Detection of *Treponema pallidum* DNA During Early Syphilis Stages in Peripheral Blood, Oropharynx, Ano-Rectum and Urine as a Proxy for Transmissibility

Silvia A. Nieuwenburg,1,a Hélène C. A. Zondag,1,a Sylvia M. Bruisten,1,a Vita W. Jongen,1 Maarten F. Schim van der Loeff,1,b,2 Alje P. van Dam,1,a,b and Henry J. C. de Vries1,b,a

1Department of Infectious Diseases, Public Health Service Amsterdam, Amsterdam, The Netherlands; 2Amsterdam Institute for Infection and Immunity (AII), Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; 3Department of Internal Medicine, Division of Infectious Diseases, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; 4Department of Medical Microbiology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; 5Department of Dermatology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

**Background.** Syphilis diagnosis may be challenging, especially in the asymptomatic and early clinical stages. We evaluated the presence of *Treponema pallidum* DNA (TP-DNA) in various sample types to elucidate transmissibility during various syphilis stages.

**Methods.** The study was conducted at the Amsterdam Centre for Sexual Health. We included adult men who have sex with men (MSM), who were suspected of having syphilis. The 2020 European guidelines definitions were followed for the diagnosis and staging of syphilis. Using a polymerase chain reaction (PCR) targeting the *polA* gene of *Treponema pallidum* (TP-PCR), we tested the following study samples on TP-DNA: peripheral blood, oropharyngeal swab, ano-rectal swab, and urine.

**Results.** From November 2018 to December 2019 we included 293 MSM. Seventy clients had primary syphilis, 73 secondary syphilis, 86 early latent syphilis, 14 late latent syphilis, 23 treated syphilis, and 27 had no syphilis. TP-DNA was detected in at least 1 study sample in 35/70 clients with primary syphilis (2/70 peripheral blood, 7/70 oropharynx, 13/70 ano-rectum, and 24/70 urine); in 62/73 clients with secondary syphilis (15/73 peripheral blood, 47/73 oropharynx, 37/73 ano-rectum, and 26/73 urine); and in 29/86 clients with early latent syphilis (5/86 peripheral blood, 21/86 oropharynx, 11/86 ano-rectum, and 6/86 urine). TP-DNA was not detected in clients with late latent syphilis or treated syphilis, nor in clients without syphilis.

**Conclusions.** TP-DNA was frequently detected in various sample types in the absence of lesions. This is in line with the high transmissibility of syphilis and opens diagnostic opportunities for early presymptomatic syphilis stages.

**Keywords.** *Treponema pallidum*; syphilis; homosexuality; male.
Detection of Treponema pallidum DNA During Early Syphilis Stages

**METHODS**

**Study Population and Study Design**

The Center for Sexual Health of the Amsterdam Public Health Service, The Netherlands, is a low-threshold sexually transmitted infection (STI) clinic performing approximately 50,000 consultations annually. Consultations are at the client's own initiative, anonymous and free of charge. From November 2018 through December 2019 participants were recruited among clients aged 18 years and older visiting the STI clinic.

Clients were eligible for participation if they were MSM and (1) had signs and symptoms suggestive of primary syphilis or secondary syphilis or (2) were diagnosed with early latent or late latent syphilis. Clients belonging to the first group were invited on their first visit (Supplementary Figure 1). Patients belonging to the second group were invited at their follow-up clinic visit.

Upon consent, we collected peripheral blood, client-obtained oropharyngeal and ano-rectal swab, and urine (as proxy for urethra) in patients with early syphilis. This to improve syphilis diagnostic options and elucidate transmissibility during various syphilis stages.

In the event of a symptomatic participant with a negative initial DFM, negative PCR ulcer swabs, and negative serology but 1 or more positive PCR results in the study samples, such patients were followed up to exclude incubating syphilis. Follow-up would in that case be at 3, 6, and 12 weeks after inclusion with tests at each visit by PCR on peripheral blood, oropharyngeal swab, ano-rectal swab, urine, and by serology.

Every client included in this study was examined by a physician for the staging of syphilis. The oral cavity was examined under direct visualization. External examination of the anus was done. If clinically indicated, proctoscopy was done.

**Treponema Pallidum Diagnostics**

In patients with suspected primary syphilis, DFM on ulcer exudate was performed. Moreover, an ulcer swab for DNA extraction and real-time PCR targeting the polA gene was routinely collected [13, 14]. This TP-PCR was considered positive when the fluorescent signal was clearly visible, and the cycle threshold (Ct) was <36. For a Ct value between 36 and 40 the DNA extraction was repeated, and both the first and the second DNA extract were (re)tested in the TP-DNA assay. When the Ct value was ≤40, the result was positive for *Treponema pallidum* DNA detection. A Ct value >40 was considered a negative result.

