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Performance of the 47-Kilodalton Membrane Protein versus DNA Polymerase I Genes for Detection of *Treponema pallidum* by PCR in Ulcers

Angèle Gayet-Ageron,[a] Frédéric Laurent,[b] Jacques Schrenzel,[c,d] Béatrice Charton,[b] Gisela Jimenez-Getaz,[c] Manuela Tangomo,[d] Tristan Ferry,[e] Patrice Sednaoui,[f] Stephan Lautenschlager,[g] Laurence Toutous-Trellu,[h] Begoña Martinez de Tejada,[i] Matthias Cavassini,[j] Stéphane Emonet,[c] Thomas Perneger,[a] Hélène Salord[b]

Division of Clinical Epidemiology, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland[a]; Laboratory of Bacteriology, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, France[b]; Bacteriology Laboratory and Division of Infectious Diseases, University of Geneva Hospitals, Geneva, Switzerland[c]; Genomic Research Laboratory, University of Geneva Hospitals, Geneva, Switzerland[d]; Service de Maladies Infectieuses et Tropicales, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, France[e]; Institut Alfred Fournier, Laboratoire CNR Gonocoques, Paris, France[f]; Triemlispital, Dermatologisches Ambulatorium Triemli, Zürich, Switzerland[g]; Department of Dermatology and Venereology, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland[h]; Department of Obstetrics and Gynaecology, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland[i]; Service de Maladies Infectieuses, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland[j]

***Treponema pallidum* PCR (*Tp*-PCR) is a direct diagnostic method for primary and secondary syphilis, but there is no recommendation regarding the best choice of target gene. In this study, we sequentially tested 272 specimens from patients with sexually transmitted ulcers using *Tp*-PCR targeting the *tpp47* and then *polA* genes. The two methods showed similar accuracies and an almost-perfect agreement.**

Syphilis has been making a worldwide comeback since the early 2000s (1–3) and was reintroduced in 2006 in Switzerland as being among the notifiable infectious diseases (4, 5). Since 2008 (6, 7), European guidelines have considered *Treponema pallidum* PCR (*Tp*-PCR) to be a definitive direct diagnostic tool for primary and secondary syphilis. Similarly, the U.S. Centers for Disease Control and Prevention (CDC) has changed their case definitions and now considers *Tp*-PCR to be capable of confirming early syphilis when clinical manifestations are present (8). However, no specific gene was recommended as a target for *Tp*-PCR. The two most widely used genes are *tpp47* and *polA* (9). *tpp47* encodes a *T. pallidum* cytoplasmic membrane protein (10) involved in cell wall synthesis (11) but that is partly specific to *T. pallidum* subsp. *pallidum* (12–14). *polA* encodes DNA polymerase I involved in DNA repair and the replication of most bacteria and shows a number of unique features in *T. pallidum* subsp. *pallidum* (15). Other targets have been tested sporadically. However, it remains unclear if one of these targets is preferred for use.

(The results from this study were presented at the 24th European Congress of Clinical Microbiology and Infectious Diseases, 10 to 13 May 2014, Barcelona, Spain, poster no. 1757 [16].)

We conducted a multicenter prospective study between 2011 and 2013 in five European cities, which were Geneva, Lausanne, and Zürich in Switzerland, and Paris and Lyon in France (17). Every patient presenting with a sexually transmitted ulcerative disease suggestive of syphilis was invited to participate. Each patient received the conventional diagnostic tests for syphilis (18), either dark-field microscopy (DFM) or a combination of the following serological assays: enzyme immunoassay (EIA), Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin (RPR) assay, a treponemal microhemagglutination assay (MHA-TP), or a fluorescent treponemal antibody absorption test (FTA-ABS). We distinguished between (i) a confirmed case (positive DFM) and (ii) a probable case (reactive VDRL or RPR result and reactive MHA-TP, FTA-ABS, or EIA result). Finally, we used (iii) an enhanced definition combining clinical information and the results from DFM and serology (17). All patients categorized as having syphilis benefited from standard treatment and were followed at 3, 6, and 12 months after treatment. The treatment response was defined by a 4-fold decline in the VDRL or RPR titer (19). Regarding *Tp*-PCR, swabs from the ulcers were collected and then analyzed sequentially. First, *Tp*-PCR targeting the *tpp47* gene (*tpp47*-*Tp*-PCR) was performed at the laboratory of bacteriology at the Geneva University Hospitals (20, 21). The test was considered positive if two of the three replicates had cycle thresholds (C_T) of <40. Next, all frozen DNA extracts were sent to Lyon (Department of Bacteriology, Hôpital de la Croix-Rousse), where *Tp*-PCR targeting the *polA* gene (*polA*-*Tp*-PCR) was performed using the primers and probes described elsewhere (22). A single *polA*-*Tp*-PCR was performed and was considered positive if the C_T was <40. The limits of detection of the two *Tp*-PCRs were blindly compared using the same

