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Incidental Syphilis Diagnosed by Real-Time PCR Screening of Urine Samples

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A dramatic increase in the incidence of sexually transmitted diseases (STDs), such as gonorrhea (1), syphilis (1, 2), and HIV seroconversion (3, 4), has been reported worldwide. The gold standard for syphilis diagnosis remains the serology test, particularly the Venereal Disease Research Laboratory (VDRL) test or rapid plasma reagin (RPR) test (5, 6). Since August 2013, following the reports of increases in STDs (7, 8), every sample addressed for detection of any one of the following pathogens has been automatically screened for all of them: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Treponema pallidum*. DNA extraction as well as quality evaluation was performed as previously described (9). The possible presence of inhibitors in the samples was also tested using primers targeting a synthetic inhibition control (9). *Treponema pallidum* real-time PCR (RT-PCR) was performed using a CFX96 device according to the manufacturer's recommendations (Bio-Rad Clinical Diagnostics, Marnes-la-Coquette, France) targeting the *polA* gene (Tpa_polA_F, GAGTGTGCAGT CCGCTATGC; Tpa_polA_R, AGGCAAAAGCGGCATTTCTA; Tpa_polA_P, TCCGCTTGGAAACAGCAGGATTG). A synthetic positive control of 381 bp from the *polA* gene produced with a pUC57 plasmid was used, as well as a negative control for each run. Confirmation PCR targeting the *flaA* gene was performed as previously described (10). Over a period of 13 months, 1,706 samples from 1,409 patients were received, of which 887 (52%) were urine samples. Seven of these samples from five patients, including four urine samples and three lesion swabs, were positive for syphilis. The treponemal load in samples from patient 2 was 2 to 3 orders of magnitude higher than those from other subjects (Table 1). *Treponema pallidum* PCR was not requested for two of the five patients, making these syphilis diagnoses incidental. These last findings were confirmed by follow-up serology performed at 1 and 4 months, respectively. Finally, all patients had both a positive VDRL test and a positive *T. pallidum* hemagglutination (TPHA) test, except patient 2, for whom serology was not performed.

During the same period, 337 sera from 147 patients were positive for syphilis by serology, defined by both a positive VDRL test and a positive TPHA test. Only 36 patients had further PCR screening. Forty-eight specimens were collected, including 25 urine samples, 16 swabs, and 7 others. Syphilis RT-PCR sensitivity was estimated at 16% (4/25) in urine and at 18.75% (3/16) in swabs.

Molecular amplification of *Treponema pallidum* DNA by RT-PCR has been proven useful in the diagnosis of early syphilis, particularly before the serological response has fully developed (11). Although genital ulcers or lesion swabs seem to be the more appropriate samples (12, 13), the sensitivity of urine specimens was previously estimated at 29% in cases of primary syphilis and at 44% in cases of secondary-stage disease (14) and offers the benefit of being noninvasive. Our experience also suggests the value of

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TABLE 1 Characteristics of the 7 samples positive for *Treponema pallidum* detection[a]

Patient no.	Age (yr)	Sex	Specimen	Initial query	Syphilis RT-PCR result (C_T)		No. of days between serology and PCR	Serology result by test:	
					polA gene	*flaA* gene		VDRL titer	TPHA
1	33	M	Urine	*C. trachomatis*	Positive (34)	Positive (36)	−3	1/32	R
			Endobuccal swab	*T. pallidum*	Positive (28)	Positive (31)			
2	24	M	Pharynx swab	*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum*	Positive (26)	Positive (28)			
			Urine	*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum*	Positive (24)	Positive (25)			
3	**43**	**M**	Urine	*N. gonorrhoeae*	Positive (31)	Positive (30)	124	1/512	R
4	**53**	**M**	Urine	*N. gonorrhoeae*, *C. trachomatis*	Positive (35)	Positive (34)	31	1/32	R
			Rectal swab	*N. gonorrhoeae*, *C. trachomatis*	Negative	Negative			
5	60	M	Genital swab	*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum*	Positive (32)	Positive (33)	0	1/8	R
			Urine	*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum*	Negative	Negative			

[a] Patients whose demographic characteristics are in bold are the two incidental diagnoses. Abbreviations: M, male; C_T, cycle threshold; R, reactive.

screening multiple anatomical sites (15), as shown by discordant multisite RT-PCR results (Table 1).

Finally, this report highlights the usefulness of RT-PCR for the detection of *Treponema pallidum* from noninvasive samples, such as urine, in the context of PCR testing for STDs. The systematic screening reduces the risk of missing a diagnosis for a disease that tends to be overlooked.

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