Enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)
National Infection Service
Food Water and Environmental Microbiology
Standard Method

FNES8 [F12]

Issued by
PHE National Infection Service
Food, Water & Environmental Microbiology
Methods Working Group
About Public Health England

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Public Health England
Wellington House
133-155 Waterloo Road
London SE1 8UG
Tel: 020 7654 8000
http://www.gov.uk/phe
Twitter: @PHE_uk
Facebook: www.facebook.com/PublicHealthEngland

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Status of National Infection Service Food, Water and Environmental Microbiology methods

These methods are well referenced and represent a good minimum standard for food, water and environmental microbiology. However, in using Standard Methods, laboratories should take account of local requirements and it may be necessary to undertake additional investigations.

The performance of a standard method depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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Citation for this document:
Amendment history

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<tr>
<td>10</td>
<td>5 Culture media and reagents</td>
<td>Removed reference to D1 and replaced with FNES26 (F2). (CR9203)</td>
</tr>
<tr>
<td>11</td>
<td>6.1 Sample preparation and wording</td>
<td>Removed repeated wording and incorrect citation to reference. (CR9203)</td>
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<td>Hazards noted colour coded (CR9204)</td>
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<td>9.0 Reporting of results</td>
<td>Removed repeated wording and incorrect citation to reference. (CR9203)</td>
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<td>Amended citation reference. (CR9203)</td>
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<td>10.0 Reference facilities and referral of cultures</td>
<td>Removed phage typing and replaced with molecular typing. (CR9205)</td>
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<td>21</td>
<td>References</td>
<td>Correction to link to reference 7 made. (CR9203) Update to references made</td>
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Introduction

Scope

The method described is applicable to the enumeration of coagulase-positive staphylococci in all food types, dairy products and environmental samples using a surface colony count technique and incubation at 37°C.

Background

The coagulase-positive staphylococci include *Staphylococcus aureus*, *S. hyicus* and *S. intermedius*. While *S. hyicus* and *S. intermedius* are mainly veterinary pathogens, *S. aureus* is a predominantly human pathogen. The majority of cases of staphylococcal food poisoning are due to the production of pre-formed enterotoxin in a food. Although most cases of infection are due to *S. aureus*, other coagulase-positive staphylococci can also produce enterotoxin and cause foodborne disease.

The presence of coagulase-positive staphylococci in ready-to-eat food is generally considered to be unsatisfactory if the count is equal to or greater than $10^4$ colony forming units per gram (CFU per g). Counts between 20 and less than or equal to $10^4$ CFU per g represent borderline quality. Low numbers indicate poor handling whereas high counts may be associated with toxin production and food poisoning. This method allows for the enumeration of *S. aureus* and other coagulase-positive staphylococci at counts of 10 CFU per g or more. The method is based on BS EN ISO 6888-1:1999 + A1:2003 and is also described in Practical Food Microbiology. EC Regulation 2073:2005 requires the examination of certain dairy and shellfish products for coagulase-positive staphylococci.

This method differs from BS EN ISO 6888-1:1999 + A1:2003 in the following ways:

<table>
<thead>
<tr>
<th>Culture media</th>
<th>PHE method F12</th>
<th>BS EN ISO 6888-1:1999 + A1:2003</th>
<th>Justification for variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sulfamethazine included in Baird Parker medium (BPM)</td>
<td>Baird Parker agar includes sulfamethazine if Proteus spp. expected in sample</td>
<td>Use of BS EN ISO 6888-1 most appropriate for the majority of sample received in PHE laboratories. Additional</td>
<td></td>
</tr>
</tbody>
</table>
| **Enumeration of coagulate-positive staphylococci**  
*(Staphylococcus aureus* and other species) |
|---|

