"Are national and international standardizations necessary for new molecular detection methods in food industry?"

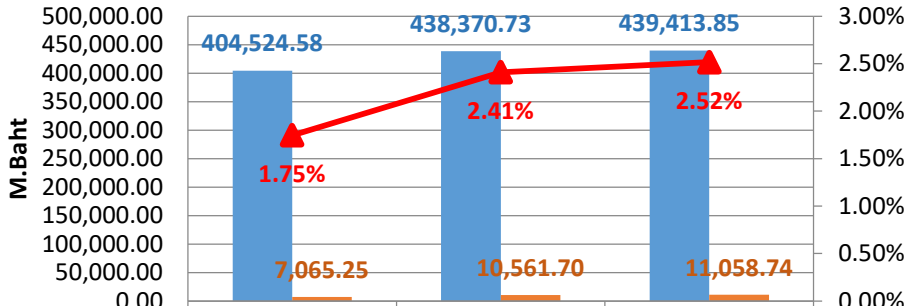
Mongkol Vesaratchavest, Ph.D.

Research and Development Center

Betagro Group

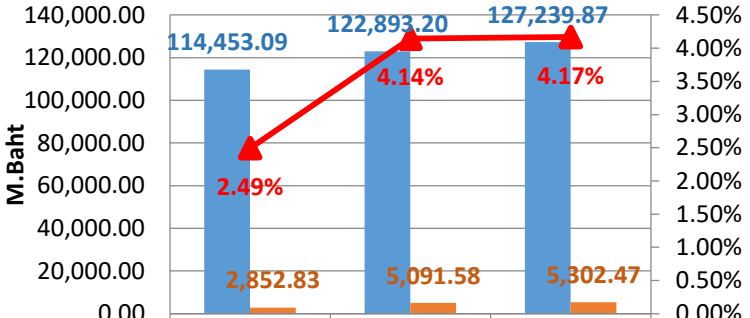
Consolidated Sales and % Net Profit of Thai Listed Food Companies : 2013-2015

CPF



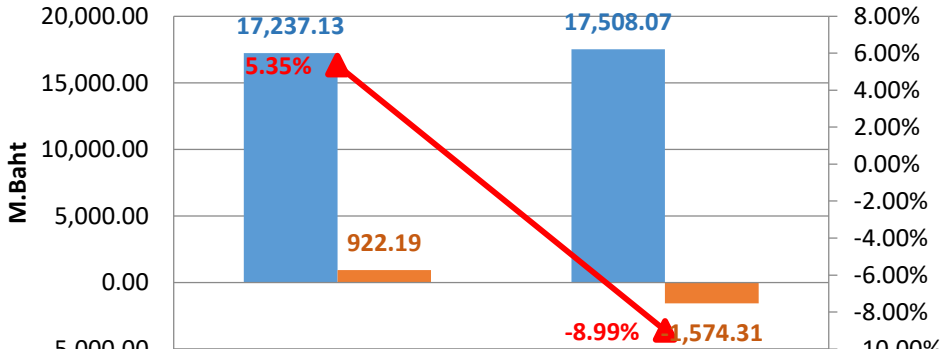
	2013	2014	2015
R	404,524.58	438,370.73	439,413.85
NP	7,065.25	10,561.70	11,058.74
%NP	1.75%	2.41%	2.52%

TU



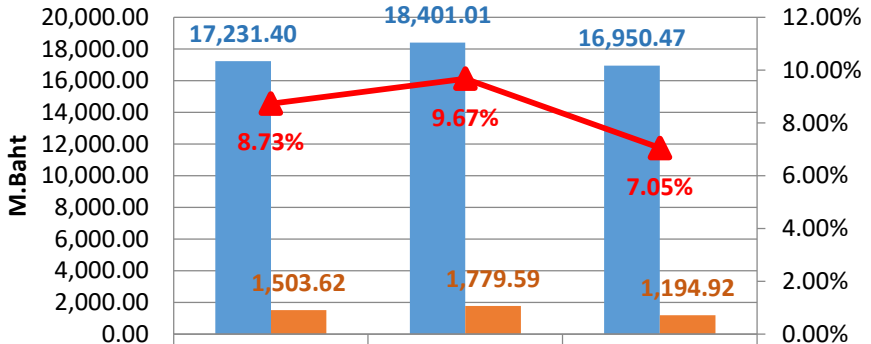
	2013	2014	2015
R	114,453.09	122,893.20	127,239.87
NP	2,852.83	5,091.58	5,302.47
%NP	2.49%	4.14%	4.17%

TFG



	2014	2015
R	17,237.13	17,508.07
NP	922.19	-1,574.31
%NP	5.35%	-8.99%

GFPT



	2013	2014	2015
R	17,231.40	18,401.01	16,950.47
NP	1,503.62	1,779.59	1,194.92
%NP	8.73%	9.67%	7.05%

(http://www.set.or.th/set/mainpage.do?language=th&country=TH)

E.coli Homepage
General Information
Enterotoxigenic *E. coli* (ETEC)
Diarrheagenic *E. coli*
Timeline for Reporting Cases of *E. coli* O157 Infection
List of Selected Outbreaks
2016 Outbreaks +
2015 Outbreaks −
O157:H7 Infections Linked to Costco Rotisserie Chicken Salad +
O26 Infections Linked to Chipotle Mexican Grill −
Advice to Food Industries & Consumers
Case Count Maps
Epi Curves
Signs & Symptoms
Key Resources
2014 Outbreaks +
2013 Outbreaks +

CDC > E.coli Homepage > 2015 Outbreaks

Multistate Outbreaks of Shiga toxin-producing *Escherichia coli* O26 Infections Linked to Chipotle Mexican Grill Restaurants (Final Update)

Posted February 1, 2016 12:00 PM ET

These two outbreaks appear to be over. However, *E. coli* is still an important cause of human illness in the United States. More information about *E. coli*, and steps people can take to reduce their risk of infection, can be found on the CDC *E. coli* web page.

Highlights

- **Read the Advice to Food Industries & Consumers »**
- These two outbreaks appear to be over. The most recent illness reported to CDC started on December 1, 2015.
- CDC, the U.S. Food and Drug Administration, the U.S. Department of Agriculture's Food Safety and Inspection Service, and public health officials in several states investigated two separate outbreaks of Shiga toxin-producing *Escherichia coli* O26 (STEC O26) infections.
  - In the initial, larger outbreak, 55 people infected with the outbreak strain of STEC O26 were reported from 11 states. Twenty-one ill people were hospitalized.
  - In the second, smaller outbreak, 5 people infected with a different strain of STEC O26 were reported from 3 states. One ill person was hospitalized.
  - There were no reports of hemolytic uremic syndrome and no deaths in either outbreak.
- Investigators used whole genome sequencing (WGS), an advanced laboratory technique,

At A Glance

Initial, Larger Outbreak

- Case Count: 55
- States: 11
- Deaths: 0
- Hospitalizations: 21

Second, Smaller Outbreak

- Case Count: 5
- States: 3
- Deaths: 0
- Hospitalizations: 1

More Information

Multistate Outbreak of Listeriosis Linked to Packaged Salads Produced at Springfield, Ohio Dole Processing Facility (Final Update)

Posted March 31, 2016 9:00 AM ET

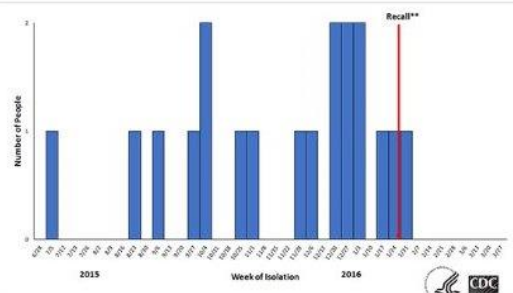
This outbreak appears to be over. However, *Listeria* remains an important cause of serious, life-threatening human illness in the United States. For more information about *Listeria* and steps that people can take to reduce their risk of infection, visit CDC's *Listeria* webpage.

Highlights

- This outbreak appears to be over. However, *Listeria* remains an important cause of serious, life-threatening human illness in the United States. For more information about *Listeria* and steps that people can take to reduce their risk of infection, visit CDC's *Listeria* webpage.
- CDC, several states, and the U.S. Food and Drug Administration (FDA) investigated a multistate outbreak of *Listeria monocytogenes* infections (listeriosis).
  - A total of 19 people infected with the outbreak strain of *Listeria* were reported from nine states.
  - All 19 people were hospitalized, and one person from Michigan died as a result of listeriosis. One illness was reported in a pregnant woman.
  - Whole genome sequencing (WGS) performed on *Listeria* isolates from all 19 ill people showed that the isolates were closely related genetically.
- According to the Public Health Agency of Canada, ill people in Canada were infected with the same outbreak strain of *Listeria*

At a Glance:

- **Case Count: 19**
- **States: 9**
- **Deaths: 1**
- **Hospitalizations: 19**
- **Recall: Yes**



Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado (FINAL UPDATE)

Posted August 27, 2012 10:30 AM ET

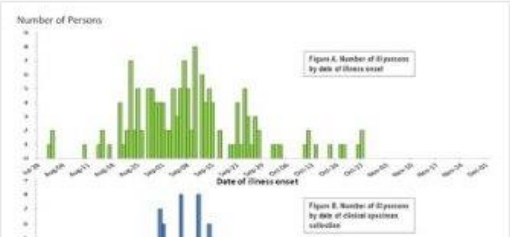
This investigation is closed. *Listeria monocytogenes* infection (listeriosis) is an important cause of illness in the United States. More information about listeriosis, and steps people can take to reduce their risk of infection, can be found on the CDC *Listeria* website.

Highlights

- This multistate outbreak of *Listeria monocytogenes* infections (listeriosis) ended in October 2011; however, on December 8, a fifth outbreak-associated subtype of *Listeria* was isolated from a sample of cantaloupe collected during the investigation.
- The fifth subtype matches that of *Listeria* isolated from one patient. This patient's illness had been reported, but had not previously been linked to the outbreak. Therefore, this case has been added to the number of outbreak-associated illnesses, bringing the total number of outbreak-associated illnesses to 147 persons infected with any of the five outbreak-associated subtypes of *Listeria*. These persons lived in 28 states.
- The number of outbreak-associated deaths has increased by three since December 8, 2011. In total, 33 deaths from outbreak-associated cases of listeriosis have been reported to CDC. In addition, one woman pregnant at the time of illness had a miscarriage.
- Ten other deaths not attributed to listeriosis occurred among persons who had been infected with an outbreak-associated subtype. State and local public health officials

At a Glance:

- **Case Count: 147**
- **States: 28**
- **Deaths: 33**
- **Hospitalizations: 143**
- **Recall: Yes**
- **Media Telebriefing: September 28, 2011**
  Transcript and Audio (MP3, 8.58 MB)



Multistate Outbreak of *Salmonella* Poona Infections Linked to Imported Cucumbers (Final Update)

Posted March 18, 2016 2:30 PM ET

This outbreak appears to be over. However, *Salmonella* remains an important cause of human illness in the United States. For more information about *Salmonella* and steps that people can take to reduce their risk of infection, visit CDC's *Salmonella* webpage.

Highlights

- This outbreak appears to be over. However, *Salmonella* remains an important cause of human illness in the United States. For more information about *Salmonella* and steps that people can take to reduce their risk of infection, visit CDC's *Salmonella* webpage.
- CDC, multiple states, and the U.S. Food and Drug Administration (FDA) investigated a multistate outbreak of *Salmonella* Poona infections.
  - A total of 907 people infected with the outbreak strains of *Salmonella* Poona were reported from 40 states.
  - A total of 204 ill people were hospitalized, and six deaths were reported from Arizona (1), California (3), Oklahoma (1), and Texas (1). *Salmonella* infection was not considered to be a contributing factor in two of the three deaths in California.

At A Glance

- Case Count: 907
- States: 40
- Deaths: 6
- Hospitalizations: 204
- Recall: Yes

- Advice to Retailers & Consumers
- Signs & Symptoms
- Key Resources

Food Safety News
Breaking news for everyone's consumption

350 sick in outbreak traced to Polish eggs; trade regs eyed

By News Desk | December 7, 2016

The number of people infected by Salmonella traced to a Polish egg farm has topped 350 with 10 countries now reporting confirmed and probable cases.

The victim count has increased substantially since October when the European Food Safety Authority reported 260 illnesses across seven countries between May 1 and Oct. 12 this year.

Wozniak Poultry Farms in Poland has been identified as the egg producer. Recalls were initiated across Europe and in Hong Kong and the outbreak has stirred questions about trade regulations.

A member of the European Parliament representing Croatia put his concerns in writing in November when he asked the European Commission if a member state can impose "stricter standards and checks than those provided for under the EU

Science News

from research organizations

Cause of Maryland food poisoning outbreak traced to Asia

Date: April 6, 2016

Source: American Society for Microbiology

Summary: Vibrio parahaemolyticus caused an outbreak of food poisoning in Maryland in 2010. The pathogen strain sequenced from patients proved to be the same strain as one of those found in raw oysters from local restaurants, strong evidence that the oysters were the source of the illness. That particular strain of V. parahaemolyticus was not local, but was traced to Asia, report investigators.

