

## BSAC Methods for Antimicrobial Susceptibility Testing

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Highlighted text indicates a change in content

1. Pages 1-3

<b>Contents</b>		<b>Page</b>
<b>Working Party members</b>		<b>4</b>
<b>Disc Diffusion Method for Antimicrobial Susceptibility Testing</b>		
<b>1. Preparation of plates</b>		<b>5</b>
<b>2. Selection of control organisms</b>		<b>6</b>
<b>3. Preparation of inoculum</b>		<b>7</b>
3.1	Comparison with 0.5 McFarland standard	7
3.1.1	Preparation of the McFarland standard	7
3.1.2	Inoculum preparation by the growth method	8
3.1.3	Inoculum preparation by the direct colony suspension method	8
3.1.4	Adjustment of the organism suspension to the density of the 0.5 McFarland standard	8
3.1.5	Dilution of suspension equivalent to 0.5 McFarland standard in distilled water before inoculation	8
3.2	Photometric standardisation of turbidity of suspension	9
3.3	Direct susceptibility testing of urines and blood cultures	10
<b>4. Inoculation of agar plates</b>		<b>11</b>
<b>5. Antimicrobial discs</b>		<b>11</b>
5.1	Storage and handling of discs	11
5.2	Application of discs	11
<b>6. Incubation</b>		<b>12</b>
6.1	Conditions of incubation	12
<b>7. Measuring zones and interpretation of susceptibility</b>		<b>12</b>
7.1	Acceptable inoculum density	12
7.2	Measuring zones	13
7.3	Use of templates for interpreting susceptibility	13
<b>8. Methicillin/oxacillin/cefoxitin testing of staphylococci</b>		<b>14</b>

8.1	Detection of methicillin/oxacillin resistance in <i>Staphylococcus aureus</i> and coagulase negative staphylococci	14
8.2	Detection of methicillin/oxacillin resistance in <i>Staphylococcus aureus</i> with cefoxitin as test agent	15

## Interpretative tables

Table	MIC and zone breakpoints for:	Page
6	Enterobacteriaceae and <i>Acinetobacter</i> spp.	17
7	<i>Pseudomonas</i> and <i>Stenotrophomonas maltophilia</i>	19
8	Staphylococci	20
9	<i>Streptococcus pneumoniae</i>	22
10	Enterococci	23
11	$\alpha$ -haemolytic streptococci	24
12	$\beta$ -haemolytic streptococci	25
13	<i>Moraxella catarrhalis</i>	26
14	<i>Neisseria gonorrhoeae</i>	27
15	<i>Neisseria meningitidis</i>	28
16	<i>Haemophilus influenzae</i>	29
		Page
17	<i>Pasteurella multocida</i>	30
18	<i>Campylobacter</i> spp.	31
19	Coryneform organisms	32
20	<i>Bacteroides fragilis</i> , <i>Bacteroides thetaiotaomicron</i> and <i>Clostridium perfringens</i>	33
21	Urinary tract infections (Gram-negative rods)	34
22	Urinary tract infections (Gram-positive cocci)	35

## Appendices

1	Advice on testing the susceptibility to co-trimoxazole	36
2	Efficacy of cefaclor in the treatment of respiratory infections caused by <i>Haemophilus influenzae</i>	37

Acknowledgment	38
References	38

## Additional information

1	Susceptibility testing of <i>Helicobacter pylori</i>	39
2	Susceptibility testing of <i>Brucella</i> species	39
3	Susceptibility testing of <i>Legionella</i> species	39
4	Susceptibility testing of topical antibiotics	40
5	Development of MIC and zone diameter breakpoints	40

## Control of disc diffusion antimicrobial susceptibility testing

1	Control strains	41
2	Maintenance of control strains	41
3	Calculation of control ranges for disc diffusion	41
4	Frequency of testing	41
5	Use of control data to monitor the performance of susceptibility testing	41
6	Recognition of atypical results	42
7	Investigation of possible sources of error	42
8	Reporting susceptibility results when controls indicate problems	43

Table	Acceptable ranges for control strains for:	
2	Iso-Sensitest agar incubated at 35-37°C in air for 18-20h	44
3	Iso-Sensitest agar supplemented with 5% defibrinated horse blood, with or without the addition of NAD, incubated at 35-37°C in air for 18-20h	46
4	Detection of methicillin/oxacillin/cefoxitin resistance in staphylococci	46
5	Iso-Sensitest agar supplemented with 5% defibrinated horse blood, with or without the addition of NAD, incubated at 35-37°C in 10% CO <sub>2</sub> /10% H <sub>2</sub> /80% N <sub>2</sub> for 18-20 h	46
6	Iso-Sensitest agar supplemented with 5% defibrinated horse blood, with or without the addition of NAD, incubated at 35-37°C in 4-6% CO <sub>2</sub> for 18-20 h	47

		Page
Control of MIC determinations		
Table	Target MICs for:	
7	<i>Haemophilus influenzae</i> , <i>Enterococcus faecalis</i> , <i>Streptococcus pneumoniae</i> , <i>Bacteroides fragilis</i> and <i>Neisseria gonorrhoeae</i>	48
8	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i>	50
9	<i>Pasteurella multocida</i>	52
10	<i>Bacteroides fragilis</i> , <i>Bacteroides thetaiotaomicron</i> and <i>Clostridium perfringens</i>	52
References		53
Suppliers		54
Useful web sites		55

2. Page 5.

## 1. Preparation of plates

- 1.1 Prepare Iso-Sensitest agar (ISA) (see list of suppliers) or media shown to have the same performance as ISA, according to the manufacturer's instructions. Supplement media for fastidious organisms with 5% defibrinated horse blood or 5% defibrinated horse blood and 20 mg/L β-nicotinamide adenine dinucleotide (NAD) as indicated in Table 1. Use Columbia agar with 2% NaCl for methicillin/oxacillin susceptibility testing of staphylococci.

