# Supplementary Material S1

## Checklist MIQE

| Item to check | Importance | |
|---|---|---|
| **Experimental design** | | |
| Definition of experimental and control groups | E | **ISE females, ISE males, WR females, WR males**; albendazole treatment (all four groups), ivermectin treatment (all four groups), controls (treament with DMSO only, all four groups) |
| Number within each group | E | N=3 (three biological replicates in each group) One biological replicate represents 10 sexed adults. |
| Assay carried out by the core or investigator's laboratory? | D | investigator´s laboratory |
| Acknowledgment of authors' contributions | D | Conceived and designed the experiments: LL, PM, BS, LS. Performed the experiments: LL, MR, PM, LP, BS. Analyzed the data: LL, PM. Contributed reagents/materials/analysis tools: IV, JL, RL, HV. Wrote the paper: PM, LS. All authors read and approved the final manuscript. |
| **Sample** | | |
| Description | E | Haemonchus contortus, adult worms, isolated from sheep abomasum, *ex vivo* ABZ, IVM treatment |
| Volume/mass of sample processed | D | 10 adults |
| Microdissection or macrodissection | E | Macrodisssection |
| Processing procedure | E | Adult worms were isolated from sheep abomasum using agar method. |
| If frozen, how and how quickly? | E | Samples were washed with saline buffer, after sex discrimination, ten worms in each replicate were counted and soaked in 300 µl of TriReagent and immediately frozen in powdered dry ice and stored in -80 freezer (similarly after anthelmintics treatment) |
| If fixed, with what and how quickly? | E | Not fixed |
| Sample storage conditions and duration | E | Samples were held at -80°C for up to one month before RNA isolation |

| **Nucleic acid extraction** | | |
|---|---|---|
| Procedure and/or instrumentation | E | Homogenization of samples was performed using pestle microhomogenizator in 1.5 ml Eppendorf tube using 200 ml of TriReagent per 10 adults. Total RNA was extracted using the TriReagent (Molecular Research Center, USA) following manufacture's protocol. The purified RNA was dissolved in 40/30 ul DEPC-Treated Water (0.01%DEPC in HPLC water, autoclaved; females/males) and stored at -80°C. |
| Name of kit and details of any modifications | E | TriReagent (Molecular Research Center, USA, TR-118). We exactly followed manufacture's protocol. |
| Source of additional reagents used | D | Chloroform (Chemapol, Czech Republic); 2-propanol, absolute Ethanol, DEPC (Sigma-Aldrich, USA) |
| Details of DNase or RNase treatment | E | Samples were not treated by DNAse I |
| Contamination assessment (DNA or RNA) | E | Reverse transcription controls (noRT, without enzyme) were performed in order to assess the absece of DNA in the RNA sample - done for all samples in the RT step using random hexamers, samples were processed along with the normal RT samples, except that no reverse transcriptase was added to the reaction mixture. noRT control samples were analyzed by qPCR using gpd primers, total difference in Ct values obtained from cDNA with respective noRT controls was always bigger than 10 cycles. |
| Nucleic acid quantification | E | *RNA concentration was determined by measuring the absorbance at 260 nm UV light* |
| Instrument and method | E | NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Scientific) |
| Purity (A260/A280) | D | The absorbance ratio 260/280 of all samples proceed was higher than 1.9 |
| Yield | D | approx. 40 µg from females and 30 µg from males (10 adults each) |
| RNA integrity: method/instrument | E | The RNA integrity was assessed by visual inspection on 2% agarose gel visualized by SYBR Safe Staining |
| RIN/RQI or Cq of 3' and 5' transcripts | E | modified of 3´-5´assay, two amplicons from long (3kb) fusion transcript were analyzed and Cq vales were compared |
| Electrophoresis traces | D | - |
| Inhibition testing (Cq dilutions, spike, or other) | E | The standard curve has been considered sufficient to rule out the presence of inhibitors of reverse-transcription activity or PCR, also taking into account the high quality of starting RNAs. |
| **Reverse transcription** | | |
| Complete reaction conditions | E | 1 µg of RNA mixed with DEPC $H_2O$ (in 5 µl in total) was firstly incubated with 1 ul random hexamers 0.1 mM. This mixture was heated to 65°C for 5 min and then incubated on ice for at least 1 min for primer annealing and spin down shortly. Addition of 14 µl of master mix followed (composition: 4 ul dNTP Mix, 2.5 mM; 4 ul 5X ProtoScript II RT Reaction Buffer, 2 ul 10x DTT, and 0.5 µl ProtoScript II 200 U/µl and 3.5 ul $H_2O$), the sample was mixed and reactions were incubated in a PCR MJ Mini (Bio-Rad) at 25 °C for 10 min, 42 °C for 50 min and 80°C for 5 min to stop the reaction. The same reaction mixture with water intead of Protoscript II was used for the No-RT control. |
| Amount of RNA and reaction volume | E | total RNA: **1ug**; Reaction volume: **20 ul**; |
| Priming oligonucleotide (if using GSP) and concentration | E | random hexamers: 100 uM |
| Reverse transcriptase and concentration | E | ProtoScript II (NEB) in final concentration: 10 U/ul |
| Temperature and time | E | mRNA: 65°C for 5min, 25°C for 10 min, 42°C for 50 min and 80°C for 5 min |
| Manufacturer of reagents and catalogue numbers | D | dNTP Mix with dTTP (Eurogentec, NU-0010-10); ProtoScript II (NEB, Cat. M0368L) |
| Cqs with and without reverse transcription | D | for gpd primer set in all samples: average Cq=18; noRT controls Cq>30 |
| Storage conditions of cDNA | D | -20°C |

