Supporting Information

The *E*nvironmental *M*icrobiology *M*inimum *I*nformation (EMMI) Guidelines: qPCR and dPCR Quality and Reporting for Environmental Microbiology

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Description of Supporting Information

Systematic Review Methods

On 22 June 2020, we searched Web of Science Core Collection via iknowledge.com using the following search terms in a title search: (virus or *virus or salmonella or campylobacter or “O157:H7” or “microbial source tracking” or Cryptosporidium or Giardia) AND (water or *water or formite* or fomes or surface* or air). The search returned 10,549 publications. Starting with the most recent publication, we performed full text review of the publication to determine whether it (1) provided measurements of pathogens or microbial source tracking markers in water or on surfaces or in air using the molecular approach of real-time qPCR or dPCR (studies on RNA viruses that performed reverse transcription before qPCR or dPCR were included); the measurements had to be on matrices with endogenous (unseeded) target, (2) was in English, and (3) was peer reviewed. Using the most recent 100 papers that fit those inclusion criteria, we recorded whether the paper reported on various controls and methods that we deem essential for the interpretation of their results. The list of information extracted from each publication discussed in this paper is provided in Table S1. These 100 papers were published between 2015 and 2020. The distribution among those years is 14 in 2020, 23 in 2019, 24 in 2018, 18 in 2017, 10 in 2016, and 11 in 2015. Only 3 of the 100 papers used digital PCR and the rest used real-time quantitative PCR methods.

Table S1. Information extracted from 100 most recent papers in health-related environmental microbiology that used qPCR and dPCR.

Question	Total w YES	Total N	% w YES
State that sampling recovery control was run?	33	100	33%
Report results of sampling recovery control	29	100	29%
State that sampling equipment negative control was run?	10	100	10%
Report results of sampling equipment negative control?	8	100	8%
Did the authors use an “elution step” (from a filter, for example)?	67	100	67%
If authors use an elution step, do they report using an elution positive control?	21	67	31%
Report the results of an elution positive control?	10	67	15%
State nucleic acid extraction negative controls were run?	20	100	20%
Report results of extraction negative controls?	9	100	9%
State nucleic acid extraction positive control was run?	38	100	38%
Report results of extraction positive controls?	17	100	17%
State reverse transcription (RT) inhibition control was run?	36	86	42%
Report results of RT inhibition control?	26	86	30%
State PCR inhibition control was run?	43	100	43%
Report results of PCR inhibition control?	26	100	26%
State RT negative control (no template control) was run?	31	86	36%
Report results of RT negative control?	11	86	13%
State RT positive control was run?	36	86	42%
Report results of RT positive control?	12	86	14%
State PCR negative control (no template control) was run?	46	100	46%
Report results for PCR negative control?	13	100	13%
Report standards / reference positive control for PCR?	59	100	59%
Report standard source?	56	100	56%
Report standard quantification methods?	41	98	42%
Report standard curve slope? R^2?	26	93	28%
Report lowest standard measured?	29	94	31%
Report Cq determination method?	23	97	24%
Reported how Cq converted to reported concentrations?	19	96	20%

Dimensional Analysis

Examples of Unit Conversions for qPCR or dPCR from Instrument Output to Target Concentration in Sample

Environmental aqueous sample where the dimension is volume

DNA or one-step RNA targets:

Target sample concentration $\left(\frac{gc}{mL\ sample}\right)$ *= Instrument output* $\left(\frac{gc}{\mu L\ rxn}\right)$ *X PCR rxn dilution* $\left(\frac{\mu L\ rxn}{\mu L\ NA\ extract\ added}\right)$ *X Extraction conc factor* $\left(\frac{\mu L\ NA\ extract}{\mu L\ sample\ concentrate\ extracted}\right)$ *X*

Inhibition dilution factor $\left(\frac{\mu L\ NA\ extract\ diluted}{\mu L\ NA\ extract}\right)$ *X Volume conversion factor 1000* $\left(\frac{\mu L}{mL}\right)$ *X Sample conc factor* $\left(\frac{mL\ sample\ concentrate}{mL\ sample}\right)$

When a two-step RT assay is performed the term PCR rxn dilution is substituted with: PCR Rxn dilution $\left(\frac{\mu L\ rxn}{\mu L\ RT\ rxn\ (i.e.,cDNA)}\right)$ *X RT dilution* $\left(\frac{\mu L\ RT\ rxn}{\mu L\ NA\ extract\ added}\right)$

When inhibition is absent the dilution factor equals 1.

