BSAC Methods for Antimicrobial Susceptibility Testing

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Disc Diffusion Method for Antimicrobial Susceptibility Testing

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
Enterobacteriaceae	ISA
Pseudomonas spp.	ISA
Stenotrophomonas maltophilia	ISA
Staphylococci (tests other than methicillin/oxacillin)	ISA
Staphylococcus aureus (tests using cefoxitin to detect methicillin/oxacillin/cefoxitin resistance)	ISA
Staphylococci (tests using methicillin or oxacillin for the detection of methicillin/oxacillin/cefoxitin resistance)	Columbia agar (*see suppliers*) with 2% NaCl[1]
Enterococci	ISA
Streptococcus pneumoniae	ISA + 5% defibrinated horse blood[2]
α-Haemolytic streptococci	ISA + 5% defibrinated horse blood + 20 mg/L NAD
β-Haemolytic streptococci	ISA + 5% defibrinated horse blood[2]
Moraxella catarrhalis	ISA + 5% defibrinated horse blood[2]
Haemophilus spp.	ISA + 5% defibrinated horse blood + 20 mg/L NAD
Neisseria gonorrhoeae	ISA + 5% defibrinated horse blood[2]
Neisseria meningitidis	ISA + 5% defibrinated horse blood[2]
Pasteurella multocida	ISA + 5% defibrinated horse blood + 20 mg/L NAD
Bacteroides fragilis, Bacteroides thetaiotaomicron, Clostridium perfringens	ISA + 5% defibrinated horse blood + 20 mg/L NAD
Campylobacter spp.	ISA + 5% defibrinated horse blood[2]
Coryneform organisms	ISA + 5% defibrinated horse blood + 20 mg/L NAD

[1]See Section 8.
[2]ISA supplemented with 5% defibrinated horse blood + 20mg/L NAD may be used.

1.2 Pour sufficient molten agar into sterile Petri dishes to give a depth of 4 mm ± 0.5 mm (25 mL in 90 mm diameter Petri dishes).

1.3 Dry the surface of the agar to remove excess moisture before use. The length of time needed to dry the surface of the agar depends on the drying conditions, e.g. whether a fan-assisted drying cabinet or 'still air' incubator is used, whether plates are dried before storage and storage conditions. **It is important that plates are not over dried.**

1.4 Store the plates in vented plastic boxes at 8-10°C prior to use. Alternatively the plates may be stored at 4-8°C in sealed plastic bags. Plate drying, method of storage and storage time should be determined by individual laboratories as part of their quality assurance programme. In particular, quality control tests should confirm that excess surface moisture is not produced and that plates are not over-dried.

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°C on beads in glycerol broth. Non-fastidious organisms may be stored at –20°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.

Table 2: Control strains for antimicrobial susceptibility testing

Organism	Strain		Characteristics
	Either	Or	
Escherichia coli	NCTC 12241 (ATCC 25922)	NCTC 10418	Susceptible
Escherichia coli	NCTC 11560		TEM-1 ß-lactamase-producer
Staphylococcus aureus	NCTC 12981 (ATCC 25923)	NCTC 6571	Susceptible
Staphylococcus aureus	NCTC 12493		*MecA*-positive, methicillin resistant
Pseudomonas aeruginosa	NCTC 12934 (ATCC 27853)	NCTC 10662	Susceptible
Enterococcus faecalis	NCTC 12697 (ATCC 29212)		Susceptible
Haemophilus influenzae	NCTC 11931		Susceptible
Haemophilus influenzae	NCTC 12699 (ATCC 49247)		Resistant to ß-lactams (ß-lactamase-negative)
Streptococcus pneumoniae	NCTC 12977 (ATCC 49619)		Low-level resistant to penicillin
Neisseria gonorrhoeae	NCTC 12700 (ATCC 49226)		Low-level resistant to penicillin
Pasteurella multocida	NCTC 8489		Susceptible
Bacteroides fragilis	NCTC 9343 (ATCC 25285)		Susceptible
Bacteroides thetaiotaomicron		ATCC 29741	Susceptible
Clostridium perfringens	NCTC 8359 (ATCC 12915)		Susceptible

3. Preparation of inoculum

The inoculum should give semi-confluent growth of colonies after overnight incubation. Use of an inoculum that yields semi-confluent growth has the advantage that an incorrect inoculum can easily be observed. A denser inoculum will result in reduced zones of inhibition and a lighter inoculum will have the opposite effect. The following methods reliably give semi-confluent growth with most isolates.

NB. Other methods of obtaining semi-confluent growth may be used if they are shown to be equivalent to the following.

3.1 Comparison with a 0.5 McFarland standard

3.1.1 *Preparation of the 0.5 McFarland standard*

Add 0.5 mL of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2$. $2H_2O$) to 99.5 mL of 0.18 M H_2SO_4 (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.

3.1.2 *Inoculum preparation by the growth method* (for non-fastidious organisms, e.g. Enterobacteriaceae, *Pseudomonas* spp. and staphylococci)
Touch at least four morphologically similar colonies (when possible) with a sterile loop. Transfer the growth into Iso-Sensitest broth or an equivalent that has been shown not to interfere with the test. Incubate the broth, with shaking at 35-37°C, until the visible turbidity is equal to or greater than that of a 0.5 McFarland standard.

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).
Colonies are taken directly from the plate into Iso-Sensitest broth (or equivalent) or sterile distilled water. The density of the suspension should match or exceed that of a 0.5 McFarland standard.

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.

3.1.4 *Adjustment of the organism suspension to the density of a 0.5 McFarland standard*
Adjust the density of the organism suspension to equal that of a 0.5 McFarland standard by adding sterile distilled water. To aid comparison, compare the test and standard suspensions against a white background with a contrasting black line.

NB. Suspension should be used within 15 min.

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.

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
β-Haemolytic streptococci	Staphylococci	*Neisseria gonorrhoeae*
Enterococci	*Serratia* spp.	*Campylobacter spp.*
Enterobacteriaceae	*Streptococcus pneumoniae*	
Pseudomonas spp.	*Neisseria meningitidis*	
Stenotrophomonas maltophilia	*Moraxella catarrhalis*	
Acinetobacter spp.	α-haemolytic streptococci	
Haemophilus spp.	*Clostridium perfringens*	
Pasteurella multocida	Coryneform organisms	
Bacteroides fragilis		
Bacteroides thetaiotaomicron		

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

3.2 Photometric standardization of turbidity of suspensions

A photometric method of preparing inocula was described by Moosdeen *et al* (1988)[1] and from this the following simplified procedure has been developed. The spectrophotometer must have a cell holder for 100 x 12 mm test tubes. A much simpler photometer would also probably be acceptable. The 100 x 12 mm test tubes could also be replaced with another tube/cuvette system if required, but the dilutions would need to be recalibrated.

3.2.1 Suspend colonies (touch 4-5 when possible) in 3 mL distilled water or broth in a 100 x 12 mm glass tube (note that tubes are not reused) to give just visible turbidity. It is essential to get an even suspension.

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

3.2.2 Zero the spectrophotometer with a sterile water or broth blank (as appropriate) at a wavelength of 500 nm and measure the absorbance of the bacterial suspension.

3.2.3 From table 4 select the volume to transfer (with the appropriate fixed volume micropipette) to 5 mL sterile distilled water.

3.2.4 Mix the diluted suspension to ensure that it is even

NB. Suspension should be used within 15 min. of preparation

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

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

NB. As spectrophotometers may differ, it may be necessary to adjust the dilutions slightly to achieve semi-confluent growth with any individual set of laboratory conditions.

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 μL 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 diptheroids.
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.

4. Inoculation of agar plate

Use the adjusted suspension within 15 min to inoculate plates by dipping a sterile cotton-wool swab into the suspension and remove the excess liquid by turning the swab against the side of the container. Spread the inoculum evenly over the entire surface of the plate by swabbing in three directions. Allow the plate to dry before applying discs.

NB. If inoculated plates are left at room temperature for extended times before the discs are applied, the organism may begin to grow, resulting in reduced zones of inhibition. Discs should therefore be applied to the surface of the agar within 15 min of inoculation.

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.

5.2 Application of discs

Discs should be firmly applied to the dry surface of the inoculated susceptibility plate. The contact with the agar should be even. A 90 mm plate will accommodate six discs without unacceptable overlapping of zones.

6. Incubation

If the plates are left for extended times at room temperature after discs are applied, larger zones of inhibition may be obtained compared with zones produced when plates are incubated immediately. Plates should therefore be incubated within 15 min of disc application.

6.1 Conditions of incubation
Incubate plates under conditions listed in table 5.

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

Organisms	Incubation conditions
Enterobacteriaceae	35-37°C in air for 18-20 h
Pseudomonas spp.	35-37°C in air for 18-20 h
Stenotrophomonas maltophilia	30°C in air for 18-20 h
Staphylococci (other than methicillin/oxacillin/cefoxitin)	35-37°C in air for 18-20 h
Staphylococcus aureus 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
Moraxella catarrhalis	35-37°C in air for 18-20 h
α-Haemolytic streptococci	35-37°C in 4-6% CO_2 in air for 18-20 h
β-Haemolytic streptococci	35-37°C in air for 18-20 h
Enterococci	35-37°C in air for 24 h[1]
Neisseria meningitidis	35-37°C in 4-6 % CO_2 in air for 18-20 h
Streptococcus pneumoniae	35-37°C in 4-6 % CO_2 in air for 18-20 h
Haemophilus spp.	35-37°C in 4-6 % CO_2 in air for 18-20 h
Neisseria gonorrhoeae	35-37°C in 4-6 % CO_2 in air for 18-20 h
Pasteurella multocida	35-37°C in 4- 6% CO_2 in air for 18-20 h
Coryneform organisms	35-37°C in 4-6% CO_2 in air for 18-20 h
Campylobacter spp.	35-37°C in microaerophilic conditions for 18-20 h
Bacteroides fragilis, Bacteroides thetaiotaomicron, Clostridium perfringens	35-37°C in 10% CO_2/10% H_2/80% N_2 for 18-20 h(Anaerobic cabinet or jar)

[1]It is essential that plates are incubated for at least 24 h before reporting a strain as susceptible to vancomycin or teicoplanin.

