Public Health England

NHS

UK Standards for Microbiology Investigations

Review of Users' Comments received by Working Group for Microbiology Standards in Clinical Bacteriology

B 27 Investigation of Cerebrospinal Fluid

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

Consultation: 21/09/2012 – 14/12/2012

Version of document consulted on: B 27dc+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	11/12/2012	**Lab Name**	on behalf of the UK Clinical Mycology Network
Section	Various		
Comment			

a. The reference for normal CSF RBC and WBC values is a 20 year old review article, and the original article includes a 'caution' about the interpretation of these values (http://cmr.asm.org/content/5/2/130.full.pdf). It is rather simplistic to state that 0-30 is a 'normal' WBC value for a neonate, when, in fact, meningitis may be diagnosed in neonates with pretty much any CSF WBC value. Furthermore, it appears to be inaccurate, in the light of (for instance) the article at http://pediatrics.aappublications.org/content/125/2/257.long, which is a modern attempt to define CSF WBC values in infants. This whole table needs to be revised according to recent literature.

b. 2.5.3 recommends a sab plate is put up in the immunocompromised although this would miss cryptosporidium as a presenting feature of HIV and also cases in non-immunocompromised.

   A sab plate would miss cryptosporidium as a presenting feature of HIV.

c. Also it is to be read at 40hrs and kept for UP TO 8 WEEKS! A sab plate cannot usefully be kept up for this long. If you have evidence that cultures need to be so prolonged, then slopes would have to be kept.

d. Is it still considered correct that C. neoformans doesn't show up well on Gram stain (see 1980 ref. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC273699/)? If so this should be made clear (with a reference), otherwise there would be no point in doing an India ink test in the presence of a negative the Gram stain. Notwithstanding the above, does the India Ink test add anything to a combination of Gram stain + CRAG, especially now that there is a lateral flow immunochromatography test available for CRAG? The India ink test is probably not very sensitive (microscopy generally isn't) and may result in a false sense of security.

e. It is very important that the CRAG test is carried out on a supernatant rather than native CSF - although this may be obvious I'm not sure the document makes it clear.

f. There should be some attempt made in this document to propose how Gram stain, India ink and CRAG fit in with each other, rather than simply mentioning all three.

g. Cryptococcal antigen (CRAG) may also be detected by LAT, although testing of serum is more sensitive than testing CSF alone'. There are a number of different types of CRAG test available, and this document should not dwell on any particular method. Also, it sort of implies that testing serum is more sensitive than

testing CSF. I doubt if many laboratories in the UK are using latex agglutination and most will use ELISA or have moved to the lateral flow device. Also an SOP should give an indication of when to perform the test. We suggest 'CSF Cryptococcalantigen testing should be carried out in all cases of suspected cryptococcal meningitis, and all cases of meningitis in immunocompromised patients in which there is an elevated CSF white cell count and no alternative diagnosis has been made. In these cases serum should also be tested for CRAG. h. Re. B27:1. The paragraph that starts 'Leukaemic meningitis' is a bit strangely worded. If leukaemia is mentioned it would more appropriately be described as 'Meningitis is rare in association with leukaemia, but...' However, I suggest that this paragraph is redundant, and it is sufficient to refer to Cryptococcus neoformans in the general paragraph about immunosuppression.	
Recommended Action	a. **NONE** The valves listed in the table represent the approximate upper and lower limits of normality particularly in neonates and children. b. **NONE** Methods for the diagnosis cryptosporidium is covered in other parts of the document. c. **ACCEPT** Document amended to include Sabouraud slope if longer incubation time is required. d. **NONE** It is the opinion of the working group that India Ink remains as a test. e. **ACCEPT** The document has been amended. f. **NONE** This should be decided at a local level. g. **ACCEPT** Recommended text inserted in to the document. h. **ACCEPT** Paragraph removed.

