Getting the measure of syphilis: qPCR to better understand early infection

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ABSTRACT

Objectives Until recently, PCR had been used to detect but not quantify *Treponema pallidum*. To understand infection kinetics of this uncultivable organism, a real-time PCR assay was developed to quantify 47 kDa membrane lipoprotein gene DNA (*tpp47*).

Methods Assay specificity was determined against DNA from humans, skin organisms and sexually transmitted pathogens. *tpp47* DNA (Nichols strain) was used to construct a standard curve for *T pallidum* quantification. Blood and ulcer samples were obtained from 99 patients being investigated or screened for syphilis and *tpp47* was quantified.

Results The assay was specific, not cross-reactive with other organisms tested and sensitive, with a detection limit of a single copy of *tpp47* DNA. For ulcer samples, the assay was 100% sensitive and 97.14% specific. Sensitivity fell to 34.1% for blood samples but specificity remained high (100%). *tpp47* DNA was more commonly detected, and at a higher copy number, in blood of patients with secondary infection (sensitivity 57.89%) compared with primary infection. Quantity of *tpp47* DNA was higher in primary infection ulcers, especially in HIV-1-positive patients, than in ulcers persisting into secondary disease.

Conclusions Quantifying *T pallidum* provides insight into syphilis infection kinetics: Ulcers of primary disease in HIV-1-positive patients are perhaps more infectious and the presence and load of *T pallidum* bacteraemia is variable, with a peak in the secondary stage. Quantitative PCR has the potential to map *T pallidum* infection and to highlight the impact of HIV on syphilis.

INTRODUCTION

A better understanding of syphilis may be obtained by quantitatively mapping the course of infection. However, the causative organism, *Treponema pallidum* subspecies *pallidum* (*T pallidum*), cannot be easily cultured, so detection relies on direct visualisation, molecular detection and, rarely, rabbit infectivity testing. None of these methods is ideal for studying *T pallidum* pathogenesis or infection kinetics. Dark ground microscopy (DGM) to count organisms in ulcer exudate or cerebrospinal fluid is highly operator dependent and cannot be used for blood.[1] Rabbit infectivity testing, often described as the gold standard for diagnosing syphilis,[2] is highly sensitive but impractical. The non-specific anti-cardiolipin-based tests, for example, rapid plasma reagin (RPR), can be used as a surrogate marker of *T pallidum* burden to stage infection and monitor treatment response but are prone to false-positive results and do not reflect the kinetics of the infection since they take months to resolve and may never become negative. Furthermore, HIV-1 infection, which can alter the course of *T pallidum* infection and response to treatment, is also known to have an impact on treponemal serology.[3 4]

Molecular detection of *T pallidum* DNA by PCR is now established for the diagnosis of syphilitic ulcers.[5 6] These assays have been based on the detection of various *T pallidum* genes, including *polA*,[7–9] *flaA*,[10] *tmpA*[11] and *tpp47*,[12–14] which are highly conserved across *T pallidum* subspecies. Until recently, molecular studies were qualitative and little was known about the quantity of *T pallidum* in clinical samples during the course of syphilis.

To study *T pallidum* infection kinetics in humans, a real-time PCR assay has been developed to detect and quantify *T pallidum tpp47* DNA in samples from patients with syphilis.

METHODS

Population and specimens

A prospective, cross-sectional study was conducted at St Mary's Hospital, London, between July 2006 and January 2008. The study had research ethics approval and samples were collected after obtaining written consent. All patients being investigated for syphilis (including routine screens) were eligible. Investigation and treatment for sexually transmitted infections (STIs) were performed in accordance with national guidelines.[15]

Six millilitres of blood were collected into EDTA for *T pallidum* DNA detection and quantification from all participants. Samples collected between July and December 2006 were processed immediately and DNA was extracted as described below. Samples collected after this period were stored at −80°C until DNA was extracted. Ulcers were cleaned and abraded with sterile water and gauze prior to sampling. After 1–2 min, the accumulated exudate was absorbed onto Sno Strips filter paper (Laboratoire Chauvin, Aubenas, France) held at the ulcer edge until dry. DGM was performed on genital ulcers according to clinic policy prior to the collection of study samples.

