

DNA Extraction Procedures for Real-time PCR Detection of *Listeria monocytogenes* and *Listeria* spp. from Artificially Contaminated Food Samples

Table 1. Listeria spp./L. monocytogenes real-time (LIS) PCR assay- Undiluted

SUMMARY

Identification of Listeria species and specifically Listeria monocytogenes by real-time PCR allows rapid detection of the pathogen from contaminated food.

The objective of the current research is to evaluate DNA extraction protocols from food enrichments for detection of *Listeria monocytogenes* by two real-time PCR procedures.

Four DNA extraction protocols from Buffered Listeria Enrichment Broth (BLEB) and DemiFraser/Fraser enrichments of artificially contaminated foods (Asadero cheese, Queso Fresco, Brie cheese, guacamole, coleslaw, and smoked salmon) were evaluated with two real-time PCR procedures for detection of Listeria monocytogenes and Listeria spp. For DNA extraction, a semi-automated magnetic particle-based extraction instrument (MagNA Pure Compact), with and without enzymatic treatment, PrepSEQ[™] Rapid Spin columns and wash-spin-boil (WSB) sample preparation techniques were compared. Real-time PCR analysis of the DNA extracts were conducted on the ABI 7500 fast platform using a multiplex Listeria spp./L. monocytogenes real-time (LIS) PCR assay targeting regions of the iap gene and designed to simultaneous detect L. monocytogenes as well as all Listeria species and the MicroSEQ® Listeria monocytogenes Pathogen Detection ki

MagNA Pure Compact DNA extraction with or without enzymatic digestion of the samples was effective for template preparation from BLEB and Fraser Broth enrichments for both PCR procedures for all of the foods tested. The rapid spin columns did not work well with most food samples, causing numerous false positive reactions in uninoculated food sample (16-100%) and inhibition of DNA amplification particularly in Fraser broth enrichments. A 1:10 dilution of the template improved its performance with the LIS assay but not the MicroSEQ® *Listeria monocytogenes* assay. The WSB preparations worked well with the LIS PCR assay, but even 1:10 dilutions of the preparations gave poor results with the MicroSEQ[®] Listeria monocytogenes assay for most foods.

These results show that template preparation is important for reliable real-time PCR screening of food enrichments for Listeria monocytogenes. The MagNA Pure Compact procedure without additional enzyme treatment of the sample resulted in template preparations that were suitable for both PCR assays. The LIS PCR assay was the most reliable test for detection of Listeria spp./L. monocytogenes from both BLEB and Fraser broth food enrichments.

INTRODUCTION

Identification of *Listeria* species and specifically *Listeria monocytogenes* by real-time PCR allows rapid detection and accurate confirmation of presumptive isolates. A real-time 5'-nuclease assay PCR assay has been developed targeting regions of the *iap* gene and designed to simultaneously detect *L. monocytogenes* as well as all *Listeria* species. The *iap* gene is present in all *Listeria* spp.with conserved and variable regions specific for each species (Bubert et al., 1992).

The purpose of study was to

- Evaluate DNA extraction protocol from food enrichments using a semi-automated magnetic particle-based extraction instrument (MagNA Pure Compact, Roche, Indianapolis, IN) with and without an enzyme pretreatment, the PrepSEQTM Rapid Spin columns and a wash-spin-boil (WSB) sample preparation technique.
- Evaluate enrichment screening by a multiplex real-time PCR specific for *Listeria monocytogenes* and *Listeria* spp with internal positive control (LIS) on a high throughput real-time thermalcycler, AB7500 Fast (Applied Biosystems/Life Technologies, Foster City, CA) and the ABI MicroSEQ[®] Listeria monocytogenes Pathogen Detection System on the same thermalcycler platform.

MATERIALS AND METHODS

Sample preparation and enrichment

Asadero cheese, brie cheese, coleslaw, queso fresco, guacamole, and smoked salmon were prepared. Food samples were artificially contaminated with the *Listeria* species at low (~0.1 cfu/g), medium (~1.0 cfu/g) and high (~10 cfu/g) levels. Uninoculated control samples were also prepared.

