Internal Validation of iQ-Check™ *Listeria* spp. Kit When Analyzing Five-Sponge Wet Pooled Environmental Samples

Tech Note

Introduction

Pooling, also referred to as wet pooling, is the combining of multiple post-enriched samples into one sample to run on a rapid detection method. The advantage of sample pooling is that it can significantly reduce the costs per test while allowing the individual isolation of swabs/sponges for presumptive positives. It is recommended that only samples from the same environmental zone be pooled for testing.

The objective of this study was to test the effectiveness of iQ-Check *Listeria* spp. at detecting various strains of *Listeria* in five-sample post-enriched pooled environmental sponge samples and determine the fractional positive detection limit of the kit with diluted pooled samples. The iQ-Check *Listeria* spp. is a real-time PCR assay for detection of all species of *Listeria* in food and environmental samples. The kit is approved by AOAC, AFNOR and Health Canada.

Material and Methods

Wet Pooling

Four strains of *Listeria* and three competitor organisms were used in this study.

1. *Listeria monocytogenes*
2. *Listeria innocua*
3. *Listeria welshimeri*
4. *Listeria ivanovii*
5. *Staphylococcus aureus*
6. *Enterococcus gallinerum*
7. *Leuconostoc pseudomesenteroides*

Listeria strains were cultured overnight at 30°C in *Listeria* Special Broth (LSB). *L. pseudomesenteroides* was cultured overnight at 30°C in Buffered Peptone Water (BPW). *S. aureus* and *E. gallinerum* were cultured overnight at 37°C in BPW. All strains were diluted in BPW, plated to Plate Count Agar (PCA) for counts and kept at 4°C while titers were verified. Inoculum was targeted at 1–10 cfu per sample for both *Listeria* and competitor strains (Table 1). All sponges were inoculated with each competitor strain. Four sponges were inoculated each with a different *Listeria* strain. The samples were labeled with the *Listeria* strain and number 1–5. Sample number 1 of each pool was the inoculated sample and numbers 2–5 were the uninoculated samples. Cultures were inoculated directly into the sponge. To each sponge, 60ml of pre-warmed LSB was added. Samples were stomached for 60 sec and incubated for 24 hr at 30°C. Incubation was continued for an additional 24 hr after initial sampling for a total of 48 hr.

A 100µl aliquot of four uninoculated samples was added to a 1.2ml cluster tube. A 100µl aliquot of an inoculated sample was added to each cluster tube and mixed by pipetting up and down 10 times. This represented a pool consisting of 4 uninoculated and 1 inoculated sample. DNA extraction in a deepwell microplate and real-time PCR were performed according to iQ-Check *Listeria* spp. package insert. Pooled and individual samples were tested after 24 and 48 hr enrichment.

iQ-Check *Listeria* spp kit

Fractional Positive Dilution Series

Fractional positive level refers to the level at the detection limit of the test in which a portion of the samples tested are positive and a portion are negative. A 100µl aliquot of each dilution (10^{-4} to 10^{-9}) of the *L. monocytogenes* inoculum culture was added to 400µl of sterile LSB and mixed by pipetting up and down 10 times. DNA extraction in a deepwell microplate and real-time PCR were performed according to iQ-Check *Listeria* spp. package insert. Each dilution pool was tested four times to get to a fractional positive level.

BIO-RAD

Table 1. Inoculum culture counts

Strain	Plate count	Inoculum count[1]
L. monocytogenes	6.0 x 10^8	7
L. innocua	6.1 x 10^8	8
L. welshimeri	3.0 x 10^8	33
L. ivanovii	4.1 x 10^8	8
S. aureus	1.9 x 10^9	8
E. gallinerum	2.6 x 10^8	68
L. pseudomesenteroides	no growth	100

1 Direct plating of inoculum to PCA

Results

All inoculated samples were positive as were all wet pooled samples (Table 2). While there was a 2-3 Cq difference between the individual sample run on its own and the pooled sample, all samples were still positive. The Cq or quantification cycle, is the fractional cycle number where fluorescence increases above the threshold. When samples were tested at 48 hr, all individual samples and pools were still positive. With this sample set, there were no harmful effects of letting the samples incubate an additional 24 hr before testing.

