

# Confident Listeria detection – new regulations, smart solutions

Real-time PCR strategies for safe food production





Lysis & real-time PCR in ONE kit



Simultaneous detection of *L. monocytogenes & Listeria* spp.



Open system



## Regulatory testing of Listeria in food production

Recent updates in food safety regulations are increasing pressure on food producers and laboratories to improve *Listeria* spp. and *L. monocytogenes* detection.

## Regulation EC 2073/2005 (Microbiolocal criteria of food)

#### Listeria monocytogenes

Original regulation (2005)



#### **Product specific limits**

- ≤ 100 CFU/g if no growth throughout shelf-life
- If growth is possible: not detected in 25 g before leaving producer control

Amended (EU 2024/2895)



#### New criteria for food that supports growth

Not detected in 25 g throughout shelf-life unless proven < 100 CFU/g

#### Listeria control now applies across all stages



#### **USDA & FDA (FSIS Programs)**

#### Listeria monocytogenes

**Original regulation** 



Primarily tested in RTE products, environmental samples, and food contact surfaces. Now (from January 2025)



Expanded testing to include all **Listeria species** in both ready-to-eat products and environmental samples.



### Where real-time PCR makes sense

Practical implementation areas for Listeria detection using real-time PCR to support compliance, traceability and release procedures.

Listeria spp. → Environmental early warning

L. monocytogenes → Critical product safety indicator

## Preventive controls



Environmental monitoring (swab testing)



Temperature control (cold chain)



Sanitation procedure



Employee hygiene



Raw material control

## **Process validation**



Thermal processing validation



Hurdle technology (ph,  $\alpha_w$  preservatives)



Shelf-life studies



Challenge testing

## Real-time PCR implementation points



- · Raw material screening
- Environmental monitoring
- In-process testing

- Finished product testing
- Shelf-life validation

Application area	Purpose	Benefit	
Product release testing	Rapid RTE food clearance	Next day results	
Environmental monitoring	Detect <i>Listeria</i> spp.	Early risk indicator	
Raw material screening	Trace <i>L. monocytogenes</i> early	Source control	
Hygiene validation	Check cleaning effectiveness	Documented assurance	
Sanitation zones	Trend analysis	Avoid persistent contamination	

## **SureFast® ONE –** simple and straightforward

## Streamlined protocol



Incubate e.g. 25 g food/feed in enrichment medium\*



Transfer 5 µL DNA samples (controls) to 20 µL master mix



Take sample from the stomacher bag and add lysis buffer



Start the qPCR run



Mix briefly and incubate for 10 minutes at 95 °C



Result analysis

#### ONE system qPCR products

Product	Description	Tests	Art. No.
SureFast® Listeria 3plex ONE AOAC-RI 062501; MicroVal 2023LR114	ROX: <i>Listeria</i> spp. Cy5: <i>Listeria monocytogenes</i> HEX: IAC	100 reactions incl. DNA prep	F5217
SureFast® Salmonella ONE AOAC-RI 81803 ; MicroVal 2014LR43	FAM: <i>Salmonella</i> spp. HEX: IAC	100 reactions incl. DNA prep	F5211
SureFast® STEC 4plex ONE	FAM: <i>E. coli stx1</i> (subtype a-d) & <i>stx2</i> (subtype a-g) Cy5: <i>eae</i> ROX: <i>E. coli</i> O157 HEX: IAC	100 reactions incl. DNA prep	F5265

<sup>\*</sup> Salmonella: BPW for 16 - 24 h; E. coli: BPW / mTSB for 18 - 24 h; Listeria: Half Fraser broth for 26 - 28 h