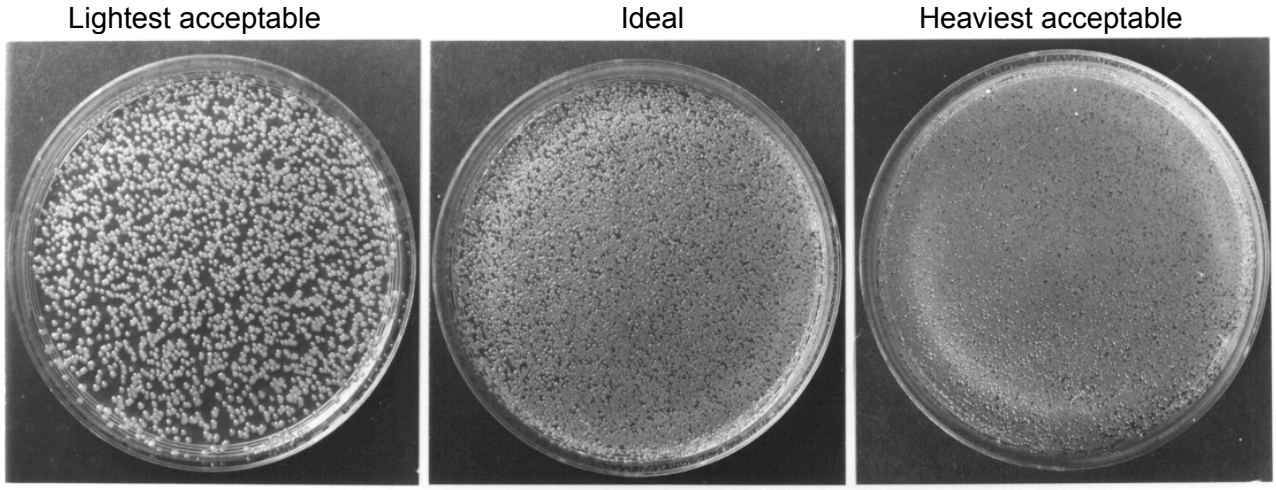
NB. Stacking plates too high in the incubator may affect results owing to uneven heating of plates. The efficiency of heating of plates depends on the incubator and the racking system used. Control of incubation, including height of plate stacking, should therefore be part of the laboratory's Quality Assurance programme.

7. Measuring zones and interpretation of susceptibility

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.

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)

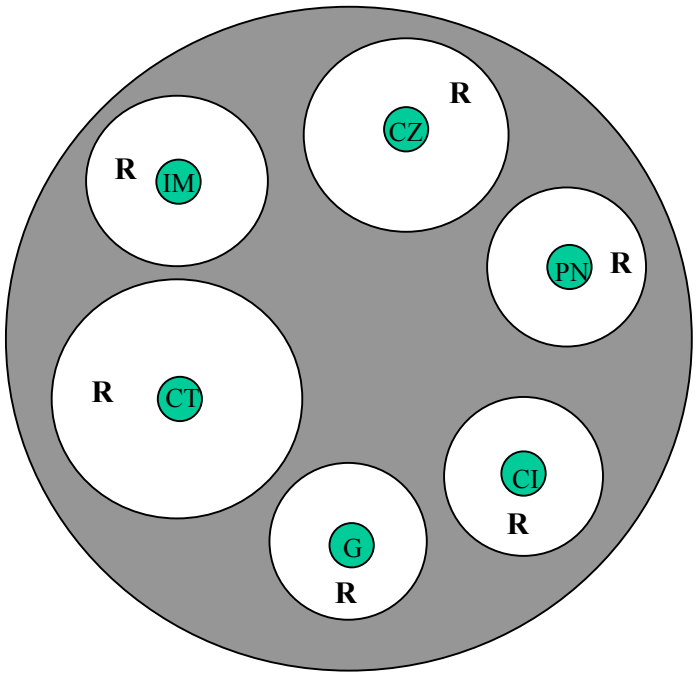
7.2.5 Confirm that the zone of inhibition for the control strain falls within the acceptable ranges in Tables 20-23 before interpreting the test (see section on control of the disc diffusion method).

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.

Figure 2: Template for interpreting zone diameters



8. Methicillin/oxacillin/cefoxitin testing of staphylococci

Methicillin susceptibility testing is difficult with some strains. Expression of resistance is affected by test conditions and resistance is often heterogeneous, with only a proportion of cells showing resistance. Adding NaCl or lowering incubation temperatures increases the proportion of cells showing resistance. Methicillin susceptibility testing of coagulase-negative staphylococci is further complicated as some strains do not grow well on media containing NaCl and are often slower-growing than *Staphylococcus aureus*. Detection of methicillin resistance in coagulase-negative staphylococci may require incubation for 48 h.

8.1 Method for detection of methicillin/oxacillin resistance in *S. aureus* and coagulase-negative 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 autoclaving, mix well to distribute the sodium chloride. Pour plates to give a depth of 4 mm (± 0.5 mm) in a 90 mm sterile Petri dish (25 ml). Dry and store plates as previously described (section 1).

8.1.2 Inoculum

Prepare inoculum as previously described (section 3).

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).

8.1.4 Discs
Place a methicillin 5 μg or an oxacillin 1 μg disc on to the surface of inoculated agar. Discs should be stored and handled as previously described (section 5).

8.1.5 Incubation
Incubate plates for 24 h at 30°C.

8.1.6 Zone measurement
Measure zone diameters (mm) as previously described (section 7).
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 methicillin/oxacillin if necessary.

8.1.7 Interpretation
For both methicillin and oxacillin interpretation is as follows:
Susceptible = ≥ 15 mm diameter, resistant = ≤ 14 mm diameter.

NB. Some hyper-producers of β-lactamase give zones within the range of 7-14 mm and, if possible, such isolates should be checked by a PCR method for *mec*A or by 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.

8.2 Detection of methicillin/oxacillin/cefoxitin resistance in *Staphylococcus aureus* by use of cefoxitin as the test agent

8.2.1 Medium
Prepare Iso-Sensitest agar as previously described (section 1).

8.2.2 Inoculum
Prepare inoculum as previously described (section 3).

8.2.3 Control
Use control strains as previously described (section 8.1.3).

8.2.4 Discs
Place a 10 μg cefoxitin disc on the surface of inoculated agar.
Discs should be stored and handled as previously described (section 5).

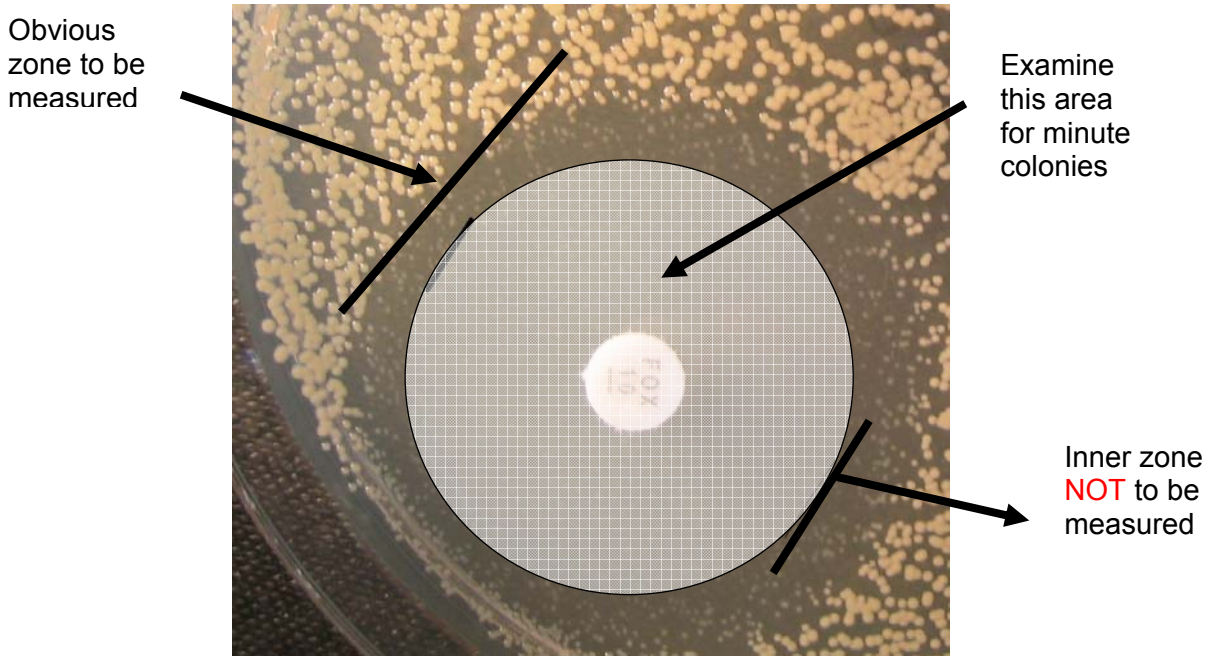
8.2.5 Incubation
Incubate plates at 35°C for 18-20 h.

NB. It is important that the temperature does not exceed 36°C, as tests incubated at higher temperatures are less reliable.

8.2.6 Zone measurement
Measure zone diameters as previously described (section 7), reading the obvious zone edge (see Figure 3).
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 = ≥22 mm diameter, resistant = ≤21 mm diameter.

