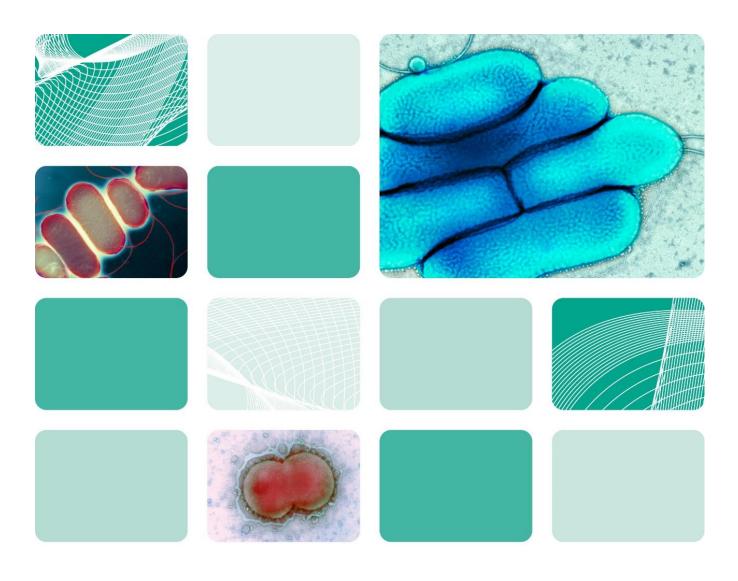


UK Standards for Microbiology Investigations

Investigation of bone marrow



Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

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UK SMIs are produced in association with:

Applied Microbiology International











































Displayed logos correct as of December 2024

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Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	4/31.10.25
Issue number discarded	2
Insert issue number	2.1
Section(s) involved	Amendment
	This is an administrative point change.
	The content of this UK SMI document has not changed.
	The last scientific and clinical review was conducted on 12/10/2015.
	Hyperlinks throughout document updated to Royal College of Pathologists website.
Whole document.	Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms.
	Partner organisation logos updated.
	Broken links to devolved administrations replaced.
	References to NICE accreditation removed.
	Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.
	'Public health responsibilities of diagnostic laboratories' section added.

Amendment no/date.	3/12.10.15
Issue no. discarded.	1.2
Insert issue no.	2
Section(s) involved	Amendment
Page 2.	Updated logos added.

Investigation of bone marrow

Scope.	Text updated for clarity.				
Introduction.	Reorganised for clarity.				
	Rapid methods section added.				
Technical information/limitations.	Addition of section on anticoagulants.				
	Reviewed and updated.				
Safety considerations.	If Hazard Group 3 organisms are not suspected, consider processing under Containment Level 2 conditions.				
	Information added regarding thermally dimorphic fungi.				
	Use of blood culture bottles recommended.				
Specimen collection.	Additional specimens for direct culture, microscopy and molecular techniques should be collected in appropriate CE marked leak proof containers.				
	Addition of direct culture and molecular techniques.				
Specimen	Section 4.5.1 culture media, conditions and organisms updated.				
processing/procedure.	Incubate blood culture broths for 5 days.				
	Incubate FAA for 5 days.				
	Incubate Sabouraud agar for 14 days.				
Reporting procedure.	Addition of reporting for molecular methods.				
Appendix 1.	Updated in line with section 4.5.1.				

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

Type of specimen

Bone marrow

This UK SMI describes the processing and microbiological investigation of bone marrow samples submitted for clinical diagnostic purposes. Techniques covered by this UK SMI include culture of bone marrow for the identification of bacteria and fungi, as well as molecular methods and rapid techniques. Other methods of investigation are available for the identification of parasites and viruses but are not covered in this UK SMI.

For the investigation of bone marrow for *Mycobacterium* species refer to UK SMI B 40 – Investigation of specimens for Mycobacterium species.

This UK SMI should be used in conjunction with other UK SMIs.

4 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 indications¹. 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 abnormalities². 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 viruses³⁻⁵.

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 however the preferred specimen for microbiological studies.

4.1 Infection 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 immunosuppressed⁶. 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 pathogens more likely⁷. In these cases, culture

of bone marrow may be useful in the investigation of pyrexia of unknown origin (PUO)^{2,8-10}. *Mycobacterium* species, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Talaromyces marneffei* (formerly *Penicillium marneffei*) and *Leishmania* species are likely to cause disseminated infection in the setting of immunosuppression^{6,11}.

4.2 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 (for example *Brucella* species and *Salmonella* Typhi) compared to blood culture. However, in some studies similar yields and turnaround times were observed^{5,12-14}. Bone marrow cultures may be positive in patients with acute or chronic infection, whereas blood cultures are more likely to be positive in patients with acute infections¹³. Bone marrow aspirates are also more likely than blood culture to be positive in patients who have been treated with antibiotics^{5,15}.

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.