Samples (25g portions) were enriched in BLEB containing pyruvate and, after 4 hour at 30°C, acriflavin, cycloheximide and nalidxiic acid added. Samples were further incubated at 30°C for a total of 48h. A parallel set of samples was enriched in Demi-Fraser broth at 30°C for 24h and subcultured into Fraser broth and incubated an additional 24h at at 30°C.

For details see poster P3-03.

PCR Procedure

Samples were screened from the 48 hr enrichment broths by four template preparation methods. Two PCR procedures (Listeria spp. and L. Monocytogenes Internal control (LIS) Multiplex PCR assay and the ABI MicroSEQ[®] assay) were used for evaluation of the extraction procedures listed below.

Wash Spin Boil Preparation (WSB)

One mL of each enrichment was transferred to a microcentrifuge tube and centrifuged at 12,000 x g for 3 minutes. The supernatant was removed and the pellet was resuspended in 0.85% saline and centrifuged again at 12,000 x g for 3 minutes. After removing the supernatant, the pellet was resuspended in 1 mL of sterile water, boiled for 10 minutes and centrifuged at 12,000 x g for one minute. The supernatant was used for PCR analysis.

PrepSEQTM Rapid Spin Preparation (RS)

Enrichment broth (750 µl) was loaded onto a PrepSEQ[™] Rapid spin column and centrifuged for 3 min at 12,000 x g. The used spin column was discarded and the supernatant removed. Proteinase K lysis buffer (55 µl) was added to the pellet and resuspended. The mixture was incubated at 56°C for 30 min. followed by heating at 95°C for 10 minutes. After heating, samples were centrifuged for one minute at 12,000 x g and the supernatant was used for PCR.

MATERIALS AND METHODS

MagNA Pure Standard Extraction (MagNA Pure) An aliquot (250 µl) of enrichment broth plus 250 µl of MagNA Pure Bacteria Lysis Buffer (BLB) were combined and vortexed briefly. The sample was boiled for 10 minutes and centrifuged for 3 minutes at 12,000 x g. Boiled sample (400 µl) was transferred to a MagNA Pure Compact Sample Tube for the DNA Bacteria purification protocol with a setting of 50 µL elution volume.

MagNA Pure Standard Extraction plus Enzymatic Digestion (MagNA Pure +)

An aliquot (250 µl) of enrichment broth plus 250 µl of MagNA Pure Bacteria Lysis Buffer (BLB) were combined. An enzyme Cocktail (lysozyme and lysostaphin) were added and and vortex briefly. After 20 min incubation at 37°C. 20 µl Proteinase K solution was added and samples were incubated at 65°C for 10 min. Samples were boiled for 10 min. and centrifuged for 3 minutes at 12,000 x g. Boiled sample (400 µl) was transferred to a MagNA Pure Compact Sample Tube for the DNA Bacteria purification protocol with a setting of 50 µl. elution volume.

Real-Time PCR detection of *Listeria* from food enrichments The ABI 7500 FAST PCR (Applied Biosystems/Life Technologies, Foster City, CA) was used for real-time PCR of the extracted DNA template samples by both the *Listeria spp./L. monocytogenes real-time (LIS)* PCR assay and the MicroSEQ[®] Listeria monocytogenes assay.

Multipex Listeria spp./L. monocytogenes real-time (LIS) PCR assay The composition of the Master mix for real-time PCR is given below.

Master Mix Component	Volume (µl) per Reaction	Volume (µl) for 42 reactions
Molecular Grade Water	5.74	241.08
Express qPCR Universal	15	630
LIS Primer/Probe Mix	3.6	151.2
IPC Primer/Probe Mix	3	126
IPC DNA	0.6	252
ROX	0.06	2.52

ABI PCR strip tubes were used for conducting the assays. For each test, 28µl of *Listeria* Master Mix and 2 µl of template were used.

Computer Set-Up for ABI 7500 FAST: Assay: Standard Curve (Absolute Quantitation) Run Mode: Fast 7500

The Listeria PRL assay	targets the <i>iap</i>	gene
Target Name	<u>Reporter</u>	
L. mono iap	Cy5	
Listeria spp. iap	FAM	
IPC	VIC	
The passive reference w	as ROX.	

