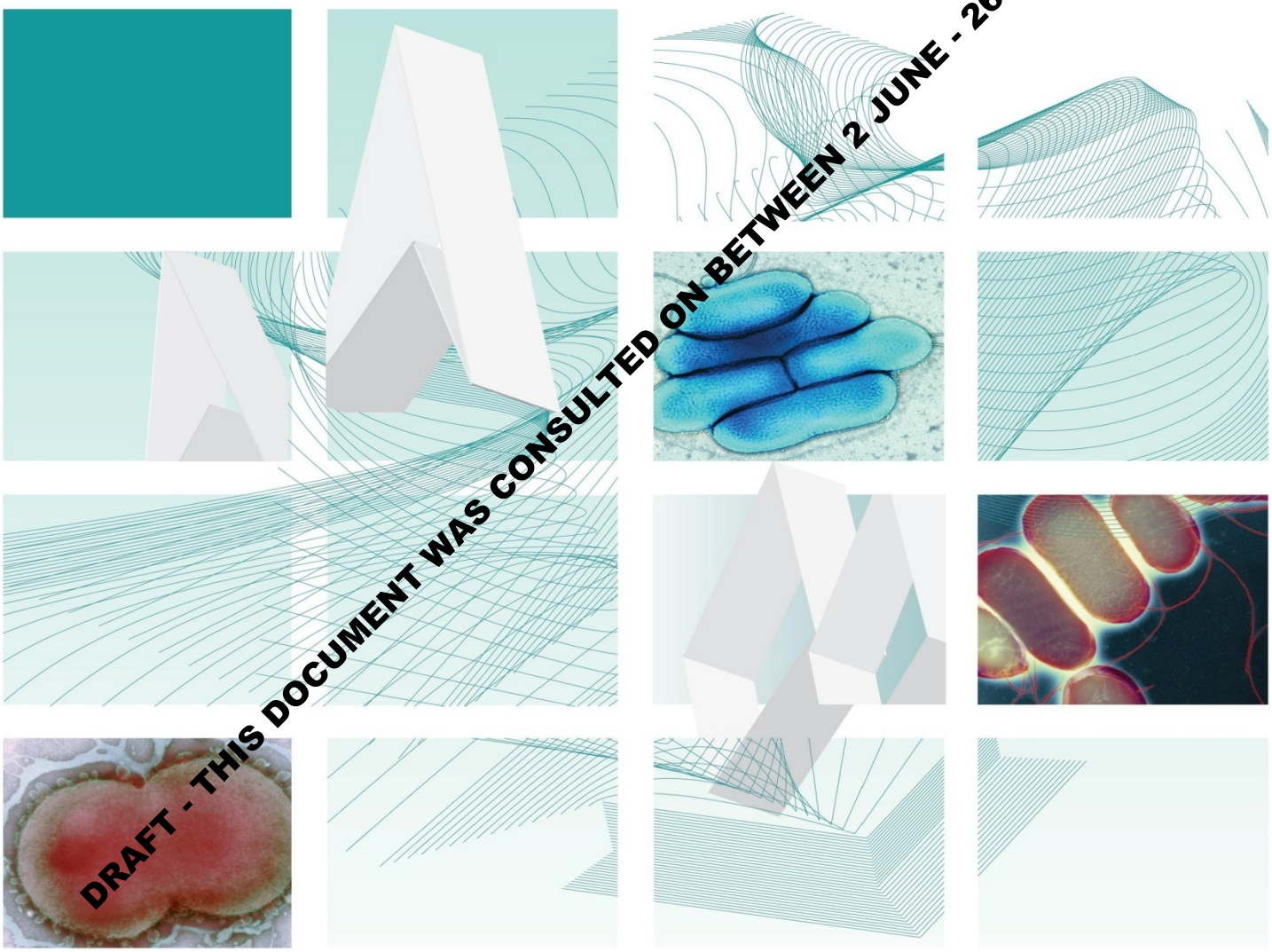
Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of Superficial Mouth Samples



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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.

We also acknowledge the Association of Clinical Oral Microbiologists for their considerable specialist input.

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UK Standards for Microbiology Investigations are produced in association with:

Contents

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.	9/dd.mm.yy<tab+enter>
Issue no. discarded.	6.2
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Section(s) involved	**Amendment**

Amendment No/Date.	8/12.04.12
Issue no. discarded.	6.1
Insert Issue no.	6.2
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. The term “CE marked leak proof container” replaces “sterile leak proof container” (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC[1,2]. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also 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. The documents also 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 participating societies may be found at http://www.hpa.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.

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

[#] 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.