Table 2. Sample wet pooling results

Sample	Cq (24hr)	Cq (48hr)
L. monocytogenes 1	19.17	17.64
L. monocytogenes 2	N/A	N/A
L. monocytogenes 3	N/A	N/A
L. monocytogenes 4	N/A	N/A
L. monocytogenes 5	N/A	N/A
L. monocytogenes POOL	22.63	20.00
L. innocua 1	18.32	18.24
L. innocua 2	N/A	N/A
L. innocua 3	N/A	N/A
L. innocua 4	N/A	N/A
L. innocua 5	N/A	N/A
L. innocua POOL	20.86	19.93
L. welshimeri 1	20.41	18.46
L. welshimeri 2	N/A	N/A
L. welshimeri 3	N/A	N/A
L. welshimeri 4	N/A	N/A
L. welshimeri 5	N/A	N/A
L. welshimeri POOL	23.23	20.58
L. ivanovii 1	24.99	20.09
L. ivanovii 2	N/A	N/A
L. ivanovii 3	N/A	N/A
L. ivanovii 4	N/A	N/A
L. ivanovii 5	N/A	N/A
L. ivanovii POOL	27.70	21.87

Assay runs for 40 cycles. Lower Cq values indicate stronger positive samples.
N/A indicates no amplification or a negative result.

The *L. monocytogenes* dilution series showed the fractional positive level of the assay to be between 10^2–10^3 cfu/ml of the sample prior to pooling (Table 3). Internal R&D testing during assay development demonstrate the (fractional positive) detection limit of the iQ-Check *Listeria* spp kit is between 10^2–10^3 cfu/ml. This matches the results with pooled samples.

Table 3. *L. monocytogenes* dilution series (5-pooled samples, four replicates tested) – fractional positive level highlighted in blue

Sample	Cq	Estimated count
LM 10^{-4}A	32.57	10^5–10^4
LM 10^{-4}B	32.28	10^5–10^4
LM 10^{-4}C	32.42	10^5–10^4
LM 10^{-4}D	32.72	10^5–10^4
LM 10^{-5}A	35.73	10^4–10^3
LM 10^{-5}B	35.63	10^4–10^3
LM 10^{-5}C	35.32	10^4–10^3
LM 10^{-5}D	36.15	10^4–10^3
LM 10^{-6}A	38.43	10^3–10^2
LM 10^{-6}B	38.64	10^3–10^2
LM 10^{-6}C	N/A	10^3–10^2
LM 10^{-6}D	38.22	10^3–10^2
LM 10^{-7}A	N/A	10^2–10^1
LM 10^{-7}B	N/A	10^2–10^1
LM 10^{-7}C	N/A	10^2–10^1
LM 10^{-7}D	N/A	10^2–10^1
LM 10^{-8}A	N/A	10 - 1
LM 10^{-8}B	N/A	10 - 1
LM 10^{-8}C	N/A	10 - 1
LM 10^{-8}D	N/A	10 - 1
LM 10^{-9}A	N/A	<1
LM 10^{-9}B	N/A	<1
LM 10^{-9}C	N/A	<1
LM 10^{-9}D	N/A	<1

Conclusions

The results for the pooled samples tested on iQ-Check *Listeria* spp. demonstrates the kit is capable of detecting low-level contamination in any single sample represented in a pool of five samples. Furthermore, this study shows the sensitivity of the kit (LOD 10^2–10^3 cfu/ml) is not compromised when wet pooling is performed with five environmental sponges.

For more information regarding our iQ-Check *Listeria* spp. kit or to learn about all of our food testing products, visit our website at **www.foodscience.bio-rad.com.**

13-0356