

# Common questions on antimicrobial susceptibility testing

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## 1. Publications on BSAC Methods of Antimicrobial Susceptibility Testing

Title	Author
A Guide to Sensitivity Testing	British Society for Antimicrobial Chemotherapy. A guide to sensitivity testing <i>J Antimicrob Chemother</i> 1991; <b>27</b> Suppl. D: 1-50
History and development of antimicrobial susceptibility testing methodology	Philip F. Wheat <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 1-4
Determination of minimum inhibitory concentrations	Jennifer M. Andrews <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 5-16
Establishing MIC breakpoints and the interpretation of <i>in vitro</i> susceptibility tests	Alasdair P. MacGowan and Richard Wise <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 17-28
The development of the BSAC standardized method of disc diffusion testing	Jennifer M. Andrews <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 29-42
BSAC standardized disc susceptibility testing method	Jennifer M. Andrews for the BSAC Working Party on Susceptibility Testing <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 43-57
Detection of $\beta$ -lactamase-mediated resistance	David M. Livermore and Derek F. J. Brown <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 59-64
Detection of methicillin/oxacillin resistance in staphylococci	Derek F. J. Brown <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 65-70
Quality assurance of antimicrobial susceptibility testing by disc diffusion	Anna King and Derek F. J. Brown <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 71-76
Recommendations for susceptibility tests on fastidious organisms and those requiring special handling	Anna King <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 77-80
Instrumentation in antimicrobial susceptibility testing	David Felmingham and Derek F. J. Brown <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 81-85
Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes	David M. Livermore, Trevor G. Winstanley and Kevin Shannon <i>J Antimicrob Chemother</i> 2001; <b>48</b> , Suppl. S1, 87-102
BSAC standardized disc susceptibility testing method (version 3)	Andrews J.M. <i>J Antimicrob Chemother</i> 2004; <b>53</b> , 713-28
BSAC standardized disc susceptibility testing method (version 4)	Andrews J.M. <i>J Antimicrob Chemother</i> 2005; <b>56</b> , 60-76
BSAC standardized disc susceptibility testing method (version 5)	Andrews J.M. <i>J Antimicrob Chemother</i> 2006; <b>58</b> , 511-29

2. Information on BSAC website (<http://www.bsac.org.uk>)

2.1 **Versions of the BSAC Disc diffusion method for antimicrobial susceptibility testing**

Title	Date
BSAC Standardized Disc Susceptibility Testing Method (version 2)	July 2001
BSAC Standardized Disc Susceptibility Testing Method (version 2.1)	August 2001
BSAC Standardized Disc Susceptibility Testing Method (version 2.1.1)	January 2002
BSAC Standardized Disc Susceptibility Testing Method (version 2.1.2)	August 2002
BSAC Standardized Disc Susceptibility Testing Method (version 2.1.3)	February 2003
BSAC Standardized Disc Susceptibility Testing Method (version 2.1.4)	May 2003
BSAC Standardized Disc Susceptibility Testing Method (version 2.1.5)	November 2003
BSAC Standardized Disc Susceptibility Testing Method (version 3)	January 2004
BSAC Standardized Disc Susceptibility Testing Method (version 3.1)	July 2004
BSAC Standardized Disc Susceptibility Testing Method (version 4)	January 2005
BSAC Standardized Disc Susceptibility Testing Method (version 5)	January 2006
BSAC Standardized Disc Susceptibility Testing Method (version 6)	January 2007
BSAC Standardized Disc Susceptibility Testing Method (version 6.1)	February 2007

2.2 **Additional methodology**

Title
<i>Stenotrophomonas maltophilia</i>
HPA Document – Laboratory Detection & Reporting of Bacteria with Extended-Spectrum Beta-Lactamases
Detection of Extended Spectrum Beta-lactamases (ESBLs) in <i>E. coli</i> and <i>Klebsiella</i> spp.
The use of Etests with BSAC Methodology
MIC Testing of <i>M. catarrhalis</i> with ampicillin/amoxicillin
Testing for dissociated resistance in staphylococci

### 2.3 **Susceptibility Testing Guide**

Updated versions of articles in the Journal of Antimicrobial Chemotherapy Supplement (JAC [2001] **48**, Suppl. S1)

