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MIA-RP

<b>ANALYST WORKSHEET</b>		1. PRODUCT <b>Crabmeat</b>	2. SAMPLE NUMBER <b>202758</b>
3. SEALS <input checked="" type="checkbox"/> NONE <input type="checkbox"/> INTACT <input type="checkbox"/> BROKEN	4. DATE RECEIVED <b>11-15-02</b>	5. RECEIVED FROM <b>Gianna R. Costo</b>	6. DISTRICT OR LAB <b>DEN-DO</b>

7. DESCRIPTION OF SAMPLE  
One cardboard box identified as "202758" by attached form FDA 525 containing 12 intact cans of product.

8. NET CONTENTS	<input type="checkbox"/> NOT APPLICABLE	DECLARE/UNIT	<u>1 LB. - 453.6 GRS</u>	9. LABELING	<u>1</u>	ORIGINAL(S) SUBMITTED
	<input checked="" type="checkbox"/> NOT DETERMINED	AMOUNT FOUND	_____		<u>2</u>	COPIES SUBMITTED
	UNITS EXAMINED	% OF DECLARED	_____		<input type="checkbox"/>	NONE

10. SUMMARY OF ANALYSIS

**CONTAINER:** Cylindrical metal can with a diameter of 10.0 cm and a height of 8.5 cm.

**LABELING:** Multi-colored printing sides and top of can. Back printing on bottom of can.

**CODE:**  
 "709202" in black ink on bottom of subs 1-3.  
 "609212" in black ink on bottom of subs 4-5.  
 "209212" in black ink on bottom of subs 6-7.  
 "209192" in black ink on bottom of sub 8.  
 "110012" in black ink on bottom of sub 9.  
 "109132" in black ink on bottom of sub 10.  
 "509212" in black ink on bottom of sub 12.  
 Code on bottom of sub 11 is not legible due to smudged ink.  
 "USE BY:" date on sides not legible due to smudged ink.

**PRODUCT:** Crabmeat.

**ANALYSIS:** Chloramphenicol. 12 subs each individually analyzed.

**METHOD:** LIB 4289, "Confirmation of Chloramphenicol Residues in Crawfish by Electrospray LC/MS," modified for crabmeat (See Attachment C).

**RESULTS:** 1ppb, 2ppb positive control met confirmation criteria for chloramphenicol. Blanks and negative control failed to meet confirmation criteria for chloramphenicol.

Sample met confirmation criteria for chloramphenicol.  
 Sample failed to meet confirmation criteria for chloramphenicol.

**RECEIVED**  
NOV 26 2002  
**FLA-CE**

11. RESERVE SAMPLE  
One cardboard box officially sealed "202758 11-15-02 Lori E. Hibbard" and identified as in Block 7, containing 1 plastic bag of product, identified "202758 sub 1 11-15-02 LEH" and 11 cans of product, each identified "202758 LEH 11-15-02" and by sub number 2-12, respectively. Each of 12 subs contains approximately 350 g of product. Also contained in outer box is one plastic bag containing 12 smaller bags of ground product (about 50 g), each identified "202758 11/15/02 LEH" and designated as reserve of subs 1-12.

12. a. ANALYST SIGNATURE (Break Seal <input type="checkbox"/> <i>Lori E. Hibbard</i>	13. WORK-SHEET CHECK	a. BY <i>Muh2 Mubon</i>
b. <i>[Signature]</i>		b. DATE <b>11/22/02</b>
c. <i>[Signature]</i>	14. DATE REPORTED <b>11-22-02</b>	

Denver District Laboratory Electronic Form 431  
2002 Version 1.0

Route to: **FLA-DO**  
**PRM/DCB**

**RECEIVED** 8 PAGES  
DEC 5 2002  
ATTACHMENT(S): A,B,C  
**FDA MIAMI IMPORTS**

Entry #: **AM 6-0040393-J**

## GENERAL CONTINUATION SHEET

PRODUCT

Crabmeat

SAMPLE NUMBER

202758

Standard Preparations (done by RHL)

USP reference standard Chloramphenicol (CAP), Lot N

5.088 mg CAP → 50.00ml acetonitrile (ACN)

C = 101.76 ug/ml STOCK

↓  
0.500ml → 50.00ml ACN

C = 1017.6 ng/ml INTERMEDIATE

↓  
1.00ml → 10.00ml ACN

C = 101.76 ng/ml FINAL

Balance used: Sartorius M5P Microbalance (FDA #1701450)

Original data kept in logbook in lab.

