Public Health England

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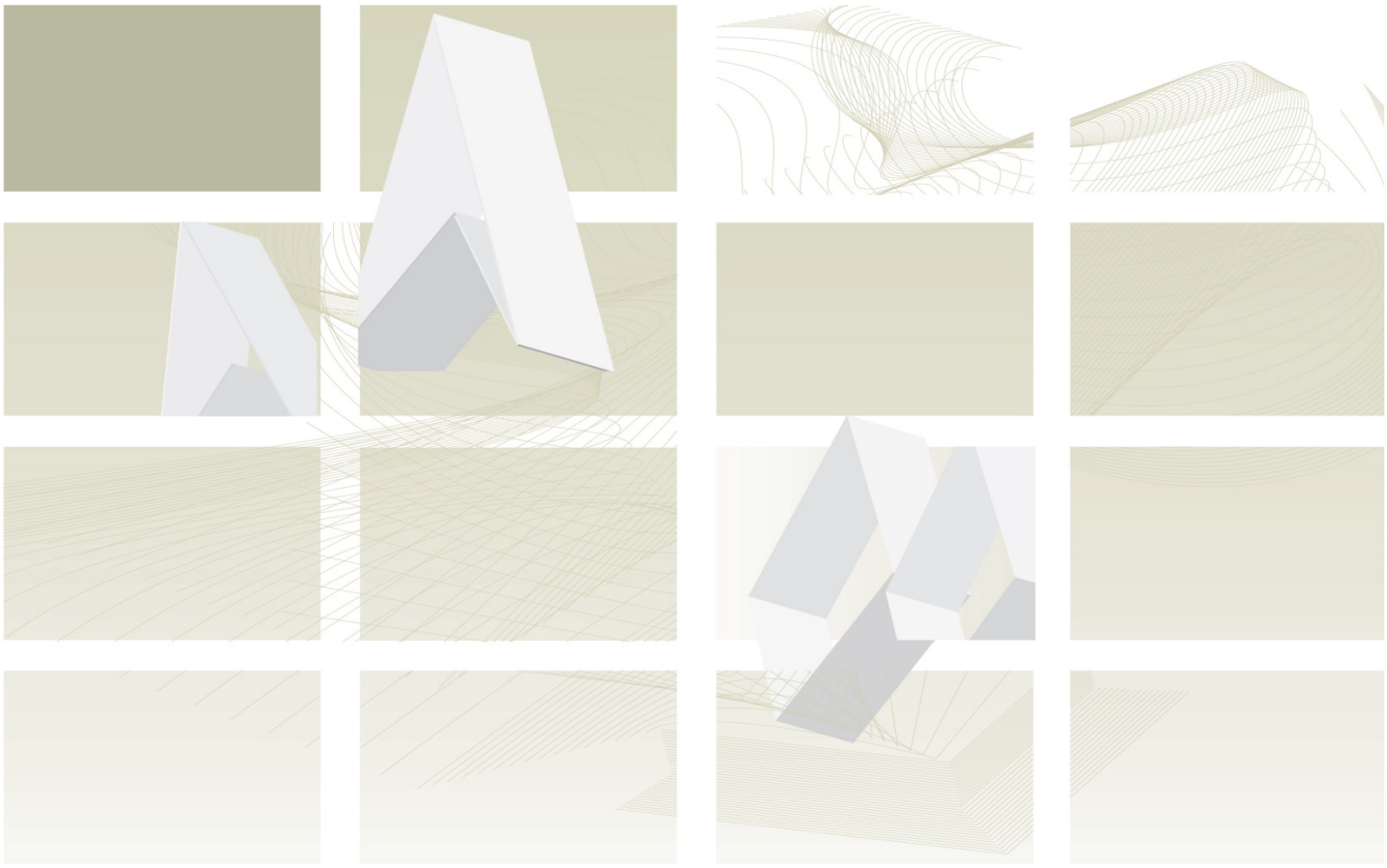
UK Standards for Microbiology Investigations

Review of Users' Comments received by
Working group for microbiology standards in clinical bacteriology

B 57 Investigation of bronchoalveolar lavage, sputum and associated specimens



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

1st Consultation 28.01.13 – 22.04.13

Version of document consulted on – B 57dd+

Proposal for changes

Comment number	1		
Date received	10/04/2013	**Lab name**	Imperial College Healthcare NHS Trust
Section	2.5.1		
Comment			
Routine dilution of sputum samples was recommended in this guideline. Only one reference (no. 57) which was published by Dixon and Miller in 1965 was used to support this method. Are there any evidence-based and more recent publications in the medical literature to back this method up?			
Recommended action	**ACCEPT** This section of the document has been re written and updated.		

