

NATIONAL STANDARD METHOD

# IDENTIFICATION OF *STAPHYLOCOCCUS* SPECIES, *MICROCOCCUS* SPECIES AND *ROTHIA* SPECIES

BSOP ID 7

Issued by Standards Unit, Evaluations and Standards Laboratory  
Centre for Infections



Association of Medical Microbiologists  
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IDENTIFICATION OF *STAPHYLOCOCCUS* SPECIES, *MICROCOCCUS* SPECIES AND *ROTHIA* SPECIES  
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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>BSOP ID 7</b>
<b>Controlled document title</b>	<b>Identification of <i>Staphylococcus</i> species, <i>Micrococcus</i> species and <i>Rothia</i> species</b>

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On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
3/ 14/09/2007	2	2.1	1	<b>Front Page</b>	Northern Ireland logo added
			All	<b>Whole document</b>	Document put in to a standard format
			All	<b>All</b>	PDF links inserted to cross- reference NSM documents

# IDENTIFICATION OF *STAPHYLOCOCCUS* SPECIES, *MICROCOCCUS* SPECIES AND *ROTHIA* SPECIES

## SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the procedure for the identification and differentiation of *Staphylococcus aureus*, *Staphylococcus* species, *Micrococcus* species and *Rothia* species. For the identification of catalase-negative Gram-positive cocci see [BSOPID 4 - Identification of Streptococcus Species, Enterococcus Species and Morphologically Similar Organisms](#)

## INTRODUCTION

The staphylococci most frequently associated with human infection are *S. aureus*, *S. epidermidis* and *S. saprophyticus*<sup>2</sup>. Other *Staphylococcus* species may also be associated with human infection<sup>3,4</sup>.

### Taxonomy<sup>5</sup>

More than thirty species of staphylococci have been recognised, most of which are found only in lower mammals. *Staphylococcus aureus* is coagulase positive; *Staphylococcus intermedius*, *Staphylococcus hyicus* and *Staphylococcus schleiferi* may also be coagulase positive. The coagulase-negative staphylococci (CNS) can be divided into six major groups, but the species found on humans are located within only two of those groups.

### Characteristics

*Staphylococcus* species are Gram-positive, non-motile, non-spore-forming cocci occurring singly, in pairs and in irregular clusters: size may be variable. Colonies are opaque and may be white or cream and are occasionally yellow or orange. The optimum growth temperature is 30°C - 37°C. They are facultative anaerobes and have a fermentative metabolism. *Staphylococcus* species are usually catalase-positive and oxidase-negative. Nitrate is often reduced to nitrite. Some species are susceptible to lysis by lysostaphin but not by lysozyme and are usually able to grow in 10% sodium chloride. Some species produce extracellular toxins<sup>6</sup>. Staphylococci may be identified by the production of deoxyribonuclease (DNase) and/or a heat-stable DNase (thermostable nuclease).

### Coagulase-positive staphylococci

#### *Staphylococcus aureus*

*Staphylococcus aureus* is a primary pathogen, which may be associated with severe infection and it is important to distinguish it from the opportunistic coagulase-negative staphylococci. In routine laboratory practice, the production of coagulase is frequently used as the sole criterion to distinguish *S. aureus* from other staphylococci. Other coagulase-positive staphylococcal species such as *S. hyicus*, *S. schleiferi* subspecies *coagulans* or *S. intermedius* may be coagulase positive but have been found only occasionally in human infection or carriage. The production of coagulase and thermostable nuclease by these staphylococci may lead to their misidentification as *S. aureus*. It is also important to note that coagulase-negative strains of *S. aureus* have been reported<sup>7</sup>.

*S. aureus* subspecies *anaerobius* is rarely isolated from clinical specimens. It grows poorly aerobically and growth may be CO<sub>2</sub> dependent. It is slide coagulase-negative and thermonuclease-negative. It may be catalase-negative. Strains may be identified by better growth anaerobically and they may give a positive coagulase test result. However, because growth may be poor the coagulase result may be negative and suspected isolates should be referred to the Reference Laboratory.

*S. hyicus* may be coagulase-positive (11 - 89% of strains) and thermostable nuclease-positive. *S. intermedius* is coagulase-positive and thermostable nuclease-positive. *S. schleiferi* subspecies

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*coagulans* is coagulase-positive and thermostable nuclease-positive, and subspecies *schleiferi* is coagulase-negative and thermostable nuclease-positive.

*S. aureus* produces virulence factors such as protein A, capsular polysaccharides and  $\alpha$  toxin. Some strains of *S. aureus* produce toxic shock syndrome 1 toxin (TSST-1), Panton Valentine Leucocidin or other toxins. Multi-resistance to antibiotics may be associated with methicillin resistant strains. It is thermostable nuclease-positive.

