Additional File 1

Method 1: Restriction digestion of the TB Control Plasmid

Approximately 100 ng of the control plasmid was linearised with 10 Units of HindIII restriction enzyme (New England Biolabs), in a final reaction volume of 50 µL. The restriction digestion was carried out at 37 °C for 3 hours, followed by a 20 minute heat deactivation at 80 °C. Linearisation was confirmed by microfluidic capillary electrophoresis using the Agilent Bioanalyzer in conjunction with a DNA 12000 assay. Quantification of the linearised plasmid was performed using a Qubit 2.0 fluorometer with a High sensitivity DNA Kit (Thermo Fisher Scientific).

Method 2: dPCR analysis using QuantStudio 3D platform (NMI inter-laboratory study)

The following instrumentation (Life Technologies) was used for analysis using the QuantStudio 3D (former Life Technologies, present supplier is Thermo Fisher Scientific; the following catalog numbers are given from Life Technologies): Dual Flat Block GeneAmp® PCR System 9700 (Cat# 4486414) and QuantStudio™ 3D Digital PCR Chip Loader (Cat# 4482592). The following materials/reagents were used according to the manufacturer's instructions: QuantStudio™ 3D Digital PCR Chip (Cat# 4485507 for v1, as the new version of the chips were released as version 2), QuantStudio™ 3D Digital PCR 20K Chip Spares Kit (Cat#4485510), QuantStudio™ 12K FlexOpenArray® Immersion Fluid and UV-Activated Chip Sealant Syringe (Cat# 4484475) and QuantStudio™ 3D Digital PCR Master Mix (Cat#4482710 for v1).

Three independent experiments were performed with a single *rpoB* or 16S rRNA dPCR assay (chip) for each unit of study material per experiment (compared to 2 for BioMark and QX100 dPCR users). Each 15 µL assay contained 3 µL template sample diluted gravimetrically according to the protocol in Supplementary Information 1. Two to three readings were performed for each chip using the QuantStudio™ 3D Digital PCR Instrument. Filled wells and negative wells were identified by setting a quality threshold of 0.5. The fluorescence threshold was set manually. The number of qualified wells, number of negative wells and DNA copies/µL of the reaction were reported.

Method 3: Short term stability testing

Short term stability of the BCG/ASM, plasmid and *M. tuberculosis* H37Rv genomic gDNA materials was evaluated by storing three replicate vials of each material at each of three storage conditions/ durations (dry ice, 4 °C and 40 °C for 7 and 14 days). To ensure that the storage terms concluded simultaneously, 'seven day' samples were stored seven days after the corresponding '14 day' samples. Plasmid and gDNA materials were quantified by BioMark dPCR, in parallel with control samples stored at -20 °C. Storage of the replicate vials of BCG/ASM material commenced on three consecutive days, while all replicate vials of the plasmid and gDNA material were stored on the same day.

Genomic DNA was extracted from each BCG/ASM sample along with reference samples stored at -80 °C, using the CTAB/NaCl protocol. A single replicate set of samples (comprising of one reference sample and one vial for each of the storage conditions and durations) were extracted on three consecutive days. gDNA extracts were stored at -20 °C immediately after elution until quantification by BioMark dPCR.

Table S1: dPCR Primers

Assay name	Oligonucleotide name	Oligo sequence	Reporter, Quencher	Amplicon length	BioMark [oligos] (nM)	QX100 [oligos] (nM)	QuantStudio™ 3D [oligos] (nM)	qPCR efficiency (R^2)*	Reference
UCL_16S	UCL_16S_F	GTGATCTGCCCTGCACTTC	[FAM]/NFQ	106	200	300	300	95% (> 0.99)	Honeyborne et al. [1]
	UCL_16S_R	ATCCCACACCGCTAAAGCG			200	300	300		
	UCL_16S_P	AGGACCACGGGATGCATGTCTTGT			200		300		
GN_rpoB1	RPOB_FW1	CAAAACAGCCGCTAGTCCTAGTC	[FAM]/NFQ	84	900		900	97% (> 0.99)	Devonshire et al. [2]
	RPOB_REV1	AAGGAGACCCGGTTTGGC			900		900		
	RPOB_PROBE1	AGTCGCCCGCAAAGTTCCTCGAA			200		300		

Abbreviations: NFQ – non-fluorescent quencher. *rpoB* and 16S rRNA (alternative nomenclature: *rrs*) sequence information based on *M. tuberculosis* H37Rv, NC_018143 (regions 1471862..1473381 and 759810..763328, respectively).

