

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

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## Additional file 3: in-house 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 in-house, please refer to additional file 2)

Outer Primer: (5 µM)		Inner Primer: (20µM)	
RLEP F3	5'CGCACCTGATGTTATCCCTT'3	RLEP FIP	5'ATGCCTGCTTGCTGGCTGAG CACCATTTCTGCCGCTGG'3
RLEP B3	5'GGTTTGGGTGGTGTGTGG'3	RLEP BIP	5'CAGTGCATCGATGATCCGGCC GTGTGGGTGGTTGATCTGC'3

Amount of samples: 6 samples, 1 positive control, 1 negative control = 8 (+10% additive due to pipetting lost)

Reagent	Single reaction [µl]	Master mix [µl]
Dryed master mix	-	1 tube
Buffer ISO-DR-004	15.00	135.00
H <sub>2</sub> O	8.00	72.00
Total vol. reaction mix [µl]	23.00	207.00
Template	2.00	
Final volume	25.00	

### Amplification/ Annealing

1	65°C	30 min.
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### Melting curve

2	80°C – 98.5°C
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Detection: FAM channel  
 Device: Genie III

Date:		Description:				
Well	Sample	Material	Result	Time to positivity [min:ss]	Fluorescence [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]