinfection.

Molecular Detection

- Recently, assays that can confirm infection by directly detecting ***T. pallidum* DNA** have been developed.
- Most commonly used to detect *T. pallidum* DNA in active ulcerations.
- *T. pallidum* DNA can also be detected in **whole blood and ear lobe scraping samples**. In two studies, DNA was detected in 56-76% of ear lobe scrapings, and 38.1-59.1% of whole blood samples from patients infected with syphilis[6,7].
- *T. pallidum* has a predilection for hiding in capillaries during the latent phase of syphilis, and since the ear lobe is rich in capillaries and poor in sensory nerves, it is a promising site for collection of *T. pallidum* DNA[6].
- A number of genes have been used as targets for detection, including the TpN47*, polA, bmp, tmpC, tmpA,* and 16SrRNA genes[8].

AIM:

In this study, we set out to detect *T. pallidum* DNA in whole blood and ear lobe samples from MSM and TW diagnosed with syphilis in two STI clinics in Lima, Peru.

METHODS

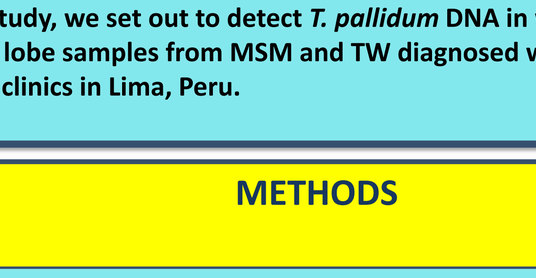
Participants:

Data were obtained from baseline samples collected for a prospective cohort study of 401 men who have sex with men (MSM) and transgender women (TW) from 2 STI clinics in Lima, Peru.

Testing and specimen collection:

1. HIV testing: Participants were tested using rapid HIV testing (Alere Determine, USA), a combined Antigen/Antibody HIV EIA, and Western blot confirmation (Genscreen ULTRA HIV Ag-Ab and Genetic Systems HIV-1 Western Blot, Bio Rad, USA).
2. Syphilis infection was assessed with RPR (BD Macro-Vue, USA) and TP-PA (Fujirebio, Japan).
3. Whole blood samples and earlobe capillary blood were collected from patients with a positive RPR and TP-PA (Figure 1).

FIGURE 1:



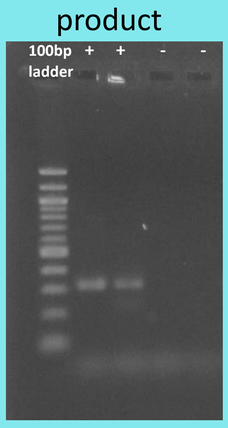
Earlobe is pierced with a lancet, capillary blood is expressed and collected on a sterile dacron swab, then placed in buffer.

Molecular detection of *T. pallidum* DNA:

1. DNA extraction was performed using the QIAamp mini kit (Qiagen, Valencia, CA), and samples were then concentrated.
2. Using specific primers for the TpN47 gene[9], an aliquot of the DNA sample was amplified using conventional PCR for participants with high RPR titers (≥1:16).
3. Positivity was determined by visual detection of PCR product on 1% agarose gel (Figure 2). Positive (*T.pallidum* Nichols strain) and negative (water) controls were also run to verify the success of the PCR reaction. Positive samples were repeated four times for confirmation of DNA detection.
4. PCR against the beta globin gene was also run as a positive control to verify the success of DNA extraction (not shown).

FIGURE 2:

Sample gel showing 310bp TpN47 PCR product



Negative and Positive Controls

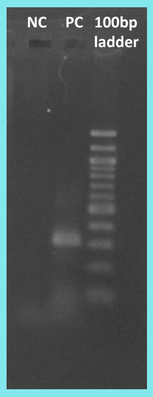


FIGURE 3:

