FLAG:

ANALYST WORKSHEET	1. PRODUCT		2. SAMPLE NUMBER
	Listeria Isolate		1040830
3. SEALS ☒ INTACT ☐ NONE ☐ BROKEN	4. DATE REC'D	5. RECEIVED FROM	6. DISTRICT OF LABORATORY
	4-11-18	**Drusilla Morrison**	DEN-LAB

7. DESCRIPTION OF SAMPLE

One whirl-pak bag officially sealed, "INV 1040830 4/6/18 Jose A. Lopez Canelaria" containing one plastic bacterial shipping container containing one intact glass test tube of agar with a screw-cap, identified, "INV 1040830 4/16/18 JAL" and "L. mono 4/6/18 DK AA09328".

8. NET CONTENTS			9. LABELING	
☐	See Orig. w/s		☐	See Orig. w/s
☐	NOT APPLICABLE	DECLARE/UNIT _____	_____	ORIGINAL(S) SUBMITTED
☒	NOT DETERMINED	AMOUNT FOUND _____	_____	COPIES SUBMITTED
_____	UNITS EXAMINED	% OF DECLARED _____	☒	NONE

10. SUMMARY OF ANALYSIS

CONTAINER: 13x100 glass test tube with a screw-cap.

RECEIVED
JUN 1 2018
FY-18 CB#423

LABELING: None.

CODE: None.

PRODUCT: Appearance of bacterial growth on an agar slant.

ANALYSIS: One isolate analyzed for verification of *Listeria monocytogenes*.

METHOD: **FDA BAM Online:**

Chapter 10- Detection of *Listeria monocytogenes* in Foods and Environmental Samples, Sections A-C, G (A), H, I.

Chapter 11, January 2001- Serodiagnosis of *Listeria monocytogenes*, Sections A-D.

Official Methods of Analysis of AOAC INTERNATIONAL (OMA) Online:

Official Method 992.19-Listeria Species, Biochemical Identification Method (Vitek GPI and GNI+).

RESULTS: *Listeria monocytogenes* Type 1 confirmed in one isolate tested.

11. RESERVE SAMPLE

No reserve. Original official seal submitted with analytical package as Attachment D. Isolate submitted to DEN-LAB for PFGE and WGS, isolate number 1040830-S001-001.

12. a. ANALYST SIGNATURE (Broke Seal ☒)	DATE	13. WORK-SHEET CHECK	a. BY
Wicia M. Brown	4-18-18		MS Nucci
b.			b. DATE 4-27-18
c.			14. DATE REPORTED 4-18-18

FORM FDA 431 (1/15) PREVIOUS EDITION IS OBSOLETE

ATTACHMENTS A-C

Class 3
Domestic / Import
HAFW4 / DCB

PCS Publishing Services (301) 443-6740 EF

Denver Laboratory Electronic Form 431
Denver Laboratory 431.dot
2017 Version 1.0

GENERAL CONTINUATION SHEET | PRODUCT: Listeria Isolate | SAMPLE NUMBER: 1040830

Listeria spp. Worksheet: Direct Plates / Confirmation

Enrichment	BLEB (24 & 48 h) N/A	Date/Initials/Time In	Acriflavin	Date/Initials/Time Added
☐ N/A Refer to VIDAS sheet	Add antibiotics after 4 h	4-11-18 CWB 1300	Cyclohexamide Sodium Nalidixate	N/A

COMP/SUB	Selective Agar	TSA-YE	Catalase	Gram Stain	Beta Hemolysis	Motility	ID	Summary
	24 HR STREAK: N/A 48 HR STREAK: 4-11-18 CWB 24 HR READ 1 48 HR READ 1: 4-12-18 CWB 24 HR READ 2 48 HR READ 2: 4-13-18 CWB	STREAK: 4-13-18 CWB READ: 04114117 SG	04114117 SG	04114117 SG	STAB: 04114118 SG READ: 4-16-18 CWB	STAB (TUBE ONLY) READ (TUBE/SLIDE): 04114117 SG	IN (API) OUT: 04/15/18 SG ☒ Vitek ☐ API Listeria	4-16-18 CWB

				1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	ID	Summary
1	24 Hr	Palcam																											Page or Attach:	☐ No *Listeria spp.* detected. ☒ *Listeria monocytogenes* detected.
	48 Hr	Palcam	TY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	24 Hr	Palcam																											Page or Attach:	☐ No *Listeria spp.* detected. ☐ *Listeria* _____ detected.
	48 Hr	Palcam																												
Controls: Sterile Media	24 Hr	Palcam																												☒ Controls performed properly ☐ Controls did not perform properly
	48 Hr	Palcam	NG	—															—					—					
E. coli ATCC 8739	24 Hr	Palcam																												
	48 Hr	Palcam	NG											—															
L. innocua ATCC 33090	24 Hr	Palcam																											Page or Attach:	
	48 Hr	Palcam	TY	+					+					+					—					+						
L. monocytogenes ATCC 19115																			+											
S. pyogenes ATCC 19615									—																					
S. aureus ATCC 25923																								—						

