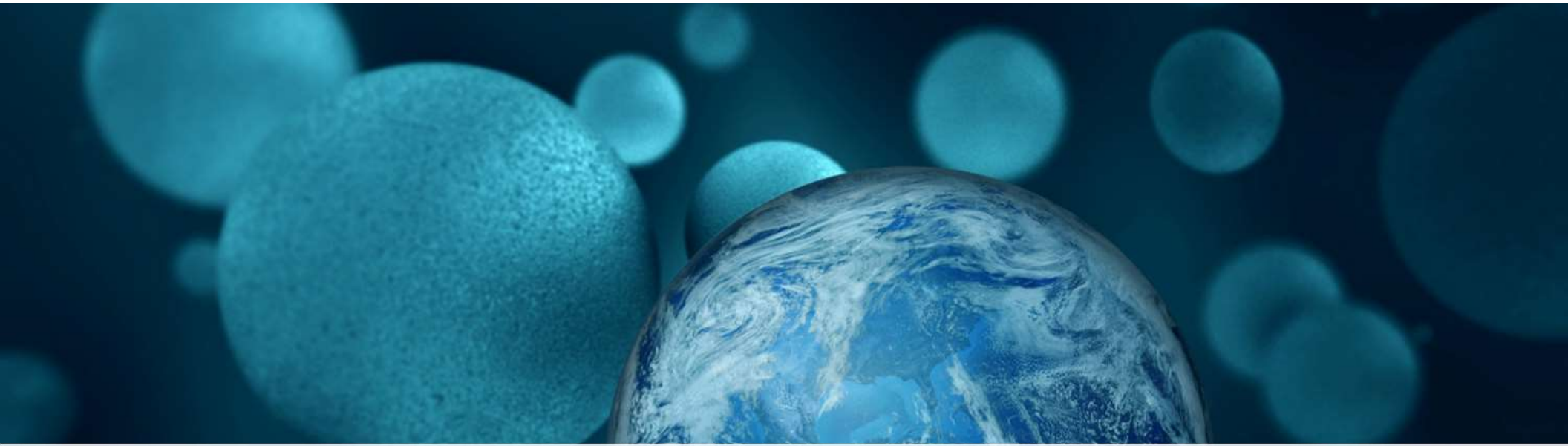
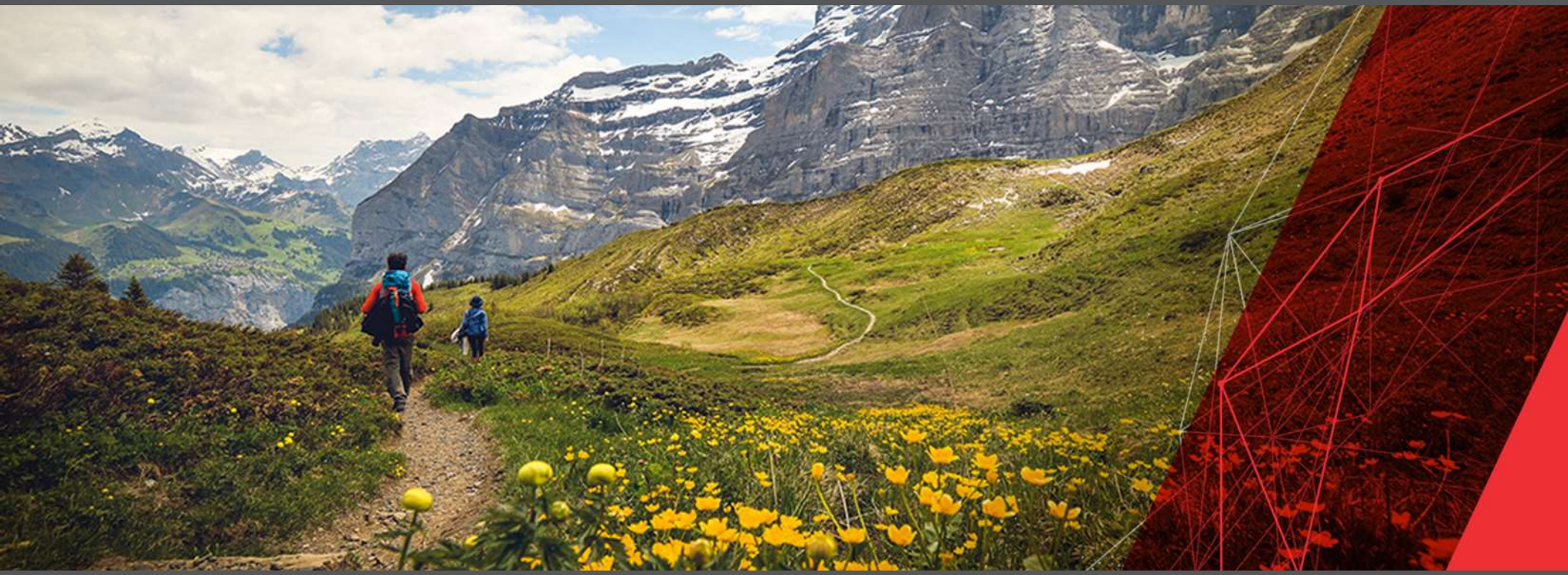


ThermoFisher SCIENTIFIC

Thermo Scientific
Molecular Technology & SureTect Real-Time PCR System

The world leader in serving science

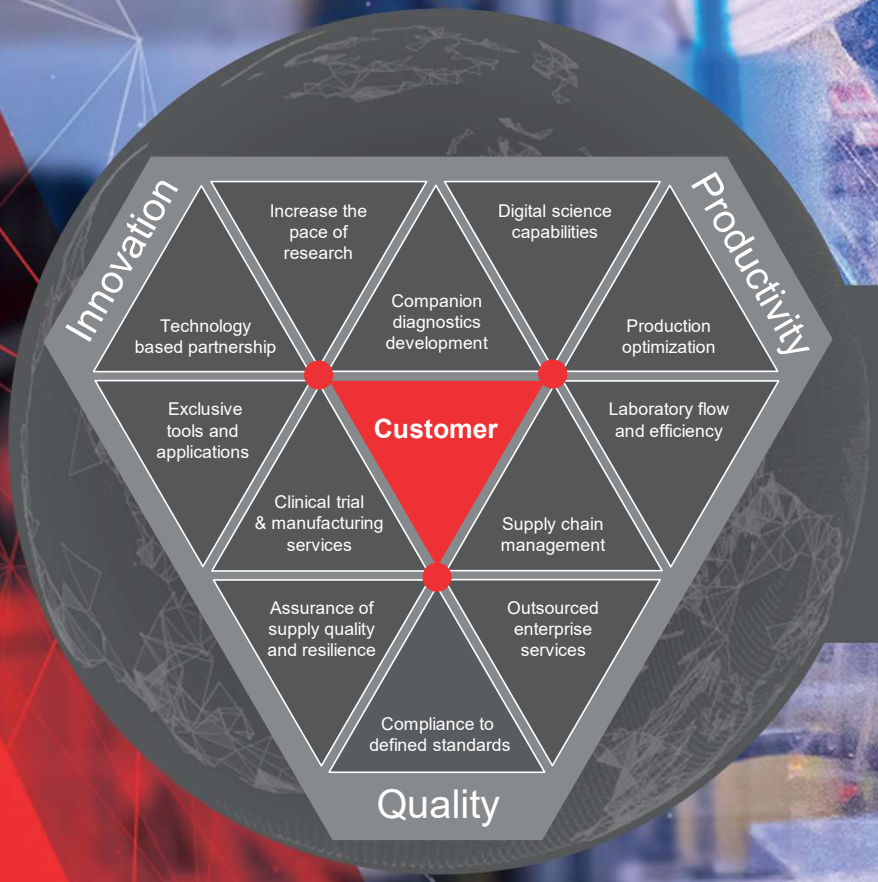
We Take Pride in Our Mission



We enable our customers to make the world healthier, cleaner and safer

ThermoFisher SCIENTIFIC

Our customer value proposition



We help our customers **accelerate innovation** and **enhance productivity**, underpinned by **quality**.

ThermoFisher SCIENTIFIC

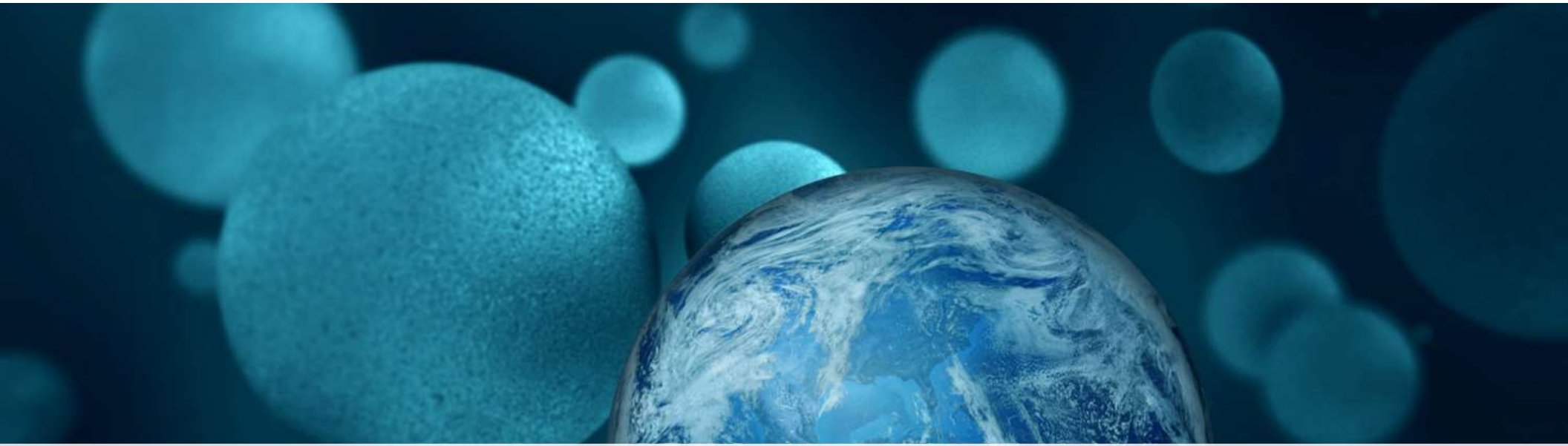
Food Protection: What Customers Tell Us They Need

Help your business meet the needs of the rapidly expanding global demand for food

- Ensure transparency, traceability and trust in the food industry
- Fast identification of unwanted contaminants (raw material to post-consumption) to manage brand perceptions
- Strategic partnerships that advance technology and digital capabilities
- Leadership and support to meet global food standards and safety regulations
- De-skilling within labs driving requirement for workflow simplicity and ease of use.