* This value is reported in Devonshire et al. [2]

Table S2: dPCR thermal cycling conditions

dPCR platform	Mastermix	Catalogue #	UNG incubation	PCR (temperature, time)			# Cycles	Droplet stabilisation/Final extension	Additional information
				Enzyme activation	Temperature 1	Temperature 2			
BioMark 37K qdPCR IFC	Taqman Gene Expression Master Mix (Life Technologies)	4369016	50°C, 120s	95°C, 600s	95°C, 15s	60°C, 60s	40	N/A	N/A
QX100 ddPCR System	2X ddPCR Super Mix for probes (Bio-Rad)	186-3010	N/A	95°C, 600s	94°C, 30s	60°C, 30s	40	98°C, 600s (followed by maintenance at 4°C).	Ramp rates were restricted to 2.5 °C/s
QuantStudio™ 3D	QuantStudio™ 3D Digital PCR Master Mix	4482710 for v1	N/A	96°C, 600s	60°C, 60s	96°C, 30s	39	60°C, 120 sec	N/A

Table S3: dPCR partition volume, partition number and mean λ values

Template	dPCR platform	Partition volume (nL)	Mean λ	Mean* total partition number
Plasmid	BioMark	0.85	1.12	770
H37Rv	BioMark		0.76	
BCG/ASM	BioMark		1.01	
Total MTB Control	BioMark		0.02	
Plasmid	QX100	0.83	1.07	14606
H37Rv	QX100		0.79	14485
BCG/ASM	QX100		0.94	14707
Plasmid	QS3D	0.809	0.90	18189
H37Rv	QS3D		0.65	18374

*Mean values per template type for QX100 (number of accepted droplets) and QS3D (following application of quality threshold). Partition number is fixed for BioMark.

