Table of contents

Section **Page**

Preface to the Second Edition.

Although we presented updates to the CDS Manual at the last three Annual Meetings of the Australian Society for Microbiology and included them subsequently on the CDS web site, we have been persuaded to put out a second edition of the manual. The manual has been renamed "A Manual for Medical and Veterinary laboratories" to reflect its use in both types of laboratories. Much of the information that was presented at the last three workshops has been added to the manual and we have included an expanded section on Quality Assurance with a guide for CDS Users participating in the Royal College of Pathologists of Australasia Quality Assurance Program.

There is now a new section describing the basis of the CDS Test and detailing some features unique to the CDS Test. Also included are the results of additional calibrations that have been carried out with *Corynebacterium* species. In collaboration with Dr. Hazel Mitchell we have developed a susceptibility test for *Helicobacter pylori* and we hope this may be of interest to some members of the CDS Users Group.

We would like to thank all of the CDS Users for their feedback and encouragement in the production of this edition.

Foreword to the First Edition.

Recent surveys of Antibiotic Susceptibility Testing by diagnostic laboratories in Australia demonstrate a high standard in the performance of this important laboratory test. The vast majority of the well performing laboratories use the CDS Method of Antibiotic Susceptibility Testing. The highly satisfactory results are in marked contrast to the poor performance of laboratories in the late sixties and early seventies. It was this unsatisfactory situation which prompted the development of the CDS Method.

The method has been expanded and modified over the last twenty-four years to meet the requirements of modern diagnostic laboratories. Additions and modifications have been presented each year to the CDS Users Group and over the last ten years the Group's meeting has become a regular feature of the ASM Annual Scientific Meetings. It is both fitting and pleasing to see the publication of the Concise Laboratory Manual for Antibiotic Susceptibility Testing by the CDS Method published under the banner of the Australian Society for Microbiology.

With over 65% of laboratories using it, the CDS Method is now accepted as the Australian national method of Antibiotic Susceptibility Testing and it continues to gain recognition internationally. It is also appropriate, therefore, that this Laboratory Manual should be launched at the IXth International Congress of Bacteriology and Applied Microbiology.

Dick Groot Obbink

Past President, ASM

1. *Introduction.*

The first published description of the CDS Test appeared in "Pathology" almost 27 years ago. The method was quickly adopted by diagnostic laboratories in Australasia and it is now the most commonly used method of susceptibility testing in this country. Some years ago the CDS Users Group was formed and the feedback from this group stimulated and assisted in further development of the test. Since the original description of the CDS Test there have been, in addition to the published updates of the method, eleven CDS Newsletters that have been distributed to members of the CDS Users Group.

Over time, several refinements have been introduced into the method and the scope of the CDS Test has been broadened to enable the vast majority of organisms encountered in a diagnostic laboratory to be tested using all of the currently available antimicrobials. Despite these changes the principles underlying the test remain the same. They include, first, the requirement that before any antibiotic can be tested by the method, it must be calibrated, that is, the size of the zones of inhibition observed with each species must be correlated with quantitative values (MIC). Secondly, that in the performance of the test, the operator must adhere closely to the method, as described, thereby reproducing the conditions that pertained at the time of calibration.

Whilst a section on quality assurance is included in the manual, the operator should remember that the most effective single quality assurance measure is to follow the technique assiduously. There is no doubt that somebody will find a simpler or more effective way of performing one or more of the steps in the CDS Test. However, before any improvement can be incorporated into the method it is necessary to confirm that it does not disturb the correlation between zone size and MIC. Therefore we would appreciate comments and suggestions in regard to any aspect of the method. Our contact address and numbers are shown on the front cover of the Manual.

This Manual attempts to put the essential elements contained in previous publications and Newsletters into a concise handbook for the bench worker. It relies heavily on the Tables and Illustrations to assist the reader in following the method and gain maximum information from the CDS Test.

We would welcome criticism of and comments on the Manual as many of the modifications and additions already made were in response to feedback from members of the CDS Users Group. The authors would like to acknowledge the contributions that the Group has made to the development of the CDS Test. Finally, we wish to thank Dr. Groot Obbink who kindly wrote the foreword for the Manual. Over the years, Dr. Groot Obbink has been a great supporter of the CDS Users Group and we sincerely appreciate his assistance in the promotion of the CDS Test with the Australian Society for Microbiology.

2. *Unique features and Basis of the CDS Test.*

Readers are referred to the original monograph on the CDS Test in "Pathology", which is also reproduced on the CDS web site, for a description of the theoretical basis of antibiotic disc testing. Some of the unique features of the CDS Test are described here and in the course of this it is hoped that the derivation of the CDS name will become evident.

Calibration (C) of the Test.

As the "gold standard" of the antibiotic susceptibility of an organism is the minimum inhibitory concentration (MIC) of the antibiotic under test all the methods of susceptibility testing must relate to this value. Moreover, the MIC must be determined by an internationally standardised technique.

The agreed gold standard test is the agar dilution technique originally proposed by Ericcson and Sherris (Ericsson H M & Sherris J C. 1971 Antibiotic Sensitivity Testing. *Acta path. Microbiol. Scand.* **217**, suppl.). Before any antibiotic or bacterial species is included in the CDS Test the test must be calibrated for that particular antibiotic and the targeted species. Calibration consists of plotting the zone sizes observed with a large number of strains of the species included in the CDS Test against the log MIC of each antibiotic.

Dichotomous (D) Separation.

The CDS Test divides and reports antibiotics simply into two categories, "susceptible" and "resistant". We do not recognise "intermediate" as a valid category in the CDS Test. The reasons that we advanced early in the development of the test, and which are still valid, was that, when it varied, the susceptibility of the common pathogens to the then available antibiotics was distributed bi-modally. In those rare cases where some strains were less resistant than others we were able to demonstrate that no method of disc testing had sufficient precision to reliably define an "intermediate" group. In the present era of Evidence Based Medicine (EBM) the strongest case against reporting "intermediate" susceptibility is the dearth of evidence relating to the response to antibiotic therapy of infection caused by these strains. As far as the CDS Test is concerned they are classed as resistant.

Susceptibility (S) and Break Points.

Over time we have adopted the term "susceptibility and susceptible" in preference to "sensitivity and sensitive" when these relate to CDS testing. The reason for this was the introduction of statistical analyses into CDS testing along with most other tests we perform in the clinical laboratory (see below). So as to avoid confusion between "antibiotic sensitivity" and "statistical sensitivity" we changed the former to "antibiotic susceptibility" and the categories of susceptibility to "resistant" and "susceptible".

With many bacterial species if susceptibility to a particular antibiotic varies it naturally divides into one or two groups. In these cases, the MIC's are bi-modally distributed into widely separated values and this is no problem in defining susceptible and resistant categories. With other species and particularly with many newer antibiotics the distribution of MIC's is continuous and separation into categories of susceptibility is made on the basis of an arbitrary break point, irrespective of the method used.

Although there may be some supporting evidence such as clinical response, accepted tissue levels and extrapolation from experience and studies with closely related antibiotics, in the majority of cases break points still are arbitrary values. The break point MIC's of the CDS Test generally are similar to those of other methods. Where we do differ is that we tend to have a more conservative approach and we will select the lower end of the range of break point MIC's as the CDS break point.

Even so, argument about a twofold difference in break points in different methods can only be considered as pseudo-exactitude when it is remembered that the values are determined by a gold standard method of MIC determination which uses doubling dilutions.

Interpretation of Results.

Where possible the CDS Test uses a uniform zone size to define susceptible strains. The susceptible zone size of 6 mm annular radius (18 mm diameter) was not chosen at random but was that point of the diffusion sigmoid curve that enabled the greatest discrimination between susceptible and resistant strains with the majority of antibiotics having a similar diffusion constant. It is worthwhile revisiting here the Humphrey and Lightbown's formula (Humphrey, J. H. & Lightbown, J. W. 1952: A general theory for plate assay of antibiotics with some practical applications. *J. Gen. Microbiol.* **7**: 129) describing diffusion in agar that is reproduced in the original CDS monograph

$$\mathbf{r^2 = 9.21\ Dt\ (logM - log\ 4\pi hDtc)}$$

Where, for our purposes, **r** is the radius of the inhibitory zone, **t** is time from start, **c** is the MIC, **D** is the diffusion constant, **M** is the disc potency and **h** is depth of agar.

The simplest interpretation of this is that the zone size is directly proportional to the diffusion constant and the log of the disc potency and inversely proportional to the log of the MIC.

It can be seen from this formula that with antibiotics of a similar diffusion constant an appropriate adjustment of the disc potency with each antibiotic will result in isolates with different susceptible MIC's to each of the antibiotics yielding a uniform zone size for all susceptible strains.

On the other hand, if the diffusion constant of the antibiotic is markedly reduced and it is not possible to increase the disc potency eg. polymyxin, then the zone cut-off point will need to be reduced.

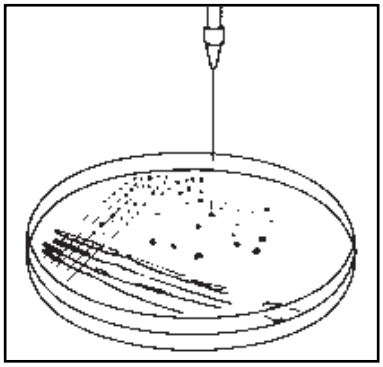
Where one species has a susceptible MIC different from that of the predominant species when tested against a particular antibiotic, the designer of the test has two choices. Either the susceptible zone size can be adjusted eg. gentamicin with *Pseudomonas* versus the *Enterobacteriaceae* (4 mm v/s 6 mm), or the potency of the disc can be changed for that species alone, eg. ampicillin with *Haemophilus influenzae* versus the *Enterobacteriaceae* (5 μg v/s 25 μg).

Performance Characteristics of the CDS Test.

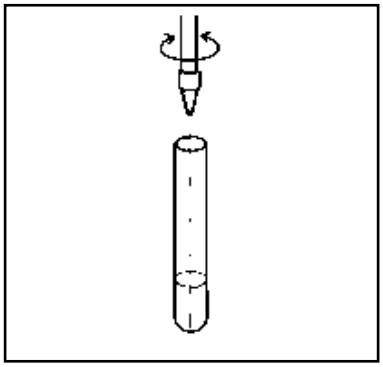
In common with other laboratory tests an assessment can be made of the performance characteristics of the CDS Test. Statistics such as sensitivity, specificity and the predictive value of the CDS Test can be calculated by relating the test results to those obtained with a standardised quantitative method. In susceptibility testing statistical sensitivity measures how well the test correctly identifies "true susceptible" strains whereas specificity refers to the ability of the test to correctly categorise "true resistant" strains. The CDS Test is designed to achieve maximum specificity, ie. the conditions of the test are set to avoid reporting a resistant strain as susceptible.

Laboratory tests rarely can achieve 100 percent sensitivity and specificity. Similarly with the CDS Test it may be necessary to sacrifice some statistical sensitivity to achieve maximum specificity. In practical terms this means that with some calibrations a few marginally susceptible strains may not be correctly identified as such by susceptibility testing. With each calibration we also calculate the positive predictive value (PPV) of the test that measures the percentage of "true susceptibles" versus all susceptibles (true plus false) reported by the test. An acceptable calibration is one where the positive predictive value is over 98 percent.

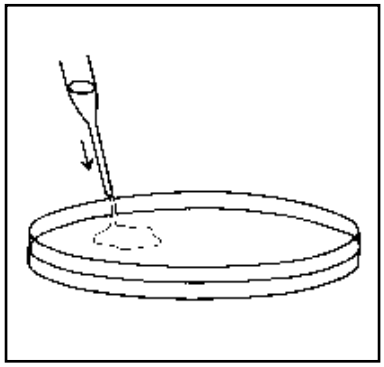
Figure 1. Performance of the CDS Test.



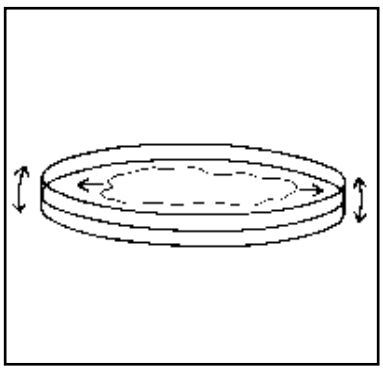
1. Stab the colony



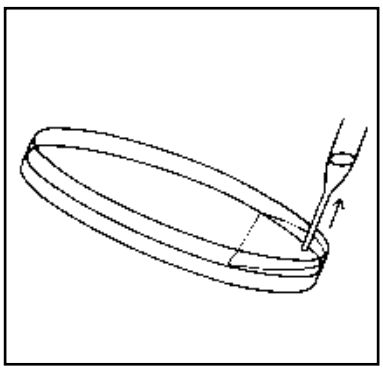
2. Rotate the straight wire



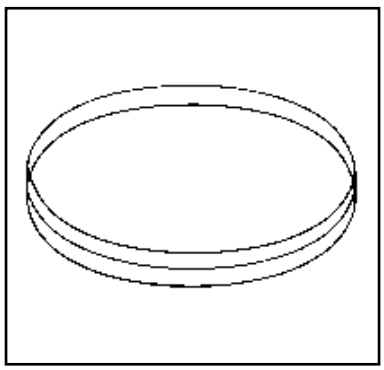
3. Inoculate pre-dried plate



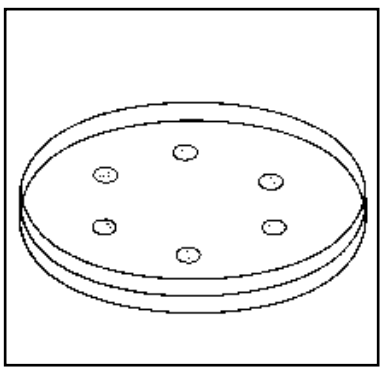
4. Distribute inoculum by rocking



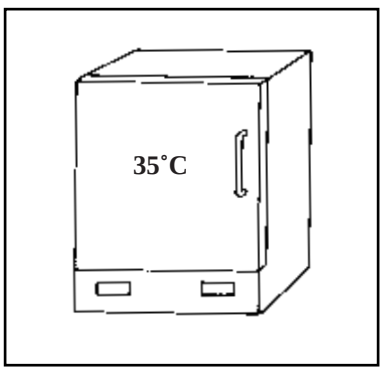
5. Remove excess inoculum



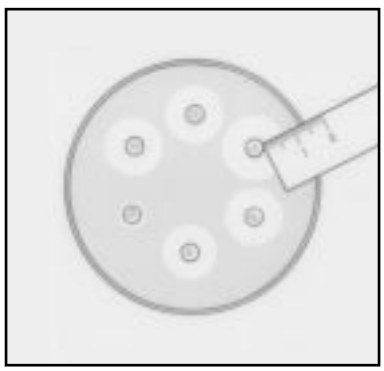
6. Let dry at room temperature (max. 30 min.)



7. Load plate with antibiotic discs



8. Incubate for 18 hours



9. Measure annular radii

3. *Performance of the CDS Test.*

3.1 *Materials.*

The basic medium used in the CDS Test is Sensitest Agar but three other media, Sensitest Agar with 5% horse blood, supplemented Haemophilus Test Medium and chocolate Columbia Blood Agar may be used. The situations where these media are used are listed in Tables 1a, 1b, 1c & 1d. The materials required for the CDS Test are listed below:

- Sensitest Agar (Oxoid CM409).
- Haemophilus Test Medium (HTM) Base (Oxoid CM898B).
- Columbia Blood Agar Base (Oxoid CM331).
- Defibrinated horse blood.
- Fresh or deep frozen solutions of Haematin and Nicotinamide Adenine Dinucleotide (NAD) to supplement HTM Base.
- 90 mm diameter plastic Petri dishes.
- 2.5 ml of sterile isotonic saline in 13 mm x 100 mm test tubes.
- 10 cm of 0.56 mm diameter nichrome wire in a loop holder.

  Available from: Australia Electrical Electronics, 342-350 Parramatta Road, Petersham, NSW 2049, Australia, Tel: (02) 9568 3888, Fax: (02) 9568 3144
- Pasteur pipettes.
- 6 mm diameter antibiotic discs supplied only by Oxoid Pty Ltd or Mast.
- Disc dispenser (maximum of 6 discs) available from Oxoid Pty Ltd or Mast.
- Max/min thermometer.
- Clear plastic mm ruler.

3.2 *Methods.*

The nine steps followed in performing the CDS Test are represented diagrammatically in Figure 1. Further details of particular aspects of the method, including preparations necessary before the performance of the actual test, are set out below:

3.2.1 *Preparation of agar plates.*

- Handle dehydrated media strictly according to the manufacturer's instructions.
- Dispense 20 ml of agar into 90 mm diameter Petri dishes.
- Store agar plates at 4ºC for a maximum of 4 weeks in plastic bags.
- Dry plates face down with the lid removed in an incubator at 35ºC. This will usually take 1 hour in a fan-forced incubator or 2 hours in an ordinary incubator.
- Do not keep any unused dried plates for longer than 2 days and store only in the refrigerator.
- Sensitest Agar containing 5% horse blood is prepared by adding defibrinated horse blood to sterilised Sensitest Agar kept at 50ºC.

- HTM is prepared by adding fresh or deep frozen solutions of Haematin and Nicotinamide Adenine Dinucleotide (NAD) to sterilised HTM Base kept at 50ºC to obtain HTM containing 15 mg/L of each of the two supplements.
- Chocolate Columbia Blood Agar is prepared by adding 8% sterile defibrinated horse blood to Columbia Blood Agar Base sterilised and cooled to 50ºC. The mixture is kept at 70ºC for 30 min for each 1L of agar to obtain "chocolate agar". The medium is cooled to 50ºC and poured.

  Note: Users may purchase commercially prepared media recommended by the CDS Test provided that the preparation by the suppliers complies strictly with the above description.

3.2.2 *Preparation of the inoculum.*

3.2.2.1 *The preferred method.*

- Use an overnight culture preferably grown on blood agar to prepare the CDS inoculum of 10^7cfu/ml. With the straight wire, stab 1 colony (1 to 2 mm in diameter). That should result in bacterial material being visible on the tip of the straight wire as shown in Fig. 2.
- Inoculate the saline by rotating the straight wire at least 10 times with the tip in contact with the bottom of the tube.
- Mix up and down at least 10 times using a Pasteur pipette.

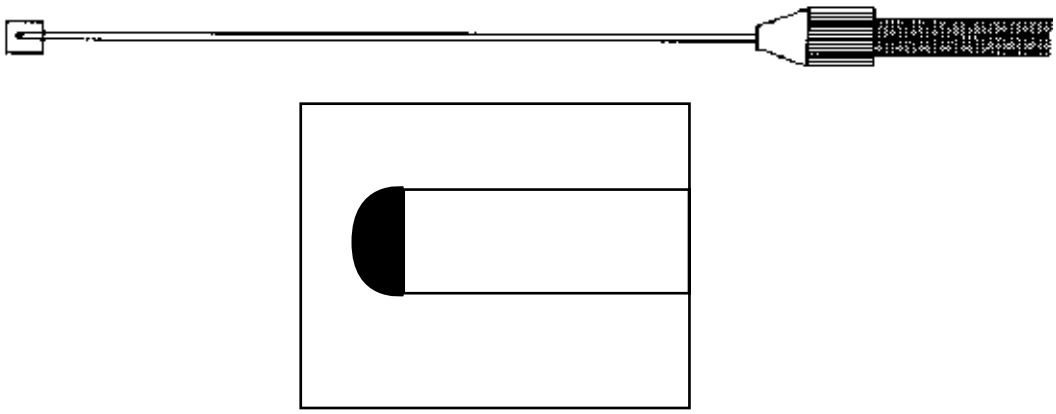


Fig. 2: Diagram showing bacterial material on the tip of the straight wire.

