UK Health Security Agency

UK Standards for Microbiology Investigations

Inoculation of culture media for bacteriology

Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Quality Guidance | Q 5 | Issue no: 2.1 | Issue date: 20.03.25 | Page: 1 of 21

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 SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

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	6/20.03.25
Issue number discarded	2
Insert issue number	2.1
Section(s) involved	**Amendment**
Whole document.	**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 09/01/2017.** Hyperlinks throughout document updated to Royal College of Pathologists website. 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.

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.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 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 8: primary culture methods.

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

4 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 following[1-3]:

- 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

5 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.

6 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 discarded[4].

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 report[7].

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[7].

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[8-10].

7 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

8 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
Dry /Liquid swabs - plate culture - liquid culture - smear See appendix 3 for information on how to inoculate both liquid and dry swabs onto culture media.	UK SMI B 4 - Investigation of superficial mouth samples UK SMI B 5 - Investigation of nasal samples UK SMI B 6 - *Culture of specimens for Bordetella pertussis and Bordetella parapertussis* UK SMI B 9 - Investigation of throat related specimens UK SMI B 11 - Investigation of swabs from skin and superficial soft tissue infections UK SMI B 14 - Investigation of pus and exudates UK SMI B 20 - Investigation of intravascular cannulae and associated specimens UK SMI B 28 - Investigation of genital tract and associated specimens UK SMI B 29 - Investigation of specimens for screening for MRSA UK SMI B 31 - Investigation of specimens other than blood for parasites UK SMI B 51 - *Screening for Neisseria meningitidis* UK SMI B 58 - Detection of carriage of group B streptococci
Fluid specimens and pus	UK SMI B 22 - Investigation of cerebrospinal fluid shunts UK SMI B 25 - Investigation of continuous ambulatory peritoneal dialysis fluid UK SMI B 26 - Investigation of fluids from normally sterile sites UK SMI B 27 - Investigation of cerebrospinal fluid UK SMI B 28 - Investigation of genital tract and associated specimens UK SMI B 38 - Investigation of bone marrow UK SMI B 40 - Investigation of specimens for Mycobacterium species UK SMI B 44 - Investigation of orthopaedic implant associated infections UK SMI B 57 - Investigation of bronchoalveolar lavage, sputum and associated specimens

Types of clinical samples	Types of culture methods and their associated UK SMIs
Faeces	UK SMI B 30 - Investigation of faecal specimens for enteric pathogens For more information on how to process faeces for parasites, refer to UK SMI 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 UK SMI B 39 -Investigation of dermatological specimens for superficial mycoses
Urine - calibrated loop, surface streak method[3,11] - filter paper method[12]	UK SMI B 41 - Investigation of urine
Tissue and biopsy specimens	UK SMI B 17 - Investigation of tissues and biopsies from deep seated sites and organs UK SMI B 42 - Investigation of bone and soft tissue associated with osteomyelitis UK SMI B 55 - Investigation of gastric biopsies for *Helicobacter pylori*
Intravascular cannulae	UK SMI B 20 - Investigation of intravascular cannulae and associated specimens

9 Subculture methods

9.1 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.

9.2 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.

9.3 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 UK SMI 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°C ± 2°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.

10 Different inoculation methods used in bacteriology

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

10.1 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 plate[13]:

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

10.2 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

10.3 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 minutes[14]

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.

11 Public health responsibilities of diagnostic laboratories

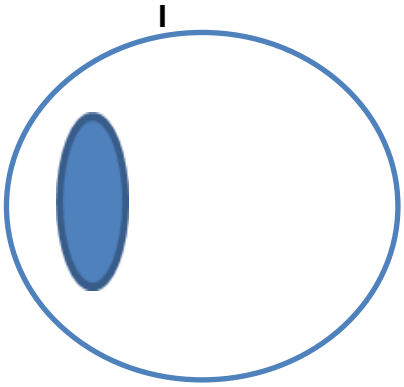
Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; 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.

