

Development of a new High Resolution Melting (HRM) assay for identification and differentiation of *Mycobacterium tuberculosis* complex samples

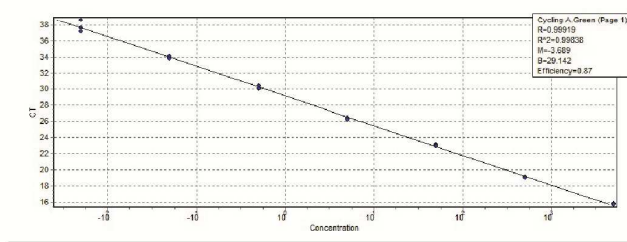
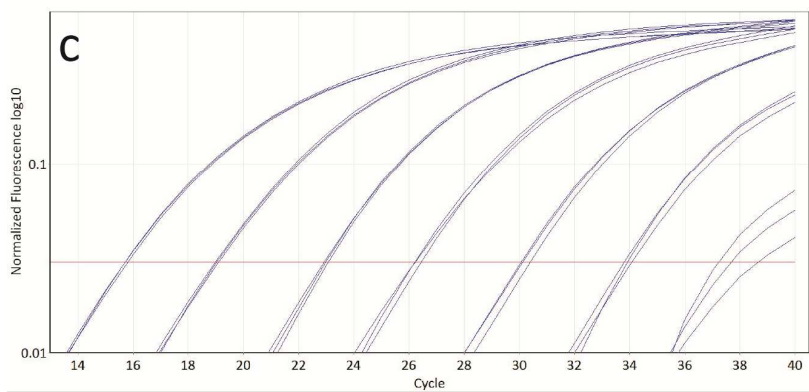
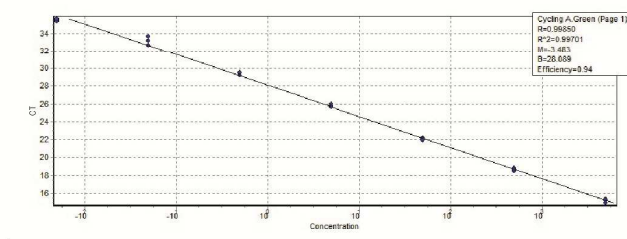
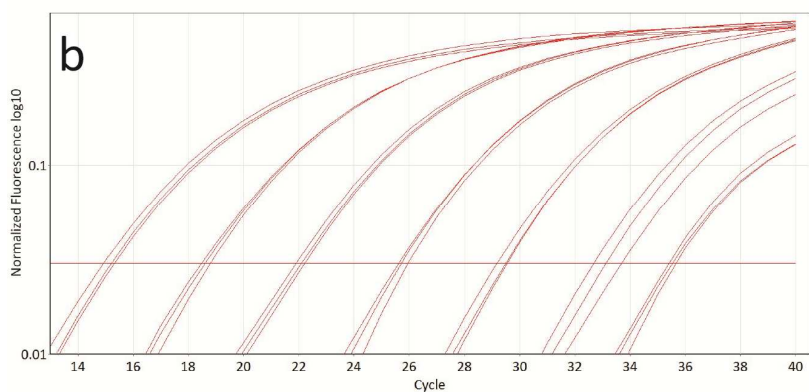
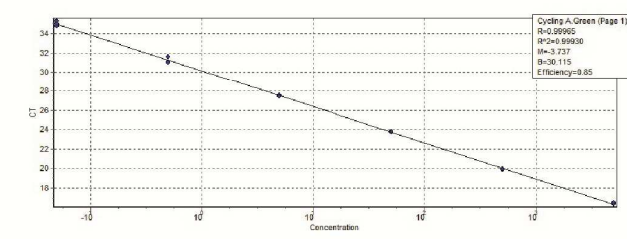
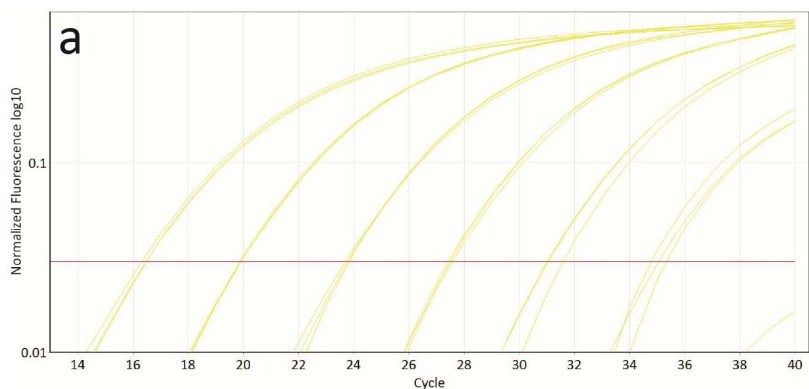
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Supplementary information

Supplementary Table ^{S1}. Raw data set and statistical parameters generated from the intra- and inter-assay using a randomly chosen subset of 22 cultured samples.

