



3MSM Health Care Academy

3M Food Safety

Uso y ventajas de amplificación isotérmica en la detección de microorganismos patógenos en alimentos y control ambiental

Introducción

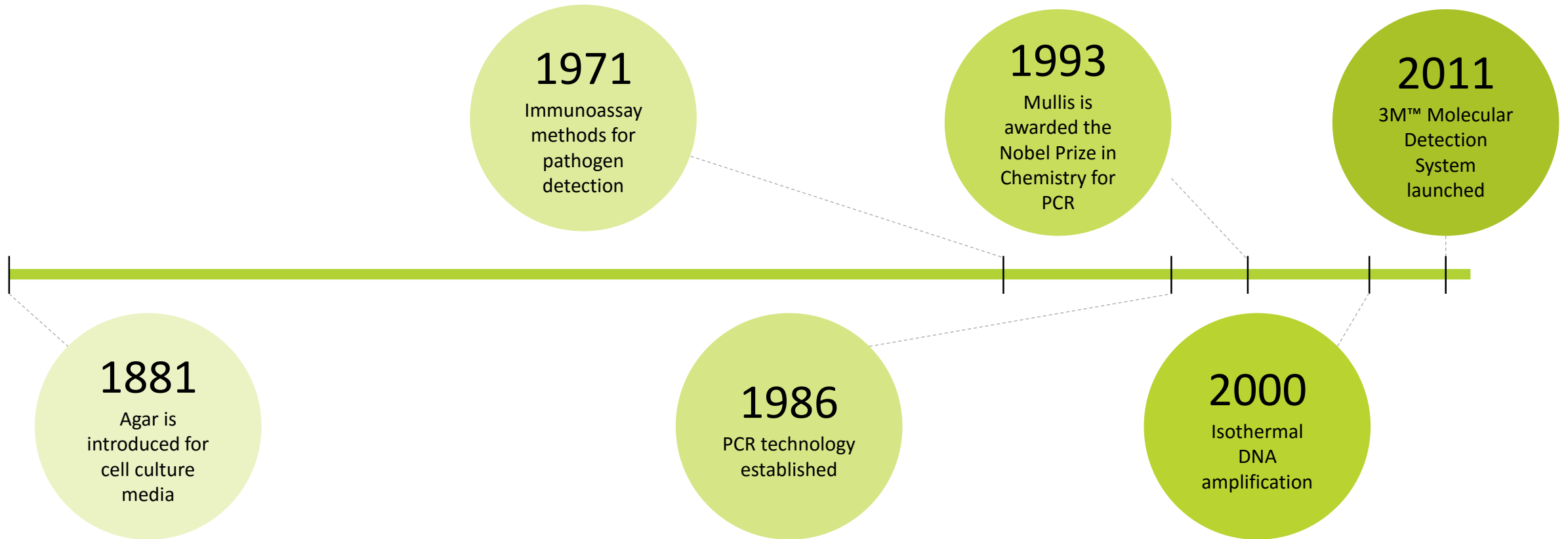
Los avances en biología molecular han permitido desarrollar varias técnicas de detección basados en genomas, en general la mayoría de estas técnicas requiere alta capacidad analítica, personal altamente capacitado y son elevadas en costo.

Dentro de los criterios que se evalúan al implementar una técnica tanto en laboratorios de planta de proceso como en laboratorios de servicio destacan la sensibilidad, especificidad, costo, simplicidad y rapidez

La técnica de amplificación isotérmica medida por bucle o LAMP destaca por ser eficaz y fácil de realizar a pesar de que el principio y mecanismo de acción es complejo

Ha sido utilizada con éxito para detectar enfermedades virales así como de agentes bacterianos

Un poco de historia...



Pirámide de metodologías actuales

TIER 1

Genético/ Molecular

**Automated:
Transcription
Mediated**

PCR

Isothermal

TIER 2

Inmunoensayo

Automated: ELFA

ELISA

Immuno Chromatography / Lateral Flow

TIER 3

Cultivo, Bioquímica

Automated: Prepare, Inoculate, Incubate, Read

Sample Ready Plates

Powdered Agar & Chromo Agar

Migrando hacia sistemas moleculares...

- ✓ Rapidez
 - Resultados más rápidos que los de método tradicional
- ✓ Alta especificidad
 - Detección basada en identificadores genéticos
- ✓ Sensibilidad
 - Capacidad de detectar muy bajas concentraciones del patógeno target
- ✓ Facilidad de uso
 - Opciones de contar con un sistema automatizado
- ✓ Mayor potencial de desarrollo
 - Nuevas tecnologías prometen flexibilidad y versatilidad en los ensayos

Lo que se espera es que los metodos moleculares sobrepasen a los metodos tradicionales y se conviertan en los Gold Standar o metodos de referencia!

**rapidmicrobiology**
focused on microbiology

Eliminating Risk & Human Error In Endotoxin Testing
Wednesday, January 24 11:00 AM – 12:00 PM EST
SIGN UP TO ATTEND

[Home](#) [News](#) [Events](#) [Test Methods](#) [Suppliers](#) [Videos](#) [Find products](#)

search rapidmicrobiology

Search

Molecular Testing for Food Pathogens

Using Molecular Based Kits to Detect Food Pathogens

Key Points

- Rapid - results usually available within 24 hours
- Highly specific - detection based on genetic identifiers
- Sensitive - capable of detecting a single cell in a 25g sample
- User friendly - suitable for full automation and able to run simultaneous assays for several pathogens
- Development potential - new technologies promise more rapid and flexible detection methods



Food microbiology laboratories have changed considerably during the last 30 years. In the early 1980s the focus was on quality control testing, much of which was done in-house by manufacturers. But as the industry began to adopt the HACCP approach to food safety management, quality control was replaced by quality assurance. Prevention, rather than detection of contamination, became the goal of food safety control and much of the remaining microbiological testing was outsourced. This trend was in part driven by the limitations of traditional microbiological methods, especially for foodborne pathogens. Conventional methods continued to rely on culturing the microorganisms in a sample to produce sufficient cells for detection. That culture step meant that the total time taken for any microbiological test is at least 24 hours – for some pathogens it is five days or more – limiting the role that microbiological testing can play in food safety and quality assurance. Conventional tests can never provide results quickly enough to monitor critical control points in a HACCP system effectively, and are now used mainly to verify that the system is working correctly.

Nevertheless, the demand for microbiological testing in the food industry has never been greater. Common foodborne pathogens like *Campylobacter* and *Salmonella* continue to cause widespread illness, while less common organisms, notably *Listeria monocytogenes* and *Shigatoxin/Vero toxin producing E. coli* (STEC/VTEC) have been responsible for very serious foodborne disease outbreaks in Europe and North America. Increasing food production, concerns over food safety, legislative requirements and the demands of large retailers have all driven an increase in microbiological testing, especially for specific pathogens. According to recent reports by market analysts Strategic Consulting, an estimated 275 million food microbiology tests were carried out in the EU in 2011, while in the USA over 213 million tests were performed in 2010. This is thought to account for over half of the entire microbiological testing market and the proportion of that testing that is pathogen-specific is rising at a rate three times greater than that of the total. This demand for more testing has inevitably created a growing need for test methods that provide results more quickly, yet the standard reference methods used for enforcement testing are still mostly based on conventional culture techniques.

In the last ten years, culture based pathogen detection has faced a growing challenge from molecular methods, especially those based on detecting elements of the microbial genome. While molecular testing has yet to overhaul conventional methods in food microbiology, the pace of technological change is driving the development of new techniques that will soon allow that to happen. Some of these already exist as commercial products.

[Click here to see the latest PCR kits for Food Pathogen Testing in our Special Focus - What's on Show at IAFP 2016](#)

Molecular detection technologies

PCR - The key discovery in the development of molecular biology in the 1980s was the polymerase chain reaction or PCR. The PCR provides a means of synthesising multiple copies of (amplifying) a specific piece of DNA. It made the speedy detection of very small quantities of DNA in a sample not only possible, but also practical. A more detailed description of the PCR and the development of practical microbial detection methods employing it can be found in our Method Guide PCR for Food Microbiology. In brief, a PCR-based detection method consists of three stages as follows:

- 1. DNA extraction** - A process designed to lyse any cells of the target pathogen in the sample and release bacterial DNA. This can be difficult in a food matrix, especially when pathogens are present in low numbers. This step typically includes a DNA purification procedure and many commercial test protocols still require an enrichment culture stage before extraction to increase the number of bacterial cells.
- 2. DNA amplification** - During this step the PCR is used to produce multiple copies of the target DNA sequence (often a single gene highly specific to the pathogen concerned). This is done by alternately raising and lowering the temperature (thermo-cycling) to facilitate the binding of primers to the target sequence and its duplication by a DNA-polymerase enzyme. By repeating this process 30-40 times enough DNA for detection can be generated in a few hours.
- 3. DNA detection** - Once sufficient copies of the target sequence have been produced they can be detected. Some methods rely on end-point detection, where the amplified target sequences are separated by electrophoresis and visualised by a staining technique. However, many commercial test protocols employ real-time detection, in which the amplification and detection steps are combined, typically by using a fluorescent reporter probe to monitor the amplification process and indicate a detection when the number of copies produced reaches a threshold level. Real-time detection also allows testing for more than one pathogen in the same assay by using probes labelled with different coloured dyes.

