



Real-Time Quantitative PCR Detection of Food Pathogenic Microorganisms with a New Master Mix Optimized to Resist PCR Inhibitors



Tanner, July 19, 2005



Topics

1. Background Information
2. New Environmental Master Mix
3. Results: *Salmonella* with Environmental Master Mix
4. Master mix comparisons
5. *Salmonella* & *Listeria* in food samples



EMM Team





- **Project Leader:** **Christine Fair**
- **AM Research:** **Maura Barbisin**
Michael Malicdem
Stephen Scharf
Lily Wong
Olga Petrauskene
Manohar Furtado
- **AM Development:** **Vanessa Chan**
Ellen Kuo
Eleonora Scarpati
Peter McNamara
- **AM Marketing:** **Thomas Schild**
Paula Stonemetz

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Overview

■ Description:

- Four Real-Time PCR assays to detect food pathogens
 - > Based on TaqMan[®] MGB probe based technology & containing Environmental Master Mix (EMM)
 - > *Listeria monocytogenes*

 - > *Salmonella enterica*

 - > *Escherichia coli* 0157:H7

 - > *Campylobacter jejuni*

- Conveys certain specific PCR and 5' nuclease service testing rights for applied markets
- Application specific software
 - > User interface walks user through plate set up and assay protocol
 - Software module that “hooks” into existing SDS platform software

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Design of EMM

- Need a master mix to work in samples with low levels of PCR inhibitors common to food samples
- Optimal master mix needed to run with duplex assay (FAM[™]/VIC[®] Dyes)



Proposed Kit Components:

- Kits use MGB probes only
 - Target: **FAM Dye**
 - Internal Positive Control (IPC): **VIC Dye**
- Environmental Master Mix
 - Designed to compensate for low levels of PCR inhibitors common to food samples
 - Contains AmpliTaq Gold[®] Low DNA
 - > Integrated Hot Start
- IPC
 - Included for two reasons:
 - > to detect PCR inhibition (reduces number of false negatives)
 - > to demonstrate that kit amplifies
 - IPC-signal designed to be independent from target starting-copy number
 - > IPC signal won't be influenced significantly if Ct of target is 15



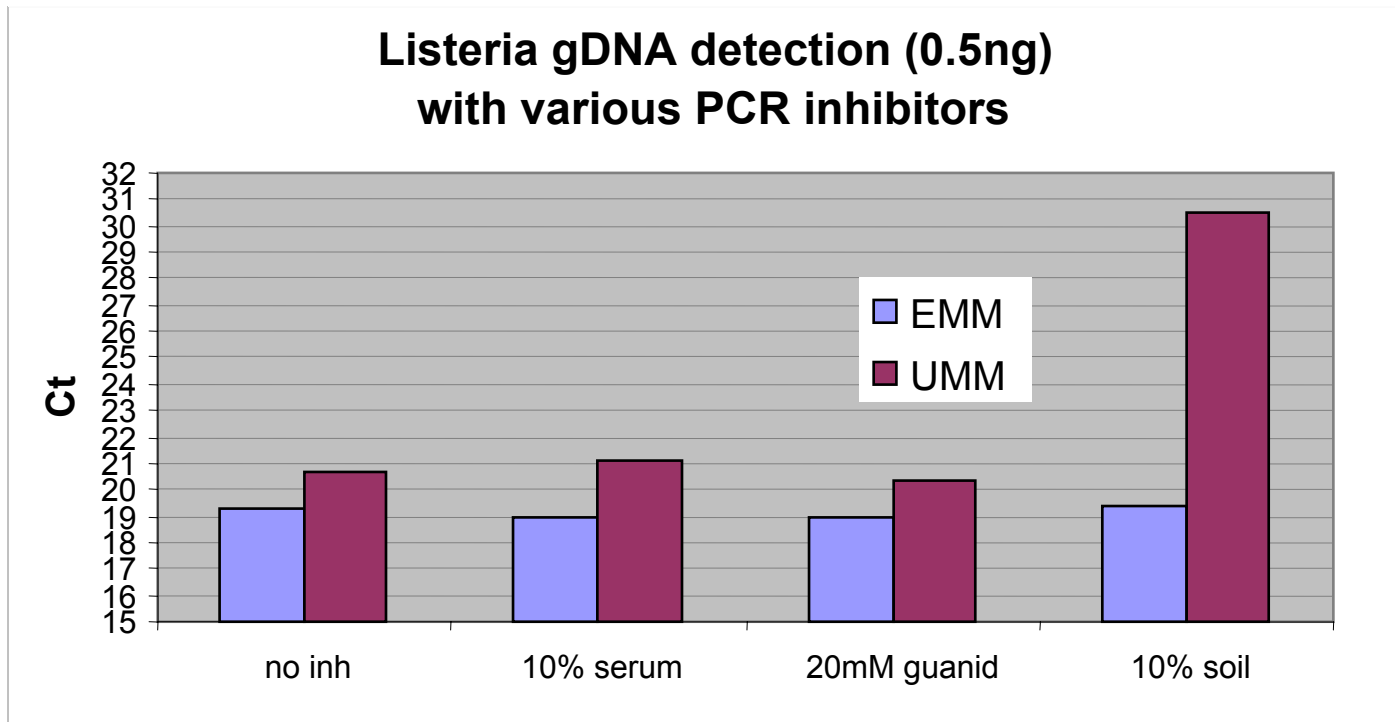
Overview of protocol

- Reaction volume: 30 μ l
- **Reaction setup:**
 - 15 μ l 2x Environmental Master Mix
 - 3 μ l 10x Assay Mix
 - 12 μ l of genomic DNA
- 10x Assay Mix contains dUTP instead of dTTP
 - Allows customers to modify protocol to use UNG either to prevent or get rid of carryover contamination
- **Thermal Cycle Protocol:**
 - Pre-PCR
 - > 50°C – 2 min
 - > 95°C – 10 min
 - PCR, 45 cycles
 - > 95°C – 15 sec
 - > 60°C – 1 min



