

**Manufacturers' protocol for testing production lots of prepared or pre-poured plated media for use with BSAC methodology.**

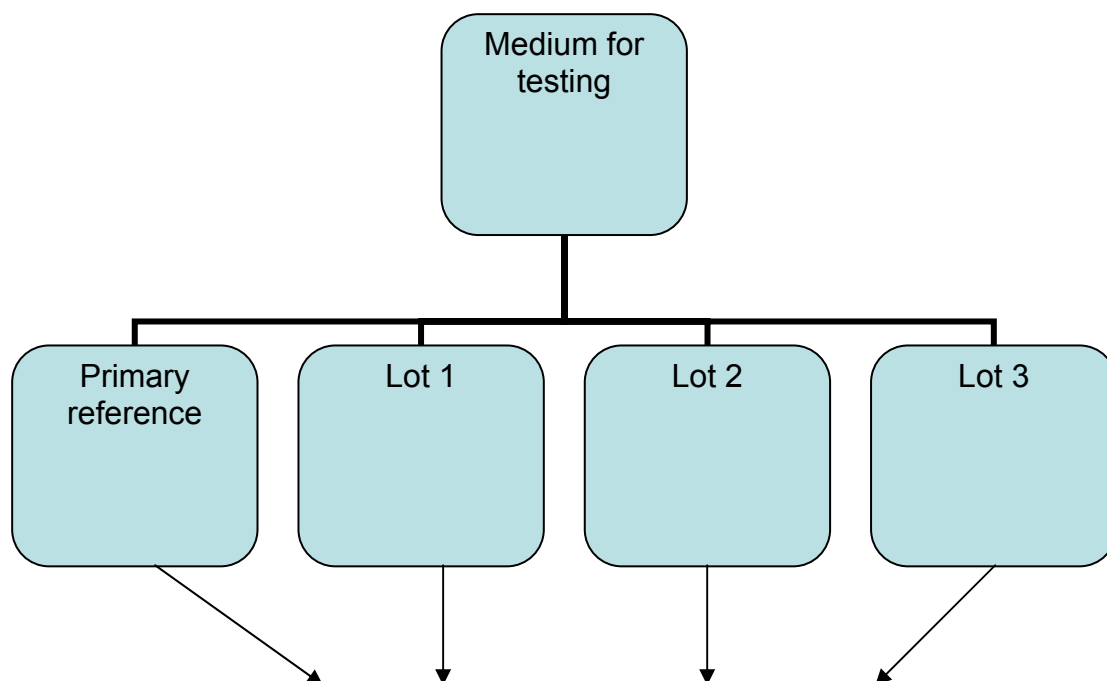
Manufacturers are asked to provide ten zone diameters from replicate tests for each agent from three manufactured lots of their product. Dehydrated medium used to manufacture lots of prepared medium must be from batches designated as conforming to BSAC requirements. The procedure for testing is as described 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  $\pm 2$  mm of the mid-point of the acceptable range, for all agents except the following antibiotic/control combination where 70% is satisfactory:

<b>Control strain</b>	<b>Number</b>	<b>Antibiotic</b>	<b>Disc content (<math>\mu\text{g}</math> unless stated)</b>
<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

**NB.**

The media manufacturer may be asked to provide a sample of one of the lots for examination by the BSAC.

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



### Study design

1. Plates (90mm petri dishes) poured to a depth of 4mm ( $\pm 0.5$  mm) will be prepared from each of the test lots within 1 day. So that uniformity of media processing can also be assessed, the 3 lots of test plates should be prepared from no more than 2 different batches of dehydrated powder (Only dehydrated medium conforming to BSAC requirements to be used).
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. Plates of each of the test lots to 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. Pour sufficient plates to a depth of 4 mm ( $\pm 0.5$  mm) and allow to set following the standard manufacturing protocol for commercially prepared plates, including those requiring supplementation (NAD and defibrinated horse blood)<sup>1</sup>.
3. Record the pH of the media when set at room temperature (25°C).
4. If necessary, remove excess moisture from the surface of the media by drying.
5. Store plates in the standard packaging used for commercially supplied batches at 4°C until use.

<sup>1</sup> All media: 3 lots of test media to be poured within 1 day

Day 2

1. Examine plate 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 suspension (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 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 1 µg

*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 <sup>0</sup> C in air for 18-20h
<i>E. faecalis</i> ATCC 29212	35-37 <sup>0</sup> C in air for 18-20h
<i>P. aeruginosa</i> NCTC 10662	35-37 <sup>0</sup> C in air for 18-20h
<i>S. aureus</i> NCTC 6571	35-37 <sup>0</sup> C in air for 18-20h
<i>S. aureus</i> ATCC 25923	35-37 <sup>0</sup> C in air for 18-20h
<i>S. pneumoniae</i> ATCC 49619	35-37 <sup>0</sup> C in 4-6% CO <sub>2</sub> in air for 18-20h

<b>Control strain</b>	<b>Incubation conditions</b>
<i>H. influenzae</i> NCTC 11931	35-37 <sup>0</sup> C in 4-6% CO <sub>2</sub> in air for 18-20h

*Day 3*

1. Plates must be placed at 4 - 8<sup>0</sup>C 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. Zones on blood containing media should be read from the front of the plate.

**Manufacturers' data sheet for recording zone diameters for control strains  
obtained on 3 test lots of prepared or pre-poured plated media**

P. aeruginosa NCTC 10662		Amikacin 30 µg	Imipenem 10 µg	Ceftazidime 30 µg	Colistin 25 µg	Gentamicin 10 µg	Ciprofloxacin 1 µg
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 3 test lots of prepared or pre-poured plated media**

E. coli NCTC 10418	Ampicillin 10 µg	Cefuroxime 30 µg	Ceftazidime 30 µg	Trimethoprim 2.5 µg	Gentamicin 10 µg	Ciprofloxacin 1 µg
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 3 test lots of prepared or pre-poured plated media**

S. aureus NCTC 6571 10418	Vancomycin 5 µg	Teicoplanin 30 µg	Penicillin 1 unit	Erythromycin 5 µg	Gentamicin 10 µg	Rifampicin 2 µg
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 3 test lots of prepared or pre-poured plated media**

S. aureus ATCC 25923	Vancomycin 5 µg	Teicoplanin 30 µg	Penicillin 1 unit	Erythromycin 5 µg	Gentamicin 10 µg	Rifampicin 2 µg
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 3 test lots of prepared or pre-poured plated media**

<i>E. faecalis</i> ATCC 29212	Vancomycin 5 µg	Teicoplanin 30 µg	Ampicillin 10 µg	Gentamicin 200 µg
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 3 test lots of prepared or pre-poured plated media**

S. pneumoniae ATCC 49619	Oxacillin 1 µg	Ciprofloxacin 1 µg	Chloramphenicol 10 µg	Tetracycline 10 µg
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 3 test lots of prepared or pre-poured plated media**

H. influenzae NCTC 11931	Ampicillin 2 µg	Cefuroxime 5 µg	Cefotaxime 5 µg	Chloramphenicol 10 µg	Tetracycline 10 µg
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				