3.2.2.2 *Acceptable, alternative methods of preparation of the inoculum.*

If bacterial material is not visible on the tip of the wire using the preferred method, one of the following methods may be used:

- Stab 3-5 colonies (suitable for small colonies such as streptococci, haemophili etc.).
- Tease the colony apart and pick up bacterial material (suitable for sticky colonies).
- Hold the straight wire at an angle of approximately 45º, move it in one direction along the edge of confluent growth until cellular material is just visible on the tip of the wire. This may be necessary with *Strep. milleri (anginosus)* and *Strep. pneumoniae* and is the least preferable method since there is a possibility that the inoculum may not be pure.

3.2.2.3 *Special circumstances.*

In situations where growth of *Strep. pneumoniae* is scanty ie. there are only a few colonies, the operator can grow the pneumococci in 3 ml peptone water (10 g of peptone plus 5 g sodium chloride in 1 L) prior to testing. To obtain the CDS inoculum of 10^7cfu/ml, suspend 3 colonies (1 mm in diameter) or 6 colonies (0.5 mm in diameter) or 8 colonies (< 0.5 mm in diameter) in 3 ml peptone water and incubate at 35ºC for 4 hours. The turbidity of the bacterial suspension should be visible to the naked eye.

In laboratories without Bunsen burners, to obtain the CDS inoculum of 10^7cfu/ml, CDS Users can proceed as follows. Prepare a suspension in saline to achieve a turbidity equivalent to a 0.5 McFarland Standard or use a spectrophotometer and adjust the suspension to obtain an absorbance of 0.1 at 640 nm. Dilute the suspension 1 in 5 in normal saline and proceed with flooding the plate as usual.

3.2.3 *Inoculation of plates.*

- Flood agar plate, rock the plate to distribute and remove excess.
- Remove the lid and place the plate, uncovered, next to a Bunsen burner to dry. This will usually take 5 to 10 min. Plates must **NOT** be left longer than 45 min.
- Apply no more than 6 antibiotic discs. See Tables 1a, 1b, 1c & 1d for correct disc potencies and QANTAS checklist for correct storage and handling of stock and in use antibiotic discs.

3.2.4 *Incubation of plates.*

Most organisms are incubated at 35ºC overnight in air but there are some exceptions (Tables 1a, 1b, 1c & 1d) and these include the species shown below:

Strep. pneumoniae, Strep. milleri (anginosus), N. meningitidis, H. influenzae, Corynebacterium spp. and *M. catarrhalis*: 35 - 37ºC in 5% CO_2.

Campylobacter spp.: 42ºC in microaerophilic conditions.

Y. enterocolitica: 30ºC in air.

Helicobacter pylori: 35ºC in microaerophilic conditions for 72 hours.

3.2.5 *Reading the zones.*

- Measure the zones from the back of the plate where possible.
- Measure the annular radius, which is the shortest distance from the edge of the disc to the edge of confluent growth. This usually corresponds to the sharpest edge of the zone (Fig. 3).

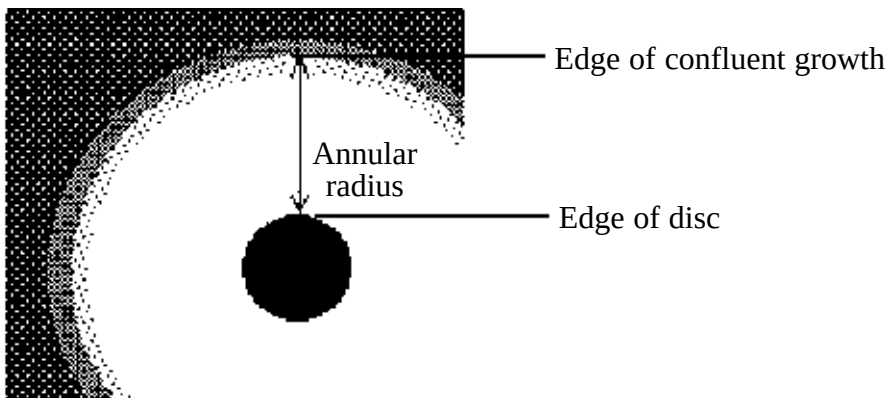


Fig. 3: Diagram showing the annular radius of the zone of inhibition.

3.2.6 *Organisms with special growth requirements.*

Cysteine, thymidine or *glutamine* requiring strains of *Enterobacteriaceae* and *pyridoxal* requiring streptococci can be tested by adding 5 drops of a sterile aqueous solution containing one of the following: cysteine (2000 mg/L), thymidine (5000 mg/L), glutamine (1000 mg/L) or pyridoxal (1000 mg/L) to 2.5 ml of saline before inoculation. Sulphafurazole and trimethoprim cannot be tested in the presence of thymidine.

CO_2 dependent staphylococci and streptococci can be incubated in 5% CO_2 at 35 - 37ºC. The effect of CO_2 on the zone sizes is not sufficient to influence the susceptibility test results.

3.2.7 *Interpretation of results.*

See Tables 1a, 1b, 1c & 1d for the MIC breakpoints and the annular radii of the zones of inhibition for susceptible strains.

Consult Tables 2a, 2b & 2c for the reporting of some antibiotics that are not calibrated.

Standard Interpretation:

Annular radius	**≥ 6 mm**	**=**	**SUSCEPTIBLE**
	< 6 mm	**=**	**RESISTANT**

Exceptions:

Pseudomonas aeruginosa v/s aminoglycosides and polymyxin. *Campylobacter* v/s erythromycin. *Neisseria meningitidis* v/s penicillin. Enterococci v/s ampicillin, chloramphenicol, gentamicin (200 µg) and nitrofurantoin. Streptococci v/s moxifloxacin and gatifloxacin. The interpretation with these species is:

Annular radius	**≥ 4 mm**	**=**	**SUSCEPTIBLE**
	< 4 mm	**=**	**RESISTANT**

The other exception is with vancomycin and teicoplanin where special interpretations apply (Tables 1a & 1 b).

Note: Special applications of the CDS Test are stated in detail in Section 5 and those circumstances where the results of the CDS Test may yield presumptive evidence of the identity of the isolate are included in that section. A summary of the antibiotic testing of each of the major groups of clinically relevant pathogens can be found in Section 6.

4. *Quality Assurance.*

Quality assurance (QA) measures in the CDS Test are incorporated in the method and references to these measures are included in each section of the description. As mentioned earlier, the most important single QA measure is *strict compliance* with the described technique. This attitude is extended to the care and use of proprietary consumables used in the CDS Test. The manufacturer's directions, recommended storage times and conditions must be followed carefully. Many of the QA checks used in the CDS Test are now part of the general routine of NATA accredited laboratories, eg. measuring and recording of refrigerator and incubator temperatures. We have included in this section a trouble shooting guide or checklist to which we gave a catchy name "CDS-QANTAS" in the belief that this would encourage laboratories to copy it and use it where necessary.

4.1 *Reference Strains.*

The performance of a CDS Test with appropriate reference strains is a critical QA measure. Laboratories should regard this aspect as they do "controls" in other types of laboratory testing and an unsatisfactory result with a reference strain will invalidate the results obtained with test strains. It is highly recommended that antibiotic susceptibility tests with the relevant reference strains are performed on the same day that isolates are tested. In laboratories where antibiotic susceptibility tests are performed infrequently, all discs "in use" should be tested with the relevant reference strain at least once a week.

4.1.1 *Obtaining Reference Strains.*

Reference strains used for quality assurance in the CDS Test:

Bacteroides fragilis	ATCC 25285
Campylobacter jejuni	NCTC 11168
Enterococcus faecalis	POW 1994
Escherichia coli	NCTC 10418
Escherichia coli	NCTC 11560 (β-lactamase positive)
Haemophilus influenzae	NCTC 4560
Haemophilus influenzae	NCTC 11315 (β-lactamase positive)
Pseudomonas aeruginosa	NCTC 10662
Staphylococcus aureus	NCTC 6571
Streptococcus pneumoniae	ARL 10582

The reference strains may be obtained from CDS representatives (see list of CDS representative in Australia, New Zealand and South East Asia, Section 9) or from

The Antibiotic Reference Laboratory
Department of Microbiology
The Prince of Wales Hospital
Randwick NSW 2031
Australia.
Tel: (02) 9382 9053
Fax: (02) 9382 9098
Email: smbell@unsw.edu.au
phamj@sesahs.nsw.gov.au

Note: NATA has notified us they intend to make it mandatory that laboratories distributing reference strains are to be accredited. This will result in significant cost and increased workload to the CDS reference and the CDS representatives laboratories. This would be an unfair imposition on these laboratories so that it has become necessary to make alternative arrangements. The Australian Collection of Microorganisms (ACM) at The University of Queensland has kindly agreed to keep the reference strains used in the CDS Test for distribution to CDS Users (see Section 9, CDS Representatives, for the details of the University of Queensland culture repository).

All enquires in regard to this service may be directed to Lucy Rivas or Dr. Lindsay Sly on 07 3365 3211 or via e-mail on l.rivas@mailbox.uq.edu.au

Details of the ACM accession numbers of the reference strains are:

Accession number	Reference Strain	
ACM 5196	*Bacteroides fragilis*	ATCC 25285
ACM 5183	*Campylobacter jejuni*	NCTC 11168
ACM 5184	*Enterococcus faecalis*	POW 1994
ACM 5185	*Escherichia coli*	NCTC 10418
ACM 5186	*Escherichia coli*	NCTC 11560 (β-lactamase positive)
ACM 5187	*Haemophilus influenzae*	NCTC 4560
ACM 5188	*Haemophilus influenzae*	NCTC 11315 (β-lactamase positive)
ACM 5189	*Pseudomonas aeruginosa*	NCTC 10662
ACM 5190	*Staphylococcus aureus*	NCTC 6571
ACM 5191	*Streptococcus pneumoniae*	ARL 10582

4.1.2 *Handling Reference Strains.*

- Upon receipt, immediately subculture the strains onto blood agar (haemophili on chocolate agar).
- Prepare a heavy suspension of an overnight culture in sterile 20% glycerol in nutrient broth.
- Store in cryogenic vials at -20ºC or preferably at -70ºC.
- To recover the culture, use the tip of a Pasteur pipette and take a sample aseptically from the *frozen* suspension, inoculate a suitable medium and return the tube to the freezer immediately.

4.1.3 *Testing Reference Strains.*

Ideally, the appropriate reference strain should be tested at the same time as the clinical isolate or at least once a week to ensure that all components of the test are in good working condition eg. for members of the *Enterobacteriaceae, Acinetobacter* spp. and *Vibrionaceae* test *Escherichia coli* NCTC 10418 and *Escherichia coli* NCTC 11560 (for Timentin, Augmentin and Tazocin only). With daily use, the reference strains are subcultured on artificial medium up to 30 times in a month. Laboratories need to subculture the reference strains from the stock culture kept at -70ºC once a month. ISO 17025 recommends the monthly subculture of reference strains from the stock culture kept at -70ºC to minimise genetic changes. Smaller laboratories without a -70ºC freezer usually perform QA once a week and this is the minimum requirement. The fresh culture plates used for QA are kept at 4ºC and subcultured again one week later for subsequent use. Reference strains will retain their properties if maintained as described. Unlike storage at room temperature, storage at 4ºC results in a decrease in bacterial metabolism and lessens the probability of mutations occurring.

Subculture may be repeated up to 30 times at the end of which the reference strains need to be subcultured from the stock kept at -70ºC. The deep frozen stock cultures must be accessed when the plate culture of the reference strain fails to give zone sizes within the recommended ranges.

QA is also performed with each new batch of antibiotic discs and with each new batch of agar plates. It is unnecessary to duplicate controls, for example, if gentamicin 10 µg or ceftazidime 10 µg discs…etc. are tested against *Escherichia coli* NCTC 10418, there is no need to test them against *Pseudomonas aeruginosa* NCTC 10662 as well and vice versa. Similarly, if antibiotics such as benzylpenicillin 0.5 u, erythromycin 5 µg etc. are tested against *Staph. aureus* NCTC 6571, there is no need to test these against *Strep. pneumoniae* ARL 10582.

4.1.3.1 *Special note on reference strain Bacteroides fragilis ATCC 25285 when performing antibiotic susceptibility testing of Helicobacter pylori.*

Bacteroides fragilis ATCC 25285 is the reference strain used to test metronidazole 5 µg that is calibrated for testing *Helicobacter pylori.*

- Upon receipt, immediately subculture the strain onto blood agar and incubate anaerobically at 35ºC for 24 h or 48 h.
- Prepare a heavy suspension of an overnight culture in sterile 20% glycerol in nutrient broth.
- Store in cryogenic vials at -20ºC or preferably at -70ºC.
- To recover the culture, use the tip of a Pasteur pipette and take a sample aseptically from the frozen suspension, inoculate a suitable medium and return the tube to the freezer immediately.

4.1.3.2 *Testing Bacteroides fragilis ATCC 25285.*

- *Bacteroides fragilis* ATCC 25285 can be tested weekly and each time this is done, cultures must be obtained fresh from the deep frozen stock.
- Subculture the strain onto blood agar and incubate anaerobically at 35ºC for 24 h or 48 h.
- One of the following methods may be used to obtain the inoculum of 10^7 cfu/ml. Stab 3-5 colonies or hold the straight wire at an angle of approximately 45º and move it in one direction along the edge of confluent growth until cellular material is just visible on the tip of the wire.
- Inoculate a blood Sensitest Agar plate and after removal of the excess suspension and allowing the surface of the agar to dry, apply the antibiotic discs and incubate anaerobically at 35ºC for 24 h.

4.1.4 *Measuring and Recording Results with Reference Strains*.

CDS Users are advised to record the actual measurement of the annular radius of each zone of inhibition each time a reference strain is tested. If the records are kept in a cumulative fashion they will draw attention to changing conditions that in their early stages may not lead to results outside the acceptable range but will eventually do so. Disc potency deterioration is the most common example of this and it is possible to detect this before it becomes a problem by observing the gradual reduction in zone sizes on successive observations.

The following table is an example of the method of recording used in the Microbiology Department at SEALS, Randwick and may be of use to some laboratories. The antibiotic disc, its potency and the acceptable zone sizes are shown in bold type.

***Staphylococcus aureus* NCTC 6571** Annular radii (mm)*						
Date	**P 0.5 8.7–13.5**	**MET 5 8.8-12.0**	**E 5 8.6–11.2**	**TE 30 10.6-16.2**	**C 30 7.8–11.4**	**CIP 2.5 9.2–12.4**
6.2.02	12	9.5	9	12.5	9	11
13.2.02	10.5	10	10	13	10	11

*Please circle zone sizes landing outside the acceptable limits.

4.2 *CDS-QANTAS Checklist.*

The CDS Quality Assurance Notations when Testing Antimicrobial Susceptibility checklist is used in "trouble shooting" to define problems revealed by the results observed with the appropriate reference strains in internal QA ie. the annular radius of the zone of inhibition is not within the acceptable range (Tables 3a, 3b & 3c).

QA is performed with the reference organisms under the conditions described in Sections 4.1.3 and 4.1.4. If the QA fails, go through the checklist carefully to define the problem.

CDS-QANTAS CHECKLIST

Organism tested: ..... [Y] or [N]

Medium	Appropriate medium used	[ ]
	90 mm diameter Petri dish used	[ ]
	Dehydrated media used within expiry date	[ ]
	Manufacturer's instructions followed	[ ]
	20 ml of medium in Petri dish	[ ]
	4 mm depth of medium in Petri dish	[ ]
	Poured plate with lid weighs approx. 35 g	[ ]
	Poured plates are stored at 4ºC	[ ]
	Plates used within 4 weeks of preparation	[ ]
Inoculum	0.56 mm diameter wire used	[ ]
	Colony sampled less than 36 hours old	[ ]
	Material visible on tip of wire	[ ]
	Tip of wire not pointed	[ ]
	Tip of wire not corroded	[ ]
	Wire allowed to cool before stabbing colony	[ ]
	Homogeneous suspension	[ ]
	Suspension turbidity visible	[ ]
	Whole plate flooded	[ ]
	Excess suspension removed	[ ]
	Flooded plate should dry within 15 min	[ ]
Antibiotic discs	Stock discs stored at -20ºC	[ ]
	Discs in use stored at 4ºC with active desiccant	[ ]
	Packaging of discs not damaged	[ ]
	Discs used within expiry date	[ ]
	Dispenser at *room temperature* before opening	[ ]
	Desiccant in dispenser *active* *	[ ]
	Positions in dispensers not shared	[ ]
	Correct disc potencies	[ ]
	No more than 6 discs on plate	[ ]
	Antibiotic discs applied within 45 min of flooding	[ ]
	Discs flat on medium	[ ]
Incubation conditions	Correct incubation temperature	[ ]
	Correct atmosphere of incubation	[ ]
	Incubated overnight (min.16 hours)	[ ]
	No more than 5 plates per stack when possible	[ ]
Measuring zones of inhibition	Homogeneous lawn of growth	[ ]
	Satisfactory growth of organism	[ ]
	Measured from edge of disc	[ ]
	Measured to edge of confluent growth	[ ]
	Measured from back of plate (where possible)	[ ]
	Not measured adjacent to another antibiotic disc	[ ]
	Check antibiotics with 2 or 4 mm cut-off	[ ]

* Timentin, Augmentin, and Tazocin discs are highly susceptible to deactivation by humidity and ambient temperature. These discs need to be stored with active desiccant (Bacto Lab. Ph: 02 9602 5499, Merck Microbiology. Ph: 1800 335 571).

4.3 *Notes on External Quality Assurance organised by the Royal College of Pathologists of Australasia Quality Assurance Program.*

CDS Users are reminded to follow the guidelines listed below when participating in the Quality Assurance program (QAP).

- Do not test antibiotics or use discs that have NOT been calibrated for use with the CDS Test.
- If the antibiotic required is not calibrated, look up the "Surrogate Disc Testing" table for the surrogate disc and report **S** or **R** based on the results obtained with the surrogate disc.
- Do not report the susceptibility of any antibiotic that is not calibrated or is not on the "Surrogate Disc Testing" table.
- Read the section relevant to the type of organism or mechanism of resistance in Section 5 when dealing with uncommon mechanisms of resistance.
- Example: If the organism is a member of the *Enterobacteriaceae* such as *Enterobacter cloacae* or *Serratia marcescens* expressing an inducible β-lactamase (flattened zone between cefotaxime 5 µg and imipenem 10 µg), it is known that resistant mutants producing large amounts of the enzyme are present at a high frequency. The report should be R for penicillins, penicillin/inhibitor combinations, cephalosporins (except cefpirome and cefepime), cephamycins and monobactams irrespective of the size of the inhibitory zone. Test and report cefpirome, cefepime, imipenem and meropenem, the antibiotics marked as T in Table 4.

5. *Special applications of the CDS Test.*

The CDS Test has been adapted to handle those circumstances where, because of unusual mechanisms of resistance, testing the susceptibility of isolates may present some difficulties or interpretation of the result is more complex than usual. Special applications of the CDS test are described in detail in this section and those circumstances where the results of the CDS Test may yield presumptive evidence of the identity of the isolate also are included in this section.

5.1 *Streptococci and β-lactam antibiotics.*

5.1.1 *Streptococcus pneumoniae.*

Five enzymes in the cell wall of *Strep. pneumoniae*, the penicillin-binding-proteins (PBP 1A, 1B, 2A, 2B and 2X) are the target sites for β-lactam antibiotics. Increases in the MIC of benzylpenicillin and cefotaxime/ceftriaxone are the result of changes in one or more of the PBPs. Although *Strep. pneumoniae* that is resistant to cefotaxime/ceftriaxone is often resistant to benzylpenicillin, the correlation is not perfect. A similar situation applies with oxacillin and benzylpenicillin. Benzylpenicillin (not oxacillin) and cefotaxime/ceftriaxone are tested separately. Isolates of *Strep. pneumoniae* are divided into 2 categories, those isolated from CSF and those isolated from sites other than CSF ie. sputum, ear and eye swabs and blood cultures not associated with meningitis. Testing and interpretation of the results are different with the two categories:

CSF:

- Benzylpenicillin: Isolates are tested using a benzylpenicillin 0.5 u disc.
  Isolates with an annular radius of the zone of inhibition < 6 mm are reported resistant.
  The MIC is ≥ 0.25 mg/L

- Cefotaxime or ceftriaxone: Isolates are tested using a cefotaxime or a ceftriaxone 0.5 µg disc. Isolates with an annular radius of the zone of inhibition < 6 mm are reported resistant. The MIC is ≥ 0.5 mg/L.

