

Help with moving disc diffusion methods from BSAC to EUCAST

This document sets out the main differences between the BSAC and EUCAST disc diffusion methods with specific emphasis on preparation prior to implementation and consumables.

Media:

Media	BSAC	EUCAST
For non fastidious organisms	IsoSensitest Agar (ISO)	Mueller Hinton Agar (MHA)
For fastidious organisms	IsoSensitest Agar + 5% defibrinated horse blood + 20mg/L β -NAD (ISON)	Mueller Hinton Agar + 5% defibrinated horse blood* + 20mg/L β -NAD (MH-F)

* Mechanically defibrinated

Storage and drying of media plates are the same for both methods with one exception:

EUCAST: For *Campylobacter jejuni* & *Campylobacter coli*: In order to reduce swarming, the MH-F plates should be dried prior to inoculation (at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min).

Inoculum:

Inoculum confluence

The table below is a comparison of BSAC and EUCAST inoculum confluence using Table 3 from the BSAC guidelines.

Organism	BSAC	EUCAST
For Enterobacteriaceae, <i>Acinetobacter</i> spp., <i>Pseudomonas</i> spp., <i>S. maltophilia</i> , enterococci, β -haemolytic streptococci, <i>H. influenzae</i> , <i>P. multocida</i> , <i>B. fragilis</i> & <i>B. thetaiotaomicron</i> .	<u>Semi-confluent</u> 1:100 dilution of 0.5 McFarland density	<u>Confluent</u> 0.5 McFarland density (See exception 1)
For staphylococci, <i>Serratia</i> spp., <i>S. pneumoniae</i> , α -haemolytic streptococci, <i>M. catarrhalis</i> , <i>N. meningitidis</i> , <i>Corynebacterium</i> spp., <i>Clostridium perfringens</i> .	<u>Semi-confluent</u> 1:100 dilution of 0.5 McFarland density	<u>Confluent</u> 0.5 McFarland density (except <i>S. pneumoniae</i> from Chocolate agar: McFarland 1) (See exception)
For <i>N. gonorrhoeae</i> , <i>Campylobacter</i> spp.	<u>Semi-confluent</u> 0.5 McFarland density	<u>Confluent</u> 0.5 McFarland density (See exception)

Exception: Disc diffusion criteria for antimicrobial susceptibility testing of anaerobes, *C. difficile*, *N. meningitidis* & *N. gonorrhoeae* have not yet been defined and an MIC method should be used.

Inoculum preparation:

Inoculum preparation	BSAC	EUCAST
Inoculum fluid	Sterile water or IsoSensitest broth	Sterile saline
Preparation method	Direct colony suspension or growth method	Direct colony suspension

Both methods recommend use of the inoculum within 15 minutes.

Inoculation of media:

Both methods use a similar method for inoculating media & recommend application of discs within 15 minutes.

Antimicrobial disc concentrations:

Concentrations of most antimicrobials in discs are the same for BSAC and EUCAST. The following table shows the antimicrobial discs with different concentrations to those used for BSAC:

Antimicrobial	BSAC content (ug)	EUCAST content (ug)	Comment
<u>Enterobacteriaceae</u>			
Ampicillin/sulbactam	Not available	10/10	
Amoxicillin	10	Not available	Use ampicillin
Piperacillin	75	30	
Piperacillin/tazobactam	75/10	30/6	
Temocillin	30	Not available as yet	
Ticarcillin	Not available	75	
Cefadroxil	Not available	30	
Cefotaxime	30	5	
Ceftazidime	30	10	
Ceftibuten	Not available	30	
Ciprofloxacin	1	5	
Pefloxacin	Not available	5	Cipro screen for