Chapter	Title	Date
1	History and development of antimicrobial susceptibility testing methodology	July 2001
2	Determination of minimum inhibitory concentrations	March 2006 (under review)
3	Establishing MIC breakpoints and the interpretation of <i>in vitro</i> susceptibility tests	January 2005
4	The development of the BSAC standardized method of disc diffusion testing	July 2001
5	BSAC standardized disc susceptibility testing method	Version 5 July 2006
6	Detection of $\beta$ -lactamase-mediated resistance	August 2005
7	Detection of methicillin/oxacillin resistance in staphylococci	July 2001 (under review)
8	Quality assurance of antimicrobial susceptibility testing by disc diffusion	July 2001
9	Recommendations for susceptibility tests on fastidious organisms and those requiring special handling	July 2001
10	Instrumentation in antimicrobial susceptibility testing	July 2001
11	Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes	March 2004

**3. Advice on susceptibility testing not currently available in the formal recommendations**

Topic	Comment
<i>Bacillus</i> spp.	See Andrews J. M. and Wise R. Susceptibility testing <i>Bacillus</i> species. <i>J Antimicrob Chemother</i> 2002; <b>49</b> : 1040-42
<i>Helicobacter</i> spp.	See Appendix 3 in version 6.1, Susceptibility testing of <i>Helicobacter pylori</i> , which gives information on the use of Etest and tentative MIC breakpoints
<i>Listeria</i> spp.	SRGA recommendations (same technique as BSAC) are available, but using disc contents that are higher than those used in the UK. This is under review by the Susceptibility Testing Working Party
<i>B. cepacia</i>	This is under review by the Susceptibility Testing Working Party
Topical antibiotics	There are currently no recommendations (other than mupirocin) because there are few published clinical data to support interpretative criteria. However, this is under review by the Susceptibility Testing Working Party and guidance is likely to be developed based on systemic breakpoints when available and epidemiological breakpoints for other agents. This is in line with the views of the European Medicines Evaluation Agency (EMA)
Susceptibility testing fungi	Not within the remit of the current Susceptibility Testing Working Party. Advice is available from the HPA Fungal Reference Laboratory, Bristol
Susceptibility testing <i>M. tuberculosis</i>	Not within the remit of the current Susceptibility Testing Working Party. Advice is available from the HPA Mycobacterial Reference Laboratory, London

#### 4. Urinary Tract Infections

Question	Answer
Which organisms are included in the recommendations?	Organisms associated with uncomplicated urinary tract infections, i.e. <i>E. coli</i> , <i>P. mirabilis</i> , <i>S. saprophyticus</i> , <i>Enterococcus</i> spp., Group B streptococci
What should be done with complicated infections including <i>S. epidermidis</i> and <i>S. aureus</i> ?	These are usually associated with more serious infections; therefore interpretative criteria for systemic antibiotics should be used.
Are there breakpoints for cotrimoxazole for the treatment of UTIs	See Appendix 1 version 6, “ <i>Testing antimicrobial susceptibility to co-trimoxazole</i> ”, for the UK Committee on Safety of Medicines recommendations.
Can trimethoprim be used for the treatment of UTIs caused by enterococci?	Recommendations were not given previously because it was thought that exogenous folate, present <i>in vivo</i> , but not present <i>in vitro</i> , would lead to false susceptible reports (Washington J. The role of the microbiology laboratory in antimicrobial susceptibility testing. <i>Infect Med</i> 16(8): 531-2 SCP Communications, Inc). However, an exhaustive literature search did not find data to support this hypothesis. Recommendations are now included in the guidelines but the subject is being reviewed again as part of the EUCAST process of harmonisation of breakpoints in Europe.
How should we deal with gaps in the recommendations for “Coliforms”?	Identification to species level is essential for correct interpretation of susceptibility and for applying expert rules. In the absence of a definitive identification, the recommendations most appropriate for the presumptive identification can be used, accepting that on some occasions the interpretation may be incorrect. A more cautious approach is to use the systemic recommendations.
Should laboratories test nalidixic acid alone to detect fluoroquinolone resistance?	Using nalidixic acid alone 25-40% of isolates with LLR to the fluoroquinolones will be reported resistant to ciprofloxacin. Organisms with low level resistance mechanisms are probably susceptible in uncomplicated infections because the concentration of drug in urine is high.