  The MIC may be necessary to define adequately the susceptibility of the strain.

  Sites other than CSF:

  As well as testing the lower potency discs, isolates are tested with an ampicillin 5 µg disc and a higher potency cefotaxime or ceftriaxone 5 µg disc (Table 1b).

- *Benzylpenicillin 0.5 u /ampicillin 5 µg*: If the inhibitory zone is **< 6 mm** with a benzylpenicillin 0.5 u disc and ≥ **6 mm** with an ampicillin 5 µg disc, report the susceptibility as follows: "There is reduced susceptibility to penicillin with the MIC between 0.25 and 2.0 mg/L".

- *Cefotaxime or ceftriaxone 0.5 µg /cefotaxime or ceftriaxone 5 µg*: If the inhibitory zone is **< 6 mm** with a cefotaxime or a ceftriaxone 0.5 µg disc and ≥ **6 mm** with a cefotaxime or a ceftriaxone 5 µg disc, report the susceptibility as follows: "There is reduced susceptibility to cefotaxime (or ceftriaxone) with the MIC between 0.5 and 2.0 mg/L".

5.1.2 *Other streptococci.*

For β-haemolytic streptococci of groups A, B, C, G and *Strep. anginosus (milleri)*, the susceptibility to the penicillins and cephalosporins (except ceftazidime) is extrapolated from the testing of benzylpenicillin 0.5 u (Table 2a).

For all other *Streptococcus* species eg. *Strep. sanguis, Strep. mitis*, if the isolate is resistant to benzylpenicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg, it can be tested against ampicillin 5 µg, cefotaxime 5 µg or ceftriaxone 5 µg. The interpretation of susceptibility is the same as it is for *Strep. pneumoniae*.

The susceptibility to the cephalosporins (other than ceftazidime) eg. cephalothin, cephalexin is extrapolated from the results obtained from the testing of cefotaxime or ceftriaxone 0.5 µg (Table 2a).

Notes.

1. **Gram-positive organisms are resistant to ceftazidime.**
2. **If infective endocarditis is present, the MIC should be determined.**

5.2 *Staphylococci*.

5.2.1.1 *Multiple-resistant, methicillin-resistant Staphylococcus aureus (MRSA).*

With strains of *Staph. aureus* susceptible to methicillin, the inhibitory zone around methicillin 5 µg is clear with the annular radius approximately 8 mm (Plate 1a). By contrast, resistance to methicillin is clearly demonstrated with most clinical isolates of multiple resistant MRSA ie. there is no inhibitory zone or the annular radius of the zone of inhibition is < 6 mm when methicillin 5 µg is tested (Plate 1b). Occasionally, heterogeneous resistance to methicillin may be present where the annular radius of the zone of inhibition is slightly more than 6 mm but there are resistant colonies within the zone of inhibition around methicillin 5 µg. Such isolates should be reported resistant to methicillin irrespective of the zone size. If there is any doubt, incubate the plate for a further 24 h and re-examine for the presence of resistant colonies after a total of 48 h of incubation (Plate 1c).

Note: There are rare strains of MRSA with reduced inhibitory zones ie. 5.5 to 6 mm in annular radius around methicillin 5 µg discs without resistant colonies within the inhibitory zones. These strains only show full resistance to methicillin in the presence of a high salt concentration. When tested on Mannitol Salt Agar (MSA), there is no zone around a methicillin 5 µg disc.

5.2.1.2 *Non multiple-resistant methicillin-resistant Staphylococcus aureus (NMR-MRSA).*

MRSA resistant only to benzylpenicillin and methicillin or non multiple-resistant MRSA (NMR-MRSA) are being isolated more frequently from patients in the community and have been implicated in hospital acquired infections. When tested against methicillin 5 µg there is usually no inhibitory zone or a zone reduced to 2 to 4 mm in annular radius (Plate 2).

5.2.1.3 *Vancomycin resistant Staphylococcus aureus (VRSA).*

The first strain of VRSA was isolated in the USA in 2002 (Center for Disease Control and Prevention. 2002. *Staphylococcus aureus* resistant to vancomycin - United States, 2002. *Morbidity and Mortality Weekly Report*. **51 (26)**: 565-567). It was resistant to oxacillin/methicillin and vancomycin (MIC > 128 mg/L). The isolate contained the *vanA* vancomycin resistance gene from enterococci. With such strains there will be no zone of inhibition around a vancomycin 5 µg disc.

5.2.1.4 *Vancomycin intermediate Staphylococcus aureus (VISA/GISA).*

MRSA with reduced susceptibility to vancomycin and teicoplanin known as VISA or GISA (vancomycin or glycopeptide intermediate *Staphylococcus aureus*) has been described overseas and recently in Australia (Hiramatsu, K. *et al*. 1997. Methicillin-resistant *Staphylococcus aureus* clinical strain with vancomycin reduced susceptibility. *J. Antimicrob Chemother*. **40**:135-6; Ward, P. *et al*. 2001. Treatment failure due to methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to vancomycin, *Med. J. Aust*. **175**: 480-483). These strains do not have the same mechanism of resistance to glycopeptides that occurs in *Enterococcus faecalis* and *Enterococcus faecium.* Electron microscopy shows that the cell wall is thickened and this results in glycopeptide molecules being trapped and prevented from reaching their target sites. The MIC of vancomycin determined by agar dilution for such strains is 4 to 8 mg/L.

Note: On CDS testing, vancomycin and teicoplanin inhibitory zones of vancomycin susceptible staphylococci have a **sharp edge** and an annular radius > 2 mm. With VISA strains, the edge of inhibitory zone around vancomycin 5 µg and teicoplanin 15 µg discs is **hazy** ie. there is fine growth at the edge of the zone and the annular radius of the zone of inhibition is reduced to < 2 mm. The hazy edge and fine growth within the inhibitory zone is more obvious with teicoplanin than vancomycin. If in doubt, incubate for a further 24 h (Plate 3).

With h-VISA, a very low number of cells (hence the name hetero or h-VISA) in the bacterial population has reduced susceptibility to vancomycin but the MIC of vancomycin determined by standard agar dilution is only 2 mg/L. Therefore, detection of the sub-population with reduced susceptibility to vancomycin is a problem. If cells are exposed to a concentration gradient of vancomycin, cells with reduced susceptibility to vancomycin will be visible after 48 or 72 h of incubation. During routine laboratory testing using the CDS Test, h-VISA strains will not be readily detected after the conventional 24 or 48 hours of incubation. It is important to be aware that treatment with a glycopeptide has failed and suspicious that h-VISA is present. In these cases, refer the isolate to specialised laboratories.

5.2.2 *Erythromycin and clindamycin v/s Staphylococcus aureus and MRSA.*

With the CDS Test, *Staph. aureus* is not tested against clindamycin, instead, erythromycin 5 µg is the surrogate disc for reporting the susceptibility to clindamycin (Table 2a). If the organism is **susceptible** to erythromycin, it is reported **susceptible** to clindamycin but if it is **resistant** to erythromycin it is reported **resistant** to clindamycin.

The use of erythromycin 5 µg as the surrogate disc for reporting the susceptibility to clindamycin in *Staph. aureus* is based on four patterns observed when this organism was tested against erythromycin and clindamycin.

1. The isolate was susceptible to erythromycin but when the bacterial population was exposed to erythromycin, resistant mutants arose at a high frequency of 10^{-5} to 10^{-6}. On the contrary, no mutants resistant to clindamycin were selected when 10^9 cfu were exposed to clindamycin. It is possible that certain clinical infections may respond to clindamycin.
2. The isolate was resistant to both erythromycin and clindamycin. Both clindamycin and erythromycin cannot be used for therapy. The resistance to both antibiotics is constitutive and is due to methylation of bacterial 23 S ribosomal RNA.
3. The isolate was resistant to erythromycin whilst it retained apparent susceptibility to clindamycin and only showed resistance to this antibiotic in the presence of erythromycin ie. there was flattening of the inhibitory zone around a clindamycin disc adjacent to that of an erythromycin disc (Plate 4). This type of resistance is also known as dissociated or inducible clindamycin resistance (ICR). When the bacterial population was exposed to clindamycin, mutants resistant to clindamycin arose at a high frequency of 10^{-5} to 10^{-6}. The MIC of clindamycin recorded with such mutants was 16 mg/L. Neither clindamycin nor erythromycin therapy will be successful.
4. The isolate was resistant to erythromycin without ICR and no mutants resistant to clindamycin were selected when 10^9 cfu were exposed to clindamycin. This pattern of susceptibility is present in a small percentage (1 to 2 %) of strains of *Staph. aureus* and is most likely due to selective efflux of erythromycin but not clindamycin from bacterial cells. Clindamycin therapy may be successful under certain clinical circumstances.

5.2.3 *Unusual Staphylococcus aureus.*

5.2.3.1 *Staphylococcus aureus with low β-lactamase activity.*

CDS Users need to remember that the annular radius of the inhibitory zone around benzylpenicillin 0.5 u with β-lactamase negative S*taph. aureus* eg. *Staph. aureus* NCTC 6571, is around 12 mm (Plate 5a).

Some rare strains of *Staph. aureus* produce low levels of β-lactamase and as a result the annular radius of the zone of inhibition around benzylpenicillin 0.5 u may be 4 - 5 mm and the edge of the zone is **sharp** (Plate 5b). However, if the inoculum is low ie. semi confluent, the inhibitory zone may be 6 mm or 7 mm but the edge of the zone is still **sharp**.

5.2.3.2 *Borderline oxacillin-resistant Staphylococcus aureus (BORSA).*

Some strains of *Staph. aureus* with high β-lactamase activity give inhibitory zones approximately 7 mm in annular radius when tested against methicillin 5 µg. Resistant mutant colonies are NOT present within the inhibitory zone after 48 h of incubation and the *mecA* gene is absent. These strains might be found to be resistant when oxacillin is tested, hence the name borderline oxacillin-resistant *Staph. aureus* (BORSA).

Note: Methicillin 5 µg and NOT oxacillin is used for the CDS Test and the CDS Test correctly reports them as susceptible.

5.2.4 *Coagulase-negative staphylococci (CNS).*

5.2.4.1 *Resistance to benzylpenicillin and heterogeneous resistance to methicillin.*

Unlike *Staph. aureus*, heterogeneous resistance to methicillin is very common in coagulase-negative staphylococci. It is important to use the correct CDS inoculum of 10^7 cfu/ml (cellular material must be visible on the tip of the wire) as a light inoculum, will not show the presence of resistant colonies within the inhibitory zone around methicillin. The correct CDS inoculum should give rise to resistant colonies within the zone of inhibition around methicillin when highly heterogeneous methicillin-resistant coagulase negative staphylococci are tested. Isolates that first appear to be susceptible to methicillin 5 µg should be re-incubated for a further 24 hours. Resistance to β-lactams is due to the presence of the *mecA* gene. **The isolate should be reported resistant to penicillin and methicillin.**

5.2.4.2 *Susceptible to benzylpenicillin AND susceptible to methicillin.*

It would appear these isolates are susceptible to both benzylpenicillin and methicillin. However, the MIC of benzylpenicillin is in the order of 0.015 mg/L whilst that of methicillin is in the order of 2 mg/L for such strains. The isolate is more susceptible to penicillin and this should be the antibiotic of choice for treating infections with these strains. It might be prudent to add a comment to the report "Benzylpenicillin or amoxycillin rather than flucloxacillin/dicloxacillin are more likely to be effective against this strain."

5.2.4.3 *Susceptible to benzylpenicillin BUT resistant to methicillin.*

Clinical isolates of coagulase-negative staphylococci may be susceptible to benzylpenicillin (β-lactamase negative) ie. with an inhibitory zone of 7 mm in annular radius around a penicillin 0.5 u disc and the edge of the zone is hazy. The inhibitory zone around a methicillin 5 µg disc is reduced to 4 mm even though the *mecA* gene is absent (Plate 6). ***The isolate is reported susceptible to benzylpenicillin but resistant to methicillin.*** The mechanism of this type of resistance to methicillin is unknown.

5.2.4.4 *Resistant to benzylpenicillin BUT susceptible to methicillin.*

There is no inhibitory zone around a benzylpenicillin 0.5 u disc and the inhibitory zone around a methicillin 5 µg disc is 7-8 mm in annular radius. The isolate appears susceptible to methicillin.

Clinically, we found that deep seated infections such as those associated with prosthetic devices due to penicillin-resistant, apparently methicillin-susceptible, coagulase-negative staphylococci failed to have a sustained response to isoxazolyl penicillins or cephalosporins because of the emergence of resistance.

5.2.4.5 *Resistant to benzylpenicillin AND resistant to methicillin.*

The annular radius of the inhibitory zone around benzylpenicillin 0.5 u and methicillin 5 µg discs is obviously < 6 mm. The isolate is resistant to both antibiotics because of the presence of the mecA gene.

5.2.5 *Rifampicin and fusidate v/s staphylococci.*

Rifampicin and fusidate can be used to treat infections caused by (MRSA) and coagulase-negative staphylococci. The mutation rate to resistance with each antibiotic is high in the order 10^{-5} to 10^{-7} and colonies may be observed within the zones of inhibition around rifampicin 1 µg and fusidate 2.5 µg discs. If the zones of inhibition around rifampicin and fusidate are ≥ 6 mm, report the isolate susceptible to the individual antibiotics. However, it is advisable that a warning such as **"Rifampicin and fusidate must be given in combination since resistance will develop rapidly to either agent if used alone"** be issued when reporting the susceptibility of these two antibiotics.

5.2.6 *Staphylococcus saprophyticus from urine.*

It is recommended that a novobiocin 5 µg disc is included for testing staphylococci isolated from urine specimens. Urine isolates of coagulase-negative staphylococci resistant to novobiocin (annular radius < 4 mm) may be presumptively identified as *Staph. saprophyticus*. *Staph. saprophyticus* is a special case where penicillin 0.5 u and methicillin 5 µg discs are not used for testing. The MIC of benzylpenicillin and methicillin with wild strains of *Staph. saprophyticus* isolated from urine is relatively high when compared with other staphylococci ie. they are intrinsically less susceptible to all penicillins and cephalosporins. Also, some isolates produce very low levels of a non-inducible penicillinase. For these reasons, the annular radius of the inhibitory zone around penicillin 0.5 u and methicillin 5 µg discs recorded with susceptible strains of *Staph. saprophyticus* may be < 6 mm. Ampicillin 5 µg (instead of penicillin 0.5 u) and cephalexin 100 µg (instead of methicillin 5 µg) discs are therefore used for the testing of this species. (Plates 7a, 7b, 7c).

5.3 *Enterococci.*

5.3.1 *Enterococci and ampicillin.*

The annular radius of the zone of inhibition around ampicillin 5 µg discs is ≥ **4 mm** for susceptible strains with the corresponding MIC of ampicillin being ≤ **4 mg/L** for such strains (Table 1a).

In recent years, the appearance of rare β-lactamase producing *E. faecalis* requires CDS Users to follow carefully the instructions when testing ampicillin 5 µg against these strains. There is a **hazy edge** of the zone of inhibition around an ampicillin 5 µg disc with the reference strain *E. faecalis* POW 1994 (Plate 8a) that is very obvious when compared with β-lactamase producing strains where there is a **sharp edge** of the inhibitory zone (Plate 8b).

Note: β-Lactamase-producing isolates have a characteristic zone of inhibition with a **sharp edge** but the annular radius of the zone of inhibition may be > 4 mm. Perform a nitrocefin based test to confirm the presence of β-lactamase and if the enzyme is present, report the isolate **resistant** to ampicillin.

The majority of *E. faecium* are resistant to ampicillin with growth up to the 5 µg disc (Plate 8c). The resistance to ampicillin/benzylpenicillin in *E. faecium* is associated with low affinity penicillin binding proteins (Williamson *et al*. 1985. One or two low affinity penicillin-binding-proteins may be responsible for the range of susceptibility of *Enterococcus faecium* to benzylpenicillin. *J. Gen. Microbiol.* **131**: 1933-1940).

5.3.2 *Vancomycin-resistant enterococci (VRE).*

As a result of the emergence of "low level" vancomycin-resistance in enterococci, important modifications have been introduced into the CDS Test for determining the susceptibility to vancomycin. "Low-level" vancomycin-resistant enterococci are those where greater than 90 % of cells are inhibited at a concentration of 1-2 mg/L of vancomycin whilst the remaining 5-10 % are inhibited at a concentration of 8 mg/L. As a result, there is a marked inoculum effect ie. the higher the inoculum, the higher the MIC. For these reasons, a low inoculum may lead to an error when determining the susceptibility to vancomycin. Therefore, it is essential to use the correct CDS inoculum of 10^7 cfu/ml (cellular material must be visible on the tip of the wire) or a dilution 1 in 5 of a suspension equivalent to McFarland Standard 0.5.

Also, it is essential to **compare** the test strain with the reference strain *Enterococcus faecalis* POW 1994 that has an inhibitory zone > 2 mm around the vancomycin 5 µg disc and the **edge of the zone is sharp**. The interpretation of the susceptibility is based on the characteristics of the inhibitory zone edge as well as the size of the zone. Remember it is important to **examine the edge** of the zone of inhibition around vancomycin.

The 4 patterns seen with enterococci are:

1. The test strain has an inhibitory zone similar to or larger than that of the reference strain and a **sharp edge**. It is **susceptible** to vancomycin (Plate 9a).
2. The test strain has an inhibitory zone with a **hazy edge** ie. fine growth is visible at the edge of the zone. The isolate is a **VRE** with ***vanB*** type resistance. *E. faecium* with low level resistance to vancomycin may have an inhibitory zone up to 4 or 5 mm in annular radius when measured from the edge of confluent growth. (Plate 9b). If in doubt, incubate the plate for a further 24 h. At 48 h, the hazy edge and the fine growth within the zone of inhibition are more obvious when compared with the reference strain.
3. There is growth up to the disc and if the organism is resistant to teicoplanin also, it is a **VRE** with ***van A*** type resistance (Plate 9c).
4. An inhibitory zone **smaller** than that of the reference strain with a **sharp edge** of the zone are typical of *E. gallinarum* and *E. casseliflavus* that possess the natural ***vanC*** type resistance (Plate 9d). Provisionally, these strains are considered resistant to vancomycin although this status is still under discussion. *E. gallinarum, E. casseliflavus* and *E. faecium* are pyruvate negative whilst *E. faecalis* is pyruvate-positive.

   Note: The term VRE refers only to *E. faecalis* and *E. faecium* that have acquired resistance to vancomycin.

   Leuconostoc and *Pediococcus* species have high inherent resistance to both vancomycin and teicoplanin, ie. there is no zone of inhibition observed around either a vancomycin 5 µg or a teicoplanin 15 µg disc (Plate 9e). Unlike enterococci, *Leuconostoc* and *Pediococcus* species lack pyrrolidonyl arylamidase (PYR) activity and therefore, this test can be used to differentiate these species from VRE.

5.3.3 *Nitrofurantoin disc testing and the presumptive identification of Enterococcus faecium.*

Include a nitrofurantoin 200 µg disc when testing enterococci because the appearance of the zone of inhibition around the disc (only applied to susceptible strains ie. those where the annular radius of the zone of inhibition is ≥ 4 mm) can assist in differentiating *E. faecium* from other enterococci.