Partner expectations

- Efficient workflow solutions to drive productivity
- Deliver new technologies to bring testing closer to the sample
- Accessible data to make better decisions across supply chain and processes



ThermoFisher SCIENTIFIC

Thermo Scientific Molecular Detection

The world leader in serving science

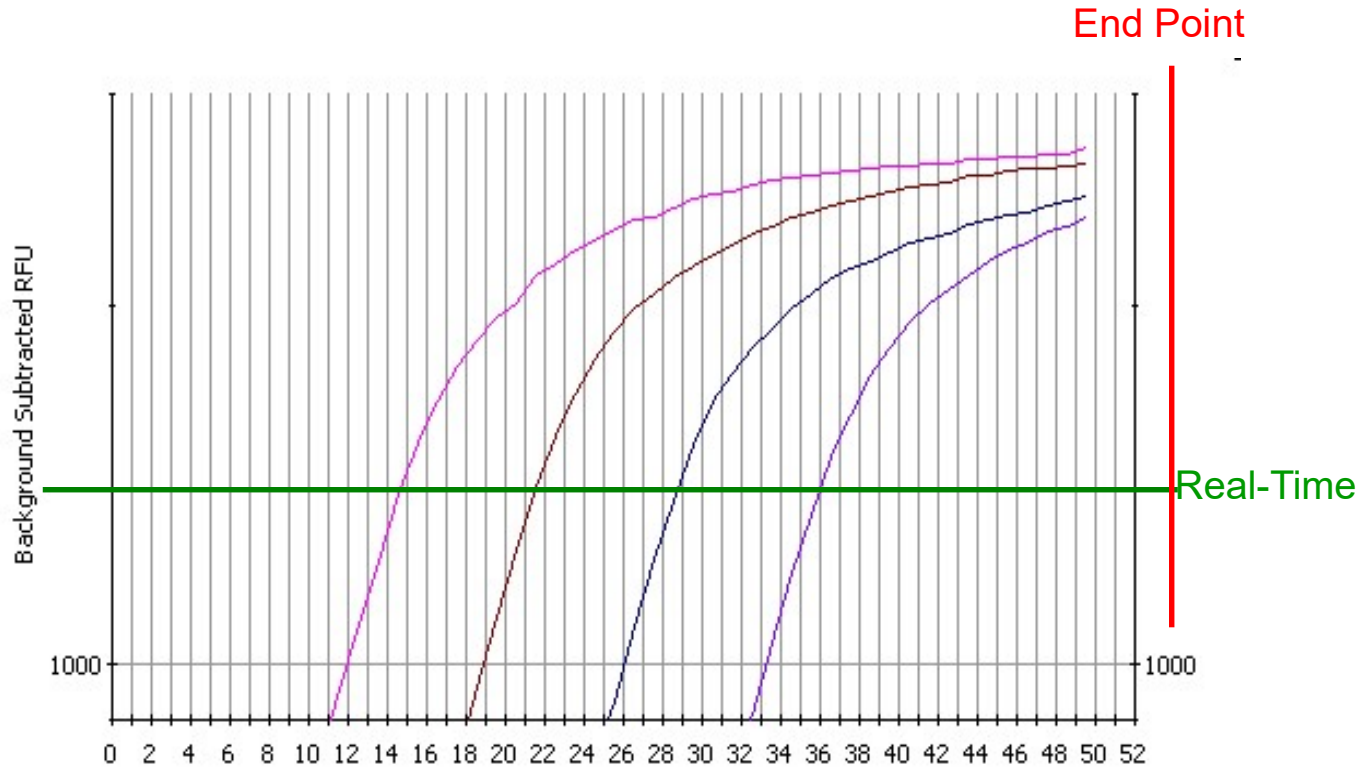
Polymerase Chain Reaction (PCR)

- For detection, DNA needs to be amplified (multiple copies produced)
- Template DNA is amplified by thermal cycling
- PCR was invented 1983 by Kary Mullis, who received a Nobel Prize in Chemistry in 1993
- The most important components in PCR are:
  - Template – the purified DNA from the sample
  - Primers – short synthetic DNA fragments that are complimentary to the template DNA and define the area to be amplified
  - DNA polymerase – the enzyme used to synthesize a new strand complimentary to the template
  - Nucleotides – DNA building blocks (A, T, C and G)

ThermoFisher SCIENTIFIC

Real Time PCR and End Point PCR

- Mechanism to see the amplified result



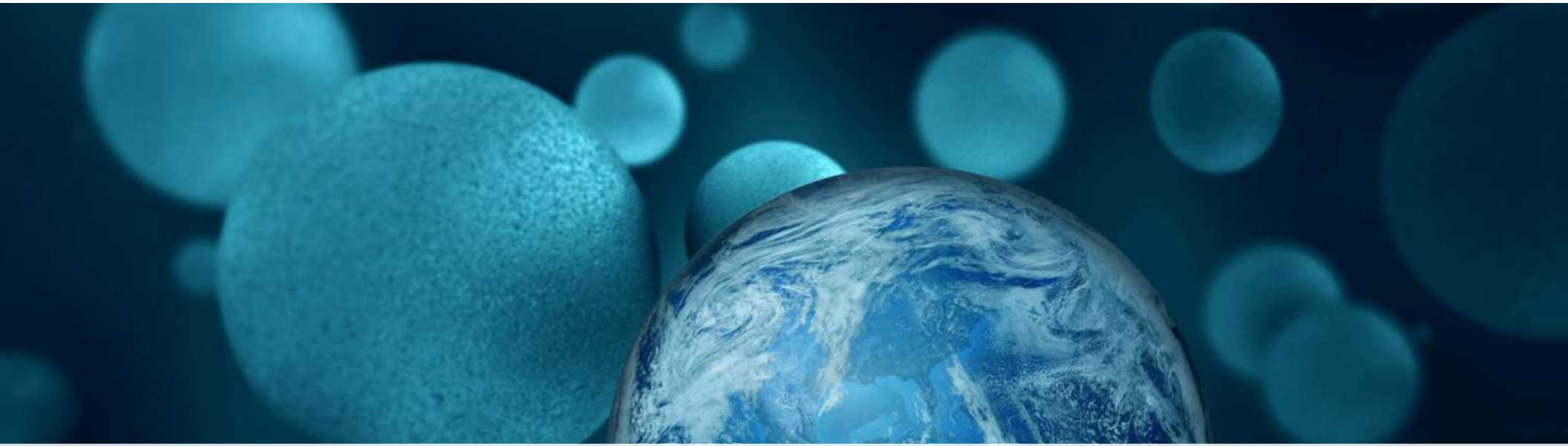
ThermoFisher SCIENTIFIC

Internal Amplification Control (IAC)

- **ONLY** with an IAC present can you have a reliable result
- **WHY?** How else would you know if the reaction in the tube has occurred?

- Potential errors:
  - Volumes are very low - bubbles or no liquid transferred
  - Pipetting errors do occur - reagents/sample may not be added
  - Enzymes stop working – due to incorrect storage or un-noticed expiry
  - Equipment may not perform correctly- without an assay control this wouldn't be recognised

ThermoFisher SCIENTIFIC



ThermoFisher SCIENTIFIC

Thermo Scientific SureTect Real-Time PCR System

The world leader in serving science

Detection: Thermo Scientific SureTect Assays and Applied Biosystems Instruments

- Thermo Scientific™ SureTect™ Pathogen Detection System comprises:
  - Applied Biosystems™ SimpliAmp™ Thermal Cycler (Sample Preparation/Lysis)
  - Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument (Real-time PCR)
  - Thermo Scientific™ RapidFinder™ Analysis Software v 1.1 (for QuantStudio 5 Food Safety Instrument) & Design Analyis
- Simple to implement and run for more efficient lab workflow
  - Minimal transfer and hands-on steps
  - Pre-filled lysis reagents and lyophilized PCR reagents
  - Universal PCR cycling parameters for all assays
  - Intuitive software for set-up and result interpretation
  - Single enrichment for some dual targets,

    e.g. Salmonella spp./Cronobacter spp. with infant formula

    Salmonella spp./E. coli O157:H7 with raw beef

SureTect PCR Food Pathogen Assays

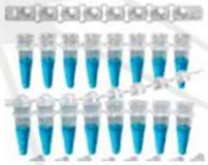
- SureTect™ Cronobacter Species PCR Assay
- SureTect™ E. coli O157:H7 PCR Assay
- SureTect™ Listeria monocytogenes PCR Assay
- SureTect™ Listeria Species PCR Assay
- SureTect™ Salmonella Species PCR Assay

- SureTect™ Campylobacter jejuni, C. coli and C. lari multiplex PCR Assay
- SureTect™ Escherichia coli O157:H7 and STEC Screening multiplex PCR Assay
- SureTect™ Escherichia coli STEC Identification multiplex PCR Assay
- SureTect™ Staphylococcus aureus PCR Assay
- SureTect™ Vibrio cholerae, V. parahaemolyticus and V. vulnificus multiplex PCR Assay

ThermoFisher SCIENTIFIC

Suretect™ Workflow

Sample Enrichment (8 – 24 hours) → **Cell Lysis & DNA Release (15 minutes)**

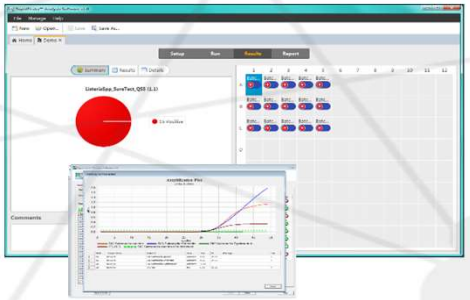


SimpliAmp: Automatic incubator (DNA lysis)

DNA Amplification → **Result Interpretation**

QuantStudio 5 PCR instrument

80 min!



QuantStudio 5 Real-Time Food Protection PCR System

Sensitive gesture touch-screen provides stand-alone capabilities:
- Unlimited PIN-protected **user accounts**
- dye calibration/RNaseP functionality

10Gb On-board memory can store 2000-5000 run files

New Blocks:
- **Faster Ramps**
- **Up to 100ul rxns**
- **1.5-fold sensitivity**

Motorized block drawer allows for **Programmable Run Pause**

Thermo Fisher Cloud online ecosystem for data analysis, storage, and monitoring

WiFi adapter port (optional use)

Narrow footprint (27cm/10.6in) takes up less space on the bench

OptiFlex technology offers maximum multiplexing:
- QS5 96: 6 colors

VeriFlex blocks offer better-than-gradient temperature zone control

NEW SureTect System Workflow Instrumentation

ThermoFisherCloud

Applied Biosystems SimpliAmp Thermal Cycler

- Small footprint, replaces two dry bath heaters & blocks for SureTect workflow
- VeriFlex™ blocks enable precise temperature control
- Three unique heat zones for optimized use
- 96 well format for high sample throughput
- Minimal maintenance required
- Two-year warranty
- Order code: A24811

Lysis/Sample Preparation

Applied Biosystems QuantStudio 5 Food Safety Instrument

- Newer system to market versus 7500 Fast System
- Fast cycling mode, 0.1 mL block
- Open platform system: use with SureTect assays, RapidFinder Salmonella Multiplex assay, other Thermo Fisher validated PCR workflows, non-pathogen and custom PCR assays
- 6 -dye color channels
- Arrives factory pre-calibrated for all 5 channels- ready to go out of the box;
- Minimal maintenance required
- Supplied with 12-month warranty
- Extended Service Agreements with loaners available.
- Order codes: A36320, A36328, or A36329