The Newmarket IgM/IgG enzyme immunoassay (EIA) (Alere, Stockport, UK) was used to screen for *T pallidum* infection. Fujirebo *T pallidum* particle agglutination (TPPA) (Mast Diagnostics, Bootle, UK) and Bio-kit (RPR (Launch Diagnostics, Longfield, UK) were used to confirm and quantify the serological response, respectively.

Baseline and convalescent syphilis serology were documented in addition to STI results. Patients' final clinico-microbiological diagnoses were recorded as one of the following: herpes simplex

virus (HSV), primary syphilis, secondary syphilis, other syphilis (comprising latent and symptomatic late disease), neurosyphilis and asymptomatic contacts of syphilis and non-specific genital ulceration. Case definitions are given in table 1.

Demographic data, sexual orientation and HIV-1 status, if known, were also recorded. Ninety-nine patients were recruited of whom 44 had a final clinical diagnosis of syphilis: 14 had primary syphilis, 19 secondary syphilis, 8 early latent syphilis and 3 neurosyphilis (all during the secondary stage). Of the 55 non-syphilis cases, 17 (31%) were contacts of patients with syphilis and 38 had non-syphilitic ulcers of which half were confirmed as genital herpes. The remaining 19 (35%) patients had no STI and were diagnosed with non-specific genital ulceration, which is consistent with studies examining the cause of genital ulceration.[17 18]

The study population is representative of the current UK syphilis epidemic.[19] Ninety-seven (98%) of the patients were men, 88 (88.7%) were Caucasian with a median age of 36 years and just over half (52, 52.5%) were HIV positive. Neither of the two women were HIV positive (full data shown in online supplementary table 1).

Sample handling and DNA extraction

Aliquots of 400 µl of EDTA whole blood (WB) were either immediately treated with 60 µl of proteinase K (Qiagen, Crawley, UK) or frozen at −80°C for future processing. DNA was extracted using the Qiagen QIAamp DNA Mini kit (blood spin protocol) with the following modifications: 400 µl of sample and buffer AL were used; the first incubation was for 3 h or overnight; final elution was into 120 µl of buffer AE following 5 min incubation. Extraction was performed in a separate laboratory to the PCR and new aliquots of reagent were used for each batch.

Whole Sno Strips were placed in microcentrifuge tubes with 400 µl phosphate buffered saline and 20 µl proteinase K. DNA was extracted using the Qiagen QIAamp DNA Mini kit, buccal swab spin protocol with the above modifications. Quantitative PCR (qPCR) results for ulcer samples are presented as copies/strip.

Real-time PCR

A 178 bp segment of the 1377 bp *T pallidum tpp47* gene (GenBank accession number M88769.1) was amplified using the following primers: forward 5′CGAGGAATACAAGATTACG AACG3′, reverse 5′ACGTGCAGAAAAACTATCCTCAG3′ (nucleotides 525–547 and 702–680, respectively). Primer sequences, product size and reaction conditions were optimised for sensitivity and reproducibility (data not shown). Each reaction contained 10 µl extracted DNA, 0.6 units LightCycler Uracil-DNA glycosylase (UDG) (Roche, Mannheim, Germany), 0.5 µm each of forward and reverse primers (Invitrogen, Paisley, UK), 4 µl FastStart Plus reaction mixture (Roche, Mannheim, Germany) (containing FastStart Taq DNA polymerase, reaction buffer, $MgCl_2$, SYBR Green I dye and dNTP mix) and PCR-grade water to a final 20 µl volume.

Real-time PCR was performed using a Roche LightCycler 2, software version 4.0, with the following conditions: UDG activation at 40°C for 10 min, preincubation at 95°C for 10 min and then 46 cycles of 95°C for 10 s, 60°C for 5 s, 72°C for 8 s and 81°C for 10 s (acquisition). Two no-template controls were

