Identification of the different phylogroups from the *Klebsiella pneumoniae* species complex by real-time PCR

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Klebsiella Research and Surveillance

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KpSC phylogroup ID

We developed and optimized a real-time PCR for the identification of the different phylogroups (Kp1 to Kp6) belonging to *Klebsiella pneumoniae* species complex (KpSC) by amplification of five specific gene targets.

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Klebsiella ; phylogroups; PCR ; identification

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Primers and Probes

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The **KpSC phylogroup ID** : Primers, Probes and Amplicon length

A	B	C	D	E	F	G
PhG -Target	**Forward (5' – 3')**	**Reverse (5' – 3')**	**Probe**	**Quencher**	**Reporter**	**Amplicon length** (bp)
Kp1 - group_8970	CCTCAAACACGCCAATATGC	TACCGCGACGAGTAAAGTGG	GATCCATTGATTCCATTCGAACCGG	BHQ-1	JOE	85
Kp2 - group_10724	TTGTTGATTGGCAGGCCTTC	AAATGCTGTGACCACCGTTG	CGCCTGGCGTCAGTGGCCCCGAGC	BHQ-1	JOE	70
Kp3/Kp5 - *tetR*	GCGGGCCGGCTTTTC	CGCATCCCAGGGTATATTCG	AGGCCGAAGCCGCAATGATATTACTCA	BHQ-1	FAM	71
Kp4 - *nanK*	AAACAGCGGATGCTCTGAAC	ATGAGTCCGCCAAGATTCTG	ATAGCCTCGACCGGGATTATTCGTGA	BHQ-2	TAMRA	145
Kp6 - *mdtG_1*	GCGTTTCAATGATCCTGTCC	ATCTGCGTCTGGAAAACAG	GTGCGCTGATTGGTATTGGATATGGC	BHQ-2	TAMRA	76

PCR mix preparation

2 PCR mix preparation for each set of primers and probe

A	B
Mix Reagents	**Volume per well (µL)**
Takyon ROX Probe MasterMix dTTP blue	12.5
Forward (500 nM)	1
Reverse (500 nM)	1
Probe (250 nM)	0.5
DNA template	2.5
PCR grade water	2.5

Master Mix: Takyon™ ROX Probe MasterMix dTTP blue(Ref.UF-RPMT-B0701, Eurogentec, Belgium)

Amplification program

3

A	B	C
	Temperature (°C)	**Time (minutes:seconds)**
Holding stage	95.0	03:00
Cycling stage (x40)	95.0	00:15
60.0	01:00	

Thermocycler: ABI StepOne Real-Time PCR system (Thermo Fischer Scientific)

Positive and negative controls

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Negative control: PCR grade water
Positive controls:

- *Kp*1 DNA (*Klebsiella pneumoniae* ATCC 13883^T) at concentration 2 ng/µl ; 2.5 µl used *i.e.*, 5 ng.
- Kp2 DNA (*Klebsiella quasipneumoniae* subsp. *quasipneumoniae* 01A030^T)
- Kp3 or Kp5 DNA (*Klebsiella variicola* subsp. *variicola* F2R9^T; *Klebsiella variicola* subsp. *tropica* 1266^T)
- Kp4 DNA (*Klebsiella pneumoniae*07A044^T)
- Kp6 DNA (*Klebsiella quasivariicola* KPN1705^T)

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