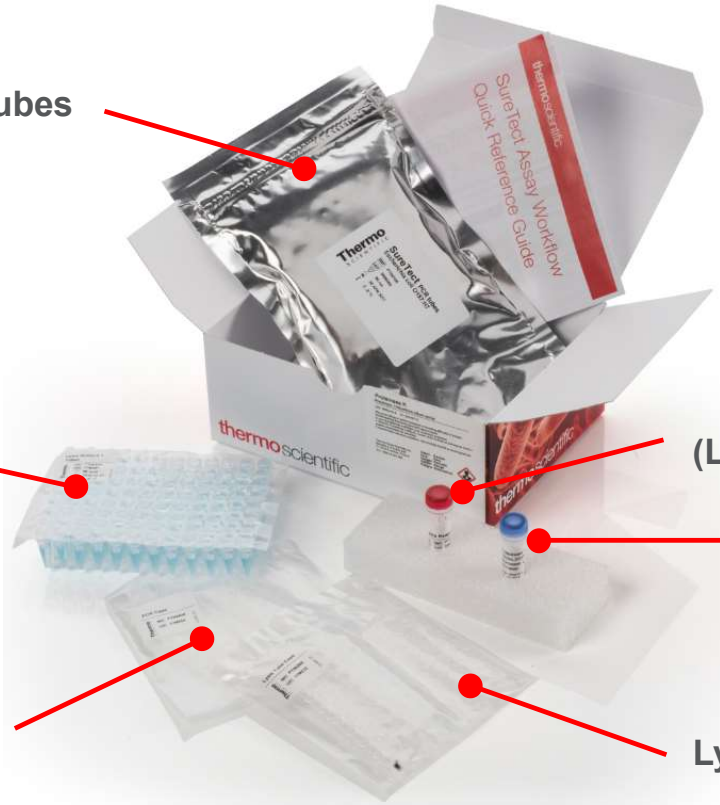
Real-Time PCR

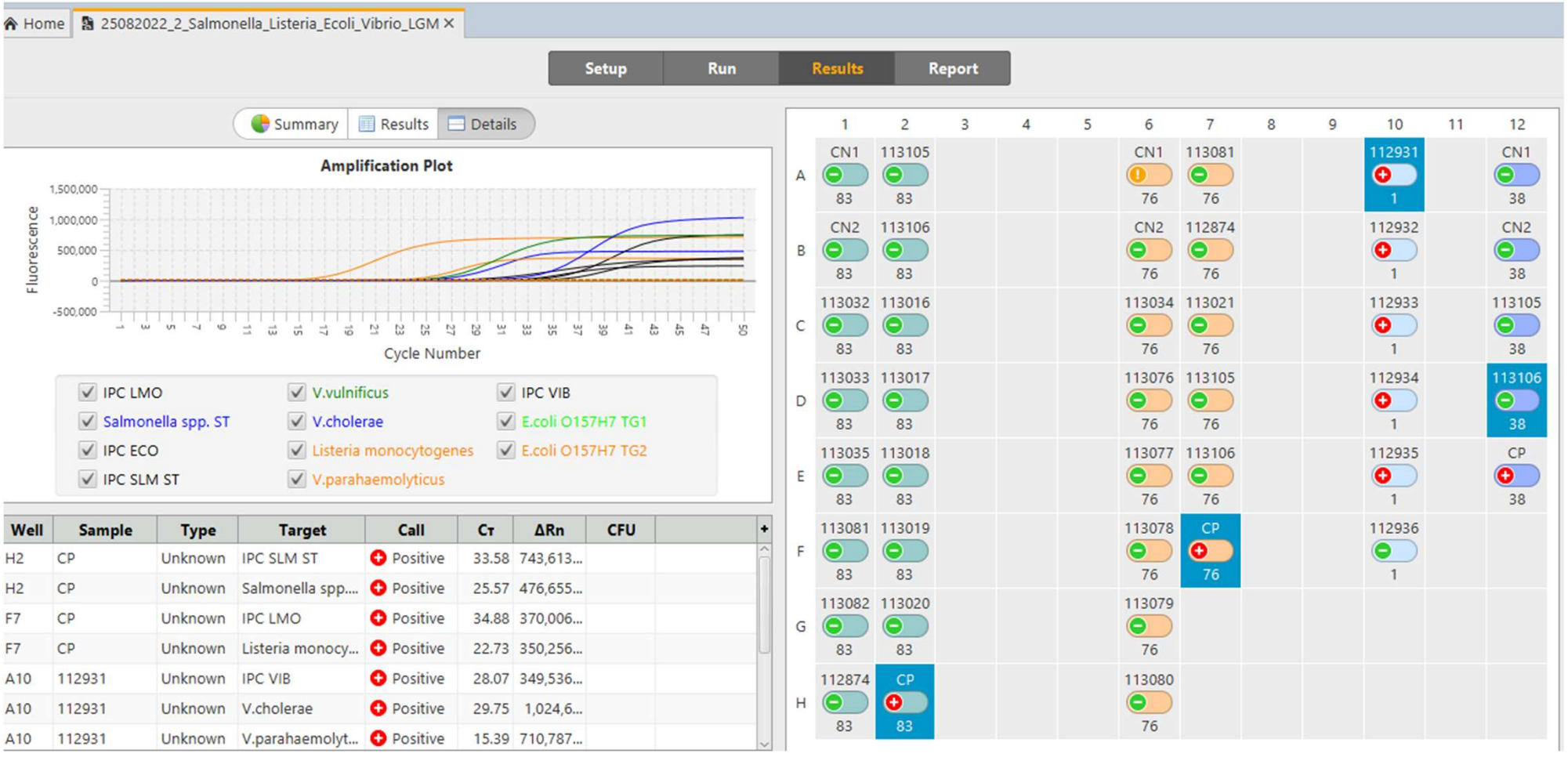
Proprietary & Confidential

ThermoFisher SCIENTIFIC

SureTect Assay Contents

Ship RT
Store 4 °C





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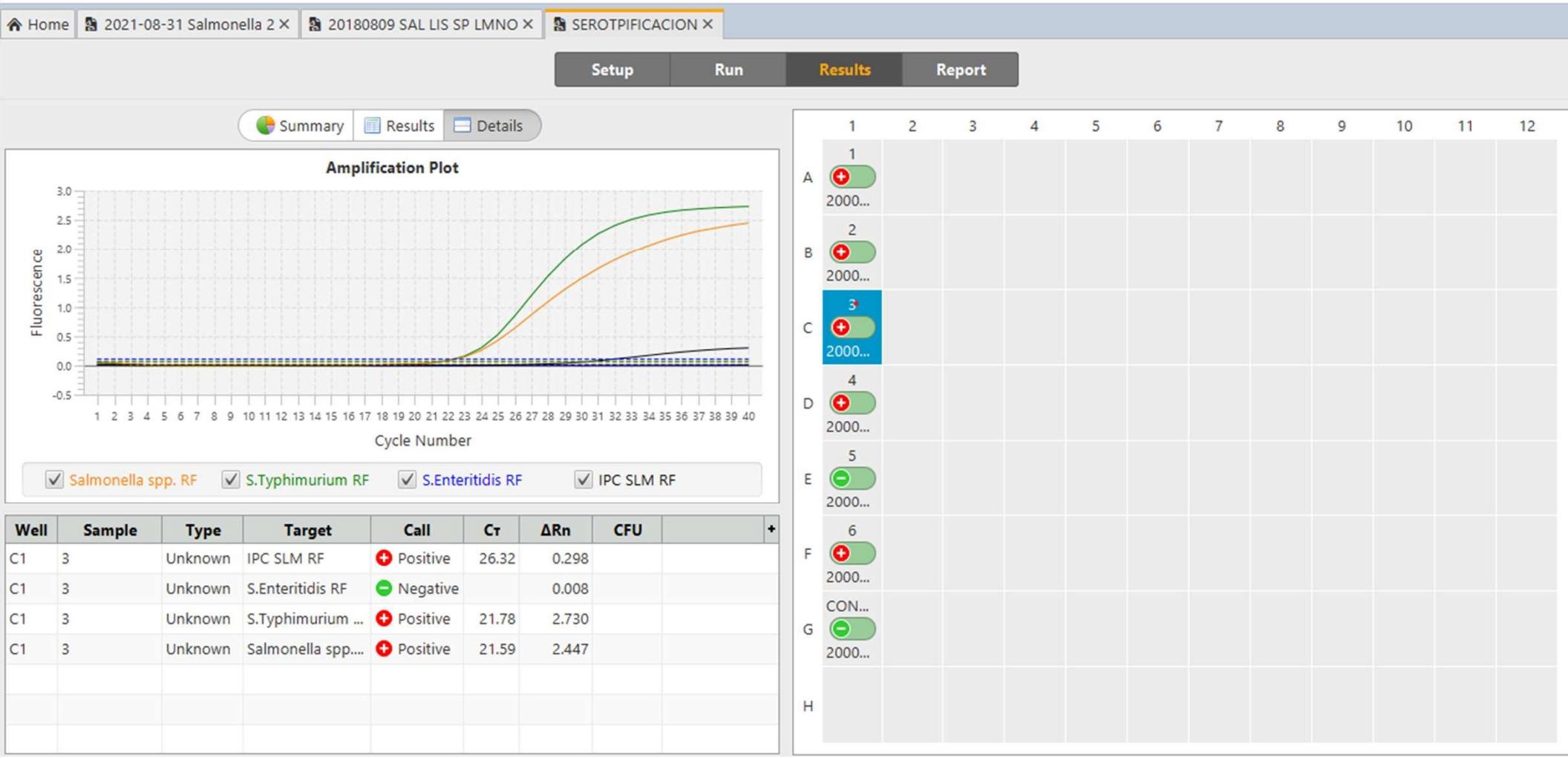
Result Analysis Using the New RapidFinder Analysis Software v 2.03



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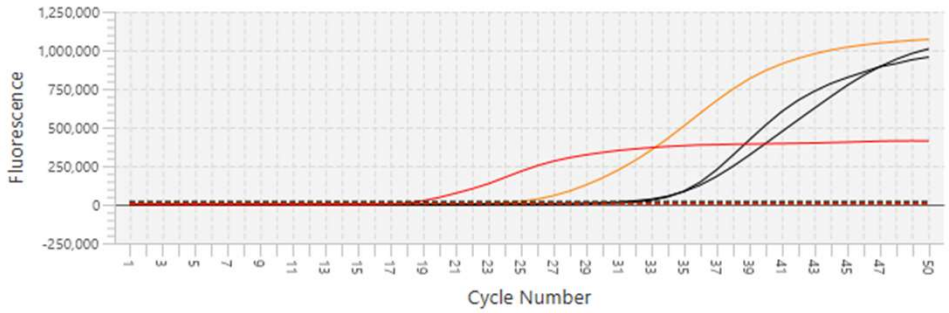
ThermoFisher SCIENTIFIC

Home | 25082022_2_Salmonella_Listeria_Ecoli_Vibrio_LGM | SEROTPIFICACION | 23032021_L_SPP_LMONO_SAL_INSA_MARCO 2021

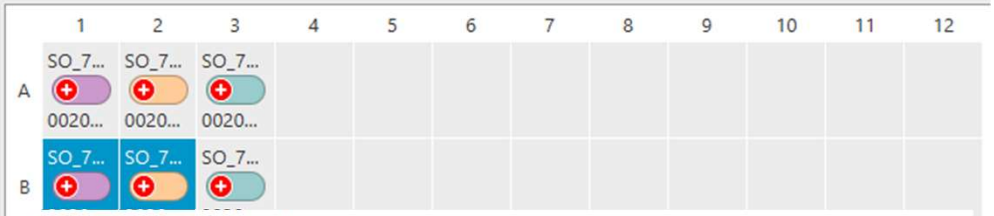
Setup | Run | Results | Report

Summary | Results | Details

Amplification Plot



Well	Sample	Type	Target	Call	Cт	ΔRn	CFU
B1	SO_705_II	Unknown	IPC LSP	Positive	32.36	955,274...	
B1	SO_705_II	Unknown	Listeria spp.	Positive	17.22	412,671...	
B2	SO_705_II	Unknown	IPC LMO	Positive	30.56	1,008,1...	
B2	SO_705_II	Unknown	Listeria monocy...	Positive	24.13	1,070,0...	



Subject: NF VALIDATION mark

La Plaine Saint-Denis, October 6th ,2022

Dear Sir,

Following the positive agreement expressed on October 6th 2022, by the Technical Board of the NF VALIDATION mark (NF102), in its application to the food industry, I beg to inform you that the **extension of NF VALIDATION certification** has been granted to OXOID ThermoFisher for the following alternative method:

Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay

Certificate reference No. UNI 03/08-11/13, with end of validity 04th-Nov.-2025

This validation extension study, performed by comparison to the reference method NF EN ISO 11290-1 (2017) and according to the validation protocol NF EN ISO 16140-2 (2016), allow to validate a new enrichment protocol using the broth 24LEB at 24±4h and to validate a new formulation of Brillance™ Listeria Agar.