## 5. Respiratory Tract Infections

Question	Answer
How should laboratories interpret the susceptibility of <i>S. pneumoniae</i> to penicillin?	Organisms with penicillin MICs $\leq 1$ mg/L are considered susceptible to $\beta$ -lactam antibiotics except in infections of the central nervous system. Cefotaxime or ceftriaxone MIC determination is advised for strains isolated from meningitis or other invasive infections.
Why are there no disc diffusion test recommendations for <i>S. pneumoniae</i> with trimethoprim?	The trimethoprim MIC <sub>50</sub> and MIC <sub>90</sub> for <i>S. pneumoniae</i> are 8 mg/L and >128 mg/L respectively. The MIC breakpoint is 0.5 mg/L and therefore this organism would not be considered susceptible.
Why are <i>H. influenzae</i> resistant to cefaclor by BSAC disc methodology?	See Appendix 2 version 6, " <i>Efficacy of cefaclor in the treatment of respiratory infections caused by H. influenzae</i> " The conclusion was that <u>the</u> pharmacodynamic data indicate that cefaclor has borderline activity against <i>H. influenzae</i> , even for community use. The outcome of infection will be difficult to predict and susceptibility testing is likely to be of limited value.
How should we deal with borderline results for <i>H. influenzae</i> with ampicillin, amoxicillin, co-amoxiclav and cefuroxime? Zone diameters are often very close to the breakpoint, which means that organisms can be interpreted as susceptible to ampicillin or amoxicillin yet resistant to co-amoxiclav.	This is inevitable when the tail of the susceptible population is very close to the zone diameter breakpoint. Isolates susceptible to ampicillin and amoxicillin will be susceptible to co-amoxiclav.

## 6. Staphylococci

Question	Answer
Can cefoxitin be used to detect resistance in coagulase negative staphylococci?	Currently the recommendations are for <i>S. aureus</i> only.
Can all $\beta$ -lactams be used to detect methicillin resistance in staphylococci?	Only methicillin, oxacillin or cefoxitin should be used for detecting resistance in staphylococci; BSAC only give recommendations for these agents.
If an organism is resistant to methicillin/oxacillin/cefoxitin is it resistant to all of the $\beta$ -lactam antibiotics?	Staphylococci exhibiting resistance to methicillin/oxacillin/cefoxitin should be regarded as resistant to other penicillins, cephalosporins, carbapenems and combinations of $\beta$ -lactam and $\beta$ -lactamase inhibitors.
Is there a need to detect LLR to mupirocin?	Harbath <i>et al</i> (Risk factors for persistent carriage of methicillin-resistant <i>Staphylococcus aureus</i> . Harbath <i>et al</i> <i>Clin Infect Dis</i> 2000 Dec; <b>31</b> (6):1380-5) suggest that there is a need to detect LLR because there is an association with persistence of carriage.
What mupirocin disc content does the BSAC recommend?	A 20 $\mu$ g mupirocin disc which will detect HLR and LLR. An MIC is not needed to determine the level of resistance.
Who supplies 20 $\mu$ g mupirocin discs?	Mast and Oxoid.
Why are there no recommendations for disc diffusion tests for susceptibility of coagulase-negative staphylococci to teicoplanin?	Studies undertaken by the BSAC have shown that in disc diffusion tests there is unacceptable merging of the susceptible and resistant populations. An MIC determination is therefore advised.

## 7. Susceptibility of Enterobacteriaceae to ampicillin, cefalexin and co-amoxiclav

Various combinations of susceptibility of Enterobacteriaceae to ampicillin, cefalexin and co-amoxiclav may be observed, depending on the species and resistance mechanisms involved. The following descriptions are a guide to indicate whether observed phenotypes are likely to be anomalous.

Ampicillin	Phenotype		Comment
	Co-amoxiclav	Cefalexin	
S	S	S	Wild type phenotype for <i>E. coli</i> , <i>P. mirabilis</i> , <i>Salmonella</i> spp. and <i>Shigella</i> spp. Exceptional phenotype for other common species, but occurs in around 2-5% of <i>Enterobacter</i> spp. via mutational loss of AmpC chromosomal $\beta$ -lactamase inducibility.
S	S	R	Uncommon but occurs in <i>E. coli</i> permeability mutants, conferring low level/borderline susceptibility (zone sizes of all may be reduced compared with susceptible isolates). Such mutants should also be resistant to cefoxitin and cefuroxime, though not to third-generation cephalosporins.
S	R	S	Unlikely phenotype. May arise if a <i>Citrobacter freundii</i> or <i>Enterobacter</i> spp. has anomalous AmpC inducibility such that (atypically) cefalexin and ampicillin do not induce AmpC whereas clavulanate does induce. If seen in <i>E. coli</i> , refer to a Reference Laboratory.
S	R	R	Uncommon pattern mostly seen in <i>Providencia</i> spp., but also rarely in <i>Enterobacter</i> spp. and <i>C. freundii</i> isolates. Reflects a situation where clavulanate and cefalexin are strong inducers but ampicillin (for whatever reason) fails to induce as strongly as normally occurs for these species. If seen in other genera or species, refer to a Reference Laboratory.
R	S	S	Low-level penicillinase producer. Wild phenotype of <i>Klebsiella</i> spp., <i>Citrobacter koseri</i> , <i>Citrobacter amalonaticus</i> and <i>Escherichia hermanii</i> . Occurs also for isolates of <i>E. coli</i> , <i>P. mirabilis</i> , <i>Salmonella</i> spp. and <i>Shigella</i> spp. that have low or moderate expression of acquired plasmid-mediated penicillinases.