Routine diagnostics at the Public Health Laboratory in Amsterdam include 3 routine serological tests: a treponemal antibody test (CLIA; Diasorin) used for screening and 2 confirmatory tests: the non-treponemal quantitative rapid plasma reagin (RPR) flocculation test (RPR-Nosticon II; bioMérieux) and the immunoblot (Inno-LIA). The immunoblot was only done if the CLIA value was ≤30. A previous analysis in our department showed that immunoblots were always positive in serum samples with a CLIA value >30 (unpublished data). All serological tests were performed according to the specifications of the manufacturers.
DNA Isolation and PCR Testing
DNA was extracted from oropharyngeal and ano-rectal dry swab samples using isopropanol precipitation after which the DNA pellet was dissolved in 50 µL of T10 buffer (10 mM Tris-HCl, pH 8.0) [30]. From March 2019, the DNA from the swabs was isolated using the MagNA Pure 24 (Roche Molecular Systems), according to the specifications of the manufacturers, after in-house validation to ensure fully comparable results.

DNA from urine and peripheral blood samples was extracted within 24 hours of collection. From the urine samples 1ml was centrifuged at 14 000 rpm for 10 minutes. The residual fluid was removed and the pellet resuspended with 200 µL PBS and 500 µL easyMAG Lysisbuffer (BioMerieux) containing 0.2% glycogen after which isopropanol precipitation was performed [31]. DNA was extracted from peripheral blood using the QIAamp Blood Mini Kit following the protocol from the manufacturer (Qiagen).

All extracted DNA samples were tested using the TP-DNA PCR assay. Simultaneously, all samples were spiked with pho-cine herpesvirus 1 (PhHV) and tested as inhibition control in the PCR. PCR on DNA extracted from anal swabs frequently showed inhibition and was therefore always simultaneously tested in a 1:10 dilution.

Statistical Analysis
We described categorical variables using count data and proportions. Continuous variables were described using their median and interquartile range (IQR). The Fisher exact test was used to compare proportions within categorical variables. The Wilcoxon rank-sum test was used to compare the distribution of continuous variables between groups. We estimated odds ratios (OR) and their 95% confidence intervals (CI) using univariable and multivariable logistic regression to assess associations between determinants and TP-DNA detection in at least 1 sample type.

We carried out analyses using Stata (v15.1, StataCorp, College Station, Texas, USA).

Ethical Statement
This study was approved by the Medical Ethics Committee of the Amsterdam University Medical Center (NL66419.018.18, 2018_236).