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Address correspondence to Angèle Gayet-Ageron, angele.gayet-ageron@hcuge.ch.

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TABLE 1 Patient characteristics

Variable[a]	Patient data ($n = 272$)
Center	
Paris	140 (51.5)
Lyon	59 (21.7)
Geneva	40 (14.7)
Lausanne	17 (6.2)
Zurich	16 (5.9)
Male gender	251 (92.3)
Age at the time of diagnosis (mean ± SD) (yr)	39.1 ± 12.3
Delay since appearance of ulcer (mean ± SD) (days)	20.4 ± 34.0
Localization of the ulcer	
Penile/vaginal	148 (54.4)
Anorectal	97 (35.7)
Oral	27 (9.9)
Route of contamination	
Homosexual	184 (71.3)
Heterosexual	70 (27.1)
Unknown	4 (1.6)
Coinfection with HIV	
Yes	53 (19.5)
No	172 (63.2)
Unknown	47 (17.3)
Concomitant HIV diagnosis during current episode of ulcerative disease	9 (17.0)
Patients treated with antiretroviral therapy	
Yes	36 (67.9)
No	14 (26.4)
Unknown	3 (5.7)
Other comorbidities	
Chronic lymphoid leukemia	1 (0.4)
Diabetes	1 (0.4)
Under corticotherapy	1 (0.4)
Diagnosis of syphilis with the reference tests	
Dark-field microscopy	
Positive	31 (18.3)
Negative	138 (81.7)
Serological assays	
Positive	87 (34.2)
Negative	151 (59.5)
Undetermined	16 (6.3)
Enhanced definition	
Positive	47 (27.8)
Negative	122 (72.2)
Combination of DFM and serological assays	
Positive	93 (34.8)
Negative	174 (65.2)

[a] The data are presented as the no. (%), unless otherwise indicated.

positive control (mixed DNA from rabbit tissues and *T. pallidum* Nichols strain DNA) at dilution rates from 1:10 to 1:100,000.

During a 2-year period, 273 patients were recruited, and 272 specimens for *Tp*-PCR were collected (Table 1). Most patients were men presenting with a genital ulceration after a mean of 20 days following homosexual intercourse. Nine patients were diagnosed with human immunodeficiency virus at the initial consultation. Globally, we obtained 77 concordant-positive and 191 concordant-negative *Tp*-PCR results; two specimens resulted in negative *polA*-*Tp*-PCR but positive *tpp47*-*Tp*-PCR results, and conversely, two had negative *tpp47*-*Tp*-PCR but positive *polA*-*Tp*-PCR results. The kappa coefficient was 0.96 (exact 95% confidence interval, 0.93 to 0.99). The two *Tp*-PCR results had the same indices of diagnostic performance according to the three case definitions (Table 2) ($P = 0.99$ for all comparisons, McNemar's test). When we considered *Tp*-PCR to be positive whenever one of the two *Tp*-PCRs was positive, sensitivity increased, especially in the enhanced definition group.