Isolate			Run 1					Run 2					Run 3					Inter-Assay						
MTBC member	sample no	origin	Ct	Tm	Mean Tm	SD	CV%	Intra-assay CV%	Ct	Tm	Mean Tm	SD	CV%	Intra-assay CV%	Ct	Tm	Mean Tm	SD	CV%	Intra-assay CV%	Mean Tm	SD	CV%	Inter-assay CV%
<i>M. microti</i>	ATCC 19422		23.08	86.75					23.15	86.72					23.38	86.73					86.73	0.02	0.02	
<i>M. bovis</i>	BCG Pasteur	ATCC 35734	28.46	86.60					28.63	86.58					29.46	86.58					86.59	0.01	0.01	
<i>M. tuberculosis</i>	H37Rv		25.80	86.93					26.06	86.98					26.36	86.95					86.95	0.03	0.03	
<i>M. tuberculosis</i>	15-961 2B	elephant	18.47	86.97	86.96	0.01	0.01		18.50	87.00	87.00	0.00	0.00		18.89	86.98	86.98	0.02	0.02		86.98	0.02	0.03	
			18.49	86.95					18.41	87.00					18.97	86.97								
			18.47	86.95					18.53	87.00					18.95	87.00								
<i>M. tuberculosis</i>	15-1115-2	elephant	20.38	86.95	86.96	0.02	0.02		20.53	87.00	87.00	0.00	0.00		20.78	87.00	87.01	0.01	0.01		86.99	0.03	0.03	
			19.94	86.98					20.53	87.00					20.65	87.00								
			20.08	86.95					20.30	87.00					20.60	87.02								
<i>M. tuberculosis</i>	15-1221-1	elephant	23.48	86.97	86.99	0.03	0.03	0.02	23.74	86.98	86.97	0.02	0.02	0.01	23.98	86.98	87.00	0.02	0.02	0.02	86.99	0.02	0.02	0.02
			23.83	86.97					24.01	86.95					24.20	87.02								
			23.53	87.02					23.58	86.98					23.85	87.00								
<i>M. caprae</i>	14-13	cow	26.57	86.57	86.59	0.02	0.02		27.27	86.55	86.56	0.01	0.01		27.72	86.60	86.61	0.01	0.01		86.59	0.03	0.03	
			26.82	86.60					27.37	86.57					27.44	86.62								
			26.52	86.60					27.24	86.55					28.26	86.62								
<i>M. caprae</i>	22914	cow	23.99	86.58	86.58	0.00	0.00		24.29	86.57	86.55	0.02	0.02		24.46	86.65	86.64	0.01	0.01		86.59	0.04	0.05	
			23.87	86.58					24.23	86.53					24.75	86.63								
			23.97	86.58					24.33	86.55					24.41	86.63								
<i>M. caprae</i>	22848	cow	26.57	86.58	86.59	0.01	0.01		26.63	86.57	86.56	0.01	0.01		26.98	86.60	86.60	0.00	0.00		86.58	0.02	0.03	
			26.73	86.60					26.48	86.55					27.23	86.60								
			26.55	86.58					26.64	86.55					27.14	86.60								
<i>M. caprae</i>	22966	cow	23.75	86.55	86.58	0.03	0.03		23.66	86.60	86.58	0.02	0.02		24.22	86.58	86.59	0.01	0.01		86.58	0.00	0.00	
			23.00	86.60					23.61	86.58					24.03	86.60								
			23.44	86.60					23.75	86.57					23.91	86.58								
<i>M. caprae</i>	13-450	cow	21.29	86.58	86.58	0.01	0.01		21.39	86.57	86.60	0.03	0.03		21.48	86.58	86.60	0.02	0.02		86.59	0.01	0.01	
			21.23	86.57					21.35	86.60					21.47	86.60								
			21.29	86.58					21.31	86.62					21.57	86.62								
<i>M. caprae</i>	13-162	cow	25.11	86.58	86.56	0.03	0.03	0.02	25.41	86.63	86.63	0.01	0.01	0.02	25.82	86.58	86.60	0.02	0.02	0.01	86.60	0.03	0.04	0.03