Table 1: Media and supplementation for antimicrobial susceptibility testing of different groups of organisms

Organisms	Medium
<i>Bacteroides fragilis</i> , <i>Bacteroides thetaiotaomicron</i> , <i>Clostridium perfringens</i>	ISA + 5% defibrinated horse blood + 20 mg/L NAD

Organisms	Medium
<i>Campylobacter spp.</i>	ISA + 5% defibrinated horse blood <sup>2</sup>
Coryneform organisms	ISA + 5% defibrinated horse blood + 20 mg/L NAD

3. Page 6.

## 2. Selection of control organisms

2.1 The performance of the tests should be monitored by the use of appropriate control strains (see section on control of antimicrobial susceptibility testing). The control strains listed (Table 2) include susceptible strains that have been chosen to monitor test performance and resistant strains that can be used to confirm that the method will detect a mechanism of resistance.

2.2 Store control strains at  $-70^{\circ}\text{C}$  on beads in glycerol broth. Non-fastidious organisms may be stored at  $-20^{\circ}\text{C}$ . Two vials of each control strain should be stored, one for an 'in-use' supply, the other for archiving.

2.3 Every week subculture a bead from the 'in-use' vial on to appropriate non-selective media and check for purity. From this pure culture, prepare one subculture on each of the following 5 days. For fastidious organisms that will not survive on plates for 5/6 days, subculture the strain daily for no more than 6 days.

4. Page 7.

Table 2: Control strains for antimicrobial susceptibility testing

Organism	Strain		Characteristics
	Either	Or	
<i>Escherichia coli</i>	NCTC 12241 (ATCC 25922)	NCTC 10418	Susceptible
<i>Escherichia coli</i>	NCTC 11560		TEM-1 $\beta$ -lactamase-producer
<i>Staphylococcus aureus</i>	NCTC 12981 (ATCC 25923)	NCTC 6571	Susceptible
<i>Staphylococcus aureus</i>	NCTC 12493		MecA-positive, methicillin resistant
<i>Pseudomonas aeruginosa</i>	NCTC 12934 (ATCC 27853)	NCTC 10662	Susceptible
<i>Enterococcus faecalis</i>	NCTC 12697 (ATCC 29212)		Susceptible
<i>Haemophilus influenzae</i>	NCTC 11931		Susceptible
<i>Haemophilus influenzae</i>	NCTC 12699 (ATCC 49247)		Resistant to $\beta$ -lactams ( $\beta$ -lactamase-negative)
<i>Streptococcus pneumoniae</i>	NCTC 12977 (ATCC 49619)		Low-level resistant to penicillin
<i>Neisseria gonorrhoeae</i>	NCTC 12700 (ATCC 49226)		Low-level resistant to penicillin
<i>Pasteurella multocida</i>	NCTC 8489		Susceptible

Organism	Strain		Characteristics
	Either	Or	
<i>Bacteroides fragilis</i>	NCTC 9343 (ATCC 25285)		Susceptible
<i>Bacteroides thetaiotaomicron</i>		ATCC 29741	Susceptible
<i>Clostridium perfringens</i>	NCTC 8359 (ATCC 12915)		Susceptible

### 3. Preparation of inoculum

The inoculum should give semi-confluent growth of colonies after overnight incubation.

#### 5. Page 8.

##### 3.1.1 Preparation of the 0.5 McFarland standard

Add 0.5 mL of 0.048 M BaCl<sub>2</sub> (1.17% w/v BaCl<sub>2</sub> · 2H<sub>2</sub>O) to 99.5 mL of 0.18 M H<sub>2</sub>SO<sub>4</sub> (1% w/v) with constant stirring. Thoroughly mix the suspension to ensure that it is even. Using matched cuvettes with a 1 cm light path and water as a blank standard, measure the absorbance in a spectrophotometer at a wavelength of 625 nm. The acceptable absorbance range for the standard is 0.08-0.13. Distribute the standard into screw-cap tubes of the same size and volume as those used in growing the broth cultures. Seal the tubes tightly to prevent loss by evaporation. Store protected from light at room temperature. Vigorously agitate the turbidity standard on a vortex mixer before use. Standards may be stored for up to six months, after which time they should be discarded. Prepared standards can be purchased (See list of suppliers), but commercial standards should be checked to ensure that absorbance is within the acceptable range as indicated above.

3.1.3 *Inoculum preparation by the direct colony suspension method* (the method of choice for fastidious organisms, i.e. *Haemophilus* spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, α and β-haemolytic streptococci, *Clostridium perfringens*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Campylobacter* spp., *Pasteurella multocida* and Coryneform organisms).

**NB.** With some organisms production of an even suspension of the required turbidity is difficult and growth in broth, if possible, is a more satisfactory option.

