BAX® System Real-Time PCR Assay for Genus *Listeria*

Inclusivity and Exclusivity

Internal validation studies were completed for submission to the AOAC Research Institute (AOAC- RI) to demonstrate the inclusivity and exclusivity capabilities of the BAX® System Real-Time PCR Assay for Genus *Listeria*. For the inclusivity panel, 97 different *Listeria* strains of significance to the food industry were tested. The exclusivity panel included 46 non-*Listeria* strains representing a wide range of organisms identified as potential cross-reactors to the target *Listeria*.

The results from the inclusivity and exclusivity testing with the BAX System Real-Time PCR Assay for Genus *Listeria* were shown to be 100% consistent with expected results. The inclusivity panel demonstrated >99% sensitivity for the *Listeria* species tested, while the exlusivity panel demonstrated 100% specificity.

Methodology

Equipment, reagents and supplies
- BAX System Real-Time PCR Assay for Genus *Listeria* (KIT2016)
- BAX System Q7 instrument, equipment and supplies
- 24 LEB Complete
- Brain-heart infusion (BHI) broth
- Incubator (37 °C)
- *Listeria* and non-*Listeria* microorganism strains
- Heat blocks (set at 55 °C and 95 °C)

Sample Enrichment and Preparation
All inclusivity and exclusivity strains used in this study were selected from the Hygiena® Qualicon Culture Collection. *Listeria* strains selected for inclusivity testing included a variety of pathogenic *L. monocytogenes* and *L. ivanovii* strains, as well as strains from the non-pathogenic serogroups *L. seeligeri, L. welshimeri, L. grayii, L. marthii* and *L. innocua*.

For inclusivity testing, a pure culture of each *Listeria* strain was inoculated into BHI broth and incubated at 37 °C overnight to an assumed concentration of 10^9 CFU/mL. Each strain was then serially diluted in prepared 24 LEB Complete media to approximately 10^5 CFU/mL, or one log above the claimed limit of detection (LOD) for the assay.

For exclusivity testing, a pure culture of each non-*Listeria* strain was inoculated into BHI broth and incubated at 37 °C overnight to an assumed concentration of 10^9 CFU/mL.

BAX System Method
BAX System lysis reagent was prepared by adding 150 µL of protease and 200 µL of Lysing Agent 2, to one 12-mL bottle of lysis buffer. Then, 200 µl of prepared lysis reagent was added to each cluster tube, followed by 5 µL of sample. Lysis was performed by heating tubes for 30 minutes at 55 °C and 10 minutes at 95 °C, then cooling tubes for at least 5 minutes at 4 °C. PCR tablets were hydrated with 30 µL of lysate, and a full process was run in the BAX System Q7 instrument according to the instructions in the BAX System User Guide.

Results

The results of the inclusivity panel are summarized in Table 1. The BAX System returned positive results for all selected *Listeria* test strains except one strain of *L. grayi* at the limit of detection. This strain was re-tested with the BAX System method at a higher concentration (10^6 CFU/mL) and returned positive results.

The results of the exclusivity panel are summarized in Table 2. The BAX System returned negative results for all 46 non-*Listeria* strains tested.

Conclusion

The results of the inclusivity panel demonstrate that the BAX System Real-Time PCR assay for Genus *Listeria* detects the target species with >99% sensitivity. The results of the exclusivity panel demonstrate that the assay is not affected by potential cross-reactors with 100% specificity.

Table 1. BAX System Inclusivity Results

Strain ID	Strain Name	BAX Result	Strain ID	Strain Name	BAX Result
566	*Listeria monocytogenes*	POS	1165	*Listeria ivanovii*	POS
605	*Listeria monocytogenes*	POS	1167	*Listeria ivanovii*	POS
643	*Listeria grayi*	POS	1171	*Listeria ivanovii*	POS
644	*Listeria innocua*	POS	1172	*Listeria welshimeri*	POS
647	*Listeria monocytogenes*	POS	1174	*Listeria welshimeri*	POS
648	*Listeria monocytogenes*	POS	1175	*Listeria welshimeri*	POS
649	*Listeria ivanovii*	POS	1176	*Listeria welshimeri*	POS
650	*Listeria seeligeri*	POS	1177	*Listeria welshimeri*	POS
652	*Listeria monocytogenes*	POS	1179	*Listeria welshimeri*	POS
653	*Listeria monocytogenes*	POS	1281	*Listeria monocytogenes*	POS
654	*Listeria welshimeri*	POS	1282	*Listeria monocytogenes*	POS
892	*Listeria innocua*	POS	1283	*Listeria monocytogenes*	POS
898	*Listeria innocua*	POS	1285	*Listeria monocytogenes*	POS
921	*Listeria innocua*	POS	1286	*Listeria monocytogenes*	POS
922	*Listeria innocua*	POS	1287	*Listeria monocytogenes*	POS
924	*Listeria innocua*	POS	1288	*Listeria monocytogenes*	POS

Table 1. BAX System Inclusivity Results (Continued)