Real-time PCR assays for food microbiology have been developed into commercial products whereby some or all of the steps can be automated to minimise the number of operations involved and reduce the risk of contamination. Automation requires a higher capital investment and so will depend on the sample throughput required. The reaction usually takes place inside a computer-controlled combined thermocycler/fluorescence detection instrument and uses pre-prepared reagents. For foodborne pathogen detection tests, the entire process can be completed within 20-30 hours with sufficient sensitivity to detect a single cell in a 25g sample.

Choose test method guide:

Select by Organism:

Select by Test Method:



Pharmaceutical Microbiology
22-23 JAN 2016
www.pharma-microbiology.com



Looking for Listeria in Food?
Read our Test Method Guide

Get our eNewsletter

Get the latest microbiology news every week - straight to your inbox

LATEST MICROBIOLOGY NEWS

- Use MWE Transwab® to Ensure Compliance with ISO 15189
- Bio-Rad RAPID/Campylobacter/Media Fits New European Regulation
- Remote Maintenance/Diagnosis Option for Astel AutoCubes
- FDA-Cleared LioCheck MIC Test Strip (MTS™) Erythromycin (E)
- How to Choose the Right Rapid Microbiology Method for Your Lab
- Data Integrity Considerations for Conventional and Rapid Microbiological Methods

Ref: Food Safety Info, www.rapidmicrobiology.com

Métodos de detección

Métodos tradicionales

Usar características metabólicas para su aislamiento e identificación.



- Capacidad para crecer en presencia de ciertos nutrientes, sales, químicos o antibióticos

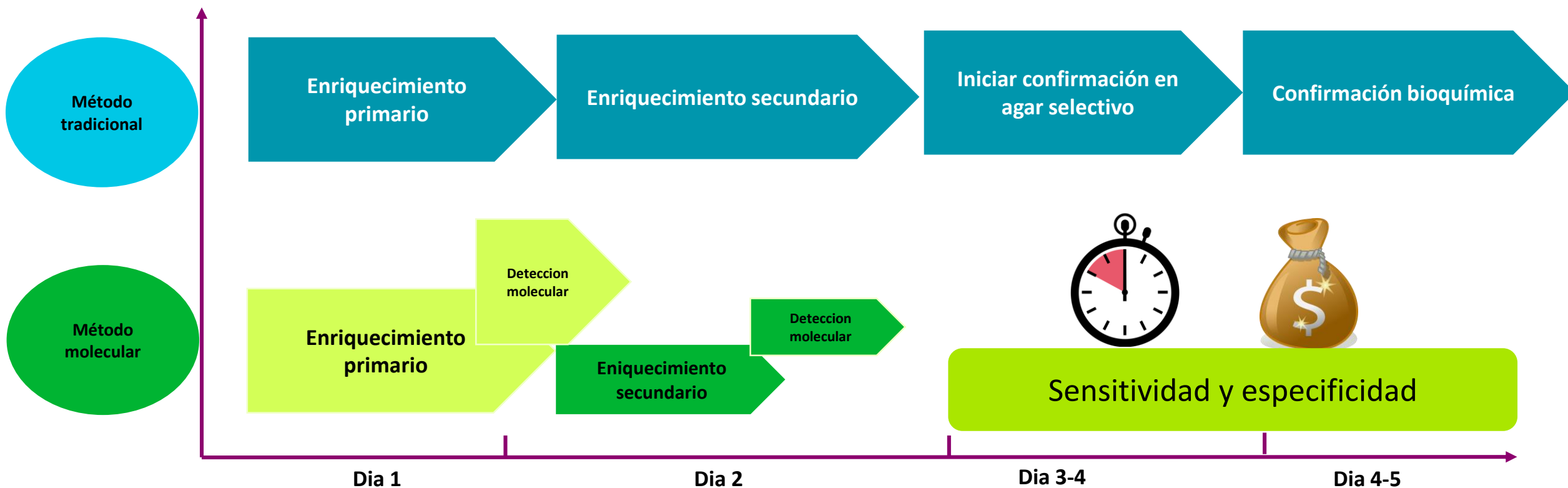
Moleculares

Busca la presencia de moléculas específicas que solo se encuentran en un microorganismo o grupo de microorganismos

Métodos rápidos de detección molecular

- *Permiten liberación de producto más rapido*
- *Permiten identificar rápidamente la posible presencia de contaminación microbiana*
- *Implementación rápida de medidas correctivas*

Beneficio de métodos moleculares para la detección de patógenos



LAMP Amplificación Isotérmica de Ácidos nucleicos

LAMP (Loop mediated isothermal amplification)

- ✓ La técnica LAMP al igual que PCR tiene la capacidad de amplificar fragmentos específicos de ADN lo que permite la detección altamente sensible de patógenos.
- ✓ La efectividad de LAMP se basa en el diseño de primers que deben ser muy específicos. A diferencia de PCR, LAMP requiere de 4 a 6 primers
- ✓ El método utiliza una ADN polimerasa con actividad de desplazamiento de cadena. Esta propiedad de amplificación por desplazamiento no requiere el paso de PCR para denaturalizar (90°C)
- ✓ Es isotérmica, es decir la reacción ocurre a una misma temperatura 60°C en forma continua, lo que es una importante ventaja sobre PCR tradicional
- ✓ Alta eficiencia de amplificación: ADN se amplifica 10^9 – 10^{10} veces en 15 a 75 minutos
- ✓ Una de las grandes ventajas está relacionado con la mayor tolerancia a inhibidores

LAMP en la comunidad científica

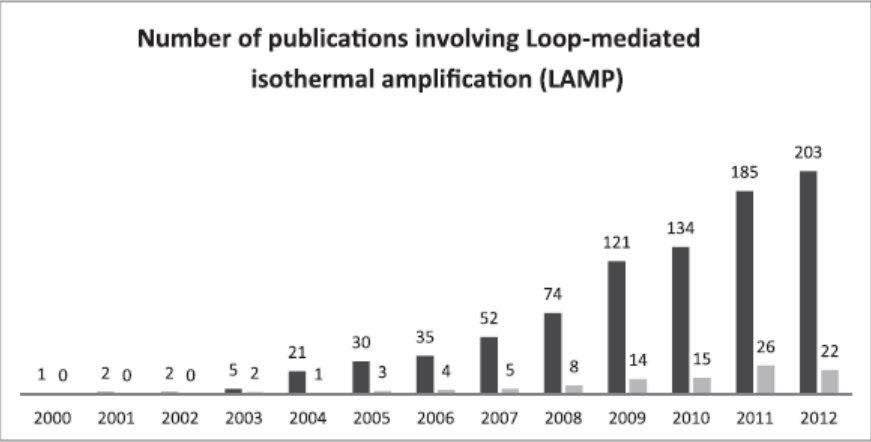
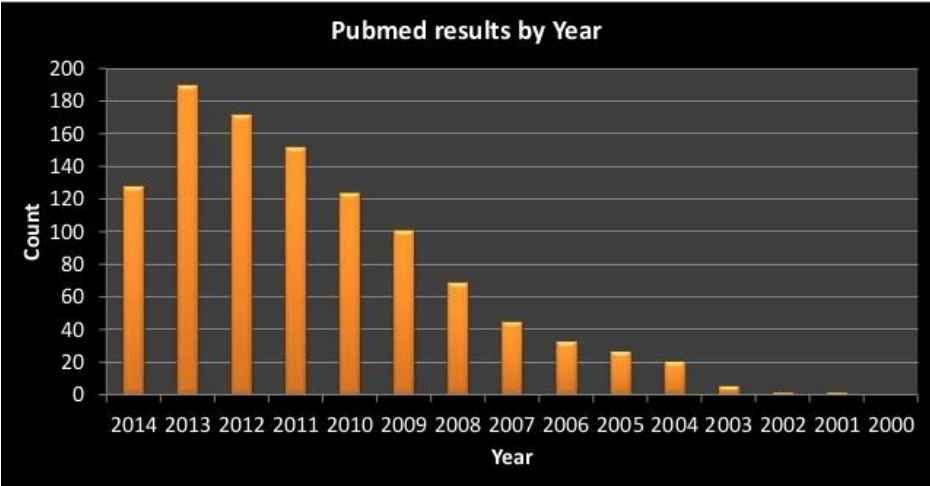


Fig. 1. Total annual number of English speaking publications (patents excluded) involving loop-mediated isothermal amplification (LAMP) (black bar) and number of publications involving LAMP in food sources (grey bar), patents excluded, between 1. January 2000 through 31 December 2012 according to Science Citation Index Expanded as of 12. March 2013.



Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Review

The application of loop-mediated isothermal amplification (LAMP) in food testing for bacterial pathogens and fungal contaminants



Ludwig Niessen*, Jie Luo, Carla Denschlag, Rudi F. Vogel

Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Weihenstephaner Steig 16, 85350 Freising, Germany

ARTICLE INFO

Article history:
Received 17 October 2012
Received in revised form
23 April 2013
Accepted 25 April 2013
Available online 9 May 2013

Keywords:
Food safety
Nucleic acid amplification

ABSTRACT

Bacterial pathogens and toxicants, parasites as well as mycotoxin producing fungi are the major biotic factors influencing the safety of food. Moreover, viral infections and prions may be present as quasi biotic challenging factors. A vast array of culture dependent analytical methods and protocols for food safety testing has been developed during the past decades. Presently, protocols involving molecular biological techniques such as PCR-based nucleic acid amplification and hybridization have become available for many of the known pathogens with their major advantages being rapidness, high sensitivity and specificity. However, this type of assays is still quite labor- and cost intensive and mostly cannot be operated directly in the field. Recently, loop-mediated isothermal amplification (LAMP) of DNA has emerged as an alternative to the use of PCR-based methods not only in food safety testing but also in a wide array of applications. Its advantages over PCR-based techniques are even shorter reaction time, no need for expensive

Loop-Mediated Isothermal Amplification (LAMP)

Especificidad

- + de 6 (primers) capaces de reconocer 8 regiones diferentes del AND target
- Bst DNA polimerasa, 5' → 3' polymerase y desplazamiento de la cadena de ADN

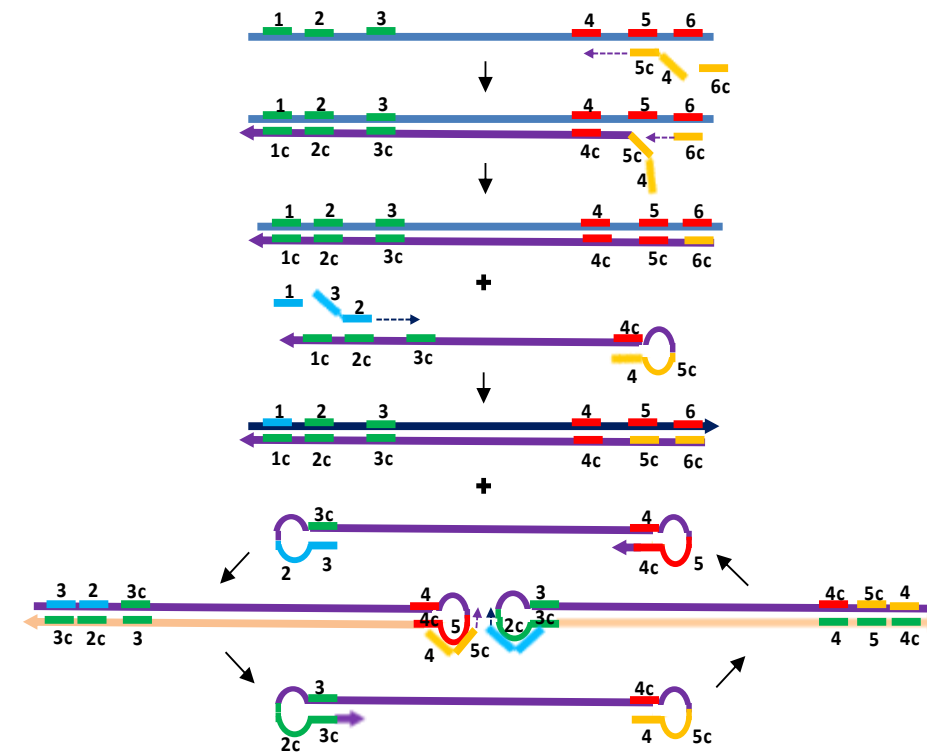
Sensibilidad

- Amplificación rápida y continua de las secuencias target de ADN
- Detección de 1-5 zonas o secuencias lógicas target por reacción

Consistencia

- Excelente tolerancia a frecuentes sustancias inhibidoras para PCR
- Reacción continua y temperatura constante

<http://loopamp.eiken.co.jp/e/lamp/anim.html>



LAMP

- Reacción isotérmica (60 to 65 °C)
- Usa de 4 a 6a primers
- **4 primers reconocen 6 regiones distintas del gen target**
- Primers “loop” aceleran la reacción e incrementan la especificadas (**reconocimiento de 2 sitios adicionales**)
- Simple, equipo alta tecnología menor costo
- Menos interferencia causado por inhibidores
- LAMP-Bioluminiscencia permite una fácil detección (puede detectar la amplificación durante la reacción)

PCR

- Reacción cíclica
- Tempertura Variable
 - Denaturación (95 °C)
 - Alineamiento/extension (50 to 60 °C)
- **Usa solo 2 primers**
- Requiere termociclados (heating and cooling)
- Utiliza sondas en el Real-time PCR que permite mayor especificidad

Tecnología superior al PCR

TABLE 3 | Advantages and limitations of molecular methods for the detection of *Listeria monocytogenes* in food

Molecular methods	Advantages	Limitations
Simple PCR	• High sensitivity and specificity • Accurate and reliable results	• Se - and • Re
Multiplex PCR	• High sensitivity and specificity • Enables simultaneous detection of multiple foodborne pathogens	• Se - PCR • Pr
Real-time/quantitative PCR	• Higher sensitivity and specificity than PCR • More rapid than simple PCR • No post-amplification • An	• Co
LAMP	• Higher sensitivity and specificity than PCR • Cost-effective • Simple • Operates without thermal cycling system	• Co

Ventajas de LAMP:

- Mayor sensibilidad y especificidad que PCR
- Económico
- Simple
- Funciona sin termociclador

An insight into the isolation, enumeration, and molecular detection of *Listeria monocytogenes* in food. Law et al., *Frontiers in Microbiology*, November 2015, Volume 6, Article 1227

3M Sistema de Detección Molecular

3M ha desarrollado una Avanzada Tecnología de Amplificación Isotérmica (LAMP) y de Detección a través de la Bioluminiscencia, para brindarte un Sistema de Detección Molecular de 3M™ fiable, preciso que incrementará su productividad.

Mejor tecnología...

**Amplificación
Isotérmica ADN**



**Bioluminiscencia
Detección**



Combinación de 2 tecnologías para brindar la precisión, rapidez esperada de una forma costo efectiva.

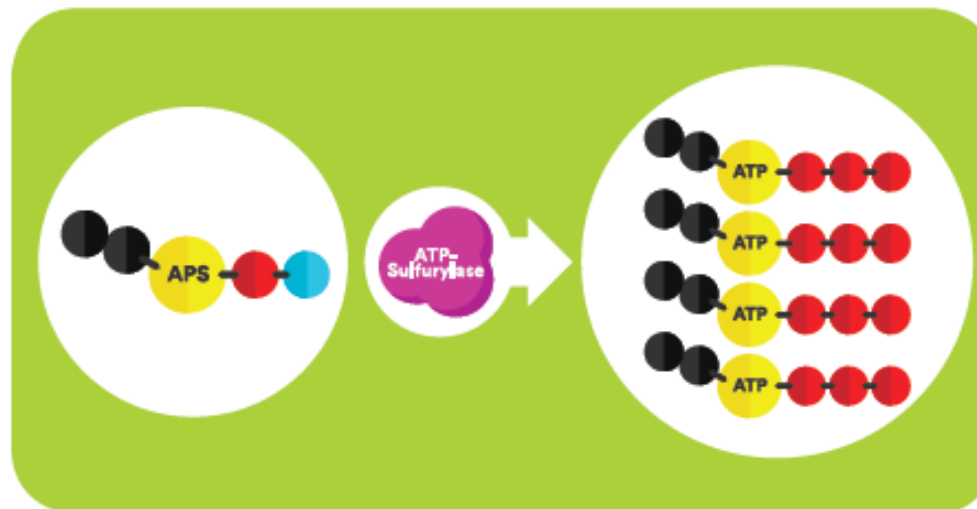
¿Cómo funciona?

Amplificación Isotérmica del ADN

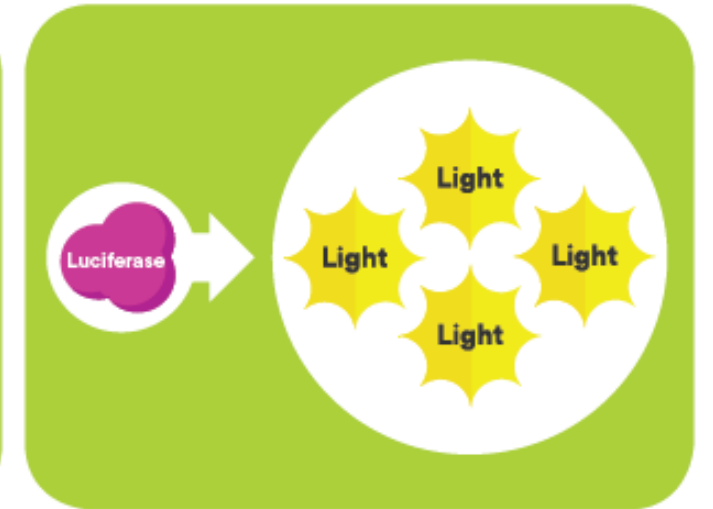


Amplificación Isotérmica Mediada por Bucle (LAMP) de ácidos nucleicos. Dirigida por la enzima Bst ADN Polimerasa y el uso de 6 diferentes cebadores para permitir una alta especificidad.