Share:

FULL STORY

Vibrio parahaemolyticus caused an outbreak of food poisoning in Maryland in 2010. The pathogen strain sequenced

Food Safety News
Breaking news for everyone's consumption

Chipotle Outbreak Illness Count Hits 514 as CMG Stock Dives Below $500

By Dan Flynn | December 23, 2015

Less than 24 hours after the federal Centers for Disease Control and Prevention (CDC) in Atlanta announced the existence of five cases of E. coli O26 associated with Chipotle Mexican Grill with different DNA fingerprints than others in their ongoing multiple state outbreak investigation, "CMG" stock crashed through the $500 per share floor.

The way the market read the latest CDC report was to assume the Denver burrito maker is likely responsible for a sixth outbreak of food borne illnesses since July because CDC found a second E. coli strain, a rare variant of O26.

And clearly it was that CDC report that dropped Chipotle's stock at closing on Tuesday to $495.62 per share or down 5.06 percent for a loss of $26.30 on the day. Chipotle has not traded at such levels since late 2013. And it was trading above $750 per share when the outbreaks began six months ago.

Outbreaks with differing strains of a pathogen do occur and CDC could decide to include the new cases within the larger nine-state outbreak involving 53 cases with the original O26 strain. The five new cases involve customers of two Chipotle

Food Safety News
Breaking news for everyone's consumption

Listeria victim seeks jury trial in federal civil case against Dole

By Coral Beach | March 10, 2016

The daughter of an Ohio woman who is fighting her way back from a coma after eating a contaminated salad has filed the first lawsuit against Dole Food Co. related to a deadly, seven-month-long international Listeria outbreak.

In her federal court complaint, Constance Georgostathis seeks unspecified damages for her mother Angeliki "Kiki" Christofield. Christofield fell ill in January after eating part of a packaged salad from Dole's Springfield, OH, production facility.

Later in January the company recalled all salads produced at that plant because a routine government sampling program found Listeria monocytogenes in a packaged salad collected from a retail location. Dole officials reported to the U.S. Food and Drug Administration on Jan. 21 that the Springfield plant had been shut down for investigation and cleaning.

"Testing by the Ohio Department of Health on the same bag of Dole prepackaged salad mix that Mrs. Christofield had consumed showed that it was positive for Listeria," according to the civil complaint.

Dole's corporate policies do not allow for public comments on pending litigation, according to company spokesman William Goldfield. The status of operations at Dole's Springfield, OH, salad plant is unknown.

Food Safety News
Breaking news for everyone's consumption

Settlement Reached for 66 Victims of 2011 Cantaloupe Listeria Outbreak

By News Desk | February 11, 2015

A settlement was recently reached between 66 victims of the 2011 Listeria outbreak linked to Jensen Brothers cantaloupe and some of the 20 defendants.

The terms of the settlement are confidential, said Williams Marler of Seattle-based Marler Clark, a food-safety law firm.

"The matter was resolved by mutual agreement of the parties," Marler said. (Marler Clark underwrites **Food Safety News**, and Marler is publisher of the site.)

Marler represented the plaintiffs in their lawsuits against Kroger, which sold some of the cantaloupe at its retail stores, a cantaloupe broker, and a third-party auditing company, among others. Walmart, another defendant, had already settled.

Will Steele, president of Frontera, Edinburg, Texas, said the company is "focused on strengthening the industry's traceability efforts."

"The matter is in the process of being resolved," Steele said.

Food Safety News
Breaking news for everyone's consumption

Outbreak tied to strawberries slowing; victims sue Sysco Corp.

By Coral Beach | October 15, 2016

New cases of Hepatitis A infections linked to strawberry smoothies have slowed so much that the state hardest hit, Virginia, is no longer providing daily updates.

In a weekly update, posted Thursday, the Virginia Department of Health reported 107 people who have tested positive for Hepatitis A reported drinking smoothies Tropical Smoothie Café prior to becoming ill.

Nationwide there were 131 confirmed victims as of Sept. 29, according to the Centers for Disease Control and Prevention, which has not posted an update on the outbreak since Sept. 30. A CDC spokeswoman said Friday afternoon the agency will likely update the public next week on the status of the outbreak.

Of the 131 sick people, who live in eight states, about 40 percent — 52 people as of Sept. 29 — have had symptoms so severe that they required hospitalization.

Virginia officials say the first illnesses began in early May, with new victims continuing to be identified through September. It can take up to 50 days for symptoms to develop after exposure to Hepatitis A.

To prevent outbreak incidents and business losses from happening

- Due to its primarily direct contact to consumers, food product can cause consumers a serious problem if the quality of food product goes wrong.

- Enhancing food safety measures are needed in order to prevent outbreak incidents and business losses.

- One of food safety measures that can be enhanced is doing more food testing or food monitoring.

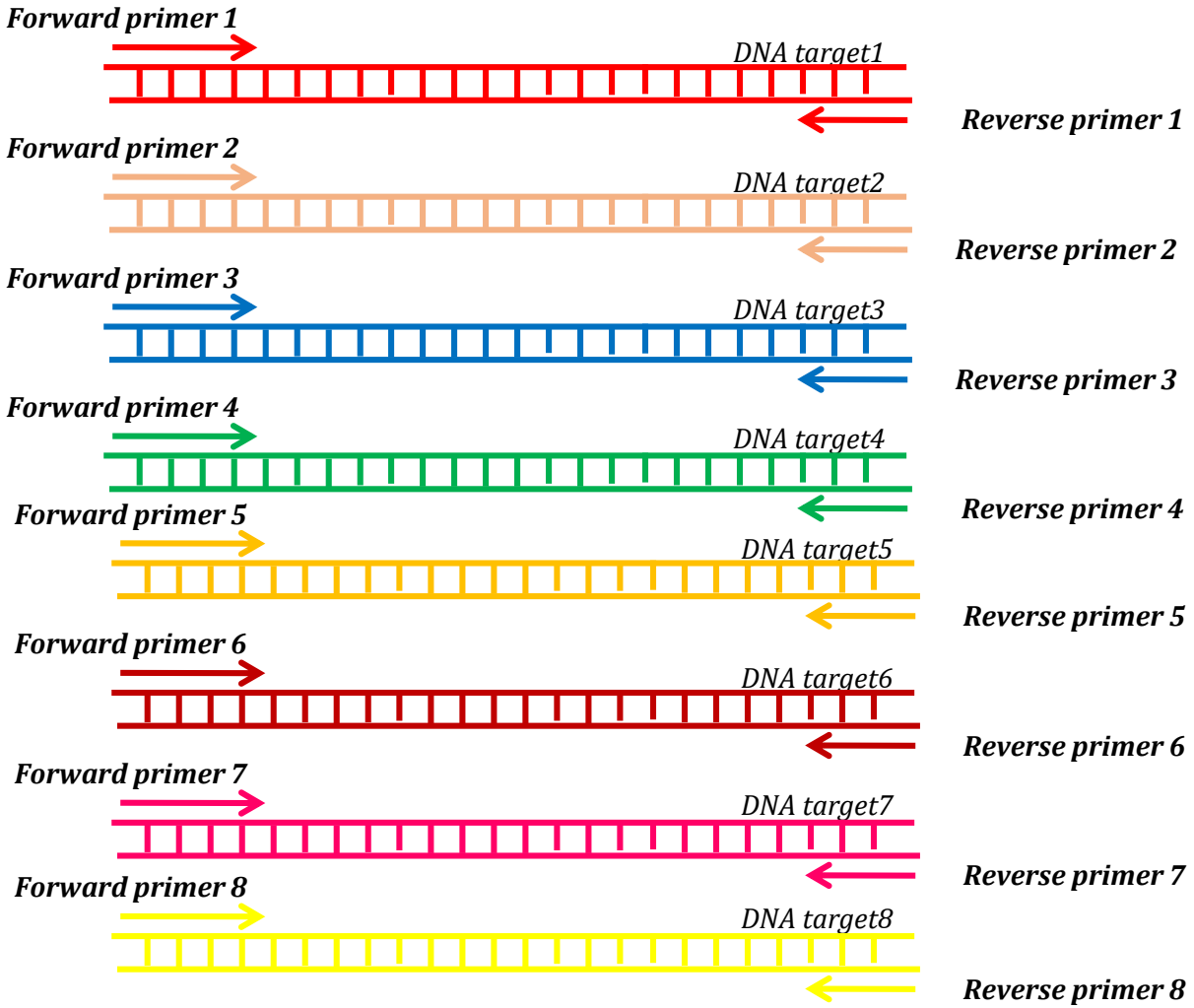
- With the conventional food testing, increasing cost of food production can not be avoided when more food monitoring are done

- A molecular (DNA) method has a lot to offer such as a cost effectiveness, a faster time to result, a simultaneous detection of multiple targets (multiplex bio-analysis), and etc., when compared with conventional method.

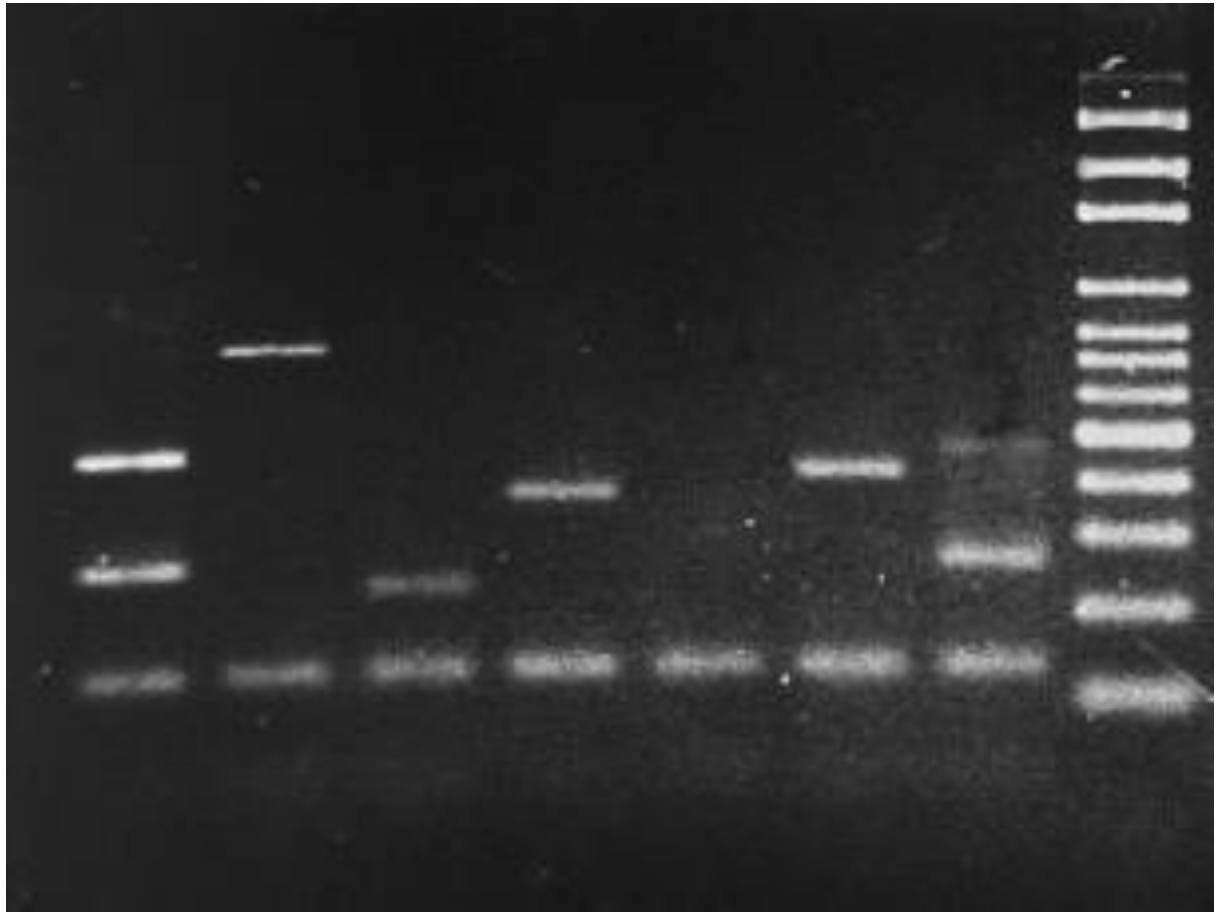
- With a faster time to result and more detailed information generated by multiplex bio-analysis, food producers are able to better manage and promptly intervene in order to enhance their food safety measures and limit business losses.