Food pathogen assays

Listeria-Inhibitors study



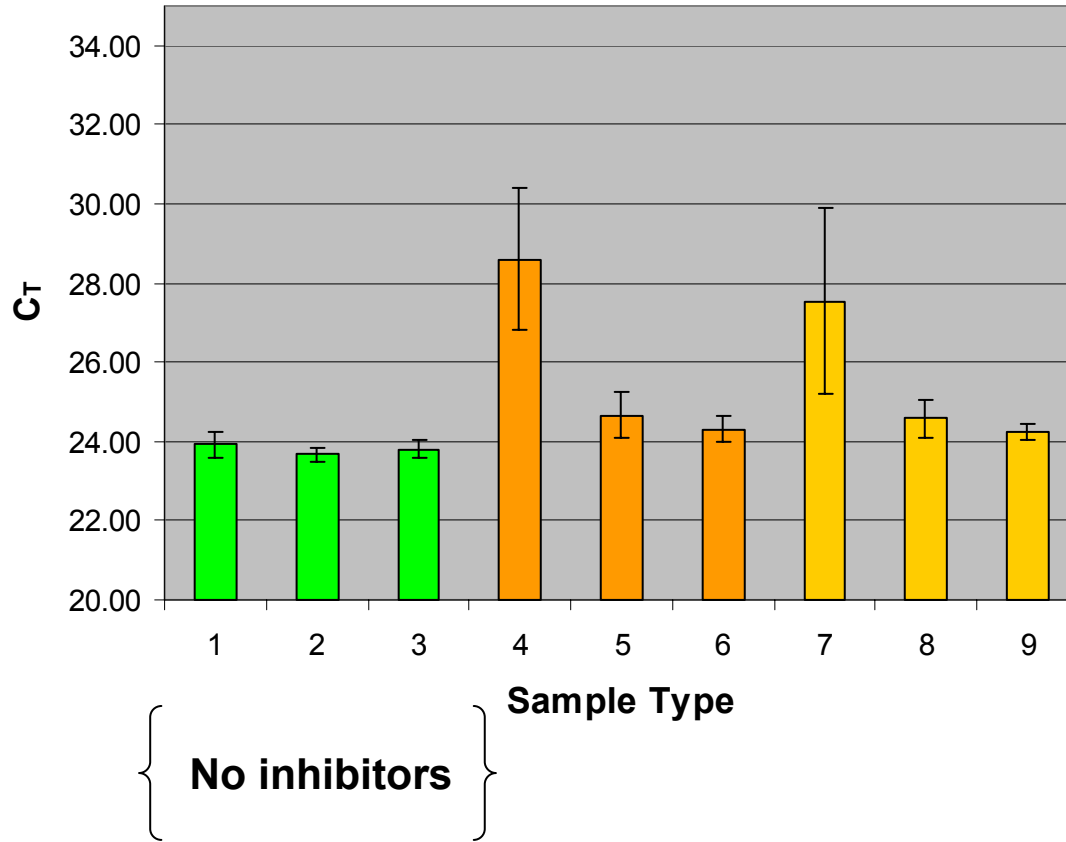
We optimized a new PCR Master Mix (EMM) that performs better than the standard TaqMan® Universal PCR Master Mix (UMM) in the presence of various inhibitors

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Optimization of Master Mix: Inhibition with Milk

A Std MM
B improved
C final version

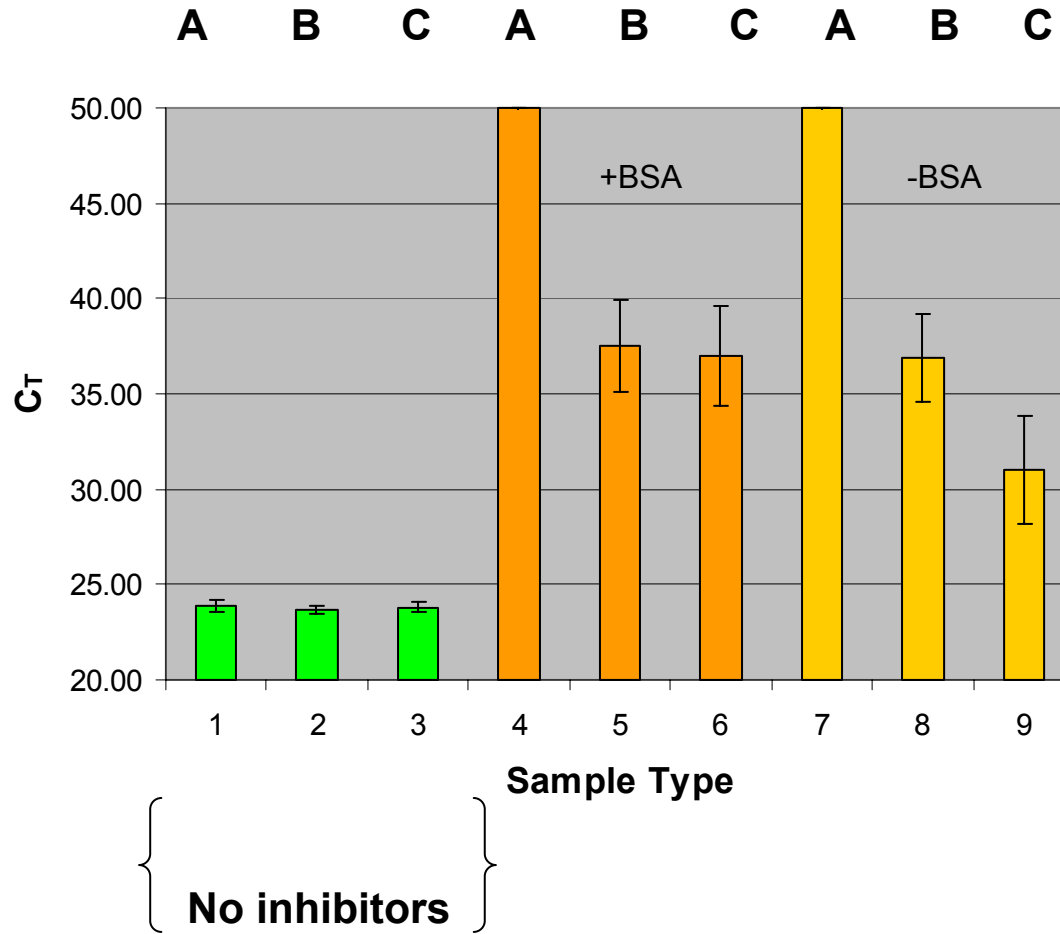


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Optimization of Master Mix: Inhibition with FBS

