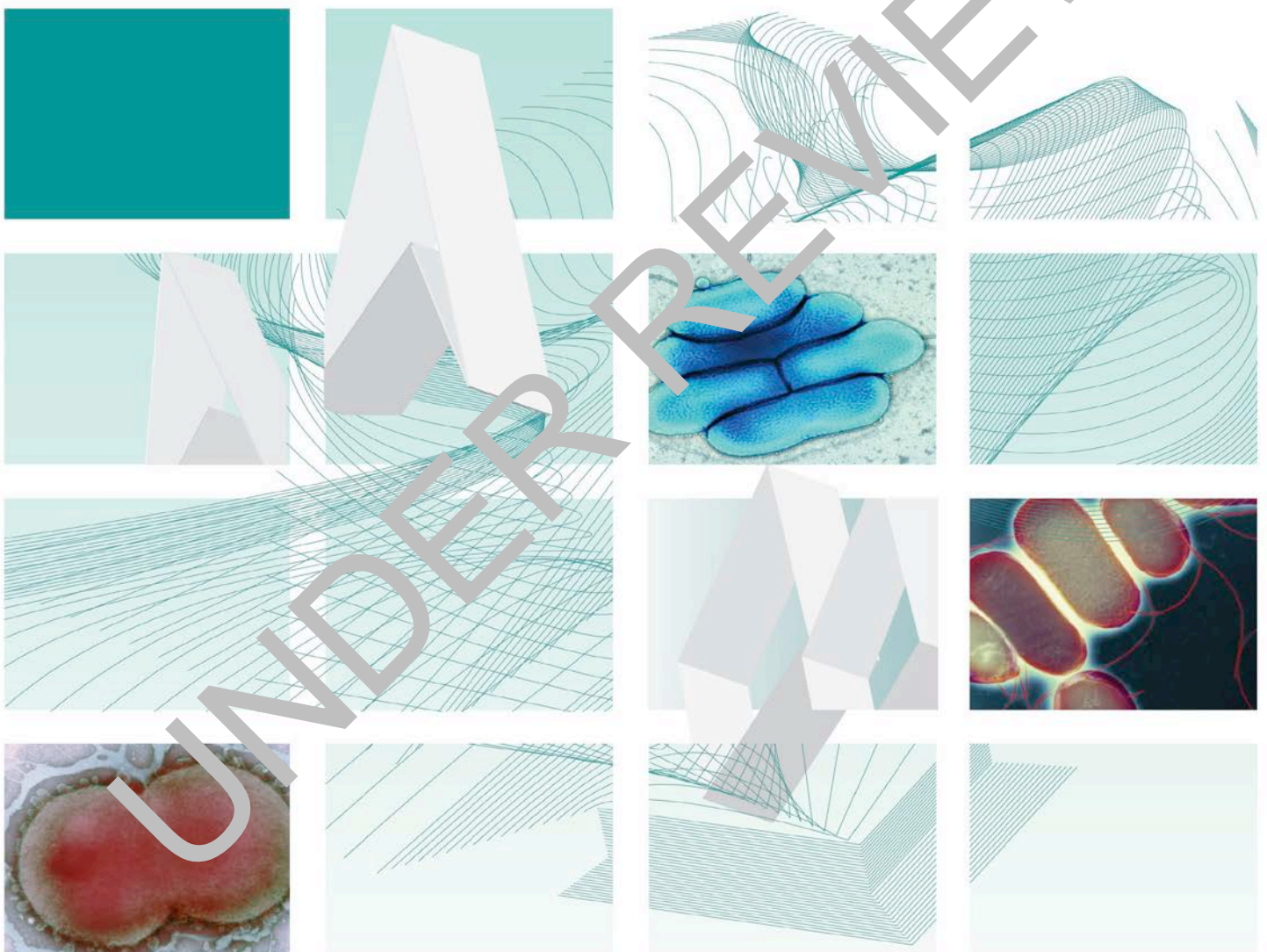




UK Standards for Microbiology Investigations

Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website

<http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	4/10.03.14
Issue no. discarded.	2.1
Insert Issue no.	2.3
Section(s) involved	Amendment
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Last sentence of scope removed.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	3/14.10.11
Issue no. discarded.	2
Insert Issue no.	2.1
Section(s) involved	Amendment
Whole document.	Document presented in a new format.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of nominating societies may be found at <http://www.npa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2014). Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species. UK Standards for Microbiology Investigations. ID 7 Issue 2.3. <http://www.hpa.org.uk/SMI/pdf>.

