

EZ-Check™ Method Performance Summary

Introduction

EZ-Check Kits are tests based on gene amplification and real-time PCR detection following enrichment of food and environmental samples. The ready-to-use PCR reagents contain oligonucleotides (primers and probes) that are highly specific to target DNA. A synthetic DNA internal control is included in the reaction mix and is critical for monitoring inhibitors and validating negative results. EZ-Check methods have been rigorously tested and validated by internationally recognized validation agencies (Table 1), with sample sizes of 25 g, 125 g, and 375 g, depending on the kit and matrix.

Table 1. EZ-Check Kits international validations.

EZ-Check Kit	Validation	Certificate Number
<i>Salmonella</i> spp.	AOAC	PTM 082501
	NF Validation	BRD 07/27-07/25
<i>Listeria</i> spp.	AOAC	PTM 012604
	MicroVal	2025LR135
<i>Listeria monocytogenes</i>	AOAC	PTM 012605
	MicroVal	2025LR136

Inclusivity/Exclusivity Testing

Inclusivity testing is performed to verify that the method can detect target bacteria, while exclusivity testing evaluates non-target strains to ensure there is no cross-reactivity. Exclusivity strains were enriched in non-selective broth for 20–24 hr at $37 \pm 1^\circ\text{C}$ and tested at high concentrations. For inclusivity testing, 10–100 colony-forming units (CFU) of each strain were cultured using the enrichment protocol validated for the specific kit. Results are shown in Table 2.

Limit of Detection

Limit of detection (LOD_{50}) is an estimate of the contamination level required to achieve positive detection in 50% of cases. This is measured by inoculating food matrices with target bacteria and carrying out the validated enrichment, extraction, and detection protocols.

The average LOD_{50} of the EZ-Check *Salmonella* spp. Kit was determined to be 0.8 [0.7–1.0] (Table 3). The average LOD_{50} of the EZ-Check *Listeria* spp. Kit was determined to be 0.8 [0.7–1.0] (Table 4). The average LOD_{50} of the EZ-Check *Listeria monocytogenes* Kit was determined to be 0.8 [0.7–1.0] (Table 5).

Table 2. Results of Inclusivity/Exclusivity Testing.

EZ-Check Kit <i>Salmonella</i> spp.	EZ-Check Kit <i>Listeria</i> spp.	EZ-Check Kit <i>Listeria monocytogenes</i>
Strains Tested: 200 <i>Salmonella</i> strains Positives Detected: 200/200 Results: 100% inclusivity Strains Tested: 30 non- <i>Salmonella</i> strains Positives Detected: 0/30 Results: 100% exclusivity	Strains Tested: 55 <i>Listeria</i> strains Positives Detected: 55/55 Results: 100% inclusivity Strains Tested: 30 non- <i>Listeria</i> strains Positives Detected: 0/30 Results: 100% exclusivity	Strains Tested: 55 <i>Listeria monocytogenes</i> strains Positives Detected: 200/200 Results: 100% inclusivity Strains Tested: 30 non- <i>Listeria monocytogenes</i> strains Positives Detected: 0/30 Results: 100% exclusivity



Table 3. LOD₅₀ for the EZ-Check *Salmonella* spp. Kit.

