MASSARRAY-BASED METHOD FOR DETECTION AND IDENTIFICATION OF FOODBORNE BACTERIA

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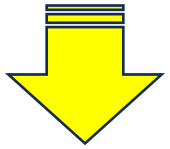
Product specialist
Lifomics Co., Ltd.

Contents

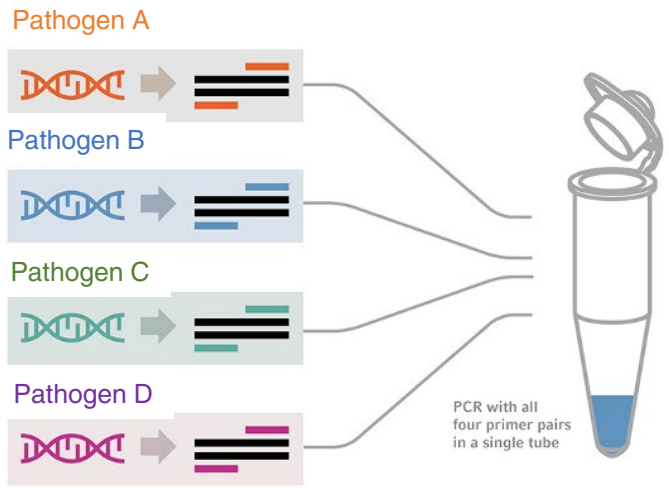
KEY MESSAGE

เหนื่อยไหมกับการเลี้ยงเชื้อจำนวนมากเพื่อตรวจสอบการปนเปื้อนของจุลชีพก่อโรคในสิ่งส่งตรวจ??



การใช้เทคโนโลยีการตรวจ SNPs เพื่อการตรวจเชื้อก่อโรค

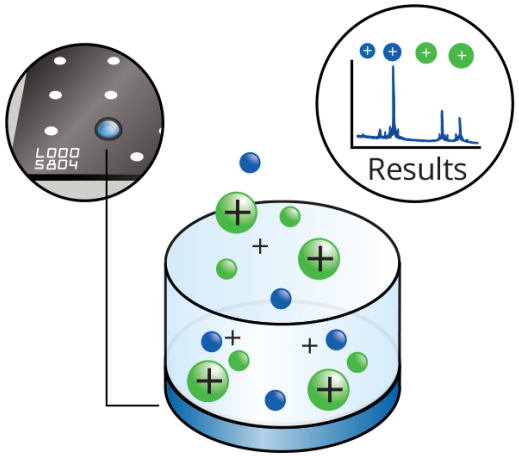
PRINCIPLE OF MASSARRAY TECHNOLOGY



Cytosine (C)	Adenine (A)	Guanine (G)	Thymine (T)
247.2 Da	271.2 Da	287.2 Da	327.1 Da

