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IMPROVED SPECIFICITY OF PATH-CHEK LISTERIA AND RAPID SCREENING FOR *LISTERIA* SPECIES.

One of the major problems when screening environmental surfaces for the presence of *Listeria* spp. is the potential presence of *Enterococcus faecalis* and to a less degree mesophilic *Bacillus* species, all of which produce similar positive reactions in most commonly employed Listeria Screening Media. This phenomenon can result in significantly high numbers of false positive reactions when samples are taken from surfaces which are unclean or have not been adequately cleaned and sanitized.

From an operational point of view, the incidence of false positive results from Listeria environmental testing can be advantageous in highlighting poor cleaning methods or poor cleaning practices. However, the confirmation of false positive Listeria screening tests does result in significant work and cost and delays in reporting final results.
Microgen Bioproducts have made a number of changes to its Path-Chek Listeria test which has resulted in increased specificity, significantly reducing the incidence of false positive tests.

These changes combined with a recommendation to incubate tests at 25ºC have increased the specificity of Path-Chek Listeria by as much as 10^4 when challenged with *E. faecalis* while its sensitivity for the detection of *Listeria* species remains unchanged (See Table 1).

Table 1. SUMMARY OF INCUBATION STUDIES

TEST ORGANISM		10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1	1
Bacillus spp (16)	25°C									
	30°C									
E. faecalis (6)	25°C									
	30°C									
L. monocytogenes (11)	25°C									
	30°C									
L. innocua (11)	25°C									
	30°C									
L. seeligeri (3)	25°C									
	30°C									
L. welshimeri (3)	25°C									
	30°C									
L. ivanovii (2)	25°C									
	30°C									

POSITIVE AT 48 HOURS

NEGATIVE AT 48 HOURS

In further studies, we have investigated the feasibility of screening presumptive positive Path-Chek Listeria tests to determine which are due to the presence of *Listeria* species. The standard bacterial test screens for the catalase enzyme, which is produced by micro organisms that live in oxygenated environments. This enzyme neutralizes toxic forms of oxygen metabolites such as hydrogen peroxide. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. *Listeria* species produce the catalase enzyme, while *E. faecalis* does not. *Bacillus* species are catalase positive.

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Our investigations have determined that when 100 - 200µl or 4 drops of 6% Hydrogen Peroxide are slowly added to positive Path-Chek Listeria tests containing *Listeria* species, the development of bubbles occurs in the top of the Path-Chek tube within 2 minutes. This is indicative of the formation of oxygen as a result of the breakdown of the hydrogen peroxide by the catalase enzyme produced by *Listeria* species. The failure of bubbles of oxygen to be produced is indicative of the absence of a catalase enzyme i.e. *E. faecalis.*

Our investigations have shown that the combination of the modifications made by Microgen to the Path-Chek Listeria, combined with the recommendation to incubate at 25ºC almost totally eliminates the possibility of false positive results. The development of any false positive tests are therefore highly indicative of heavy contamination of surfaces which in turn is indicative of poor cleaning methods or performance, both of which should be investigated.

In addition, the performance of a Positive Catalase Test on all positive Path-Chek Listeria tests has been demonstrated to provide a simple, low cost confirmation test for the presence of *Listeria* species.

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