4.2.1 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 water⁵. Enteric fever is the only bacterial infection for which bone marrow is routinely recommended¹⁶. Culture of bone marrow is considered to be the 'gold standard' method for diagnosis of typhoid fever. Blood culture may lack sensitivity and culture of bone marrow aspirates has been shown to produce a higher yield even when following antimicrobial treatment^{5,16}. In one study it was shown that 1mL of bone marrow gave an equivalent result to 15mL of blood¹⁷. Serology is available but has low sensitivity and specificity due to cross reactions with other Salmonella species and Enterobacteriaceae¹⁷. Nucleic acid amplification tests (NAATs) on culture positive bone marrow aspirates have been reported but are not yet in routine use¹⁸. 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 under diagnosed. 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 (as well as liver tissue and lymph nodes) may improve the recovery rate within a shorter time frame^{12,13,19}.

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

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laboratory diagnosis, however incubation times may be long³. The use of continuous blood culturing systems reduces culture time; positive results may be available within five to seven days¹⁹. Bone marrow culture assists in aiding diagnosis in uncertain cases of disseminated disease, particularly in those with HIV^{14,20-22}. Molecular methods for detection are currently under development (refer to <u>UK SMI B 40 – Investigation of specimens for Mycobacterium species</u>)³.

4.2.2 Fungi

Infection with dimorphic fungi such as *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* or *Talaromyces marneffei* (formerly *Penicillium marneffei*) may occasionally be diagnosed by bone marrow examination, but culture sensitivity varies^{11,23}. 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 *Talaromyces marneffei* to about four days²⁴⁻²⁶. 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 specimens¹⁴.

4.2.3 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. Coinfection 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 Europe¹.

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 immunochromatographic tests (ICT) have been developed and evaluated^{1,28}. 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^{4,27}. 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%^{1,27}.

4.2.4 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.

4.3 Rapid techniques

Molecular methods²⁹⁻³¹

NAAT - 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^{32,33}

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.

5 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.

Specimen containers^{34,35}

UK 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".

Anticoagulants³⁶

Specimens for direct culture, microscopy and molecular techniques should be collected in appropriate CE marked leak-proof containers. Various tubes containing anticoagulants may be used, manufacturer's instructions should be consulted prior to use.

Specimens for direct culture and microscopy may be submitted in a plain sterile tube, or a sterile heparinised tube. Specimens for NAAT may be submitted in sterile tubes containing heparin or EDTA.

6 Safety considerations^{34,35,37-51}

6.1 Specimen collection transport and storage^{34,35,37-40}

Use aseptic technique.

Ideally, specimens for culture should be collected directly into blood culture bottles and transported in sealed plastic bags.

Additional bone marrow specimens should be submitted in an appropriate CE marked leak-proof containers and transported in sealed plastic bags.

Compliance with postal, transport and storage regulations are essential.

6.2 Specimen processing^{34,35,37-51}

Where Hazard Group 3 organisms (eg *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.

If Hazard Group 3 organisms are not suspected, consider processing under Containment Level 2 conditions.

All laboratory procedures (including the examination of plates and cultures) must be conducted in a microbiological safety cabinet⁴³.

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

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 UK SMI.

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

7 Specimen collection

7.1 Type of specimens

Bone marrow, bone marrow sample inoculated in a blood culture bottle

7.2 Optimal time and method of collection⁵²

For safety considerations refer to Section 6.

Collect specimens before starting antimicrobial therapy where possible⁵².

Specimens for culture should ideally be collected in blood culture bottles.

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Additional specimens for direct culture, microscopy and molecular techniques should be collected in appropriate CE marked leak-proof containers. For information regarding appropriate use of anticoagulants refer to technical information/limitations.

7.3 Adequate quantity and appropriate number of specimens⁵²

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

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

8 Specimen transport and storage^{34,35}

8.1 Optimal transport and storage conditions

For safety considerations refer to Section 6.

Specimens should be transported and processed as soon as possible⁵².

9 Specimen processing/procedure^{34,35}

9.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species.

Refer to UK SMI B 40 – Investigation of specimens for Mycobacterium species.

9.2 Appearance

N/A

9.3 Sample preparation

9.3.1 Pre-treatment

Standard

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.

Optional

N/A

9.3.2 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.

Optional

Specimens collected into appropriate CE marked leak proof containers should be used for microscopy and may be used for the following tests:

Direct culture

Where clinically indicated, direct plate culture may be required.

Molecular techniques

Specimens for molecular testing should be processed according to manufacturer's instructions.

9.4 Microscopy

9.4.1 Standard

Giemsa stain

Giemsa stains should be carried out for Leishmaniasis as indicated by local protocols; a smear maybe made at the patient's bedside or at the receiving laboratory.

Refer to <u>UK SMI TP 39 – Staining procedures</u>

9.4.2 Optional

Gram stain

Refer to UK SMI TP 39 – Staining procedures

9.4.3 Supplementary

See <u>UK SMI B 40 - Investigation of specimens for Mycobacterium species</u>, and <u>UK SMI TP 39 - Staining procedures</u>

9.5 Culture and investigation

Inoculate each agar plate using a sterile pipette (<u>UK SMI Q 5 - Inoculation of culture media for bacteriology</u>).