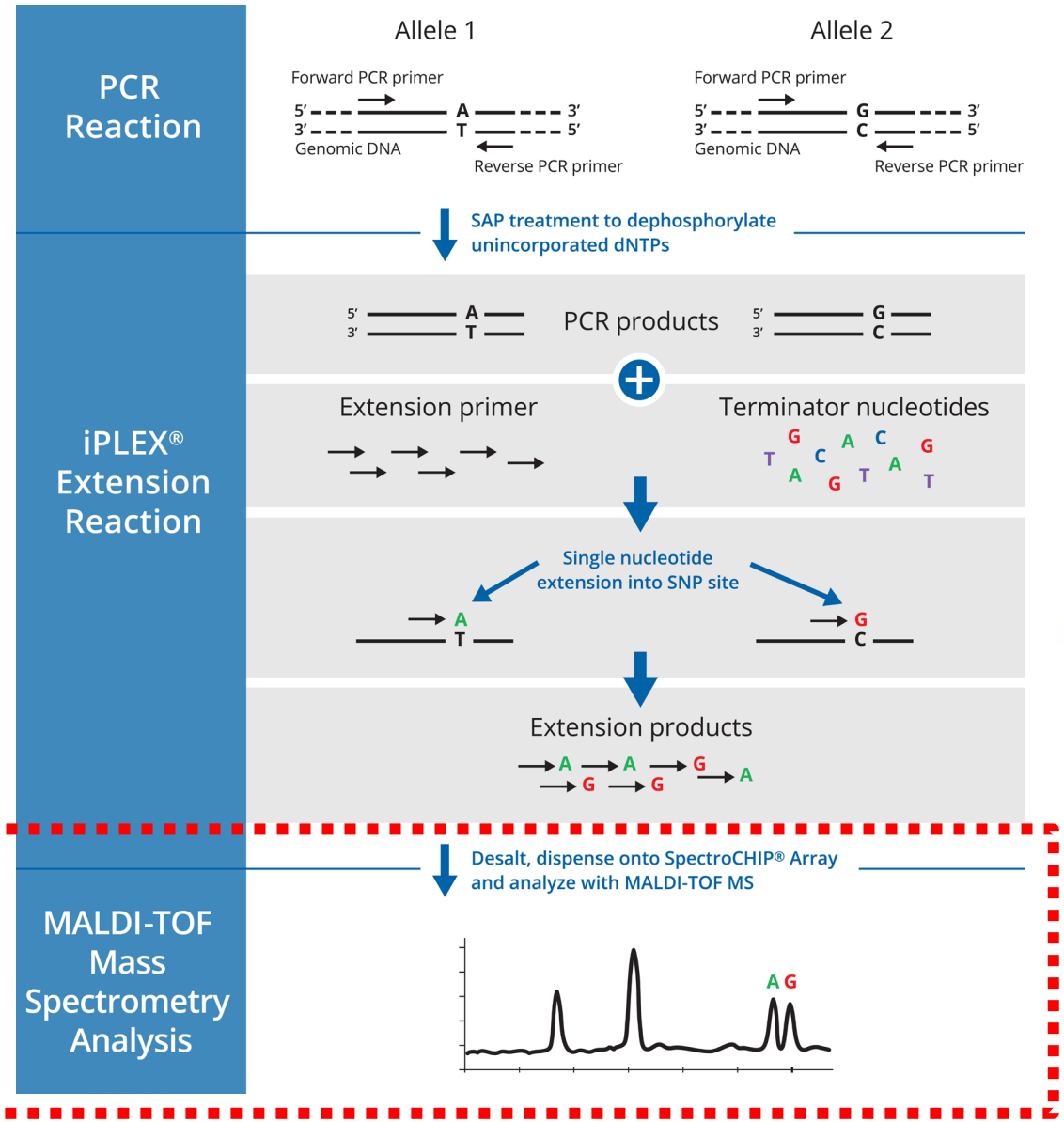
Multiplex PCR

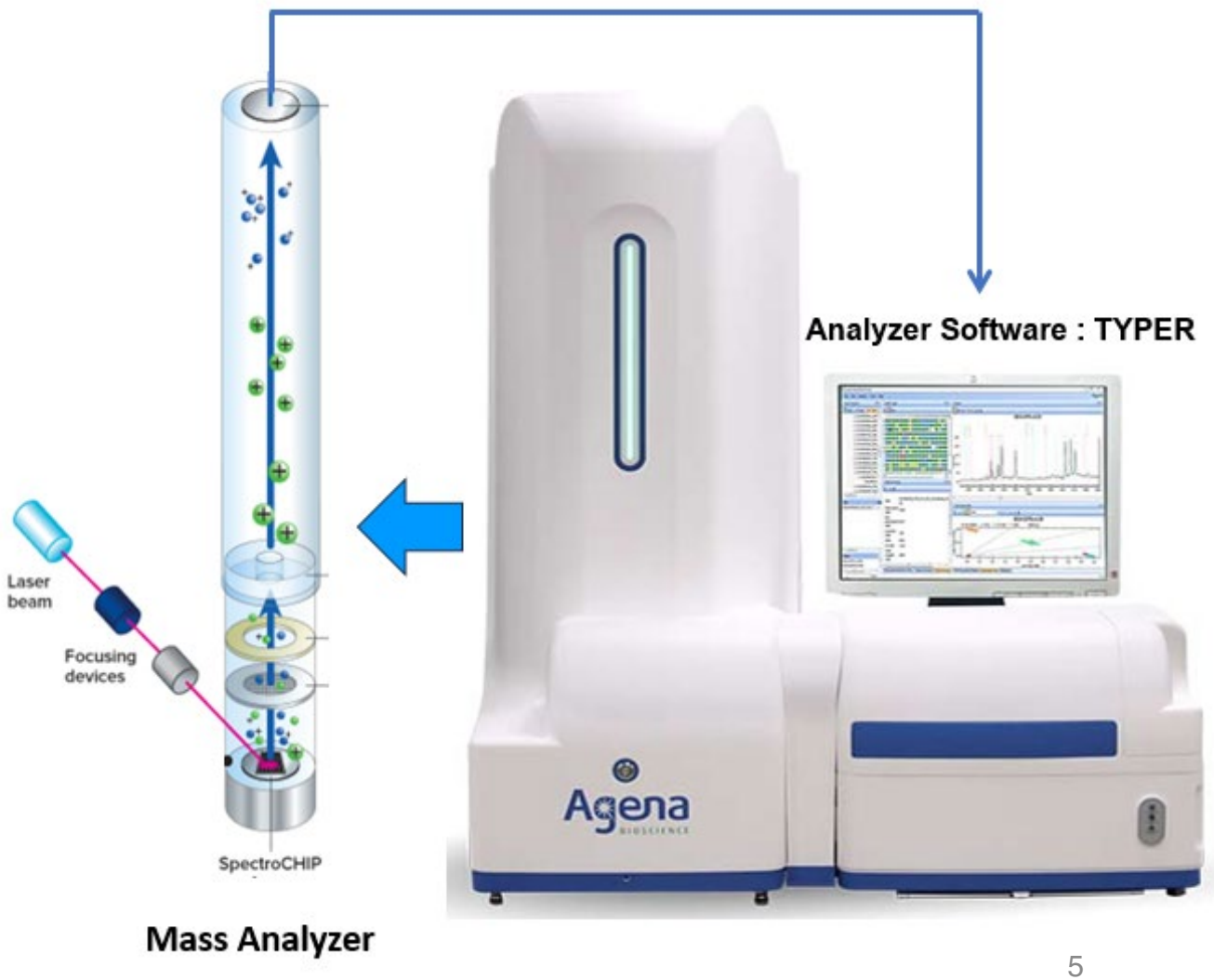
MALDI-TOF
Mass Spectrometry



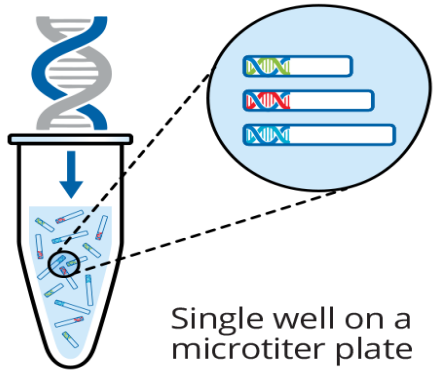
MassARRAY Detection Range
4,000 – 9,000 Daltons

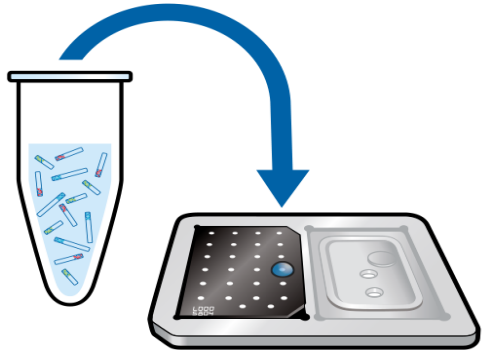
SYSTEM WORKFLOW

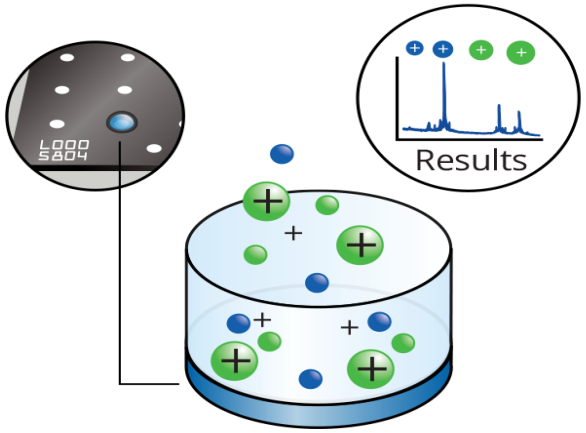




SAMPLE PROCESSES JOURNEY







PCR/SAP/Extension

Multiplex end-point PCR followed by a single base extension reaction

DNA SAMPLING 5-10 ng/ well

Target Sequence Detection

Automated analyte transfer and data acquisition

Data Analysis

Data display and report generation

96 FORMAT

Run Time: 5.5 Hrs*
Hands-on Time: 25 Min

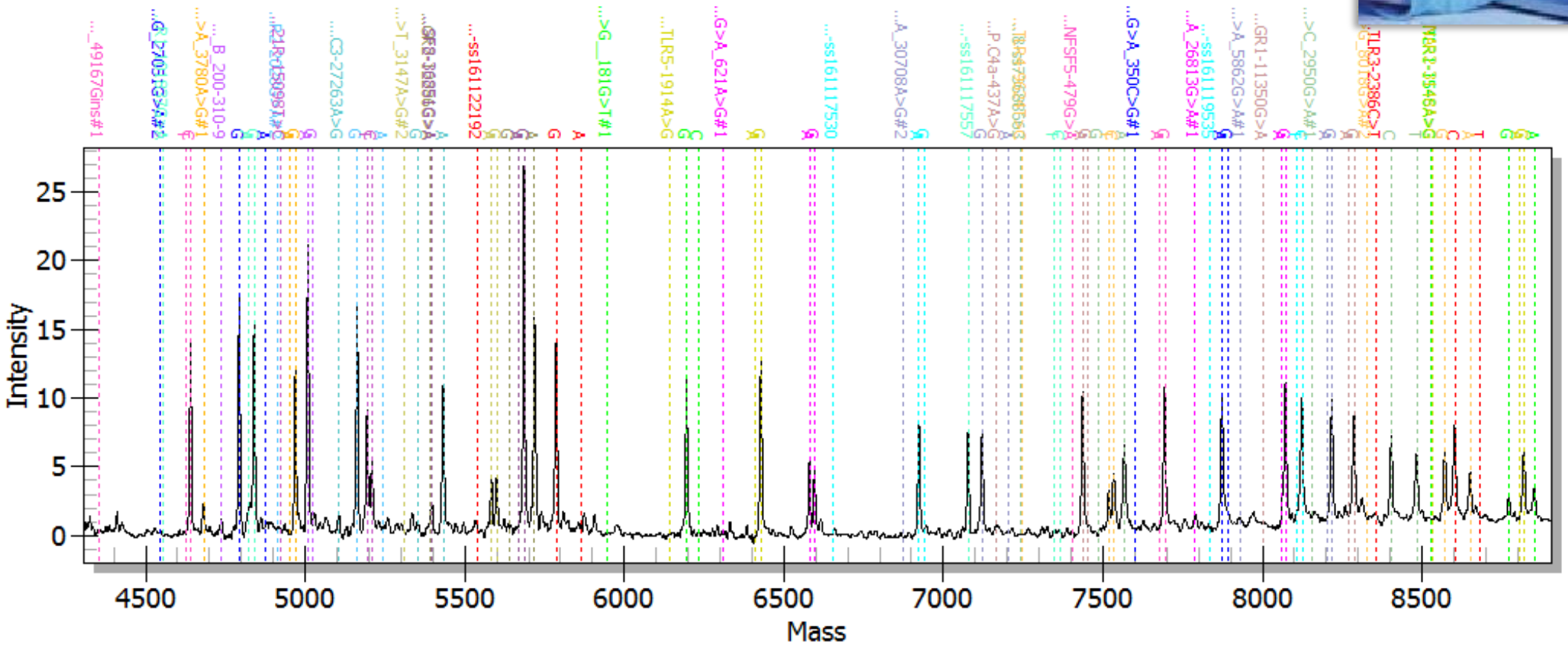
Run Time: 100 Mins*
Hands-on Time: 5 Min

Hands-on Time:
5-10 Min

ADVANCED SOFTWARE

Report & data analysis

The Power of Multiplexing Multiple Markers in a Single Well



frontiers Q1, IF 5.8

Suebwongsa et al., 2024

MassARRAY: A High-Throughput Solution for Rapid Detection of Foodborne Pathogens in Real-World Settings

Namfon Suebwongsa[1*], Surasak Jiemsup[1], Pannita Santiyanont[1], Piyapha Hirunpatrawong[2], Pornsiri Assawapirin[2], Monthatip Thongkum[2], Prakaymars Panumars[2], Nipa Chokesajjawatee[1], Supaporn Wongsrichai[3], Pichet Koompa[3], Suganya Yongkiettrakul[1*]

[1]National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand, [2]Lifomics Co., Ltd., Bang Khae, Bangkok, THAILAND, Thailand, [3]Department of Livestock Development (Thailand), Thailand

FOODBOURNE DISEASES

Foodborne diseases in the WHO South-East Asia Region

Every year

>150 million people fall **ill** } **>175 000** **die**

including

60 million children <5 fall **ill** } **50 000** **die**

Diarrhoeal diseases are responsible for majority of deaths. Key causes:

- Norovirus
- Non-typhoidal *Salmonella*
- Pathogenic *E. coli*

Region has >1/2 the people globally who are infected and die from **typhoid fever or hepatitis A**

FOODBORNE DISEASES ARE PREVENTABLE.
EVERYONE HAS A ROLE TO PLAY.

