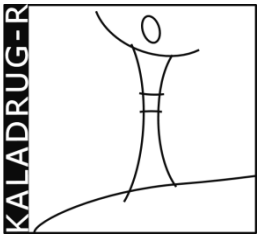
KALADRUG-R: Laboratory SOP#16



***Leishmania donovani* diagnostic PCR on SSU rDNA**

Date of preparation: November 10th 2010

Author: ITMA and BPKIHS

AIM: this protocol is designed to amplify *Leishmania donovani* DNA from bone marrow/pellet/ blood samples.

IMPORTANT NOTE: *if you use a different polymerase enzyme then Qiagen HotstarTaq or a different PCR cycler, you need to retest the analytical sensitivity to check if you obtain comparative results. This can be done by testing a dilution series of extracted parasite spiked blood samples or even quantified genomic DNA as described for the Leishmania SSU PCR in Deborggraeve S et al Journal of Infectious Diseases: 198 p1565-1572, 2008*

A. Reagents used

component	brand + type	catalogue no.	stock concentration*	concentration unit
PCR water	--	--	--	--
buffer	Qiagen Coralload	1032517	10	X
$MgCl_2$ solution	Qiagen	1005482	25	mM
dNTPs	Eurogentec	NU-0010-50	5	mM each
BSA	Promega	R396A	10	mg/mL
Polymerase	Qiagen HotStarTaq Plus	1032502	5	units/µL

** this is the concentration in the tube used to make the mastermix*

B. Primers

name	sequence	stock conc. (µM=pmol/µL)*
M18S-L-F	5' CGTAGTTGAACTGTGGGCTGTGC 3'	100
M18S-L-R	5' ACTCCCGTGTTTCTTGTTTCTTTGAA 3'	100

** this is the concentration in the tube used to make the master mix*

C. PCR master mix

components	volume per PCR (µL)	unit	final PCR conc.
water	18,75	--	--
buffer	2,5	X	1
$MgCl_2$ solution	1	mM	1
dNTPs	1	mM each	0,2
BSA	0,25	mg/mL	0,1
M18S-L-F	0,2	pmol/µL	0,8
M18S-L-R	0,2	pmol/µL	0,8
polymerase	0,1	units/µL	0,02
template	1	--	--
total	25		

D. Cycler program

Temp (°C)	Time		Cycles
95	5	min	1
94	30	sec	40
60	30	sec	
72	30	sec	
72	5	min	
4	∞	min	1

**cycling parameters were optimised to work on Eppendorf Mastercycler or Biometra T3000*

E. Control samples to include in each PCR

1) positive control 1 (0.4 ng/µL)

2) positive control 2 (0.04 pg/µL)

3) negative control N1 = add 1 µL MilliQ water instead as template

4) negative control N2 = add 1 µL of DNA extraction control as template

F. Sample description and analysis

1) Prepare 2,5 % agarose gel and immerse in 0.5x TAE buffer for electrophoresis

2) Load 5 µL (0.5 µg/µL) of Fermentas gene ruler 100 bp or 100 bp + molecular weight marker

3) Load 12 µL of each PCR sample on the gel, there is no need to add loading dye to the PCR product since the coralloid buffer already contains loading dye

4) Run the gel at 100 volt for 30 min

5) Stain the gel with Ethidium Bromide and visualise the results.

6) Evaluate the gel: check the signal of the positive and negative controls. The size of the expected amplicon should be 115 bp. Fill out the reporting form for all samples (see next page).

SSU PCR report for asymptomatic study Kaladrug-R **date: ../../..**

Lane no. gel	Sample code	Gel result (positive/negative)		Lane no. gel	Sample code	Gel result (positive/negative)
1	pos. contr 1			1	pos. contr 1	
2	pos. contr 2			2	pos. contr 2	
3	neg. contr 1			3	neg. contr 1	
4	neg. contr 2			4	neg. contr 2	
5				5		
6				6		
7				7		
8				8		
9				9		
10				10		
11				11		
12				12		
13				13		
14				14		
15				15		
16				16		
17				17		
18				18		
19				19		
20				20		

PASTE GEL HERE