



## **Case Study: Free DNA Removal of Listex-Treated Cheese**

**BIO-RAD**

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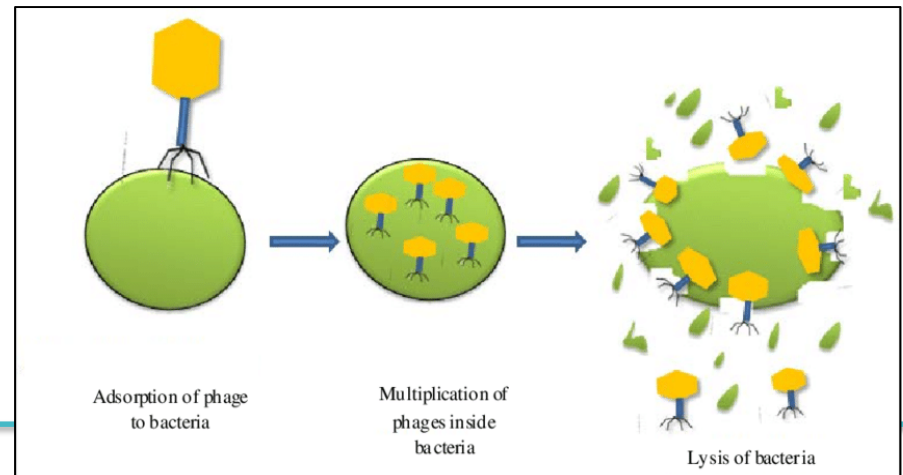
# Case Study

## Situation

- Largest cheese manufacturer using Bio-Rad's iQ-Check real-time PCR to test cheese products, observed a high rate of false positive results with *Listeria* spp.
- Cheese manufacturers routinely used Listex for targeted *Listeria* control.
- Current methods to remove free DNA are hazardous and time consuming.

## What is Listex?

- Listex is an antimicrobial processing aid (Phage P100) used to prevent *Listeria* in food products including meat, fish and cheese.
- Application of Listex is either by spraying or by immersion.

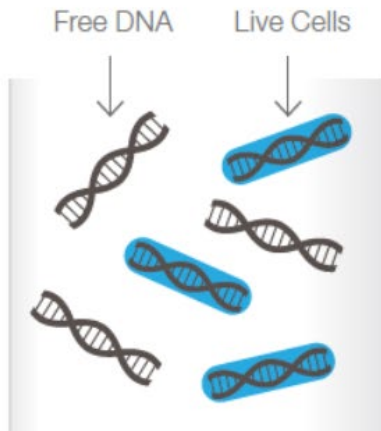


# Free-DNA Removal Solution (FDRS)

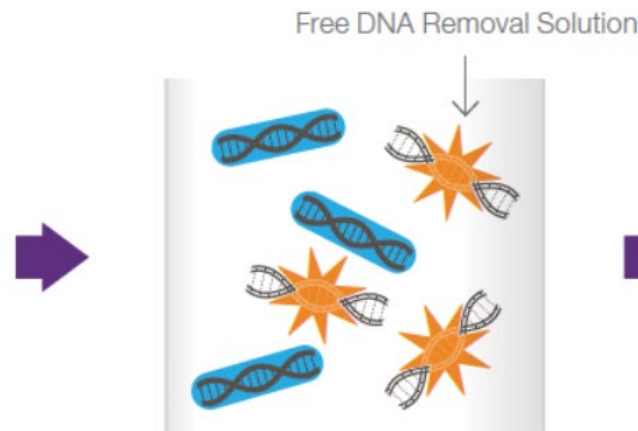
- Free DNA is degraded by an enzymatic treatment of the enrichment broth prior to DNA extraction.
- Reagent is inactivated by the lysis buffer allowing for the extraction of DNA from intact and living cells.