Every year foodborne diseases cause:

almost **in 10** people to fall ill

33 million healthy life years lost

Foodborne diseases can be deadly, especially in children <5

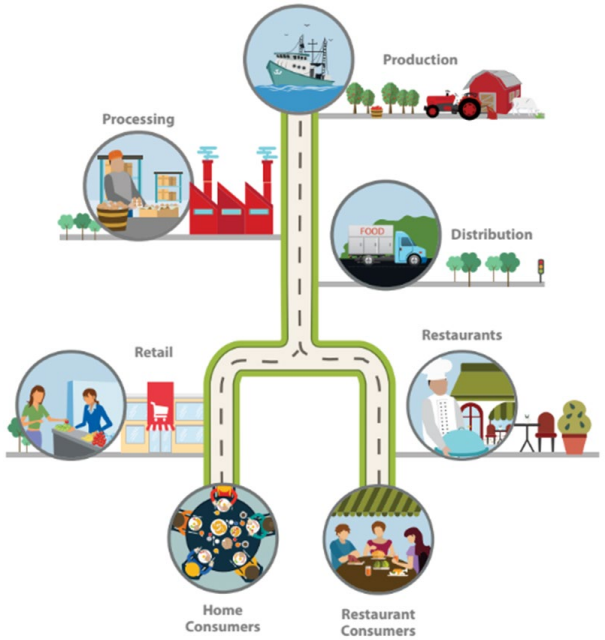
420 000 deaths

Children account for almost **1/3** **of deaths from foodborne diseases**

For more information: **www.who.int/foodsafety**
#SafeFood
Source: WHO Estimates of the Global Burden of Foodborne Diseases. 2015.

World Health Organization

FOOD SUPPLY CHAIN



Food supply chain

Food businesses

Food producers → **Food processors & manufacturers** → **Warehouses and Distributors** → **Retailers and food services** → **Consumers**

Food safety monitoring and regulation

Food safety regulators

Food safety auditors

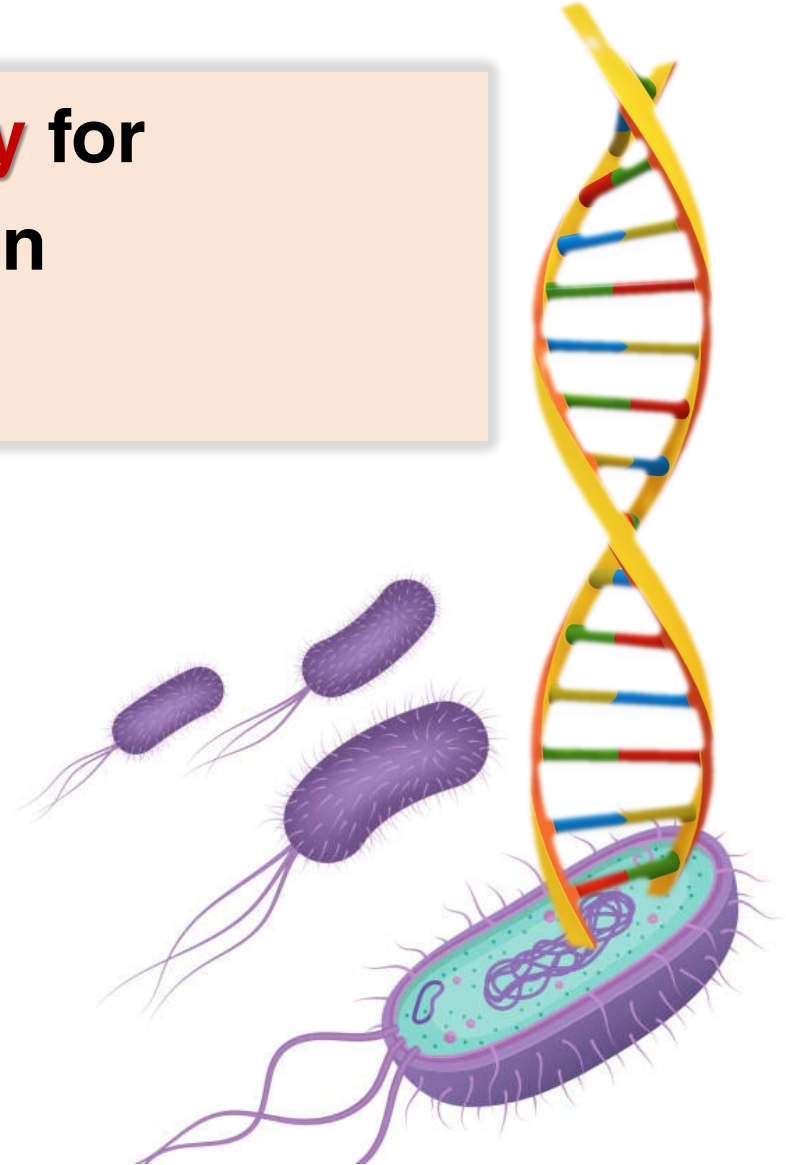
Food safety legislation, regulation and standards

Other legislation, regulation and standards

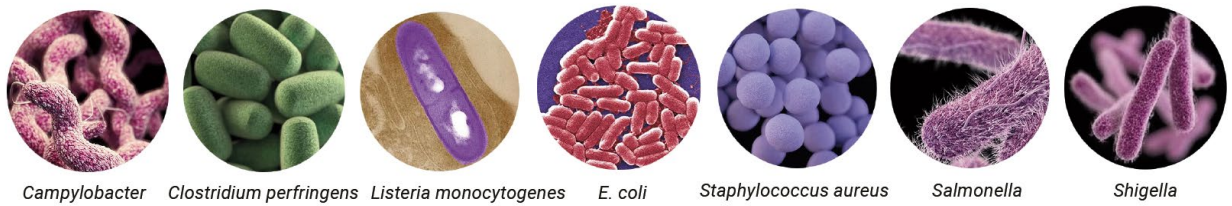
OBJECTIVE

To develop an efficient high-throughput assay for bacterial detection and identification based on MassARRAY® technology

- *Reliable and feasible*
- *Non labor intensive*
- *Less time consuming*
- *More cost effectiveness*



BACTERIAL FOODBORNE PANEL



Campylobacter *Clostridium perfringens* *Listeria monocytogenes* *E. coli* *Staphylococcus aureus* *Salmonella* *Shigella*

10 BACTERIAL FOODBORNE PATHOGENS IN A SINGLE REACTION

- *Salmonella* spp.
- *Campylobacter coli*
- *Campylobacter jejuni*
- *Listeria monocytogenes*
- *Escherichia coli and Shigella* spp.
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Staphylococcus aureus*
- *Clostridium perfringens*

Design	Assay Development			Validation	
12 pairs of multiplex PCR primers for 10 species	MagPurix Pathogen kit B High efficiency Cost relevant	Automated DNA extraction High-throughput, High efficiency, Cost effectiveness	EconoTaq Polymerase Specific polymerase, Cost relevant	Sensitivity 99%*	Specificity 100%**

World Health Organization

U.S. FOOD & DRUG ADMINISTRATION

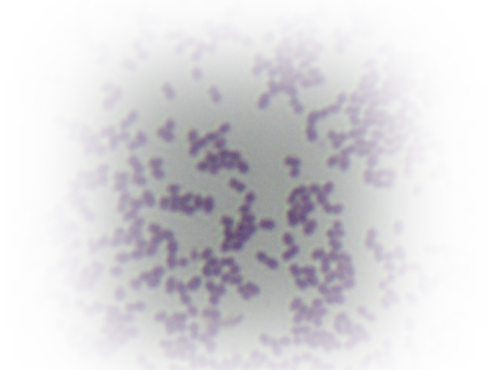
CDC Centers for Disease Control and Prevention

$$*\%\text{Sensitivity} = \frac{true\ positive}{true\ positive + false\ negative} \times 100$$

$$**\%\text{Specificity} = \frac{true\ negative}{true\ negative + false\ negative} \times 100$$

MassARRAY-based bacterial detection assay

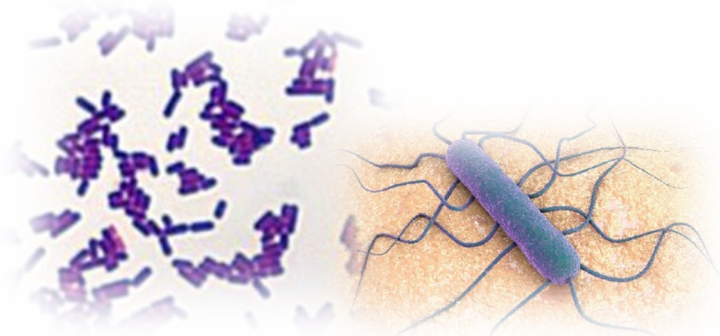
Detection of 10 bacterial foodborne pathogens in a single reaction



