

Report on an unusual case of leprosy from Germany: just an exception of the rule?

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Supplementary material 1: 16S rRNA RT qPCR run protocol

Assay: 16S rRNA RT qPCR
Analysis: Detection of *Mycobacterium leprae* cDNA (16S rRNA)
Type of PCR: Reverse transcriptase- quantitative real-time PCR

Primer: (10 μ M)		Probe: (5 μ M)	
16S TaqF	5' GCA TGT CTT GTG GTG GAA AGC 3'	16TP2	5' 6FAM- CCA TCC TGC ACC GCA AAA A -BBQ 3'
16S TaqR	5' CAC CCC ACC AAC AAG CTG AT 3'		

Reagent	Number of samples:		plus 10%:		Remarks
	Single reaction [μ l]	Mastermix [μ l]	Concentration of solution	Final concentration	
DEPC Water	8,60				Carl Roth
16S TaqF	1,00		10 μ M	0,5 μ M	TibMolBiol - HPLC
16S TaqR	1,00		10 μ M	0,5 μ M	TibMolBiol - HPLC
16S TP2	1,00		5 μ M	0.2 μ M	TibMolBiol - HPLC
qPCR Mix Plus	4,00		5 U/ μ l	1 U/ μ l	Solis BioDyne
Exo IPC Mix	2,00		10x	1x	Applied Biosystems*
Exo IPC DNA	0,40		50x	1x	Applied Biosystems*
Total vol. Mastermix [μl]	18,00				
Template	2,00				
Final volume	20,00				

*formerly Life Technologies

Step:	Temperature:	Time:
1	95°C	15 min.
2	95°C	15 s
3	60°C	60 s

(Step 2-3: 40x)

Duration 1:38h

Detection in VIC and FAM channels of the CFX96 real-time PCR detection system (BioRad, Munich, Germany) after each step 3.

Sample number	Sample (Patient/ID)	Result	Dilution
1			10 [^]
2			10 [^]
3			10 [^]
4			10 [^]
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6			10 [^]
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8			10 [^]
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43			10 [^]
44			10 [^]
45			10 [^]
46			10 [^]
47			10 [^]
48			10 [^]

Remarks: _____