RLEP LAMP for the laboratory confirmation of leprosy: towards a point-of-care test

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Malkin Saar, Marcus Beissner, Fatih Gültekin, Issaka Maman, Karl-Heinz Herbinger and Gisela Bretzel

Additional file 1: in-house "wet" RLEP LAMP run protocol

Assay:	**RLEP LAMP**
Analysis:	**Detection of *Mycobacterium leprae* DNA (RLEP)**
Type of NAAT:	**Loop-mediated isothermal amplification**
Type of Primers:	**Wet**

Outer Primer: (5 µM)	
RLEP F3	5'CGCACCTGATGTTATCCCTT'3
RLEP B3	5'GGTTTGGGTGGTGTTGTGG'3

Inner Primer: (20µM)	
RLEP FIP	5'ATGCCTGCTTGCTGGCTGAG CACCATTTCTGCCGCTGG'3
RLEP BIP	5'CAGTGCATCGATGATCCGGCC GTGTGGGTGGTTGATCTGC'3

	Amount of samples:	**6 samples, 1 positive control, 1 negative control = 8 (plus 20% additive due to pipetting lost)**		
Reagent	**Single reaction [µl]**	**Master mix [µl]**	**Concentration of stock solution**	**Final concentration**
Isothermal Master mix ISO-DR-004 [Optigene; Horsham, UK]	15.00	**150.00**	-	1x
Primer F3	1.00	**10.00**	5 µM	0.2 µM
Primer B3	1.00	**10.00**	5 µM	0.2 µM
Primer FIP	2.00	**20.00**	20 µM	1.6 µM
Primer BIP	2.00	**20.00**	20 µM	1.6 µM
H_2O	2.00	**20.00**	-	-
Total vol. reaction mix [µl]	23.00	**230.00**		
Template	2.00			
Final volume	25.00			

Amplification/ Annealing

1	65°C	30 min.

Melting curve

2	80°C – 98.5°C

Detection: **FAM channel**

Device: **Genie III**

Date:	**Description:**					
Well	**Sample**	**Material**	**Result**	**Time to positivity [min:ss]**	**Fluoroscence [k]**	**Annealing temperature [°C]**
1						
2						
3						
4						
5						
6						
7						
8						

Results interpretation: **Result positive if amplification ≤25 min AND fluorescence ≥ 5 [k]**

Result negative if amplification >25min OR fluorescence < 5 [k]