Enterococcus faecalis
Enterococcus faecium

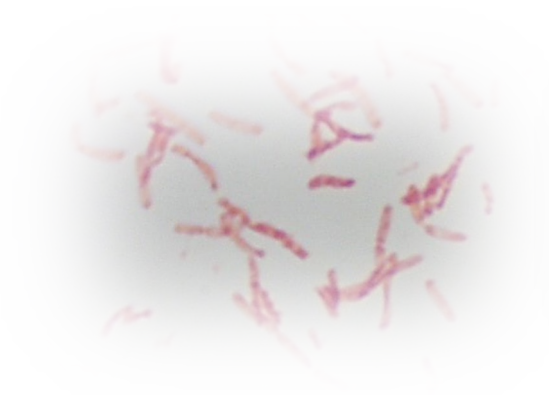
Clostridium perfringens

Stapylococcus aureus

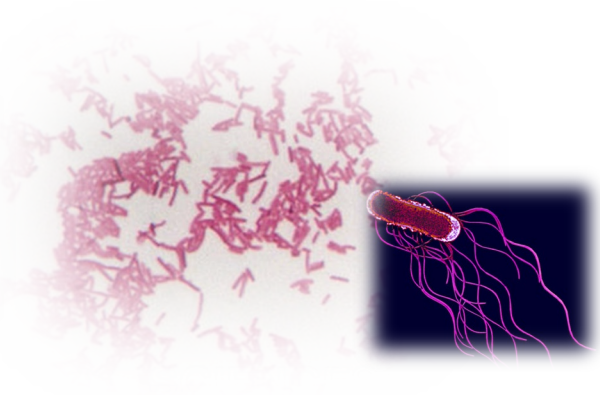


Listeria monocytogenes

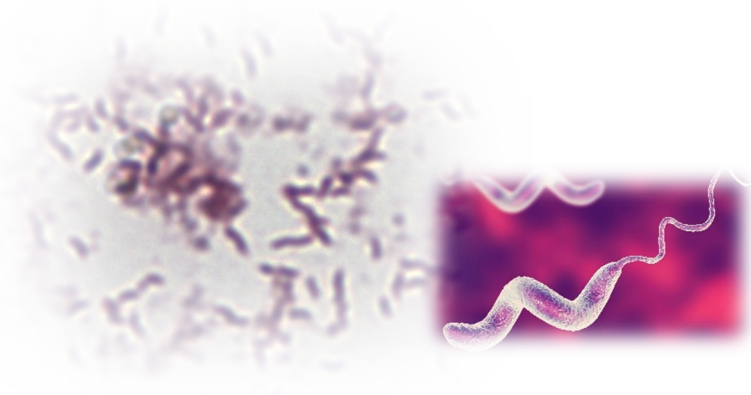
Escherichia coli



Shigella spp.

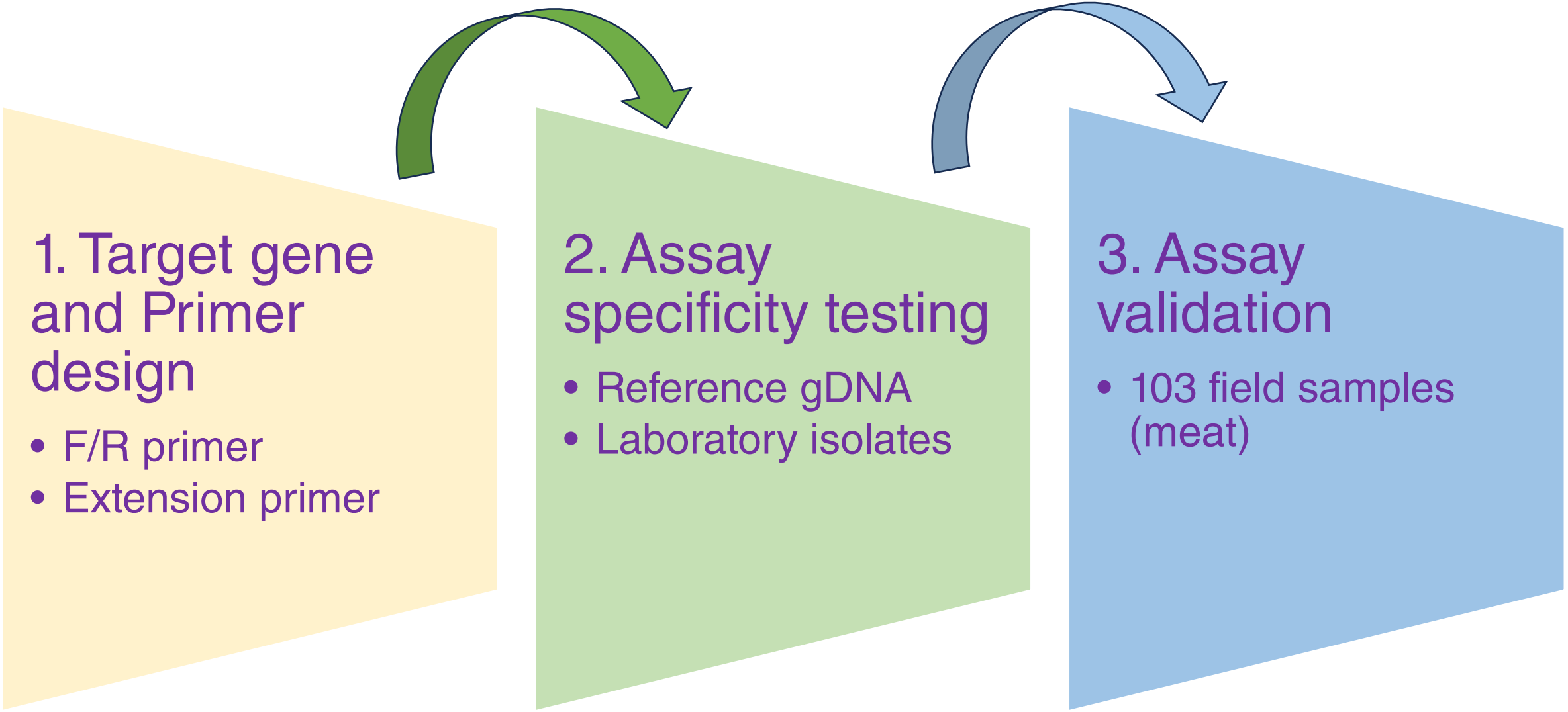


Salmonella enterica



Campylobacter jejuni
Campylobacter coli

Research workflow



Primers and Assay designed

Table 1. Primers used in this study

Targeted species	Primers	PCR primers (forward, reverse)		PCR amplicon size (bp)	Extension primers			Mass of SBE products (Da)
		Tm (°C)	% GC		Tm (°C)	% GC	Mass (Da)	
Bacteria	Bac16 1–1	62.8, 66.9	54.5, 48.4	105	45.8	38.9	5545.6	5816.8
Campylobacter coli	Camp005	63.5, 61.9	50.0, 45.2	150	50.0	42.9	6747.4	7034.6
	Camp006	63.6, 59.8	50.0, 43.3	105	49.6	39.1	7511.9	7783.1
Campylobacter jejuni	Camp002	64.1, 63.5	50.0, 50.0	108	46.5	27.3	6781.5	7108.5
Clostridium perfringens	Clos001	61.7, 63.8	46.7, 50.0	101	46.2	21.7	7078.7	7365.9
Escherichia coli/Shigella spp.	Eco001N	62.0, 63.9	46.7, 50.0	108	48.6	36.8	5795.8	6043.0
Enterococcus faecium	Ent001	61.2, 63.9	46.7, 50.0	99	48.4	28.0	7670.0	7957.2
Enterococcus faecalis	Ent003	62.6, 63.9	46.7, 50.0	141	49.3	50.0	5425.5	5752.6
Listeria monocytogenes	Lis001	62.5, 61.8	50.0, 46.7	99	45.7	56.2	4955.2	5282.3
	LisG	–	–	99	52.1	62.5	4979.2	5306.3
Salmonella spp.	Sal002	63.4, 63.8	50.0, 50.0	106	51.1	42.9	6365.2	6652.4
Staphylococcus aureus	Stap001	58.6, 59.3	38.7, 43.3	94	46.0	35.0	6238.1	6565.2

Table 2. Genomic DNA samples used as reference DNA templates in this study.