NB. Hyper-producers of β-lactamase give zones within the ranges of the susceptible population.

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

	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
Antibiotic	R >	I	S ≤	Disc content (µg)	R ≤	I	S ≥
Amikacin	16	16	8	30	15	16-18	19
Amoxicillin[1]	16	16	8	10	11	12-14	15
Ampicillin[1]	16	16	8	10	11	12-14	15
Aztreonam[2]	1	-	1	30	23	-	24
Cefaclor	1	-	1	30	34	-	35
Cefamandole[3,4]	8	-	8	30	19	-	20
Cefepime	1	-	1	30	31	-	32
Cefixime	1	-	1	5	19	-	20
Cefoperazone[3]	4	-	4	30	24	-	25
Cefotaxime	1	-	1	30	29	-	30
Cefotetan[3]	4	-	4	30	23	-	24
Cefoxitin[4]	8	-	8	30	19	-	20
Cefpirome	1	-	1	20	24	-	25
Cefpodoxime[5,6]	1	-	1	10	**19**	-	**20**
Ceftazidime	2	-	2	30	27	-	28
Ceftazidime[7] *E. coli* & *Klebsiella* spp.	2	-	2	30	21	-	22
Ceftibuten	1	-	1	10	27	-	28
Ceftizoxime	1	-	1	30	29	-	30
Ceftriaxone	1	-	1	30	27	-	28
Cefuroxime (axetil)	1	-	1	30	24	-	25
Cefuroxime (parenteral)	8	-	8	30	19	-	20
Cefalothin[4]	8	-	8	30	26	-	27
Cefradine[4]	8	-	8	30	11	-	12
Chloramphenicol	8	-	8	30	20	-	21
Ciprofloxacin[8,9]	1	1	0.5	1	16	17-19	20
Co-amoxiclav[1]	16	16	8	20/10	11	12-14	15
Colistin[10]	4	-	4	25	14	-	15
Co-trimoxazole[11,12]	32	-	32	25	15	-	16
Doxycycline	1	-	1	30	28	-	29
Ertapenem	2	-	2	10	**27**	**-**	**28**
Gatifloxacin	1	-	1	2	19	-	20
Gemifloxacin	0.25	-	0.25	1	19	-	20
Gentamicin[13]	4	4	2	10	16	17-19	20
Imipenem[14]	4	-	4	10	22	-	23
Levofloxacin	2	2	1	1	13	14-16	17
Meropenem	4	-	4	10	22	-	23
Mezlocillin	16	-	16	75	21	-	22
Moxifloxacin	1	1	0.5	1	16	17-19	20
Ofloxacin	1	1	0.5	5	25	26-28	29
Piperacillin /Tazobactam	16	-	16	75/10	21	-	22
Piperacillin	16	-	16	75	23	-	24
Streptomycin[3]	8	-	8	10	12	-	13
Sulfamethoxazole	32	-	32	100	13	-	14
Timentin	16	-	16	85	20	-	21
Tobramycin[13]	4	4	2	10	17	18-20	21
Trimethoprim	2	1-2	0.5	2.5	14	15-19	20

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

Some problems with testing *Acinetobacter* and *Serratia* spp. have been related to difficulties in achieving the correct inoculum. Once a clinically significant isolate of *Acinetobacter* sp. or *Serratia* sp. has been identified, it might be prudent to determine the susceptibility by an MIC method, or the disc diffusion test must be repeated if the inoculum density is outside the acceptable range.

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.

[1] These interpretative standards apply only to *Escherichia coli* and *Proteus mirabilis*

[2] The MIC breakpoint for aztreonam has been set to ensure that ESBL producers with aztreonam MIC values of 4 mg/L are not interpreted as susceptible to this agent.

[3] Zone diameter breakpoints are valid only for *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis*.

[4] The MIC breakpoints has been adjusted to take account of the MIC distribution for the population lacking a mechanism of resistance.

[5] All Enterobacteriaceae isolates should be tested with cefpodoxime or both cefotaxime (or ceftriaxone) and ceftazidime. Enterobacteriaceae with resistance to cefpodoxime, ceftriaxone, cefotaxime or ceftazidime should be tested for the presence of ESBLs. Organisms inferred to have ESBLs should be reported resistant to all penicillins (except temocillin) and cephalosporins, including the fourth-generation cephalosporins cefepime and cefpirome. For serious infections, carbapenems (imipenem, meropenem and ertapenem) are the treatment of choice.

[6] 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.

[7] Isolates of *Escherichia coli* and *Klebsiella* spp. have been identified with ceftazidime MICs of 1 mg/L, which is higher than those for the `wild susceptible' population (c. 0.12 mg/L). These isolates do not possess extended-spectrum β-lactamases and until a mechanism of resistance has been identified zone diameter breakpoint is tentative.

[8] Isolates of *Escherichia coli* and *Klebsiella* spp. with ciprofloxacin MICs of 0.25 and 0.5 mg/L may be reported as resistant. These MICs are higher than those for the `wild susceptible' populations for the species and may indicate a mechanism of resistance with clinical significance.

[9] 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.

[10] Some strains of Enterobacteriaceae (particularly *Serratia*, *Providencia*, *Citrobacter* and *Enterobacter* spp.) produce clear zones of inhibition with small colonies around the colistin disc. These isolates are resistant as the MICs typically exceed 128 mg/L.

[11] Based on sulfamethoxazole MIC.

[12] For advice on testing susceptibility to co-trimoxazole, see Appendix 1.

[13] Individual aminoglycoside agents must be tested; susceptibility to other aminoglycosides cannot be inferred from the gentamicin result and *vice versa*.

[14] *Proteus* spp. and *Morganella morganii* are considered poor targets for imipenem.

Table 7: MIC and zone diameter breakpoints for *Pseudomonas* spp. and *Stenotrophomonas maltophilia*[1].

	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
Antibiotic	R >	I	S ≤	Disc content (µg)	R ≤	I	S ≥
Amikacin	16	16	8	30	15	16-18	19
Aztreonam	8	-	8	30	22	-	23
Carbenicillin	128	-	128	100	12	-	13
Cefotaxime	1	-	1	30	26	-	27
Cefpirome	1	-	1	20	19	20-24	25
Ceftazidime	8	-	8	30	23	-	24
Ceftriaxone	1	-	1	30	29	-	30
Ciprofloxacin	1	1	0.5	1	12	13-22	23
Ciprofloxacin	1	1	0.5	5	19	20-29	30
Colistin	4	-	4	25	13	-	14
Gatifloxacin	1	-	1	2	19	-	20
Co-trimoxazole[1,2]	32	-	32	25	19	-	20
Gemifloxacin	0.25	-	0.25	5	19	-	20
Gentamicin	4	-	4	10	17	-	18
Imipenem[3]	4	-	4	10	21	-	22
Levofloxacin	2	2	1	5	16	17-21	22
Meropenem[3]	4	-	4	10	21	-	22
Moxifloxacin	4	2-4	1	5	17	18-24	25
Netilmicin	4	2-4	1	30	15	16-18	19
Piperacillin	16	-	16	75	23	-	24
Piperacillin /tazobactam	16	-	16	75/10	21	-	22
Ticarcillin	64	32-64	16	75	19	-	20
Timentin	64	32-64	16	85	19	-	20
Tobramycin	4	-	4	10	19	-	20

[1] For *Stenotrophomonas maltophilia*, susceptibility testing is not recommended except for co-trimoxazole (see www.bsac.org.uk BSAC Standardized Susceptibility Testing Method, Additional Methodology, *Stenotrophomonas maltophilia*).

[2] MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

[3] The detection of resistance mediated by carbapenemase is difficult, particularly if resistance is not fully expressed. Consideration should be given to testing ceftazidime and carbapenem resistant isolates for the presence of carbapenemases.

Table 8: MIC and zone diameter breakpoints for staphylococci.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg unless stated)	R ≤	I	S ≥
Amikacin *Staphylococcus aureus*	16	16	8	30	15	16-18	19
Amikacin coagulase-negative staphylococci	16	16	8	30	21	22-24	25
Azithromycin	1	-	1	15	19	-	20
Cefoxitin[1] *Staphylococcus aureus*	4	-	4	10	21	-	22
Chloramphenicol	8	-	8	10	14	-	15
Ciprofloxacin[2]	1	-	1	1	17	-	18
Clarithromycin	0.5	-	0.5	2	19	-	20
Clindamycin[3]	0.5	-	0.5	2	25	-	26
Co-amoxiclav[1]	1	-	1	3	17	-	18
Co-trimoxazole[4,5]	32		32	25	16	-	17
Erythromycin	0.5	-	0.5	5	19	-	20
Fusidic acid	1	-	1	10	29	-	30
Gatifloxacin	1	-	1	2	19	-	20
Gemifloxacin	0.25	-	0.25	1	19	-	20
Gentamicin	1	-	1	10	19	-	20
Linezolid[6]	4	-	4	10	19	-	20
Methicillin[1,7]	4	-	4	5	14	-	15
Moxifloxacin	1	-	1	1	19	-	20
Mupirocin[8,9]	4	-	4	5	21	-	22
Mupirocin[9]	256	8-256	4	20	6	7-26	27
Neomycin	-	-	-	10	16	-	17
Ofloxacin	1	-	1	5	27	-	28
Oxacillin[1,7,10]	2	-	2	1	14	-	15
Penicillin[11]	0.12	-	0.12	1 unit	24	-	25
Quinupristin/ Dalfopristin[12]	2	-	2	15	19	-	20
Rifampicin	0.06	-	0.06	2	29	-	30
Teicoplanin[13,14]	8	8	4	30	14	-	15
Telithromycin	0.5	-	0.5	15	26	-	27
Tetracycline	1	-	1	10	19	-	20
Tobramycin for *Staphylococcus aureus*	1	-	1	10	20	-	21
Tobramycin for coagulase - negative staphylococci	1	-	1	10	29	-	30
Trimethoprim[15]	0.5	-	0.5	5	19	-	20
Vancomycin[14]	8	8	4	5	11	-	12

[1] 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 *mec*A 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.

