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***Listeria monocytogenes* Shelf-Life Challenge Study on  
Modified Atmosphere-Packaged Roasted Split Chicken  
Breast**

**For**

**Mionix Corporation**

**The National Food Laboratory**

**MW6693**

**October 16, 2003**

**By**

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# ***Listeria monocytogenes* Shelf-Life Challenge Study on Modified Atmosphere-Packaged Roasted Split Chicken Breast**

## **BACKGROUND:**

Mionix Corporation and a top five USA poultry processor wished to determine the “anti-listerial” effects of Mionix Safe<sub>2</sub>O-RTE 01 on roasted split chicken breast packaged under a modified atmosphere of (O<sub>2</sub> (0.6-1.2%), CO<sub>2</sub>(32-35%), N<sub>2</sub>(balance)) and stored at 4.4°C.

## **OBJECTIVES:**

To determine the ability of modified atmosphere packaged roasted split breast to support the growth of *Listeria monocytogenes* during the course of an inoculated pack study.

## **MATERIALS AND METHODS:**

**Organisms.** Five strains of *L. monocytogenes* were provided by the top five USA poultry processor (PP).

### **Strains of *Listeria monocytogenes* used in this study.**

<b>Strain #</b>	<b>Source</b>
ATCC # 19111	Serovar 1, Poultry isolate
ATCC # 19115	Serovar 4b, Human isolate
PP # 400	Human isolate from lunchmeat
PP # 1778	Human isolate from lunchmeat
PP # 2926	Human isolate from lunchmeat

The five-strain cocktail was crioadapted and prepared as follows: All five strains were incubated separately at 30°C for 18 hours. The cultures were diluted 1:5 into a fresh, pre-warmed medium and incubated for 4 hours at 30°C. The tubes were transferred to a 5°C incubator for 24 hours and enumerated via Direct Microscopy Count (DMC) by means of a Petroff-Hauser counting chamber. Equal concentrations of each culture were combined to obtain an inoculum cocktail with a target level of 100 cfu/per serving size. Plate counts of the cocktail were performed as a confirmation of the DMC before, during and after inoculation using Modified Oxford agar containing Modified Oxford Supplement. The MOx plates were incubated at 35°C for 2 days before results were recorded.

**Media.** Brain Heart Infusion (BHI) broth was used as the culture medium. UVM broth and Fraser broth were used as the MPN media. Modified Oxford medium (MOx) and Horse Blood agar were used in confirmations.

**Product.** The top five USA poultry processor provided product for testing. The product tested was roasted split breast.

**Inoculation and Packaging.** The weight of the retail sample packages were recorded. The packaging weight and the actual sample weights were noted. Weights before and after treatment were also noted. Intact retail packages were aseptically opened.

Each roasted split breast was challenged at the target inoculum of 100 cfu/serving. Individual split breasts (one breast per package) were inoculated on the surface of the skin with the culture cocktail and spread with a sterile hockey stick. The tray was refrigerated for 2 hours to allow attachment of *L. monocytogenes*. After the end of two hours, three treatments were provided to the breasts. **Treatment 1** consisted of inoculated control samples, where the breasts were transferred to another tray and the tray was packaged under a modified atmosphere of (O<sub>2</sub>(0.6-1.2%), CO<sub>2</sub>(32-35%), N<sub>2</sub>(balance)) and stored at 4.4°C. For **treatment 2** the Mionix Solution **Safe<sub>2</sub>O™** brand-**RTE 01** was diluted, one part solution with two parts deionized H<sub>2</sub>O before application. Each split breast was sprayed with diluted Mionix treatment solution for 20 seconds with an atomizing spray equipment, with 1.5 feet of distance between the spray nozzle and chicken breasts. The flow rate was about 180ml/min. Treated chicken breasts from each tray were transferred into a clean tray immediately after the spray. For **treatment 3**, sterile deionized H<sub>2</sub>O was used to spray the chicken breasts the same way as with the Mionix treatment group. All chicken breasts (including Mionix treated and water treated) were kept in a 35°F refrigerator for 4 hours before packaging under a modified atmosphere of (O<sub>2</sub>(0.6-1.2%), CO<sub>2</sub>(32-35%), N<sub>2</sub>(balance)) and sealing with a Multivac vacuum packager. The product was sealed in packaging film supplied by the top five USA poultry processor.