The mean C_T values among all the positive *Tp*-PCR results were significantly lower for *polA*-*Tp*-PCR than for *tpp47*-*Tp*-PCR (28.3 ± 3.7 versus 32.1 ± 5.6, $P < 0.001$ using Wilcoxon signed-rank test). The limits of detection of the two *Tp*-PCR results were identical at a threshold 1:100,000. Among the four discordant results, none had been examined by DFM, and all had clearly positive serology results (Table 3). Of note, *tpp47*-*Tp*-PCR was considered negative in one patient (one C_T value at 39.0 and two <40), but this patient had a *polA*-*Tp*-PCR result that was clearly positive; this suggests a false-negative *tpp47*-*Tp*-PCR result. All four patients were considered to have syphilis and were treated for syphilis using 2.4 million units of penicillin G benzathine administered intramuscularly, and all responded to treatment, suggesting that all were true-positive cases.

We demonstrated that the diagnostic performances of the two currently used targets for *Tp*-PCR were comparable with ulcer specimens, irrespective of the case definition of syphilis. The agreement between the two *Tp*-PCRs was almost perfect (23). To our knowledge, only one previous study reported a high agreement between *tpp47*-*Tp*-PCR and *polA*-*Tp*-PCR using a smaller collection of 112 paired specimens from patients with ulcers suggestive of primary syphilis (24). Another study reported similar sensitivities of three types of *Tp*-PCR (*tpp47*-*Tp*-PCR, *polA*-*Tp*-PCR, and a combination of the two) in the blood samples from latent syphilis cases, but it did not assess the agreement between the three methods (25). Our results also confirmed the accuracy and clinical value of *tpp47*-*Tp*-PCR (26) and *polA*-*Tp*-PCR (27) with primary syphilis ulcers. The added value of our study is that we assessed the two *Tp*-PCR methods with a large number of paired specimens selected by a standardized procedure. Any discrepancies between the two techniques could not be explained by a difference in DNA extraction, since the testing procedures were rigorous and fully standardized. The two *Tp*-PCR methods had the same analytical limits of detection and thus had comparable sensitivities. However, our study was pragmatic, and the mean number of replication cycles performed was higher for *tpp47*-*Tp*-PCR than for *polA*-*Tp*-PCR; this might explain why the two discrepancies favored *tpp47*-*Tp*-PCR. If two additional replications had been done for *polA*-*Tp*-PCR, the chance of a positive result would have been greater.

In conclusion, we confirmed that *Tp*-PCR is a useful diagnostic tool for ulcers that suggest primary syphilis and that the results do not depend on the target gene, suggesting that either *tpp47* or *polA* can be used in practice. Combining the results of the two *Tp*-PCRs did not drastically improve the clinical utility of the test. Therefore, the supplemental cost of performing an additional *Tp*-PCR targeting another gene is not justified.

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TABLE 2 Description of the results of the two *Tp*-PCRs according to the three case definitions, corresponding indices of accuracy and clinical utility for each *Tp*-PCRs taken separately and combining the results of the two methods

	Tp-PCR result with case definition in (*n*):					
	Dark-field microscopy (169)		Serological assays (254)		Enhanced definition (169)	
Tp-PCR assessment	Positive	Negative	Positive	Negative	Positive	Negative
Tp-PCR targeting either *tpp47* or *polA* gene[a]						
Positive	29	13	68	10	41	1
Negative	2	125	19	141	6	121
Accuracy and clinical utility of *Tp*-PCR targeting *tpp47* or *polA* gene (% [95% CI])[a]						
Sensitivity	93.6 (78.6–99.2)		77.0 (66.8–85.4)		87.2 (74.3–95.2)	
Specificity	90.6 (84.4–94.9)		93.4 (88.2–96.4)		99.2 (95.5–99.9)	
Positive predictive value	69.1 (52.9–82.4)		87.0 (77.4–93.6)		97.6 (87.4–99.9)	
Negative predictive value	98.4 (94.4–99.6)		87.6 (81.6–91.8)		95.3 (90.1–97.8)	
Positive likelihood ratio	9.9 (5.9–16.8)		11.6 (6.3–21.4)		106.4 (15.1–751.8)	
Negative likelihood ratio	0.07 (0.02–0.27)		0.25 (0.17–0.36)		0.13 (0.06–0.27)	
Accuracy and clinical utility of *Tp*-PCR combining the results of the two methods (% [95% CI])[b]						
Sensitivity	93.8 (79.2–99.2)		79.6 (69.6–87.4)		97.7 (87.7–99.9)	
Specificity	90.6 (84.4–94.9)		93.4 (88.2–96.8)		95.3 (90.0–98.2)	
Positive predictive value	69.8 (53.9–82.8)		87.5 (78.2–93.8)		87.5 (74.8–95.3)	
Negative predictive value	98.4 (94.4–99.8)		88.7 (82.7–93.2)		99.2 (95.5–99.9)	
Positive likelihood ratio	44.3 (11.0–177.7)		7.7 (5.0–12.0)		106.8 (15.1–754.0)	
Negative likelihood ratio	0.31 (0.20–0.48)		0.14 (0.08–0.25)		0.13 (0.06–0.27)	