Thermal cycler Profile

A 2-step PCR protocol was	used:
Initial Activation	60 sec at 95°
45 cycles	10 sec at 94°
	45 sec at 60°

ABI MicroSEQ[®] Listeria monocytogenes Pathogen Detection System The MicroSEQ® Listeria monocytogenes Pathogen Detection Kit was used according to the manufacturer's instructions, using 30µL of undiluted or diluted extracts from the *Listeria* enrichment broths. The MicroSEQ[®] Listeria monocytogenes assay beads are contained in 8-tube strips, with one tube for each reaction.

Run Mode: Fast 7500Data was analyzed with ABI 7500 FAST PCR Rapid Finder Express.

The MicroSEQ® Listeria mo	nocytogenes assay is specific f	for <i>Listeria monocytogenes</i> onl	ly.
<u>Target Name</u>	<u>Reporter</u>	<u>Quencher</u>	<u>Color</u>
L. monocytogenes	FAM	(none)	Red
IPC	VIC	(none)	Black
The passive reference was R	OX.		

Thermal cycler Profile

A 2-step PCR protocol	was used:
Initial Activation	2 minutes at 95°
40 cycles	3 sec at 95
	30 sec at 60

K.C. Jinneman¹, K. J. Yoshitomi¹, P.A. Orlandi², S.D. Weagant³, R. Zapata⁴, P.E. Browning⁴, and W.M. Fedio⁴ ¹ Food and Drug Administration, Bothell, WA 98021; ² Food and Drug Administration, Rockville, MD 20857; ³ Weagant Consulting, Poulsbo, WA 98370 ⁴ New Mexico State University, Food Safety Laboratory, Las Cruces, NM 88003

Listeria PCR Master Mix Composition

<u>Quencher</u>	Color
(none)	Red
(none)	Green
(none)	Black

	Inoculum		BLI	EB			DF
Sample	Level	MagNA Pure	MagNA Pure+	RS	WSB	MagNA Pure	MagNA Pure-
0	Uninoculated	0/6	0/6	0/6	0/6	0/6	0/6
der	Low	6/6	6/6	5/6	5/6	4/6	6/6
Asa	Medium	6/6	6/6	6/6	6/6	6/6	6/6
ł	High	6/6	6/6	6/6	6/6	6/6	6/6
	Uninoculated	0/6	0/6	0/6	0/6	0/6	0/6
ie	Low	1/6	1/6	1/6 no amp	2/6	5/6	6/6
Bı	Medium	6/6	6/6	$5/6_{no\ amp}$	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	5/6 L.spp only	6/6
N,	Uninoculated	0/6	0/6	0/6	0/6	0/6	0/6
ssla	Low	5/6	5/6	5/6	5/6	6/6	6/6
Cole	Medium	6/6	6/6	6/6	6/6	6/6	6/6
0	High	6/6	6/6	6/6	6/6	6/6	6/6
ole	Uninoculated	0/6	0/6	0/6	0/6	0/6	0(2)/6
am	Low	6/6	6/6	5/6	5/6	5/6	6/6
uac	Medium	6/6	6/6	6/6	6/6	5/6	6/6
Ċ	High	6/6	6/6	6/6	6/6	6/6	6/6
~ ~	Uninoculated	0/6	0/6	0(1)/6	0/6	0(1)/6	0/6
lesc	Low	4/6	4/6	5/6	4/6	6/6	6/6
Qu Fre	Medium	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6
п	Uninoculated	0/6	0/6	0(1)/6	0/6	0/6	0/6
mo	Low	6/6	6/6	6/6	6/6	6/6	5/6
Sal	Medium	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6
Tota	l Uninoculated	0/36	0/36	0(2)/36	0/36	0(1)/36	0(2)/36
	Total Low	28/36	28/36	27/36	27/36	32/36	35/36
	Total Medium	36/36	36/36	35/36	36/36	35/36	36/36
	Total High	36/36	36/36	36/36	36/36	35/36	36/36
(#) indi	cates false posi	itive result					

Table 2. Listeria spp./L. monocytogenes real-time (LIS) PCR assay-- 1:10 Dilution