Comment Number	2		
Date Received	10/12/2012	**Lab Name**	Department of Medical Microbiology, Conquest Hospital
Section	1.2.2		
Comment			
CSFs should be examined immediately. Evidence shows that to obtain accurate cell counts they should be examined within one hour of the sample being taken from a patient and certainly within two hours (by which time up to 50% of cells can lyse). The time taken between receipt in the laboratory and processing is largely irrelevant and meaningless as it doesn't take into account when the sample was taken/transport times. A transport time of two to four hours plus up to another two hours to process a sample is not going to yield accurate results. Increasing the time for samples to be processed in the laboratory from two to four hours is an astounding change given the evidence for decline in WBC with time (you already quote evidence for this in the old document). If you want to promote quality/accuracy, then I can't see that you have any choice other than to recommend that samples are processed within one hour of the sample being taken and certainly within two hours as recommended by eg the EFNS task force. I don't have access to CLSI documents, but I believe they also recommend this.			
Evidence			
Your own evidence stated in the original document.-Guidelines on routine CSF analysis. Report from an ENFS task force. Europ. J. Neurol. 2006; 13:913-22.-Effect of delay in analysis of CSF parameters. Arch Dis Child Feta Neonatal Ed 2010;95:25-29			
Recommended Action	**ACCEPT** Document has been amended and brought in to line with B 37.		

Comment Number	3		
Date Received	10/12/2012	**Lab Name**	Virus Lab, Aberdeen Royal Infirmary
Section	Introduction, top of page 8		
Comment			
I am pleased you have put aseptic in brackets of inverted commas, other than the 1st word on this page. Suggest delete aseptic here.			
Recommended Action	**NONE** This change has already been made.		

Comment Number	4		
Date Received	07/12/2012	**Lab Name**	Sunderland Royal Hospital
Section			
Comment			
It is about the reporting of the WBC count in CSF, CAPD, peritoneal fluid and other fluids. For blood specimens the WBC is always reported as x 10^9/L as by standard practice and in line with the Pathology Harmony recommendations. see http://www.pathologyharmony.co.uk/ But how do you report the WBC count for other body fluids such as CSF or peritoneal fluid? Currently the National Microbiology SOPs for CAPD fluids (UK Standards for Microbiology Investigations B 25) as well as the national SOP for CSF investigations (UK Standards for Microbiology Investigations B 27) as well as the national SOP for sterile fluids (UK Standards for Microbiology Investigations B 26) all relate to WBC counts expressed as x 10^6/L. Thus a CSF is abnormal if the WBC is > 5 x 10^6/L or > 0.005 x 10^9/L (same result in different units) but we (humans) tend to handle and understand better integer numbers rather than decimal numbers, thus reporting as x 10^6/L seems preferable to me. Our Microbiology lab uses x 10^6/L in these reports but we are merging with another lab using x 10^9/L: they want us to change! Can I assume that a lot of thinking as gone into the UK Standards B 25, B 26 and B 27 and thus there is no plan to harmonise WBC reports from body fluids to a different unit?			
Recommended Action	**NONE** There are no plans to change the UK SMIs at this time.		

Comment Number	5		
Date Received	06/12/2012	**Lab Name**	Clyde Microbiology Laboratory
Section	Microscopy 2.4.1 b		
Comment			
a. Use of white cell diluting fluid is used where there are a reasonable red blood cell count, not only when heavily bloodstained. Unless there is a thought that this also destroys the white cells. b. Also 2 hours can be an impossible time-frame to receive CSFs for microscopy in this era of merged labs.			
Evidence			
Samples to travel 25 miles by porter, taxi and porter to lab - may well be >2 hours. Transport even within sites can be a challenge and some samples can be deemed none emergency by clinician.			

Recommended Action	a. **ACCEPT** The word heavily will be removed from the document. b. **NONE** These are high priority samples.

Comment Number	6		
Date Received	05/12/2012	**Lab Name**	Belfast HSCT
Section	General		
Comment			
I think the SMI can be less congested and simpler by making a separate SMI for the investigation of mycobacteria plus or minus other rare pathogens.			
Recommended Action	**NONE** These organisms are mentioned only for background and are covered in more depth in other documents.		

