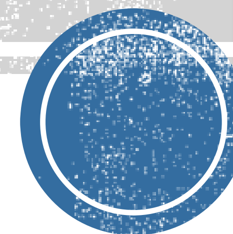
IZSAM G.CAPORALE TERAMO

Listeria monocytogenes Laboratorio Nazionale di Riferimento

UN NUOVO SISTEMA LAMP (LOOP-MEDIATED ISOTHERMAL AMPLIFICATION) PER LA RICERCA DI LISTERIA MONOCYTOGENES IN CAMPIONI ALIMENTARI



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Università degli Studi di Milano,

Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare (VESPA)

Tecnologia LAMP *(Loop-mediated isothermal AMPlification)*

Tecnica isotermica di amplificazione genetica che permette la rivelazione del DNA ad alta sensibilità (*Notomi et al., 2000*).

LAMP combina:

- **Semplicità di esecuzione ed interpretazione del dato (non necessario personale specializzato);**
- **Rapidità di esecuzione del test;**
- **Alta specificità e sensibilità.**

Inoltre non necessita di strumentazioni sofisticate e costose (es. termociclatori)

Facilità di esecuzione: permette di analizzare campioni anche direttamente *in situ* ottenendo risultati in tempi rapidi (~ un'ora)

POCT: POINT OF CARE TESTING

Tecnologia LAMP *(Loop-mediated isothermal AMPlification)*

Applicazioni molteplici: ***diagnostica umana, animale e vegetale, certificazione genetica in ambito agroalimentare***.

Journal of Clinical Microbiology, June 2003, p. 2616–2622 Vol. 41,
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.6.2616–2622.2003
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Loop-Mediated Isothermal Amplification for Direct Detection of *Mycobacterium tuberculosis* Complex, *M. avium*, and *M. intracellulare* in Sputum Samples

Tomotada Iwamoto,[1]* Toshiaki Sonobe,[1] and Kozaburo Hayashi[2]
Department of Bacteriology[1] and Department of Parasitic Agents,[2] Kobe Institute of Health, 4-6 Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan

Received 23 December 2002/Returned for modification 4 February 2003/Accepted 7 March 2003

Water Research 122 (2017) 62–69
Contents lists available at ScienceDirect
Water Research
journal homepage: www.elsevier.com/locate/watres
ELSEVIER

A loop-mediated isothermal amplification (LAMP) assay for the rapid detection of *Enterococcus* spp. in water

Roland Martzy [a,g], Claudia Kolm [a,g], Kurt Brunner [a], Robert L. Mach [b], Rudolf Krska [c], Hana Šinkovec [d], Regina Sommer [e,g], Andreas H. Farnleitner [b,f,g], Georg H. Reischer [a,b,*]

Food Sci. Biotechnol. 23(2): 467-474 (2014)
DOI 10.1007/s10068-014-0064-x
RESEARCH ARTICLE

Development of a Loop-mediated Isothermal Amplification Assay for Detecting *Listeria monocytogenes prfA* in Milk

Ae-Ri Cho, Hee-Jin Dong, Kun-Ho Seo, and Seongbeom Cho

DOI: 10.1111/j.1745-4565.2009.00196.x

DEVELOPMENT AND EVALUATION OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) METHOD FOR DETECTING *LISTERIA MONOCYTOGENES* IN RAW MILK

DEGUO WANG[1,2], GUICHENG HUO[1,3], DAXI REN[1] and YONGGANG LI[1]

ELSEVIER
FEMS Microbiology Letters 253 (2005) 155–161
FEMS MICROBIOLOGY Letters
www.fems-microbiology.org

Loop-mediated isothermal amplification for the rapid detection of *Salmonella*

Yukiko Hara-Kudo [a,*], Manabu Yoshino [b], Tadashi Kojima [b], Masanari Ikedo [b]

ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 62 (2008) 357–365
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Evaluation of loop-mediated isothermal amplification for detection of *Toxoplasma gondii* in water samples and comparative findings by polymerase chain reaction and immunofluorescence test (IFT)

Isaia Sotiriadou[a,b], Panagiotis Karanis[a,c,*]

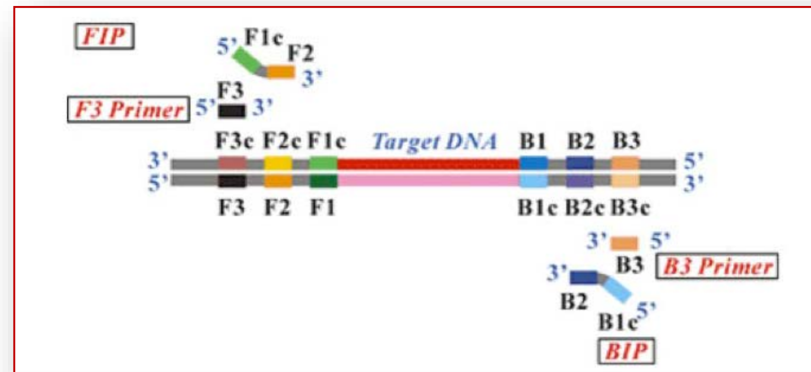
Journal of Applied Microbiology
sfam
Journal of Applied Microbiology ISSN 1364-5072
ORIGINAL ARTICLE

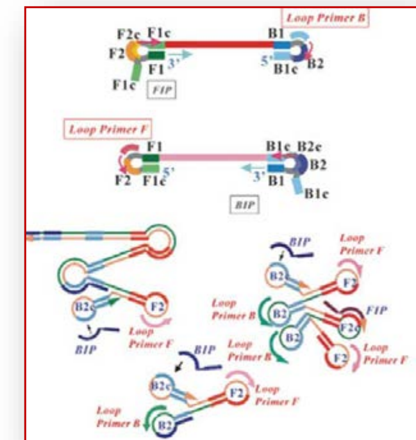
A loop-mediated isothermal amplification method for rapid direct detection and differentiation of nonpathogenic and verocytotoxigenic *Escherichia coli* in beef and bovine faeces

A.Ch. Stratakos[1], M. Linton[2], S. Millington[3] and I.R. Grant[1]

LAMP: meccanismo della reazione

La LAMP è una tecnologia che si basa su **un'amplificazione isotermica** di acidi nucleici, **sull'attività di una polimerasi termostabile** con attività di '*strand displacement*' ricavata da *Bacillus stearothermophilus (BSt polimerasi)* e su **6 primer** disegnati in modo peculiare che riconoscono 8 regioni sul DNA target prescelto (F3c, F2c e F1c in direzione 3' e B1, B2 e B3 in direzione 5')





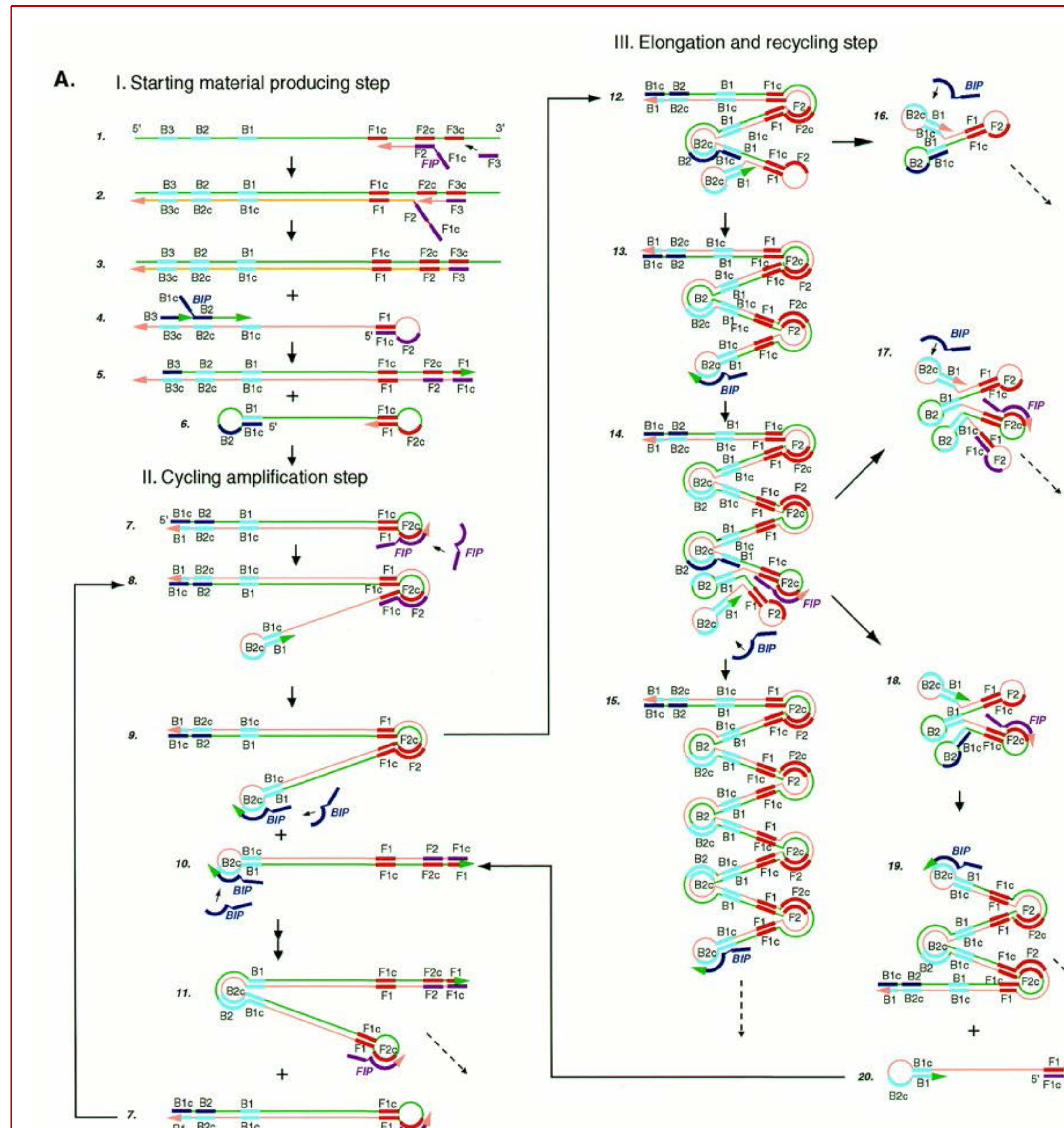
La reazione e composta da **due steps**:

- durante la prima parte della reazione sono coinvolte le due coppie di primers FIP-BIP e F3-B3 (+ 2 opzionali LF-LB)
- successivamente per lo spiazzamento dei filamenti e per la sintesi del DNA vengono coinvolti soltanto i primers esterni (FIB-BIP).

