

1 Supplementary Table S1: *Mycobacterium tuberculosis complex* isolates used in this study

Species	Strain	Country of Isolation	Origin ^a	Remark
<i>M. tuberculosis</i>	22	Mongolia	RIVM	Beijing lineage
<i>M. tuberculosis</i>	53	Argentina	RIVM	Haarlem lineage
<i>M. tuberculosis</i>	112	The Netherlands	RIVM	CAS lineage
<i>M. tuberculosis</i>	67	Comoro Islands	RIVM	EAI lineage
<i>M. tuberculosis</i>	41	Chile	RIVM	LAM lineage
<i>M. tuberculosis</i>	103	China	RIVM	T-family lineage
<i>M. tuberculosis</i>	12594_02	Former Soviet Union	Borstel	Beijing lineage
<i>M. tuberculosis</i>	1500_03	Former Soviet Union	Borstel	Beijing lineage
<i>M. tuberculosis</i>	1934_03	Former Soviet Union	Borstel	Beijing lineage
<i>M. tuberculosis</i>	1428_02	Ghana	Borstel	Cameroon lineage
<i>M. tuberculosis</i>	5390_02	Ghana	Borstel	Cameroon lineage
<i>M. tuberculosis</i>	5400_02	Ghana	Borstel	Cameroon lineage
<i>M. tuberculosis</i>	2637_02	Germany	Borstel	Delhi/CAS lineage
<i>M. tuberculosis</i>	7936_01	Germany	Borstel	Delhi/CAS lineage
<i>M. tuberculosis</i>	1797_03	Germany	Borstel	EAI lineage
<i>M. tuberculosis</i>	4850_03	Germany	Borstel	EAI lineage
<i>M. tuberculosis</i>	947_01	Germany	Borstel	EAI lineage
<i>M. tuberculosis</i>	2336_02	Germany	Borstel	Haarlem lineage
<i>M. tuberculosis</i>	9532_03	Germany	Borstel	Haarlem lineage
<i>M. tuberculosis</i>	7968_03	Germany	Borstel	LAM lineage
<i>M. tuberculosis</i>	8885_03	Germany	Borstel	LAM lineage
<i>M. tuberculosis</i>	946_03	Germany	Borstel	LAM lineage
<i>M. tuberculosis</i>	2151_03	Germany	Borstel	S-type lineage
<i>M. tuberculosis</i>	2318_06	Germany	Borstel	S-type lineage
<i>M. tuberculosis</i>	10469_01	NA ^c	Borstel	Ghana lineage
<i>M. tuberculosis</i>	10493_01	NA ^c	Borstel	Ghana lineage
<i>M. tuberculosis</i>	2570_02	NA ^c	Borstel	Ghana lineage
<i>M. tuberculosis</i>	2201_99	Uganda	Borstel	Uganda I lineage
<i>M. tuberculosis</i>	2333_99	Uganda	Borstel	Uganda I lineage
<i>M. tuberculosis</i>	2176_99	Uganda	Borstel	Uganda II lineage
<i>M. tuberculosis</i>	2191_99	Uganda	Borstel	Uganda II lineage
<i>M. tuberculosis</i>	4412_04	Germany	Borstel	X-type lineage
<i>M. tuberculosis</i>	9953_04	Germany	Borstel	X-type lineage
<i>M. tuberculosis</i>	11313_03	Germany	Borstel	Tur lineage
<i>M. tuberculosis</i>	1657_03	Germany	Borstel	Ural lineage
<i>M. tuberculosis</i>	10264_03	Germany	Borstel	Tur lineage
<i>M. tuberculosis</i>	10529_03	Germany	Borstel	Tur lineage
<i>M. tuberculosis</i>	8431_03	Germany	Borstel	Ural lineage
<i>M. tuberculosis</i>	3493_07		Borstel	Hamburg lineage
<i>M. tuberculosis</i>	10707_07		Borstel	Hamburg lineage
<i>M. tuberculosis</i>	9679_00	NA ^c	Borstel	Laboratory strain ATCC
H37Rv	-	NA ^c	Mario Vaneechoutte	Clinical isolates
(19 clinical				

isolates)

