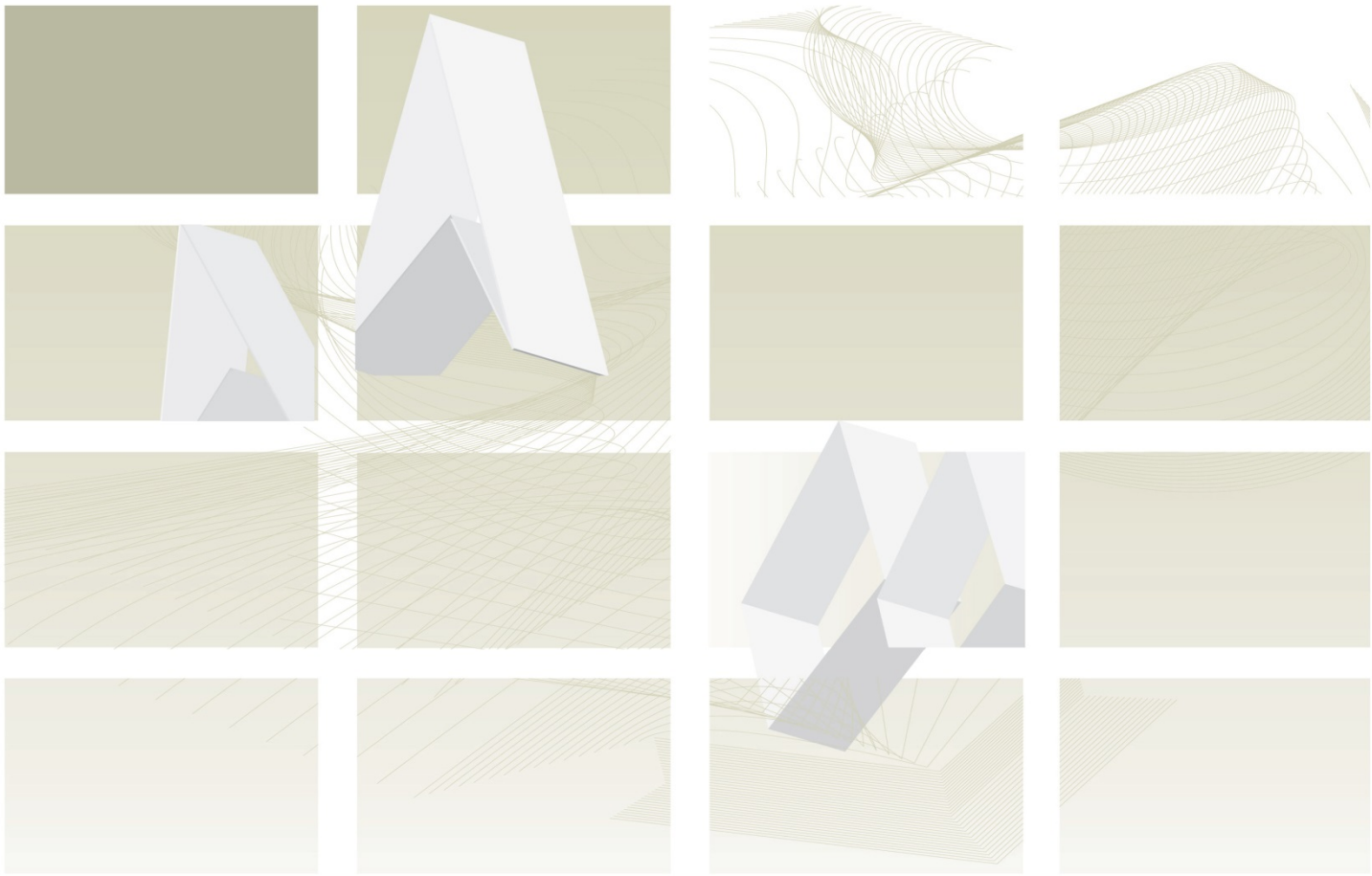
Public Health England

NHS

UK Standards for Microbiology Investigations

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

TP 39 Staining procedures



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

Issued by the Standards Unit, National Infection Service, PHE

RUC | TP 39 | Issue no: 3 | Issue date: 17.05.19

Consultation: 07/01/2019 – 21/01/2019

Version of document consulted on: TP 39dn+

Proposal for changes

Comment number	1		
Date received	08/01/2019	**Lab name/Professional body**	Microbiology dept., Sheffield Teaching Hospitals FT, Northern General Hospital, Sheffield
Section	**Acridine orange stain (*Trichomonas vaginalis*)** Quality control on page 33		
Comment			
Hard to control as TV is difficult to maintain, and breaks down on prepared slides after a few days. No particular control strain is recommended and no EQA is available. This makes this technique a problem with UKAS accreditation.			
Evidence			
Not completed.			
Financial barriers			
Not completed.			
Health benefits			
Not completed.			
Are you aware of any interested parties we should consider consulting with on the development of this document?			
UKAS			
Recommended action	**NONE** Many thanks for the information. We recognise the difficulty of the use of this positive control as mentioned in this section of the document but it does not detract from the validity of the test.		

Comment number	2		
Date received	14/01/2019	**Lab name/Professional body**	Public Health laboratory, Birmingham
Section	a. Fungal stains section 2 page 26		

	b. Fungal stains section 5 page 30
Comment	
a. We use sellotape (single sided) directly onto a drop of LCPB on a slide, which is not mentioned in this SMI. The method suggested uses double-sided scotch tape and a coverslip. However, the wording does not make a sense. It is possible that using a coverslip and double-sided tape in a microscopic preparation to examine cultures could give a clearer preparation. However, the wording in the document needs improvement. b. Method for skin and hair is the same as we use, however TP39 says to pre-soften nail samples with KOH and refers to B39. B39 says to inoculate nail scrapings directly into a drop of KOH on a slide then add a drop of calcofluor. We pre-soften nail scrapings in a few drops of KOH in a sarsedt tube. This is an extra step introduced after a staff member attended the training course run by the Mycology Reference unit in Bristol and gives a more homogenous preparation of material on the slide.	
