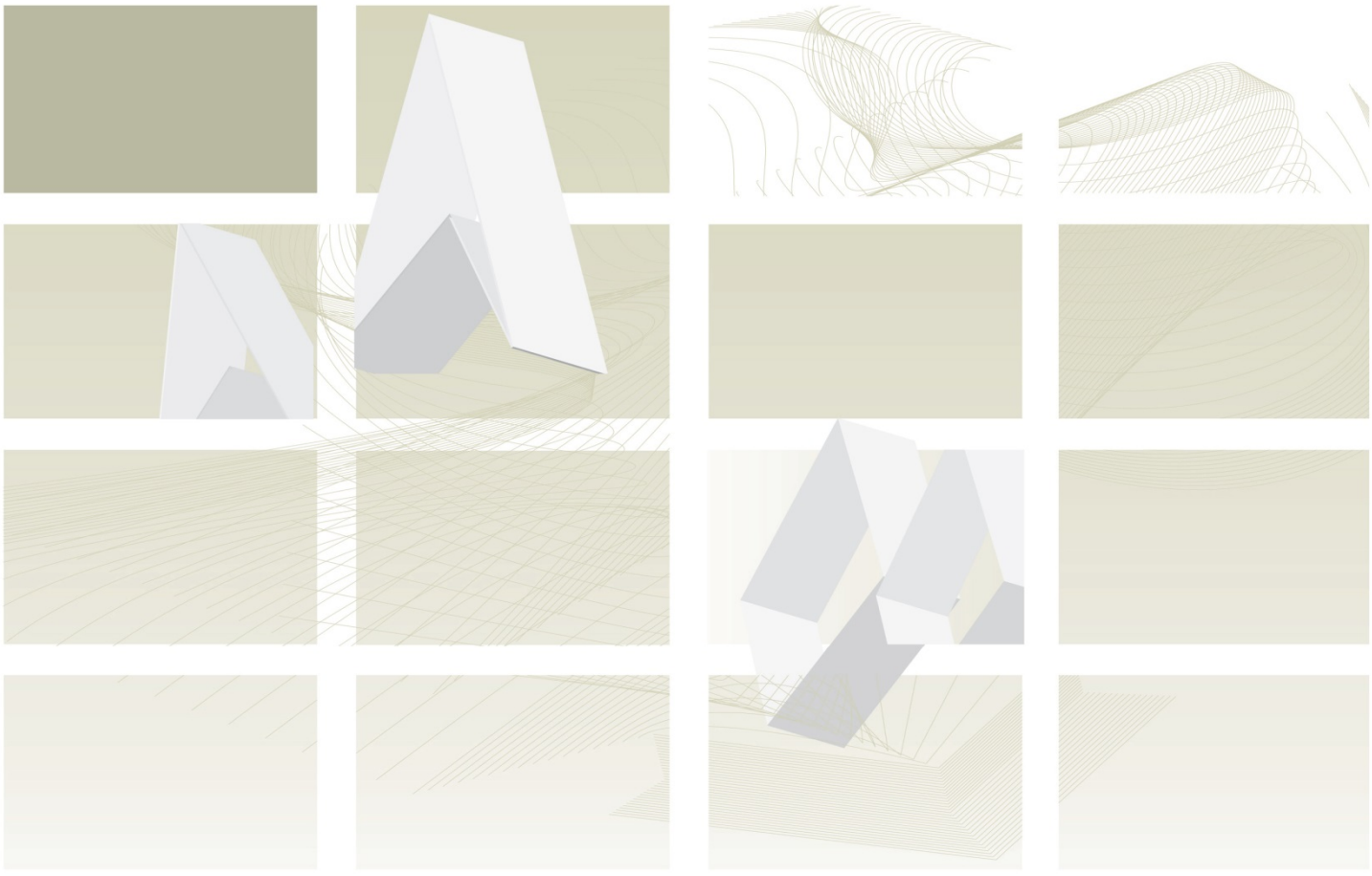
Public Health England

NHS

UK Standards for Microbiology Investigations

Review of users' comments received by Working group for microbiology standards in clinical bacteriology

Q 5 Inoculation of culture media for bacteriology



NICE accredited www.nice.org.uk/accreditation

"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**."

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

Consultation: 08/12/2015 – 04/01/2016

Version of document consulted on: Q 5do+

Proposal for changes

Comment number	1		
Date received	17/12/2015	**Lab name**	Microbiology, Macclefield DGH
Section	Appendix 2 - use of wire loops		
Comment			
The appendix states that 'the use of wire loops are prohibited in UK microbiology laboratories' but there is no reference for this. I cannot find any HSE document prohibiting their use; can you clarify where this comes from please?			
Financial barriers			
Disposable loops would be more expensive though probably acceptable.			
Health benefits			
No.			
Recommended action	**ACCEPT** This has been amended to explain that wire loops are not used in many UK laboratories, however a few still use them for certain procedures. Although, there are no references to support this but it has been a common practice in the last few years in many UK laboratories.		

