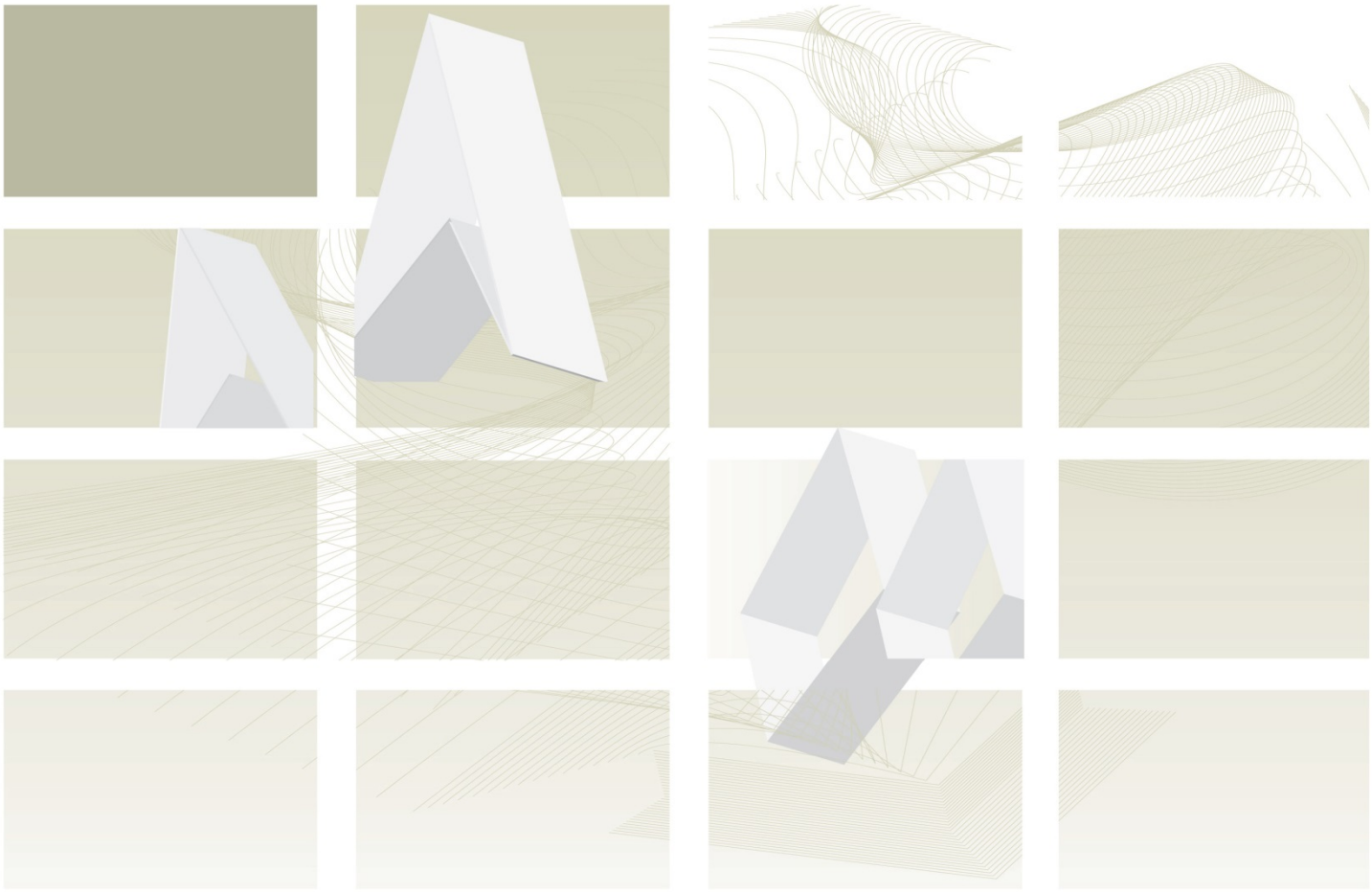
Public Health England

NHS

UK Standards for Microbiology Investigations

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

B 40 Investigation of specimens for *Mycobacterium* species



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: 13/03/2015 – 13/04/2015

Version of document consulted on: B 40dx+

Proposal for changes

Comment number	1		
Date received	18/03/2015	**Lab name**	Sheffield Teaching Hospitals, Microbiology Laboratory
Section	Various		
Comment			
a. 4.5.1.1 Decontamination of specimens using 4% NaOH i. The decontamination time has changed from 30 minutes in the previous SMI to 15 minutes in the current version. Is there any evidence to support this change? ii. There is no mention of how much phosphate buffer or water to neutralise with. Equal volume, equal volume of original specimen or fill up the universal. b. 4.5.1.2 Decontamination of specimens using (0.5N) H_2SO_4 i. If the deposit has already been re-suspended in phosphate buffer is addition of NaOH with phenol red still required? Clarification required.			
Recommended action	a. i. **NONE** There are 3 references to support the statement. The decontamination time has been changed because of the harmful effect of NaOH to the tubercle bacilli. ii. **ACCEPT** This has been updated accordingly. b. i. **NONE** NaOH with phenol red indicator is still added to the deposit already re-suspended in phosphate buffer so that neutralisation can be verified visually.		

Comment number	2		
Date received	19/03/2015	**Lab name**	Crosshouse Hospital
Section	4.5.1 and 4.5.1.3		
Comment			
Initial list of decontamination methods show 3% oxalic acid but methods later only show 5% oxalic acid.			
Evidence			
5. Decontamination of specimens using oxalic acid (3%) and 4.5.1.3 Decontamination of specimens using 5% oxalic acid[113]			
Recommended action	**ACCEPT** This has been updated in the document accordingly.		

Comment number	3		
Date received	27/03/2015	**Lab name**	Medical Microbiology Department, Northern Health and Social Care Trust
Section	Introduction		
Comment			
In the section on non-tuberculous mycobacteria (NTM), it states that they are not transmitted from person to person. However there is evidence of transmission between cystic fibrosis patients.			
Evidence			
https://www.cysticfibrosis.org.uk/media/381091/CC15%20-20NTM%20guidelinesv2.pdf			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** This section of the document has been updated accordingly.		

Comment number	4		
Date received	28/03/2015	**Lab name**	Pathology Laboratory - Cayman Islands
Section	4.5.5		
Comment			
Questioning whether the target organism for pulmonary tuberculosis in the matrix should say *M. tuberculosis* instead of *M. xenopi*.			
Recommended action	**ACCEPT** This has been updated in the document both in section 4.5.5 and the flowchart accordingly.		

Comment number	5		
Date received	08/04/2015	**Lab name**	Public Health Wales Microbiology