			25.17	86.53					25.55	86.63					25.72	86.60								
			24.82	86.57					25.54	86.62					25.88	86.62								
<i>M. bovis</i>	20482	cow	26.55	86.55	86.56	0.01	0.01		26.61	86.62	86.61	0.02	0.03		26.86	86.62	86.63	0.02	0.02		86.60	0.03	0.04	
			26.39	86.57					26.64	86.62					26.96	86.65								
			26.31	86.57					26.28	86.58					26.81	86.62								
<i>M. bovis</i>	20594	cow	28.22	86.55	86.54	0.01	0.01		28.52	86.60	86.61	0.01	0.01		29.66	86.65	86.65	0.02	0.02		86.60	0.05	0.06	
			27.85	86.53					28.53	86.62					28.72	86.63								
			27.40	86.55					28.42	86.60					29.28	86.67								
<i>M. bovis</i>	20175	cow	22.06	86.55	86.58	0.03	0.03		21.80	86.58	86.57	0.02	0.02		22.20	86.62	86.64	0.02	0.02		86.60	0.04	0.04	
			21.30	86.58					21.72	86.58					22.22	86.65								
			22.02	86.60					21.95	86.55					22.43	86.65								
<i>M. bovis</i>	20606	cow	24.19	86.62	86.60	0.02	0.02		24.73	86.60	86.59	0.01	0.01		24.43	86.65	86.65	0.00	0.00		86.61	0.03	0.04	
			24.30	86.58					24.27	86.58					24.58	86.65								
			24.23	86.60					24.56	86.60					24.78	86.65								
<i>M. bovis</i>	22667	cow	22.92	86.60	86.61	0.01	0.01		22.91	86.60	86.62	0.03	0.03		23.33	86.65	86.64	0.01	0.01		86.62	0.02	0.02	
			22.87	86.62					23.20	86.65					23.46	86.65								
			23.06	86.60					23.03	86.60					23.43	86.63								
<i>M. bovis</i>	22539	cow	24.09	86.62	86.61	0.01	0.01	0.02	24.39	86.60	86.61	0.02	0.02	0.02	24.79	86.60	86.61	0.02	0.02	0.02	86.61	0.00	0.00	0.03
			23.91	86.60					24.59	86.60					24.67	86.63								
			24.31	86.62					24.24	86.63					25.08	86.60								
<i>M. microti</i>	22928	cat	20.62	86.70	86.73	0.03	0.03		20.57	86.75	86.76	0.01	0.01		20.79	86.73	86.73	0.03	0.03		86.74	0.02	0.02	
			20.63	86.75					20.76	86.77					20.53	86.70								
			20.72	86.73					20.86	86.75					20.74	86.75								
<i>M. microti</i>	15-1765	cat	24.74	86.72	86.72	0.02	0.02		24.84	86.78	86.76	0.02	0.02		25.31	86.75	86.75	0.00	0.00		86.74	0.02	0.03	
			24.48	86.73					24.74	86.75					25.42	86.75								
			24.96	86.70					24.76	86.75					25.15	86.75								
<i>M. microti</i>	14-58	cat	28.43	86.67	86.71	0.03	0.04		28.04	86.77	86.77	0.01	0.01		28.91	86.75	86.77	0.02	0.02		86.75	0.04	0.04	
			28.38	86.73					28.55	86.78					28.36	86.78								
			28.48	86.72					28.53	86.77					29.15	86.77								
<i>M. microti</i>	15-1955	cat	24.62	86.73	86.75	0.02	0.02		24.81	86.77	86.76	0.01	0.01		25.08	86.80	86.78	0.02	0.02		86.76	0.02	0.02	
			24.08	86.77					24.45	86.77					24.69	86.77								
			23.90	86.75					24.62	86.75					24.87	86.77								
<i>M. microti</i>	16-2156	cat	26.87	8																				

