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Multiplex PCR for detection of *bla* KPC, *bla* OXA-48-like, *bla* VIM, *bla* IMP and *bla* NDM *genes in* gram negative bacilli

Primer name	Gene	Sequence 5´--3´
VIM-F	VIM	AGT GGT GAG TAT CCG ACA G
VIM-R		ATG AAA GTG CGT GGA GAC
IMP-UF1	IMP	GGY GTT TWT GTT CAT ACW TCK TTY GA
IMP-UR1		GGY ARC CAA ACC ACT ASG TTA TCT
NDM-F	NDM	AGC ACA CTT CCT ATC TCG AC
NDM-R		GGC GTA GTG CTC AGT GTC
KPC-F	KPC	AAC AAG GAA TAT CGT TGA TG
KPC-R		AGA TGA TTT TCA GAG CCT TA
OXA48-F	OXA-48-like	ATGCGTGTATTAGCCTTATCGG
OXA48-R2		TGAGCACTTCTTTTGTGATG

Amplicon size	Cycling program
VIM= 261 bp IMP = 404 bp NDM = 512 bp KPC = 916 bp OXA-163 = 763 bp (*)	Initial denaturation = 94ºC for 5min; Cycling= 30-35 cycles: 94ºC 30sec -- 54º 30sec -- 72ºC 60sec; Final extension = 72ºC for 10min

PCR Reagents--> Final volume= 50ul	
Reagent	**Volume**
ADN	5 ul
Buffer 10X	5 ul
MgCl2 (50 mM)	1,5 ul
dNTP´s (10 mM)	1 ul
Taq pol (5U/ul)	0,4 ul
Primers vim-F/R (10 µM)	1 ul each
Primers ndm-F/R (10 µM)	1 ul each
Primers impU-1/2 (10 µM)	2,4 ul each
Primers kpc-F/R (10 µM)	2 ul each
Primers oxa48-F/R2 (10 µM)	1 ul each
H2O	22,3 ul
Final volume	**50 ul**

Reference
Servicio Antimicrobianos, INEI-ANLIS "Dr. Carlos G. Malbrán".
(*) 763bp amplicon size corresponds to the product obtained for blaOXA-163 gene. This gene has a 12 nucleotide deletion with respect to blaOXA-48 within the amplified region. Therefore, the size of the amplification product for the blaOXA-48 gene is 775bp.