##### 3.1.5 Dilution of suspension in distilled water before inoculation

Dilute the suspension (density adjusted to that of a 0.5 McFarland standard) in distilled water as indicated in Table 3.

#### 6. Page 9.

6. Table 3: Dilution of the suspension (density adjusted to that of a 0.5 McFarland standard) in distilled water

Dilute 1:100	Dilute 1:10	No dilution
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Dilute 1:100	Dilute 1:10	No dilution
<i>β</i> -Haemolytic streptococci	Staphylococci	<i>Neisseria gonorrhoeae</i>
Enterococci	<i>Serratia</i> spp.	<i>Campylobacter</i> spp.
Enterobacteriaceae	<i>Streptococcus pneumoniae</i>	
<i>Pseudomonas</i> spp.	<i>Neisseria meningitidis</i>	
<i>Stenotrophomonas maltophilia</i>	<i>Moraxella catarrhalis</i>	
<i>Acinetobacter</i> spp.	<i>α</i> -haemolytic streptococci	
<i>Haemophilus</i> spp.	<i>Clostridium perfringens</i>	
<i>Pasteurella multocida</i>	Coryneform organisms	
<i>Bacteroides fragilis</i>		
<i>Bacteroides thetaiotaomicron</i>		

**NB.** These suspensions should be used within 15 min of preparation.

7. page 10 and 11.

Table 4: Dilution of suspensions of test organisms according to absorbance reading

Organisms	Absorbance reading at 500 nm	Volume ( $\mu$ L) to transfer to 5 mL sterile distilled water
Enterobacteriaceae	0.01 - 0.05	250
Enterococci	>0.05 - 0.1	125
<i>Pseudomonas</i> spp.	>0.1 - 0.3	40
Staphylococci	>0.3 - 0.6	20
	>0.6 - 1.0	10
<i>Haemophilus</i> spp.	0.01 - 0.05	500
Streptococci	>0.05 - 0.1	250
Miscellaneous fastidious Organisms	>0.1 - 0.3	125
	>0.3 - 0.6	80
	>0.6 - 1.0	40

### 3.3 Direct antimicrobial susceptibility testing of urine specimens and blood cultures

Direct susceptibility testing is not advocated as the control of inoculum is very difficult. Direct testing is, however, undertaken in many laboratories in order to provide more rapid test results. The following methods have been recommended by laboratories that use the BSAC method and will achieve the correct inoculum size for a reasonable proportion of infected urines and blood cultures. If the inoculum is not correct (i.e. growth is not semi-confluent) or the culture is mixed, the test must be repeated.

#### 3.3.1 Urine specimens

##### 3.3.1.1 Method 1

Thoroughly mix the urine specimen, then place a 10  $\mu$ L loop of urine in the centre of the susceptibility plate and spread evenly with a dry swab.

##### 3.3.1.2 Method 2

Thoroughly mix the urine specimen, then dip a sterile cotton-wool swab in the urine and remove excess by turning the swab against the inside of the container. Use the swab to make a cross in the centre of the susceptibility plate and spread evenly with another sterile dry swab. If only small

numbers of organisms are seen in microscopy, the initial cotton-wool swab may be used to inoculate and spread the susceptibility plate.

### 3.3.2 Positive blood cultures

The method depends on the Gram reaction of the infecting organism.

#### 3.3.2.1 Gram-negative bacilli.

Using a venting needle, place one drop of the blood culture in 5 mL of sterile water, then dip a sterile cotton-wool swab in the suspension and remove excess by turning the swab against the inside of the container. Use the swab to spread the inoculum evenly over the surface of the susceptibility plate.

#### 3.3.2.2 Gram-positive organisms.

It is not always possible accurately to predict the genera of Gram-positive organisms from the Gram's stain. However, careful observation of the morphology, coupled with clinical information, should make an "educated guess" correct most of the time.

Staphylococci and enterococci.

Using a venting needle, place three drops of the blood culture in 5 mL of sterile water, then dip a sterile cotton-wool swab in the suspension and remove excess by turning the swab against the inside of the container. Use the swab to spread the inoculum evenly over the surface of the susceptibility plate.

Pneumococci, "viridans" streptococci and diphtheroids.

Using a venting needle, place one drop of the blood culture in the centre of a susceptibility plate, and spread the inoculum evenly over the surface of the plate.

## 5. Antimicrobial discs

Refer to interpretation tables 6-23 for the appropriate disc contents for the organisms tested.

### 5.1 Storage and handling of discs.

Loss of potency of agents in discs will result in reduced zones of inhibition. To avoid loss of potency due to inadequate handling of discs the following are recommended:

5.1.1 Store discs in sealed containers with a desiccant and protected from light (this is particularly important for some light-susceptible agents such as metronidazole, chloramphenicol and the quinolones).

5.1.2 Store stocks at -20°C except for drugs known to be unstable at this temperature. If this is not possible, store discs at <8°C.