Evidence	
Not completed.	
Financial barriers	
No.	
Health benefits	
No.	
Are you aware of any interested parties we should consider consulting with on the development of this document?	
No.	
Recommended action	a. **ACCEPT** The method has been updated with appropriate information to make it clearer. The use of Sellotape has been removed as it is a brand name and not recommended by UK SMIs. The use of adhesive tape has been added and updated accordingly. b. **NONE** Both the TP39 and B39 documents both reflect that nail specimens should be pre-softened which is what is recommended in the method for preparation of nail specimens for microscopy.

Comment number	3		
Date received	14/01/2019	**Lab name/Professional body**	Salisbury District Hospital

Section	Technical Information/Limitations and the stains
Comment	
a. Decolourising step - In some laboratories, laboratory staff are taught to add the decolourising reagent drop by drop until it runs clear. This is ambiguous - are you recommending this? If so, say that this is good practice, otherwise it is not clear if you are saying this practice is good or not. b. In 1. Auramine Phenol Stain Auramine stain show high sensitivity and specificity than Ziehl-Neelsen's method. This reads odd. Do you mean 'higher'? c. For fluorescent stains might it be useful to put the wavelength needed in LED microscopes? It is listed for *Cryptosporidium*, but not others	
Evidence	
Not completed.	
Financial barriers	
No.	
Health benefits	
No.	
Are you aware of any interested parties we should consider consulting with on the development of this document?	
No.	
Recommended action	a. **NONE** Many thanks for the information but it has been made clear that laboratories should follow manufacturer's instructions when performing any staining technique. b. **ACCEPT** This has been updated in the document accordingly. c. **ACCEPT** This has been updated in the document accordingly.

