Detection of *Listeria* in Yeast Samples with foodproof® *Listeria* plus *L. monocytogenes* LyoKit

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One Health Diagnostics™

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BAX® System Q7 | BAX® System X5 | foodproof® | microproof®

INTRODUCTION:

According to the European Food Safety Authority (EFSA), *Listeria monocytogenes* infections are among the five most common zoonoses with 2,738 human cases of illness in 2022. In addition, *L. monocytogenes* caused the most severe zoonotic diseases in humans with 1,330 hospitalizations and 286 deaths. (European Food Safety Authority and European Centre for Disease Prevention and Control, 2023, The European Union One Health 2022 Zoonoses Report, *EFSA Journal*).

Yeast is one products that can be highly contaminated with *L. monocytogenes* and should be therefore closely monitored.

This data summarizes the results of an application study for the detection of *L. monocytogenes* in active dry and compressed yeast samples with an alternative PCR detection method. The scope of this study was to optimize the enrichment time, temperature, enrichment media, and DNA isolation method for more sensitive detection of *L. monocytogenes* in a high background flora of yeast.

The method should guarantee the detection of a contamination level of 1-10 CFUs/25 g sample. In a paired study, this ability was evaluated with the following alternative method: real-time PCR foodproof® *Listeria* plus *L. monocytogenes* LyoKit (KIT230129, Hygiena Diagnostics) in combination with the DNA isolation kit foodproof® StarPrep Two Kit (KIT230177, Hygiena Diagnostics) or alternatively with the easy-to-handle BAX® Lysis for *Listeria* (KIT2005).

PURPOSE:

The objective of this study was to optimize the enrichment of *L. monocytogenes* in yeast samples and to verify that the foodproof *Listeria* plus *L. monocytogenes* LyoKit produces qualitative results comparable to the plate-count-based reference method according to ISO 11290-1:2017-05 in dried and compressed yeast samples.

REGISTERED TRADEMARKS/ GLOBAL CERTIFICATIONS:

PCR: **food**proof® *Listeria* plus *L. monocytogenes* LyoKit (KIT230129)
AOAC RI certified (070401).
DNA Extraction 1: **food**proof® StarPrep Two (KIT230177)
DNA Extraction 2: BAX® System Real-time *L. monocytogenes* (KIT2005)

METHOD:

Active dry and compressed yeast samples (25 g sample size) were inoculated with a low-level spiking of dry-stressed *L. monocytogenes* (1-10 CFU/25 g). Samples were enriched with Actero™ Elite *Listeria* Enrichment Media, or Tryptic Soy Broth with different sample dilutions, supplements, temperatures and incubation times. For the optimized enrichment method, samples were enriched for 24 or 48 hours at 37 °C. When an enrichment time of 48 hours was used, a sub-cultivation was conducted after the first 24 hours of enrichment. The optimized enrichment methods are listed in Table 1. All samples were analyzed with the alternative and the reference method in a paired study. For the reference method, enrichments were plated on agar plates according to ISO 11290-1:2017-05. DNA isolation was conducted with the foodproof StarPrep Two, (KIT230177) and easy-to-handle BAX Lysis for *Listeria* (KIT2005). The real-time PCR foodproof *Listeria* plus *L. monocytogenes* LyoKit (KIT230129) was followed according to the manufacturer's instructions. For the controls, 25 µL of water was used for PCR Negative Control and 25 µL of foodproof Control Template for PCR Positive Control. The PCR was performed on LightCycler® 96 (Roche Diagnostics) and the analysis mode "Abs Quant" was used for baseline correction and the calculation of crossing points. DNA Extraction and PCR protocols are listed in table 2.

Table 1: Enrichment Protocols of optimized method

	Dry Yeast		Compressed Yeast	
Sample size	25 g		25 g	
Strain	*Listeria monocytogenes* (NCTC 11994)			
Stress Conditions	Dry Stress			
Medium	Actero™ Elite *Listeria* Enrichment Media	Trypticase Soy Broth (TSB)	Actero™ Elite *Listeria* Enrichment Media	Trypticase Soy Broth (TSB)
Dilution Factor for first and Second Enrichment	1:7	1:20	1:7	1:20
First Enrichment	24 h		24 h	
Second Enrichment	1 ml of First Enrichment, 24 hours		no	
Incubation Temperature	37 °C			

Table 2: DNA Extraction and PCR Protocols

		Process foodproof®	Process BAX®
DNA Extraction	Kit	foodproof StarPrep Two	DNA Extraction of BAX System Real-time *L. monocytogenes*
	Sample Volume Extraction	800 µL	5 µL
	Protocol	According to manual "Standard Protocol"	According to manual
PCR	Kit	foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit	
	Sample Volume PCR	5 µL	25 µL

RESULTS:

DRIED YEAST: For active dried yeast samples, an enrichment for 2 x 24 hours at 37 °C in 1:7 Actero™ Elite *Listeria* Enrichment Media results in a sensitive detection of *Listeria* sensu stricto and *L. monocytogenes*. After the first 24 hours incubation, a sub-culture in a 1:7 dilution of Actero Media for max. 24 hours at 37 °C is necessary to reach 100% agreement with the results of the reference method if the sample is contaminated with a low-level concentration of 4.3 CFUs/25 g. As an alternative Trypticase Soy Broth (TSB) can be used in a 1:20 dilution for first and second enrichment (48 hours in total) at 37 °C. The enrichment in Actero Elite *Listeria* Enrichment Media shows eight positive samples out of eight tested replicates. With TSB, seven positives out of eight tested replicates could be generated. All qualitative results of the alternative method were in 100% agreement with the reference (Table 3), regardless of the enrichment broth or extraction method used.