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

9.5.1 Culture media, conditions and organisms

Clinical details/	Specimen Standard	Standard media	Incubati	on		Cultures read	Target organism(s)
conditions		illeula	Temp °C	Atmos	Time	reau	organism(s
All clinical conditions	Bone marrow	Blood culture broths (aerobic and anaerobic) Subculture all bottles onto subculture plates below.	35 – 37	Air	5 d + terminal subculture	Continuous monitoring	Any organism
Subculture plates	Bone marrow	Blood agar	35 – 37	5 – 10% CO ₂	40 – 48hr	≥40hr	Any organism
		Chocolate agar	35 - 37	5 – 10% CO ₂	40 - 48hr*	≥40hr	Any organism
		FAA	35-37	Anaerobic	5 d	3 d and 5 d	Anaerobes
For these situa	ations, add the f	ollowing:					
Clinical details/			Incubation			Cultures read	Target organism(s
conditions		,	Temp °C	Atmos	Time		J - (-)
Systemic fungal infection	Bone marrow	Sabouraud agar (slopes)	28 - 30	Air	14 d	Daily	Yeast and Mould
Where clinically indicated	Bone marrow	Direct Culture: Blood agar	35 – 37	5 – 10% CO ₂	40 – 48hr	≥40hr	Any organism
		Chocolate agar	35 – 37	5 – 10% CO ₂	40 - 48hr*	≥40hr	Any organism
		FAA	35-37	Anaerobic	5 d	3 d and 5 d	Anaerobes
Optional Mole	cular Technique	S					
Optional Molece Clinical details/ conditions	cular Technique	S Molecular Technique	Instructi	ons			Target organism(s

Other organisms for consideration - *Mycobacterium* species (see <u>UK SMI B 40 - Investigation of specimens for Mycobacterium species</u>), fungi, parasites (see <u>UK SMI B 31 - Investigation of specimens other than blood for parasites</u>) and viruses (see https://www.rcpath.org/profession/publications/standards-for-microbiology-investigations/virology.html).

*Incubation times may be increased up to 5 days if Brucella species infection is suspected.

9.6 Identification

Refer to individual UK SMIs for organism identification.

9.6.1 Minimum level of identification in the laboratory

All organisms to species level.

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

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

9.7 Antimicrobial susceptibility testing

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

9.8 Referral for outbreak investigations

N/A

9.9 Referral to reference laboratories

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

Organisms with unusual or unexpected resistance, or associated with 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, turnaround times, transport procedure and any other requirements for sample submission:

England

Wales

Scotland

Northern Ireland

10 Reporting procedure

10.1 Microscopy

10.1.1 Standard

Giemsa stain

Report as indicated by local protocols.

10.1.2 Optional

Gram stain

Report organism detected.

Supplementary

For the reporting of microscopy for *Mycobacterium* species refer to <u>UK SMI B 40</u> – <u>Investigation of specimens for Mycobacterium species</u>.

10.1.3 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

10.2 Culture

Following results should be reported:

- · clinically significant organisms isolated
- · other growth
- · absence of growth

10.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestor.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

10.3 Molecular

Report results according to manufacturer's instructions.

10.4 Antimicrobial susceptibility testing

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

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11 Notification to UKHSA, or equivalent in the devolved administrations⁵⁵⁻⁵⁸

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify UK Health Security Agency (UKHSA) 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 UKHSA 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 UKHSA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to UKHSA and many UKHSA 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'.

https://www.gov.uk/government/organisations/uk-health-security-agency
Other arrangements exist in Scotland^{55,56}, Wales⁵⁷ and Northern Ireland⁵⁸.

12 Public health responsibilities of diagnostic laboratories

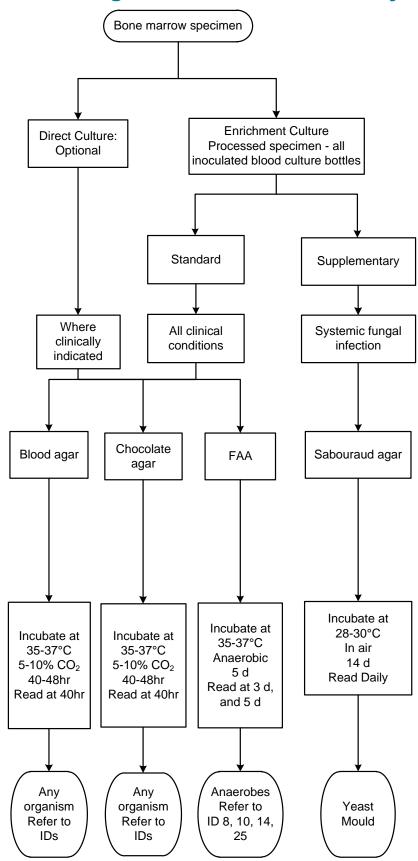
Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

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Algorithm: Investigation of bone marrow by culture



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An explanation of the reference assessment used is available in the <u>scientific</u> information section on the UK SMI website.

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