

BAX® System PCR Assay for Screening *Listeria monocytogenes*

Inclusivity/Exclusivity

An evaluation was performed to test the inclusivity/exclusivity of the BAX® System PCR assay for screening *Listeria monocytogenes*. The BAX® System demonstrated 100% inclusivity and none of the other *Listeria* species or non-*Listeria* tested gave a reproducible positive result.

Methodology

Choice of Strains

All of the isolates used in this study were chosen from the Hygiena™ culture collection. Most of these isolates were originally obtained from naturally contaminated food samples, and all were confirmed with the API® *Listeria* identification system.

A panel of 195 *L. monocytogenes* isolates was used for inclusivity testing. The exclusivity panel consisted of 78 non-*L. monocytogenes* isolates, including close genetic relatives of *L. monocytogenes*, organisms that give similar biochemical test results to *L. monocytogenes* or species that are found in the same environment and grow under the same conditions as *L. monocytogenes*.

Culture Enrichment

Isolates were collected and grown overnight at 30°C in Brain-Heart Infusion (BHI) media. All of the cultures were then streaked for purity on BHI agar. For each strain, one colony was inoculated into a tube containing 10 mL of BHI broth and incubated overnight at 30°C. The overnight cultures were diluted 1:10 in BHI broth to form test samples of approximately 10⁸ cfu/mL. Isolates were blind coded, mixed and randomized.

Sample Preparation and Processing

Lysis reagent was prepared (150 µL protease + 12 ml lysis buffer) and added in 200-µL aliquots to cluster tubes. For each sample, a 5-µL aliquot was added to a cluster tube, and lysis was performed according to the BAX® System protocol for *L. monocytogenes*. Tablets were hydrated with 50-µL of the lysate and a full process run was completed.

Results

Of 195 *L. monocytogenes* strains tested (see Table 1), all gave positive results with the BAX® System method. Of the 78 strains used for exclusivity testing (see Table 2), none was consistently positive. Six non-*L. monocytogenes* strains (*L. innocua* DD1064, *L. innocua* DD1159, *L. ivanovii* DD1168 and DD 1303, *L. seeligeri* DD2538 and *L. welshimeri* DD1172) and one non-*Listeria* strain (*Enterococcus* species DD2468) gave initial positive results that repeated four times as negative. Since the inclusivity and exclusivity organisms were randomized and run simultaneously, it is likely that cross-contamination caused those initial positive results.

Table 1. BAX® System inclusivity results

Strain No.	Serotype (if known)	Source	BAX Result	Strain No.	Serotype (if known)	Source	BAX Result
891	4b	soft cheese	Pos	3570	–	industry isolate	Pos
936	2	spinal fluid	Pos	3573	–	industry isolate	Pos
939	4a	sheep brain	Pos	3574	–	industry isolate	Pos
940	4c	fowl	Pos	3575	–	industry isolate	Pos
941	4b	spinal fluid	Pos	3576	–	industry isolate	Pos
957	4b	soft cheese	Pos	3577	–	industry isolate	Pos
1048	3	unknown	Pos	3578	–	industry isolate	Pos
1057	1a	rabbit	Pos	3579	–	industry isolate	Pos
1058	–	roast beef	Pos	3580	–	industry isolate	Pos
1072	–	cheese pancakes	Pos	3581	–	industry isolate	Pos
1144	1/2a	stilton	Pos	3582	–	industry isolate	Pos
1145	1/2a	coleslaw	Pos	3583	–	industry isolate	Pos
1148	1/2a	soft cheese	Pos	3592	–	industry isolate	Pos
1149	1/2a	raw milk	Pos	3593	–	industry isolate	Pos
1151	1/2b	pate	Pos	3594	–	industry isolate	Pos
1152	1/2b	pate	Pos	3595	–	industry isolate	Pos
1153	1/2b	food	Pos	3596	–	industry isolate	Pos
1281	3c	cooked chicken	Pos	3597	–	industry isolate	Pos
1282	3c	unknown	Pos	3598	–	industry isolate	Pos
1283	3b	cooked turkey	Pos	3599	–	industry isolate	Pos
1284	3c	ham	Pos	4553	–	smoked ham	Pos
1286	3c	cooked chicken	Pos	4560	–	honey ham	Pos
1287	3a	unknown	Pos	4561	–	fruit salad	Pos
1288	3a	cooked turkey	Pos	4564	–	chocolate pavlova	Pos
1293	3a	pate	Pos	4567	–	pizza	Pos
1294	4b	ice cream	Pos	4568	–	factory environment	Pos
1295	3b	pepper quiche	Pos	4569	–	factory environment	Pos
1299	1/2b	pork liver pate	Pos	4571	–	honey ham	Pos
1305	3a	boiled ham	Pos	4589	–	fisherman's pie	Pos
1306	3b	liver pate	Pos	4590	–	buffalo wings	Pos
1307	3b	pate	Pos	4613	–	dairy environment	Pos
1310	3b	chicken	Pos	4616	–	ham	Pos
1313	4b	cheese	Pos	4617	–	dairy environment	Pos
1316	3a	cooked chicken	Pos	4618	–	roast ham	Pos
1320	4 (not 4b)	egg	Pos	4640	–	waldorf salad	Pos
1322	3c	chicken sandwich	Pos	4641	–	fisherman's pie	Pos
1324	3a	chicken	Pos	4646	–	ham line swab	Pos
1325	1/2b	fish fingers	Pos	4651	–	Leicester cheese	Pos
1326	4b	cooked turkey	Pos	4675	–	cheese factory	Pos
1327	4b	chicken salad	Pos	4676	–	cheese scrapes	Pos

