



STANDARDS ASSOCIATION OF AUSTRALIA

Australian Standard

METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOOD

PART 2—EXAMINATION FOR SPECIFIC ORGANISMS

AS 1766.2.4 COAGULASE-POSITIVE STAPHYLOCOCCI

1 SCOPE. This standard sets out methods for the detection and enumeration of coagulase-positive staphylococci in foods other than dairy products and eggs and egg products.

NOTE: Procedures for dairy products are given in AS 1095* and for eggs and egg products in AS 1142†.

2 APPLICATION. The methods are intended as reference methods suitable for checking that products comply with microbiological requirements specified in regulations.

3 REFERENCED DOCUMENTS. The following standards are referred to in this standard:

- AS 1766 Methods for the Microbiological Examination of Food
- AS 1766.1 General Procedures and Techniques
- AS 1766.3 Examination of Specific Products
- AS 1766.5 Preparation of Media, Diluents and Reagents

4 OUTLINE OF THE METHODS. Coagulase-positive staphylococci are isolated quantitatively using the surface spread method or qualitatively using the triplicate tube enrichment method. Production of coagulase is confirmed by means of the tube test.

5 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES.

5.1 Culture media. The following culture media are required:

- Baird-Parker (BP) medium.
- Giolitti and Cantoni (G&C) medium (see Appendix A).
- Brain heart infusion (BHI) broth.

5.2 Reagents. The following reagents are required:

- Sodium chloride solution, 8.5 g/L.
- Rabbit blood plasma, oxalated or heparinized but not citrated. When purchased in freeze-dried form, prepare as directed by the manufacturer.

5.3 Reference cultures. The following reference cultures are required:

- Staphylococcus aureus* NCTC 6571 (coagulase-positive).
- Staphylococcus epidermidis* NCTC 6513 (coagulase-negative).

6 TEST PROCEDURES.

6.1 Surface spread method. The procedure shall be as follows:

- Using the spread method described in AS 1766.1, inoculate prepared plates of BP medium with appropriate dilutions of the product under test.
- Prepare controls for each series of tests by streaking the two reference strains specified in Clause 5.3 on to separate sectors of a plate of BP medium.
- Incubate tests and controls at $37 \pm 1^\circ\text{C}$ for 48 h.
- Examine the plates for typical colonies of coagulase-positive staphylococci.

Count the number of typical colonies on the test plates.

NOTE: If colonies are insufficiently developed after 48 h, incubation may be continued to 72 h before counting.

- Confirm that typical colonies produce coagulase using the test described in Clause 7.
NOTE: Some coagulase positive strains may be egg yolk negative. Therefore, some non-typical colonies should also be selected for coagulase testing.
- From the proportion of selected colonies confirmed as coagulase producers and the dilution factor, estimate the number of coagulase-positive staphylococci per millilitre or gram of the test sample.

6.2 Triplicate tube enrichment method. The procedure shall be as follows:

- To each of three tubes containing 19 mL of G&C medium, add 1 mL of a suitable dilution of the sample containing the amount of product to be tested, or where appropriate, 1 g of the original undiluted sample.
- Inoculate the two reference cultures specified in Clause 5.3 into separate tubes of G&C medium.
- Seal the inoculated tubes with a layer, 2 cm to 3 cm thick, of melted 2 percent agar cooled to 45°C , incubate at $37 \pm 1^\circ\text{C}$ and examine for black discolouration after 24 h and 48 h.
- From each tube showing a black discolouration or a black precipitate, streak a loopful on to prepared plates of BP medium. Also prepare plates from the two reference G&C broth cultures.

* AS 1095, Microbiological Methods for the Dairy Industry

† AS 1142, Methods for the Microbiological Examination of Eggs and Egg Products