Table 1 Case definitions

Stage/disease	Definition
HSV	Typical single or multiple genital ulcers from which HSV-1 or HSV-2 DNA is isolated using an in-house PCR.
Primary syphilis	Diagnosis requires the presence of a chancre on examination and microbiological confirmation of *Treponema pallidum* with any of the following: dark ground microscopy, PCR provided by the sexually transmitted bacteria reference laboratory, Health Protection Agency, London, or positive treponemal serology (as per national guidelines).[16] Primary diagnoses to include dark-ground negative ulcers and initially negative serology, which becomes positive up to 3 months later with no sign of secondary disease.
Secondary syphilis	Defined as syphilis within the first 2 years of infection and characterised by both clinical and microbiological findings. Clinical findings include typical rash, condylomata lata, muco-cutaneous lesions, generalised lymphadenopathy, anterior uveitis, hepatitis and splenomegaly. Microbiological findings must include serological tests consistent with secondary disease and may, if available, include PCR of secondary lesions.[16] If anogenital examination reveals persisting chancres, the case is still counted as secondary.
Other syphilis	*Latent syphilis* Positive serological tests in the absence of any symptom or sign. Classified as early within the first 2 years of infection and late thereafter. *Symptomatic late syphilis* Positive serology including a negative or low and unchanging RPR titre together with symptoms and clinical signs of cardiovascular or gummatous disease. Late disease with neurological involvement is classed as neurosyphilis.
Neurosyphilis	Any stage of disease with clinical neurological involvement* (including meningitis, cranial nerve palsies, parenchymatous general paresis, tabes dorsalis), serological findings consistent with the stage of disease and a relevant CSF abnormality (as per national guidelines).[16] NB: For analysis, cases with neurological involvement were counted as neurosyphilis not by stage.
Asymptomatic contact of syphilis	Patient reports sexual contact with a suspected or confirmed case of syphilis. Examination reveals no sign of disease and serological results (baseline, 6 weeks and 3 months after exposure) are not consistent with current infection.
Non-specific genital ulceration	Genital ulceration is present on examination, but no microbiological diagnosis is made. PCR for HSV is negative, DGM is negative, syphilis serology is not consistent with current infection and diagnostic *T pallidum* PCR (if performed) is negative.

*Patients without neurological symptoms do not routinely undergo CSF examination at our centre.
CSF, cerebrospinal fluid; DGM, dark ground microscopy; HSV, herpes simplex virus; RPR, rapid plasma reagin.

included in each experiment, and standard precautions to avoid contamination were employed.

Samples were run in duplicate and discordant results were repeated. Negative samples were diluted 1:10 and repeated if inhibition was suspected.

Assay specificity

During assay development, PCR product identity was confirmed using both agarose gel electrophoresis (2% agarose gel containing Tris-acetate-EDTA buffer, 0.6 mg ethidium bromide) and DNA sequencing using the Applied Biosystems dRhodamine kit and 3100 capillary sequencer. Using NCBI BLAST software, the 178 bp amplicon showed 100% sequence homology in comparison with published *T pallidum* 47 kDa gene sequences.

Melting curve analysis was performed on all amplicons from clinical samples and compared with an in-run standard in order to confirm amplification of the correct target. Fluorescence emission in the 530 nm channel at a linear temperature transition rate of 0.1°/s from 60°C to 95°C was monitored with continuous acquisition. The melting temperature (T_m) of the correct 178 bp amplicon was 82.5°C.

In order to confirm non-cross-reactivity with commensal treponemes, the infective agents of other STIs, common skin organisms or human DNA, the assay was tested against DNA from these sources (see online supplementary table 2).

Preparation of the standard curve and limit of assay detection

T pallidum (Nichols strain) bacteria were enumerated using DGM to give an initial concentration of 6×10^8 organisms/ml (suspended in glycerol and phosphate buffer). DNA was extracted from 10 µl aliquots using Qiagen QIAamp DNA Mini kit as per the manufacturer's protocol and eluted into 60 µl of molecular-grade water to produce a concentration of 10^6 *T pallidum* genome equivalents/10 µl. Serial fivefold dilutions were made using molecular-grade water from 1/5 to $1/10^8$. DNA detection was unreliable beyond $1/10^6$ dilution (C_T 38.04), thus 1 copy/10 µl was determined to be the lowest limit of reliable detection. An example of an in-run standard curve used for *T pallidum* DNA quantification in clinical samples is shown in the online supplementary data.

