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

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Additional file 4: ready-to-use RLEP DRB LAMP run protocol

Assay:	**RLEP LAMP**
Analysis:	**Detection of *Mycobacterium leprae* DNA (RLEP)**
Type of NAAT:	**Loop-mediated isothermal amplification**
Type of Primers:	**Dry reagent based (lyophilization by Amplexdiagnostics GmbH, Germany)**

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**
Reagent	**Single reaction [µl]**	No reaction mix, pipette directly into the wells of the delivered strips.
dryed master mix strips	Already prepared in the well	
resuspension solution RS	12.50	
H_2O	7.50	
Total vol. in well [µl]	20.00	
Template	5.00	
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 annealing temperature 91.5 ±1°C AND Tp >10 min AND fluorescence ≥ 5 [k]

If fluorescence >2: Use the Genie Explorer: Result positive if annealing temperature 91.5 ±1°C AND starting fluorescence ≥ 20 [k] AND Tp >10 min (threshold 0.002 ratio)

Result negative if otherwise