TY = Typical *Listeria spp.* Growth NT = Not Typical NG = No Growth + = Positive − = Negative
N/A = Not Applicable + (Gram Stain) = Typical *Listeria* morphology - (Gram Stain) = Not typical *Listeria* morphology

Palcam	040618 E1	Poly O Type 1	6155977	~~API Listeria~~ Centrifuge	4-17-18 CWB NC1173	30 °C incubator	NC91854
Chromogenic		Poly O Type 4	6204847	Saline	032218C2	35 °C incubator	1702159
TSA-YE	195976	Vitek System	170 2096	~~ZYM B~~ O Typ antisera	4-17-18 CWB 158452	Pipettor #	NC1080
TSA II 5% SB	8046774	Vitek Saline	032618C2	~~TSB-YE~~ O Type 4 antisera	4-17-18 CWB 158460	Tips	163191
Motility		Vitek Card	242051103	~~Tryptose Broth~~ Form Saline	4-17-18 CWB 120717C1	Serological Pipets	
Microscope	1700533	Loops	1700689	H_2O_2:	0360961		TPB 188354

ANALYST(S): Alicia M. Brown

PAGE 2 OF 2 PAGES

Attachment A

1040830
4-16-18
CWB

Denver District Food and Drug Admin

bioMerieux Customer: 02821
System #: C23158

Laboratory Report

Printed Apr 14, 2018 15:40 MDT
Autoprint
Report Version: 1 of 1

#1040830, Isolate 1

Isolate Group: 1040830-1

Card Type: GP Testing Instrument: 000011DD7B0C (4639)

Serology Result: Type 1 4-17-18 CWB

Bionumber: 342210224733621
Organism Quantity:

Comments:	

Identification Information	**Card:**	GP	**Lot Number:**	2420551103	**Expires:**	May 31, 2019 13:00 MDT
	Completed:	Apr 14, 2018 15:56 MDT	**Status:**	Final	**Analysis Time:**	7.00 hours
Selected Organism	99% Probability		**Listeria monocytogenes**			
	Bionumber:	342210224733621			**Confidence:**	Excellent identification

SRF Organism	
Analysis Organisms and Tests to Separate:	
Analysis Messages: Critical Pathogen, check Camp test and beta-hemolysis	
Contraindicating Typical Biopattern(s)	

Biochemical Details

2	AMY	+	4	PIPLC	+	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	-	16	BGAR	-	17	AMAN	+	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Attachment B

Denver District Food and Drug Admin

1040830
4-16-18
CWB

bioMerieux Customer: 02821
System #: C23158

Laboratory Report

Printed Apr 14, 2018 16:38 MDT
Autoprint
Report Version: 1 of 1

L. innocua Control
ATCC # 33090

Isolate Group: L.innocua 33090-1

Card Type: GP Testing Instrument: 000011DD7B0C (4639)

Bionumber: 142200224733621
Organism Quantity:

Comments:	

Identification Information	**Card:**	GP	**Lot Number:**	2420551103	**Expires:**	May 31, 2019 13:00 MDT	
	Completed:	Apr 14, 2018 16:56 MDT	**Status:**	Final	**Analysis Time:**	8.00 hours	
Selected Organism	99% Probability		**Listeria innocua**				
	Bionumber:	142200224733621			**Confidence:**	Excellent identification	
SRF Organism							
Analysis Organisms and Tests to Separate:							
Analysis Messages:							
Contraindicating Typical Biopattern(s)							

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	-	16	BGAR	-	17	AMAN	+	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	(+)	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Attachment C

1040830
4-16-18
CWB

Denver District Food and Drug Admin

bioMerieux Customer: 02821
System #: C23158

Laboratory Report

Printed Apr 14, 2018 15:40 MDT
Autoprint
Report Version: 1 of 1

L. monocytogenes Control
ATCC # 19115

Isolate Group: L. mono 19115-1

Card Type: GP Testing Instrument: 000011DD7B0C (4639)

4-17-18 CWB
Serology result: Type 4

Bionumber: 342200224733621
Organism Quantity:

Comments:	

Identification Information	**Card:**	GP	**Lot Number:**	2420551103	**Expires:**	May 31, 2019 13:00 MDT
	Completed:	Apr 14, 2018 15:56 MDT	**Status:**	Final	**Analysis Time:**	7.00 hours
Selected Organism	99% Probability		**Listeria monocytogenes**			
	Bionumber:	342200224733621			**Confidence:**	Excellent identification

