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UK Standards for Microbiology Investigations

Inoculation of culture media for bacteriology

Issued by the Standards Unit, Microbiology Services, PHE

Acknowledgments

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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: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

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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.	5/dd.mm.yy <tab+enter>
Issue no. discarded.	1.3
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Section(s) involved	**Amendment**

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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. 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

[#] 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.

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

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Scope of document

This SMI describes the basic methods of inoculating primary culture media with clinical specimens including swabs, fluid, urine, faeces, tissue and cannulae; as well as subsequent sub-culturing of organisms from one medium (solid or liquid) to another using aseptic techniques.

This SMI should be used in conjunction with other SMIs.

Introduction

This quality guidance describes the methods of inoculating culture media and sub-culturing of organisms using aseptic techniques.

To process clinical specimens satisfactorily for bacteriological culture, consideration must be given to[1,2]:

- samples (where possible) are taken before antimicrobial therapy is started
- the need to process specimens within appropriate timescale for organism viability and clinical need
- the safety aspects of specimen processing
- the specimen type and its anatomical origin
- the requirement for pre-treatment before inoculation (eg centrifugation, homogenisation and dilution as is the case with TB clinical samples such as sputum)
- the selection of primary isolation media
- the incubation temperature and atmosphere

1 General principles

Solid media

In general, media should be inoculated in a logical order (see below) from least selective to most selective to avoid the inhibition of organisms by the selective agent:

1. Media without inhibitors (eg blood agar)
2. Indicator media (eg CLED agar)
3. Selective media (eg XLD agar, GC selective agar)
4. Smears for staining

There may be occasions where it may not be advisable to inoculate media in this way. For example, swabs for gonococcal (GC) culture may contain only small numbers of organisms. This will make the inoculation of the GC selective agar the priority. Where specimens are insufficient for a full range of culture plates, priorities should be based on origin of specimen and the range of likely organisms to be encountered.

For the isolation of individual colonies, the inoculum should be spread, usually by using a sterile loop, taking care to avoid the edges of the plate where contaminants are more likely to be located.

Liquid media

Liquid media may be inoculated first when processing fluid specimens. This reduces the chances of carry-over from contaminated solid media. However, liquid media should be inoculated after the solid media when swabs and faeces are examined, to avoid diluting the organisms contained on or in the sample and to avoid any organisms (whether viable or non-viable) present in a liquid medium being transferred to other liquid media, solid media or to slides.

Smears

Smears for staining are usually made after all culture media have been inoculated to avoid carry-over of contaminants that may be on the surface of the slide. However, there may be occasions where the smear is required prior to culture and then a sterile slide should be used.

Slides may be sterilised by flooding the slide with alcohol, discarding the excess and drying on a hot plate. Under no circumstance should the alcohol be burned off in a Bunsen flame.

Antimicrobial discs

Antimicrobial discs for identification (eg optochin, bacitracin) may be added as appropriate. Discs should be placed near the edge of the plate, between the areas covered by the first and second spread, to avoid total inhibition of very susceptible organisms.

Labelling of culture media

As a minimum requirement, all culture plates and containers must be labelled to identify the patient name or laboratory number or barcode. Additional labelling, including date of culture or sub-culture will be necessary for selected specimens, such as those requiring prolonged incubation or sub-culture from enrichment broth.

To work safely and minimise risks of cross contamination, suitable racks should also be used when inoculating, incubating or storing liquid cultures or culture plates[3].

2 Inoculation of culture media

For the effective detection of the bacterial content of specimens, it is important to achieve growth of individual colonies by using a good technique to inoculate the specimen on culture media. There are many variations and personal preferences for "plating out", some of which are described in this document.

All culture media should be checked before use for contamination and expiry date. Culture media should have an identifiable batch or quality control number and have passed QC tests before use. Plates that are beyond their expiry date, contaminated plates, and broth media appearing unusually turbid should be discarded.

