iQ-Check Free DNA Removal Solution: A Study to Demonstrate the Impact on Viable Target Cells

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Introduction

PCR results in processed foods and environmental samples frequently encounter challenges with false positives, primarily arising from the amplification of DNA derived from non-viable cells of target pathogens. Bio-Rad developed the iQ-Check Free DNA Removal Solution (Bio-Rad Laboratories, Inc., catalog #3594970) in 2016 to solve this problem that was observed in environmental samples collected from a dairy product manufacturing plant that was using a phage-based pathogen treatment solution. The iQ-Check Free DNA Removal Solution or FDRS, is a non-toxic, enzymatic reagent designed to treat a variety of food, cannabis, water, and environmental samples to specifically denature extra-cellular DNA that could result from the application of commonly used antimicrobial treatments in the food and beverage industry such as heat-processing, dessication, irradiation, disinfectants, etc. The application of FDRS in the iQ-Check real-time PCR kit workflow with cannabis matrices has been AOAC approved for the detection of *Aspergillus*, *Salmonella*, and STEC. This internal study was performed as a proof of concept using inoculated environmental sponge samples to determine the impact of FDRS on living cells.

Study Design: Impact of FDRS on viable cells

To challenge the limits of the current validated protocol (10 µl for 15-30 min), a study was conducted to assess the impact that FDRS has on viable/live cells (Fig. 1). Environmental sponges enriched with *Listeria* Special Broth (LSB, Bio-Rad, catalog #3564703) and Buffered Peptone Water (BPW, Bio-Rad, catalog #12013259) were inoculated with 2.05 x 10^6 of *Listeria monocytogenes* (ATCC 13932) and 3.05 x 10^6 of *Salmonella* Typhimurium (ATCC 14028) respectively. The final concentration of *L. monocytogenes* was 3.41 x 10^4 and 5.08 x 10^4 for S. Typhimurium. Analysis was performed by measuring the effect that various volumes and treatment times of FDRS has on target microorganisms after enrichment.

FDRS was dispensed in triplicate at volumes of 0, 10, 50, 100 µl into a deep well microplate. To the 0 µl wells, 100 µl of sterile water was added to ensure equal dilution volumes was achieved. 100 µl of enriched samples were added to each well and incubated without shaking at 37 ± 2°C for 0, 15, 30, 45, and 60 minutes. After incubation, samples were extracted using the Easy I or II DNA extraction protocols and tested using the iQ-Check *Salmonella* II PCR and the iQ-Check *Listeria* spp. kits following the Fast APFs.

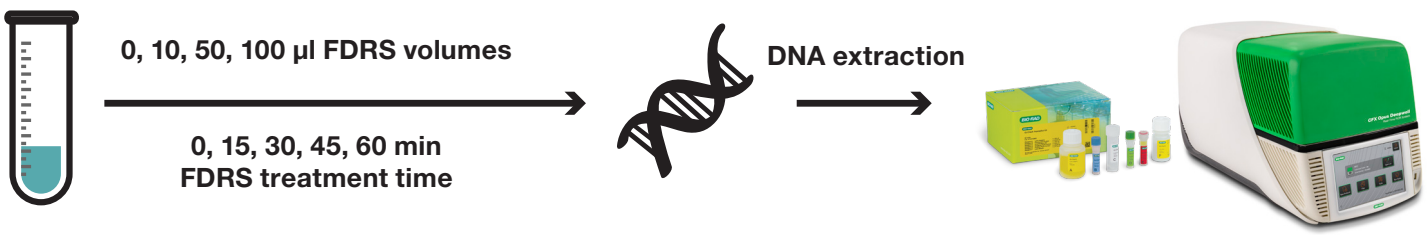

Fig. 1. FDRS treatment conditions assessed in the iQ-Check real-time PCR workflow.

Results

The iQ-Check *Listeria* spp. Cq values for different volumes of FDRS added during varied treatment times were similar with little variation demonstrating no impact on sensitivity. When the volume of FDRS was increased over all time points tested, there were only minor differences in average Cq value (Fig. 2). At 0 µl FDRS, the average difference was 1.86; at 10 µl FDRS, the average difference was 0.55; at 50 µl FDRS, the average difference was 0.46; at 100 µl FDRS, the average difference was 0.53. When the incubation time was increased over all volumes tested, there were only minor differences in average Cq value (Fig. 3). At 0 min, the average difference was 1.0; at 15 min, the average difference was 1.44; at 30 min, the average difference was 2.5; at 45 min, the average difference was 2.3; at 60 min, the average difference was 2.85. All positive samples remained positive in all rounds of testing.