Uninoculated samples were also packaged under a modified atmosphere of (O<sub>2</sub>(0.6-1.2%), CO<sub>2</sub>(32-35%), N<sub>2</sub>(balance)) and sealed with a Multivac vacuum packager. These samples served as uninoculated controls for headspace composition analysis, and pH and a<sub>w</sub> analysis on the sampling dates.

**Incubation.** Samples were incubated at 4.4 °C for up to 40 days.

**MPN Analysis and Confirmations.** Samples were tested by using a nine tube MPN of 10, 1 and .1 gram series initially; during the study the MPN dilutions were changed as cell counts increased per gram of product. Samples were enriched in the UVM broth tubes at 30°C for 22 +/- 2 hours. At the end of the incubation period, an aliquot of 0.1 ml from each UVM broth tube was transferred to a corresponding labeled 10ml tube of Fraser broth supplemented with Ferric Ammonium Citrate and incubated at 35°C for 18-24 hours. If the tube appeared negative, tubes were reincubated at 35°C until a total incubation time of 48 +/- 2 hours was reached. All nine tubes were then streaked onto

Modified Oxford (MOx) agar and examined for appropriate medium reaction. Every positive colony from the MOx plate was streaked onto Horse Blood agar plates and incubated for 24 hours at 35°C. All confirmations (beta hemolysis reaction, catalase reaction & tumbling motility) were looked at/performed using colonies from the blood agar plates.

**Time 0 Testing.** On the day of inoculation, 3 uninoculated samples, 3 Mionix treated and three water treated samples were enumerated using MPN method. Two samples were analyzed for headspace composition, pH and  $a_w$ . Two uninoculated samples were also analyzed for aerobic plate count and lactic acid bacteria.

**Sampling/Enumeration.** Samples incubated at 4.4°C were tested on 0, 5, 8, 14, 20, 22, 26, 27, 28, 29, 32, 34, and 40 days after inoculation. On each sampling date, 3 uninoculated samples, 3 Mionix treated and three water treated samples were enumerated using MPN method. Two uninoculated samples were also analyzed for headspace composition, pH &  $a_w$  on the sampling dates.

## **RESULTS AND DISCUSSION:**

### **Results of the entire study are presented in the tables of Appendix A.**

The results for Day 0 initial microbiological analysis samples are presented in Table 1. Chemistry results (Headspace gas composition) are presented in Table 2. The pH and aw results are presented in Table 3. Microbiological analysis (inoculated controls, Mionix treated and water treated) are presented in Table 4. Weight gain/loss for uninoculated (boned) Mionix treated samples are presented in Table 5. Weight gain/loss for inoculated Mionix treated samples are presented in Table 6. Weight gain/loss for water treated samples are presented in Table 7.

The untreated chicken breasts had < 10 cfu/gm of both aerobic plate count and lactic acid bacteria on day 1. As depicted in Table 2, hydrogen gas was detected from day 11 onwards on testing for the head space gas composition. Mionix (Mionix Solution **Safe<sub>2</sub>O<sup>TM</sup><sub>brand</sub>-RTE 01**) treated samples had target populations of less than 2 logs at the end of the 40 day shelf life study.

## **RESULTS AND DISCUSSION:**

This 40 day shelf life study demonstrates that Mionix Solution (**Safe<sub>2</sub>O<sup>TM</sup><sub>brand</sub>-RTE 01**), used in the concentrations mentioned above is effective in inhibiting the growth of *Listeria monocytogenes* on MAP packaged chicken breasts when compared to inoculated control samples and inoculated water treated samples. *Listeria monocytogenes* populations increased by less than 2 log cycles by the end of the 40-day study period.

