



UK Standards for Microbiology Investigations

Inoculation of culture media for bacteriology



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Acknowledgments

UK Standards for Microbiology Investigations (UK 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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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

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Amendment table

Each UK 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 number/date	5/09.01.17
Issue number discarded	1.3
Insert issue number	2
Anticipated next review date*	09.01.20
Section(s) involved	Amendment
Whole document.	Document has been strengthened to include the updated Appendix 1: Illustration of inoculation methods, added Appendix 2: Technical limitations/information as well as Appendix 3: Inoculation of swabs on culture media. Section 6 has been added to explain the different inoculation methods used in bacteriology.
References.	References updated.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. 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 UK SMIs

UK 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 UK 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

UK 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 UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] 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.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date 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.

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Suggested citation for this document

Public Health England. (2017). Inoculation of culture media for bacteriology. UK Standards for Microbiology Investigations. Q 5 Issue 2. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Scope of document

This UK 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.

Refer to the appropriate UK SMI for mycological samples in section 4.

This UK SMI should be used in conjunction with other UK 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 the following¹⁻³:

- samples (where possible) are taken before antimicrobial therapy is started
- the quality of specimens taken and the transport conditions when in transit to the laboratory
- the need to process specimens within appropriate time scale 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 (for example, centrifugation, homogenisation and dilution as is the case with acid fast bacilli (AFB) 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 carry over of the selective agent:

1. Media without inhibitors (for example, blood agar)
2. Indicator media (for example, CLED agar)
3. Selective media (for example, XLD 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 (or if available, disposable loop), taking care to avoid the edges of the plate where contaminants are more likely to be located.

Liquid media

Liquid media should be inoculated first when processing fluid specimens ensuring that media are inoculated in a sequence that minimises the risk of cross contamination. 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, for example in the case where specimens for investigation for acid fast bacilli are received. Great care should be taken to avoid contamination for example by not placing the loop back into the specimen after touching the slide.

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 minimise transcription errors.

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.

2 Inoculation of culture media

All culture media must be checked visually before use for contamination, significant physical imperfections (for example, uneven distribution of media, variable amounts of medium in petri dishes/tubes/bottles, colour, gross deformation of the surface on the media) 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 discarded⁴.

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.

The initial area inoculated should cover between a quarter and a third of the total area of agar used (Figure 1). Whole plates, half plates, or quarter plates can be used depending on the circumstances (Figures 2, 3 and 4). Specimens may be plated out for individual colonies, or seeded directly over an entire segment of a plate and incubated without further spreading.

Antimicrobial discs for identification (for example, 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 (see figures 2 and 3).

Inoculation loops are designed for quantitative procedures such as sampling, serial dilutions, as well as for bacterial inoculation. Inoculation loops can be ‘wire or disposable loops’. Disposable loops were initially used in safety cabinets to avoid sterilisation with Bunsen burners but now their use is common practice to comply with the health and safety regulations. Disposable loops are also desirable for quantitative purposes. Wire loops are rarely used in clinical microbiology laboratories in the UK to reduce the risk of infection from aerosols of pathogenic organisms and, cross-contamination from improper sterilisation of the wire loops. Therefore, disposable loops are recommended in this document.

For polymicrobial clinical specimens, 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 between streaks.

All media should be incubated as soon as possible after inoculation. In particular, plates for anaerobic incubation should be incubated as soon as possible to prevent loss of viability (<15 minutes)^{5,6}. After inoculation, the specimen, or a portion of it, should be retained for at least 48 hours after the laboratory has issued the final report⁷.

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 laboratory⁷.

Highly automated machines have been introduced in many clinical microbiological laboratories worldwide to contribute to more accurate, rapid, and cost-effective management of patient samples⁸⁻¹⁰.

3 Aseptic technique

When handling specimens or cultures, the use of an aseptic technique is crucial to avoid contamination and to protect the worker from infection.

In-house training to develop these skills should be given to staff processing the specimens or cultures.

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

- 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
- lids from agar media should be placed on the bench to face upwards and after the plates are inoculated, the lids should be replaced immediately to avoid any contamination
- 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 discard the loops
- keeping samples away from the face when opening culture containers. This can be achieved by wearing the appropriate PPE when handling cultures
- aerosol production should be minimised by:
 - opening caps of clinical specimens 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 by mixing the sample gently
 - avoiding expelling the last drop from a pipette
 - removing excess fluid from a swab put in a suspension (to be inoculated on an agar plate) by turning the swab against the inside of the container
- when forceps or scissors are used for handling specimens, they should be autoclaved and sterilised before use. Use disposable forceps or scissors if available, and dispose into a sharps bin after use

4 Primary culture methods

There are many different culture methods used for inoculation of clinical samples on culture media. Refer to the appropriate UK SMIs on the processing of different clinical samples, see table below.

