



Improved Specificity of Path-Chek Hygiene Listeria

All internationally accepted standards for the isolation of *Listeria* spp. and *L. monocytogenes* from food samples recommend that all pre-enrichment and sometimes enrichment broths are incubated at 30°C rather than 35 - 37°C, (ISO 11290 -1:1996, AS/NZS 1766.2.16: 1998, US FDA BAM/AOAC). One rationale for this initial lower incubation temperature is that *Listeria* spp. and in particular *L. monocytogenes* may not grow well in these media when incubated at 35 - 37°C and that in cases of mixed populations of *Listeria* spp., *L. innocua* may in fact overgrow *L. monocytogenes* at this incubation temperature but not at 30°C.

A study was performed in an independent Public Health Food Testing Laboratory to ascertain the potential effects of incubating the Path-Chek Hygiene Listeria at 30°C rather than 35 - 37°C. This study was performed using a range of environmental samples taken from a local food manufacturer who is known to have Listeria in their work environment.

In this trial each sample area was swabbed in triplicate, one swab was processed in Path-Chek Hygiene at an incubation temperature of 37°C, a second swab was processed similarly, but incubated at 30°C. The third swab was transferred into 10ml UVM broth which was incubated for 48 hours at 30°C after which a sample of the broth was examined for the presence of *Listeria* spp. using an AOAC Approved Listeria EIA.

All Path-Chek Listeria tests were subcultured onto Oxford Agar and incubated at 37°C for 48 hours and examined for colonies resembling *Listeria* spp. after 48 hours incubation. All suspicious colonies were Gram stained and subjected to appropriate identification tests to include or exclude *Listeria* spp.

RESULTS

A total of 35 environmental samples were examined for the presence of *Listeria* spp.. Three samples were found by all 3 methods to contain *L. monocytogenes*, Path-Chek Hygiene Listeria was able to detect the presence of these 3 positive samples after only 24 hours incubation, more than 24 hours earlier than the testing laboratories control method (Table 1.).

Although the results of this trial did not demonstrate any differences between the isolation of *Listeria* spp. in the 3 positive samples, this investigation did demonstrate a significant decrease in the number of false positive results in the Path-Chek Hygiene Listeria tests that were incubated at 30°C when compared to those incubated at 37°C.

After 24 hour incubation at 30°C, only 2 samples produced false positive results, whilst the same samples incubated at 37°C produced 9 false positive results. After a total of 48 hours incubation, the tests incubated at 37°C produced 13 false positive results, whilst those incubated at 30°C produced only 6 false positive tests. 6 positive results at 48 hours at both 30°C and 37°C, showed no growth once sub-cultured on the Oxford Agar plates, producing no identification as the organism in the broth did not grow on the Oxford Agar plates.

Based on this investigation it is clear optimum results for Path-Chek Hygiene Listeria are obtained at 30°C and should be examined for a colour change indicative of the presence of *Listeria* spp. after both 24 and 48 hours.

Swab Source	Path Chek Listeria 30C Incubation Esculin reaction		Typical colonies on Oxford Agar	Identification	Path Chek Listeria 37C Incubation Esculin reaction		Typical colonies on Oxford Agar	Identification	EIA result	Identification
	24hr	48hr			24hr	48hr				
1. Floor near sliding door	-	+	No	GPC	+	+	No	GPC	-	
2. Drain in holding room	+	+	No Growth		+	+	No Growth		-	
3. Rubber on sliding door	+	+	No	GPC	+	+	No	GPC	-	
4. Production room drain	-	+	No	GPB	+	+	No Growth		-	
5. Production room floor	-	-			-	+	No	GPC	-	
6. Floor under pump	-	-			-	-			-	
7. Floor in corner	-	-			+	+	No Growth		-	
8. Floor mat near pack line	-	-			-	+	No Growth		-	
9. Coolroom floor	-	-			+	+	No	GPB	-	
10. Blast room sliding door	-	-			+	+	Yes	GPB	-	
11. Wheels on pump frame	-	-			+	+	No Growth		-	
12. Wheels on pump track 1	-	-			-	-			-	
13. Wheels on pump track 2	-	-			-	-			-	
14. Wheels on pump track 4	-	-			-	-			-	
15. Wheels on pump track 3	+	+	Yes	L. monocytogenes	+	+	Yes	L. monocytogenes	+	L. monocytogenes
16. Wheels on pump 2	+	+	Yes	L. monocytogenes	+	+	Yes	L. monocytogenes	+	L. monocytogenes
17. Wheels on pump 3	+	+	Yes	L. monocytogenes	+	+	Yes	L. monocytogenes	+	L. monocytogenes
20. Chiller slide door	-	-			-	-			-	
26. Open drain	-	-			-	-			-	
27. Chiller slide door	-	-			-	-			-	
28. Undemeath bench	-	-			-	-			-	
29. Floor near pallet	-	-			-	-			-	
30. Drain near foot bath	-	-			-	+	No	GPB	-	
31. Drain in batching area	-	+	No Growth		-	+	No Growth		-	
32. Flor in blast chiller	-	-			-	-			-	
33. Wall in thawing room	-	-			-	-			-	
34. Door in thawing room	-	+	No Growth		+	+	No Growth		-	
35. Door in chiller	-	-			-	-			-	

Table 1. Comparison of Path-Chek Hygiene Listeria tests incubated at 30°C and 37°C and control EIA System for environmental samples.

GPC = gram-positive cocci, presumptively identified as *E. faecalis*
 GPB = gram-positive bacilli, presumptively identified as *Bacillus* spp.