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.

Suggested Citation for this Document

Public Health England. (YYYY <tab+enter>). Investigation of Superficial Mouth Samples. UK Standards for Microbiology Investigations. B 4 Issue #.# <tab+enter>. http://www.hpa.org.uk/SMI/pdf

Scope of Document

Type of Specimen

Mouth swab, saliva and oral rinse

Scope

This SMI describes the processing, and bacteriological and mycological investigation of superficial mouth samples. Predominately mouth swabs but saliva and oral rinses are also covered. Infections of salivary glands (parotid, submandibular and sub-lingual) include bacterial and viral infections and are not covered in this SMI.

This SMI should be used in conjunction with other SMIs.

Introduction

Infections of the oral mucosa usually present as acute conditions. Usually these arise from the colonising oral flora but can also result from a flare-up of a chronic low-grade infection.

Oral mucosal infections are typically associated with biofilms formed on the inanimate surfaces present in the oral cavity such as the teeth and dentures.

Infections of the gingiva (gingivitis, including acute ulcerative gingivitis) and periodontal tissues (periodontitis) are the most common forms of oral infection but processing specimens from these infections is covered by a separate SMI B 17 – Investigation of Tissues and Biopsies.

Infections of the oral mucosa

Oral Mucositis

Oral mucositis is a painful complication of chemotherapy or head and neck radiotherapy, caused by direct cytotoxicity of the treatment regime. Super-infection usually with yeasts and oral bacteria can exacerbate the problem and microbiological examination can help to guide symptomatic treatment.

Erythematous and Pseudomembranous Candidosis[3,4]

Erythematous and pseudomembranous candidosis are the most frequent clinical presentations of oral fungal infection. The infections may involve the mucosal surfaces of the cheeks, tongue (dorsal and ventral surfaces) and both hard and soft palates. The most common cause is *Candida albicans*, but Candida species other than *C. albicans* species such as *Candida glabrata* may also be isolated, either alone or in combination with *C. albicans*, especially in the medically compromised or those with a history of prolonged antifungal therapy[5,6]. Atrophic candidosis (denture stomatitis) may occur in the palatal mucosa below the fitting surface of dentures, especially when patients sleep with their dentures in place and/or have xerostomia. *Candida* species other than *C. albicans* species are important to identify, since they may demonstrate reduced susceptibility and clinical resistance to the first line anti-fungal agents and may be responsible for refractory or recurrent infections. Rarely, moulds may colonise and infect sinuses and result in palatal erosion. Specimens in the form of an oral rinse (known volume of sterile saline) are used to quantitatively determine colonisation or infection[7].

Angular Cheilitis and Peri-Oral Infections

Angular cheilitis and peri-oral infections are common infections affecting the angles of the mouth and lips, usually caused by an intra-oral reservoir of infection, typically biofilms associated with denture stomatitis. Infection may be due to *S. aureus*, *Candida* species and/or Group A streptococci. It is common for dentate patients with angular cheilitis to have infection with both *S. aureus* and *C. albicans* in the labial commissure region. In addition to swabs from the lesions themselves, swabs should also be collected from relevant intra-oral sites eg denture-fitting surface and the anterior nares to identify sites of colonisation to be treated with eradication therapy, to reduce relapse rates.

Staphylococcal Mucositis[8]

Patients who are severely medically compromised and have reduced salivary flow, together with parenteral feeding, may develop staphylococcal mucositis caused by *S. aureus. Enterobacteria* may also play a role in severe cases. The erythematous changes in the oral mucosa may be indistinguishable clinically from candidosis, requiring the need for microbiological investigation. Results should be interpreted in a clinical context since asymptomatic carriage of *S. aureus* or Enterobacteria may occur. Strict regular oral hygiene measures are usually sufficient to resolve clinical symptoms. Systemic antibiotics are not usually required, though may play an important role in the management of severe oral mucositis in some patient groups such as the terminally ill.

Oral Ulceration

There are many non-infective causes of oral ulceration such as traumatic ulcers, recurrent aphthous ulcers, inflammatory conditions and malignant lesions. Infective causes of oral ulceration are commonly viral in origin (eg Herpes simplex). Uncommon bacterial causes of ulceration are syphilis and tuberculosis whilst other rare causes of oral ulceration include fungal infections such as histoplasmosis.

Abscess and Deeply Seated Infections

Abscess and deeply seated infections (dental abscesses, and salivary gland abscesses) are dealt with in B 14 - Investigation of Abscesses and Deep-Seated Wound Infections.