5.1.3 Store working supplies of discs at <8°C.

5.1.4 To prevent condensation, allow discs to warm to room temperature before opening containers.

5.1.5 Store disc dispensers in sealed containers with an indicating desiccant.

5.1.6 Discard discs on the expiry date shown on the side of the container.

Table 5: Incubation conditions for antimicrobial susceptibility tests on various organisms

Organisms	Incubation conditions
Enterobacteriaceae	35-37°C in air for 18-20 h
<i>Pseudomonas</i> spp.	35-37°C in air for 18-20 h
<i>Stenotrophomonas maltophilia</i>	30°C in air for 18-20 h
Staphylococci (other than methicillin/oxacillin/cefoxitin)	35-37°C in air for 18-20 h
<i>Staphylococcus aureus</i> using cefoxitin for the detection of methicillin/oxacillin/cefoxitin resistance	35°C in air for 18-20 h
Staphylococci using methicillin or oxacillin to detect resistance	30°C in air for 24 h
<i>Moraxella catarrhalis</i>	35-37°C in air for 18-20 h
$\alpha$ -Haemolytic streptococci	35-37°C in 4-6% CO <sub>2</sub> in air for 18-20 h
$\beta$ -Haemolytic streptococci	35-37°C in air for 18-20 h
Enterococci	35-37°C in air for 24 h <sup>1</sup>
<i>Neisseria meningitidis</i>	35-37°C in 4-6 % CO <sub>2</sub> in air for 18-20 h
<i>Streptococcus pneumoniae</i>	35-37°C in 4-6 % CO <sub>2</sub> in air for 18-20 h
<i>Haemophilus</i> spp.	35-37°C in 4-6 % CO <sub>2</sub> in air for 18-20 h
<i>Neisseria gonorrhoeae</i>	35-37°C in 4-6 % CO <sub>2</sub> in air for 18-20 h
<i>Pasteurella multocida</i>	35-37°C in 4- 6% CO <sub>2</sub> in air for 18-20 h
Coryneform organisms	35-37°C in 4-6% CO <sub>2</sub> in air for 18-20 h
<i>Campylobacter</i> spp.	35-37°C in air for 18-20 h
<i>Bacteroides fragilis</i> , <i>Bacteroides thetaiotaomicron</i> , <i>Clostridium perfringens</i>	35-37°C in 10% CO <sub>2</sub> /10% H <sub>2</sub> /80% N <sub>2</sub> for 18-20 h( Anaerobic cabinet or jar)

## 7.1 Acceptable inoculum density

The inoculum should give semi-confluent growth of colonies on the susceptibility plate, within the range illustrated in Figure 1.

## 9. Page 13

10.

Figure 1: Acceptable inoculum density range for a Gram-negative rod

## 7.2 Measuring zones

7.2.1 Measure the diameters of zones of inhibition to the nearest millimetre (zone edge should be taken as the point of inhibition as judged by the naked eye) with a ruler, callipers or an automated zone reader.

7.2.2 Tiny colonies at the edge of the zone, films of growth as a result of the swarming of *Proteus* spp. and slight growth within sulphonamide or trimethoprim zones should be ignored.

7.2.3 Colonies growing within the zone of inhibition should be subcultured and identified and the test repeated if necessary.

7.2.4 When using cefoxitin for the detection of methicillin/oxacillin/cefoxitin resistance in *S. aureus*, measure the obvious zone, taking care to examine zones carefully in good

light to detect minute colonies that may be present within the zone of inhibition (see Figure 3)

7.2.5 Confirm that the zone of inhibition for the control strain falls within the acceptable ranges in Tables 20-23 before interpreting the test (see section on control of the disc diffusion method).

### 7.3 Use of templates for interpreting zone diameters

A template may be used for interpreting zone diameters (see Figure 2). A program for preparing templates is available from the BSAC (<http://www.bsac.org.uk>).

The test plate is placed over the template and the zones of inhibition are examined in relationship to the template zones. If the zone of inhibition of the test strain is within the area marked with an 'R', the organism is resistant. If the zone of inhibition is equal to or larger than the marked area, the organism is susceptible.

10. Page 14

Figure 2: Template for interpreting zone diameters

## 8. Methicillin/oxacillin/cefoxitin testing of staphylococci

### 8.1.1 Medium

Prepare Columbia (See list of suppliers) or Mueller-Hinton agar (See list of suppliers) following the manufacturer's instructions and add 2% NaCl. After