Strain ID	Strain Name	BAX Result	Strain ID	Strain Name	BAX Result
927	*Listeria innocua*	POS	1289	*Listeria seeligeri*	POS
944	*Listeria grayi*	POS*	1291	*Listeria seeligeri*	POS
1063	*Listeria innocua*	POS	1292	*Listeria seeligeri*	POS
1064	*Listeria innocua*	POS	1293	*Listeria monocytogenes*	POS
1069	*Listeria monocytogenes*	POS	1294	*Listeria monocytogenes*	POS
1072	*Listeria monocytogenes*	POS	1295	*Listeria monocytogenes*	POS
1144	*Listeria monocytogenes*	POS	1297	*Listeria seeligeri*	POS
1145	*Listeria monocytogenes*	POS	1298	*Listeria seeligeri*	POS
1146	*Listeria monocytogenes*	POS	1299	*Listeria monocytogenes*	POS
1147	*Listeria monocytogenes*	POS	1300	*Listeria seeligeri*	POS
1149	*Listeria monocytogenes*	POS	1302	*Listeria monocytogenes*	POS
1152	*Listeria monocytogenes*	POS	1305	*Listeria monocytogenes*	POS
1156	*Listeria innocua*	POS	1306	*Listeria monocytogenes*	POS
1164	*Listeria ivanovii*	POS	1307	*Listeria monocytogenes*	POS
1308	*Listeria monocytogenes*	POS	3376	*Listeria ivanovii*	POS
1309	*Listeria monocytogenes*	POS	3555	*Listeria grayi*	POS
1310	*Listeria monocytogenes*	POS	3572	*Listeria innocua*	POS
1311	*Listeria monocytogenes*	POS	3573	*Listeria monocytogenes*	POS
1312	*Listeria monocytogenes*	POS	3574	*Listeria monocytogenes*	POS
1313	*Listeria monocytogenes*	POS	3576	*Listeria monocytogenes*	POS
1314	*Listeria monocytogenes*	POS	3577	*Listeria monocytogenes*	POS
1315	*Listeria monocytogenes*	POS	3578	*Listeria monocytogenes*	POS
1316	*Listeria monocytogenes*	POS	3579	*Listeria monocytogenes*	POS
1321	*Listeria monocytogenes*	POS	3580	*Listeria monocytogenes*	POS
2874	*Listeria seeligeri*	POS	3581	*Listeria monocytogenes*	POS
3244	*Listeria innocua*	POS	3582	*Listeria monocytogenes*	POS
3327	*Listeria seeligeri*	POS	3678	*Listeria ivanovii*	POS
3329	*Listeria seeligeri*	POS	4553	*Listeria monocytogenes*	POS
3351	*Listeria welshimeri*	POS	4568	*Listeria monocytogenes*	POS
3354	*Listeria welshimeri*	POS	4571	*Listeria monocytogenes*	POS
3359	*Listeria welshimeri*	POS	5425	*Listeria monocytogenes*	POS
3363	*Listeria grayi*	POS	7644	*Listeria monocytogenes*	POS
			13529	*Listeria marthii*	POS

* Positive result obtained at 10^6 CFU/mL

Table 2. BAX System Exclusivity Results

Strain ID	Strain Name	BAX Result	Strain ID	Strain Name	BAX Result
379	*Bacillus subtilus*	NEG	1111	*Staphylococcus capitis*	NEG
383	*Citrobacter freundii*	NEG	1112	*Staphylococcus xylosus*	NEG
659	*Lactococcus lactis*	NEG	1113	*Staphylococcus sciuri*	NEG
691	*Streptococcus thermophilus*	NEG	2392	*Rhodococcus equi*	NEG
692	*Streptococcus bovis*	NEG	2552	*Enterococcus faecium*	NEG
695	*Streptococcus pyogenes*	NEG	2553	*Enterococcus faecium*	NEG
707	*Salmonella* Newport	NEG	2554	*Enterococcus faecalis*	NEG
713	*Bacillus thuringiensis*	NEG	2558	*Citrobacter freundii*	NEG
714	*Bacillus thuringiensis*	NEG	2560	*Citrobacter koseri*	NEG
715	*Bacillus cereus*	NEG	2561	*Citrobacter koseri*	NEG
716	*Bacillus thuringiensis*	NEG	2624	*Enterococcus gallinarum*	NEG
721	*Bacillus cereus*	NEG	2625	*Enterococcus durans*	NEG
863	*Staphylococcus aureus*	NEG	2626	*Enterococcus hirae*	NEG
877	*Bacillus cereus*	NEG	2628	*Salmonella kentucky*	NEG
878	*Bacillus cereus*	NEG	2636	*Staphylococcus felis*	NEG
879	*Bacillus cereus*	NEG	3981	*Enterococcus faecalis*	NEG
912	*Staphylococcus aureus*	NEG	3992	*Streptococcus mutans*	NEG
1011	*Bacillus subtilus*	NEG	3996	*Streptococcus equi*	NEG
1024	*Bacillus cereus*	NEG	4063	*Carnobacterium gallinarum*	NEG
1096	*Staphylococcus aureus*	NEG	4064	*Carnobacterium divergens*	NEG
1098	*Staphylococcus aureus*	NEG	7332	*Lactobacillus curvatus*	NEG
1105	*Staphylococcus warneri*	NEG	7344	*Lactobacillus acidophilus*	NEG
1107	*Staphylococcus xylosus*	NEG	9174	*Micrococcus luteus*	NEG