Detección de la Bioluminiscencia



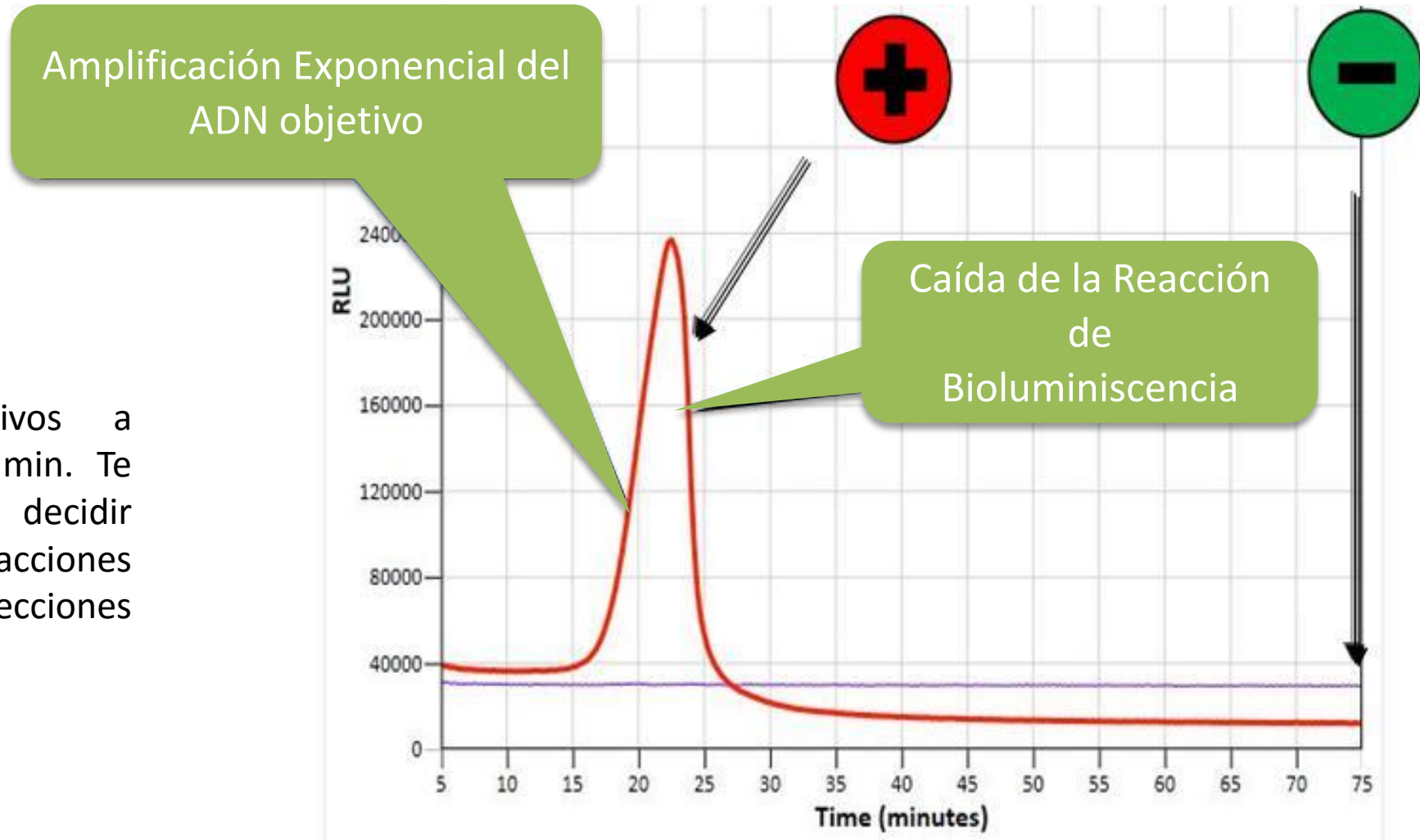
Generación Exponencial de Pirofosfatos Inorgánicos (Ppi) son convertidos a Adenosine Tri Fosfato (ATP) mediado para la enzima ATP-Sulfurilasa.



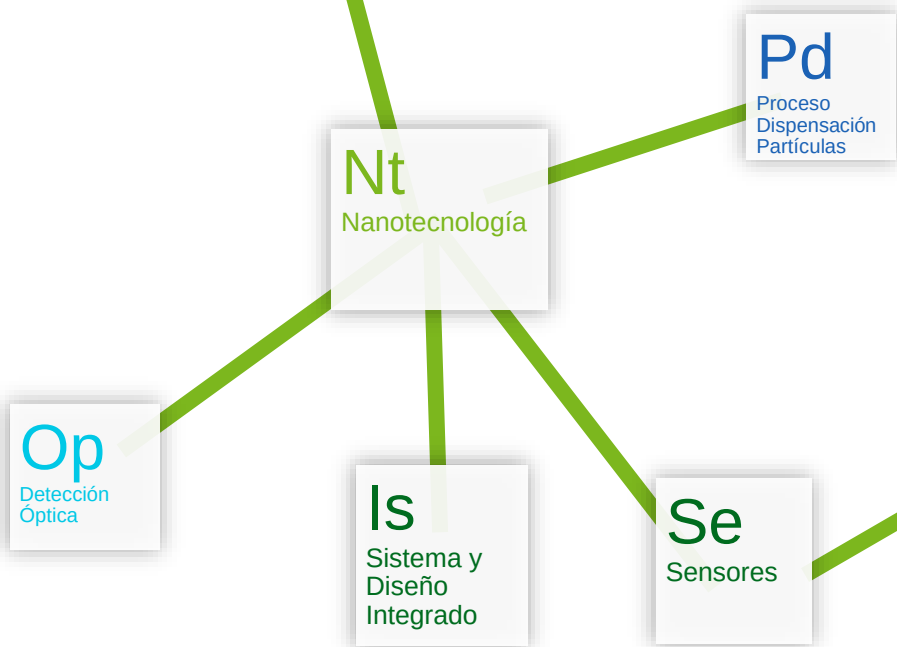
La enzima luciferasa permite mediar la reacción de bioluminiscencia en presencia de ATP, permitiendo la generación de luz y su detección en tiempo real a partir de los 15 min.

Detección Y Resultados en Tiempo Real

Resultados Positivos a partir de los 15 min. Te permite decidir rápidamente las acciones correctivas y correcciones en tus procesos.



¿Cómo se obtiene el Sistema de Detección Molecular?



Ab Abrasives	Bi Biotech	Em Electronic Materials		Pm Polymer Melt Processing	Sm Specialty Materials
Ac Acoustics	Ce Ceramics	Es Electronics & Software		Nt Nano-technology	Po Porous Materials & Membranes
Ad Adhesives	Dd Drug Delivery	Fc Flexible Coating & Packaging		Mi Microbial Detection and Control	Su Surface Modification
Am Advanced Materials	Di Display	Fe Flexible Electronics	Is Integrated Systems & Design	Op Opto-electronics	Pp Precision Processing
An Analytical	Do Dental & Orthodontic Materials	Fi Films	Im Imaging	Me Mechanical Fasteners	Pr Process Design & Control
As Application Software	Ec Energy Conversion	Fl Fluoro-materials	In Inspection & Measurement	Mr Micro-replication	Rp Radiation Processing
				Pe Predictive Engineering & Modeling	We Accelerated Weathering
				Se Sensors	Wo Wound Management

Las Placas Petrifilm™ aprovechan las 9 plataformas tecnológicas de 3M.

Am Advanced Materials	Nt Nano-technology	Pd Particle & Dispersion Processing	Es Electronics & Software	Is Integrated Systems & Design	Se Sensors	Bi Biotech	Mi Microbial Detection and Control	Op Opto-electronics
--------------------------	-----------------------	--	------------------------------	-----------------------------------	---------------	---------------	---------------------------------------	------------------------



¿Qué sigue?

Propuesta de Valor

3M™ Sistema de Detección Molecular Next Gen (2)



Fácil de usar
menos
traspasos
Reactivos listos
para usar



TTR –
Resultados
al día
siguiente



Amplio
Portafolio
Protocolo único



Alcance:
diferentes
alimentos y
muestras
ambientales

Amplificación
Isotérmica DNA



Detección por
Bioluminiscencia



Tecnología de
avanzada



Equivalente a
ISO, medios
estándares



Sustentabilidad

Provee una detección cualitativa en 24 horas con un protocolo único y eficiente

Simplified for Productivity

3M Method *Salmonella*

37°C ±1°C
or 41.5°C ±1°C
18-30 hrs



3M Method *E. coli* O157 (including H7)

41.5°C ±1°C
8-24 hrs



3M Method *Listeria*

37°C ±1°C
24-32 hrs



3M Method *Listeria monocytogenes*

37°C ±1°C
24-32 hrs*



*Raw dairy matrices
require 40 hrs.

3M Method *Cronobacter*

37°C ±1°C
18-24 hrs



3M Method *Campylobacter*

41.5°C ±1°C
22-28 hrs



Transfer 20 µL enriched sample to lysis tube.



Heat 15 minutes – 100°C ±1°C.
Cool 5 minutes on block at room temperature.



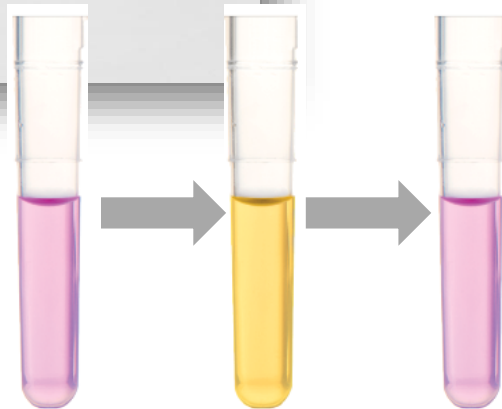
Transfer 20 µL lysate to reagent tubes
containing lyophilized pellet.



Place tubes in instrument.
Start run.
Amplification & detection in 15-75 minutes.
Automated & color-coded real-time results.

