

UK Standards for Microbiology Investigations

Investigation of Bone Marrow



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.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: <http://www.hpa.org.uk/SMI>

UK Standards for Microbiology Investigations are produced in association with:



The Association for
Clinical Biochemistry
Microbiology Group



The Royal College of Pathologists
Pathology: the science behind the cure



UK Standards for Microbiology Investigations[#]: Status

Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the standards 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 essential laboratory methodologies which underpin quality, for example assay validation, quality assurance and understanding uncertainty of measurement.

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 interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within PHE in partnership with the NHS, Public Health Wales and with professional organisations.

The list of participating organisations may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of an organisation's logo in an SMI implies support for the objectives and process of preparing SMIs. Representatives of professional organisations are members of the steering committee and working groups which develop SMIs, although the views of participants are not necessarily those of the entire organisation they represent.

SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at <http://www.hpa.org.uk/SMI> as controlled documents in Adobe PDF format.

[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

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

The process for the development of SMIs is certified to ISO 9001:2008.

NHS Evidence has accredited the process used by PHE to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in PHE's Standard Operating Procedure SW3026 (2009) version 6.

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 well referenced 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. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. 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.

Whilst every care has been taken in the preparation of SMIs, PHE, its successor organisation(s) 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.

SMIs are the copyright of PHE which should be acknowledged where appropriate.

Microbial taxonomy is up to date at the time of full review.

Equality and Information Governance

An Equality Impact Assessment on UK Standards for Microbiology Investigations is available at <http://www.hpa.org.uk/SMI>.

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.

Suggested citation for this document:

Public Health England. (2012). Investigation of Bone Marrow. UK Standards for Microbiology Investigations. B 38 Issue dl + <http://www.hpa.org.uk/SMI/pdf>.

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ISO 9001:2008

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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.	1/dd.mm.yy <tab+enter>
Issue no. discarded.	1
Insert Issue no.	## <tab+enter>
Section(s) involved.	Amendment.

Amendment No.	-/09.11.07
Issue no. discarded.	-
Insert Issue no.	1
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} . Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Section on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

Scope of Document

Type of Specimen

Bone Marrow

Scope

This SMI describes the processing and microbiological investigation of bone marrow sent for clinical diagnostic purposes. This SMI concentrates on the culture of bone marrow samples for the identification of bacteria and fungi. Other methods of investigation are also available for the identification of bacteria, fungi, parasites and viruses from bone marrow samples, but are not covered in this SMI.

This SMI should be used in conjunction with other SMIs.

Introduction

Microbiological examination of bone marrow is an invasive technique infrequently performed for the investigation of pyrexia of unknown origin (PUO) and occasionally for other indications³. It is sometimes undertaken when other less invasive investigations and diagnostic imaging have failed to determine a cause, or, more frequently, when infection is part of the differential diagnosis in the investigation of haematological abnormalities⁴. The demonstration of microorganisms in bone marrow by microscopy, culture or nucleic acid amplification techniques is useful for diagnosis of infection with a limited number of bacteria, fungi, parasites and viruses⁵⁻⁷.

Bone marrow is aspirated from the posterior iliac crest or the sternum; a core biopsy may also be collected, and this can be examined histologically for evidence of granulomata and microorganisms. The aspirate is the preferred specimen for microbiological studies.

Infections in patients who are immunocompromised

It has been suggested that bone marrow cultures should not be used for immunocompetent patients, but should be reserved for patients who are severely immunosuppressed⁸. Conditions leading to significant immunosuppression such as advanced HIV infection, bone marrow or solid organ transplant or high dose corticosteroid therapy predispose patients to infection with opportunistic pathogens and make disseminated infection with other pathogens more likely⁹. In these cases culture of bone marrow may be useful in the investigation of pyrexia of unknown origin (PUO)^{4,10}. *Mycobacterium* species, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffeii* and *Leishmania* species are likely to cause disseminated infection in the setting of immunosuppression⁸.

