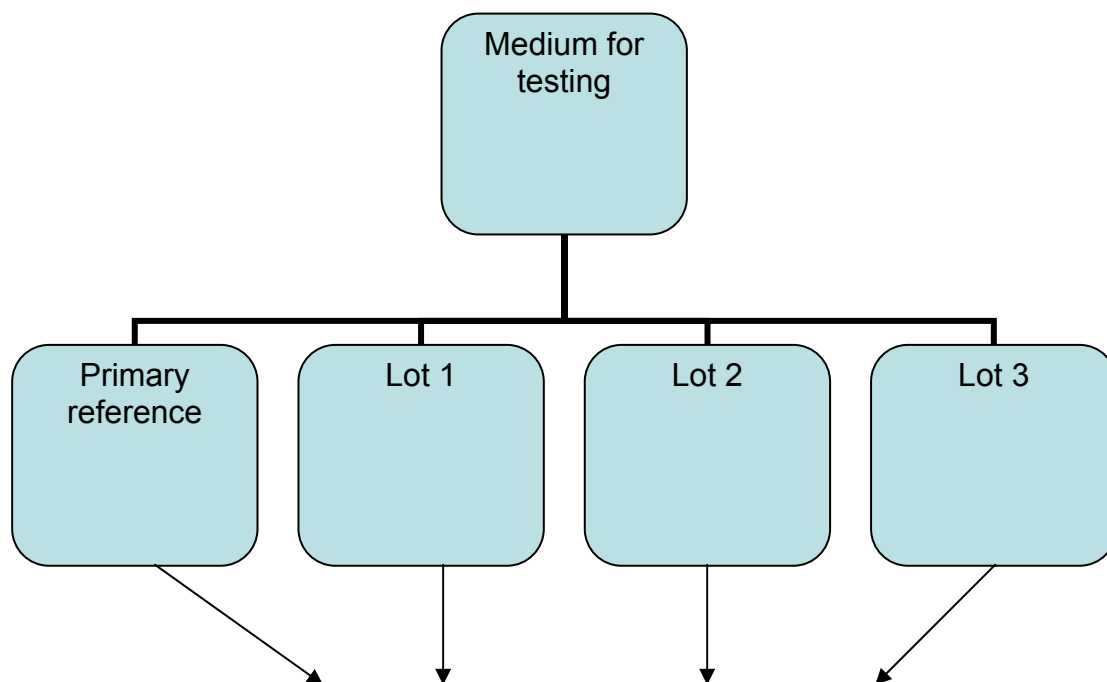


**Manufacturers' protocol for testing production lots of dehydrated media
For use with BSAC methodology**

Manufacturers will be provided with sufficient primary reference media to undertake testing. Manufacturers are asked to provide ten zone diameters from replicate tests for each agent from three lots of their medium and from the primary reference medium. The procedure for testing is as described in the Journal of Antimicrobial Chemotherapy (2001) **48**, Suppl. S1, 43-57. *BSAC standardized disc susceptibility testing method*. Medium is considered satisfactory if 80% of zones are within ± 2 mm mid-point of the acceptable range, for all agents except the following antibiotic/control combination where 70% is satisfactory:

Control strain	Number	Antibiotic	Disc content (μg unless stated)
<i>E. faecalis</i>	ATCC 29212	Gentamicin	200
"	"	Ampicillin	10
<i>E. coli</i>	NCTC 10418	Cefuroxime	30
<i>P. aeruginosa</i>	NCTC 10662	Ciprofloxacin	1
<i>S. aureus</i>	NCTC 6571	Gentamicin	10
<i>S. aureus</i>	ATCC 25923	Penicillin	1 unit
<i>H. influenzae</i>	NCTC 11931	Tetracycline	10
"	"	Chloramphenicol	10
<i>S. pneumoniae</i>	ATCC 49619	Erythromycin	5
"	"	Tetracycline	10

Having submitted satisfactory results for three production lots of medium to the BSAC Working Party on Antimicrobial Susceptibility Testing the manufacturer may label his media as 'conforms to BSAC requirements'. Having submitted acceptable performance data the media manufacturer may continue to label any lot of media meeting the acceptance criteria by 'in-house' testing without submitting further results to the BSAC Working Party on Antimicrobial Susceptibility Testing.