One
Protocol
for All
Pathogen
Targets

Nueva Química de Lisis

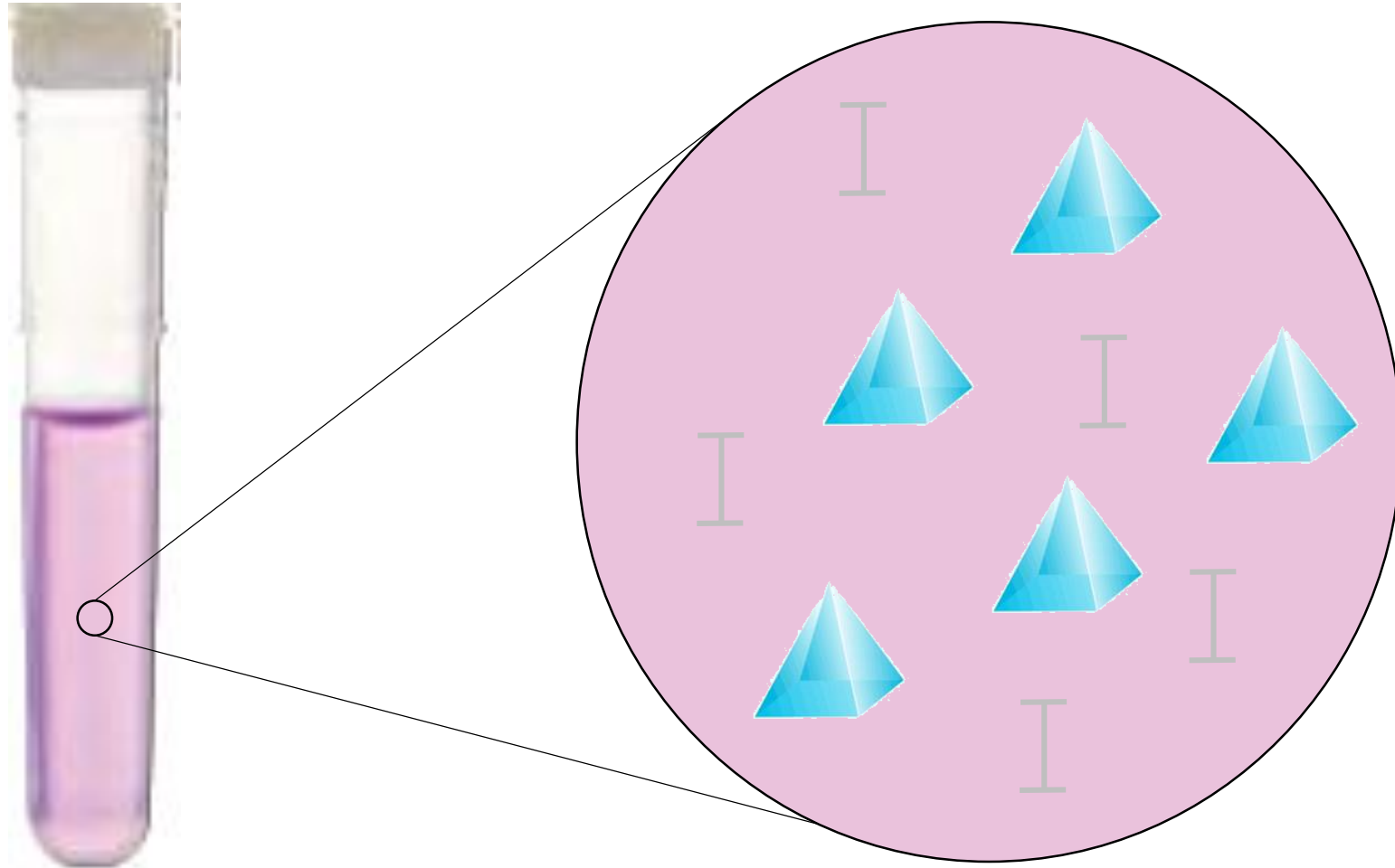


Nueva Bioquímica de lisis basada en la nanotecnología de 3M

- Soluciones listas para usar
- Fase líquida de quelación – no requiere uso resinas
- Asociación efectiva con inhibidores

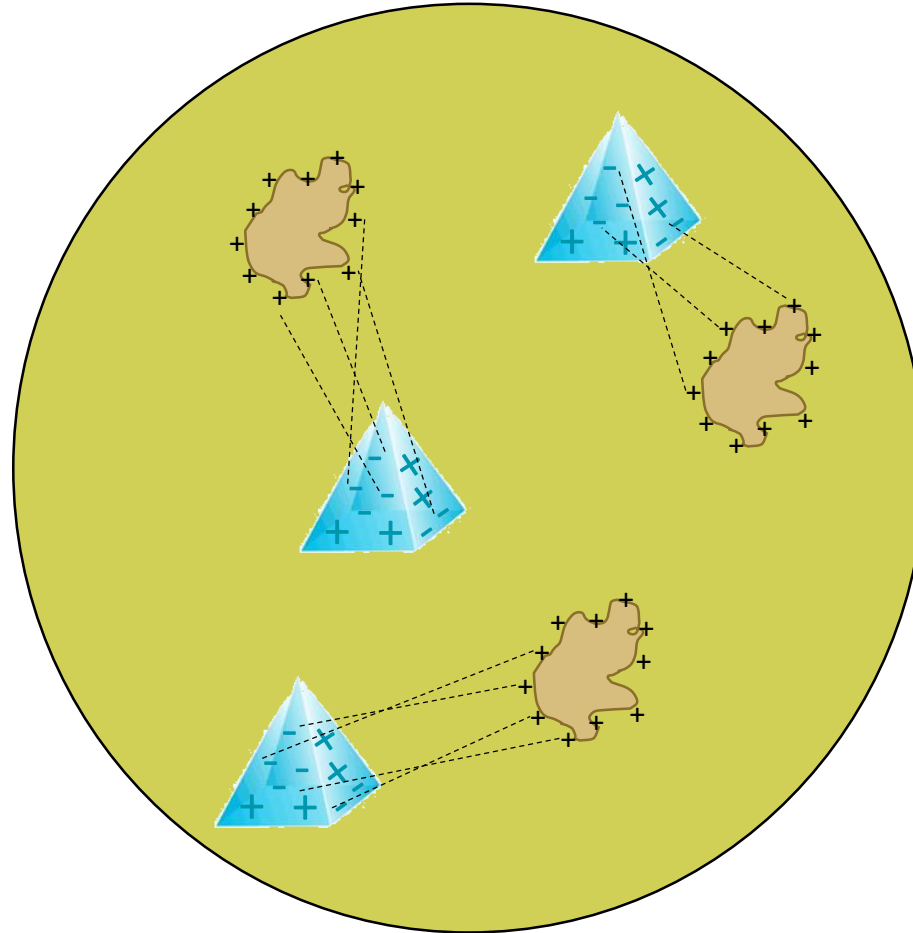
Control de proceso por especial cambio de color

- Indicador temperatura: Frío (Rosado) y caliente (Amarillo)
- Muestra visual indica cuándo pasar al siguiente paso de la reacción



3M Nanotechnology

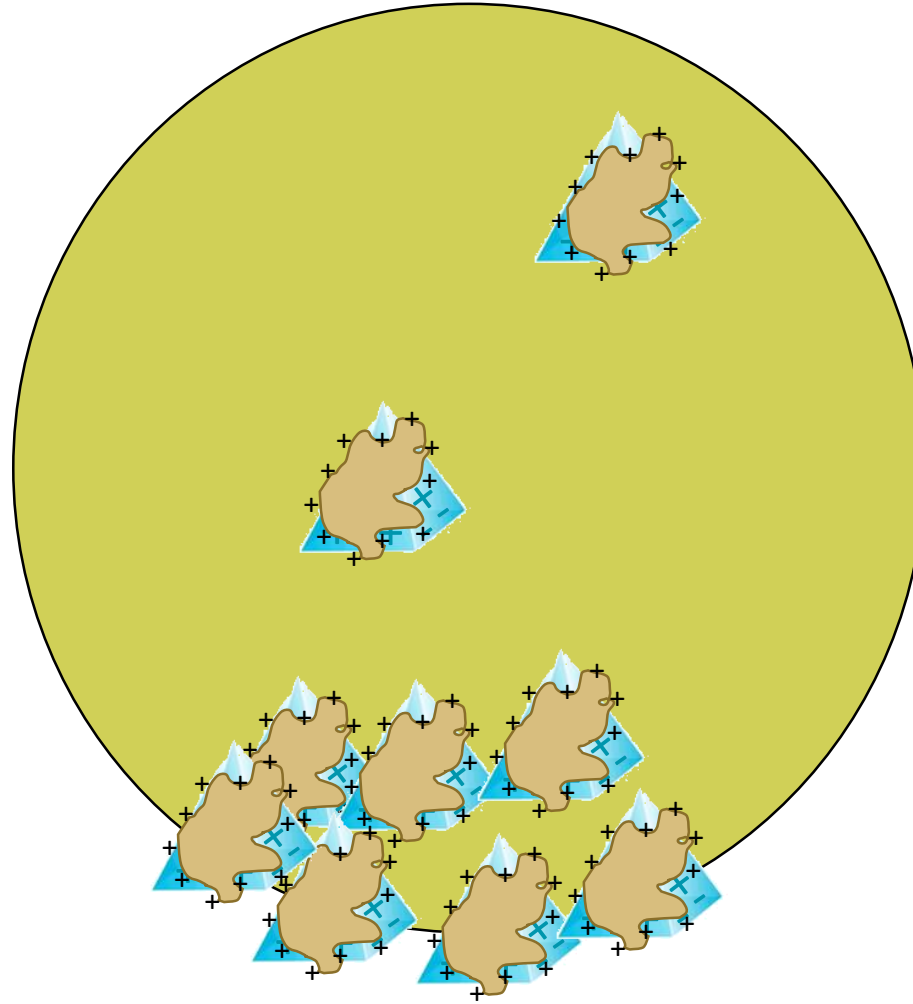
3. 3M Nanotechnology



a) Hace que las proteínas que se encuentran en la muestra y que podrían interferir con la amplificación del ADN

