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| Table1. MIQE checklist for authors, reviewers, andeditors. | | |
| Item to check | Importance | Line number or section |
| Experimental design | | |
| Definition of experimental and control groups | E | Line 60 |
| Number within each group | E | Line 60 |
| Assay carried out by the coreor investigator’s laboratory? | D | Line 56 |
| Acknowledgment of authors’ contributions | D | Line 293 |
| Sample | | |
| Description | E | Line 61 |
| Volume/mass of sample processed | D | Line 123 |
| Microdissection or macrodissection | E | Line 123 |
| Processing procedure | E | Line 60-76 and Line 123 |
| If frozen,how and how quickly? | E | Line 124 |
| If fixed,with what and how quickly? | E | Line 124 |
| Sample storage conditions and duration (especially for FFPEb samples) | E | Line 124 |
| Nucleic acid extraction | | |
| Procedure and/or instrumentation | E | Line 125 |
| Name of kit and details of any modifications | E | Line 125 |
| Source of additional reagents used | D | Not appliable |
| Details of DNase or RNase treatment | E | Line 125 |
| Contamination assessment (DNA or RNA) | E | Line 125-128 |
| Nucleic acid quantification | E | Line 129 |
| Instrument and method | E | Line 123-134 |
| Purity(A260/A280) | D | Line 310 |
| Yield | D | Line 310 |
| RNA integrity: method/instrument | E | Line 125-128 |
| RIN/RQI or Cq of 3’ and 5’ transcripts | E | Line 125-128 |
| Electrophoresis traces | D | None |
| Inhibition testing (Cq dilutions, spike,or other) | E | Line 125-128 |
| Reverse transcription | | |
| Complete reaction conditions | E | Line 126-127 |
| Amount of RNA and reaction volume | E | Line 126-127 |
| Priming oligonucleotide (if using GSP) and concentration | E | Line 126-127 |
| Reverse transcriptase and concentration | E | Line 126-127 |
| Temperature and time | E | Line 126-127 |
| Manufacturer of reagents and catalogue numbers | D | Line 126-127 |
| Cqs with and without reverse transcription | Dc | Line 310 |
| Storage conditions of cDNA | D | Line 310 |
| qPCR target information | | |
| Gene symbol | E | Table S2 |
| Sequence accession number | E | Table S2 |
| Location of amplicon | D | Table S2 |
| Amplicon length | E | Table S2 |
| In silico specificity screen (BLAST, and soon) | E | Line 310 |
| Pseudogenes, retropseudogenes,or other homologs? | D | None |
| Sequence alignment | D | None |
| Secondary structure analysis of amplicon | D | None |
| Location of each primer by exon or intron (if applicable) | E | Table S2 |
| What splice variants are targeted? | E | Table S2 |
| qPCR oligonucleotides | | |
| Primer sequences | E | Table S2 |
| RTPrimerDB identification number | D | Table S2 |
| Probe sequences | Dd | Table S2 |
| Location and identity of any modifications | E | Table S2 |
| Manufacturer of oligonucleotides | D | Line 128 |
| Purification method | D | Not applicable |
| qPCR protocol | | |
| Complete reaction conditions | E | Line 130-132 |
| Reaction volume and amount of cDNA/DNA | E | Line 130-132 |
| Primer, (probe),Mg2+ ,and dNTP concentrations | E | Line 130-132 |
| Polymerase identity and concentration | E | Line 130-132 |
| Buffer/kit identity and manufacturer | E | Line 130-132 |
| Exactchemical composition of the buffer | D | Not applicable |
| Additives (SYBR Green I, DMSO, and so forth) | E | Line 130-132 |
| Manufacturer of plates/tubes and catalog number | D | Not applicable |
| Complete thermocycling parameters | E | Line 130-132 |
| Reaction setup (manual/robotic) | D | Line 130-132 |
| Manufacturer of qPCR instrument | E | Line 130-132 |
| qPCR validation | | |
| Evidence of optimization (from gradients) | D | None |
| Specificity (gel, sequence, melt, ordigest) | E | Line 310 |
| For SYBR Green I, Cq of the NTC | E | Line 310 |
| Calibration curves with slope and *y* intercept | E | Line 310 |
| PCR efficiency calculated from slope | E | Line 310 |
| CIs for PCR efficiency or SE | D | Line 310 |
| r2 of calibration curve | E | Line 310 |
| Linear dynamic range | E | Line 310 |
| Cq variation at LOD | E | Line 310 |
| CIs throughout range | D | Line 310 |
| Evidence for LOD | E | Line 310 |
| If multiplex, efficiency and LOD of each assay | E | Line 310 |
| Data analysis | | |
| qPCR analysis program (source, version) | E | Statistical analysis |
| Method of Cq determination | E | Statistical analysis |
| Outlier identification and disposition | E | Statistical analysis |
| Results for NTC | E | Statistical analysis |
| Justification of number and choice of reference genes | E | Statistical analysis |
| Description of normalization method | E | Statistical analysis |
| Number and concordance of biological replicates | D | Line 60 |
| Number and stage (reverse transcription or qPCR) of technical replicates | E | Line 132 |
| Repeatability (intraassay variation) | E | Line 132 |
| Reproducibility (interassay variation, CV) | D | Line 132 |
| Power analysis | D | None |
| Statistical methods for results significance | E | Statistical analysis |
| Software (source, version) | E | Statistical analysis |
| Cq or raw data submission with RDML | D | Line 310 |

aAllessential information (E) must be submitted with the manuscript. Desirableinformation (D) should be submitted if available. If primers are from RTPrimerDB, information on qPCR target, oligonucleotides, protocols, and validation is available from that source.

bFFPE, for malin-fixed, paraffin-embedded; RIN, RNA integrity number; RQI, RNA quality indicator; GSP, gene-specificpriming; dNTP, deoxynucleoside triphosphate.

cAssessing the absence of DNA with a no–reverse transcription assay isessential when first extracting RNA. Once the sample has been validated as DNAfree, inclusion of a no–reverse transcription control isdesirable but no longer essential.

dDisclosure of the probe sequence is highly desirable and strongly encouraged; however, because no tallvendors of commercial predesigned assays provide this information, it cannot be an essential requirement. Use of such assays is discouraged.