RESULTS
Between November 2018 and December 2019, we included 322 participants in the study, of whom 29 participants did not meet the inclusion criteria (Figure 1). Thus, 293 MSM were included
| Table 1. Sociodemographic and Clinical Characteristics of Male Patients Included in the Trepoli study (n = 293) by Syphilis Stage, Amsterdam, The Netherlands, November 2018 to December 2019 |
|---|---|---|---|---|---|---|
| | No Syphilis (n = 27) | Primary (n = 70) | Secondary (n = 73) | Early Latent (n = 86) | Late Latent (n = 14) | Treated Syphilis (n = 23) |
| Demographics | | | | | | |
| Age, y | | | | | | |
| Median [IQR] | 35 [27–43] | 41 [33–50] | 39 [33–48] | 40 [30–52] | 35 [26–42] | 41 [35–48] |
| <35 y | 13 | 48% | 22 | 31% | 24 | 33% | 30 | 35% | 7 | 50% | 5 | 22% |
| 35–44 y | 8 | 30% | 21 | 30% | 25 | 34% | 21 | 24% | 6 | 43% | 9 | 39% |
| ≥45 y | 6 | 22% | 27 | 39% | 24 | 33% | 35 | 41% | 1 | 7% | 9 | 39% |
| Country of origin | | | | | | |
| The Netherlands | 16 | 59% | 46 | 66% | 36 | 50% | 51 | 59% | 5 | 36% | 13 | 57% |
| Other | 11 | 41% | 24 | 34% | 36 | 50% | 35 | 41% | 9 | 64% | 10 | 43% |
| Education | | | | | | |
| None/primary/secondary | 2 | 8% | 15 | 23% | 16 | 27% | 20 | 25% | 4 | 3% | 3 | 15% |
| College/university | 24 | 92% | 49 | 75% | 41 | 68% | 60 | 74% | 8 | 67% | 16 | 80% |
| Other | 0 | 0% | 1 | 2% | 3 | 5% | 1 | 1% | 0 | 0% | 1 | 5% |
| Sexual behavior | | | | | | |
| Gender of sex partners | | | | | | |
| Men | 26 | 96% | 68 | 97% | 73 | 100% | 85 | 99% | 13 | 93% | 22 | 96% |
| Men and women | 1 | 4% | 2 | 3% | 0 | 0% | 1 | 1% | 1 | 7% | 1 | 4% |
| No. of sexual partners (6 mo) | | | | | | |
| Median [IQR] | 4 [3–15] | 8 [4–20] | 6 [4–15] | 10 [5–20] | 8 [4–12] | 8 [5–15] |
| <5 | 14 | 52% | 19 | 27% | 23 | 32% | 16 | 20% | 5 | 36% | 5 | 22% |
| 5–9 | 4 | 15% | 18 | 26% | 18 | 25% | 21 | 26% | 2 | 14% | 7 | 30% |
| 10–14 | 2 | 7% | 11 | 16% | 11 | 15% | 14 | 18% | 4 | 29% | 3 | 13% |
| ≥15 | 7 | 26% | 22 | 31% | 21 | 29% | 29 | 36% | 3 | 21% | 8 | 35% |
| Health and biometrics | | | | | | |
| HIV status | | | | | | |
| Negative | 21 | 78% | 52 | 74% | 44 | 60% | 49 | 57% | 13 | 93% | 11 | 48% |
| Positive | 6 | 22% | 18 | 26% | 29 | 40% | 37 | 43% | 1 | 7% | 12 | 52% |
| cART use* | | | | | | |
| No | 1 | 17% | 0 | 0% | 3 | 11% | 2 | 6% | 0 | 0% | 0 | 0% |
| Yes | 5 | 83% | 18 | 100% | 25 | 89% | 33 | 94% | 1 | 100% | 11 | 100% |
| Most recent CD4 count (cells/µL)* | | | | | | |
| <350 | 0 | 0% | 0 | 0% | 1 | 5% | 0 | 0% | 0 | 0% | 0 | 0% |
| 350–499 | 0 | 0% | 0 | 0% | 2 | 10% | 1 | 3% | 0 | 0% | 0 | 0% |
| ≥500 | 4 | 100% | 14 | 100% | 17 | 85% | 29 | 97% | 1 | 100% | 8 | 100% |
| PrEP use* | | | | | | |
| No | 20 | 95% | 44 | 85% | 37 | 84% | 42 | 86% | 11 | 85% | 9 | 82% |
| In the past 3 months | 1 | 8% | 8 | 15% | 7 | 16% | 6 | 12% | 1 | 8% | 2 | 18% |
| In the past 4–12 months | 0 | 0% | 0 | 0% | 0 | 0% | 1 | 2% | 1 | 8% | 0 | 0% |
| Chlamydia diagnosis* | | | | | | |
| No | 23 | 85% | 65 | 93% | 69 | 95% | 86 | 100% | 13 | 93% | 19 | 83% |
| Yes | 4 | 15% | 6 | 7% | 4 | 5% | 0 | 0% | 1 | 7% | 4 | 17% |
| LGV diagnosis* | | | | | | |
| No | 24 | 89% | 69 | 99% | 73 | 100% | 88 | 100% | 14 | 100% | 22 | 96% |
| Yes | 3 | 11% | 1 | 1% | 0 | 0% | 0 | 0% | 0 | 0% | 1 | 4% |
| Gonorrhea diagnosis* | | | | | | |
| No | 22 | 81% | 64 | 91% | 67 | 92% | 84 | 98% | 13 | 93% | 19 | 83% |
| Yes | 5 | 19% | 6 | 9% | 6 | 8% | 2 | 2% | 1 | 7% | 4 | 17% |
| Herpes simplex diagnosis* | | | | | | |
| No | 21 | 78% | 70 | 100% | 72 | 99% | 84 | 98% | 14 | 100% | 18 | 78% |
| Yes | 6 | 22% | 0 | 0% | 1 | 1% | 2 | 2% | 0 | 0% | 5 | 22% |
| Notified for syphilis | | | | | | |
| No | 23 | 85% | 58 | 83% | 62 | 85% | 86 | 100% | 14 | 100% | 19 | 83% |
| Yes | 4 | 15% | 12 | 17% | 11 | 15% | 0 | 0% | 0 | 0% | 4 | 17% |
in the analysis. Among these participants 27 (9%) had no syphilis, 70 (24%) were diagnosed with primary syphilis, 73 (25%) with secondary syphilis, 86 (29%) with early latent syphilis, 14 (5%) with late latent syphilis, and 23 (8%) with treated syphilis. There were no participants with incubating syphilis. Median age was 40 years (IQR 31–48) (Table 1). Of the 293 participants, 167 (57%) were born in the Netherlands. Among the 103 men prophylaxis (PrEP) in the preceding year. Thirty-one (11%) of the 293 participants had been notified for syphilis by a sexual partner.