Section	Non-tuberculous Mycobacteria		
Comment			
In this section it states “NTM are not transmitted from person-to-person.” Although current (old) guidance still states this is the case, there is growing evidence to suggest this may not be so black and white and I would be wary of making such a definite statement. I am thinking of work performed in Cambridge and Seattle, references below.			
Evidence			
1. Bryant JM, Grogono DM, Greaves D, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. Lancet 2013; published online March 29. http://dx.doi.org/10.1016/S0140-6736(13)60632-7. 2. Aitken ML, Limaye A, Pottinger P, et al. Respiratory outbreak of *Mycobacterium abscessus* subspecies *massiliense* in a lung transplant and cystic fibrosis center. Am J Respir Crit Care Med 2012; 185: 231-32.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** This section of the document has been updated accordingly. The references have been used to solidify the evidence that NTM (only *M. abscessus* so far) can be transmitted from person		

	to person.

Comment number	6		
Date received	10/04/2015	**Lab name**	Truro
Section	Pages 17, 18, 21, 26, 30, 32 and 40		
Comment			
a. Pg 17 Quality Control section needs a change in font size to match the rest of the document. b. Pg 18 1.2 Paragraph 7 - Air flow readings must be taken with the hot plate off - Please provide references and evidence. May not be applicable for Class 1 cabinet. c. Pg 21 Blood section - This SOP refers to B37 for Blood cultures but B37 refers to B40 (old MAI SOP is merged into TB SOP not blood cultures) d. Pg 26 - Decontamination of specimens - We decontaminate for 25 mins (Newcastle method) e. Pg 30 4.5.4 Point 2 - Not clear what the role is in TBM f. Pg 32 - Pulmonary tuberculosis incubation temperature is not routine at 42C g. Pg 40 - This flowchart does not include a section on *M. xenopi* at 42C shown on 4.5.5 table - this should be an option if required.			
Recommended action	a. **ACCEPT** Font size has been increased in line with the UK SMI template. b. **NONE** This is recommended as good practice. This reference (British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14) discusses the use of appropriate equipment in the MSC but not specifically on the use of hot plates. This was discussed at the Bacteriology Working Group meeting and a form of words has been agreed and this has been updated in the document. c. **NONE** This section refers to B 37 for further information on collection and processing of blood cultures while in B 37 document; users are referred to B 40 to use this document when investigating specimens for *Mycobacterium* species. d. **ACCEPT**		

	This has been updated and references added accordingly. e. **NONE** This information is already covered in the document. f. **ACCEPT** This has been moved to the optional section in table 4.5.5 if needed. g. **ACCEPT** The flowchart has been amended to include the optional section on *M. xenopi* if needed.

