

Weston Solutions qPCR Report - Sample Results

Client: County of San Diego

Project: SDR MST DW

Survey: Event 3 - 8

Date Received: 5/17, 7/5, 7/26, 8/3, 8/16 and 8/31/18

n Samples: 12

Date Filtered: 5/17, 7/5, 7/26, 8/3, 8/16 and 8/31/18

Date Extracted: 9/7/18

Date PCR: 10/4/18

Assay: HumanBacteroidales-HF183TaqmanCAMan

µL Template per Reaction: 5

Lab Blanks: passed n= 2

No Template Controls: passed n= 3

Positive Extraction Controls^A: passed n= 3

Inhibition Control^B: HF183 (B.dorei)

File name(s): Plate 387 HF183TMCaMan ka.pcrd

Sample ID	Site ID	Weston DNA ID	Date Sampled	Time Sampled	Matrix	Sample Result ^C	Qualifier ^D	Sample Concentration ^E	Sample Stdev ^F	Units ^G	SLOD ^H	SLLOQ ^H	cpr	Inhibition Result ^I
MST-SDR-780	MST-SDR-780	3966MST-SDR-780	07/05/18	1005	FW	Detected, DNQ		50	3	copies/100mL	29	57	5	0
MST-SDR-207	MST-SDR-207	3967MST-SDR-207	07/05/18	1035	FW	ND	§<	3	0	copies/100mL	29	57	0	0
MST-SDR-098	MST-SDR-098	3968MST-SDR-098	07/05/18	1055	FW	BDL		36	25	copies/100mL	43	86	3	0
MST-SDR-780	MST-SDR-780	4074MST-SDR-780	05/17/18	1000	FW	BDL		15	1	copies/100mL	29	57	2	0
MST-SDR-207	MST-SDR-207	4075MST-SDR-207	05/17/18	1030	FW	ND	§<	3	0	copies/100mL	29	57	0	0
Field Blank	MST-SDR-780	4076Field Blank	05/17/18	1000	FB	ND	§<	3	0	copies/100mL	29	57	0	0
MST-SDR-780	MST-SDR-780	4260MST-SDR-780	07/26/18	0915	FW	BDL	§<	7	5	copies/100mL	29	57	1	0
MST-SDR-207	MST-SDR-207	4261MST-SDR-207	07/26/18	0955	FW	ND	§<	3	0	copies/100mL	29	57	0	0
MST-SDR-098	MST-SDR-098	4262MST-SDR-098	07/26/18	1020	FW	ND	§<	3	0	copies/100mL	29	58	0	0
MST-SDR-098	MST-SDR-098	4335MST-SDR-098	08/03/18	0425	FW	ND	§<	3	0	copies/100mL	29	57	0	0
MST-SDR-098	MST-SDR-098	4397MST-SDR-098	08/16/18	0430	FW	BDL	§<	6	3	copies/100mL	29	57	1	0
MST-SDR-098	MST-SDR-098	4568MST-SDR-098	08/31/18	0430	FW	BDL	§<	11	13	copies/100mL	29	57	1	0

Abbreviations: Avg = Average; BDL = Below Detection Limit; cpr = copies per reaction; Cq = quantification (threshold) cycle; DNQ = Detectable But Not Quantifiable; FB = Field Blank; FW: Fresh Water; GW: Ground Water; L; SLT: Salt Water; SW: Storm Water; LOQ = 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; TSC = Target Sequence Copies; ROQ = Range of Quantification; SLLOQ = Sample Specific Lower Limit of Quantification; SLOD = Sample Specific Limit of Detection.

Footnotes: ^ASample Process Control (SPC), Sketa assay for salmon sperm. ^BInhibition Control = assay used for 2 well spike with DNA dilution method. ^CSuggestion for conversion of sample result into categorical results: ROQ and DNQ = positive; ND = negative; BDL = equivocal (see explanation on Part B). ^DIf shown: §Average computed for ND result by substituting Cq with maximum number of cycles (Boehm et al., 2013). ^EConcentration = mean of at least 3 technical replicates. ^FStandard Deviation of at least 3 technical replicates. ^GFor enterococci, results are given in Target Sequence Copies (TSC), as per EPA Method 1611 (standard concs in TSC/ul = copies/ul x 4). ^HSLOD and SLLOQ: sample specific detection and quantification limits calculated based on sample specific processing volumes see more information on Part B. ^IInhibition: 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. See Part B for additional comments.



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Weston Solutions qPCR Report - Standard Curve Metrics

Client: County of San Diego

Project: SDR MST DW

Survey: Event 3 - 8

Date Received: 5/17/18 - 8/31/18

Comments:

Standard Curve Metrics*

Assay HumanBacteroidales-HF183TaqmanCAMan

% Efficiency 99.32

r^2 0.992

slope -3.34

y-intercept 38.52

curve source Weston Master Curve

standard source genomic

Site Conclusion Values	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.

AVG Filtration Volume 292 mL

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

ND: Cq=maximum cycle number, negative result.

BDL: $0 < Cq \leq LOD$, Equivocal result.

DNQ: $LOD < Cq \leq LLOQ$, positive binary result.

ROQ: $Cq > LLOQ$, positive result.

LLOQ : 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 limit of detection. 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.

Abbreviations: Avg = Average; BDL = Below Detection Limit; cpr = copies per reaction; Cq = quantification (threshold) cycle; DNQ = Detectable But Not Quantifiable; FB = Field Blank; 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; TSC = Target Sequence Copies; ROQ = Range of Quantification; SLLOQ = Sample Specific Lower Limit of Quantification; SLOD = Sample Specific Limit of Detection.

QC Officer (Satomi Yonemasu) Date

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