For 35/70 (50%) participants with primary syphilis, TP-DNA was detected in at least one study sample: 2 (3%) peripheral blood, 7 (10%) pharyngeal, 13 (19%) anal, and 24 (34%) urine samples (Table 2, Figure 2). For 62/73 (85%) participants with secondary syphilis, TP-DNA was detected in at least one study sample: 15 (21%) peripheral blood, 47 (64%) pharyngeal, 37 (51%) anal, and 26 (36%) urine samples. For 29/86 (34%) participants with early latent syphilis, TP-DNA was detected in at least one study sample: 5 (6%) peripheral blood, 21 (24%) pharyngeal, 11 (13%) anal, and 6 (7%) urine samples. No TP-DNA was detected in participants with late latent syphilis or treated syphilis, nor those without syphilis. TP-DNA was detected in 2 or more sample types in 7 (10%) participants with primary syphilis, in 39 (53%) participants with secondary syphilis, and in 9 (11%) participants with early latent syphilis (Supplementary Figure 2). Irrespective of syphilis stage, TP-DNA was less

### Table 1. Continued

| Syphilis Stage       | No Syphilis (n = 27) | Primary (n = 70) | Secondary (n = 73) | Early Latent (n = 86) | Late Latent (n = 14) | Treated Syphilis (n = 23) |
|---------------------|---------------------|------------------|--------------------|-----------------------|-----------------------|--------------------------|
| n                   | %                   | n                | %                  | n                     | %                     | n                        |
| RPR                 |                     |                  |                    |                       |                       |                          |
| Median [IQR]        | NA                  | NA               | 2 [0–8]            | 16 [16–32]            | 8 [4–32]              | 6 [0–8]                  |
| Negative            | 27 100%             | 21 30%           | 0 0%               | 10 12%                | 4 29%                 | 17 74%                   |
| Low (1:1–1:4)       | NA                  | NA               | 24 34%             | 4 5%                  | 15 15%                | 5 21%                    |
| Middle (1:8–1:16)   | NA                  | NA               | 15 21%             | 38 52%                | 39 45%                | 7 50%                    |
| High (1:32:1:128)   | NA                  | NA               | 10 14%             | 31 42%                | 24 28%                | 0 0%                     |

Abbreviations: cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; LGV, lymphogranuloma venereum; NA, not applicable; no., number; PrEP, pre-exposure prophylaxis; RPR, rapid plasma reagin.

Data missing for: country of origin (n = 1), education level (n = 29), number of sexual partners (n = 6), ART use (n = 4), and most recent CD4 count (n = 26).

*Unless otherwise stated.

*In the 6 months before the consultation.

*In patients with HIV.

*In HIV negative men only.

*At current visit.

### Table 2. TP-DNA Detection in Peripheral Blood, Throat and Anal Swabs and Urine, by Syphilis Stage, in MSM with Early Syphilis, Amsterdam, The Netherlands, November 2018 to December 2019

| Syphilis Stage       | No Syphilis (n = 27) | Primary (n = 70) | Secondary (n = 73) | Early Latent (n = 86) | Late Latent (n = 14) | Treated Syphilis (n = 23) |
|---------------------|---------------------|------------------|--------------------|-----------------------|-----------------------|--------------------------|
| n                   | %                   | n                | %                  | n                     | %                     | n                        |
| Anatomical location |                     |                  |                    |                       |                       |                          |
| Peripheral blood    | 0 0%                | 2 3%             | 15 21%             | 5 6%                  | 0 0%                  | 0 0%                     |
| Throat              | 0 0%                | 7 10%            | 47 64%             | 21 24%                | 0 0%                  | 0 0%                     |
| Anus                | 0 0%                | 13 19%           | 37 51%             | 11 13%                | 0 0%                  | 0 0%                     |
| Urine               | 0 0%                | 24 34%           | 26 36%             | 6 7%                  | 0 0%                  | 0 0%                     |
| No. of locations positive |           |                  |                    |                       |                       |                          |
| 0                   | 27 100%             | 35 50%           | 11 15%             | 57 66%                | 14 100%               | 23 100%                  |
| 1                   | 0 0%                | 28 40%           | 23 32%             | 20 23%                | 0 0%                  | 0 0%                     |
| 2                   | 0 0%                | 4 6%             | 21 29%             | 6 7%                  | 0 0%                  | 0 0%                     |
| 3                   | 0 0%                | 2 3%             | 12 16%             | 1 1%                  | 0 0%                  | 0 0%                     |
| 4                   | 0 0%                | 1 1%             | 6 8%               | 2 2%                  | 0 0%                  | 0 0%                     |
| ≥1 location positive| 0 0%                | 35 50%           | 62 85%             | 29 34%                | 0 0%                  | 0 0%                     |

Abbreviations: MSM, men who have sex with men; No., number; TP-DNA, Treponema pallidum DNA.
frequently detected in peripheral blood samples than in pharyngeal, anal, or urine samples (Figure 2). In primary and early latent syphilis the median RPR titer in TP-DNA positive participants was higher than in TP-DNA negative participants, but this difference was significant only in participants with early latent syphilis ($P = .006$) (Supplementary Figure 3).

