Possible Rapid Screening of Positive Path-Chek Hygiene Listeria Tests

An earlier investigation (Technical Bulletin No.108) provided a strong indication that the specificity of Path-Chek Hygiene Listeria was significantly improved when the tests were incubated at 30°C rather than 35 - 37°C. One of the other potential benefits of incubating the Listeria tests at this lower temperature is that it may be possible to screen any positive tests (those turning black) using the Microgen® Listeria Latex test as a simple and rapid early confirmation test prior to further confirmation by sub-culturing these broths and identification of any suspect colonies.

A brief trial was performed using 10 strains of *Listeria* spp and a range of 11 cultures that may be potential cross-reacting organisms in Path-Chek Hygiene Listeria. In this trial, all cultures were inoculated onto sheep blood agar plates and incubated aerobically at 37°C, after which serial 1:10 dilutions were prepared and standard 100µl samples were absorbed onto Path-Chek Hygiene Swabs such that each test organism was inoculated into the Path-Chek Hygiene Detection Broth at alevel of ≤ 100 cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Listeria Detection Broths which were incubated at 30°C and examined for the development of a black colour after 48 hours incubation. A Microgen® Listeria Latex agglutination test was then performed on a single drop of the Path-Chek Hygiene Listeria Detection Broth with all tests that developed this black colour indicative of a positive Listeria test.

Results:

The results of this evaluation are found in Table 1.

ORGANISM	Colour Reaction	Listeria Latex
L. monocytogenes 305	Black	Positive
L. monocytogenes 551	Black	Positive
L. innocua 306	Black	Positive
L. innocua 555	Black	Positive
L. seeligeri 310	Black	Positive
L. seeligeri 552	Black	Positive
L. ivanovii 325	Black	Positive
L. ivanovii 111	Black	Positive
L. welshimeri 308	Black	Positive
L. welshimeri 560	Black	Positive
Carnobacterium divergens 968	Straw	
Bacillus mycoides 974	Straw	
Bacillus cereus 991	**Black**	**Negative**
Bacillus licheniformis 992	Straw	
Enterococcus avium 1136	Straw	
Enterococcus durans 988	Straw	
Enterococcus faecalis 1137	**Black**	**Negative**
Enterococcus faecum 1138	**Black**	**Negative**
Enterococcus gallenarum 1139	Straw	
Enterococcus hirae 1140	**Black**	**Negative**
Enterococcus faecium 1058	Straw	

All of the *Listeria* spp. grown in Path-Chek Hygiene Listeria Detection Broth, incubated at 30°C developed a black colour either at 24 or 48 hours. As soon as a positive reaction was observed, a Microgen® Listeria Latex was performed. All of the *Listeria* spp. tested produced a positive latex agglutination reaction. All of the potential cross reacting organisms that produced a false positive reaction in Path-Chek Hygiene Listeria produced a negative latex reaction.

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On the basis of this brief study it appears that Path-Chek Hygiene Listeria tests incubated at 30°C can be screened to confirm the presence of *Listeria* spp. using the Microgen® Listeria Latex test. All positive agglutination reactions should be sub-cultured onto an appropriate differential Listeria isolation media and the identification of suspect colonies confirmed.

The Microgen® Listeria latex kit contains latex particles coated with antibodies raised against flagella antigens of Listeria spp. incubation at 30°C optimises the development of flagella by Listeria species thus improving the reactivity of the organism in the latex test.

The detection of *Listeria* spp. in certain food processing environments can have significant ramifications. The ability to detect positive samples as early as 24 hours and perform a simple 2 minute latex agglutination test to provide initial confirmation or to exclude false positive reactions can offer major advantages to companies performing this type of environmental testing.