Comment number	2		
Date received	22/04/2013	**Lab name**	Mycology Reference Laboratory, Bristol
Section	Various		
Comment			

Overall

Suggest that we need a section specifically related to mycological diagnosis where we make different recommendations for fungal culture from different patient groups. When there is little fungus present in the BAL or sputum (immunocompromised setting) you can maximise isolation and microscopic detection by processing the entire sample. Whereas with the CF patients if you process the entire sample you grow the majority organism which is usually *Aspergillus fumigatus* but you often miss accompanying *Scedosporium* and *Exophiala* species.

Non-CF patients ie immunocompromised and others:

After treating with mucolytic agent if required, spin entire sample. Examine part of residue with KOH and calcofluor staining and culture the remainder.

CF patients:

After treating with a mucolytic agent plate 1uL and spread well over plate. Spin the remainder and examine part of the residue with KOH and calcofluor staining and culture the remainder.

a. There is no mention of the fact that respiratory samples from patients with travel history to areas where dimorphic fungi are endemic, however long ago, may grow such fungi. Any mould grown from such a patient should be processed at CL3 as

soon as it is detected, and until a dimorphic fungus is excluded. I think this is a significant missed safety opportunity! Page 7: scope should include fungal respiratory infection.

b. Page 11, line 3: spelling of coccidioidomycosis.

c. Page 11, line 4: spelling of Coccidioides plus change *C. pedrosii* to *C . posadasii.*

d. Page 11: Add *Penicillium marneffei* (South east Asia, southern China) and *Blastomyces dermatitidis* (North America, Central and South America and Africa).

e. Page 11: *Crytococcus neoformans* and *C. gatti*i are unusual causes of pneumonia in immunocompetent individuals and are mainly encountered in HIV-infected individuals. These need to be distinguished from commensal Candida species.

f. Page 11 Paracoccidioidomycosis caused by *Paracoccidioides brasiliensis* (Central and South America) usually causes asymptomatic primary pulmonary infection that may reactivate if immune function declines. This applies to all the fungi mentioned above not just Paracoccidioidomycosis.

g. Page 13 Under 'Technical information/limitations': - 'Mucorales' should be 'mucoraceous moulds'.

h. Page 14 2.1 containment level 3 - not clear if this is for all respiratory samples.

i. Page 14 2.2 Test selection/Additional comments for BAL: - Patients considered to be at risk of pulmonary aspergillosis, or in whom fungal infection is suspected, should have a portion of BAL fluid tested for Aspergillus galactomannan (or perhaps this goes in 2.5 before Molecular methods).

j. Page 14 2.2 Induced sputum may be sent for investigation for *P. jirovecii* --- (B 31 - Investigation of specimens other than blood for parasites). *P. jirovecii* is a fungus.

k. Page 15 2.4.2 Gram staining may identify yeasts or (not of) hyphae

l. Page 16 2.4.2 Salivary specimens may be rejected before homogenisation or on the basis of a ratio of <2:1 WBCs:SECs determined by a Gram stain at low power magnification (x100). - Need a reference.

m. Page 16 2.4.2 KOH preparation or Calcofluor for fungi.

BAL

Indirect immunofluorescent antibody test for *P. jirovecii.*

Where are the sections for these?

n. Page 16 2.5.1 Dilute 10µL of homogenised sputum in 5mL of sterile distilled water.

This is small sample when at least 1.0ml was recommended earlier - will miss fungi.

Page 17 Supplementary

o. Fungi, Mycobacterium species (B 40 - Investigation of specimens for Mycobacterium species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

The fungi section seems to have been missed.