Organisms which have been demonstrated in bone marrow

Some organisms invade bone marrow as part of a multi-system infection, whereas others have a tropism for bone marrow or the cell lines therein. In several studies, culture of bone marrow has been shown to be a faster and more sensitive method of isolation of certain organisms (eg *Brucella* species and *Salmonella* Typhi) compared to blood culture, however in some, similar yields and turnaround times were observed^{7,11-13}. Bone marrow cultures may be positive for patients with acute or chronic infection; whereas blood cultures are more likely to be positive in patients with acute infections¹². Bone marrow aspirates are also more likely than blood culture to be positive in patients who have been treated with antibiotics^{7,14}.

Bone marrow examination is most likely to be performed for the organisms below. The list is not exhaustive; other organisms may be detected or isolated.

Bacteria

Salmonella Typhi and *Salmonella* Paratyphi

Salmonella Typhi and *Salmonella* Paratyphi (groups A, B, and C) are the causative organisms of enteric (typhoid) fever and are usually carried by humans, and transmitted via contaminated food or water⁷. Enteric fever is the only bacterial infection for which bone marrow is routinely recommended¹⁵. Culture of bone marrow is considered to be the 'gold standard' method for diagnosis of typhoid fever; compared to blood culture which may lack sensitivity, culture of bone marrow aspirates has been shown to produce a higher yield even when following antimicrobial treatment^{7,15}. In one study it was shown that 1mL of bone marrow gave equivalent result to 15mL of blood¹⁶. Serology is available, but has a low sensitivity and specificity due to cross reactions with other *Salmonella* species and Enterobacteriaceae¹⁶. Nucleic acid amplification tests (NAATs) on culture positive bone marrow aspirates have been reported, but are not yet in routine use¹⁷. Cultures of *S. Typhi* and of *S. Paratyphi* A, B or C, known or suspected, must be handled at Containment Level 3.

Brucella species

Brucella is a zoonotic disease which has a wide range of symptoms and is thought to be greatly undiagnosed. Laboratory diagnostic techniques include culture, NAATs and antibody detection (the presence of antibodies is not always indicative of active brucellosis). Recovery from blood is suboptimal and it has been suggested that culture of bone marrow (liver tissue and lymph nodes) may improve the recovery rate within a shorter time frame^{11,12,18}.

Mycobacterium species

Mycobacterium species are considered an important cause of pyrexia of unknown origin. Tuberculosis is primarily caused by *Mycobacterium tuberculosis*; a number of non-tuberculous mycobacterial species have been isolated from systemic infections in patients who are HIV positive. Culture is considered the 'gold standard' method for laboratory diagnosis, however incubation times may be long⁵. The use of continuous blood culturing systems reduces culture time; positive results may be available within five to seven days¹⁸. Bone marrow culture assists in aiding diagnosis in uncertain cases of disseminated disease, particularly in those with HIV^{13,19,20}. Molecular methods for detection are currently under development (refer to [B 40 – Investigation of specimens for *Mycobacterium* species](#))⁵.

Fungi

Infection with dimorphic fungi such as *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* or *Penicillium marneffe* may occasionally be diagnosed by bone marrow examination^{21,22}. Culture sensitivity varies. Culture for *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* may take between two and six weeks; continuous monitoring blood culture systems have been shown to reduce culture time of *Penicillium marneffe* to about four days²³⁻²⁵. It has been suggested that culture of bone marrow samples may be more sensitive than other tests; however, diagnosis is more frequently made by detection of these organisms in respiratory and tissue specimens^{13,21,22,26}.

Parasites

Leishmania species

There are over 20 species of the protozoan parasite *Leishmania*. Humans are infected by the bite of infected female sandflies. The disease is endemic in five continents and over eighty countries. Leishmaniasis presents as three distinct syndromes, visceral (also known as Kala-azar),

cutaneous and mucosal. Visceral Leishmaniasis, for which bone marrow investigation may be performed, can be fatal if untreated and is characterised by fever, weight loss, hepatosplenomegaly and pancytopenia²⁷. Co-infection with HIV in endemic areas is associated with a more rapid progression to AIDS and infection has been transmitted through needle-sharing by infected drug users in south west Europe³.