La reazione avviene mediante l'interazione dei reagenti con il DNA bersaglio all'interno di una provetta, a una **temperatura costante compresa tra i 60°C e 65°C**.

LAMP

Il prodotto finale della LAMP è una miscela di strutture di DNA a cavolfiore composte da varie anse (la struttura di DNA è anche detta manubrio).



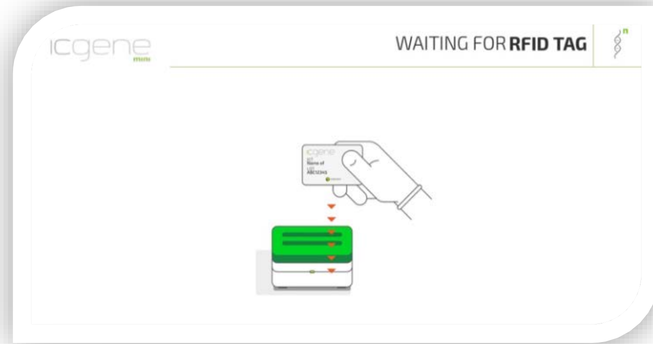
Sistema di Real time-LAMP miniaturizzato

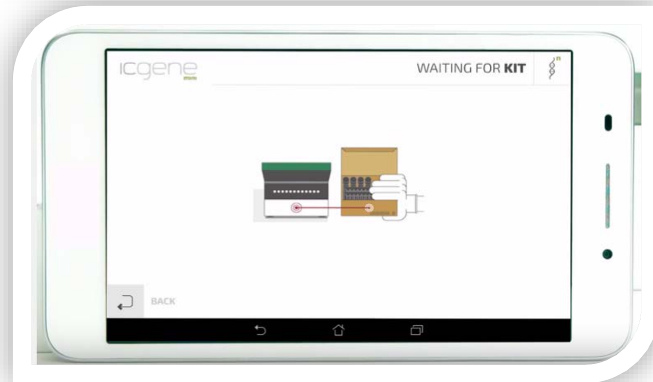
Kit pronti all’uso e monouso, lettore di fluorescenza e tablet

FASE 1: **Estrazione preliminare dell’acido nucleico** dal campione da analizzare

FASE 2:
a) **Amplificazione genetica** a singolo test con tecnologia LAMP
b) Contemporanea **rilevazione della fluorescenza emessa dal campione in tempo reale** e **interpretazione automatica del risultato finale mediante lo strumento attraverso la formazione di curve sigmoidi**

Riconoscimento del Kit e dell'operatore





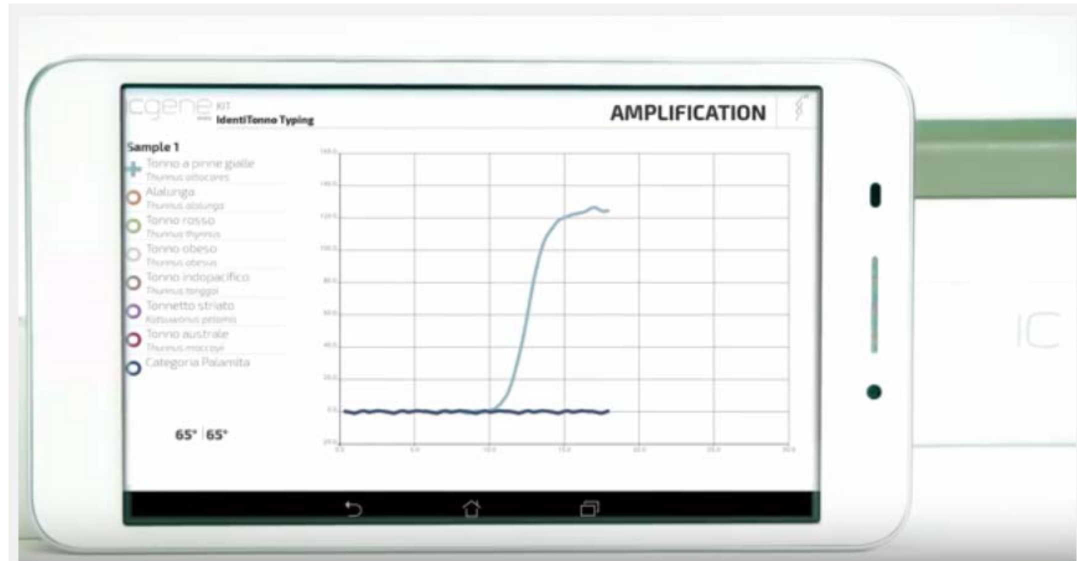
- Le operazioni necessarie per effettuare le analisi sono pre-programmate all'interno di badge riconosciuti in modalità contactless dallo strumento mediante **tecnologia RFID**.

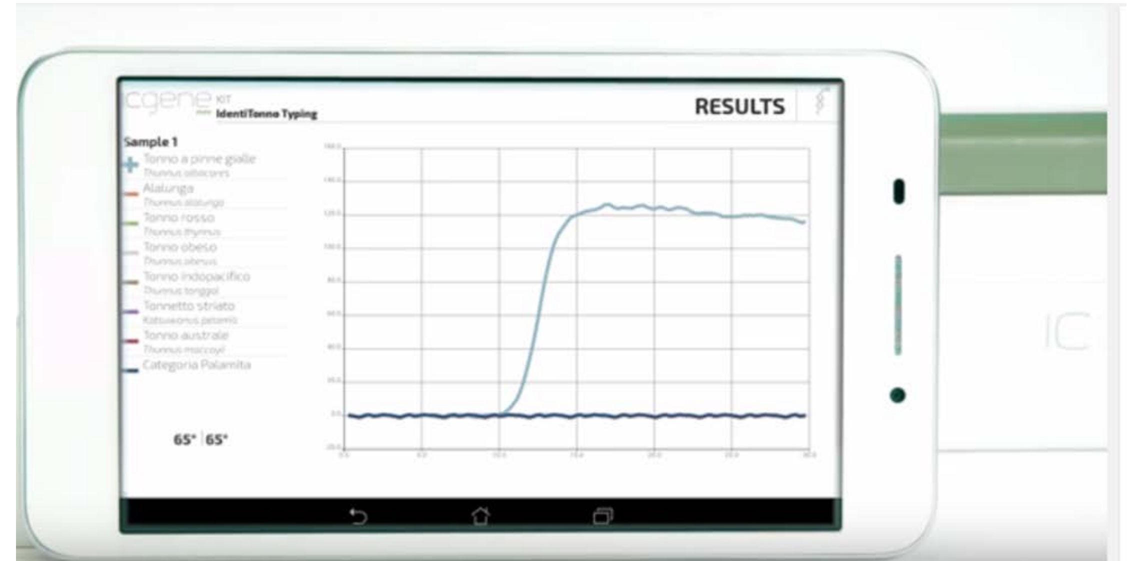
- Anche l'operatore che esegue il test viene tracciato, consentendo l'accensione dello strumento e l'avvio delle analisi.

- **Tracciabilità:**

✓ **del test eseguito;**
✓ **dell'operatore;**
✓ **del lotto e data di scadenza dei reagenti utilizzati;**
✓ **del risultato finale.**

Rivelazione





Lo strumento amplifica e legge la fluorescenza del campione, formando curve sigmoidi in tempo reale.

Effettua simultaneamente l'analisi su 12 campioni.

Campi applicazione

Bacteria
- Xylella fastidiosa
- Erwinia amylovora

Phytoplasmas
- Flavescence doree
- Bois noir

Virus
- Citrus tristeza virus (CTV)
- Tomato leaf curl New Delhi virus (ToLCNDV)

Fungus
- Guignardia citricarpa

Insects
- Popillia japonica
- Anoplophora chinensis
- Aromia bungii

Listeria monocytogenes ←

Salmonella spp.

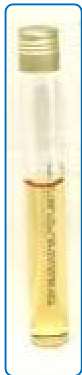
Campylobacter spp.