**APPENDIX A (TABLES 1-7)**

**Table 1.**

Analysis of Lactic Acid Bacteria and Aerobic Plate Count on untreated Chicken Breasts

<b>Samples</b>	<b>Aerobic Plate Count (Cfu/gm)</b>	<b>Lactic Acid Bacteria (Cfu/gm)</b>
1	<10	<10
2	<10	<10

Analysis of *Listeria monocytogenes*----- Initial, Middle and Final plate count

<b>Initial Cfu/ml</b>	<b>Middle Cfu/ml</b>	<b>Final Cfu/ml</b>
<b>520</b>	<b>720</b>	<b>730</b>

**Table 2. Headspace Gas Composition (O<sub>2</sub>(0.6-1.2%), CO<sub>2</sub>(32-35%), N<sub>2</sub>(balance))**

Day	Sample1 CO <sub>2</sub>	Sample 1 O <sub>2</sub>	Sample1 N <sub>2</sub>	Sample1 H <sub>2</sub>	Sample 2 CO <sub>2</sub>	Sample 2 O <sub>2</sub>	Sample 2 N <sub>2</sub>	Sample 2 H <sub>2</sub>
0	23.3	3.0	72.7	BDT*	28.3	1.4	70.4	BDT*
5	26.0	1.0	73.0	BDT*	22.2	0.1	77.7	BDT*
11	38.4	0.1	50.6	10.9	29.4	0.0	68.9	1.7
14	31.3	0.0	65.2	3.5	28.8	0.2	69.5	1.5
20	30	0.0	62.1	7.9	38.7	0.1	48.2	13.1
22	35.8	0.0	52.3	11.8	37.6	0.1	48.1	14.2
26	42.3	0.1	38.4	19.3	36.9	0.0	57.1	5.9
27	42.2	0.1	44.3	13.4	44.9	0.1	41.6	13.4
28	53.2	0.0	24.0	22.8	40.0	0.0	42.1	17.8
29	44.7	0.0	39.6	15.6	41.7	0.0	39.3	18.9
32	50.4	0.0	24.4	25.2	53.8	0.0	24.0	22.1
34	48.6	0.0	34.5	16.8	49.1	0.0	29.9	21.0
40	50.8	0.0	27	22.2	49.6	0.1	30.5	19.8

BDT\*: Below detection threshold

**Table 3. pH & Water Activity**

<b>Day</b>	<b>Samples</b>	<b>pH</b>	<b>Average pH</b>	<b>A<sub>w</sub></b>	<b>Average A<sub>w</sub></b>
0	1	5.70	5.85	0.979	0.981
	2	6.00		0.983	
5	1	5.49	5.55	0.989	0.989
	2	5.61		0.99	
8	1	5.67	5.77	0.99	0.989
	2	5.88		0.989	
14	1	6.12	5.92	0.988	0.989
	2	5.71		0.989	
20	1	5.59	5.64	0.988	0.988
	2	5.69		0.987	
22	1	5.79	5.80	0.984	0.985
	2	5.82		0.987	
26	1	5.85	5.77	0.985	0.986
	2	5.70		0.986	
27	1	5.75	5.82	1.0	0.999
	2	5.90		0.997	
28	1	5.88	5.79	1.006	1.006
	2	5.71		1.007	
29	1	6.21	6.08	0.982	0.983
	2	5.95		0.984	
32	1	5.95	5.96	0.983	0.983
	2	5.97		0.983	
34	1	6.05	5.96	0.996	0.999
	2	5.87		1.003	
40	1	5.95	5.88	0.986	0.984
	2	5.81		0.983	



**Table 4. Microbiological Analysis****Product: Roasted Chicken Breast**

A = Inoculated samples B = Inoculated and then treated with water, C = Inoculated and then

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value
0	A1	0.43	1.25	0.10
	D1	+		
	A2	0.93		
	D1	+		
	A3	2.40		
	D1	+		
	B1	0.072	0.073	-1.13
	D2	+		
	B2	0.074		
	D2	+		
	B3	0.074		
	D2	+		
	C1	<0.03	<0.044	<-1.36
	D3	-		
	C2	<0.03		
D3	-			
C3	0.072			
D3	-			
5	A1	>11	>11	>1.04
	D1	+		
	A2	11		
	D1	+		
	A3	>11		
	D1	+		
	B1	2.4	5.27	0.72
	D2	+		
	B2	11		
	D2	+		
	B3	2.4		
	D2	+		
	C1	0.29	0.91	-0.04
	D3	-		
	C2	2.4		
D3	-			
C3	0.036			
D3	-			

treated with Mionix solution, D = Bag (1:10, remainder of the sample)