Yours Sincerely.

Proprietary & Confidential

ThermoFisher SCIENTIFIC

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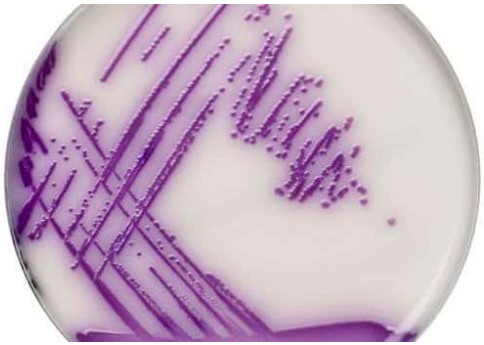
Yours Sincerely.

Precis™ Chromogenics Workflows

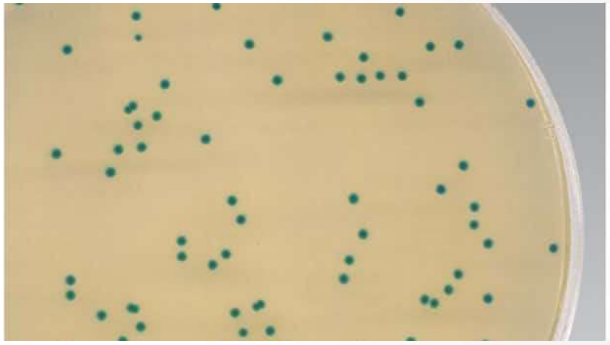
As easy than 1, 2, 3

Salmonella Precis™



- Broad range of food, feeding stuffs, environmental samples

Cronobacter Precis™



- Infant formula & cereals, environmental samples

Listeria Precis™

New formulation
New workflow
Approval Oct 2022

- Broad range of food, environmental samples

Proprietary & Confidential
silvia.carnio@thermofisher.com | 5-October-2022

Listeria Precis™

NEW workflow

① 24 Listeria Enrichment Broth (24 LEB) to boost Listeria recovery and growth

② New Brilliance Listeria for more selectivity and specificity

③ PrecisCheck™ lateral flow tests to confirm *Listeria* and *L. monocytogenes* characteristic colonies within 15 min

Case study run at a routine testing laboratory (FR)

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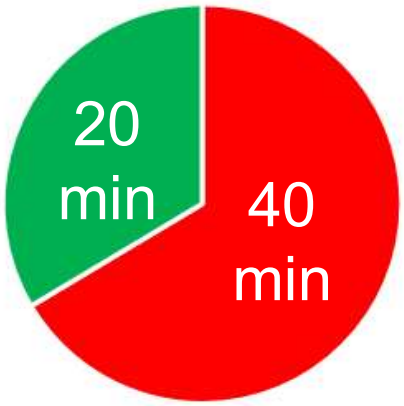
silvia.camilo@thermofisher.com | 5-October-2022

24 LEB to boost Listeria recovery and growth

70 routine testing samples

- Competitors workflows all used 100 µL pipetting before streaking onto the chromogenic agar
- 10 µL streaking with a basic loop saves 21 minutes time for 70 samples.



- **33% time saving = 20 min saving in one hour**
- **Human power saving and error limitation**

Free up 33% of handling time in the screening step

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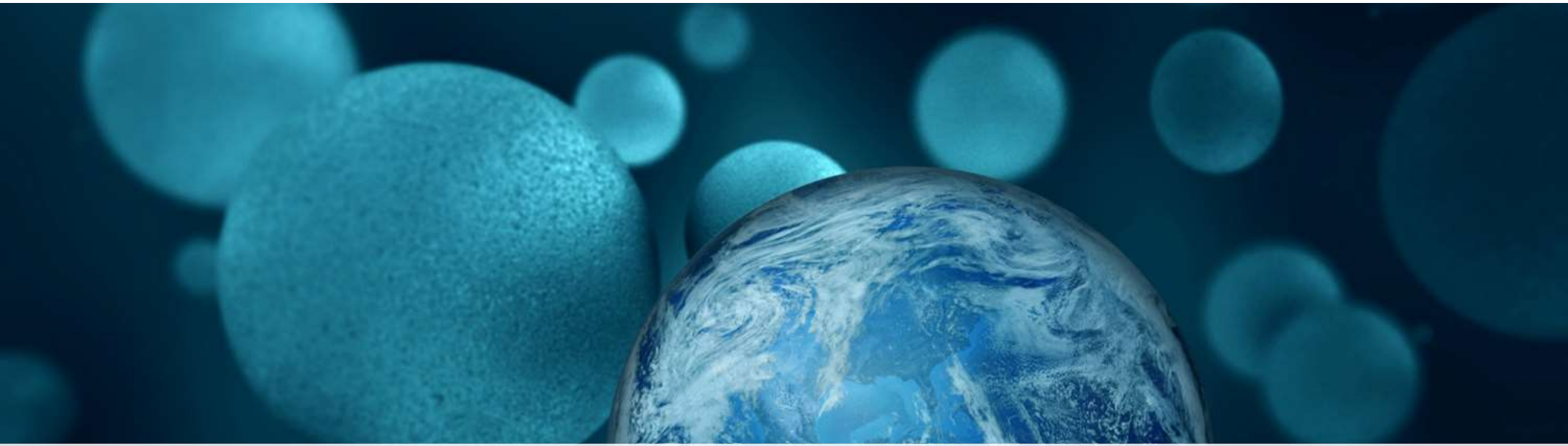
silvia.carnio@thermofisher.com | 5-October-2022



ThermoFisher SCIENTIFIC

Traditional detection vs. SureTect

Traditional Salmonella Detection EN ISO 6579:2002

BPW

RVS

MKTTn

XLD

Second medium

XLD

Second medium

Purify

Confirm

Non selective media, Biochem, serological

Purify

Confirm

Non selective media, Biochem, serological

Purify

Confirm

Non selective media, Biochem, serological

Purify

Confirm

Non selective media, Biochem, serological

4-5 days

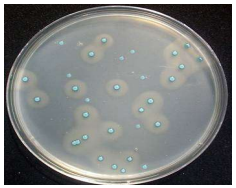
ThermoFisher SCIENTIFIC

Traditional Listeria detection ISO 11290-1/A1:2004

Half Fraser broth

Fraser Broth

OCLA (ISO) or ALOA

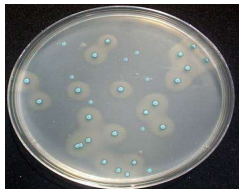


Palcam or Oxford

Purify

5-7 days

OCLA (ISO) or ALOA

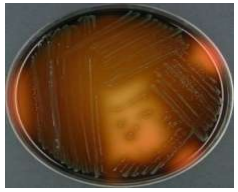


Purify

Confirm

Gram, Catalase, Heamolyse, Test Camp ,Biochemical gallery

Palcam or Oxford



Purify

Confirm

Gram, Catalase, Heamolyse, Test Camp ,Biochemical gallery

Confirm

Gram, Catalase, Heamolyse, Test Camp ,Biochemical gallery

Confirm

Gram, Catalase, Heamolyse, Test Camp ,Biochemical gallery

ThermoFisher SCIENTIFIC

SureTect PCR

Day 1

Day 2

Enrichment

Lysis

Amplification

25g 20-26 hrs
Except 25g Salmonella spp
beef (8-10hrs)

3-6 days faster results

Add ProK & (LR2) and 10 µL enriched sample or negative control to lysis tube.

Place in Simpliamp 25mins

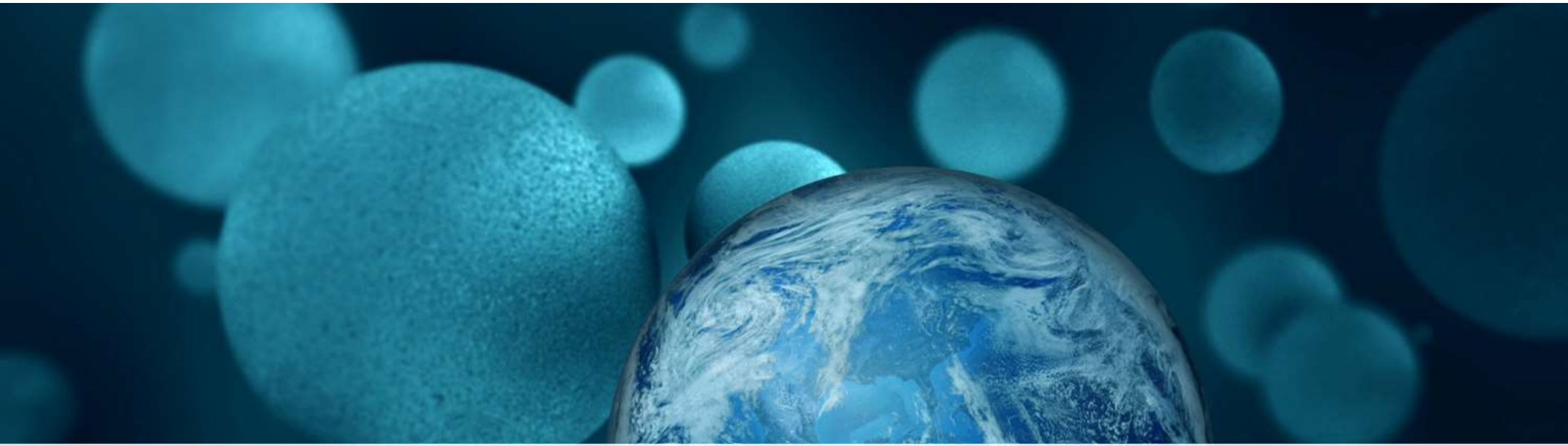
Transfer 20ul lysate into PCR tubes

Mix well

(10) 20-28hrs

Place tubes in instrument. Start run
Automated results Run Time 80 min

ThermoFisher SCIENTIFIC



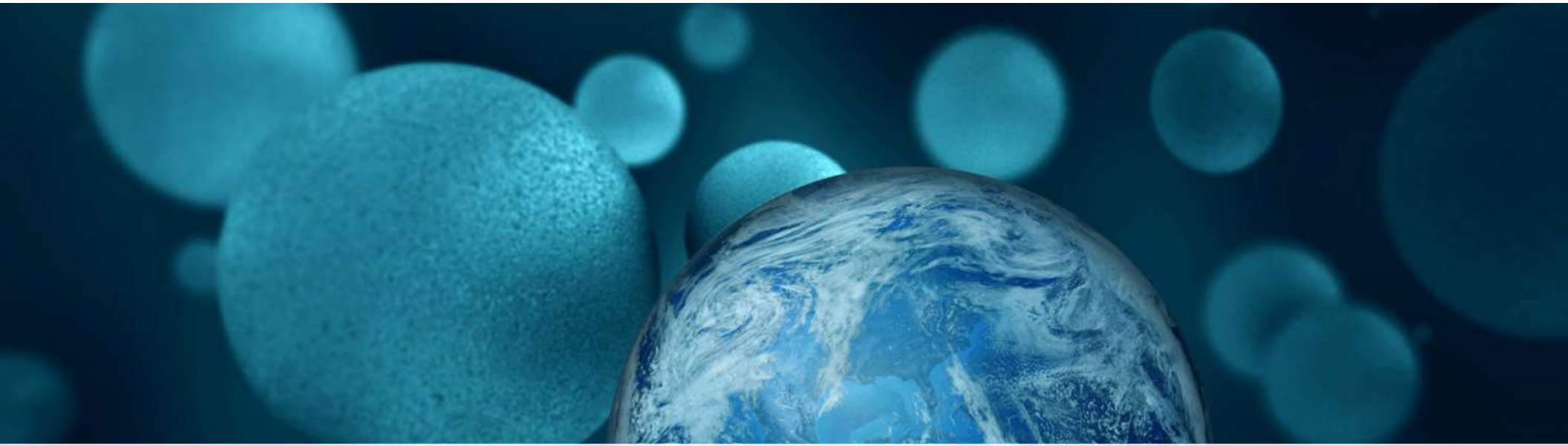
ThermoFisher SCIENTIFIC

Are all Rapid Detection Assays the Same?