To assess whether TP-DNA positivity in oropharyngeal and ano-rectal swabs and in urine was due to TP-DNA from lesions in patients with ulcerative disease at the site of collection, we separately analyzed those with and without localized ulcerative disease. Among primary syphilis patients without a penile ulcer, 8/29 (28%) urine samples were TP-DNA positive; among secondary syphilis patients without a penile ulcer this was 22/68 (39%) (Table 3, Supplementary Figure 4). Among primary syphilis patients without an anal ulcer, 7/60 (12%) ano-rectal samples were TP-DNA positive; among secondary syphilis patients without an anal ulcer this was 32/68 (47%). Among primary syphilis patients without a pharyngeal ulcer, 6/70 (9%) pharyngeal samples were TP-DNA positive; among secondary syphilis patients without a pharyngeal ulcer this was 46/71 (65%).

We assessed the determinants of TP-DNA detection in one or more sample types. In the multivariable model secondary syphilis was significantly associated with TP-DNA positivity (adjusted odds ratio [aOR] 6.83 (95% CI 2.94–15.90, Table 4). Also, fewer sex partners and a negative HIV status were significantly associated with TP-DNA positivity. There were no significant associations between the Ct value (as a measure of sample TP-DNA content) of the various sample types and syphilis stage. The TP-DNA content of the peripheral blood samples however was markedly lower (higher Ct values) than those of the other sample types (Supplementary Table 1). We assessed study sample TP-DNA positivity by duration of symptoms (dichotomized into ≤2 weeks or >2 weeks) in the primary and secondary syphilis subgroups. In almost all combinations of stage and sample type TP-PCR positivity was higher in participants with symptoms lasting >2 weeks, reaching statistical significance for anal TP-PCR positivity in participants with primary syphilis ($P = .042$) and throat TP-PCR positivity in participants with secondary syphilis ($P = .038$, Supplementary Table 2).

**DISCUSSION**

In this study we detected TP-DNA in anal and pharyngeal swabs, in urine samples (as a proxy of urethral mucosa) and in peripheral blood in patients with early stages of syphilis (primary, secondary and early latent syphilis). These findings support the following assumptions about the nature of syphilis: (1) The presence of TP-DNA in mucosal tissue and body fluids of patients with early latent syphilis supports the notion that syphilis is transmissible in the absence of signs or symptoms [2]. In contrast, the absence of TP-DNA in patients with late latent syphilis is in line with the assumption that the late stages are considered not infectious [3, 5]. The presence of TP-DNA in peripheral blood samples in all early syphilis stages is in line with the assumption that hematogenous dissemination of the infection occurs soon after inoculation [4, 31, 32].
In comparison to patients with either primary or early latent syphilis, patients with secondary syphilis significantly more often had at least 1 or more TP-DNA positive study sample, significantly more often a TP-DNA positive oropharyngeal and peripheral blood sample. This finding supports the notion that secondary syphilis is the most contagious stage of syphilis [5].

Finally, the absence of TP-DNA in patients with treated syphilis and those without syphilis confirms the specificity of the test used to detect TP-DNA [14]. In none of the study participants an extra diagnosis of syphilis was found based on TP-DNA positivity of study samples, compared to routine diagnostics.

Several studies have shown that PCR can be used to detect TP-DNA in whole blood at various syphilis stages [15–17, 24, 25]. Here, we used peripheral blood and confirmed TP-PCR positivity, although this was infrequently detected. The presence of TP-DNA in pharyngeal and anal samples in the presence of a lesion has been reported before [19–21]. In this study, we also detected TP-DNA in the absence of a lesion. Oral and anal shedding could therefore play a role in the transmission of syphilis. We also detected TP-DNA in urine samples as was reported previously [23, 27]. In both Dubourg et al and Towns et al, urine samples were positive for TP-DNA in 4/25 (16%) and 12/198 (6%) samples, respectively. Although urine samples are easy to obtain, they are hardly ever used for syphilis diagnosis, as judged by the low number of studies. Whether TP-DNA in urine originates from uro-genital tract lesions, such as penile ulcers, or from renal filtration or asymptomatic mucosal lesions is unknown.