A Std MM
B improved
C final version

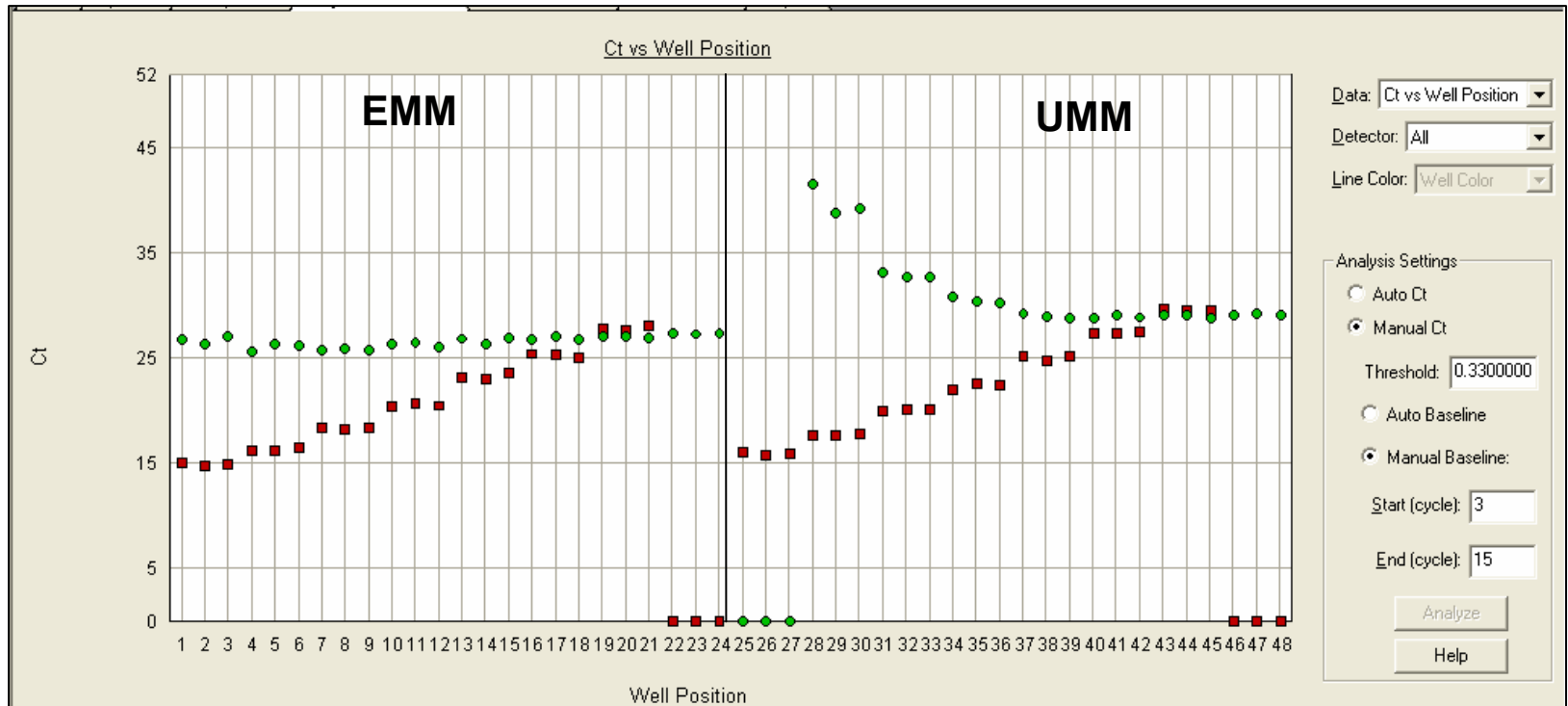


23% FBS



Listeria monocytogenes target titration

1:10 dilutions, 10 ng to 10 fg



7300 data

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Salmonella Kit

Key features tested:

sensitivity (limit of detection)

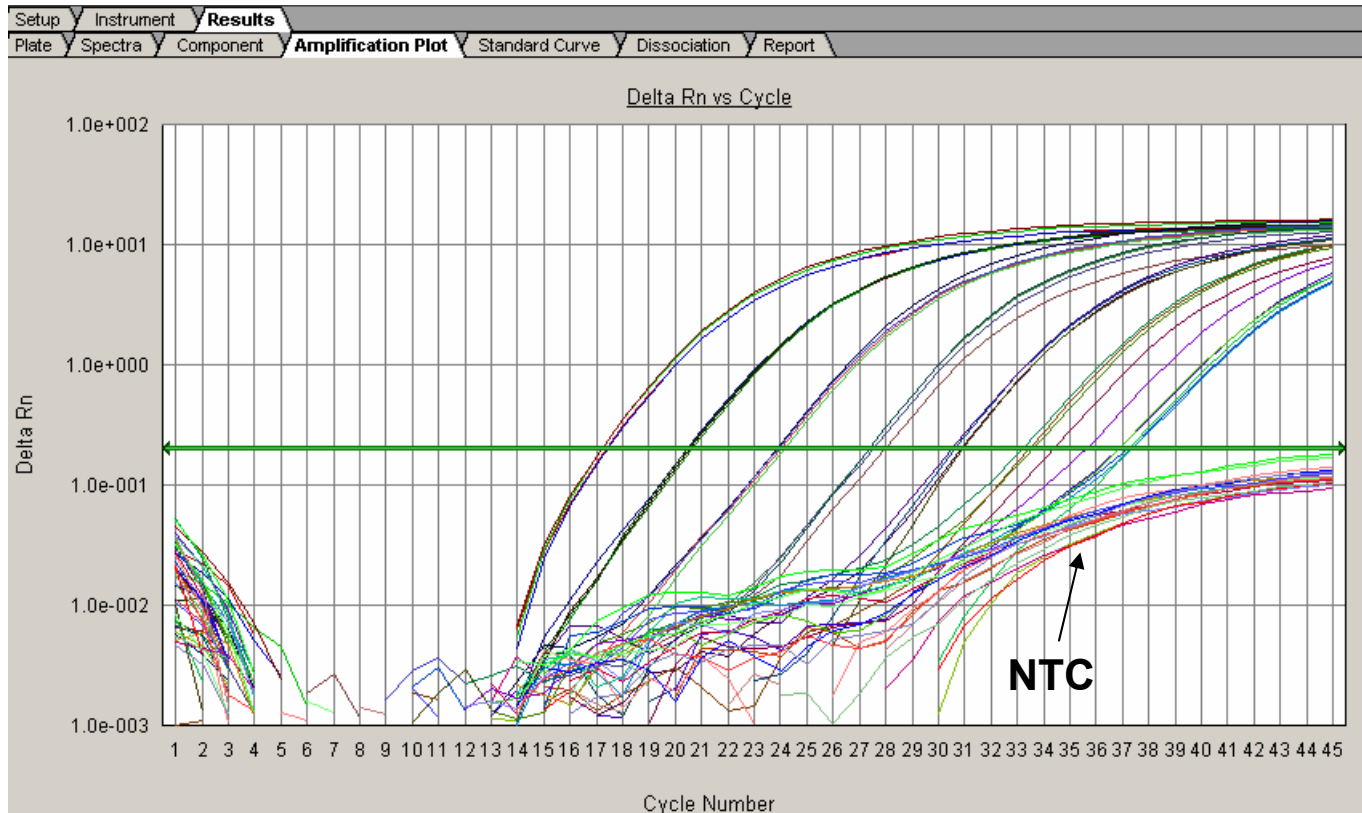
efficiency

specificity

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Amplification Plot: ΔR_n vs Cycle Number