Comment number	4		
Date received	17/01/2019	**Lab name/Professional body**	SRUC Veterinary Services
Section	a. Gram stain b. Gram stain fixation		
Comment			
a. We have used sodium carbonate as per Kopelhoff for staining of anaerobes and occasionally other bugs as it retains dye for Gram positives.			

b. Some laboratories have commented that fixing in alcohol can improve morphology. Are there any comments for or against this?
Evidence
Benefits of bicarbonate have been recognised for a long time.
Financial barriers
No.
Health benefits
No.
Are you aware of any interested parties we should consider consulting with on the development of this document?
I was first informed of this at the SAM anaerobic training course around 25 years ago. It may be worth asking the Anaerobic Reference Lab

Recommended action	a. **ACCEPT** The technical limitations section has been updated with information on the different modifications of Gram stain for anaerobes. b. **NONE** Many thanks for the information. The Anaerobe Reference Laboratory was contacted with regards to the Kopeloff modification of Gram stain and they worked with us to ensure that accurate information was added in the review of this document.

Comment number	5		
Date received	21/01/2019	**Lab name/Professional body**	Department of Clinical Microbiology, Royal Cornwall Hospitals Trust
Section	Fungal stains section 2, Bacteria stains 4		
Comment			
Fungal stain Section 2 Lactophenol: a. magnification low power states x100 (should this be x10) and high power states x430 should this be x40? Or is this including eyepiece magnification b. Sellotape as this is a brand name - adhesive tape is generic Bacteria stains Section 4 Mc Fadyean stain:			

c. inconsistent wording for Class 1 biological safety cabinet through the document. (detailed as exhaust protective cabinet / protective safety cabinet)	
Evidence	
Not completed.	
Financial barriers	
No.	
Health benefits	
No.	
Are you aware of any interested parties we should consider consulting with on the development of this document?	
No.	
Recommended action	a. **NONE** Many thanks for the information. The magnification for both the low power and high power includes the eyepiece magnification. b. To be discussed at the BWG meeting. c. **ACCEPT** This has been updated in the document accordingly.

Comment number	6		
Date received	21/01/2019	**Lab name/Professional body**	SfAM
Section	2. Gram stain		
Comment			
Staining young broth-grown cultures can provide clearer morphology c.f. plate -grown cultures, especially for cocco-bacillary forms.			
Evidence			
Personal experience gained in PHLS labs. Think Dr N. Preston, Manchester University, demonstrated this. It may be in older versions of Bergey's Manual of Determinative Bacteriology or Cowan and Steel.			
Financial barriers			
No.			
Health benefits			
No.			

Are you aware of any interested parties we should consider consulting with on the development of this document?	
No.	
Recommended action	**NONE** Many thanks for the information. Similar information has already been added in the technical information under the Gram stain.

Comment received outside of consultation

Comment number	1		
Date received	25/01/2019	**Lab name/Professional body**	Birmingham Clinical
Section	section 9		
Comment			
Flood the slide with strong carbol fuchsin (ie 100mL of 10x Concentrated carbol fuchsin which should be diluted in 900mL distilled water before use) heat the underside of the slide gently until steam rises but not boiling (Caution: overheating causes spattering of the stain and may crack the slide). This is not a safe method of carrying out staining. We keep the Stain on a hot plate so it remains warm. Stain is added to the fixed slide on a staining rack.			
Evidence			
This would reduce the risk of the phenol, having a heat source in the laboratory and the spattering of the stain. This method has been done in Birmingham NMRS- N&C laboratory for over 8 years.			
Financial barriers			
Not completed.			
Health benefits			
Not completed.			
Are you aware of any interested parties we should consider consulting with on the development of this document?			
Not completed.			
Recommended action	**ACCEPT** The use of hotplate has already been mentioned under safety considerations for the Ziehl - Neelsen stain (acid fast bacilli). This has also been made clearer in the "*Method*" subheading.		

Respondents indicating they were happy with the contents of the document

Overall number of comments: 3			
Date received	15/01/2019	**Lab name/Professional body**	Keith Shuttleworth and Associates Ltd
Health benefits			
None to my knowledge.			
Date received	17/01/2019	**Lab name/Professional body**	The Holly Pathology
Health benefits			
Not completed.			
Date received	21/01/2019	**Lab name/Professional body**	SfAM
Health benefits			
Not completed.			