Appendix 1: Illustrations of inoculation methods

Figure 1
Inoculation of agar



Figures 2 and 3
Streaking inoculum for individual colonies

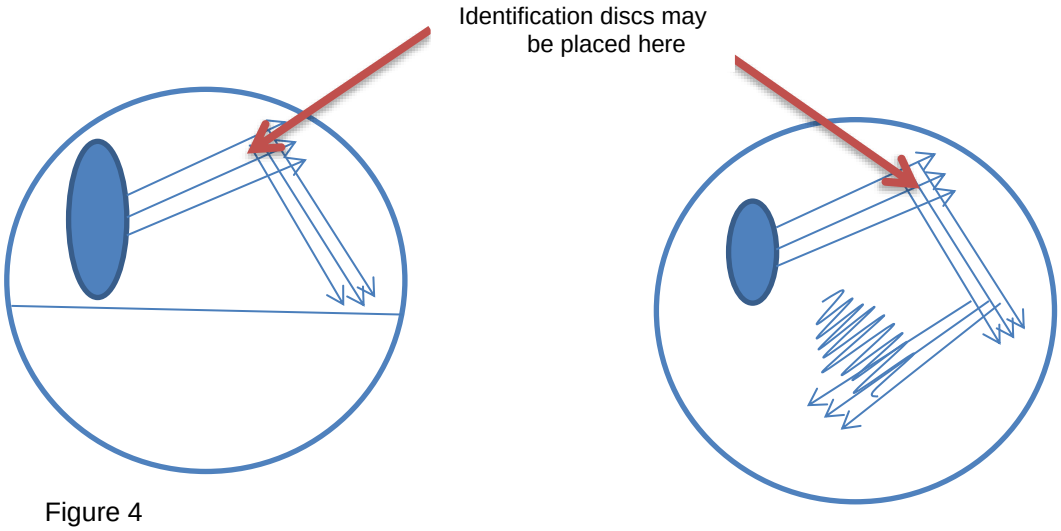


Figure 4
Using quarter plates

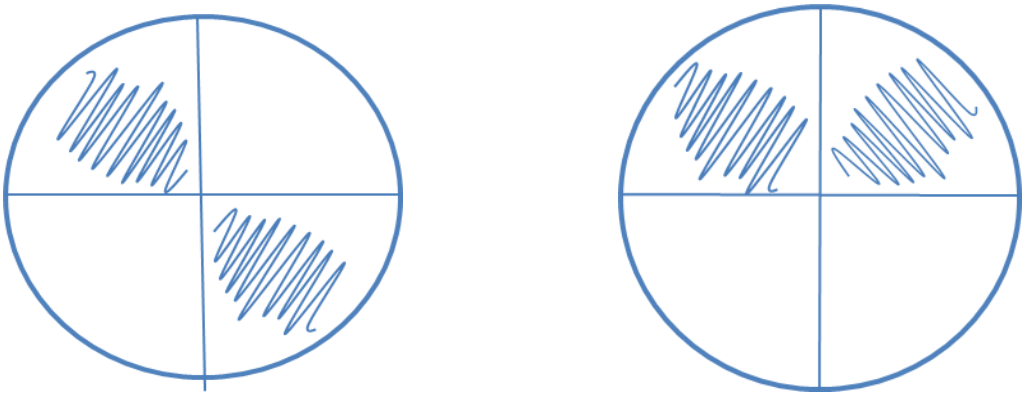


Figure 5

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 UK SMI 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 agar[14].

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 programme[15].

Commercial automated instruments

Maintenance costs for automated instruments need to be considered before their introduction into the laboratories[16].

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 - 100µL of specimen to each plate or broth and to transfer 1-2 drops on a slide. It should be

noted that about 30µL 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

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

1. Cintron F. Initial processing, inoculation, and incubation of aerobic bacteriology specimens. Clinical Microbiology Procedures HandbookVol 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**

3. Baron E. Specimen Collection, Transport, and Processing: Bacteriology. In: Jorgensen JH, Pfaller MA, Carroll K, Funke G, Landry M, Richter S et al., editors. Manual of Clinical Microbiology. 11th ed. Washington DC: ASM press; 2015. p. 270-315. **B, III**

4. The Australian Society for Microbiology Guidelines for Assuring Quality of Medical Microbiological Culture Media: Australian Society for Microbiology, Inc; 2012. 1-32. **A, V**

5. Jousimies-Somer H, Summanen P, Citron D et al. Anaerobic Bacteriology Manual. In: Jousimies-Somer H, Summanen P, Citron D et al, editors. 6th ed.: Star Publishing Company; 2002. p. 54. **B, III**

6. Garcia LS. Examination of Primary Culture Plates for Anaerobic Bacteria. Clinical Microbiology Procedures Handbook. 3rd ed. Washington DC: ASM Press; 2010. p. 703-8. **B, III**

7. The Royal College of Pathologists. Report of the Working Party of the Royal College of Pathologists and the Institute of Biomedical Science. The Retention and Storage of Pathological Records and Archives. London 2015. 1-59. **A, V**

8. Glasson JH, Guthrie LH, Nielsen DJ, Bethell FA. Evaluation of an Automated Instrument for Inoculating and Spreading Samples onto Agar Plates. J Clin Microbiol 2008;46:1281-4. **B, II**

9. Mischnik A, Mieth M, Busch CJ, Hofer S, Zimmermann S. First evaluation of automated specimen inoculation for wound swab samples by use of the Previ Isola system compared to manual inoculation in a routine laboratory: finding a cost-effective and accurate approach. J Clin Microbiol 2012;50:2732-6. **B, II**

10. Mischnik A, Trampe M, Zimmermann S. Evaluation of the impact of automated specimen inoculation, using Previ Isola, on the quality of and technical time for stool cultures. Ann Lab Med 2015;35:82-8. **B, II**

11. Stevens M. Screening urines for bacteriuria. Med Lab Sci 1989;46:194-206. **C, III**

12. Leigh DA, Williams JD. Method for the detection of significant bacteriuria in large groups of patients. J Clin Pathol 1964;17:498-503. **B, II**

13. Katz S. The Streak Plate Protocol. American Society for Microbiology. http://www.asmscience.org/content/education/protocol/protocol.3160. **B, III**

14. Wise K. Preparing Spread Plates Protocols. American Society for Microbiology. http://www.asmscience.org/content/education/protocol/protocol.3085. **B, III**

15. BS EN ISO 11133:2014 - Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media. 1st ed.: BSI; 2014. p. 14, Section 4.6 **A, V**

16. Croxatto A, Prod'hom G, Faverjon F, Rochais Y, Greub G. Laboratory automation in clinical bacteriology: what system to choose? Clin Microbiol Infect 2016;22:217-35. **B, III**