

Reduction of Cross-Contamination Risk With the EZ-Check Solution

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Abstract

Food pathogen testing is crucial for ensuring food safety. By regularly testing products, businesses can protect consumers, comply with regulations, and safeguard their brand reputation. Current DNA extraction and PCR setup processes for routine pathogen testing are highly reproducible and efficient, yet concerns about cross-contamination persist due to the high sensitivity of molecular methods. The easy-to-use EZ-Check Solution, combined with good laboratory practices, is specifically designed to considerably prevent cross-contamination throughout its workflow. Here, we examine the ability of the EZ-Check *Salmonella* spp. Kit to minimize cross-contamination by testing highly contaminated samples alongside negative controls and uninoculated samples. Post-run analyses included swabbing critical labware and bench areas to confirm the absence of cross-contamination. The results demonstrate the effectiveness of the design and workflow of the kit to reduce the risk of cross-contamination in routine food pathogen testing.

Introduction

The EZ-Check Solution is a next-generation real-time PCR platform designed for rapid and accurate food pathogen detection. Given the sensitivity inherent in PCR systems, cross-contamination is a critical concern. The EZ-Check Solution has been engineered to significantly reduce this risk through several key design principles of the manual workflow:

- Minimized pipetting steps and using pre-aliquoted reagents—the EZ-Check workflow requires only two pipetting steps, which is achieved through the use of pre-aliquoted and lyophilized reagents included in reaction strips. This design inherently limits opportunities for human error and aerosol generation during the addition of samples
- Single volume extraction and PCR—a single 25 μL volume is used for both the extraction and PCR steps, simplifying handling and reducing multiple pipetting actions and use of various tip sizes
- Optimized labware design—EZ-Check Lysis strips are designed for open-tube extraction, eliminating the need for manual closing and opening steps, in which cross-contamination can occur. The strip height is engineered to maintain an air gap of 1/3 of the tube height during shaking, further minimizing droplet generation and dispersal

- Controlled agitation parameters—during thermomixer shaking, the speed increases and decreases smoothly at the start and end of the cycle, specifically reducing aerosolization

Materials and Methods

Pure Culture Test

For highly contaminated samples, a pure culture of *Salmonella enterica* Enteritidis (ATCC #13076) was grown in Tryptic Soy Broth (TSB; Bio-Rad Laboratories, Inc., catalog #3553454) overnight at 37°C. The overnight culture was diluted (1/10) in Buffered Peptone Water Plus (BPW; Bio-Rad, #3554179) to achieve a titer of 1×10^6 CFU/mL (200 μL culture in 1,800 μL BPW).

Twenty-five microliters of the above dilution was directly transferred to EZ-Check Lysis strips containing pre-aliquoted lysis reagent, and the strips were placed in an EZ-Check Lysis Rack. Negative controls (fresh BPW) were also prepared and included adjacent to the positive samples according to a plate layout constructed specifically to test for cross-contamination (Table 1).



Table 1. Plate layout alternating positive and negative samples to test for cross-contamination.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BPW	BPW	BPW									
B	Pos 1/10	BPW	BPW									
C	BPW	BPW	BPW									
D	Pos 1/10	BPW	BPW									
E	BPW	BPW	BPW									
F	Pos 1/10	BPW	BPW									
G	BPW	BPW	BPW	BPW	BPW	BPW	BPW	BPW	BPW	BPW	BPW	Negative control
H	Pos 1/10	BPW	BPW	Pos 1/10	BPW	BPW	Pos 1/10	BPW	BPW	Pos 1/10	BPW	Positive control

Following the EZ-Check workflow, the Lysis Rack was incubated in a heating thermoshaker (Bio-Rad, #17010254) for 15 min at 1,300 rpm and 95–100°C. The extracted DNA was added to the ReadyPCR Strips of the EZ-Check *Salmonella* spp. kit. Positive and negative kit controls were also added. The PCR plate was sealed with optical caps, and PCR was performed using the CFX Opus Real-Time PCR System (Bio-Rad, #12011319).

Environmental Swab Test

Swabs of the Lysis rack, thermoshaker, and surrounding areas were taken before the start of the experiment; the equipment and areas were then cleaned and swabbed again. Swabbing was performed less than 1 min after DNA extraction and PCR plate setup by sweeping the swab over a 5 cm² surface area for 10–15 sec. The swabbing locations included the EZ-Check Lysis Rack (top, front side, rear side, left side, and right side), thermoshaker top, thermoshaker screen, and lab bench (Figure 1).

The swabs were placed in 500 µL of distilled water in a tube and manually twisted. Excess liquid was removed from the swab by pressing it against the wall of the tube. The swab was removed, and the tube was covered and vortexed. Twenty-five microliters of each sample was used for DNA extraction and subsequent PCR.



Fig. 1. Location of swabs taken on the heating thermoshaker and EZ-Check Lysis Rack. Red circle (•) represents sample number.