<table>
<thead>
<tr>
<th><strong>PHE method F12</strong></th>
<th><strong>BS EN ISO 6888-1:1999 +A1:2003</strong></th>
<th><strong>Justification for variation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>samples</td>
<td>Use of 6888-2 recommended for products made from raw milk and raw meat</td>
<td>dilution performed on samples likely to have a high background.</td>
</tr>
<tr>
<td><strong>Inoculum volume</strong></td>
<td>Either a 0.5 mL spread plate, a 50 µL spiral plate or 2 x 0.5 mL spread plates, depending on the detection limit required</td>
<td>Duplicate 0.1 mL spread plates or duplicate volumes of 1 mL distributed over 3 plates</td>
</tr>
<tr>
<td><strong>Incubation</strong></td>
<td>Plates incubated for 48 ± 4 h (but may be examined at 24 ± 2 h where high levels of background flora is likely)</td>
<td>Plates incubated for 24 ± 2 h and then a further 24 ± 2 h</td>
</tr>
<tr>
<td><strong>Incubation</strong></td>
<td>Plates incubated at 37°C</td>
<td>Plates may be incubated at 35 or 37°C</td>
</tr>
<tr>
<td><strong>Confirmation procedures</strong></td>
<td>Confirmation is by the tube coagulase test for Official Control samples, but by DNase and slide coagulase tests only for other sample types.</td>
<td>Confirmation is by the tube coagulase test, but recognises weak reactions other methods including thermostable nucleases are allowed</td>
</tr>
<tr>
<td><strong>Confirmation procedure</strong></td>
<td>Tube coagulase method specified is specific for Remel coagulase plasma reagent</td>
<td>Reagent is not specified</td>
</tr>
<tr>
<td><strong>Formulation of Blood Agar</strong></td>
<td>Horse blood is used in blood agar</td>
<td>Sheep blood is specified</td>
</tr>
</tbody>
</table>
1. Principle

The enumeration of coagulase-positive staphylococci by this method involves inoculation of the surface of a selective agar medium with a specified volume of a $10^{-1}$ dilution and other appropriate decimal dilutions of the test sample followed by incubation at $37^\circ C$ for 48 h. Calculation of the number of coagulase-positive staphylococci (CFU per g or mL or sample) is made from the number of typical and/or atypical colonies obtained on the selective medium and subsequently confirmed by coagulase and DNase testing. Isolates from Official Control samples are confirmed by tube coagulase, whilst those from other sample types are confirmed using the DNase and slide coagulase tests.

2. Definitions

For the purpose of this method, the following definitions apply:

Coagulase-positive staphylococci
Bacteria which form typical and/or atypical colonies on the surface of the selective agar medium described in this method and which show positive reactions in the confirmatory tests specified.

Enumeration of the coagulase-positive staphylococci
Determination of the number of coagulase-positive staphylococci found per millilitre or per gram of sample (or per cm$^2$, per swab or per cloth) when the test is carried out in accordance with this method.

3. Safety considerations

3.1 General safety considerations

Normal microbiology laboratory precautions apply
All laboratory activities associated with this SOP must be risk assessed to identify hazards. Appropriate controls must be in place to reduce the risk to staff or other groups. Staff must be trained to perform the activities described and must be provided with any personal protective equipment (PPE) specified in this method. Review of this method must also include a review of the associated risk.
assessment to ensure that controls are still appropriate and effective. Risk assessments are site specific and are managed within safety organiser.

**Information Note:** Throughout this method hazards are identified using **red text**. Where a means of controlling a hazard has been identified this is shown in **green text**.

### 3.2 Specific safety considerations

**Safety glasses** and **protective gloves** must be worn when handling **hydrochloric acid** used for the DNase test.

### 3.3 Laboratory containment

All procedures can be performed in a containment level 2 (CL2) laboratory.

### 4. Equipment

Usual laboratory equipment and in addition:

- top pan balance capable of weighing to 0.1g
- gravimetric diluter (optional)
- stomacher
- vortex mixer
- spiral plater (optional)
- incubator: 37 ± 1°C
- colony counter (optional)
- stomacher bags (sterile)
- automatic pipettors and associated sterile plugged pipette tips capable of delivering up to 10 mL and 1 mL amounts (optional)
- pipettes (sterile total delivery) 10 mL and 1 mL graduated in 0.1 mL volumes (optional)
- spreaders - sterile, disposable
- sterile round-bottom tubes
5. Culture media and reagents

Equivalent commercial dehydrated media may be used; follow the manufacturer’s instructions.