SRF Organism	
Analysis Organisms and Tests to Separate:	
Analysis Messages: Critical Pathogen, check Camp test and beta-hemolysis	
Contraindicating Typical Biopattern(s)	

Biochemical Details

2	AMY	+	4	PIPLC	+	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	-	16	BGAR	-	17	AMAN	+	19	PHOS	-
20	LeuA	(-)	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

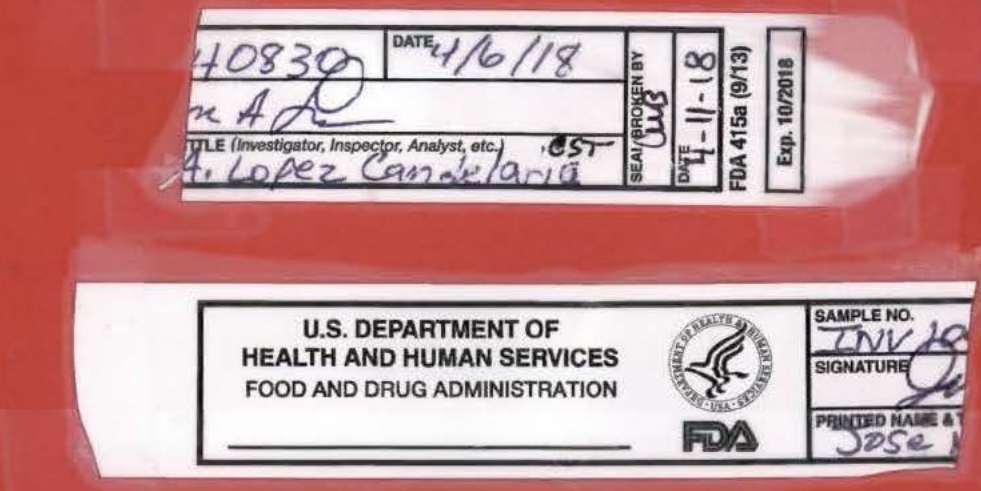
Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Attachment D

1040830
4-11-18
CMB

Original Official Seal



FLAG:

ANALYST WORKSHEET	1. PRODUCT: Isolate		2. SAMPLE NUMBER: 1040830
3. SEALS ☐ INTACT ☒ NONE ☐ BROKEN	4. DATE REC'D: 4/17/2018	5. RECEIVED FROM: Celicia M. Brown	6. DISTRICT OF LABORATORY: DEN-LAB

7. DESCRIPTION OF SAMPLE

Blood agar plate identified as "1040830" and further identified by sub number and isolate number.

8. NET CONTENTS			9. LABELING	
☐	See Orig. w/s		☐	See Orig. w/s
☒	NOT APPLICABLE	DECLARE/UNIT _____	_____	ORIGINAL(S) SUBMITTED
☐	NOT DETERMINED	AMOUNT FOUND _____	_____	COPIES SUBMITTED
_____	UNITS EXAMINED	% OF DECLARED _____	☒	NONE

10. SUMMARY OF ANALYSIS

CONTAINER: Blood agar plate.

RECEIVED
MAY 09 2018
CB

LABELING: None.

CODE: None.

PRODUCT: *Listeria* species isolates.

ANALYSIS: Whole Genome Sequencing of isolates within a pooled library of isolates.

METHOD: Nextgen seq protocol_12032015 r003v2 GenomeTrakr Whole Genome Sequencing: Genomic DNA extraction using the Qiagen QIAcube, DNA sample preparation using the Nextera XT Kit, and Sequencing using the Illumina MiSeq.

RESULTS: Whole Genome Sequencing run "FDN_042718" was uploaded to the Isilon1-FDN drive for analysis and submission to NCBI on 5/1/2018. (Isolate FDA00013037).

11. RESERVE SAMPLE

Microcentrifuge tubes of extracted DNA identified as "1040830" and further identified by isolate number. These are kept for frozen stock storage.

12. a. ANALYST SIGNATURE (Broke Seal ☐) Andrew L. Kaiser	DATE	13. WORK-SHEET CHECK	a. BY
Andrew L. Kaiser	5/1/2018		Margaret Brinsky
b.			b. DATE 5/2/18
c.			14. DATE REPORTED 5/1/2018

FORM FDA 431 (1/15) PREVIOUS EDITION IS OBSOLETE

ATTACHMENTS No Attachments.