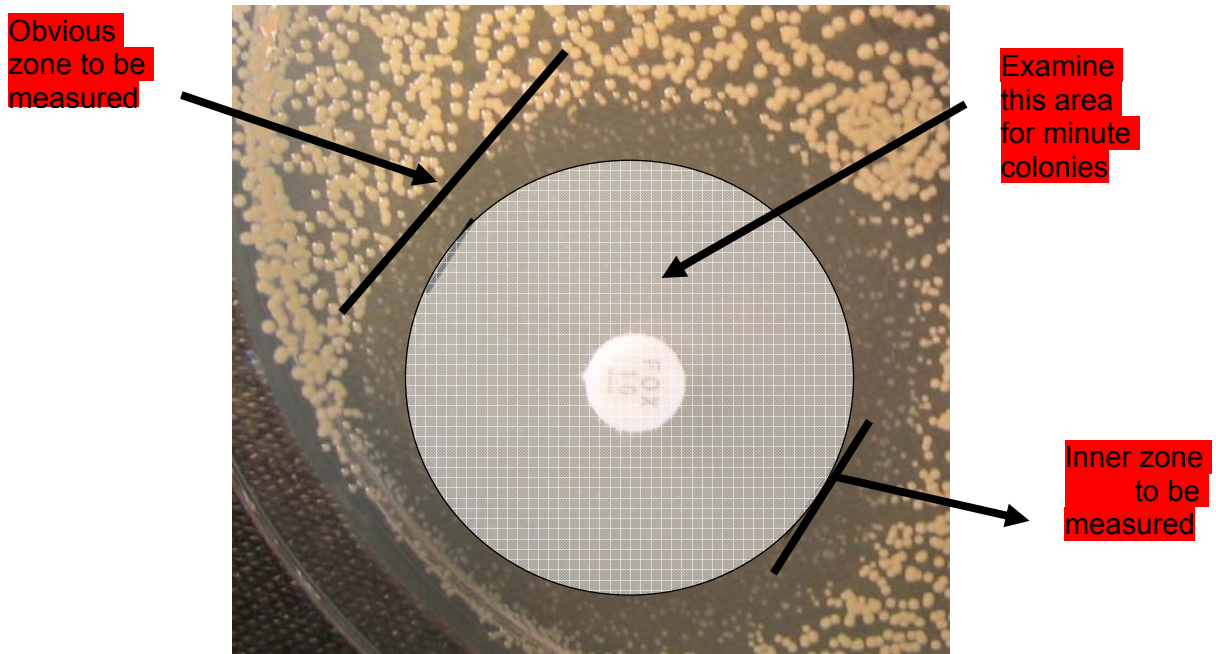
### 8.1.3 Control

Susceptible control strains (*Staphylococcus aureus* ATCC 25923 or NCTC 6571) test the reliability of disc content.

*Staphylococcus aureus* NCTC 12493 is a methicillin resistant strain and is used to check that the test will detect resistant organisms (although no strain can be representative of all the MRSA types in terms of their response to changes in test conditions).

Examine zones carefully in good light to detect colonies, which may be minute, in zones. If there is suspicion that the colonies growing within zones are contaminants they should be identified and the isolate re-tested for resistance to cefoxitin if necessary.

Figure 3: Reading cefoxitin zones of inhibition with *Staphylococcus aureus*



### 8.2.7 Interpretation

Susceptible =  $\geq 22$  mm diameter, resistant =  $\leq 21$  mm diameter.

**NB.** Hyper-producers of  $\beta$ -lactamase give zones within the ranges of the susceptible population.

12. Page 17

Table 6: MIC and zone breakpoints for Enterobacteriaceae (including *Salmonella* and *Shigella* spp.) and *Acinetobacter* spp.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg)	R ≤	I	S ≥
Cefpodoxime <sup>5,6</sup>	1	-	1	10	19	-	20
Ertapenem	2	-	2	10	27	-	28

13. Page 18

The identification of Enterobacteriaceae to species level is essential for the application of expert rules for the interpretation of susceptibility. Species that typically have inducible AmpC enzymes (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Morganella morganii* and *Providencia* spp.) readily mutate to stably derepressed AmpC production during treatment (in 20% cases with *Enterobacter* spp), conferring resistance to all first, second and third generation cephalosporins.

<sup>6</sup> Organisms with cefpodoxime zone diameters of < 20 mm have a substantive mechanism of resistance. Organisms with zone diameters of 21-25 mm are uncommonly ESBL-producers and may require further investigation.

<sup>9</sup> For ciprofloxacin, there is clinical evidence to indicate a poor response in systemic infections caused by *Salmonella* spp. with reduced susceptibility to fluoroquinolones (ciprofloxacin MICs 0.125-1 mg/L). This reduced susceptibility is most reliably detected with nalidixic acid 30 µg discs as isolates with reduced susceptibility show no zone of inhibition.

<sup>13</sup> Individual aminoglycoside agents must be tested; susceptibility to other aminoglycosides cannot be inferred from the gentamicin result and vice versa.

<sup>14</sup> *Proteus* spp. and *Morganella morganii* are considered poor targets for imipenem.

14. Page 19.

Table 7: MIC and zone diameter breakpoints for *Pseudomonas* spp. and *Stenotrophomonas maltophilia*<sup>1</sup>.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg)	R ≤	I	S ≥
Co-trimoxazole <sup>1,2</sup>	32	-	32	25	19	-	20

<sup>2</sup> MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

15. Page 20

<sup>1</sup> Staphylococci exhibiting resistance to methicillin/oxacillin/cefoxitin should be regarded as resistant to other penicillins, cephalosporins, carbapenems and combinations of β-lactam and β-lactamase inhibitors. Some hyper-producers of β-lactamase give zones within the range of 7-14 mm and if possible, should be checked by a PCR method for *mecA* or a latex agglutination test for PBP2a. Increase in methicillin/oxacillin zone size in the presence of clavulanic acid is not a reliable test for hyper-producers of β-lactamase as zones of inhibition with some MRSA also increase in the presence of clavulanic acid. Rarely, hyper-producers of β-lactamase give no zone in this test and would therefore not be distinguished

from MRSA.

16. Page 21

<sup>3</sup> Organisms that appear resistant to erythromycin, but susceptible to clindamycin should be checked for the presence of inducible resistance (see [www.bsac.org.uk/Susceptibility Testing/BSAC Standardized Disc Susceptibility Method/Additional Methods](http://www.bsac.org.uk/Susceptibility_Testing/BSAC_Standardized_Disc_Susceptibility_Method/Additional_Methods)).