Peptone saline diluent (Maximum recovery diluent)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>8.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
<tr>
<td>pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

Buffered peptone water (optional)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of casein</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate dodecahydrate or anhydrous disodium hydrogen phosphate</td>
<td>9.0 g or 3.5 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
<tr>
<td>pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>

**Information Note:** Additional diluents may be required for dairy products please refer to SOP FNES26 (F2)\(^8\) for media formulations.

Baird-Parker agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Meat extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>10.0 g</td>
</tr>
<tr>
<td>L-Glycine</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Egg yolk emulsion</td>
<td>50 mL</td>
</tr>
<tr>
<td>Potassium tellurite</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
<tr>
<td>pH 6.8 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>
Blood agar
Columbia agar base with 5% horse blood

DNase agar

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Deoxyribonucleic acid</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

pH 7.3 ± 0.2 at 25°C

Brain Heart Infusion broth (BHI)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of animal tissues</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Dehydrated calf brain infusion</td>
<td>12.5 g</td>
</tr>
<tr>
<td>Dehydrated beef heart infusion</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Di sodium hydrogen phosphate anhydrous</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

pH 7.4±0.2 at 25°C

Hydrochloric acid (1N)

Staphylococcus latex test kit (commercially available)

Rabbit plasma
Commercially available dehydrated rabbit plasma e.g. Remel Coagulase Plasma (R21052) rehydrated and used according to manufacturer instructions
6. Sample processing

6.1 Sample preparation and dilutions

Following the procedure described in Standard Method FNES26 (F2)\(^8\)
Preparation of samples and dilutions, plating and sub-culture, prepare a 10\(^{-1}\) homogenate of the sample in either peptone saline diluent (PSD) or buffered peptone water (BPW) and further decimal dilutions as required in PSD. For swabs refer to Standard Method FNES4 (E1)- Detection and Enumeration of Bacteria in Swabs and Other Environmental Materials\(^9\).

Homogenise for between 30 seconds and three minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions and the type of sample being examined.

6.2 Inoculation and incubation

If possible, dilutions should be selected that will give colony counts of between 10 and 300 colonies per plate.

Starting with the highest dilution inoculate 0.5 mL of each dilution onto the centre of a dried Baird Parker (BP) plate. If a detection limit of 10 CFU per g is required inoculate two plates each with 0.5 mL of the initial 10\(^{-1}\) dilution. Liquid samples may be inoculated directly onto agar plates without prior dilution. Using a sterile spreader and starting with the highest dilution, spread the inoculum carefully over the surface of each plate as soon as possible taking care not to touch the sides of the plate. If testing samples for the purpose of official control or in the case of formal samples inoculate all dilutions in duplicate.

If high counts of coagulase-positive staphylococci (greater than 10\(^3\) CFU per g) are expected, use a spiral plater to inoculate 50 µL of each dilution onto BP plates.

Plating of the medium with the test portion must be performed within 45 minutes of preparation of the sample homogenate.

Leave the plates in an upright position on the bench for 15 minutes or until the inoculum has been absorbed into the surface of the agar. Invert the plates so that the bottom is uppermost and place in an incubator at 37 ± 1 °C for 48 ± 4 h. If high
levels of background flora are likely, plates may be examined at 24 ± 2 h and again after a further 24 ± 2 h.

6.3 Counting of colonies

Examine the plates for typical colonies of coagulase-positive staphylococci.

Typical colonies are black or grey, shiny and convex (1 - 1.5 mm diameter after incubation for 24 h and 1.5 - 2.5 mm after 48 h), surrounded by a zone of clearing. After at least 24 h incubation an opalescent ring may appear in this zone immediately in contact with the colonies. Count and record the number of typical colonies.