Towns et al [27]. reported the presence of TP-DNA in both lesion and nonlesional samples. In their study, oral rinses, urine and anal swabs were also analyzed, as well as semen from some participants. However, they did not analyze peripheral blood samples. Similar to our study, they frequently detected TP-DNA in the oral (44%) and anal (29%) cavity of patients with secondary syphilis, although we found higher proportions (64% and 51%, respectively). We also more frequently detected TP-DNA in extra-lesional samples of participants with primary syphilis (50% in our study vs 30% by Towns et al) and early latent syphilis (34% in our study vs 8% by Towns et al). We could not confirm a relation between RPR titer and extra-lesional TP-DNA as found by Towns et al. They did not include late latent and treated infections nor persons with a negative syphilis diagnosis. In addition, compared to Towns et al we diagnosed fewer patients with extra-genital ulcers which may explain their lower positivity rates.

The strength of this study was that we systematically screened peripheral blood, oropharynx and ano-rectum samples, and urine which may play a role in the transmission of *Treponema pallidum*. This study also has several limitations. The detection of TP-DNA does not prove the presence of viable and potentially infectious

| Penile Ulcer Present | Penile Ulcer Absent |
|----------------------|---------------------|
| N        | n (%)  | N        | n (%)  |
| Urine    |         |         |         |
| Primary syphilis | 41 | 16 (39%) | 29 | 8 (28%) |
| Secondary syphilis| 5  | 4 (80%) | 68 | 22 (33%) |
| Early latent syphilis| 0 | 0 (0%) | 86 | 6 (7%) |
| Anal Ulcer Present | Anal Ulcer Absent |
| N        | n (%)  | N        | n (%)  |
| Anus     |         |         |         |
| Primary syphilis | 10  | 6 (60%) | 60 | 7 (12%) |
| Secondary syphilis| 5  | 5 (100%) | 68 | 32 (47%) |
| Early latent syphilis| 1 | 0 (0%) | 85 | 11 (13%) |
| Pharyngeal Ulcer Present | Pharyngeal Ulcer Absent |
| N        | n (%)  | N        | n (%)  |
| Pharyngeal |         |         |         |
| Primary syphilis | 1  | 1 (100%) | 69 | 6 (9%) |
| Secondary syphilis| 2  | 1 (50%) | 71 | 46 (65%) |
| Early latent syphilis| 2 | 1 (50%) | 84 | 20 (24%) |

Abbreviations: MSM, men who have sex with men; No., number; TP-DNA, *Treponema pallidum* DNA.
bacteria. Future studies should investigate the viability of TP-DNA positive samples, especially found in the oral and anal cavity. Presently this has not been possible because TP culture of direct patient samples has proven to be very difficult. Furthermore, we found that fewer sex partners and a negative HIV status were as-sociated with TP-DNA positivity. This counterintuitive finding could possibly be explained assuming that those with fewer sex partners and/or a negative HIV status seek care at an earlier (ie, more infectious) stage and respond better to partner notification calls. In patients with early syphilis, we might have missed lesions. Proctoscopy was only done if clinically indicated (eg, proctitis or intra-anal lesion) and during throat examination ulcers might have been overlooked. Furthermore, the number of patients without syphilis was small, so the demonstrated precision of the specificity of the test is limited. In addition, this study was conducted among an MSM population; it would be worthwhile to examine whether women may have a different pattern of TP-DNA shedding. Finally, we do not know if patients with 2 or more positive TP-DNA samples acquired this from the same sexual partner. However, we found intra-patient homogeneity in an investigation of the T. pallidum molecular variation within patients [33].

Performing TP-PCR on extra-genital samples could be useful as an additional diagnostic tool, especially to identify early incubating infections within the serological window phase. Blood samples are rarely PCR positive and thus may not be useful in routine screening. Urine samples, and anal and pharyngeal swabs are frequently routinely collected for STI screening for molecular diagnosis of C. trachomatis and N. gonorrhoeae infections. An additional TP-NAAT assay could possibly help to diagnose early syphilis infections in patients with non-reactive serological test results, who are nevertheless suspected of having syphilis [34]. The diagnostic value of TP-DNA assays in routine STI screening of asymptomatic persons at increased risk for syphilis should be further evaluated in larger cohorts and other routine clinic settings.