Table 3: Method comparison in dried yeast samples - reference method according to ISO 11290-01:2017-05 versus alternative method with different options for extraction: Spiking *L. monocytogenes*.

Results of foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit													
Inoculum: *Listeria monocytogenes* NCTC 11994						25 g Dried Yeast							
						DNA Extraction Kit: foodproof StarPrep Two				DNA Extraction Kit: BAX System Real-time *L. monocytogenes*			Reference
						PCR KIT: foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit				PCR KIT: foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit			OCLA & PALCAM Agar
Enrichment Media	Enrichment Temperature in °C	First enrichment	Second enrichment	Inoculation Level CFU/ 25 g	Enrichment time hours (first/second)	L. mono-cytogenes		L. sensu stricto		L. mono-cytogenes		L. sensu stricto	
						Pos/Rep	Mean Cq	Pos/Rep	Mean Cq	Pos/Rep	Mean Cq	Pos/Rep	Mean Cq	Pos/Rep
Actero	37	1:7	1:7	4.3	24/24	8/8	17.99	8/8	16.13	8/8	21.16	8/8	18.82	8/8
TSB	37	1:20	1:20	4.3	24/24	7/8	29.72	7/8	28.86	7/8	31.76	7/8	31.06	7/8

Pos/Rep = positive Results of tested Replicates; Actero = Actero Elite *Listeria* Enrichment Media; TSB = Tryptic Soy Broth

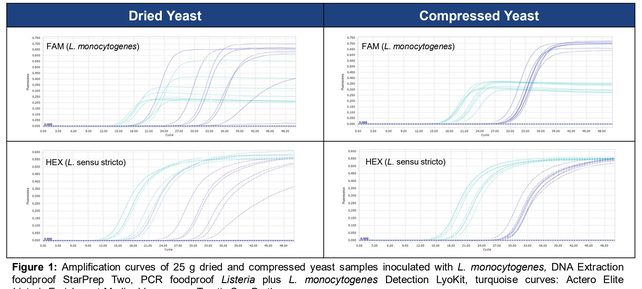

Figure 1: Amplification curves of 25 g dried and compressed yeast samples inoculated with *L. monocytogenes*, DNA Extraction foodproof StarPrep Two, PCR foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit, turquoise curves: Actero Elite *Listeria* Enrichment Media, blue curves: Tryptic Soy Broth

COMPRESSED YEAST: An optimized enrichment method for *Listeria* in active compressed yeast samples could be shown with an enrichment time of only 24 hours at 37 °C either with a dilution of 1:7 in Actero Elite *Listeria* Enrichment Media or alternatively, Tryptic Soy Broth (Dilution 1:20). The reference and the alternative method both detect eight positive samples out of eight tested replicates with a low contamination concentration. For the alternative PCR method, conducted with the foodproof *Listeria* plus *L. monocytogenes* LyoKit, the results were independent of the DNA extraction method, StarPrep Two or BAX Lysis. Even with a very low inoculation level of 2.3 CFUs/25 g sample, two out of three replicates were positive, which is also in 100% agreement with the reference method (see Table 4).

Table 4: Method comparison in compressed yeast samples - reference method according to ISO 11290-01:2017-05 versus alternative method with different options for extraction: Spiking *L. monocytogenes*

Results of foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit						25 g Compressed Yeast								
Inoculum: *Listeria monocytogenes* NCTC 11994						DNA Extraction: foodproof StarPrep Two Kit				DNA Extraction: BAX System Real-time *L. monocytogenes*				Reference
						PCR KIT: foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit				PCR KIT: foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit				OCLA & PALCAM Agar
Enrichment Media	Enrichment Temperature in °C	Enrichment dilution	Inoculation Level CFU/ 25 g	Enrichment time hours		L. mono-cytogenes		L. sensu stricto		L. mono-cytogenes		L. sensu stricto		
						Pos/Rep	Mean Cq	Pos/Rep	Mean Cq	Pos/Rep	Mean Cq	Pos/Rep	Mean Cq	Pos/Rep
Actero	37	1:7	2.3*	24		2/3	19.77	2/3	18.69	2/3	18.81	2/3	17.72	2/3
Actero	37	1:7	4.3 **	24		8/8	19.41	8/8	17.97	8/8	23.09	8/8	20.18	8/8
TSB	37	1:20	4.3 **	24		8/8	30.15	8/8	29.80	8/8	33.16	8/8	32.51	8/8

Pos/Rep = positive Results / Replicates; Actero = Actero Elite *Listeria* Enrichment Media; TSB = Tryptic Soy Broth
* sample inoculated with 2.3 CFUs/25 g sample
* * with *L. monocytogenes* natural contaminated sample (low-level concentration), inoculated with 4.3 CFUs/25 g sample

The enrichment methods was successfully optimized in time, temperature and media dilution to suppress the growth of yeast, which led to an increased growth and a more sensitive detection of *Listeria sensu stricto* and *Listeria monocytogenes* with the alternative real-time PCR System of Hygiena.

SIGNIFICANCE:

The results of this study demonstrate that the real-time PCR system foodproof *Listeria* plus *L. monocytogenes* LyoKit can successfully analyze low concentrations (< 10 CFUs/sample) of *Listeria monocytogenes* in 25 g active dry and compressed yeast samples with the same sensitivity as the reference method when the optimized enrichment procedure is used for the growth of *Listeria monocytogenes* in yeast.