[2] MIC breakpoints relate to high-dose therapy (750 mg).

[3] 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).

[4] For advice on testing susceptibility to co-trimoxazole see Appendix 1.

[5] MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

[6] Information on clinical response in patients with serious staphylococcal infections is not yet available. In such patients an MIC determination might be appropriate.

[7] Recommendations for tests on Mueller-Hinton or Columbia agars with 2% NaCl.

[8] An Etest or other MIC method should be performed on any strain designated mupirocin resistant when tested with a 5 µg disc. The MIC will indicate whether the strain has low-level (MIC 8 - 256 mg/L) or high-level (MIC ≥512 mg/L) resistance.

[9] Isolates with low-level resistance to mupirocin (MICs 8-256 mg/L) may be eradicated more slowly than susceptible isolates.

[10] MIC breakpoint for coagulase-negative staphylococci is currently under review.

[11] Penicillin; check for heaped zone edge (= resistant)

[12] The presence of blood has a marked effect on the activity of quinupristin/dalfopristin. On the rare occasions when blood needs to be added to enhance the growth of staphylococci, susceptible = ≥15 mm, resistant ≤14 mm.

[13] Teicoplanin - disc testing not recommended for coagulase-negative staphylococci. An MIC method should be used to determine susceptibility.

[14] 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.

[15] Amended zone diameter breakpoints are microbiological breakpoints based on the MIC distribution for the wild type population. However, there is no clear evidence correlating these breakpoints with clinical efficacy.

Table 9: MIC and zone diameter breakpoints for *Streptococcus pneumoniae*.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R>	I	S ≤	Disc content (µg)	R ≤	I	S ≥
Azithromycin	1	-	1	15	19	-	20
Cefaclor[1]	1	-	1	30	24	-	25
Cefixime[1]	1	-	1	5	19	-	20
Cefotaxime[1]	1	-	1	5	29	-	30
Cefpodoxime[1]	1	-	1	1	21	-	22
Ceftibuten[1]	1	-	1	10	27	-	28
Ceftizoxime[1]	1	-	1	30	29	-	30
Ceftriaxone[1]	1	-	1	30	27	-	28
Cefuroxime[1]	1	-	1	5	24	-	25
Cefadroxil[1]	1	-	1	30	24	-	25
Cefalexin[1]	2	-	2	30	24	-	25
Chloramphenicol	8	-	8	10	17	-	18
Ciprofloxacin[2]	2	0.25-2	0.12	1	9	10-24	25
Clarithromycin	0.5	-	0.5	2	19	-	20
Co-trimoxazole[3,4]	32	-	32	25	16	-	17
Ertapenem[1]	1	0.06-1	0.03	10	27	28-39	40
Erythromycin	0.5	-	0.5	5	19	-	20
Gatifloxacin	1	-	1	2	19	-	20
Gemifloxacin	0.25	-	0.25	1	19	-	20
Imipenem[1]	4	-	4	10	24	-	25
Levofloxacin	2	-	2	1	9	-	10
Linezolid	4	4	2	10	19	-	20
Meropenem[1]	4	-	4	10	27	-	28
Moxifloxacin	0.5	-	0.5	1	17	-	18
Ofloxacin[2]	4	0.25-4	0.12	5	15	16-27	28
Penicillin[5]	1	0.12-1	0.06	Oxacillin 1	19	-	20
Quinupristin/ Dalfopristin	2	-	2	15	19	-	20
Rifampicin	1	-	1	5	21	-	22
Telithromycin	0.5	-	0.5	15	28	-	29
Tetracycline	1	-	1	10	19	-	20
Vancomycin	4	-	4	5	12	-	13

[1] Organisms with reduced susceptibility to penicillin: confirm resistance with a test for penicillin MIC. Organisms with an MIC ≤ 1 mg/L are considered susceptible to β-lactam agents except in infections of the central nervous system. In addition, cefotaxime MIC determination is advised for strains isolated from meningitis or other invasive infections.

[2] Isolates with ciprofloxacin or ofloxacin MICs of ≤2 mg/L are considered as having intermediate susceptibility.

[3] For advice on testing susceptibility to co-trimoxazole see Appendix 1.

[4] MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

[5] Penicillin resistance in *Streptococcus pneumoniae* is detected with an oxacillin 1 µg disc.

Table 10: MIC and zone diameter breakpoints for enterococci.

Antibiotic	MIC breakpoint (mg/L) R >	I	S ≤	Disc content (µg)	Interpretation of zone diameters (mm) R ≤	I	S ≥
Ampicillin	8	-	8	10	19	-	20
Azithromycin	1	-	1	15	29	-	30
Gentamicin[1]	128	-	128	200	**14**	-	**15**
Imipenem	4	-	4	10	19	-	20
Linezolid	4	-	4	10	19	-	20
Meropenem	4	-	4	10	19	-	20
Quinupristin/ dalfopristin[2]	2	-	2	15	19	-	20
Teicoplanin[3]	8	8	4	30	19	-	20
Vancomycin[3]	8	8	4	5	12	-	13

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

[1] High-level gentamicin-resistant enterococci usually give no zone or only a trace of inhibition around gentamicin 200 µg discs. Occasionally, however, the plasmid carrying the resistance gene may be unstable and the resistance is seen as a zone of inhibition with a few small colonies within the zone. Retesting of resistant colonies results in growth to the disc or increased numbers of colonies within the zone. Zones should be carefully examined to avoid missing such resistant organisms. If in doubt, isolates may be sent to the reference laboratory for confirmation.

[2] The presence of blood has a marked effect on the activity of quinupristin/dalfopristin. On the rare occasions when blood needs to be added to enhance the growth of enterococci, susceptible = ≥15 mm, resistant = ≤14 mm.

[3] It is essential that plates be incubated for at least 24 h before reporting a strain as susceptible to vancomycin or teicoplanin.

Table 11: MIC and zone diameter breakpoints for α-haemolytic streptococci

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg unless stated)	R ≤	I	S ≥
Amoxicillin	1	-	1	2	19	-	20
Cefotaxime	1	-	1	5	20	-	21
Clindamycin	0.5	-	0.5	2	19	-	20
Erythromycin	0.5	-	0.5	5	19	-	20
Linezolid	2	-	2	10	19	-	20
Penicillin	0.12	-	0.12	1 unit	21	-	22
Teicoplanin	4	-	4	30	15	-	16
Tetracycline	1	-	1	10	23	-	24
Vancomycin	4	-	4	5	13	-	14

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

Antibiotic	MIC breakpoint (mg/L)			Disc content (µg unless stated)	Interpretation of zone diameters (mm)		
	R >	I	S ≤		R ≤	I	S ≥
Azithromycin	1	-	1	15	19	-	20
Cefadroxil	1	-	1	30	24	-	25
Cefixime	1	-	1	5	19	-	20
Cefotaxime	1	-	1	5	27	-	28
Cefalexin	2	-	2	30	24	-	25
Cefalothin	1	-	1	30	28	-	29
Clarithromycin[1]	0.5	-	0.5	2	19	-	20
Co-trimoxazole[2,3]	32	-	32	25	16	-	17
Ertapenem	2	-	2	10	34	-	35
Erythromycin	0.5	-	0.5	5	19	-	20
Linezolid	4	4	2	10	19	-	20
Penicillin	0.12	-	0.12	1 unit	19	-	20
Telithromycin	0.5	-	0.5	15	25	-	26
Tetracycline	1	-	1	10	19	-	20

[1] Active metabolite not taken into consideration
[2] For advice on testing susceptibility to co-trimoxazole see Appendix 1.
[3] MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

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

Antibiotic	MIC breakpoint (mg/L) R >	I	S ≤	Disc content (µg)	Interpretation of zone diameters (mm) R ≤	I	S ≥
Ampicillin[1]	1	-	1	2	29	-	30
Cefaclor	1	-	1	30	22	-	23
Cefuroxime	1	-	1	5	19	-	20
Chloramphenicol	2	-	2	10	22	-	23
Ciprofloxacin[2]	0.5	-	0.5	1	17	-	18
Clarithromycin[3]	0.5	-	0.5	2	19	-	20
Co-amoxiclav	1	-	1	2/1	18	-	19
Co-trimoxazole[4,5]	32		32	25	11	-	12
Ertapenem	2	-	2	10	34	-	35
Erythromycin	0.5	-	0.5	5	27	-	28
Gatifloxacin[2]	1	-	1	2	19	-	20
Gemifloxacin[2]	0.25	-	0.25	1	19	-	20
Levofloxacin	1	-	1	1	19	-	20
Linezolid	4	-	4	10	19	-	20
Moxifloxacin[2]	0.5	-	0.5	1	17	-	18
Ofloxacin[2]	0.5	-	0.5	5	34	-	35
Telithromycin	0.5	-	0.5	15	29	-	30
Tetracycline	1	-	1	10	21	-	22

[1] Test for β-lactamase. β-lactamase positive isolates of *Moraxella catarrhalis* are often slow to become positive and tests for β-lactamase production must be examined after the longest recommended time for the test before being interpreted as negative.
[2] Quinolone resistance is most reliably detected with nalidixic acid discs. Isolates with reduced susceptibility to fluoroquinolones show no zone of inhibition with nalidixic acid.
[3] Active metabolite not taken into consideration.
[4] For advice on testing susceptibility to co-trimoxazole, see Appendix 1.
[5] MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.