In order to be sure that the standard curve was biologically relevant, detection of *T pallidum* DNA in WB and water were compared. Serial 10-fold dilutions of *T pallidum* DNA were added to syphilis-seronegative WB, quantified and found to be similar to dilutions in molecular-grade water described above (see online supplementary table 3).

Bacteria and DNA

T pallidum (Nichols) organisms were obtained through passage in rabbit testes and donated by Nigel Appleton (Newmarket Laboratories Ltd, Suffolk, UK). DNA from *T pallidum* subspecies *pertenue* and *Treponema denticola* was donated by Sheila Lukehart (University of Washington, Seattle, USA). All other samples were donated by Stuart Philip (St Mary's Hospital, London, UK). Human DNA samples were obtained from blood donated for research by patients seronegative for *T pallidum*.

Statistical analysis was performed using PASW statistics, V.18, SPSS, Chicago, IL. Significance levels were calculated using the Mann–Whitney U test (two sided, 95% CIs).

RESULTS

During the validation, as previously described in a rabbit model,[10] extraction and quantification of *tpp47* DNA from WB gave more consistent results than from peripheral blood mononuclear cells or plasma (data not shown). Thus, only ulcer and WB results are presented.

Table 2 details all patients included in the study and gives serological, DGM and PCR results.

Species specificity

Amplification was observed neither with DNA extracted from the panel of STI and other organisms nor with human DNA. *T pallidum* subspecies *pertenue* was detected by the assay. This result was expected, as there are very few genetic differences between *T pallidum* subspecies.

Sensitivity and specificity of *tpp47* detection in ulcer and WB samples

Patients' final clinico-microbiological diagnoses (as detailed in table 1) were compared with *T pallidum* qPCR results.

The sensitivity and specificity for *T pallidum* detection in syphilitic ulcers, primary or secondary, were 100% and 97·14%, respectively. The details of a potential false-positive result are given below. With the exception of secondary syphilis (57.89%), sensitivity for *T pallidum* in WB samples was low (34.09% for all stages overall) while specificity was 100%. The sensitivity and specificity of WB PCR compared with serology for the diagnosis of primary disease were 27.27% and 100%, respectively.

All 14 patients with primary syphilis had PCR-positive ulcers and 10 were DGM positive. All four patients with DGM-negative ulcers had positive serology at enrolment. Compared with the final diagnosis, the sensitivity and specificity of DGM for syphilis ulcers (primary and secondary combined) were 70.59% and 87.88%, respectively, with no significant difference observed for primary or secondary cases analysed individually. The sensitivity of PCR compared with DGM for the diagnosis of primary disease was 100%.

Primary syphilis

tpp47 DNA was detected in ulcer exudates of all 14 patients diagnosed with primary syphilis. Median copy number of *tpp47* DNA from ulcers was 1832 copies/strip (range 251–14 244), which did not differ significantly between those processed immediately ((n=9) median 2577 copies/strip (427–14 244)) and those processed after storage ((n=5) median 1064 copies/strip (251–2115) (p=0.162)). The median number of ulcer *tpp47* copies/strip in HIV-1-positive and HIV-1-negative patients were 2115 (757–14 244) and 648 (251–2742) (p=0.048), respectively. Mean CD4 count of the seven HIV-1-infected patients was 501 (range 270–920) and CD4 count was not associated with *T pallidum* load.

Three patients had *tpp47* DNA detectable in WB with a copy number 1–2 logs lower (median 127 copies/ml, 127–168) than for ulcers. It did not differ according to HIV status and was associated with a modal RPR of 4. The mean duration of symptoms for these patients was 8 days (range 7–10 days), compared with 30.5 days (1–90) for those who were *tpp47* DNA PCR negative in WB.

Secondary syphilis

tpp47 DNA was detected in the blood of 11/19 (58%) patients with secondary syphilis. This rate of detection was higher (86%) in samples that had been processed immediately compared with 42% when DNA extraction was performed after storage (p=0.02) (see online supplementary figures). The mean *tpp47* DNA copy number in WB samples extracted immediately was 516 copies/ml and the modal RPR for these patients was 128.