<i>M. canettii</i>	116	Somalia	RIVM	Smooth growing strain described by van Soolingen <i>et al.</i> 1997
<i>M. canettii</i>	1997-1549	Switzerland	RIVM	Swiss isolate described in Pfyffer <i>et al.</i> 1998
<i>M. canettii</i>	NLA000701671	Somalia	RIVM	Characterised on the basis of their spoligotype, IS6110 RFLP type and smooth growth as
<i>M. canettii</i>	NLA000200937	Eritrea	RIVM	Characterised on the basis of their spoligotype, IS6110 RFLP type and smooth growth
<i>M. canettii</i>	1996-46	France	RIVM	Canetti strain
<i>M. canettii</i>	3040_99	The Netherlands	Borstel	
<i>M. canettii</i>	3151_08	NA ^c	Borstel	
<i>M. canettii</i>	3041_99	The Netherlands	Borstel	
<i>M. bovis</i>	117	Argentina	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i>	126	Argentina	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i>	73	The Netherlands	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i>	130	The Netherlands	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i>	24	Saudi Arabia	RIVM	Isolated from an oryx, Antelope clade, see also Smith <i>et al.</i> 2006
<i>M. bovis</i>	4258_00	Germany	Borstel	
<i>M. bovis</i>	751_01	Germany	Borstel	
<i>M. bovis</i>	7540_01	Germany	Borstel	
<i>M. bovis</i>	-	NA ^c	Mario	Clinical isolates
(6 isolates)			Vaneechoutte	
<i>M. bovis</i> BCG	48 (2)	The Netherlands	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i> BCG	71	Japan	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i> BCG	83	Russia	RIVM	See Kremer <i>et al.</i> 2005
<i>M. bovis</i> BCG	2008-714 ^b	NA ^c	RIVM	Identified on basis of characteristic IS6110/IS1081 RFLP patterns according to van Soolingen <i>et al.</i> 1992
<i>M. bovis</i> BCG	2008-1601 ^b	NA ^c	RIVM	Identified on basis of characteristic IS6110/IS1081 RFLP patterns according to van Soolingen <i>et al.</i> 1992
<i>M. bovis</i> BCG	DSM 43990	NA ^c	DSMZ	<i>Mycobacterium bovis</i> Karlson and Lessel 1970
<i>M. bovis</i> BCG	DSM 45071	NA ^c	DSMZ	<i>Mycobacterium bovis</i> Karlson and Lessel 1970
<i>M. caprae</i>	2006-1960 ^b	The Netherlands	RIVM	Characterised using Hain genotype MTBC kit
<i>M. caprae</i>	2007-0039 ^b	The Netherlands	RIVM	Characterised using Hain genotype MTBC kit
<i>M. caprae</i>	1694_00	Germany	Borstel	
<i>M. caprae</i>	8986_99	Germany	Borstel	
<i>M. caprae</i>	9577_99	Germany	Borstel	
<i>M. microti</i>	62	United Kingdom	RIVM	see van Soolingen <i>et al.</i> 1998
<i>M. microti</i>	25	United Kingdom	RIVM	see van Soolingen <i>et al.</i> 1998

<i>M. microti</i>	15274 ^b	United Kingdom	RIVM	see van Soolingen <i>et al.</i> 1998
<i>M. microti</i>	15912 ^b	Belgium	RIVM	see van Soolingen <i>et al.</i> 1998
<i>M. microti</i>	15911 ^b	Netherlands	RIVM	see van Soolingen <i>et al.</i> 1998
<i>M. microti</i>	417/01	Germany		Llama lineage
<i>M. pinnipedii</i>	76	Argentina	RIVM	See Kremer <i>et al.</i> 2005
<i>M. pinnipedii</i>	81	Argentina	RIVM	See Kremer <i>et al.</i> 2005
<i>M. pinnipedii</i>	101	Argentina	RIVM	See Kremer <i>et al.</i> 2005
<i>M. pinnipedii</i>	7011_02	Germany	Borstel	
<i>M. pinnipedii</i>	7739_01	Germany	Borstel	
<i>M. africanum</i>	6	The Netherlands	RIVM	<i>M. africanum</i> clade 2
<i>M. africanum</i>	128 (85)	The Netherlands	RIVM	<i>M. africanum</i> clade 2
<i>M. africanum</i>	2007-1386 ^b	The Netherlands	RIVM	<i>M. africanum</i> clade 2
<i>M. africanum</i>	2007-1154 ^b	The Netherlands	RIVM	<i>M. africanum</i> clade 2
<i>M. africanum</i>	2007-1073 ^b	The Netherlands	RIVM	<i>M. africanum</i> clade 2
<i>M. africanum</i>	1449_02	Ghana	Borstel	<i>M. africanum</i> clade 1
<i>M. africanum</i>	1473_02	Ghana	Borstel	<i>M. africanum</i> clade 1
<i>M. africanum</i>	10473_01	Ghana	Borstel	<i>M. africanum</i> clade 1
<i>M. africanum</i>	10494_01	Ghana	Borstel	<i>M. africanum</i> clade 1
<i>M. africanum</i>	1443_02	Ghana	Borstel	<i>M. africanum</i> clade 1
<i>M. africanum</i>	10476_01	Ghana	Borstel	<i>M. africanum</i> clade 2
<i>M. africanum</i>	10514_01	Ghana	Borstel	<i>M. africanum</i> clade 2
<i>M. africanum</i>	5468_02	Ghana	Borstel	<i>M. africanum</i> clade 2
<i>M. africanum</i>	9550_99	Ghana	Borstel	<i>M. africanum</i> clade 2 ATCC

^a RIVM = National Tuberculosis Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; *Borstel = National Reference Center for Mycobacteria, Forschungszentrum Borstel, Germany; *DSM = The German Collection of Microorganisms

^b Represent RIVM strains not previously described in literature, however have been characterised to the species level using techniques outlined in references supplied in the manuscript.

^c This information was not available (NA) for this study.

Supplementary Table S2: Non *tuberculosis* mycobacteria and other strains of bacteria used in this study

Non <i>tuberculosis</i> mycobacteria	Strain designation ^a	Remark
<i>Mycobacterium aichiense</i>	DSM 44147	Type strain, isolated from soil
<i>Mycobacterium alvei</i>	DSM 44176	Type strain, isolated from water sample
<i>Mycobacterium arupense</i>	DSM 44942	Type strain, isolated from a tendon
<i>Mycobacterium asiaticum</i>	ITG 8182	See De Baere <i>et al.</i> 2002
<i>Mycobacterium avium</i>	ITG 7886	See Vaneechoutte <i>et al.</i> 1993
<i>Mycobacterium boenickei</i>	DSM 44677	Type strain, isolated from a leg wound
<i>Mycobacterium branderri</i>	DSM 44624	Type strain, isolated from human sputum
<i>Mycobacterium brisbanense</i>	DSM 44680	Type strain, isolated from a sinus
<i>Mycobacterium brumae</i>	DSM 44177	Type strain, isolated from water sample
<i>Mycobacterium canariasense</i>	DSM 44828	Type strain, isolated from human blood
<i>Mycobacterium celatum</i>	ITG 6147	See De Baere <i>et al.</i> 2002
<i>Mycobacterium chelonae</i>	ITG 4975	NA ^b
<i>Mycobacterium chelonae</i> subsp. <i>abscessus</i>	DSM 44196	Type strain
<i>Mycobacterium confluentis</i>	DSM 44017	Type strain, isolated from human sputum