Atypical colonies of coagulase-positive staphylococci may occur, particularly in dairy products, shrimps and giblets. For foods of this type, also count and record atypical colonies.

These may present as:

- shining black colonies with or without a narrow white edge (the zones of clearing and opalescence are absent or barely visible)
- grey colonies free of clear zones

Spread plates
Take for enumeration only those plates that contain a maximum of 300 colonies with up to 150 typical colonies.

Spiral plates
Count the number of colonies on the plates either manually, in conjunction with a viewing grid, or using an automated colony counter.

If counting manually, centre the plate over the counting grid ensuring that appropriate lighting is available. Choose any segment and count the colonies from the outer edge into the centre until 20 colonies have been counted. Continue to count the remaining colonies in the subdivision of the segment containing the twentieth colony. For colonies on the dividing line count the colonies on the outermost line of the segment and on one side only. Record this count together with the number assigned to the subdivision of the segment (eg “4a”).
Count in the same area on the opposite side of the plate and record the count. If there are less than 20 colonies in the entire segment count all colonies on the plate. Calculate the count per mL of dilution plated by adding together the counts from the two segments (or total plate) and dividing the total by the volume constant for the segment counted. Alternatively, use the tables supplied by the manufacturer. If there are less than 35 colonies on the total plate and a manually inoculated surface spread plate is not available report the result as less than 7x10³.

6.4 Confirmation tests

Subculture five colonies of each type (or all colonies if less than five) for confirmatory testing.

6.4.1 Routine samples

Confirm identity of suspect colonies using DNase and coagulase production. Spot inoculate each colony onto a segment of a DNase agar plate and spread for discrete colonies onto a segment of a blood agar plate. Transfer the plates to an incubator at 37 ± 1°C for 21 ± 3 h. Examine the blood agar plates for purity and colonial morphology consistent with coagulase-positive staphylococci i.e. cream or golden colonies, up to 3 mm in diameter.

**DNase production**

Wearing gloves and safety glasses, flood the DNase plate with normal hydrochloric acid (HCl). After about 30 seconds, discard the excess HCl into a chemical waste container. A positive reaction is shown by a defined zone of clearing surrounding the inoculated spot.

**Staphylococcus latex agglutination**

Wearing gloves and safety glasses and using the growth from blood agar, perform a staphylococcus latex agglutination test on the colonies giving a positive DNase result, following the kit manufacturer’s instructions.

Colony types are confirmed as coagulase-positive staphylococci if they show typical colonial morphology on blood agar and give positive reactions in the staphylococcus latex agglutination test. If colonies give a positive DNase reaction but negative latex agglutination result, perform a tube coagulase test as described below. A positive tube coagulase test confirms the colonies as coagulase-positive staphylococci.
6.4.2 Outbreak, formal and Official Control samples

Confirm the identity of colonies using the tube coagulase method.

**Tube coagulase**
Inoculate each colony into 5-10 mL Brain Heart Infusion (BHI) broth and incubate at 37 ± 1°C overnight. Reconstitute the Remel coagulase plasma by adding 5 mL of sterile water and mixing gently until clear. Transfer 0.5 mL of this to the required number of sterile round bottom tubes. Aseptically add 0.5 mL of culture to the tubes. Incubate at 37 ± 1°C. Examine tubes for clotting every 30 minutes for 4 h by carefully tilting the tubes: if negative after 4 h, re-examine at 24 h. The test is positive if the volume of the clot occupies more than half of the original volume of the liquid. If using coagulase plasma from other suppliers follow the manufacturer instructions.

For Official Control purposes, confirmation is based on a positive tube coagulase reaction. However, it is recognised that some strains of *Staphylococcus aureus* give a weakly positive coagulase reaction. In these cases, a positive DNase reaction should be considered to confirm their identity as coagulase-positive staphylococci.