The initial area inoculated should cover between a quarter and a third of the total area of agar used. Whole plates, half plates, or quarter plates can be used depending on the circumstances. Specimens may be plated out for individual colonies, or seeded directly over an entire segment of a plate and incubated without further spreading.

Inoculation loops are designed for quantitative procedures such as sampling, serial dilutions, as well as for bacterial inoculation. There are various types of inoculation loops – wire loops or the disposable alternative. The disposable loops were initially used in situations where flaming is not practical, such as in safety cabinets but is common practice for health and safety purposes. The use of wire loops is rarely seen in use in microbiology laboratories in the UK but a few clinical laboratories may still use these. This is due to some limitations in its use such as the risk of infection due to aerosol formation of pathogenic organisms, as well as cross-contamination due to improper sterilisation of the wire loops. Therefore, disposable loops are recommended in this document.

For a potentially heavily contaminated sample, the disposable loop should either be changed between each series of streaks, or the loop may be rotated to make the next series of streaks with the unused side of the loop. For semi-quantitative analysis of urine, the loop should be changed.

All media should be incubated as soon as possible after inoculation. Plates for anaerobic incubation should be incubated as soon as possible to prevent loss of viability (<15 minutes)[4]. After inoculation, the specimen, or a portion of it, should be retained for at least 48 hours after the laboratory has issued the final report[5].

Most positive culture plates can be discarded within 24-48 hours of issuing a final authorised report. Cultures of particular epidemiological value may be retained for longer as organisms may need further work or referral to a reference laboratory[5].

Retained routine microscope slides should be kept for seven days after issue of the final report. Slides for examination for *Mycobacterium* species and positive cultures of *Mycobacterium* species should be retained in a locked cupboard in a Category 3 laboratory until the final report from the Reference Laboratory has been received.

3 Aseptic technique

When handling specimens or cultures, aseptic technique is important to avoid contamination and to protect the worker from infection from the sample.

In-house training to demonstrate the skills of aseptic technique should be given to staff who will process specimens or cultures.

The following points should be observed when culturing specimens or performing subcultures:

- with the exception of urine specimens, caps and lids from containers should not be placed on the workbench, but retained in the hand while the sample is being processed, taking care not to contaminate the hand or cap. Caps and lids should be replaced as soon as possible
- if the work is being carried out on the open bench, a disposable jar should be in close proximity to the operator in order to place the loops
- keep samples away from the face when opening culture containers
- aerosol production should be minimised by:
  - opening caps slowly in a microbiological safety cabinet as the contents of containers are sometimes under pressure
  - avoiding vigorous swirling or shaking of the sample prior to opening
  - avoiding expelling the last drop from a pipette
- if forceps or scissors are used when handling specimens, they should be autoclaved and then sterilised before use. If available, use disposable forceps or scissors and dispose in a disposable waste jar after use

4 Primary culture methods

4.1 Swabs - plate culture

Initial inoculum should cover between a quarter and a third of the plate to be used (Figure 1).

The swab should be rolled over the inoculation area to maximise transfer of organisms, taking care to avoid the edges of the plate.

Inoculation of samples to selective media such as Sabouraud agar (when usually only a quarter plate will be used) may not require spreading with a loop (Figure 4).

4.2 Swabs - liquid culture

Using aseptic technique, remove the broth container cap, place the swab in the broth, break off (or cut) the swab-stick and replace the cap. The swab may be placed in the broth directly, or after inoculating solid culture media (consideration should be given to the possibility that contaminants may be transferred into the broth from contaminated culture plates).

4.3 Fluid specimens and pus

The centrifuged deposit of any fluid is re-suspended in approximately 0.5mL supernatant, and then transferred to the appropriate culture media with a sterile pipette. Thick pus may require inoculation with the aid of a swab/swab stick. If a semi-quantitative method is required, inoculate the media with a standard loop (1µL, 10µL etc) or a piston-operated pipette as appropriate.

4.4 Urine - calibrated loop, surface streak method[6]

(See B 41 – Investigation of urine)

The urine is mixed gently to avoid foaming.