### **Coagulase negative staphylococci<sup>8</sup>**

The CNS are opportunistic pathogens which lack many of the virulence factors associated with *S. aureus*. There are more than 30 species of CNS. *S. epidermidis* and *S. saprophyticus* are the species most often associated with infection but *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus lugdenensis*, *S. schleiferi* subspecies *schleiferi*, *Staphylococcus simulans* and *Staphylococcus warneri* have also been implicated<sup>9,10</sup>. Many of these species are also thermostable nuclease-negative. Multi-resistance is associated with some strains of *S. epidermidis* which is thermostable nuclease-negative. *S. haemolyticus* is often multi-resistant and frequently demonstrates reduced susceptibility to teicoplanin<sup>11</sup>. *S. saprophyticus* is novobiocin resistant. *Staphylococcus pasteurii* can be phenotypically distinguished from all of the other novobiocin-susceptible staphylococci except *S. warneri*, from which it can only be differentiated by genotyping<sup>12</sup>.

*S. saccharolyticus* was previously known as *Peptococcus saccharolyticus*.

### **Micrococcus species**

*Micrococcus* species are strictly aerobic. *Micrococcus luteus* produces yellow colonies. Cells are Gram-positive cocci arranged in tetrads. Micrococci may be distinguished from staphylococci by a modified oxidase test<sup>13,14</sup>. *Staphylococcus* species, with the exception of *S. sciuri*, *S. lentus* and *S. vutulus* are oxidase-negative and *Micrococcus* species are oxidase-positive.

### **Rothia species**

*Rothia* species are weakly catalase-positive. Growth is facultatively anaerobic. The species associated with infection is *Rothia mucilaginosus* which was previously known as *Micrococcus mucilaginosus* or *Staphylococcus salivarius*<sup>15</sup>.

### **Principles of identification**

*Staphylococcus aureus* has traditionally been identified by tube coagulase tests that detect staphylocoagulase or "free coagulase". However, detection of surface proteins such as clumping factor (slide coagulase test) and/or protein A (commercial latex tests) may be used for rapid identification. Inclusion of latex particles sensitized with antibodies against specific capsular antigens has enabled commercial manufacturer's to improve the sensitivity of latex tests to detect atypical strains of *S. aureus* and MRSA that fail to express the major characteristics listed above<sup>16</sup>. Positive results or suspected erroneous slide tests may be confirmed by a tube coagulase test.

## **TECHNICAL INFORMATION**

N/A

# 1 SAFETY CONSIDERATIONS<sup>17-27</sup>

Refer to current guidance on the safe handling of all organisms documented in this NSM.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

# 2 TARGET ORGANISMS

## **Staphylococcus species reported to have caused human infection**<sup>2-6,8-13,16,28-61</sup>

<b>Species</b>		<b>subspecies</b>	
<i>Staphylococcus aureus</i>	<i>aureus</i>	<i>aureus</i>	
<i>Staphylococcus aureus</i>	<i>aureus</i>	<i>anaerobius</i>	
<i>Staphylococcus epidermidis</i>	<i>epidermidis</i>		] <i>S. epidermidis</i> group
<i>Staphylococcus capitis</i>	<i>capitis</i>	<i>capitis</i>	
<i>Staphylococcus capitis</i>	<i>capitis</i>	<i>ureolyticus</i>	
<i>Staphylococcus hominis</i>	<i>hominis</i>	<i>hominis</i>	
<i>Staphylococcus hominis</i>	<i>hominis</i>	<i>novobiosepticus</i>	
<i>Staphylococcus haemolyticus</i>	<i>haemolyticus</i>		
<i>Staphylococcus lugdunensis</i>	<i>lugdunensis</i>		
<i>Staphylococcus saccharolyticus</i>	<i>saccharolyticus</i>		
<i>Staphylococcus warneri</i>	<i>warneri</i>		
<i>Staphylococcus saprophyticus</i>	<i>saprophyticus</i>		] <i>S. saprophyticus</i> group
<i>Staphylococcus cohnii</i>	<i>cohnii</i>	<i>cohnii</i>	
<i>Staphylococcus cohnii</i>	<i>cohnii</i>	<i>ureolyticus</i>	
<i>Staphylococcus caprae</i>	<i>caprae</i>		
<i>Staphylococcus hyicus</i>	<i>hyicus</i>		
<i>Staphylococcus intermedius</i>	<i>intermedius</i>		
<i>Staphylococcus schleiferi</i>	<i>schleiferi</i>	<i>coagulans</i>	
<i>Staphylococcus schleiferi</i>	<i>schleiferi</i>	<i>schleiferi</i>	
<i>Staphylococcus simulans</i>	<i>simulans</i>		

## **Other species reported to have caused human infection**<sup>15,62-70</sup>

*Micrococcus luteus*  
*Rothia mucilagenosus*

## 3 IDENTIFICATION

### 3.1 MICROSCOPIC APPEARANCE

**Gram stain** ([BSOFTP 39 - Staining Procedures](#))

Gram-positive cocci occurring singly, in pairs, tetrads and in irregular clusters.