Page 18 Table 2.5.2

p. Why are Sab plates read at 40 hours?

Where it says 'Mycological investigations' we suggest that SAB is cultured at both 35-37C and 42-44C for 5 days. q. Footer suggest: ‘ Keep cultures up for longer (up to six weeks) if dimorphic fungal pathogens are suspected’ ..... delete specific mention of *P. brasiliensis.* Page 20 2.6 Minimum level of ID r. 'Fungi' and 'Yeasts' are listed separately (this is universal in SOPs I think). This should either say 'Yeasts' and 'Moulds' (preferred) or just 'Fungi'. s. Page 21 Notification to the PHE.	
Recommended action	a. **ACCEPT** This part of the document has been strengthened. b. **ACCEPT** Correction made. c. **ACCEPT** Correction made. d. **ACCEPT** These fungi have been added to the document. e. **ACCEPT** Candida has been moved out of the more unusual fungal causes part of the document. f. **ACCEPT** Sentence moved and made more general. g. **ACCEPT** Wording changed. h. **NONE** Document refers to level 3. i. **ACCEPT** Section inserted in to 2.2. j. **ACCEPT** Cross reference removed. k. **ACCEPT** Correction made. l. **NONE** This is accepted as good practice and is not documented m. **ACCEPT** These sections of the document have been strengthened.

	n. **ACCEPT** This will be covered in the strengthening of the fungal section. o. **ACCEPT** This will be covered in the strengthening of the fungal section. p. **ACCEPT** Document amended. q. **ACCEPT** Document amended. r. **ACCEPT** All UK SMIs will be changed to Yeasts and Moulds as part of PHE rebranding exercise. s. **ACCEPT** All UK SMIs will be changed as part of PHE rebranding exercise.

Comment number	3		
Date received	18/11/2013	**Lab name**	University Hospital Limerick,
Section			
Comment			
We follow the PHE guidance for the processing of process our sputa and cystic fibrosis specimens- HPA SOP 57 Investigation of Bronchoalveolar Lavage Sputum and Associated Specimens. However, we have learned that some centres use an additional cetrimide agar and incubate for up to 5D to increase detection of Pseudomonas. Can you advise if there are any plans to revise the PHE guidelines as our issue is that we have recently missed detection of Pseudomonas as a consequence of partaking in an EU study. I don't believe NEQAS utilises specific resp. samples from the CF population?			
Recommended action	**NONE** The document recommends the minimum requirement. Additional plates can be added depending on local requirements.		

2nd Consultation 02.06.14 – 26.08.14

Version of document consulted on – B 57dr+

Proposal for changes

Comment number	1		
Date received	02/06/2014	**Lab name**	PHE/RCPath
Section	a. Pneumonia section Community acquired subsection p8 b-e. Cystic Fibrosis/ Fungal Infection c. 5.1 Microscopy/ f-j. General Comments		
Comment			
a. *Chlamydia pneumoniae*, *Chlamydia psittaci* should be *Chlamydophila pneumoniae*, *Chlamydophila psittaci*. In the line 'Respiratory viruses, such as Respiratory syncytial virus (RSV), influenza and adenoviruses may occasionally cause primary viral pneumonia' suggest lower case 'r' for respiratory syncytial virus, and remove 'occasionally'. Respiratory viruses are thought to cause at least 10% of CAP in adults and more in children, so you might replace 'occasionally' with 'commonly'; in the study from Canada cited as evidence below viruses alone were found in 15%, bacteria alone 20%. CF section: b. *H. influenza* should be *H. influenzae*. c. The importance of viruses in exacerbations of CF might be mentioned. d. *Mycobacterium abscesses* infection might be included in this section (or the next) Fungal Infection section: e. BAL is a good specimen for *P. jirovecii* PCR as is sputum (induced or expectorated). f. Microscopy: *P. jirovecii* 'oocysts' - should be 'cysts'. Additional Comments: g. Some suggestion of considering viruses should be added to the algorithms. h. There is no mention of *B. pertussis*. i. Should there be a paragraph about other less common pathogens such as Q fever and dangerous pneumonia pathogens such as *B. anthracis*? j. Wouldn't 'calcofluor white' be preferable to 'calcofluor' throughout document? k. The method of aspergillus culture should be specified, suggest using the Manchester Mycology Reference Centre methodology to increase yield.			
Evidence			
Johnstone J et al Viral infection in adults hospitalized with community acquired pneumonia. Chest 2008; 134: 1141-8 Fraczek MG et al. Volume dependency for culture of fungi from respiratory secretions and increased sensitivity of Aspergillus quantitative PCR Mycoses 2014;57:69-			