Comment Number	7		
Date Received	04/12/2012	**Lab Name**	EUROIMMUN UK Ltd.
Section	General		
Comment			
The documents contain no reference to antibody diagnostics of CSF/serum pairs in accordance with the recommendations of Reiber, which provide decisive guideleines on the diagnosis of inflammatory process in the CNS. The significance of CSF diagnostics has been described in various publications of Prof. Reiber.			
Evidence			
For example: Reiber H (1994) Flow rate of cerebrospinal fluid (CSF) a concept common to normal blood-CSF barrier function and dysfunction in neurological diseases. J Neurol Sci 122:189 203 Reiber H (1995a) External quality assessment in clinical neurochemistry: Survey of analysis for cerebrospinal fluid (CSF) proteins based on CSF/serum quotients. Clin Chem 41:256 263 Reiber H, Lange P (1991) Quantification of virus-specific antibodies in cerebrospinal fluid and serum: Sensitive and specific detection of antibody synthesis in brain. Clin Chem 37:1153 1160 Reiber H, Peter JB (2001) Cerebrospinal fluid analysis disease-related data patterns and evaluation programs. J Neurol Sci 184:101			
Recommended Action	**PARTIAL ACCEPT** The scope of this document will be amended to make it clear that viruses and immunological conditions are not covered.		

Comment Number	8		
Date Received	30/11/2012	**Lab Name**	Great Ormond Street Hospital
Section	2.5.2 2.5.3		
Comment			
Broth culture should be used for neurosurgical infections, especially when antimicrobials have already been started and to investigate late shunt related infection. The use of a 7 day FAA plate may be insufficient to detect the slow growing propionibacteria in shunt related infections; minimum 14 day incubation should be advised, but ideally there should be a prolonged anaerobic enrichment broth and then subculture.			
Evidence			
HPA Standard protocol recommends- a primary anaerobic plate for 7 - 14 days (and then says read at 40hr and 5 days, so that is a bit confusing). - but no broth but I am not convinced by that advice. I quite see the rational for no broth in non-neurosurgical infection (Shah SS PIDJ 2012 - Cerebrospinal fluid enrichment broth cultures rarely contribute to the diagnosis of bacterial meningitis). However the two references quoted both actually recommend broths in shunt CSFs (Meredith F 1997, and Dunbar SA 1998)- Meredith FT J Clin Micro 1997. Clinical Utility of Broth Cultures of Cerebrospinal Fluid from Patients at Risk of Shunt Infection Concluded: 'suspected CSF shunt infection may be one of the few remaining clinical scenarios in which the use of a broth medium for culture may be helpful to the clinician. Consequently, we recommend the continued use of broth medium for the culture of CSF from patients with CSF shunts to exclude the possibility of an infection caused by Propionibacterium sp.'- Dunbar SA 1998 J Clin Micro 1998 Microscopic Examination and Broth Cultures of Cerebrospinal Fluid in Diagnosis of Meningitis Concludes that: CSF specimens should be cultured in broth in special cases only, such as patients with CNS shunts....The more recent published literature on culture of Propionibacterium from shunts (or prosthetic joints) supports prolonged anaerobic culture eg Kai Arnell et al Journal of Neurosurgery: Pediatrics May 2008 / Vol. 1 / No. 5 / Pages 366-372 Cerebrospinal fluid shunt infections in children over a 13-year period: anaerobic cultures and comparison of clinical signs of infection with Propionibacterium acnes and with other bacteria. Which says: The addition of cultures for anaerobic bacteria and prolonged observation time of the cultures led to an increase in the diagnostic yield by more than one third. Infection with P. acnes resulted in a mild clinical picture that may easily be overlooked if adequate anaerobic cultures are not obtained.Likewise culture from PJI suggest the same eg Butler-Wu J Clin Micro 2011 Optimization of Periprosthetic Culture for Diagnosis of Propionibacterium acnes Prosthetic Joint Infection In our own experience, we introduced the Robertson's Cooked Meat extended incubation (5-7 days) and anaerobic subculture (further 7 days) a few years ago and find Propionibacterium in the broth that was not on the 5 day anaerobic plate and we feel are clinically significant. (The majority of broths are no growth, even setting up on the open bench).This may partially explain the higher % yield of GPRs between a historical (non-impregnated shunts) 1993 - 2003 cohort compared to a recent Bactiseal (clindamycin and rifampicin impregnated) cohort, and lower yield of 'no growths' treated as infection; possibly the antimicrobial impregnated shunt has 'uncovered' a cohort of late GPR infection by reducing the CoNSs. Organisms isolated from shunt CSF infections at GOSH 1993-2003-1592 shunts (non bactiseal)- 8.4% infection rate; 133 infections, only 2 propionibacteria but 7 no growth (treated as			