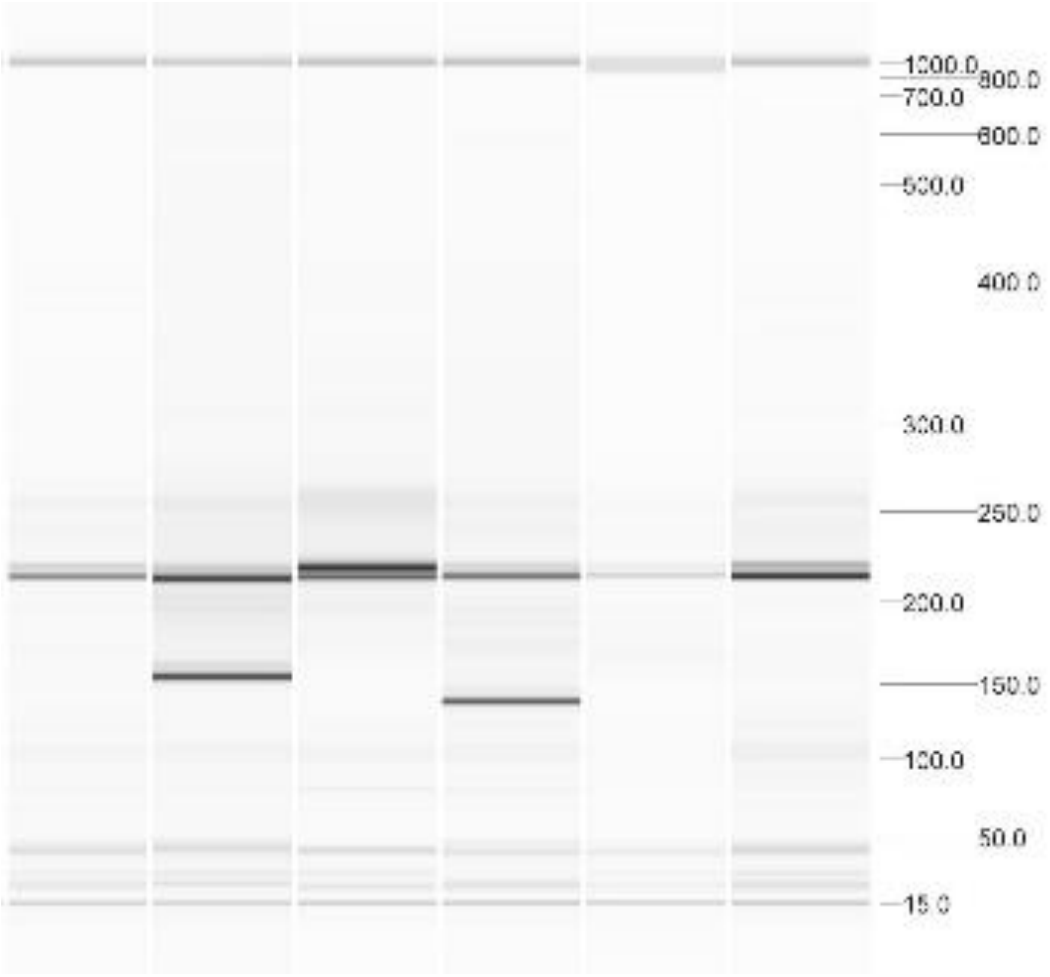
Multiplex-bio analysis

Multiplex PCR



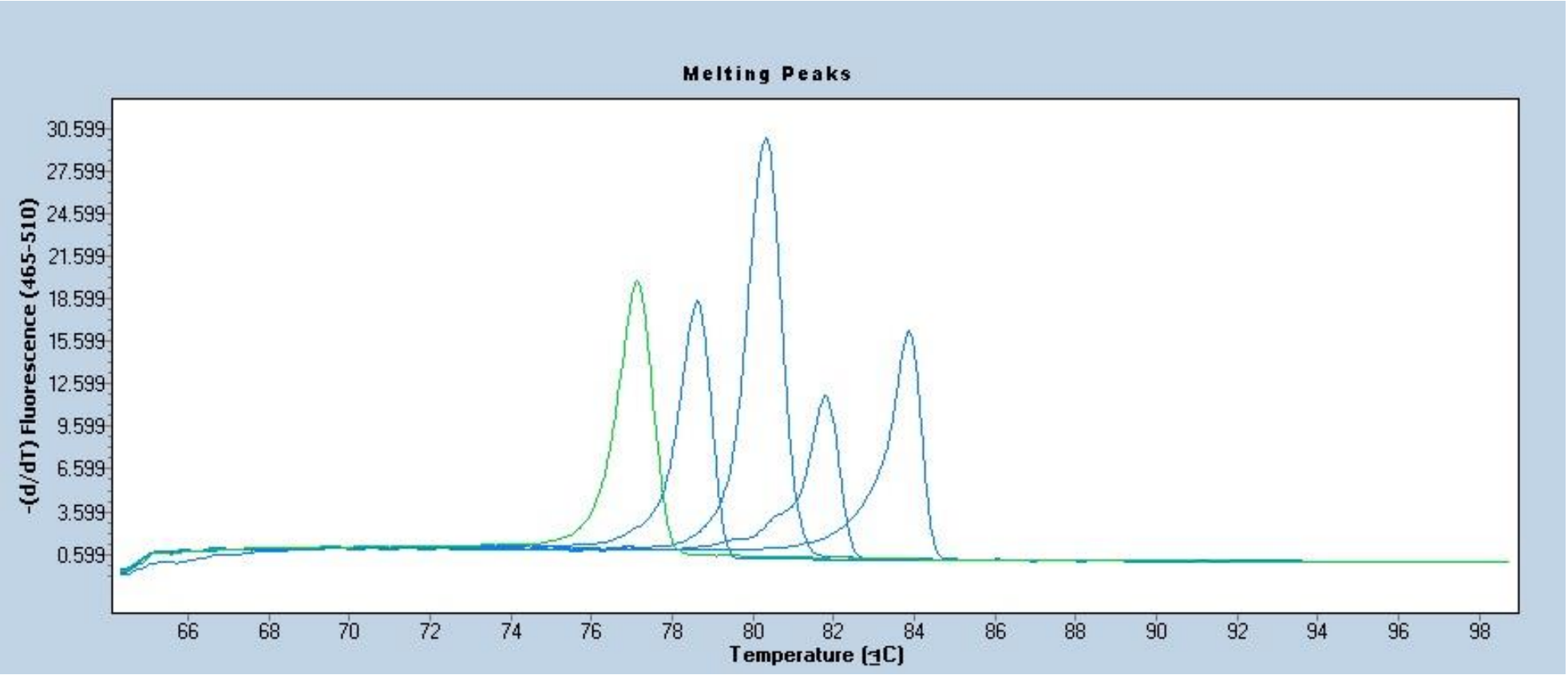
Gel Electrophoresis Analysis





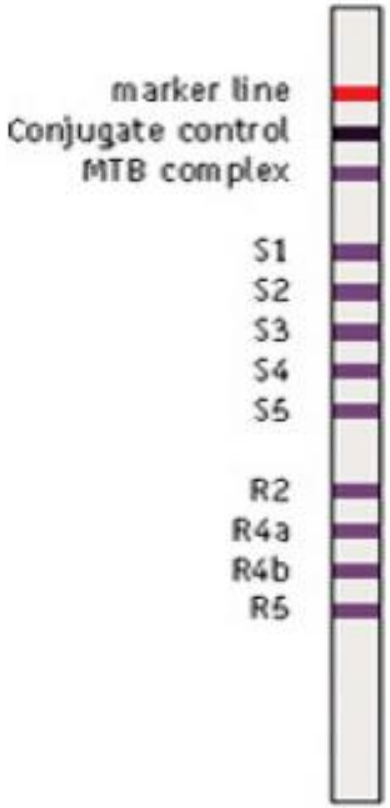
High Resolution Melt (HRM) Analysis

Melting Temperature (Tm)



DNA Probe and DNA Target Hybridization Analysis

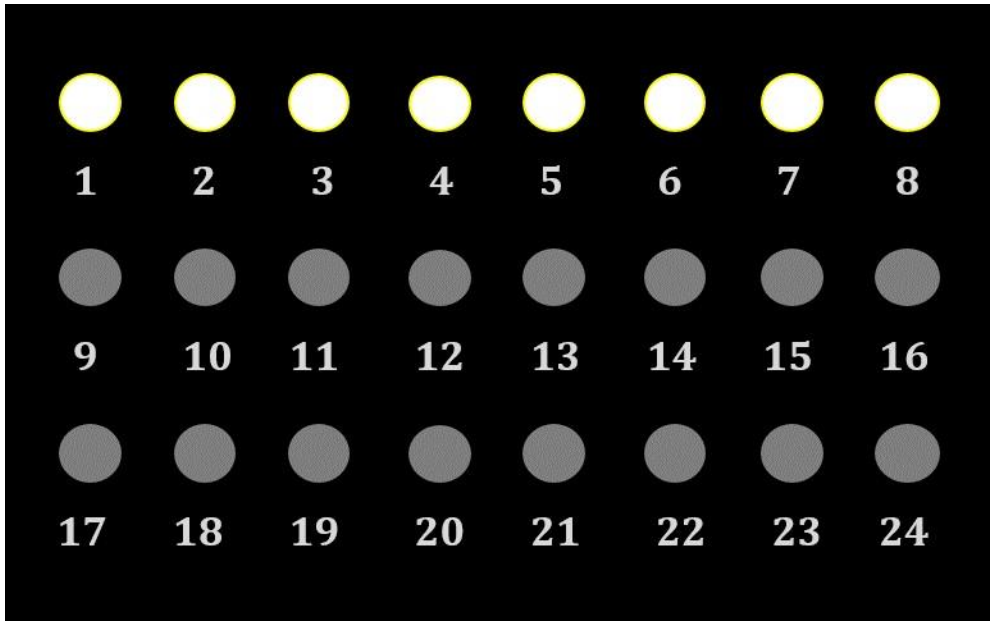
Figure 1.2. Position of the oligonucleotide probes on the INNO-LiPA Rif.TB strip.