If the zone of inhibition has a hazy edge with an annular radius of 5 to 7 mm, the isolate is likely to be *E. faecium* (Plate 10a).

If the zone of inhibition has a sharp edge with an annular radius > 6 mm, the isolate is not *E. faecium* (Plate 10b).

Note: With the CDS Test, an enterococcal isolate with no zone of inhibition around an ampicillin 5 µg disc and a hazy edge of the inhibitory zone around a nitrofurantoin 200 µg disc is likely to be *E. faecium*.

5.3.4 *Enterococci and trimethoprim.*

With the CDS Test, the testing of enterococci against trimethoprim is not recommended. *In vitro*, enterococci may appear to be susceptible to trimethoprim but this may not be the case *in vivo*. Enterococci can utilise exogenous dihydrofolate, folinic acid, tetrahydrofolate and thymidine that may be present in the urine and these compounds may antagonise the antibacterial activity of co-trimoxazole or trimethoprim. This may result not only in the failure of therapy of enterococcal urinary infections but also in the development of bacteraemia (Murray, B. E. 1990. The life and time of the *Enterococcus. Clinical Microbiology Reviews*. **3**: 46-65).

5.4 *Corynebacterium species.*

Corynebacterium species have been calibrated against a large number of antibiotics commonly used for the treatment of Gram-positive infections (Table 1a). All species are tested on Sensitest Agar supplemented with 5% horse blood at 35 - 37ºC, in an atmosphere of 5 % CO_2. Slow growing isolates are incubated for 48 h.

If the isolate is resistant to benzylpenicillin 0.5 u, it can be tested against ampicillin 5 µg. If the annular radius of the zone of inhibition is < 6 mm with benzylpenicillin 0.5 u but ≥ 6 mm with ampicillin 5 µg then its susceptibility is reported as "There is reduced susceptibility to penicillin with the MIC between 0.25 mg/L and 2 mg/L."

5.5 *Disc approximation tests.*

CDS Users are advised to use disc approximation tests when testing members of the *Enterobacteriaceae* and *Vibrionaceae*. This involves a strategic positioning of β-lactam antibiotic discs so that extended spectrum β-lactamases (ESBLs) and inducible β-lactamases can be detected.

5.5.1 *Detection of extended-spectrum β-lactamases (ESBLs).*

Most ESBLs are readily inhibited by clavulanic acid, one of the two components in Augmentin (amoxycillin/clavulanate) and Timentin (ticarcillin/clavulanate). If an Augmentin or Timentin disc is placed near a cephalosporin disc eg. cephalexin, cefotaxime or ceftriaxone, the clavulanic acid diffuses out from the Augmentin or Timentin discs and inhibits the ESBL produced by the organism. This allows the cephalosporin to act more effectively and results in an enhanced clear inhibitory zone resembling a "keyhole" or a clear elliptical area between the discs (Plates 11a, 11b).

5.5.2 *Detection of inducible chromosomal β-lactamases.*

Imipenem is an efficient inducer of chromosomal β-lactamases. If an imipenem disc is placed adjacent to a cephalosporin disc eg. cephalexin, cefotaxime, ceftriaxone or a cephamycin disc eg. cefotetan, β-lactamase production is induced by imipenem. This results in enhancement of growth around the cephalosporin/cephamycin disc and the edge of the inhibitory zone is flattened.

Some organisms with an inducible chromosomal β-lactamase may possess an ESBL also. Both types of β-lactamase can be detected by disc approximation tests. Flattening of the zone of inhibition around for example, cefotetan, adjacent to imipenem is indicative of induction of the chromosomal β-lactamase. A "key hole" effect between Augmentin and cefotaxime is indicative of the presence of an ESBL (Plates 12a, 12b).

5.6 *The β-lactamases of members of the Enterobacteriaceae.*

The resistance to ampicillin is due primarily to the production of penicillinase type β-lactamases TEM-1 or TEM-2 in *E. coli, Citrobacter diversus (koseri)* and *Proteus mirabilis* or SHV-1 in *Klebsiella pneumoniae*. These β-lactamases are inhibited by clavulanic acid (Plates 13a, 13b), tazobactam and sulbactam and belong to the *functional group 2b* enzymes (Bush, K, Jacoby, G.A. & Medeiros A.A. 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob. Agents Chemother*. **39**: 1211-1233). *Klebsiella oxytoca* produces a chromosomally mediated β-lactamase (K1). Clavulanic acid inhibits this enzyme but not as well as with most ESBL's (Plate 14a). Some strains of *K. oxytoca* expressing high levels of this enzyme are resistant to ampicillin, cephalexin, the third generation cephalosporins (except ceftazidime) and aztreonam and are therefore similar to members of the *Enterobacteriaceae* that express the extended-spectrum β-lactamases (ESBLs). The susceptibility to ceftazidime and the modest synergy of clavulanic acid contained in either Augmentin or Timentin with cefotaxime resulting in a "tiny keyhole effect" suggest the presence of the K1 β-lactamase (Plate 14b).

5.6.1 *Extended spectrum β-lactamases (ESBLs).*

ESBLs belong to the *functional group 2be* β-lactamases and are derived from the *functional group 2b* enzymes TEM-1, TEM-2 and SHV-1. These β-lactamases are capable of hydrolysing the extended-spectrum cephalosporins and aztreonam but not the cephamycins eg. cefotetan and cefoxitin. However, with cefoxitin, mutants intrinsically resistant to the antibiotic are selected at a high frequency of 10^{-4} to 10^{-5}. The MIC of the third generation cephalosporins (eg. cefotaxime, ceftriaxone, ceftazidime) recorded with the majority of clinical isolates expressing an ESBL is ≥ 2 mg/L. Therefore, with the CDS Test, the MIC of cefotaxime, ceftriaxone and ceftazidime for susceptible strains is ≤ 1 mg/L and most ESBL producing *Enterobacteriaceae* will be found to be **resistant during routine disc testing**. Furthermore, with strategic positioning of a cefotaxime 5 µg disc near an Augmentin 60 µg disc or a Timentin 85 µg disc, the CDS Test will detect the presence of an ESBL, indicated by a clear zone of synergy (a "key hole effect") or an elliptical area of clearing between the two discs (Plates 15a, 15b).

Note: For systemic infections, even if an isolate appears susceptible to Augmentin/ Timentin/Tazocin, DO NOT REPORT IT AS SUSCEPTIBLE. Resistant mutants may be selected during therapy (Table 4).

5.6.2 *Inducible cephalosporinases.*

There are two groups of chromosomally mediated inducible cephalosporinases that are expressed by members of the *Enterobacteriaceae*.

5.6.2.1 *Inducible cephalosporinases of Bush functional group 1 or AmpC β-lactamases.*

Inducible cephalosporinases of *functional group 1*, also known as AmpC β-lactamases, are inhibited by aztreonam but not by clavulanic acid, tazobactam or sulbactam. They are produced by *Enterobacter cloacae*, *Enterobacter aerogenes*, *Serratia marcescens*,

Citrobacter freundii, *Hafnia alvei*, *Aeromonas hydrophila*, *Aeromonas cavia*, *Providencia stuartii*, *Providencia rettgeri* and *Morganella morganii*: the so-called "ESCHAPPM" group of organisms with inducible cephalosporinases. In all the above species, resistant mutants with high β-lactamase activity (is derepressed production of the chromosomal encoded enzyme) are present at a high frequency. (Plates 16a, 16b). As a result, therapy with cephalosporins, cephamycins and monobactams may fail because of the selection of such mutants (Sanders, C.C. & Sanders, W.E. 1979. Emergence of resistance during therapy with newer β-lactam antibiotics: role of inducible β-lactamases and implication for the future. *Review of Infectious Diseases*. **5**: 639-648). With the CDS Test, disc approximation is used to demonstrate the presence of inducible cephalosporinases during routine antibiotic susceptibility testing. The flattened edge of the inhibitory zone around a cefotaxime 5 µg disc adjacent to an imipenem 10 µg disc reveals the presence of an inducible cephalosporinase. Imipenem induces the cells to produce increased levels of β-lactamase that destroys cefotaxime in the area between the two discs. The result is a flattened edge of the inhibitory zone around cefotaxime adjacent to that of imipenem (Plate 16a).

The reporting of β-lactam antibiotic susceptibility with the "ESCHAPPM" group of organisms should follow the recommendations found in Table 4. CDS Users are reminded that, in order to obtain full marks in the Quality Assurance Program (QAP), the reporting of the susceptibility of the "ESCHAPPM" group should be in accord with the recommendations found in this table. Additionally, the detection of an inducible cephalosporinase may assist with the identification of an organism.

5.6.2.2 *Inducible cephalosporinases of Bush functional group 2e.*

Inducible cephalosporinases of *functional group 2e* are inhibited by clavulanic acid, tazobactam and sulbactam and are produced by *Proteus vulgaris* and *Proteus penneri*. These inducible cephalosporinases may be recognised by the flattened edge of the inhibitory zone around a cefotaxime 5 µg disc adjacent to an imipenem 10 µg disc. With the CDS Test, the susceptibility of *Proteus vulgaris* and *Proteus penneri* to Augmentin 60 µg is a useful characteristic to differentiate these species from those producing a β-lactamase of *functional group 1* that confers resistance to Augmentin (Plate 17a). Another useful feature is that derepressed mutants of *Proteus vulgaris* and *P. penneri* producing large quantities of *functional group 2e* β-lactamase are susceptible to ceftazidime (Plate 17b). This is not the case with derepressed mutants of other bacterial species that produce *functional group 1 β-lactamase*.

5.6.3 *Enterobacteriaceae producing an inducible cephalosporinase and an ESBL.*

Members of the *Enterobacteriaceae* that produce an inducible cephalosporinase of *functional group 1* may also possess an ESBL. In strains with low levels of inducible cephalosporinase, there is a typical "key-hole" area of clearing between a cefotaxime 5 µg and an Augmentin 60 µg or a Timentin 85 µg disc. However, the inhibitory zone around either Augmentin or Timentin is < 6 mm in annular radius because of the presence of an inducible cephalosporinase that is not inhibited by clavulanic acid (Plate 18a). On the other hand, high levels of a cephalosporinase may interfere with the detection of an ESBL. In this case, place a cefepime 10 µg disc or an aztreonam 30 µg disc near an Augmentin 60 µg or Timentin 85 µg disc or in the centre of the plate if there is no room on the disc dispenser for an extra disc. The interaction between cefepime or aztreonam and the clavulanic acid in either the Augmentin or Timentin discs reveals the presence of an ESBL (Plate 18b).

5.6.4 *Non-inducible cephalosporinases (plasmid mediated AmpC β-lactamases).*

Plasmid-mediated cephalosporinases of *functional group 1* have arisen through the transfer of genes coding for the chromosomal AmpC β-lactamase onto plasmids. This transfer has resulted in plasmid-mediated cephalosporin resistance in *E. coli*, *Klebsiella pneumoniae* and *Salmonella* spp. (Thomson, K. 2001. Controversies about extended spectrum and AmpC β-lactamases. *Emerging Infectious Diseases*. **7**: 333-336). The substrate profiles of a plasmid-mediated cephalosporinase of *functional group 1* are similar to those of derepressed mutants of *E. cloacae* or *C. freundii* that hyperproduce β-lactamase but this enzyme is not inducible. Amongst the *Enterobacteriaceae, E. coli* is the species most commonly found to carry the plasmid mediated AmpC β-lactamase. *E. coli* may possess low activity of the plasmid mediated AmpC β-lactamase. With these isolates, there is no inhibitory zone around a cephalexin 100 µg disc and one just under 6 mm in annular radius around a cefotaxime 5 µg disc (Plate 19a). With other strains that have high β-lactamase activity, there is no inhibitory zone around the cephalexin 100 µg or cefotaxime/ceftriaxone 5 µg discs (Plate 19b).

5.6.5 *Inhibitor-resistant TEM β-lactamases (IRTs).*

Recently, resistance to Augmentin in some strains of *E. coli* was found to be due to the production of mutant forms of TEM β-lactamase. These new TEM β-lactamases are far less susceptible to clavulanic acid than the original TEM enzyme and are called inhibitor resistant TEM β-lactamases or IRTs. IRT producing *E. coli* are resistant to Augmentin but remain susceptible to cephalexin.

5.7 *Aeromonas spp. and β-lactam antibiotics.*

Two inducible β-lactamases A1 and A2 have been described in *Aeromonas* species. A1 is a cephalosporinase and A2 is a penicillinase/carbapenemase that hydrolyses imipenem and meropenem. A1 is readily identified by the disc approximation test used to detect an inducible cephalosporinase (Plate 20a) and the enzyme is usually found in *A. hydrophila* and *A. caviae*. These species are considered resistant to cephalosporins and cephamycins (cefoxitin and cefotetan). However, aztreonam can be tested since in all strains we have examined so far, we have not observed derepressed mutants on exposure to this β-lactam antibiotic. *A. sobria* usually lacks the inducible cephalosporinase A1 as indicated by the absence of a flattened edge of the inhibitory zone around a cefotaxime 5 µg disc. This species can be tested against cephalosporins, cephamycins and aztreonam (Plate 20b). By contrast, the expression of the A2 enzyme may be heterogeneous and resistance to imipenem and meropenem may not be detectable by any conventional method including determination of the MIC. As a result, false susceptibility to carbapenems might be reported. We recommend that *Aeromonas* spp. should be reported as **resistant** to carbapenems (Table 4).

5.8 *Acinetobacter species.*

For antibiotic susceptibility testing, *Acinetobacter* species can be separated into two groups: the ampicillin-susceptible *A. lwoffi /A. lwoffi*-like group and the ampicillin resistant *A. baumannii /A. baumannii*-like group where a **non-inducible cephalosporinase** is present but derepressed β-lactamase producing mutants are not.

Both groups have a degree of resistance to cephalosporins and this is observed even with the β-lactamase-negative *A. lwoffi /A. lwoffi*-like group where there is an inhibitory zone with an annular radius approximately 8 mm around an ampicillin 25 µg disc but a much smaller zone around a cefotaxime 5 µg disc. This pattern is typical of the *A. lwoffi / A. lwoffi*-like group and may assist with identification (Plates 21a, 21b).

The *A. baumannii /A. baumannii*-like group resistant to ß-lactam antibiotics is readily recognised. Isolates from urine can be recognised by the susceptibility observed with an Augmentin 60 µg disc and the resistance to cephalexin 100 µg together with the resistance to trimethoprim (Plates 22a, 22b).

Notes: Cephalosporinase produced by *Acinetobacter* spp. is not inhibited by clavulanic acid. The susceptibility to Augmentin or Timentin often observed in *Acinetobacter* spp. is due to the combined antibacterial activities of clavulanate and amoxycillin or ticarcillin. The MIC of clavulanic acid ranges from 1 to 2 mg/L with *A. lwoffi /A. lwoffi*-like isolates and from 4 to 32 mg/L with *A. baumannii/A. baumannii*-like isolates.

5.9 *Stenotrophomonas maltophilia.*

There is a high mutation rate (10^{-4} to 10^{-6}) of resistance to β-lactams, aminoglycosides and quinolones with *Steno. maltophilia*. The organism possesses two different β-lactamases: L1, a penicillinase/carbapenemase and L2 that is primarily a cephalosporinase but is able to hydrolyse aztreonam and penicillins also. L2 is inhibited effectively by clavulanic acid whilst L1 is not. ALL isolates of this species should be considered resistant to all antibiotics intended to be used as monotherapy. The drug of choice is co-trimoxazole since wild strains of *Steno. maltophilia* usually are resistant to trimethoprim and susceptible to sulphafurazole but there is still a marked synergy between the two antibiotics. Note that there is usually light growth within the inhibitory zone around sulphafurazole. If this inhibitory zone cannot be seen readily, then repeat the test using a 1/10 dilution of the CDS inoculum. There should be a typical *pear shape zone* of inhibition indicating synergy between sulphafurazole and trimethoprim. The pattern of no zone around an imipenem (or meropenem) disc and the marked synergy between trimethoprim and sulphafurazole suggests that the isolate is likely to be a *Steno. maltophilia* (Plate 23a). If the isolate is resistant to sulphafurazole (Plate 23b), it can be tested against Timentin, aztreonam, ciprofloxacin and moxifloxacin using the criteria set out for *Pseudomonas* species ie. the annular radius of the inhibitory zones for susceptible strains is ≥ 6 mm. A warning such as **"A combination of antibiotics is necessary for successful therapy"** should be issued with the susceptibility report.

5.10 *Haemophilus influenzae and Haemophilus species.*

H. influenzae is tested on Haemophilus Test Medium (HTM) (Oxoid CM 898) supplemented with haematin and NAD prepared freshly from haemin and NAD pure substance, both at a concentration of 15 mg/L in agar. The plates are incubated at 35ºC - 37ºC, in an atmosphere of 5% CO_2.

Rare strains of *H. influenzae* may grow very poorly or not at all on HTM. In that case the organism is tested on chocolate Columbia Blood Agar, not chocolate Sensitest Agar.

5.10.1 *Haemophilus species other than H. influenzae.*

Other *Haemophilus* species such as *H. aphrophilus, H. paraphrophilus* and some *H. parainfluenzae* isolated from blood culture may not grow on Haemophilus Test Medium. These organisms are tested on chocolate Columbia Blood Agar, at 35ºC - 37ºC, in an atmosphere of 5% CO_2.

5.11 *Neisseria meningitidis.*

Neisseria meningitidis is tested on Sensitest Agar supplemented with 5% horse blood, at 35ºC - 37ºC, in an atmosphere of 5% CO_2.

5.11.1 *Neisseria meningitidis and benzylpenicillin.*

Benzylpenicillin 0.5 u has been calibrated for the testing of *N. meningitidis* and the annular radius of the zone of inhibition for susceptible strains is ≥ 4 mm. The MIC of benzylpenicillin for susceptible strains is ≤ 0.25 mg/L and this is lower than the commonly acceptable MIC of 1 mg/L. The benzylpenicillin 0.5 u disc and the low MIC breakpoint of 0.25 mg/L may be used for epidemiological "screening" purposes. Invasive isolates of *N. meningitidis* should be sent to a reference centre for serotyping and confirmation of the identity and antibiotic susceptibility.

5.11.2 *Neisseria meningitidis and rifampicin.*

Rifampicin 1 µg has been calibrated for the testing of *N. meningitidis* and the annular radius of the zone of inhibition for susceptible strains is ≥ 6 mm. The MIC of rifampicin for susceptible strains is ≤ 0.5 mg/L and this is lower than the commonly acceptable MIC. The rifampicin 1 µg disc and the low MIC breakpoint may be used for epidemiological "screening" purposes. Invasive isolates of *N. meningitidis* should be sent to a reference centre for serotyping and confirmation of the identity and antibiotic susceptibility.

5.12 *Helicobacter pylori.*

Amoxycillin, clarithromycin, metronidazole and tetracycline have been calibrated for testing *Helicobacter pylori* on chocolate Columbia Blood Agar in a microaerophilic atmosphere at 35ºC for 72 h. The inoculum is prepared in Brain Heart Infusion broth (not saline) using a 72 h culture of *H. pylori* grown on blood agar. The bacterial suspension should be adjusted to an equivalent 2 McFarland standard and this inoculum gives a lawn of confluent growth (not semi-confluent). Two dried chocolate Columbia Blood Agar plates are flooded with the suspension and the excess is removed. Only 3 antibiotic discs maximum should be placed on a plate since very large zones of inhibition occur with each antibiotic with susceptible strains of *H. pylori*. It is important that the discs be positioned 1 cm from the edge of the plate because if they are any further towards the centre, the large inhibitory zones might run together.

The disc potencies used in the CDS Test are amoxycillin 2 µg, erythromycin 5 µg (this is the surrogate disc for reporting the susceptibility to clarithromycin), metronidazole 5 µg and tetracycline 30 µg.