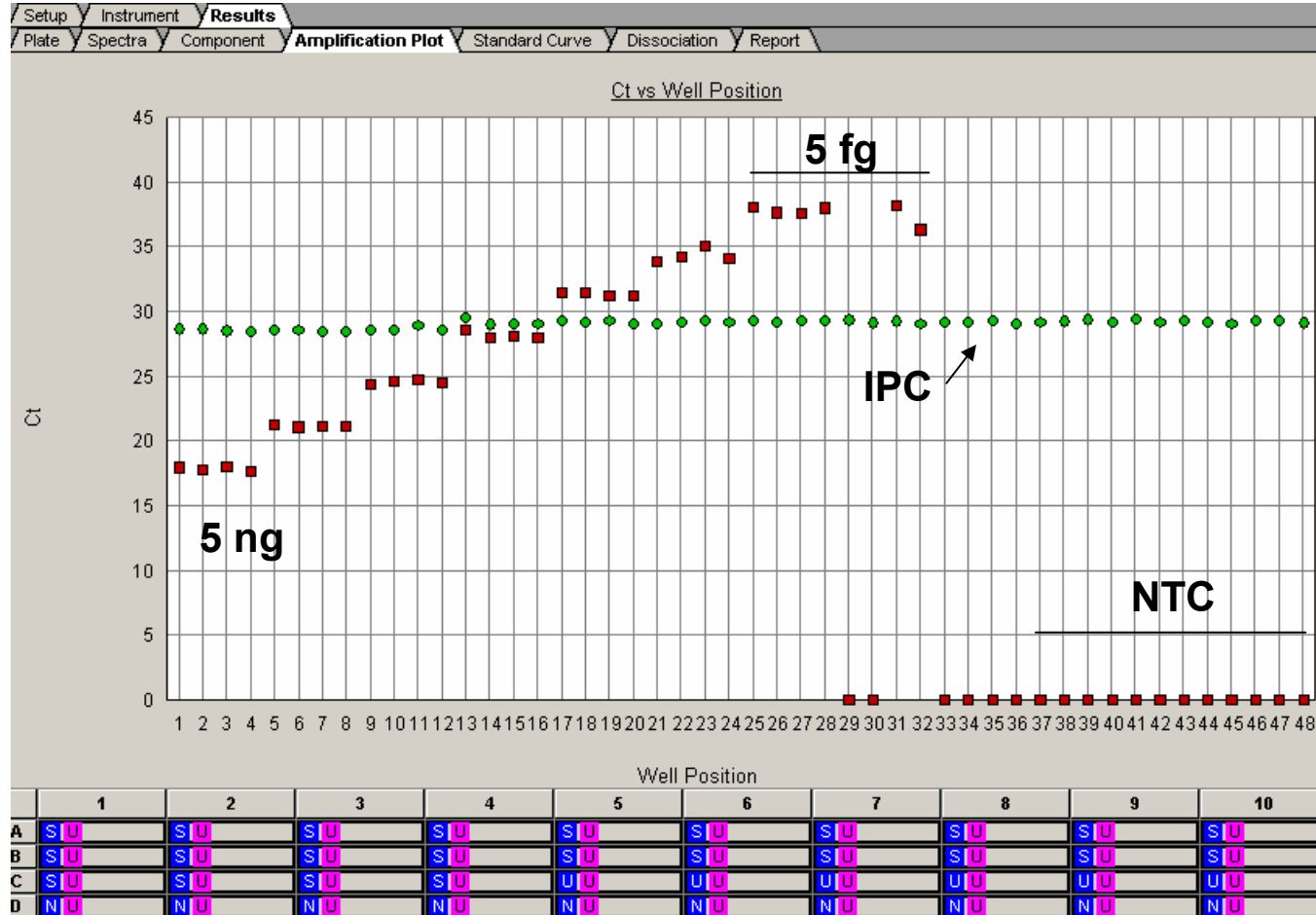


Titration of *Salmonella* genomic DNA starting with 5 ng followed by 1:10 dilutions

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C_T Values of Target and IPC

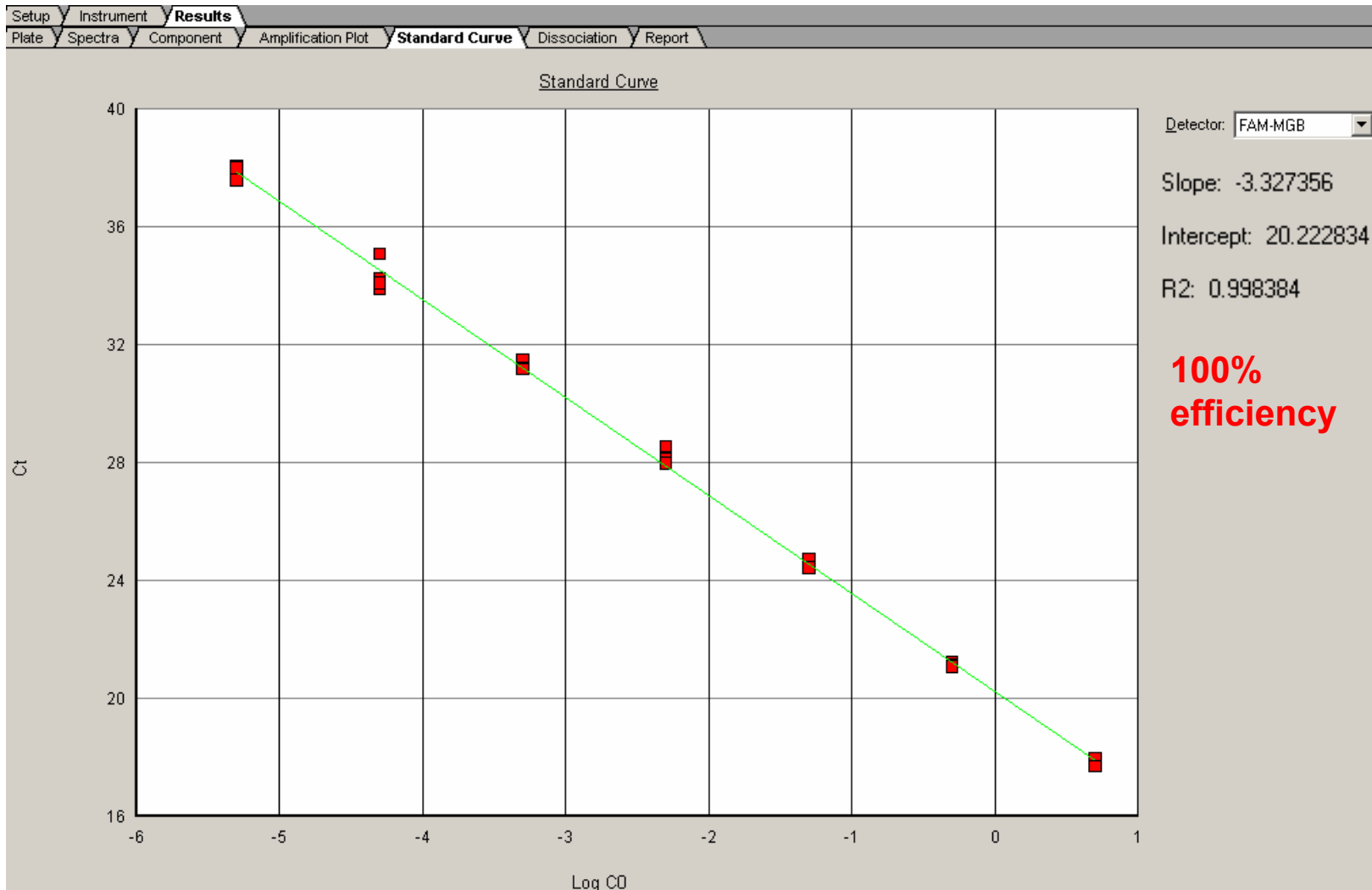


One copy of *Salmonella* genomic DNA \cong 5 fg

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Standard Curve Plot and Efficiency