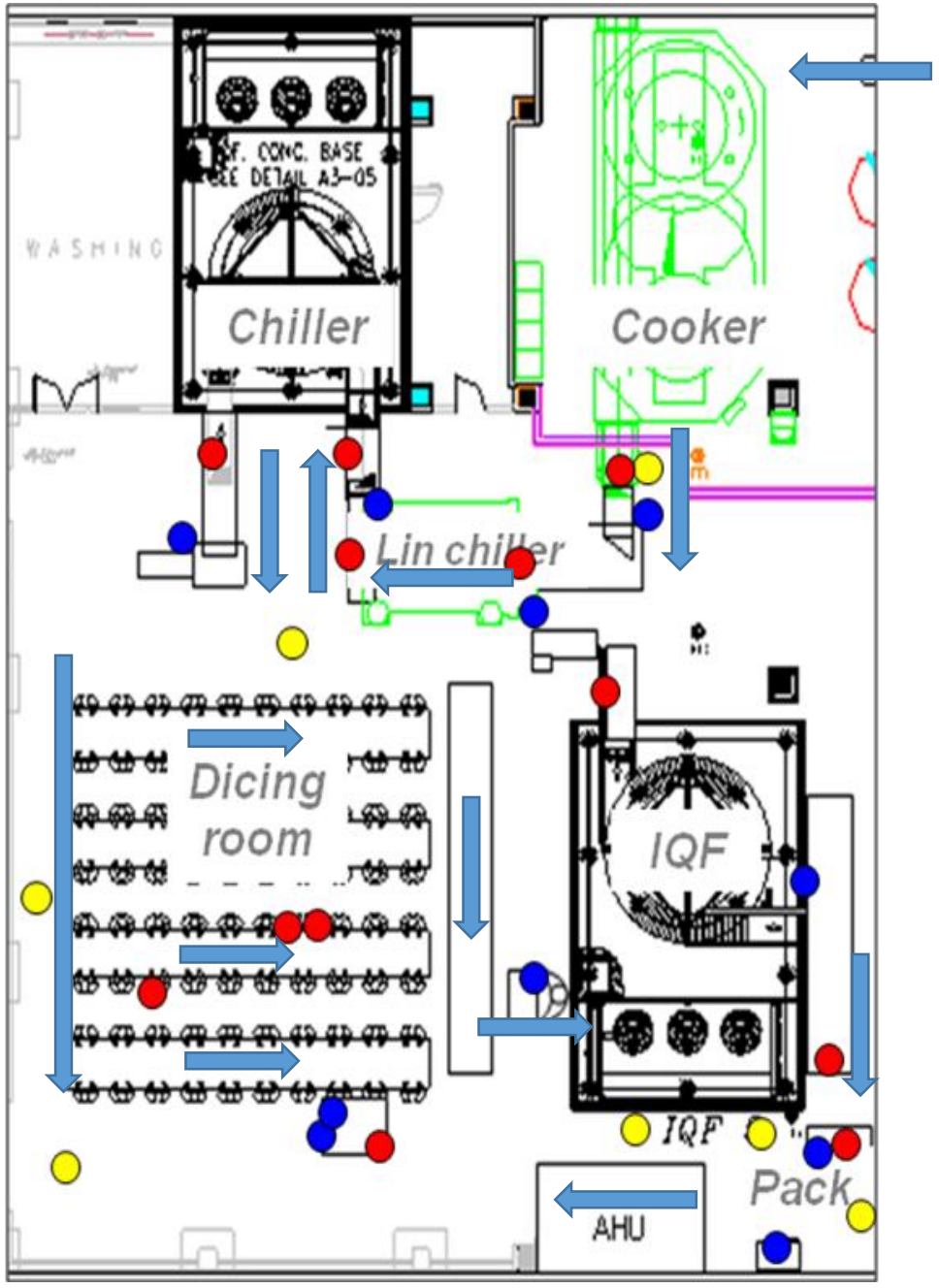


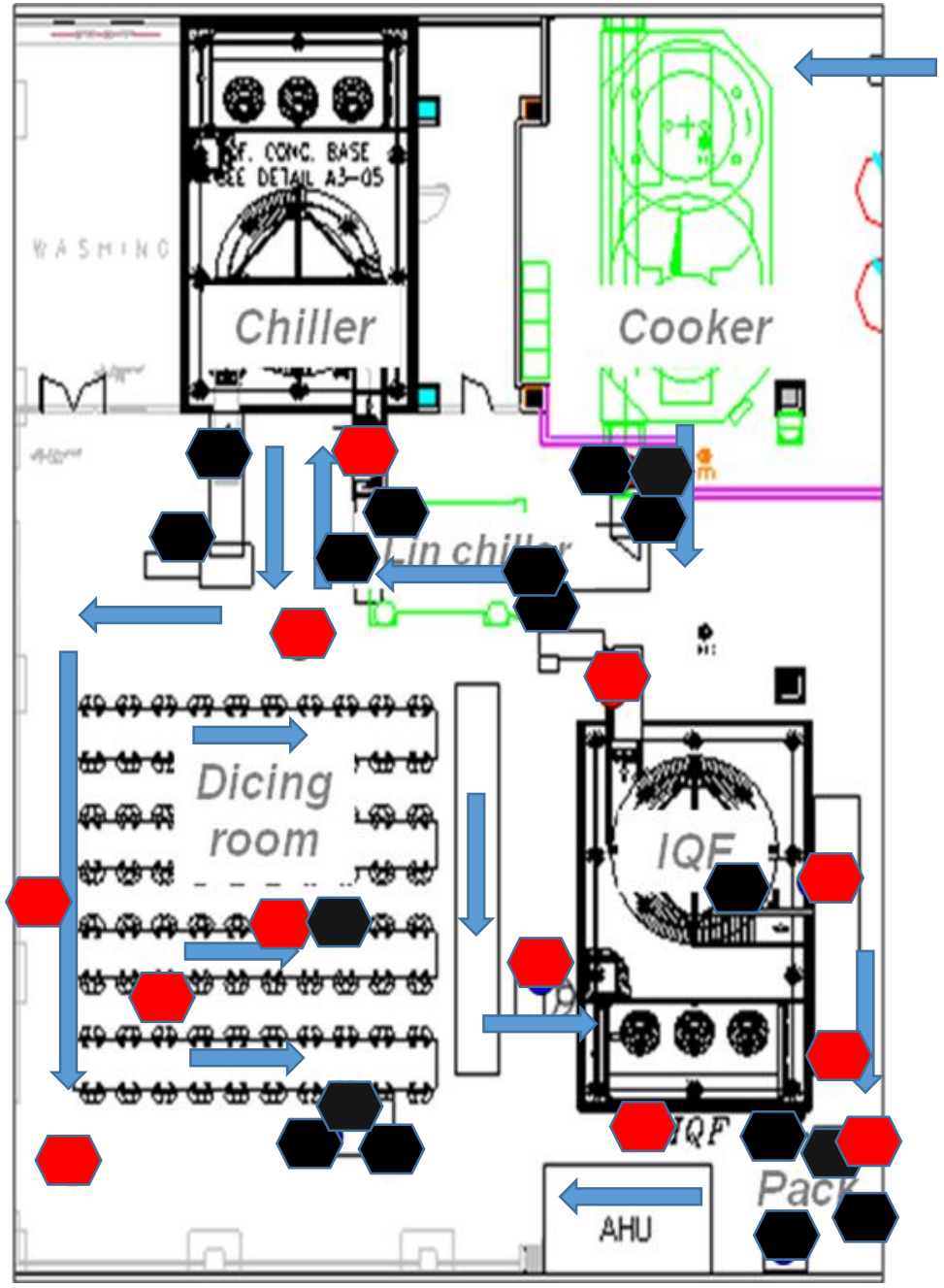
A red Marker line is drawn at the top of the INNO-LiPA Rif.TB strip for orientation. The conjugate control (Conj. Control) provides an internal control for the colour development reaction.

The MTB complex line is a specific probe for *M.tuberculosis* complex.

S1-S5 are wild-type probes for the *rpoB* gene, and R2, R4a, R4b and R5 are specific *rpoB* mutation probes.
The INNO-LiPA Rif.TB assay is validated for use on bacteria grown on solid media.




Chiller
Cooker
Lin chiller
Dicing room
IQF
IQF
Pack
AHU


Chiller
Cooker
Dicing room
IQF
AHU
Negative
Target1
Target2
Target3
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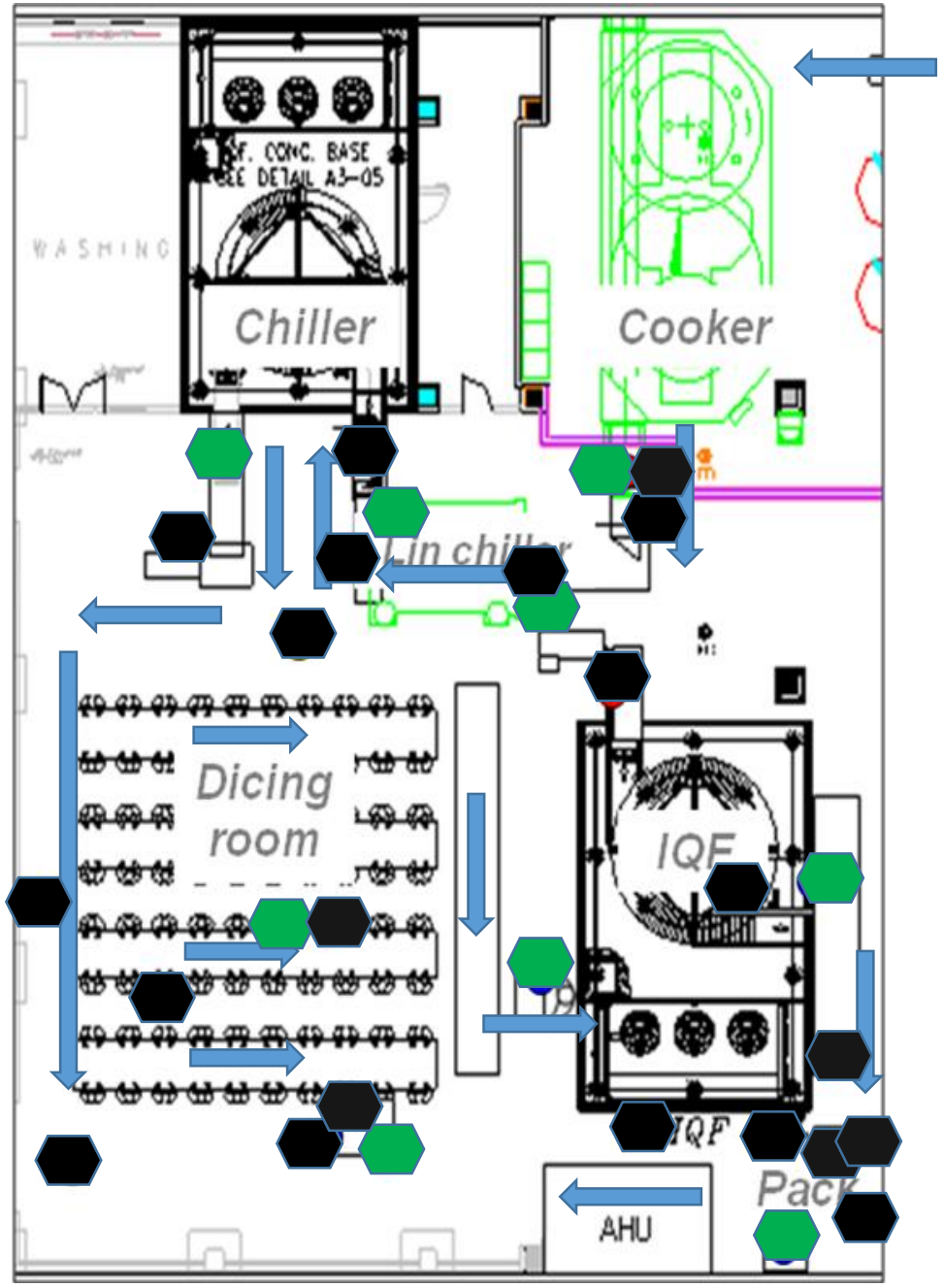
Target6
Target7
Target8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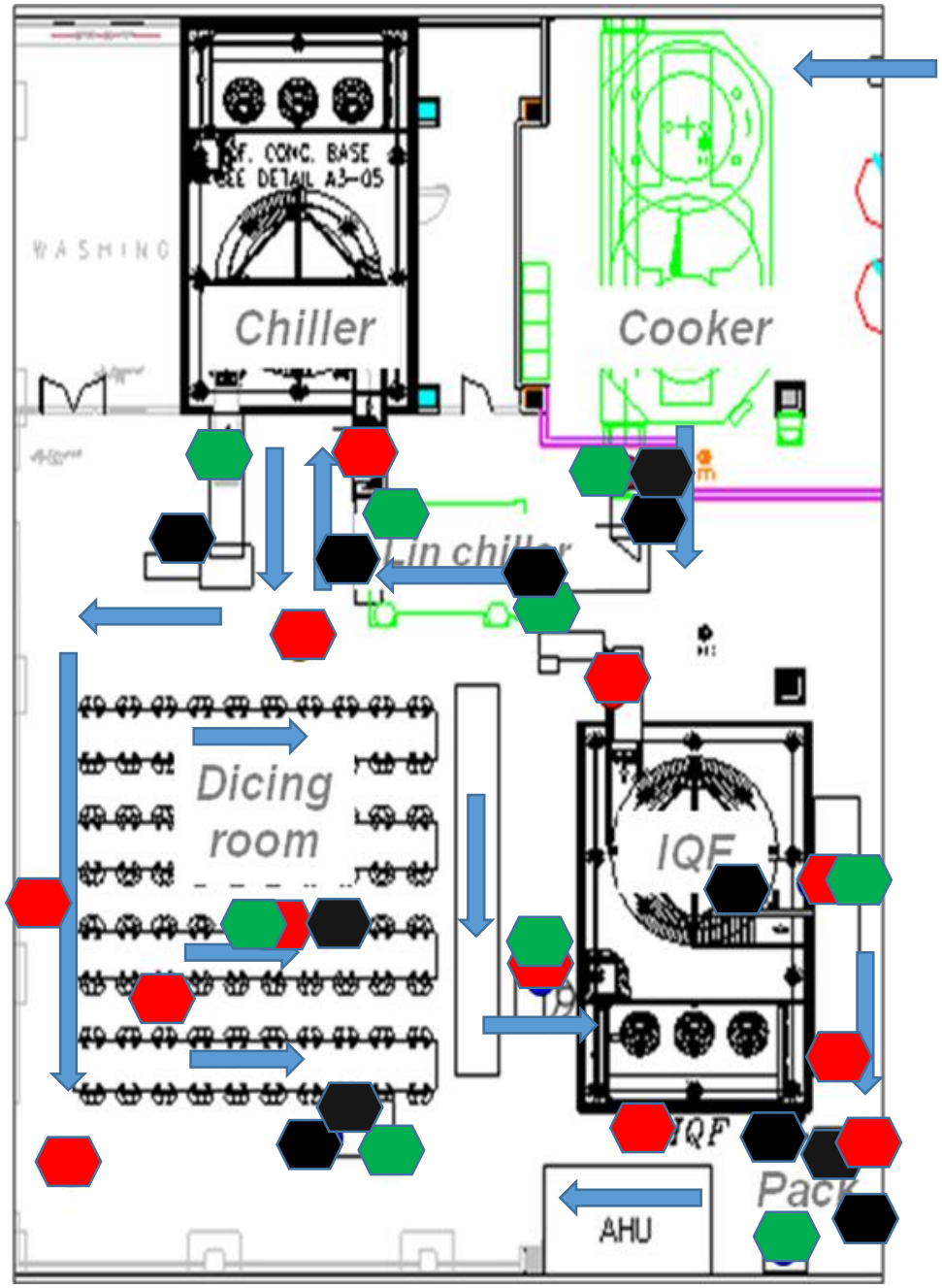
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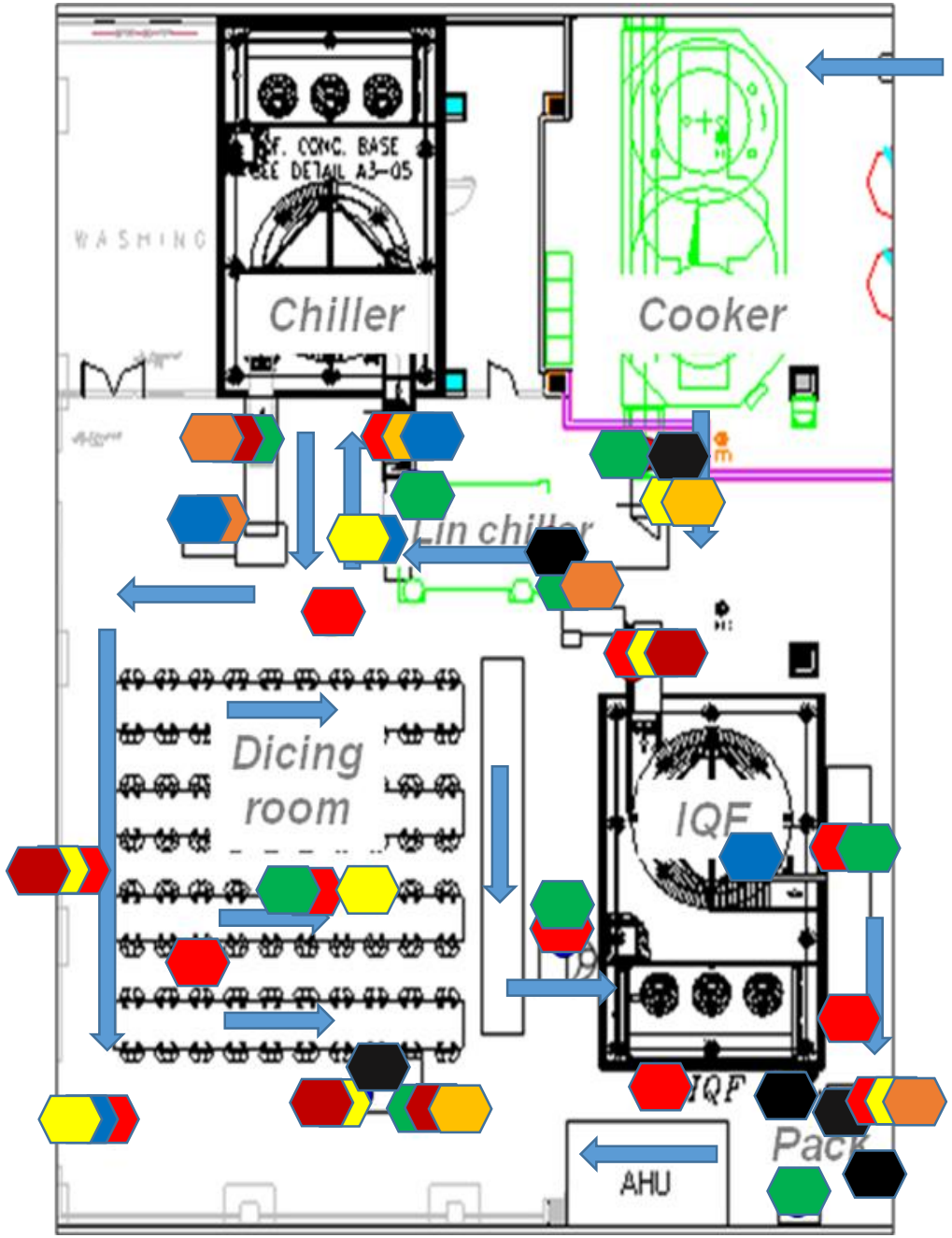
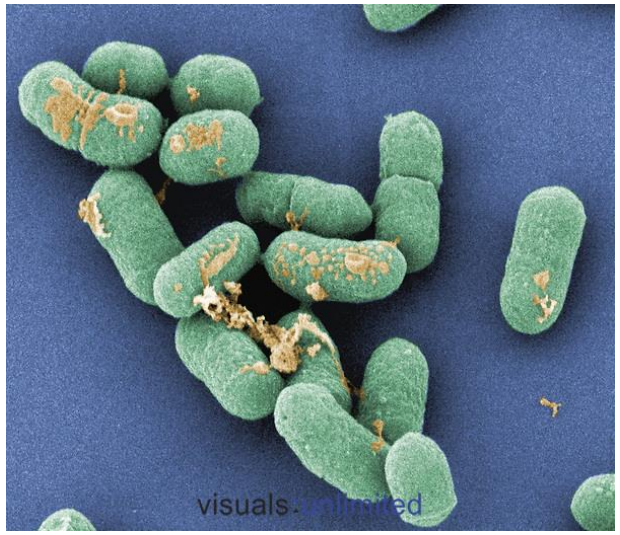