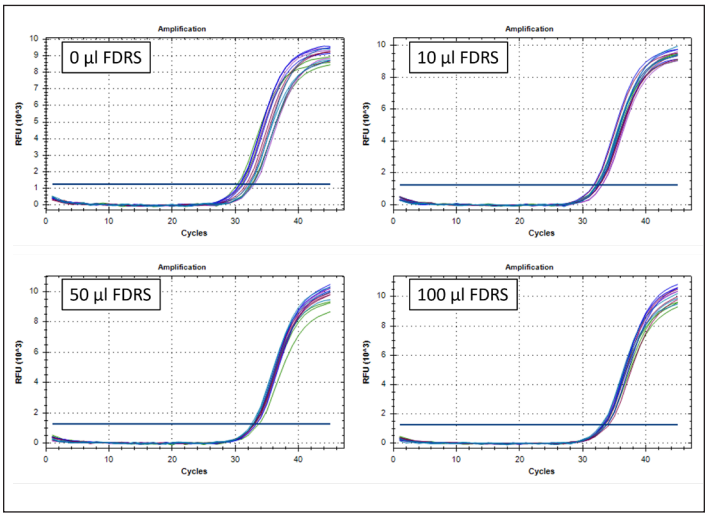

Fig. 2. iQ-Check *Listeria* spp. PCR Curves by Volume. FDRS treatment timepoints are indicated by the following PCR curves: 0 min (light blue), 15 min (maroon), 30 min (dark blue), 45 min (purple), 60 min (green).

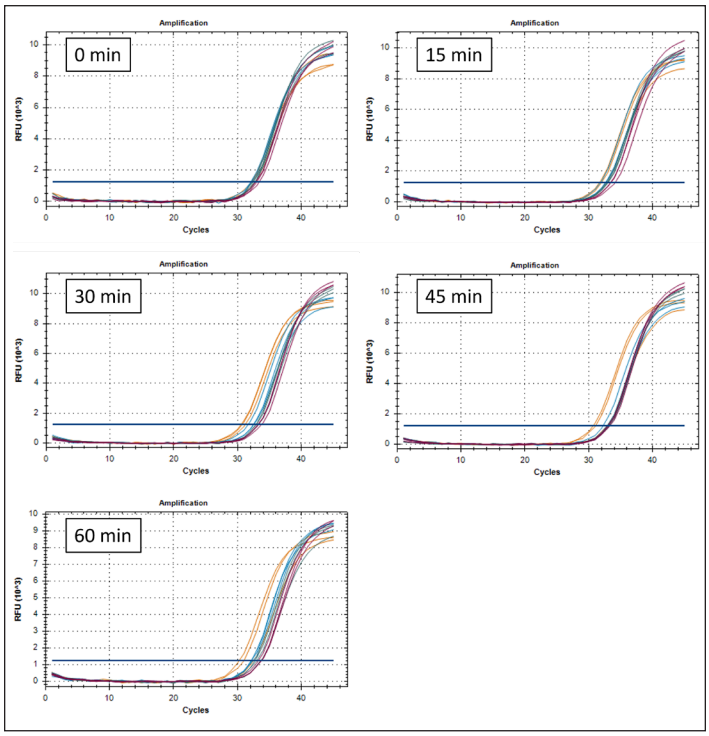

Fig. 3. iQ-Check *Listeria* spp. PCR Curves by Time. FDRS treatment volumes are indicated by the following PCR curves: 0 µl (orange), 10 µl (light blue), 50 µl (green), 100 µl (maroon).

For the iQ-Check *Salmonella* II kit, an impact on the sensitivity was observed with a 100 µl volume and an incubation time above 45 min. When the volume of FDRS was increased over all time points tested, only the 100 µl test volume demonstrated significant differences in average Cq value (Fig. 4). At 0 µl FDRS, the average difference was 0.57; at 10 µl FDRS, the average difference was 0.71; at 50 µl FDRS, the average difference was 1.2; at 100 µl FDRS, the average difference was 3.05. When the incubation time was increased over all volumes tested, the 45 min and 60 min time points demonstrated a significant difference in average Cq value (Fig. 5). At 0 min, the average difference was 1.49; at 15 min, the average difference was 2.56; at 30 min, the average difference was 2.95; at 45 min, the average difference was 4.44; at 60 min, the average difference was 4.96. All positive samples remained positive in all rounds of testing.