| Table 4. Univariable and Multivariable Analysis of Determinants of TP-DNA Detection in one or more Sample types in MSM With Early Syphilis, Amsterdam, The Netherlands, November 2018 to December 2019 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | TP-DNA Detected ≥ 1 Sample Type | Univariable Model | Multivariable Model |
|                                | n/N (%) | OR | 95% CI | P-Value | aOR | 95% CI | P-Value |
| **Syphilis stage**             |          |    |        |         |     |        |         |
| Primary                        | 35/70 (50%) | Ref |        | <.001 | Ref |        | <.001 |
| Secondary                      | 62/73 (85%) | 5.64 | 2.55–12.47 | .001 | 6.83 | 2.94–15.90 | .001 |
| Early latent                   | 29/66 (34%) | 0.51 | .27–.97 | .051 | 0.55 | .27–1.12 | .051 |
| Age, median [IQR]              | 38 [31–48] | 0.81 | .03–1.36 | .084 | 0.86 | .06–1.14 | .0304 |
| Age <35 y                      | 48/76 (63%) | Ref |        | .140 |     |        |         |
| 35–44 y                        | 37/67 (55%) | 0.72 | .37–1.41 | .084 | 0.68 | .36–1.31 | .046 |
| ≥45 y                          | 41/86 (48%) | 0.53 | .28–1.00 | .084 | 0.69 | .48–1.00 | .046 |
| **Country of birth**           |          |    |        |         |     |        |         |
| The Netherlands                | 74/133 (56%) | Ref |        | .770 | Ref |        | .770 |
| Other                          | 51/95 (54%) | 0.92 | .54–1.57 | .246 | 0.68 | .36–1.31 | .246 |
| **No. of sex partners, median [IQR]** | 6 [4–15] | 0.65 | .47–.89 | .008 | 0.69 | .48–1.00 | .046 |
| HIV status                     |          |    |        |         |     |        |         |
| Negative                       | 88/145 (61%) | Ref |        | .024 | Ref |        | .024 |
| Positive                       | 38/84 (45%) | 0.54 | .31–.92 | .041 | 0.50 | .25–.98 | .041 |
| Any C. trachomatis infection   |          |    |        |         |     |        |         |
| No                             | 120/220 (55%) | Ref |        | .468 | Ref |        | .468 |
| Yes                            | 6/9 (67%) | 1.67 | .41–6.83 | .468 | 1.46 | .27–7.85 | .468 |
| Any N. gonorrhoeae infection   |          |    |        |         |     |        |         |
| No                             | 118/215 (55%) | Ref |        | .869 | Ref |        | .869 |
| Yes                            | 8/14 (57%) | 1.09 | .37–3.27 | .059 | 0.59 | .16–2.19 | .059 |

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range ; MSM, men who have sex with men; No., number; OR, odds ratio; Ref, reference; TP-DNA, Treponema pallidum DNA.

*Unless otherwise indicated.

aOR per 10 year increase in age.

1 missing.

1 Odds ratio (OR) for each log + 1 increase in number of partners.
In conclusion, we frequently found TP-DNA in peripheral blood, oropharyngeal, and ano-rectal swabs, and urine collected from MSM diagnosed with early syphilis. Detection of TP-DNA in these sample types provides a better understanding of the infectious nature of early syphilis stages.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors would like to acknowledge Michelle Kroone and Ertna Ersan for creating the database used in this study. Furthermore, they would like to thank Belle Toussaint and Michelle Himschoot for their technical assistance.

Disclaimer. A current study on syphilis at the Public Health Service has received research use only T. pallidum Transcription Mediated Assay kits by the company Hologic.

Patient consent. Patient consent was obtained for every individual participating in this study

Financial support. This work was supported by the Public Health Service of Amsterdam.

Potential conflicts of interest. The authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Golden MR, Marra CM, Holmes KK. Update on syphilis: resurgence of an old problem. JAMA 2003; 290:1510–4.
2. Stamm LV. Syphilis: re-emergence of an old foe. Microb Cell 2003; 6:363–70.
3. Unemo M, Bradshaw CS, Hocking JS, et al. Sexually transmitted infections: challenges ahead. Lancet Infect Dis 2017; 17:e235–e79.
4. Starisky LE, Visser M, van Aar M, et al. Sexually transmitted infections in the Netherlands in 2020. RIVM. Available at: https://rivm.openrepository.com/bitstream/handle/10029/625007/2021-0052.pdf?sequence=1&isAllowed=y.
5. Ghanem KG, S R, Rice PA. The modern epidemiology of syphilis. N Engl J Med 2020; 382:845–54.
6. Lafond RE, Lukehart SA. Biological basis for syphilis. Clin Microbiol Rev 2006; 19:29–49.
7. Patient consent. Patient consent was obtained for every individual participating in this study.

Financial support. This work was supported by the Public Health Service of Amsterdam.