UNDER REVIEW

Scope of Document

This SMI describes the procedure for the identification and differentiation of *Staphylococcus aureus*, *Staphylococcus* species, *Micrococcus* species and *Rothia* species.

This SMI should be used in conjunction with other SMIs.

Introduction

The staphylococci most frequently associated with human infection are *S. aureus*, *S. epidermidis* and *S. saprophyticus*¹. Other *Staphylococcus* species may also be associated with human infection^{2,3}.

Taxonomy⁴

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-sporing 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 toxins⁵. 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 reported⁶.

S. aureus subspecies *anaerobius* is rarely isolated from clinical specimens. It grows poorly aerobically and growth may be CO₂ 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 *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 α 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 staphylococci⁷

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 lugdunensis*, *S. schleiferi* subspecies *schleiferi*, *Staphylococcus simulans* and *Staphylococcus warneri* have also been implicated^{8,9}. 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 teicoplanin¹⁰. *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.

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^{12,13}. *Staphylococcus* species, with the exception of *S. sciuri*, *S. lentus* and *S. vulvulus* 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 mucilagenosus* which was previously known as *Micrococcus mucilagenosus* or *Staphylococcus salivarius*¹⁴.

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 sensitised 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 Methicillin-resistant *Staphylococcus aureus* (MRSA) that fail to express the major characteristics listed above¹⁵. Positive results or suspected erroneous slide tests may be confirmed by a tube coagulase test.

Technical Information/Limitations

N/A

UNDER REVIEW

1 Safety Considerations¹⁶⁻³²

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

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^{1-5,7-12,15,33-65}**

Species		Subspecies	
<i>Staphylococcus</i>	<i>aureus</i>	<i>aureus</i>	
<i>Staphylococcus</i>	<i>aureus</i>	<i>anaerobius</i>	
<i>Staphylococcus</i>	<i>epidermidis</i>		} <i>S. epidermidis</i> group
<i>Staphylococcus</i>	<i>capitis</i>	<i>capitis</i>	
<i>Staphylococcus</i>	<i>capitis</i>	<i>ureolyticus</i>	
<i>Staphylococcus</i>	<i>hominis</i>	<i>hominis</i>	
<i>Staphylococcus</i>	<i>hominis</i>	<i>novobiosepticus</i>	
<i>Staphylococcus</i>	<i>haemolyticus</i>		
<i>Staphylococcus</i>	<i>lugdunensis</i>		
<i>Staphylococcus</i>	<i>macularolyticus</i>		
<i>Staphylococcus</i>	<i>warneri</i>		
<i>Staphylococcus</i>	<i>saprophyticus</i>		} <i>S. saprophyticus</i> group
<i>Staphylococcus</i>	<i>cohnii</i>	<i>cohnii</i>	
<i>Staphylococcus</i>	<i>cohnii</i>	<i>ureolyticus</i>	
<i>Staphylococcus</i>	<i>caprae</i>		
<i>Staphylococcus</i>	<i>hyicus</i>		
<i>Staphylococcus</i>	<i>intermedius</i>		

<i>Staphylococcus</i>	<i>schleiferi</i>	<i>coagulans</i>
<i>Staphylococcus</i>	<i>schleiferi</i>	<i>schleiferi</i>
<i>Staphylococcus</i>	<i>simulans</i>	

Other species reported to have caused human infection^{14,66-74}

<i>Micrococcus luteus</i>		
<i>Rothia mucilagenosus</i>		

3 Identification

3.1 Microscopic Appearance

Gram stain ([TP 39 - Staining Procedures](#))

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

3.2 Primary Isolation Media

Blood agar 16-48hr incubation in 5-10% CO₂ at 30°C-37°C.