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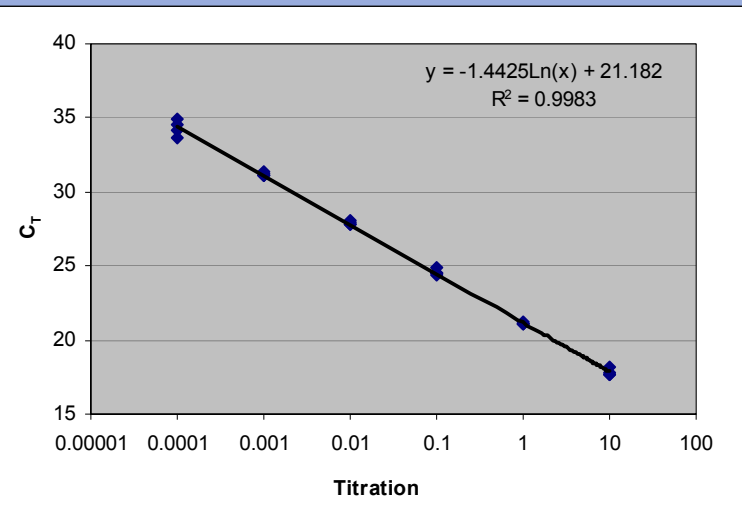
Food pathogen assays

Listeria-Efficiency

<i>Listeria</i> + IPC	x	y
10	1	18.12
10	1	17.73
10	1	17.67
10	1	17.64
1	0	21.15
1	0	21.10
1	0	21.14
1	0	21.17
0.1	-1	24.56
0.1	-1	24.55
0.1	-1	24.39
0.1	-1	24.89
0.01	-2	27.90
0.01	-2	27.83
0.01	-2	27.83
0.01	-2	28.10
0.001	-3	31.24
0.001	-3	31.38
0.001	-3	31.10
0.001	-3	31.07
0.0001	-4	34.22
0.0001	-4	33.72
0.0001	-4	34.92
0.0001	-4	34.53

The assay is highly efficient

Efficiency 100.02
lower 97.54
upper 102.62



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Food pathogen assays Efficiency Summary

Organism	Sensitivity (genomic copies)	Percent efficiency	Confidence level
<i>Salmonella</i>	1-10 copies	101.95	>95%
<i>Listeria</i>	1-10 copies	100.02	>95%
<i>E.coli</i> O157:H7	1-10 copies	100.09	>95%
<i>Campylobacter</i>	1-10 copies	101.04	>95%

All the assays have near 100 % efficiency.



Product overview

Instrumentation, sensitivity & efficiency

- Kits tested to perform on the following instruments
 - ABI PRISM[®] 7000 SDS
 - Applied Biosystems 7300 System
 - Applied Biosystems 7500 System
 - Applied Biosystems 7900 HT System
- Sensitivity of kits
 - Kits detect 1-10 copies of target DNA/rxn
 - Kits detect 1 CFU in 25 g of food after pre-enrichment
- Kit efficiency:
 - All kits have near 100% efficiency



Food pathogen assays

Salmonella-Specificity

Organism	Strain designation	Result	Ct	Organism	Strain designation	Result	Ct
<i>Salmonella ser. typhimurium</i>	ser. typhimurium	+	20.35	<i>Salmonella ser. eimsbutter</i>	1017/94	+	17.31
<i>Salmonella ser. enterica</i>	l ser. infantus	+	20.59	<i>Salmonella ser. give</i>	458/89	+	17.29
<i>Salmonella ser. anatum</i>	10/12	+	20.69	<i>Salmonella ser. havana</i>	SL5749	+	17.23
<i>Salmonella ser. panama</i>	10/13	+	20.40	<i>Salmonella ser. isangi</i>	1066/94	+	17.50
<i>Salmonella ser. hemo</i>	2/CI	+	19.53	<i>Salmonella ser. montgomery</i>	457/89	+	17.62
<i>Salmonella ser. enterica</i>	9/B6	+	19.54	<i>Salmonella ser. naestyed</i>	1131/95	+	17.24
<i>Salmonella ser. typhi</i>		+	21.47	<i>Salmonella nienstedten</i>	1018/94	+	17.07
<i>Salmonella ser. typhimurium</i>	6765	+	20.31	<i>Salmonella ser. agona</i>	SARB1	+	17.46
<i>Salmonella ser. infantis</i>	6064	+	19.71	<i>Salmonella ser. decatur</i>	SARB8	+	17.51
<i>Salmonella ser. hadar</i>		+	17.88	<i>Salmonella ser. miami</i>	SARB28	+	17.32
<i>Salmonella</i>	MF350	+	17.13	<i>Salmonella ser. 18:Z4Z32</i>	11594	+	23.32
<i>Salmonella ser. abortus-equi</i>	SL5414	+	18.58	<i>Citrobacter freundii</i>	6879	-	nd
<i>Salmonella ser. abortus-ovis</i>	1132/95	+	18.18	<i>Shigella</i>	Sfla 395	-	nd
<i>Salmonella ser. adeleide</i>	859/93	+	17.59	<i>Shigella</i>	SFL153	-	nd
<i>Salmonella ser. austin</i>	SL5747	+	17.77	<i>Enterobacter aerogenes</i>	Q87	-	nd
<i>Salmonella ser. boccker</i>	1012/94	+	17.94	<i>Staphylococcus aureus aureus</i>	Q87	-	nd
<i>Salmonella ser. borum</i>	1016/94	+	17.31	<i>Pseudomonas aeruginosa</i>	ATCC #27853	-	nd
<i>Salmonella ser. bovisorbificans</i>	SL5747	+	17.49	<i>Vibrio cholerae</i>	036	-	nd
<i>Salmonella ser. carrau</i>	1011/94	+	18.62	<i>Bacillus cereus</i>		-	nd
<i>Salmonella ser. chandans</i>	456/89	+	17.16	<i>Shigella dysenteriae</i>		-	nd