Matrix/Sample Pair	LOD ₅₀ CFU/Sample Size [Confidence Interval]
Ground beef (25 g) / <i>S. Typhimurium</i>	0.7 [0.4–1.1]
Poultry / <i>S. Kentucky</i>	1.8 [0.9–3.5]
Raw milk / <i>S. Ohio</i>	0.8 [0.4–1.4]
Deli salad / <i>S. Enteritidis</i>	0.8 [0.4–1.4]
Bagged spinach / <i>S. Lexington</i>	0.6 [0.4–1.1]
Pasteurized whole liquid eggs / <i>S. Havana</i>	0.9 [0.5–1.5]
Dry kibbles for dogs / <i>S. Derby</i>	1.2 [0.6–2.1]
Process water / <i>S. Livingstone</i>	0.8 [0.5–1.4]
Raw cocoa powder (375 g) / <i>Salmonella enterica</i> GIVE	0.5 [0.3–0.9]
Raw ground beef (375 g) / <i>S. Senftenberg</i>	0.8 [0.4–1.4]
Raw ground turkey / <i>S. Saintpaul</i>	0.6 [0.3–1.1]
Raw chicken breast / <i>S. Hadar</i>	0.8 [0.4–1.4]
Raw ground pork / <i>S. Newport</i>	0.8 [0.4–1.5]
Non-fat dry milk / <i>S. Ohio</i>	0.7 [0.4–1.2]
Whey protein powder / <i>S. Montevideo</i>	0.5 [0.3–1.0]
All-purpose flour / <i>S. Infantis</i>	0.8 [0.4–1.5]
Cheddar cheese / <i>S. Heidelberg</i>	0.7 [0.4–1.4]
Romaine lettuce / <i>S. Mbandaka</i>	0.9 [0.5–1.6]
Frozen vegetable blend / <i>S. Typhimurium</i>	0.9 [0.5–1.6]
Fresh cut cantaloupe / <i>S. Oranienburg</i>	0.7 [0.4–1.3]
Cocoa powder / <i>S. Thompson</i>	0.7 [0.4–1.4]
Creamy peanut butter / <i>S. Tennessee</i>	0.6 [0.3–1.1]
Dry dog food / <i>S. Schwarzengrund</i>	0.7 [0.4–1.2]
Infant formula w/probiotics / <i>S. Poona</i>	0.7 [0.3–1.3]
Garlic powder / <i>S. Agona</i>	0.9 [0.5–1.6]
Bean sprouts / <i>S. Enteritidis</i>	0.6 [0.3–1.2]
Stainless steel 4" x 4" / <i>S. Bredeney</i>	71 [35–144] *
Rubber 1" x 1" / <i>S. Chester</i>	35 [19–62] *
Sealed concrete 4" x 4" / <i>S. Thompson</i>	36 [20–63] *
Raw beef trim sampling cloth / <i>S. Anatum</i>	39.8 [21.1–74.8] *
Chicken carcass rinse / <i>S. Indiana</i>	43.4 [23.4–80.3] *

Table 4. LOD₅₀ for the EZ-Check *Listeria* spp. Kit.

