Alaska Water Laboratories

281 N Main St., STE # 101
Wasilla AK, 99654
907-373-6130

Alaska Laboratory# AK01000

Client	ADEC Nonpoint Source Pollution Program
Contact	Sarah Apsens, Laura Eldred
Project Name	SOA Bristol Bay Pathogen - MST #1 2022
AWL #	AWL-22-02075
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.

Mary Curry Digitally signed by Mary Curry Date: 2022.08.10 12:04:35 -08'00'

Signature of Laboratory Management Date

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Client Project Name	SOA Bristol Bay Pathogen - MST #1 2022	AWL #	AWL-22-02075
Receipt Date and Time	6/28/2022 17:30	Due Date	7/27/2022
Cooler/Sample Temp (C)	4.34	Sampler Initials	BM

Sample Receipt Comments: Sample received by MCC on 6/28/2022 at 4.34C (RT#1), on frozen ice.

Samples Received

Microbiological

Sample Location	AWL ID	Collection Date/ Time	Analysis Date/Time	Analysis	Notes
Snag Point-062822-003	AWL-22-02075-001	6/28/2022 12:00	7/26/2022 0:00	MST	AWL-00310
Scandinavian-062822-003	AWL-22-02075-002	6/28/2022 12:30	7/26/2022 0:00	MST	AWL-00271
Kanak-062822-003	AWL-22-02075-003	6/28/2022 13:00	7/26/2022 0:00	MST	AWL-00301

Analytical Methods

Analyte	Method	Comments
MST	MST	Samples prepped in-house for analysis 6-29-22 11:20 - AKS; Subcotnracted to Lumin Ultra

Cert Required None
CMDP #

Log In Initials: MCC 6-29-22
DQO Initials: JKR 6-29-22

Comments: Samples part of RUSH reports AWL-22-02072, AWL-22-02073, AWL-22-02074 -MST logged under AWL-22-02075 due to extended analytical turn around time.

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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.

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 vlaues 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 microornanism prescence. However, the probabality 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 (coeffecient of variation, CV) of under 25%.

Inhibition check
A 1:10 dilution of the original sample is analyzed togther 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.

Qualitification 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.

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. Devitations 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

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Contact	Sarah Apsens, Laura Eldred		
Project	SOA Bristol Bay Pathogen - MST #1 2022	**Collection**	
DW Y/N	N	Date / time	6/28/2022 12:00
PWS#	None		
AWL #	AWL-22-02075		
Sample Location	Snag Point-062822-003		
AWL ID/ Fraction	AWL-22-02075-001	Matrix	AQ

SM #	Date Collected	Time Collected	Analysis	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD	LOQ	Result Unit
SM22G20006	6/28/2022	12:00 PM	Human_HF183	33.42	33.62	3.87E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml
SM22G20012	6/28/2022	12:00 PM	Human_HumM2(EPA)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20018	6/28/2022	12:00 PM	Gull_Gull-4	33.81	33.79	3.53E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml

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Project	SOA Bristol Bay Pathogen - MST #1 2022	**Collection**	
DW Y/N	N	Date / time	6/28/2022 12:30
PWS#	None		
AWL #	AWL-22-02075		
Sample Location	Scandinavian-062822-003		
AWL ID/ Fraction	AWL-22-02075-002	Matrix	AQ

SM #	Date Collected	Time Collected	Analysis	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD	LOQ	Result Unit
SM22G20007	6/28/2022	12:30 PM	Human_HF183	35.96	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20013	6/28/2022	12:30 PM	Human_HumM2(EPA)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20019	6/28/2022	12:30 PM	Gull_Gull-4	32.78	33.07	6.43E+02	ROQ	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 #1 2022
DW Y/N N
PWS# None

Collection
Date / time 6/28/2022 13:00

AWL # AWL-22-02075
Sample Locati Kanak-062822-003
AWL ID/ Frac AWL-22-02075-003
Matrix AQ

SM #	Date Collected	Time Collected	Analysis	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD	LOQ	Result Unit
SM22G20008	6/28/2022	1:00 PM	Human_HF183	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20014	6/28/2022	1:00 PM	Human_HumM2(EPA)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM22G20020	6/28/2022	1:00 PM	Gull_Gull-4	34.43	35.03	1.91E+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

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AWL-22- 02075

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Client/Company Name & Address: ADEC - Non Point Pollution Program	**Public Water System (PWS) ID:**	**AWL Staff**
	Project Name/ID : SOA BB Pathogen - MST	**Quote Number** **AWL #**
	Turnaround Time (TAT) for Results	**Account #:** **Check** **Credit**
Contact Person:	___ **Standard** ___ **Expedited** (prior authorization required) *Specify Requested Due Date if not standard	**Invoice Contact Name & Address & Phone:**
Phone No:		
Fax No:	**Requested Date for Results:**	
E-mail:	**Results to STATE: Yes No**	**Routine Non-Routine**
Special Instructions/Requirements: MST Sampling #1 in 2022	Specify if REPEAT sample	**PO/Contract No.:**

Requested Analysis/Method

Kit Preparation/Shipping Charge:

	Client Sample Identification (Name, Designation, Location, etc.)	Date Sampled	Time Sampled	Matrix (DW, WW, SO)	No. of Containers	Cl Residual	MST Preservative Lot#	Preservative Lot#	Preservative Lot#	Preservative Lot#	Preservative Lot#	Preservative Lot#	Preservative Lot#	State Pt. Sampling ID	Facility ID	Trigger/ Repeat/ Special
1	Snag Point-062822-003	6-28-22	12:00				AWL-00310									
2	Scandinavian-062822-003	6-28-22	12:30				AWL-00271									
3	Kanakanak-062822-003	6-28-22	13:00				AWL-00301									
4																
5																
6																
7																
8																
9																
10																

Relinquished by:	Date	Time	Received by:	Date	Time
COC's relinquished AWL-22-02072,			MCC	6-28-22	17:30
Relinquished by: AWL-22-02073, AWL-22-02074	Date	Time	Received by:	Date	Time
Relinquished by:	Date	Time	Received by:	Date	Time
Name of Sampler: (printed)	Ben Matlock				

Section To Be Completed by AWL

Custody Seal (circle): Intact Broken (Absent)

Temperature on arrival: 4.34 °C

Thermo ID RT #1

Recv Notes:

Temp (TB) Cooler

Ice Frozen Wet

Delivery Method (Circle) Courier USPS/Mail (Hand)