These organisms may be isolated from other media including Cysteine Lactose.

Electrolyte Deficient agar (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 or orange, on blood agar. Haemolysis may be detected. They appear as yellow-green, 2mm 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 ([TP 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* ([TP 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 ([TP 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

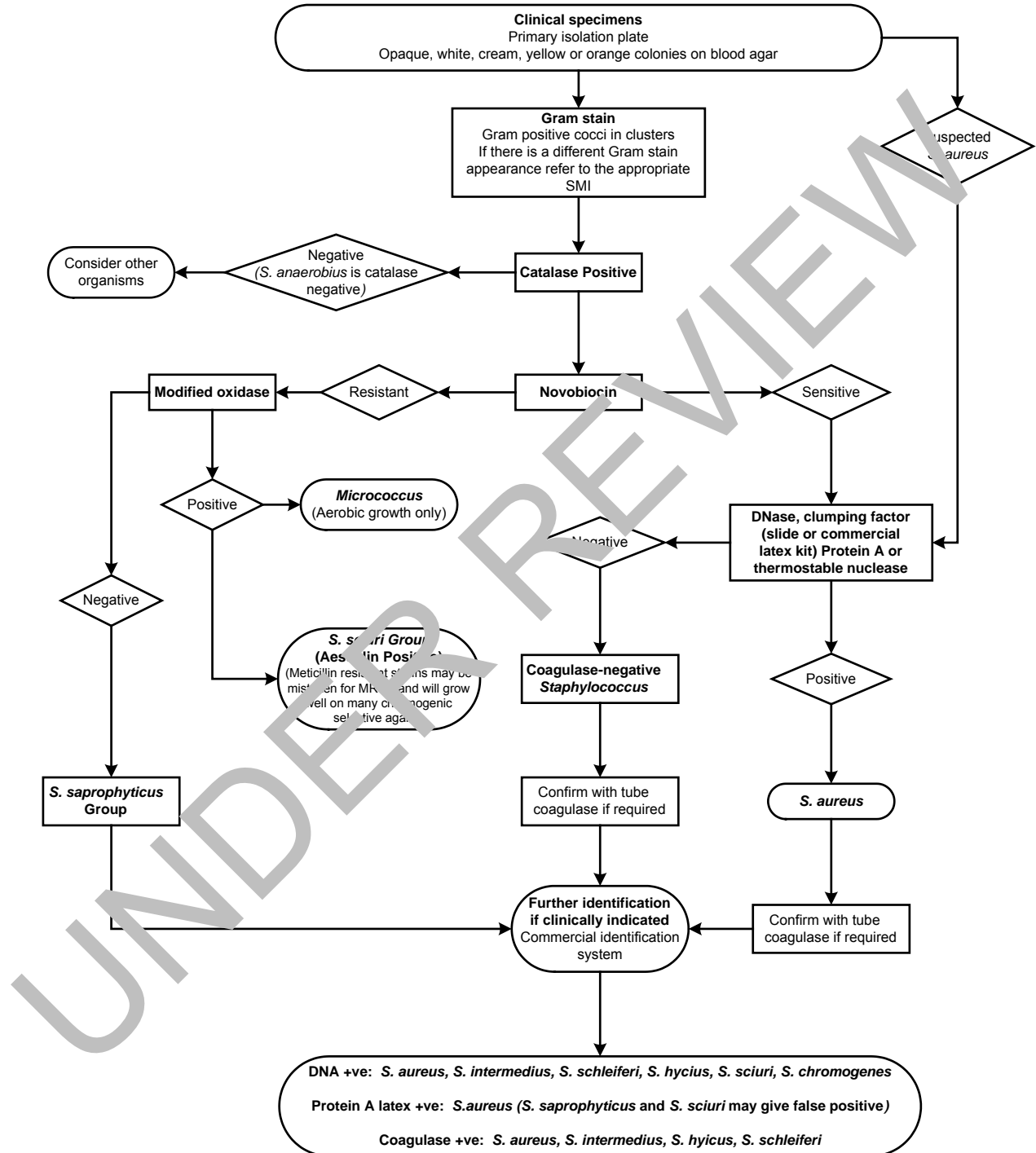
N/A

3.6 Storage and Referral

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

UNDER REVIEW

4 Presumptive Identification of *Staphylococcus* species



This flowchart is for guidance only.

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 presumed 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 presumed 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 CDSC

N/A

5.5 Public Health England⁷⁵

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

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

6 Referrals

6.1 Reference Laboratory

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory refer to:

Staphylococcus Reference Service
Antimicrobial Resistance and Healthcare Associated Infections Reference Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5HT
<http://www.hpa.org.uk/cfi/lhcai/default.htm>

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

Contact appropriate devolved nation reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales
<http://www.hpa.org.uk/webw/HPAweb&Page8=HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Scotland
http://www.hps.scot.nhs.uk/re_lab/index.aspx

Northern Ireland
<http://www.belfasttrust.nhs.uk/Internet/Laboratory-MortuaryServices.htm>

7 Notification to PHE^{75,76} or Equivalent in the Devolved Administrations⁷⁷⁻⁸⁰

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily

report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

Other arrangements exist in Scotland^{77,78}, Wales⁷⁹ and Northern Ireland⁸⁰.

UNDER REVIEW

References

1. Kloos W. Taxonomy and systematics of staphylococci indigenous to humans. In: Archer GL, Crossley KB, editors. *The Staphylococci in Human Disease*. New York: Churchill Livingstone; 1997. p. 113-7.
2. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev* 1994;7:117-40.