infection) 499 recent bacteseal shunts with extended anaerobic culture- 5% infection; 25; 4 propionibacteria/other GPR and 1 no growth. Hence, I would suggest extended anaerobic culture as standard minimum 14 days ideally with a broth enrichment first. Additionally, if not prepared to use a broth in all, I would recommend essential if antimicrobials have already been started (no evidence started, but good common sense and clinical experience!)	
Recommended Action	**ACCEPT** With a caveat that they are only useful in certain circumstances.

Consultation: 18/03/2013 – 07/06/2013

Version of document consulted on: B 27df+

PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	18/03/2013	**Lab Name**	Microbiology, Pilgrim Hospital, Boston, Lincs
Section			
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?			
Yes. This is a reasonable time scale to allow for growth of slow-growers and is in line with yeast/fungal culture incubation times set out in other UK SMI's.			
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?			
Yes. However, in Section 3.1.1 the time quoted for microscopy reporting is 2 hours. This is too long. I would take a serious look at a laboratory organisational and prioritisation structure that allowed two hours for such an important result to be produced. A 1 hour microscopy reporting time would seem much more reasonable and should be easily achievable by a well organised laboratory with competent staff.			
Do you have any views on the use of broth cultures for diagnosing shunt infections?			
No.			
Recommended Action	N/A		

Comment Number	2		
Date Received	05/04/2013	**Lab Name**	Microbiology Department, Freeman Hospital, Newcastle Hosp Trust

Section	2.7 and 2.8
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?	
Our laboratory protocol is 10 day incubation for extended anaerobic culture and 21 days for extended fungal investigations.	
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?	
Yes.	
Do you have any views on the use of broth cultures for diagnosing shunt infections?	
Not routinely performed; may be performed as the request of microbiologist.	
Comment	
a. 2.7 - Please consider inclusion of referral to EUCAST guidance as well as BSAC b. 2.8 - Web link to the reference lab user manual and request forms is a very useful inclusion	
Recommended Action	a. **ACCEPT** This is being done as part of the transfer to the PHE template. b. **NONE**

Comment Number	3		
Date Received	24/04/2013	**Lab Name**	Nottingham
Section	2.5.3		
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?			
No What is the evidence for 14 days, aware of extended incubation until 10 days for more fastidious and Proprionobacteria. Beyond that will the plates still be moist enough for organisms to grow?			
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?			
Yes.			
Do you have any views on the use of broth cultures for diagnosing shunt infections?			
Aware may increase yield, but difficult to interpret and distinguish from contamination during sampling and processing if broth only positive.			
Comment			
Table 2.5.3 For the neurosurgical samples it's unclear of what point extended plates are read and results reported. As FAA plate incubated for 14 days but states read >40hr and 5 days			

Recommended Action	**ACCEPT** UK SMI amended to make clearer and amended to ten days.

Comment Number	4		
Date Received	03/06/2013	**Lab Name**	Western Sussex Hospitals Microbiology Laboratory
Section			
Do you agree with a 14 day incubation time for Sabouraud or anaerobic agar?			
Yes.			
Do you agree with a time between collection to microscopy and culture of a maximum of 4 hours?			
Yes.			
Do you have any views on the use of broth cultures for diagnosing shunt infections?			
Should be used - original paper specified whilst not useful in general they were for abscesses and shunt infections. J Clin Microbiol 1997;35:3109-11. Consequently, we recommend the continued use of broth medium for the culture of CSF from patients with CSF shunts to exclude the possibility of an infection caused by Propionibacterium sp.			
Comment			
I would have liked to see a worked example of calculating an uncertainty of Measurement for the cell count.			
Recommended Action	**NONE** This comment will be considered as part of the review of the Quality SMI documents in 2015.		

Comment Number	5		
Date Received	06/06/2013	**Lab Name**	Kingston Hospital
Section			
Comment			
a. The CSF WCC reference ranges for neonates and age 1- 4 appear higher than most paediatric reference ranges. They are also contradictory to the figures proposed in the NICE 2010 Meningitis Guidance for Children. May I know what is the rationale behind this? b. Also there is currently no CSF reference range for age between > 7 days to 12 months old. May I know why?			