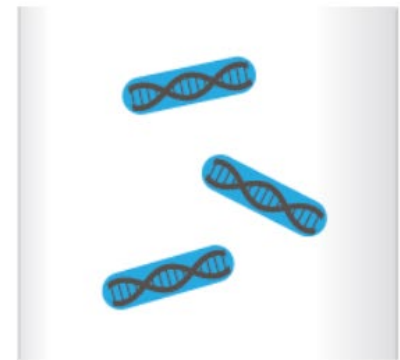
Enriched sample



Heat to 37°C for 15–30 min

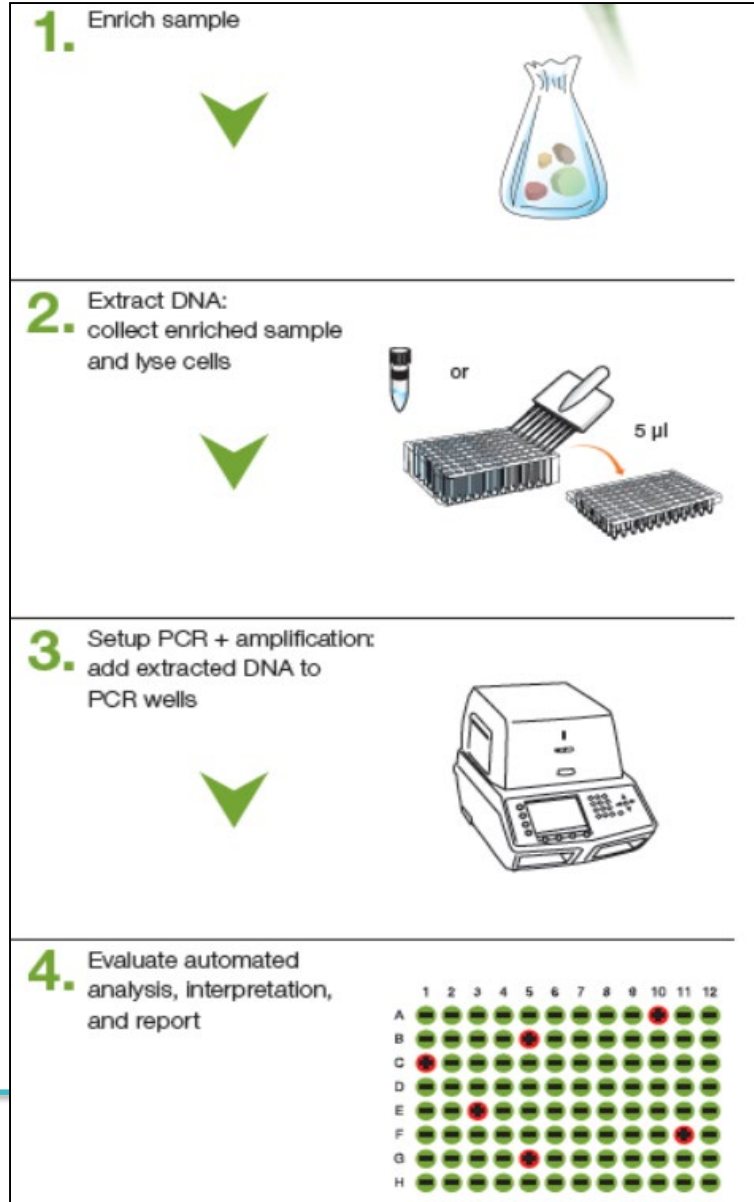


Sample ready for DNA extraction with the free DNA removed





# Internal Study



25 g Listex treated samples  
225 ml LSB (enrichment broth)  
24-25 hr (enrichment time)

iQ-Check *Listeria* spp.  
iQ-Check *Listeria monocytogenes*

# Observations

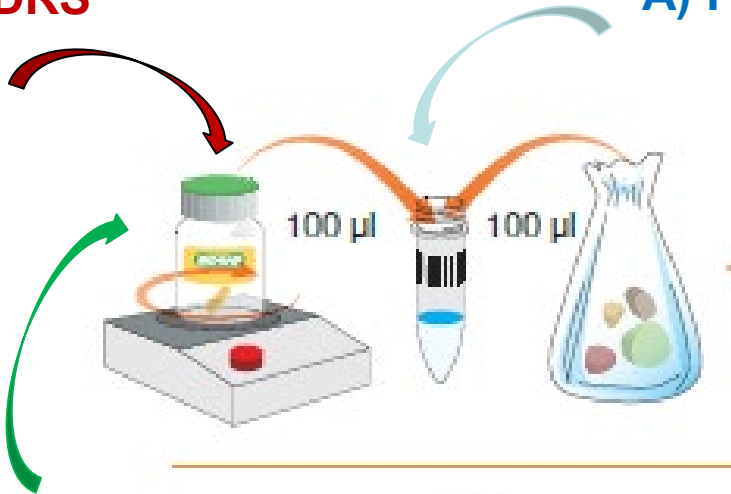
- *Listeria* DNA was detected in all Listex treated samples.
- DNA present in the Listex treated cheese was negative for *Listeria monocytogenes*
- Sample centrifugation before extraction reduced the amount of contaminating DNA by approximately 10-fold but did not lead up to a complete extinction of the signal.

