



USE PROTOCOL FOR R & F® *ANTHRACIS* CHROMOGENIC AGAR

- 1 Prepare the R & F® *Anthraxis* Chromogenic Agar according to the instruction 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. After surface drying, the plates can be placed in Petri plate sleeves (cutting a hole in the sleeves to allow condensation to escape) and stored at 2-8°C for up to 45 days.
- 2 Inoculate the plates using spread-plating or streak for isolation, as preferred. Allow the inoculum to completely soak into the agar before inversion and incubation.
- 3 Incubate the plates inverted at 35-37°C and observe at 24 and 48 hours.
- 4 *Bacillus anthracis* colonies appear cream-colored, or cream with a pale blue-teal center after 24 hours' incubation. The colonies should be 2-6 mm diameter, and have a rough, ground-glass texture. The colonies may or may not show "medusa-head" morphology. After 24 hours of incubation *Bacillus cereus*/*Bacillus thuringiensis* colonies will be a very dark teal-blue, with a thin cream rim, and 6-7 mm in diameter. With a marking pen, circle the dark teal *Bacillus cereus*/*Bacillus thuringiensis* colonies, and the cream or pale blue *Bacillus anthracis* colonies. Mark the *Bacillus cereus*/*Bacillus thuringiensis* colonies with an "X" or other label to differentiate them from the *Bacillus anthracis* colonies, and re-incubate for an additional 24 hours. After 48 hours of incubation, the *Bacillus anthracis* colonies will now have blue-teal centers and a large white rim, while *Bacillus cereus*/*Bacillus thuringiensis* colonies will be unchanged. Any cream colony that has not developed a blue or teal center is not *Bacillus anthracis*. You may prefer to develop a different system for differentiating the two colony types at these two time periods, or find the 24 hour incubation sufficient.
- 5 Use additional standard or approved methods for colony confirmation.