Technology Used in Rapid Detection Assays

Technology Type		Technology name
Molecular	Probe based (PCR)	TaqMan™ & Solaris™
		Scorpions®
		FRET
	Florescent Dye based (PCR)	SYBR® Green
	Iso-Thermal Amplification	L-AMP
Immunological	Protein / antibody based	ELFA
		ELISA
		Lateral Flow

ThermoFisher SCIENTIFIC

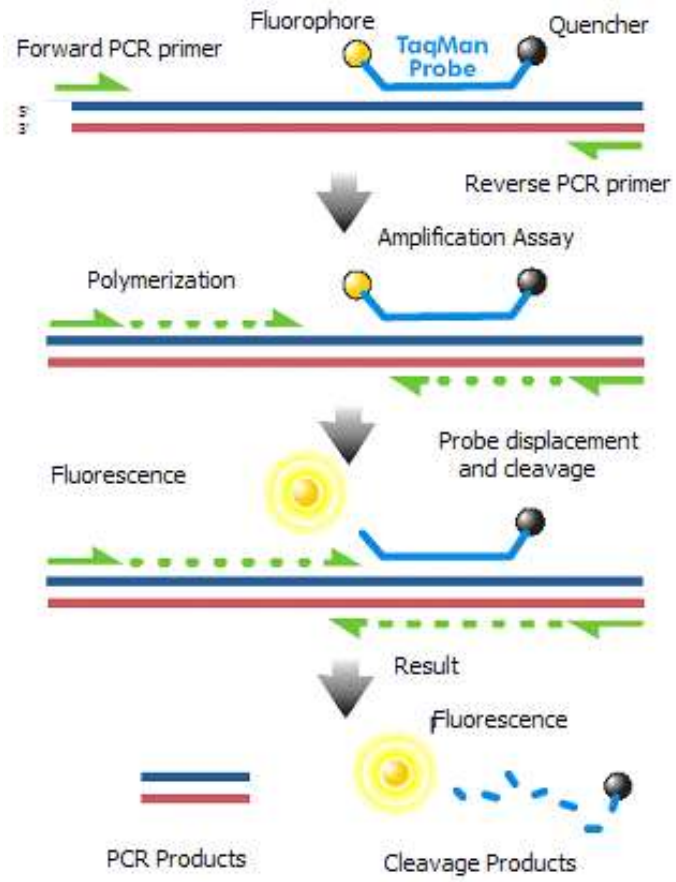


ThermoFisher SCIENTIFIC

Probe based chemistry

Probe based chemistry

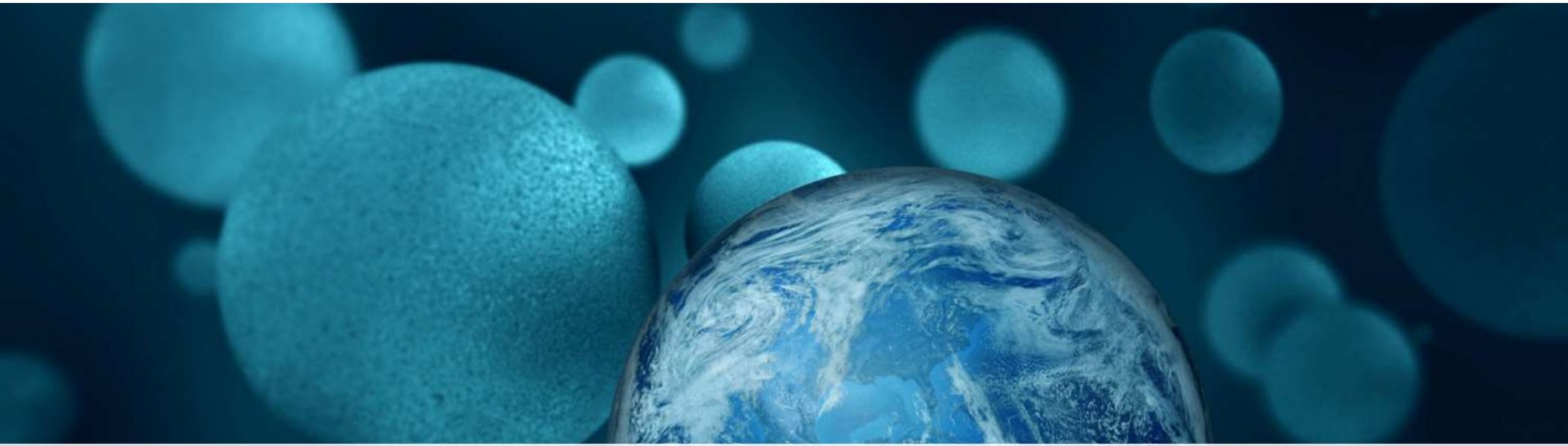
- TaqMan is the best known and most widelyused probe based chemistry used in the real-time PCR technique
- Probes with fluorescence labels
  - Fluorescence on a probe has a specific wavelength
  - Different channels of the qPCR instrument detect different wavelengths
- Multiple targets in one reaction
  - Different filters in the qPCR instrument



ThermoFisher SCIENTIFIC

Probe based chemistry

- Excellent sensitivity and specificity: robust and reliable
- Easy to multiplex assays: few limitations for development of new assays
- IAC / internal amplification control in each tube as standard



ThermoFisher SCIENTIFIC

SureTect Validations, Certifications & Additional Studies

Validations and Certifications: ISO 16140-2

SureTect Assay workflow on QuantStudio 5 Food Safety Real-Time PCR System for detection of:

Salmonella species	*Listeria* species	*Listeria monocytogenes*	*E. coli* O157:H7	*Cronobacter* species
UNI 03/07 - 11/13 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS	UNI 03/09 - 11/13 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS	UNI 03/08 - 11/13 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS	UNI 03/10 - 03/15 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS	UNI 03/11 - 12/15 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
All foods and environmental samples	All foods and Environmental samples	All foods and Environmental samples	Raw beef (seasoned and unseasoned)	Powdered Infant Formula with and without probiotics Production environment samples

ThermoFisher SCIENTIFIC

Validations and Certifications: AOAC RI

SureTect Assay workflow on QuantStudio 5 Food Safety Real-Time PCR System for detection of:

Salmonella species	*Listeria* species	*Listeria monocytogenes*	*E. coli* O157:H7
#051303	#071304	#061302	#021501
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 051303	PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 071304	PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 061302	PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 021501
AOAC RI Performance Test Methods℠ www.aoac.org	AOAC RI Performance Test Methods℠ www.aoac.org	AOAC RI Performance Test Methods℠ www.aoac.org	AOAC RI Performance Test Methods℠ www.aoac.org
Raw ground beef, Pork frankfurters, Raw ground pork, 375g (1:5) ,Raw ground beef, Raw chicken breast, Non-fat milk powder, Pasteurized 2% milk, Cooked shrimp, Bagged lettuce, Cantaloupe melon, Bean sprouts, Stainless steel, Plastic surface, Chilled ready-to-eat dinner, Pasteurized liquid whole egg, Chilled pizza dough, Black pepper corns, Dog food, Cat food, Chocolate	Raw ground beef, Raw ground pork, Raw pork sausage, Pork frankfurters, Salami, Cooked slice ham, Raw ground turkey, Cooked sliced turkey, Fresh bagged spinach, Bagged lettuce, Cantaloupe, Pasteurized 2% milk, Pasteurized brie cheese, Processed cheese, Raw cod, Smoked salmon, Cooked prawns, Ice-cream, Stainless steel, Plastic	Raw ground beef, Raw ground pork, Raw pork sausage, Pork frankfurters, Salami, Cooked sliced ham, Raw ground turkey, Cooked sliced turkey, Fresh bagged spinach, Bagged lettuce, Cantaloupe, Pasteurized 2% milk, Pasteurized brie Cheese, Processed cheese, Ice-cream, Raw cod, Smoked salmon, Cooked prawns, Stainless steel and Plastic	Raw ground beef (1:4/1:5, 375g/325g), Raw beef trim (1:4/1:5, 375g/325g), Bagged spinach and Apple juice

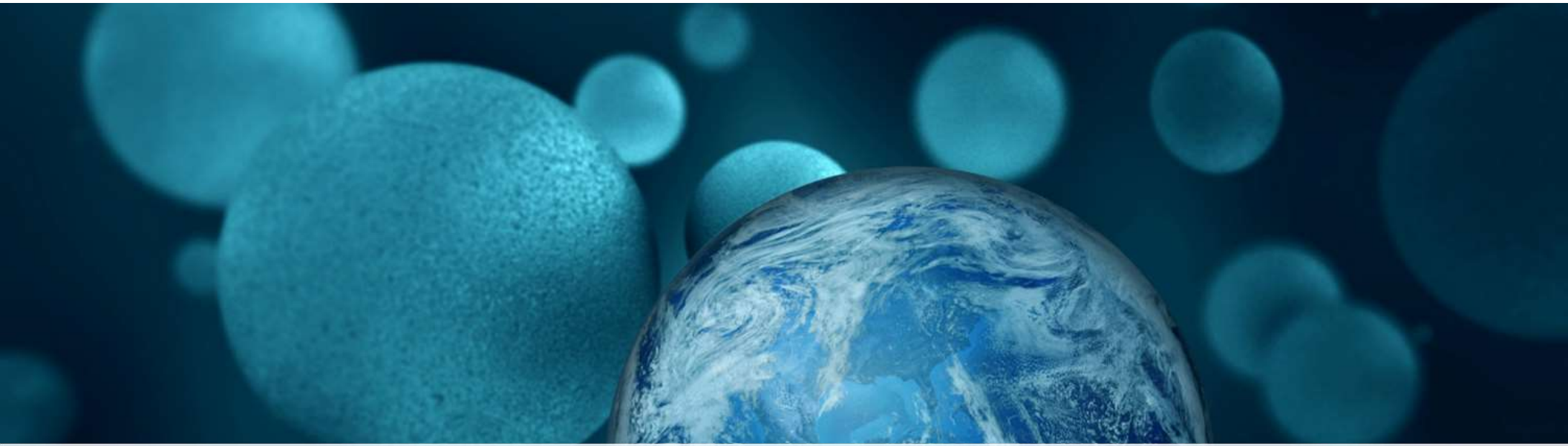
All validated samples sizes are 25 g/mL unless otherwise stated

ThermoFisher SCIENTIFIC

- **AOAC Official Method of Analysis (OMA) approval for the Thermo Scientific™ SureTect™ Salmonella species PCR Assay with a broad range of food and selected environmental surfaces claim.**

- **AOAC Official Method of Analysis (OMA) approval for the Thermo Scientific™ SureTect™ Listeria monocytogenes and Listeria species PCR Assay with a broad range of food and selected environmental surfaces claim.**

-



ThermoFisher SCIENTIFIC

ISO 16140-part 3 what is it and what is the scope

ISO 16140 — Microbiology of the food chain — Method validation

Part 3

Protocol for the VERIFICATION of VALIDATED reference methods and VALIDATED ALTERNATIVE methods in a single laboratory

ICS › 03 › 03.120 › 03.120.20

ISO/IEC 17025:2017

General requirements for the competence of testing and calibration laboratories

ThermoFisher SCIENTIFIC

Which methods can be adopted under ISO 16140 –part 3

1. **Reference methods**, with interlaboratory study

2. **Alternative methods;** with interlaboratory study

SureTect –RTPCR pathogen detection

ThermoFisher SCIENTIFIC

Method implementation in the past

ISO standards

Salmonella Detection (ISO 6579:2002)

Sample + **BPW**
18 hours at 37°C

Subculture 0.1ml into 10ml **RVS Broth**
24 hours at 41.5°C

Subculture 1.0ml into 10ml **MKTTn Broth**
24 hours at 37°C

Subculture each broth onto **XLD Agar** + **2nd Medium**
24 hours at 37°C

Confirm suspect colonies
(6-24 hours)

4-5 days

Vs.