b) Se unan a través de las cargas superficiales a estas proteínas inhibidoras

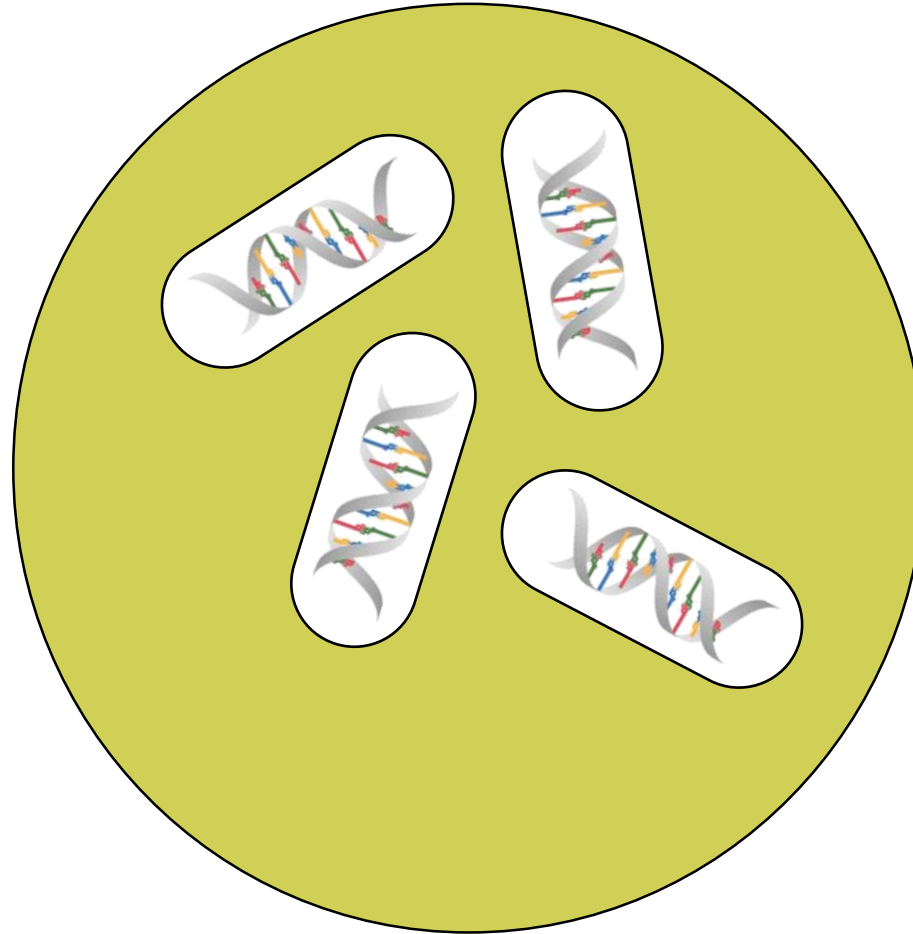
3. 3M Nanotechnology



c) Haciendo que se inactiven y agrupen

d) Y que precipiten al fondo del tubo y no interfieran en el ensayo

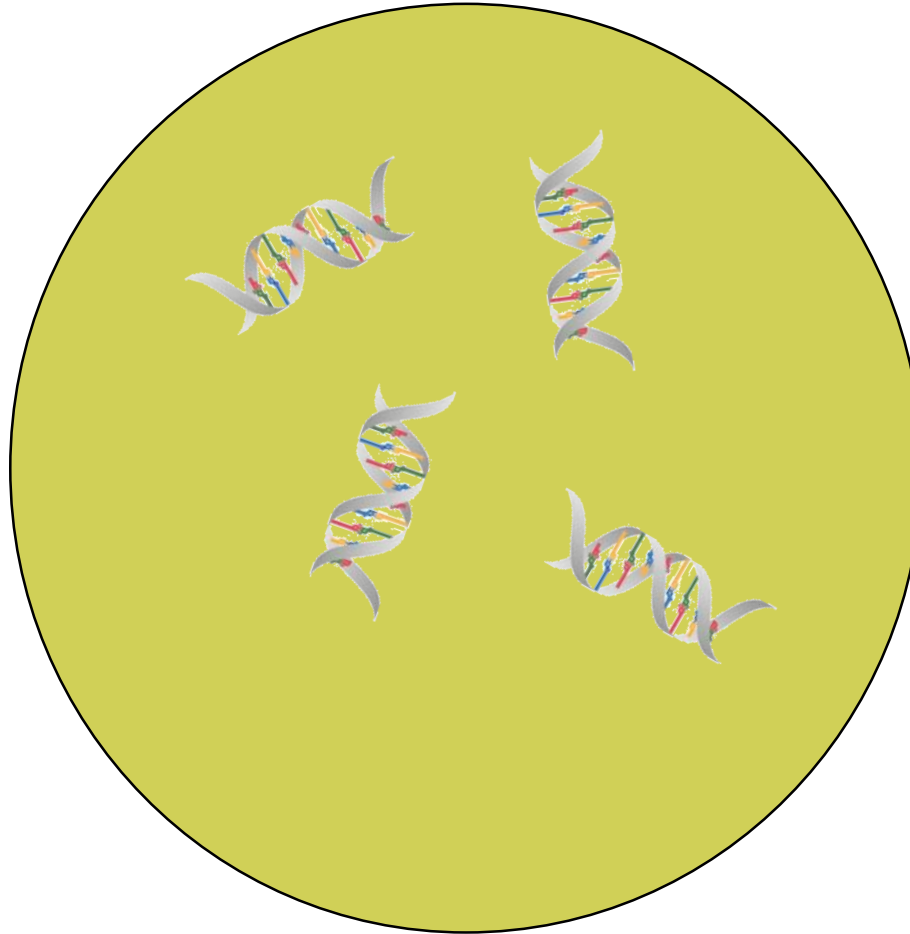
4. Cell Lysis



a) El ADN del patgeno objetivo que se encuentra dentro de la celula bacteriana

b) Es liberado al romperse la pared celular

4. Cell Lysis



c) Quedando libre y dirigiéndose a la parte superior de la solución en el tubo

3M Molecular Detection System gives you more control with:



Control your...

✓ Time	✓ Productivity
✓ Protocol	✓ Quality
✓ Process	✓ Brand

Environmental zoning conversation – 3M Environmental Monitoring Webinars with Dr. Wiedmann on [3M Food Safety Environmental Monitoring Landing Page](#)
✓ Zoning concept is in webinar 3.

Method certifications & validations

Recognized by organizations and government agencies around the world

International recognition

AOAC® Performance Tested MethodSM

MDA2 – <i>Salmonella</i> ••Certificate #091501	MDA2 – <i>Listeria</i> ••Certificate #111501	MDA2 – <i>Listeria monocytogenes</i> ••Certificate #081501
<i>Salmonella</i> ••Certificate #031208	<i>E. coli</i> O157 ••Certificate #71202	<i>Listeria</i> ••Certificate #081203
<i>Listeria monocytogenes</i> ••Certificate #051401	<i>Cronobacter</i> ••Certificate #101703	

AOAC® Official Method of AnalysisSM

MDA2 – <i>Salmonella</i> ••AOAC 2016.01	MDA2 – <i>Listeria</i> ••AOAC 2016.07	MDA2 – <i>L. monocytogenes</i> ••AOAC 2016.08	MDA2 – <i>E. coli</i> O157 ••AOAC 2017.01
<i>Salmonella</i> ••AOAC 2013.09	<i>Listeria</i> ••AOAC 2014.06	<i>Listeria monocytogenes</i> ••AOAC 2014.07	

NF VALIDATION certificate granted by AFNOR Certification

MDA 2 – <i>Salmonella</i> ••3M 01/16-11/16	MDA 2 – <i>Listeria</i> ••3M 01/14-05/16	MDA 2 – <i>Listeria monocytogenes</i> ••3M 01/15-09/16
MDA 2 – <i>E. coli</i> O157 ••3M 01/18-05/17		
<i>Salmonella</i> ••3M 01/11-11/12		
<i>E. coli</i> O157 ••3M 01/12-03/13		



Recognition by country

Australia

Department of Agriculture/Australian Quarantine and Inspection Service Approved Methods.

Salmonella
••AOAC 031208

E. coli O157
••AOAC 71202

Listeria
••AOAC 081203

Brazil

Ministry of Agriculture, Livestock and Supply (MAPA) Official Method.

Salmonella

Canada

Heath Canada Compendium of Analytical Methods.

Salmonella
••MFLP-06

Listeria
••MFLP-05

E. coli O157

Listeria monocytogenes



The Scientific Association Dedicated to Analytical Excellence®



3M wins USDA-FSIS contract for pathogen detection



USDA-FSIS has awarded 3M the contract for pathogen detection instruments. (photo: 3M)

07.10.2018 By Bob Sims



ST. PAUL, Minn. – After thorough evaluations of many commercially available methods of pathogen detection, the US Dept. of Agriculture Food Safety and Inspection Service (USDA FSIS) has awarded 3M the contract for pathogen detection instruments. 3M's Molecular Detection System will be used to detect *Salmonella*, *Listeria monocytogenes* and *E. coli* O157 (including H7), the three major pathogenic organisms threatening the safety of meat, poultry and egg-related products.

"Protecting food, consumers and businesses with innovative and reliable technologies has been at the core of everything we do, so the USDA FSIS' selection of 3M as a partner is validation of the science and the spirit of our work," Polly Foss, 3M Food Safety global vice president, said in a statement. "The 3M Molecular Detection System has proven to be a highly accurate and efficient tool for many food producers globally."