	No.	Samples (gDNA)	Strains	Sources
Bacteria	1	*Campylobacter coli*	JV20	ATCC
	2	*C. jejuni*	D3071	ATCC
	3	*Clostridium perfringens*	WAL-14572	ATCC
	4	*Escherichia coli*	B171	ATCC
	5	*Enterococcus faecalis*	TX0104	ATCC
	6	*E. faecalis*	TUSoD Ef11	ATCC
	7	*Enterococcus faecium*	TX0133a04	ATCC
	8	*Listeria marthii*	FSL S4-120	ATCC
	9	*L. monocytogenes*	F6900	ATCC
	10	*L. monocytogenes*	Li21	ATCC
	11	*Salmonella enterica*	15/5	ATCC
	12	*S. enterica*	LT2	ATCC
	13	*Shigella* spp.	NMICID504	Human[a]
	14	*Staphylococcus aureus*	CM05	ATCC
Protozoa	15	*Crithidia fasciculata*	ND	Human[b]
	16	*Leishmania martiniquensis*	ND	Human[b]
Virus	17	Severe acute respiratory syndrome coronavirus 2	ND	Human[b]
Human	18	Human	No.4312660	Applied Biosystems, California, United States.
Plant	19	Plant	ND	Teak leaf[c]

Validation and evaluation of MassARRAY-based assay

Determination of sensitivity and specificity of MassARRAY-based assay with gDNA

Table 3. Bacterial isolates used

Samples (gDNA)	Reference (no.)	Lab isolate (no.)	Total (no.)
Staphylococcus aureus	2	7	9
Salmonella spp.	5	13	18
Shigella spp./Escherichia coli	2	6	8
Listeria monocytogenes	4	4	8
Enterococcus faecium	1	4	5
Enterococcus faecalis	1	10	11
Campylobacter jejuni	0	8	8
Campylobacter coli	0	3	3
Clostridium perfringens	0	5	5
Others species	3	7	10
Mixed templates	4	0	4
Total	22	67	89

