

Weston Solutions Lab Report - Sample Results

Project: Summit City WW Feasibility Study

Client: Pace Engineering

Survey: No. 2

Date Received: 02/22/23

n Samples: 7

Assay: Human Bacteroidales - HF183TMCaMan

µL Template per Reaction: 2

Method: qPCR

Method Blanks: passed

n= 3

No Template Controls: passed

n= 6

Positive Extraction Controls^A: passed

n= 7

Inhibition Control^B: HF183 (B.dorei) / Sketa

Sample ID	Site ID	Date Sampled	Time Sampled	Matrix	Volume Filtered (mL)	Sample Result ^C	Qualifier ^D	Sample Concentration ^E	Sample Stdev ^F	Units ^G	SLOD ^H	SLLOQ ^H	cpr	Inhibition Result ^I
11A	11A	02/21/23	0925	FW	100	BDL	§<	111	137	copies/100 mL	214	429	2	0
8C	8C	02/21/23	0935	FW	100	Detected, ROQ		5,941	2,267	copies/100 mL	214	429	83	0
23	23	02/21/23	0945	FW	100	Detected, ROQ		194,244	34,103	copies/100 mL	214	429	2,719	0
8B	8B	02/21/23	0955	FW	100	ND	§<	26	0	copies/100 mL	214	429	0	0
8A (24)	8A (24)	02/21/23	1005	FW	100	ND	§<	26	0	copies/100 mL	214	429	0	0
Field Blank	Field Blank	02/21/23	0950	FW	100	ND	§<	26	0	copies/100 mL	214	429	0	0
Filter Blank	Filter Blank	02/21/23	1105	Filtration Blank	25	ND	§<	103	0	copies/100 mL	857	1714	0	0



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Weston Solutions Lab Report - Lab Control Results

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Survey: No. 2

Date Received: 02/22/23

n Samples: 7

Assay: Human Bacteroidales - HF183TMCaMan

µL Template per Reaction: 2

Method Blanks: passed

No Template Controls: passed

Positive Extraction Controls^A: passed

Inhibition Control^B: HF183 (B.dorei) / Sketa

Method: qPCR

n= 3

n= 6

n= 7

Sample ID	Site ID	Matrix	Sample Result ^C	Qualifier ^D	cpr	QC Result ^E
Extraction Blank 1	Weston	Lab Blank	ND	§<	0.36	PASS
Extraction Blank 2	Weston	Lab Blank	ND	§<	0.36	PASS
Extraction Blank 3	Weston	Lab Blank	ND	§<	0.36	PASS
NTC 1900	Weston	Lab Blank	ND	§<	0.36	PASS
NTC 1906	Weston	Lab Blank	ND	§<	0.36	PASS

Sample ID	Site ID	Matrix	Sample Result ^C	Qualifier ^D	cpr	QC Result



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Weston Solutions Lab Report - Standard Curve Metrics

Project: Summit City WW Feasibility Study
Client: Pace Engineering

Survey: No. 2
Date Received: 02/22/23

Comments:

Standard Curve Metrics

Assay: Human Bacteroidales - HF183TMCaMan

% Efficiency: 99.32

R²: 0.992

Slope: -3.34

Y-Intercept: 38.52

curve source: Weston Master Standard Curve

standard source: genomic DNA

Method Detection Limits	ND sub	LOD	LLOQ
cpr (copies per reaction):	0.36	3.0	6.0
Ct equivalent:	40.00	36.93	35.93
LOD > % amplification:	83		
LLOQ StdDev:	0.85		

Copies per genome 7

* based on a master standard curve with a minimum of 50 data points.

Sample result calculations use cpr values based on the following definitions:

ND: Cq=maximum cycle number, negative result.

BDL: Max cycle number>Sample Cq≥LOD(Cq), Equivocal result.

DNQ: LOD(Cq)>Sample Cq≥LLOQ(Cq), positive binary result.

ROQ: Sample Cq<LLOQ (Cq), positive result.

LLOQ (EPA Methods 1696/97): Upper 95% Prediction Interval at 10 cpr

LLOQ (all other methods): lowest concentration with amplification rate of 100% (>20 reps)

In addition, SLOD and SLLOQ values are provided. These are sample specific detection limits which take into account sample processing, for example volumes or mass.

Categorical Results:

ROQ and DNQ = positive; ND = negative

BDL results are categorized as "equivocal" because a signal was observed below the reporting limit (usually LOD, EPA Method 1696:LLOQ). The result can therefore not be classified as either a negative or positive with great confidence. Weston uses BDL concentration values to compute averages unless directed otherwise by Client. Sites with chronic BDL results may warrant additional monitoring. EPA Method 1696: BDL = ND.

Abbreviations: BDL = Below Detection Limit; cpr = copies per reaction; Cq = quantification (threshold) cycle; DNQ = Detectable But Not Quantifiable; LLOQ = Lower Limit of Quantification; LOD = Limit of Detection; n=number; N/A = Not Applicable; ND = Not Detected; NDsub = substitution value for nondetects; PCR = Polymerase chain reaction; rxs = reactions; StdDev = Standard Deviation; sub = substitution; ROQ = Range of Quantification; SLLOQ = Sample Specific Lower Limit of Quantification; SLOD = Sample Specific Limit of Detection.

Alexander Schriewer

Laboratory Director (Alexander Schriewer)

03/07/2023

Date

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Abbreviations

Avg	Average
BDL	Below Detection Limit
cpr	Copies Per Reaction
Final Cq	Quantification cycle used to make concentration estimate
ddPCR	Droplet Digital Polymerase Chain Reaction
DNQ	Detectable, not quantifiable
FB	Field Blank
FW	Fresh Water
GW	Ground Water
IAC	Internal Amplification Control
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
MB	Method Blank
n	Number
N/A	Not Applicable
ND	Not Detected
NTC	No Template Control
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
R ²	Correlation Coefficient
ROQ	Range of Quantification
rxns	Reactions
SLLOQ	Sample Specific Lower Limit of Quantification
SLOD	Sample Specific Limit of Detection
SLT	Salt Water
StdDev	Standard Deviation
Std. Error	Calibration model slope and intercept standard error
sub	Substitution
SW	Storm Water
TSC	Target Sequence Copies
WW	Wastewater

Footnotes

- ^A Sample Process Control (SPC), Sketa assay for salmon sperm
- ^B Inhibition Control: If not EPA Method 1696/1697: assay used for 2-well spike with DNA dilution method
- ^C see explanation for ND, BDL, DNQ, and ROQ on Part C
- ^D If shown: \bar{x} : Average computed for ND result by
- a) qPCR: substituting Cq with maximum number of cycles (Boehm et al., 2013) or
 - b) ddPCR: substituting with 1 cpr; the result can be therefore interpreted as less than the given value.
- ϕ : QC flag, see notes on Part C
- ^E Concentration = mean of at least 3 technical replicates.
- ^F Standard Deviation of at least 3 technical replicates.
- ^G For enterococci, results are given in Target Sequence Copies (TSC), as per EPA Method 1611 (standard concs in TSC/ul = copies/ul x 4).
- ^H SLOD and SLLOQ: sample specific detection and quantification limits calculated based on sample specific processing volumes see more information on Part C.
- ^I Inhibition Categories:
- 0 = no inhibition observed
 - 1 = inhibition observed, but overcome in diluted sample
 - 2 = inhibition not overcome in diluted sample: The given concentration may be underestimated for positive samples
 - 3 = Dilution needed to overcome inhibition did not yield amplification. Given concentration may be underestimated.
 - NT = not tested.