Alternative ISO validated methods

SureTect Salmonella PCR

Sample + **BPW OR One Broth Salmonella**
20-24hours at 37°C

Lysis DNA extraction
20-30mins

Streak on to **Brilliance Salmonella**
24hrs at 37°C

Confirmation **Oxoid Salmonella Latex kit** or traditional ISO

24-26hrs

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No requirement to run a reference method

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Which items to select for implementation verification and item verification?

Scope of the validation
Broad range of food

Food categories	Food types	Examples of items
Meat & meat products	Raw meat Raw delicatessen Cooked delicatessen	Ground beef Raw sausage Cooked ham
Milk & dairy products	Pasteurized milks and yoghurts Pasteurized cheese Dairy dessert	Yoghurts Camembert Tiramisu
Vegetables & fruits	Bagged vegetables and fruits Deli-salad Sprouts & fresh produces	Bagged lettuce Guacamole Soja
Egg products	Pasteurized eggs Egg powder Ready-to-eat and reheat products	Liquid white egg Whole egg powder Omelet
Fish & seafood	Raw fish and seafood Cured and smoked fish Ready-to-eat & reheat products	Fish filet Smoked salmon Surimi

Scope of the laboratory application
Broad range of food

Implementation verification or **Item verification**

Usual lists of samples tested by the laboratory	Examples of items	Reasons
Meat and meat products	Raw meat **Raw delicatessen** Cooked delicatessen	In the validation scope **Microflora & low moisture** In the validation scope
Milk and dairy products	**Raw milk & cheese** **Milk powders** Dairy desserts and yoghurts **Ice creams** **Probiotic milks**	**Microflora** **Freeze drying** In the validation scope **Freezing** **Probiotics**
Vegetables and fruits	Bagged vegetables and fruits Deli-salad Sprouts and fresh produces	In the validation scope In the validation scope In the validation scope
Chocolates, confectionary & bakery products	**Cocoa beans, cocoa powder, cocoa butter, chocolates** **Syrups, candies, honey, sugar** **Cookies, cakes**	**Low moisture foods with high fat content** **Low moisture** **Low moisture**
Infant formula and infant cereals	**Non probiotic formulations** **Probiotic formulations** **Ingredients (Maltodextrin, lactose...)**	**Low moisture** **Low moisture + probiotics** **Low moisture + chemical properties**

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Method verification

Verification of SureTect *Salmonella* PCR Assay

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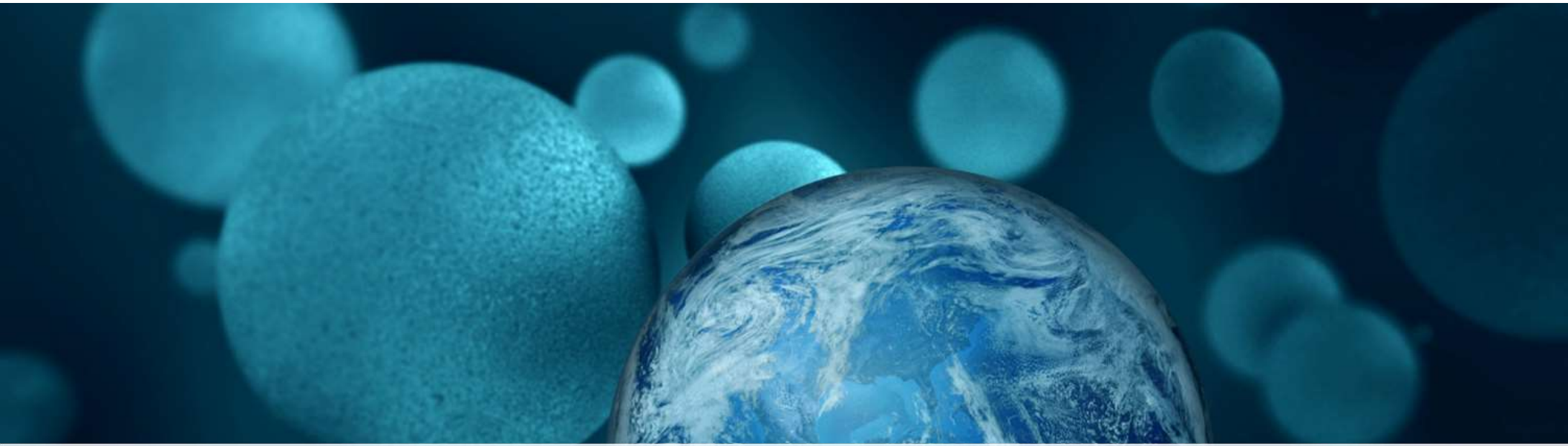
Ensure you have all required documentation readily available to ensure a succesful verification:

- the user guide
- the ISO 16140-2 validation study report
- the certificate
- the user guide of the
  - 7500 Fast Thermocycler

  OR
  - QuantStudio 5 Thermocycler
- the ISO 16140-part 3 standard

Verification tools



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Molecular Biology-qPCR Applications

The world leader in serving science

Examples of Real-Time PCR Applications in Food and Water Testing

- Foodborne pathogens detection
- GMOs detection & quantification
- Species (e.g. Fish and meat) identification & quantification
- SARS-CoV-2 (causal agent of COVID-19) on Food Packaging and Environmental Surfaces
- Bio-threat species
- *Legionella* sp

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Beneficios:

Velocidad Menos de 4 horas desde el muestro hasta el risultadot

Alto rendimiento Validado y probado en agua sanitaria, térmica y procedente de torres de refrigeración

Fiabilidad Normas relacionadas con el material de referencia

Máxima especificidad probada al 100 % de conformidad con la norma ISO / TS 12869

Conveniencia Solución completa que incluye la preparación de la muestra y el análisis de la PCR en tiempo real

Flexibilidad Capacidad para detectar y cuantificar *Legionella spp* . y *L. pneumophila simultáneamente*

Compatibilidad Sistema abierto y flexible compatible con los principales termocicladores de PCR en tiempo real del mercado

alizadas para la introducción a la ogía PCR:
rmociclador PCR y formación

1. Filtración | **2. Extracción del ADN** | **3. Configuración** | **4. PCR en** | **5. Análisis de los**

10 min	60 min	10 min	100 min	10 min
Filtración de muestras de agua para concentrar las bacterias	Aislamiento y purificación rápida del ADN con el DNApure Water Isolation kit	Adición de ADN extraído de las muestras y de los controles a los pocillos de PCR que contienen reactivos de amplificación	Amplificación por PCR de *Legionella spp* . / *L. pneumophila* y los controles internos	

Tiempo total de análisis: menos de 4 horas

DI-Check *Legionella pneumophila*
Número de referencia del certificado DTV 41/01- 12/19, con fin de vigencia 04-dic-2023 MÉTODOS DE ANÁLISIS DE AGUAS
El método está validado para la detección y cuantificación de *L. pneumophila* en todo tipo de muestras de agua, por comparación con el método de referencia NF T90-471 (2015) e ISO/ TS 12869 (2019) y según el protocolo de validación de la certificación AFNOR para la detección y recuento de *L. pneumophila* por PCR. https://nf-validation.afnor.org/

Especificaciones técnicas

	Método cuantitativo	Método cuantitativo
Límite de detección	5 GU/pocillo PCR	5 GU/pocillo PCR
Límite de cuantificación	25 GU/PCR well	na
Rango de cuantificación	25-25 000 GU/pocilloPCR	na
Especificidades	Amplia lista de especies bacterianas analizadas para la inclusión y la exclusividad	
ADN estándar	Curva estándar relacionada con el material de referencia	
InControl interno de amplificación en cada muestra	Detecta la presencia de inhibidores en la muestra y comprueba la exactitud de los resultados negativos	
Cicladores térmicos compatibles	Requiere un ciclador qPCR con juego de filtros para FAM™ y dyes VIC/HEX/JOE	

Detalles del producto

Código	Producto	Descripción	Formato
MBK0080	DNApure Water Isolation kit (AFNOR)	Kit de extracción de ADN	100 extracciones
MBK0058	DI-Check Legionella spp. kit	real-time PCR kit	100 pruebas
MBK0081	DI-Check L. pneumophila kit (AFNOR)	real-time PCR kit	100 pruebas

DIATHEVA DI-Check
Legionella pneumophila

Environment
Water testing

Quality solutions for the detection and quantification of pathogenic microorganisms in water

DI-Check Legionella pneumophila
Certificate reference No. DTV 41/01-12/19, with end of validity 04-Dec-2023
WATER ANALYSIS METHODS
The method is validated for the detection and quantification of *L. pneumophila* in all types of water samples, by comparison to the reference method NF T90-471 (2015) and ISO/TS 12869 (2019) and according to the AFNOR certification's validation protocol for the detection and enumeration of *L. pneumophila* by PCR.
https://nf-validation.afnor.org/

Code	Product	Description	Size
MBK0080	DNApure Water Isolation kit (AFNOR)	DNA extraction kit	100 extractions
MBK0058	DI-Check Legionella spp. kit	real-time PCR kit	100 tests
MBK0081	DI-Check L. pneumophila kit (AFNOR)	real-time PCR kit	100 tests

QuantStudio™ Design & Analysis Software v1.5.1

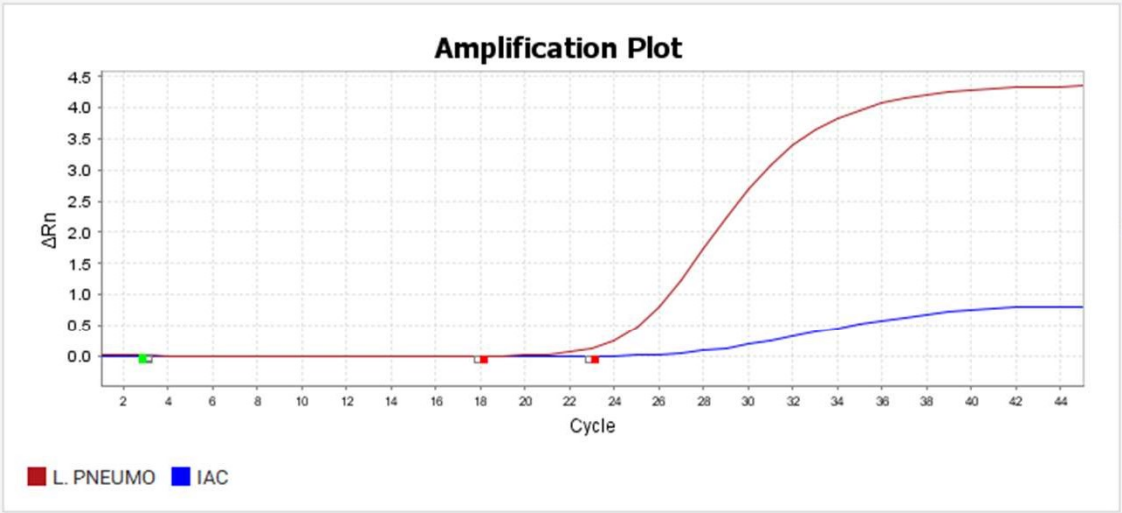
File Edit Analysis Tools Help

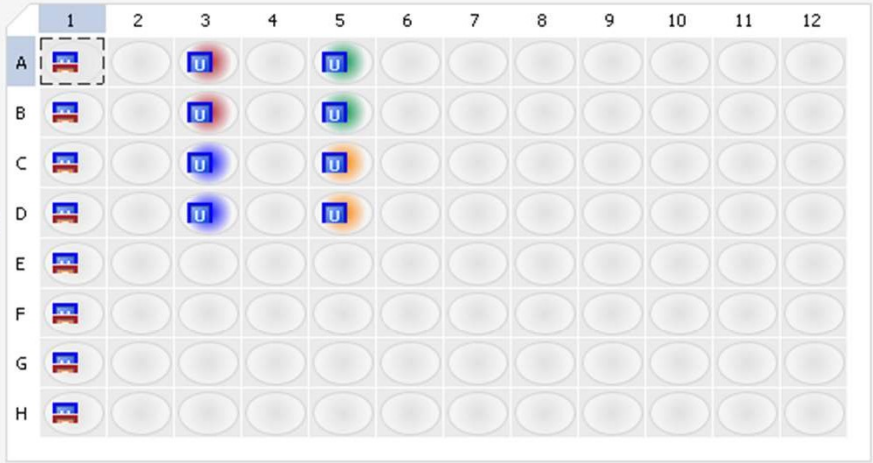
Properties Method Plate Run Results Export Analyze