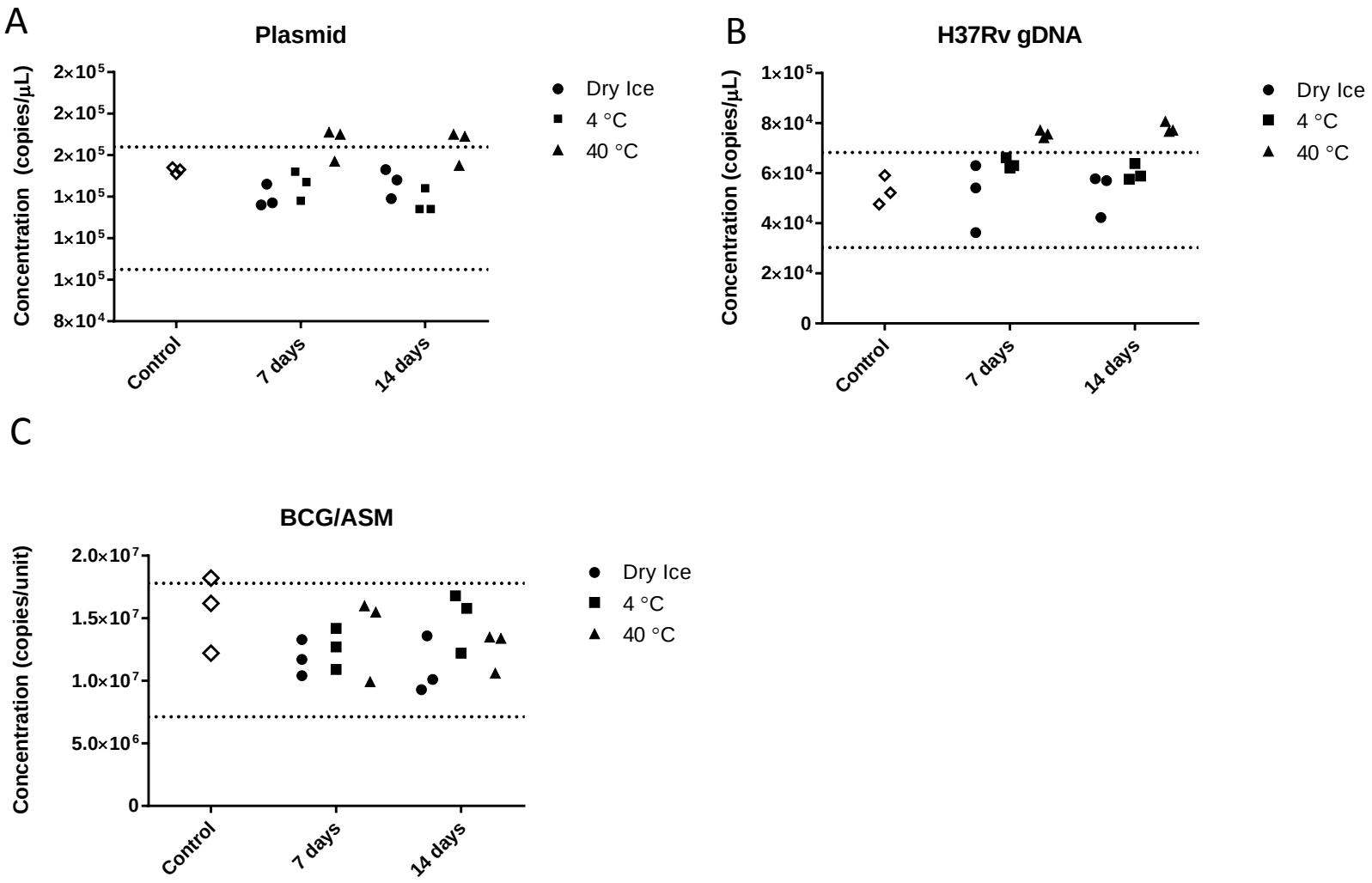

Figure S1: Stability testing of EQA materials. Materials were stored at three conditions (dry ice, 4°C, 40°C) for 7 or 14 days (n = 3) prior to analysis and compared with control samples maintained at -20°C (Plasmid, H37Rv gDNA) or -80°C (BCG/ASM). Individual datapoints are displayed for each condition. Dotted lines indicate values assigned to materials from NMI inter-laboratory study (Table 1).