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 ≥
Azithromycin	1	-	1	15	27	-	28
Cefixime[1]	1	-	1	5	29	-	30
Cefotaxime[1]	1	-	1	5	29	-	30
Ceftriaxone[1]	0.25	-	0.25	5	34	-	35
Cefuroxime	1	-	1	5	19	-	20
Ciprofloxacin[2,3]	0.06	0.06	0.03	1	28	-	29
Erythromycin	0.5	-	0.5	5	11	-	12
Nalidixic acid[2]	-	-	-	30	**6**	**7-31**	**32**
Penicillin[4]	1	0.12-1	0.06	1 unit	17	18-25	26
Rifampicin	1	-	1	2	20	-	21
Spectinomycin	64	-	64	25	13	-	14
Tetracycline[5]	1	-	1	10	13		27

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

[1] Resistance to ceftriaxone, cefotaxime and cefixime has not been described. Isolates with chromosomally encoded reduced susceptibility to penicillin have slightly reduced zones of inhibition with these agents but they remain susceptible. Results for isolates with reduced zones around ceftriaxone, cefotaxime and cefixime discs should be confirmed by MIC determinations.

[2] 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.

[3] The MIC breakpoint has been lowered to ensure that isolates with reduced susceptibility to ciprofloxacin are detected.

[4] Test for β-lactamase.

[5] Use the tetracycline result to infer susceptibility to doxycycline. For epidemiological purposes, isolates with plasmid-mediated resistance to tetracycline may be distinguished from those with chromosomal resistance on the basis of zone diameters; isolates with plasmid-mediated resistance have no zones of inhibition and those with low-level chromosomal resistance have zone diameters 14-26 mm.

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

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg unless stated)	R ≤	I	S ≥
Cefotaxime	1	-	1	5	29	-	30
Chloramphenicol	2	-	2	10	19	-	20
Ciprofloxacin[1]	0.06	0.06	0.03	1	31	-	32
Erythromycin	0.5	-	0.5	5	26	-	27
Penicillin	0.06	-	0.06	1 unit	24	-	25
Rifampicin	1	-	1	2	29	-	30
Tetracycline	1	-	1	10	21	-	22

NB. *Neisseria meningitidis* is a category 2 pathogen, but has a derogation to 3 when heavy suspensions are used (DoH Hazard 29 January 1993). Consequently suspension and dilution of organisms and inoculation of plates for susceptibility tests must be carried out in a class 1 safety cabinet.

[1] Quinolone resistance is most reliably detected with nalidixic acid. Isolates with reduced susceptibility to fluoroquinolones have no zone of inhibition with nalidixic acid discs.

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

Antibiotic	MIC breakpoint (mg/L) R >	I	S ≤	Disc content (µg unless stated)	Interpretation of zone diameters (mm) R ≤	I	S ≥
Amoxicillin[1,]	1	-	1	2	16	-	17
Ampicillin[1,]	1	-	1	2	17	-	18
Azithromycin[2]	4	0.5-4	0.25	15	19	20-34	35
Cefaclor [3]	1	-	1	30	36	-	37
Cefotaxime	1	-	1	5	24	-	25
Ceftazidime	2	-	2	30	29	-	30
Ceftriaxone	1	-	1	30	34	-	35
Cefuroxime	1	-	1	5	16	-	17
Chloramphenicol	2	-	2	10	24	-	25
Ciprofloxacin[4]	0.5	-	0.5	1	27	-	28
Clarithromycin[5]	16	1-16	0.5	5	9	10-24	25
Co-amoxiclav	1	-	1	2/1	16	-	17
Co-trimoxazole[6,7]	32		32	25	21	-	22
Ertapenem	2	-	2	10	29	-	30
Erythromycin	8	1-8	0.5	5	14	15-27	28
Gatifloxacin[4]	1	-	1	2	19	-	20
Gemifloxacin[4]	0.25	-	0.25	1	19	-	20
Imipenem	4	-	4	10	19	-	20
Levofloxacin[4]	1	-	1	1	19	-	20
Meropenem	4	-	4	10	27	-	28
Moxifloxacin[4]	0.5	-	0.5	1	17	-	18
Nalidixic acid[4]	-	-	-	30	-	-	-
Ofloxacin[4]	0.5	-	0.5	5	36	-	37
Telithromycin[8]	2	1-2	0.5	15	15	16-19	20
Tetracycline	1	-	1	10	21	-	22
Trimethoprim	0.5	-	0.5	2.5	20	-	21

[1] Test for β-lactamase.
[2] No resistant strains yet described.
[3] See Appendix 2.
[4] Quinolone resistance is most reliably detected with nalidixic acid. Strains with reduced susceptibility to fluoroquinolones give no zone of inhibition with a 30µg nalidixic acid disc.
[5] Active metabolite not taken into consideration.
[6] For advice on testing susceptibility to co-trimoxazole see Appendix 1.
[7] MIC breakpoint based on sulfamethoxazole concentration in 19:1 combination with trimethoprim.
[8] The mode telithromycin MIC for these organisms is 1 mg/L; therefore the majority of isolates will be interpreted as having intermediate susceptibility.

Table 17: MIC and zone diameter breakpoints for *Pasteurella multocida*.

Antibiotic	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)		
	R >	I	S ≤	Disc content (µg unless stated)	R ≤	I	S ≥
Ampicillin	1	-	1	10	29	-	30
Cefotaxime	1	-	1	5	33	-	34
Ciprofloxacin[1]	1	-	1	1	28	-	29
Nalidixic acid	-	-	-	30	27	-	28
Penicillin	0.12	-	0.12	1 unit	21	-	22
Tetracycline	1	-	1	10	25	-	26

[1] Quinolone resistance is most reliably detected with nalidixic acid discs.

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[1]	1	-	0.5	1	17	-	18

[1] Quinolone resistance is most reliably detected with nalidixic acid discs.

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

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

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

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

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

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

Table 21: MIC and zone diameter breakpoints for Gram-negative rods isolated from urinary tract infections[1-4].

	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)								
					Coliforms			*Escherichia coli*			*Proteus mirabilis*		
Antibiotic	R >	I	S ≤	Disc content (µg)	R≤	I	S≥	R ≤	I	S≥	R ≤	I	S≥
Amoxicillin[5]	32	-	32	25	11	-	12	11	-	12	11	-	12
Ampicillin[5]	32	-	32	25	11	-	12	11	-	12	11	-	12
Cefalexin[6]	32	-	32	30	-	-	-	15	-	16	11	-	12
Ciprofloxacin	4	-	4	1	19	-	20	19	-	20	19	-	20
Co-amoxiclav[5]	32	-	32	20/10	11	-	12	11	-	12	11	-	12
Fosfomycin[7,8]	128	-	128	200/50	-	-	-	19	-	20	33	-	34
Mecillinam[9]	8	-	8	10	-	-	-	13	-	14	13	-	14
Nalidixic acid	16	-	16	30	17	-	18	17	-	18	17	-	18
Nitrofurantoin	32	-	32	200	-	-	-	19	-	20	-	-	-
Norfloxacin	4	-	4	2	15	-	16	15	-	16	15	-	16
Trimethoprim	2	-	2	2.5	16	-	17	16	-	17	16	-	17

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

1 If an organism is isolated from multiple sites, for example from blood and urine, interpretation of susceptibility should be made with regard to the systemic site (e.g., if the blood isolate is resistant and the urine isolate susceptible, both should be reported resistant irrespective of the results obtained using interpretative criteria for urine isolates).

2 For agents not listed criteria given for systemic isolates may be used for urinary tract isolates (see Tables 1 and 2).

3 Direct susceptibility tests on urine samples may be performed as long as the inoculum gives semi-confluent growth.

4 In the absence of definitive organism identification, use the recommendations most appropriate for the presumptive identification, accepting that on some occasions the interpretation may be incorrect. A more cautious approach is to use the systemic recommendations.

5 These interpretative standards apply only to *Escherichia coli* and *Proteus mirabilis* and not to species that have chromosomal penicillinases (*Klebsiella* spp.) or those that typically have inducible AmpC enzymes (e.g. *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp.). The identification of Enterobacteriaceae to species level is essential before applying expert rules for the interpretation of susceptibility.

6 Cefalexin results may be used to report susceptibility to cefadroxil.

7 Fosfomycin/glucose-6-phosphate

8 Fosfomycin – the susceptibility of *Proteus* spp. that swarm up to the disc can be difficult to interpret.

9 Isolates of *Escherichia coli* and *Klebsiella* spp. that produce ESBLs often appear susceptible to mecillinam *in vitro* but clinical efficacy against these organisms is unproven.

Table 22: MIC and zone diameter breakpoints for Gram-positive cocci isolated from urinary tract infections[1,2.]

	MIC breakpoint (mg/L)				Interpretation of zone diameters (mm)								
					Enterococci			Staphylococcus saprophyticus			Group B streptococci		
Antibiotic	R >	I	S ≤	Disc content (µg)	R ≤	I	S ≥	R ≤	I	S ≥	R≤	I	S ≥
Ampicillin	32	-	32	25	19	-	20	25	-	26	25	-	26
Cefalexin[3]	32	-	32	30	-	-	-	-	-	-	23	-	24
Ciprofloxacin	4	-	4	1	11	-	12	17	-	18	12	-	13
Ciprofloxacin	4	-	4	5	15	-	16	-	-	-	18	-	19
Co-amoxiclav	32	-	32	20/10	20	-	21	27	-	28	27	-	28
Fosfomycin[4]	128	-	128	200/50	19	-	20	19	-	20	-	-	-
Mecillinam	64	-	64	50	-	-	-	9	-	10	-	-	-
Nalidixic acid	16	-	16	30	17	-	18	-	-	-	-	-	-
Nitrofurantoin	32	-	32	200	14	-	15	19	-	20	19	-	20
Norfloxacin	4	-	4	2	15	-	16	-	-	-	-	-	-
Trimethoprim[5]	2	-	2	2.5	21	-	22	14	-	15	15	-	16

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.