Prove preliminari

STEP 1: verifica della specificità su ceppi di *Listeria monocytogenes* e *Listeria* spp. in brodo di coltura

Listeria monocytogenes
32/32 ceppi identificati-100%
• ***Ceppi da Panel Centro Referenza (25)***
• ***Ceppi isolati da alimenti (6)*** (***salame, prosciutto crudo, prosciutto di Praga, taleggio, cocktail gamberi, sandwich***)
• ***Ceppi ATCC 7644 (1)***

42 ceppi



7-8 Log UFC/mL

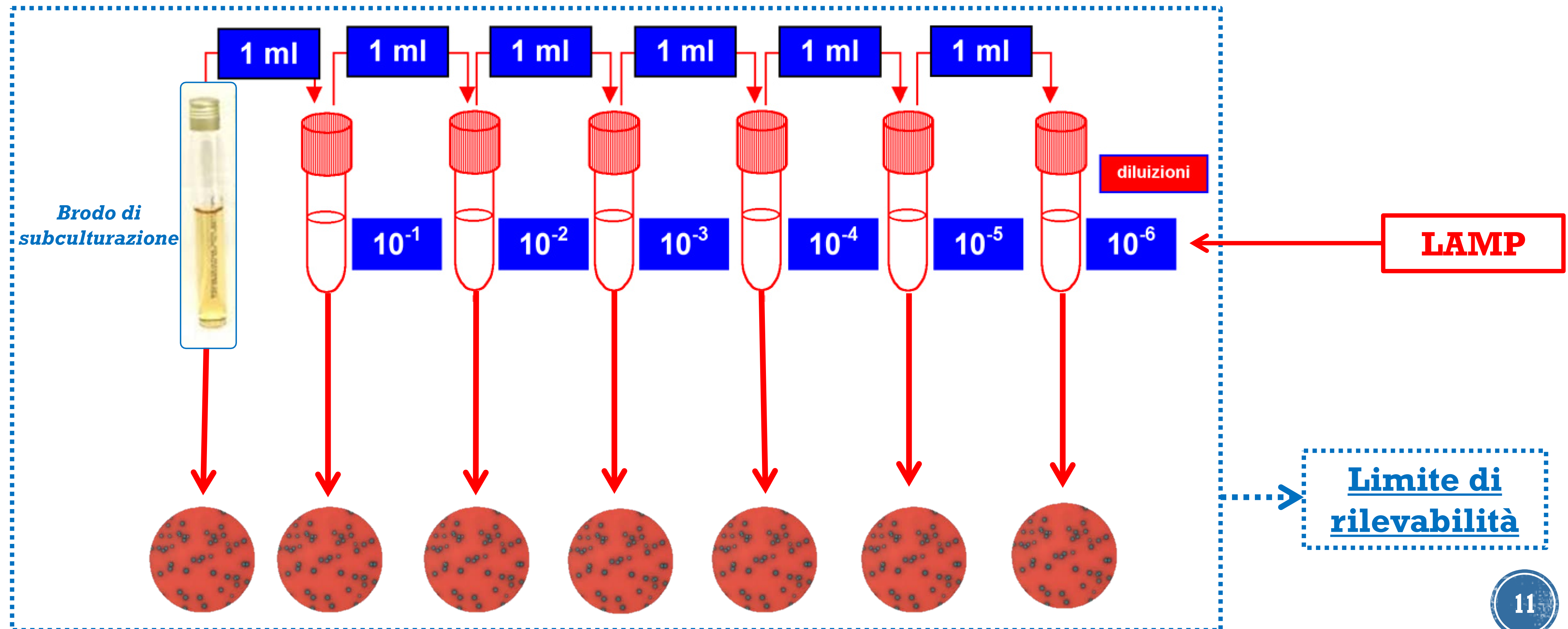
Listeria* spp. non *monocytogenes
10/10 ceppi non identificati

Codice	***ID***
ATCC 33090	***L. innocua***
C16-M	***L. ivanovii***
A4-O	***L. ivanovii***
A14-O	***L. grayi***
A15-O	***L. welshimeri***
A17-L	***L. seeligeri***
A19-L	***L. innocua***
A26-M	***L. innocua***
B14-O	***L. welshimeri***
B15-M	***L. grayi***

Prove preliminari

STEP 2: verifica della **sensibilità** su ceppi di *Listeria monocytogenes* in brodo di coltura

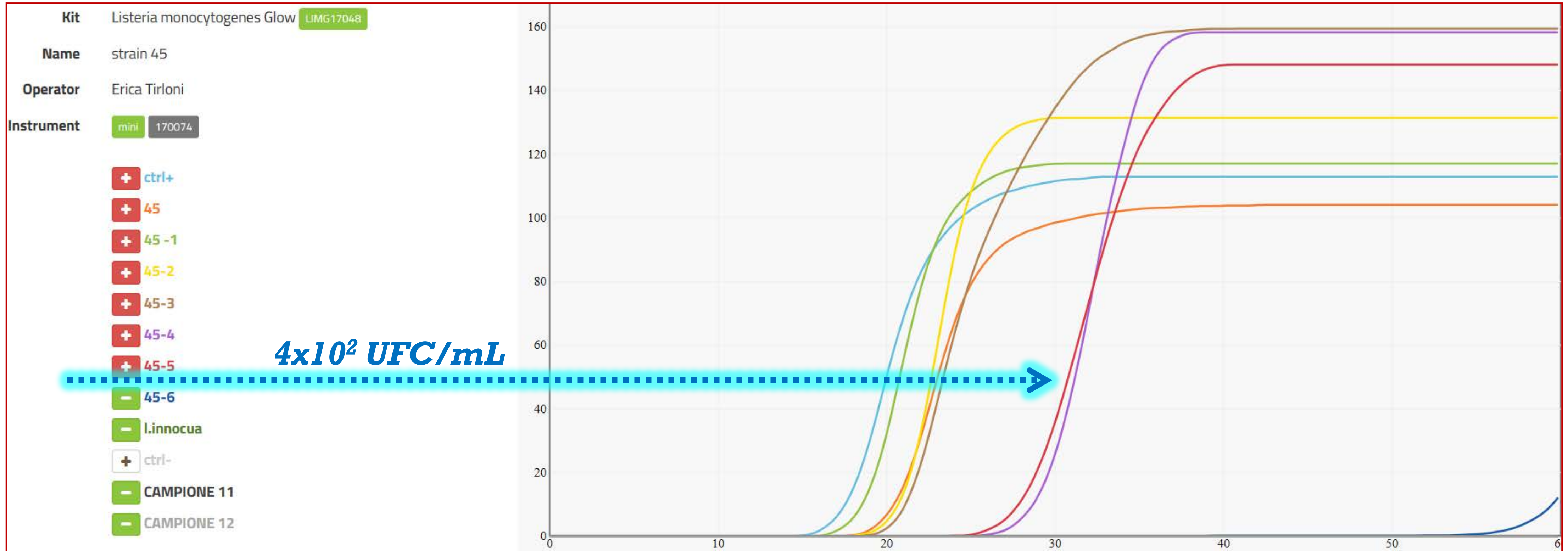
***4 ceppi**: 45 (substrato carneo), 99 (substrato ittico), 118 (substrato lattiero-caseario), 49 (altro substrato)*



Prove preliminari

STEP 2: verifica della **sensibilità** su ceppi di *Listeria monocytogenes* in brodo di coltura

45

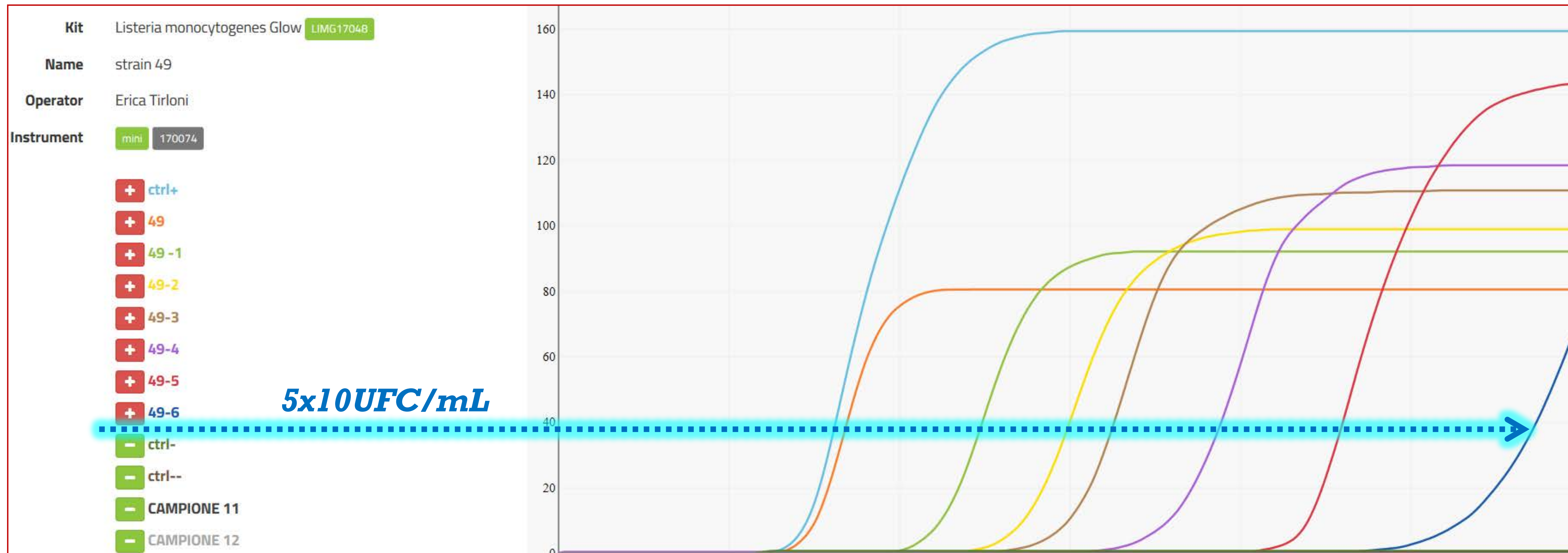


Tempi di amplificazione: 16- 40 minuti

Prove preliminari

STEP 2: verifica della **sensibilità** su ceppi di *Listeria monocytogenes* in brodo di coltura