Spiking Level

1ppb spike-

100ul Final Std → 10.0g

$$\frac{100\text{ul} \times 101.76\text{ng/ml} \times 1\text{ml}/1000\text{ul}}{10.0\text{g}} = 1.02\text{ppb}$$

2ppb spike-

200ul Final Std → 10.0g

$$\frac{200\text{ul} \times 102.4\text{ng/ml} \times 1\text{ml}/1000\text{ul}}{10.0\text{g}} = 2.04\text{ppb}$$

Chromatographic Standard Preparation (done by RHL)

0.500ml Stock Std → 50.00ml 0.1% formic acid C = 1017.6 ng/ml INTERMEDIATE

↓  
400ul → 10.00ml 0.1% formic acid C = 40.7 ng/ml FINAL

Standard level:

$$\frac{40.7\text{ng/ml} \times 250\text{ml (final volume of samples)}}{10.0\text{g (sample size)}} = 1.02\text{ng/g or } 1.02\text{ppb}$$

Solvent Blank

0.1% formic acid used to reconstitute dried extracts.

ANALYST(S)

*Rebecca A. Lee*

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## GENERAL CONTINUATION SHEET

PRODUCT

LEH 12 CRODMeat  
11-6 ~~Shrimp~~

SAMPLE NUMBER

202758

SAMPLE PREPARATION (done by LEH on 11-15-08)

Aliquot of tissue taken from each subsample and weighed out to approximately 100g. Individual aliquots ground and split into two bags – half for the analysis and half for reserve.

Balance used: Sartorius 3713.MP (FDA #NC90882)

Weight of Aliquots:

sub 1 = 101.76g

sub 2 = 101.49g

sub 3 = 100.30g

sub 4 = 100.61g

sub 5 = 101.44g

sub 6 = 102.99g

sub 7 = 100.70g

sub 8 = 102.82g

sub 9 = 101.22g

sub 10 = 101.11g

sub 11 = 100.77g

sub 12 = 100.25g

ANALYST(S)

J. E. Howard

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## GENERAL CONTINUATION SHEET

PRODUCT

Crabmeat

SAMPLE NUMBER

202758

WEIGHINGS (done by LEH on 11-18-02)

10g of tissue weighed out for analysis.

Sample

Sub 1: 10.01g	Sub 7: 9.99g
Sub 2: 10.00g	Sub 8: 10.01g
Sub 3: 10.01g	Sub 9: 10.01g
Sub 4: 10.00g	Sub 10: 10.01g
Sub 5: 10.00g	Sub 11: 10.01g
Sub 6: 10.00g	Sub 12: 10.01g

Spike

Negative control weight (1ppb spike): 10.00g

Negative control weight (2ppb spike): 10.00g

Control

Negative control weight: 10.00g

\* Balance used for weighings:

Sartorius 3713 MP  
NC90930EXTRACTION (done by <sup>GIN</sup> LEH on 11-18-02)

Reagent blank, negative control, spike, and each subsample individually extracted and taken through analysis.

Spike Preparation (done by LEH on 11-18-02)

Negative control wet-spiked with final standard solution before taken through extraction. See Spiking Levels for amounts.

Reagent Blank Preparation

Reagents of analysis taken through extraction minus tissue.

LEH 11-22-02  
Data not used. See page 5.

ANALYST(S)