Following presumptive identification using Giemsa stain to detect amastigotes, samples should be sent to the reference laboratory for confirmation. Rapid diagnostic tests including direct agglutination and immunochromographic tests (ICT) have been developed and evaluated^{3,28}. Serological diagnosis is available but it is significantly less sensitive in those with advanced HIV coinfection than for HIV negative individuals; negative results should not therefore be used to rule out a diagnosis in those with HIV^{6,29}. Cross-reactions can occur in patients with prior exposure to *Trypanosoma cruzi*. Splenic puncture is the most sensitive test, but bone marrow examination is safer and has a sensitivity of around 70 – 80%^{3,27}.

Viruses

Many viruses can be detected in bone marrow samples. Viral detection indicates infection, but does not necessarily confirm diagnosis of disease. The clinical significance of a positive bone marrow result is dependent on the immune status of the patient and the disease/illness under investigation; positive results from bone marrow samples must therefore be interpreted with caution. Routinely, NAATs or serology on peripheral blood is used for diagnosis of acute viral infection. In the immunocompromised, blood serology results may be negative at the onset of clinical disease. If there is a high clinical suspicion of viral infection, but peripheral blood NAATs results are negative, diagnosis may be confirmed by bone marrow examination.

Rapid techniques

Molecular methods³⁰⁻³²

NAATs - Nucleic Acid Amplification Techniques (eg PCR) for the identification of bacteria, fungi, parasites and viruses from clinical samples have been shown to be highly specific and sensitive. PCR targets conserved genes of the genome, and enables the rapid identification of organisms including those that are slow to grow or are unculturable. Results are available within a short timeframe particularly if multiplex real-time PCR is used.

MALDI-TOF Mass Spectrometry^{33,34}

Recent developments in identification of bacteria and yeast include the use of 16s ribosomal protein profiles obtained by Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) mass spectrometry. Mass peaks achieved by the test strains are compared to those of known reference strains. It is possible for an organism to be identified from an isolate within a short time frame and it is increasingly being used in laboratories to provide a robust, rapid and effective identification system for bacterial and yeast isolates.

Technical Information/Limitations

Specimen containers^{1,2}

SMLs 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 Specimen Collection, Transport and Storage^{1,2}

1.1 Safety considerations³⁵⁻⁴⁶

Use aseptic technique.

Ideally should be collected directly into blood culture bottles however appropriate CE marked leak proof containers and transport in sealed plastic bags may be used in some circumstances.

Compliance with postal and transport regulations is essential.

1.2 Achieving optimal conditions

1.2.1 Time between specimen collection and processing

Collect specimens before antimicrobial therapy where possible.

Specimens should be transported and processed as soon as possible.

1.2.2 Special considerations to minimise deterioration

If processing is delayed, refrigeration is preferable to storage at ambient temperature⁴⁶.

Delays of over 48hr are undesirable.

1.3 Correct specimen type and method of collection

Specimens should ideally be collected in blood culture bottles. However, in accordance with local requirements additional specimens may be collected in appropriate CE marked leak proof containers containing anti-coagulants and placed in sealed plastic bags.

1.4 Adequate quantity and appropriate number of specimens

As large a sample as possible should be obtained, with the caveat that volumes of >3 mL are likely to be contaminated with peripheral blood which may have a dilution effect.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

2 Specimen Processing^{1,2}

2.1 Safety considerations³⁵⁻⁴⁶

Containment Level 2.

All specimens must be processed in a microbiological safety cabinet including the examination of plates and cultures.

Where Hazard Group 3 organisms e.g. *Mycobacterium tuberculosis*, *Salmonella* Typhi, *Salmonella* Paratyphi, dimorphic fungi and *Brucella* species are suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

Some of the Hazard Group 3 fungi are thermally dimorphic and will grow as yeast forms in blood culture bottles and sub-cultures at 37 °C but as the highly infective mould form when sub-cultured onto agar incubated at 28-30 °C. Care should be taken with yeast isolates if there is a relevant travel history especially in HIV-infected individuals.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet³⁸.