1 If an organism is isolated from multiple sites, for example from blood and urine, interpretation of susceptibility should be made with regard to the systemic site (e.g., if the blood isolate is resistant and the urine isolate susceptible, both should be reported resistant irrespective of the results obtained using interpretative criteria for urine isolates).

2 Direct susceptibility tests on urine samples may be performed as long as the inoculum gives semi-confluent growth.

3 Cefalexin results may be used to report susceptibility to cefadroxil.

4 Fosfomycin/glucose-6-phosphate.

5 There is some doubt about the clinical relevance of testing the susceptibility of enterococci to trimethoprim

Appendix 1: Testing antimicrobial susceptibility to co-trimoxazole

Breakpoints for testing susceptibility to co-trimoxazole are provided. However, the following recommendations from the UK Committee on the Safety of Medicines (CSM) should be noted.

”Co-trimoxazole should be limited to the role of drug of choice in *Pneumocyctis carinii* pneumonia, it is also indicated for toxoplasmosis and nocardiasis. It should now only be considered for use in acute exacerbations of chronic bronchitis and infections of the urinary tract when there is good bacteriological evidence of sensitivity to co-trimoxazole and good reason to prefer this combination to a single antibiotic; similarly it should only be used in acute otitis media in children when there is good reason to prefer it. Review of the safety of co-trimoxazole using spontaneous adverse drug reaction data has indicated that the profile of reported adverse reactions with trimethoprim is similar to that with co-trimoxazole; blood and generalised skin disorders are the most serious reactions with both drugs and predominantly have been reported to occur in elderly patients. A recent large post-marketing study has demonstrated that such reactions are very rare with co-trimoxazole; the study did not distinguish between co-trimoxazole and trimethoprim with respect to serious hepatic, renal, blood or skin disorders.”

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 β-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_{50} 2 mg/L; MIC_{90} 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.

Acknowledgment

The BSAC acknowledges the assistance of the Swedish Reference Group for Antibiotics (SRGA) in supplying some breakpoint data for inclusion in this document.

References

1. Moosdeen, F., Williams, J.D. & Secker, A. (1988). Standardization of inoculum size for disc susceptibility testing: a preliminary report of a spectrophotometric method. J. Antimicrob Chemother **21**, 439-43.

Additional information

1. Susceptibility testing of *Helicobacter pylori*

Disc diffusion methods are not suitable for testing *Helicobacter pylori* as this species is slow growing and results may not be accurate. The recommended method of susceptibility testing is Etest (follow technical guide instructions).

Suspend colonies from a 2-3 day culture on a blood agar plate in sterile distilled water and adjust the density to equal a McFarland 3 standard.

Use a swab dipped in the suspension to inoculate evenly the entire surface of the plate. The medium of choice is Mueller-Hinton agar or Wilkins-Chalgren agar with 5-10% horse blood.

Allow the plate to dry and apply Etest strip.

Incubate at 35°C in microaerophilic conditions for 3-5 days.
Read the MIC at the point of complete inhibition of all growth, including hazes and isolated colonies. Tentative interpretative criteria for MICs are given in Table 1.

Table 1: Tentative MIC breakpoints for *Helicobacter pylori*

	MIC breakpoint (mg/L)	
Antimicrobial agent	R >	S ≤
Amoxicillin	1	1
Clarithromycin	1	1
Tetracycline	2	2
Metronidazole	4	4

2. Susceptibility testing of *Brucella* species

Brucella spp. are Hazard Group 3 pathogens and all work must be done in containment level 3 accommodation. The antimicrobial agents most commonly used for treatment are doxycycline, rifampicin, ciprofloxacin, tetracycline and streptomycin and, from the limited information available, there is little or no resistance to these drugs. *Brucella* spp. are uncommon isolates and interpretative standards are not available. Since *Brucella* spp. are highly infectious, susceptibility testing in routine laboratories is not recommended.

3. Susceptibility testing of *Legionella* species

Legionella spp. are slow growing and have particular growth requirements. Disc diffusion methods for susceptibility testing are unsuitable. Susceptibility should be determined by agar dilution MICs on buffered yeast extract agar with 5% water-lysed horse blood[1]. The antimicrobial agents commonly used for treatment are macrolides, rifampicin and fluoroquinolones. Validated MIC breakpoints are not established for *Legionella* spp. If results for test isolates are within range of the normal wild type distribution, given in table 2, clinical susceptibility may be assumed.

Table 2: MIC ranges for wild type *Legionella* spp.

Antimicrobial agent	MIC range for wild-type *Legionella* spp. (mg/L)
Erythromycin	0.06-0.5
Clarithromycin	0.004-0.06
Rifampicin	0.004-0.06
Ciprofloxacin	0.016-0.06

4. Susceptibility testing of topical antibiotics

MIC breakpoints specifically for topical antibiotics are not given because there are no pharmacological, pharmacodynamic or clinical response data on which to base recommendations. [Relevant data would be gratefully received].

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). Any changes will be dated.

Ad hoc modifications to breakpoints by users are not acceptable.

Control of Antimicrobial Susceptibility Testing

1. Control strains

Control strains include susceptible strains to monitor test performance (not for the interpretation of susceptibility), and resistant strains to confirm that the method will detect particular mechanisms of resistance, for example, *Haemophilus influenzae* ATCC 49247 is a β-lactamase negative, ampicillin resistant strain (see table 2 of disc diffusion method). Tables 2-6 provide zone diameters for recommended control organisms under a range of test conditions.

Control strains can be purchased from the National Collection of Type Cultures (NCTC; HPA Centre for Infections, 61 Colindale Avenue, London NW9 5HT). Alternatively, some may be obtained commercially (see section on suppliers)

2. Maintenance of control strains

Store control strains by a method that minimises the risk of mutations, for example, at -70^{0}C, on beads in glycerol broth. Ideally, two vials of each control strain should be stored, one as an ”in-use” supply, the other for archiving. Every week a bead from the ”in-use” vial should be subcultured on to appropriate non-selective media and checked for purity. From this pure culture, prepare one subculture for each of the following 7 days. Alternatively, for fastidious organisms that will not survive on plates for 7 days, subculture the strain daily for no more than 6 days.

3. Calculation of control ranges for disc diffusion tests

The acceptable ranges for the control strains have been calculated by combining zone diameter data from `field studies' and from multiple centres supplying their daily control data, from which cumulative distributions of zones of inhibition have been prepared. From these distributions, the 2.5 and 97.5 percentiles were read to provide a range that would contain 95% of observations. If distributions are normal, these ranges correspond to the mean $\pm$ 1.96 SD. The percentile ranges obtained by this method are, however, still valid even if the data do not show a normal distribution.

4. Frequency of routine testing with control strains

When the method is first introduced, daily testing is required until there are acceptable readings from 20 consecutive days (this also applies when new agents are introduced or when any test component changes). This provides sufficient data to support once weekly testing.

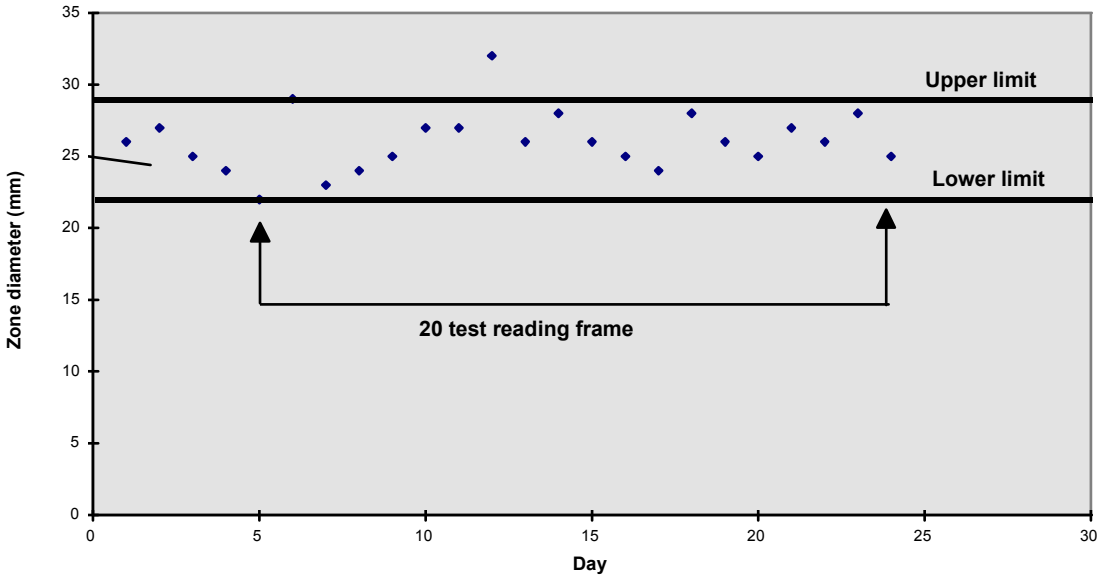
5. Use of control data to monitor the performance of disc diffusion tests

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 than1 in every 20 results is outside the limits of acceptability. If 2 or more results fall out of the acceptable range this is 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.

Figure 1. Use of control strains to monitor performance of disc susceptibility testing



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 β-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 noculum 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.

Table 2: Acceptable zone diameter ranges for control strains on Iso-Sensitest agar, plates incubated at 35-37 ^{0}C in air for 18-20 h.