Environmental solid sample where the dimension is mass

DNA or one-step RNA targets:

Target sample concentration $\left(\frac{gc}{g\ sample}\right)$ *= Instrument output* $\left(\frac{gc}{\mu L\ rxn}\right)$ *X PCR rxn dilution* $\left(\frac{\mu L\ rxn}{\mu L\ NA\ extract\ added}\right)$ *X Inhibition dilution factor* $\left(\frac{\mu L\ NA\ extract\ diluted}{\mu L\ NA\ extract}\right)$

X Mass conversion factor 1000 $\left(\frac{mg}{g}\right)$ *X Extraction factor* $\left(\frac{\mu L\ NA\ extract}{mg\ sample\ extracted}\right)$

When a two-step RT assay is performed the term PCR rxn dilution is substituted with: PCR Rxn dilution $\left(\frac{\mu L\ rxn}{\mu L\ RT\ rxn\ (i.e.,cDNA)}\right)$ *X RT dilution* $\left(\frac{\mu L\ RT\ rxn}{\mu L\ NA\ extract\ added}\right)$

When inhibition is absent the dilution factor equals 1.

gc = gene copies
rxn = reaction

Environmental Microbiology Minimum Information Checklist

Study Description

Study: [Study Name]
Date: 04-Jan-1904
Completed by: [Filled By...]

Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection	Analysis
(notes)	☐ Performed (notes)	☐ Performed (notes)	(notes)	☐ Performed (notes)	☐ qPCR ☐ dPCR (notes)	(notes)

Control Checklist

	Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection
Step performed	☐	☐	☐	☐	☐	☐
Negative Controls						
Step has control info	☐	☐	☐	☐	☐	☐
# control replicates	0	0	0	0	0	0
Control result reported	☐	☐	☐	☐	☐	☐
Data handling reported	☐	☐	☐	☐	☐	☐
Positive Controls						
Control introduced	☐	☐	☐	☐	☐	☐
Internal/External	Internal	Internal	Internal	Internal	Internal	Internal
Independent/Parallel	Independent	Independent	Independent	Independent	Independent	Independent
Step has control info	☐	☐	☐	☐	☐	☐
# control replicates	0	0	0	0	0	0
Control result reported	☐	☐	☐	☐	☐	☐
Data Handling reported	☐	☐	☐	☐	☐	☐

Process Checklist

Environmental Sampling

- ☐ Sampling Procedure
- ☐ Number of samples
- ☐ Sample amount, mean, range
- ☐ Sampling locations, dates, times

Sample Treatment

- ☐ Performed
- ☐ Treatment procedure
- ☐ Reagents

Sample Reduction

- ☐ Performed
- ☐ Reduction procedure
- ☐ Reagents
- ☐ Concentration Factor

Nucleic Acid Extraction

- ☐ Extraction procedure
- ☐ Amount extracted, amount obtained
- ☐ Extract storage conditions

qPCR or dPCR

- ☐ Target gene name, amplicon length
- ☐ Thermocycling temperatures and times
- ☐ Master mix: composition, vendors, concentrations
- ☐ Additives: vendors, concentrations
- ☐ Template amount added, pre-treatment (if any)
- ☐ Primers: sequences, concentrations, vendors, references
- ☐ Amplicon confirmation method (probe, melt curve, etc)
- ☐ Probe sequence, concentration, vendor, reference
- ☐ Instrumentation
- ☐ Equivalent volume of sample analyzed by PCR
- ☐ Inhibition assessment procedure
- ☐ Inhibition control description (if used)
- ☐ Number samples tested and found inhibited

Reverse Transcription

- ☐ Performed
- ☐ One or two step
- ☐ cDNA storage conditions (if two step)
- ☐ Reaction temperatures and times
- ☐ Reaction reagents and concentrations
- ☐ Priming method
- ☐ Reaction volume, added template amount
- ☐ Inhibition assessment procedure
- ☐ Inhibition control description (if used)
- ☐ Number samples tested and found inhibited

Analysis – dPCR

- ☐ Threshold settings
- ☐ Technical replicates, number, well merging
- ☐ Partitions measured, number, mean, variance
- ☐ Partition volume
- ☐ Target copies per partition, mean, variance
- ☐ Program used for dPCR analysis
- ☐ Explanation of control results, example plots

Analysis – qPCR

- ☐ Method for handling failed negative controls
- ☐ Technical replicates, number, calculations
- ☐ Calibration standards: description and source
- ☐ Method of quantifying standards
- ☐ Calibration curve slope
- ☐ Calibration curve R2
- ☐ Lowest standard measured or 95% LOD
- ☐ Cq value determination method