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Alaska Laboratory# AK01000

Client ADEC Nonpoint Source Pollution Program
Contact Sarah Apsens, Laura Eldred

Project Name SOA Bristol Bay Pathogen MST Sampling 2 2022
AWL # AWL-22-02228
PWS # None

Please direct any questions regarding the final report to Mary@AKWaterLabs.com or Matt@AKWaterLabs.com, or call 907-373-6130.

The results presented in this report meet the requirement of the laboratory's certifications and internal QC processes. Any exceptions will be noted in the case narratives attached. Subcontract Data has been entered into the AWL Final Report, however the full subcontract report is available upon request.

The attached should contain analytical results for the analyses submitted on the client chain of custody. The information includes no opinions of the analysts or labs, data is represented after meeting certified testing requirements, and quality control measures.

Reproduction of the report in full requires the written approval of the laboratory.

Signature of Laboratory Management Date

Alaska Laboratory# AK01000

Client Project Name SOA Bristol Bay
 Pathogen MST AWL # AWL-22-02228
 Sampling 2 2022

Receipt Date and Time 7/12/2022 17:53 Due Date 8/2/2022

Cooler/Sample Temp (C) 2.09 Sampler Initials BM

Sample Receipt Comments Received by MCC 7/12/22 2.09C #1RT on frozen ice.

Samples Received

Microbiological

Sample Location	AWL ID	Collection Date/ Time	Analysis Date/Time	Analysis	Notes
Kanak-071222-003	AWL-22-02228-001	7/12/2022 12:55	7/26/2022	MST	Freeze and Filter at AWL
Scandinavian-071222-003	AWL-22-02228-002	7/12/2022 12:25	7/26/2022	MST	Freeze and Filter at AWL
Snag Point-071222-003	AWL-22-02228-003	7/12/2022 12:05	7/26/2022	MST	Freeze and Filter at AWL

Analytical Methods

Analyte	Method	Comments
MST	MST	Subcontracted to Lumin Ultra

Cert Required None
CMDP #

Log In Initials: MCC 7-13-22
 DQO Initials: JKR 7-13-22

Comments: Samples received with RUSH samples under AWL-22-02224, AWL-22-02225, AWL-22-02226. Logged under AWL-22-02228.

Definitions:

DUP	Sample Duplicate
LCS/LCSD	Laboratory Control Sample/Laboratory Control Sample Duplicate
MRL	Method Reporting Limit
MB	Method Blank
MCL	Maximum Contaminant Level
MDL	Method Detection Limit
MS/MSD	Matrix Spike/Matrix Spike Duplicate
N/A	Not Applicable
TNTC	Count is Too Numerous To Count
<MDL	Result recovery is below the detectable laboratory limit, listed as the MDL

Data Qualifiers:

B	The result of both the method blank and the target sample are above the MDL.
D	Sample analysis accomplished through dilution.
J	The reported result is an estimated value above the LOD but below the LOQ, or above the MDL but below the PQL.
U	Result is below the MDL, PQL, LOD, or LOQ
*	LCS/LCSD or Sample DUP fails all Duplicate criteria.
H	Holding time exceeded
E	Exceeds MCL
Q	One or more quality control criteria failed.

General Comments:

1.0)	Basis: "As Received" = analyzed as received from client; "Dry" = dried prior to being analyzed; "Dry Weight Corrected" = analyzed as received; result corrected for percent moisture.
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Laboratory Comments

Submitter: Alaska Water Labs
Report Generated: 7/27/2022

Non-Detect (ND) Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification (LOQ, see below). This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations, and the confidence of such quantification will be lower than that declared by the definition of LOQ.

Quantifiable Results (ROQ)

Sample results are within the range of quantification of calibration curves (standard curves) of a validation qPCR method. For most qPCR assays, the range is 1E1 to 1E5 copies/reaction. Copy number measurements reported are relative, not absolute, quantification.

LOD (Limit of Detection, lower)

A general consensus was reached around the definition of the LOD as the lowest amount of analyte, which can be detected with more than a stated percentage of confidence (95%), but, not necessarily quantified as an exact value. It must be noted that LOD is not a limiting value and therefore, that Ct values below the LOD cannot automatically be considered as negative. From the definition of LOD, it is evident that values below LOD are absolutely valid in terms of microorganism presence. However, the probability of their repeated detection is lower than 95%.

LOQ (Limit of Quantification, lower)

The LOQ was defined as the smallest amount of analyte, which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation. Numerically, the LOQ is defined as the lowest concentration of analyte, which gives a predefined variability (coefficient of variation, CV) of under 25%.

Inhibition check

A 1:10 dilution of the original sample is analyzed together each time with the undiluted sample to evaluate the effect of PCR inhibition. If the sample is inhibited, where 1:10 dilution produces a high signal than undiluted sample, the 1:10 dilution results will be used for quantification. The use of 1:10 dilution sample results will be reflected in Analytical Volume(ul). For example, if the analytical volume for undiluted sample is 2ul, the analytical volume for 1:10 dilution will be 0.2ul.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. The most reliable way to accurately test for contamination is to combine genetic testing with scientifically sound and adequate study design appropriate for the environmental quality questions to be answered or issues to be resolved.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination.