49

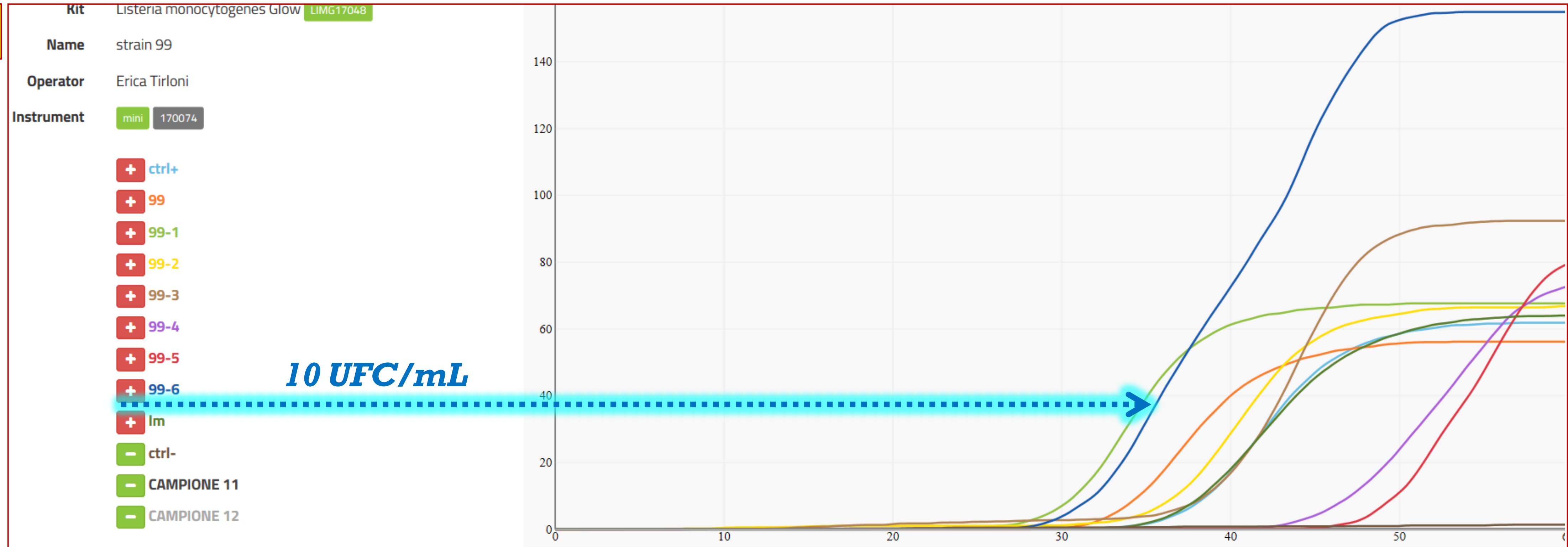


Tempi di amplificazione:
14- 50 minuti

Prove preliminari

STEP 2: verifica della **sensibilità** su ceppi di *Listeria monocytogenes* in brodo di coltura

99

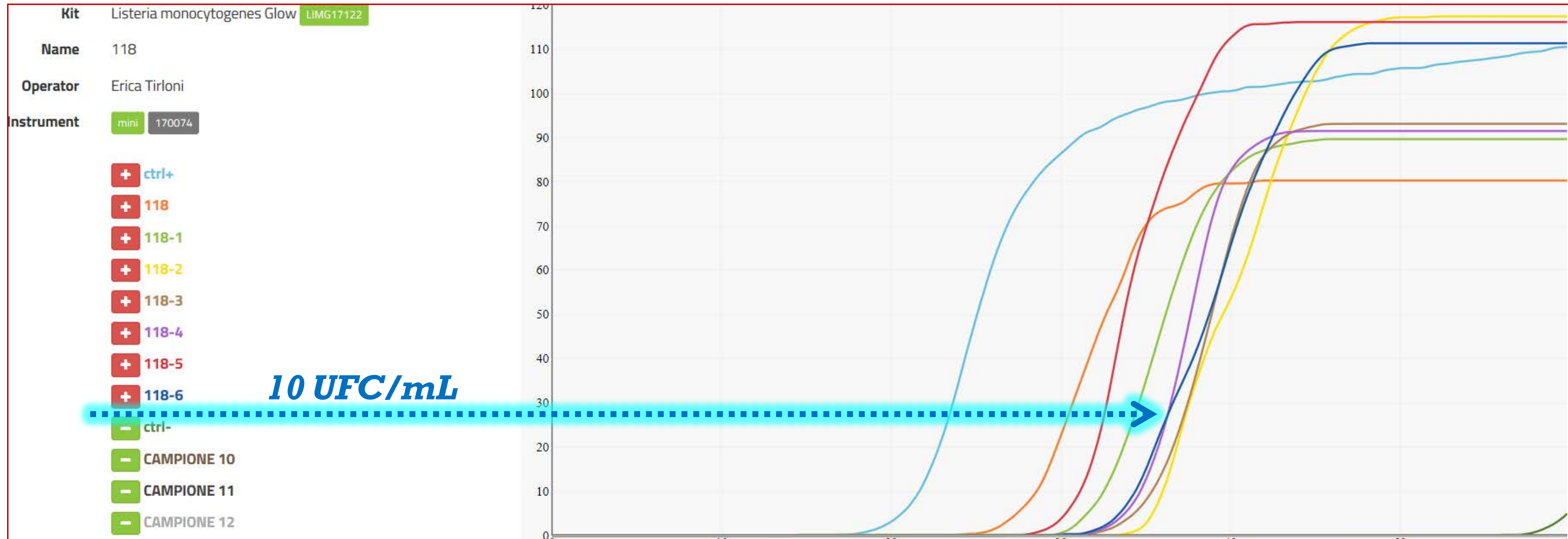


Tempi di amplificazione: 27- 45 minuti

Prove preliminari

STEP 2: verifica della **sensibilità** su ceppi di *Listeria monocytogenes* in brodo di coltura

118



Tempi di amplificazione:
25- 35 minuti

Confronto con risultati in letteratura-brodi

	Limite rilevabilità brodo
Presente studio	10-400 UFC/mL
Cho et al. (2014)	2,22 UFC/mL
Tang et al. (2011)	20 UFC/mL
Wang et al. (2012)	$1.0 *10^4$ UFC/mL

Prove preliminari

STEP 3: verifica della **sensibilità su prodotti lattiero caseari volontariamente contaminati con *Listeria monocytogenes* e sottoposti ad analisi qualitativa**

Latte

Grasso 3.6%

Latte intero

Grasso 1.6%

Latte scremato

Formaggi con limitata struttura e assenza microflora

Grasso 27.5%

Cream cheese

Grasso 5%

ricotta

Grasso 42%

mascarpone

Formaggi con struttura e presenza microflora

Grasso 22%

mozzarella

Grasso 4.5%

cottage cheese

Grasso 25%

crescenza

Formaggi stagionati con struttura e microflora

Grasso 26%

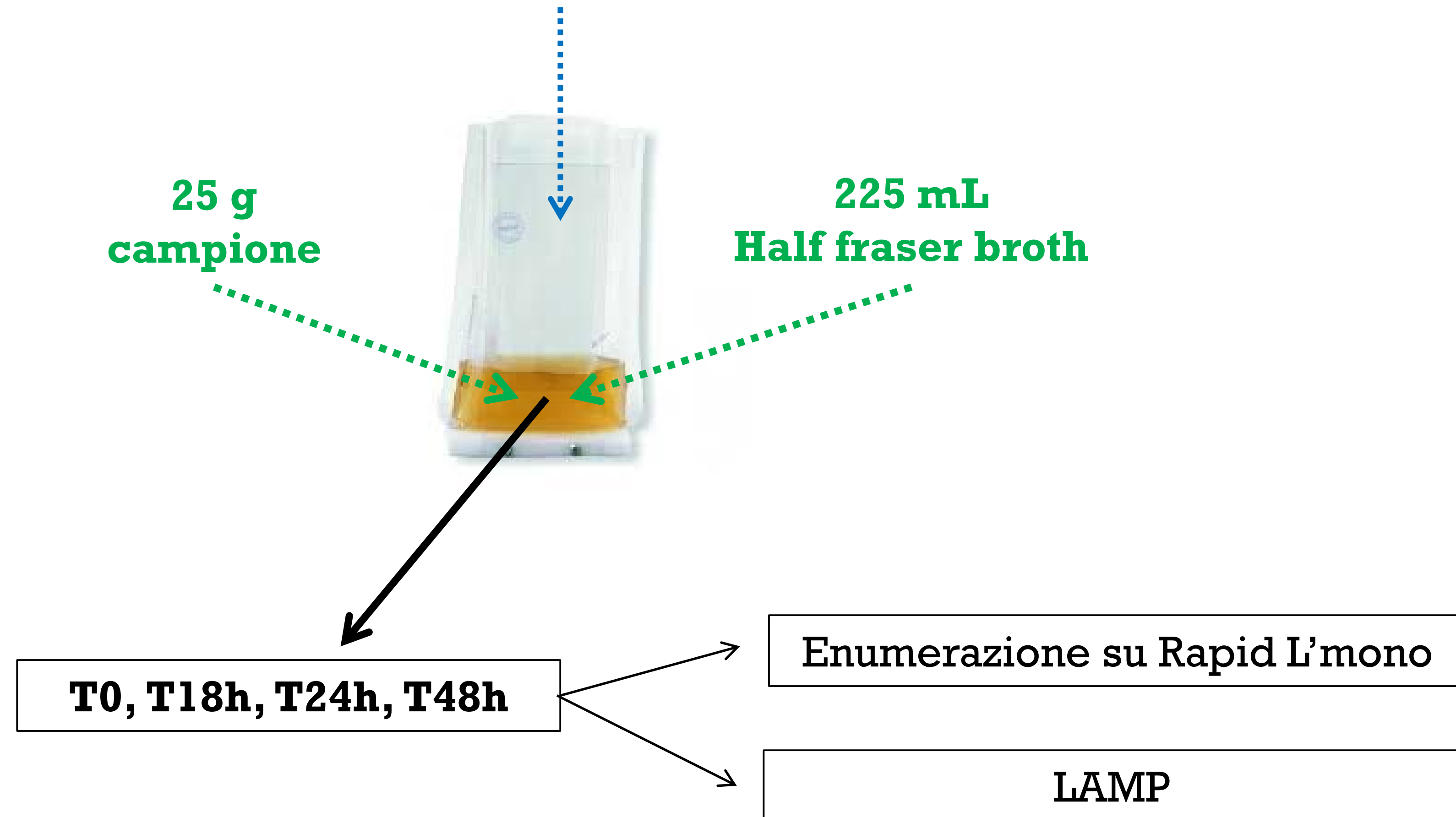
gorgonzola

Grasso 27%

taleggio

Prove preliminari

<u>STEP 3</u>: verifica della sensibilità **su alimenti contaminati da *Listeria monocytogenes* e sottoposti ad analisi qualitativa brodo di coltura**