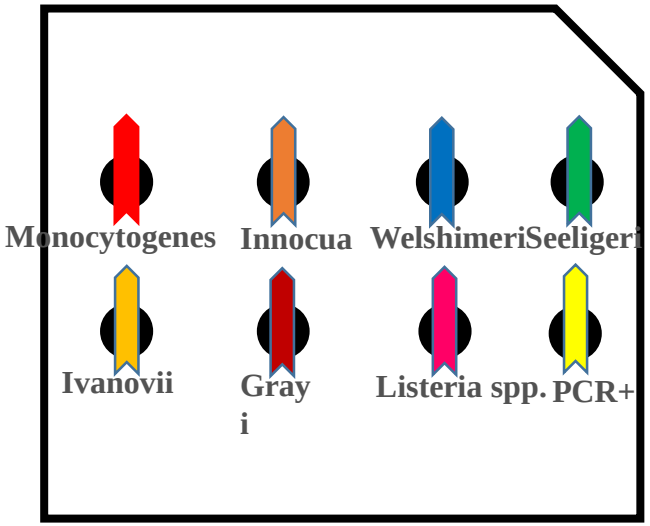
Negative
Target1
Target2
Target3
Target4
Target5
Target6
Target7
Target8

Listeria



- *Listeria* species are Gram-positive, rod-shaped bacteria.
- Listeriosis is a serious infection usually caused by eating food contaminated with *Listeria monocytogenes.*
- The disease primarily affects older adults, pregnant women, adults with weakened immune systems.
- This pathogen can cause meningitis and spontaneous abortion.
- At present, the major 6 species of genus *Listeria* are concerned by many food industries
- The present study is to develop probes array-based method for the rapid detection and identification of six species of genus *Listeria*
- *L. monocytogenes*
- *L. innocua*
- *L. welshimeri*
- *L. seeligeri*
- *L. ivanovii*
- *L. grayi*
- *Listeria spp.*

		Conventional ISO11290-1 (Phenotypic)	Probes Array Pre-enrichment culture (Genotypic)
Day 1	Pre-enrichment	25 g+ 225mL Half Fraser 30℃, 24-26h	18 hr cell for culture and Probe Array method (6h)
Day 2	Enrichment	1mL of culture 10mL of Fraser broth 30°C, 20-26h 	
Day 3	Identification	VIDAS LIS test	
Day 4-5	Isolation	Plating out on Oxford, Palcum agar and ALOA agar 37 ℃, 24-48h	
Day 6	confirmation	TSYEA 35℃, 18-24 , catalase test 	
Day 7-8	confirmation	TSYEB 25℃ 18-24h medium 25℃ 48h BA 37℃ 24h for hemolysis, Camp test 37℃ 24h	
Day9-10	confirmation	Rha and Xyl 35℃ 24-48h	



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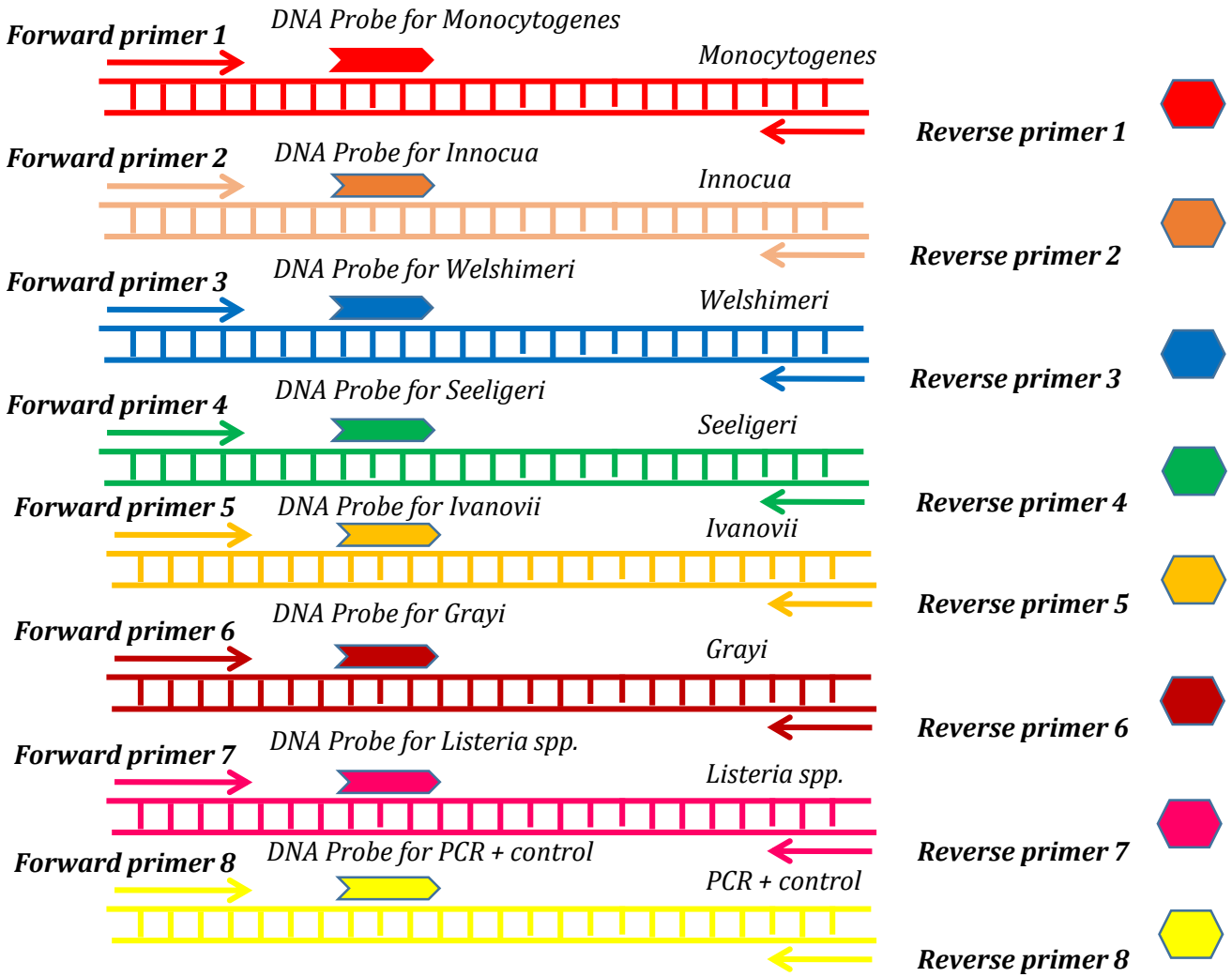
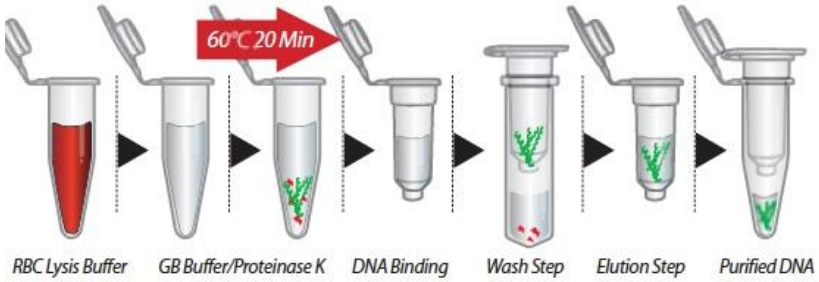
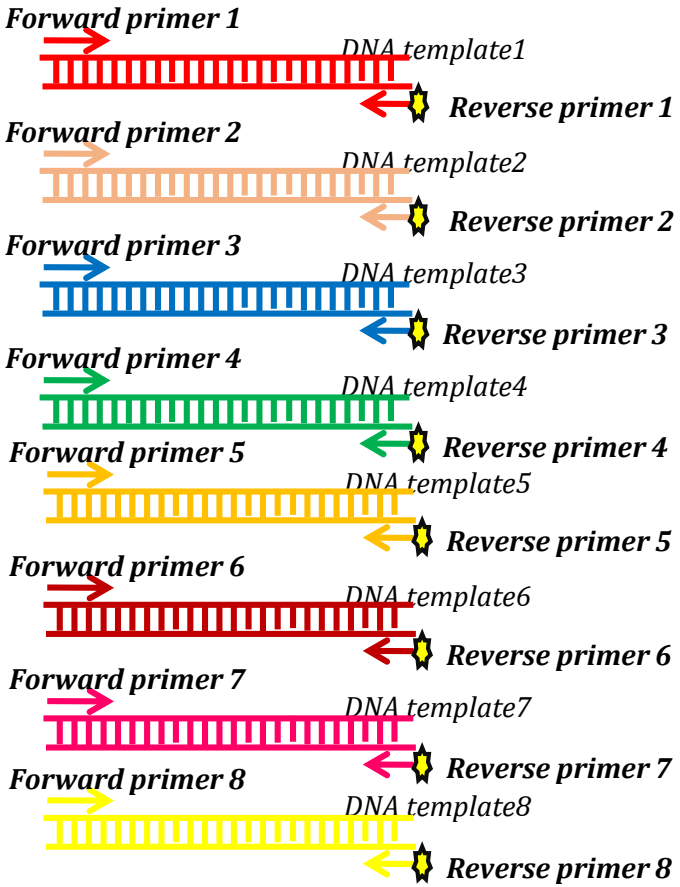
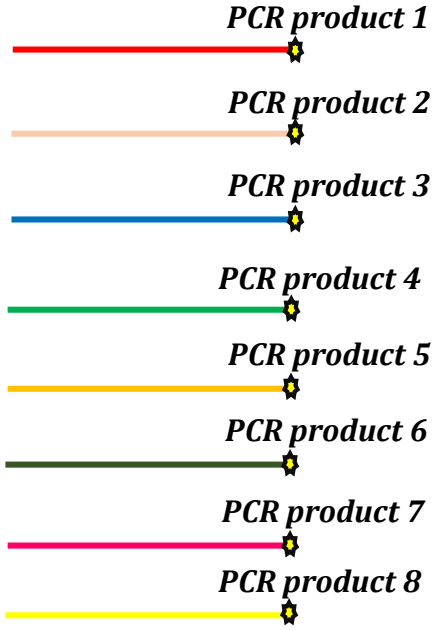
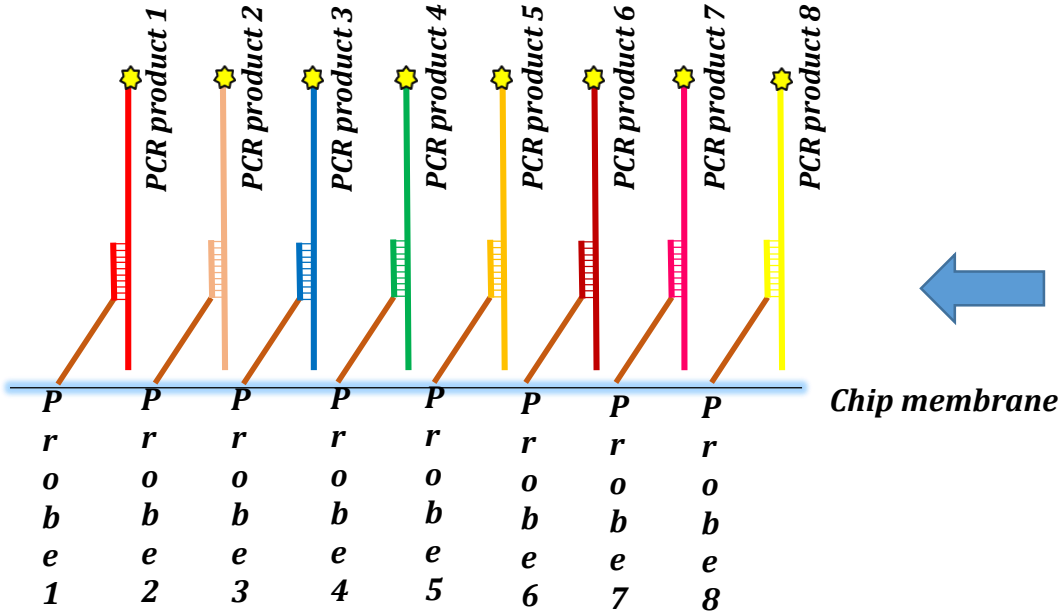
- *L. ivanovii*
- *L. grayi*
- *Listeria spp.*

