

ITEM TO CHECK	PROVIDED	COMMENT
	Y/N	
1. SPECIMEN		
Detailed description of specimen type and numbers	Y	Methods of Main Text
Sampling procedure (including time to storage)	Y	Methods of Main Text
Sample aliquotation, storage conditions and duration	Y	Methods of Main Text
2. NUCLEIC ACID EXTRACTION		
Description of extraction method including amount of sample processed	Y	Methods of Main Text
Volume of solvent used to elute/resuspend extract	Y	Methods of Main Text
Number of extraction replicates	Y	Methods of Main Text
Extraction blanks included?	Y	Methods of Main Text
3. NUCLEIC ACID ASSESSMENT AND STORAGE		
Method to evaluate quality of nucleic acids	N	Not Done
Method to evaluate quantity of nucleic acids (including molecular weight)	N	Not Done
Storage conditions: temperature, concentration, duration, buffer, aliquots	Y	Methods of Main Text
Clear description of dilution steps used to prepare working DNA solution	Y	Methods of Main Text
4. NUCLEIC ACID MODIFICATION		
Template modification (digestion, sonication, pre-amplification, Details of repurification following modification if performed)	N	NA
	Y	Zymo Column in Methods of Main Text
5. REVERSE TRANSCRIPTION		
cDNA priming method and concentration	N	NA
One or two step protocol (include reaction details for two step)	Y	Methods of Main Text
Amount of RNA added per reaction	Y	Methods of Main Text
Detailed reaction components and conditions	Y	Methods of Main Text, also provided in protocols.io protocol referenced in main text
Estimated copies measured with and without addition of RT*	N	Not Done
Manufacturer of reagents used with catalogue and lot numbers	N	Reagents, Manufacturers, and Catalogue Numbers Reported in Supplemental Material. Lot Numbers are Not Reported
Storage of cDNA: temperature, concentration, duration, buffer and	Y	NA
6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION		
Sequence accession number or official gene symbol	Y	Provided in reference in paper to Huisman et al.
Method (software) used for design and <i>in silico</i> verification	Y	Provided in reference in paper to Huisman et al.
Location of amplicon	Y	Provided in reference in paper to Huisman et al.
Amplicon length	Y	Provided in reference in paper to Huisman et al.
Primer and probe sequences (or amplicon context sequence)**	Y	Methods in Main Text
Location and identity of any modifications	Y	NA
Manufacturer of oligonucleotides	Y	Methods in Main Text
7. dPCR PROTOCOL		
Manufacturer of dPCR instrument and instrument model	Y	Methods of Main Text, also provided in protocols.io protocol
Buffer/kit manufacturer with catalogue and lot number	Y	Methods of Main Text, also provided in protocols.io protocol
Primer and probe concentration	Y	Methods of Main Text, also provided in protocols.io protocol
Pre-reaction volume and composition (incl. amount of template and if	Y	Methods of Main Text, also provided in protocols.io protocol
Template treatment (initial heating or chemical denaturation)	N	NA
Polymerase identity and concentration, Mg++ and dNTP concentrations***	N	Included in Kit Manuals
Complete thermocycling parameters	Y	Methods of Main Text, also provided in protocols.io protocol referenced in main text
8. ASSAY VALIDATION		
Details of optimisation performed	N	Commercial Kit, Followed Manufacturer's Instructions
Analytical specificity (vs. related sequences) and limit of blank (LOB)	N	Provided in reference in paper to Huisman et al.
Analytical sensitivity/LoD and how this was evaluated	Y	Methods of Main Text
Testing for inhibitors (from biological matrix/extraction)	Y	Methods of Main Text
9. DATA ANALYSIS		
Description of dPCR experimental design	Y	Methods of Main Text
Comprehensive details negative and positive of controls (whether applied	Y	Methods of Main Text
Partition classification method (thresholding)	Y	Methods of Main Text, also provided in protocols.io protocol referenced in main text
Examples of positive and negative experimental results (including	Y	Supplemental Information
Description of technical replication	Y	Methods of Main Text
Repeatability (intra-experiment variation)	Y	Supplemental Information
Reproducibility (inter-experiment/user/lab etc. variation)	N	Assays were only completed within one laboratory
Number of partitions measured (average and standard deviation)	Y	Supplemental Information
Partition volume	Y	Reported by Manufacturer
Copies per partition (λ or equivalent) (average and standard deviation)	Y	Supplemental Information
dPCR analysis program (source, version)	Y	Methods of Main Text
Description of normalisation method	Y	Methods of Main text when applicable
Statistical methods used for analysis	Y	Methods of Main Text
Data transparency	raw data uploaded to online repository with ID:	https://purl.stanford.edu/yn042kx5009