

Multiplex real-time PCR protocol for detection of carbapenemase genes: *kpc*, *oxa-48*, *ndm*, *imp* y *vim*

Multiplex	Gen	Range T°m	Alleles
CBP	<i>bla</i> KPC	88,25-89,57	KPC-2; KPC-3
	<i>bla</i> VIM	83,27-84,04	VIM-2; VIM-36
	<i>bla</i> IMP	76,26-77,12	IMP-8
	<i>bla</i> OXA-48	78,05-79,15	OXA-48; OXA-163
	<i>bla</i> NDM	81,33-82,14	NDM-1; NDM-5

PCR
95°C 10 min / 35 cycles: 95°C 20 seg - 55°C 20 seg - 72°C 45 seg
Melting Curve
95°C 30seg / 65°C 30 seg - 95°C 30 seg Resolution: 0,2 °C/10 seg

Reagent	Final Concentration	Volume (20ul)
MeltDoctor™ HRM		
Master Mix 2X	1X	10 µl
Primers VIM	0,2 µM	0,4 µl (c/u)
Primers NDM	0,4 µM	0,8 µl (c/u)
Primers IMP	0,6 µM	1,2 µl (c/u)
Primers OXA-48	0,2 µM	0,4 µl (c/u)
Primers KPC	0,2 µM	0,4 µl (c/u)
H2O	-	1,6 µl
DNA	-	2 µl

Gen	Name	Secuence	Amplicon size
<i>bla</i> KPC	KPC-RT-F	5'-TCGCTAAACTCGAACAGG	785 pb
	KPC-RT-R	5'-TTACTGCCCGTTGACGCCCAATCC	
<i>bla</i> VIM	VIM-F	5'-AGTGGTGAGTATCCGACAG	261 pb
	VIM-R	5'-ATGAAAGTGCCTGGAGAC	
<i>bla</i> IMP	IMP-RT-F	5'-GAGTGGCTTAATTCTCRATC	188 pb
	IMP-UR1	5'-GGYARCCAAACCACTASGTTATCT	
<i>bla</i> OXA-48	O48-RT-F	5'-TGTTTTTGGTGGCATCGAT	177 pb
	O48-RT-R	5'-GTAAMRATGCTTGGTTCGC	
<i>bla</i> NDM	NDM-RT-F	5'-ACACCAGTGACAATATCACCG	82 pb
	NDM-RT-R	5'-TTGGCCTTGCTGCCTTG	

Reference
Primers KPC-RT-F; KPC-RT-R; IMP-RT-F; O48-RT-F; O48-RT-R; NDM-RT-F; y NDM-RT-R del trabajo Monteiro et al. JAC,2012 (10.1093/jac/dkr563)