<i>Mycobacterium conspicuum</i>	DSM 44136	Type strain, isolated from patient with disseminated infection
<i>Mycobacterium flavescens</i>	VUB A016	See De Baere <i>et al.</i> 2002
<i>Mycobacterium fortuitum</i>	ITG 8020	See Vaneechoutte <i>et al.</i> 1993
<i>Mycobacterium genavense</i>	ITG 97-102	See De Baere <i>et al.</i> 2002
<i>Mycobacterium gilvum</i>	DSM 9487	Isolated from soil
<i>Mycobacterium goodii</i>	DSM 44492	Type strain
<i>Mycobacterium gordonaie</i>	ITG 7704	See Vaneechoutte <i>et al.</i> 1993
<i>Mycobacterium heckeshornense</i>	DSM 44428	Type strain, isolated from human respiratory tract
<i>Mycobacterium houstonense</i>	DSM 44676	Type strain, isolated from a facial abscess
<i>Mycobacterium intracellulare</i>	DSM 43223	Type strain
<i>Mycobacterium kansasii</i>	ITG 7727	See Vaneechoutte <i>et al.</i> 1993
<i>Mycobacterium kubiciae</i>	DSM 44627	Type strain, isolated from human sputum
<i>Mycobacterium lacus</i>	DSM 44577	Type strain, isolated from human elbow
<i>Mycobacterium mageritense</i>	DSM 44476	Type strain, isolated from human sputum
<i>Mycobacterium malmoense</i>	ITG 940611	See De Baere <i>et al.</i> 2002
<i>Mycobacterium marinum</i>	ITG 1727	See Vaneechoutte <i>et al.</i> 1993
<i>Mycobacterium massiliense</i>	DSM 45103	Type strain, isolated from human blood
<i>Mycobacterium moriokaense</i>	DSM 44221	Type strain, isolated from soil
<i>Mycobacterium mucogenicum</i>	DSM 44625	Type strain, isolated from human cyst
<i>Mycobacterium nebraskense</i>	DSM 44803	Type strain, isolated from human sputum
<i>Mycobacterium neworleansense</i>	DSM 44679	Type strain, isolated from human scalp
<i>Mycobacterium paratuberculosis</i>	ITG 2666	See De Baere <i>et al.</i> 2002
<i>Mycobacterium scrofulaceum</i>	DSM 43992	Type strain, isolated from human cervical lymph node Type strain, isolated from sputum of patient with tuberculosis-like disease
<i>Mycobacterium shimoidei</i>	DSM 44152	
<i>Mycobacterium simiae</i>	ITG 4485	See Vaneechoutte <i>et al.</i> 1993
<i>Mycobacterium smegmatis</i>	DSM 43756	Type strain
<i>Mycobacterium szulgai</i>	ITG 4979	NA ^b
<i>Mycobacterium tusciae</i>	DSM 44338	Type strain, isolated from human cervical lymph node
<i>Mycobacterium ulcerans</i>	ITG 96-1439	NA ^b
<i>Mycobacterium xenopi</i>	ITG 4986	See De Baere <i>et al.</i> 2002
Other bacteria	Strain designation	Remark
<i>Staphylococcus aureus</i>	DSM 20231	Type strain, isolated from human pleural fluid
<i>Listeria monocytogenes</i>	DSM 20600	Type strain, isolated from a rabbit
<i>Escherichia coli</i>	DSM 301	Disinfectant test strain
<i>Klebsiella oxytoca</i>	ATCC 43086	NA ^b
<i>Enterococcus faecalis</i>	DSM 20371	Isolated from pleural fluid
<i>Proteus mirabilis</i>	DSM 4479	Type strain

<i>Bacillus cereus</i>	DSM 31	Type strain
<i>Bordetella pertussis</i>	CCUG 13475	Isolated from patient suffering from whooping cough
<i>Streptococcus agalactiae</i>	DSM 2134	Type strain
<i>Rhodococcus equi</i>	DSM 20307	Type strain, isolated from lung abscess of foal
<i>Streptomyces albidoflavus</i>	DSM 40455	Type strain
<i>Nocardoides sp.</i>	DSM 17401	Proposed type strain, isolated from marine sediment
<i>Nocardia salmonicida</i>	DSM 40472	Type strain, isolated from blueback salmon
<i>Nocardia asiatica</i>	clinical isolate	Isolated from human wound, see Wauters <i>et al.</i> 2005
<i>Nocardia nova</i>	clinical isolate	Isolated from human abscess, see Wauters <i>et al.</i> 2005
<i>Nocardia cyriacigeorgica</i>	clinical isolate	Isolated from human bronchial aspirate, see Wauters <i>et al.</i> 2005
<i>Nocardia farcinica</i>	clinical isolate	Isolated from human abscess, see Wauters <i>et al.</i> 2005

^a DSM = The German Collection of Microorganisms; *ITG = Institute of Tropical Medicine, Antwerp; *VUB = Department of Microbiology, Academic Hospital of the Free University of Brussels, Brussels, Belgium Germany; *ATCC = American Type Culture Collection; *CCUG = Culture Collection, University of Göteborg, Sweden.

^b This information was not available (NA) for this study.

