

DNA Hydrolysis (DNase Test)

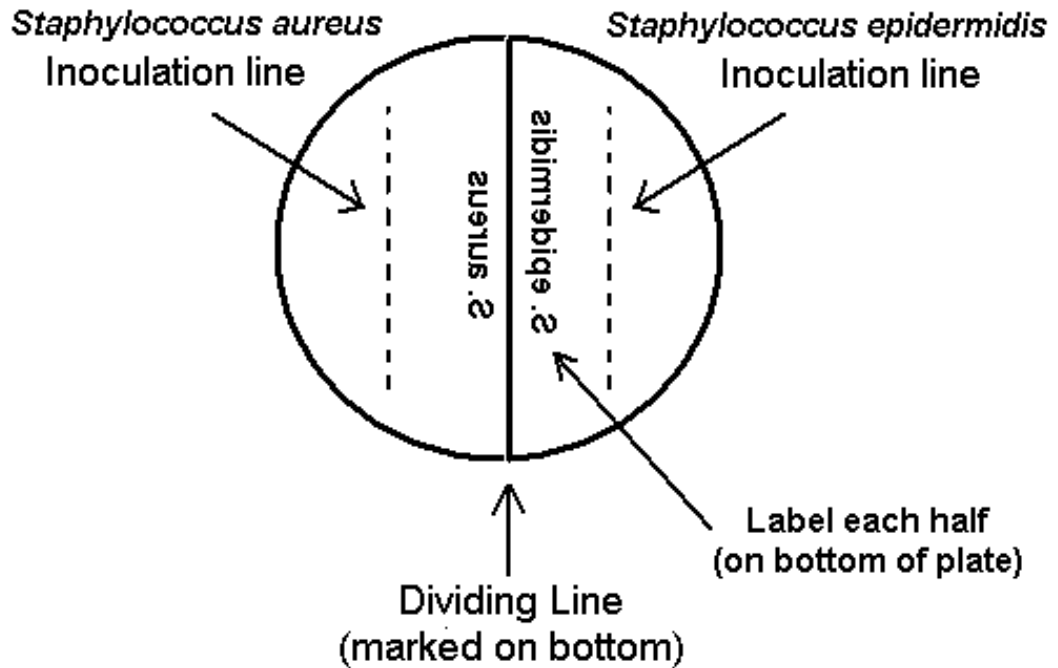
Most pathogenic strains of *Staphylococcus* (staphylococci) produce a nuclease enzyme called **Deoxyribonuclease (DNase)** (Fig. 6-31 of the Atlas, p. 55). DNase degrades host DNA and increases the pathogenicity of staphylococci that possess it. To demonstrate the presence of DNase, agar containing dissolved DNA is inoculated with a line of staphylococci. A zone of clearing around the colony indicates a positive DNase test. This clearing occurs because the large DNA molecule has been degraded by the enzyme, and the end products dissolve in the added acid (1 N HCl). Intact DNA does not dissolve in the acid but rather is precipitated by it; thus, the medium around colonies that do not produce DNase becomes opaque. This procedure is different from the procedure listed in the Atlas.

Procedure:

To demonstrate the presence or absence of DNase activity we will use *Staphylococcus aureus* and *Staphylococcus epidermidis* as the test organisms. **Caution: *Staphylococcus aureus* is a known pathogen. Wash hands thoroughly with disinfectant soap and wipe down bench with Amphyl disinfectant immediately after use. Use proper aseptic technique when making culture transfers.**

First Period

1. With a marker, divide the bottom of a gelatin agar plate in half and label half the plate *Staphylococcus aureus* and the other half *Staphylococcus epidermidis*. Place your name and date on the plate. Do not make a mark on the plate indicating where the bacteria will be inoculated because this may interfere with interpretation of the results.
2. Using aseptic technique, streak the respective bacteria onto the plate in a straight line within the section.
3. Incubate the plate in an inverted position for 24 to 48 hours at 35 °C.



Second Period

1. Cover the plate with just enough 1 N HCl to barely cover the plate. Use the HCl reagent sparingly. It is a strong acid so avoid contact with skin and clothing. Rinse exposed skin immediately with large volumes of water. Localize the HCl to the area of the bacterial lines of growth. Allow the plate to sit (right side up, lid up) on the bench for approximately 15 – 20 minutes. Do not invert the plate. Examine the plate for evidence of DNA hydrolysis. Hydrolysis is evidenced by a non-opaque clear zone around the bacterial growth. If no DNA hydrolysis has taken place, the zone around the bacterial growth will be opaque.
2. Record your results in the space below.