Prove preliminari - 18 e 24 h

STEP 3: verifica della sensibilità **su alimenti contaminati da *Listeria monocytogenes* e sottoposti ad analisi qualitativa brodo di coltura**

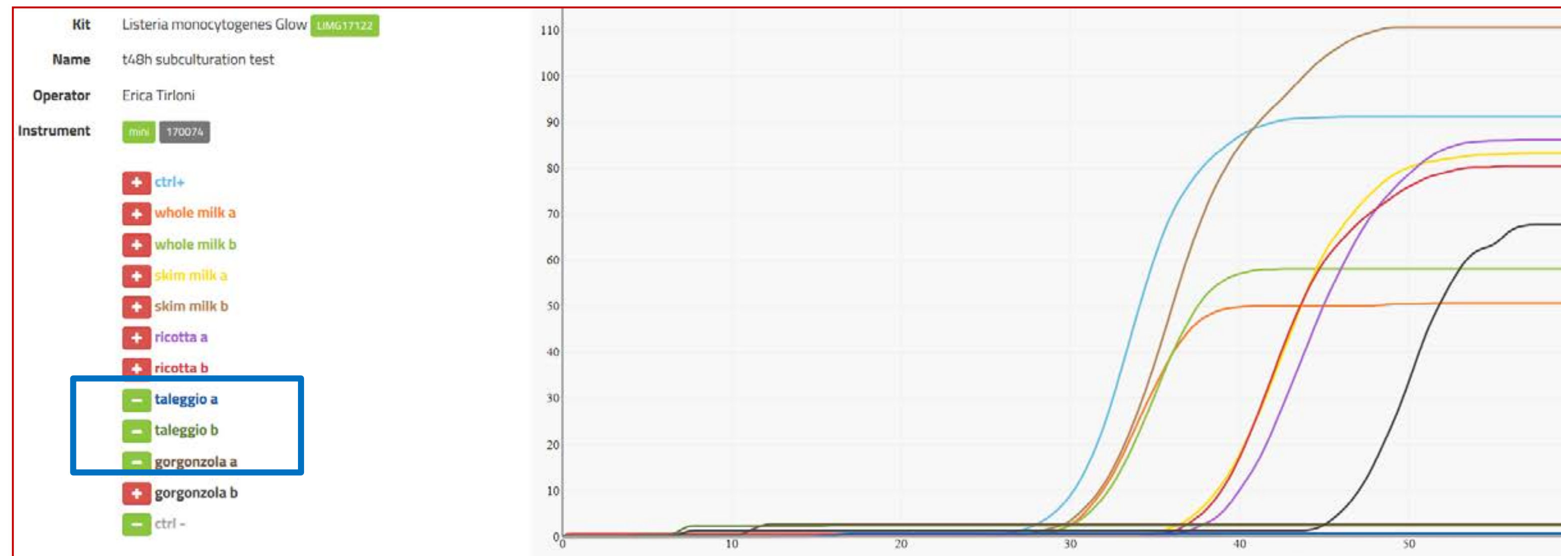
Prodotto	T18h		T24h	
	LAMP	Conta microbica Log UFC/g	LAMP	Conta microbica Log UFC/g
Latte intero A	-	3.4	-	4.7
Latte intero B	-	3.3	-	4.7
Latte scremato A	-	3.4	-	4.7
Latte scremato B	-	3.3	-	4.7
Ricotta A	-	2.5	-	4.3
Ricotta B	-	2.3	-	3.4
Mascarpone A	-	2.3	-	4.1
Mascarpone B	-	2.0	-	4.3
Taleggio A	-	2.3	-	2.5
Taleggio B	-	2.6	-	2.6
Gorgonzola A	-	3.2	-	4.2
Gorgonzola B	-	1.0	-	2.3
Cream cheese A	-	2.3	-	4.2
Cream Cheese B	-	2.4	+	4.2
Crescenza A	-	2.6	-	4.3
Crescenza B	-	1.8	-	4.3
Mozzarella A	-	2.6	-	4.2
Mozzarella B	-	2.9	-	4.3
Cottage cheese A	-	3.3	-	4.5
Cottage cheese B	-	3.2	-	4.6

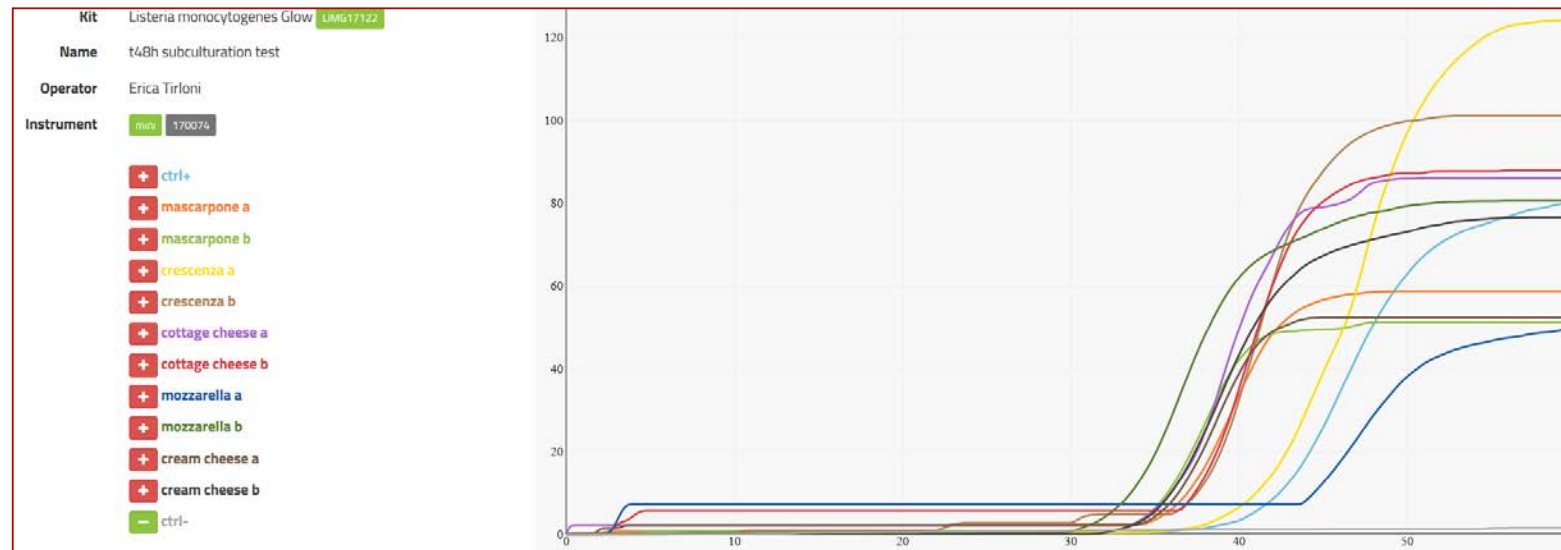
Cariche < 5 Log UFC/g

Prove preliminari - 48 h

STEP 3: verifica della sensibilità **su alimenti contaminati da *Listeria monocytogenes* e sottoposti ad analisi qualitativa brodo di coltura**

- **< crescita LM per condizioni del substrato? pH?**
- **Microflora naturale?**
- **Effetto mascherante della matrice?**





Prodotto	T48h	
	LAMP	Conta microbica Log UFC/g
Latte intero A	+	8.0
Latte intero B	+	8.1
Latte scremato A	+	8.3
Latte scremato B	+	8.1
Ricotta A	+	7.9
Ricotta B	+	8.0
Mascarpone A	+	8.3
Mascarpone B	+	8.1
Taleggio A	-	3.3
Taleggio B	-	3.5
Gorgonzola A	+	5.8
Gorgonzola B	-	2.9
Cream cheese A	+	8.2
Cream Cheese B	+	8.4
Crescenza A	+	7.7
Crescenza B	+	8.1
Mozzarella A	+	8.1
Mozzarella B	+	8.1
Cottage cheese A	+	8.2
Cottage cheese B	+	8.1

Possibile interferenza delle microflora naturale presente/substrato non permissivo

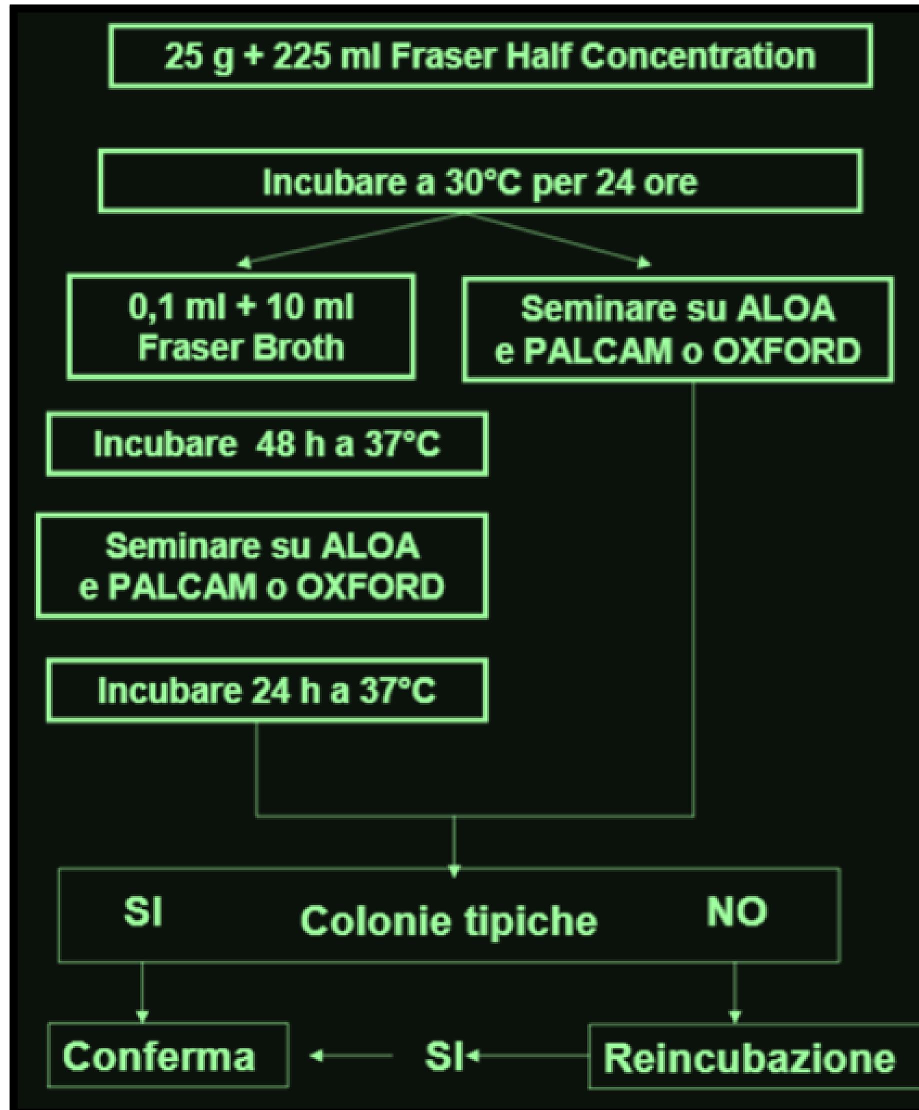
inibizione della crescita di *Listeria monocytogens*

