STAPHYLOCOCCUS AUREUS

LIM PANG YONG

INTRODUCTION

Staphylococcus aureus is a common organism on the skin and in the nasal passages of approximately 50% of the population. Heat treated seafood may become contaminated with this organism by poor handling, then storage at improper temperatures allows the organism to multiply and produce its toxin.

This type of food poisoning may be avoided by practising strict personal hygiene, thorough cleaning and disinfection of equipment, and storage of susceptible food at temperatures below 10°C or above 60°C.

Examination of a product for S. aureus does not guarantee protection against staphylococcal food poisoning because the organism may be killed, without destruction of the heat stable enterotoxin produced during growth of the organism. A direct microscopic smear of the food may be helpful, as direct detection of toxin in food requires methods which are too involved for routine use. A smear reveals viable and killed cells of staphylococci.

I CULTURE MEDIA*

Baird Parker medium
Brain heart infusion broth (BHI)
Citrated human plasma
Trypticase soy broth + 10% NaCl (TSB)
Butterfield's buffered phosphate diluent

* Refer to Appendix B for methods of media preparation.

II APPARATUS

'Waring' blender & flasks
Pipettes
Scissors & forceps
Alcohol lamps
Alcohol (70% v/v) swabs
Autoclave
Incubator
Water-bath
Weighing balance
Laminar flow chamber
Test-tubes
Plating loops

III SAMPLING PROCEDURE

Refer to 'AEROBIC PLATE COUNT' (E-2) SECTION III

IV SAMPLE PREPARATION

Refer to 'AEROBIC PLATE COUNT' (E-2) SECTION IV

V PROCEDURE

1. Select appropriate dilutions and for every dilution, transfer 1 ml aliquots into each of 3 TSB tubes.

2. Incubate the tubes at 35°C for 48 hrs.

3. The presence of turbidity indicates presumptive S. aureus.

4. Streak a loopful of the culture from a positive tube onto Baird Parker agar plate.

5. Incubate the tubes at 35°C for 48 hrs.

6. Typical colonies of S. aureus on Baird Parker agar appear as smooth, black, convex and shiny with narrow white entire margins and are surrounded by clear zones extending into the opaque medium.

7. Subculture all suspected colonies in BHI broth and incubate at 35°C for 24 hrs.

8. Transfer 0.5 ml of the broth culture into a test-tube and add 1 ml of citrated human plasma. Mix by gentle rotation of the tube.

9. Incubate at 35°C for about 6 hrs, and if negative, examine again after 24 hrs.

10. A 3+ or 4+ clot formation is considered a positive reaction for S. aureus. A 3+ reaction refers to formation of a large organized clot and a 4+ reaction is when the entire content of the tube coagulates and is not displaced when the tube is inverted. (See illustration overleaf).

11. Using the MPN Tables (Appendix A), calculate the MPN of S. aureus based on the proportion of confirmed turbid TSB tubes for 3 consecutive dilutions.

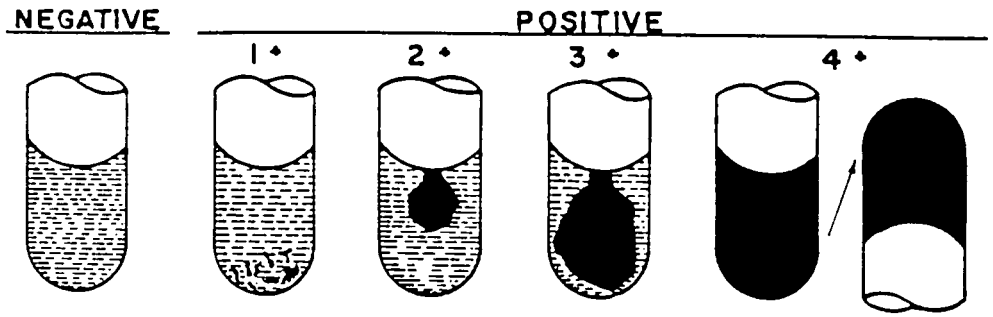
VI CALCULATION OF MPN

$$\text{Most Probable Number (MPN)} = \frac{\text{Index}}{10} \times (450 + W) \times \frac{1}{W}$$

where W : weight of sample in g

Index : from MPN Tables

TYPES OF COAGULASE TEST REACTIONS



	NEGATIVE	NO EVIDENCE OF FIBRIN FORMATION
1+	POSITIVE	SMALL UNORGANIZED CLOTS
2+	POSITIVE	SMALL ORGANIZED CLOT
3+	POSITIVE	LARGE ORGANIZED CLOT
4+	POSITIVE	ENTIRE CONTENT OF TUBE COAGULATES AND IS NOT DISPLACED WHEN TUBE IS INVERTED

VII BACTERIOLOGICAL LIMITS OF S. AUREUS FOR FISH/FISHERY PRODUCTS (COOKED & RAW)

Cooked products : 100 MPN/g

Raw products : 250 MPN/g

REFERENCE

A. Hazzard. 1985. ASEAN Training Course in Fish Quality Control. Training course organised by HAWKAID, Hawkesbury Agricultural College Research and Development Co. Ltd. Chapter: Microbiology in seafood quality control. Section: 68, 114 & 115

FLOW DIAGRAM OF EXAMINATION PROCEDURES FOR STAPHYLOCOCCUS AUREUS