Osteomyelitis

Osteomyelitis, including bacterial, mycobacterial and fungal osteomyelitis are dealt with in B 12 - Investigation of Bone and Soft Tissue Associated with Osteomyelitis.

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers[1,2]

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”.

1 Safety Considerations[1,2,9-23]

1.1 Specimen Collection, Transport and Storage[1,2,9-12]

Use aseptic technique.

Collect saliva and oral rinse specimens into appropriate CE marked leak proof containers and transport specimens in sealed plastic bags.

Use tubes with transport medium for transporting swabs and transport in sealed plastic bags[24].

Transport each swab in transport medium in a CE marked container in a sealed plastic bag.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[1,2,9-23]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[15].

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

If histoplasma (and/or other relevant dimorphic pathogens causing oral ulceration) risk then contaminant level 3 is required.

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

2 Specimen Collection

2.1 Type of Specimens

Mouth swab, saliva and oral rinse

2.2 Optimal Time and Method of Collection[25]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[25].

To assure that the preconditions of the sampling for oral infections are comparable it is advised that patients should not:

1. Eat or drink within 2 hours
2. Brush their teeth within 2 hours
3. Use any mouth rinse of disinfectant within 2 hours prior to sampling

If possible samples should be taken in the morning under fasting conditions.

Unless otherwise indicated collect each swab for bacterial and/or fungal culture and place in appropriate transport medium[24,26-29].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

Sample any lesions or inflamed areas using cotton tipped swabs. Samples of denture fitting surfaces should also be swabbed as these are more sensitive sites than the palatal mucosa to recover *Candida* sp. The use of a tongue depressor or spatula may be helpful. Oral rinses can be useful to follow up level of colonisation. These are collected by rinsing with 10mL of sterile saline for one minute.

2.3 Adequate Quantity and Appropriate Number of Specimens[25]

Numbers and frequency of specimens collected depend on the clinical condition of patient.

3 Specimen Transport and Storage[1,2]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[25].

Collect mucosal swabs in transport medium which should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature.

Oral rinses should be transported in a CE marked leak proof containers and placed in sealed plastic bags and processed as soon as possible

4 Specimen Processing/Procedure[1,2]

4.1 Test Selection

Most mouth samples are swabs unless the patient is immunocompromised or has other clinical indications.

Saliva samples may be collected for microbiological investigation and for other types of assessment. Increasingly saliva is being used as a sample for new diagnostic techniques, but also for assessing xerostomia and risk of dental caries. Care is needed to avoid contamination of these specimens and cross infection from these specimens. Sometimes culture is done with an exact volume of saliva in order to assess the count of a particular organism (eg *S. mutans* or lactobacilli per mL of the original saliva sample.

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

For oral rinses (saliva/mouth washings) centrifuge at 3200 rpm for 10 minutes.

Decant supernatant into disinfectant and re suspend the deposit in 1mL PBS.

This is now the neat sample.

Inoculate 50µL onto a sabouraud agar plate using a hockey stick to spread out and a Columbia agar plate for single colonies.

For comparison it is sometimes useful to dilute neat sample 1:100 (0.1mL + 9.9mL PBS). Inoculate 50µL onto a Columbia Blood Agar and use a hockey stick to spread out. A MacConkey/CLED plate may also be useful.

4.4 Microscopy

Direct microscopic examination with Calcofluor staining may be helpful if histoplasma or mould infection is suspected.

4.5 Culture and Investigation

Inoculate each agar plate using a sterile loop or a loopful of liquid (Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Oral candidosis Fungal infection Oral erythema Denture stomatitis Angular cheilitis Mouth ulcer	Mouth swab, Saliva and oral rinse	Sabouraud agar	35-37	Air	40-48hr*	Daily	*C. albicans,* Non-albicans yeasts
		Blood agar	35-37	5-10% CO_2	16-24hr	daily	Group A, strep, *S. aureus,* Coliforms
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Oral mucositis Immunocompromised patients	Mouth swab, Saliva and oral rinse	MacConkey/CLED agar	35-37	Air	16-24hr	daily	*Coliforms* and non-fermentative gram negatives
		Chomogenic agar	35-37	Air	16-24hr	daily	Candida spp
*If Histoplasmosis is suspected the length of incubation should be extended and carried out in Category 3 conditions. If an unusual fungal infection is suspected a second Sabouraud plate should be set up at 30°C and incubation time extended.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Candida species	species level
Staphylococcus aureus	species level
Lancefield group A streptococcus	species level
Enterobacteriaceae species	"coliform" level if dominant growth

Organisms may be further identified if this is clinically or epidemiologically indicated.

