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Inhibitory Effects of Enrichment Media on the Accuprobe Test for *Listeria monocytogenes*

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During an evaluation of the Accuprobe kit for the detection of *Listeria monocytogenes*, some of the enrichment media used were found to interfere with the test. Microscopic examination during the lysis step of the test revealed that media containing high salt greatly reduced or prevented cell lysis. This prevented the probe from binding to the cellular RNA, resulting in false-negative results.

The Accuprobe kit for the detection and confirmation of *Listeria monocytogenes* enables the detection from culture in 45 min. An overnight culture (50 μl) is added to a tube containing the lysis reagent. After the cells are lysed, a probe which has a chemiluminescence label is added. The probe hybridizes to rRNA specific to *L. monocytogenes*. The label on the unbound probe is destroyed during the hydrolysis step, and the bound probe is detected via a chemiluminescence reaction. The light emission is measured with a luminometer. Values greater than 1,500 PLU (PAL/AccuLDR light units) indicate a positive result (2).

During an evaluation of the Accuprobe kit in this laboratory, it was found that when University of Vermont broth (UVM) and Fraser broth (FB) were used for sample enrichment, false-negative results occurred. This problem did not occur when tryptone soya broth (TSB) and *Listeria* enrichment broth (LEB) were used.

Since UVM and FB are more selective than the other broths, it was initially thought that cell numbers did not reach the required sensitivity levels for this kit. However, negative results were obtained even from cultures in both UVM and FB estimated by plate counts to contain up to 10^9 CFU of *L. monocytogenes* per ml. A similar problem was also observed by Ninet et al. (4) with UVM and PALCAMY broths.

Removing the broths by centrifugation and resuspending the cells in the lysis buffer provided with the kit allowed the tests to be conducted on cells propagated in both UVM and FB. Bobbitt and Betts (1) did not report any problems with the test, since they routinely concentrated cells by centrifugation, thus removing the broths and the inhibitory substances.

When a culture of *L. monocytogenes* in UVM (which had tested negative with the kit) was diluted 10-fold with sterile distilled water and then tested, the result was positive. Presumably, a component of the broth was being diluted below its threshold for inhibition. The reductions in signal were thus related to an inhibitory substance in the UVM medium which affected the test itself.

Components that were present in UVM and FB but either absent or at reduced levels in LEB and TSB were investigated as possible inhibitors of the test. These included esculin, acriflavine HCl, and salts (NaCl and Na_2HPO_4).

The effects of adding esculin and acriflavine HCl to TSB and LEB were investigated. Esculin and acriflavine HCl are present in UVM (at levels of 1.0 and 0.012 g/liter, respectively) and in FB (at 1.0 and 0.025 g/liter, respectively). Only acriflavine HCl is present in LEB (at 0.015 g/liter), and neither is present in TSB. Fifty-microliter portions of a culture (approximately 10^9 CFU/ml) of *L. monocytogenes* in TSB were added to 950 μl of TSB, LEB, UVM, and FB. These were the controls for each of the broths to be investigated. At the cell concentrations used, the signals produced for the LEB and TSB controls were positive, and the UVM and FB signals were negative. Various concentrations of esculin and acriflavine HCl (to the levels present in UVM and FB) were added to both TSB and LEB containing the same quantity of culture as the controls. The assay was performed directly on 50 μl of each broth (Table 1). The percent decreases in signal were measured relative to culture in TSB.

The signals for the culture suspended in UVM and FB were reduced (relative to TSB) by 92 and 89%, respectively, giving a negative test result as before. The addition of 1.0 g of esculin per liter to TSB resulted in a decrease in signal of 7% (Table 1). A decrease of 20% occurred when 0.012 g of acriflavine HCl per liter was added to TSB. It was interesting to note that a combination of esculin and acriflavine HCl (concentrations as described above) affected the signal to a lesser degree (14% decrease) than acriflavine HCl alone. Trends similar to those found with TSB were observed for LEB when these chemicals were added. The signal decrease for LEB was 28%, showing it to be more inhibitory than TSB but still allowing a positive test result.

Even when TSB and LEB were fortified with esculin and acriflavine HCl at the levels present in UVM and FB, positive results were produced. It appears, therefore, that although esculin and acriflavine HCl contribute to a decrease in signal, they are not responsible for the large decrease in signal observed for UVM and FB.

The inhibitory effects of UVM were investigated by titrating it with a noninhibitory broth (TSB). Mixed broths containing various proportions of TSB and UVM were prepared, and 50 μl of culture (10^9 CFU/ml) was added to each combination. The Accuprobe assay was performed with 50 μl of the mix.

When 20% UVM was added to TSB, a signal reduction of 53% occurred. The addition of 70% UVM produced a reduction in signal of greater than 90%, which was the same as that observed for UVM alone.