3. Weinstein MP, Mirrett S, Van Pelt L, McKinnon M, Zimmer BL, Kloos W, et al. Clinical importance of identifying coagulase-negative staphylococci isolated from blood cultures: evaluation of MicroScan Rapid and Dried Overnight Gram-Positive panels versus a conventional reference method. *J Clin Microbiol* 1998;36:2089-92.
4. Holt JG. Gram-Positive cocci. In: Holt JG, Krieg NR, Sneath PHA, Staley JL, Williams ST, editors. *Bergey's Manual of Determinative Bacteriology*. 9th ed. Baltimore; 1994. p. 527-37.
5. Jarlov JO, Hojbjerg T, Busch-Sorensen C, Scheibel J, Moller JK, Kolm S-C, et al. Coagulase-negative Staphylococci in Danish blood cultures: species distribution and antibiotic susceptibility. *J Hosp Infect* 1996;32:217-27.
6. Vandenesch F, Lebeau C, Bes M, McDevitt D, Greenlan T, Novick R, et al. Coagulase deficiency in clinical isolates of *Staphylococcus aureus* involves both transcriptional and post-transcriptional defects. *J Med Microbiol* 1994;40:344-9.
7. Rupp ME, Archer GL. Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis* 1994;19:231-43.
8. Christensen GD, Parisi JT, Bisno AL, Simpson W, Beachey EH. Characterization of clinically significant strains of coagulase-negative staphylococci. *J Clin Microbiol* 1983;18:258-69.
9. Jansen B, Schumacher-Perdreau F, Petersen G, Pulverer G. New aspects in the pathogenesis and prevention of polymer-associated foreign-body infections caused by coagulase-negative staphylococci. *J Invest Surg* 1999;2:361-80.
10. Bannerman TL, Wadick DL, Kloos WE. Susceptibility of *Staphylococcus* species and subspecies to teicoplanin. *Antimicrob Agents Chemother* 1991;35:1919-22.
11. Vandenesch F,ARRIER-GROS-CLAUDE JD, Bes M, Fuhrmann C, Delorme V, Mouren C, et al. *Staphylococcus aureus*-specific oligonucleotide probes derived from a random amplified DNA fragment. *FEMS Microbiol Lett* 1995;132:147-52.
12. Baker J. Comparison of various methods for differentiation of staphylococci and micrococci. *J Clin Microbiol* 1984;19:875-9.
13. Faller A, Schleifer KH. Modified oxidase and benzidine tests for separation of staphylococci from micrococci. *J Clin Microbiol* 1981;13:1031-5.
14. van Tiel FH, Slangen BF, Schouten HC, Jacobs JA. Study of *Stomatococcus mucilaginosus* isolated in a hospital ward using phenotypic characterization. *Eur J Clin Microbiol Infect Dis* 1995;14:193-8.
15. Personne P, Bes M, Lina G, Vandenesch F, Brun Y, Etienne J. Comparative performances of six agglutination kits assessed by using typical and atypical strains of *Staphylococcus aureus*. *J Clin Microbiol* 1997;35:1138-40.

Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species

16. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".
17. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-17.
18. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.
19. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
20. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.
21. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).
22. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32
23. Advisory Committee on Dangerous Pathogens. Infectious at work. Controlling the risks. Her Majesty's Stationery Office. 2003.
24. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
25. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.
26. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.
27. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
28. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
29. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
30. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
31. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
32. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14
33. Laughlin TJ, Armstrong DG, Caporusso J, Lavery LA. Soft tissue and bone infections from puncture wounds in children. West J Med 1997;166:126-8.

Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species

34. Patel SR, Oleginski TP, Perruquet JL, Harrington TM. Pyomyositis: clinical features and predisposing conditions. *J Rheumatol* 1997;24:1734-8.
35. Santos KR, Fonseca LS, Bravo Neto GP, Gontijo Filho PP. Surgical site infection: rates, etiology and resistance patterns to antimicrobials among strains isolated at Rio de Janeiro University Hospital. *Infection* 1997;25:217-20.
36. Fayon MJ, Tucci M, Lacroix J, Farrell CA, Gauthier M, Lafleur L, et al. Nosocomial pneumonia and tracheitis in a pediatric intensive care unit: a prospective study. *Am J Respir Crit Care Med* 1997;155:162-9.
37. Abele-Horn M, Dauber A, Bauernfeind A, Russwurm W, Seyfarth-Metzger I, Gleich P, et al. Decrease in nosocomial pneumonia in ventilated patients by selective oropharyngeal decontamination (SOD). *Intensive Care Med* 1997;23:187-95.
38. Osterlund A, Nordlund E. Wound infection caused by *Staphylococcus hyicus* subspecies *hyicus* after a donkey bite. *Scand J Infect Dis* 1997;29:95.
39. Lee J. *Staphylococcus intermedius* isolated from dog-bite wounds. *J Infect* 1994;29:105.
40. Celard M, Vandenesch F, Darbas H, Grandjean J, Jean-Pierre H, Kirkerian G, et al. Pacemaker infection caused by *Staphylococcus schleiferi*, a member of the human preaxillary flora: four case reports. *Clin Infect Dis* 1997;24:1014-5.
41. Ozturkeri H, Kocabeyoglu O, Yergok YZ, Kosan E, Yenen GS, Keskin K. Distribution of coagulase-negative staphylococci, including the newly described species *Staphylococcus schleiferi*, in nosocomial and community acquired urinary tract infections. *Eur J Clin Microbiol Infect Dis* 1994;13:1076-9.
42. Latorre M, Rojo PM, Unzaga MJ, Cisterna R. *Staphylococcus schleiferi*: a new opportunistic pathogen. *Clin Infect Dis* 1993;16:588-90.
43. Mancao M, Miller C, Cochrane J, Hoffman Sauter K, Weber E. Cerebrospinal fluid shunt infections in infants and children in Mobile, Alabama. *Acta Paediatr* 1998;87:667-70.
44. Ludlam H, Tremlett CH, Wilson AP. Preventing infection with *Staphylococcus aureus* in CAPD. *Perit Dial Int* 1997;17:405-8.
45. Henke PK, Benamini TM, Gamson JR, Brittan KR, Peyton JC, Lam TM. *Staphylococcus epidermidis* graft infections associated with locally suppressed major histocompatibility complex class II and elevated MHC-1 expression. *Arch Surg* 1997;132:894-902.
46. Karanikli M, Kokou A, Panagiotopoulou HS, Anastassiou ED, Dimitracopoulos G. The major 20 kDa polysaccharide of *Staphylococcus epidermidis* extracellular slime and its antibodies as powerful agents for detecting antibodies in blood serum and differentiating among slime-positive and -negative *S. epidermidis* and other staphylococci species. *Arch Biochem Biophys* 1997;341:389-95.
47. Schneider PF, Riley TV. *Staphylococcus saprophyticus* urinary tract infections: epidemiological data from Western Australia. *Eur J Epidemiol* 1996;12:51-4.
48. Mehta G, Kumari S. Multi-resistant *Staphylococcus haemolyticus* in a neonatal unit in New Delhi. *Ann Trop Paediatr* 1997;17:15-20.
49. Burnie JP, Naderi-Nasab M, Loudon KW, Matthews RC. An epidemiological study of blood culture isolates of coagulase-negative staphylococci demonstrating hospital-acquired infection. *J Clin Microbiol* 1997;35:1746-50.