Supplementary Figure S1. Real-time-PCR amplification curves and standard curves of the serial dilution using (a) *M. tuberculosis* H37Rv; (b) *M. bovis* BCG Pasteur ATCC 35734 and (c) *M. microti* ATCC 19422.



Supplementary Table ^{S3}. MTBC positive isolates used for the development of the high resolution melting assay.

Sample	Species	Origin	Specimen	Culture?	Clinical specimen?
<i>M. bovis</i> (15)					
20175	<i>M. bovis</i>	cow	lung	cultured	not available
20482	<i>M. bovis</i>	cow	lung, lymph node pool	cultured	not available
20531	<i>M. bovis</i>	cow	lymph node pool	cultured	not available
20593	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20594	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20596	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20597	<i>M. bovis</i>	cow	lymph node pool	cultured	not available
20599	<i>M. bovis</i>	cow	lymph node pool	cultured	not available
20600	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20606	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20608	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20609	<i>M. bovis</i>	cow	lymph node pool	cultured	clinical specimen
20665	<i>M. bovis</i>	cow	lymph node pool	cultured	not available
22539	<i>M. bovis</i>	cow	lymph node	cultured	not available
22667	<i>M. bovis</i>	cow	lymph node	cultured	not available
<i>M. caprae</i> (8)					
14-13	<i>M. caprae</i>	Cow	lymph node	cultured	clinical specimen
22914	<i>M. caprae</i>	Cow	lymph node pool	cultured	clinical specimen
22971	<i>M. caprae</i>	Cow	lymph node pool	cultured	clinical specimen
22848	<i>M. caprae</i>	Cow	liver, lung, lymph node pool	cultured	clinical specimen
22966	<i>M. caprae</i>	Cow	lymph node pool	cultured	not available
13-450	<i>M. caprae</i>	Cow	lymph node	cultured	not available
13-162	<i>M. caprae</i>	Cow	lymph node	cultured	not available
20948	<i>M. caprae</i>	Cow	lymph node	not available	clinical specimen
<i>M. microti</i> (15)					
22928	<i>M. microti</i>	cat	lung, lymph node pool	cultured	clinical specimen
15-1765	<i>M. microti</i>	cat	lung	cultured	clinical specimen
15-342	<i>M. microti</i>	alpaca	lymph node	cultured	clinical specimen
14-58	<i>M. microti</i>	cat	lung	cultured	clinical specimen
14-690	<i>M. microti</i>	alpaca	liver	cultured	clinical specimen
17-2287	<i>M. microti</i>	alpaca	spleen	cultured	clinical specimen
15817	<i>M. microti</i>	lama	lymph node	cultured	clinical specimen
15-1955	<i>M. microti</i>	cat	lymph node	cultured	clinical specimen
16-2156	<i>M. microti</i>	cat	bronchoalveolar lavage	cultured	not available
1522744	<i>M. microti</i>	cat	lung, lymph node pool	cultured	not available
16-1347	<i>M. microti</i>	cat	lung	cultured	not available
17-1084	<i>M. microti</i>	cat	lymph node, skin	culture ongoing	clinical specimen
17-549	<i>M. microti</i>	lama	liver	cultured	clinical specimen
17-1063	<i>M. microti</i>	alpaca	lymph node, lung, heart, liver, cervical vertebra	culture ongoing	clinical specimen
MI16	<i>M. microti</i>	wildboar (Spain)	unknown	cultured	not available
<i>M. tuberculosis</i> (4)					
15-961-2	<i>M. tuberculosis</i>	elephant 1	lung	cultured	clinical specimen
15-961-1	<i>M. tuberculosis</i>	elephant 1	pharyngeal swab	not cultivated	clinical specimen
15-1115-2	<i>M. tuberculosis</i>	elephant 2	lung	cultured	not available
15-1221-1	<i>M. tuberculosis</i>	elephant 3	lung	cultured	not available

Supplementary Table ^{S4}. An exclusivity panel consisting of 41 non-tuberculous mycobacteria and 3 non-mycobacterial species were tested for specificity of the high resolution melting assay.

Species	No. of isolates	Species	No. of isolates
<i>M. abscessus</i> sp.	2	<i>M. malmoense</i>	1
<i>M. avium</i> subsp. <i>avium</i>	2	<i>M. marinum</i>	1
<i>M. avium</i> subsp. <i>hominissuis</i>	32	<i>M. monacense</i>	3
<i>M. avium</i> subsp. <i>paratuberculosis</i>	1	<i>M. nebraskense</i>	1
<i>M. avium</i> subsp. <i>silvaticum</i>	1	<i>M. neoaurum</i>	5
<i>M. bourgelatii</i>	1	<i>M. nonchromogenicum</i>	7
<i>M. celatum</i>	1	<i>M. palustre</i>	1
<i>M. chelonae</i> subsp. <i>chelonae</i>	1	<i>M. parafortuitum</i>	2
<i>M. chimaera/intracellulare/youngonense</i>	1	<i>M. paragordoniae</i>	6
<i>M. chitae</i>	1	<i>M. peregrinum</i>	2
<i>M. elephantis</i>	1	<i>M. persicum</i>	2
<i>M. engbaekii</i>	1	<i>M. phlei</i>	3
<i>M. europaeum</i>	1	<i>M. scrofulaceum</i>	1
<i>M. fortuitum/porcinum</i>	1	<i>M. simiae</i>	1
<i>M. goodii</i>	1	<i>M. smegmatis</i>	1
<i>M. gordonae</i>	2	<i>M. szulgai</i>	1
<i>M. hassiacum</i>	1	<i>M. terrae</i>	1
<i>M. interjectum/paraense</i>	1	<i>M. vaccae</i>	4
<i>M. intermedium</i>	1	<i>M. xenopi</i>	5
<i>M. intracellulare</i>	3	<i>Nocardia paucivorans</i>	1
<i>M. kansasii</i>	10	<i>Escherichia coli</i>	1
<i>M. lymphaticum</i>	1	<i>Streptococcus suis</i>	1