The annular radius of the inhibitory zone for susceptible strains is ≥ 6 mm for the four antibiotics calibrated (Table 1d).

H. pylori is a difficult organism to work with and dies readily. For this reason, it is not used as a reference strain for Quality Assurance. *Bacteroides fragilis* ATCC 25285 is used as the reference strain to test metronidazole 5 µg on blood Sensitest Agar incubated anaerobically (Table 3c) whilst *Staphylococcus aureus* NCTC 6571 is used as the reference strain to test amoxycillin 2 µg, erythromycin 5 µg and tetracycline 30 µg on Sensitest Agar in air (Table 1b).

6.1. *A Guide to the Use of the Tables.*

There are twenty-one tables (including nine for Veterinary Laboratories) in the manua, each containing information essential for the performance of the CDS Test:

Tables 1a, 1b, 1c, 1d and Veterinary Tables 1a, 1b, 1c, & 1d that are headed "Calibrations 2002" collectively summarise much of the basic information used in the CDS Test. They list all the organisms and antimicrobials tested by the method, the media used, conditions of incubation, disc potencies, cut off sizes for unusual annular radii and the MICs for susceptible strains (breakpoints). The tables are updated regularly and operators should ensure that they are using the latest versions of these tables. It is most important to pay particular attention to the footnotes included with each table as these highlight exceptions, restrictions and some specific directions.

Tables 2a, 2b, 2c and Veterinary Tables 2a, 2b, & 2c that we call "Surrogate Disc Testing 2002", list those antimicrobials where the susceptibility can be inferred from the results obtained with a closely related agent, the "surrogate disc". The table is arranged according to bacterial species and the relationship between the antimicrobials is valid only for the species indicated. This table is also updated regularly as data are accumulated which either invalidate the relationship or enable us to add agents to the list. Laboratories may find it useful to include a comment on the susceptibility report that the result reported with a particular antimicrobial indicates the susceptibility to another.

Tables 3a, 3b, 3c and Veterinary Tables 3a, 3b and 3c list the 10 reference strains, the media and conditions of testing and the expected range of zone sizes observed with each disc of a stated potency. The footnotes explain how the acceptable ranges of zone sizes were derived and recommend the indications for and the frequency of testing the reference strains.

Table 4 is a guide through the maze of testing and reporting the susceptibility of Gram negative species to β-lactam antibiotics. The elaboration of one or more β-lactamases is an important and common mechanism of resistance in these species but for several reasons, resistance may be difficult or impossible to demonstrate by the usual methods of antibiotic susceptibility testing. The table sets out, in some detail, those species where resistance should be assumed on the basis of previous documentation of the presence of a stable mechanism of resistance and those where susceptibility can be reliably demonstrated by the disc test. This table also relies heavily on the footnotes to draw attention to exceptions and special circumstances.

6. TABLES

Table 1a. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
***Corynebacterium* species**			
(Sensitest, CO_2, 35°C) *			
Ampicillin •	5		≤ 2.0
Benzylpenicillin	0.5 u		≤ 0.125
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Rifampicin	1		≤ 0.5
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Vancomycin	5	2 mm	≤ 4.0
Enterococci			
(Blood Sensitest, air, 35°C)			
Ampicillin	5	4 mm Ø	≤ 4.0
Chloramphenicol	30	4 mm	≤ 8.0
Gentamicin	200	4 mm	≤ 512
Linezolid	10		≤ 4.0
Nitrofurantoin +	200	4 mm	≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Vancomycin	5	(See foot note) #	≤ 4.0
***Listeria* spp.**			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		≤ 1.0
Gentamicin	10		≤ 1.0

Ø Perform a nitrocefin based test to detect β-lactamase activity if the zone of inhibition has a sharp edge and an annular radius > 4 mm. β -Lactamase-positive isolates are reported as resistant.

A zone of inhibition with a hazy edge indicates low level resistance to vancomycin (*VanB* type), irrespective of the size of the inhibitory zone.

* Slow growers are incubated for 48 h.

• If a *Corynebacterium* spp. is resistant to penicillin 0.5 u, test ampicillin 5 µg.

\+ For testing urine isolates only.

Table 1b. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
Staphylococci (Sensitest, air, 35°C)			
Ampicillin •	5		≤ 0.5
Benzylpenicillin $	0.5 u		≤ 0.06
Cephalexin •	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Gentamicin	10		≤ 1.0
Kanamycin	50		≤ 8.0
Linezolid	10		≤ 4.0
Methicillin $	5		≤ 4.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Mupirocin	5		≤ 2.0
Nitrofurantoin +	200		≤ 32.0
Rifampicin	1		≤ 0.5
Sulphafurazole	300		≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Trimethoprim	5		≤ 2.0
Vancomycin	5	2 mm	≤ 4.0
Streptococci (Blood Sensitest, air, 35°C) @			
Ampicillin †	5		≤ 2.0
Benzylpenicillin	0.5 u		≤ 0.125
Cefotaxime	0.5		≤ 0.25
Ceftriaxone	0.5		≤ 0.25
Cefotaxime †	5		≤ 2.0
Ceftriaxone †	5		≤ 2.0
Chloramphenicol	30		≤ 8.0
Co-trimoxazole	25		≤ 0.5/9.5
Erythromycin	5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5	4 mm	≤1.0
Nitrofurantoin +	200		≤ 32.0
Rifampicin	1		≤ 0.5
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Vancomycin	5	2 mm	≤ 4.0

+ For testing urine isolates only

$ NOT for testing *Staph. saprophyticus*.

@ *Strep. pneumoniae* & *Strep. anginosus* (*milleri*) are incubated in 5% CO_2.

• ONLY for testing isolate of *Staph. saprophyticus*.

† NOT for testing *Strep. pneumoniae* from CSF. If *Strep. pneumoniae* or any other *Streptococcus* species from a site other than CSF is resistant to penicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg , test ampicillin 5 µg, cefotaxime 5 µg and ceftriaxone 5 µg.

Table 1c. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (μg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
***Enterobacteriaceae*, *Vibrionaceae*, & *Acinetobacter* spp. (Sensitest, air, 35°C)** [#]			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Augmentin •	60		≤16.0/8.0
Aztreonam	30		≤ 8.0
Cefazolin	30		≤16.0
Cefepime	10		≤ 2.0
Cefotaxime	5		≤ 1.0
Cefotetan	30		≤ 8.0
Cefoxitin	30		≤ 8.0
Cefpirome	10		≤ 2.0
Cefpodoxime	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ceftriaxone	5		≤ 1.0
Cefuroxime	30		≤ 8.0
Cephalexin	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin	2.5		≤ 1.0
Enoxacin	10		≤ 4.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Kanamycin	50		≤ 8.0
Meropenem	5		≤ 2.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Nalidixic acid [+]	30		≤ 4.0
Netilmicin	30		≤ 2.0
Nitrofurantoin [+]	200		≤ 32.0
Norfloxacin [+]	10		≤ 4.0
Sulphafurazole	300		≤ 64.0
Tazocin •	55		≤ 16.0/2.0
Tetracycline	30		≤ 4.0
Timentin •	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0
***Pseudomonas* spp. *& Burkholderia* spp. (Sensitest, air, 35°C)**			
Amikacin	30	4 mm	≤ 16.0
Aztreonam	30		≤ 8.0
Cefepime	10		≤ 2.0
Cefpirome	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ciprofloxacin	2.5		≤ 1.0
Gentamicin	10	4 mm	≤ 4.0
Imipenem	10		≤ 4.0
Meropenem	5		≤ 2.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Netilmicin	30	4 mm	≤ 8.0
Norfloxacin [+]	10		≤ 4.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm	≤ 1.0
Sulphafurazole	300		≤ 64.0
Tazocin	55		≤ 16.0/2.0
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm	≤ 4.0
Trimethoprim	5		≤ 2.0

[#] *Yersinia enterocolitica* is incubated in air at 30^0 C. [+] For testing urinary isolates only

• If an ESBL is present, report Augmentin, Timentin and Tazocin for isolates from URINE ONLY.

Table 1d. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

MISCELLANOUS GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
Campylobacter spp. **(Blood Sensitest, microaerophilic, 42°C)**			
Ciprofloxacin	2.5		≤ 1.0
Erythromycin	5	4 mm	≤ 0.5
Gentamicin	10		≤ 1.0
Tetracycline	30		≤ 4.0
Haemophilus influenzae/Haemophilus **spp (HTM[@] agar, 5% CO_2, 35 - 37°C)**			
Ampicillin	5		≤ 1.0
Augmentin	15		≤ 2.0/1.0
Cefaclor	30		≤ 4.0
Cefotaxime	0.5		≤ 0.25
Cefpodoxime	10		≤ 2.0
Ceftriaxone	0.5		≤ 0.25
Cefuroxime	30		≤ 4.0
Chloramphenicol	10		≤ 2.0
Ciprofloxacin	2.5		≤ 1.0
Co-trimoxazole	25		≤ 1.0/19.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
Helicobacter pylori **(Chocolate Columbia Blood Agar, microaerophilic, 35°C**			
Amoxycillin	2		≤ 0.25
Erythromycin	5		≤ 0.5
Metronidazole	5		≤ 4.0
Tetracycline	30		≤ 4.0
Moraxella catarrhalis **(Blood Sensitest, 5% CO_2, 35 - 37°C)**			
Benzylpenicillin	0.5 u		≤ 0.25
Cefaclor	30		≤ 4.0
Cefpodoxime	10		≤ 2.0
Cefuroxime	30		≤ 4.0
Ciprofloxacin	2.5		≤ 1.0
Co-trimoxazole	25		≤ 1.0/19.0
Erythromycin	5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
Neisseria meningitidis **(Blood Sensitest, 5% CO_2, 35 - 37°C)**			
Benzylpenicillin	0.5 u	4 mm	≤ 0.25
Cefotaxime	0.5		≤ 0.25
Ceftriaxone	0.5		≤ 0.25
Chloramphenicol	10		≤ 2.0
Ciprofloxacin	2.5		≤ 1.0
Rifampicin	1		≤ 0.5
Pasteurella multocida **(Blood Sensitest, air, 35°C)**			
Ampicillin	5		≤ 1.0
Ciprofloxacin	2.5		≤ 1.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Tetracycline	30		≤ 4.0
Stenotrophomonas maltophilia **(Sensitest, air, 35°C)**			
Sulphafurazole	300		≤ 64.0

[@] Haemophilus Test Medium containing 15mg/L of freshly prepared haematin and NAD.

Table 2a. Surrogate disc testing 2002. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-POSITIVE ORGANISMS

Antibiotic reported	Surrogate disc used	Disc potency (µg)
Staphylococci (except *S. saprophyticus from urine)*		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Methicillin	5
Azithromycin	Erythromycin	5
Cephalosporins [&]	Methicillin	5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Cloxacillin	Methicillin	5
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Dicloxacillin	Methicillin	5
Flucloxacillin	Methicillin	5
Lincomycin	Erythromycin	5
Norfloxacin [$]	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
***Staphylococcus saprophyticus* from urine**		
Amoxycillin	Ampicillin	5
Augmentin	Cephalexin	100
Benzylpenicillin	Ampicillin	5
Cephalosporins [&]	Cephalexin	100
Cloxacillin	Cephalexin	100
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Dicloxacillin	Cephalexin	100
Flucloxacillin	Cephalexin	100
Norfloxacin [$]	Ciprofloxacin	2.5
Penicillin V	Ampicillin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Streptococci *		
Amoxycillin	Benzylpenicillin	0.5 u
Amoxycillin	Ampicillin †	5
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Benzylpenicillin	Ampicillin †	5
Cephalosporins [&]	Cefotaxime/Ceftriaxone	0.5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Lincomycin	Erythromycin	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30

[&] Ceftazidime is inactive against Gram-positive organisms.

[+] Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

[$] Reporting of norfloxacin is for urine isolates ONLY.

***** For streptococci groups A, B, C, G and *Strep. anginosus*, the susceptibility to penicillin and cephalosporin antibiotics (except ceftazidime) is extrapolated from the testing of benzylpenicillin 0.5 u.

† NOT for testing *Strep.pneumoniae* from CSF. Test if isolate is resistant to penicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg.

Table 2b. Surrogate disc testing 2002. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-POSITIVE ORGANISMS

Antibiotic reported	Surrogate disc used	Disc potency (μg)
***Corynebacterium* species**		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Cephalosporins[&]	Benzylpenicillin	
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Lincomycin	Erythromycin	5
Norfloxacin [$]	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
Enterococci		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
***Listeria* spp.**		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5

[&] Ceftazidime is inactive against Gram-positive organisms.
[$] Reporting of norfloxacin is for urine isolates ONLY.

Table 2c. Surrogate disc testing 2002. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-NEGATIVE ORGANISMS

Antibiotic reported	Surrogate disc used	Disc potency (μg)
***Campylobacter* spp.**		
Tetracyclines	Tetracycline	30
Enterobacteriaceae/ Vibrionaceae/ Acinetobacter spp.		
Amoxycillin	Ampicillin	25
Cephalothin [@]	Ampicillin	25
Ceftriaxone	Cefotaxime	5
Cefotaxime	Ceftriaxone	5
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Piperacillin	Ampicillin	25
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Ticarcillin	Ampicillin	25
***Haemophilus influenzae*/*Haemophilus* spp.**		
Amoxycillin	Ampicillin	5
Cefepime	Cefotaxime/Ceftriaxone	0.5
Cefotaxime	Ceftriaxone	0.5
Cefpirome	Cefotaxime/Ceftriaxone	0.5
Ceftazidime	Cefotaxime/Ceftriaxone	0.5
Ceftriaxone	Cefotaxime	0.5
Cephalexin	Cefuroxime/Cefaclor	30
Tetracyclines	Tetracycline	30
Helicobacter pylori		
Clarithromycin	Erythromycin	5
Moraxella catarrhalis		
Azithromycin	Erythromycin	5
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Cefuroxime/Cefaclor	30
Cephalosporins	Cefuroxime/Cefaclor	30
Clarithromycin	Erythromycin	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
Neisseria meningitidis		
Ampicillin	Benzylpenicillin	0.5 u
Amoxycillin	Benzylpenicillin	0.5 u
Cefotaxime	Ceftriaxone	0.5
Ceftriaxone	Cefotaxime	0.5
Pasteurella multocida		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
Tetracyclines	Tetracycline	30
***Pseudomonas* spp & *Burkholderia* spp.**		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u
Co-trimoxazole [+]	Trimethoprim	5
Co-trimoxazole [+]	Sulphafurazole	300
Stenotrophomonas maltophilia		
Co-trimoxazole	Sulphafurazole	300

[@] Not for *Acinetobacter* spp.

[+] Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

Table 3a. Reference strains 2002. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
***Enterococcus faecalis* POW 1994**		
(Blood Sensitest, air 35°C)		
Ampicillin	5	5.9 - 9.2
Gentamicin	200	6.6 - 9.9
Nitrofurantoin	200	6.1 - 8.7
Teicoplanin	15	3.1 - 5.3
Vancomycin	5	2.0 - 3.7
***Staphylococcus aureus* NCTC 6571**		
(Sensitest, air 35°C)		
Amoxycillin˙	2	9.1 - 11.9
Benzylpenicillin	0.5 u	8.7 - 13.5
Chloramphenicol	30	7.8 - 11.4
Ciprofloxacin	2.5	9.2 - 12.4
Erythromycin	5	8.6 - 11.2
Fusidic acid	2.5	8.6 - 12.6
Gatifloxacin	2.5	10.1 - 14.9
Gentamicin	10	6.6 - 9.4
Kanamycin	50	7.8 - 9.6
Linezolid	10	7.9 - 13.1
Methicillin	5	8.8 - 12.0
Moxifloxacin	2.5	10.9 - 14.5
Nitrofurantoin	200	6.7 - 10.3
Rifampicin	1	9.3 - 12.5
Sulphafurazole	300	9.3 - 13.7
Teicoplanin	15	3.4 - 6.1
Tetracycline	30	10.6 - 16.2
Trimethoprim	5	8.5 - 11.3 &
Vancomycin	5	2.8 - 4.9
***Streptococcus pneumoniae* ARL 10582**		
(Blood Sensitest, 5% CO_2, 35 - 37°C)		
Ampicillin	5	10.8 - 15.2 &
Benzylpenicillin	0.5u	8.3 - 14.8
Cefotaxime	0.5	9.3 - 14.8
Ceftriaxone	0.5	9.1 - 14.3
Chloramphenicol	30	8.0 - 13.2
Co-trimoxazole	25	7.0 - 9.2
Erythromycin	5	7.1 - 12.9
Gatifloxacin	2.5	5.6 - 8.4
Moxifloxacin	2.5	5.6 - 8.6
Rifampicin	1	7.5 - 10.8
Teicoplanin	15	5.1 - 8.0
Tetracycline	30	9.2 - 14.5
Vancomycin	5	5.1 - 8.6

***** The acceptable range (95% confidence limits) is the mean ± 2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

NOTE: Additional testing with reference strains must be performed when:

a. A new batch of medium is used.

b. A new batch of discs is used.

c. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range. ˙ For *Helicobacter pylori* only.

Table 3b. Reference strains 2002. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
Escherichia coli **NCTC 10418** # **(Sensitest, air, 35˚C)**		
Amikacin	30	6.7 - 10.3
Ampicillin	25	7.5 - 10.7
Aztreonam	30	13.7 - 15.9
Cefazolin	30	6.7 - 12.7
Cefepime	10	11.9 - 15.3
Cefotaxime	5	9.7 - 13.7
Cefotetan	30	11.9 - 14.8
Cefoxitin	30	9.8 - 13.0
Cefpirome	10	11.9 - 14.6
Cefpodoxime	10	10.3 - 12.7
Ceftazidime	10	9.3 - 14.1&
Ceftriaxone	5	10.5 - 14.3
Cefuroxime	30	7.5 - 10.1
Cephalexin	100	6.9 - 10.9
Chloramphenicol	30	8.7 - 11.9
Ciprofloxacin	2.5	12.4 - 15.8
Gatifloxacin	2.5	11.2 - 14.8
Enoxacin	10	9.7 - 15.7
Gentamicin	10	6.2 - 9.4
Imipenem	10	10.3 - 13.5
Kanamycin	50	6.2 - 11.8
Meropenem	5	11.0 - 14.4
Moxifloxacin	2.5	10.0 - 13.4
Nalidixic acid	30	8.9 - 12.1
Netilmicin	30	7.7 - 11.3
Nitrofurantoin	200	6.3 - 9.5
Norfloxacin	10	10.4 - 16.4
Sulphafurazole	300	5.0 - 9.4
Tetracycline	30	5.8 - 11.0
Tobramycin	10	6.4 - 8.4
Trimethoprim	5	8.8 - 13.6 &
Escherichia coli **NCTC 11560 (Sensitest, air, 35°C)**		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
Tazocin	55	7.4 - 9.2

* The acceptable range (95% confidence limits) is the mean ± 2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

If antibiotics are tested with *Escherichia coli* NCTC 10418, there is no need to test these against *Pseudomonas aeruginosa* NCTC 10662 as well and vice versa.