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16 Supplementary Table S3: Oligonucleotide primers and probes used in this study

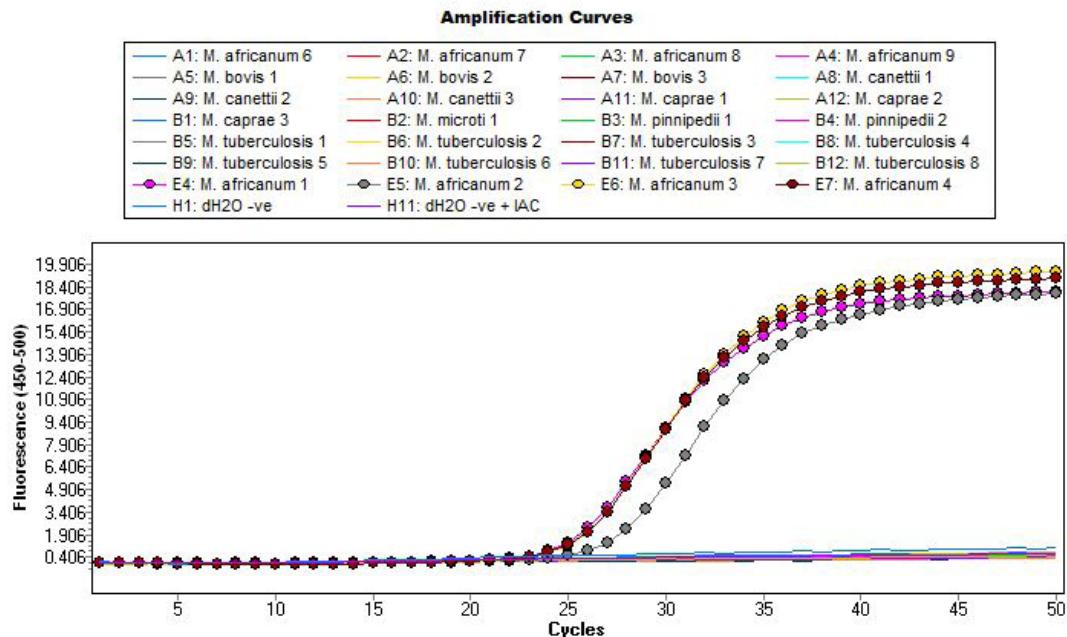
Name	Function	Sequence 5'→3'
MTC_Fw	Forward Sequencing primer, forward MTC real-time PCR assay primer	AGACCGTGC GGATCTTG
MTC_Rv	Reverse Sequencing primer, Reverse MTC real-time PCR assay primer	CATGGAGATCACCCGTGA
MTC Probe	MTC probe	HEX-ATTGGTCACCCGGATTCGGT-BHQ1
wbblI_Fw	Forward sequencing primer, Forward real-time PCR assay primer	TACCAGCTTCAGTTCCGT
wbblI_Rv	Reverse sequencing primer, Reverse real-time PCR assay primer	GCACCTATATCTTCTTAGCCG
wbblI probe	wbblI probe	FAM-ATGGTGC GCAGTTC ACTGC
<i>M. canetti</i> sp Fw	Forward <i>M. canetti</i> specific primer	ATGTGGTTTCAGTACGACTTC
<i>M. canetti</i> sp Rv	Reverse <i>M. canetti</i> specific primer	GATGGCAGTGTCTTATCCAA
<i>M. canetti</i> sp probe	<i>M. canetti</i> specific probe	ROX-TGAGAGGTGTTGGCACGCAA-BHQ2
lpqT Fw	Forward sequencing primer, Forward real-time PCR assay primer	ACGAATCCGGCGATGATC
lpqT_Rv	Reverse sequencing primer, Reverse real-time PCR assay primer	CGACTGCACACCTGGAA
lpqT probe	lpqT Probe	FAM-TTGGCCGGCGCCGGTT-BHQ1
RD1_Fw	Forward sequencing primer, Forward real-time PCR assay primer	CATCGCTGATGTGCTTGC
RD1_Rv	Reverse sequencing primer, Reverse real-time PCR assay primer	TGCGCCGAGCTGTATT
RD1_probe	RD1 Probe	ROX-ACACTAGCGTCAATGCGGTCA-BHQ2
<i>M. caprae</i> lepA_Fw	Forward sequencing primer, Forward real-time PCR assay primer	AGACCGTGC GGATCTTG
<i>M. caprae</i> lepA_Rv	Reverse sequencing primer, Reverse real-time PCR assay primer	CATGGAGATCACCCGTGA
<i>M. caprae</i> lepA probe	<i>M. caprae</i> lepA Probe	Cyan 500-TATCGGGTACACAAAGACGA -BBQ
RD713_Fw S	RD713 Forward sequencing primer	CCATCTGCGCTTCGGTGCTTCT
RD713_RvS	RD 713 Reverse sequencing primer	CTGCCAGTCGTCTCCCCATTGTG

RD713_Fw	Forward real-time PCR assay primer	ACGGAACGGTCAAGAAC
RD713_Rv	Reverse real-time PCR assay primer	GCTCAAGAATCGTCGCTA
RD713 probe	RD 713 Probe	Cyan 500-ACGTCTTGACCGCGAC- BBQ
RD701_FwS	RD 701 Forward sequencing primer	CTGTGCAGGTGGTCGTTTC
RD701_RvS	RD 701 Reverse sequencing primer	CGAATTGCTCATCCCGTAAC
RD701_Fw	Forward real-time PCR assay primer	AACGGGTCGGATTCTCC
RD701_Rv	Reverse real-time PCR assay primer	CCGAAACCCTCGTTGATC
RD701 probe	RD 701 Probe	ROX-TCAGCCGCCGCCAACC-BHQ2
IAC MSMEG_0660_Fw	Forward sequencing primer, Forward real-time PCR assay primer	TCACCGACCATGTCCAG
IAC MSMEG_0660_Rv	Reverse sequencing primer, Reverse real-time PCR assay primer	CGTTGCCCAATCCGTATG
IAC MSMEG_0660 probe	IAC MSMEG_0660 probe	Cy5-CAGCAGTACCATGCCATCG-BHQ2
<i>ssrA</i> _Fw	<i>ssrA</i> Forward sequencing primer	TGGGGCTGAAAGGTTCGA
<i>ssrA</i> _Rv	<i>ssrA</i> Reverse sequencing primer	TGGAGCTGCCGGGAAT