The end of a sterile calibrated loop (eg 1µL, 2µL or 10µL) is dipped to just below the surface of the urine and removed vertically, taking care not to carry over any urine on the shank. See Figure 2.

4.5 Urine - filter paper method[7]

(See B 41 - Investigation of urine)

Commercially prepared sterile filter paper strip is dipped in the urine up to the mark indicated.

Remove excess urine by touching the side of the strip against the side of the container and allow the urine time to absorb into the strip before inoculating a CLED or chromogenic agar plate.

Bend the inoculated end of the strip and press it flat against the agar for a few seconds.

Several specimens may be inoculated on one CLED or chromogenic agar plate using this technique, but it is important to ensure adequate spacing to minimise the risk of any antibacterial effect.

4.6 Faeces

Initial inoculum should be made on the appropriate agar media (using swabs) used to cover between a quarter and a third of the plate to be used as shown in Figure 1. This should then be spread using loops over the inoculation area taking care to avoid the edges of the plate. The faecal material may be placed in the broth directly, or after inoculating solid culture media for subculture. Inoculation of a broth medium is optional. Using aseptic technique, remove the broth container cap, place the faecal material in the broth using a loop or a swab, break off (or cut) the swab-stick and replace the cap. If using the loop, mix the faecal material gently in the broth and then dispose of the loop in a disposable waste jar.

4.7 Tissue and biopsy specimens

Homogenise tissue with a sterile tissue grinder (Griffith's tube or sterile glass (ballotini) beads or a pestle and mortar) and inoculate 1 or 2 drops of the homogenate on appropriate media (Figure 4).

Tissue may also be cut or sliced with a sterile scalpel or, preferably, sterile scissors. Using sterile forceps, smear the sliced portion directly on the culture medium. If enrichment culture is performed to avoid possible contamination, the medium should

be inoculated with pieces of specimen that have not been spread over the surface of solid culture media.

All homogenisation and grinding procedures involving tissue or biopsy specimens must be performed in a Class 1 safety cabinet.

4.8 Intravascular cannulae

Inoculate culture media directly by rolling the cannulae across the surface of a whole agar plate five times (avoiding the edges of the plate) or culture any blood, fluid or material contained in or on the specimen (see B 20 - Investigation of intravascular cannulae and associated specimens).

5 Subculture methods

5.1 Subculture of liquid media to a solid or liquid medium

Obtain samples for subculture with a sterile loop (1µL, 10µL etc) or a plastic pipette. Immerse the loop in the fluid to be subcultured, and remove carefully without allowing excess fluid to remain on the shank of the loop. Care should be taken not to contaminate the loop holder with liquid culture as this will be difficult to sterilize and may cause subsequent problems with cross contamination.

Either inoculate a loopful of fluid on an appropriate agar plate, streaking out for individual colonies (Figure 2), or inoculate 2-3 drops from the pipette on appropriate agar plates or to further fluid culture media. The use of a pipette is particularly recommended when sub-culturing organisms to multiple culture media, including those used for biochemical tests.

Subculture blood culture bottles according to manufacturer's instructions. Most continuous monitoring systems require the use of sub-vent units or sheathed needles.

Note: Caution must be observed when subculturing bottles under obvious increased pressure.

Before subculture of broth that may contain mixed organisms including anaerobes, gently agitate to give an even distribution of organisms throughout the broth with as little disturbance as possible.

Subculture Selenite F broth and alkaline peptone water by inserting a sterile loop or pipette to the broth and sampling from just below the surface.

5.2 Subculture from a solid medium to a liquid medium

Select a representative colony or colonies of the organism to be subcultured and using aseptic technique transfer to an appropriate broth with a sterile loop. Gently agitate before incubation to distribute the organisms throughout the broth.

5.3 Subculture from a solid medium to a solid medium

Picking colonies for subculture may be carried out with a sterile disposable loop. It is recommended that a sterile loop be used when dealing with mixed cultures to ensure the sampling of the single colonies.