**Product: Roasted Chicken Breast**

A = Inoculated samples, B = Inoculated and then treated with water, C = Inoculated and then treated with Mionix solution, D = Bag (1:10, remainder of the sample)

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value
8	A1	110	67.33	1.83
	D1	+		
	A2	46		
	D1	+		
	A3	46		
	D1	+		
	B1	9.3	47.77	1.68
	D2	+		
	B2	24		
	D2	+		
	B3	110		
	D2	+		
	C1	2.3	2.30	0.36
	D3	-		
	C2	2.3		
D3	-			
C3	2.3			
D3	-			
14	A1	>1100	>1100	>3.04
	D1	+		
	A2	>1100		
	D1	+		
	A3	>1100		
	D1	+		
	B1	120	>773	>2.89
	D2	+		
	B2	>1100		
	D2	+		
	B3	>1100		
	D2	+		
	C1	<0.03	<0.03	<-1.52
	D3	-		
	C2	<0.03		
D3	-			
C3	<0.03			
D3	-			

**Product: Roasted Chicken Breast**

A = Inoculated samples, B = Inoculated and then treated with water, C = Inoculated and then treated with Mionix solution, D = Bag (1:10, remainder of the sample)

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value
20	A1	24000	26433	4.42
	D1	+		
	A2	46000		
	D1	+		
	A3	9300		
	D1	+		
	B1	4600	6000	3.78
	D2	+		
	B2	2400		
	D2	+		
	B3	11000		
	D2	+		
	C1	<0.03	<0.03	<-1.52
	D3	-		
	C2	<0.03		
D3	-			
C3	<0.03			
D3	-			
22	A1	24000	60000	4.78
	D1	+		
	A2	110000		
	D1	+		
	A3	46000		
	D1	+		
	B1	46000	33167	4.52
	D2	+		
	B2	46000		
	D2	+		
	B3	7500		
	D2	+		
	C1	<0.03	<0.03	<-1.52
	D3	-		
	C2	<0.03		
D3	-			
C3	<0.03			
D3	-			

**Product: Roasted Chicken Breast**

A = Inoculated samples, B = Inoculated and then treated with water, C = Inoculated and then treated with Mionix solution, D = Bag (1:10, remainder of the sample)

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value		
26	A1	1100000	758333	5.88		
	A2	75000				
	A3	1100000				
	B1	23000			374410	5.57
	B2	230				
	B3	1100000				
	C1	<0.03	<0.03	<-1.52		
	D3	-				
	C2	<0.03				
	D3	-				
	C3	<0.03				
	D3	-				
	27	A1	110000	110000	5.04	
		D1	+			
		A2	110000			
D1		+				
A3		110000				
D1		+				
B1		24000	28333	4.45		
D2		+				
B2		46000				
D2		+				
B3		15000				
D2		+				
C1		0.03	<0.03	<-1.52		
D3		-				
C2		<0.03				
D3	-					
C3	<0.03					
D3						

**Product: Roasted Chicken Breast**

A = Inoculated samples, B = Inoculated and then treated with water, C = Inoculated and then treated with Mionix solution, D = Bag (1:10, remainder of the sample)

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value		
28	A1	230000	117333	5.07		
	A2	92000				
	A3	30000				
	B1	230			33543	4.53
	B2	93000				
	B3	7400				
	C1	<0.03	<0.03	<-1.52		
	D3	-				
	C2	<0.03				
	D3	-				
	C3	<0.03				
	D3	-				
	29	A1	230000	953333	5.98	
		A2	2400000			
		A3	230000			
B1		23000	11933			4.08
B2		9200				
B3		3600				
C1		<0.03		<0.03	<-1.52	
D3		-				
C2		<0.03				
D3		-				
C3		<0.03				
D3		-				

**Product: Roasted Chicken Breast**

A = Inoculated samples, B = Inoculated and then treated with water, C = Inoculated and then treated with Mionix solution, D = Bag (1:10, remainder of the sample)