Table 2. Plate layout for environmental swabs taken after DNA extraction and PCR and before and after disinfection.

	1	2	3	4
A	Sample 1 before cleaning	Sample 1 after cleaning	Sample 1 after DNA extraction	Water blank
B	Sample 2 before cleaning	Sample 2 after cleaning	Sample 2 after DNA extraction	Water + swab blank
C	Sample 3 before cleaning	Sample 3 after cleaning	Sample 3 after DNA extraction	BPW blank
D	Sample 4 before cleaning	Sample 4 after cleaning	Sample 4 after DNA extraction	Sample 4B after DNA extraction
E	Sample 5 before cleaning	Sample 5 after cleaning	Sample 5 after DNA extraction	Sample 5B after DNA extraction
F	Sample 6 before cleaning	Sample 6 after cleaning	Sample 6 after DNA extraction	Sample 6B after DNA extraction
G	Sample 7 before cleaning	Sample 7 after cleaning	Sample 7 after DNA extraction	Negative control
H	Sample 8 before cleaning	Sample 8 after cleaning	Sample 8 after DNA extraction	Positive control

Results

For the cross-contamination plate testing the pure culture, all negative samples tested negative despite being placed next to highly contaminated samples (Figure 2). The Cq values of the contaminated wells were between 19.58 and 20.05, indicating an *S. Enteritidis* concentration of 1×10^6 CFU/mL.

In addition, results from the environmental swab test of the specific labware used for the EZ-Check workflow and surrounding areas indicated no detectable contamination of the surfaces (Figure 3).

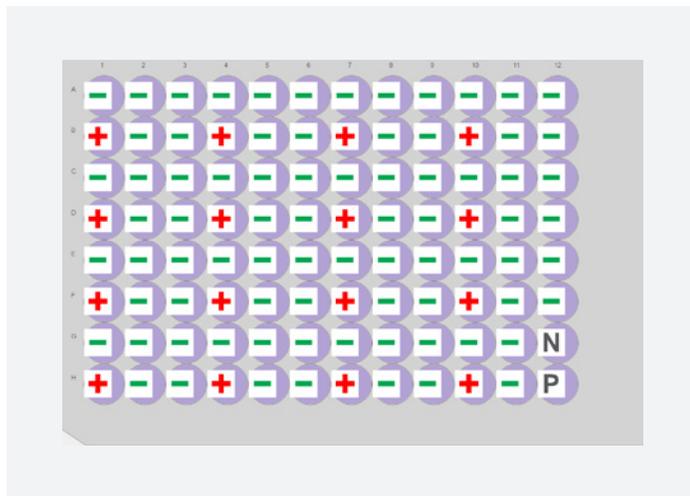


Fig. 2. EZ-Check *Salmonella* spp. Kit pure culture test results. No cross-contamination was observed. The wells are labeled as (+) positive result, (-) negative result, (N) negative control, or (P) positive control.

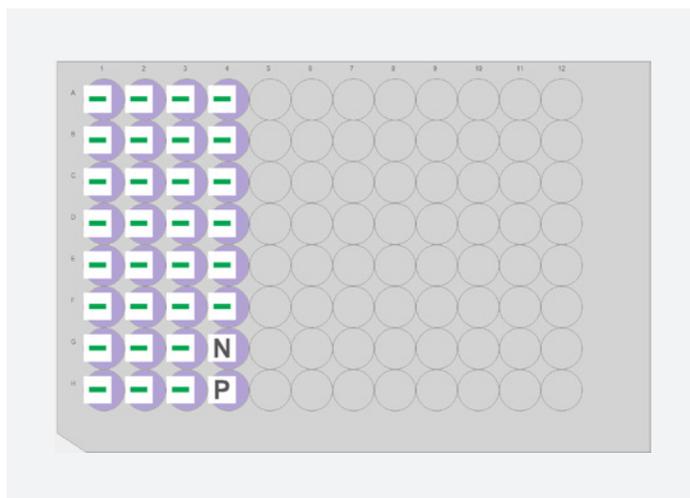


Fig. 3. Swab test results for the EZ-Check *Salmonella* spp. Kit. No contamination of the extraction labware and instrument during the DNA extraction process was detected. The wells are labeled as (+) positive result, (-) negative result, (N) negative control, or (P) positive control.

Conclusions

When testing highly contaminated samples (up to 1×10^6 CFU/mL), cross-contamination was not detected within the scope of this study using the EZ-Check Solution workflow. The inherent design features of the EZ-Check Solution, including minimized pipetting steps (only two required), single volume usage, optimized labware design (open lysis strips, controlled air gap), and smooth shaking speed transitions, collectively and significantly reduce the risk of cross-contamination in routine food pathogen testing. This powerful combination provides robust and reliable results, enhancing confidence in laboratory operations.

For more information on the EZ-Check Solution,
visit bio-rad.com/EZCheck.

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