MassARRAY® System workflow

1 PCR AMPLIFICATION → 2 SAP → 3 SINGLE-BASE EXTENSION (SBE)

4 SAMPLE TRANSFER & DATA ECQUISITION

5 DATA ANALYSIS & REPORTING

28 MINS HAND-ON TIME

135 MINS | 25 MINS | 260 MINS | 60 MINS | 8.3 HRS. ELAPS TIME

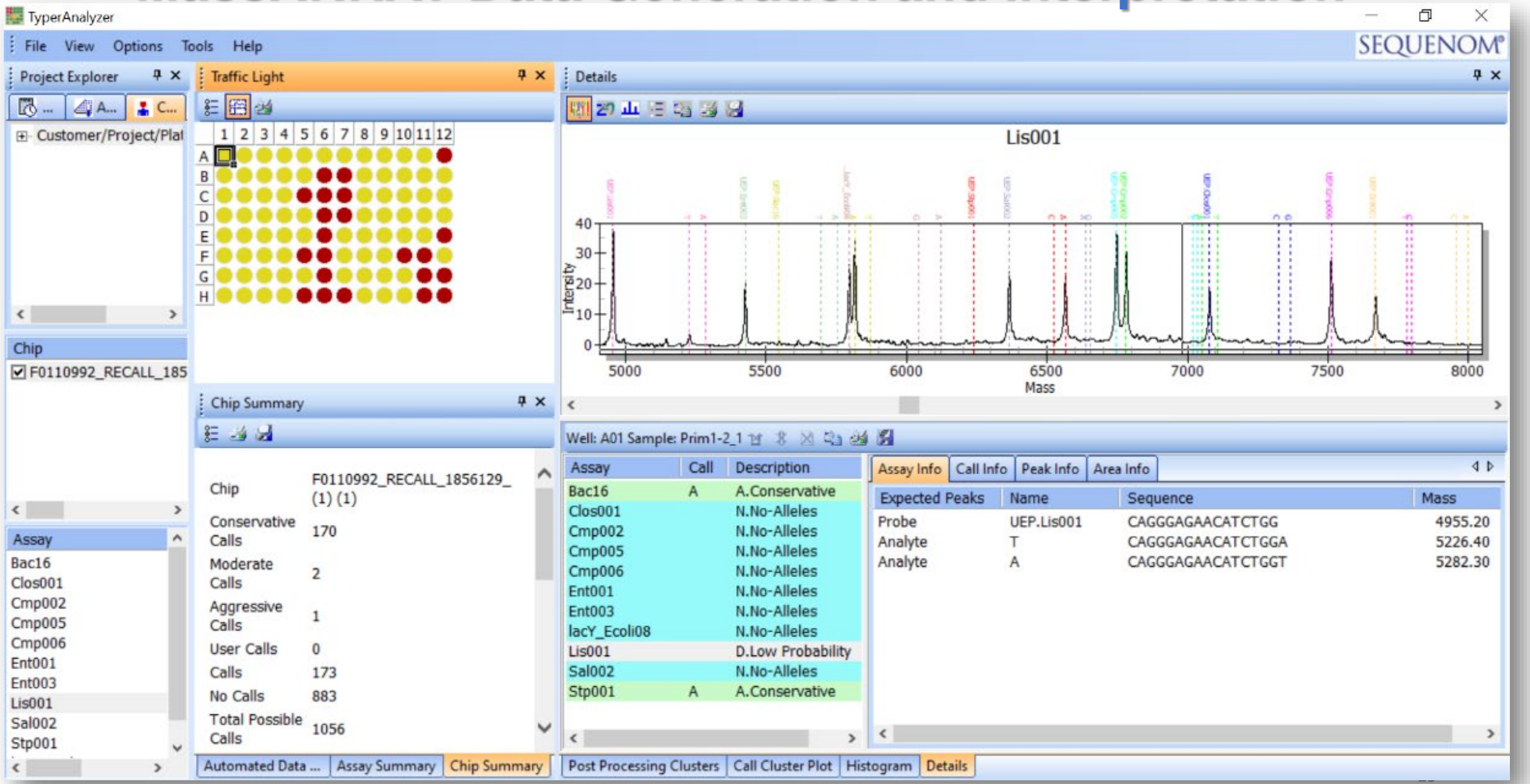
15 MINS Hands-on Time

3 MINS Hands-on Time

5 MINS Hands-on Time

5 MINS Hands-on Time

MassARRAY Data Generation and Interpretation



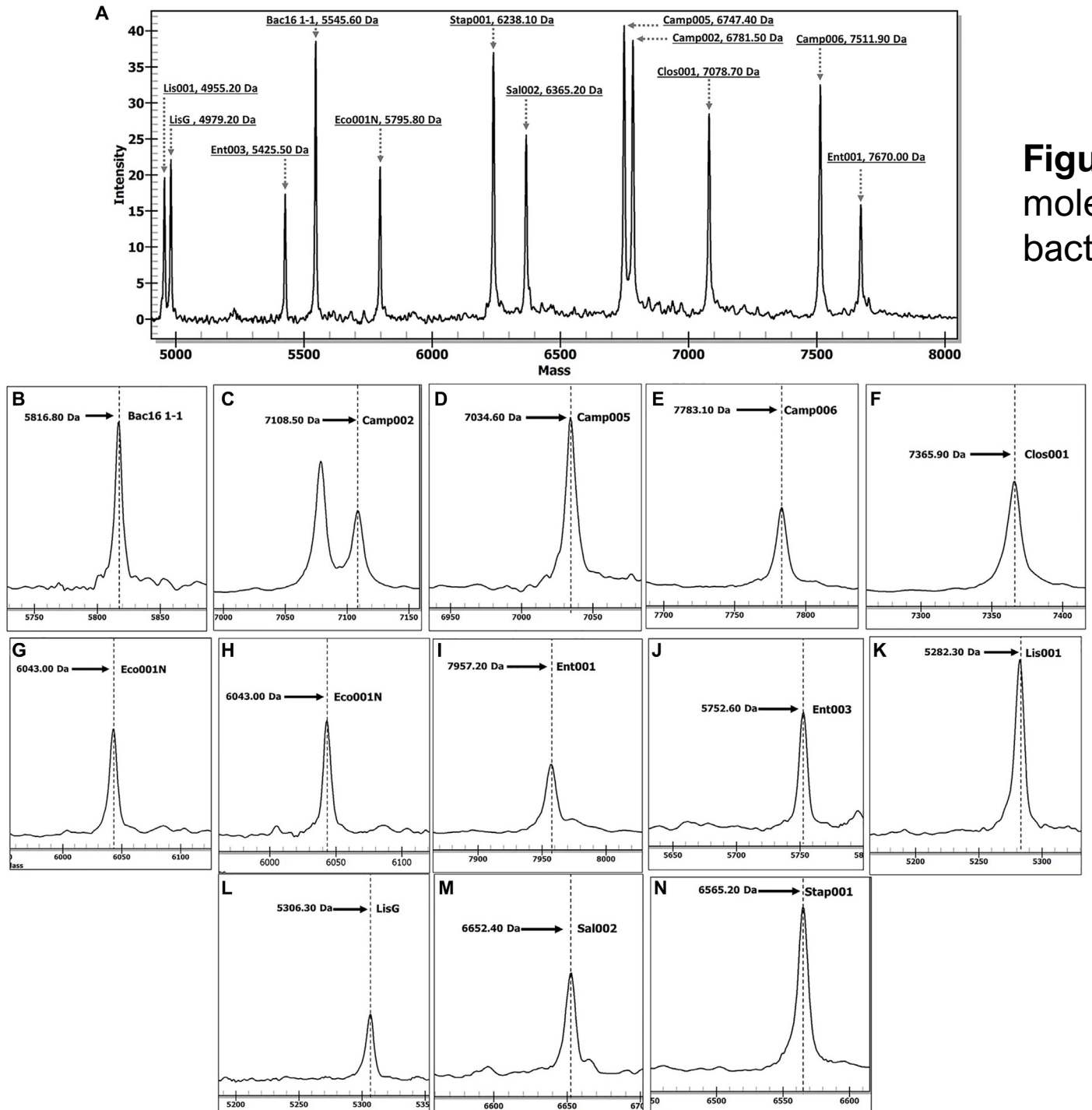


Figure 1. The chromatograms illustrate the specific molecular masses of SBE products corresponding to bacterial targets.

(A) a negative control (DW).
(B) SBE product of bacteria (Bac16 1–1).
(C) SBE products of *C. jejuni*
(D,E) SBE products of *C. coli.*
(F) SBE products of *Cl. perfringens*
(G) SBE products of *E. coli*
(H) SBE product of *Shigella spp*.
(I)SBE products of *E. faecium.*
(J) SBE products of *E. faecalis.*
(K) SBE products of *L. monocytogenes* variant 1.
(L) SBE products of *L. monocytogenes* variant 2
(M) SBE products of *Salmonella spp.*
(N) SBE products of *S. aureus.*

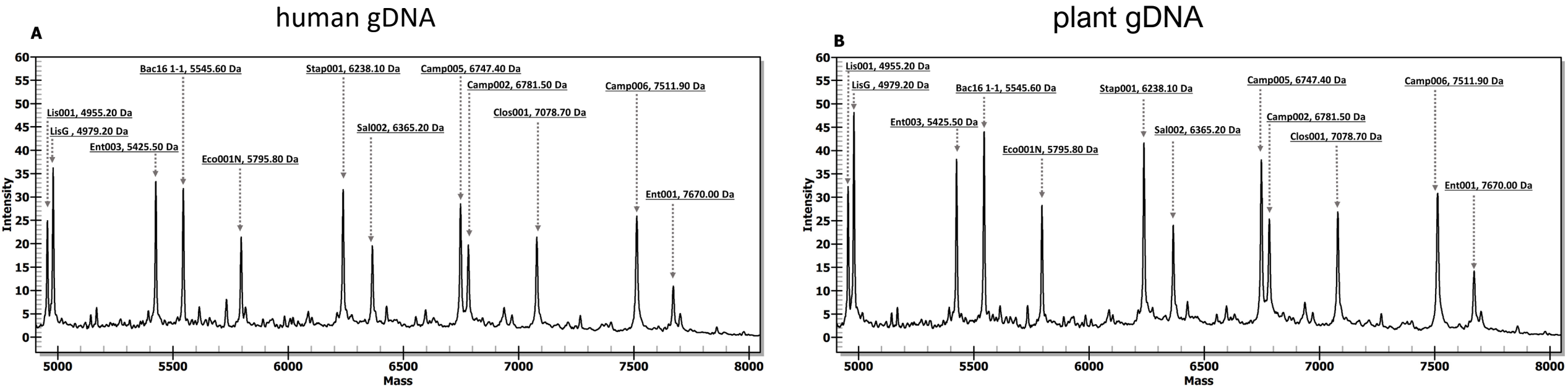
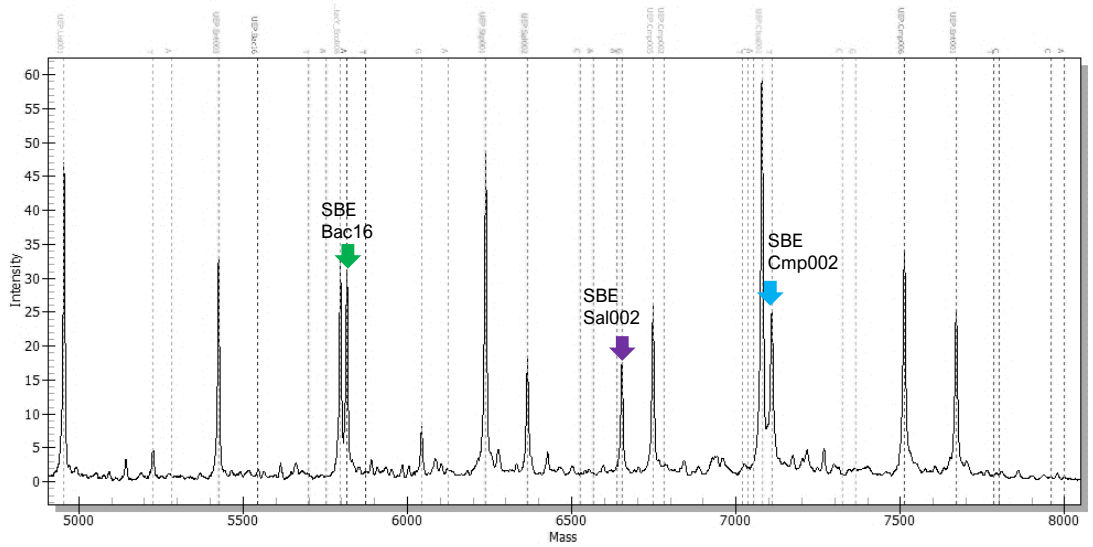


Figure 2. The chromatograms representing negative results clearly illustrate specific molecular mass of the extension primers when human gDNA (A) and plant gDNA (B) were used templates in the MassARRAY-based assay. Similar chromatograms were observed for additional negative controls, including *Listeria marthii*, *Crithidia fasciculata*, *Leishmania martiniquensis*, and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). These chromatograms indicated consistent negative results across different non-targeted organisms, thus confirming the absence of cross-reaction in the MassARRAY-based assay system.

Salmonella* spp./*C. jejuni



- *16s rRNA*
- *Salmonella spp.*
- *C. jejuni*
- *E. faecium*

Salmonella* spp./*C. jejuni/E. faecium

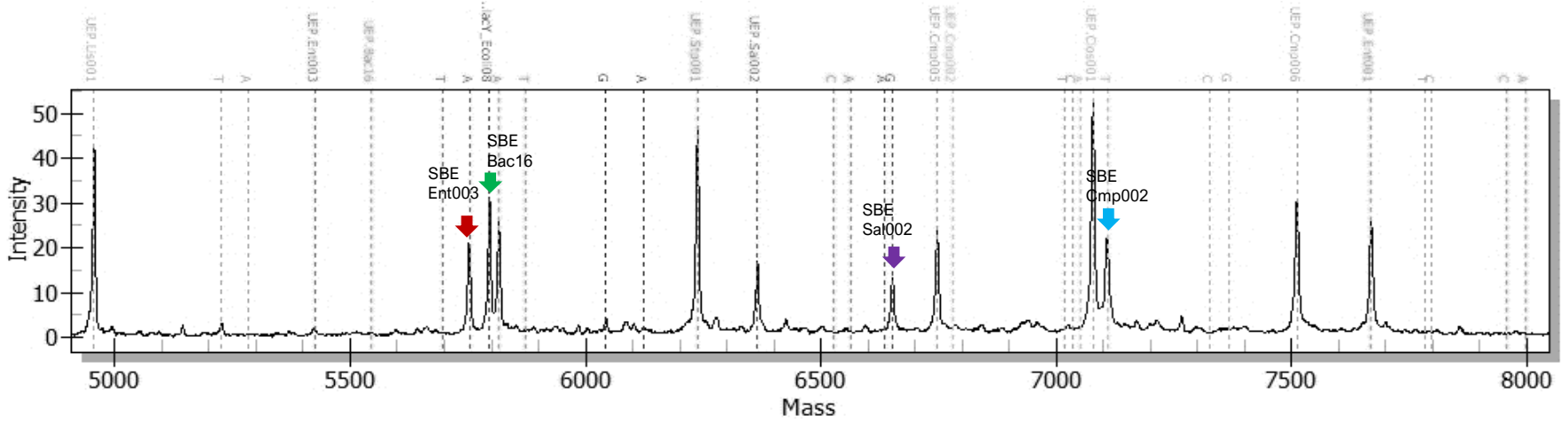


Figure 3. The chromatograms representing negative results clearly illustrate specific molecular mass of the extension primers when using mixture template gDNA