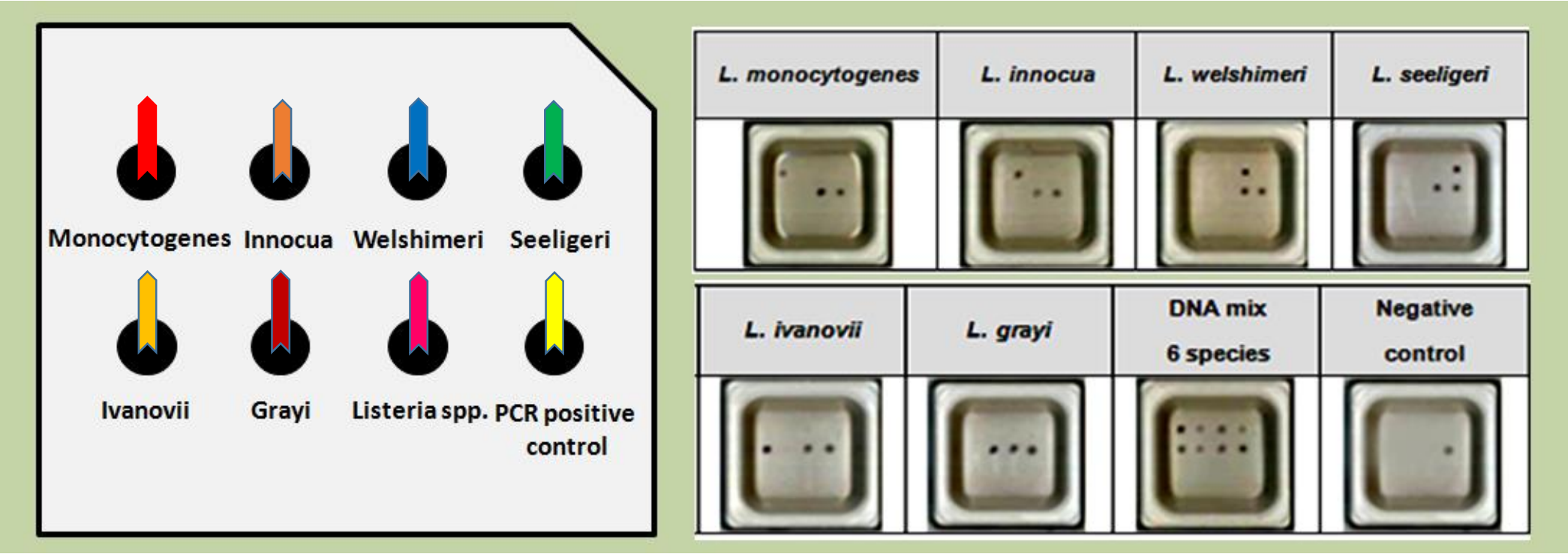



60°C 20 Min
RBC Lysis Buffer
GB Buffer/Proteinase K
DNA Binding
Wash Step
Elution Step
Purified DNA


Forward primer 1
DNA template1
Reverse primer 1
Forward primer 2
DNA template2
Reverse primer 2
Forward primer 3
DNA template3
Reverse primer 3
Forward primer 4
DNA template4
Reverse primer 4
Forward primer 5
DNA template5
Reverse primer 5
Forward primer 6
DNA template6
Reverse primer 6
Forward primer 7
DNA template7
Reverse primer 7
Forward primer 8
DNA template8
Reverse primer 8

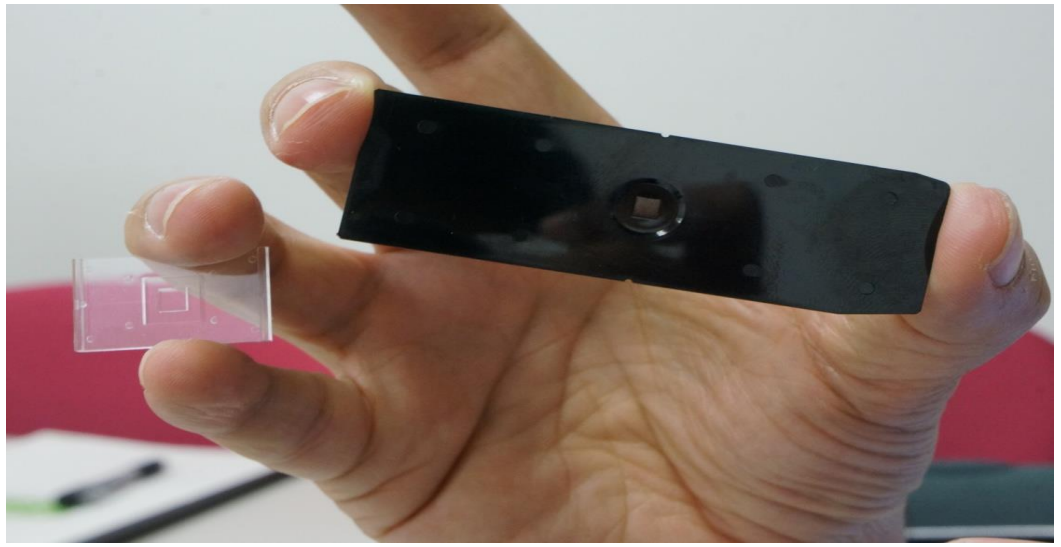

PCR product 1
PCR product 2
PCR product 3
PCR product 4
PCR product 5
PCR product 6
PCR product 7
PCR product 8


PCR product 1
PCR product 2
PCR product 3
PCR product 4
PCR product 5
PCR product 6
PCR product 7
PCR product 8
Probe 1
Probe 2
Probe 3
Probe 4
Probe 5
Probe 6
Probe 7
Probe 8
Chip membrane

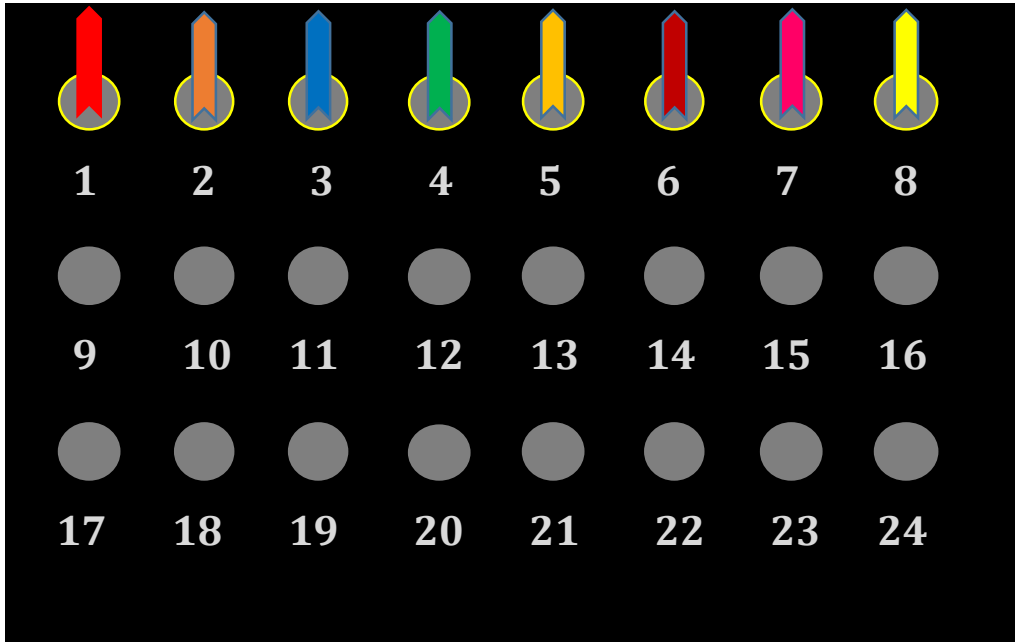


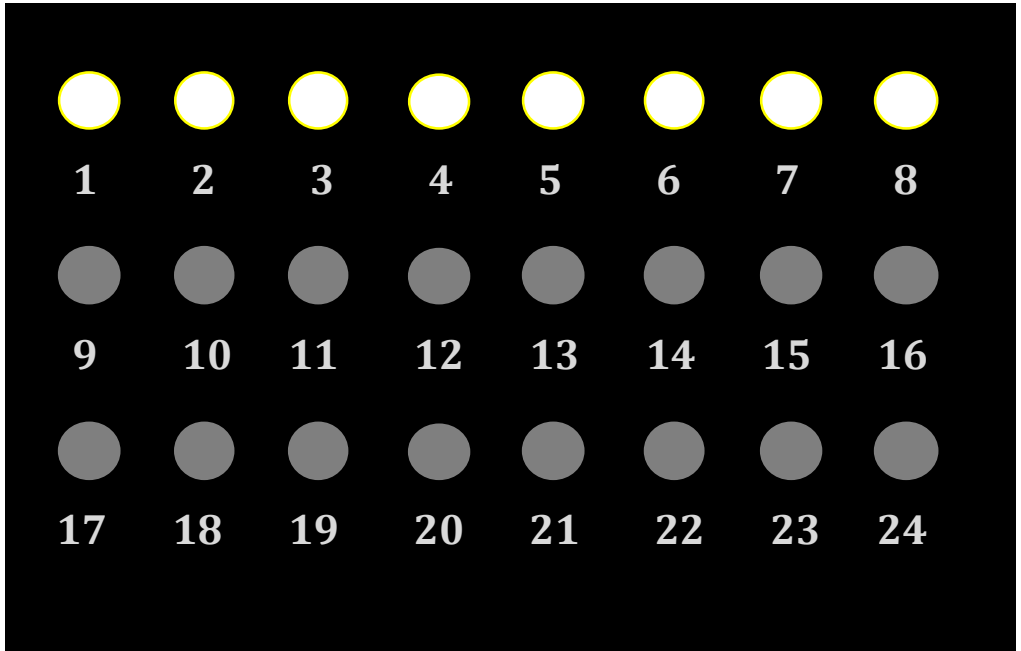
Labelled with Biotin

TOYO SEIKAN GROUP



Labelled with Cy5





Specificity test

- **Specificity test → 100%**

Pure Culture (60 isolates)	Non-*Listeria*	*Listeria*
Non-*Listeria*	**33**	-
Listeria	-	**27**