<sup>4</sup> For advice on testing susceptibility to co-trimoxazole see Appendix 1.

<sup>5</sup> MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

<sup>14</sup> Glycopeptide intermediate *Staphylococcus aureus* (GISA) cannot be detected by this method or any other disc diffusion method. The Etest “macro-method” may be used to screen for GISA and GISA with heterogenous resistance to vancomycin (hetero-GISA) but positive results require confirmation. Population analysis is the most reliable method for confirming resistance and for distinguishing susceptible, hetero-GISA and GISA isolates. If, on clinical grounds, resistance to vancomycin is suspected, it is recommended that the organism be sent to a specialist laboratory, such as Southmead Hospital in Bristol or the Antibiotic Resistance Monitoring and Reference Laboratory at Colindale for further investigation.

17. Page 22

<sup>4</sup> MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

18. Page 23

Table 10: MIC and zone diameter breakpoints for enterococci.

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg)	Interpretation of zone diameters (mm)		
	R >	I	S ≤		R ≤	I	S ≥
Gentamicin <sup>1</sup>	128	-	128	200	14	-	15

19. Page 25

20. Table 12: MIC and zone diameter breakpoints for β-haemolytic streptococci

<sup>3</sup> MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

20. Page 26

Table 13: MIC and zone diameter breakpoints for *Moraxella catarrhalis*

<sup>5</sup> MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

21. Page 27

Table 14: MIC and zone diameter breakpoints for *Neisseria gonorrhoeae*

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg unless stated)	R ≤	I	S ≥
Nalidixic acid <sup>2</sup>	-	-	-	30	<b>6</b>	<b>7-31</b>	<b>32</b>

The information in bold is tentative. Breakpoints will remain tentative for one year from when first published.

<sup>2</sup> Quinolone resistance is generally reliably detected with nalidixic acid, however there have been a few isolates that are resistant to ciprofloxacin yet susceptible to nalidixic acid in disc diffusion tests. The mechanism of resistance and the prevalence of these isolates in the UK is still under investigation. Isolates with reduced susceptibility to fluoroquinolones normally have no zone of inhibition with a 30 µg nalidixic acid disc. For organisms with nalidixic acid zone diameters 7-31 mm a ciprofloxacin MIC should be determined if the patient is to be treated with this agent.

22. Page 28

Table 15: MIC and zone diameter breakpoints for *Neisseria meningitidis*.

**NB. *Neisseria meningitidis* is a category 3 pathogen. Consequently suspension and dilution of organisms and inoculation of plates for susceptibility tests must be carried out in a class 1 safety cabinet.**

23. Page 29

Table 16: MIC and zone diameter breakpoints for *Haemophilus influenzae*.

<sup>7</sup> MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

24. Page 31

Table 18: MIC and zone diameter breakpoints for *Campylobacter* spp.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg unless stated)	R ≤	I	S ≥
Erythromycin	0.5	-	0.5	5	19	-	20
Ciprofloxacin <sup>1</sup>	1	-	0.5	1	17	-	18

<sup>1</sup> Quinolone resistance is most reliably detected with nalidixic acid discs.

25. Page 32

Table 19: MIC and zone diameter breakpoints for Coryneform organisms.

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg unless stated)	Interpretation of zone diameters (mm)		
	R >	I	S ≤		R ≤	I	S ≥
Ciprofloxacin	<b>1</b>	-	<b>0.5</b>	<b>1</b>	<b>11</b>	<b>12-16</b>	<b>17</b>
Penicillin	<b>0.12</b>	-	<b>0.12</b>	<b>1 unit</b>	<b>19</b>	-	<b>20</b>
Vancomycin	<b>8</b>	-	<b>4</b>	<b>5</b>	<b>19</b>	-	<b>20</b>

The information in bold is tentative. Breakpoints will remain tentative for one year from when first published.

26. Page 33

Table 20: MIC and zone diameter breakpoints for *Bacteroides fragilis*, *Bacteroides thetaiotaomicron* and *Clostridium perfringens*.

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg unless stated)	Interpretation of zone diameters (mm)		
	R >	I	S ≤		R ≤	I	S ≥
Metronidazole	<b>8</b>	-	<b>8</b>	<b>5</b>	<b>17</b>	-	<b>18</b>

The information in bold is tentative. Breakpoints will remain tentative for one year from when first published.

27. Page 34

Table 21: MIC and zone diameter breakpoints for Gram-negative rods isolated from urinary tract infections<sup>1-4</sup>.

NB. These recommendations are for organisms associated with uncomplicated urinary tract infections. For complicated infections systemic recommendations should be used.

28. Page 35

Table 22: MIC and zone diameter breakpoints for Gram-positive cocci isolated from urinary tract infections<sup>1,2</sup>.

NB. These recommendations are for organisms associated with uncomplicated urinary tract infections. For complicated infections and infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are associated with more serious infections, systemic recommendations should be used.

## Appendix 2: Efficacy of cefaclor in the treatment of respiratory infections caused by *Haemophilus influenzae*

Concerns have been expressed, particularly by laboratories moving from Stokes' method to the BSAC disc diffusion method, about the interpretation of susceptibility of *Haemophilus influenzae* to cefaclor. When using Stokes' method the majority of isolates appeared susceptible; but with the BSAC disc diffusion method most isolates are now reported resistant. The following comments explain the BSAC rationale for interpretation of cefaclor susceptibility.