Sensitivity and Specificity of MassARRAY-based assay

Assay sensitivity 98.8%
Assay specificity 100 %

Sample (genomic DNA)	Number of tested samples		Total	Base calling			%Sensitivity	%Specificity
	Reference strians	Lab isolated strains		Detected	Not detected	Cross-reaction		
Staphylococcus aureus	2	7	9	9	0	0	100	100
Salmonella spp.	5	13	18	18	0	0	100	100
Shigella spp. / *E. coli*	2	6	8	8	0	0	100	100
Listeria monocytogenes	4	4	8	8	0	0	100	100
Enterococcus faecium	1	4	5	5	0	0	100	100
Enterococcus faecalis	1	10	11	11	0	0	100	100
Campylobacter jejuni	0	8	8	8	0	0	100	100
Campylobacter coli	0	3	3	3	0	0	100	100
Clostridium perfringens	0	5	5	5	0	0	100	100
Others species								
Clostridium spp.	0	4	4	3 [a]	1	0	75	100
Klebsiella pneumoniae	0	3	3	3 [a]	0	0	100	100
Listeria inocua	2	0	2	2 [a]	0	0	100	100
Campyrobacter lari	1	0	1	1 [a]	0	0	100	100
Total	18	67	85	84	1	0	98.8	100

Data summarized from two independent experiments.

[a] Base calling for *16s rRNA* gene only.

Validation and evaluation of MassARRAY-based assay

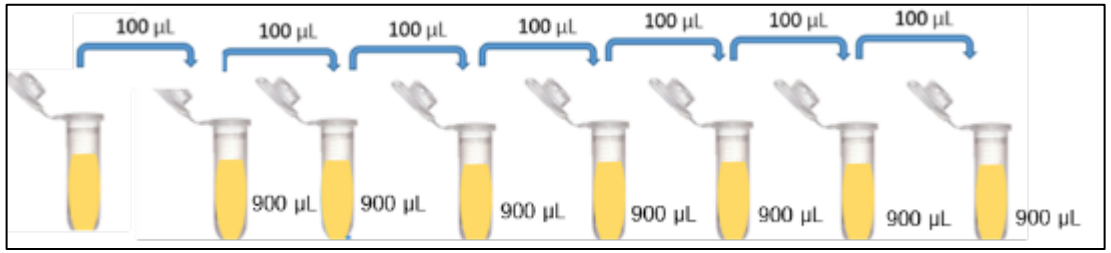
Determination of limit of detection (LOD) with bacterial cells

Bacterial culture incubated at 37° C or 41 ° C for 18 h

↓

Adjusted OD 600 = 1.85 and collected bacterial cells

↓



→ 100 µL for plate count

↓

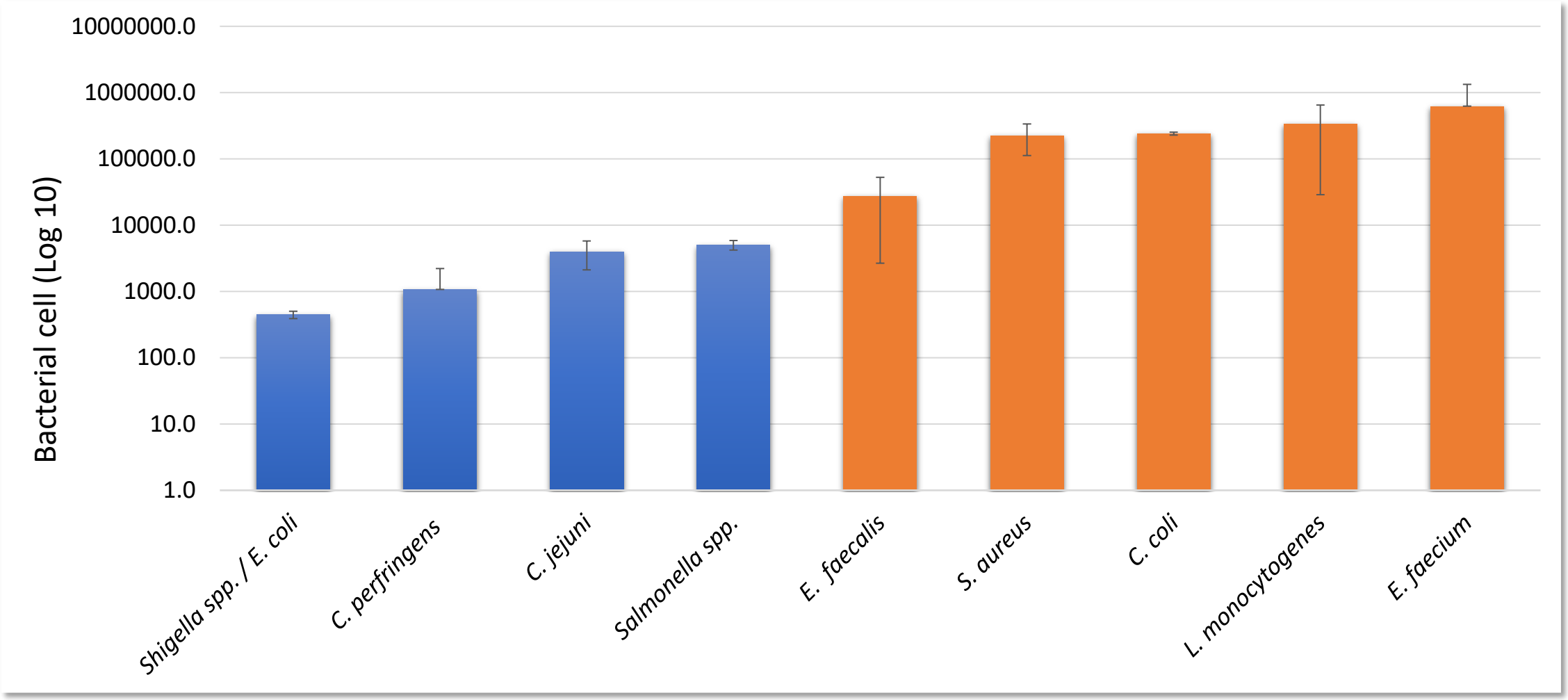
100 µL for gDNA extraction using QuickExtract™

↓

MassARRAY detection

LOD of MassARRAY-based assay

- LOD ~ 10^2 – 10^3 cells of *E. coli, C. perfringens, C. jejuni,* and *Salmonella* spp.
- LOD ~ 10^4 – 10^5 cells of *E. faecalis, S. aureus, C. coli, L. monocytogenes,* and *E. faecium*