Sampla	Inoculum		BLE	EB			DF
Sample	Level	MagNA Pure	MagNA Pure+	RS	WSB	MagNA Pure	MagNA Pure+
0	Uninoculated	0/6	0/6	0(2)/6	0/6	0/6	0/6
der	Low	6/6	6/6	6/6	5/6	4/6	5/6
Asa	Medium	6/6	6/6	6/6	5/6	6/6	6/6
7	High	6/6	6/6	6/6	6/6	6/6	6/6
	Uninoculated	0/6	0/6	0(1)/6	0/6	0/6	0/6
ie	Low	1/6	2/6	1/6	1/6	5/6	5/6
\mathbf{B}_{1}	Medium	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6
8	Uninoculated	0/6	0/6	0/6	0/6	0/6	0/6
sla	Low	4/6	5/6	5/6	5/6	6/6	6/6
ole	Medium	6/6	6/6	6/6	6/6	6/6	6/6
0	High	6/6	6/6	6/6	6/6	6/6	6/6
ole	Uninoculated	0(1)/6	0/6	0/6	0(2)/6	0/6	0/6
am	Low	5/6	6/6	5/6	5/6	5/6	6/6
ıac	Medium	6/6	6/6	6/6	6/6	6/6	6/6
G	High	6/6	6/6	6/6	6/6	6/6	6/6
	Uninoculated	0(1)/6	0/6	0(1)/6	0/6	0/6	0/6
eso sco	Low	4/6	4/6	4/6	4/6	6/6	6/6
Qu Fre	Medium	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6
-	Uninoculated	0/6	0/6	0/6	0/6	0/6	0/6
noi	Low	6/6	6/6	6/6	5/6	6/6	5/6
Salı	Medium	6/6	6/6	6/6	5/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6
Tota	al Uninoculated	0(2)/36	0/36	0(4)/36	0(2)/36	0/36	0/36
	Total Low	26/36	29/36	27/36	25/36	32/36	33/36
	Total Medium	36/36	36/36	36/36	34/36	36/36	36/36
	Total High	36/36	36/36	36/36	36/36	36/36	36/36
(#) indi	cates false pos	itive result					



Figure 1: ABI 7500 Fast Real-Time PCR System



Figure 2: MagNA Pure Compact System

			Table 3.	MicroSEQ [®]	Listeria r	nonocytoz	genes assay	Undiluted	b
	Commle	Inoculum		BLEF	3			DF	
WSB	Sample	Level	MagNA Pure	MagNA Pure+	RS	WSB	MagNA Pure	MagNA Pure+	RS
0(1)/6	0	Uninoculated	0/6	0/6	0(2)/6	0(2)/6	0(1)/6	0(3)/6	0(1)/6
5/6	der	Low	6/6	6/6	0/6	3/6	5/6	6/6	0(2)/6
5/6	Asa	Medium	6/6	6/6	0/6	5/6	6/6	6/6	0(4)/6
6/6	~	High	6/6	6/6	1/6	3/6	6/6	6/6	0(4)/6
0/6		Uninoculated	0/6	0(1)/6	0(6))/6	0/6	0/6	0(3)/6	0(4)/6
5/6	ie	Low	1/6	1(4)/6	0(5)/6	1/6	5/6	6/6	0(5)/6
6/6	Br	Medium	6/6	6/6	0(5)/6	1/6	6/6	6/6	0(4)/6
6/6		High	6/6	6/6	0(3)/6	1/6	6/6	6/6	0(6)/6
0/6	3	Uninoculated	0/6	0/6	0(5)/6	0(1)/6	0(1)/6	0/6	0(6)/6
6/6	sla	Low	6/6	5/6	5(1)/6	5(1)/6	6/6	6/6	6/6
6/6	ole	Medium	6/6	6/6	6/6	6/6	6/6	6/6	6/6
6/6	0	High	6/6	6/6	5/6 false neg	6/6	6/6	6/6	6/6
0/6	ole	Uninoculated	0/6	0(1)/6	0(6)/6	0(6)/6	0(3)/6	0(4)/6	0(1)/6
5/6	ame	Low	6/6	6/6	1/6 no amp	6/6	6/6	6/6	4/6 no an
6/6	lac	Medium	6/6	6/6	4/6 no amp	6/6	6/6	6/6	4/6 no am
6/6	Ğ	High	6/6	6/6	4/6 no amp	6/6	6/6	6/6	4/6 no an
0/6		Uninoculated	0(1)/6	0/6	0/6	0(1)/6	0(5)/6	0(4)/6	0(1)/6
6/6	eso	Low	4/6	4/6	0/6 no amp	1/6 no amp	6/6	6/6	4/6
6/6	Que Fre	Medium	6/6	6/6	0/6 no amp	1/6 no amp	6/6	6/6	5/6
6/6		High	6/6	6/6	0/6 no amp	1/6 no amp	6/6	6/6	3(1)/6
0(1)/6	-	Uninoculated	0/6	0(5)/6	0(3)/6	0(5)/6	0/6	0(3)/6	0(1)/6
5/6	noi	Low	6/6	6/6	2(2)/6	6/6	6/6	5/6	3/6 no an
6/6	Salı	Medium	6/6	6/6	3/6 no amp	6/6	6/6	6/6	2/6 no an
6/6	01	High	6/6	6/6	2(4)/6	6/6	6/6	6/6	4(2)/6
0(2)/36	Tota	l Uninoculated	0(1)/36	0(7)/36	0(22)/36	0(15)/36	0(10)/36	0(17)/36	0(14)/3
32/36		Total Low	29/36	28(4)/36	8(8)/36	22(1)/36	34/36	35/36	17(7)/3
35/36		Total Medium	36/36	36/36	13(5)/36	25/36	36/36	36/36	17(8)/3
36/36		Total High	36/36	36/36	12(7)/36	23/36	36/36	36/36	17(13)/3
	(#) indi	antan falan man	itive magualt		. ,				. ,