Antimicrobial agent	Disc content (μg unless stated)	*Escherichia coli*			*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Enterococcus faecalis*
		NCTC 10418	ATCC 25922	NCTC 11560[1]	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29212
Amikacin	30	24-27	23-27	-	21-30	26-32	-	-	-
Ampicillin	10	21-26	16-22	-	-	-	-	-	26-35
Ampicillin	25	24-30	21-28	-	-	-	-	-	-
Aztreonam	30	39-44	36-40	-	27-30	26-30	-	-	-
Azithromycin	15	-	-	-	-	-	-	-	15-21
Cefixime	5	32-36	27-30	-	-	-	-	-	-
Cefoxitin	30	28-33	26-30	-	-	-	-	-	-
Cefotaxime	30	36-45	34-44	-	20-29	20-24	-	-	-
Ceftazidime	30	32-40	31-39	-	29-37	27-35	-	-	-
Cefuroxime	30	25-32	24-29	-	-	-	-	-	-
Cefalexin	30	21-28	16-21	-	-	-	-	-	-
Cefradine	30	19-25	16-22	-	-	-	-	-	-
Chloramphenicol	10	21-27	20-29	-	-	-	20-26	19-27	-
Ciprofloxacin	1	31-40	31-37	-	21-28	24-30	25-32	17-22	14-19
Ciprofloxacin	5	-	-	-	29-37	31-37	-	-	21-27
Clindamycin	2	-	-	-	-	-	30-35	26-33	No zone
Co-amoxiclav	3	-	-	-	-	-	-	27-32	-
Co-amoxiclav	30	18-31	20-26	12-18	-	-	-	-	-
Colistin	25	15-19	16-20	-	17-20	16-20	-	-	-
Ertapenem	10	35-41	35-39	-	-	-	-	-	-
Erythromycin	5	-	-	-	-	-	22-31	22-29	-
Fusidic acid	10	-	-	-	-	-	32-40	30-37	-
Gentamicin	10	21-27	21-27	-	20-26	22-28	24-30	20-26	-
Gentamicin	200	-	-	-	-	-	-	-	22-27
Imipenem	10	32-37	33-37	-	20-27	23-28	-	-	28-32
Levofloxacin	1	30-33	28-34	-	-	-	-	-	-

Antimicrobial agent	Disc content (μg unless stated)	*Escherichia coli*			*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Enterococcus faecalis*
		NCTC 10418	ATCC 25922	NCTC 11560[1]	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29212
Levofloxacin	5	-	-	-	22-29	23-29	-	-	-
Linezolid	10	-	-	-	-	-	31-35	26-30	24-29
Meropenem	10	38-42	27-39	-	32-39	32-39	-	-	22-28
Mupirocin	5	-	-	-	-	-	26-35	24-34	-
Mupirocin	20	-	-	-	-	-	30-38	27-35	-
Nalidixic acid	30	28-36	26-32	-	-	-	-	-	-
Neomycin	10	-	-	-	-	-	-	21-27	-
Netilmicin	10	22-27	22-26	-	17-20	20-24	-	22-28	-
Nitrofurantoin	200	25-30	23-27	-	-	-	21-25	20-26	-
Norfloxacin	2	34-37	32-36	-	-	-	-	-	-
Ofloxacin	5	31-37	31-38	-	18-26	18-25			
Penicillin	1 unit	-	-	-	-	-	32-40	29-36	-
Piperacillin	75	30-35	27-32	-	27-35	27-34	-	-	-
Pip/tazobactam	85	30-35	26-31	-	28-35	28-35	-	-	26-32
Quinupristin/ Dalfopristin	15	-	-	-	-	-	27-31	-	12-19
Rifampicin	2	-	-	-	-	-	27-39	29-36	-
Streptomycin	10	18-24	17-22	-	-	-	-	-	-
Teicoplanin	30	-	-	-	-	-	17-23	16-20	19-25
Tetracycline	10	23-29	22-28	-	-	-	31-40	26-35	-
Ticarcillin	75	32-35	27-30	-	24-28	23-27	-	-	-
Ticarcillin/ clavulanic acid	85	33-37	27-31		25-29	24-27	-	-	-
Tobramycin	10	24-27	-	-	23-30	26-32	-	29-35	-
Trimethoprim	2.5	30-37	25-31	-	-	-	25-30	20-28	28-35
	5	-	-	-	-	-	24-34	-	-
Vancomycin	5	-	-	-	-	-	14-20	13-17	13-19

[1]β-Lactamase producing strain

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Table 3: Acceptable zone diameter ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood, with or without the addition of NAD, plates incubated at 35-37^{0}C in air for 18-20 h.

Antimicrobial agent	Disc content (μg unless stated)	*Staphylococcus aureus* NCTC 6571	*Staphylococcus aureus* ATCC 25923
Erythromycin	5	22-29	23-29
Penicillin	1 unit	30-41	27-35
Tetracycline	10	30-38	28-36

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

Antimicrobial agent	Medium	Disc content (μg)	*Staphylococcus aureus* NCTC 6571	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* NCTC 12493[a]
Methicillin	Columbia/Mueller Hinton agar + 2% NaCl	5	18-30	18-28	No zone
Oxacillin	Columbia/Mueller Hinton agar + 2% NaCl	1	19-30	19-29	No zone
Cefoxitin	ISA	10	26-31	24-29	13-19

[a] 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^{0}C in 10% CO_2 /10% H_2/80% N_2 for 18-20 h.

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

Table 6: Acceptable zone diameter ranges for control strains on Iso-Sensitest agar supplemented with 5% defibrinated horse blood with or without the addition of NAD, plates incubated at 35-37^{0}C in 4-6% CO_2 for 18-20 h.

Antimicrobial agent	Disc content (μg unless stated)	*Staphylococcus aureus*		*Haemophilus influenzae* (with NAD)		*Streptococcus pneumoniae*	*Pasteurella multocida*
		NCTC 6571	ATCC 25923	NCTC 11931	ATCC 49247[a]	ATCC 49619	NCTC 8489
Amoxicillin	2	29-34	-	-	-	-	-
Ampicillin	2	-	-	22-30	6-13	-	-
Ampicillin	10	-	-	-	-	-	32-37
Azithromycin	15	-	-	24-36	20-30	-	-
Cefotaxime	5	26-32	-	33-45	27-38	-	35-41
Cefuroxime	5	22-29	24-29	22-28	6-16	-	-
Chloramphenicol	10	21-26	-	30-40	30-38	21-29	-
Ciprofloxacin	1	22-29	18-23	32-40	33-44	14-21	31-37
Clindamycin	2	21-25	-	-	-	-	-
Co-amoxiclav	3	-	-	20-27	10-20	-	-
Ertapenem	10	-	-	30-38	25-34	35-40	-
Erythromycin	5	25-29	-	12-23	9-16	23-36	-
Linezolid	10	22-26	-	-	-	-	-
Nalidixic acid	30	9-17	9-17	33-38	-	-	-
Oxacillin	1	-	-	-	-	8-16	-
Penicillin	1 unit	37-44	29-36	-	-	-	24-28
Quinupristin/Dalfopristin	15	-	-	-		21-29	-
Rifampicin	2	32-37	-	-	-	-	-
Rifampicin	5	-	-	-	-	28-35	-
Teicoplanin	30	14-19	-	-	-	-	-
Tetracycline	10	33-40	27-34	27-35	9-14	26-36	29-34
Trimethoprim	2.5	-	-	30-40	28-36	-	-
Vancomycin	5	12-16	-	-	-	-	-

[a]β-Lactamase-negative, ampicillin-resistant strain

9. Control of MIC determination

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

Table 7: Target MICs for *Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Bacteroides fragilis* and *Neisseria gonorrhoeae* control strains by BSAC methods

Antimicrobial agent	*Haemophilus influenzae*		*Enterococcus faecalis*	*Streptococcus pneumoniae*	*Bacteroides fragilis*	*Neisseria gonorrhoeae*
	NCTC 11931	ATCC 49247	ATCC 29212	ATCC 49619	NCTC 9343	ATCC 49226
Amikacin	-	-	128	-	-	-
Amoxicillin	0.5	4	0.5	0.06	32	0.5
Ampicillin	-	-	1	0.06	32	-
Azithromycin	2	2	-	0.12	-	-
Azlocillin	-	-	-	-	4	-
Aztreonam	-	-	>128	-	2	-
Cefaclor	-	128	>32	2	>128	-
Cefamandole	-	-	-	-	8	-
Cefixime	0.03	0.25	-	1	64	-
Cefotaxime	-	0.25	32	0.06	4	-
Cefoxitin	-	-	-	-	4	-
Cefpirome	0.06	0.5	16		16	-
Cefpodoxime	0.12	0.5	>32	0.12	32	-
Ceftazidime	0.12	-	>32	-	8	-
Ceftriaxone	-	-	>32	0.06	4	-
Cefuroxime	2	16	>32	0.25	32	-
Cephadroxil	-	-	>32	-	32	-
Cephalexin	-	-	>32	-	64	-
Cephalothin	-	-	16	-	-	-
Chloramphenicol	-	-	4	4	4	-
Ciprofloxacin	0.008	0.008	1	1	2	0.004
Clarithromycin	8	4	-	0.03	0.25	0.5
Clindamycin	-	-	8	0.12	0.5	-
Co-amoxiclav	0.5	8	0.5	0.06	0.5	0.5
Cotrimoxazole	-	1	2	4		-
Dalfopristin/ quinupristin	-	-	1	0.5	16	-
Enoxacin	-	-	-	-	1	-
Ertapenem	0.12	0.5	-	0.12	0.25	-
Erythromycin	8	8	4	0.12	1	0.5
Faropenem	-	-	-	0.06	1	-
Fleroxacin	-	-	-	-	4	-
Flucloxacillin	-	-	-	-	16	-
Fucidic acid	-	-	2	-	-	-
Gatifloxacin	0.008	-	0.25	0.25	0.5	0.004
Gemifloxacin	0.12	-	0.03	0.03	0.25	0.002
Gentamicin	-	-	8	-	128	-
Grepafloxacin	-	0.004	-	0.25	-	-
Imipenem	-	-	0.5	-	0.06	-
Levofloxacin	0.008	0.015	1	0.5	0.5	0.008
Linezolid	-	-	-	2	4	-
Loracarbef	-	128	>32	2	>128	-
Mecillinam	-	-	>128	-	>128	-
Meropenem	-	-	2	-	0.06	-