Types of clinical samples	Types of culture methods and their associated UK SMIs
<p>Dry /Liquid swabs</p> <ul style="list-style-type: none"> - plate culture - liquid culture - smear <p>See appendix 3 for information on how to inoculate both liquid and dry swabs onto culture media.</p>	<p>B 1 - Investigation of ear infections and associated specimens</p> <p>B 2 - Investigation of bacterial eye infections</p> <p>B 4 - Investigation of superficial mouth samples</p> <p>B 5 - Investigation of nasal samples</p> <p>B 6 - Culture of specimens for <i>Bordetella pertussis</i> and <i>Bordetella parapertussis</i></p> <p>B 9 - Investigation of throat related specimens</p> <p>B 11 - Investigation of swabs from skin and superficial soft tissue infections</p> <p>B 14 - Investigation of pus and exudates</p> <p>B 20 - Investigation of intravascular cannulae and associated specimens</p> <p>B 28 - Investigation of genital tract and associated specimens</p> <p>B 29 - Investigation of specimens for screening for MRSA</p> <p>B 31 - Investigation of specimens other than blood for parasites</p> <p>B 51 - Screening for <i>Neisseria meningitidis</i></p> <p>B 58 - Detection of carriage of group B streptococci</p>
<p>Fluid specimens and pus</p>	<p>B 22 - Investigation of cerebrospinal fluid shunts</p> <p>B 25 - Investigation of continuous ambulatory peritoneal dialysis fluid</p> <p>B 26 - Investigation of fluids from normally sterile sites</p> <p>B 27 - Investigation of cerebrospinal fluid</p> <p>B 28 - Investigation of genital tract and associated specimens</p> <p>B 37 - Investigation of blood cultures (for organisms other than <i>Mycobacterium</i> species)</p> <p>B 38 - Investigation of bone marrow</p> <p>B 40 - Investigation of specimens for <i>Mycobacterium</i> species</p> <p>B 44 - Investigation of orthopaedic implant associated infections</p> <p>B 57 - Investigation of bronchoalveolar lavage, sputum and associated specimens</p>

Faeces	B 30 - Investigation of faecal specimens for enteric pathogens For more information on how to process faeces for parasites, refer to B 31 - Investigation of specimens other than blood for parasites
Skin scrapings, nail and hair	For more information on how to culture these samples onto culture media, refer to B 39 -Investigation of dermatological specimens for superficial mycoses
Urine <ul style="list-style-type: none"> - calibrated loop, surface streak method^{3,11} - filter paper method¹² 	B 41 - Investigation of urine
Tissue and biopsy specimens	B 17 - Investigation of tissues and biopsies from deep seated sites and organs B 42 - Investigation of bone and soft tissue associated with osteomyelitis B 55 - Investigation of gastric biopsies for <i>Helicobacter pylori</i>
Intravascular cannulae	B 20 - Investigation of intravascular cannulae and associated specimens

5 Subculture methods

5.1 Subculture of liquid media onto a solid or liquid medium

Obtain samples for subculture with a sterile disposable 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 (if using wire loops) with liquid culture as this will be difficult to sterilise 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 subculturing organisms to multiple culture media, for example, 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. If an anaerobe is suspected for example when the anaerobic blood culture bottle is positive, it is advisable to subculture as soon as possible onto appropriate media with anaerobic incubations, as a needle puncture will introduce oxygen into the blood culture bottles.

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

Before subculture of the 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 disposable 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 disposable loop. Emulsify the organism using the inside surface of the container and 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 disposable loop be used when dealing with mixed cultures to ensure sampling of each individual colony.

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 (see Appendix 1).