### Cefaclor pharmacokinetics

Cefaclor is dosed at 250-500 mg TDS po: 250 mg TDS is probably the most common dose but data is absent to confirm this. The expected  $C_{max}$  for 250 mg is 5-10 mg/L and 10-20 mg/l for 500 mg; the half life is 1 h; drug concentration in blood is <1 mg/L at 4 h and the protein binding is 25-50%. Tissue penetration is similar to other  $\beta$ -lactams.

### Cefaclor potency against *Haemophilus influenzae*

Data from the BSAC surveillance programme 2003-2004 (n= 899) indicates that the cefaclor MIC range is 0.12-128 mg/L; MIC<sub>50</sub> 2 mg/L; MIC<sub>90</sub> 8 mg/L.

### Pharmacodynamics

An average patient with an *Haemophilus influenzae* infection will have a free drug Time>MIC of 25% with 250 mg dosing and 37% with 500 mg dosing. A conservative Time>MIC target for cephalosporins in community practice is 40-50%, but this is not achieved with cefaclor. Therefore, it is likely that cefaclor will have at best borderline activity against *Haemophilus influenzae*.

### Conclusion

The pharmacodynamic data indicate that cefaclor has borderline activity against *Haemophilus influenzae*, even for community use. The outcome of infection will be difficult to predict and susceptibility testing is likely to be of limited value.

## 5. Development of MIC and zone diameter breakpoints

All breakpoints are subject to review in the light of additional data and any data relating to breakpoints, control zone ranges or any other aspect of antimicrobial susceptibility testing would be welcome (contact the Working Party secretary or any member listed at the front of this document).

The BSAC is part of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and is actively involved in the process of harmonization of MIC breakpoints in Europe. This process will undoubtedly lead to some small breakpoint adjustments, and these will be incorporated into the BSAC method as European breakpoints are agreed.

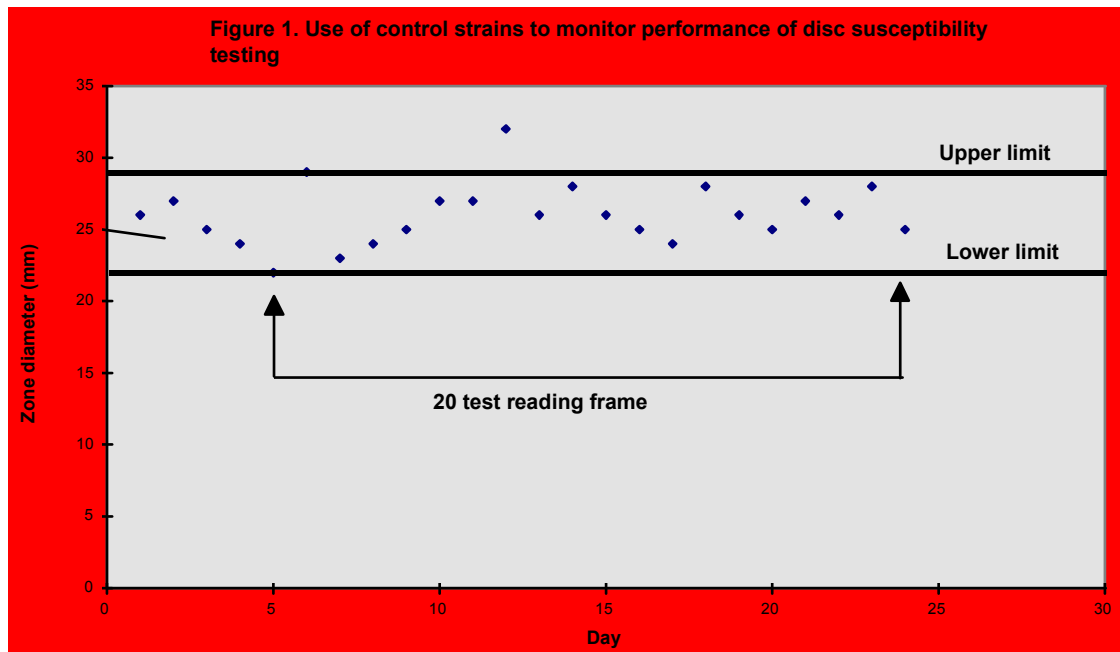
The BSAC has a mechanism to modify and publish changes to breakpoints on an annual basis via the BSAC www site ([www.bsac.org.uk](http://www.bsac.org.uk)). Any changes will be dated.

*Ad hoc* modifications to breakpoints by users are not acceptable.+

## 5. Use of control data to monitor the performance of disc diffusion tests

Use a reading frame of 20 consecutive results (remove the oldest result when adding a new one to make a total of 20) as illustrated in Figure 1. Testing is acceptable if no more than 1 in every 20 results is outside the limits of acceptability. If 2 or more results fall out of the acceptable range this requires immediate investigation.

Look for trends within the limits of acceptability e.g. tendency for zones to be at the limits of acceptability; tendency for zones to be consistently above or below the mean; gradual drift in zone diameters. Quality Assurance will often pick up trends before the controls go out of range.