NOTE: Additional testing with reference strains must be performed when:

a. A new batch of medium is used.

b. A new batch of discs is used.

c. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

Table 3c. Reference strains 2002. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS CONTINUED

Antibiotic	Disc content (µg)	Acceptable range* (mm)
***Bacteroides fragilis* ATCC 25285 (Blood Sensitest, anaerobic, 35°C)**		
Metronidazole	5	7.1 - 13.5
***Campylobacter jejuni* NCTC 11168 (Blood Sensitest, microaerophilic, 42°C)**		
Ciprofloxacin	2.5	9.2 - 16.9
Erythromycin	5	6.4 - 12.4
Gentamicin	10	7.0 - 11.0
Tetracycline	30	10.3 - 16.0
***Haemophilus influenzae* NCTC 4560 (HTM@ agar, 5% CO_2, 35 - 37°C)**		
Ampicillin	5	7.0 - 11.1
Cefaclor	30	8.1 - 12.1
Cefotaxime	0.5	9.2 - 12.8
Cefpodoxime	10	10.9 - 14.1
Ceftriaxone	0.5	9.1 - 12.9
Cefuroxime	30	8.3 - 12.8
Chloramphenicol	10	11.1 - 14.3 &
Ciprofloxacin	2.5	11.1 - 15.9
Co-trimoxazole	25	9.0 - 12.5
Gatifloxacin	2.5	13.5 - 17.1
Moxifloxacin	2.5	10.6 - 15.2
Tetracycline	30	9.9 - 13.3
***Haemophilus influenzae* NCTC 11315 (HTM@ agar, 5% CO_2, 35 - 37°C)**		
Augmentin	15	7.7 - 10.1
***Pseudomonas aeruginosa* NCTC 10662 # (Sensitest, air, 35°C)**		
Amikacin	30	7.4 - 10.6
Aztreonam	30	8.3 - 13.1
Cefepime	10	8.1 - 11.3
Cefpirome	10	8.1 - 10.6
Ceftazidime	10	7.5 - 11.9
Ciprofloxacin	2.5	8.9 - 14.5
Gatifloxacin	2.5	7.8 - 11.4
Gentamicin	10	5.5 - 9.5
Imipenem	10	7.9 - 10.3
Meropenem	5	9.7 - 14.8
Moxifloxacin	2.5 $	- -
Netilmicin	30	6.4 - 10.4
Piperacillin	50	8.1 - 12.9
Polymyxin	300 u	5.2 - 7.2
Ticarcillin	75	7.3 - 12.1
Tobramycin	10	7.0 - 10.6

***** The acceptable range (95% confidence limits) is the mean ± 2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

@ Haemophilus Test Medium containing 15 mg/L freshly prepared Haematin and NAD.

\# If antibiotics are tested with *Escherichia coli* NCTC 10418, there is no need to test these against *Pseudomonas aeruginosa* NCTC 10662 as well and vice versa.

NOTE: Additional testing with reference strains must be performed when:

a. A new batch of medium is used.

b. A new batch of discs is used.

c. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

$ Moxifloxacin MIC recorded with *Ps. aeruginosa* NCTC 10662 is 2 mg/L and is above the susceptible MIC. Test *E. coli* NCTC 10418 instead.

Table 4, 2002. **A guide for the testing/reporting of β-lactam antibiotics for *Enterobacteriaceae*/ *Aeromonas* spp., *Pseudomonas* spp. /*Burkholderia* spp. and *Stenotrophomonas maltophilia*.**

R = The organism is resistant to the antibiotic because it possesses a mechanism of resistance that may not be demonstrated by disc testing.
T = Can be tested.

Organism/species	Antibiotic								
	AMP	AMC	ATM	CAZ	CXM/ CL	CPD	CPO	CRO	CTT
Inducible β-lactamases present									
Ent. cloacae / Ent. aerogenes	R	R	R	R	R	R	**T**	R	R
Cit. freundii	R	R	R	R	R	R	**T**	R	R
Ser. marcescens	R	R	R	R	R	R	**T**	R	R
Prov. stuartii / Prov. rettgeri	R	R	**T**	R	R	R	**T**	R	R
Morg. morganii	R	R	**T**	R	R	R	**T**	R	R
Prot. vulgaris / Prot. penneri[1]	R	**T**	R	**T**	R	R	**T**	R	**T**
Aeromonas /A2 (most *A. sobria*)	R	R	**T**	**T**	**T**	**T**	**T**	**T**	**T**
Aeromonas /A1 & A2	R	R	**T**	R	R	R	**T**	R	R
Hafnia alvei	R	R	R	R	R	R	**T**	R	R
Enterobacteriaceae with ESBL	R	**T**[2]	R	R	R	R	R	R	**T**
Enterobacteriaceae with inducible β-ses and ESBL	R	R	R	R	R	R	R	R	R
Pseudomonas / *Burkholderia* spp.	R	R	**T**	**T**	R	R	**T**	R	R
Steno. maltophilia	R	R	R	R	R	R	R	R	R

AMP=ampicillin, AMC=Augmentin, ATM=aztreonam, CAZ=ceftazidime, CXM=cefuroxime, CL=cephalexin, CPD=cefpodoxime, CPO=cefpirome, CRO=ceftriaxone, CTT=cefotetan

1. Isolates with high β-lactamase activity may give no zone around CTX 5 μg but show a "key-hole" effect that may be mistaken as an indication of the presence of an ESBL. However, they are susceptible to ceftazidime that can be tested.
2. Test isolates from urine ONLY. Isolates from other sites are considered RESISTANT.

Table 4, 2002 (continued). A guide for the testing/reporting of β-lactam antibiotics for *Enterobacteriaceae*/ *Aeromonas* spp., *Pseudomonas* spp. /*Burkholderia* spp. and *Stenotrophomonas maltophilia*.

R = The organism is resistant to the antibiotic because it possesses a mechanism of resistance that may not be demonstrated by disc testing.
T = Can be tested.

Organism/species	Antibiotic								
	CTX	FEP	FOX	IPM	KZ	MEM	PRL	TIM	TZP
Inducible _-lactamases present									
Ent. cloacae / Ent. aerogenes	R	**T**	R	**T**	R	**T**	R	R	R
Cit. freundii	R	**T**	R	**T**	R	**T**	R	R	R
Ser. marcescens	R	**T**	R	**T**	R	**T**	R	R	R
Prov. stuartii / Prov. rettgeri	R	**T**	R	**T**	R	**T**	R	R	**T**
Morg. morganii	R	**T**	R	**T**	R	**T**	R	R	**T**
Prot. vulgaris / Prot. penneri[1]	R	**T**	**T**	**T**	R	**T**	R	**T**	**T**
Aeromonas /A2 (most *A. sobria*)	**T**	**T**	**T**	R	**T**	R	R	R	R
Aeromonas /A1 & A2	R	**T**	R	R	R	R	R	R	R
Hafnia alvei	R	**T**	R	**T**	R	**T**	R	R	R
Enterobacteriaceae with ESBL	R	R	R	**T**	R	**T**	R	**T**[2]	**T**[2]
Enterobacteriaceae with inducible β-ses and ESBL	R	R	R	**T**	R	**T**	R	R	R
Pseudomonas / *Burkholderia* spp.	R	**T**	R	**T**	R	**T**	**T**	**T**	**T**
Steno. maltophilia	R	R	R	R	R	R	R	R	R

CTX=cefotaxime, FEP=cefepime, FOX=cefoxitin, IPM=imipenem, KZ=cefazolin, MEM=meropenem, PRL=piperacillin, TIM=Timentin, TZP=Tazocin.

1. Isolates with high β-lactamase activity may give no zone around CTX 5 but show a "key-hole" effect that may be mistaken as an indication of the presence of an ESBL. However, they are susceptible to ceftazidime that can be tested.
3. Test isolates from urine ONLY. Isolates from other sites are considered RESISTANT.

7. *Special section for Veterinary Laboratories.*

Veterinary Tables, 2002.

These tables are prepared for veterinary laboratories by adding antibiotics that are used only in veterinary medicine to the general tables of calibrations.

Veterinary Tables 1a & 1b. Calibrations for Gram-positive organisms.

These tables list the antibiotics calibrated for the Gram-positive organisms. Note that not all antibiotics calibrated are used in veterinary medicine. For each group of organisms, the media and the incubation conditions are shown in brackets under the group name. For example "Blood Sensitest, air, 35ºC @" for streptococci means the media and the incubation conditions recommended for streptococci are Sensitest Agar supplemented with horse blood and incubation is in air at 35ºC. The flag @ indicates that *Strep. pneumoniae & Strep. anginosus (milleri)* are incubated in 5% CO_2. For each antibiotic, the disc potency and the MIC for susceptible strains are indicated clearly. If requested, the MIC ≤ … mg/L may also be reported for susceptible strains. When there is an exception to the standard interpretation, it is clearly marked. For example, when testing enterococci against vancomycin, CDS Users need to see the foot note # that says: A zone of inhibition with a hazy edge indicates low level resistance to vancomycin (*Van B* type) irrespective of the size of the inhibitory zone.

Veterinary Tables 1c & 1d. Calibrations for Gram-negative organisms.

These tables list the antibiotics calibrated for Gram-negative organisms, namely members of the *Enterobacteriaceae, Vibrionaceae & Acinetobacter* spp., *Pseudomonas* spp. *& Burkholderia* spp., *Pasteurella multocida, Campylobacter* spp., *Haemophilus* spp., *Helicobacter pylori, Moraxella catarrhalis, Neisseria meningitidis* and *Stenotrophomonas maltophilia*.

For organisms not included in the Table of Calibrations, extrapolate the testing from that established for similar organisms. Examples: *Erysipelothrix* spp. from streptococci in CO_2. *Pasteurella* spp. from *Pasteurella multocida*.

Veterinary Tables 2a, 2 b & 2c. Surrogate Disc Testing.

Surrogate disc testing is unique to the CDS method. It allows the reporting of other antibiotics of the same class based on the susceptibility results of an antibiotic disc known as the surrogate disc. For example, the susceptibility of staphylococci to amoxycillin/ampicillin is inferred from the results recorded with benzylpenicillin 0.5 u. If the annular radius of the zone of inhibition is < 6 mm, the organism is **resistant** to benzylpenicillin, amoxycillin and ampicillin. Do not use an ampicillin disc since this is not calibrated for testing staphylococci. Similarly, when testing staphylococci, if the annular radius of the zone of inhibition around a methicillin 5 µg disc is > 6 mm, the organism is **susceptible** to methicillin, cloxacillin, flucloxacillin, Augmentin, ceftiofur and other cephalosporins (except ceftazidime) such as cephalothin and cefazolin.

The susceptibility to **ceftiofur** is obtained from surrogate testing a variety of other antibiotics (Veterinary Tables 2a, 2b & 2c.)

The susceptibility to **enrofloxacin** is obtained from surrogate testing ciprofloxacin (Veterinary Tables 2a, 2b & 2c.).

Veterinary Table 1a. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
***Corynebacterium* species**			
(Sensitest, CO_2, 35°C) *			
Ampicillin •	5		≤ 2.0
Benzylpenicillin	0.5 u		≤ 0.125
Chloramphenicol	30		≤ 8.0
Ciprofloxacin (surrogate for enrofloxacin also)	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Rifampicin	1		≤ 0.5
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Vancomycin	5	2 mm	≤ 4.0
Enterococci			
(Blood Sensitest, air, 35°C)			
Ampicillin	5	4 mm Ø	≤ 4.0
Chloramphenicol	30	4 mm	≤ 8.0
Gentamicin	200	4 mm	≤ 512
Linezolid	10		≤ 4.0
Nitrofurantoin +	200	4 mm	≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Vancomycin	5	(See foot note) #	≤ 4.0
***Listeria* spp.**			
(Blood Sensitest, air, 35°C)			
Ampicillin	5		≤ 1.0
Gentamicin	10		≤ 1.0

Ø Perform a nitrocefin based test to detect β-lactamase activity if the zone of inhibition has a sharp edge and an annular radius > 4 mm. β-Lactamase-positive isolates are reported as resistant.

A zone of inhibition with a hazy edge indicates low level resistance to vancomycin (*VanB* type), irrespective of the size of the inhibitory zone.

* Slow growers are incubated for 48 h.

• If a *Corynebacterium* spp. is resistant to penicillin 0.5 u, test ampicillin 5 µg.

\+ For testing urine isolates only.

Veterinary Table 1b. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc potency (µg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
Staphylococci (Sensitest, air, 35°C)			
Ampicillin •	5		≤ 0.5
Benzylpenicillin $	0.5 u		≤ 0.06
Cephalexin •	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin (surrogate for enrofloxacin also)	2.5		≤ 1.0
Erythromycin	5		≤ 0.5
Fusidic acid	2.5		≤ 0.5
Gentamicin	10		≤ 1.0
Kanamycin	50		≤ 8.0
Linezolid	10		≤ 4.0
Methicillin $ (surrogate for ceftiofur also)	5		≤ 4.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Mupirocin	5		≤ 2.0
Nitrofurantoin +	200		≤ 32.0
Novobiocin*	5		≤ 1.0
Rifampicin	1		≤ 0.5
Sulphafurazole	300		≤ 64.0
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Trimethoprim	5		≤ 2.0
Vancomycin	5	2 mm	≤ 4.0
Streptococci (Blood Sensitest, air, 35°C) @			
Ampicillin †	5		≤ 2.0
Benzylpenicillin (surrogate for ceftiofur also)	0.5 u		≤ 0.125
Cefotaxime	0.5		≤ 0.25
Ceftriaxone	0.5		≤ 0.25
Cefotaxime †	5		≤ 2.0
Ceftriaxone †	5		≤ 2.0
Chloramphenicol	30		≤ 8.0
Co-trimoxazole	25		≤ 0.5/9.5
Erythromycin	5		≤ 0.5
Moxifloxacin/Gatifloxacin	2.5	4 mm	≤1.0
Nitrofurantoin +	200		≤ 32.0
Rifampicin	1		≤ 0.5
Teicoplanin	15	2 mm	≤ 8.0
Tetracycline	30		≤ 4.0
Vancomycin	5	2 mm	≤ 4.0

+ For testing urine isolates only

$ NOT for testing *Staph. saprophyticus*.

@ *Strep. pneumoniae* & *Strep. anginosus* (*milleri*) are incubated in 5% CO_2.

• ONLY for testing isolate of *Staph. saprophyticus*.

† NOT for testing *Strep. pneumoniae* from CSF. If *Strep. pneumoniae* or any other *Streptococcus* species from a site other than CSF is resistant to penicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg , test ampicillin 5 µg, cefotaxime 5 µg and ceftriaxone 5 µg.

* Antibiotic calibrated specifically for veterinary medicine.

Veterinary Table 1c. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc potency (μg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
***Enterobacteriaceae*, *Vibrionaceae,* & *Acinetobacter* spp. (Sensitest, air, 35°C)** [#]			
Amikacin	30		≤ 4.0
Ampicillin	25		≤ 8.0
Apramicin*	15		≤ 8.0
Augmentin •	60		≤16.0/8.0
Aztreonam	30		≤ 8.0
Cefazolin (surrogate for ceftiofur also)	30		≤16.0
Cefepime	10		≤ 2.0
Cefotaxime	5		≤ 1.0
Cefotetan	30		≤ 8.0
Cefoxitin	30		≤ 8.0
Cefpirome	10		≤ 2.0
Cefpodoxime	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ceftriaxone	5		≤ 1.0
Cefuroxime	30		≤ 8.0
Cephalexin	100		≤ 16.0
Chloramphenicol	30		≤ 8.0
Ciprofloxacin (surrogate for enrofloxacin also)	2.5		≤ 1.0
Enoxacin	10		≤ 4.0
Gentamicin	10		≤ 1.0
Imipenem	10		≤ 4.0
Kanamycin	50		≤ 8.0
Neomycin*	30		≤ 4.0
Meropenem	5		≤ 2.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Nalidixic acid [+]	30		≤ 4.0
Netilmicin	30		≤ 2.0
Nitrofurantoin [+]	200		≤ 32.0
Norfloxacin [+]	10		≤ 4.0
Spectinomycin*	25		≤ 32.0
Streptomycin*	25		≤ 16.0
Sulphafurazole	300		≤ 64.0
Tazocin •	55		≤ 16.0/2.0
Tetracycline	30		≤ 4.0
Timentin •	85		≤ 32.0/2.0
Tobramycin	10		≤ 1.0
Trimethoprim	5		≤ 2.0
***Pseudomonas* spp. *& Burkholderia* spp. (Sensitest, air, 35°C)**			
Amikacin	30	4 mm	≤ 16.0
Aztreonam	30		≤ 8.0
Cefepime	10		≤ 2.0
Cefpirome	10		≤ 2.0
Ceftazidime	10		≤ 4.0
Ciprofloxacin (surrogate for enrofloxacin also)	2.5		≤ 1.0
Gentamicin	10	4 mm	≤ 4.0
Imipenem	10		≤ 4.0
Meropenem	5		≤ 2.0
Moxifloxacin/Gatifloxacin	2.5		≤ 1.0
Netilmicin	30	4 mm	≤ 8.0
Norfloxacin [+]	10		≤ 4.0
Piperacillin	50		≤ 16.0
Polymyxin	300 u	4 mm	≤ 1.0
Sulphafurazole	300		≤ 64.0
Tazocin	55		≤ 16.0/2.0
Ticarcillin	75		≤ 32.0
Timentin	85		≤ 32.0/2.0
Tobramycin	10	4 mm	≤ 4.0
Trimethoprim	5		≤ 2.0

[#] *Yersinia enterocolitica* is incubated in air at 30°C. [+] For testing urinary isolates only

• If an ESBL is present, report Augmentin, Timentin and Tazocin for isolates from URINE ONLY.

* Antibiotic calibrated specifically for veterinary medicine.

Veterinary Table 1d. Calibrations 2002. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

MISCELLANOUS GRAM-NEGATIVE ORGANISMS

Antibiotic		Disc potency (μg)	Exception to standard interpretation	MIC for susceptible strains (mg/L)
Campylobacter spp.				
(Blood Sensitest, microaerophilic, 42°C)				
Ciprofloxacin	(surrogate for enrofloxacin also)	2.5		≤ 1.0
Erythromycin		5	4 mm	≤ 0.5
Gentamicin		10		≤ 1.0
Tetracycline		30		≤ 4.0
***Haemophilus influenzae/Haemophilus* spp**				
(HTM[@] agar, 5% CO_2, 35 - 37°C)				
Ampicillin		5		≤ 1.0
Augmentin		15		≤ 2.0/1.0
Cefaclor	(surrogate for ceftiofur also)	30		≤ 4.0
Cefotaxime		0.5		≤ 0.25
Cefpodoxime		10		≤ 2.0
Ceftriaxone		0.5		≤ 0.25
Cefuroxime	(surrogate for ceftiofur also)	30		≤ 4.0
Chloramphenicol		10		≤ 2.0
Ciprofloxacin	(surrogate for enrofloxacin also)	2.5		≤ 1.0
Co-trimoxazole		25		≤ 1.0/19.0
Moxifloxacin/Gatifloxacin		2.5		≤ 1.0
Tetracycline		30		≤ 4.0
Helicobacter pylori				
(Chocolate Columbia Blood Agar, microaerophilic, 35°C)				
Amoxycillin		2		≤ 0.25
Erythromycin		5		≤ 0.5
Metronidazole		5		≤ 4.0
Tetracycline		30		≤ 4.0
Moraxella catarrhalis				
(Blood Sensitest, 5% CO_2, 35 - 37°C)				
Benzylpenicillin		0.5 u		≤ 0.25
Cefaclor	(surrogate for ceftiofur also)	30		≤ 4.0
Cefpodoxime		10		≤ 2.0
Cefuroxime	(surrogate for ceftiofur also)	30		≤ 4.0
Ciprofloxacin	(surrogate for enrofloxacin also)	2.5		≤ 1.0
Co-trimoxazole		25		≤ 1.0/19.0
Erythromycin		5		≤ 0.5
Moxifloxacin/Gatifloxacin		2.5		≤ 1.0
Tetracycline		30		≤ 4.0
Neisseria meningitidis				
(Blood Sensitest, 5% CO_2, 35 - 37°C)				
Benzylpenicillin	(surrogate for ceftiofur also)	0.5 u	4 mm	≤ 0.25
Cefotaxime		0.5		≤ 0.25
Ceftriaxone		0.5		≤ 0.25
Chloramphenicol		10		≤ 2.0
Ciprofloxacin	(surrogate for enrofloxacin also)	2.5		≤ 1.0
Rifampicin		1		≤ 0.5
Pasteurella multocida				
(Blood Sensitest, air, 35°C)				
Ampicillin	(surrogate for ceftiofur also)	5		≤ 1.0
Ciprofloxacin	(surrogate for enrofloxacin also)	2.5		≤ 1.0
Moxifloxacin/Gatifloxacin		2.5		≤ 1.0
Tetracycline		30		≤ 4.0
Stenotrophomonas maltophilia				
(Sensitest, air, 35°C)				
Sulphafurazole		300		≤ 64.0

[@] Haemophilus Test Medium containing 15mg/L of freshly prepared haematin and NAD.

