

**STAPHYLOCOCCUS AUREUS
(CULTURE METHOD)**

PRINCIPLE

Coagulase positive *Staphylococci* are isolated from samples by using an enrichment procedure. The group is confirmed through the use of selective and differential media, followed by testing presumptive colonies for coagulation. Caution must be exercised when applying the method, since isolates may be pathogenic.

SCOPE

The method is applicable to starches, sugars, syrups and most coproducts of the corn wet milling industry.

MEDIA AND REAGENTS

1. Tryptic Soy Broth. Prepare medium according to the manufacturer's directions. Dispense 100 mL into 250 mL Erlenmeyer flasks. Sterilize by autoclaving at 121°C for 15 mins.
2. Use one of the following:
 - a. Baird-Parker Agar Base. Prepare medium according to manufacturer's directions. Sterilize by autoclaving at 121°C for 15 mins. Cool to 45-50°C, add prewarmed (45-50°C) Egg Yolk Tellurite Enrichment (EYTE) to the agar base, 50 mL EYTE per 950 mL agar base. Mix completely and dispense in 15 mL volumes into Petri dishes and solidify.
 - b. Vogel-Johnson Agar. Prepare medium according to manufacturer's directions. Sterilize by autoclaving at 121°C for 15 mins. Cool to 45-50°C, add Potassium Tellurite Solution 1%. Mix completely and dispense in 15 mL volumes into Petri dishes and solidify.
 - c. Mannitol Salt Agar. Prepare medium according to the

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manufacturer's directions. Sterilize by autoclaving at 121°C for 15 mins. Cool to 45-50°C, then dispense in 15 mL volume in Petri dishes and solidify.

3. Rabbit Coagulase Plasma EDTA. Rehydrate according to manufacturer's directions with sterile water. Dispense 0.5 mL into 10 × 75 mm tubes.

PROCEDURE

Aseptically weigh 10 g of sample into 100 mL of Tryptic Soy Broth (TSB), mix well and incubate for 46-50 hrs. at 35-37°C. Using a 3 mm inoculating loop, streak from TSB onto medium of choice. Cover, invert and incubate for 46-50 hrs. at 35-37°C. After incubation, examine plates for colonies which conform to the morphological descriptions below. Inoculate the suspect colony into individual tubes which contain 0.5 mL coagulase plasma (Note 1). Incubate at 35-37°C, examining the tubes at 3 hrs. and subsequently at suitable intervals up to 24 hrs. for any degree of coagulation.

Selective Medium	Colony Characteristics Morphology	Gram Stain
Vogel-Johnson Agar Medium	Black Surrounded by Yellow Zone	Positive cocci (in clusters)
Mannitol Salt Agar Medium	Yellow Colonies with Yellow Zone	Positive cocci (in clusters)
Baird-Parker Agar Medium	Black, Shiny, surrounded by clear zones	Positive cocci (in clusters)

CALCULATION

Report as Coagulase Positive *Staphylococci* per 10 g of sample, if any tubes are positive.

NOTES AND PRECAUTIONS

1. The coagulase test must be performed with a positive control.

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REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.