Phenotype			Comment
Ampicillin	Co-amoxiclav	Cefalexin	
R	S	R	Wild type pf <i>P. vulgaris</i> , <i>P. penneri</i> and <i>C. diversus</i> , reflecting activity of chromosomal $\beta$ -lactamases. May arise for <i>E. coli</i> , <i>Klebsiella</i> spp. <i>P. mirabilis</i> , <i>Salmonella</i> spp. and <i>Shigella</i> spp. with high-level expression of acquired penicillinases, in which case co-amoxiclav zone, though implying susceptibility, is likely to be borderline. May be a low to moderate-level ESBL producer (most or all ESBLs are inhibited by clavulanate, and confer resistance to cefalexin). Not, however, a reliable identification of ESBL production by itself.
R	R	S	May be high-level penicillinase producer, overwhelming co-amoxiclav but leaving cefalexin as borderline susceptible see above also for ampicillin R, co-amoxiclav S cefalexin R. Also arises in <i>E. coli</i> , <i>Klebsiella</i> spp. and <i>P. mirabilis</i> that have acquired penicillinases that are naturally resistant to inhibition by clavulanate ( e.g. OXA types, which account for 1-5% of ampicillin resistance in <i>E. coli</i> ) or which have undergone mutations that confer inhibitor resistance (e.g. inhibitor resistant TEM types, sometimes called IRT enzymes).
R	R	R	Wild phenotype of <i>Enterobacter</i> spp., <i>C. freundii</i> , <i>Serratia</i> spp., <i>Morganella morganii</i> , <i>Providencia</i> spp. owing to inducible AmpC $\beta$ -lactamases. May arise in <i>E. coli</i> , <i>Klebsiella</i> spp., <i>P. mirabilis</i> , <i>C. koseri</i> , <i>C. amalonaticus</i> and <i>E. hermannii</i> owing to strongly expressed acquired penicillinases or ESBLs or to acquired (plasmid-mediated) AmpC enzymes.

## 8. Detection of mechanisms of resistance

Mechanism	Recommendation
ESBLs	See Detection of Extended Spectrum beta-lactamases (ESBLs) in <i>E. coli</i> and <i>Klebsiella</i> spp. ( <a href="http://www.bsac.org.uk">www.bsac.org.uk</a> ) and link to the HPA Document- Laboratory Detection & Reporting of Bacteria with Extended-Spectrum beta-lactamases.
Fluoroquinolone resistance	Generally, a 30 µg nalidixic acid disc will detect resistance in Enterobacteriaceae, <i>H. influenzae</i> , <i>N. gonorrhoeae</i> , <i>N. meningitidis</i> , <i>M. catarrhalis</i> . However, there are isolates of <i>S. typhi</i> and <i>N. gonorrhoeae</i> that are nalidixic acid susceptible but ciprofloxacin resistant. An MIC determination should be undertaken on all Salmonellae isolated from invasive infections and if ciprofloxacin is to be used for treatment of <i>N. gonorrhoeae</i> , that susceptibility to ciprofloxacin should be confirmed for all nalidixic acid susceptible isolates.
β-Lactamase	For <i>H. influenzae</i> , <i>M. catarrhalis</i> and staphylococci see Susceptibility testing Guide ( <a href="http://www.bsac.org.uk">www.bsac.org.uk</a> ) Chapter 6 Detection of β-lactamase mediated resistance. David Livermore or David Livermore's Power Point Presentation from the User Group meeting of October 2004 ( <a href="http://www.bsac.org.uk">www.bsac.org.uk</a> ).
Dissociated resistance to lincosamides	See testing for dissociated resistance, additional methods ( <a href="http://www.bsac.org.uk/-db/-documents/Testing-fordisscit.pdf">www.bsac.org.uk/-db/-documents/Testing-fordisscit.pdf</a> ) For advice on interpretation see footnotes to tables 9, 12 & 13 in the disc diffusion method.