Select a representative colony or colonies of the organism to be subcultured with a sterile disposable loop, and subculture on the appropriate medium by touching the loop on to the surface of the agar, and plate out.

To ensure even inoculation of biochemical test systems and multiple media, colonies should be picked and transferred to an appropriate suspension fluid or medium (eg approximately 2mL peptone water or nutrient broth). The use of a densitometer or McFarland standards may be required to adjust inoculum density. Gently agitate the suspension. Use a loopful, or a drop from a pipette of the inoculated broth, or a swab immersed in the broth suspension to inoculate the plate or test system.

The use of a pipette is recommended when subculturing fluid to more than one culture medium.

Multipoint inoculators are convenient when many replicate cultures are needed. They may be semi or fully automatic and can spot-inoculate approximately 20 cultures on a standard 9cm Petri dish, or up to 96 cultures to a microtitre tray. Multipoint inoculators have been used for urine culture, identification testing, and antimicrobial susceptibility testing. For more information refer to B 41 - Investigation of urine.

Shake tube cultures are useful for observing colony formation in deep agar cultures, and are especially useful for microaerophilic and anaerobic organisms. Agar in bottles and tubes is melted and maintained at a temperature of approximately 45°C. The agar is allowed to cool slightly and a sterile disposable loop is used to inoculate the culture into the molten agar. The tube is incubated after gentle mixing. Submerged colonies will develop at different levels in the medium according to their respiratory requirements.

Stab cultures can be used to observe motility, acid and gas production, and biochemical activity (eg gelatin liquefaction, DNAse). A representative colony can be picked with a sterile inoculating stab needle and then stabbed on the appropriate agar.

6 Different inoculation methods used in bacteriology

There are different methods of inoculation used in bacteriology. However, the most common methods are described below:

Streak plating technique

The purpose of this procedure (outlined below) is to obtain well isolated colonies from an inoculum by creating areas of increasing dilution on a single plate[8]:

1. Inoculate clinical specimen using a sterile inoculation loop onto agar media. Gently spread bacteria over a portion of the culture media surface
2. Drag loop into section 1 to obtain bacteria. Then spread it out into a second section
3. Drag loop into section 2 to obtain bacteria. Then spread it out into the third section. Do the same for the same for the third and the fourth section. Ensure that sections 1 and 4 do not overlap. Dispose of the inoculation loop used
4. Replace the lid and then incubate the streaked agar plate at the appropriate temperature in an inverted position

Agar stab technique

The method (outlined below) is used to prepare stab cultures (to observe motility or oxygen usage, or when inoculating certain types of solid medium) and to pick single colonies from a plate:

1. Using aseptic technique pick a single well isolated colony with a sterile inoculating stab needle and stab the needle several times through the agar to the bottom of the vial or tube
2. Replace the cap and tighten loosely when incubating to allow gas exchange
3. Incubate the stabbed agar plate/slant at the appropriate temperature

Spread plate technique

The purpose of this procedure (outlined below) is to distribute cells evenly so that well isolated individual colonies can be grown. These are then counted or used for further tests such as serial dilutions:

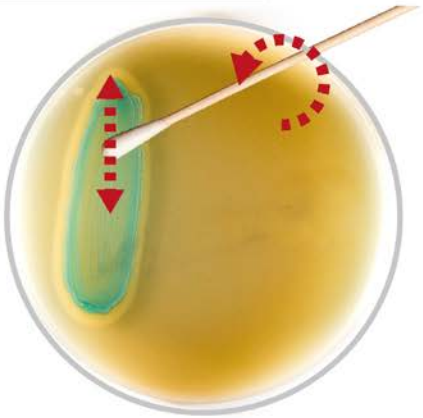
1. Inoculate clinical specimen using a sterile spreader or alternative onto agar media. Gently spread bacteria over the entire culture media surface backward and forward while rotating the plate. Avoid the spreader touching the corners of the agar media
2. Replace the lid and allow the plate to stand in an upright position to dry for 10 to 20 minutes[9]
3. Incubate the spread agar plate at the appropriate temperature in an inverted position

Note: Other culture methods that may be used include the pour plate, liquid culture methods and anaerobic culture methods.