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50. Akiyama H, Kanzaki H, Tada J, Arata J. Coagulase-negative staphylococci isolated from various skin lesions. *J Dermatol* 1998;25:563-8.
51. Kessler RB, Kimbrough RC, III, Jones SR. Infective endocarditis caused by *Staphylococcus hominis* after vasectomy. *Clin Infect Dis* 1998;27:216-7.
52. Barcs I, Herendi A, Lipcsey A, Bogнар C, Hashimoto H. Phage pattern and antibiotic resistance pattern of coagulase-negative staphylococci obtained from immunocompromised patients. *Microbiol Immunol* 1992;36:947-59.
53. Kloos WE, George CG, Olgiate JS, Van Pelt L, McKinnon ML, Zimmer BL, et al. *Staphylococcus hominis* subsp. *novobiosepticus* subsp. nov., a novel trehalose- and N-acetyl-D-glucosamine-negative, novobiocin- and multiple-antibiotic-resistant subspecies isolated from human blood cultures. *Int J Syst Bacteriol* 1998;48 Pt 3:799-812.
54. Jarlov JO, Prag J, Rosdahl VT, Espersen F. Evaluation of staphylococci isolated from a blood culture system (colorbact). *APMIS* 1995;103:383-7.
55. Crichton PB, Anderson LA, Phillips G, Davey PG, Rowley DI. Subspecies discrimination of staphylococci from revision arthroplasties by ribotyping. *J Hosp Infect* 1995;30:439-47.
56. al Rashdan A, Bashir R, Khan FA. *Staphylococcus capitis* causing aortic valve endocarditis. *J Heart Valve Dis* 1998;7:518-20.
57. Vandenesch F, Eykyn SJ, Bes M, Meugnier H, Fleurette J, Etienne J. Identification and ribotypes of *Staphylococcus caprae* isolates isolated as human pathogens and from goat milk. *J Clin Microbiol* 1995;33:888-92.
58. Shuttleworth R, Behme RJ, McNabb A, Colby WF. Human isolates of *Staphylococcus caprae*: association with bone and joint infections. *J Clin Microbiol* 1997;35:2537-41.
59. Schnitzler N, Meilicke R, Conrad G, Frank D, Haase G. *Staphylococcus lugdunensis*: report of a case of peritonitis and an easy-to-perform screening strategy. *J Clin Microbiol* 1998;36:812-3.
60. De Hondt G, Ieven M, Vandermerck C, Colaert J. Destructive endocarditis caused by *Staphylococcus lugdunensis*. Case report and review of the literature. *Acta Clin Belg* 1997;52:27-30.
61. Koh TW, Brecher SJ, Layton CA. Successful treatment of *Staphylococcus lugdunensis* endocarditis complicated by multiple emboli: a case report and review of the literature. *Int J Cardiol* 1996;55:193-7.
62. Gidley RW, Johnson BY, Stiernberg CM. Contemporary management of deep neck space infections. *Otolaryngol Head Neck Surg* 1997;116:16-22.
63. Orrett F, Shurland SM. Significance of coagulase-negative staphylococci in urinary tract infections in a developing country. *Conn Med* 1998;62:199-203.
64. Kolawole DO, Shittu AO. Unusual recovery of animal staphylococci from septic wounds of hospital patients in Ile-Ife, Nigeria. *Lett Appl Microbiol* 1997;24:87-90.
65. BATTERY JP, Easton M, Pearson SR, Hogg GG. Pediatric bacteremia due to *Staphylococcus warneri*: microbiological, epidemiological, and clinical features. *J Clin Microbiol* 1997;35:2174-7.
66. Peces R, Gago E, Tejada F, Lares AS, Alvarez-Grande J. Relapsing bacteraemia due to *Micrococcus luteus* in a haemodialysis patient with a Perm-Cath catheter. *Nephrol Dial Transplant* 1997;12:2428-9.

Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species

67. Rabitsch W, Brugger SA, Pirker W, Baumgartner C, Reiter E, Keil F, et al. Symmetrical necrosis of globus pallidus with severe gait disturbance in a patient with myelodysplastic syndrome given allogeneic marrow transplantation. *Ann Hematol* 1997;75:235-7.
68. Kern W, Kurrle E, Vanek E. Ofloxacin for prevention of bacterial infections in granulocytopenic patients. *Infection* 1987;15:427-33.
69. Kiehn TE, Armstrong D. Changes in the spectrum of organisms causing bacteremia and fungemia in immunocompromised patients due to venous access devices. *Eur J Clin Microbiol Infect Dis* 1990;9:869-72.
70. Abraham J, Bilgrami S, Dorsky D, Edwards RL, Feingold J, Hill DR, et al. *Stomatococcus mucilaginosus* meningitis in a patient with multiple myeloma following autologous stem cell transplantation. *Bone Marrow Transplant* 1997;19:639-41.
71. Park MK, Khan J, Stock F, Lucey DR. Successful treatment of *Stomatococcus mucilaginosus* meningitis with intravenous vancomycin and intravenous ceftriaxone. *Clin Infect Dis* 1997;24:278.
72. McWhinney PH, Kibbler CC, Gillespie SH, Patel S, Morrison D, Hoffmann A, et al. *Stomatococcus mucilaginosus*: an emerging pathogen in neutropenic patients. *Clin Infect Dis* 2002;14:641-6.
73. Vasishtha S, Isenberg HD, Sood SK. *Gemella morbillorum* as a cause of septic shock. *Clin Infect Dis* 1996;22:1084-6.
74. Kloos W, Bannerman TL. *Staphylococcus and Micrococcus*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, editors. *Manual of Clinical Microbiology*. 7th ed. Washington DC: American Society for Microbiology; 1999. p. 267-82.
75. Public Health England. *Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories*. 2013. p. 1-37.
76. Department of Health. *Health Protection Legislation (England) Guidance*. 2010. p. 1-112.
77. Scottish Government. *Public Health (Scotland) Act*. 2008 (as amended).
78. Scottish Government. *Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States*. 2009.
79. The Welsh Assembly Government. *Health Protection Legislation (Wales) Guidance*. 2010.
80. Home Office. *Public Health Act (Northern Ireland) 1967 Chapter 36*. 1967 (as amended).