78.Fraczek MG(1), Kirwan MB, Moore CB, Morris J, Denning DW, Richardson MD	
Financial barriers	
No.	
Recommended action	a. **ACCEPT** Amended. b. **ACCEPT** Amended. c. **ACCEPT** Amended. d. **ACCEPT** Placed in the document where applicable. e. **ACCEPT** The document has been amended and references added. f. **ACCEPT** Amended. g. **ACCEPT** References have been added. h. **ACCEPT** A cross reference to B 6 – Culture of specimens for *Bordetella pertussis* and *Bordetella parapertussis*. i. **ACCEPT** The document has been amended to include this information. j. **ACCEPT** This has been changed throughout the document and brought in line with TP 39. k. **PARTIAL ACCEPT** The reference has been inserted in to the document.

Comment number	2		
Date received	03/06/2014	**Lab name**	Wexham Park Hospital
Section	Appendix (both)		
Comment			
Standard media states chocolate plus bacitracin disc or incorporated into medium.			

Should add blood agar plate as standard if it is incorporated in order to isolate *S. pneumoniae*.	
Evidence	
Referred to in section 4.5.3.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	**ACCEPT** This media has been added as an option.

Comment number	3		
Date received	26/08/2014	**Lab name**	UKCMN
Section	Introduction - fungal infection		
Comment			
a. Paragraph 2 Line 1 - Aspergillosis should be aspergillosis. b. Line 2 - contribute should be contributes. c. Paragraph 5 Line 3 - need a space between in and immunocompetent. d. Line 4 - individuals but reported should be are reported. e. Line 8 - need to add Africa, Australia and eastern Asia after Central America. f. Line 9 - Coccidioide simmitis should be Coccidioides immitis. g. Line 10 - should be eastern USA, Central and South America and Africa. h. Line 14 - Talaromyces (previously Penicillium) marneffei. i. Line 14 - Blastomyces is already mentioned above in the paragraph. j. Paragraph 6 Line 1 - should be immunocompromised host. k. Line 4 - Circulating antigen in the serum or BAL.			
Recommended action	**ACCEPT** These edits (a-k) have been accepted and made.		

Comment number	4		
Date received	26/08/2014	**Lab name**	Cambridge PHE
Section	a. 3		

	b. 4
Comment	
a. Delays in processing are a particular concern for respiratory samples where the CFU of potential pathogens such as *Haemophilus influenzae* and *Strep pneumoniae* may decrease if left at fridge temperature and organisms such as the pseudomonads may multiple at room or even fridge temperature. A BAL is not undertaken lightly and should be transported to the laboratory promptly and processed optimally (I appreciate it is difficult to set standards for this and this needs local agreement). This is becoming more of an issue with the centralisation of microbiology services and the resulting delays in processing. Suggest clearer wording such as: 'BAL and sputum should be processed promptly to give the best opportunity to culture pathogenic organisms and reduce the risk of overgrowth with contaminants. If processing has to be delayed, refrigeration (of up to 24 hours) is preferable to storage at ambient temperature. If specimens are not processed on the same day that they are collected, this should be noted on the report and interpretation of results should be made with care.' b. 4.3 Why 15 minutes DTT at 35-37C, many labs use room temperature and leave until homogenised is that no longer acceptable? c. 4.4.2 PCP what about a PCR method (not my area of expertise so unable to give evidence but I am sure that others will have commentated). d. 4.5.3 This is not the same as the summary in Appendix 1 and 2. For example: 1. Different incubation temperatures quoted for culture of Bcc.2. MSA for *Staph aureus* for CF in Appendix but not in section. e. 4.5.3. 3. Different incubation times for fungi. f. If no blood agar plate is recommended, are you confident that staff will recognise beta haemolytic streptococci? - group A strep are an important cause of serious post flu pneumonia. Will staff be able to recognise *S. pneumoniae* on chocolate agar - when they may appear indistinguishable from viridians-type streptococci? g. 4.6.1 Identification only refers to *Burkholderia cepacia* complex. Should also include reference to *B. gladioli* (which is not part of the *Burkholderia cepacia* complex), also to *B. pseudomallei*. Suggest change *Burkholderia cepacia* complex in left hand column to *Burkholderia* spp., with recommendation to identify to species level as already stated 'pseudomonads' is not a suitable level of identification for CF or bronchiectasis patients. Unusual morphotypes of *P. aeruginosa* may be missed. Organisms such as *Ralstonia*, *Achromobacter* and *Pandoraea* are emerging pathogens in chronic structural lung disease. Suggest a minimum of identification to genus level.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **ACCEPT** This section has been updated.