Veterinary Table 2a. Surrogate disc testing 2002. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc – updated 25.11.2002.

GRAM-POSITIVE ORGANISMS

Antibiotic reported	Surrogate disc used	Disc potency (µg)
Staphylococci (except *S. saprophyticus from urine)*		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Methicillin	5
Azithromycin	Erythromycin	5
Ceftiofur / other cephalosporins [&]	Methicillin	5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Cloxacillin	Methicillin	5
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Dicloxacillin	Methicillin	5
Enrofloxacin	Ciprofloxacin	2.5
Flucloxacillin	Methicillin	5
Lincomycin	Erythromycin	5
Neomycin	Kanamycin	50
Norfloxacin [$]	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
***Staphylococcus saprophyticus* from urine**		
Amoxycillin	Ampicillin	5
Augmentin	Cephalexin	100
Benzylpenicillin	Ampicillin	5
Ceftiofur / other cephalosporins [&]	Cephalexin	100
Cloxacillin	Cephalexin	100
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Dicloxacillin	Cephalexin	100
Enrofloxacin	Ciprofloxacin	2.5
Flucloxacillin	Cephalexin	100
Norfloxacin [$]	Ciprofloxacin	2.5
Penicillin V	Ampicillin	5
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Streptococci *		
Amoxycillin	Benzylpenicillin	0.5 u
Amoxycillin	Ampicillin [↓]	5
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Benzylpenicillin	Ampicillin [↓]	5
Ceftiofur	Benzylpenicillin	0.5 u
Cephalosporins (except ceftiofur)[&]	Cefotaxime/Ceftriaxone	0.5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Lincomycin	Erythromycin	5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30

[&] Ceftazidime is inactive against Gram-positive organisms.

[+] Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

[$] Reporting of norfloxacin is for urine isolates ONLY.

* For streptococci groups A, B, C, G and *Strep. anginosus*, the susceptibility to penicillin, ampicillin,amoxicillin, cloxacillin and cephalosporins (except ceftazidime) is extrapolated from the results of testing with a benzylpenicillin 0.5 u disc.

[↓] NOT for testing *Strep.pneumoniae* from CSF. Test if isolate is resistant to penicillin 0.5 u, cefotaxime 0.5 µg or ceftriaxone 0.5 µg.

Veterinary Table 2b. Surrogate disc testing 2002. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-POSITIVE ORGANISMS

Antibiotic reported	Surrogate disc used	Disc potency (µg)
***Corynebacterium* species**		
Amoxycillin	Benzylpenicillin	0.5 u
Ampicillin	Benzylpenicillin	0.5 u
Azithromycin	Erythromycin	5
Ceftiofur / other cephalosporins [&]	Benzylpencillin	5
Clarithromycin	Erythromycin	5
Clindamycin	Erythromycin	5
Enrofloxacin	Ciprofloxacin	2.5
Lincomycin	Erythromycin	5
Norfloxacin [$]	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
Enterococci		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5
***Listeria* spp.**		
Amoxycillin	Ampicillin	5
Benzylpenicillin	Ampicillin	5

[&] Ceftazidime is inactive against Gram-positive organisms.

[$] Reporting of norfloxacin is for urine isolates ONLY.

Veterinary Table 2c. Surrogate disc testing 2002. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-NEGATIVE ORGANISMS

Antibiotic reported	Surrogate disc used	Disc potency (µg)
Campylobacter spp.		
Enrofloxacin	Ciprofloxacin	2.5
Tetracyclines	Tetracycline	30
***Enterobacteriaceae/ Vibrionaceae/ Acinetobacter* spp.**		
Amoxycillin	Ampicillin	25
Ceftiofur	Cefazolin	30
Cephalothin [@]	Ampicillin	25
Ceftriaxone	Cefotaxime	5
Cefotaxime	Ceftriaxone	5
Co-trimoxazole [+]	Sulphafurazole	300
Co-trimoxazole [+]	Trimethoprim	5
Enrofloxacin	Ciprofloxacin	2.5
Piperacillin	Ampicillin	25
Sulphonamides	Sulphafurazole	300
Tetracyclines	Tetracycline	30
Ticarcillin	Ampicillin	25
Haemophilus influenzae/Haemophilus spp.		
Amoxycillin	Ampicillin	5
Cefepime	Cefotaxime/Ceftriaxone	0.5
Cefotaxime	Ceftriaxone	0.5
Cefpirome	Cefotaxime/Ceftriaxone	0.5
Ceftazidime	Cefotaxime/Ceftriaxone	0.5
Ceftiofur	Cefuroxime/Cefaclor	30
Ceftriaxone	Cefotaxime	0.5
Cephalexin	Cefuroxime/Cefaclor	30
Enrofloxacin	Ciprofloxacin	2.5
Tetracyclines	Tetracycline	30
Helicobacter pylori		
Clarithromycin	Erythromycin	5
Moraxella catarrhalis		
Azithromycin	Erythromycin	5
Amoxycillin / Ampicillin	Benzylpenicillin	0.5 u
Augmentin	Cefuroxime/Cefaclor	30
Ceftiofur	Cefuroxime/Cefaclor	30
Cephalosporins	Cefuroxime/Cefaclor	30
Clarithromycin	Erythromycin	5
Enrofloxacin	Ciprofloxacin	2.5
Penicillin V	Benzylpenicillin	0.5 u
Roxithromycin	Erythromycin	5
Tetracyclines	Tetracycline	30
Neisseria meningitidis		
Ampicillin / Amoxycillin	Benzylpenicillin	0.5 u
Cefotaxime	Ceftriaxone	0.5
Ceftiofur	Benzylpenicillin	0.5 u
Ceftriaxone	Cefotaxime	0.5
Enrofloxacin	Ciprofloxacin	2.5
Pasteurella multocida		
Amoxycillin / Benzylpenicillin	Ampicillin	5
Ceftiofur	Ampicillin	5
Enrofloxacin	Ciprofloxacin	2.5
Tetracyclines	Tetracycline	30
***Pseudomonas* spp & *Burkholderia* spp.**		
Azlocillin	Piperacillin	50
Colistin	Polymyxin	300 u
Co-trimoxazole [+]	Trimethoprim	5
Co-trimoxazole [+]	Sulphafurazole	300
Enrofloxacin	Ciprofloxacin	2.5
Stenotrophomonas maltophilia		
Co-trimoxazole	Sulphafurazole	300

[@] Not for *Acinetobacter* spp.

[+] Resistance to co-trimoxazole is indicated only by resistance to both sulphafurazole and trimethoprim.

Veterinary Table 3a. Reference strains 2002. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-POSITIVE ORGANISMS

Antibiotic	Disc content (µg)	Acceptable range* (mm)
***Enterococcus faecalis* POW 1994**		
(Blood Sensitest, air 35°C)		
Ampicillin	5	5.9 - 9.2
Chloramphenicol	30	6.6 - 9.9
Gentamicin	200	6.6 - 9.9
Nitrofurantoin	200	6.1 - 8.7
Teicoplanin	15	3.1 - 5.3
Vancomycin	5	2.0 - 3.7
***Staphylococcus aureus* NCTC 6571**		
(Sensitest, air 35°C)		
Amoxycillin •	2	9.1 - 11.9
Benzylpenicillin	0.5 u	8.7 - 13.5
Chloramphenicol	30	7.8 - 11.4
Ciprofloxacin	2.5	9.2 - 12.4
Erythromycin	5	8.6 - 11.2
Fusidic acid	2.5	8.6 - 12.6
Gentamicin	10	6.6 - 9.4
Kanamycin	50	7.8 - 9.6
Linezolid	10	7.9 - 13.1
Methicillin	5	8.8 - 12.0
Novobiocin	5	6.1 - 12.5
Rifampicin	1	9.3 - 12.5
Sulphafurazole	300	9.3 - 13.7
Teicoplanin	15	3.4 - 6.1
Tetracycline	30	10.6 - 16.2
Trimethoprim	5	8.5 - 11.3&
Vancomycin	5	2.8 - 4.9
***Streptococcus pneumoniae* ARL 10582**		
(Blood Sensitest, 5% CO_2, 35 - 37°C)		
Ampicillin	5	10.8 - 15.2&
Benzylpenicillin	0.5u	8.3 - 14.8
Chloramphenicol	30	8.0 - 13.2
Co-trimoxazole	25	7.0 - 9.2
Erythromycin	5	7.1 - 12.9
Rifampicin	1	7.5 - 10.8
Teicoplanin	15	5.1 - 8.0
Tetracycline	30	9.2 - 14.5
Vancomycin	5	5.1 - 8.6

* The acceptable range (95% confidence limits) is the mean ± 2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

NOTE: Additional testing with reference strains must be performed when:

a. A new batch of medium is used.

b. A new batch of discs is used.

c. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

• For *Helicobacter pylori* only.

Veterinary Table 3b. Reference strains 2002. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS

Antibiotic	Disc content (μg)	Acceptable range* (mm)
Escherichia coli **NCTC 10418** # **(Sensitest, air, 35˚C)**		
Amikacin	30	6.7 - 10.3
Ampicillin	25	7.5 - 10.7
Apramicin	15	5.3 - 7.9
Aztreonam	30	13.7 - 15.9
Cefazolin	30	6.7 - 12.7
Cefepime	10	11.9 - 15.3
Cefotaxime	5	9.7 - 13.7
Cefotetan	30	11.9 - 14.8
Cefoxitin	30	9.8 - 13.0
Cefpirome	10	11.9 - 14.6
Cefpodoxime	10	10.3 - 12.7
Ceftazidime	10	9.3 - 14.1&
Ceftriaxone	5	10.5 - 14.3
Cefuroxime	30	7.5 - 10.1
Cephalexin	100	6.9 - 10.9
Chloramphenicol	30	8.7 - 11.9
Ciprofloxacin	2.5	12.4 - 15.8
Gatifloxacin	2.5	11.2 - 14.8
Enoxacin	10	9.7 - 15.7
Gentamicin	10	6.2 - 9.4
Imipenem	10	10.3 - 13.5
Kanamycin	50	6.2 - 11.8
Meropenem	5	11.0 - 14.4
Moxifloxacin	2.5	10.0 - 13.4
Nalidixic acid	30	8.9 - 12.1
Neomycin	30	6.0 - 8.6
Netilmicin	30	7.7 - 11.3
Nitrofurantoin	200	6.3 - 9.5
Norfloxacin	10	10.4 - 16.4
Spectinomycin	25	5.0 - 7.8
Streptomycin	25	6.2 - 7.8
Sulphafurazole	300	5.0 - 9.4
Tetracycline	30	5.8 - 11.0
Tobramycin	10	6.4 - 8.4
Trimethoprim	5	8.8 - 13.6 &
Escherichia coli **NCTC 11560 (Sensitest, air, 35°C)**		
Augmentin	60	6.4 - 9.6
Timentin	85	6.0 - 8.4
Tazocin	55	7.4 - 9.2

***** The acceptable range (95% confidence limits) is the mean ± 2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

If antibiotics are tested with *Escherichia coli* NCTC 10418, there is no need to test these against *Pseudomonas aeruginosa* NCTC 10662 as well and vice versa.

NOTE: Additional testing with reference strains must be performed when:

a. A new batch of medium is used.

b. A new batch of discs is used.

c. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

Veterinary Table 3c. Reference strains 2002. Antibiotic disc content and the acceptable range (mm) of the annular radii of the zones of inhibition with the reference strains used in the CDS method.

GRAM-NEGATIVE ORGANISMS CONTINUED

Antibiotic	Disc content (µg)	Acceptable range* (mm)
***Bacteroides fragilis* ATCC 25285 (Blood Sensitest, anaerobic, 35°C)**		
Metronidazole	5	7.1 - 13.5
***Campylobacter jejuni* NCTC 11168 (Blood Sensitest, microaerophilic, 42°C)**		
Ciprofloxacin	2.5	9.2 - 16.9
Erythromycin	5	6.4 - 12.4
Gentamicin	10	7.0 - 11.0
Tetracycline	30	10.3 - 16.0
***Haemophilus influenzae* NCTC 4560 (HTM@ agar, 5% CO_2, 35 - 37°C)**		
Ampicillin	5	7.0 - 11.1
Cefaclor	30	8.1 - 12.1
Cefotaxime	0.5	9.2 - 12.8
Cefpodoxime	10	10.9 - 14.1
Ceftriaxone	0.5	9.1 - 12.9
Cefuroxime	30	8.3 - 12.8
Chloramphenicol	10	11.1 - 14.3 &
Ciprofloxacin	2.5	11.1 - 15.9
Co-trimoxazole	25	9.0 - 12.5
Gatifloxacin	2.5	13.5 - 17.1
Moxifloxacin	2.5	10.6 - 15.2
Tetracycline	30	9.9 - 13.3
***Haemophilus influenzae* NCTC 11315 (HTM@ agar, 5% CO_2, 35 - 37°C)**		
Augmentin	15	7.7 - 10.1
***Pseudomonas aeruginosa* NCTC 10662 # (Sensitest, air, 35°C)**		
Amikacin	30	7.4 - 10.6
Aztreonam	30	8.3 - 13.1
Cefepime	10	8.1 - 11.3
Cefpirome	10	8.1 - 10.6
Ceftazidime	10	7.5 - 11.9
Ciprofloxacin	2.5	8.9 - 14.5
Gatifloxacin	2.5	7.8 - 11.4
Gentamicin	10	5.5 - 9.5
Imipenem	10	7.9 - 10.3
Meropenem	5	9.7 - 14.8
Moxifloxacin	2.5 $	- -
Netilmicin	30	6.4 - 10.4
Piperacillin	50	8.1 - 12.9
Polymyxin	300 u	5.2 - 7.2
Ticarcillin	75	7.3 - 12.1
PPTobramycin	10	7.0 - 10.6

***** The acceptable range (95% confidence limits) is the mean ± 2 standard deviations. The mean was derived from >120 measurements with different operators using different batches of both agar and discs.

@ Haemophilus Test Medium containing 15 mg/L freshly prepared Haematin and NAD.

\# If antibiotics are tested with *Escherichia coli* NCTC 10418, there is no need to test these against *Pseudomonas aeruginosa* NCTC 10662 as well and vice versa.

NOTE: Additional testing with reference strains must be performed when:

a. A new batch of medium is used.

b. A new batch of discs is used.

d. The appropriate reference strain must be tested at the same time as the clinical isolate or at least ONCE weekly.

& Adjusted acceptable range.

$ Moxifloxacin MIC recorded with *Ps. aeruginosa* NCTC 10662 is 2 mg/L and is above the susceptible MIC. Test *E. coli* NCTC 10418 instead.

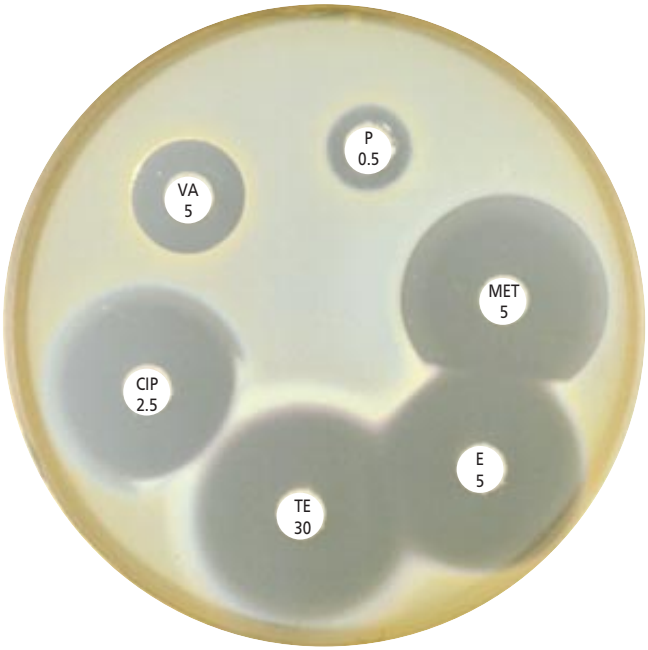


Plate 1a:

A common isolate of *Staph. aureus* with resistance to penicillin (P 0.5 u) only.

Note the annular radius of 10 mm around the methicillin (MET 5) disc.

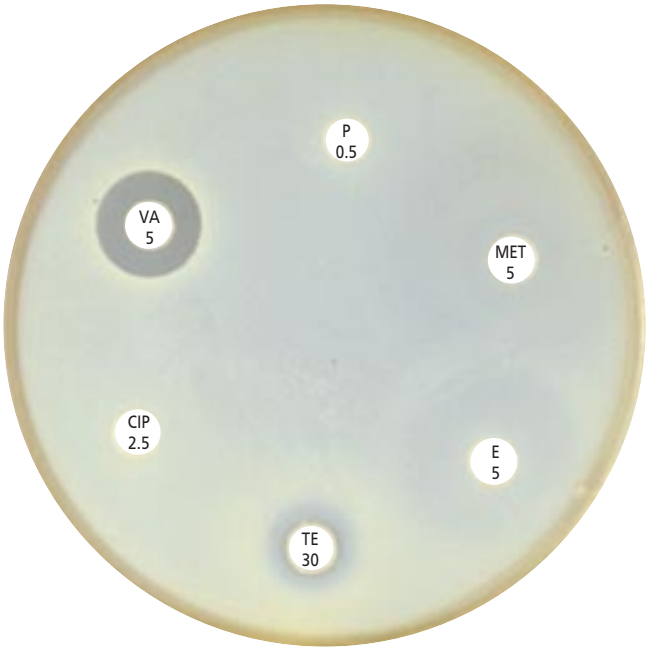


Plate 1b:

A commonly isolated multiple-resistant methicillin-resistant *Staph. aureus* (MRSA) with no inhibitory zone around the methicillin (MET 5) disc.

Plate 1c:

MRSA with heterogeneous resistance to methicillin. There are resistant colonies in the zone of inhibition around methicillin (MET 5) when tested on Sensitest Agar incubated at 35˚C for 24 h. If in doubt, incubate for 48 h.

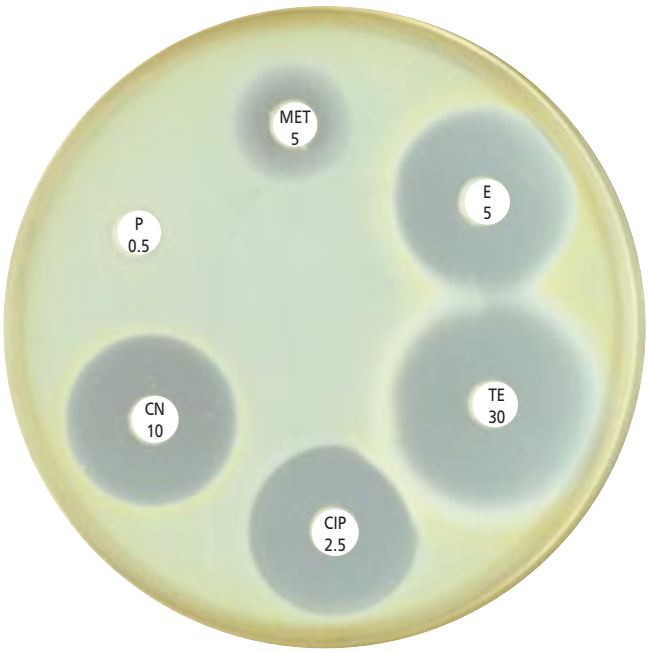


Plate 2:

Non-multiple resistant MRSA with resistance to methicillin and penicillin only.