Table 1. BAX® System inclusivity results (continued)

Strain No.	Serotype (if known)	Source	BAX Result	Strain No.	Serotype (if known)	Source	BAX Result
1328	1/2c	cooked turkey	Pos	4684	–	cheese factory	Pos
1419	4d	sheep	Pos	4693	–	seafood sandwich	Pos
1423	7	unknown	Pos	4695	–	bacon & egg sand.	Pos
1464	–	dairy product	Pos	4698	–	chicken sandwich	Pos
1961	–	clinical	Pos	4700	–	cheddar	Pos
1964	–	raw milk	Pos	4854	–	spaghetti bolognese	Pos
2077	–	cheese	Pos	4856	–	cream cake	Pos
2365	1/2b	ice cream	Pos	5821	4b	processed meat	Pos
2882	–	chipped ham	Pos	5823	4b	dried sausage	Pos
3075	–	environmental	Pos	5830	–	dairy environment	Pos
3323	1	raw milk	Pos	5839	–	salt whey	Pos
3333	4	Mexican cheese	Pos	5855	–	cooked turkey	Pos
3334	4b	ice cream	Pos	5857	–	frozen gateau	Pos
3335	3b	brie	Pos	5858	–	demerara sugar	Pos
3339	4	environmental	Pos	5862	–	salmon pate	Pos
3350	1	environmental	Pos	6130	–	vegetable protein	Pos
3365	1a	ice cream	Pos	6138	–	dairy environment	Pos
3366	4b	shrimp	Pos	6139	–	cheese	Pos
3379	1	environmental	Pos	6366	–	salami	Pos
3385	1	environmental	Pos	6367	–	smoked trout	Pos
3390	–	unknown	Pos	6369	–	smoked salmon	Pos
3391	1a	environmental	Pos	6415	3b	ice cream	Pos
3392	1a	lobster	Pos	6420	3a	meat	Pos
3393	1a	shrimp	Pos	6439	–	cheese	Pos
3394	1a	milk	Pos	6442	4b	French cheese	Pos
3395	1b	cheese	Pos	6444	–	Jarlsberger cheese	Pos
3401	–	ground lamb	Pos	6447	–	pepperoni	Pos
3402	–	unknown	Pos	6450	–	check sample	Pos
3403	–	fish	Pos	6455	–	cheese	Pos
3405	–	chicken patties	Pos	6474	–	smoked salmon	Pos
3406	–	unknown	Pos	6478	–	salami	Pos
3407	–	ground lamb	Pos	6535	–	ground pork	Pos
3408	–	veal patties	Pos	6610	–	hamburger	Pos
3410	–	ground veal	Pos	6611	–	brie	Pos
3544	–	industry isolate	Pos	6612	–	raw milk	Pos
3545	–	industry isolate	Pos	6616	–	sausage	Pos
3546	–	industry isolate	Pos	6618	–	monk fish	Pos
3547	–	industry isolate	Pos	6754	–	custard powder	Pos
3548	–	industry isolate	Pos	6760	–	chocolate dessert	Pos
3549	–	industry isolate	Pos	6763	–	prawn coleslaw	Pos
3556	–	ground veal	Pos	6764	–	lamb casserole	Pos
3559	–	ground veal	Pos	6766	–	satay chicken	Pos
3560	–	industry isolate	Pos	6783	–	carbonara sauce	Pos
3561	–	industry isolate	Pos	6891	–	brie	Pos
3563	–	industry isolate	Pos	7304	–	chicken Kiev	Pos
3564	–	industry isolate	Pos	7822	1/2c	unknown	Pos
3565	–	industry isolate	Pos	7856	4a,b	unknown	Pos
3566	–	industry isolate	Pos	7923	–	guacamole	Pos
3567	–	industry isolate	Pos	8810	–	beef franks	Pos
3568	–	industry isolate	Pos	8816	–	orange sherbet	Pos
3569	–	industry isolate	Pos				