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value
32	A1	2100000	1576667	6.20
	A2	2400000		
	A3	230000		
	B1	240000	446787	5.65
	B2	360		
	B3	1100000		
	C1	4.3	<1.45	<0.16
	D3	-		
	C2	<0.03		
	D3	-		
C3	<0.03			
D3	-			
34	A1	43000	3686000	6.57
	A2	15000		
	A3	11000000		
	B1	23000	35766.67	4.55
	B2	75000		
	B3	9300		
	C1	0.92	0.44	-0.36
	D3	-		
	C2	0.36		
	D3	-		
C3	0.03			
D3	-			

**Product: Roasted Chicken Breast**

A = Inoculated samples, B = Inoculated and then treated with water, C = Inoculated and then treated with Mionix solution, D = Bag (1:10, remainder of the sample)

Pull Date	Treatment Type	MPN (g/ml)	Average MPN	Log Value
40	A1	1100000	1100000	6.04
	A2	1100000		
	A3	1100000		
	B1	150	38150	4.58
	B2	110000		
	B3	4300		
	C1	240	<80.2	<1.90
	D3	-		
	C2	<0.3		
D3	-			
C3	<0.3			
D3	-			

**Table 5. Weight (in grams) of uninoculated boned Mionix treated Chicken Breast**

<b>Samples</b>	<b>Initial Wt of Breasts (without tray weight)</b>	<b>Weight of Breast 1</b>	<b>Weight of Breast 2</b>	<b>Weight of Breast 1&amp; 2</b>	<b>Weight gain/loss</b>
1	547.4	257.06	292.06	549.12	1.72
2	572.97	315.92	253.51	569.43	-3.54
3	456.26	286.02	171.96	457.98	1.72
4	448.7	174.83	277.08	451.91	3.21
5	468.7	226.54	246.02	472.56	3.86
6	508.22	240.27	266.05	506.32	-1.9
7	533.12	300.57	233.13	533.7	0.58
8	542.96	231.07	292.27	523.34	-19.62
9	428.89	274.86	163.68	438.54	9.65
10	462.27	216.11	250.73	466.84	4.57
11	541.47	251.27	284.33	535.6	-5.87
12	495.24	198.03	293.22	491.25	-3.99
13	405.31	209.42	193.57	402.99	-2.32
14	490.66	276.82	214.72	491.54	0.88
15	472.74	213.41	260.49	473.9	1.16
16	538.41	247.33	288.37	535.7	-2.71
17	539.16	285	256.01	541.01	1.85
18	486.48	251.63	237.36	488.99	2.51
19	534.67	320.19	217.16	537.35	2.68
20	542.74	253.4	291.88	545.28	2.54
21	510.48	267.57	229.96	497.53	-12.95
22	496.03	268.93	227.69	496.62	0.59
23	489.64	242.53	258.46	500.99	11.35
24	505.21	280	245.66	525.66	20.45
25	547.99	283.72	262.6	546.32	-1.67
26	542.42	238.66	268.21	506.87	-35.55
27	467.97	232.68	242.7	475.38	7.41
28	420.99	203.08	222.98	426.06	5.07
29	547.4	257.06	292.06	549.12	1.72
30	572.97	315.92	253.51	569.43	-3.54
31	456.26	286.02	171.96	457.98	1.72
32	448.7	174.83	277.08	451.91	3.21
33	468.7	226.54	246.02	472.56	3.86