Comment number	7		
Date received	13/04/2015	**Lab name**	Public Health Wales
Section	Various		
Comment			

a. P8 “Introduction”.

   For a complete list of MTBC members, there is at least one novel species, *M. mungi* from Alexander KA, Laver PN, Michel AL, Williams M, van Helden PD, Warren RM, Gey van Pittius NC. Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. Emerg Infect Dis. 2010 Aug;16(8):1296-9. doi: 10.3201/eid1608.100314. Erratum in: Emerg Infect Dis. 2010 Dec;16(12):2024.

b. P9 “Pulmonary Tuberculosis.

   Perhaps the requirement for two or three specimens needs clarification here and later on on P19?

c. P12 - *Mycobacterium avium intracellulare* group (MAI):

   There is also *M. chimaera* within the MAI group?

d. P16 - MALDI-TOF Identification of *Mycobacterium* species

   MALDI-TOF is now widely available.

e. P19: 2.2.1 Correct specimen type and method of collection Sputum specimens

   Clarification needed on 2/3 specimens within the UK/globally?

f. P30 - 4.5.4 Nucleic acid amplification tests.

   Perhaps it should be emphasised that if there is not enough specimen volume for PCR and culture, then only culture should be done. Also, that PCR positive specimens should ideally be subsequently confirmed by a corresponding positive culture.

g. P32 - 4.6 Identification

   Is there an individual SMI for ID of *Mycobacterium* species?

h. P32 4.9 Referral to Reference Laboratories

This link is specifically for PHE NMRL. For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms. England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

This PHE link needs clarification as the link for the Wales Centre for Mycobacteria is: http://www.wales.nhs.uk/sites3/page.cfm?orgId=457&pid=25286.

The current address for the Welsh TB lab is: Wales Centre for Mycobacteria (WCM) Public Health Wales Microbiology Cardiff Llandough Hospital Penlan Road Penarth CF64 2XX Tel: 029 2071 6408

i. 5.4 Strain typing reporting

MycoNet is now ETS STM?

j. P38 References

i. Is reference 4 still valid in light of the 2015 PHE strategy?

ii. Should this be included as a reference? Centre for Disease Prevention and Control. Mastering the basics of TB control: Development of a handbook on TB diagnostic methods. Stockholm: ECDC; 2011.

Financial barriers

No.

Health benefits

No.

Recommended action	a. **NONE** This reference will not be accepted because *M. mungi* according to Euzeby, JP 2015 does not have standing in nomenclature and so is not included in the 169 species of *Mycobacterium*. b. **ACCEPT** This has been updated with references and reasons as to why the WHO has recommended that at least 2 samples should be processed in a TB case. c. **ACCEPT** This has been updated accordingly. d. **NONE** MALDI-TOF MS is used but it is not yet widely available in many clinical laboratories due to costs and lack of expertise. e. **ACCEPT** See comment b.

	f. **ACCEPT** This has been updated in section 4.5.4. g. **NONE** There is no proposal for an individual SMI for Identification of *Mycobacterium* species. Isolates should be sent to appropriate Reference laboratory for further identification. h. **ACCEPT** The link has been added to the Welsh TB lab and address amended. i. **NONE** MycobNet is the correct name and not MycoNet. j. i. **NONE** The validity of the reference in light of PHE strategy remains unclear at this stage. *Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci U S A 2002; 99:3684-9.* ii. **ACCEPT** This reference has been added in the appropriate sections of the document.

Comment number	8		
Date received	13/04/2015	**Professional body**	IBMS
Section	Various		
Comment			
a. Safety considerations in performing MALDI-TOF analysis for the identification of any mycobacteria strains may be worth considering. b. Report time of auramine or ZN slide should be in line with Gram strain. Reporting an AFB microscopy is suggested to be within the next working day whereas a Gram strain, on a CSF for example (SMI B27) must be verbally reported within 2hrs with a hard copy available within 24hrs. c. There is the safety briefing circulated very recently by PHE regarding *M. chimaera* which should also be considered for inclusion in the policy. This is obviously related to a risk of post-operative infections following use of by-pass machines (see attached). The organism belongs to the *M. avium* complex however it would seem sensible to mention it as a separate species and list the new risks related to cardio surgery.			

d.

i. In the introduction it states “The genus *Mycobacterium* is a member of the family Mycobacteriaceae and consists of 168 species and 10 subspecies of which a few have been reclassified to other genera within the family” Ref 3. Euzeby,JP. List of prokaryotic names with standing in nomenclature - Genus *Mycobacterium*.