### 3.2 PRIMARY ISOLATION MEDIA

Blood agar 16 - 48 h incubation in 5 - 10% CO<sub>2</sub> at 35°C - 37°C

These organisms may be isolated from other media including CLED, Staph/Strep selective and Mannitol Salt agar (MSA).

### 3.3 COLONIAL APPEARANCE

Colonies of *Staphylococcus* species are usually opaque and may be white or cream and sometimes yellow to orange on blood agar. Haemolysis may be detected. They appear as yellow-green, 1 - 2 mm, lactose-fermenting colonies on CLED. *Micrococcus* species produce yellow or red-pigmented colonies on blood agar. *Rothia* species are round, convex, mucoid and adhere to the agar. Colonial morphology varies with species and is not fully described here.

### 3.4 TEST PROCEDURES

**Catalase test** (see [BSOFTP 8 - Catalase Test](#))

*Staphylococcus*, *Micrococcus* and *Rothia* species are catalase-positive.

*S. aureus* subspecies *anaerobius* and *S. capitis* may be catalase-negative.

**Coagulase and other tests to detect *S. aureus*** (see [BSOFTP 10 - Coagulase Test](#))

Protein A, clumping factor (slide coagulase or latex), thermostable nuclease or tube coagulase tests may be used. Positive results or suspected erroneous slide tests (listed above) may be confirmed by a tube coagulase test.

*S. aureus*, some strains of *S. hyicus*, *S. intermedius*, and *S. schleiferi* subspecies *coagulans* are coagulase-positive and thermostable nuclease-positive. Other species of staphylococci are coagulase-negative and thermostable nuclease-negative or weak positive.

**Modified oxidase test** (see [BSOFTP 26 - Oxidase Test](#))

A 6% solution of tetra-methyl-phenylene-diamine in dimethyl sulphoxide may be used to differentiate micrococci from most staphylococci.