Number of participants by RPR titer

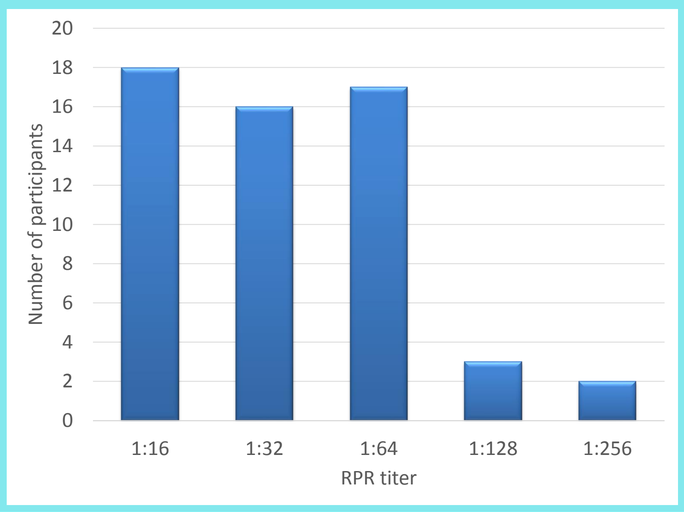
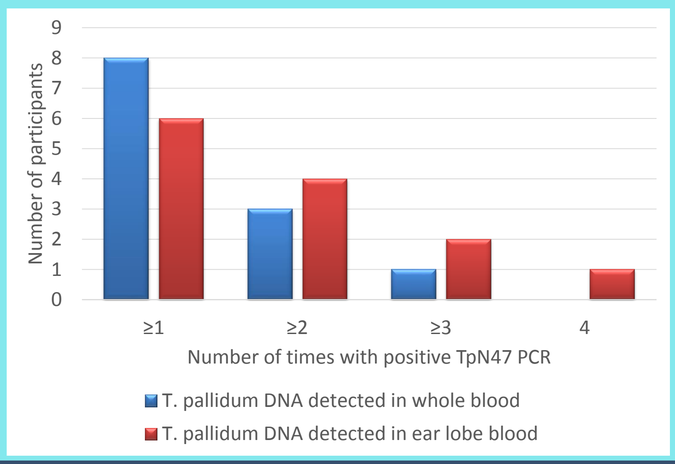


FIGURE 4:

Number of participants with *T. pallidum* DNA detected in whole blood and ear lobe blood, stratified by number of positive TpN47 PCR reactions



RESULTS

56 participants had RPR titer ≥1:16 (1:16(n=18), 1:32(n=16), 1:64(n=17), 1:128(n=3), and 1:256(n=2)) (Figure 3). All had positive TP-PA tests.

Depending on the threshold for positivity (≥1-4 positive TpN47 PCRs), the number of participants with detectable TpN47 DNA ranged from 0-8 for whole blood, and 1-6 for ear lobe blood (Figure 4).

A total of 8 (14.3%) participants had *T.pallidum* DNA detected from whole blood, and 6 (10.7%) from earlobe capillary blood.

Because there was *T.pallidum* DNA detected in both the whole blood and earlobe samples for some participants, the total number of participants with detectable DNA from either source in at least 1 PCR reaction was 10. Of these 10 participants, 5 were HIV positive, and 5 were HIV negative.

All beta globin PCR reactions (positive controls) were positive.

DISCUSSION

Overall, only **a small proportion (17.9%)** of our participants had *T. pallidum* DNA detected by TpN47 PCR in either whole blood or ear lobe blood. This is less than other studies which have found greater success in isolating *T. pallidum* DNA from both whole blood and ear lobe scrapings[6,7,8].

Some **possible explanations for the low yield** could include:

- **Methods of specimen collection**. We used capillary blood collected from the earlobe instead of ear lobe scrapings[6,7]. Ear lobe scrapings were avoided because of fear of causing a stigmatizing scar on our MSM and TW participants.

- **Small amounts of DNA in tissue**. Because we anticipated that very small amounts of DNA would be present in the earlobe and blood specimens, we concentrated the extracted DNA samples before using them in the PCR reactions. Nevertheless, the yield still proved to be low.

In addition to low yield, there was an issue of **inconsistent results** when TpN47 PCR was repeated on the same specimen. This could be due to:

- Low amounts of DNA in the original sample
- Degradation of the small amount of DNA over the time that the repeated reactions were run.

Further attempts to verify the amplified PCR product could be undertaken through other techniques such as sequencing or restriction enzyme digest.

Next Steps:

- Optimize collection of samples, extraction of DNA, and detection of *T. pallidum* DNA to improve yield
- Analysis with a larger number of samples may allow determination of clinical/participant characteristics that may predict likelihood of DNA detection.
- Detection of *T. pallidum* DNA continues to be an informative technique because it is the first step in the molecular characterization of *T. pallidum*, including macrolide resistance and subtyping.

CONCLUSION:

We were able to isolate *T. pallidum* DNA from a small number of whole blood and ear lobe samples collected from patients with syphilis. Further work is needed to determine how to improve this yield and to determine factors associated with DNA detection.

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