



Urine as Sample Type for Molecular Diagnosis of Natural Yellow Fever Virus Infections

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KEYWORDS PCR, diagnostics, urine, yellow fever

Yellow fever virus (YFV; genus *Flavivirus*) is a reemerging threat to public health in Africa and South America. Despite the availability of an effective, safe, and affordable vaccine (1), YFV has caused multiple outbreaks in recent years. Recent outbreaks included urban yellow fever (YF) in Angola and the Democratic Republic of Congo in 2015 to 2016 and a significant increase in sylvatic YF cases in South America since December 2016. A total of 792 confirmed cases, among which there were 435 deaths, occurred from December 2016 to 31 May 2017 in Brazil alone (2, 3). This recent increased activity of YFV in South America is reflected in an unusual number (four) of reported cases in European Union travelers since August 2016, which included a patient returning to the Netherlands from Surinam in March 2017 (4, 5).

Laboratory diagnosis of YFV infection relies heavily on molecular testing, as accurate diagnosis based on serology is complicated by cross-reactivity with other flaviviruses (such as dengue virus and Zika virus) and the inability to discriminate between naturally acquired immunity and vaccine-acquired immunity. International guidelines indicate whole blood and serum as specimens of choice for reverse transcription-PCR (RT-PCR) detection with a typical detection window of 1 to 7 days after the onset of symptoms (6, 7). Increasing amounts of evidence point at other bodily fluids such as saliva, semen, or urine as complementary or alternative sample types for some flaviviruses (8–16). Although the additional value of urine as a diagnostic sample type for detection of YFV RNA has been demonstrated for vaccinees with suspected adverse events upon vaccination, no data have been reported yet on the detection of YFV in urine from naturally infected cases (17).

To explore the strong suggestion that urine can be a valuable sample type for YFV diagnostics, we investigated the presence of YFV RNA in a longitudinal set of urine samples from the aforementioned Dutch YF patient (5). Total nucleic acids were extracted (MagnaPureLC; Roche, Almere, the Netherlands) with input and output volumes of 200 μ l and 100 μ l, respectively. YFV and internal control (phocine distemper virus [PDV] [18]) real-time RT-PCR (LC480-II; Roche) was performed using 8 μ l of extract in a 20- μ l final volume, 1 \times TaqMan Fast Virus 1-Step master mix (Life Technologies, Nieuwerkerk aan den IJssel, the Netherlands), 0.5 U uracil-N-glycosylase (Life Technologies), and the following primers (0.4 μ l each) and probe (adapted from Domingo et al. [18]): YFV_{wdD} (5'-GCTAATTGAGGTGYATTGGTCTG-3') (45 pmol/ μ l), YFV_{probeD} (5'-ATCGAGTTGCTAGGCAATAAACACATTTGGATT-3') (5 pmol/ μ l), and YFV_{rev} (5'-CTGCTAATCGCTCAAMGAACG-3') (45 pmol/ μ l). Assay validation was performed according to ISO

Accepted manuscript posted online 30 August 2017

Citation Reusken CBEM, Knoester M, GeurtsvanKessel C, Koopmans M, Knapen DG, Bierman WFW, Pas S. 2017. Urine as sample type for molecular diagnosis of natural yellow fever virus infections. *J Clin Microbiol* 55:3294–3296. <https://doi.org/10.1128/JCM.01113-17>.

Editor Alexander J. McAdam, Boston Children's Hospital

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TABLE 1 Longitudinal analysis of serum and urine samples of a naturally infected yellow fever patient

Parameter	Result ^a								
	Serum			Urine					
Day of sampling ^b	6	14	20	9	17	20	24	31	45
Dilution factor ^c	10×	None	None	2×	None	None	None	None	None
C _T value ^d	28.60	31.95	35.23	26.50	35.40	32.20	36.60	>40.00	>40.00
Interpretation	P	P	P	P	P	P	P	ND	ND

^aNone, no dilution input RNA; ND, not detected; P, positive.

^bData are indicated as day after onset of symptoms.

^cData represent dilution factor-extracted input RNA in RT-PCR.

^dC_T, threshold cycle.

15189:2012 guidelines (19). Informed consent was obtained from the patient according to national medical ethical regulations.

We report the presence of YFV RNA in urine samples from a naturally infected YF patient, with real-time RT-PCR-based YFV detection until 24 days after the onset of symptoms (Table 1). This observation has potential importance for the identification of clinical YF cases, as urine collection is noninvasive, cheap, and easy to perform. The latter factor is especially relevant under conditions in which blood collection can be problematic (e.g., collection from neonates and patients with hemorrhages) and in situations where trained personnel and/or proper facilities are unavailable (17, 20). Urine sampling might provide a long window of opportunity to confirm YF diagnosis in suspected cases, thereby decreasing the reliance on cross-reactive serology in the first weeks of illness. While viremia was atypically detected until at least day 20 in the naturally infected YF patient, we could not compare the detection windows between serum and urine as no serum samples were available beyond day 20. Although our study data prove the presence of YFV in urine of a wild-type YF case, further studies in a large cohort of such naturally infected patients are needed to establish the extent of our observation and the typical window of detection in urine in relation to detection in plasma/serum and to circulating YFV strains and severity of disease.

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