Table 2. BAX® System exclusivity results

Strain No.	Strain ID	Source	BAX Result	Strain No.	Strain ID	Source	BAX Result
3361	<i>Listeria grayi</i>	unknown	Neg	2090	<i>Listeria seeligeri</i>	unknown	Neg
3355	<i>Listeria grayi</i>	unknown	Neg	949	<i>Listeria seeligeri</i>	unknown	Neg
10698	<i>Listeria grayi</i>	unknown	Neg	1289	<i>Listeria seeligeri</i>	crab pate	Neg
1157	<i>Listeria innocua</i>	hamburger	Neg	1300	<i>Listeria seeligeri</i>	cooked ham	Neg
927	<i>Listeria innocua</i>	chopped ham	Neg	1304	<i>Listeria seeligeri</i>	chicken	Neg
1161	<i>Listeria innocua</i>	salami	Neg	1292	<i>Listeria seeligeri</i>	cooked chicken	Neg
1063	<i>Listeria innocua</i>	chopped ham	Neg	1303	<i>Listeria seeligeri</i>	pate	Neg
1156	<i>Listeria innocua</i>	lettuce	Neg	2538	<i>Listeria seeligeri</i>	unknown	Neg
1066	<i>Listeria innocua</i>	stilton	Neg	1179	<i>Listeria welshimeri</i>	food	Neg
1154	<i>Listeria innocua</i>	pate	Neg	1174	<i>Listeria welshimeri</i>	raw chicken	Neg
1158	<i>Listeria innocua</i>	black pudding	Neg	943	<i>Listeria welshimeri</i>	unknown	Neg
1155	<i>Listeria innocua</i>	camembert	Neg	3233	<i>Listeria welshimeri</i>	unknown	Neg
2087	<i>Listeria innocua</i>	unknown	Neg	1177	<i>Listeria welshimeri</i>	smoked mackerel	Neg
1064	<i>Listeria innocua</i>	chopped ham	Neg	1176	<i>Listeria welshimeri</i>	chicken	Neg
1159	<i>Listeria innocua</i>	celery salad	Neg	3351	<i>Listeria welshimeri</i>	unknown	Neg
1465	<i>Listeria ivanovii</i>	unknown	Neg	1173	<i>Listeria welshimeri</i>	unknown	Neg
1466	<i>Listeria ivanovii</i>	unknown	Neg	1175	<i>Listeria welshimeri</i>	sausage	Neg
1170	<i>Listeria ivanovii</i>	unknown	Neg	1180	<i>Listeria welshimeri</i>	food	Neg
1166	<i>Listeria ivanovii</i>	salami	Neg	1172	<i>Listeria welshimeri</i>	salami	Neg
1171	<i>Listeria ivanovii</i>	unknown	Neg	1024	<i>Bacillus cereus</i>	pasteurized milk	Neg
1165	<i>Listeria ivanovii</i>	salami	Neg	9204	<i>Bacillus subtilis</i>	unknown	Neg
1164	<i>Listeria ivanovii</i>	radishes	Neg	7329	<i>Carnobacterium piscicola</i>	vacuum pack beef	Neg
1169	<i>Listeria ivanovii</i>	unknown	Neg	1991	<i>E. coli</i> O157:H7	beef	Neg
1167	<i>Listeria ivanovii</i>	soft cheese	Neg	2122	<i>E. coli</i>	bleu cheese	Neg
1168	<i>Listeria ivanovii</i>	unknown	Neg	4088	<i>E. coli</i>	fish	Neg
3364	<i>Listeria murrayi</i>	unknown	Neg	2468	<i>Enterococcus species</i>	cole slaw	Neg
5907	<i>Listeria murrayi</i>	unknown	Neg	3981	<i>Enterococcus faecalis</i>	unknown	Neg
3363	<i>Listeria murrayi</i>	unknown	Neg	2468	<i>Enterococcus faecalis</i>	unknown	Neg
3357	<i>Listeria murrayi</i>	unknown	Neg	8031	<i>Enterococcus faecium</i>	cheese powder	Neg
3815	<i>Listeria murrayi</i>	unknown	Neg	958	<i>Enterococcus faecium</i>	brie	Neg
3802	<i>Listeria murrayi</i>	unknown	Neg	9111	<i>Kurthia gibsoni</i>	unknown	Neg
3803	<i>Listeria murrayi</i>	unknown	Neg	10836	<i>Lactobacillus delbruecki</i>	Emmental cheese	Neg
4095	<i>Listeria murrayi</i>	unknown	Neg	10205	<i>Lactococcus lactis</i>	Danish cheese starter	Neg
4323	<i>Listeria murrayi</i>	unknown	Neg	9178	<i>Micrococcus luteus</i>	unknown	Neg
5861	<i>Listeria murrayi</i>	unknown	Neg	1095	<i>Staphylococcus carnosus</i>	fermented sausage	Neg
1301	<i>Listeria seeligeri</i>	corned beef	Neg	1106	<i>Staphylococcus xylois</i>	Italian salami	Neg
1298	<i>Listeria seeligeri</i>	chicken roll	Neg	1396	<i>Staphylococcus aureus</i>	unknown	Neg
1297	<i>Listeria seeligeri</i>	pate	Neg	3515	<i>Staphylococcus epidermis</i>	unknown	Neg
1291	<i>Listeria seeligeri</i>	lettuce	Neg	3990	<i>Streptococcus equi</i>	unknown	Neg