Comment: According to ref 3 there are 170 species and 13 sub species yet this SMI states 168 and 10 sub species. This requires some clarification to avoid confusion.

ii. In addition, within ref 3 states “Note: In 2009, Leao et al. proposed the union of ¤ *Mycobacterium bolletii* and *Mycobacterium massiliense*, and the recognition of two subspecies within *Mycobacterium abscessus*: *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *massiliense*. The proposal of *Mycobacterium abscessus* subsp. *massiliense* was not in accordance with the Rules of the Bacteriological Code, because the epithet *bolletii* has priority over the epithet *massiliense*. Consequently, the name *Mycobacterium abscessus* subsp. *massiliense* was illegitimate. In 2011 Leao et al. propose the correct name *Mycobacterium abscessus* subsp. *bolletii* (Adékambi et al. 2006) Leao et al. 2011.”

Comment: There are several publications that do not agree that *M abscessus* subsp *massiliense* is an illegitimate name, see references below:

a) Comparing *Mycobacterium massiliense* and *Mycobacterium abscessus* lung infections in cystic fibrosis patients Journal of Cystic Fibrosis 2015 Jan;14(1):63-9 Anne-Laure Roux et al.

b) Molecular Fingerprinting of *Mycobacterium abscessus* Strains in a Cohort of Pediatric Cystic Fibrosis Patients J. Clin. Microbiol. 2012, 50(5):1758. Kathryn A. Harris,a Dervla T. D. Kenna,b Cornelis Blauwendraat,a John C. Hartley,a Jane F. Turton,b Paul Aurora,cand Garth L. J. Dixona Department of Microbiology, Virology and Infection Control, Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom[a]; Laboratory for Healthcare Associated Infection, HPA Centre for Infections, London, United Kingdom[b]; and Paediatric Respiratory Medicine and Lung Transplantation, Great Ormond Street Hospital.

c) Cohort Study of Molecular Identification and Typing of *Mycobacterium abscessus, Mycobacterium massiliense*, and *Mycobacterium bolletii* JOURNAL OF CLINICAL MICROBIOLOGY, July 2009, p. 1985–1995 Vol. 47, No. 7 Adrian M. Zelazny,[1,2*] Jeremy M. Root,[2] Yvonne R. Shea,[1] Rhonda E. Colombo,[2] Isdore C. Shamputa,[3]Frida Stock,[1] Sean Conlan,[4] Steven McNulty,[5] Barbara A. Brown-Elliott,[5] Richard J. Wallace, Jr.,[5]Kenneth N. Olivier,[2] Steven M. Holland,[2] and Elizabeth P. Sampaio.

e. In the section Non-Tuberculous Mycobacteria (NTM) it states “ NTM are ubiquitous in nature, have a varied spectrum of pathogenicity for humans, are not transmitted from person to person and are often resistant to classical anti-tuberculous chemotherapy[41,42].

Comment: While this may be true of most NTM, there are several publications confirming the transmission of *M abscessus* complex, in particular *M massiliense* in the CF population but also in other clinical settings: see references below.

d) The growing threat of non-tuberculous mycobacteria in CF Journal of Cystic Fibrosis (2014) Volume 14, Issue 1, Pages 1–2 R. Andres Floto * Charles S

Haworth

e) Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study: Lancet 2013; 381: 1551–60 R Andres Floto.

f) Molecular Characterization of *Mycobacterium massiliense* and *Mycobacterium bolletii* in Isolates Collected from Outbreaks of Infections after Laparoscopic Surgeries and Cosmetic Procedures_ J CLIN MICRO, March 2008, p. 850–855 Vol. 46, No. 3 Cristina Viana-Niero,1 Karla Vale´ria Batista Lima,2 Maria Luiza Lopes,

g) Phenotypic and molecular characterization of quinolone resistance in *Mycobacterium abscessus* subsp. *bolletii* recovered from postsurgical infections. J Med Microbiol. 2012 Jan;61(Pt 1):115-25.de Moura VC1, da Silva MG, Gomes KM, Coelho FS, Sampaio JL, Mello FC, Lourenço MC, Amorim Ede L, Duarte RS.