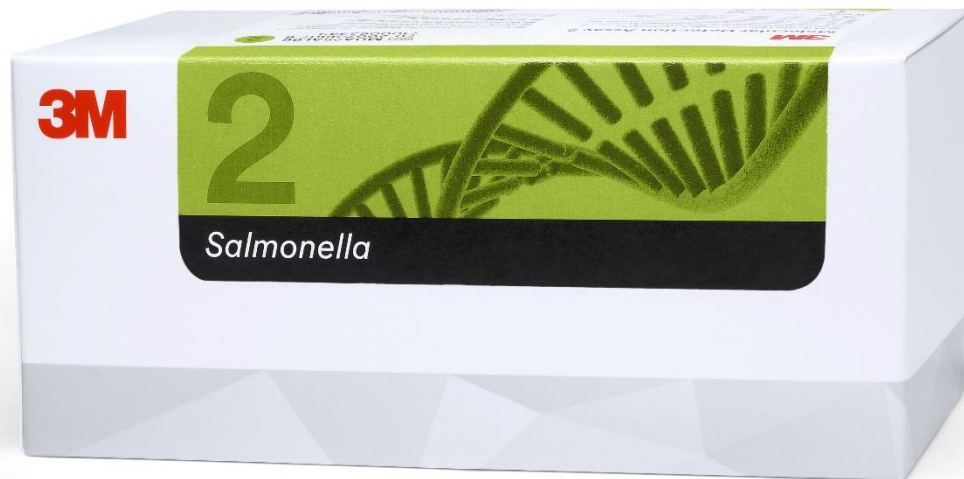
Equivalent Testing Methodologies for *Listeria* species and *Listeria monocytogenes* in Environmental Samples

FDA has determined that the following methods are "scientifically valid" and "at least equivalent to the method of analysis in § 112.152(a) in accuracy, precision, and sensitivity"¹ in detecting *Listeria* species and *L. monocytogenes*. The method of analysis in § 112.152(a) is "[Testing Methodology for *Listeria* species or *L. monocytogenes* in Environmental Samples](#)" (October 2015, Version 1).

1. AOAC Official Method 2013.10. VIDAS® UP *Listeria* (LPT), *Listeria* in select foods and environmental surfaces.
2. AOAC Official Method 2016.07. 3M Molecular Detection Assay (MDA) 2, *Listeria* in select foods and environmental surfaces.
3. AOAC Official Method 996.14. Assurance polyclonal enzyme immunoassay method (EIA), *Listeria* in select foods and environmental surfaces.

AOAC[®] Official Method of AnalysisSM

Ensayo Detección Molecular 2 3M MDA2 – Salmonella



AOAC OMA 2016.01



Validation of alternative
analysis methods
NF102 – Application to the food industry

Certificate

Certificate No.: **3M 01/16-11/16**
Validation decision dated: 25-11-2016
Expiry date: 25-11-2020

The Company:

3M Health Care
Food Safety Department
2501 Hudson Road, Building 275 5W 05
St Paul, Minnesota 55144 - United States

Is authorized to affix the NF VALIDATION mark on the alternative analysis method cited below, in accordance with the NF VALIDATION general rules and the certification rules NF102 - Validation of alternative analysis methods (Application to the food industry):

3M[™] Molecular Detection Assay 2 – *Salmonella*

Validated for the detection of *Salmonella* spp.

Technical sh
reference's

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

EN ISO 16140-2:2016 validation study of
the 3M[™] Molecular Detection Assay 2 -
Salmonella for the detection of *Salmonella* spp
in food products and environmental samples

Qualitative method

AOAC[®] Official Method of AnalysisSM

Ensayo Detección Molecular 2 3M MDA2 – *Listeria monocytogenes*



AOAC OMA 2016.08



Validation of alternative analysis methods
NF102 – Application to the food industry

Certificate

Certificate No.: 3M 01/15-09/16
Validation decision dated: 30-09-2016
Expiry date: 30-09-2020

The Company:

3M Health Care
Food Safety Department
2501 Hudson Road, Building 275 5W 05
St Paul, Minnesota 55144 - United States

Is authorized to affix the NF VALIDATION mark on the alternative analysis method cited below, in accordance with the NF VALIDATION general rules and the certification rules NF102 - Validation of alternative analysis methods (Application to the food industry):

3MTM Molecular Detection Assay 2 – *Listeria monocytogenes*

Validated for the detection of *Listeria monocytogenes*

Summary report

EN ISO 16140-2:2016 validation study of
the 3MTM Molecular Detection Assay 2 -
Listeria monocytogenes for detection of
Listeria monocytogenes in food products
and environmental samples

Qualitative method

Instrucciones específicas para los Métodos validados de Performance Tested MethodSM de AOAC® #091501



En los estudios de PTM de AOAC, se descubrió que el Ensayo de Detección Molecular 2 para *Salmonella* 3M era un método efectivo para la detección de la *Salmonella*. Las matrices que se analizaron en el estudio se muestran en la Tabla 3.

Tabla 3. Protocolos de enriquecimiento conforme a PTM de AOAC #091501.

El volumen de la muestra transferida a los tubos de Solución de Lisis (LS) es de 20 µL.

Matriz de la muestra	Tamaño de la muestra	Volumen del caldo de enriquecimiento	Temperatura de enriquecimiento (±1°C)	Tiempo de enriquecimiento (h)
Carne de res molida cruda	25 g	225 ml de BPW ISO (precalentada)	41,5	10-24
	325 g	975 ml de BPW ISO (precalentada)		
Carne de pollo molida cruda	25 g	225 ml de BPW ISO (precalentada)	41,5	10-24
	325 g	975 ml de BPW ISO (precalentada)		
Pollo empanizado cocido	325 g	2925 ml de BPW ISO	37	18-24
Alimento seco para perros	25 g	225 ml de BPW ISO	37	18-24
	375 g	1500 ml de BPW ISO		
Pimienta negra, langostino entero crudo, espinaca cruda en bolsa, queso estadounidense procesado pasteurizado	25 g	225 ml de BPW ISO	37	18-24
Enjuague de carcasa de pollo	30 ml	30 ml de BPW ISO (precalentada)	41,5	18-24
Esponja de carcasa de pollo	1 esponja	50 ml de BPW ISO (precalentada)	41,5	18-24
Leche en polvo descremada instantánea	25 g	225 ml de BPW ISO	37	20-24
Cacao en polvo	25 g	225 ml de BPW ISO	37	24-28
Huevo entero líquido pasteurizado	100 ml	900 ml de BPW ISO	37	18-24
Agua de riego de brote gastado	375 ml	3375 ml de BPW ISO	37	18-24
Mantequilla de mani cremosa	25 g	225 ml de BPW ISO	37	18-24
	375 g	3375 ml de BPW ISO		
Ambiental	Horizonte sellado	225 ml de BPW ISO (precalentada)	41,5	18-24
	Acero inoxidable	10 ml de BPW ISO (precalentada)	41,5	18-24
	Azulejos de cerámica sellados	50 ml de BPW ISO (precalentada)	41,5	18-24

Table 2: Enrichment protocols according to AOAC Official MethodsSM 2014.07 and AOAC Performance TestedSM Certificate #051401.

	Sample Matrix	Sample Size	Primary Enrichment Demi-Fraser Broth (no FAC)			Secondary Enrichment Fraser Broth Base (no FAC)		
			Enrichment Broth Volume (mL)	Enrichment Temperature (±1°C)	Enrichment Time (hr)	Enrichment Medium (mL)	Enrichment Temperature (±1°C)	Enrichment Time (hr)
AOAC OMA 2014.07 AOAC PTM #051401	Full fat cottage cheese	25 g	225	37	24-28	Not required		
	Chocolate milk	25 g	225	37	24-28	Not required		
	Beef hot dogs	25 g	225	37	26-30	Not required		
		125 g	1225					
	Deli turkey	25 g	225	37	26-30	Not required		
		125 g	1225					
	Cold smoked salmon	25 g	225	37	26-30	Not required		
Environmental surfaces	Concrete	1 sponge	100	37	26-30	Not required		
	Concrete, Stainless Steel	1 sponge	225					
AOAC PTM #051401	Raw bagged Spinach	25 g	225	37	24-26	Transfer 0.1 mL into 10 mL Fraser Broth Base	37	24-26
	Romaine lettuce	25 g	225	37	24-26	Transfer 0.1 mL into 10 mL Fraser Broth Base	37	24-26
	Whole cantaloupe	1 melon	Enough volume to allow melon to float [volume may be 1.5 times the weight of the cantaloupe]	37	24-26	Transfer 0.1 mL into 10 mL Fraser Broth Base	37	24-26
	Environmental	1 swab	10	37	26-30	Not required*		
For Other Matrices		1 sponge	100					
	Heat-processed dairy	1 sponge	225	37	24-28	Not required*		
		25 g	225					
	Other foods**	25 g	225	37	24-26	Fraser Broth Base: 0.1 mL into 10 mL	37	24-26

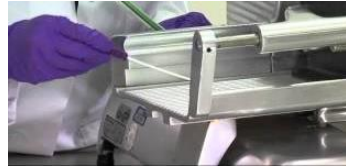
* A 24-26 hr primary enrichment in Demi-Fraser Broth Base (no FAC) followed by a 24-26 hr secondary enrichment in Fraser Broth Base (no FAC) is also acceptable for these matrices.

** Other Foods includes meat, poultry, fish, seafood, produce, vegetable and vegetable-based products, and raw dairy.

Validaciones oficiales para métodos moleculares como NF y AOAC OMA están ligadas al proceso de enriquecimiento.