Study design

[modify as other document]

1. Plates poured to a depth of 4mm (± 0.5 mm) will be prepared within 1 day; for the primary reference medium and each of the test lots.
2. Plates will be stored under the same conditions before use.
3. Testing by disc diffusion for the antibiotic/control strain combinations stipulated by the BSAC will be tested on all media on the same day.
4. Ten separate inocula will be prepared following BSAC recommendations.
5. Four plates for the primary reference medium and each of the lots will be inoculated with each inoculum.
6. Discs from the same batch (Oxoid) will be applied to the surface of the agar.
7. Incubation will be as detailed in the BSAC recommendations.

Test procedure

Day 1

1. Thaw frozen stock cultures and subculture onto appropriate media to give single colonies.
2. Prepare primary reference and candidate lots of media following the manufacturers' instructions and cool media to 50⁰C before plates are poured¹. For the preparation of supplemented media (NAD and defibrinated horse blood) it must be ensured that the supplements are added when the media has cooled to 42⁰C.
3. Pour sufficient plates to a depth of 4 mm (± 0.5 mm) and allow to set. Record the pH of the media when set at room temperature (25⁰C).
4. Remove excess moisture from the surface of the media by drying.
5. Store plates in sealed plastic bags (10 plates per bag) at 4⁰C until used.

¹ All media, primary reference and 3 lots of media to be poured within 1 day

Day 2

1. Examine plated cultures of the control strains for purity.
2. Remove agar plates and discs from the refrigerator and allow them to warm to room temperature.
3. Prepare 10 suspensions of the control strains equivalent to a 0.5 McFarland standard as described in the Journal of Antimicrobial Chemotherapy (2001) **48**, Suppl. S1, 43-57. *BSAC standardized disc susceptibility testing method*. Dilute suspensions (see below) before inoculation.

1:100	1:10
<i>E. coli</i> NCTC 10418	<i>S. aureus</i> NCTC 6571
<i>E. faecalis</i> ATCC 29212	<i>S. aureus</i> ATCC 25923
<i>P. aeruginosa</i> NCTC 10662	<i>S. pneumoniae</i> ATCC 49619
<i>H. influenzae</i> NCTC 11931	

NB. Suspensions should be applied to plates within 15 min of preparation

4. Dip a sterile cotton-wool swab into the suspension and remove the excess liquid by turning the swab against the side of the tube. Spread the inoculum evenly over the entire surface of 10 plates each of the primary reference and 3 lots of media, by swabbing in three directions. Allow the plates to dry before applying discs. Discs should be applied to the surface of the agar within 15 min of inoculation.
5. The following discs should be applied:

E. coli NCTC 10418

Ampicillin 10 µg; cefuroxime 30 µg; ceftazidime 30 µg ; trimethoprim 2.5 µg; gentamicin 10 µg ; ciprofloxacin 1µg

P. aeruginosa NCTC 10662

Gentamicin 10 µg; amikacin 30 µg; ceftazidime 30 µg; imipenem 10 µg; colistin 25µg ; ciprofloxacin 1 µg

S. aureus NCTC 6571

Vancomycin 5 µg; teicoplanin 30µg; gentamicin 10 µg; penicillin 1 unit; erythromycin 5 µg; rifampicin 2 µg

S. aureus ATCC 25923

Vancomycin 5 µg; teicoplanin 30µg; gentamicin 10 µg; penicillin 1 unit; erythromycin 5 µg; rifampicin 2 µg

E. faecalis ATCC 29212

Vancomycin 5µg; teicoplanin 30 µg; ampicillin 10 µg; gentamicin 200µg

S. pneumoniae ATCC 49619

Ciprofloxacin 1 µg; oxacillin 1 µg; chloramphenicol 10 µg; tetracycline 10 µg

H. influenzae NCTC 11931

Ampicillin 2 µg; cefuroxime 5 µg; cefotaxime 5 µg; chloramphenicol 10 µg; tetracycline 10 µg