| **qPCR target information** | | |
|---|---|---|
| Gene symbol | E | Table 1 |
| Sequence accession number | E | Table 1 |
| Location of amplicon | D | - |
| Amplicon length | E | Table 1 |
| In silico specificity screen (BLAST, and so on) | E | NCBI primer BLAST ruled out non-specificities within sequenced genome |
| 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 | Table 1 |
| RTPrimerDB identification number | D | n/a |
| Probe sequences | D | n/a |
| Location and identity of any modifications | E | No modifications were done |
| Manufacturer of oligonucleotides | D | Generi Biotech, Hradec Králové, Czech Republic |
| Purification method | D | desalted |
| **qPCR protocol** | | |
| Complete reaction conditions | E | PCR reactions were performed in an iQ5 Real-Time PCR Detection System (Bio-Rad) using SYBR Green I in final volume of 20 ul. Reaction mix consisted of 2 ul 10X PCR Buffer, 1.4 ul 50mM MgCl2, 0.8 ul 5mM dNTP Mix, 0.6 ul SYBR Green I, 0.1 ul 5 U/ul HotGoldStar DNA Polymerase (Eurogentec core kit), 0.4 ul of each primer (5uM forward and reverse primers) to final concentration 100 nM and 5 ul of diluted cDNA (corresponding to 5 µg RNA). The PCR reactions were initiated with 10 minute incubation at 95°C, followed by 40 cycles of 95°C for 10 seconds, 60°C for 20 seconds and 72°C for 20 seconds. All reactions were performed in duplicates. |
| Reaction volume and amount of cDNA/DNA | E | Reaction volume: 20ul; amount of cDNA: 5ul of **1/50** dilution |
| Primer, (probe), MgCl2, and dNTP concentrations | E | 200uM each dNTP, 3.5mM MgCl2, final primers concentration was 100 mM |
| Polymerase identity and concentration | E | HotGoldStar 5 U/µl (Eurogentec) |
| Buffer/kit identity and manufacturer | E | qPCR Core kit for SYBR® Green I No ROX (10x buffer, MgCl2 50mM, dNTP mix 5mM each, HotGoldStar, SYBR® Green I stock and DMSO tubes)-Eurogentec (RT-SN10-05NR) |
| Exact chemical composition of the buffer | D | - |
| Additives (SYBR Green I, DMSO, and so forth) | E | SYBR Green I diluted in DMSO, both components of the qPCR core kit |
| Manufacturer of plates/tubes and catalog number | D | 8-strips, flat cap (BioRad) |
| Complete thermocycling parameters | E | Initial denaturation: 95°C for 10 minute, then 40 cycles at 95°C for 10 seconds, 60°C for 20 seconds, 72°C for 20 seconds |
| Reaction setup (manual/robotic) | D | manual |
| Manufacturer of qPCR instrument | E | Bio-Rad |

