The Overlay Method of Plating Bacteria

The "overlay method" of plating bacteria is used when a very uniform lawn of growth is needed. Spread plating often cannot produce a lawn that is homogeneous enough for antibiotic susceptibility testing by the disc diffusion method (Kirby-Bauer) or to form bacterial virus plaque assays. In the overlay method, bacteria are added to a melted agar solution which is poured onto the surface of an agar plate. During incubation, bacteria form microcolonies within the agar top layer.

1. For each plate you need a small (12X100 mm) sterile culture tube pre-warmed to 47-50°C for 10'. These tubes are conveniently pre-warmed by placing them in a thermoregulated aluminum alloy block.

2. Pipette approximately 4 ml of melted top agar solution into each of the pre-warmed tubes. This may be done in advance and the tubes maintained at 47-50°C for several hours before use.

The top agar solution is similar to regular agar medium except that the agar concentration is 7 g/l rather than the usual 16 g/l.
Warm the pipette by flaming before transferring the top agar solution. Return the bottle of top agar to the water bath when you are finished with it.

3. Add 0.1 ml of bacterial suspension (usually an overnight culture) to the top agar. Quickly mix the tube and pour the contents onto a pre-warmed (37°C) agar plate. Immediately rock the plate gently so that the top agar solution evenly covers the entire surface. The top agar solidifies within a minute or two.