Matrix/Sample Pair	LOD ₅₀ CFU/Sample Size [Confidence Interval]
Raw whole milk / <i>Listeria innocua</i>	0.2 [0.1–0.4]
Raw whole milk cheese / <i>Listeria fleishmanii</i> & <i>Listeria monocytogenes</i> (1/2b)	0.5 [0.3–0.8]
Mexican soft cheese / <i>L. monocytogenes</i> (4b)	1.0 [0.5–1.9]
Ice cream / <i>Listeria seeligeri</i> & <i>L. monocytogenes</i> (4ab)	0.7 [0.4–1.3]
Cheddar cheese / <i>L. monocytogenes</i> (1/2b)	0.9 [0.5–1.6]
Beef hot dogs / <i>Listeria welshimeri</i> & <i>L. monocytogenes</i>	0.7 [0.3–1.2]
Turkey hot dogs / <i>Listeria grayi</i> & <i>L. monocytogenes</i> (1)	0.8 [0.5–1.5]
Raw fermented sausage / <i>L. monocytogenes</i> (1/2a)	1.0 [0.5–1.9]
Frozen chicken nuggets / <i>L. monocytogenes</i> (4e)	0.8 [0.4–1.3]
RTE deli ham / <i>Listeria marthii</i>	0.7 [0.4–1.2]
Salami / <i>L. monocytogenes</i>	0.8 [0.4–1.4]
RTE deli turkey / <i>L. innocua</i> & <i>L. monocytogenes</i> (1/2a)	0.7 [0.4–1.3]
Smoked salmon / <i>Listeria ivanovii</i>	0.6 [0.3–1.1]
Frozen cooked shrimp / <i>L. monocytogenes</i> (4a)	0.5 [0.3–0.9]
Fresh cut cantaloupe / <i>Listeria seeligeri</i>	0.8 [0.4–1.5]
Bagged salad (Romaine) / <i>L. monocytogenes</i> (3c)	0.8 [0.5–1.5]
Frozen vegetable blend / <i>L. ivanovii</i> & <i>L. monocytogenes</i> (1/2c)	0.6 [0.3–1.0]
Frozen green peas / <i>L. monocytogenes</i> (7)	0.7 [0.4–1.2]
Egg salad / <i>Listeria rocourtiae</i> & <i>L. monocytogenes</i> (4b)	0.7 [0.4–1.3]
Hummus / <i>L. monocytogenes</i> (3b)	0.6 [0.3–1.1]
RTE deli salad / <i>L. monocytogenes</i> (4b)	0.8 [0.5–1.5]
Pasteurized whole liquid egg / <i>L. monocytogenes</i> (4c)	0.5 [0.3–1.0]
Stainless steel / <i>L. monocytogenes</i> & <i>Enterococcus faecalis</i>	51.7 [29.3–91.3] *
Sealed concrete / <i>Listeria newyorkensis</i> & <i>L. monocytogenes</i>	48.3 [28.5–81.9] *
Rubber / <i>Listeria booriae</i> & <i>L. monocytogenes</i>	65.4 [38.2–111.9] *
Process water / <i>L. monocytogenes</i> (1/2c)	77.5 [44.7–134.5] *

RTE, ready to eat

*Higher LOD values are expected for environmental surfaces and sampling cloths. This is related to the protocol used in the validation study, which is designed to achieve fractional recovery (5–15 positive results) from 20 test portions. The inoculum applied to surfaces generally needs to be significantly higher than the fractional levels used for food matrix test portions. Since the environmental surfaces must dry for 16–24 hr after inoculum is added, a higher initial titer is required to achieve fractional results at time of sampling to account for cell die-off.

Table 5. LOD₅₀ for the EZ-Check *Listeria monocytogenes* Kit.

Matrix/Sample Pair	LOD ₅₀ CFU/Sample Size [Confidence Interval]
Raw whole milk / <i>Listeria monocytogenes</i> (3a)	0.7 [0.4–1.2]
Raw whole milk cheese / <i>L. monocytogenes</i> (1/2b)	0.6 [0.3–1.0]
Mexican soft cheese / <i>L. monocytogenes</i> (4b)	1.0 [0.5–1.9]
Ice cream / <i>L. monocytogenes</i> (4ab)	0.7 [0.4–1.4]
Cheddar cheese / <i>L. monocytogenes</i> (1/2b)	0.9 [0.5–1.6]
Beef hot dogs / <i>L. monocytogenes</i>	0.9 [0.5–1.6]
Turkey hot dogs / <i>L. monocytogenes</i> (1)	1.1 [0.6–2.0]
Raw fermented sausage / <i>L. monocytogenes</i> (1/2a)	1.0 [0.5–1.9]
Frozen chicken nuggets / <i>L. monocytogenes</i> (4e)	0.8 [0.4–1.3]
RTE deli ham / <i>L. monocytogenes</i> (1/2a)	1.0 [0.6–1.9]
Salami / <i>L. monocytogenes</i>	0.8 [0.4–1.4]
RTE deli turkey / <i>L. monocytogenes</i> (1/2a)	0.9 [0.4–1.7]
Smoked salmon / <i>L. monocytogenes</i>	0.7 [0.4–1.3]
Frozen cooked shrimp / <i>L. monocytogenes</i> (4a)	0.5 [0.3–0.9]
Fresh cut cantaloupe / <i>L. monocytogenes</i> (4b)	0.9 [0.6–1.6]
Bagged salad (Romaine) / <i>L. monocytogenes</i> (3c)	0.8 [0.5–1.5]
Frozen vegetable blend / <i>L. monocytogenes</i> (1/2c)	0.8 [0.4–1.6]
Frozen green peas / <i>L. monocytogenes</i> (7)	0.7 [0.4–1.2]
Egg salad / <i>L. monocytogenes</i> (4b)	0.8 [0.4–1.5]
Hummus / <i>L. monocytogenes</i> (3b)	0.6 [0.3–1.1]
RTE deli salad / <i>L. monocytogenes</i> (4b)	0.8 [0.5–1.6]
Pasteurized whole liquid egg / <i>L. monocytogenes</i> (4c)	0.5 [0.3–1.0]
Stainless steel / <i>L. monocytogenes</i>	51.7 [29.3–91.3] *
Sealed concrete / <i>L. monocytogenes</i>	67.2 [38.1–118.7] *
Rubber / <i>L. monocytogenes</i>	69.0 [38.2–124.9] *
Process water / <i>L. monocytogenes</i> (1/2c)	77.5 [44.7–134.5] *