Basse cariche (~3 log cfu/g) non rilevate

In 48 h di subculturazione + 1h e 30min di esecuzione test ottenimento del risultato finale (vs 72h del metodo classico) dell'analisi qualitativa: risparmio di almeno 24 ore

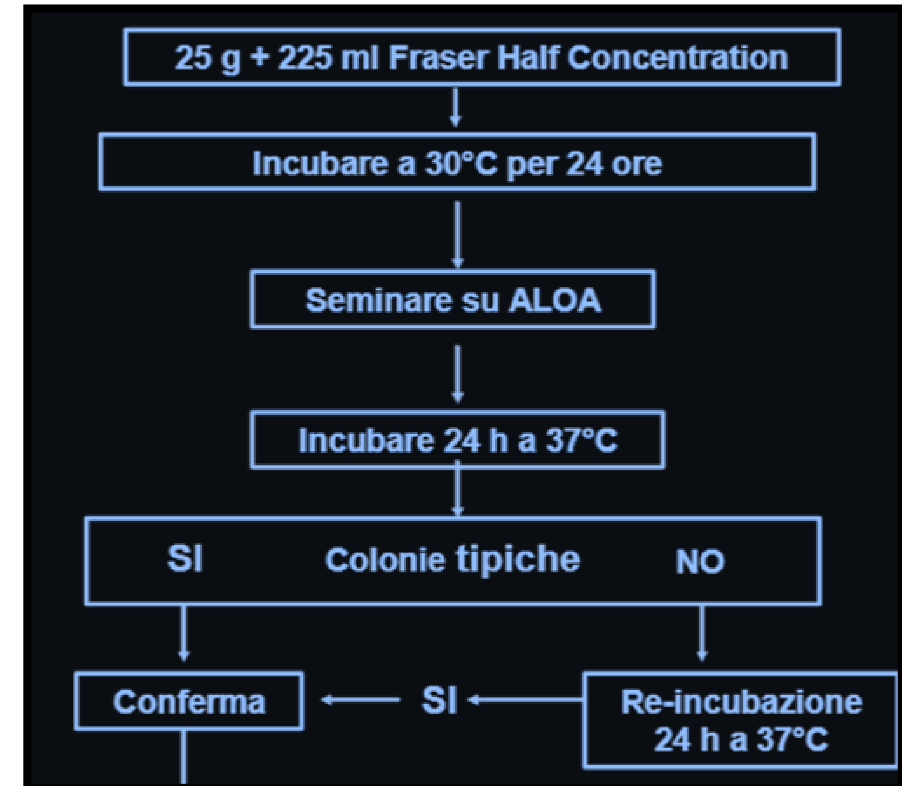
Metodiche classiche di ricerca di *Listeria monocytogenes*

ISO



Tempo minimo:
24+24+24=72h

AFNOR



Tempo minimo:
24+24+24=72h

Conclusioni

	Limite rilevabilità
Ceppo 118 Brodo	**10 UFC/ml (=1 Log UFC/mL)**
Prodotti inoculati con 118	**4.2-5.8 Log UFC/g**

Interferenza substrato (alimento):

- ***grasso;***
- ***microflora naturale presente;***
- ***struttura.***

Conclusioni

Studio	Substrato	LOD
Wang et al., (2010)	Latte crudo volontariamente contaminato	**$1.86*10^2$ UFC/mL**
Wang et al. (2015)	Latte crudo volontariamente contaminato	**$2.4*10^4$ UFC/mL**
Cho et al., (2014)	Latte crudo volontariamente contaminato	**$2.22*10^0$ UFC/mL**
Shan et al., (2012)	Alimenti volontariamente contaminati	**$>2.8*10^3$ UFC/g**
Mikŝ-Krajnik et al., (2016)	Superfici a contatto con alimenti	**$>10^2$ UFC/100 cm^2**
Applicazioni		
Tang et al., (2011)	Pollame naturalmente contaminato	**100% concordanza con metodi culturali classici**
Tang et al., (2017)	Vegetali naturalmente contaminati	**-**
Wu et al., (2014)	Alimenti naturalmente contaminati *(carne bovina, RTE carnei)*	**100% concordanza con metodi culturali classici**
Wang et al. (2015)	Carne suina naturalmente contaminata	**100% concordanza con metodi culturali classici**

In futuro…

Nuovi test…su nuove matrici alimentari

Nuovi test per verificare la possibilità di riduzione del periodo di incubazione a 36 h

In futuro…

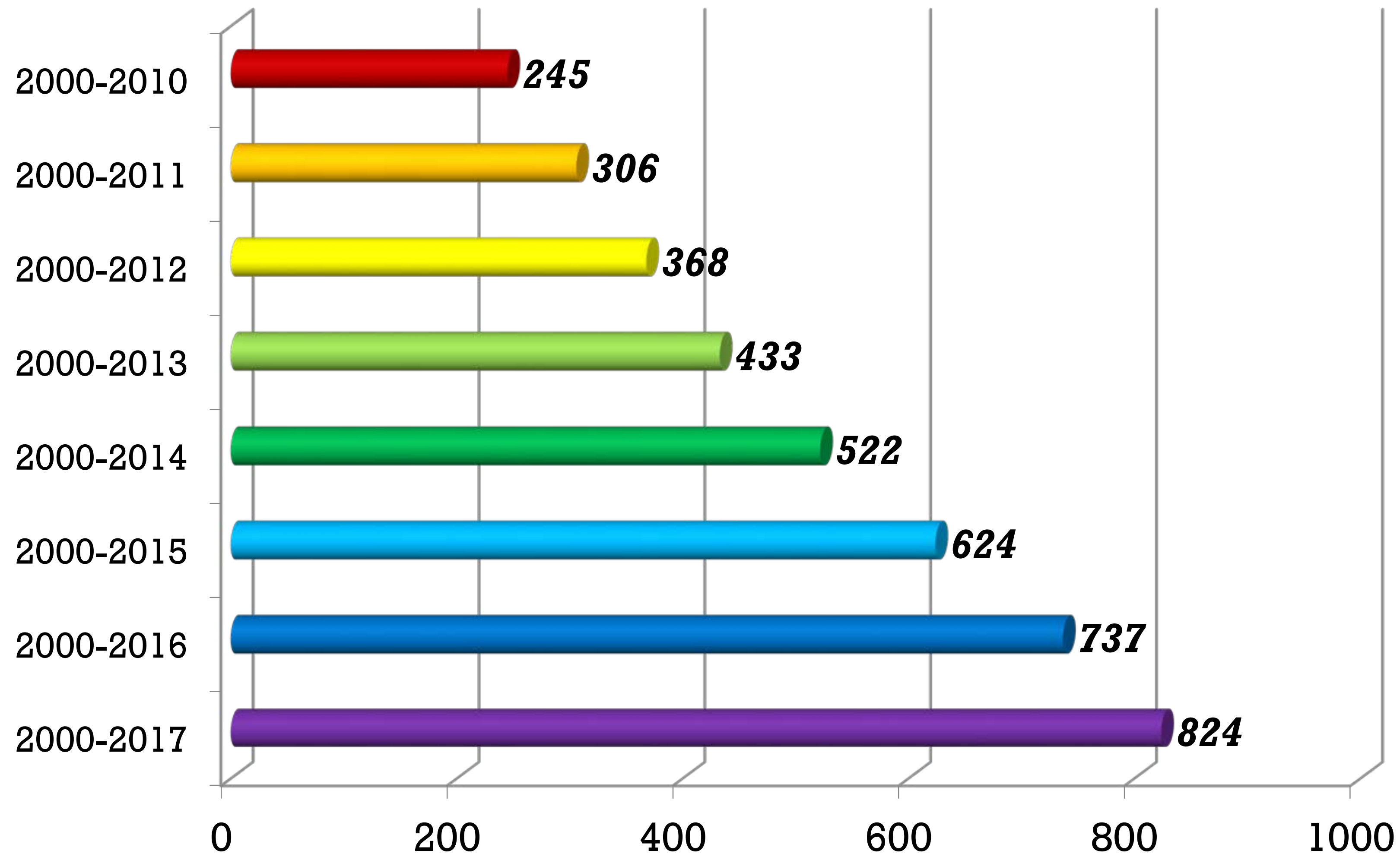
Capitolo 1. Criteri di sicurezza alimentare

Categoria alimentare	Microrganismi/loro tossine, metaboliti	Piano di campionamento (1)		Limiti (2)		Metodo d'analisi di riferimento (3)	Fase a cui si applica il criterio
		n	c	m	M		
1.1 Alimenti pronti per lattanti e alimenti pronti a fini medici speciali (4)	*Listeria monocytogenes*	10	0	Assente in 25 g		EN/ISO 11290-1	Prodotti immessi sul mercato durante il loro periodo di conservabilità
1.2 Alimenti pronti che costituiscono terreno favorevole alla crescita di *L* diversi da quelli destinati a medici speciali	*Listeria monocytogenes*	5	0	100 ufc/g (5)		EN/ISO 11290-2 (6)	Prodotti immessi sul mercato iodo di conser-
							enti non siano llo diretto del- ore alimentare
1.3 Alimenti pronti che non cos terreno favorevole alla crescit *monocytogenes*, diversi da quel lattanti e a fini medici specia							ercato di conser-

L'impiego di metodi d'analisi alternativi è accettabile quando tali metodi sono validati in base al metodo di riferimento di cui all'allegato I e se è utilizzato un metodo proprietario

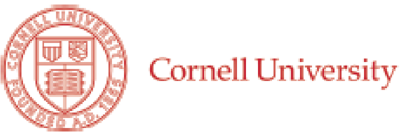
certificato da una terza parte in base al protocollo definito nella norma EN/ISO 16140 o ad altri protocolli analoghi accettati a livello internazionale.