| **qPCR validation** | | |
|---|---|---|
| Evidence of optimization (from gradients) | D | - |
| Specificity (gel, sequence, melt, or digest) | E | Melting curve analysis, ramping from 55°C to 95°C, in 0.5°C steps where fluorescence data are measured every 30s. Gene-specific amplification was confirmed by a single band in 2% agarose gel electrophoresis stained with SYBR Safe. No template controls (no cDNA in PCR) were run for each gene to detect unspecific amplification and primer dimerization. |
| For SYBR Green I, Cq of the NTC | E | The signal of the amplification plot in NTC samples was late (Cq>34). The difference between the negative control and all the cDNA sample was > 9 cycles. |
| Calibration curves with slope and y intercept | E | gpd: y = -3.206x + 34.547; far: y = -3.325x + 35.285; sod: y = -3.265x + 40.467; rpl:y = -3.302x + 38.044; eef: y = -3.324x + 40.542; pfk: y = -3.3595x + 43.299; tnt: y = -3.267x + 38.542; act: y = -3.5452x + 36.283; ama: y = -3.2262x + 37.399; 18s: y = -3.373x + 37.57; ncbp: y = -3.375x + 40.527 |
| PCR efficiency calculated from slope | E | Table 1 |
| CIs for PCR efficiency or SE | D | - |
| r2 of calibration curve | E | gpd: 0.9993; far: 0.9995; sod: 0.9976 ; rpl: 0.9994; eef: 0.9982; pfk: 0.9931; tnt: 0.9927; act: 0.9923; ama: 0.9996; 18s: 0.9993; ncbp: 0.9953 |
| Linear dynamic range | E | The linear dynamic range was considered taking into account the linearity of the standard curves; For mRNA: from 1/5 dilution of cDNA to 1/5000 dilution |
| Cq variation at LOD | E | not detected |
| CIs throughout range | D | - |
| Evidence for LOD | E | not detected, the dilutions of cDNA performed in linear range defined by standard curve |
| If multiplex, efficiency and LOD of each assay | E | n/a |
| **Data analysis** | | |
| qPCR analysis program (source, version) | E | Bio-Rad iQ5 |
| Method of Cq determination | E | The threshold is set manualy to the level where the fluorescence rises above detection limit. The threshold specify Cq values. |
| Outlier identification and disposition | E | None of the Cq values was discarded |
| Results for NTCs | E | The signal of the amplification plot was late (Cq>34). The difference between the negative control and all the cDNA sample was > 9 cycles. |
| Justification of number and choice of reference genes | E | This is a study for the selection of reference genes |
| Description of normalization method | E | Described in text |
| Number and concordance of biological replicates | D | Three biological replicates |
| Number and stage of technical replicates | E | qPCR reactions were performed in duplicate |
| Repeatability (intraassay variation) | E | Mean standard deviation of duplicates: 0.15 |
| Reproducibility (interassay variation, CV) | D | - |
| Power analysis | D | - |
| Statistical methods for results significance | E | - |
| Software (source, version) | E | Refinder (http://www.leonxie.com/referencegene.php), Genorm v3, Bestkeeper v1 , Normfinder v 0.953 (details in supplementary material S2) |
| Cq or raw data submission with RDML | D | - |