A free, supported QC programme is available from the following website:  
<http://www.thehealthcarenet.com/shareware.htm>

## 6. Recognition of atypical results for clinical isolates

Atypical results with clinical isolates may indicate problems in testing that may or may not be reflected in zone diameters with control strains.

An organism with inherent resistance appears susceptible e.g. *Proteus* spp. susceptible to colistin or nitrofurantoin.

Resistance is seen in an organism when resistance has previously not been observed, e.g. penicillin resistance in Group A streptococci.

Resistance is seen in an organism when resistance is rare or has not been seen locally, e.g. vancomycin resistance in *Staphylococcus aureus*.

Incompatible susceptibilities are reported, e.g. a methicillin resistant staphylococcus reported susceptible to a  $\beta$ -lactam antibiotic.

In order to apply such rules related to atypical results it is useful to install an 'expert' system for laboratory reporting to avoid erroneous interpretation,

## 7. Investigation of possible sources of error

If the control values are found to be outside acceptable limits on more than one occasion during a reading frame of twenty tests, investigation into the possible source of error is required. Possible problem areas are indicated in table 1.

Table 1: Potential sources of error in disc diffusion antimicrobial susceptibility testing.

Possible source of error	Detail to check
Test conditions	Excessive pre-incubation before discs applied Excessive pre-diffusion before plates incubated Incorrect incubation temperature Incorrect incubation atmosphere Incorrect incubation time Inadequate illumination of plates when reading Incorrect reading of zone edges
Medium	Required susceptibility testing agar not used Not prepared as required by the manufacturer's instructions Batch to batch variation Antagonists present (eg with sulphonamides and trimethoprim) Incorrect pH Incorrect divalent cation concentration Incorrect depth of agar plates Agar plates not level Expiry date exceeded
Antimicrobial discs	Wrong agent or content used Labile agent possibly deteriorated Light sensitive agent left in light Incorrect storage leading to deterioration Disc containers opened before reaching room temperature Incorrect labelling of disc dispensers Expiry date exceeded
Control strains	Contamination Mutation Incorrect inoculum density Uneven inoculation Old culture used

## 8. Reporting susceptibility results when controls indicate problems

Microbiologists must use a pragmatic approach, as results from repeat testing are not available on the same day. If results with control strains are out of range the implications for test results need to be assessed.

**Control results out of range**

If control zones are below range but test results are susceptible, or control zones are above range but test results are resistant, investigate possible sources of error but report the test results. Otherwise it may be necessary to suppress reports on affected agents, investigate and retest.

**Atypical results**

If results are atypical with clinical isolates, the purity of the isolate and identification should be confirmed and the susceptibility repeated. Suppress the results for individual agents and retest.

**32. Page 45**

Antimicrobial agent	Disc content ( $\mu\text{g}$ unless stated)	Staphylococcus aureus	
		NCTC 6571	ATCC 25923
<b>Mupirocin</b>	<b>20</b>	<b>30-38</b>	<b>27-35</b>

Table 4: Acceptable zone diameter ranges for control strains for detection of methicillin/oxacillin/cefoxitin resistance in staphylococci.

Antimicrobial agent	Medium	Disc content (µg)	<i>Staphylococcus aureus</i>		
			NCTC 6571	ATCC 25923	NCTC 12493 <sup>a</sup>
Cefoxitin	ISA	10	26-31	24-29	13-19

<sup>a</sup> Methicillin/oxacillin/cefoxitin- resistant strain.

Table 5: Acceptable zone diameter ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood and NAD, plates incubated at 35-37°C in 10% CO<sub>2</sub>/10% H<sub>2</sub>/80% N<sub>2</sub> for 18-20 h.

Antimicrobial agent	Disc content (µg unless stated)	<i>Bacteroides fragilis</i> NCTC 9343	<i>Bacteroides thetaiotaomicron</i> ATCC 29741	<i>Clostridium perfringens</i> NCTC 8359
Metronidazole	5	34-43	26-40	11-23

34. Page 48

### 9. Control of MIC determination

Tables 7-10 provide target MIC (mg/L) values for recommended control strains by BSAC methodology.<sup>1,2</sup> MICs should be within one two-fold dilution of the target values.

35. Page 52

Table 10: Target MICs (mg/L) for anaerobic control strains by BSAC methods on Iso-Sensitest agar supplemented with 5% defibrinated horse blood and 20 mg/L NAD

Antimicrobial agent	<i>Bacteroides fragilis</i> NCTC 9343	<i>Bacteroides thetaiotaomicron</i> ATCC 29741	<i>Clostridium perfringens</i> NCTC 8359
Metronidazole	0.5	4	8

36. Page 54

### Suppliers

Reagent	Suppliers (others may be available)
ISA	CM471, Oxoid, Basingstoke, UK
Columbia agar	CM331, Oxoid, Basingstoke, UK
Mueller Hinton agar	CM337, Oxoid, Basingstoke, UK
NAD	Mast Group, Merseyside, UK
McFarland turbidity standards	bioMérieux, Basingstoke, UK
Control strains	NCTC, Colindale, London Oxoid, Basingstoke, UK Mast Laboratories, Merseyside, UK Becton Dickinson, Oxford, UK TCS Biosciences Ltd. Buckingham, UK

37. Page 55

### Useful web sites

**EUCAST**      European Committee on Antimicrobial      <http://www.eucast.org>  
Susceptibility Testing