To ensure even inoculation of biochemical test systems and multiple media, colonies should be picked and transferred to an appropriate suspension medium (for example, 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, a drop from a pipette, 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 are melted and maintained at a temperature of $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$. 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 (for example, gelatin liquefaction, DNase). A representative colony can be picked with a sterile inoculating stab needle and then stabbed onto 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 a specimen or culture inoculum by creating areas of increasing dilution on a single plate¹³:

1. Inoculate clinical specimen using a sterile inoculation loop onto agar media. Spread specimen over a portion of the culture media surface gently (see figure 1)
2. Drag loop from the inoculated section and spread it out into a second section
3. Drag loop from the section 2 and then spread it out into the third section. Do the same for the third and the fourth section. Ensure that sections 1 and 4 do not overlap. Dispose of the inoculation loop used into an appropriate container (see figures 2 and 3)
4. Replace the lid and then incubate the streaked agar plate at the appropriate temperature in an inverted position to prevent condensation

Agar stab technique

The method (outlined below) is used to prepare stab cultures (to observe motility, 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

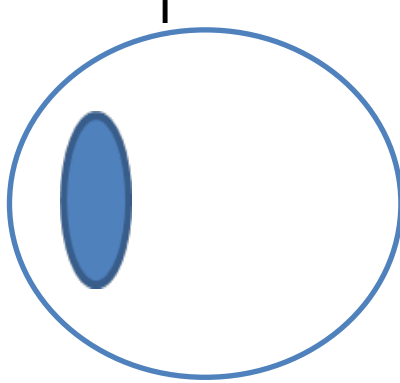
The purpose of this procedure (outlined below) is to distribute cells evenly so that well isolated individual colonies can be grown. These are then counted or used for further tests such as serial dilutions:

1. Inoculate clinical specimen using a sterile spreader or alternative onto agar media. Gently spread bacteria over the entire culture medium surface backward and forward while rotating the plate. Avoid the spreader touching the edges of the agar plate
2. Replace the lid and allow the plate to stand in an upright position (with the lid at the top) to dry for 10 to 20 minutes¹⁴
3. Incubate the spread agar plate at the appropriate temperature in an inverted position (with the lid at the bottom)

Note: There are other culture methods that may be used which include the pour plate, liquid culture methods and anaerobic culture methods. However, these are not discussed in this UK SMI document.

Appendix 1: Illustrations of inoculation methods

Figure 1
Inoculation of agar



Figures 2 and 3
Streaking inoculum for individual colonies

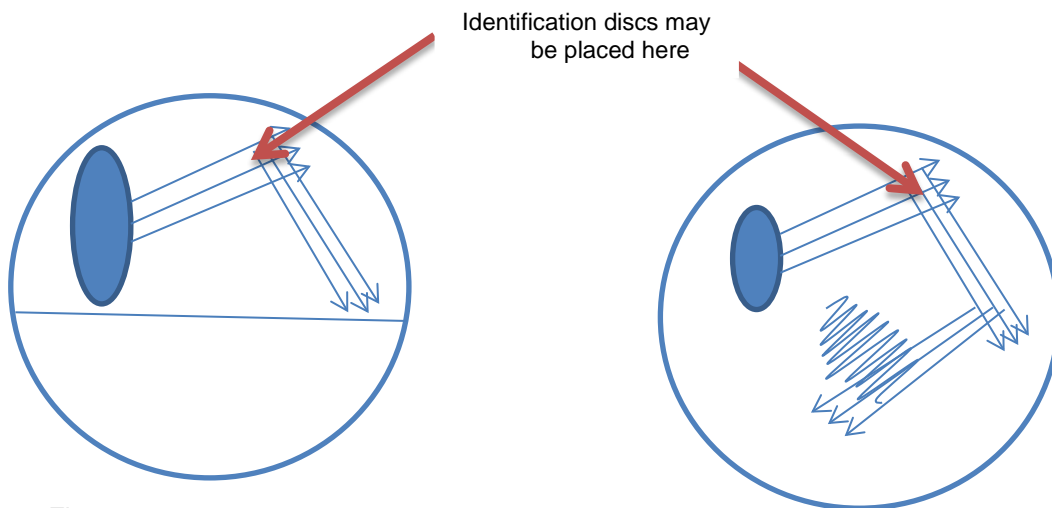


Figure 4
Using quarter plates

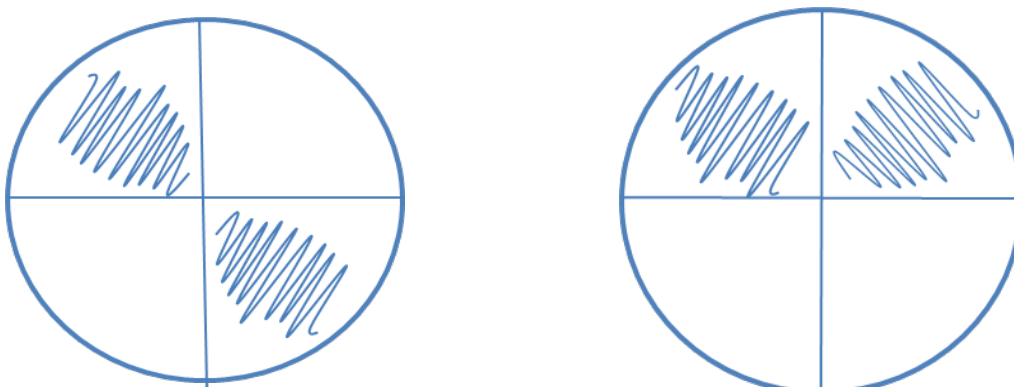


Figure 5

Using an automated inoculator

(Courtesy of Mischnik et al, 2015)¹⁰

These illustrations are for guidance only.

