CHAPTER 12

Isolation and identification of *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive spherically shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although S. aureus usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. S. aureus is one of the leading pathogens for deaths associated with antimicrobial resistance and the emergence of antibiotic-resistant strains, such as methicillin-resistant S. aureus (MRSA), is a worldwide problem in clinical medicine. 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. The presence of a large number of S. aureus organisms in a food may indicate poor handling or sanitation; however, it is not sufficient evidence to incriminate a food as the cause of food poisoning.

Methods used to detect and enumerate S. aureus depend on the reasons for testing the food and on the past history of the test material. Processed foods may contain relatively small numbers of debilitated viable cells, whose presence must be demonstrated by appropriate means. Analysis of food for S. aureus may lead to legal action against the party or parties responsible for a contaminated food.

Samples requiring enumeration of *S. aureus*

Add 225 ml of phosphate-buffered saline water to blender jar containing 25 g of sample and blend 2 min. This results in a dilution of 10^{-1}. Make dilutions of original homogenate promptly, using pipets that deliver required volume accurately. Prepare all decimal dilutions with 9 ml of sterile diluent plus 1 ml of previous dilution, unless otherwise specified. Shake all dilutions vigorously.

Not more than 15 min should elapse from the time sample is blended until all dilutions are in appropriate media.

Isolation and enumeration of *S. aureus*

For each dilution to be plated, aseptically transfer 1 ml sample suspension to 3 plates of Baird-Parker agar, distributing 1 ml of inoculum equitably to 3 plates (e.g., 0.4 ml, 0.3 ml, and 0.3 ml). Spread inoculum over surface of agar plate, using sterile bent glass streaking rod. Retain plates in upright position until inoculum is absorbed by agar (about 10 min on properly dried plates). If inoculum is not readily adsorbed, place plates upright in incubator for about 1 h. Invert plates and incubate 45-48 h at 35-37°C. Select plates containing 20-200 colonies, unless only plates at lower dilutions (>200 colonies) have colonies with typical appearance of S. aureus.

Colonies of S. aureus are circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. Occasionally from various foods and dairy products, nonlipolytic strains of similar appearance may be encountered, except that surrounding opaque and clear zones are absent. Strains isolated from frozen or desiccated foods that have been stored for extended periods frequently develop less black coloration than typical colonies and may have rough appearance and dry texture.

Count and record colonies.

If several types of colonies are observed which appear to be S. aureus on selected plates, count number of colonies of each type and record counts separately. When plates of the lowest dilution contain <20 colonies, these may be used. If plates containing >200 colonies have colonies with the typical appearance of S. aureus and typical colonies do not appear at higher dilutions, use these plates for the enumeration of S. aureus, but do not count nontypical colonies. Select > 1 colony of each type counted and test for coagulase production. Add number of colonies on triplicate plates represented by colonies giving positive coagulase test and multiply by the sample dilution factor. Report this number as number of S. aureus/g of food tested.

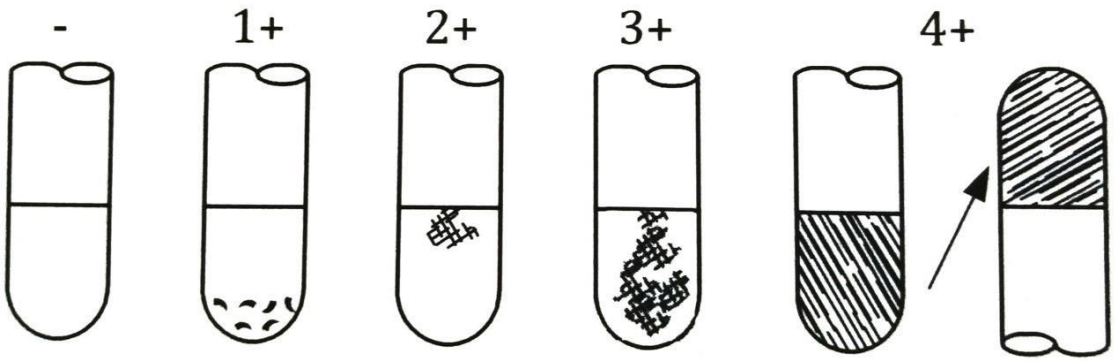
TYPICAL COLONY

ATYPICAL COLONY

TYPICAL COLONY CHARACTERISTICS	ATYPICAL COLONY CHARACTERISTICS
• Typical colonies are black or grey, shining, convex and are surrounded by a clear zone, which can be partially opaque.	• Shining black colonies with or without a narrow white edge; the clear zone is absent or barely visible and the opalescent ring is absent or hardly visible.
• 1 mm to 1.5 mm in dia after incubation for 24 h±2 h and 1.5 mm to 2.5 mm dia after incubation for 48 ±4 h	• Atypical colonies have the same size as typical colonies.
• After incubation for at least 24 h, an opalescent ring immediately in contact with the colonies can appear in this clear zone.	• The colonies can be grey in colour free of clear zone.

Coagulase test

Transfer suspect S. aureus colonies into small tubes containing 0.2-0.3 ml BHI broth and emulsify thoroughly. Inoculate agar slant of suitable maintenance medium, e.g., TSA, with loopful of BHI suspension. Incubate BHI culture suspension and slants 18-24 h at 35-37°C. Retain slant cultures at room temperature for ancillary or repeat tests in case coagulase test results are questionable. Add 0.5 ml reconstituted coagulase plasma with EDTA (B-4, above) to the BHI culture and mix thoroughly. Incubate at 35-37°C and examine periodically over 6 h period for clot formation. Only firm and complete clot that stays in place when tube is tilted or inverted is considered positive for S. aureus. Partial clotting, formerly 2+ and 3+ coagulase reactions, must be tested further (4). Test known positive and negative cultures simultaneously with suspect cultures of unknown coagulase activity. Stain all suspect cultures with Gram reagent and observe microscopically.



Score	Observation	Coagulase interpretation at 24 h
-	No clot	Negative
1+	Small unorganized clot(s)	Intermediate shall be confirmed by another method
2+	Intermediate small organized clot	Intermediate shall be confirmed by another method
3+	Large organized clot	Positive
4+	Entire contents of the tube coagulates and is not displaced when the tube is inverted	Positive