6. Incubate plates in the conditions shown below:

Control strain	Incubation conditions
<i>E. coli</i> NCTC 10418	35-37 ⁰ C in air for 18-20h
<i>E. faecalis</i> ATCC 29212	35-37 ⁰ C in air for 18-20h
<i>P. aeruginosa</i> NCTC 10662	35-37 ⁰ C in air for 18-20h
<i>S. aureus</i> NCTC 6571	35-37 ⁰ C in air for 18-20h
<i>S. aureus</i> ATCC 25923	35-37 ⁰ C in air for 18-20h
<i>S. pneumoniae</i> ATCC 49619	35-37 ⁰ C in 4-6% CO ₂ in air for 18-20h
<i>H. influenzae</i> NCTC 11931	35-37 ⁰ C in 4-6% CO ₂ in air for 18-20h

Day 3

1. Plates must be placed at 4 - 8⁰C after incubation. Groups of 10 plates should be removed from the refrigerator and left at room temperature for 15 min before zones are measured.
2. Zone diameters should be measured to within 0.1 mm using digital callipers or an automated zone reader that has been calibrated with manual measurement. The zone should be measured from the back of the plate against a black non-reflective background with a light source that is above and behind the plate.

**Manufacturer's data sheet for recording zone diameters for control strains
obtained on primary reference media and 3 test lots**

<i>P. aeruginosa</i> NCTC 10662	Amikacin 30 µg	Imipenem 10 µg	Ceftazidime 30 µg	Colistin 25 µg	Gentamicin 10 µg	Ciprofloxacin 1 µg
Primary reference	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 1	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 2	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 3	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

Manufacturers' data sheet for recording zone diameters for control strains obtained on primary reference media and 3 test lots

<i>E. coli</i> NCTC 10418	Ampicillin 10 µg	Cefuroxime 30 µg	Ceftazidime 30 µg	Trimethoprim 2.5 µg	Gentamicin 10 µg	Ciprofloxacin 1 µg
Primary reference	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 1	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 2	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 3	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

**Manufacturer's data sheet for recording zone diameters for control strains
obtained on primary reference media and 3 test lots**

<i>S. aureus</i> NCTC 6571	Vancomycin 5 µg	Teicoplanin 30 µg	Penicillin 1 unit	Erythromycin 5 µg	Gentamicin 10 µg	Rifampicin 2 µg
Primary reference	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 1	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 2	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 3	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

**Manufacturer's data sheet for recording zone diameters for control strains
obtained on primary reference media and 3 test lots**

<i>S. aureus</i> ATCC 25923	Vancomycin 5 µg	Teicoplanin 30 µg	Penicillin 1 unit	Erythromycin 5 µg	Gentamicin 10 µg	Rifampicin 2 µg
Primary reference	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 1	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 2	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
Lot 3	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					

**Manufacturer's data sheet for recording zone diameters for control strains
obtained on primary reference media and 3 test lots**

<i>E. faecalis</i> ATCC 29212	Vancomycin 5 µg	Teicoplanin 30 µg	Ampicillin 10 µg	Gentamicin 200 µg
Primary reference	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
Lot 1	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
Lot 2	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
Lot 3	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			

**Manufacturer's data sheet for recording zone diameters for control strains
obtained on primary reference media and 3 test lots**

<i>S. pneumoniae</i> ATCC 49619	Oxacillin 1 µg	Ciprofloxacin 1 µg	Chloramphenicol 10 µg	Tetracycline 10 µg
Primary reference	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
Lot 1	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
Lot 2	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			
Lot 3	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			
	9			
	10			

**Manufacturer's data sheet for recording zone diameters for control strains
obtained on primary reference media and 3 test lots**

<i>H. influenzae</i> NCTC 11931	Ampicillin 2 µg	Cefuroxime 5 µg	Cefotaxime 5 µg	Chloramphenicol 10 µg	Tetracycline 10 µg
Primary reference	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
Lot 1	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
Lot 2	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
Lot 3	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				