Recommended Action	a. **ACCEPT** This section has been made clearer b. **NONE** A caveat has been added to say that the table is just for guidelines

COMMENTS RECEIVED OUTSIDE OF CONSULTATIONS

Comment Number	1		
Date Received	11/09/2012	**Lab Name**	MSTAG
Section	a. General Comment b. Normal CSF values table c. 2.7 d. 2.5.3 e. 2.4.1 f. 2.1 g. 1.4 h. Abnormalities assoc. with bacterial meningitis.		
Comment			
a. Would like to see more detail about TB meningitis. b. Clarify neonate age range, specify type of luecocoytes. c. BSAC guidelines given, but many labs are now using other guidelines. d. First part of table clinical details etc. instead of giving list of clinical details how about stating ALL CSFs in this section get a minimum of Choc and BA Immunocompromised patients up to 8 weeks for SAB plate disputed, suggest add comment incubate up to 14 days if indicated FAA/NEO incubation time given as 7-14 d, but next column-cultures read gives 40hr and 5d. Suggest 5 days. e. Section 2-has a chunk of text been removed? It doesn't make sense. Gram stain: suggest simplify this section-instead of listing exclusions, how about something along the lines of: 'Perform Gram stain on neonates, Immunocompromised and raised cell counts'.The word sterile is used in this section regarding centrifuging in a sterile capped conical container. Clotted specimens-describes how to make a smear for Gram stain but in previous section states do not perform Gram. f. Paragraph 5-either provide more detail for TSE agents (lots of……..dots used) or provide a reference. Subsequent paragraphs regarding processing under a hood-is this the recommendation for ALL CSF samples? g. 10ml for Mycobacteria! This is an extremely large volume for CSF, the MRU recommend a minimum of 0.5ml. h. Paragraph 3 WBC: RBC ratio-would be clearer if this was added to the above table and age ranges, given ie the gap between newborn and adult is a large		

range.	
Recommended Action	a. **NONE** This is covered in more detail in B 40. b. **PARTIAL ACCEPT** Document updated. c. **NONE** The group has agreed to continue to recommend BSAC until such a time as they become EUCAST. d. **NONE** All UK SMIs start with clinical details and most users find this the most useful presentation. e. **NONE** This change has already been made. f. **NONE** This change has already been made. g. **ACCEPT** The document has been updated. h. **NONE** Information in table is standard for this area.

Comment Number	2		
Date Received	03/01/2013	**Lab Name**	Southampton General Hospital
Section	SOP numbers B 22 and B27 both concerning CSF and CSF shunts		
Comment			
I have just had one of our consultants ask why we weren't following these SOPs. We have found conflicting and confusing information. For the investigation of CSF culture the incubation times states 7 to 14 days but only states to read at >40hrs and at 5 days with no mention of 14 day reading anywhere. Please could this be clarified for us. We have only followed the reading and reporting at 5 days but will change as soon as this is understood.			
Recommended Action	**ACCEPT** This section of the document has been amended to make it clearer.		

Comment Number	3		
Date Received	02/01/2013	**Lab Name**	University Hospitals of Leicester NHS Trust
Section	Whole document		
Comment			
a. Do you know if the advice re neurosurgical type CSFs and anaerobic incubation and when you read the plates, and use of broth enrichment is going to change? b. Currently it states incubation for 7-14 days but only read at 40hr and 5 days, which is a bit confusing! c. It also doesn't mention enrichment broths, is this likely to change for this type of CSF?			
Recommended Action	a. **ACCEPT** When plates are read has been made clearer. The advice on broths remains the same. b. **ACCEPT** When plates are read has been made clearer. c. **PARTIAL ACCEPT** Broths are mentioned in the document as useful in certain circumstances, this will be strengthened.		

RESPONDENTS INDICATING THEY WERE HAPPY WITH THE CONTENTS OF THE DOCUMENT

Overall number of comments: 1			
Date Received	29/05/2013	**Lab Name**	Golden Jubilee National Hospital