		iQ-Check <i>Listeria mono.</i> assay			iQ-Check <i>Listeria spp.</i> assay		
		Target	IC	Result	Target	IC	Result
T0	Easy II	N/A	34,22	Negative	37,04	33,76	Positive
		N/A	33,19	Negative	37,62	32,96	Positive
24h no shaking	Easy II	N/A	32,67	Negative	37,59	32,87	Positive
		N/A	33,28	Negative	37,04	33,18	Positive
25h with shaking	Easy II	N/A	32,65	Negative	36,62	33,49	Positive
		N/A	32,69	Negative	36,56	33,09	Positive
Controls	C-	N/A	33,05	Negative	N/A	33,71	Negative
	C+	31,28	32,54	Positive	31,74	32,44	Positive
	C+ 1/10	34,91	32,98	Positive	34,5	33,31	Positive
		35,47	32,78	Positive	35,02	33,3	Positive
	C+ 1/100	37,91	32,59	Positive	36	33,69	Positive
		38,13	32,88	Positive	36,3	33,27	Positive

# Evaluation of 3 Protocols

**B) FDRS**

**A) FDRS**



**C) FDRS+ modified lysis buffer**



15 min



15 min

37°C  
+

95 - 100°C



# Results

		Protocol A			Protocol B			Protocol C		
		Target	IC	Result	Target	IC	Result	Target	IC	Result
<i>no reagent</i>		36,24	34,12	<b>Positive</b>	36,24	34,16	<b>Positive</b>	35,64	33,72	<b>Positive</b>
		36,33	33,75	<b>Positive</b>	37,38	34,56	<b>Positive</b>	35,92	33,96	<b>Positive</b>
DNA removal reagent		37,19	34,8	<b>Positive</b>	38,61	34,19	<b>Positive</b>	N/A	34,26	<b>Negative</b>
		36,41	34,26	<b>Positive</b>	45,29	34,03	<b>Positive</b>	N/A	33,8	<b>Negative</b>
		N/A	33,85	<b>Negative</b>	N/A	34,03	<b>Negative</b>	N/A	33,8	<b>Negative</b>
		N/A	33,96	<b>Negative</b>	N/A	34,06	<b>Negative</b>	N/A	34,1	<b>Negative</b>
		N/A	34,19	<b>Negative</b>	N/A	34,09	<b>Negative</b>	N/A	33,83	<b>Negative</b>
		N/A	34,19	<b>Negative</b>	N/A	34,37	<b>Negative</b>	N/A	34,12	<b>Negative</b>
		N/A	34,07	<b>Negative</b>	N/A	34,19	<b>Negative</b>	N/A	34,38	<b>Negative</b>
		N/A	34,14	<b>Negative</b>	N/A	33,74	<b>Negative</b>	N/A	34,31	<b>Negative</b>
		N/A	34,13	<b>Negative</b>	N/A	34,42	<b>Negative</b>	N/A	33,68	<b>Negative</b>
		N/A	34,44	<b>Negative</b>	N/A	34,2	<b>Negative</b>	N/A	33,93	<b>Negative</b>

- Protocol C yielded the best result.
- Protocol A & B were efficient with twice as much reagent.

# Summary

- A novel reagent was developed to remove free DNA from cheese samples without affecting the detection of viable bacteria.
- FDRS was integrated into the iQ-Check protocol adding only 15-30 minutes of sample handling time.
- PCR optimization and reagent stability studies were performed extensively to validate performance and efficacy of FDRS.
- Independently evaluated in accordance with AOAC guidelines for deli ham, cheese, stainless steel and sealed concrete.
- Integrated with automated method (iQ-Check Prep) for high volume testing labs.





Thank You!