HAFW4
Domestic/Import
Class 4

PCS Publishing Services (301) 443-6740 EF

Denver Laboratory Electronic Form 431
Denver Laboratory 431.dot
2017 Version 1.0

Split 2

GENERAL CONTINUATION SHEET	PRODUCT: Isolate	SAMPLE NUMBER: 1040830

Whole Genome Sequencing of Gram Positive Organisms

	DATE/INITIALS	MEDIA/REAGENTS/EQUIPMENT	
Isolate Preparation	4/17/18 ALK	BHI 197848	Loops 1707014
		Shaker NC91427	Lysis buffer 19:717
DNA Extraction	4/18/18 ALK	QIAcube 1702235	Microcentrifuge tubes 032618E1
		Qiagen Kit 188387	Centrifuge NA13964
DNA Quantitation	4/26/18 ALK	Qubit 2321612105	Tubes 0036A4C13-04
		Qubit dsDNA BR kit 1901245	Conical tube 3112726
DNA Tagmentation and PCR Amplification	4/26/18 ALK	Nextera XT kit 190043	PCR strips
		Thermal Cycler NC22298	PCR caps
		Index Primer kit 190031	Index Caps 27888
		Molecular Biology grade water 185389	
PCR Clean-Up	4/27/18 ALK	Microcentrifuge tubes 040918E1	AMPure beads 181113
		Ethanol 120065	Nextera XT kit 190043
Library Normalization	4/27/18 ALK	Nextera XT kit 189984	NaOH 041118Lot B
Library Pooling and Sample Loading/Run Start	4/27/18 ALK	MiSeq reagent kit Box 1(Cartridge) 20245616	
		MiSeq reagent kit Box 2 (Buffers) 20245705	
		Heat Block NC20301	MiSeq 1702236
Initial Metrics	NA	Cycle #	Cluster Density
		Cluster Passing Filter	≥Q30
		Estimated Yield	
Final Metrics (Run Complete)	4/30/18 ALK	Cluster Density 778 K/mm²	≥Q30 86.0% (C.1G)
		Cluster Passing Filter 93.7%	Estimated Yield 7340.5 MB
Data Transfer	5/1/18 ALK		

PIPETTORS, TIPS and MISC.

P20 Pipettor 128549	P20 tips 17016P	Pipettor
P200 Pipettor 123559	P200 tips 162639	Tips
P1000 Pipettor 123596	P1000 tips 17198P	0.2 mL Tubes VGRG3

ANALYST(S) Andrew L. Kaiser

GENERAL CONTINUATION SHEET	PRODUCT Isolate	SAMPLE NUMBER 1040830

4/26/18 ALK : DNA Quantification using the Qubit (ng/µL) 4/26/18 ALK : Index Primer combinations

Isolate #	Sample Name	Project	Read 1 (µg/mL)	Read 2 (µg/mL)	Avg. (µg/mL)	µL H_2O	Index 1 (N7_)	Index 2 (S5_)
1047919-S008-051	FDA00012931	PRJNA186035	26.8	25.2	26.0	260	N710	S515
1047018-S101-004	FDA00012986	PRJNA215355	6.62	6.56	6.59	65.9	N710	S516
1047018-S101-006	FDA00012987	PRJNA215355	38.8	37.2	38.0	380	N710	S517
1040830-S001-001	FDA00013037	PRJNA215355	19.6	19.1	19.35	193.5	N710	S518
0966732-S002-001	FDA00013038	PRJNA186035	19.1	18.7	18.9	189	N711	S515
0966732-S003-013	FDA00013039	PRJNA186035	17.2	16.0	16.6	166	N711	S516
0966732-S004-001	FDA00013040	PRJNA186035	30.4	29.4	29.9	299	N711	S517
0966732-S005-013	FDA00013041	PRJNA186035	24.4	23.4	23.9	239	N711	S518
0966732-S006-006	FDA00013042	PRJNA186035	14.8	17.8	16.3	163	N712	S515
0966732-S007-004	FDA00013043	PRJNA186035	27.2	26.8	27.0	270	N712	S516
0966732-S007-008	FDA00013044	PRJNA186035	27.8	27.8	27.8	278	N712	S517
0966732-S008-001	FDA00013045	PRJNA186035	31.6	31.6	31.6	316	N712	S518
0966732-S010-001	FDA00013046	PRJNA186035	26.0	27.0	26.5	265	N714	S515
0966733-S001-001	FDA00013047	PRJNA186035	20.2	19.8	20.0	200	N714	S516
0966733-S010-001	FDA00013048	PRJNA186035	23.0	22.2	22.6	226	N714	S517
1047296-S021-001	FDA00013049	PRJNA215355	51.8	51.8	51.8	518	N714	S518
1047296-S021-007	FDA00013050	PRJNA215355	23.2	23.2	23.2	232	N715	S515

ANALYST(S) Andrew L. Kaiser