Appendix 1: Illustration of inoculation technique

These illustrations are for guidance only.

Figure 1
Inoculation of agar



Figures 2 and 3
Streaking inoculum for individual colonies

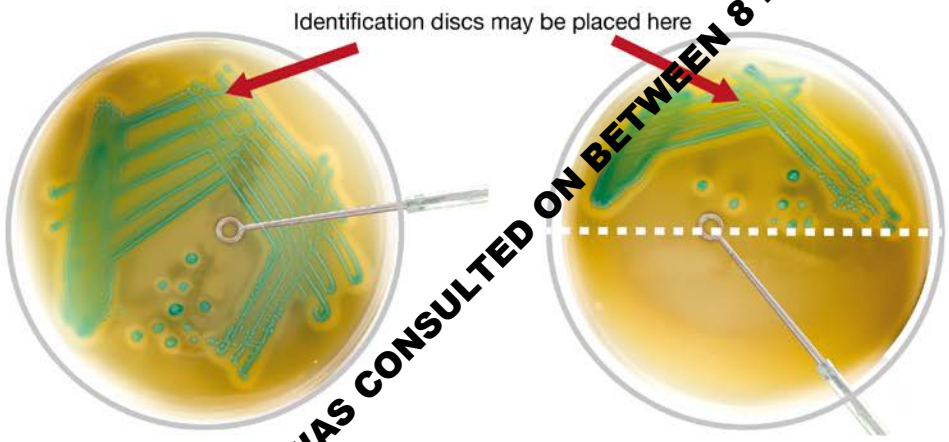
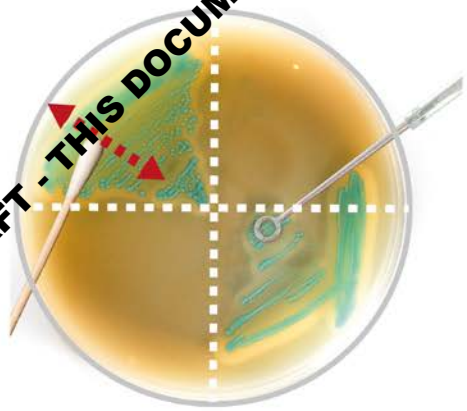


Figure 4
Using quarter plates



Appendix 2: Technical limitations/information

Use of wire loops

The use of wire loops are prohibited in UK microbiology laboratories but there might be a few laboratories that still use them. These were discouraged from being used due to the risk of infection from aerosol formation of pathogenic organisms, as well as cross-contamination due to improper sterilisation of the wire loops. The UK SMI does not recommend the use of wire loops. A typical example of an SMI where the use of wire loops is discouraged is TP 8 - Catalase test. TP8 covers the inoculating wire loops (nichrome) where reaction with the hydrogen peroxide can produce false positive reactions.

Spread plate technique

Ensure that plates are sufficiently dry prior to use. It should also be noted that there should be no delay in spreading the inoculum once it has been applied to the plate as some cells will rapidly attach to the agar[9].

Incubation temperature

Stacking plates too high in the incubator may affect results owing to uneven distribution of temperature around the plates. The efficiency of heating of plates depends on the incubator and the racking system used. Stacking of plates to a maximum height should therefore be part of the laboratory's Quality Assurance programme[3].

Commercial automated instruments

Highly automated machines have been introduced in many clinical microbiological laboratories worldwide to contribute to more accurate, rapid, and cost-effective management of patient samples. These have been shown to effectively inoculate and spread/streak samples on a variety of solid media as well as isolate more colonies than the manual methods. Its advantages also include its ease of use especially with laborious tasks such as labelling, inoculation, and streaking[10-12].

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