Comment number	2		
Date received	21/12/2015	**Lab name**	Health and Social Care trust, Northern Ireland
Section	All of it.		
Comment			
A few mainly textual comments as below: a. Cover page: Capitals for: Inoculation of Culture Media for Bacteriology b. P9: Section 2: Para 4: This might read: 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. [Del '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 [Del: 'in use'] in microbiology laboratories in the UK but a few clinical laboratories may still use [Del: these] them. [Del: This] The decline in use is due to some limitations in [Del: 'its'] their 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. P9: Section 2: Para 5: 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. [DN: I may be out of date but if the loop is dipped in the fluid would both sides not be equally contaminated?] For semi-quantitative analysis of urine, the loop should be changed.

c. P9: Section 2: Para 6: All media should be incubated as soon as possible after inoculation. [Insert] In particular, 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 report5.

d. P10: Section 3: Para 1: When handling specimens or cultures, aseptic technique is important to avoid contamination and to protect the worker from infection. [Del: 'from the sample'].

e. P10: Section 3: Bullet 2: 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 [DN: ? discard] place the loops

f. P10: Section 3: Bullet 5: 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 into a disposable jar after use

g. P10: Section 4.1 Para 1: Initial inoculum should cover between a quarter and a third of the plate [Del: 'to be used'] (Figure 1).

h. P11: Section 4.5 Para 1: Commercially prepared sterile filter paper strip is dipped in the urine up to the mark indicated. [DN: An instruction as are the next two paras, therefore might be better as: Dip a commercially prepared sterile filter paper strip into the urine up to the mark indicated].

i. P11: Section 4.6:[DN: Might be better as:]Initial inoculum should be swabbed on to appropriate agar media to cover between a quarter and a third of the plate 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 [Del: '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 a [Del 'the'] loop, mix the faecal material gently in the broth and then dispose of the loop in a disposable waste jar.

j. P13: Section 5.3; Para 3: 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, [Del 'or'] a drop from a pipette [Del: 'of the inoculated broth'], or a swab immersed in the broth suspension to inoculate the plate or test system.

k. P13: Section 6: Point 3. Drag loop into section 2 to obtain bacteria. Then spread it out into the third section. Do the same [Del: 'for the same'] for the third and the fourth section. Ensure that sections 1 and do not overlap. Dispose of the

inoculation loop used l. P15: Appendix 2: Use of wire loops. The use of wire loops is [Del: are] prohibited in UK microbiology laboratories but there might be a few laboratories that still use them [DN: If they are 'prohibited' why are some labs still using them? ?check and possibly use 'discouraged' rather than 'prohibited']. [Del: 'These'] Wire loops were discouraged from being used due to the risk of infection from aerosol formation of pathogenic organisms, as well as cross-contamination due to [Ins: their] improper sterilisation [Del: '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. TP 8 covers the inoculating wire loops (nichrome) where reaction with the hydrogen peroxide can produce false positive reactions.	
Evidence	
These are thoughts for your consideration.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **NONE** UK SMIs follow the PHE style guide for presentation of information. b. **ACCEPT** The changes have been updated in the document. c. **ACCEPT** The change has been updated in the document. d. **ACCEPT** The change has been updated in the document. e. **ACCEPT** The change has been updated in the document. f. **ACCEPT** The change has been updated in the document. g. **ACCEPT** The change has been updated in the document. h. **ACCEPT** The change has been updated in the document. i. **ACCEPT** The change has been updated in the document. j. **ACCEPT**

	The change has been updated in the document. k. **ACCEPT** The change has been updated in the document. l. **ACCEPT** The change has been updated in the document.