If blood culture bottles are employed to provide an enrichment broth then any consequential use and subsequent disposal of syringes and needles must comply with local safety protocols.

Specimen containers must also be placed in a suitable holder.

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

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

2.2 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species (see [B 40 – Investigation of specimens for *Mycobacterium* species](#))

2.3 Appearance

N/A

2.4 Microscopy

2.4.1 Standard

Gram Stain (See [TP39 – Staining Procedures](#))

Giemsa stain

Giemsa stains should be carried out as indicated by local protocols; a smear maybe made at the patient's bedside or at the receiving laboratory. (See [TP39 – Staining Procedures](#))

2.5 Culture and investigation

2.5.1 Pre-treatment

If not already done, inoculate blood culture bottles with specimen and load onto the automated continuous monitoring blood culture system. Subculture positive bottles as required (see [B 37 – Investigation of blood cultures \(for organisms other than *Mycobacterium* species\)](#)).

2.5.2 Specimen processing

Standard

Bottles that flag as positive on the automated system should be subcultured according to the same procedure as for blood culture bottles (see [B 37 – Investigation of blood cultures \(for organisms other than *Mycobacterium* species\)](#)) inoculate agar plates with specimen from blood culture bottles (see [Q 5 – Inoculation of culture media for bacteriology](#)).

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2.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmosphere	Time		
All clinical conditions	Blood culture broths Subculture positive bottles onto subculture plates below.	35 – 37	Air	7 d up to 21 d for slow growers + terminal subculture	Continuous monitoring	Any organism
Subculture plates	Blood agar	35 – 37	5 – 10% CO ₂	40 – 48 hr	40hr	Any organism
	Chocolate agar	35 - 37	5 – 10% CO ₂	40 - 48 hr*	40hr	Any organism
For these situations, add the following:						
Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Systemic fungal infection	Sabouraud agar	28 – 30	Air	5 d	3 d and 5 d	Fungi
Microscopy suggesting anaerobes	Neomycin FAA with metronidazole 5 µg disc	35-37	Anaerobic	7 d	2 d, 5 d and 7 d	Anaerobes
Other organisms for consideration - <i>Mycobacterium</i> species (see B 40 - Investigation of specimens for <i>Mycobacterium</i> species), fungi, parasites (see B 31 - Investigation of specimens other than blood for parasites) and viruses (see www.hpa.org.uk/smi/ncvirology).						
* Incubation times may be increased up to 5 days if <i>Brucella</i> infections are suspected						

2.6 Identification

2.6.1 Minimum level of identification in the laboratory

All organisms to species level.

Note: Any organism considered to be a contaminant may not require identification to species level.

Refer to individual SMLs for organism identification.

2.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) guidelines.

2.8 Referral to reference laboratories

Contact appropriate reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission. Information regarding specialist and reference laboratories is available via the following websites:

[HPA - Specialist and Reference Microbiology Tests and Services](#)

[Health Protection Scotland – Reference Laboratories](#)

[Belfast Health and Social Care Trust – Laboratory and Mortuary Services](#)

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.

Presumptive *Leishmania* species should be sent to the reference laboratory for confirmation.

2.9 Referral for outbreak investigations

N/A

3 Reporting Procedure

3.1 Microscopy

Dependent on local protocols.

3.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report 16 – 72 hr.

3.2 Culture

Report clinically significant isolates or

Report other growth or

Report absence of growth.

3.2.1 Culture reporting time

Clinically urgent results to be telephoned or sent electronically.

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

3.3 Antimicrobial susceptibility testing

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

4 Notification to PHE^{47,48}

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify 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.

