



USE PROTOCOL FOR R & F® *LISTERIA* SP./*LISTERIA* *MONOCYTOGENES* CHROMOGENIC PLATING AND CONFIRMATORY MEDIA

- 1 From a designated *Listeria* enrichment broth, streak a loopful onto R & F® *Listeria* sp./*Listeria monocytogenes* Chromogenic Plating Medium. Prepare the R & F® *Listeria* sp./*Listeria monocytogenes* Chromogenic Plating Medium according to the instructions provided on the packaging labels. After the plates have been poured, they should be stored in the dark for 48 hours at room temperature to dry the surface of the agar. The plates should be opaque white in appearance. After surface drying, the plates can be placed in Petri plate sleeves (cut several holes in the sleeves to allow condensation to escape) and stored inverted at 2-8°C for up to 60 days.
- 2 Incubate the plate(s) at 35°C for 48 hours.
- 3 Typical *Listeria monocytogenes* and *Listeria ivanovii* colonies appear blue-green to blue-violet colored with a convex colony shape, 1.0-2.0 mm in diameter with or without a blue-green halo against the opaque white background. Blue-green to blue-violet colonies may appear after 24 hours. The other nonpathogenic *Listeria* sp. (*Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, and *Listeria grayi*) colonies appear pink colored with convex colony shape, 1.0-2.0 mm in diameter without a halo against the opaque white background.
- 4 To determine the presence of *Listeria*, pick several blue-green to blue-violet and/or pink colonies and perform catalase and wet mount. If the cells are positive for tumbling and catalase, this indicates the presence of *Listeria* sp. in the sample.
- 5 To differentiate the blue-green to blue-violet colonies as either *L. monocytogenes* or *L. ivanovii* strains, R & F® *Listeria monocytogenes* Confirmatory Medium should be used.
- 6 Pick at least 3 to 5 blue-green to blue-violet colonies (the colonies previously tested for tumbling and catalase) and streak on a biplate containing R & F® *Listeria monocytogenes* Confirmatory Medium on one side and a medium showing acid from 1.0% rhamnose (instructions on the R & F® *Listeria monocytogenes* Confirmatory Medium) on the other side. One biplate can be used for the 3 to 5 colonies.
- 7 Incubate the plate 35°C for 6 to 24 hours. Most reactions occur after 6 hours.
- 8 *Listeria monocytogenes* fluoresces on R & F® *Listeria monocytogenes* Confirmatory Medium (positive for α -mannosidase indicated by a fluorogenic reaction) and/or produces acid from rhamnose, whereas, *Listeria ivanovii* shows no fluorescent reaction on R & F® *Listeria monocytogenes* Confirmatory Medium and no acid production from rhamnose. For some *Listeria monocytogenes* strains, fluorescence and/or acid production can be observed after just 6 hours.

Remember either fluorescence or acid production needs to occur (not both) for a positive *Listeria monocytogenes* confirmation.