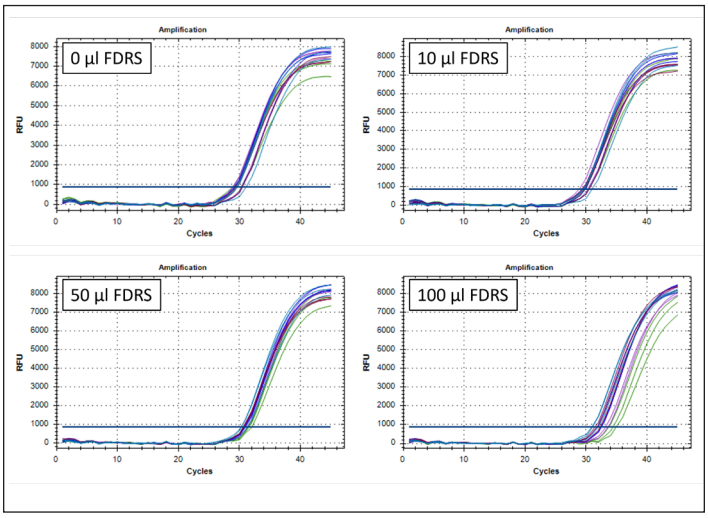

Fig. 4. iQ-Check *Salmonella* II PCR Curves by Volume. FDRS treatment timepoints are indicated by the following PCR curves: 0 min (light blue), 15 min (maroon), 30 min (dark blue), 45 min (purple), 60 min (green).

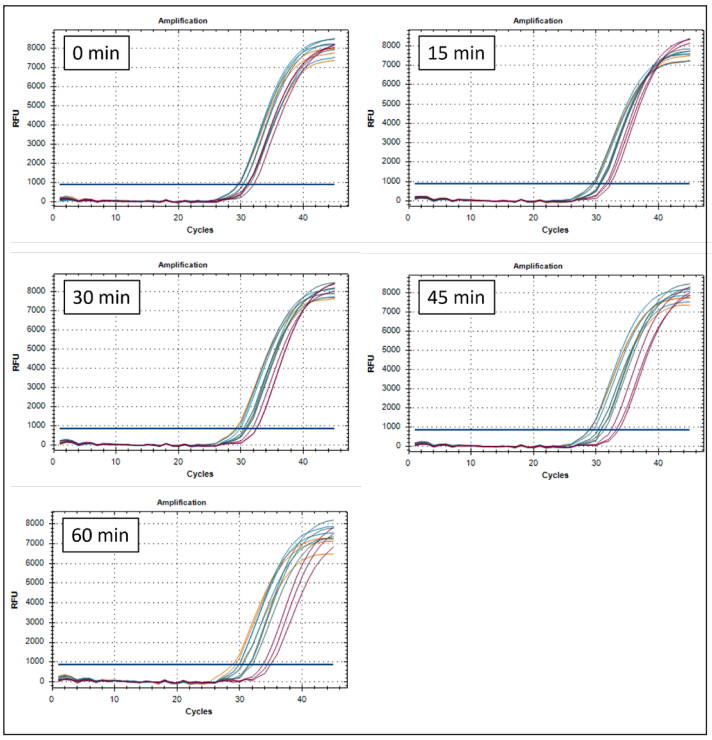

Fig. 5. iQ-Check *Salmonella* II PCR Curves by Time. FDRS treatment volumes are indicated by the following PCR curves: 0 µl (orange), 10 µl (light blue), 50 µl (green), 100 µl (maroon).

Conclusion

This study confirmed that the current AOAC and AFNOR approved FDRS conditions (10 µl for 15–30 min) do not impact the sensitivity of both iQ-Check kits tested.

The results demonstrate that FDRS used in higher amounts and with longer incubation times than the standard protocol does not affect the sensitivity of iQ-Check *Listeria* spp. method. For iQ-Check *Salmonella* II method, an impact on the sensitivity was observed with a 100 µl volume and an incubation time above 45 min. From the data generated in this study, Bio-Rad does not recommend using 100 µl of FDRS for more than 30 min for the detection of *Salmonella* environmental sponges.

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