

Supplemental Digital Content (SDC 1- Combined File)

SDC 2, Materials and Methods

Microbiological PCR diagnosis

P. jirovecii DNA was detected in induced sputum and/or BAL specimens using an ‘in-house’ real-time PCR assay adapted from Brancart (31). The *P. jirovecii* β -tubulin gene was amplified using primers 1186F (5'-GATCCGAGACATGGTCGCTATT) and 1257R (5'-TTCAACCTCCTTCATGGAAACAG), and TaqMan 1212T probe (5'-6FAM-TGTTGCAGCGATTTTCCGCGGTA-BHQ1). DNA was extracted from samples using NucliSENS[®] easyMAG[®] (bioMérieux, Castle Hill, NSW, Australia) according to manufacturer's instructions. Each 20 μ L - PCR reaction contained 1xLC FastStart reaction mix (containing FastStart *Taq* DNA polymerase, reaction buffer, dNTPs and 1mM MgCl₂), 3.5mM MgCl₂, 0.5 μ M 1186F and 1257R primers, 0.05 μ M 1212T probe and 10 μ l DNA. Cycling parameters were 95°C for 10 min, followed by 40 cycles 95°C for 5s, 58°C for 20s and 72°C for 20s. Samples with a crossing value <37.3 cycles were classified “positive” for *P. jirovecii*. (31)

SDC 3, Table 1: MLST loci information. Abbreviations: β -*TUB*, β -Tubulin, DHPS, dihydropteroate synthetase; ITS, internal transcribed region of the rRNA gene; mtLSU, mitochondrial large subunit of the rRNA gene region.

MLST gene Locus	Primers	PCR conditions	GenBank reference accession number	Reference
β - <i>TUB</i>	5'-TCATTAGGTGGTGGAAACGGG-3' 5'-ATCACCATATCCTGGATCCG-3'	94°C 3min; 40 cycles: 94°C 30s, 62°C 1min, 72°C 1min; 72°C 5min	EU979560	(7)
<i>DHPS</i>	5'-GCGCCTACACATATTATGGCCATTTTAAATC-3' 5'-GGAACCTTCAACTTGGCAACCAC-3'	95°C 10min; 10 cycles: 94°C 15s, 72°C 30s (decrease 1°C per cycle), 72°C 15s; 40 cycles: 92°C 15s, 62°C 30s, 72°C 15s; 72°C 5min	AJ586567	(32)
<i>LSU</i>	5'-GATGGCTGTTTCCAAGCCCA-3' 5'-GTGTACGTTGCAAAGTACTC-3'	94°C 3min; 40 cycles: 94°C 30s, 52°C 1min, 72°C 1min; 72°C 5min	M58605	(7)
ITS	First round 5'-AGTTGATCAAATTTGGTCATTTAGAG-3' 5'-CTCGGACGAGGATCCTCGCC-3'	First round 96°C 5min; 25 cycles: 94°C 1min, 60°C 1min, 72°C 4.5min; 72°C 7min	U07220	(33-35)
	Second round 5'-CGTAGGTGAACCTGCGGAAGGATC-3' 5'-GTTTCAGCGGGTGATCCTGCCTG-3'	Second round 94°C 5min; 20 cycles: 94°C 1min, 56°C 1min, 72°C 4.5min; 72°C 7min		