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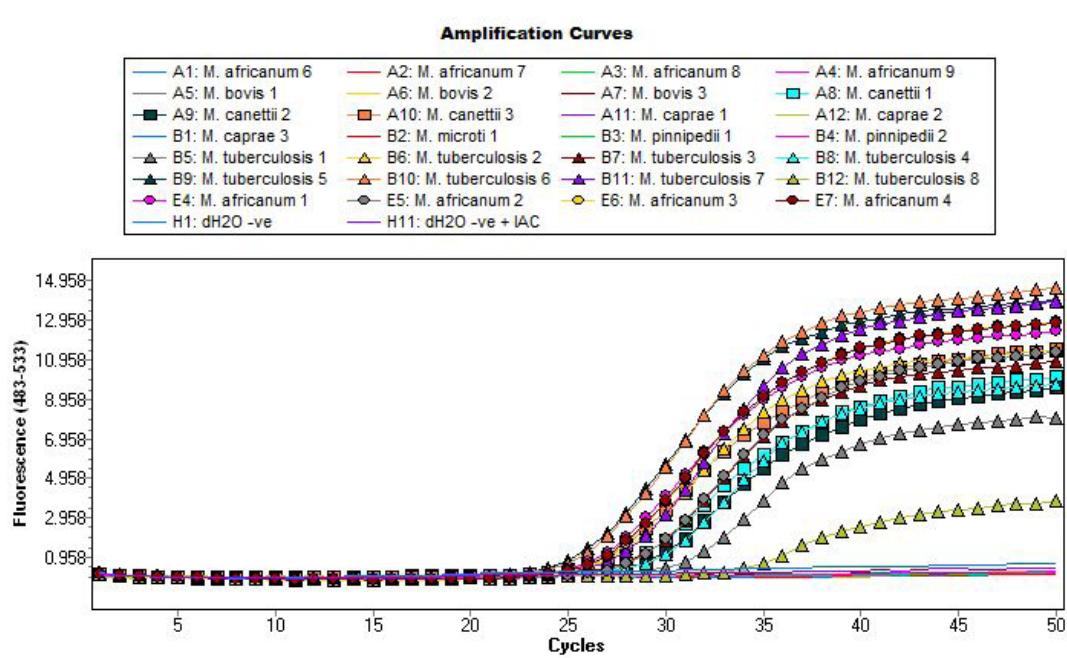
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Fig. S1A



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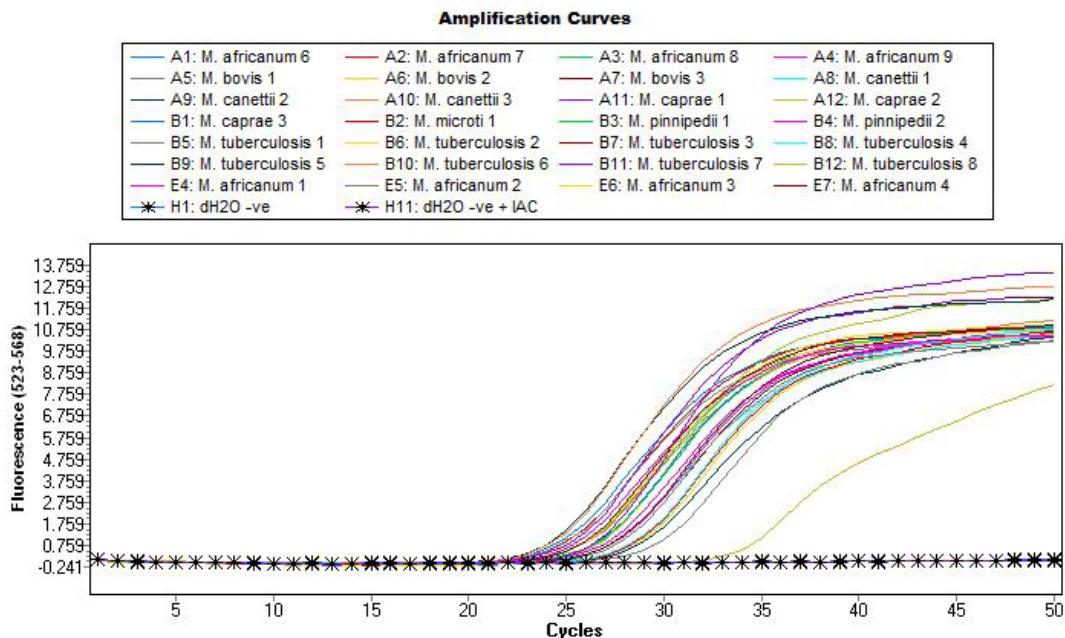
Fig. S1B