Antimicrobial agent	*Haemophilus influenzae*		*Enterococcus faecalis*	*Streptococcus pneumoniae*	*Bacteroides fragilis*	*Neisseria gonorrhoeae*
	NCTC 11931	ATCC 49247	ATCC 29212	ATCC 49619	NCTC 9343	ATCC 49226
Metronidazole	-	-	-	-	0.5	-
Moxalactam	-	-	-	-	0.25	-
Moxifloxacin	0.03	0.03	0.25	0.5	-	0.004
Naladixic acid	-	1	-	>128	64	-
Nitrofurantoin	-	-	8	-	-	-
Norfloxacin	-	-	2	-	16	-
Ofloxacin	-	-	2	-	1	-
Oxacillin	-	-	-	1	-	-
Pefloxacin	-	-	-	-	1	-
Penicillin	-	4	2	0.5	16	-
Piperacillin	-	-	2	-	2	-
Rifampicin	-	-	2	0.03	-	-
Roxithromycin	16	16	-	0.12	2	-
Rufloxacin	-	-	-	-	16	-
Sparfloxacin	-	0.002	-	0.25	1	-
Teicoplanin	-	-	0.25	-	-	-
Telithromycin	1	2	0.008	0.008	-	0.03
Tetracycline	-	16	16	0.12	0.5	-
Ticarcillin	-	-	-	-	4	-
Tobramycin	-	-	16	-	-	-
Trimethoprim	-	-	0.25	4	16	-
Trovafloxacin	0.008	0.002	0.06	0.12	0.12	-
Vancomycin	-	-	2	0.25	16	-

Table 8: Target MICs (mg/L) for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* control strains by BSAC methods

Antimicrobial agent	*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		
	NCTC 10418	ATCC 25922	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29213
Amikacin	0.5	1	2	2	1	-	2
Amoxicillin	2	4	>128	>128	0.12	0.25	0.5
Ampicillin	2	4	>128	>128	0.06	-	-
Azithromycin	-	-	-	-	0.12	0.12	0.12
Azlocillin	4	-	4	-	0.25	-	-
Aztreonam	0.03	0.25	4	2	>128	-	>128
Carbenicillin	2	-	32	-	0.5	-	-
Cefaclor	1	2	>128	>128	1	-	1
Cefamandole	0.25	-	>128	>128	0.25	-	-
Cefixime	0.06	0.25	16	-	8	8	16
Cefotaxime	0.03	0.06	8	8	0.5	-	1
Cefotetan	0.06	-	>128	>128	4	-	-
Cefoxitin	4	-	>128	>128	2	-	-
Cefpirome	0.03	0.03	4	1	0.25	-	0.5
Cefpodoxime	0.25	0.25	128	>128	1	4	2
Ceftazidime	0.06	0.25	1	1	4	-	8
Ceftizoxime	0.008	-	-	-	2	-	-
Ceftriaxone	0.03	0.06	8	8	1	-	2
Cefuroxime	2	4	>128	>128	0.5	1	1
Cephadroxil	8	8	>128	>128	1	-	2
Cephalexin	4	8	>128	>128	1	-	4
Cephaloridine	-	-	>128	>128	0.06	-	-
Cephalothin	4	8	>128	>128	0.5	-	0.25
Cephradine	-	-	>128	>128	2	-	-
Chloramphenicol	2	4	128	-	2	-	2
Ciprofloxacin	0.015	0.015	0.25	0.25	0.12	0.5	0.5
Clarithromycin	-	-	-	-	0.12	0.12	0.12
Clindamycin	-	-	-	-	0.06	0.12	0.06
Co-amoxiclav	2	4	>128	128	0.12	0.12	0.25
Colistin	0.5	-	2	-	128	-	-
Cotrimoxazole	0.25	0.25	-	-	-	-	2
Dalfopristin/ Quinupristin	-	-	-	-	0.12	0.25	0.25
Dirythromycin	-	-	-	-	1	-	1
Enoxacin	0.25	-	1	-	0.5	-	-
Ertapenem	0.008	0.015	-	-	-	-	-
Erythromycin	-	-	-	-	0.12	0.5	0.25
Farapenem	0.25	-	>128	>128	0.12	-	-
Fleroxacin	0.06	0.12	1	-	0.5	-	-
Flucloxacillin	-	-	>128	>128	0.06	-	-
Flumequine	2	-	>128	>128	-	-	-
Fosfomycin	4	-	>128	>128	8	-	-
Fusidic acid	>128	-	-	-	0.06	0.12	0.06
Gatifloxacin	0.015	0.015	1	1	0.03	0.12	0.12
Gemifloxacin	0.008	0.008	0.25	0.25	0.015	0.03	0.03
Gentamicin	0.25	0.5	1	1	0.12	0.25	0.25
Grepafloxacin	0.03	0.03	0.5	-	0.03	-	-
Imipenem	0.06	0.12	2	1	0.015	-	0.015
Kanamycin	1	-	1	-	2	-	-
Levofloxacin	0.03	0.03	0.5	0.5	0.12	0.25	0.25
Linezolid	-	-	-	-	0.5	1	-

Antimicrobial agent	*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		
	NCTC 10418	ATCC 25922	NCTC 10662	ATCC 27853	NCTC 6571	ATCC 25923	ATCC 29213
Lomefloxacin	-	-	-	-	0.5	-	-
Loracarbef	0.5	1	>128	>128	0.5	-	1
Mecillinam	0.12	0.12	8	-	8	-	64
Meropenem	0.015	0.008	2	0.25	0.03	-	0.06
Methicillin	-	-	>128	>128	1	2	2
Mezlocillin	2	-	8	-	0.5	-	-
Moxalactam	0.03	-	8	-	8	-	-
Moxifloxacin	0.03	0.03	2	2	0.06	0.06	0.06
Mupirocin	-	-	-	-	0.25	0.25	0.12
Nalidixic acid	2	4	>128	>128	>128	128	128
Neomycin	-	-	32	-	0.12	-	-
Nitrofurantoin	4	8	-	-	8	-	16
Norfloxacin	0.06	0.06	1	1	0.25	-	1
Ofloxacin	0.06	0.03	1	1	0.25	-	0.5
Oxacillin	-	-	>128	>128	0.25	0.25	0.5
Pefloxacin	0.06	-	0.5	-	0.25	-	-
Penicillin	-	-	>128	>128	0.03	0.03	0.12
Piperacillin	0.5	2	4	2	0.25	-	1
Rifampicin	16	-	-	-	0.004	0.015	0.004
Roxithromycin	-	-	-	-	0.25	0.5	0.5
Rufloxacin	0.5	-	8	-	1	-	-
Sparfloxacin	0.015	0.015	0.5	0.5	0.03	-	-
Sulphonamide	16	-	>128	>128	64	-	-
Teicoplanin	-	-	-	-	0.25	0.5	0.5
Telithromycin	-	-	-	-	0.03	0.06	0.06
Temocillin	2	-	>128	-	128	-	-
Tetracycline	1	2	-	32	0.06	-	0.5
Ticarcillin	1	-	16	-	0.5	-	-
Ticarcillin/ 4mg/L clavulanate	-	-	32	16	-	-	-
Tobramycin	0.25	0.5	0.5	0.5	0.12	-	0.5
Trimethoprim	0.12	0.25	32	-	0.25	-	0.5
Trovafloxacin	0.015	0.015	0.5	0.5	0.015	0.03	0.03
Vancomycin	-	-	-	-	0.5	0.5	1

Table 9: Target MICs (mg/L) for *Pasteurella multocida* control strain by BSAC methods

Antimicrobial agents	*Pasteurella multocida* NCTC 8489
Ampicillin	0.12
Cefotaxime	0.004
Ciprofloxacin	0.008
Penicillin	0.12
Tetracycline	0.25

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	*Bacteroides fragilis* NCTC 9343	*Bacteroides thetaiotaomicron* ATCC 29741	*Clostridium perfringens* NCTC 8359
Metronidazole	0.5	4	8

References

1. Andrews, J.M. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, Suppl S1 to Volume 48 July 2001.

2. Andrews, J. M., Jevons, G., Brenwald, N. and Fraise, A. for the BSAC Working Party on Sensitivity Testing. Susceptibility testing *Pasteurella multocida* by BSAC standardized methodology. *Journal of Antimicrobial Chemotherapy.*

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)

Useful web sites

BSAC	British Society for Antimicrobial Chemotherapy	http://www.bsac.org.uk
SRGA	The Swedish Reference Group for Antibiotics	http://www.srga.org
CDC	Centre for Disease Control (Atlanta, USA)	http://www.cdc.gov
WHO	World Health Organisation (Geneva, Switzerland)	http://www.who.int
CLSI	Clinical and Laboratory Standards Institute	http://www.clsi.org
NEQAS	National External Quality Assessment Scheme	http://www.ukneqas.org.uk
NCTC	National Collection of Type Cultures	http://www.ukncc.co.uk
JAC	The Journal of Antimicrobial Chemotherapy	http://www.jac.oupjournals.org
EUCAST	European Committee on Antimicrobial Susceptibility Testing	http://www.eucast.org