The assay is highly specific for *Salmonella* strains



Food pathogen assays

Listeria-Specificity

Organism	Strain designation	Result	Ct	Organism	Strain designation	Result	Ct
<i>Listeria monocytogenes</i>	LS24 (1b)	+	19.07	<i>Listeria monocytogenes</i>	(1a)	+	18.28
<i>Listeria monocytogenes</i>	LS113 (1/2a)	+	19.92	<i>Listeria ivanovii</i>	ATCC 19119	-	nd
<i>Listeria monocytogenes</i>	LS80 (1/2b)	+	20.09	<i>Listeria innocua</i>	33090	-	nd
<i>Listeria monocytogenes</i>	LS115 (1/2c)	+	18.61	<i>Listeria innocua</i>	LS7	-	nd
<i>Listeria monocytogenes</i>	LS111 (3a)	+	19.46	<i>Listeria innocua</i>	Q94 (4)	-	nd
<i>Listeria monocytogenes</i>	LS225 (4a)	+	19.47	<i>Listeria seeligeri</i>	LS22 (3b)	-	nd
<i>Listeria monocytogenes</i>	LS2/ScottA (4b)	+	20.16	<i>Listeria seeligeri</i>	LS56	-	nd
<i>Listeria monocytogenes</i>	LS244 (4ab)	+	18.90	<i>Listeria welshimeri</i>	LS128	-	nd
<i>Listeria monocytogenes</i>	LS21	+	22.91	<i>Listeria welshimeri</i>	LS156	-	nd
<i>Listeria monocytogenes</i>	Q91 (1)	+	22.19	<i>Listeria grayi</i>	RF4738	-	nd
<i>Listeria monocytogenes</i>	Q98 (1b)	+	24.97	<i>Listeria murrayi</i>	LS39	-	nd
<i>Listeria monocytogenes</i>	Q101 (4)	+	21.42	<i>Salmonella ser. Enterica</i>	I ser. Infantus	-	nd
<i>Listeria monocytogenes</i>	Q126 (1)	+	21.66	<i>Enterobacter aerogenes</i>	Q87	-	nd
<i>Listeria monocytogenes</i>	Q128 (4)	+	21.80	<i>Staphylococcus aureus aureus</i>	Q87	-	nd
<i>Listeria monocytogenes</i>	Q158 (4b)	+	23.82	<i>Pseudomonas aeruginosa</i>	ATCC #27853	-	nd
<i>Listeria monocytogenes</i>	Q159 (3b)	+	24.79	<i>Vibrio cholerae</i>	036	-	nd
<i>Listeria monocytogenes</i>	Q160 (1b)	+	23.65	<i>Bacillus cereus</i>		-	nd
<i>Listeria monocytogenes</i>	Q161 (1.1/2a)	+	22.03	<i>Shigella dysenteriae</i>		-	nd

The assay is highly specific for *Listeria monocytogenes*



Food pathogen assays

E.coli O157:H7-Specificity

Organism	Strain designation	Result	Ct
<i>Escherichia coli</i>	O157:H7	+	20.37
<i>Escherichia coli</i>	O157:NM	+	22.16
<i>Escherichia coli</i>	O55:H7	+	21.94
<i>Escherichia coli</i>	O157:H7	+	22.01
<i>Escherichia coli</i>	O157:H7	+	21.76
<i>Escherichia coli</i>	O157:H7	+	21.84
<i>Escherichia coli</i>	O157:H7	+	20.55
<i>Escherichia coli</i>	O157:H7	+	20.32
<i>Escherichia coli</i>	O157:H7	+	21.04
<i>Escherichia coli</i>	O157:H7	+	22.67
<i>Escherichia coli</i>	O55:H7	+	21.44
<i>Escherichia coli</i>	O55:H7	+	21.08
<i>Escherichia coli</i>	O55:H7	+	20.88
<i>Escherichia coli</i>		-	nd
<i>Escherichia coli</i>	O26:H11	-	nd
<i>Escherichia coli</i>	O145:NM	-	nd
<i>Escherichia coli</i>	O78:K80:H12	-	nd
<i>Escherichia coli</i>	O103:H2	-	nd

Organism	Strain designation	Result	Ct
<i>Escherichia coli</i>	O5:NM	-	nd
<i>Escherichia coli</i>	O137:H41	-	nd
<i>Escherichia coli</i>	O18:H21	-	nd
<i>Escherichia coli</i>	O28:H35	-	nd
<i>Escherichia coli</i>	O26:H32	-	nd
<i>Escherichia coli</i>	O55:H9	-	nd
<i>Escherichia coli</i>	O154:H25	-	nd
<i>Escherichia coli</i>	O156:H8	-	nd
<i>Citrobacter freundii</i>	6879	-	nd
<i>Shigella</i>	Sfla 395	-	nd
<i>Shigella dysenteriae</i>		-	nd
<i>Salmonella ser. enterica</i>	I ser. infantus	-	nd
<i>Listeria monocytogenes</i>		-	nd
<i>Enterobacter aerogenes</i>	Q87	-	nd
<i>Staphylococcus aureus aureus</i>	Q87	-	nd
<i>Pseudomonas aeruginosa</i>	ATCC #27853	-	nd
<i>Vibrio cholerae</i>	036	-	nd
<i>Bacillus cereus</i>		-	nd



