

***Detection, Determination and Confirmation of
Staphylococcus aureus.***

Staphylococcus aureus is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Thus, the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation. *S. aureus* can cause severe food poisoning .

Equipment:

Refer to lab1. (Equipment, Materials and Glassware).

Culture media:

- Trypticase (tryptic) soy broth with 10% sodium chloride and 1% sodium pyruvate.
- Baird Parker (**BP**) Medium
- Mannitol Salt Ager (MSA) .
- Desiccated Coagulase Plasma (rabbit) with EDTA

Procedure:

1.Preparation of food homogenate:

Aseptically weigh 50 g food sample into the sterile blender jar. Add 450ml of diluent (1:10) and homogenize 2 min at high speed (16000-18000 rpm). Alternately use stomacher for sample preparation

2. Dilution:

Pipette 10ml of the food homogenate into 90ml of diluent (or 1ml to 9ml) to make a 1:100 dilution. Mix well using a vortex-mixer. Transfer 1ml from this dilution to a fresh tube of 9ml to give a 1:1000 dilution. Repeat until the desired dilution is obtained.

A- Most probable number method:

This procedure is recommended for testing processed foods likely to contain a small number of *S. aureus*.

B-Surface Plating method :

This method is applicable for general purpose use in testing foods expected to contain > 10 cells of *S. aureus* per g.

Transfer 1ml of the food homogenate (1:10 dilution) and other dilutions to triplicate plates of **BP medium** and equitably distribute 1ml inoculum over the triplicate plates. Spread inoculum over agar surface using sterile bent glass streaking rods (hockey sticks).

Incubate plates in upright position in the 35-37 C incubator for about 1 hour or until inoculum is absorbed by medium. Then invert plates and incubate 45-48 hours.

Counting colonies:

Count colonies of typical *S. aureus* appearance . Test for coagulase production on suspected colonies. Add number of colonies on triplicate plates represented by colonies giving positive coagulate test. Multiply the count obtained by inverse of corresponding sample dilution. Report as *S. aureus* per gm or ml of the sample.

Expression of result:

$$\textit{Staphylococcus aureus} = x$$