Qualification Assay Results (Detected/Non-Detected only)

Such results are only reported as Detected or Non-Detected without quantification. Non-Detected results are defined as stated above, and Detected results are defined as detected Ct in both replicate qPCR reactions.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of LuminUltra Technologies Inc, as well as its agents or representatives, the liability of the company shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to LuminUltra Technologies Inc. The company shall not be liable for any damages, either direct or consequential. LuminUltra Technologies Inc provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request. The sample(s) cited in this report may be used for research purposes after an archiving period of 3 months from the date of this report. Research includes, but is not limited to internal validation studies and peer-reviewed research publications. Anonymity of the sample(s), including the exact geographic location will be maintained by assigning an arbitrary internal reference. These anonymous samples will only be grouped by state / province of origin for research purposes. The client must contact LuminUltra Technologies Inc in writing within 10 days from the date of this report if he/she does not wish for their submitted sample(s) to be used for any type of future research.

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for and the DNA extracted per kit manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives

Alaska Laboratory# AK01000

Client ADEC Nonpoint Source Pollution Program
 Contact Sarah Apsens, Laura Eldred
 Project SOA Bristol Bay Pathogen MST Sampling 2 2022 **Collection**
 DW Y/N N Date / time 7/12/2022 12:55
 PWS# None

AWL # AWL-22-02228
 Sample Kanak-071222-003
 Location
 AWL ID/ Matrix AQ
 Fraction AWL-22-02228-001

SM #	Date Collected	Time Collected	Analysis	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD	LOQ	Result Unit
SM22G20009	7/12/2022	12:55 PM	Human_HF183	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20015	7/12/2022	12:55 PM	Human_HumM2(EPA)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20021	7/12/2022	12:55 PM	Gull_Gull-4	34.3	34.62	2.26E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml

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Client ADEC Nonpoint Source Pollution Program
 Contact Sarah Apsens, Laura Eldred
 Project SOA Bristol Bay Pathogen MST Sampling 2 2022 **Collection**
 DW Y/N N Date / time 7/12/2022 12:25
 PWS# None

AWL # AWL-22-02228
 Sample Location Scandinavian-071222-003
 AWL ID/ Fraction AWL-22-02228-002 Matrix AQ

SM #	Date Collected	Time Collected	Analysis	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD	LOQ	Result Unit
SM22G20010	7/12/2022	12:25 PM	Human HF183	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20016	7/12/2022	12:25 PM	Human_HumM2(EPA)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20022	7/12/2022	12:25 PM	Gull Gull-4	33.93	33.69	3.17E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml

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Client ADEC Nonpoint Source Pollution Program
 Contact Sarah Apsens, Laura Eldred
 Project SOA Bristol Bay Pathogen MST Sampling 2 2022 **Collection**
 DW Y/N N Date / time 7/12/2022 12:05
 PWS# None

AWL # AWL-22-02228
 Sample Snag Point-071222-003
 Location
 AWL ID/ Matrix AQ
 Fraction

SM #	Date Collected	Time Collected	Analysis	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD	LOQ	Result Unit
SM22G20011	7/12/2022	12:05 PM	Human HF183	ND	35.49	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20017	7/12/2022	12:05 PM	Human HumM2(EPA)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20023	7/12/2022	12:05 PM	Gull Gull-4	33.97	34.18	3.41E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml

AWL Chain of Custody

Custody form MUST be signed
Please provide as much information as possible



AWL-22-02228

907-575-6130

Client/Company Name & Address: ADEC Nonpoint Source Pollution Program		Public Water System (PWS) ID: Project Name/ID: MST Sampling 2-2022		Quote Number AWL #		AWL Staff								
Contact Person:	Standard	Expedited (prior authorization required)	Invoice Contact Name & Address & Phone:		Account #:	Check	Credit							
Phone No:	Turnaround Time (TAT) for Results		Requested Date for Results:		Invoice Contact Name & Address & Phone:									
Fax No:	Results to STATE: Yes No		Requested Date for Results:		Invoice Contact Name & Address & Phone:									
E-mail:	Results to STATE: Yes No		Requested Date for Results:		Invoice Contact Name & Address & Phone:									
Special Instructions/Requirements:		Specify if REPEAT sample		PO/Contract No.:										
Kit Preparation/Shipping Charge:		Requested Analysis/Method												
Client Sample Identification (Name, Designation, Location, etc.)		Date Sampled	Time Sampled	Matrix (DW, WW, SO)	No. of Containers	CI Residual	Preservative Lot#	Preservative Lot#	Preservative Lot#	Preservative Lot#	Preservative Lot#	State Pt. Sampling ID	Facility ID	Trigger/Repeat Special
1 ∞ AWL-22-02224 / Karak-071222-003		7/12/22	12:55				MST							
2 AWL-22-02225 / Scandinavian - 071222-003		7/12/22	12:25				X							
3 AWL-22-02226 / Snag Point - 071222-003		7/12/22	12:05				X							
4														
5														
6														
7														
8														
9														
10														
Relinquished by:		Date	Time	Received by:	Date	Time	Section To Be Completed by AWL							
Relinquished by:		Date	Time	Received by: MOC	Date	Time	Custody Seal (circle):	Intact						
Relinquished by:		Date	Time	Received by:	Date	Time	Temperature on arrival:	2.09		°C		Thermo ID	Broken Absent	
Name of Sampler: (printed)		Date	Time	Received by:	Date	Time	Temp	TB		Cooler		Recv Notes:		
Ben Matlock							Ice	Frozen		Wet		Delivery Method (Circle) USPS/Mail Hand		