h) Respiratory Outbreak of *Mycobacterium abscessus* Subspecies *massiliense* in a Lung Transplant and Cystic Fibrosis Center

To the Editor: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE VOL 185 2012 Moira L. Aitken, M.D.

f. NTM Identification section 4.6 states “Refer to individual SMIs for organism identification. Organisms may be further identified if this is clinically or epidemiologically indicated”

Comments: As stated within ref 41

“ For some NTM isolates, especially rapidly growing mycobacterial (RGM) isolates (*M. fortuitum, M abscessus*, and *M. chelonae*), other identification techniques may be necessary including extended antibiotic in vitro susceptibility testing, DNA sequencing or polymerase chain reaction (PCR) restriction endonuclease assay (PRA).”

“Because of differences in antimicrobial susceptibility that determine treatment options, species-level identification of the NTM is becoming increasingly clinically important. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of an NTM isolate from a normally sterile site such as blood. For initial clinical mycobacterial isolates, however, it is sometimes difficult to determine the clinical significance of the isolate without species identification. Therefore, identification of most mycobacterial isolates to the species level and not merely as groups, such as “*M. chelonae/abscessus group*” is strongly recommended. If, after consultation between the clinician and the laboratorian and in the event that a specific laboratory does not have the necessary technology for species identification of an NTM isolate, the isolate could be sent to a reference laboratory for further analysis.”

g. Why does this SMI document refer to MALDI-TOF for identification of *Mycobacterium* spp that has not been fully validated yet does not discuss the standard HAIN assay that is currently in use for the identification of NTM, or its limitations (see ref i below and ref 111)? With reference to NTM in particular the *abscessus* complex, HAIN is unable to separate the members of the complex and is known to misidentify some strains as *M chelonae*.

i) Comparison of two methods for identification of *Mycobacterium abscessus* and

Mycobacterium chelonae by K.M. Sands, A. Nicholson, C. Rennison, A. Barrett, S. Bourke, A. Robb, K. Gould, J.G. Magee Journal of Cystic Fibrosis (Vol.11) Volume 11, Supplement 1 , Page S85, June 2012. http://www.cysticfibrosisjournal.com/article/S1569-1993%2812%2960284-7/abstract?source=aemf h. In addition there is no recommendation to refer these isolates to a specialist reference laboratory that uses molecular identification methods e.g Colindale deploys sequencing of housekeeping genes rpoB, hsp65, sodA, nor does it recommend strain typing of the *M abscessus* complex. i. Whilst this document quotes there is no evidence of person to person spread of NTM, without typing how do you know? Colindale are working toward WGS but currently strain type *M abscessus* complex using VNTR sequence cluster analysis. This is an important point especially with respect to CF isolates as without proper identification and strain typing we will not be able to monitor *M abscessus* complex effectively. See refs d, e, f, g and h. j. Rapid Growing species States "*M. abscessus* more so than the other non-tuberculous mycobacterium are an increasing problem for the cystic fibrosis patient group 49. Testing should be considered in patients who show deteriorating lung function but where no clear pathogen has been identified 50-52". Comments: The CF trust recommends annual screening of CF sputum for NTM, we would query why.	
Recommended action	a. **ACCEPT** This has been updated in the document accordingly. b. **NONE** This has not been recommended and so Gram stain and auramine or ZN slides should be reported as stated in the various SMIs. c. **ACCEPT** This has been updated in the document accordingly. d. i. **ACCEPT** The update on the taxonomy of the genus *Mycobacterium* has been made and the BWG members agreed that it should be stated that the genus *Mycobacterium* consists of over 100 species because of the continuous taxonomy update. ii. **NONE** The UK SMIs follow the approved list of bacterial names laid out in Rules of the Bacteriological Code. e. **ACCEPT** This section of the document has been updated accordingly. Some of the references have been used to prove that transmission of NTM (*M. abscessus*) between

	patients is possible. f. **ACCEPT** This section of the document has been updated accordingly. g. **ACCEPT** The standard HAIN assay has been updated in the document and its limitations are mentioned in the technical limitations. However, MALDI-TOF MS is mentioned as it is very useful in the identification for this important group of pathogens, potentially allowing accurate treatment regimens to be started earlier, although it is not widely available yet in all clinical laboratories due to lack of expertise and costs. h. **NONE** All suspected/confirmed *Mycobacterium s*amples are referred to reference laboratories. i. **ACCEPT** See comment 5. j. **NONE** We are not in a position to comment on another guideline producer's recommendation.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	30/03/2015	**Lab name**	Hairmyres Hospital Microbiology Laboratory