Supplement 4: Additional information on MIQE compliance for qPCR assays.

Table S1: Description of the modified MIQE checklist (Bustin et al. 2009, 2010) for qPCR diagnostic assays for researchers, authors, reviewers, and editors. SD = standard deviation, SE = standard error, IRC = inter-run calibrator, CV = coefficient of variation, NTC = no template control

ASSAY CHECKLIST	DESCRIPTION
Sample/Template	
Source	Sample details: tissue type, location, volume, processing
Method of preservation	Frozen with temperature or preservative (e.g., ethanol, formalin)
Storage time (if appropriate)	How long were samples preserved before DNA extraction
Extraction method	Method used to extract DNA (e.g., kit or reagents)
DNA storage and time	How was DNA stored and how long before assayed
Concentration/Purity	Method with average and variance (e.g., SD, SE, CI) of DNA concentration and purity of tested samples
Inhibition assessment	Method of assessing inhibition; preferred Cq dilution series of template DNA (efficiency and linearity, spiked if necessary, to span 5-6 orders of magnitude); acceptable if within 10% of the standard curve
Assay Optimization/Validation	
Sequence accession number	Reference sequence in GenBank
Amplicon details	Location and size of amplicon
Primer sequence	Provide primer (and probe if Taqman) sequences even if already published
In silico	Method used to develop primers (e.g., BLAST, Primer-BLAST, alignment, phylogenetic analysis)
Empirical	Assay optimization details (e.g., anneal temperature, primer concentration, PCR additives)
Priming conditions	Details of qPCR cycling conditions
Specificity	Specificity testing against closely related organisms (e.g., melt curve, gel electrophoresis, sequencing)
PCR efficiency	Standard curve dilution series (e.g., linear plasmid containing target amplicon); provide slope, efficiency, and variance (SD or SE)
Linear dynamic range	Range in which the standard curve is linear, R^2 and variance (SE)
Limits of detection (LOD)	Absolute limit of detection of the assay with < 5% failed reactions
Intra-assay variation (repeatability)	Variance (SD) of Cq values for replicate reactions of standards, IRC and samples
Inter-assay variation (reproducibility)	Copy number variance (CV) for the same samples or IRC ran from several independent runs
PCR	
Protocols	Provide detailed protocols (as supplemental materials), including set up and contamination controls
Thermocycler	Manufacturer and settings changed from default if applicable
Reagents	Supplier, concentrations, and volumes
Negative control	Cq and melt curve of NTC (amplification is acceptable if > 5 Cq from LOD)
Positive control	Inter-run calibrator (IRC) or known positive sample assessed by a different method
Replicates	Number of technical replicates (at least duplicate, triplicate recommended)
Inter-platform variation (reproducibility)	Assess performance of standard curve and the same biological material used for 'Inter-assay variation' and report copy number CV
Data Analysis	
Software	qPCR software and settings
Assay validation	External validation of tested samples by histopathology, microscopy, or other diagnostic method

Table S2: Blank version of the modified MIQE checklist for qPCR assays to be used by authors.

ASSAY CHECKLIST	qPCR Assay Details
Sample/Template	
Source	
Method of preservation	
Storage time (if appropriate)	
Extraction method	
DNA storage and time	
Concentration/Purity	
Inhibition assessment	
Assay Optimization/Validation	
Sequence accession number	
Amplicon details	
Primer sequence	
In silico	
Empirical	
Priming conditions	
Specificity	
PCR efficiency	
Linear dynamic range	
Limits of detection (LOD)	
Intra-assay variation (repeatability)	
Inter-assay variation (reproducibility)	
PCR	
Protocols	
Thermocycler	
Reagents	
Negative control	
Positive control	
Replicates	
Inter-platform variation (reproducibility)	
Data Analysis	
Software	
Assay validation	