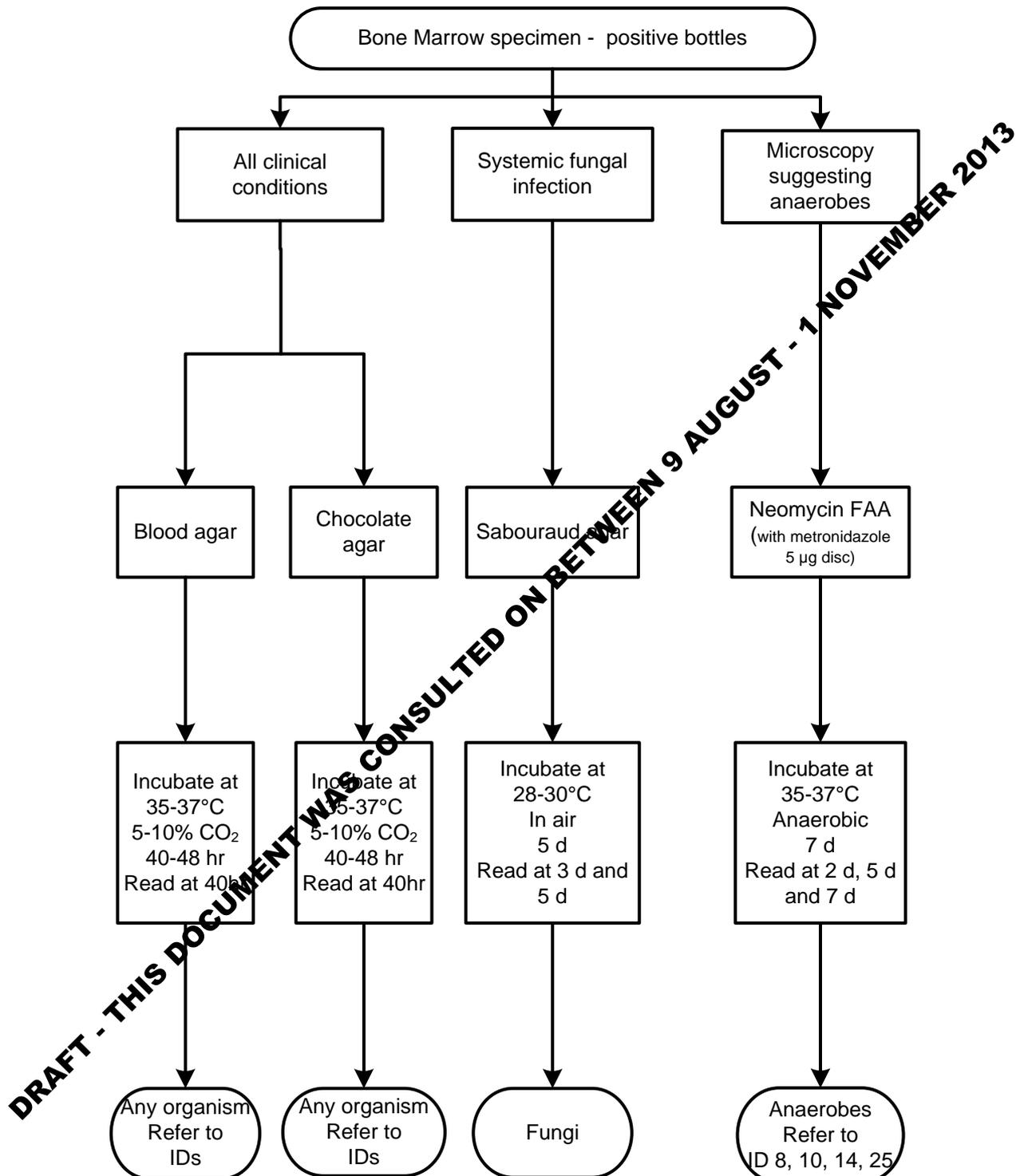
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland⁴⁹ and Wales⁵⁰.

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Appendix 1 – Investigation of bone marrow



References

1. 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".
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DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 9 AUGUST - 7 NOVEMBER 2013