7. Quality control

Quality control of media and internal quality assurance checks should be performed according to in-house procedures using the following control organisms:

Positive control:
S. *aureus* (Oxford strain)  NCTC 6571

Negative control:
S. *epidermidis*  NCTC 11047
8. Calculation of results

Calculations occur automatically in the StarLims system as described in National Method FNES6 (Q12) Sample processing and result entry in StarLims\textsuperscript{10}. Calculations are performed as described below.

8.1 Calculation of results from routine samples.

Calculate the number of coagulase positive Staphylococci per g as follows:

\[
\text{Count per g} = \frac{\text{No. of colonies confirmed}}{\text{No. of colonies tested}} \times \frac{\text{Presumptive count}}{\text{Volume tested x dilution}}
\]

For spiral plates, calculate the Colony Count per mL of dilution plated by adding together the counts from the two segments and dividing the total by the volume constant for the segment counted. Alternatively, use the tables supplied by the manufacturer. To obtain the colony count per g or mL multiply the count by the dilution factor.

**Information Note:** For samples showing less than 35 colonies on a plate the confidence limits will be wide and it is recommended that the result is reported as less than $7 \times 10^3$ CFU per gram, per mL or other sample portion.

8.2 Calculation of results from formal or official control samples.

For a result to be valid, it is considered necessary to count at least one dish containing a minimum of 15 colonies. Use the plate counts to calculate $N$, the Coagulase positive Staphylococci count in the test sample per millilitre or per g, as the weighted mean from two successive dilutions using the following equation:

\[
N = \frac{\sum a}{V (n_1 + 0.1n_2) d}
\]

when:
Enumeration of coagulase-positive staphylococci
(Staphylococcus aureus and other species)

\[ \Sigma a \] is the sum of the colonies counted on all the plates retained from two successive dilutions, at least one of which contains a minimum of 15 CFU

\[ n_1 \] is the number of plates counted at the first dilution

\[ n_2 \] is the number of plates counted at the second dilution

\[ d \] is the dilution from which the first counts were obtained \([d = 1 \text{ in the case (liquid products) where the directly inoculated test sample is retained, } d = 0.1 \text{ for } 10^{-1} \text{ dilution } \text{etc}]\)

\[ V \] is the volume of the inoculum, in millilitres, applied to each plate

Round off the result to two significant figures.

8.3 Estimation of counts in formal or official control samples (low numbers).

If both dishes at the level of the first retained dilution contain less than 15 confirmed colonies, calculate \( N_E \), the estimated Coagulase positive Staphylococcus count present in the test sample, as the arithmetical mean from two parallel plates using the following equation:

\[
N_E = \frac{\Sigma a}{V \cdot n \cdot d}
\]

when:

\[ \Sigma a \] is the sum of the confirmed colonies counted on the two plates

\[ n \] is the number of plates retained

\[ d \] is the dilution from which the first counts were obtained \([d = 1 \text{ in the case (liquid products) where the directly inoculated test sample is retained, } d = 0.1 \text{ for } 10^{-1} \text{ dilution } \text{etc}]\)

\[ V \] is the volume of the inoculum, in millilitres, applied to each plate

Information Note: Estimated counts (8.3) should be reported with the comment “Count Estimated due to low numbers”
9. Reporting of results

All results are reported using the StarLims system as described in method FNES17 (Q13) Technical Validation and release of result in StarLims. The test report must specify the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result.

**Information note:** Where high levels of background flora are present, masking of coagulase positive staphylococci may occur. Where the plates from a $10^{-1}$ show heavy growth of background flora and further dilutions eg $10^{-1}$ spiral show no evidence of coagulase positive staphylococci the LOD reported must reflect that it was not possible to read the result at lower dilutions.