Comment number	3		
Date received	21/12/2015	**Lab name**	National Convenor, Culture Media Special Interest Group, Australian Society for Microbiology
Section	References; Section 2; Section 4.4		
Comment			
a. Many of the references cited appear to have been 'rolled over' from earlier editions of the SMI and not necessarily checked to see if these are still the appropriate reference to be used. Where a text has been reissued, and the information relevant to the subject appears in the newer edition, then the newer edition should be cited, not the older version, in most cases. b. Whilst much is referenced throughout the document, QC of media is not. Page 9, Section 2, paragraph 2 sentence: "Culture media should have an identifiable batch or quality control number and have passed QC tests before use". should be cited. Suggested references: - Clinical Microbiology Procedures Handbook 3rd edition 2007. Section 14. – This reference was published in 2010 and not 2007. - Guidelines for Assuring Quality of Medical Microbiological Culture Media, 2nd edition. Australian Society for Microbiology 2012. Attached. - CLSI M22-A3. 2004 Quality Assurance of commercially prepared media. c. Page 11, section 4.4 makes reference to figure 2, but figure 2 does not correlate with the text of section 4.4. Remove 'See Figure 2' from the text, or add a new Figure that matches the text (which will become Figure 4).			
Evidence			
Reference 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. 1-19.If using this reference, then this should also cite pages 10-19, not 1-19, as pages 1-9 are outside the scope of the SMI. There is a third edition of CMPH but the one above I agree offers better info on this issue. – **Reference is kept as it is. However, the pages are correct as stated in the document and pages 1-9 are relevant for the document. This has now been**			

updated to read as it is in the handbook (1.4.1 – 1.4.19).

Manual of Clinical Microbiology 10th edition (2011) Ch.16 pp243, might be another option, of the 11th edition might be valid. – **Both editions have been looked at and the 11th edition has been added and updated in the document.**

Reference 2. Collins CH, Lyne PM, Grange JM, editors. Collins and Lyne's Microbiological Methods. 7th ed. Oxford: Butterworth-Heinemann; 1995. p. 94-6.the latest edition of this text is the 8th edition published in 2004, and this reference should be updated to Collins CH, Lyne PM, Grange JM, Falkinham JO, editors. Collins and Lyne's Microbiological Methods. 8th ed. Oxford: Arnold; 2004. p. 81-83. Attached. – **Reference used in the document is the 8th edition but it was not updated on the reference manager software used at the time. This has been updated now.**

Reference 3. Peterz ME. Temperature in agar plates and its influence on the results of quantitative microbiological food analyses. Int J Food Microbiol 1991; 14:59-66.The validity of this reference in this SMI has not been fully established. The subject matter of the reference - quantitative counts, food microbiology - are not directly relevant to the SMI. The information within the reference made is not necessarily relevant at all to clinical plates: the issue was about reaching a temperature of 44C on a given media for a given organism group. The information in this reference should not be extrapolated in this way. You may wish to keep the statement in Appendix 2, but this reference above should be removed. It should also be removed from the sentence at the top of page 9, as this is not a correct citation - the reference does not state this. **Reference has been removed from top of the page and from the document.**

The issue of humidity is far more important to good growth and performance of media, and perhaps reference should be made to this aspect as well as stack height. There are numerous reference to this, perhaps citing BN EN ISO11133:2014 section 4.6 page 14 would be best as it captures both. Attached. **This reference has been updated in the document.**

Reference 4. Jousimies-Somer H, Summanen P, Citron D et al. Anaerobic Bacteriology Manuel. In: Jousimies-Somer H, Summanen P, Citron D et al, editors. Sixth ed. Star Publishing Company; 2002. p. 54.There are later references available, but this one is VG. **Reference 4 will be kept on in the document.**

- Suggested alternatives: Clinical Microbiology Procedures Handbook 3rd edition 2007 (ASM Press); section 4.4, page 4.4.1 **This reference has been added in the document. It should be noted that this reference was published in 2010 and not 2007.**

Reference 5. OK

Reference 6. Stevens M. Screening urines for bacteriuria. Med Lab Sci 1989; 46:194-206.B41 (as cited within the text) would make a far superior reference here than the one cited. Specifically, section 4.5.2 of B41 is a superior reference here. **Reference 6 will be kept in the document.**

Other possible references include

- Manual of Clinical Microbiology 10th edition Ch.16 page 261 **The 11th edition of the Manual of Clinical Microbiology has been added and updated in the document.**
- Clinical Microbiology Procedures Handbook 3rd edition 2007, Section 3.12, pages 3.12.6 - 3.12.12. **This reference was published in 2010 and not**

2007. Reference 7. Leigh DA, Williams JD. Method for the detection of significant bacteriuria in large groups of patients. J Clin Pathol 1964; 17: 498-503.see comments and suggestions as per reference 6. **Reference 7 will be kept in the document.** References 8 and 9.as these are web-based references, it makes sense to include the weblink as a hyperlink in the document. eg, reference 8, add http://www.microbelibrary.org/component/resource/laboratory-test/3160-the-streak-plate-protoco l eg, reference 9, add http://www.microbelibrary.org/component/resource/laboratory-test/3085-preparing-spread-plates-protocols - **These web links have been cited within references 8 and 9 accordingly in the document.** References 10-12, OK.
Financial barriers
No.
Health benefits
Nil known.

