Appendix S1

MATERIAL AND METHODS

Sample preparation for PCR

From each patient approximately 20 ml of first-void urine was collected and 7–13 ml was mailed to Copenhagen in a nucleic-acid-stabilizing medium (GeneLock®, Sierra Molecular, Sonora, CA, USA). The samples were stored at 5°C for maximum 1 day before they were sent from Stockholm to Copenhagen by ordinary post. The samples spent approximately 2 days in the mail and were stored at 5°C until DNA purification within a few days.

Urine samples in GeneLock® were concentrated by centrifugation of 1,900 µl at 30,000 × g for 15 min. The pellet was dissolved in 300 µl of a 20% Chelex-100 slurry (BioRad, Hercules, CA, USA) in TE-buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) and incubated at 95°C for 10 min, as previously described (34).

The prepared samples were stored at –20°C until PCR was performed. Before PCR, samples were vortexed and centrifuged briefly, and 2 µl of the supernatant was used as template in the *N. meningitidis* and *S. pneumoniae, H. influenzae, M. catarrhalis* and adenovirus assays and 10 µl template was used in the *T. vaginalis* assay, corresponding to approximately 16 and 79 µl of the original urine sample, respectively. In the remaining PCRs 5 µl of the supernatant was used as template, corresponding to approximately 40 µl of the original urine sample.

PCR assays

All samples were tested by PCR for *C. trachomatis, N. gonorrhoeae, M. genitalium, T. vaginalis, U. urealyticum* and *U. parvum, N. meningitidis, S. pneumoniae, H. influenzae, M. catarrhalis*, adenovirus, and HSV-1 and -2 at Statens Serum Institute in Copenhagen.

C. trachomatis was detected by real-time PCR targeting the *16S rRNA* gene, and positive results were subjected to a real-time PCR targeting the *C. trachomatis* cryptic plasmid (S1) allowing to discriminate between wild-type and new-variant *C. trachomatis* (27). *M. genitalium* was detected by real-time PCR targeting the *MgPa*-gene (34) and confirmed by conventional PCR targeting the *16S rRNA* gene (S2). Macrolide resistance-mediating mutations were demonstrated by sequencing the region V of the *23S rRNA* gene in *M. genitalium* positive samples (S3).

N. gonorrhoeae was detected by a real-time PCR targeting the *porA* pseudogene (S4) with a slight modification of the probe (FAM-CAGCAAGTCCGCCTATACGCCTGCTACTT-BHQ-1). For detection of *U. urealyticum* and *U. parvum* a multiplex PCR targeting the urease gene was used (36). *N. meningitidis* and *S. pneumoniae* were detected with a multiplex PCR targeting the capsular transport (*ctrA*) and pneumolysin (*ply*) genes, respectively (S5). *H. influenzae* was detected with primerset F729 and R819 producing a 112 base-pair fragment of the *protein D* gene (S6). The target-specific *H. influenzae* probe was shortened and labelled with FAM at the 5'-end and a minor groove binder (MGB) at the 3'-end: FAM-AAA-CATCCAATCGTAATTA-MGB. *M. catarrhalis* was detected as described (S7), except that an extra base was added in the forward primer to match the *copB* outer membrane protein gene: GTGAGTGCCGCTTTTACA, and that the probe was labelled with FAM and BHQ at the 5' and 3'-end, respectively.

HSV-1 and -2 were detected by a multiplex real-time TaqMan-based PCR amplifying fragments of the Envelope Glycoprotein *G* and *D* gene in HSV-1 and -2, respectively. The HSV-1 forward and reverse primers were: HSV1-F 5´GTCTGTGGTGTTTTTGG-CATCAT and HSV1-R 5´CACCGACAAGAACCAAAAGGAA, and the HSV-2 primers were: HSV2-F 5´CGCCAAATACGC-CTTAGCA and HSV2-R 5´ GAAGGTTCTTCCCGCGAAAT. Target specific HSV-1 and -2 probes were labelled with FAM and Yakima Yellow™, respectively (unpublished data).

Thermocycling conditions for the TaqMan based PCR assays were 95°C for 10 min, then 50 cycles of 95°C for 15 s and 60°C for 1 min.

Adenovirus was detected by real-time SYBR-green assay PCR amplifying the *hexon* gene using the primerset AdF1 and AdR1 (5). Thermocycling conditions for the adenovirus assay were 95°C for 5 min, followed by 10 cycles of 95°C for 15 s, 65°C to 55°C touchdown with a 1°C decrement per cycle for 30 s, 72°C for 45 s, then 40 cycles of 95°C for 15 s, 55°C for 30 s and 72°C for 45 s, followed by a melting curve analysis. For adenovirus species-determination, positive samples were Sanger-sequenced and a BLAST search was conducted in GenBank.

All real-time PCR assays were performed on an ABI 7500 Sequence Detection Systems with 96-well conventional blocks (ABI, Life Technologies, Foster City, CA, USA).

T. vaginalis was detected by a conventional PCR with primers TVK3 and TVK7 amplifying a 261 base-pair fragment of a conserved region of a 2,000 base-pair repeat, as described previously (S8).

Positive controls for PCR

For quantification of the bacterial species, genomic DNA was extracted from cultured bacteria using DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) and quantified using a Qubit™ Assay Kit (Invitrogen, Life Technologies). Ten-fold dilutions containing 1–100,000 genome equivalents (geq) per µl of DNA from each bacterial species were made in TE buffer containing 1 µg/ml of calf thymus DNA (Sigma-Aldrich, Brøndby, Denmark). PCR results are given as DNA copies (genome equivalents, geq) per ml in the original urine sample.

SUPPLEMENTARY REFERENCES

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