Salts are a major component in both UVM and FB. They both contain 20 g of NaCl and 12 g of Na_2HPO_4 per liter. LEB and TSB contain only 5 g of NaCl and 2.5 g of potassium salts per liter. The effects of the potassium salts were not tested, since these salts are present at concentrations in the nonin-

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TABLE 1. Effect of addition of acriflavine HCl and esculin to TSB and LEB on luminometer signal

Medium	Esculin (g/liter)	Acriflavine (g/liter)	Signal (PLU)[a]	% Reduction relative to TSB	Result
TSB[b]	0	0	2,864	0	+
TSB	1.0	0	2,661	7	+
TSB	0	0.012	2,298	20	+
TSB	1.0	0.012	2,466	14	+
LEB[b]	0	0.015	2,065	28	+
LEB	1.0	0.015	2,022	30	+
LEB	0	0.027	1,623	44	+
LEB	1.0	0.027	1,822	37	+
UVM[b]	1.0	0.012	230	92	−
FB[b]	1.0	0.025	324	89	−

[a] Signals of greater than 1,500 PLU indicate a positive test result (2).
[b] Standard composition.

hibitory broths similar to (slightly higher than) those in the inhibitory broths. To investigate the effects of salt concentrations on the assay, NaCl (5 to 20 g/liter) and Na_2HPO_4 (0 to 12 g/liter) were added to TSB containing 50 μl of culture (10^9 CFU/ml). The effects of various concentrations of the two salts on the assay are indicated in Table 2.

The addition of 20 g of NaCl and 12 g of Na_2HPO_4 per liter (consistent with levels in UVM) to TSB resulted in a reduction in signal of 85% and a negative result. This confirmed that the salt concentration had a major effect on the outcome of the test. The depression of response seems to be related to the total concentration of ions in the solution, as can be seen in Fig. 1 (data obtained from Table 2). Users of the Accuprobe kit should be aware that when they use media similar to TSB, solutions with concentrations of ions of greater than 0.5 M are quite likely to produce false-negative test results.

Microscopic investigation into the effect of salt during the lysis step was conducted. Cells in TSB and TSB containing salt (same level as UVM) were added to the tube provided with the kit. The tube that was supplied contained lyophilized lysing reagents. The lysis step was performed and the cells were viewed under a phase-contrast microscope to determine whether cell lysis had occurred.

Cultures in TSB and TSB plus salts were examined, and the cells in TSB were lysed; however, intact cells were present in TSB containing salt. These results suggest that high salt levels

TABLE 2. Effect of addition of salt to TSB on the luminometer signal

NaCl (g/liter)	Na_2HPO_4 (g/liter)	Signal (PLU)[a]	Total ion concn (mol/liter)[b]	% Reduction relative to TSB
5	0	5,275 (+)	0.21	0
20	0	1,233 (−)	0.72	77
5	12	1,854 (+)	0.47	65
10	12	1,518 (+)	0.64	71
15	12	1,200 (−)	0.81	77
20	12	711 (−)	0.98	86
20	0	1,233 (−)	0.72	77
20	6	1,062 (−)	0.85	80
20	12	711 (−)	0.98	86

[a] Signals of greater than 1,500 PLU indicate a positive test result (+) (2).
[b] Total ion concentration, $[Na^+] + [Cl^-] + [HPO_4^{2-}]$ in moles per liter.

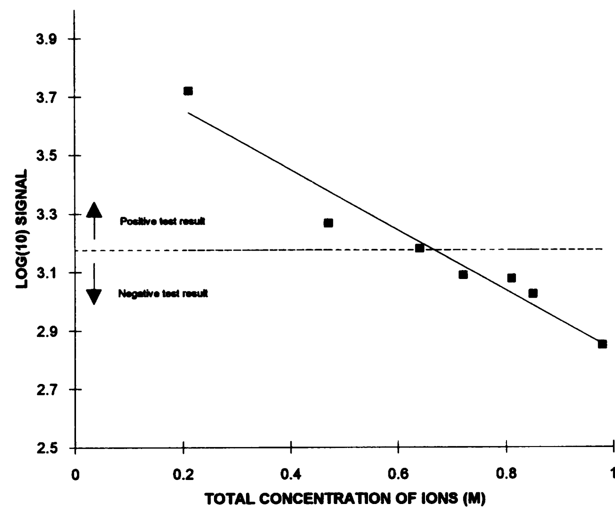

FIG. 1. Effect of ion concentration on response of Accuprobe test.

prevent cell lysis; thus, the probe cannot access the rRNA and the organism cannot be detected. The lysis reagent's identity is not revealed by Gen-Probe; therefore, it is difficult to speculate on the mechanism of inhibition. Lysis of gram-positive organisms generally requires the use either of surfactants or of enzymes such as lysozyme. We note that in a study by Metcalf and Deibel (3), the rate of lysis of *Streptococcus faecium* by lysozyme was reduced in the presence of moderate levels of salts. This was attributed to charge competition from the salt ions, which prevent the lysozyme from coating the cell surface and thus lysing the cells.

In conclusion, the salt concentrations in both UVM and FB were determined to be the major cause of false-negative results with the test. Microscopic examination of cells in the presence of high salt concentrations showed that the cells remained intact. This prevented the probe from hybridizing to the rRNA. Esculin and acriflavine HCl were also found to contribute to the reduction in signal, but this was insignificant compared with the salt effect. It is recommended that cultures in broths containing high salt levels (UVM and FB) be centrifuged and that the pellet be resuspended in the lysing reagent (which is supplied with the kit). This step will remove the salts as well as other possibly interfering substances.

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