Food pathogen assays

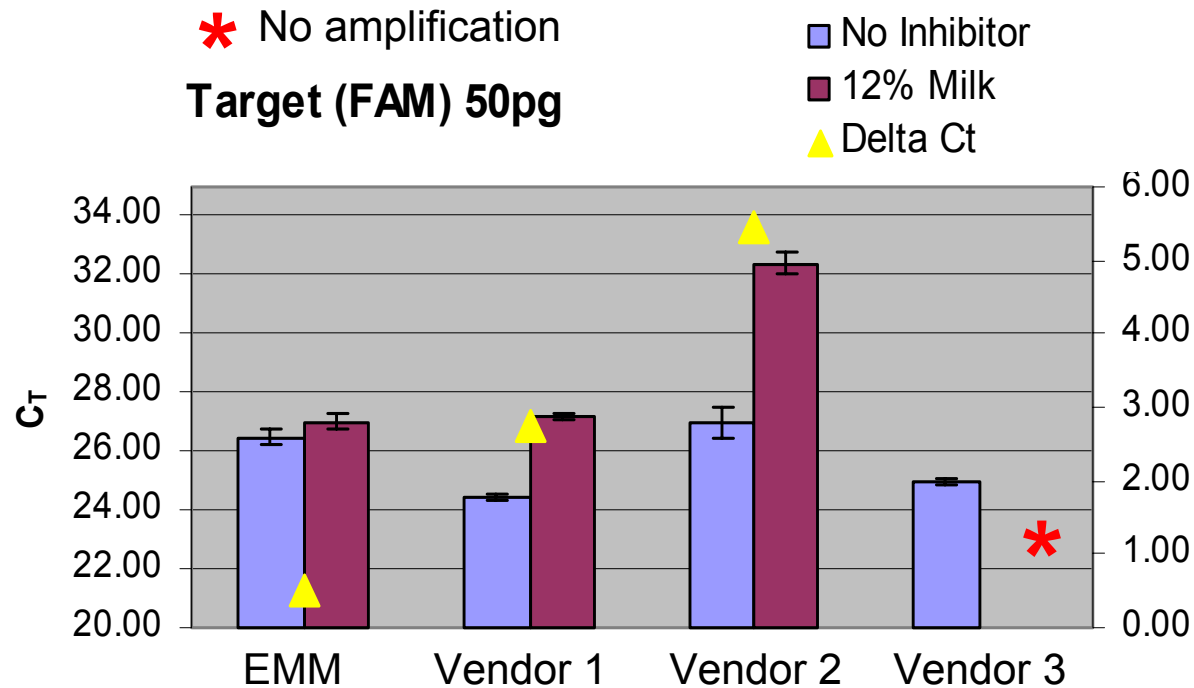
Campylobacter-Specificity

Organism	Strain designation	Result	Ct	Organism	Strain designation	Result	Ct
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	VPI H840 [CIP]	+	20.03	<i>Campylobacter coli</i>	LRA 069.05.89	+	20.65
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	AS-83-79	+	19.60	<i>Campylobacter coli</i>	Inn 183	+	35.02
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	LRA 094.06.89	+	20.23	<i>Campylobacter laridis</i>	Standford/BL	-	nd
<i>Campylobacter jejuni</i>	C31	+	19.07	<i>Campylobacter fetus</i> subsp. <i>fetus</i>	NADL 1083-2255	-	nd
<i>Campylobacter jejuni</i>		+	18.79	<i>Helicobacter pylori</i>	87A300	-	nd
<i>Campylobacter jejuni</i>	No. 1	+	19.92	<i>Helicobacter pylori</i>	RSB6 NCTC	-	nd
<i>Campylobacter jejuni</i>	No. 2	+	21.52	<i>Helicobacter pylori</i>	TX30A NCTC	-	nd
<i>Campylobacter jejuni</i>	No. 3	+	21.29	<i>Arcobacter cryaephilus</i>	LRA 077.02.87	-	nd
<i>Campylobacter jejuni</i>	No. 4	+	19.56	<i>Salmonella ser. enterica</i>	I ser. infantus	-	nd
<i>Campylobacter jejuni</i>	63R	+	20.31	<i>Listeria monocytogenes</i>		-	nd
<i>Campylobacter jejuni</i>	81	+	20.69	<i>Enterobacter aerogenes</i>	Q87	-	nd
<i>Campylobacter jejuni</i>	89	+	33.73	<i>Staphylococcus aureus aureus</i>	Q87	-	nd
<i>Campylobacter jejuni</i>	194	+	20.09	<i>Pseudomonas aeruginosa</i>	ATCC #27853	-	nd
<i>Campylobacter jejuni</i>	ATCC #33560	+	20.91	<i>Vibrio cholerae</i>	036	-	nd
<i>Campylobacter jejuni</i>	3135	+	18.78	<i>Bacillus cereus</i>		-	nd
<i>Campylobacter jejuni</i>	3138	+	19.32	<i>Shigella dysenteriae</i>		-	nd



Food pathogen assays

Salmonella-Master mix study

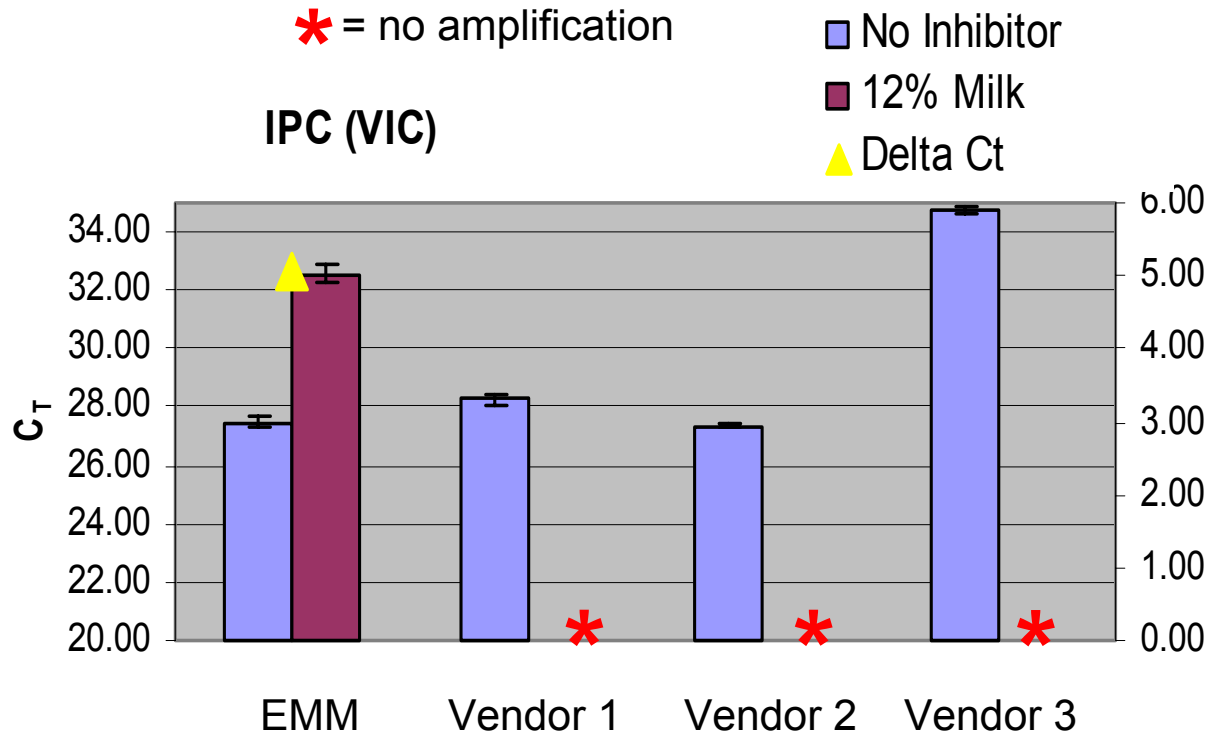


EMM is the best performer in the presence of inhibitors

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Food pathogen assays IPC-Master Mix study

