Coagulase and DNase and TNase Testing for *Staphylococcus aureus*

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This technical bulletin describes and compares the three principle tests used to identify *Staphylococcus aureus* and predict enterotoxin producing ability.

The coagulase, DNase and TNase methods found in the FDA Bacteriological Analytical Manual and in the Canadian Health Protection Branch Compendium of Methods are briefly described below.

**Coagulase**
The ability to clot plasma is a widely used method to identify pathogenic *staphylococci*. Rabbit plasma is mixed with a culture of the suspected organism; organisms that produce the coagulase enzyme clot the plasma within 6 hours. In addition to *S. aureus*, *Staphylococcus intermedius* and *S. hyicus* produce the coagulase enzyme and are thus commonly grouped with coagulase-positive staph.

**DNase**
Deoxyribonuclease (DNase) is an enzyme that breaks down DNA. Certain species of bacteria have the ability to produce the DNase enzyme – *Staphylococcus aureus*, *S. intermedius*, *S. hyicus*, Group A *Streptococcus*, and *Serratia marcescens*. This activity is demonstrated by culturing organisms on an agar medium containing DNA and a dye, which changes color in the presence of the degraded DNA.

**TNase**
Thermostable nuclease (TNase) is a specific, heat-stable DNase that breaks down DNA. Bacteria that have the ability to produce the TNase enzyme are *S. aureus*, *Staphylococcus intermedius*, and *Staphylococcus hyicus*. TNase activity is demonstrated by heating the organism in suspension to about 60°C and then putting this suspension on an agar medium containing DNA and a dye, which changes color in the presence of the degraded DNA.
There are a variety of methods that can be performed to identify *Staphylococcus aureus*, including tests for enzymes, such as coagulase, thermo-stable deoxyribonuclease (TNase) and deoxyribonuclease (DNase). Studies have shown that coagulase and TNase activities correlate with *S. aureus* isolates approximately 99-100% of the time\(^1\),\(^2\) and that 96% of TNase-producing *S. aureus* also produce DNase\(^3\).

The goal of food safety and regulatory testing is to assay for the presence of those *Staphylococcus* capable of producing enterotoxin. The following table from *Bergey's Manual of Determinative Bacteriology* summarizes the relationships between the presence of coagulase or DNase and the ability to produce enterotoxin.

<table>
<thead>
<tr>
<th></th>
<th>Coagulase</th>
<th>DNase</th>
<th>Enterotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>&gt;90%</td>
<td>&gt;90%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td><em>Staphylococcus intermedius</em></td>
<td>&gt;90%</td>
<td>&gt;90%</td>
<td>5-40%</td>
</tr>
<tr>
<td><em>Staphylococcus hyicus</em></td>
<td>&gt;90%</td>
<td>24-56%</td>
<td>pos</td>
</tr>
<tr>
<td>Other <em>Staphylococcus</em> species</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

The 3M™ Petrifilm™ Staph Express Count™ Plate contains inhibitors that prevent most DNase producing non-*S. aureus* from growing on the plate. If colony colors other than deep red-violet appear on the Petrifilm Staph Express Count Plate, the disk helps to identify *S. aureus* by using a DNase reaction.