The **MIQE** guidelines: **M**inimum **I**nformation for Publication of **Q**uantitative Real-Time PCR **E**xperiments

MIQE checklist for authors, reviewers, and editors.[a]

ITEM TO CHECK	IMPORTANCE
EXPERIMENTAL DESIGN	
Definition of experimental and control groups	E
Number within each group	E
Assay carried out by the core or investigator's laboratory?	D
Acknowledgment of authors' contributions	D
SAMPLE	
Description	E
Volume/mass of sample processes	D
Micro dissection or macrodissection	E
Processing procedure	E
If frozen, how and how quickly?	E
If fixed, with what and how quickly?	E
Sample storage conditions and duration (especially for FFPE[b] samples)	E
NUCLEIC ACID EXTRACTION	
Procedure and/or instrumentation	E
Name of kit and details of any modifications	E
Source of additional reagents used	D
Details of DNase or RNase treatment	E
Contamination assessment (DNA or RNA)	E
Nucleic acid quantification	E
Instrument and method	E
Purity (A_{260}/A_{280})	D
Yield	D
RNA integrity:method/instrument	E
RIN/RQI or C_q of 3'and 5' transcripts	E
Electrophoresis traces	D
Inhibition testing (C_q dilutions, spike, or other)	E
REVERSE TRANSCRIPTION	
Complete reaction conditions	E
Amount of RNA and reaction volume	E
Priming oligonucleotide (if using GSP) and concentration	E
Reverse transcriptase and concentration	E
Temperature and time	E
Manufacturer of reagents and catalogue numbers	D
C_qs with and without reverse transcription	D[c]
Storage conditions of cDNA	D
qPCR TARGET INFORMATION	
Gene symbol	E
Sequence accession number	E
Location of amplicon	D
Amplicon length	E
Insilico specificity screen (BLAST, and soon)	E
Pseudogenes, retropseudogenes, or other homologs?	D
Sequence alignment	D
Secondary structure analysis of amplicon	D
Location of each primer by exon or intron (if applicable)	E
What splice variants are targeted?	E
qPCR OLIGONUCLEOTIDES	
Primer sequences	E
RTPrimer D B identification number	D
Probe sequences	D[d]
Location and identity of any modifications	E
Manufacturer of oligonucleotides	D
Purification method	D

ITEM TO CHECK	IMPORTANCE
qPCR PROTOCOL	
Complete reaction conditions	E
Reaction volume and amount of cDNA/DNA	E
Primer, (probe), Mg^2+, and dNTP concentrations	E
Polymerase identity and concentration	E
Buffer/kit identity and manufacturer	E
Exact chemical composition of the buffer	D
Additives (SYBR Green I, DMSO, and so forth)	E
Manufacturer of plates/tubes and catalog number	D
Complete thermocycling parameters	E
Reaction set up (manual/robotic)	D
Manufacturer of qPCR instrument	E
qPCR VALIDATION	
Evidence of optimisation (from gradients)	D
Specificity (gel, sequence, melt, or digest)	E
For SYBR Green I, C_q of the NTC	E
Calibration curves with slope and y intercept	E
PCR efficiency calculated from slope	E
CIs for PCR efficiency or SE	D
r^2 of calibration curve	E
Linear dynamic range	E
C_q variation at LOD	E
CIs throughout range	D
Evidence for LOD	E
If multiplex, efficiency and LOD of each assay	E
DATA ANALYSIS	
qPCR analysis program (source, version)	E
Method of C_q determination	E
Outlier identification and disposition	E
Results for NTCs	E
Justification of number and choice of reference genes	E
Description of normalisation method	E
Number and concordance of biological replicates	D
Number and stage (reverse transcription or qPCR) of technical replicates	E
Repeatability (intra assay variation)	E
Reproducibility (interassay variation, CV)	D
Power analysis	D
Statistical methods for results significance	E
Software (source, version)	E
C_q or raw data submission with RDML	D

[a] All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If primers are from RTPrimer DB, information on qPCR target, oligonucleotides, protocols, and validation is available from that source.

[b] FFPE, formalin-fixed, paraffin-embedded; RIN, RNA integrity number; RQI, RNA quality indicator; GSP, gene-specific priming; dNTP, deoxynucleoside triphosphate.

[c] Assessing the absence of DNA with ano–reverse transcription assay is essential when first extracting RNA. Once the sample has been validated as DNA free, inclusion of ano–reverse transcription control is desirable but no longer essential.

[d] Disclosure of the probe sequence is highly desirable and strongly encouraged; however, because not all vendors of commercial predesigned assays provide this information, it cannot be an essential requirement.Use of such assays is discouraged.

The MIQE guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments: Clinical Chemistry 55:4 611–622 (2009): Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., Wittwer, C.