Dore E. Hilliard D. J. Miller

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Results  
1st extraction

***** (+) if meets criterium. (-) if not met*****										
Sample	RT <sup>1</sup> (±5% of std #1)	% R.A. <sup>2</sup> of 194 in MS <sup>2</sup> of 321	% R.A. of 257 in MS <sup>2</sup> of 321 <i>11/20/02 plus</i>	% R.A. of 194 in MS <sup>2</sup> of 323	% R.A. of 257/259 in MS <sup>2</sup> of 323	ST <sup>3</sup> for 194 in MS <sup>2</sup> of 321 (≥ 10X control)	ST for 257 in MS <sup>2</sup> of 321 (≥ 10X control)	ST for 194 in MS <sup>2</sup> of 323 (≥ 10X control)	ST for 257/259 in MS <sup>2</sup> of 323 (≥ 10X control)	Area abundance (≥75% of 1 ppb spike)
1ppb std #1	NA	100	<del>40</del> 42	100	36	+	+	+	+	+
Solvent Blank	-	-	-	-	-	-	-	-	-	-
Control	+	63	-	-	-	NA	NA	NA	NA	-
1 ppb spike	+	100	37	100	38	+	+	+	+	NA
2 ppb spike	+	100	37	100	37	+	+	+	+	+
Reagent Blank	-	-	-	-	-	-	-	-	-	-
Sub 1	+	57	-	-	-	-	-	-	-	-
Sub 2	+	100	37	100	36	+	+	+	+	+
Sub 3	+	21	-	-	-	-	-	-	-	-
Sub 4	+	-	-	-	-	-	-	-	-	-
1ppb std #2	+	100	36	100	36	+	+	+	+	+
Sub 5	+	79	34	9	-	-	-	-	-	-
Sub 6	+	<del>66</del> 27	-	49	13	-	-	-	-	-
Sub 7	+	<i>11/20/02 plus</i> 19	-	-	-	-	-	-	-	-
Sub 8	+	36	<del>6</del> <i>11/20/02 plus</i> 6	-	-	-	-	-	-	-
Sub 9	+	21	-	31	-	-	-	-	-	-
Sub 10	+	60	31	29	-	-	-	-	-	-
Sub 11	+	97	-	-	-	-	-	-	-	-
Sub 12	+	42	-	-	-	-	-	-	-	-
1ppb std #3	DID NOT	INJECT	-	-	-	-	-	-	-	-

<sup>1</sup> Retention Time (RT) taken from the m/z 321-194 chromatogram.  
<sup>2</sup> %R.A. is % relative abundance from spectral tabulation.  
<sup>3</sup> ST is signal threshold.

LEH 11-22-03  
 Data not used due to standard injection failure.  
 Sample re-extracted -

## GENERAL CONTINUATION SHEET

PRODUCT

Crabmeat

SAMPLE NUMBER

202758

WEIGHINGS (done by LEH on 11-19-02)

10g of tissue weighed out for analysis.

Sample

Sub 1: 10.01g	Sub 7: 10.01g
Sub 2: 10.01g	Sub 8: 9.99g
Sub 3: 10.01g	Sub 9: 10.02g
Sub 4: 10.01g	Sub 10: 10.02g
Sub 5: 10.02g	Sub 11: 10.00g
Sub 6: 10.02g	Sub 12: 10.02g

Spike

Negative control weight (1ppb spike): 9.98g

Negative control weight (2ppb spike): 10.02g

Control

Negative control weight: 10.02g

Balance used for weighings:

Sartorius 3713 MP  
NC90930EXTRACTION (done by GSW on 11-20-02)

Reagent blank, negative control, spike, and each subsample individually extracted and taken through analysis.

Spike Preparation (done by GSW on 11-20-02)

Negative control wet-spiked with final standard solution before taken through extraction. See Spiking Levels for amounts.

Reagent Blank Preparation

Reagents of analysis taken through extraction minus tissue.

ANALYST(S)