Table 2 Standard clinical investigation and study results for all participants categorised by final clinico-microbiological diagnosis

Final diagnosis	Patient	HIV-1 status	CD4 count	DGM result*	HSV PCR result*	Syphilis EIA result at enrolment	RPR result at enrolment	Timing of DNA extraction	WB *tpp47* (copies/ml whole blood)	Ulcer *tpp47* copy number (copies/strip)
Primary syphilis (n=14)	6	–	–	+	–	+	8	Immediate	0	620
	9	+	390	+		+	16	Immediate	127	2108
	11	+	480	+		+	16	Immediate	0	12 440
	18	+	270	–	–	+	0	Immediate	0	757
	23	–	–	+		+	4	Immediate	0	2742
	24	–	–	+		–	16	Immediate	0	2577
	26	+	920	+		–		Immediate	0	7426
	32	+	720	–		+	4	Immediate	168	14 244
	33	–	–	–	–	+	64	Immediate	0	427
	52	+	320	+		+	2	Later	0	2115
	69	–	–	+	–	+	32	Later	0	251
	89	+	420	+		–		Later	0	1555
	90	–	–	–		+	4	Later	127	1064
	93	–	–	+	–	+	4	Later	0	648
Secondary syphilis (n=19)	3	+	660	Unavailable		+	64	Immediate	0	2
	16	+	240	–	–	+	128	Immediate	886	630
	17	–	–	+	–	+	128	Immediate	1026	422
	22	–	–	+		+	64	Immediate	518	211†
	27	+	680			+	8	Immediate	632	
	29	–	–			+	16	Immediate	58	
	34	+	390	Unavailable	–	+	128	Immediate	495	251†
	45	+	350			+	64	Later	6	
	46	+	330			+	16	Later	0	
	47	–	–			+	256	Later	0	
	49	+	630			+	128	Later	10	
	53	+	400			+	64	Later	28	
	61	+	470			+	512	Later	91	
	63	+	790			+	32	Later	0	
	74	+	410			+	128	Later	0	
	75	+	470			+	256	Later	0	
	77	+	370			+	32	Later	0	
	81	+	430			+	128	Later	65	
	83	+	420			+	128	Later	0	Unavailable
Other syphilis (latent or symptomatic late) (n=8)	1	–	–			+	0	Immediate	0	
	12	+	400			+	1	Immediate	0	
	28	+	220			+	256	Immediate	70	
	44	–	–			+	16	Later	0	
	59	+	610			+	0	Later	0	
	67	–	–			+	0	Later	0	
	84	+	570			+	0	Later	0	
	95	–	–			+	1	Later	0	
Asymptomatic contact of syphilis (n=17)	2	–	–			+	1	Immediate	0	
	4	–	–			–		Immediate	0	
	8	–	–			–		Immediate	0	
	15	+	730			–		Immediate	0	
	25	–	–			–		Immediate	0	
	30	+	320			–		Immediate	0	
	38	+	490			+	0	Immediate	0	
	51	–	–			–		Later	0	
	57	–	–			–		Later	0	
	58	+	470			+	0	Later	0	
	65	+	490			–		Later	0	
	73	–	–			–		Later	0	
	76	+	670			+	0	Later	0	
	78	+	510			–		Later	0	
	88	+	320			+	0	Later	0	
	94	+	640			–		Later	0	
	99	–	–			–		Later	0	

