Selective Diagnostic Medium for Pathogenic *Listeria* spp.

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Pathogenic *Listeria* serovars produced complete hemolysis on agars containing 5% rabbit erythrocytes and 10 μg of acriflavin, 40 μg of nalidixic acid, and 7.5 activity units of *equi* factor per ml. Apathogenic *Listeria innocua* was nonhemolytic on this medium.

The pathogenicity of *Listeria* spp. is closely associated with hemolytic activity, but most *Listeria monocytogenes* strains display a very weak, if any, hemolytic effect on sheep erythrocytes (6, 8). A typical hemolysis is produced by strains of serovar 5, proposed as a new species, *Listeria ivanovii* (5). Based upon direct hemolysis on sheep blood agar and synergistic hemolytic action with *equi* factor from *Corynebacterium (Rhodococcus)* *equi*, strains of *Listeria* spp. can be divided in four hemolytic groups (8). Subsequent assays have illustrated the hemolytic action of *Listeria* spp. and the synergism with *equi* factor in media with man, horse, and rabbit erythrocytes (7, 9, 10). Rabbit erythrocytes are the most sensitive to the hemolytic activity of *Listeria*; nevertheless, the phenomenon of hemolysis is observed with the apathogenic species *Listeria innocua* (10), which has been described as nonhemolytic (3, 4). Unlike the positive reaction with all strains of pathogenic *Listeria* spp., the hemolytic effect of *L. innocua* on rabbit blood is not enhanced by the *equi* factor (7–10). Sheep blood agars supplemented with *equi* factor are useful for in vitro differentiation of the pathogenic *Listeria* spp.; however, the addition of substances recommended for selective cultivation of *Listeria* spp. (2) suppress the hemolytic synergism, especially of *L. monocytogenes* strains (8). The aim of our study was to prepare a selective diagnostic medium for pathogenic *Listeria*.

The trials were carried out with 69 strains of *L. innocua*, 136 strains of *L. monocytogenes* belonging to different serovars with properties of either the *m1* or *m2* hemolytic group, and 6 strains of *L. ivanovii* (or serovar 5). A detailed description of the strains has been presented (7–10).

*Equi* factor was prepared from *C. equi* NCTC 1621. The techniques for preparing and assaying the activity were those described previously (7–9).

Nutrient agar CM3, Columbia agar base CM331, brain heart infusion CM225, and brain heart infusion agar CM375 (Oxoid Ltd.) were used. All media and saline were prepared with 2.5 mg of MgSO₄ per ml.

The hemolytic effects of strains of *Listeria* spp. observed on solid media containing 5% (vol/vol) washed rabbit erythrocytes corresponded to earlier descriptions (10).

When rabbit blood agars were supplemented with 10 μg of acriflavin and 40 μg of nalidixic acid per ml, both *L. innocua* and many colonies of *L. monocytogenes* hemolytic group *m1* were nonhemolytic. The direct hemolytic effect of the *m2* strains was evidently reduced, and the hemolytic zone of *L. ivanovii* strains was also smaller (Fig. 1).

The addition of *equi* factor to the medium containing washed rabbit erythrocytes and bacterial inhibitors resulted in a positive hemolytic

![FIG. 1. R-1. Agar with washed rabbit erythrocytes, acriflavin, and nalidixic acid. Growing on quadrants are colonies of: i. *L. innocua*; *m1*, and *m2*, hemolytic groups of *L. monocytogenes*, and 5. *L. ivanovii.*](http://jcm.asm.org/download/18_6/1433_1432-1433.jpg)
FIG. 2. REI. The same medium as that shown in Fig. 1., but supplemented with equi factor. Cultivated strains are identified as described in the legend to Fig. 1.

effect by pathogenic Listeria spp. The pathogenic species, L. innocua, remained nonhemolytic (Fig. 2). The optimum amount of equi factor was 7.5 activity units per ml of medium, although the described results could be observed with amounts ranging from 3 to 50 activity units per ml.

There are divergent opinions on the hemolytic synergism of pathogenic Listeria species and C. equi. Originally, it was recommended for differentiation between L. monocytogenes and Erysipelothrix rhusiopathiae (1). Previous results (8, 9) call attention to its availability for differentiating within the genus Listeria.

A team of authors stated that the C. equi exosubstance exerted synergism merely with the hemolysin of L. ivanovii (5), and they suggested use of this synergism to discern this new species from L. monocytogenes. Our experience indicates that in the presence of equi factor, both pathogenic species of Listeria exhibit hemolytic reactions on sheep, human, equine, and particularly rabbit blood agars (8–10).

The synergism of Listeria hemolysin(s) and equi factor on agar containing rabbit erythrocytes and antimicrobial agents offers a selective diagnostic medium for pathogenic species of the genus Listeria.

LITERATURE CITED