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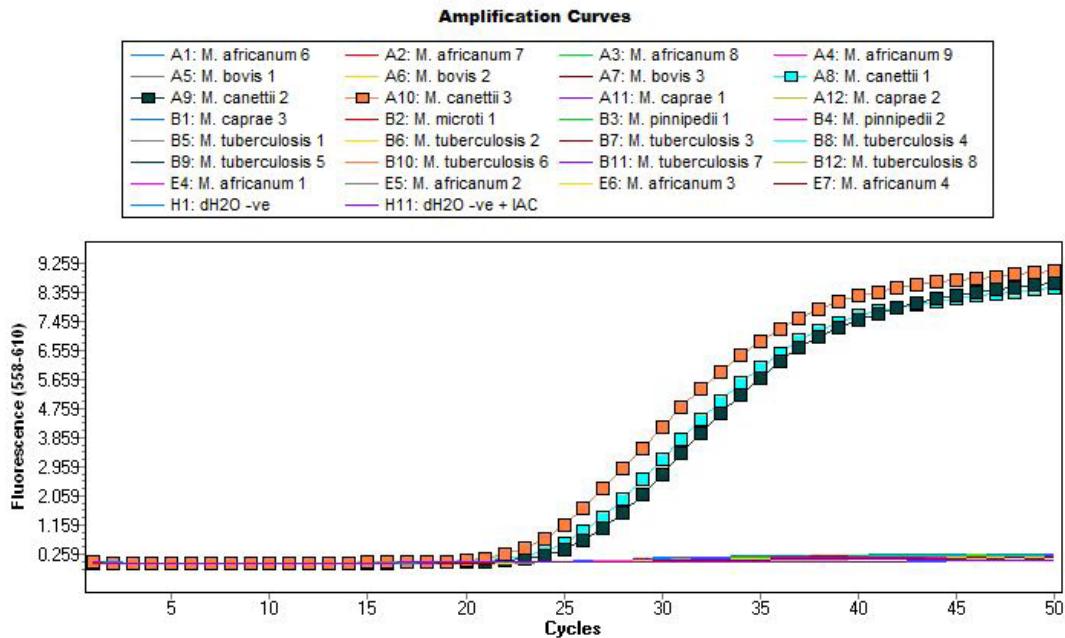
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Fig. S1C



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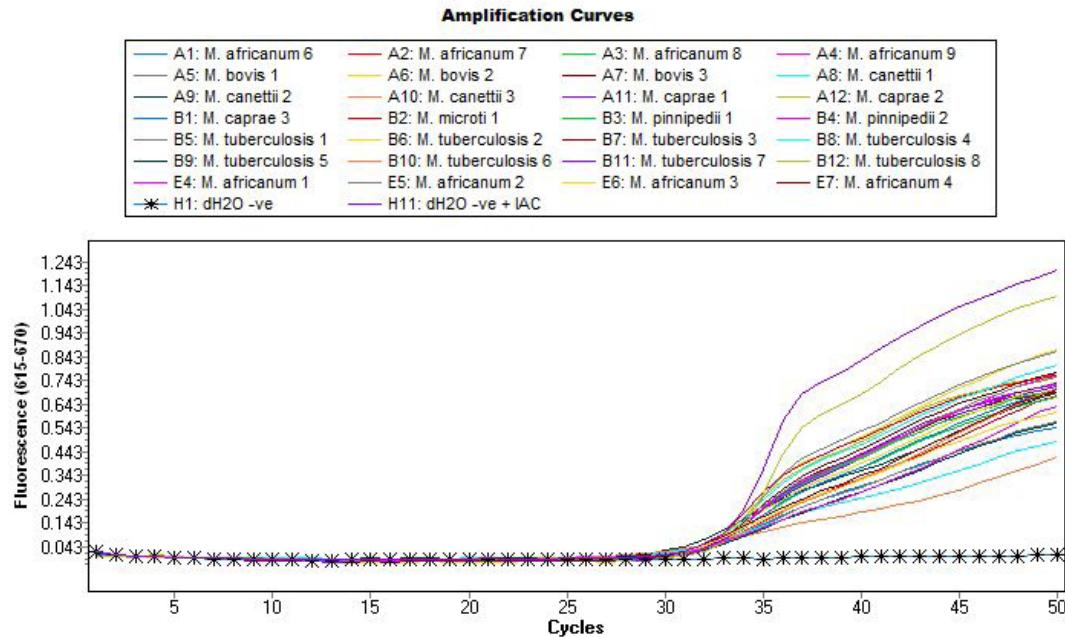
Fig. S1D



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Fig. S1E



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Supplementary Figure Legend

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Fig. S1

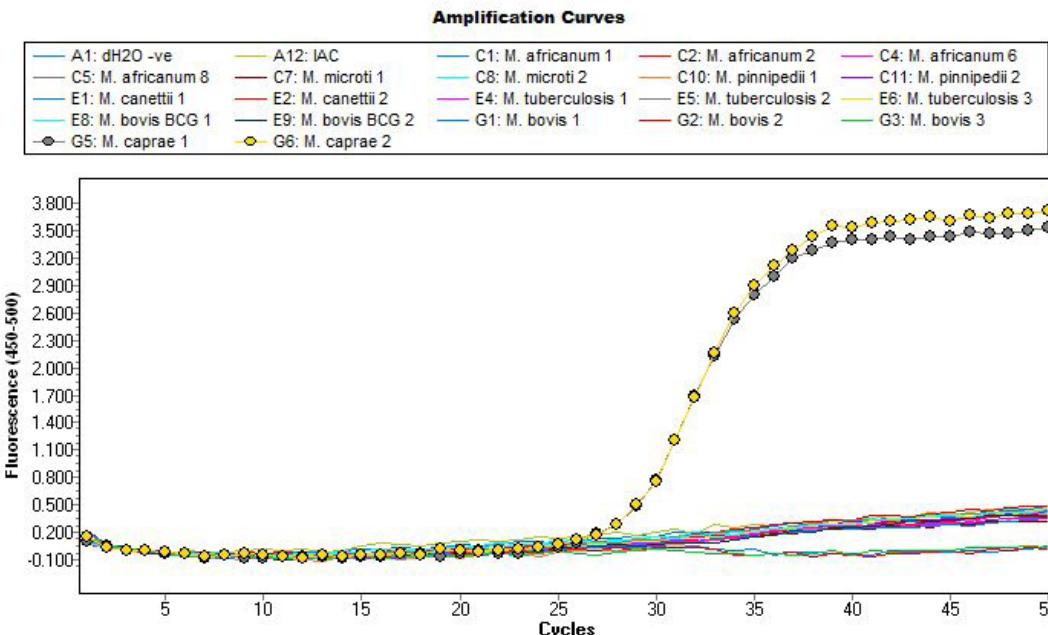
Amplification curves for multiplex 1 real-time PCR assay