			<i>Salmonella</i> spp.
Levofloxacin	1	5	
Moxifloxacin	1	5	
Nalidixic acid	30	Not available	Use ciprofloxacin for screen instead.
Norfloxacin	2	10	
Netilmicin	Not available	10	
Azithromycin	15	Not available as yet	
Tetracycline	10	Not available as yet	
Fosfomycin	200/50	Not available as yet	
Nitrofurantoin	200	100	
Trimethoprim	2.5	5	
<u><i>Acinetobacter</i> species</u>			
Piperacillin/tazobactam	75/10	Not available	Testing of β -lactams not reliable.
Ciprofloxacin	1	5	
Levofloxacin	Not available	5	
Netilmicin	Not available	10	
Tobramycin	Not available	10	
Co-trimoxazole*	Not available	1.25/23.75	
<u><i>Pseudomonas</i> species</u>			
Piperacillin	75	30	
Piperacillin/tazobactam	75/10	30/6	
Cefepime	Not available	30	
Ceftazidime	30	10	
Ciprofloxacin	1 OR 5	5	
<u><i>Staphylococcus</i> species</u>			
Ampicillin	25	2	
Cefoxitin	10	30	
Oxacillin	1	Not available	Use cefoxitin
Ciprofloxacin	1	5	
Moxifloxacin	1	5	
Norfloxacin	Not available	10	Can be used instead of ciprofloxacin to screen
Neomycin	10	Not available	Perform MIC
Netilmicin	Not available	10	
Azithromycin	15	Not available	Use erythromycin
Clarithromycin	2	Not available	Use erythromycin
Erythromycin	5	15	
Doxycycline	30	Not available	Use tetracycline
Tetracycline	10	30	

Chloramphenicol	10	30	
Trimethoprim (UTI)	2.5	5	
Fosfomycin	200/50	Not available as yet	
Mupirocin	20	200	
Nitrofurantoin (UTI)	200	100	
Rifampicin	2	5	
<u><i>S. pneumoniae</i></u>			
Cefaclor	Not available	30	
Ciprofloxacin	1	5	
Levofloxacin	1	5	
Moxifloxacin	1	5	
Norfloxacin	Not available	10	Can be used instead of ciprofloxacin to screen
Teicoplanin	Not available	30	
Azithromycin	15	Not available	Use erythromycin
Clarithromycin	2	Not available	Same as BSAC
Erythromycin	5	15	
Telithromycin	15	15	Same as BSAC
Minocycline	Not available	30	
Tetracycline	10	30	
Chloramphenicol	10	30	
<u><i>Enterococcus species</i></u>			
Amoxicillin	10	Not available	Use ampicillin
Ampicillin	10	2	
Ciprofloxacin	Not available	5	
Levofloxacin	Not available	5	
Norfloxacin (screen)	Not available	10	
Gentamicin	200	30	
Nitrofurantoin	200	100	
Trimethoprim	2.5	5	
Co-trimoxazole*	Not available	1.25/23.75	
<u>Alpha haemolytic streptococci</u>			
Amoxicillin	2	Not available	Use ampicillin
Ampicillin	Not available	2	
Cefazolin	Not available	30	
Cefepime	Not available	30	Can be used instead of Benzylpenicillin
Ceftriaxone	Not available	30	Can be used instead of Benzylpenicillin
Cefuroxime	Not available	30	Can be used instead of Benzylpenicillin
Erythromycin	5	15	

Linezolid	10	Not available as yet	
<u>Beta haemolytic streptococci</u>			
Levofloxacin	Not available	5	
Moxifloxacin	Not available	5	
Norfloxacin (screen)	Not available	10	
Teicoplanin	Not available	30	
Vancomycin	Not available	5	
Azithromycin	15	Not available	Use erythromycin
Clarithromycin	2	Not available	Use erythromycin
Erythromycin	5	15	
Minocycline	Not available	30	
Tetracycline	10	30	
Chloramphenicol	Not available	30	
Trimethoprim	2.5	5	
Nitrofurantoin	200	100	
Rifampicin	Not available	5	
<u>M. catarrhalis</u>			
Cefaclor	30	Not available	
Cefepime	Not available	30	
Cefixime	Not available	5	
Cefotaxime	Not available	5	
Cefpodoxime	Not available	10	
Ceftriaxone	Not available	30	
Cefuroxime (oral & iv)	5	30	
Doripenem	Not available	10	
Imipenem	Not available	10	
Meropenem	Not available	10	
Ciprofloxacin	1	5	
Levofloxacin	1	5	
Moxifloxacin	1	5	
Clarithromycin	2	Not available	Use erythromycin
Erythromycin	5	15	
Minocycline	Not available	30	
Tetracycline	10	30	
Chloramphenicol	10	30	
<u>H. influenzae</u>			
Ampicillin/sulbactam	Not available	10/10	
Amoxicillin	2	Not available	Use ampicillin
Benzylpenicillin (screen)	Not available	1 unit	Screen for β -lactam resistance
Cefepime	Not available	30	
Cefixime	Not available	5	
Cefpodoxime	Not available	10	