Table 4. MicroSEQ[®] Listeria monocytogenes assay-- Diluted 1:10

Sampla	Inoculum		BLEI	В			DF		
Sample	Level	MagNA Pure	MagNA Pure+	RS	WSB	MagNA Pure	MagNA Pure+	RS	WSE
0	Uninoculated	0/6	0/6	0(3)/6	0/6	0/6	0/6	0(3)/6	0/6
der	Low	5/6	6/6	5/6	5/6	4/6	6/6	4/6	4/6
Asa	Medium	6/6	6/6	6/6	5/6	6/6	6/6	6/6	6/6
~	High	6/6	6/6	5/6	5/6	6/6	6/6	6/6	6/6
	Uninoculated	0/6	0/6	0(6)/6	0(4)/6	0/6	0/6	0(6)/6	0(1)/
rie.	Low	2/6	2/6	2(4)/6	2(4)/6	5/6	6/6	5(1)/6	5/6
B	Medium	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
м	Uninoculated	0/6	0/6	0(5)/6	0/6	0/6	0/6	0(5)/6	0/6
sla	Low	5/6	5/6	5(1)/6	5/6	6/6	6/6	6/6	6/6
ole	Medium	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
0	High	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
ole	Uninoculated	0/6	0/6	0(5)/6	0(6)/6	0/6	0/6	0(5)/6	0(4)/
am	Low	6/6	6/6	5(1)/6	6/6	5/6	6/6	4(2)/6	5/6
Jac	Medium	6/6	6/6	6/6	5/6	6/6	6/6	6/6	6/6
G	High	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
	Uninoculated	0/6	0(1)/6	0(6)/6	0(5)/6	0/6	0/6	0(6)/6	0(3)
esc	Low	4/6	4/6	4(1)/6	4(2)/6	6/6	6/6	6/6	6/6
Pre Pre	Medium	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	5/6 false neg	6/6	6/6	6/6	6/6	6/6
c	Uninoculated	0/6	0(2)/6	0(1)/6	0(3)/6	0/6	0(1)/6	0(6)/6	0/6
iom	Low	6/6	6/6	6/6	6/6	6/6	5/6	6/6	5/6
Salı	Medium	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
	High	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Tota	l Uninoculated	0/36	0(3)/36	0(26)/36	0(18)/36	0/36	0(1)/36	0(31)/36	0(8)/3
	Total Low	28/36	29/36	27(7)/36	28(6)/36	32/36	35/36	31(3)/36	31/3
	Total Medium	36/36	36/36	36/36	34/36	36/36	36/36	36/36	36/3
	Total High	36/36	36/36	34/36	35/36	36/36	36/36	36/36	36/3