Results

Action Save

All Target | Amplification Plot | View

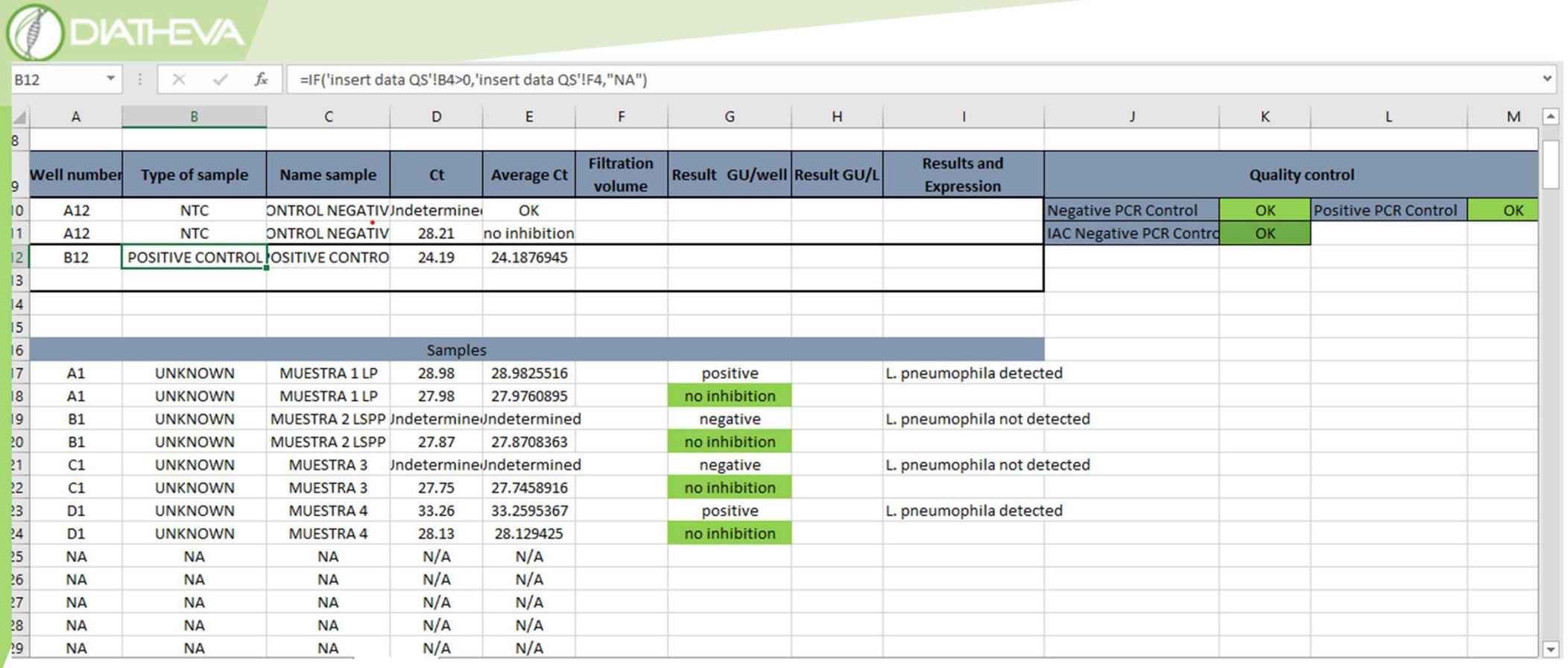




Wells: U 16 S 8 N 0 80 Empty

Previous Next

Home | 2022-11-21_LE... | LEGIONELLA P... | 2022-08-17_13... | 20181213.eds



B12 =IF('insert data QS'!B4>0,'insert data QS'!F4,"NA")

Well number	Type of sample	Name sample	Ct	Average Ct	Filtration volume	Result GU/well	Result GU/L	Results and Expression	Quality control			
A12	NTC	ONTROL NEGATIV	Undetermine	OK					Negative PCR Control	OK	Positive PCR Control	OK
A12	NTC	ONTROL NEGATIV	28.21	no inhibition					IAC Negative PCR Contro	OK		
B12	POSITIVE CONTROL	OSITIVE CONTRO	24.19	24.1876945								

Samples								
A1	UNKNOWN	MUESTRA 1 LP	28.98	28.9825516		positive		L. pneumophila detected
A1	UNKNOWN	MUESTRA 1 LP	27.98	27.9760895		no inhibition		
B1	UNKNOWN	MUESTRA 2 LSPP	Undetermine	Undetermined		negative		L. pneumophila not detected
B1	UNKNOWN	MUESTRA 2 LSPP	27.87	27.8708363		no inhibition		
C1	UNKNOWN	MUESTRA 3	Undetermine	Undetermined		negative		L. pneumophila not detected
C1	UNKNOWN	MUESTRA 3	27.75	27.7458916		no inhibition		
D1	UNKNOWN	MUESTRA 4	33.26	33.2595367		positive		L. pneumophila detected
D1	UNKNOWN	MUESTRA 4	28.13	28.129425		no inhibition		
NA	NA	NA	N/A	N/A				
NA	NA	NA	N/A	N/A				
NA	NA	NA	N/A	N/A				
NA	NA	NA	N/A	N/A				
NA	NA	NA	N/A	N/A				

Why Care About Food Authenticity and Fraud?

- In 2014 the Grocery Manufacturers Association (GMA) estimated that fraud cost the global food industry $10-$15 billion a year, with 10% of all commercially sold food products affected[1,2]
- Fraud resulting in a food safety or public health risk event could have significant financial or public relations consequences for the food industry or company
- Food fraud can tarnish the reputation of the food manufacturer or contract testing laboratory

[1] GMA, Consumer Product Fraud, Deterrence and Detection, 2010, http://www.gmaonline.org/downloads/wygwam/consumerproductfraud.pdf
[2] A. Kircher, NCFPD, Tools for Protecting the Nations Food Supply, June 5, 2012.

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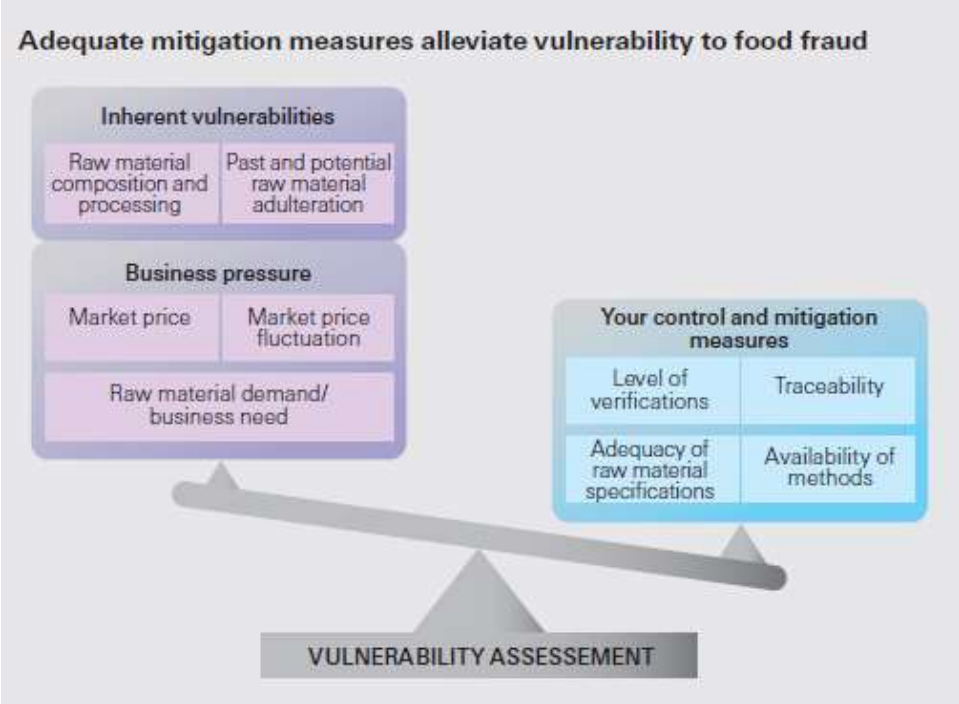
Mitigation Against Food Fraud

- Raw material specifications
- Supplier relationship
- Supplier audit
- Supply chain transparency and simplification
- Alert system
- Analytical surveillance



Excerpt from: https://www.nestle.com/asset-library/documents/library/documents/suppliers/food-fraud-prevention.pdf

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Species Quantification

RapidFinder Quant Multi-Meat

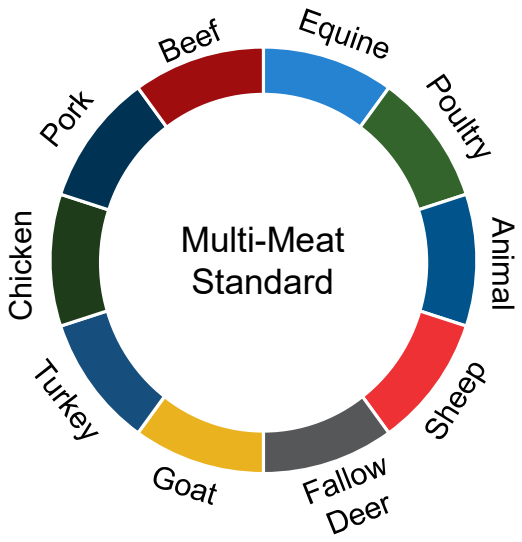
Allows the percentage of as little as 0.5% of one species DNA in a sample to be determined with respect to total animal DNA

Used in combination with one or more ID kits Beef, Pork, Equine, Poultry, Chicken, Turkey, Sheep, Goat , Fallow Deer

Multi-Meat Standard

- Plasmid DNA quantitation standard containing a copy of each of the targets used during analysis
- Used to build a standard curve of known copy numbers
- Comparison with the samples allows a relative quantification to be made