Specificity test

- **Specificity test → 60 isolates of foodborne pathogen (12 genus)**

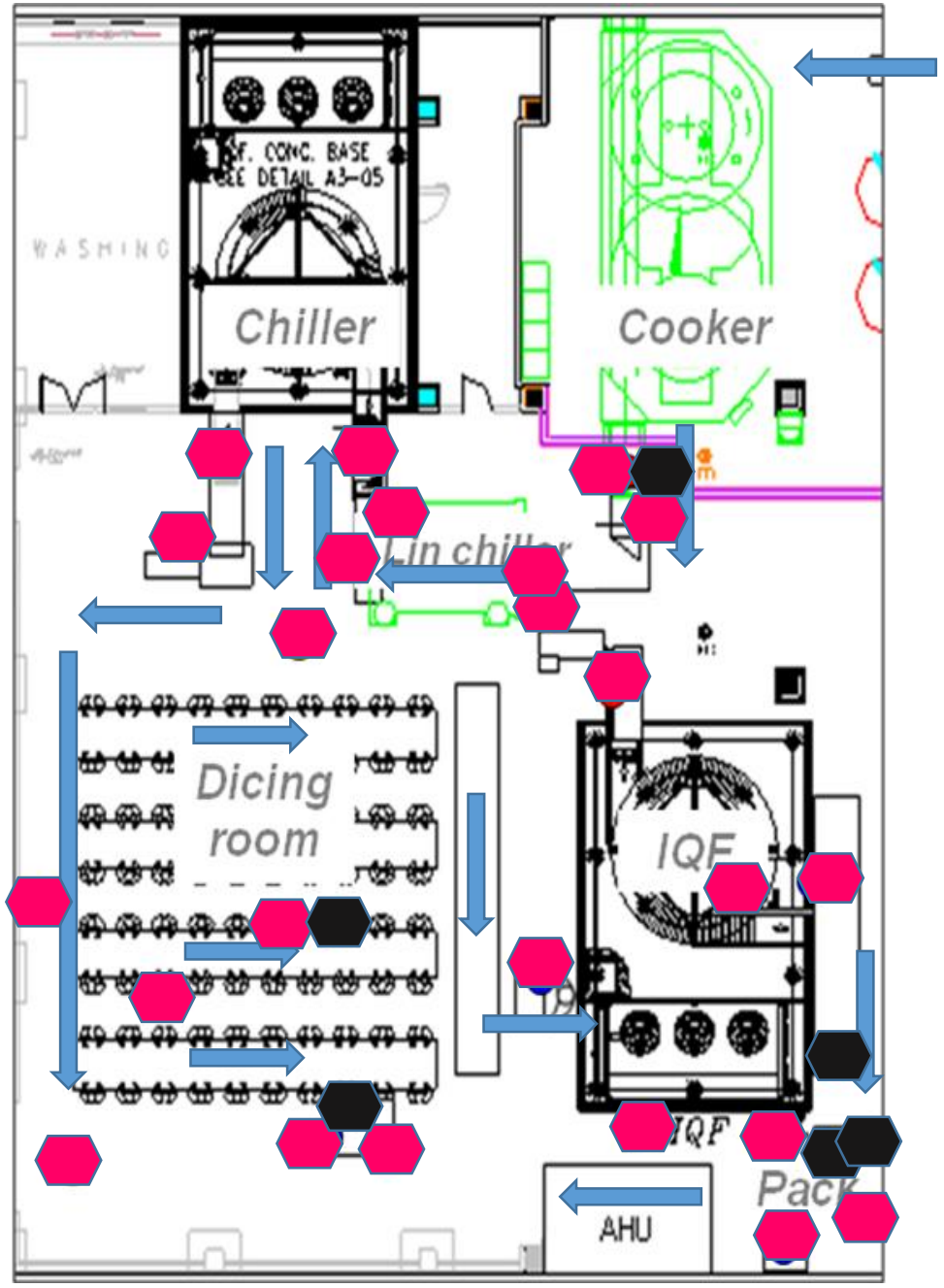
Organisms	No.of isolation	True positive	True negative	False Positive	Organisms	No.of isolation	True positive	True negative	False Positive
Listeria monocytogenes	7	7	-	-	***Streptococcus uberis***	1	-	1	-
Listeria innocua	6	6	-	-	***Mycoplasma bovis***	1	-	1	-
Listeria welshimeri	6	6	-	-	***Corynebacterium bovis***	1	-	1	-
Listeria seeligeri	6	6	-	-	***Salmonella*** **Typhimurium**	2	-	2	-
Listeria ivanovii	1	1	-	-	***Salmonella*** **Infantis**	1	-	1	-
Listeria grayi	1	1	-	-	***Salmonella*** **Hadar**	2	-	2	-
Stapphylococcus aureus	1	-	1	-	***Salmonella*** **Enteritidis**	2	-	2	-
Bacillus cereus	1	-	1	-	***Salmonella*** **Virchow**	2	-	2	-
E. coli	1	-	1	-	***Salmonella*** **Typhi**	1	-	1	-
Yersinia enterocolitica	1	-	1	-	***Salmonella*** **Paratyphi A**	1	-	1	-
Shigella dysenteriae	1	-	1	-	***Salmonella*** **Adelaide**	1	-	1	-
Vibrio alginolyticus	1	-	1	-	***Salmonella*** **Berta**	1	-	1	-
Vibrio mimiicus	1	-	1	-	***Salmonella*** **California**	1	-	1	-
Campylobacter jejuni subsp. fetus	1	-	1	-	***Salmonella*** **Chester**	1	-	1	-
Clostridium perfringens	1	-	1	-	***Salmonella*** **Coleypark**	1	-	1	
Streptococcus agalactiae	1	-	1	-	***Salmonella*** **Florida**	1	-	1	-
Streptococcus bovis	1	-	1	-	***Salmonella*** **Hartford**	1	-	1	-
Streptococcus dysgalactiae	1	-	1	-					

Sensitivity test

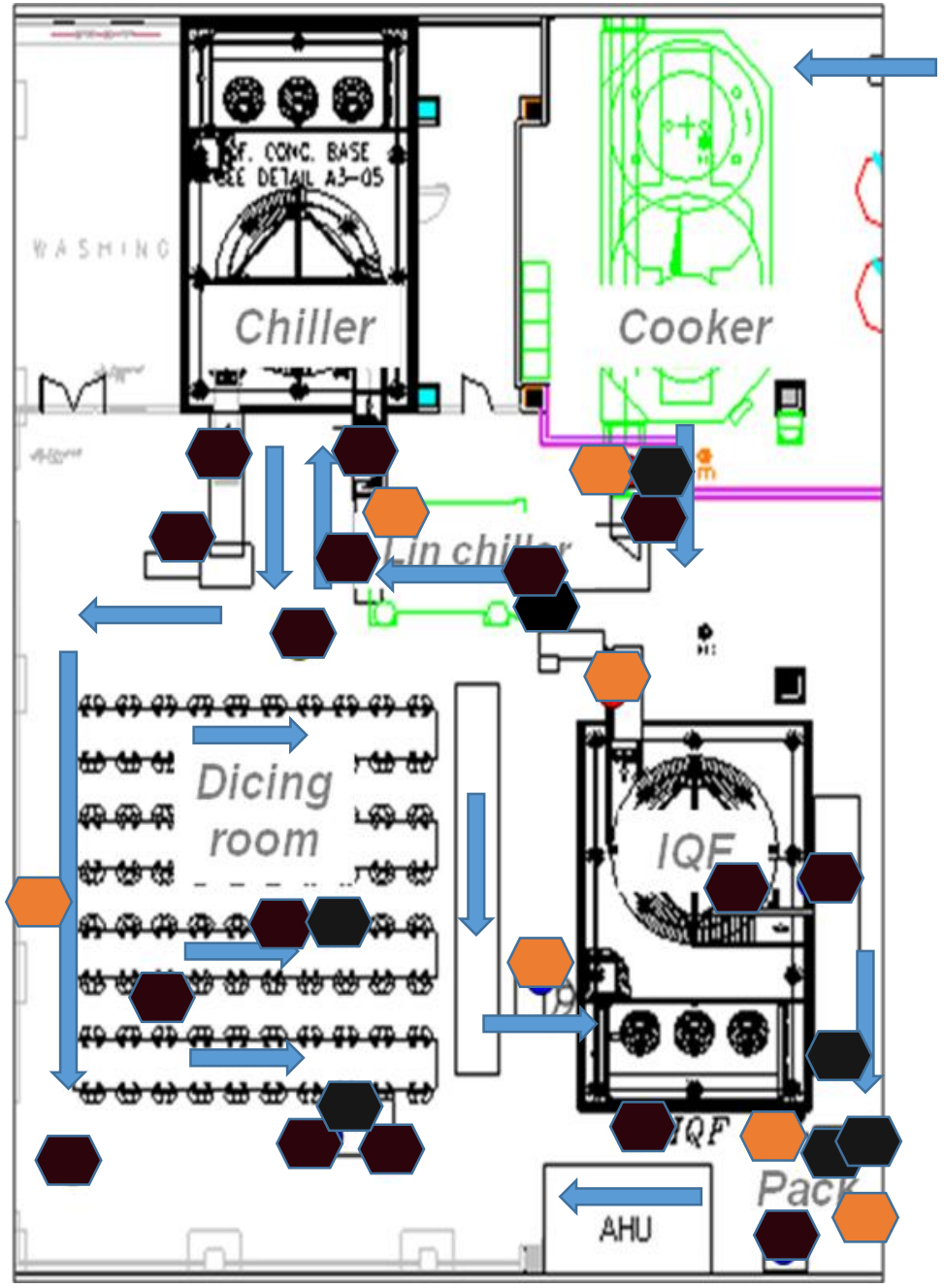
- Highly sensitivity (0.1%w/w of DNA and detection limit is 0.1 ng of DNA)

Application

- To test with swab samples from poultry plant about 1,000 samples
  - Environmental samples
  - Poultry products
- From the results, probes array-based method can help for tracking sources of contamination and it can be quickly implemented to control and monitor Listeria contamination in food production.



- *Negative*
- *Monocytogenes*
- *Innocua*
- *Welshimeri*
- *Seeligeri*
- *Ivanovii*
- *Grayi*
- *Listeria spp.*
- *PCR + control*



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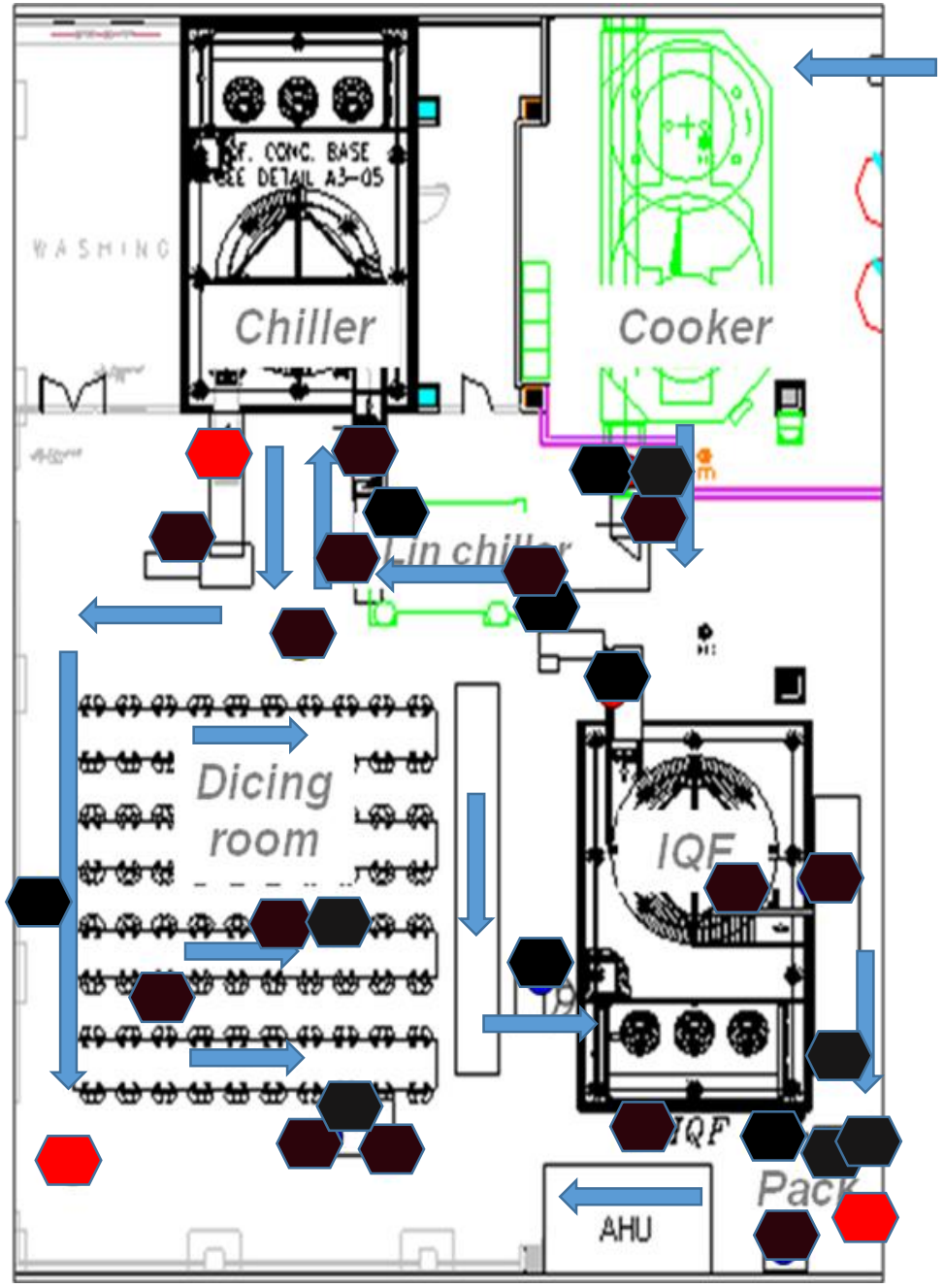