Leah P. ... John E. HillbrandPAGE 6 OF 8 PAGES

Results *2<sup>nd</sup> extraction (new crab control used)*

\*\*\*\*\*(+ if meets criterium. (-) if not met)\*\*\*\*\*

Sample	RT <sup>1</sup> (=5% of std #1)	% R.A. <sup>2</sup> of 194 in MS <sup>2</sup> of 321	% R.A. of 257 in MS <sup>2</sup> of 321	% R.A. of 194 in MS <sup>2</sup> of 323	% R.A. of 257/259 in MS <sup>2</sup> of 323	ST <sup>3</sup> for 194 in MS <sup>2</sup> of 321 (≥ 10X control)	ST for 257 in MS <sup>2</sup> of 321 (≥ 10X control)	ST for 194 in MS <sup>2</sup> of 323 (≥ 10X control)	ST for 257/259 in MS <sup>2</sup> of 323 (≥ 10X control)	Area abundance (≥75% of 1 ppb spike)
1 ppb std =1	NA	100	39	100	35	+	+	+	+	+
Solvent Blank	-	-	-	-	-	-	-	-	-	-
Control	-	-	-	-	-	NA	NA	NA	NA	-
1 ppb spike	+	100	36	100	34	+	+	+	+	NA
2 ppb spike	+	100	37	100	36	+	+	+	+	+
Reagent Blank	-	-	-	-	-	-	-	-	-	-
Sub 1	+	39	-	-	-	-	-	-	-	-
Sub 2	+	100	41	100	<del>35</del> 36	+	+	+	+	+
Sub 3	+	15	-	-	-	-	-	-	-	-
Sub 4	-	-	-	-	-	-	-	-	-	-
1 ppb std =2	+	100	42	100	33	+	+	+	+	+
Sub 5	+	-	-	-	-	-	-	-	-	-
Sub 6	+	16	-	-	-	-	-	-	-	-
Sub 7	+	-	-	-	-	-	-	-	-	-
Sub 8	+	100	36	<del>18</del> 19	33	-	-	-	-	-
Sub 9	+	100	41	96	58	-	-	-	-	-
Sub 10	+	43	21	17	-	-	-	-	-	-
Sub 11	+	37	-	-	-	-	-	-	-	-
Sub 12	+	27	-	-	-	-	-	-	-	-
1 ppb std =3	+	100	37	100	39	+	+	+	+	+

<sup>1</sup> Retention Time (RT) taken from the m/z 321→194 chromatogram.  
<sup>2</sup> %R.A. is % relative abundance from spectral tabulation.  
<sup>3</sup> ST is signal threshold.

GENERAL CONTINUATION SHEET	PRODUCT Crabmeat	SAMPLE NUMBER 202758
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Using an ion trap instrument the following criteria must be met for positive qualitative confirmation of chloramphenicol:

- 1) **Spectral criterium:** a) The ion  $m/z$  194  $[M-H-(NH_2COCCl_2H)]^-$  must be observed in the  $MS^2$  spectra from both parent ions ( $m/z$  321 and 323), and should be a predominant peak in the mass range  $m/z$  100-300. b) In addition, the ions corresponding to  $[M-H-(HCOCl)]^-$  ( $m/z$  257 in  $MS^2$  spectra of 321;  $m/z$  257 and 259 in  $MS^2$  spectra of 323) must be observed with an approximate relative abundance to the base peak  $m/z$  194 as is observed in the external standards. Other peaks that may be observed in the  $MS^2$  spectra, but are not required for confirmation, include  $m/z$  249  $[M-H-(2HCl)]^-$ ,  $m/z$  176  $[m/z$  194 -  $(H_2O)]^-$ , and  $m/z$  152. *Any other predominant ions in the spectra should be able to be explained (i.e. present in reagent or matrix blank).*
- 2) **Signal threshold criterium:** The integrated signal for the following ion transitions ( $m/z$  321 $\rightarrow$ 194, 323 $\rightarrow$ 194, 321 $\rightarrow$ 257, 323 $\rightarrow$ 257+259) at the retention for CAP should be greater than or equal to ten times the signal from these same ion transitions at the corresponding retention time from a matrix blank (control) analyzed the same day.
- 3) **Retention time criterium:** The retention time should be  $\pm$  5% of an external standard run on that day.

For lab classification purposes, the area abundance of the integrated signal for  $m/z$  321 $\rightarrow$ 194 is evaluated against the area abundance of the same signal in the 1ppb spike.

#### Instrument Parameters

Finnegan LCQ DECA Ion Trap Mass Spectrometer coupled to a modular Spectrasystem LC system. The components of the LC system include a SCM1000 degasser, P4000 LC pump, AS3000 autosampler, and a UV6000LP UV/VIS detector. The software used was Xcaliber Version 1.2.

The instrument was tuned with a 1 ng/ $\mu$ L CAP solution made by diluting 50  $\mu$ L of CAP Stock Standard solution.

The LC Column was an Xterra phenyl (2.1 x 100 mm, 3.5  $\mu$ , Waters Corp. P/N 186001180).

ANALYST(S) <i>M. E. H. ...</i>	PAGE <u>8</u> OF <u>8</u> PAGES
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