Recommended action	a. **NONE** Newer editions are checked as part of the literature review. b. **ACCEPT** This reference has been added and updated accordingly. c. **ACCEPT** This has been updated accordingly.

Comment number	4		
Date received	22/12/2015	**Lab name**	North Bristol NHS Trust Microbiology
Section	Page 8		
Comment			
P.8 (Smears): 'Slides may be sterilised by flooding the slide with alcohol, discarding the excess and drying on a hotplate.' This is disinfection not sterilization.			
Evidence			
Knowledge.			
Financial barriers			
No.			
Health benefits			

No.	
Recommended action	**ACCEPT** This has been removed from the document.

Comment number	5		
Date received	23/12/2015	**Lab name**	North Bristol Trust
Section	a. P.8 (Section 1 part 4. Smears for staining) b. Top of p.12 (Section 4, part 4.7 Tissue and biopsy specimens)		
Comment			
a. 'Slides may be sterilised by flooding the slide with alcohol, discarding the excess and drying on a hotplate.' This is disinfection not sterilization. b. All homogenisation and grinding procedures involving tissue or biopsy specimens must be performed in a Class 1 safety cabinet. Should Class 2 cabinets be considered to protect specimen/culture as well as operators?			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	a. **ACCEPT** This has been removed from the document. b. **ACCEPT** This has been updated accordingly.		

Comment number	6		
Date received	05/01/2016	**Professional body**	Institute of Biomedical Science
Section	Section 2 Inoculation of culture media and Appendix 2: Technical limitation/information, use of wire loops		
Comment			
The SMI recommends the use of plastic disposable loops throughout the main text with appendix 2 stating that the use of wire loops is prohibited in the UK. The IBMS would like clarification on the prohibition of wire loop usage in the UK. It is felt that it would be			

useful if this was referenced, identifying the body or organisation that issued the prohibition statement. It is thought that without this the document could be challenged by the companies offering commercial automated systems that use wire loops as part of their automated method. The inclusion of reference to laboratories that may still use wire loops can be construed as misleading. In view of the prohibitive status we would suggest reference to these laboratories should be removed from the text.	
Recommended action	**ACCEPT** This has been amended to read as "the use of wire loops is rarely seen in use in microbiology laboratories within the UK" and that for quantitative purposes, disposable loops should be used as they are desirable.

Comment number	7		
Date received	24/12/2015	**Professional body**	Microbiology Scientific and Technical Advisory Group
Section	Various		
Comment			

a. General comments

i. There was general concern that this SMI should be incorporated in all of the individual SMIs as if this were to be followed alone it would lead to incorrect actions.

ii. There is too much additional non-relevant information in this SMI and it was generally thought that it is not necessary.

iii. General question as to whether authors names of SMIs could be included on the documents?

iv. Throughout the document it refers to Category 3 laboratory, this should be Containment Level 3.

v. Replace the word "waste jar" with "waste container" throughout the document.

Should the SMI continue to be in existence then the MSTAG has the following comments:-

b. Page 8

Smears

i. The first paragraph should include the sentence "Where possible always do smears after the specimen has been cultured" although it is appreciated that this is listed 1-4 in the first paragraph.

ii. The end of the first paragraph should say "….and then a sterile slide may be used"

iii. The second paragraph with regards to sterilising slides by flooding with alcohol should be removed, this is unsafe practice. Ideally both paragraphs should be deleted

c. Page 9

i. 2nd paragraph " All culture media must be checked before use"

ii. 4th paragraph and whole document contradicts itself with regards to the use of wire loops.

iii. Last paragraph- not sure why this is in the SMI as it has nothing to do with inoculation of culture media, this should be taken out. It states that Slides for examination for Mtb should be kept in a locked cupboard in a Category 3 laboratory until the final report from the reference laboratory has been received. Nobody does this, isolates should be stored in locked containers but not slides? This paragraph mentions Category 3 and should be containment level 3.

iv. There is no mention of automatic spreaders or inoculators.

d. Page 10

i. There is no mention of liquid swabs which have a different technique for inoculating

ii. "With the exception of urine specimens" – either delete or explain this does not make sense

iii. Second paragraph "If the work is being carried out on the open bench, a disposable container should be in …." Replace the word jar with container throughout the document.

iv. Opening caps slowly in a microbiological safety cabinet – does this apply to all specimens this is not clear?

v. Avoid vigorous swirling or shaking…. This should be clarified by saying "gently invert the specimen."

vi. Last paragraph mentions scissors disposed of in a disposable waste jar, this should be a sharps bin.