Potential conflicts of interest. The authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

12. Scott L J, Gunson RN, Carman WF, Winter AJ. A new multiplex real-time PCR test for HSV1/2 and syphilis: an evaluation of its impact in the laboratory and clinical setting. Sex Transm Infect 2010; 86:537–9.
13. Kook AG, Bruisten SM, Diersorp M, van Dam AJ, Templeton K. Specific and sensitive diagnosis of syphilis using a real-time PCR for Treponema pallidum. Clin Microbiol Infect 2006; 12:1233–6.
14. Heymans R, van der Helm J, I, et al. Clinical value of Treponema pallidum real-time PCR for diagnosis of syphilis. J Clin Microbiol 2010; 48:497–502.
15. Gayet-Ageron A, Nitin B, Toutsou-Treluy L, et al. Assessment of a real-time PCR test to diagnose syphilis from diverse biological samples. Sex Transm Infect 2009; 85:264–9.
16. Castro R, Prieto E, Aguas MJ, Manata ML, Botas J, Pereira FM. Molecular subtyping of Treponema pallidum subsp. pallidum in Lisbon, Portugal. J Clin Microbiol 2009; 47:1668–73.
17. Martin JE, Tsang RS, Sutherland K, et al. Molecular characterization of syphilis in patients in Canada: azithromycin resistance and detection of Treponema pallidum DNA in whole-blood samples versus ulcerative swabs. J Clin Microbiol 2009; 47:1668–73.
18. Shields M, Guy RJ, Jeffreys NJ, Finlayson RJ, Donovan B. A longitudinal evaluation of Treponema pallidum PCR testing in early syphilis. BMC Infect Dis 2012; 12:353.
19. Towns JM, Leslie DE, Denham I, Azzato F, Fairley CK, Chen M. Painful and multiple anogenital lesions are common in men with Treponema pallidum PCR-positive primary syphilis without herpes simplex virus coinfection: a cross-sectional clinic-based study. Sex Transm Infect 2016; 92:110–5.
20. Gayet-Ageron A, Sednaoui P, Lautenschlager S, et al. Use of Treponema pallidum PCR in testing of ulcers for diagnosis of primary syphilis. Emerg Infect Dis 2015; 21:127–9.
21. Glatz M, Juricicev N, Altwegg M, et al. A multicenter prospective trial to assess a new real-time polymerase chain reaction for detection of Treponema pallidum, herpes simplex-1/2 and Haemophilus ducreyi in genital, anal and oropharyngeal ulcers. Clin Microbiol Infect 2014; 20:O1020–7.
22. Castro R, Prieto E, Aguas MJ, et al. Detection of Treponema pallidum sp. pallidum DNA in latent syphilis. Int J STD AIDS 2007; 18:842–5.
23. Dubourg G, Edouard S, Prudent E, Fournier PE, Raoult D. Incidental syphilis diagnosis by real-time PCR screening of urine samples. J Clin Microbiol 2015; 53:3707–8.
24. Grange PA, Gressier L, Dion PL, et al. Evaluation of a PCR test for detection of Treponema pallidum in swabs and blood. J Clin Microbiol 2012; 50:546–52.
25. Wang C, Cheng Y, B L, et al. Sensitive detection of Treponema pallidum DNA from the whole blood of patients with syphilis by the nested PCR assay. Emerg Microbes Infect 2018; 7:83.
26. Zhou C, Zhang X, Zhang W, Duan J, Zhao F. PCR detection for syphilis: diagnostic status and prospects. J Clin Lab Anal 2019; 33:e22890.
27. Towns JM, Leslie DE, Denham I, et al. Treponema pallidum detection in lesion and non-lesion sites in men who have sex with men: a prospective, cross-sectional study. Lancet Infect Dis 2021; 21:1324–31.
28. Stoltey JE, Cohen SE. Syphilis transmission: a review of the current evidence. Sex Health 2015; 12:103–9.
29. Janier M, Unemo M, Dupin N, Tiptica GS, Potocznik M, Patel R. 2020 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol 2021; 35:574–88.
30. Bon RJ, Christerson L, Schm van der Loeff ME, Coutinho RA, Herrmann B, Bruisten SM. Evaluation of high-resolution typing methods for Chlamydia trachomatis in samples from heterosexual couples. J Clin Microbiol 2011; 49:2844–53.
31. Salazar JC, Rathu A, Michael NL, Radolf JD, Jagodzinski LL. Assessment of the kinetics of Treponema pallidum dissemination into blood and tissues in experimental syphilis by real-time quantitative PCR. Infect Immun 2007; 75:2954–8.
32. Church B, Wall E, Webb JR, Cameron CE. Interaction of Treponema pallidum, the syphilis spirochete, with human platelets. PLoS One 2019; 14:e0210902.
33. Zondag H, Nieuwenburg S, van Dam A, et al. Treponema pallidum intra-patient homogeneity between various body locations in patients with infectious syphilis. Presented at: ISSTDR & HIV 2021 Conference; Amsterdam, The Netherlands. 14–17 July 2021.
34. Getman D, M L, Barakat N, et al. Analytical performance characteristics of a new transcription-mediated amplification assay for Treponema pallidum. J Clin Microbiol 2021; 59:e005121.