Continued

Table 2 Continued

Final diagnosis	Patient	HIV-1 status	CD4 count	DGM result*	HSV PCR result*	Syphilis EIA result at enrolment	RPR result at enrolment	Timing of DNA extraction	WB *tpp47* (copies/ml whole blood)	Ulcer *tpp47* copy number (copies/strip)
HSV infection (n=19)	10	−	−	−	+	−		Immediate	0	0
	19	−	−	−	+	−		Immediate	0	0
	20	−	−	Unavailable	+	−		Immediate	0	0
	31	−	−	−	+	−		Immediate	0	0
	37	−	−	−	+	−		Immediate	0	0
	39	−	−	−	+	−		Immediate	0	0
	40	−	−	−	+	+	0	Later	0	0
	41	−	−	−	+	−		Later	0	0
	42	+	780	−	−	−		Later	0	0
	43	−	−	−	+	Unavailable		Later	0	0
	55	+	550	−	−	−		Later	0	0
	70	−	−	−	+	−		Later	0	0
	71	+	680	−	+	−		Later	0	0
	72	−	−	−	+	−		Later	0	0
	79	−	−	−	+	−		Later	0	0
	86	−	−	−	+	−		Later	0	0
	91	+	370	Unavailable	+	−		Later	0	Unavailable
	92	−	−	+	+	−		Later	0	0
	98	−	−	+	+	−		Later	0	0
Non-specific genital ulceration (n=19)	5	−	−	−	−	−		Immediate	0	0
	7	+	540	+		+	0	Immediate	0	0
	13	−	−	−	−	−		Immediate	0	0
	14	−	−	−	−	−		Immediate	0	0
	21	−	−	−	−	−		Immediate	0	0
	35	−	−	−	−	−		Immediate	0	0
	48	−	−	−	−	−		Later	0	0
	50	−	−	+	−	+	0	Later	0	0
	54	+	210			−		Later	0	
	60	+	750	−	−	−		Later	0	0
	62	+	400	−	−	−		Later	0	46‡
	64	+	130			+	0	Later	0	
	66	+	590	−	−	−		Later	0	0
	68	+	580			−		Later	0	
	80	−	−	−	−	−		Later	0	0
	82	+	340	−	−	−		Later	0	0
	85	+	120	−	−	+	1	Later	0	0
	87	+	610	−	−	−		Later	0	0
	97	+	500			−		Later	0	
Neurosyphilis (n=3)	36	+	290			+	32	Immediate	0	
	56	−	−			+	64	Later	0	
	96	+	660			+	8	Later	0	

*Test performed only when clinically indicated.
†Muco-cutaneous oral lesion.
‡Patient with a 1-week history of multiple non-tender genital ulcers thought to be atypical genital herpes and treated with acyclovir. Convalescent syphilis serology and PCR for HSV types 1 and 2 were negative.
DGM, dark ground microscopy; EIA, enzyme immunoassay; HSV, herpes simplex virus; RPR, rapid plasma reagin; WB, whole blood.

T pallidum was detected in WB of 8/15 (53%) HIV-1-infected versus 3/4 (75%) uninfected (28%) patients. Mean CD4 count of HIV-1-infected patients with *tpp47*-positive WB was 448, not significantly different from those *tpp47* negative in WB (492, p=0.594).

Three patients had muco-cutaneous oral lesions, of which two were positive for *tpp47* DNA. Four patients with secondary syphilis still had anogenital ulcers of which three were sampled and all were *tpp47* DNA positive. When compared, the mean *tpp47* DNA copy number was lower in these anogenital ulcers persistent in secondary disease (351 copies/strip) than in primary chancres (3498 copies/strip) (p=0.032).

Other cases

tpp47 DNA was detected in WB of one of the eight patients with latent disease. This HIV-1-positive untreated patient, with an initial RPR titre of 1:131 072, had been lost to follow-up for 2 years prior to the study. RPR at the time of recruitment was 1:256.

One ulcer sample from a patient with a 1-week history of multiple non-tender genital ulcers thought to be atypical genital herpes and treated with acyclovir had detectable *tpp47* DNA. DGM was negative as were the contemporaneous and convalescent syphilis serology and PCR for *herpes simplex virus* types one and two. This may represent a false-positive PCR result, but it is not known whether this patient received

coincidental treponemocidal antibiotics that could have prevented seroconversion.

CONCLUSIONS AND DISCUSSION

For 20 years, PCR has been used with a variety of clinical and rabbit specimens to detect, type and quantify *T pallidum*.[5 6 10 20 21] PCR reliably detects *T pallidum* in ulcer specimens and is now established, often as part of a multiplex assay, for the diagnosis of primary syphilis.[5] The more modest success of *T pallidum DNA* PCR in other clinical samples has been attributed to their lower treponemal load.[9]

There are few data on the quantification of *T pallidum* by PCR in clinical samples, thus the timing of bacteraemia and duration of post-treatment as well as the impact of HIV-1 infection on the course of *T pallidum* infection are uncertain. Although cross-sectional, the data presented here provide insight into the range of *T pallidum* DNA copies that can be detected in samples taken at different stages of disease.