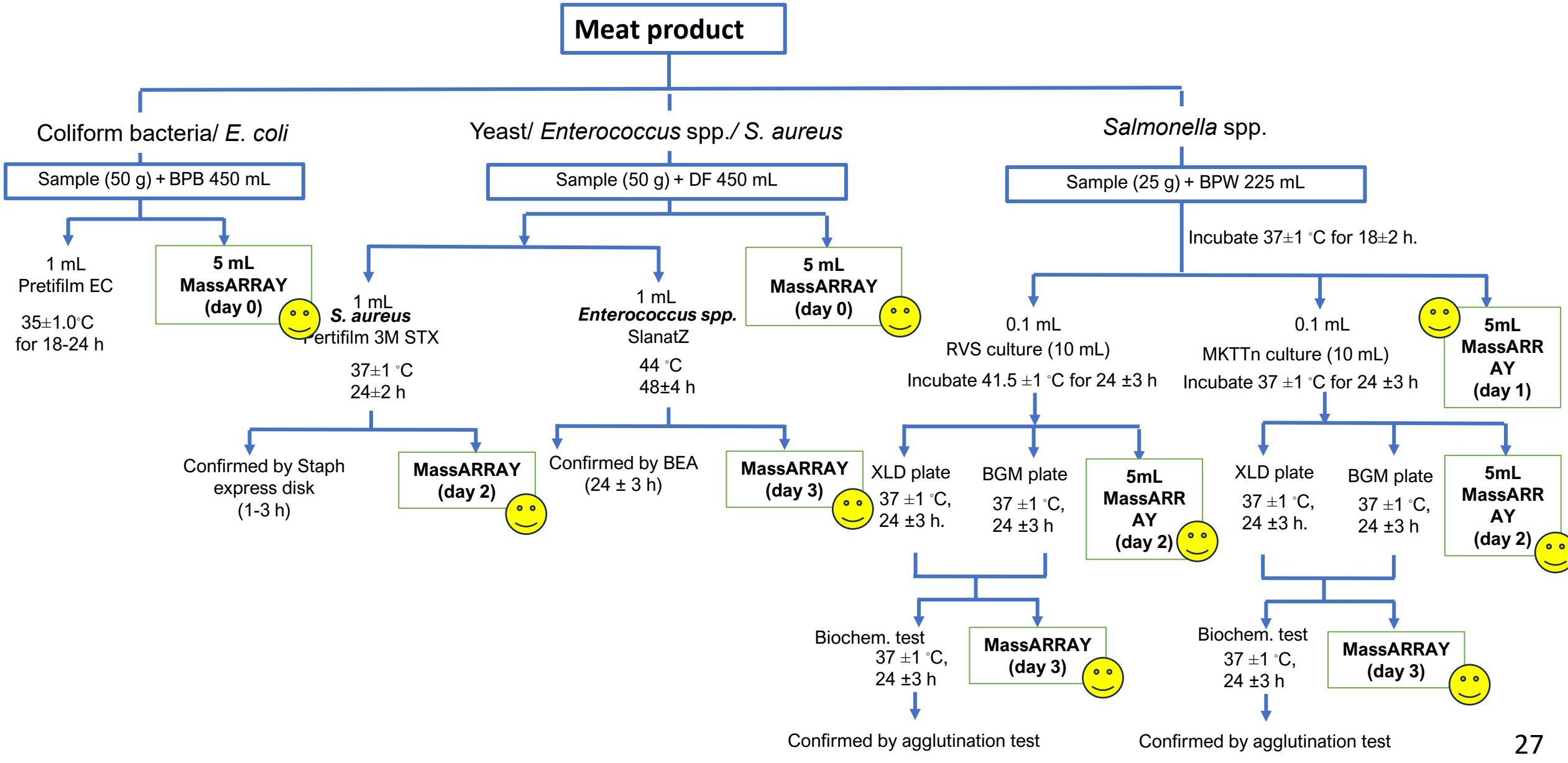


Data obtained from three independent experiments.

Validation of MassARRAY with unknown specimens

In collaboration with
Bureau of Quality Control of Livestock Products
(สำนักตรวจสอบคุณภาพสินค้าปศุสัตว์ (สตส.) กรมปศุสัตว์)

Pathogen detection procedure of Bureau of Quality Control of Livestock Products



Lab Workflow

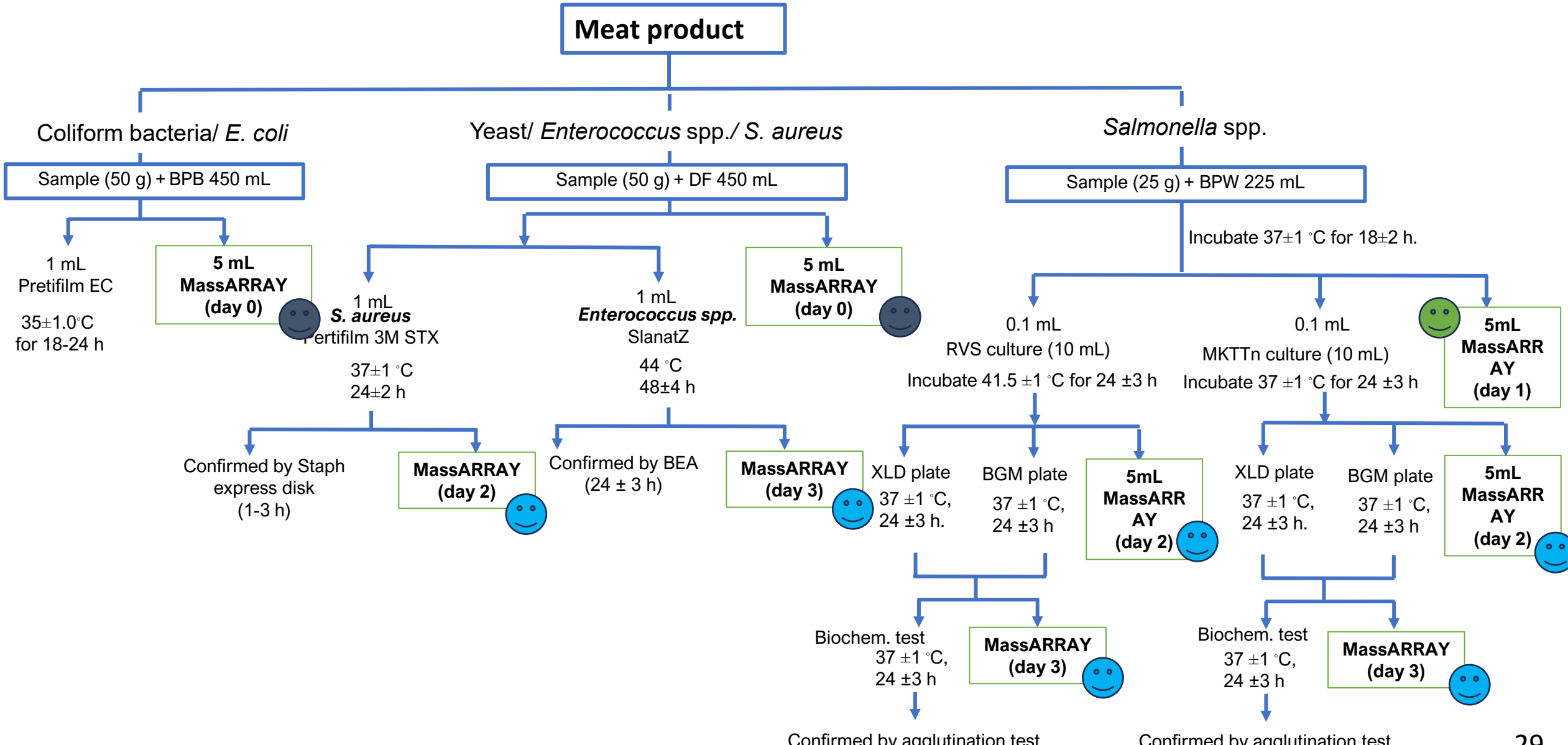
Collected 5-10 mL, centrifuged at 4,000-7,000 rpm 10 min.
and decarded media.

The DNA extraction procedure employed the
MagPurix® Bacterial DNA Extraction Kit (ZP02006),

1 µl of gDNA for PCR by using EconoTaq® Polymerase

MassARRAY

Pathogen detection procedure of Bureau of Quality Control of Livestock Products



Result: Validation of MassARRAY with unknown specimens

Sample no.	Sample ID	Bacterial culture-based method					MassARRAY-based assay				
		Bacteria	*E. coli/ Shigella* spp.	*Enterococcus* spp.	*Salmonella* spp.	*S. aureus*	Bacteria	*E. coli/ Shigella* spp.	*Enterococcus* spp.	*Salmonella* spp.	*S. aureus*
1	4,627	P	N	N	N	N	P	N	N	N	N
2	4,628	P	N	N	N	N	P	N	N	N	N
3	4,646	P	P	N	N	N	P	P	N	N	N
4	5,543	P	P	N	N	P	P	P	N	N	P
5	5,545	P	N	N	N	N	P	N	N	N	N
6	5,546	P	N	N	N	N	P	N	N	N	N
7	5,555	P	N	N	N	N	P	N	N	N	N
8	5,556	P	P	P	N	N	P	P	P*	N	N
9	5,571	P	P	N	N	N	P	P	N	N	N
10	38,532	P	P	P	P	N	P	P	P*	P	N
11	38,633	P	P	P	P	P	P	P	P*	P	P
12	38,809	P	P	P	N	N	P	P	P*	N	N

N, negative result; P, positive result. *The method specifically identified the species as *E. faecalis*.

91 samples showed positive bacteria
12 samples presented positive target bacteria

100% concordance with standard culture method

SUMMARY

- The developed MassARRAY-based assay demonstrated its high-throughput performance in simultaneously identifying targeted species in a single assay with high specificity.

- Its successful application to field samples positioned it as a compelling choice for the rapid detection of foodborne bacterial pathogens in real-world field settings.

- Furthermore, the cost effectiveness, time efficiency, and accuracy inherent to MassARRAY technology make it highly suitable for adoption in food industrial laboratories, particularly those engaged in quality control of food products.

MassARRAY-based application for food safety and security

Acknowledgement

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BIOTEC

a member of NSTDA

Thank you

Q&A
Discussion

Efficiency of MassARRAY detection

Sample	Sample type	Number
Meat sample	DF/BPB	103 (day 0)
Total 103 samples	BPW	103 (day 1)
	RVS /MKTTn broth	103 (Day 2)
	Selective plates	11 (Day 3)*

* The day of sample obtained from Bureau of Quality Control of Livestock Products.