Qualora l'operatore del settore alimentare intenda applicare metodi analitici diversi da quelli validati e certificati come indicato al terzo comma, tali metodi sono validati in base a protocolli riconosciuti a livello internazionale e il loro impiego è autorizzato dall'autorità competente.



Numero lavori con parola chiave LAMP
2000-2010
2000-2011
2000-2012
2000-2013
2000-2014
2000-2015
2000-2016
2000-2017
245
306
368
433
522
624
737
824
0
200
400
600
800
1000

Sistema LAMP 3M validato per *Listeria* spp.



P2-124: Validation of the 3M™ Molecular Detection System for the Detection of *Listeria* in Meat, Seafood, Dairy and Retail Environments

Department of Food Science, Cornell University, Ithaca, NY, United States: Esther D. Fortes; Martin Wiedmann **3M Food Safety, 3M Center, St. Paul, MN, United States:** John David; Bob Koeritzer

ABSTRACT

There is a continued need to develop improved rapid methods for detection of foodborne pathogens. The 3M™ Molecular Detection System utilizes isothermal DNA amplification and bioluminescence to detect targeted pathogens, after an enrichment step. The project aim was to evaluate the 3M Molecular Detection System and 3M™ Molecular Detection Assay *Listeria* using environmental samples obtained from retail delis and meat, seafood, and dairy processing plants. 391 environmental samples were collected using 3M™ Sponge-Sticks with D/E Neutralizing Buffer and tested for *Listeria* with the 3M Molecular Detection System after 22- and 48-hour enrichment in 3M™ Modified *Listeria* Recovery Broth; 3M Modified *Listeria* Recovery Broth enrichments were also used for cultural detection of *Listeria* spp.

Overall, 3M Molecular Detection System and culture based detection after enrichment in 3M Modified *Listeria* Recovery Broth did not differ significantly (P<0.05) with regard to the number of positive samples, when separate chi-square analyses were performed for (i) number of positive samples after 22 hours, (ii) number of positive samples after 48 hours and (iii) number of positive samples after 22 hours and/or 48 hours of enrichment in 3M Modified *Listeria* Recovery Broth. Among 288 sampling sites that were tested with duplicated sponges, 67 tested positive with the 3M Molecular Detection System and 67 also tested positive with the traditional FDA BAM method, further supporting that the 3M Molecular Detection System performs equivalent to traditional methods when used for testing of environmental sponge samples.

INTRODUCTION

PCR-based methods have revolutionized the detection of foodborne pathogens from food or environmental samples by allowing for both more rapid as well as often more specific detection than traditional cultural methods. While other amplification based methods for detection of nucleic acids, including a variety of isothermal amplification methods, have been reported in the peer-reviewed literature[1-4] and have been offered commercially for detection of pathogens in clinical settings, systems using isothermal amplification methods for detection of foodborne pathogens have only recently started to become commercially available. In particular, the 3M Molecular Detection System, which has recently been released, uses loop-mediated isothermal amplification (LAMP), an isothermal amplification method, for detection of foodborne pathogens. LAMP has previously been shown to allow for sensitive detection of *Salmonella enterica* in liquid eggs[5] and to be less susceptible to inhibition by culture media and certain biological substances as compared to PCR[6].

The goal of this project was to evaluate a pre-production version of the 3M Molecular Detection System for its ability to detect *Listeria* in environmental sponge samples, using the 3M Molecular Detection Assay *Listeria*. Detection of *Listeria* spp. in environmental samples collected in retail deli establishments may provide one avenue to help control transmission of *L. monocytogenes* in the deli meat retail environment, which is increasingly recognized as a concern, particularly since a recent risk assessment suggested that a considerable proportion of human listeriosis cases linked to consumption of RTE meat and poultry products is linked to contamination that occurs after products leave the processing environment[7].

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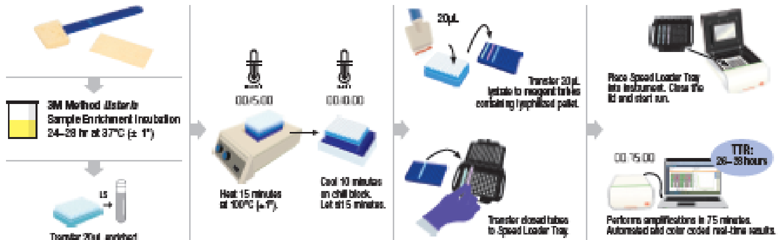
3M MOLECULAR DETECTION ASSAY *Listeria* PROTOCOL



MATERIALS AND METHODS

Environmental samples. A total of 391 samples were collected from retail (n=120), seafood processing (n=72), meat processing (n=100) and dairy processing (n=99) environments using 3M Sponge-Sticks with D/E Neutralizing Buffer. Samples were collected in conjunction with ongoing environmental sampling projects.

3M Molecular Detection System analysis and cultural *Listeria* confirmation. Sample sponges to be tested with the 3M Molecular Detection Assay *Listeria* were enriched in 225mL 3M Modified *Listeria* Recovery Broth directly in the 3M sample collection bag. Sample enrichments were incubated at 30°C and tested with the 3M Molecular Detection System at 22 hours and 48 hours, following the manufacturer's protocol. The same enrichments were used for isolation of *Listeria* spp. as a confirmation of 3M Molecular Detection System results; procedures used for isolation were similar to the plating procedures detailed in FDA BAM.

Confirmation of *Listeria* isolates by PCR and *Listeria* species identification. One putative *Listeria* colony per sample was confirmed as *Listeria* spp. using a PCR assay that amplifies a fragment of the *sigB* gene; sequencing of this PCR product allows classification of isolates into *Listeria* spp.

Detection of *Listeria* in a second sponge using standard microbiological methods. In addition to the sponge sample used for enrichment with the 3M Modified *Listeria* Recovery Broth, followed by analysis with the 3M Molecular Detection System and cultural detection, duplicate sponges from 288 sampling sites were also tested according to the FDA BAM standard method with minor modifications.

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RESULTS AND DISCUSSION

A total of 391 environmental samples from retail, seafood processing, meat processing and dairy processing were tested for *Listeria* with the 3M Molecular Detection Assay *Listeria* after 22- and 48-hour enrichment in 3M Modified *Listeria* Recovery Broth. In addition, the 3M Modified *Listeria* Recovery Broth enrichment for each sample was also tested for *Listeria* spp. using standard plating procedures similar to the FDA BAM method.

Enrichments of a variety of environmental sponge samples from different sources do not cause interference or inhibition of the 3M Molecular Detection System. Among all 391 samples tested, none were shown to inhibit the 3M Molecular Detection Assay *Listeria* results, based on the results for the Matrix Control (data not shown).

Detection with the 3M Molecular Detection System after 22- and 48-hour enrichment does not yield statistically different numbers of samples positive for *Listeria* spp. The number of samples positive at 22 and 48 hours (Table 1) was not significantly different for either the 3M Molecular Detection System or cultural detection from 3M Modified *Listeria* Recovery Broth (chi-square P<0.05).

3M Molecular Detection System results after 22 and 48 hours of sample enrichment are not significantly different from culture-based detection after 3M Modified *Listeria* Recovery Broth enrichment. Overall, 3M Molecular Detection System and culture based detection after enrichment in 3M Modified *Listeria* Recovery Broth did not differ significantly (P<0.05) with regard to the number of positive samples (Table 1).

For a total of 288 of the 387 samples included in the final data analyses, duplicate sponges taken from the same sites had also been tested using an FDA BAM protocol (Table 2).

While there were a number of samples that were positive by the 3M Molecular Detection System after enrichment in 3M Modified *Listeria* Recovery Broth and negative by FDA BAM and vice versa, these data clearly indicate that the overall methods employing enrichment in 3M Modified *Listeria* Recovery Broth and in BLEB yielded comparable results on environmental sponges.

3M Molecular Detection System positive samples represented diversity of *Listeria* species. *sigB* sequencing and allelic typing of a single isolate for each of the 74 3M Molecular Detection System positive samples yielded a diversity of Listeria species and allelic types (Fig. 1).

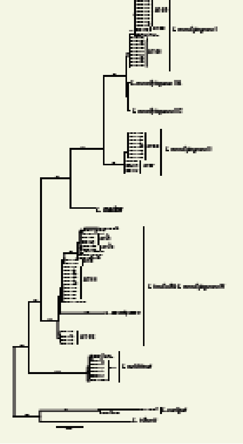
Table 1. Correlation between the 3M Molecular Detection System and traditional culture results after enrichment in 3M Modified *Listeria* Recovery Broth.