101 Figure S1A Real-time amplification curves for *M. africanum* West African 1 (circle) using
102 RD713 in Cyan500 channel (450-500). Figure S1B Amplification curves for *M. africanum*
103 West African 1 (circle), *M. tuberculosis* (triangles) and *M. canetti* (rectangles) using the
104 *wbll1* gene in FAM channel (483-533). Figure S1C Amplification curves for all members of
105 the MTC using the *lepA* gene, with the no template control highlighted (stars) in the HEX
106 channel (523-568). Figure S1D Amplification curves of *M. canetti* (rectangles) in the ROX
107 channel (558-610) using RD^{canettii1}. Figure S1E Amplification curves for IAC in Cy5 channel
108 (615-670) with the no template control highlighted with stars through amplification curve.

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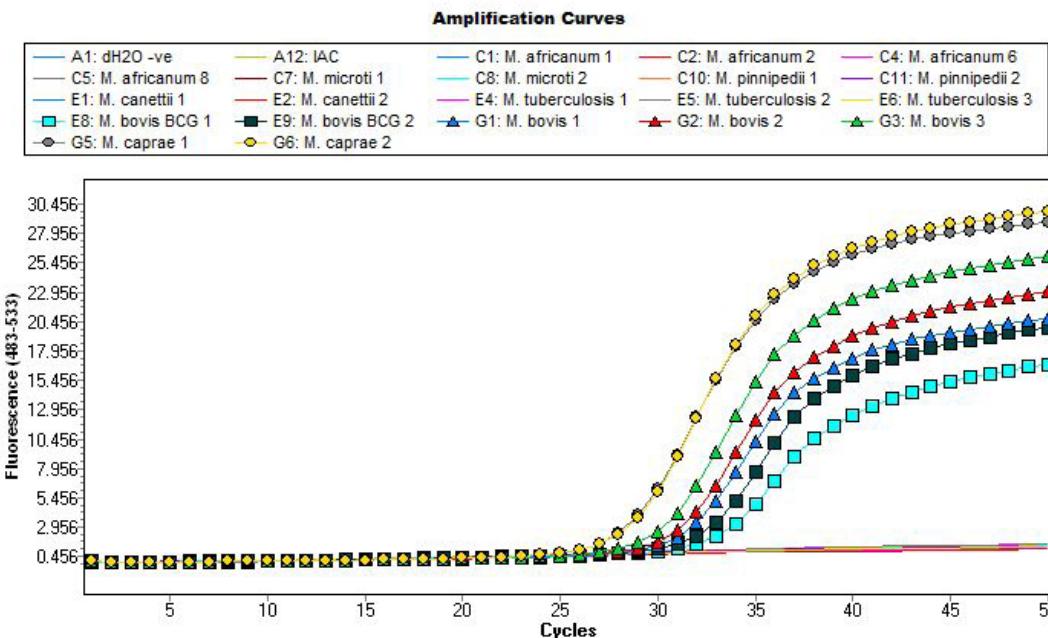
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Fig. S2A



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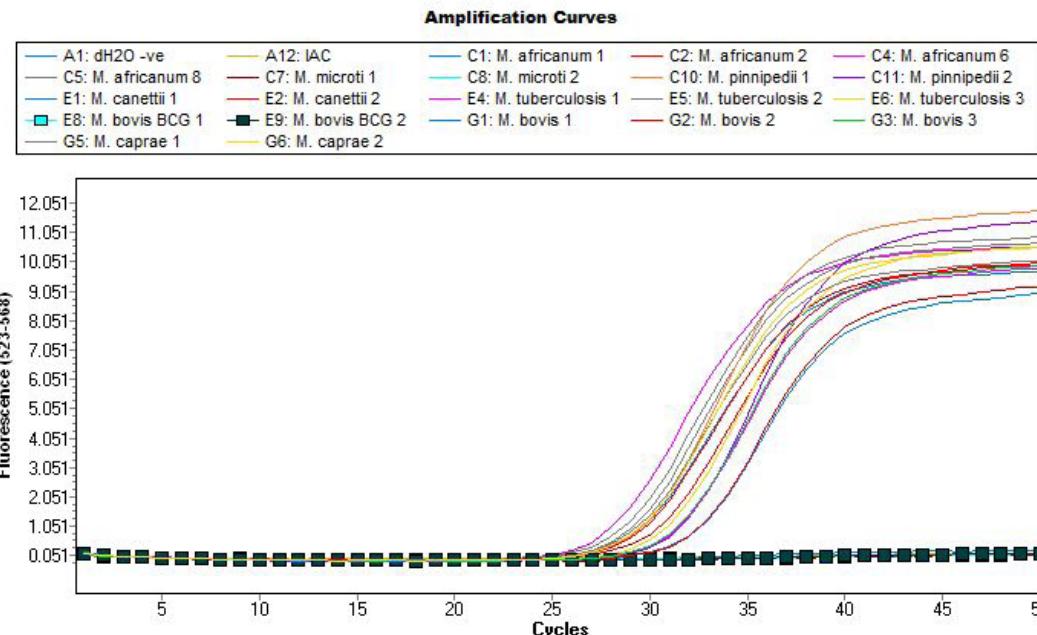
Fig. S2B