0(1)/6

6/6

5/6

4/6

 $0(1)/\epsilon$

0/6 no amp

0(2)/36

27/36

21/36

19/36

RS

0(1)/6

4/6

6/6

6/6

6/6

0/6

5/6

6/6

6/6 0/6

6/6

6/6

6/6

0/6

5/6

6/6

6/6

WSE

0/6

6/6

6/6

6/6

6/6

0(1)/6

5/6

6/6

6/6

0/6

6/6

6/6

6/6

0/6

5/6

6/6

0(1)/36 0(2/36)

31/36 30/36

36/36 36/36

36/36 36/36

6/6





Figure 5: PrepSEQ[™] Rapid Spin Column and Collection Tube





WSB

0(5)/6

4/6

4/6

6/6

0(6)/6

5/6

3/6

4/6

0/6

6/6

6/6

6/6

0(6)/6

6/6

6/6

6/6

0(6)/6

6/6

6/6

6/6

6/6

0(1)/6

0(2)/6

0(4)/6

0(4)/6

0(4)/6

0(5)/6

0(4)/6

0(6)/6

6/6

6/6

6/6

0(1)/6

4/6 no amp

4/6 no amp

0(1)/6

3(1)/6

3/6 no amp

0(17)/36 0(14)/36 0(28)/36

35/36 17(7)/36 33/36

36/36 17(8)/36 25/36

36/36 17(13)/36 33/36

0(1)/6 0(5)/6

2/6 no amp 0/6

4(2)/6 5/6



RESULTS and DISCUSSION

- Four sample preparation procedures were used for 48h Listeria enrichments (BLEB and DF/Fraser Broth) and examined by two real-time PCR detection procedures - A multiplex Listeria spp./Listeria monocytogenes PCR and MicroSEQ[®] Listeria monocytogenes assay
- Columns (Tables 1 4).
- Examination of amplification curves of the samples is necessary to ensure that the ABI software is correctly interpreting the amplification curve (Figures 6-9).
- Extraction procedures did not always remove PCR inhibitors from the samples and numerous unamplified samples were seen (example in Figure 8).
- Dilution of samples reduced the number of unreliable results for the MicroSEQ® Listeria *monocytogenes* assay (Table 4).
- For the *Listeria* (LIS) multiplex assay, a 1:10 dilution of the sample did not improve the test (Table 2). • Overall, for the *Listeria* spp./*L. monocytogenes* real-time (LIS) PCR assay the Ct values for the *L.* monocytogenes and Listeria spp. markers were lowest for MP < MP+ < RS < WSB (data not shown) • For the MicroSEQ[®] Listeria monocytogenes assay, Ct for the Listeria monocytogenes target amplification followed the same pattern
- template

CONCLUSIONS

- MagNA Pure extraction was the most effective procedure for template preparation from BLEB and DF/Fraser broth enrichments
- MagNA Pure extraction with enzymatic digestion did not improve template preparation from the
- enrichments • PrepSEQTM Rapid Spin columns did not work well for *Listeria* template preparation. • Wash Spin Boil preparations were the most simple and effective procedure for sample preparation.

REFERENCES

Bubert, A., Kohler, S., and Goebel, W. The homologous and heterologous regions within the *iap* gene allow genus- and species-specific identification of *Listeria* spp. by polymerase chain reaction. Appl Environ Micro. 1992. 58:2625-2632.

ACKNOWLEDGEMENTS

Figure 4: Nucleic Acid Isolation Kit I - Reagent Cartridge

DISCLAIMER: Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the U.S. Food and Drug Administration.





Figure 7: Salmon Rapid Spin MicroSEQ[®] - False Positive



Figure 9: Salmon MagnNA Pure *Listeria* spp./L. monocytogenes real-time (LIS) PCR assay – Good Amplification

• Numerous false positive results were observed particularly in the undiluted samples from Rapid Spin

• Of the 4 extraction procedures examined the MagNA Pure the most concentrated and cleanest DNA