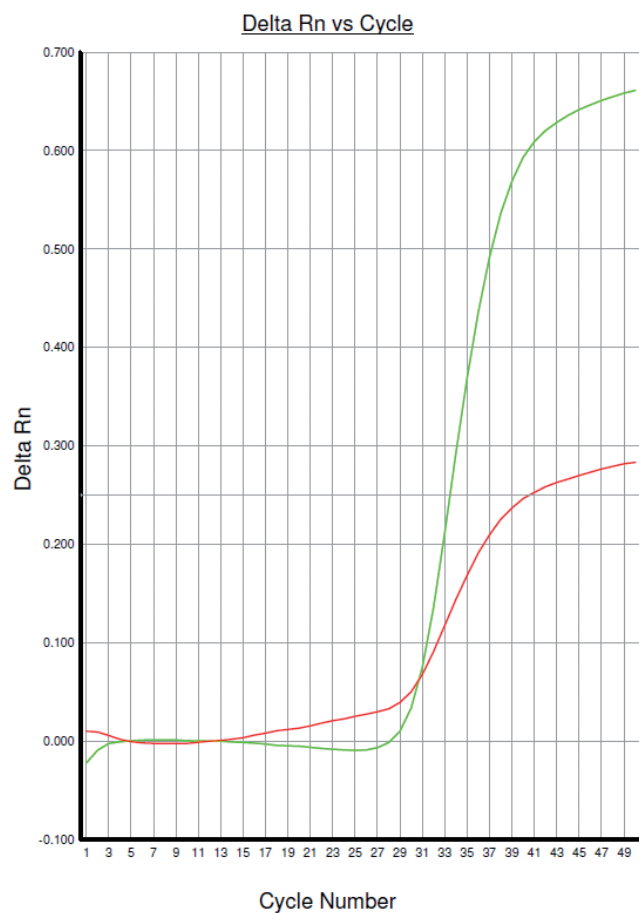


Fig. S1. Mixed *M. genitalium* strains with mutations 2071T and 2071G. Arrowhead indicates site of mutations.



Fig. S2. Mixed *M. genitalium* isolates with wild type and mutated isolate 2071G. Arrowhead indicates site of mutation.



Selected Detector: All

Well(s): A5

Document: 170306KMGNS2.sds (Standard Curve)

Fig. S3. Mixed *M. genitalium* isolates with mutations 2071T and 2071G (same sample as in Fig. S1), detected by real-time PCR for detection of wild type and of macrolide-associated mutations of the 23S rRNA gene. The mutation at position 2071T was detectable by the probe for wild type (red curve) 23S rRNA gene.

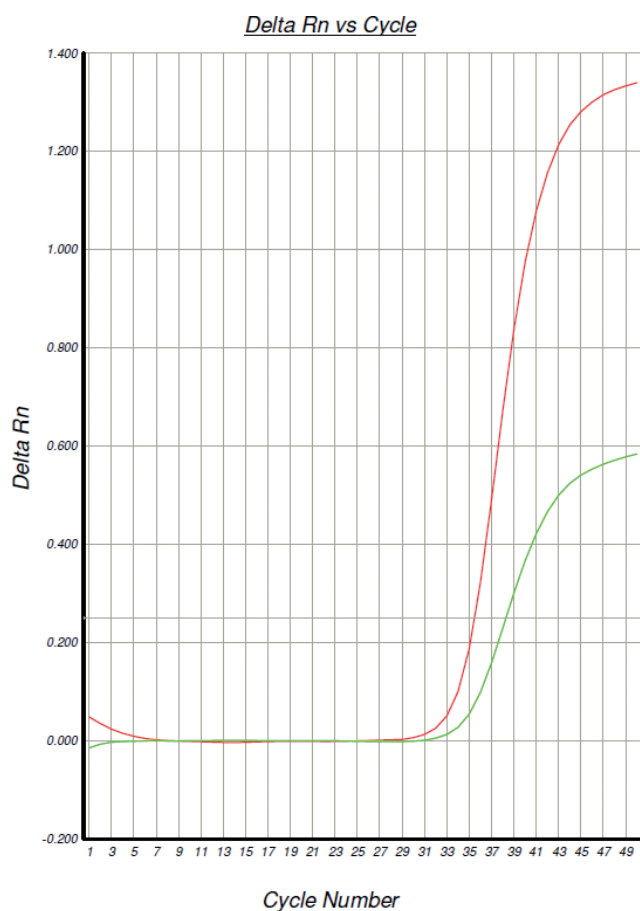


Fig. S4. Mixed *M. genitalium* isolates with wild type and 2071G mutation (same sample as in Fig. S2), detected by real time PCR for detection of wild type (red curve) and of macrolide-associated mutations of the 23S rRNA gene.

Table SI. Validation of the used 23S sequencing assay (1) of *M. genitalium* in our laboratory by testing 5 isolates with known 23S status as determined at another laboratory

Sample number	Other laboratory 23S genotype	Our laboratory 23S genotype
1	Mutant	Mutant (A2071G)
2	Wild type	Wild type
3	Mutant	Mutant (A2071G)
4	Mutant	Mutant (A2071G)
5	Mutant	Mutant (A2072G)

(1) Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis 2008; 47: 1546–1553.