Ceftibuten	Not available	30	
Cefuroxime (oral & iv)	5	30	
Doripenem	Not available	10	
Ciprofloxacin	1	5	
Levofloxacin	1	5	
Moxifloxacin	1	5	
Azithromycin	15	Not available	Use erythromycin
Clarithromycin	5	Not available	Use erythromycin
Erythromycin	5	15	
Minocycline	Not available	30	
Tetracycline	10	30	
Chloramphenicol	10	30	
Rifampicin (prophylaxis)	Not available	5	
<u><i>Pasteurella multocida</i></u>			
Ampicillin	10	2	
Co-amoxiclav*	Not available	2/1	
Cefotaxime	Not available	5	
Ciprofloxacin	Not available	5	
Levofloxacin	Not available	5	
Tetracycline (screen)	Not available	30	
<u><i>Campylobacter species</i></u>			
Ciprofloxacin	1	5	
Nalidixic acid	30	Not available	Use ciprofloxacin
Erythromycin	5	15	
Tetracycline	Not available	30	
<u><i>Corynebacterium species (except C. diphtheriae)</i></u>			
Ciprofloxacin	1	5	
Moxifloxacin	Not available	5	
Gentamicin	Not available	10	
Clindamycin	Not available	2	
Tetracycline	Not available	30	
Linezolid	Not available	10	
Rifampicin	Not available	5	
<u>Gram negative anaerobes</u>			
Co-amoxiclav	20/10	Not available as yet	
Piperacillin/tazobactam	75/10	Not available as yet	
Meropenem	10	Not available as yet	
Clindamycin	2	Not available as yet	
Metronidazole	5	Not available as yet	
<u>Gram positive anaerobes (except C. difficile)</u>			

Benzympenicillin	1 unit	Not available as yet	
Meropenem	10	Not available as yet	
Clindamycin	2	Not available as yet	
Metronidazole	5	Not available as yet	
<u><i>Listeria species</i></u>			
Benzympenicillin	Not available	1 unit	
Ampicillin	Not available	2	
Meropenem	Not available	10	
Erythromycin	Not available	15	
Co-trimoxazole*	Not available	1.25/23.75	

* The following antimicrobial combinations are referred to differently in BSAC & EUCAST:
 Co-amoxiclav (BSAC) & Amoxicillin/clavulanic acid (EUCAST)
 Co-trimoxazole (BSAC) & Trimethoprim-sulfamethoxazole
 Storage & handling of discs is similar for both methods.

Application of discs:

Application of discs is similar for both methods, including recommending incubation within 15 minutes of application.

Incubation conditions:

Below is a table of incubation conditions for BSAC and EUCAST methods.