Immunocompromised Patients

Candida species	species level
Aspergillus species and other moulds	genus level
Staphylococcus aureus	species level
Lancefield group A streptococcus	species level
Coliforms	species level
Pseudomonas species	species level if dominant growth
Acinetobacter species	species level if dominant growth
Stenotrophomonas species	species level if dominant growth

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

C. albicans is not routinely tested unless associated with recurrent infection, requested by clinician or the patient's history indicates significant immunosuppression.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

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

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

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

Report for fungi if applicable.

5.2 Culture

Report clinically significant organisms isolated **or**

Report other growth, eg: “Mixed upper respiratory tract flora” **or**

Report absence of growth or

Report presence or absence of specific named pathogens

Report quantitative growth if applicable.

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically.

Written report, 16–72hr stating, if appropriate, that a further report will be issued.

5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[30,31] or Equivalent in the Devolved Administrations[32-35]

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.

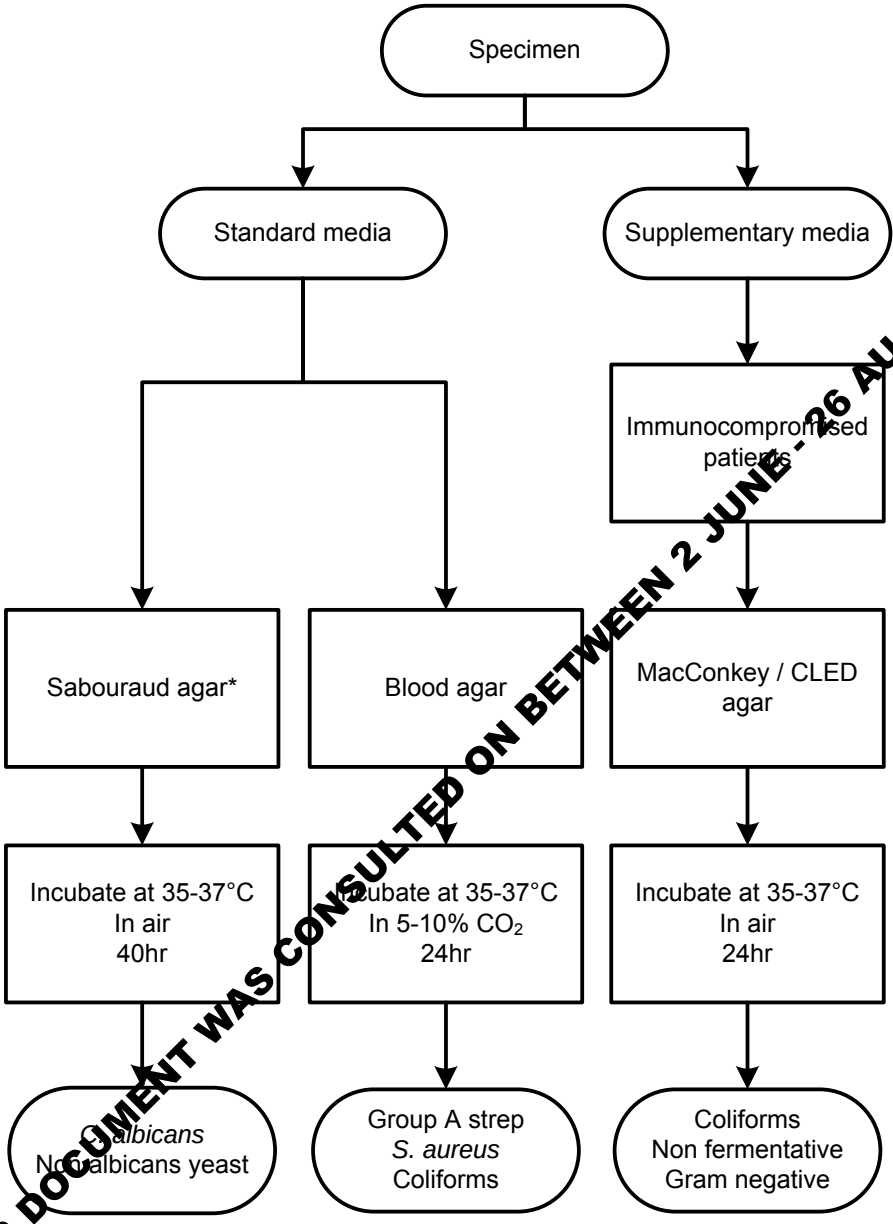
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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 (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/

Other arrangements exist in Scotland[32,33], Wales[34] and Northern Ireland[35].

Appendix: Investigation of Superficial Mouth Samples



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