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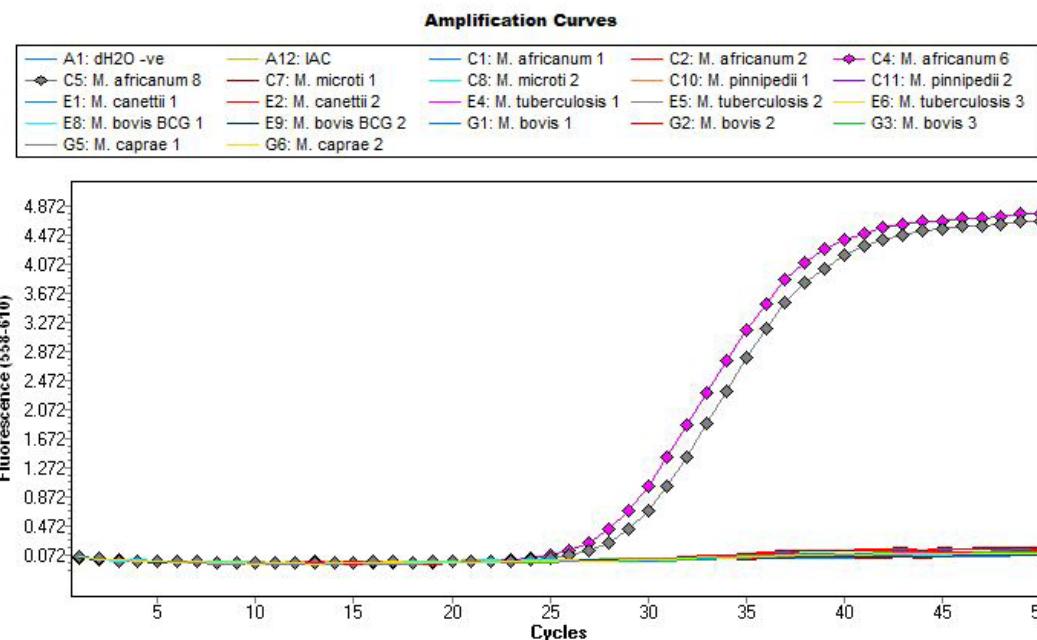
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Fig. S2C



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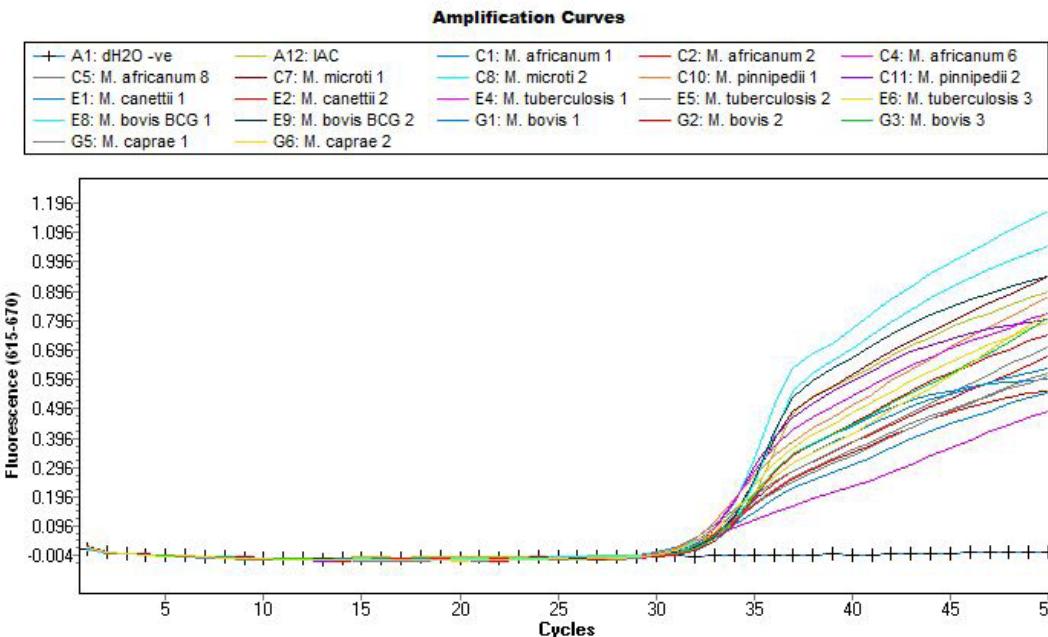
Fig. S2D



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Fig. S2 E



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156 **Fig. S2**
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158 **Amplification curves for multiplex 2 real-time PCR assay**
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160 Figure S2A Real-time amplification curves for *M. caprae* (circle) using *lepA* gene in
161 Cyan500 channel (450-500). Figure S2B Amplification curves for *M. caprae* (circle), *M.*
162 *bovis* (triangles) and *M. bovis BCG* (rectangles) using the *lpqT* gene in FAM channel (483-
163 533). Figure S2C Amplification curves for all members of the MTC with the exception of *M.*
164 *bovis BCG* (diamonds) and *M. microti* in the HEX channel (523-568). Figure S2D
165 Amplification curves of *M. africanum* West African 2 (diamonds) in the ROX channel (558-
166 610) using RD701. Figure S2E Amplification curves for IAC in Cy5 channel (615-670) with
167 the no template control highlighted with stars through amplification curve.

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