No. of Samples	3M MDS Results after Enrichment		Culture Results after 3M mLRB Enrichment	
	22 hours	48 hours	22 hours	48 hours
Concordant Results				
310	–	–	–	–
65	+	+	+	+
3	–	+	–	+
1	+	–	+	–
Discrepant Results				
2	–	+	+	+
1	–	–	+	+
3	+	+	–	+
2	+	–	–	–

Table 2. Correlation between the 3M Molecular Detection System after enrichment in 3M Modified *Listeria* Recovery Broth and FDA-BAM methodology.

No. of Samples	3M Molecular Detection System	FDA-BAM
Positive	67	67
Negative	221	221
Total	288	288

Figure 1. *sigB* phylogeny for 75 *Listeria* isolates representing one isolate from each of the 74 3M Molecular Detection System positive samples that were also positive by cultural methods (after enrichment in 3M Modified *Listeria* Recovery Broth) as well as one isolate from a sample that was positive by cultural methods (after enrichment in 3M Modified *Listeria* Recovery Broth), but not by the 3M Molecular Detection System.



CONCLUSIONS

Overall, our data show that the 3M Molecular Detection Assay *Listeria*, performs equally as well as the gold standard method when used with sponge samples collected from naturally contaminated environmental sites. The system was able to detect a diversity of *Listeria* species and reported real-time positive results in as early as 25 minutes, following enrichment and a simple lysis protocol. As samples from a variety of different food associated environments were tested, these data suggest that this assay is unlikely to experience inhibition. This is consistent with previous reports[6,8,9] that suggest that LAMP, the isothermal amplification technology used in the 3M Molecular Detection System, is highly robust and less sensitive to inhibition as compared to many PCR-based amplification methods.

ACKNOWLEDGEMENTS

This study was supported by a 3M-sponsored research contract with Cornell University. The authors thank Henk den Bakker and Steven Warchocki for help with analysis of *sigB* DNA sequence data and Laura Strawn for help with experiments.

Sistema LAMP 3M validato per *Listeria* spp.

Validation of the 3M™ Molecular Detection System for the Detection of *Salmonella*, *E. coli* O157 (including H7) and *Listeria* spp., in Dried Fruits, Nuts and Environmental Samples

www.agfoodsafety.org
www.food-safetynet.com

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INTRODUCTION

In order to ensure the safety of consumers in the food market, a much more comprehensive farm to table approach is required. For this to be a reality, faster and more accurate testing methods for pathogens are necessary, especially since traditional microbiology methods are cumbersome and take more than four days to complete. Modern developments in molecular biology have resulted in isothermal nucleic acid amplification technology, also known as loop-mediated isothermal amplification (LAMP), which is recognized as highly robust, efficient, sensitive, specific and easy to use. The 3M™ Molecular Detection System was developed, for the rapid and specific detection of pathogens using the innovative combination of LAMP and bioluminescence to report the amplification of the target DNA in real time.

PURPOSE

This study was conducted to evaluate the performance of the 3M Molecular Detection System and its assays in the detection of *Salmonella*, *E. coli* O157:H7 and *Listeria* spp., in the presence of potential interferences from seven sample matrices. The system's performance was also assessed on environmental samples. Presumptive positives were confirmed through cultural methods.

METHOD

Salmonella, *E. coli* O157:H7 and *Listeria* spp. pathogen screen tests were performed on various nuts and dried fruits; 25g samples of each matrix were enriched with the required media (3M™ Buffered Peptone Water (ISO) or 3M™ Modified *Listeria* Recovery Broth) and incubated according to the protocol table below. One set of samples was inoculated with 25 CFU; one set was not inoculated with pathogens.

Table 1: Samples

Sample Description	Sample Size	Enrichment Media Volume
Raisins	25g	225mL
Almonds	25g	225mL
Walnuts	25g	225mL
Diced Walnuts	25g	225mL
Salted Pistachios	25g	225mL
Raw Pistachios	25g	225mL
Kernel Pistachios	25g	225mL
Environmental Surface	1 swab	100mL
Pool of Raisins, Almonds, Walnuts and Raw Pistachios	4 x 10mL (Post-enrichment)	N/A
Pool of Raisins, Almonds, Walnuts, Diced Walnuts and Salted Pistachios	5 x 10mL (Post-enrichment)	N/A
Salted Pistachios and Kernel Pistachios	2 x 10mL (Post-enrichment)	N/A
Raw Pistachios and Kernel Pistachios	2 x 10mL (Post-enrichment)	N/A
Environmental Swabs	2 x 10mL (Post-enrichment)	N/A

Table 2: Protocols

Assay	Incubation Temperature	Enrichment Time (Hours)
Salmonella	37°C	18–24
E. coli O157:H7	42°C	18–24
Listeria spp.	37°C	24–28

Environmental swabs were enriched in 100mL of the required media according to the protocol for each assay; *Salmonella*, *E. coli* O157:H7 and *Listeria* spp. environmental surface samples were ran spiked and un-spiked.

Matrix Controls were used for each matrix to check for possible interference from the sample. Kit negative controls and reagent controls (known Positive Control) were run along with each test to confirm that the kits were working as designed.

A pool was performed for spiked and un-spiked *Salmonella* samples only, 10mL aliquots were taken from each enriched sample (see Table 1); then 50µL of anti-body coated magnetic beads, specific for *Salmonella*, were added to the pooled samples and placed on the Applied Biosystems Pathatrix® Immuno-magnetic Separation unit, four individual samples were processed along with the pooled samples for comparison purposes.

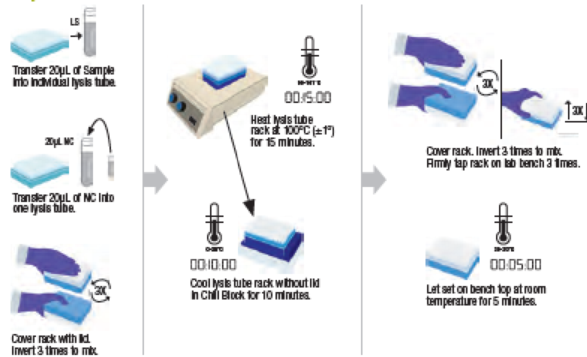
Presumptive positives were confirmed by the use of traditional cultural methods as follows:

- *Salmonella*: All presumptive positive samples were streaked onto XLD, HE, BBL™ CHROMagar™ *Salmonella* and incubated at 35 ± 1°C for 24–48 hours.
- *E. coli* O157:H7: All presumptive positive samples were streaked onto CT-SMAC and incubated at 35 ± 1°C for 24–48 hours.
- *Listeria* spp.: All presumptive positive samples were streaked onto RAPID'L.mono™ (Bio-Rad) and incubated at 35 ± 1°C for 24–48 hours.

PROTOCOL REFERENCE

1. Enrichment

2. Lysis



3. Amplification

TECHNOLOGY OVERVIEW

Powered by an innovative combination of unique technologies to bring molecular level accuracy expected by customers — without sacrificing productivity.

RESULTS

	n	*Salmonella* % Sensitivity	*Salmonella* % Specificity	*E. coli* O157:H7 % Sensitivity	*E. coli* O157:H7 % Specificity	*Listeria* spp. % Sensitivity	*Listeria* spp. % Specificity
Raisins	5	100	100	100	100	100	100
Almonds	5	100	100	100	100	100	100
Walnuts	5	100	100	100	100	100	100
Diced Walnuts	5	100	100	100	100	100	100
Salted Pistachios	5	100	100	100	100	100	100
Raw Pistachios	5	100	100	100	100	100	100
Kernel Pistachios	5	100	100	100	100	100	100
Environmental Swab	5	100	100	100	100	100	100
Pooled Samples	6	100	100	N/A	N/A	N/A	N/A

Matrix Controls were valid, confirming that there was no interference from the matrices tested.

% Sensitivity and % Specificity were calculated as follows:

$$\% \text{ Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$\% \text{ Specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

Cultural methods confirmed all presumptive positive results from the 3M Molecular Detection System.

SIGNIFICANCE

This study demonstrates that the 3M Molecular Detection System is a highly robust, efficient, sensitive and specific detection method. Compatibility with a variety of samples was demonstrated; the absence of matrix interference was confirmed through the use of the Matrix Control for each assay.

ACKNOWLEDGEMENTS

Apart from the efforts of myself, the success of any project or experiment depends largely on the encouragement and guidelines of many others. I would like to take this opportunity to express my sincere gratitude to the people who have been instrumental in the successful completion of this validation experiment. I would like to show my greatest appreciation and gratitude to 3M and Thomas Jones (DFA Laboratory Director) for allowing me the opportunity to test the 3M Molecular Detection System. I would like to express my gratitude towards Peter Benedetto for putting this validation poster together.

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Journal of Food Protection, Vol. 76, No. 5, 2013, Pages 874–878
doi:10.4315/0362-028X.JFP-12-552
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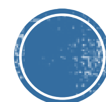
Research Note

Validation of the 3M Molecular Detection System for the Detection of *Listeria* in Meat, Seafood, Dairy, and Retail Environments

ESTHER D. FORTES,[1] JOHN DAVID,[2] BOB KOERITZER,[2] AND MARTIN WIEDMANN[1]*

GRAZIE PER L'ATTENZIONE



Protocollo (messo a punto al termine delle prove preliminari)

- **Preparazione del campione**:

Dal brodo di arricchimento (Half Fraser Broth), dopo incubazione di 48 h, viene prelevato 1 aliquota

- **Estrazione del DNA**:
  - Centrifugazione
  - Risospensione del pellet con di buffer di estrazione
  - Incubazione per consentire l'estrazione del DNA
  - Centrifugazione
  - Prelievo di un'aliquota di 3 μl di DNA estratto per la reazione LAMP
- **Preparazione della reazione LAMP:**

- In una provetta di Primer mix (primers liofili) aggiungere 22 μl di LAMP mix (enzima, cloruro di magnesio, nucleotidi e buffer di reazione), 30 μl di olio minerale e 3 μl di DNA estratto
- Inserire la provetta nello strumento e avviare l'amplificazione

Nel caso di prodotti alimentari grassi (es. formaggio ecc.) l'estratto deve essere diluito 1:5 con il buffer di estrazione