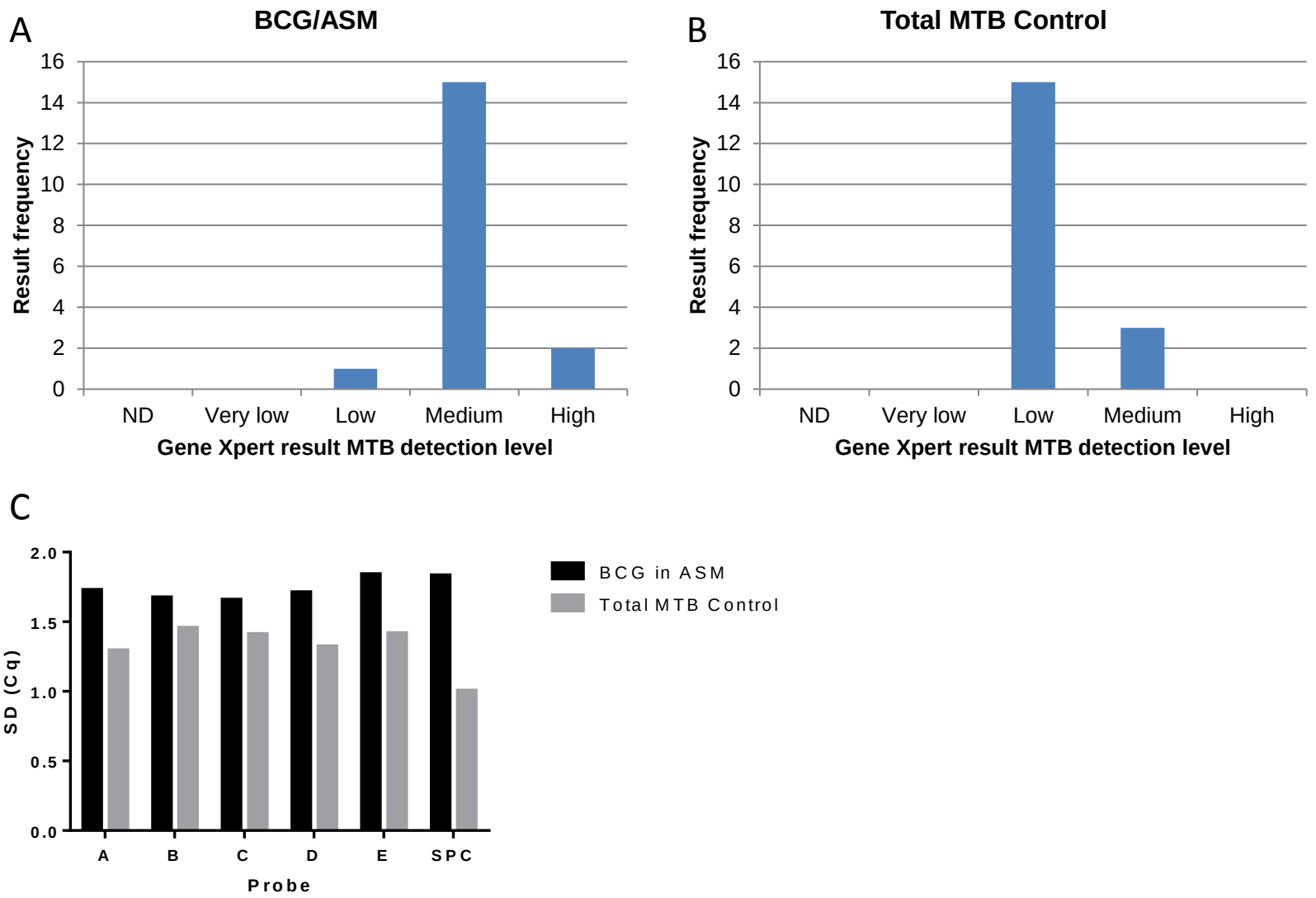

Figure S2: Results of analysis of BCG/ASM and Total MTB Control materials by Gene Xpert MTB/RIF end-user laboratories. The frequency of MTB detection levels from individual test results from all participating laboratories (n = 18) are shown for (A) BCG/ASM and (B) Total MTB Control materials. (C) Variation (SD) in Cq values for both EQA materials.

Table S4: Extraction and qPCR methods of qPCR end-user laboratories

Laboratory name	Extraction method	Gene target (qPCR)	Reference (if applicable)
Borstel	DNA mini tissue kit (Qiagen)	CRISPR-associated protein Csm	Hillemann et al. [3, 4]
GOSH	DNA Mini kit (Qiagen), with an added bead-beating step	*pks-13, ESAT-6, hupB, hsp65*	N/A
NUI Galway	IDI bead beating (3 minutes) Quick gDNA™ MiniPrep (Zymo)	*lepA*	Reddington et al. [5]

Table S5: Xpert MTB/RIF assay and instrumentation information (Xpert end-user laboratories)

Laboratory name	Gene Xpert system version	MTB/RIF Assay version
San Raffaele Scientific Institute	4.4a	5
Lancet Laboratories	6.1	5
Forschungszentrum Borstel	4.4a	5
UCL	4.6a	5
TASK Applied Science	4.4a	5
KCMC/KCRI	6.1	5

Table S6 : Cq (Ct) values from the difference instruments/ laboratories: single qPCR value and average XPERT MTB/RIF value from 5 probes. Laboratory β did not detect one of the replicate materials for the Total MTB Control. One of the Xpert MTB/RIF results from Laboratory 1 was omitted from the comparison (red cell) for the BCG/ASM analysis as it was a clear outlier result. This was further supported by a high SPC result of 30.6.

	qPCR Laboratory			Xpert MTB/RIF Laboratory					
Material	α	β	γ	1	2	3	4	5	6
Total MTB	32		36	26	25	24	22	24	24
Control	31	35	36	25	26	26	24	23	26

	32	36	36	26	25	24	24	22	24
	21	26	26	19	21	19	16	21	19
BCG/ASM	20	28	28	18	23	20	20	20	17
	20	26	29	26	20	22	19	21	20

References:

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3. Hillemann D, Warren R, Kubica T, Rüsch-Gerdes S, Niemann S. Rapid detection of *Mycobacterium tuberculosis* beijing genotype strains by real-time PCR. J Clin Microbiol. 2006;44:302-6.
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5. Reddington K, Zumla A, Bates M, van Soolingen D,Niemann S, Barry T, O'Grady J. SeekTB, a two-stage multiplex real-time-PCR-based method for differentiation of the *Mycobacterium tuberculosis* complex. J Clin Microbiol. 2012;50:2203-6.