Appendix 2: Technical limitations/information

Use of wire loops

The use of wire loops is rarely seen in microbiology laboratories within the UK although a few clinical laboratories may still use these for certain procedures. Their use has been discouraged to prevent the risk of infection from aerosol formation of pathogenic organisms, and cross-contamination from improper sterilisation of the wire loops. The UK SMI does not recommend the use of wire loops. A particular example of an UK SMI where the use of wire loops is discouraged is [TP 8 - Catalase test](#). TP 8 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 agar¹⁴.

Labelling and contamination between organisms

It is essential that laboratory staff ensure that accurate labelling of culture media is done at all times for samples inoculated on culture media. Extreme care should be taken for example, when multiple samples are plated onto culture media to avoid mislabelling or plating a wrong sample onto the portion of the plate labelled for another sample.

Laboratory staff should also ensure that inoculum is applied within the space located on the plate so as to avoid contamination between other inoculums on the plate. Figure 4 shows an example of how to inoculate a clinical specimen on a quarter plate.

Incubation temperature

The humidity of the atmosphere in the incubator as well as the incubation temperature is very important for good growth of microorganisms and performance of media. 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 type of incubator and the racking system used including the appropriate number of plates in stacks. Stacking of plates to a maximum height should therefore be part of the laboratory's Quality Assurance programme¹⁵.

Commercial automated instruments

Maintenance costs for automated instruments need to be considered before their introduction into the laboratories¹⁶.

Appendix 3: Inoculation of swabs on culture media

Dry swabs - plate culture

Initial inoculum should cover between a quarter and a third of the plate (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 MacConkey agar (when either a full or a quarter plate will be used) may not require spreading with a loop (Figure 4). Automated system of plating however uses whole plates.

Dry swabs - liquid culture

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

Liquid swabs – both plate and liquid culture

The procedure described shows how patient samples suspended in liquid transport medium are inoculated on culture media:

Vigorously shake or vortex the liquid swab tube for 5 seconds to release the sample from the swab tip, and evenly disperse and suspend the patient specimen in the liquid transport medium.

Unscrew the swab cap and remove the swab applicator.

Roll the tip of the swab applicator onto the surface of one quadrant of the culture medium plate to provide the primary inoculum.

Return the swab to the swab transport medium tube for 2 seconds to absorb more sample suspension before inoculating each additional plate, broth or smear. Unscrew the swab cap and use the swab to inoculate the plate or broth.

After the primary inoculum has been done, standard laboratory techniques should then be used to streak the primary inoculum of patient sample across the surface of the culture plate (see figure 2). For laboratories using automated inoculating and streaking instruments, specimens are automatically mixed and 30µL volumes of the swab specimen are inoculated using the robotic system (See figure 5).

Inoculate the slide last due to the potential for contamination.

Remove the contaminated swab from the cap with a sterile forceps and discard the swab. Autoclave and sterilise the forceps after use or otherwise use disposable forceps, if available. Do not return the contaminated swab to the transport liquid after slide inoculation. Save the transport liquid specimen for additional testing if needed. Alternatively, a sterile pipette can be used to transfer approximately 30 - 100 μ L of specimen to each plate or broth and to transfer 1-2 drops on a slide. It should be noted that about 30 μ L would be a suitable amount of liquid for a pre-marked 20mm diameter well slide.

Note: If no slide is inoculated, return the swab to the tube and save the specimen for additional testing if needed.

References

Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	Quality of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

1. Cintron F. Initial processing, inoculation, and incubation of aerobic bacteriology specimens. *Clinical Microbiology Procedures Handbook* Vol 1. Washington DC: American Society for Microbiology; 1992. p. 1.4.1-.4.1.9. **B, III**
2. Collins and Lyne's Microbiological Methods. In: Collins CL, PM; Grange, JM; , editor. 8th ed.: Butterworth-Heinemann; 2004. p. 81-3. **B, III**
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