[a] We found the same results with *Tp*-PCR targeting *tpp47* gene and that targeting *polA*. The presence of 2 discrepancies favoring *Tp*-PCR targeting the *tpp47* gene and 2 discrepancies favoring *Tp*-PCR targeting the *polA* gene led to the same results. 95% CI, 95% confidence interval.

[b] Global results are based on the combination of the results of the two *Tp*-PCRs; if one of the two *Tp*-PCRs or the two *Tp*-PCRs were positive, *Tp*-PCR was globally considered positive.

TABLE 3 Description of the four discordant results between the two types of *Tp*-PCR

Results	Data for case no. (center):			
	1 (Geneva)	2 (Geneva)	3 (Lyon)	4 (Zurich)
Tp-PCR (C_T) targeting:				
tpp47 gene	Positive (33.9/33.9/0)	Positive (36.0/35.0/37.0)	Negative	Negative (37.0/0/0)[a]
polA gene	Negative	Negative	Positive (35.8)	Positive (36.8)
Serological assays[b]				
VDRL/RPR titer	1:64	1:16	1:32	1:4
MHA-TP titer	1:20,480	1:2,560	1:20,480	1:10,240
FTA-ABS	NA[c]	1:3,200	NA	Reactive
EIA	NA	NA	45.06	NA
Localization of the ulcer	Anorectal	Oral	Anorectal	Penile
Titer at follow-up after treatment (mo)				
3	RPR, 1:8; MHA-TP, 1:10,240	RPR, 1; MHA-TP, 1:1,280	RPR, 1:4; MHA-TP, 1:10,240	RPR, 0; MHA-TP, 1:160
6	RPR, 1; MHA-TP, 1:2,560	RPR, 0; MHA-TP, 1:1,280	RPR, 1:2; MHA-TP, 1:10,240	NA
12	RPR, 0; MHA-TP, 1:1,280	NA	NA	NA

[a] Three replicates were performed for *Tp*-PCR targeting the *tpp47* gene. As one replicate among the three was positive, *Tp*-PCR targeting the *tpp47* gene was considered negative.

[b] VDRL, Venereal Diseases Research Laboratory; RPR, rapid plasma reagin; MHA-TP, microhemagglutination assay for antibodies to *T. pallidum*; FTA-ABS, fluorescent treponemal antibody-absorbed test; EIA, enzyme immunoassay.

[c] NA, not available.

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A.G.-A. designed and coordinated the prospective clinical study, collected and managed data, and performed the statistical analysis. P.S., S.L., L.T.-T., B.M.D.T., T.F., and M.C. were responsible for or coordinated patient recruitment at their site for the prospective clinical study. S.E., J.S., G.J.-G., and M.T. were in charge of the technical aspect of the prospective clinical study and were responsible for the interpretation of all PCR assays targeting the *tpp47* gene. F.L. and H.S. were in charge of the technical aspect of the prospective clinical study and were responsible for the interpretation of all PCR assays targeting the *polA* gene. T.P. supervised the study conduct and gave important advice regarding the interpretation and presentation of the results. A.G.-A. wrote the paper, with important contributions being made from all co-authors.

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