Report as:

Coagulase Positive-Staphylococci

9.1 Lower detection limit

**Liquid products**

Plates prepared from the undiluted ($10^0$) product are found to contain no colonies, report the result as:

Not Detected CFU per mL

**Solid food products**

If no colonies are present, report as:

Less than 10 CFU per g or mL (2 x 0.5 ml surface spread using a $10^{-1}$ dilution)

OR

Less than 20 CFU per g or mL (1 x 0.5 ml surface spread using a $10^{-1}$ dilution)

OR

Less than $7.0 \times 10^3$ CFU per g or mL (50 µl spiral plate using a $10^{-1}$ dilution)
Swabs and cloths
The lower limit of detection may vary, depending on the quantity of diluent used in the preparation of the sample. Care must be taken when reporting these results to ensure that the appropriate dilution factor is used in the calculation of results. Guidance on the calculation for results from swabs and other materials can be obtained from Standard Method FNES4 E1- Detection and Enumeration of Bacteria in Swabs and other Environmental Materials⁹.

9.2 Upper detection limit

If count is 100 or more, report counts with one figure before and one figure after the decimal point in the form of:

\[ a \times 10^b \text{ CFU per g or mL} \]

where a is never less than 1.0 or greater than 9.9 and b represents the appropriate power of ten. Round counts up if the last figure is 5 or more and down if the last figure is 4 or less.

eg: 1920 CFU per g = 1.9 x 10³ CFU per g, 235,000 CFU per g = 2.4 x 10⁵ CFU per g (where single 0.5 mL plate used)

If there are only plates containing more than 150 typical colonies or a total of 300 colonies (typical and atypical) report as greater than the upper limit for the test dilution used with the comment “Count too high to be estimated at dilution used”. Where the estimated count does not allow interpretation of the result referral of the isolate, testing of the food for the presence of enterotoxin and submission of a repeat sample should be considered.
10. Reference facilities and referral of cultures

In certain circumstances, it may be necessary to investigate isolates further. Examination of the food for the presence of enterotoxin may also be appropriate.

For outbreaks it is recommended that three separate colonies from each positive sample are referred for molecular typing and enterotoxin characterization. Referral of the original food sample for toxin testing is also recommended in these circumstances.

In the event that a routine or official control sample fails to comply with EC regulations or is considered to be unsatisfactory: potentially injurious to health or unfit for human consumption (e.g. greater than $10^4$ CFU per g), one isolate from each positive sample may be referred for further characterisation as appropriate.

Reference facilities are available at the following national reference laboratories:

Enterotoxin Testing.
Gastrointestinal Bacteria Reference Laboratory, PHE, Colindale

**Information note:** The reference laboratory tests referred isolates for enterotoxin by PCR. If detection of formed enterotoxin in food remnants is required please seek advice from GBRU.

A request form for referral to reference facilities can be obtained using the following link:

11. Acknowledgements and contacts

This Standard Method has been developed, reviewed and revised by Microbiology Services, Food, Water and Environmental Microbiology Methods Working Group.

The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

For further information please contact us at:

Public Health England
National Infection Service
Food Water & Environmental Microbiology Laboratories
Central Office
Colindale
London
NW9 5EQ

E-mail: fwelabs@phe.gov.uk
References


Appendix: Flowchart showing the process for performing the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)

1. Prepare a $10^{-1}$ dilution of sample
2. Homogenise by stomaching
3. Prepare any further dilutions in peptone saline diluent if required
4. Starting with the highest dilution inoculate 0.5 mL of each dilution onto the centre of a Baird Parker plate (and/or use spiral plater). For official control samples and formal samples inoculate plates in duplicate
5. Spread the inoculum across each plate using a sterile spreader and leave to absorb for 15 min
6. Incubate at 37°C for 48 hours in aerobic conditions
7. Count typical colonies (and atypical colonies for foods of bovine origin)
8. Subculture five suspect colonies onto DNase agar and blood agar and incubate at 37°C for 21 ± 3 h.
9. Check blood agar for typical morphology and flood DNase agar with HCL
10. Perform latex agglutination and/or tube coagulase test (formal samples) on DNase positive colonies. DNase positive colonies that give a negative latex test should be re-tested using the tube coagulase test
11. Calculate the total coagulase-positive staphylococci count per g, per mL, per swab, per cloth