Organism	BSAC	EUCAST
<i>Enterobacteriaceae</i>	35-37°C in air for 18-20h	35±1°C in air for 16-20h
<i>Pseudomonas</i> spp.	35-37°C in air for 18-20h	35±1°C in air for 16-20h
<i>Acinetobacter</i> spp.	35-37°C in air for 18-20h	35±1°C in air for 16-20h
<i>S. maltophilia</i>	30°C in air for 18-20h	35±1°C in air for 16-20h
<i>Staphylococcus</i> spp. (other than oxacillin/cefoxitin)	35-37°C in air for 18-20h	35±1°C in air for 16-20h
<i>Staphylococcus</i> spp. (using cefoxitin)	35°C in air for 18-20h	35±1°C in air for 16-20h
<i>Staphylococcus</i> spp. (using oxacillin)	30°C in air for 24h	Not applicable, use cefoxitin.
<i>Enterococcus</i> spp.	35-37°C in air for 18-20h 35-37°C in air for 24h for	35±1°C in air for 16-20h 35±1°C in air for 24h for

	glycopeptides	glycopeptides
β -haemolytic streptococci (grps A,B,C,G)	35-37°C in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h
α -haemolytic streptococci (viridans)	35-37°C in 4-6% CO ₂ in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h
<i>S. pneumoniae</i>	35-37°C in 4-6% CO ₂ in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h
<i>H. influenzae</i>	35-37°C in 4-6% CO ₂ in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h
<i>M. catarrhalis</i>	35-37°C in 4-6% CO ₂ in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h
<i>P. multocida</i>	35-37°C in 4-6% CO ₂ in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h
Coryneform bacteria	35-37°C in 4-6% CO ₂ in air for 18-20h	35±1°C in 4-6% CO ₂ in air for 16-20h. Isolates with insufficient growth after 16-20h are reincubated and read at 40-48h.
<i>Campylobacter jejuni / coli</i>	42°C in microaerophilic* conditions for 24h	41±1°C in microaerobic* environment for 24h. Isolates with insufficient growth after 24h are reincubated and read at 40-48h.
<i>Listeria monocytogenes</i>	No method available	35±1°C in 4-6% CO ₂ in air for 16-20h
<i>N. meningitidis / gonorrhoeae</i>	35-37°C in 4-6% CO ₂ in air for 18-20h	No method available
<i>B. fragilis, B. thetaiotaomicron, C. perfringens</i>	35-37°C in 10% CO ₂ /10% H ₂ /80% N ₂ for 18-20h	No method available

* Microaerophilic & microaerobic are both terms for conditions with low oxygen levels.

Control organisms:

The following lists the organisms used to control quality in the BSAC and EUCAST disc diffusion methods.

Organism	BSAC	EUCAST
<i>E. coli</i>	NCTC 10418 / ATCC 10536 or NCTC 12241 / ATCC 25922	NCTC 12241 / ATCC 25922
<i>E. coli</i> (TEM-1 β -lactamase producer)	NCTC 11560	ATCC 35218 / NCTC 11954
<i>K. pneumoniae</i>	Not available	ATCC 700603 / NCTC 13368 ESBL producer
<i>P. aeruginosa</i>	NCTC 10662 / ATCC 25668 or ATCC 27853 / NCTC 12903	ATCC 27853 / NCTC 12903
<i>S. aureus</i>	NCTC 6571 or ATCC 25923 / NCTC 12981	ATCC 29213 / NCTC 12973
<i>S. aureus</i> (MRSA, MecA positive)	NCTC 12493	NCTC 12493
<i>E. faecalis</i>	ATCC 29212 / NCTC 12697	ATCC 29212 / NCTC 12697
<i>E. faecalis</i> (HL gentamicin resistant)	Not available	ATCC 51299 / NCTC 13379 VanB positive
<i>S. pneumoniae</i>	ATCC 49619 / NCTC 12977	ATCC 49619 / NCTC 12977
<i>H. influenzae</i>	NCTC 11931	ATCC 49766 / NCTC 12975
<i>H. influenzae</i> (β -lactamase negative, ampicillin resistant)	ATCC 49247 / NCTC 12699	ATCC 49247 / NCTC 12699
<i>Campylobacter jejuni</i>	Not available	ATCC 33560 / NCTC11351
<i>N. gonorrhoeae</i>	NCTC 12700 / ATCC 49226	Not available
<i>P. multocida</i>	NCTC 8489	Use <i>H. influenzae</i> ATCC 49766 / NCTC 12975
<i>B. fragilis</i>	NCTC 9343 / ATCC 25285	Not available