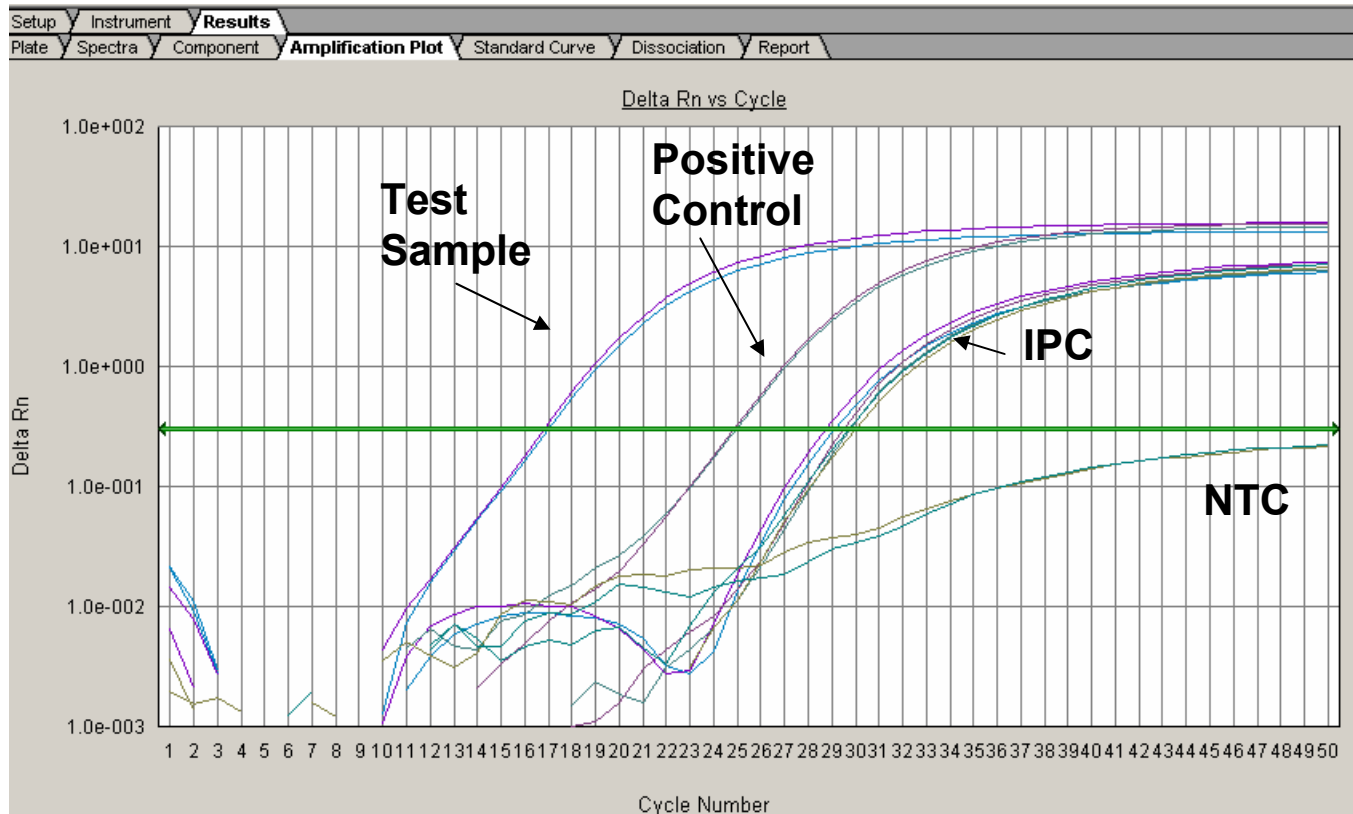


EMM is the best performer in the presence of a common food inhibitor

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Overnight pre-enrichment of eggs spiked with 1-10 CFU *Salmonella*

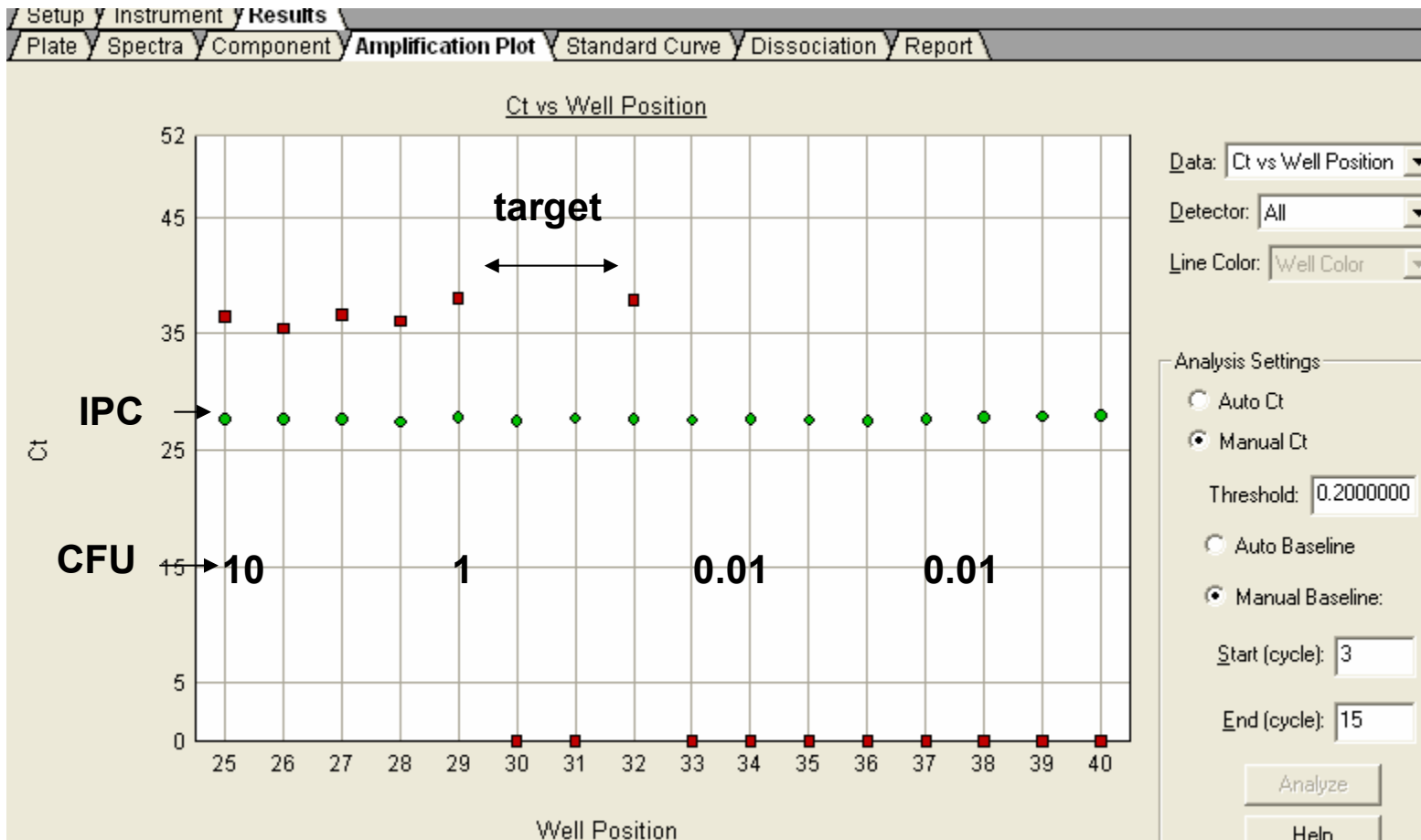


Samples were run with PrepMan Ultra Procedure

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Cheese + *Listeria monocytogens* pre-enrichment



Reps = 4

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