Since several groups had found the *tpp47* gene to be reliable and specific for *T pallidum* detection,[5 9 12 13] new primers were designed for a real-time PCR assay. These have been shown to be equally specific for *T pallidum* detection with no cross-reactivity to *T denticola*, any of the common commensal skin flora or sexually transmitted organisms tested. Sensitivity was maintained when used to detect *T pallidum* DNA extracted from human WB samples with no inhibition by the high concentration of human DNA.

Storage at −80°C prior to DNA extraction impaired the detection and quantification of *T pallidum in* WB but not in ulcer samples. Cruz *et al* reported a similar effect, with detection of *T pallidum* DNA in WB of patients with secondary syphilis just 30% compared with 63% when the samples were processed within a few hours of collection.[22] The maximum interval between blood sampling and DNA extraction, which is likely to be short, remains to be established. Additional study is also needed to optimise *T pallidum* collection from ulcer samples. While Sno Strips are shown here to be efficient at absorbing exudate and bacterial DNA, the volume (which is in part dependent on the ulcer area) of ulcer exudate will influence final copy number in the same way as the concentration of *T pallidum* in the ulcer fluid. Quantifying a human reference gene from ulcers (eg, β-globin) and comparing it with *tpp47* load may also help standardisation. It remains to be shown, however, that the amount of human DNA detected would not simply mirror that of *T pallidum*.

T pallidum DNA was detected in the ulcers of all 14 patients diagnosed with primary syphilis, a similar finding to the work of Palmer *et al* in 2002[12] and unsurprising, given that the chancre is the site of bacterial inoculation. Moreover, *tpp47* DNA PCR from ulcer exudates appears to more reliably include or exclude *T pallidum* infection than DGM. *T pallidum* DNA was also detected in ulcer samples in secondary syphilis with three out of four genital and two out of three oral lesions being positive. Fewer organisms/strip were detected in these lesions than in primary chancres (351 vs 3498), which likely represents ulcer healing and resolution.

T pallidum bacteraemia is thought to occur from the outset of the primary infection and to persist at all stages of disease thereafter,[23] and while these stages are well defined, the rate of bacterial dissemination in humans is unknown. In rabbits inoculated intratesticularly, *T pallidum flaA* DNA could be detected at low load in blood after 24 h and at much higher load after 7–10 days. The increased detection after 1 week corresponded with rising RPR titres and widespread treponemal dissemination into organs.[10] The detection rates observed in WB for patients with primary (3/14, 21.4%) and secondary (11/19, 58%) syphilis are lower than the detection rates for ulcers, but are comparable with those reported previously.[12 22] A higher detection rate in WB in secondary syphilis corresponds to a greater number of organisms and higher RPRs, similar to the picture reported in the rabbits 7–10 days post-inoculation. *T pallidum* DNA detection in blood has been reported in 0–58.3% of patients with latent disease.[9 14 22–25] Kouznetsov *et al* reported higher detection of *T pallidum* DNA in peripheral blood mononuclear cell samples in early latent (4/4) than in late latent (1/) cases, and in our study, quantifiable low-load (70 copies) bacteraemia was found in only one patient (1/7, 14.3%), who had early latent disease.[14]

Combining the qualitative and quantitative findings, a pattern of bacterial dissemination emerges. In primary disease, high numbers of organisms in ulcers but low copy number or no *tpp47* DNA were detected in blood. In secondary disease, the healing ulcers have decreased load but bacteraemia is more frequently detected and at higher load than primary disease. By the latent stage, treponemal bacteraemia is less commonly detected and when present is at low load.

Syphilis and HIV-1 are known to interact in a number of ways. Both HIV-1 transmission and acquisition are facilitated by the presence of syphilitic ulcers and there is evidence that those ulcers and the clinical course of syphilis can be affected by HIV-induced immunosuppression.[3] Rompalo *et al* studied 214 patients with genital ulcers and found HIV-1-positive men to be 11% more likely to have secondary syphilis with a concomitant primary chancre.[26] Similarly, in this study, HIV-1-positive patients with secondary syphilis were more likely to present with unhealed primary ulcers. HIV-1-positive patients had a higher number of organisms in primary ulcers than patients known to be HIV-1 uninfected. Although this association did not reach a high level of statistical significance, the presence of more organisms suggests that HIV-1-positive patients with primary syphilis are more infectious and for longer, which may help explain the high rates of syphilis–HIV co-infection.