**Lysostaphin test**

**Commercial identification kit**

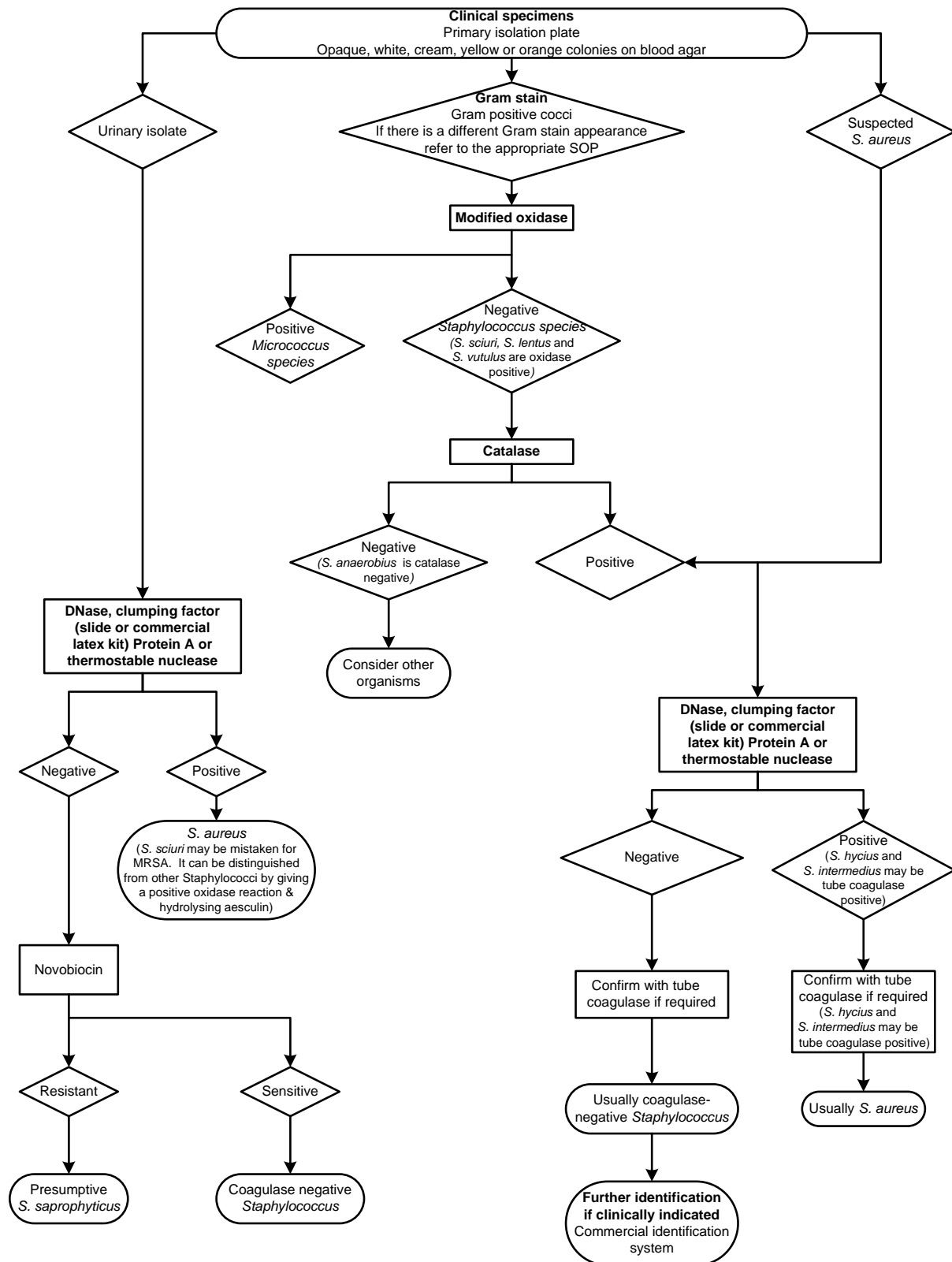
### 3.5 FURTHER IDENTIFICATION

N/A

### 3.6 STORAGE AND REFERRAL

If required, save pure isolate on a nutrient agar slope for referral to the Reference Laboratory.

# 4 PRESUMPTIVE IDENTIFICATION OF STAPHYLOCOCCUS SPECIES – FLOW CHART



## 5 REPORTING

### 5.1 PRESUMPTIVE IDENTIFICATION

If appropriate growth characteristics, colonial appearance, Gram stain of the culture, catalase and slide coagulase or latex agglutination results are demonstrated.

**NOTE:** *S. hyicus*, *S. intermedius* and *S. schleiferi* may be tube coagulase positive.

### 5.2 CONFIRMATION OF IDENTIFICATION

Following confirmatory coagulase test results.

### 5.3 MEDICAL MICROBIOLOGIST

Inform the medical microbiologist of presumptive and confirmed *Staphylococcus aureus* when the request card bears relevant information, eg:

- toxin mediated phenomena (eg Toxic Shock Syndrome, scalded skin syndrome, epidermal necrolysis, bullous impetigo, necrotising pneumonia, food poisoning)
- history of substance abuse, alcoholism, immunodeficiency or other serious underlying disorder such as cancer, or patients receiving treatment for cancer (neutropenia and/or mucositis)
- outbreaks or instances of cross-infection

The medical microbiologist should also be informed of presumptive and confirmed isolates of *Staphylococcus* species under the following circumstances:

- osteomyelitis and septic arthritis
- infections involving indwelling medical devices, eg prosthetic valves, pacemakers, CSF shunts, peritoneal or vascular catheters
- endocarditis, haematogenous dissemination of infection, septicaemia
- serious soft-tissue infections (cellulitis, erysipelas, necrotising myofasciitis, puerperal sepsis, surgical wound infection, pneumonia, peritonitis, meningitis, formation of abscesses or empyemas)

All isolates of multi-drug resistant *S. aureus*, including MRSA, should be brought to the attention of the medical microbiologist

Follow local protocols for reporting to clinician.

### 5.4 CCDC

Refer to local Memorandum of Understanding.

### 5.5 CENTRE FOR INFECTIONS<sup>71</sup>

Refer to current guidelines on CDSC and COSURV reporting.

### 5.6 INFECTION CONTROL STAFF

Inform the infection control team of isolates of methicillin resistant *Staphylococcus aureus*.

## 6 REFERRALS

### 6.1 REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to: <http://www.hpa.org.uk/cfi/lhcai/default.htm>

*Staphylococcus* Reference Laboratory Section  
Laboratory of Healthcare-Associated Hospital Infection  
Centre for Infections  
Health Protection Agency  
61 Colindale Avenue  
London  
NW9 5HT

Contact Centre for Infections main switchboard: Tel. +44 (0) 20 8200 4400

## 7 ACKNOWLEDGEMENTS AND CONTACTS

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