vii. 4.1 Swabs-plate culture…Inoculation of samples to selective media such as Sabouraud agar (when usually only a quarter plate will be used) should be altered, automated systems of plating use whole of ½ plates therefore this statement is inaccurate.

viii. 4.2 Swabs – liquid culture

It is not clear why you would perform liquid culture on a swab? Is this enrichment?

ix. 4.3 Fluid specimens and pus

The term "piston-operated pipette" is odd,

x. 4.4 This should be deleted as it is included in B41 and does not need to be repeated in this SMI

xi. 4.5 This should be deleted as it is included in B41 and does not need to be repeated in this SMI

xii. 4.6 The SMI states "Inoculation of a broth medium is optional" however the

SMI for enterics recommends inoculation of a mannitol selenite broth. xiii. 4.7 Delete the last line “All homogenisation and grinding procedures…” xiv. 5.1 “The use of a pipette is particularly recommended when sub-culturing organisms to multiple culture media, including those used for biochemical tests. A biochemical test is not a culture media which is the title of this SMI therefore this should be removed. xv. 5.2 Section should be deleted as it is not really needed xvi. 5.3 Uses different terms which is also repeated throughout the SMI “sterile disposable loop” and “sterile loop”, terminology should be consistent. xvii. 5.3 uses the term suspension fluid, medium and broth in the same paragraph, terminology should be consistent. e. Page 15 i. Figure 2 represents poor streaking, it is not necessary for 5 streaks to come out of the initial inoculum this could be limited to 3 as it represents very poor technique ii. Figure 4 is poorly produced with the bottom quarter inoculum going over the edge of the quarter it is placed in, this represents very poor technique. The top quarter has a double headed red arrow, it is not clear what this means or whether the operator should inoculate up and down the quarter plate. Both of these need to be reviewed as this is poor. f. Page 16 Appendix 2 Use of wire loops The statement that “The use of wire loops are prohibited in the UK” is in accurate and should be removed, some laboratories represented in the MSTAG still use wire loops for certain procedures. The whole of the section on “Use of wire loops” should be deleted if this statement is correct. g. Page 17 References Reference 4 Spelling correction “Anaerobic Bacteriology Manual”	
Recommended action	a. i. **NONE** The BWG members felt that it was a useful document to retain and the sections where more information is needed will be linked to the appropriate SMI in question. ii. **NONE** The BWG members felt that the information in this quality document should remain as it is. iii. **NONE** This is not within the remit of the UK SMI. UK SMIs do not mention author names except where they have written the document or contributed greatly to it. a. iv. **ACCEPT** This has been updated in the document accordingly. v. **ACCEPT**

	This has been updated in the document accordingly. b. i. **NONE** This is already in the document on page 8. ii. **NONE** The word 'should' should be used as the word 'may' makes the sentence sound as if there is another alternative to use apart from using a sterile slide. iii. **ACCEPT** This has been updated in the document accordingly. c. i. **ACCEPT** This has been updated in the document accordingly. ii. **ACCEPT** This has been updated in the document accordingly. iii. **ACCEPT** This has been removed from the document. iv. **ACCEPT** This has been discussed both in section 2 and in appendix 2: Technical limitations/Information. d. i. **ACCEPT** This has been updated in the document. ii. **ACCEPT** This has been updated in the document. iii. **ACCEPT** This has been updated in the document. iv. **NONE** This applies to all specimens received into the laboratory. v. **NONE** This will remain in the document. vi. **ACCEPT** This has been updated in the document. vii. **ACCEPT** This has been updated in the document. viii. **ACCEPT** This has been updated in the document. ix. **ACCEPT** The word has been removed and updated in the

	document. x. **ACCEPT** This has been removed from the document. xi. **ACCEPT** This has been removed from the document. xii. **ACCEPT** This has been removed from the document. xiii. **ACCEPT** This has been removed from the document. xiv. **ACCEPT** This has been updated in the document accordingly. xv. **NONE** The BWG members felt that the information in section 5.2 of this quality document should remain as it is. xvi. **ACCEPT** This has been updated in the document. xvii. **ACCEPT** This has been updated in the document. e. i. **ACCEPT** This has been updated with new pictures in the document accordingly. ii. **ACCEPT** This has been updated with new pictures in the document accordingly. f. ii. **ACCEPT** This has been updated with new pictures in the document accordingly. g. **ACCEPT** This has been updated accordingly in the document. h. **ACCEPT** This has been updated in the reference manager software.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 2			
Date received	10/12/2015	**Lab name**	Northern Trust Microbiology Department

Date received	10/12/2015	**Lab name**	Royal Stoke University Hospital