This study had small sample sizes in some groups, which limited the statistical significance of associations. It is also appreciated that longitudinal sampling of the same patients during the course of their infection would more accurately reflect infection kinetics but would not be ethical. Importantly though, we demonstrate that quantification of *T pallidum* has the potential to determine the kinetics of the infection and the impact of HIV-1 co-infection on those kinetics. In this study, a pattern of *T pallidum* load by site and stage is described with important observations related to the infectiousness of HIV-positive patients with primary syphilis.

Key messages

- Real-time PCR can be used to quantify *Treponema pallidum* in clinical samples and provide insight into the kinetics of syphilis infection.
- The amounts of *T pallidum* found in ulcers and blood at different stages of syphilis follow a biologically plausible pattern.
- Larger numbers of *T pallidum* in ulcers may mean HIV-1-positive patients can more easily transmit syphilis.

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Competing interests The corresponding author has had full access to all the data in the study and had final responsibility for the decision to submit for publication. These data were presented, in part, at the IUSTI World Congress, Cape Town, 2009.

Ethics approval This study was conducted with the approval of the Hounslow and Hillingdon Local Research Ethics Committee. Ref: 06/Q0407/29.

Contributors CT: Sample analysis, data collection and interpretation, and wrote the manuscript. MOFH: Developed and validated the tpp47 qPCR, performed sample analysis and co-wrote the manuscript. DG: Initial study design and co-wrote the manuscript. SH and JD: Recruited the patients and collated clinical data. MOMC: Designed and supervised the study and co-wrote the manuscript. GPT: Designed and supervised the study, obtained regulatory approval and co-wrote the manuscript.

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Book review

Sexually Transmitted Infections: The Facts (3rd edition)

Edited by David Barlow (with Dr Julie Fox, for chapter on 'HIV and AIDS—the clinical picture'). Published by Oxford University Press, Oxford, 2011, pp 152, £12.99 (http://ukcatalogue.oup.com/product/9780199595655.do) (softback). ISBN 978-0-19-959565-5.

This little book provides detailed information on sexually transmitted infections (STIs) and related conditions, but does not stop there. It begins by explaining what patients can expect when they go for STI testing, what a genitourinary medicine clinic is and why they might choose to attend one. A chapter entitled 'Understanding your results' explains complex concepts, including false positives and negatives and the implications of test sensitivity and specificity. It also covers issues in the interpretation of surveillance data (such as how changes in testing patterns and practices can lead to *apparently* higher rates of infection). Chapters on different infections follow, and the book concludes with two chapters on HIV/AIDS, one putting the HIV epidemic in its social and historical context, and the other offering a clinical overview of HIV testing, infectivity, postexposure prophylaxis and treatment.

Although adults and young people may increasingly seek out information on STIs online, there is certainly still a place for the third edition of this book, which is clearly written and authoritative. I would hope to see this book in college, secondary school or public libraries; despite the depth and detail provided, it is still small enough to hide under homework or revision notes! It would be a useful resource for both staff and young people in youth services and students' unions. Academic researchers new to the field of STIs may also benefit from the overview the book provides on specific infections, testing practices and how genitourinary services operate—which may seem obvious to their genitourinary clinical colleagues. At the end of each chapter, a few references are supplied: for instance, URLs for BASHH/IUSTI clinical guidelines and patient organisations such as the Herpes Virus Association and Terrence Higgins Trust.

The tone is matter-of-fact, if slightly dated in places. With considerable effort taken to convey a large amount of information in such a short volume, one can forgive the occasional lapse into technical terminology (in most cases this is explained). Light-hearted cartoons make a charming and welcome change from the graphic photos that one might expect in a book on STIs, which I felt to be appropriate, since photos may (in my view) reinforce public misconceptions that serious infections are obvious and symptomatic. Finally, historical anecdotes and patient vignettes provide human interest in this useful, educational book.

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