	b. **ACCEPT** Now say follow manufacturer's instructions. c. **ACCEPT** This has now been mentioned. d. **ACCEPT** These have now been brought in line. e. **NONE** The second temperature is for universal tubes not plates. f. **ACCEPT** A note on this situation and the suggestion of an addition of a blood plate have been added. g. **ACCEPT** This information has now been included in the introduction and the minimum level of identification section modified.

Comments received outside of consultations

Comment number	1		
Date received	31/12/2012	**Lab name**	HPA Public Health Laboratory Manchester
Section	Whole document		
Comment			
Incidentally where *P. jirovecii* is discussed in the SMIs, eg in B57, it is misspelt as *P. jiroveci* - this is a common mistake as it was the first nomenclature used but please correct it.			
Evidence			
If you want the reason see Stringer JR, Beard CB, Miller RF. Spelling *Pneumocystis jirovecii*. Emerg Infect Dis. 2009 March; 15(3): 506.			
Recommended action	**ACCEPT** Change made.		

Comment number	2		
Date received	07/01/2013	**Lab name**	MSTAG
Section	a. Molecular Detection methods b. Semi-quantative culture		

	c. Vortexing d. 2.5.2 e. 2.5.3 f. Whole document
Comment	
a. "extraction systems are specific"- not true. b. Confusing-needs simplifying. Needs clarification which is for Sputa/BAL. c. Although it affects the "air-curtain", it is just as risky to vortex outside-ie what happens if tube breaks. Also the term "curtain" is this a reference to a Type 2 cabinet as type 1s do not have an air curtain. d. i. Blood agar not in table any more, what happened to this as a basic media for minimum standards, either incubated CO_2 or anaerobically. ii. Supplementary media-add chromogenic staph media as well as mannitol salt agar. iii. Although Legionella is a separate method add in Legionella media here. iv. *B. cepacia* agar confusing, should read "35^{o} C for 2 days then 30^{o} C for 3 days not 5 days. e. This table is so similar to 2.5.2, is it required? f. General comment not necessarily relevant to this method but it was noted that pathogenic fungi can grow in liquid TB media.	
Recommended action	a. **ACCEPT** Sentence removed. b. **ACCEPT** Section has been streamlined. c. **ACCEPT** Sentences removed. d. i. **NONE** Blood agar has never been in this document as we have a chocolate agar plate. ii. **ACCEPT** This media option has been added to the document. iii. **ACCEPT** The media has been added. iv. **ACCEPT**

	The times in the table have been clarified. e. **NONE** There are subtle differences which would be lost if the tables were merged. f. **NONE**

Respondents indicating they were happy with the contents of the document

Overall number of comments: 7			
Date received	29/01/2013	**Lab name**	SRM Institute For Medical Sciences, Chennia, India
Date received	31/01/2013	**Lab name**	RIE
Date received	07/02/2013	**Lab name**	Ex Laboratorio Microbiologica Careggi Firenze
Date received	13/02/2013	**Lab name**	Golden Jubilee National Hospital
Date received	15/02/2013	**Lab name**	Microbiology, Newcastle Hospitals NHS Trust
Date received	03/06/2014	**Lab name**	Microbiology
Date received	10/06/2014	**Lab name**	Princess of Wales' hospital