% Species DNA = species DNA/total animal DNA X 100



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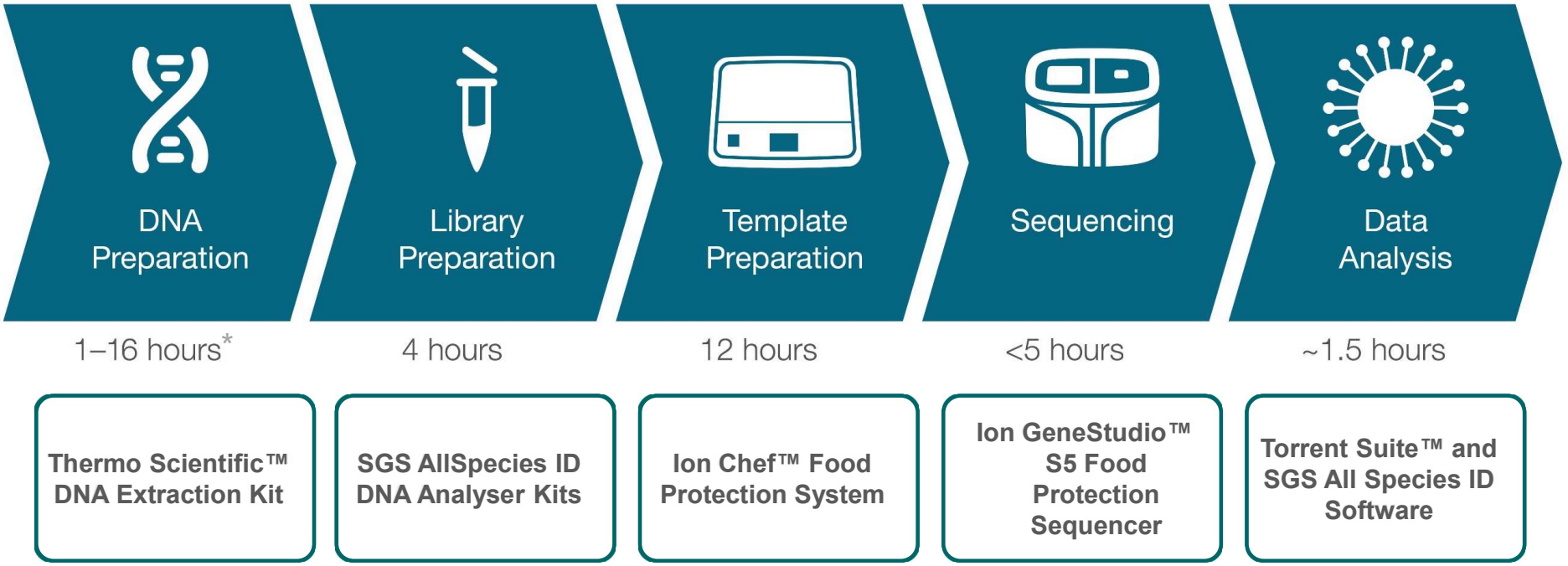
Multi-Species Screening and Identification

The new **Thermo Scientific™ NGS Food Authenticity Workflow** is a complete, automated, next generation sequencing workflow and software database for multi-species ID screening



* DNA preparation time range includes overnight incubation for select few sample types

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~30 Minutes Hands-On Time After loading the Ion Chef Food Protection Instrument

Ion Chef™
Automated template preparation

Pipet
library into Ion Chef System for templating and chip loading

Load cartridge
into Ion Chef System

15 min
hands-on time

Ion GeneStudio™ S5
Sequencing

Transfer chip
to Ion GeneStudio S5 System for sequencing

15 min
hands-on time

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Multi-Species Screening and Identification

DNA is extracted and purified from the test sample

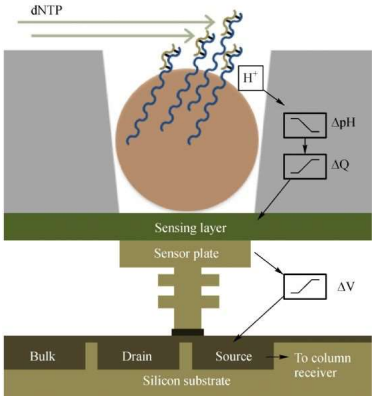
The DNA is then amplified and individually barcoded using SGS All Species ID Analyser Kits

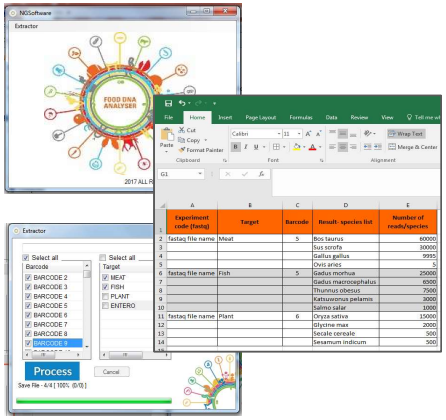
Ion Chips contain millions of microscopic wells; the barcoded DNA is copied onto beads, amplified and then loaded into the Ion Chip wells (one bead/well)

The DNA on each bead is single-stranded; in the Ion GeneStudio Sequencer a sequential flow of nucleotides is passed over the Ion Chip building a complementary DNA strand

Each extension releases H+ which is measured in each well and translated into the DNA sequence

S5 FASTQ files are uploaded to SGS All Species ID Software program
Results appear as a list of the species present in the sample
If a species is present but not identified an 'unknown' result will be recorded
No additional interpretation of the results is required by the technician





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Informative and Reliable Results

- All targets are detected in a single run (meat, fish & plant)
- Reliable results in complex (multi-ingredient) and processed samples
  - DNA sequencing – most reliable method for species confirmation
  - DNA can be highly fragmented as the regions targeted are very short, e.g. 100 nucleotides
- NGS is starting to be introduced into international standardization:
  - NWIP (new work item proposal) ISO project: ISO TC 34/SC 16 ISO 22949-1
  - Molecular biomarker analysis – Detection and identification of animal species by DNA sequencing methods (Including NGS)

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Target	Barcode	Species	Number Of Reads
FISH	1	Pangasianodon_hypophthalmus	3680
		Sander_lucioperca	1737
		Pleuronectes_platessa	1158
		Anguilla_japonica	1124
		Lophius_piscatorius	615
		Chanos_chanos	364
		Melanogrammus_aeglefinus	64
	2	Oncorhynchus_mykiss	630
	3	Abramis_brama	2086
		Leuciscus_leuciscus	756
		Leuciscus_idus	550
		Rutilus_rutilus	534
		Vimba_vimba	407
		Alburnus_istanbulensis	284
	4	Rutilus_rutilus	5417
		Pollachius_virens	2807
MEAT	22	Bos_taurus	19306
	23	Gallus_gallus	16534
		Bos_taurus	4217
	24	Sus_scrofa	10105
		Bos_taurus	158
PLANT	3	Origanum_sp./Origanum_onites	86660
		Origanum_sp./Origanum_vulgare	43666
		Origanum_sp./Origanum_majorana/Orig	42978
		Thymus_sp.	5159
		Convolvulus_arvensis	2772
		Lactuca_sp./Lactuca_sativa	1369
	4	Origanum_sp./Origanum_onites	71582
		Origanum_sp./Origanum_vulgare	36150
		Origanum_sp./Origanum_majorana/Orig	35415
		Laurus_nobilis	15076

DNA sequences databases:
GenBank (=NCBI BLAST)
BOLD (Barcode Of Life Database)

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Find Out More About Multi-Species ID for Your Laboratory Today

- Discuss your food fraud management system and needs
- Find out how Thermo Fisher Scientific and SGS can help your business protect itself from food fraud
- Review customer data: Nestlé Research evaluation study

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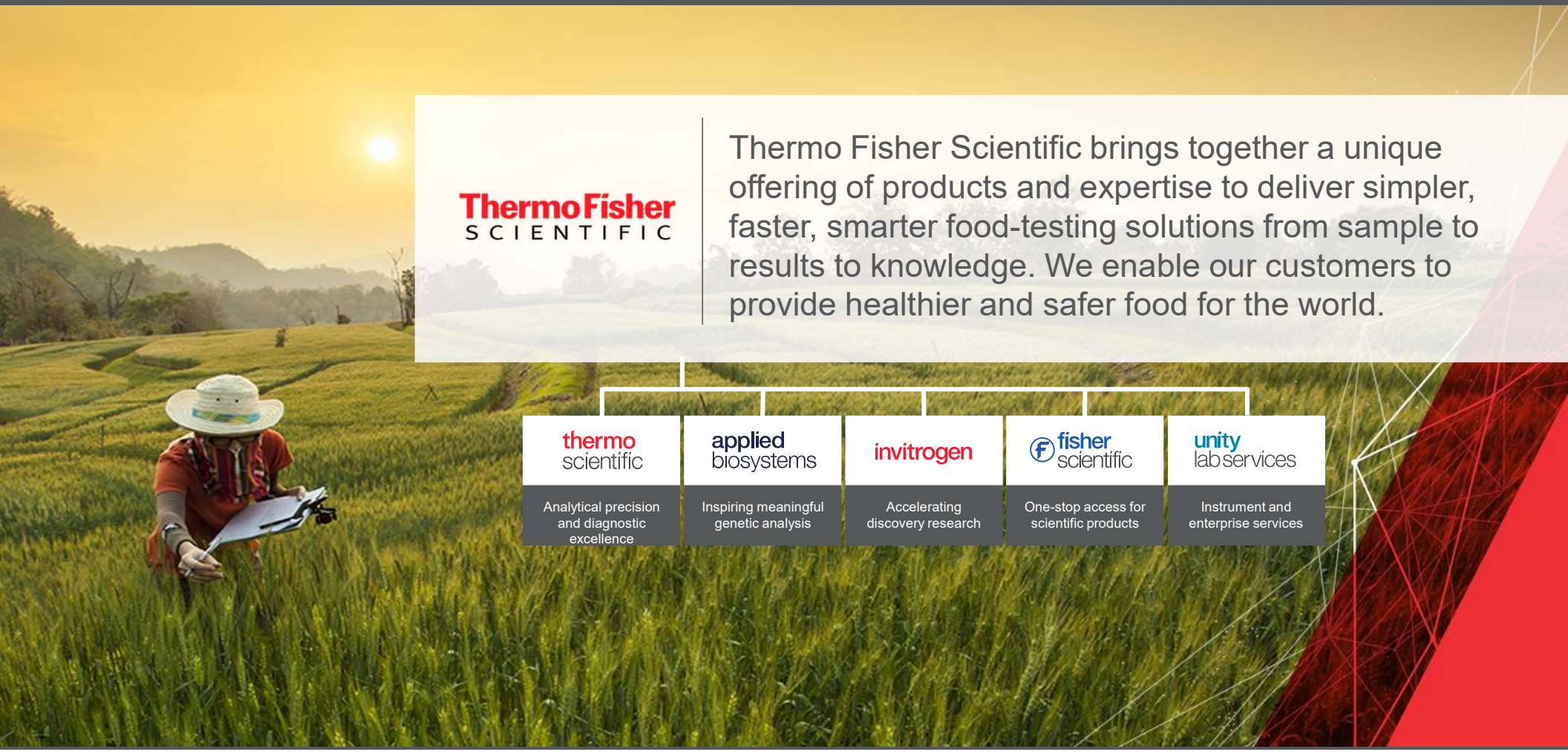
Our leading scientific products, services and workflow solutions



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Thermo Fisher Scientific brings together a unique offering of products and expertise to deliver simpler, faster, smarter food-testing solutions from sample to results to knowledge. We enable our customers to provide healthier and safer food for the world.

thermo scientific	applied biosystems	invitrogen	fisher scientific	unity lab services
Analytical precision and diagnostic excellence	Inspiring meaningful genetic analysis	Accelerating discovery research	One-stop access for scientific products	Instrument and enterprise services

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