**Table 6. Weight (in grams) of inoculated deboned Mionix treated Chicken Breast**

Samples	Initial Wt of breasts (without tray weight)	Weight of Breast 1	Weight of Breast 2	Weight of Breast 1 & 2	Weight gain/loss
1	434.59	203.65	243.71	447.36	12.77
2	450.06	204.19	258.74	462.93	12.87
3	488.76	217.31	284.49	501.8	13.04
4	476.45	267.93	221.39	489.32	12.87
5	469.78	242	238.92	480.92	11.14
6	465.28	226.92	255.58	482.5	17.22
7	529.82	218.38	320.81	539.19	9.37
8	421.22	208.51	227.01	435.52	14.3
9	478.18	243.83	249.11	492.94	14.76
10	427.48	219.22	224.6	443.82	16.34
11	484.38	274.23	230.63	504.86	20.48
12	487.29	232.58	264.89	497.47	10.18
13	431	232.82	212.45	445.27	14.27
14	490.66	188.99	310.58	499.57	8.91
15	509.64	251.94	271.29	523.23	13.59
16	522.71	236.17	303.71	539.88	17.17
17	420.05	208.2	223.04	431.24	11.19
18	445.85	236.52	225.39	461.91	16.06
19	438.61	272.6	185.26	457.86	19.25
20	442.01	220.07	232.05	452.12	10.11
21	453.43	241.33	225.59	466.92	13.49
22	630.07	245.88	393.64	639.52	9.45
23	491.56	237.73	270.59	508.32	16.76
24	439.26	230.36	224.82	455.18	15.92
25	453.05	269.95	201.07	471.02	17.97
26	446.33	218.98	241.28	460.26	13.93
27	428.86	242.8	206.16	448.96	20.1
28	508.8	283.89	242.3	526.19	17.39
29	506.91	242.8	273.37	516.17	9.26
30	484.61	265.38	237.12	502.5	17.89
31	443	255	204.42	459.42	16.42
32	460.46	251.9	224.82	476.72	16.26
33	454.79	272.69	200.14	472.83	18.04
34	404.43	206.22	211.15	417.37	12.94
35	430.71	183.63	261.62	445.25	14.54
36	421.89	190.51	241.94	432.45	10.56
37	478.5	232.62	259.71	492.33	13.83
38	475.19	233.8	254.44	488.24	13.05
39	492.96	237.24	274.36	511.6	18.64

40	498.28	267.61	248.17	515.78	17.5
41	512.54	263.63	263.46	527.09	14.55
42	481.02	189.46	303.14	492.6	11.58

**Table 7. Weight (in grams) of inoculated deboned Water treated Chicken Breast**

Samples	Initial Wt of breasts (without tray weight)	Weight of Breast 1	Weight of Breast 2	Weight of Breast 1 & 2	Weight gain/loss
1	427.93	203.56	230.45	434.01	6.08
2	497.81	251.87	251.59	503.46	5.65
3	516.85	240.2	285.84	526.04	9.19
4	441.01	225.82	225.51	451.33	10.32
5	401.46	214.33	213.95	428.28	26.82
6	467.26	235.03	239.52	474.55	7.29
7	507.23	266.31	251.61	517.92	10.69
8	544.58	260.48	294.04	554.52	9.94
9	518.55	222.01	316.1	538.11	19.56
10	459.02	224.57	245.44	470.01	10.99
11	467.24	222.82	259.94	482.76	15.52
12	456.29	271.77	197.99	469.76	13.47
13	488.04	256.51	239.46	495.97	7.93
14	525.59	235.4	298.28	533.68	8.09
15	377.19	210.55	181.25	391.8	14.61
16	447.18	201.85	256.16	458.01	10.83
17	425.96	246.9	195.63	442.53	16.57
18	497.09	181.51	325.33	506.84	9.75
19	495.24	289.02	217.81	506.83	11.59
20	491.53	218.59	283.7	502.29	10.76
21	442.77	186.42	265.68	452.1	9.33
22	473.92	240.44	242.48	482.92	9
23	496.38	274.95	237.07	512.02	15.64
24	534.79	242.36	309.87	552.23	17.44
25	430.68	251.1	192.49	443.59	12.91
26	487.86	187.88	312.43	500.31	12.45
27	476.89	213.77	270.19	483.96	7.07
28	536.54	262.9	284.93	547.83	11.29
29	509.84	242.19	280.07	522.26	12.42
30	426.32	226.62	209.69	436.31	9.99
31	423.89	209.2	227.84	437.04	13.15
32	453.46	234.62	228.45	463.07	9.61
33	516.97	258.72	240.44	499.16	-17.81
34	462.72	218.4	255.93	474.33	11.61
35	465.17	230.56	243.73	474.29	9.12
36	534.03	236.28	309.32	545.6	11.57
37	486.24	264.07	263.66	527.73	41.49
38	489.86	230.33	266.85	497.18	7.32
39	416.7	231.86	197.33	429.19	12.49
40	454.05	224.46	239.8	464.26	10.21
41	526.69	234.42	299.52	533.94	7.25
42	485.31	229.42	270.15	499.57	14.26

43	427.95	225.18	209.6	434.78	6.83
44	453.95	234.56	229.19	463.75	9.8