Detección de patógenos en alimentos y muestras ambientales

1. Colección de la muestra



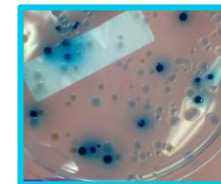
Colectar el analito y asegurar que se mantiene intacto hasta el analisis

2. Enriquecimiento



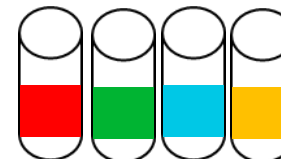
Permitir la multiplicacion del organismo patogeno para facilitar la deteccion y permitir su recuperacion si esta dañado

3. Detección



Determinar a traves de un metodo especifico si el organismo patogeno podria estar presente

4. Confirmación



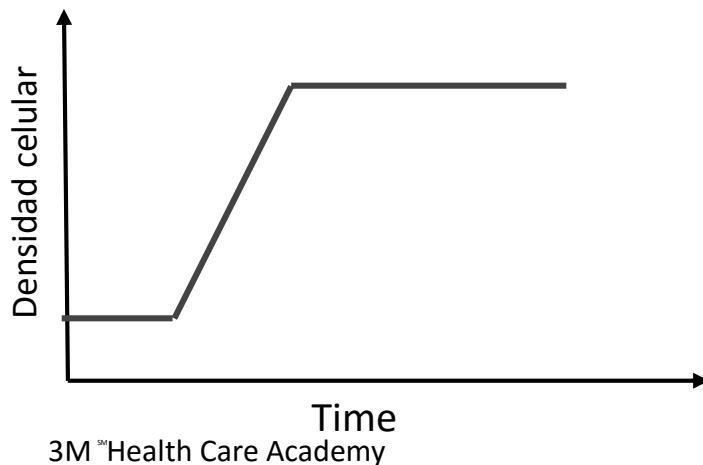
Confirmar el resultado obtenido en el paso de Deteccion

Enriquecimiento

Objetivo

Permitir la recuperación y multiplicación de células

- Dañadas
- Estresadas
- En presencia de otros microorganismos que están de manera natural en el producto (microflora asociada)
 - Mayor población
 - Mejor estado fisiológico
- En presencia de factores, inherentes al producto, que pueden tener un impacto en el crecimiento microbiano



La mayoría de los métodos moleculares requieren que la densidad celular del patógeno, después del enriquecimiento, llegue a 10^3 - 10^4 UFC/mL para poder ser detectado

Buenas practicas que permiten tener un adecuado crecimiento microbiano

Asegurarse que las condiciones requeridas para el crecimiento microbiano sean óptimas

b. Cuando se utilicen volúmenes de muestra grandes >100 g

- Asegurar que la temperatura requerida para la incubación no es afectada debido al uso de un mayor volumen de caldo de enriquecimiento.

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.09	Page 6 of 19	
Title: Isolation And Identification of <i>Salmonella</i> From Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges		
Revision: .09	Replaces: .08	Effective: 1/2/17

Table 1. Sample Preparation and Enrichment Guide

Product	Sample Preparation		Incubation Cultural or PCR rapid screen
	Portion Size	Enrichment Amount determined by volume or weight	
Ready-to-Eat Meat, Poultry and Siluriformes Foods	325 ± 6.5 g	975 ± 19.5 ml BPW	35 ± 2°C for 18-24 h
Raw Poultry Products	325 ± 32.5 g or 25 ± 2.5 g	1625 ± 32.5 ml BPW or 225 ± 4.5 ml BPW	35 ± 2°C for 20-24 h
Raw Meat and Raw Beef Mixed Products	325 ± 32.5 g or 25 ± 2.5 g	975 ± 19.5 ml mTSB or 75 ± 1.5 ml mTSB	42 ± 1°C for 15-24 h
Poultry Carcass and Environmental Sponges	1 sponge pre-moistened with 10 ml buffer	50 ± 1 ml BPW to bring total volume to 60 ml*	35 ± 2°C for 20-24 h
Meat Carcass and Environmental Sponges	1 sponge pre-moistened with 10 ml buffer	50 ± 1 ml mTSB to bring total volume to 60 ml*	42 ± 1°C for 15-24 h
Whole Bird and Parts Rinses	30 ± 0.6 ml sample rinse fluid	30 ± 0.6 ml BPW	35 ± 2°C for 20-24 h
Pasteurized Liquid, Frozen or Dried Egg Products	100 ± 2 g	900 ± 18 ml BPW	35 ± 2°C for 18-24 h
Raw Siluriformes Products	25 ± 2.5 g	225 ± 4.5 ml BPW	35 ± 2°C for 22-26 h
Fermented Products	325 ± 6.5 g + 10 g of sterilized calcium carbonate	2925 ± 58.5 ml of BPW with 1 ml of a 1% aqueous solution of crystal violet per liter	35 ± 2°C for 18-24 h
Dried Products (Breeding Mix, Dehydrated Sauce, Soup Mix, Dried Milk)	325 ± 6.5 g	2925 ± 58.5 ml BPW	35 ± 2°C for 18-24 h

* or maintain a 1:6 ratio for different project buffer volumes, e.g., 25 ml buffer + 125 ml enrichment

325 g + 2925 mL de BPW a 35 °C / 18 h

Nota: Evaluar si se requiere el uso de medio precalentado antes del enriquecimiento.

Tabla 2. Protocolos generales de enriquecimiento

Matriz de la muestra	Tamaño de la muestra ¹	Volumen del caldo de enriquecimiento ^{1,2}	Temperatura de enriquecimiento (±1 °C)	Tiempo de enriquecimiento (h)	Volumen de análisis de la muestra
La fórmula en polvo para lactantes (Powdered infant formula, PIF) y materias primas tales como leche en polvo, polvo de soja, suero en polvo, lactosa, harina de arroz y maltodextrina.	Muestra de 1X gramos	9X ml BPW ISO (1:10)	37	18-24	20 µl
Materias primas tales como sales, minerales, aminoácidos, vitaminas y DHA (ácido docosahexaenoico).	Muestra de 1X gramos	99X ml BPW ISO (1:100)	37	18-24	20 µl
Muestras ambientales secas como polvo, barreduras y suciedad aspirada	Muestra de 1X gramos	9X ml BPW ISO (1:10)	37	18-24	20 µl
Esponja ambiental con 10 ml de caldo de Letheen o D/E Caldo Neutralizante	1 dispositivo de muestreo	90 ml BPW ISO (1:10)	37	18-24	20 µl

1. 3M ha evaluado el Ensayo de Detección Molecular 2 3M - Clone Select usando las proporciones de dilución que se indican en la Tabla 2 hasta 300 g. Es responsabilidad del usuario validar las proporciones de dilución o los protocolos alternativos para garantizar que este método de prueba satisface los criterios del usuario.

2. Use caldo de enriquecimiento BPW ISO precalentado si el volumen del caldo de enriquecimiento supera los 300 ml.

Buenas practicas que permiten tener un adecuado crecimiento microbiano

Asegurarse que las condiciones requeridas para el crecimiento microbiano sean óptimas

b. Cuando se utilicen volúmenes de muestra grandes >100 g

- Asegurar que la temperatura requerida para la incubación no es afectada debido al uso de un mayor volumen de caldo de enriquecimiento.



Muy bien



Bien



!Porfavor NO!

Tener bolsas de enriquecimiento muy juntas disminuye la distribución de calor



Bien

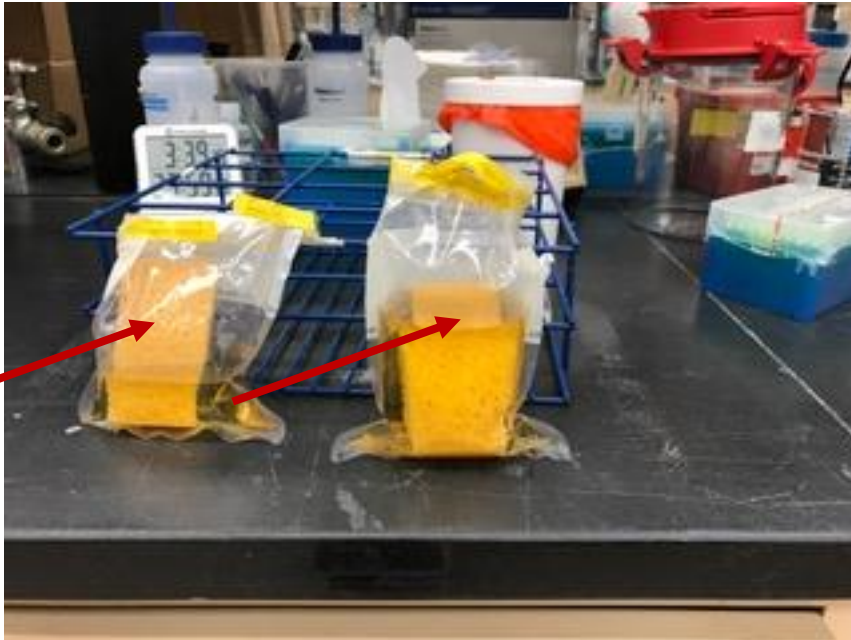


Super bien



Entre mas espacio exista entre las bolsas y contenedores hay mejor distribución de calor

Asegurar que la muestra queda completamente sumergida en el caldo de enriquecimiento



Común en esponjas, leche en polvo, vegetales frondosos, alimentos poco densos



Gracias!



Veronica Yáñez
Key Account Leader 3M Food Safety
vfyanez@mmm.com