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- *Monocytogenes*
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- *Welshimeri*
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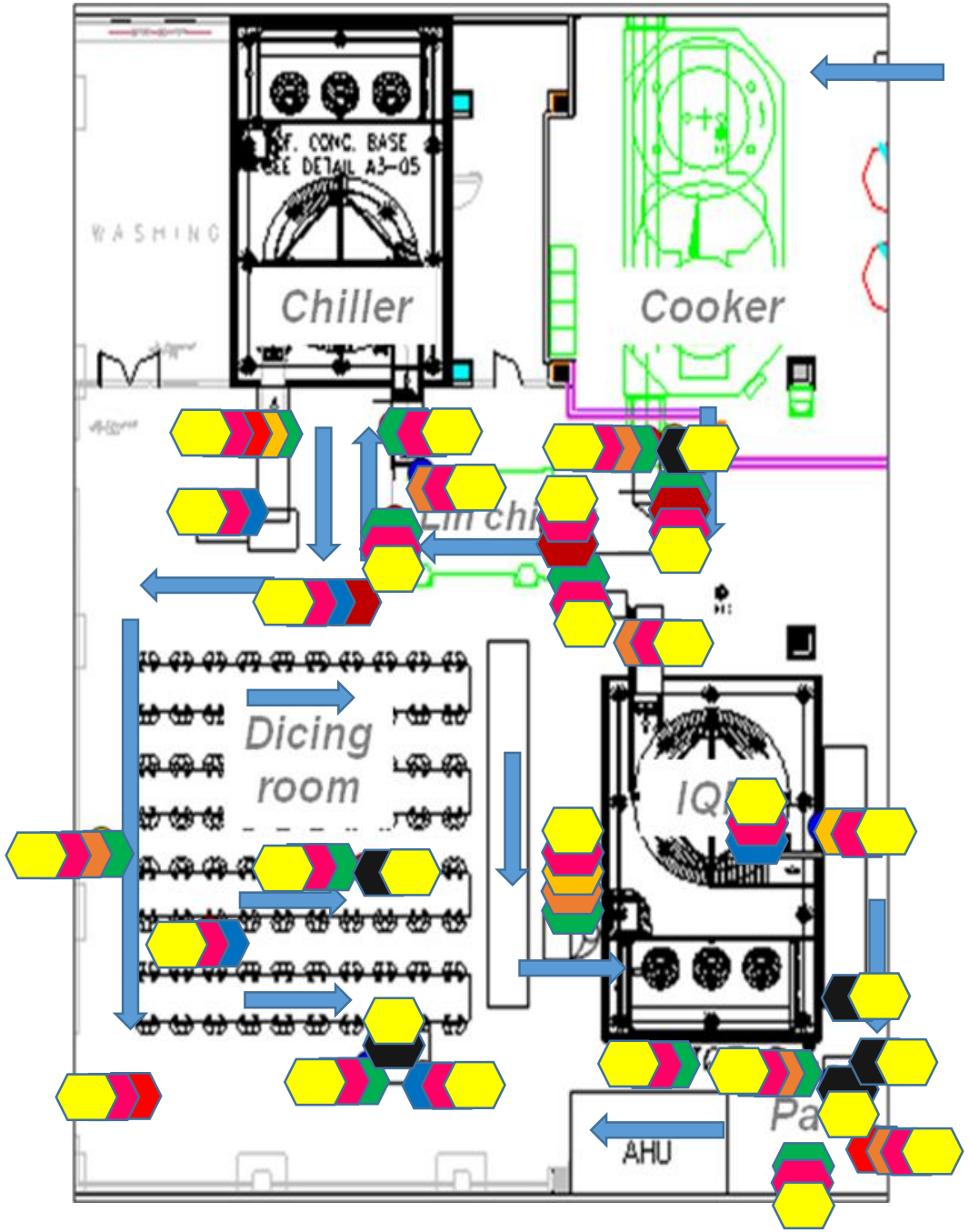
- *Ivanovii*
- *Grayi*
- *Listeria spp.*
- *PCR + control*



- *Negative*
- *Monocytogenes*
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- *Seeligeri*
- *Ivanovii*
- *Grayi*
- *Listeria spp.*
- *PCR + control*

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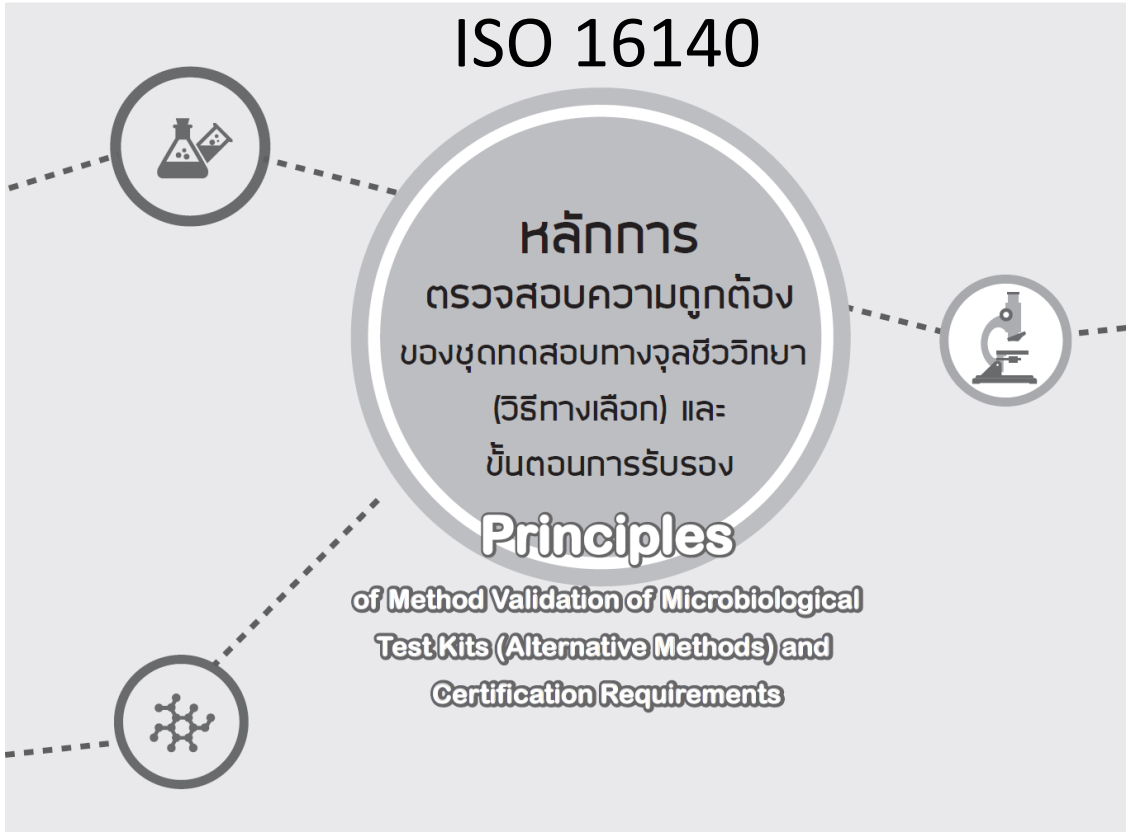
AOAC INTERNATIONAL
Thailand Section

ISO 16140



Validation of Microbiological Method

1. Qualitative methods
    - Present / absent

2. Quantitative methods

Validation of Microbiological Methods

Phase 1
Method Development

Single Lab Validation

- Method development validation study
- Inclusivity / Exclusivity study
- Limit of detection / Relative detection level
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)

Phase 2
Independent Laboratory

Independent Lab Validation

- Independent laboratory validation study (laboratory in Thailand)
- Matrix study (Food categories)
- Inclusivity / Exclusivity study
- Limit of detection / Relative detection level
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)
- Laboratory audit by globalgroup

Phase 3
Inter Laboratory

Inter Lab Validation

- Inter laboratory validation study (laboratory in Thailand)
- ≥ 10 laboratories / matrix
- ≥ 8 replicates / laboratory
- 3 different contamination levels
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)

Phase1: Method Development Validation Study

Specificity test

➢ **Specificity test → 60 isolates of foodborne pathogen (12 genus)**

Organisms	No.of isolation	True positive	True negative	False Positive	Organisms	No.of isolation	True positive	True negative	False Positive
Listeria monocytogenes	7	7	-	-	***Streptococcus uberis***	1	-	1	-
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Listeria grayi	1	1	-	-	***Salmonella*** Hadar	2	-	2	-
Stapphylococcus aureus	1	-	1	-	***Salmonella*** Enteritidis	2	-	2	-
Bacillus cereus	1	-	1	-	***Salmonella*** Virchow	2	-	2	-
E. coli	1	-	1	-	***Salmonella*** Typhi	1	-	1	-
Yersinia enterocolitica	1	-	1	-	***Salmonella*** Paratyphi A	1	-	1	-
Shigella dysenteriae	1	-	1	-	***Salmonella*** Adelaide	1	-	1	-
Vibrio alginolyticus	1	-	1	-	***Salmonella*** Berta	1	-	1	-
Vibrio mimiicus	1	-	1	-	***Salmonella*** California	1	-	1	-
Campylobacter jejuni subsp. fetus	1	-	1	-	***Salmonella*** Chester	1	-	1	-
Clostridium perfringens	1	-	1	-	***Salmonella*** Coleypark	1	-	1	
Streptococcus agalactiae	1	-	1	-	***Salmonella*** Florida	1	-	1	-
Streptococcus bovis	1	-	1	-	***Salmonella*** Hartford	1	-	1	-
Streptococcus dysgalactiae	1	-	1	-					

Specificity test

- **Specificity test → 100%**

Pure Culture (60 isolates)	Non-*Listeria*	*Listeria*
Non-*Listeria*	33	-
Listeria	-	27

Relative accuracy (AC) test

- Relative accuracy → 100%

Relative sensitivity (SE) test

- Relative sensitivity → 100%

Limit of detection test

- Limit of detection → 0.1 ng of DNA

Validation of Microbiological Methods

Phase 1
Method Development

Single Lab Validation

- Method development validation study
- Inclusivity / Exclusivity study
- Limit of detection / Relative detection level
- Relative accuracy (AC)
- Relative specificity (SP)
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Phase 2
Independent Laboratory

Independent Lab Validation

- Independent laboratory validation study (laboratory in Thailand)
- Matrix study (Food categories)
- Inclusivity / Exclusivity study
- Limit of detection / Relative detection level
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)
- Laboratory audit by globalgroup

Phase 3
Inter Laboratory

Inter Lab Validation

- Inter laboratory validation study (laboratory in Thailand)
- ≥ 10 laboratories / matrix
- ≥ 8 replicates / laboratory
- 3 different contamination levels
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)

Phase2: Independent Laboratory Validation Study

Target

- **Target microorganisms →** ***Listeria monocytogenes***
  Listeria innocua
  Listeria welshimeri
  Listeria seeligeri
  Listeria grayi
  Listeria ivanovii

Matrix study

- **Matrix study →** **Food categories (chicken)**

  →Fresh , Frozen and Processed chicken

Relative detection level

- **Relative detection level → 6 level**

  → 0, 2, 5, 10, <20, <100 cfu/25g

Inclusivity /exclusivity test

- **Inclusivity test → 50 pure strains**
- **Exclusivity test → 30 pure strains**

Relative accuracy (AC) test

- **Relative accuracy →**

Relative sensitivity (SE) test

- **Relative sensitivity →**

Limit of detection test

- **Limit of detection →**

Validation of Microbiological Methods

Phase 1
Method Development

Single Lab Validation

- Method development validation study
- Inclusivity / Exclusivity study
- Limit of detection / Relative detection level
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)

Phase 2
Independent Laboratory

Independent Lab Validation

- Independent laboratory validation study (laboratory in Thailand)
- Matrix study (Food categories)
- Inclusivity / Exclusivity study
- Limit of detection / Relative detection level
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)
- Laboratory audit by globalgroup

Phase 3
Inter Laboratory

Inter Lab Validation

- Inter laboratory validation study (laboratory in Thailand)
- ≥ 10 laboratories / matrix
- ≥ 8 replicates / laboratory
- 3 different contamination levels
- Relative accuracy (AC)
- Relative specificity (SP)
- Relative sensitivity (SE)

Phase3: Inter Laboratory Validation Study

Target

- **Target microorganisms →** ***Listeria monocytogenes***
  Listeria innocua
  Listeria welshimeri
  Listeria seeligeri
  Listeria grayi
  Listeria ivanovii

Matrix study

- **Matrix study →** **Food categories (chicken)**

  →Fresh , Frozen and Processed chicken

Number of Laboratory

- ≥ 10 laboratories / matrix
- ≥ 8 replicates / laboratory

Contamination level

- Contamination level → 3 levels

  → 1, 10, <100 cfu/25g

Relative accuracy (AC) test

- **Relative accuracy →**

Relative sensitivity (SE) test

- **Relative sensitivity →**

Limit of detection test

- **Limit of detection →**

Acknowledgement

Thanks for Your Attention