RTE, ready to eat

Method Comparison/Matrix Studies

Matrix testing is critical to demonstrating the performance of a method compared to the reference method with real-world food samples. The EZ-Check methods have been verified with external and internal testing on a wide variety of foods. No significant difference was found between the reference method and an alternative method for all matrices tested (Tables 6 & 7).

Table 6. Matrices Tested With EZ-Check *Salmonella* spp. Kit.

Category	Matrices Tested
Composite foods	RTE: Deli salad
Meat products	Raw and RTC: Raw ground beef, raw ground pork, raw ground turkey, raw chicken breast, chicken carcass rinse
Dairy products	Heat processed: Nonfat dry milk, whey protein powder, cheddar cheese Raw products: Raw milk
Egg products	Whole liquid egg
Produce	Fresh: Romaine lettuce, bean sprouts, bagged spinach Frozen: Vegetable blend, green peas Processed: Fresh cut cantaloupe
Dried cereals, fruits, nuts, seeds, and vegetables	All-purpose flour, garlic powder, creamy peanut butter
Infant formula and cereals	Infant formula with probiotics
Chocolate, bakery products and confectionary	Cocoa powder
Pet food and animal feed	Dry dog food
Environmental samples	Rubber, sealed concrete, stainless steel, process water
Primary production samples	Raw beef trim sampling cloth

RTC, ready to cook; RTE, ready to eat.

Table 7. Matrices Tested With EZ-Check *Listeria* spp. and *Listeria monocytogenes* Kits.

Category	Matrices Tested
Composite foods	RTE: Egg salad, deli salad
Meat products	RTE and RTRH: Beef hot dogs, turkey hot dogs, frozen breaded chicken nuggets Deli: Turkey, ham, salami, raw fermented sausage
Dairy products	Raw milk and products: Raw whole milk, raw whole milk cheese Heat processed: Mexican-style pasteurized soft cheese, ice cream, pasteurized cheddar cheese
Egg products	Processed: Pasteurized whole liquid egg
Fishery products	RTE and RTRH: Smoked salmon, frozen cooked shrimp
Produce	Frozen: Vegetable blend, green peas Processed: Fresh cut cantaloupe, bagged salad (Romaine)
Dried cereals, fruits, nuts, seeds, and vegetables	Hummus
Environmental samples	Stainless steel, sealed concrete, rubber, process water

RTE, ready to eat; RTRH, ready to reheat.

*Higher LOD values are expected for environmental surfaces and sampling cloths. This is related to the protocol used in the validation study, which is designed to achieve fractional recovery (5–15 positive results) from 20 test portions. The inoculum applied to surfaces generally needs to be significantly higher than the fractional levels used for food matrix test portions. Since the environmental surfaces must dry for 16–24 hr after inoculum is added, a higher initial titer is required to achieve fractional results at time of sampling to account for cell die-off.

References

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