9. *N. gonorrhoeae*

Question	Answer
Can ciprofloxacin be used as first-line therapy?	The 2002 GRASP survey (ref) showed that resistance to ciprofloxacin had risen to 9.8% indicating that the target of >95% efficacy in first-line therapy was no longer achieved.
Is cefuroxime a good choice of antibiotic for treatment?	See Which cephalosporin for gonorrhoeae? Professor Catherine Ison et al on behalf of the North Thames Audit Group (ref) .This report underscores the use of cefixime and ceftriaxone, but finds that cefuroxime is a poor alternative.  Version 6 of the BSAC recommendations states that although cefuroxime is not recommended for clinical use, it can be used as an indicator antibiotic to detect reductions in susceptibility to other oxyimino cephalosporin.
Should nalidixic acid be used to detect fluoroquinolone resistance?	Generally, a 30 µg nalidixic acid disc will detect resistance in <i>N. gonorrhoeae</i> . However, there are isolates of <i>N. gonorrhoeae</i> that are nalidixic acid susceptible but ciprofloxacin resistant. The BSAC recommend that susceptibility to ciprofloxacin should be confirmed on all nalidixic acid susceptible isolates if ciprofloxacin is to be used for treatment.

## 10. Enterococci

Problem	Comment
Failure to detect glycopeptide resistance	The problem is usually solved if plates are incubated for 24 h to allow micro-colonies within the zone edge to be seen more easily. It is helpful to use good light and a hand lens when examining zones of inhibition.

**11. Gradient tests (Etest, AB Biodisk; MICE, Oxoid) by BSAC Methodology**

<b>Question</b>	<b>Answer</b>
Is it possible to use gradient tests with BSAC methodology?	The BSAC method with increased inoculum may be used. See <a href="http://www.bsac.org.uk/db/documents/etest_may_2007.pdf">www.bsac.org.uk/ db/ documents/etest_may_2007.pdf</a>

## 12. Control strains

Question	Comment
There are gaps in the table; when will data be available?	<p>The BSAC has a rolling programme for devising acceptable ranges for control strains. Approximately 30 laboratories around the UK have agreed to test control/antibiotic combinations when requested. Zone data from these centres is combined with data obtained at the BSAC Standardized Method Development Centre (SMDC) in Birmingham to calculate acceptable ranges.</p> <p>The intention of the BSAC to eventually provide data for all control/antibiotic combinations.</p>
When there are no BSAC recommendations; what does the laboratory do?	<p>It is possible to calculate an acceptable range for <i>in-house</i> use until data is published. A programme for calculating an acceptable range is available from the SMDC by emailing <a href="mailto:jenny.andrews@swbh.nhs.uk">jenny.andrews@swbh.nhs.uk</a></p>
If control zones are repeatedly outside the acceptable range; what does the laboratory do?	<p>If laboratories collect zone data for a control/antibiotic combination where BSAC recommendations are not available, please forward the data to the SMDC (see email above) as this can be combined with data from other centres and included in the method.</p>
If control zones are repeatedly outside the acceptable range; what does the laboratory do?	<p>Refer to the section on <i>The Control of Antimicrobial Susceptibility Testing</i> in the current version of the recommendations on disc diffusion testing at <a href="http://www.bsac.org.uk">www.bsac.org.uk</a> Table 1 gives possible sources of error. If the problem is not resolved send data to the SMDC (<a href="mailto:jenny.andrews@swbh.nhs.uk">jenny.andrews@swbh.nhs.uk</a>)</p>

### **13. Templates for interpretation of disc diffusion tests**

Zone diameters in routine tests are most easily interpreted by use of templates of zone diameter breakpoints. A program to prepare customised templates has been written and supported by Trevor Winstanley on behalf of the BSAC Working Party on Antimicrobial Susceptibility Testing.

The BSAC disc diffusion template programme is available on the BSAC web site at [www.bsac.org.uk/susceptibility-testing/bsac-disc-diffusion-template-program.cfm](http://www.bsac.org.uk/susceptibility-testing/bsac-disc-diffusion-template-program.cfm)

#### 14. Miscellaneous questions about BSAC methodology

Question	Comment
Does the laboratory have to use the BSAC methods for preparing inocula?	As long as the method used achieves semi-confluent growth then it does not have to be changed.
Can direct susceptibility testing be undertaken?	Yes as long as semi-confluent growth is achieved. Methods for direct susceptibility testing are given in the current version of the recommendations ( <a href="http://www.bsac.org.uk">www.bsac.org.uk</a> )
Can media other than Oxoid Iso-Sensitest agar be used for testing?	Yes if it shown to have the same performance as Iso-Sensitest.
What does the laboratory do if there are no BSAC recommendations for disc diffusion testing?	Contact the BSAC Standardized Method Development Centre for advice ( <a href="mailto:jenny.andrews@swbh.nhs.uk">jenny.andrews@swbh.nhs.uk</a> )
Can SRGA recommendations be used with BSAC methodology?	The methods of testing are the same and therefore they can be used where there are no BSAC recommendations, but not that some disc contents are different to those used for the BSAC method.