Note the reduced inhibitory zone of approximately 4 mm around the methicillin (MET 5) disc.

Plate 3:

MRSA with reduced susceptibility to vancomycin (VISA/GISA).

Note the reduced and hazy zone around vancomycin (VA 5) and teicoplanin (TEC 15) discs.

Plate 4:

MRSA with inducible clindamycin resistance (ICR): No zone around erythromycin (E 5) and a flattened zone around clindamycin (DA 2) near the erythromycin disc.

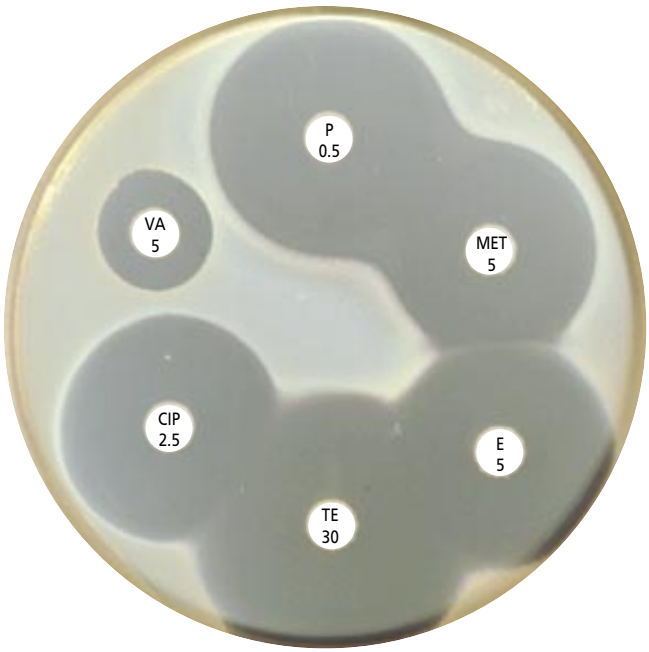


Plate 5a:

QA reference strain, *Staph. aureus* NCTC 6571.

Note the approximate zone sizes around penicillin (P 0.5 u), methicillin (MET 5) and vancomycin (VA 5) are 12 mm, 10 mm and 3 mm respectively.

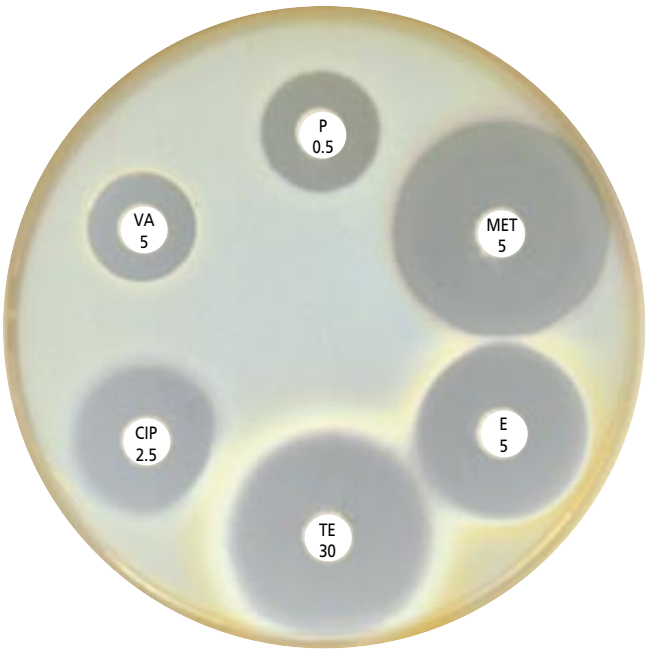


Plate 5b:

Staph. aureus with low β-lactamase activity.

Note the sharp edge of the inhibitory zone around the penicillin disc (P 0.5 u) and a reduced annular radius of 4-5 mm.

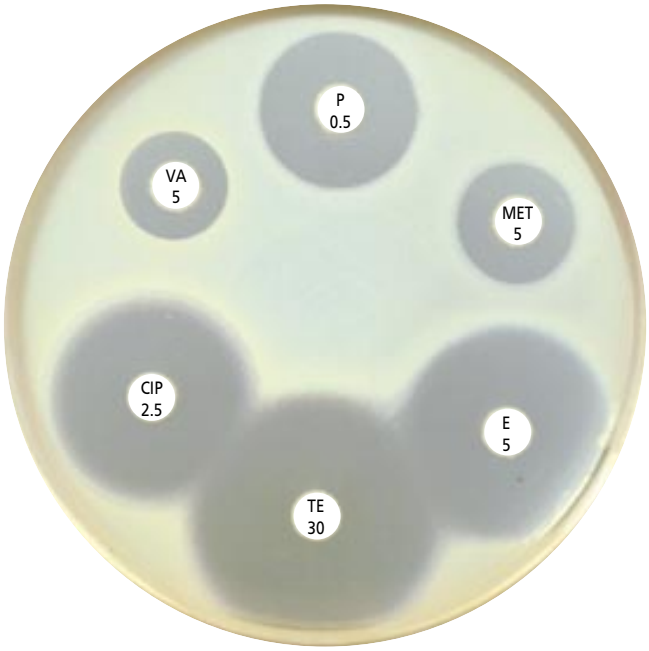


Plate 6:

Coagulase-negative *Staphylococcus* sensitive to penicillin (P 0.5 u) and resistant to methicillin (MET 5).

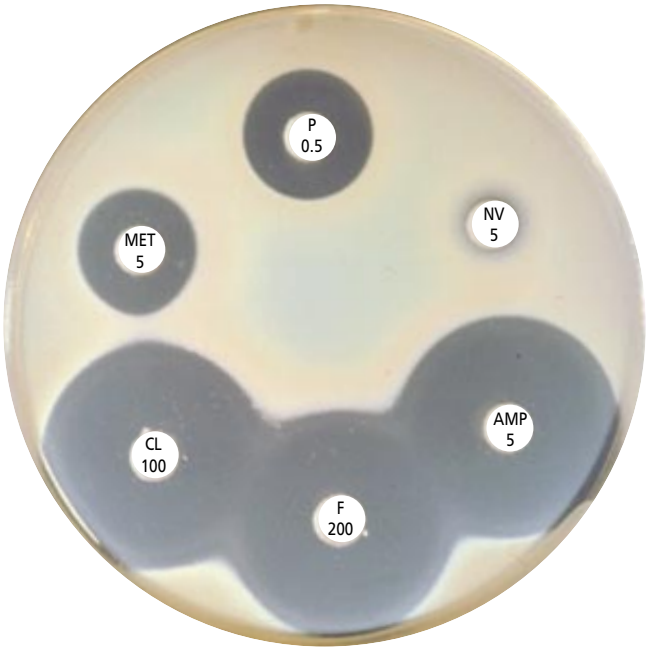


Plate 7a:

A typical *Staph. saprophyticus* from urine: Resistance to novobiocin (NV 5) and reduced zones around both penicillin (P 0.5) and methicillin (MET 5) discs. Interpret the susceptibility to penicillins from the ampicillin (AMP 5) disc and not the penicillin disc.

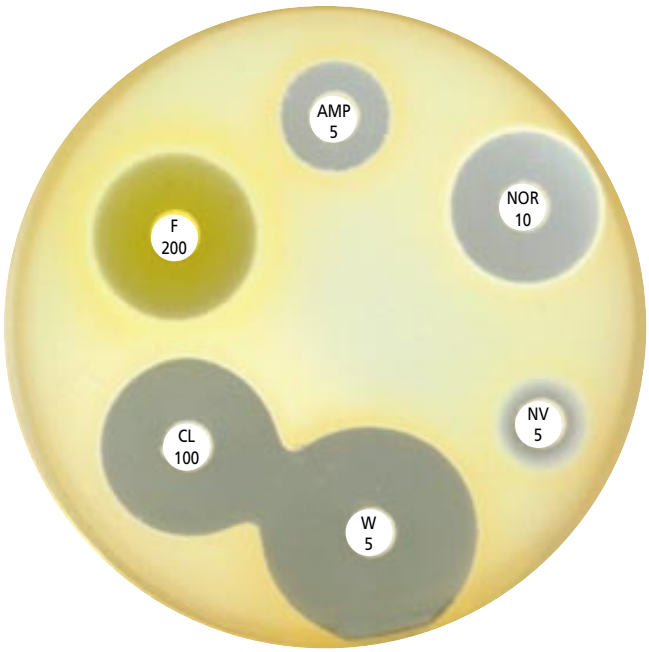


Plate 7b:

Staph. saprophyticus from urine resistant to ampicillin (AMP 5) and susceptible to cephalexin (CL 100).

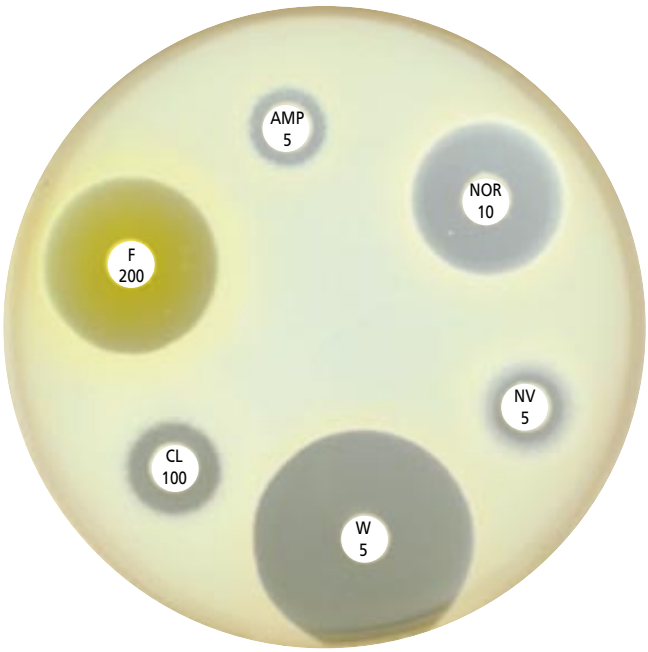


Plate 7c:

Staph. saprophyticus from urine resistant to ampicillin (AMP 5) and cephalexin (CL 100).

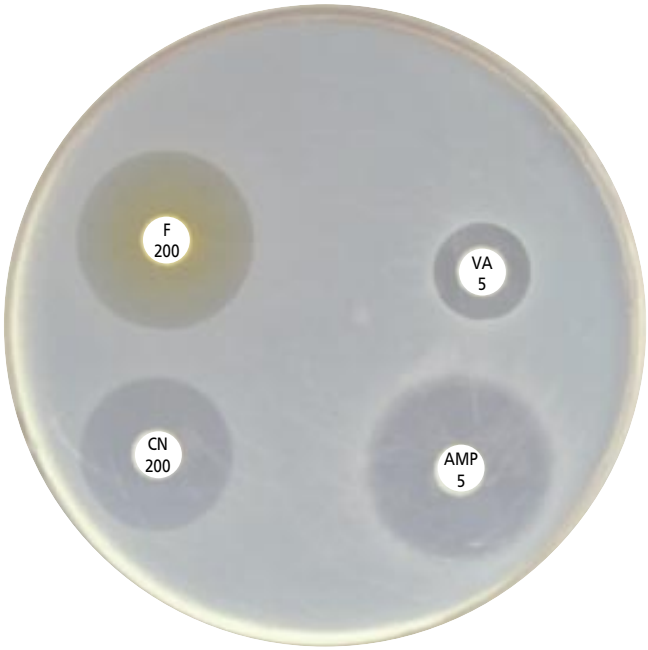


Plate 8a:

E. faecalis POW 1994.

Note the hazy edge of the zone of inhibition around the ampicillin (AMP 5) disc.

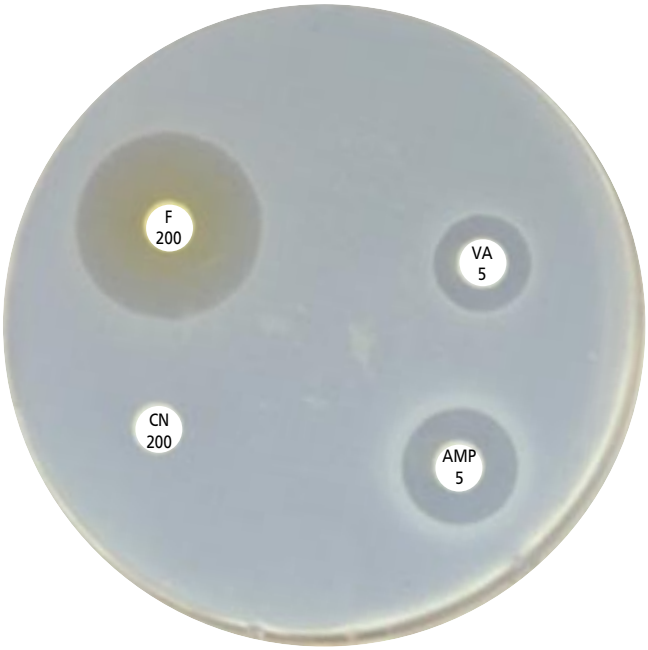


Plate 8b:

β-Lactamase producing *E. faecalis.*

Note the sharp edge of the inhibitory zone around the ampicillin (AMP 5) disc.

Plate 8c:

A common isolate of *E. faecium* with no inhibitory zone around the ampicillin (AMP 5) disc.

Plate 9a:

E. faecalis susceptible to vancomycin (VA 5) with an inhibitory zone >2 mm in annular radius and a sharp edge.

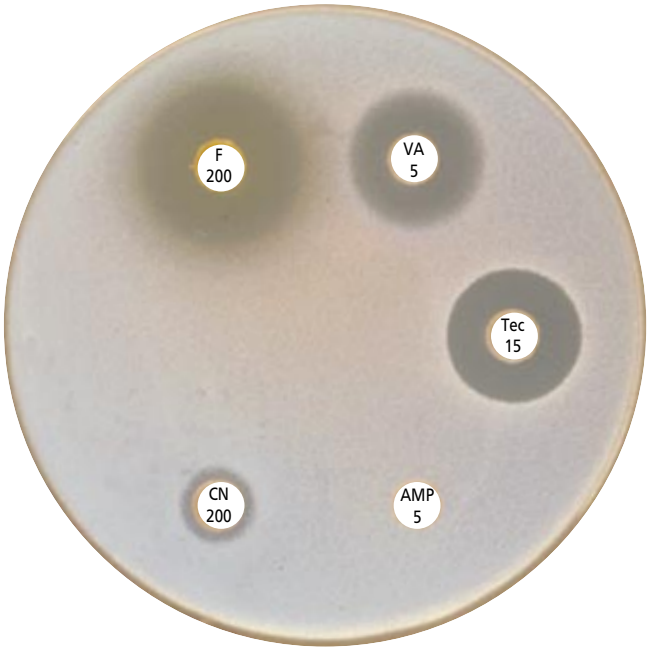


Plate 9b:

VRE *faecium* with Van B type resistance.
Note the hazy edge of the large inhibitory zone around the vancomycin (VA 5) disc.

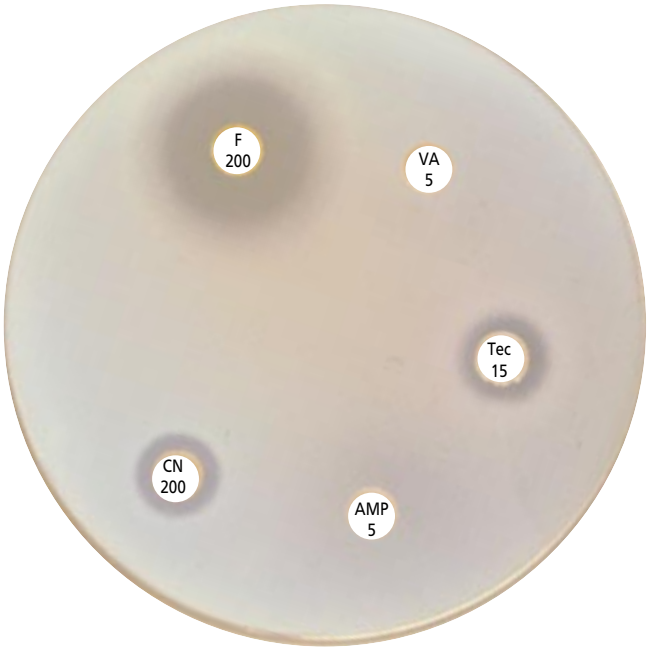


Plate 9c:

VRE *faecium* with Van A type resistance ie. resistant to both vancomycin (VA 5) and teicoplanin (TEC 15).

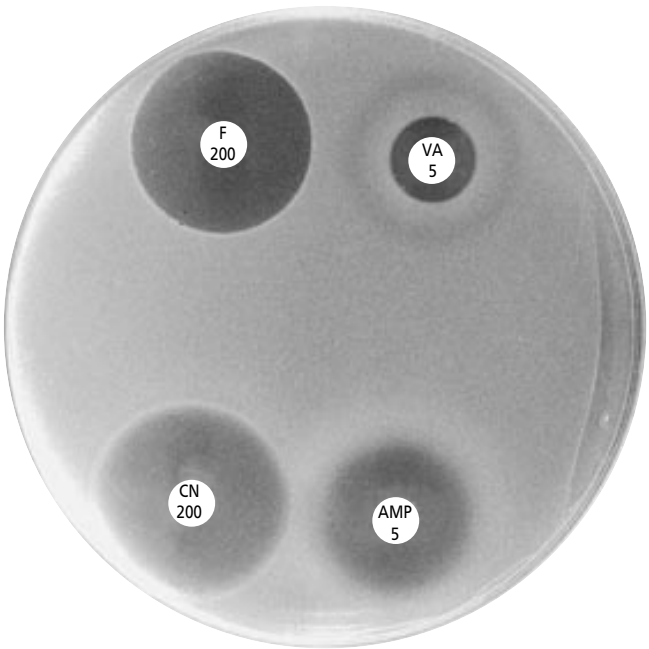


Plate 9d:

E. gallinarum with intrinsic resistance to vancomycin (VA 5).

Note the sharp edge of the reduced inhibitory zone around the vancomycin disc.

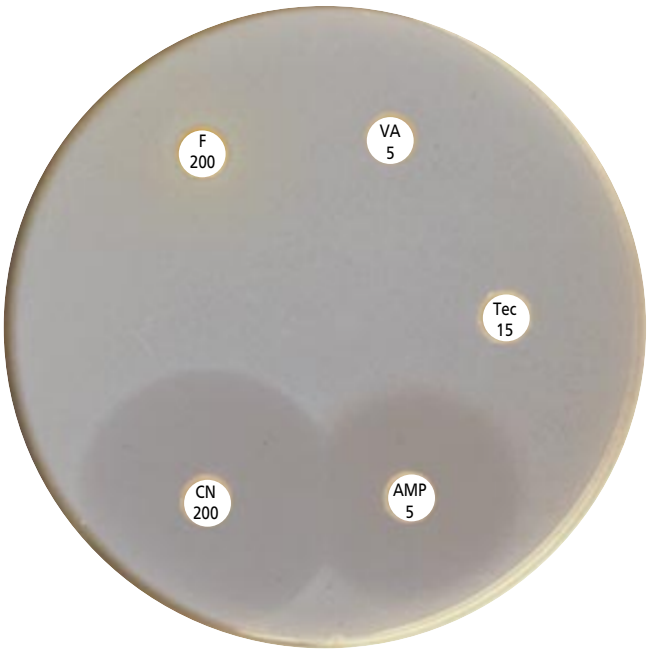


Plate 9e:

Leuconostoc species with high inherent resistance to both vancomycin and teicoplanin: No zone around vancomycin (VA 5) and teicoplanin (TEC 15).

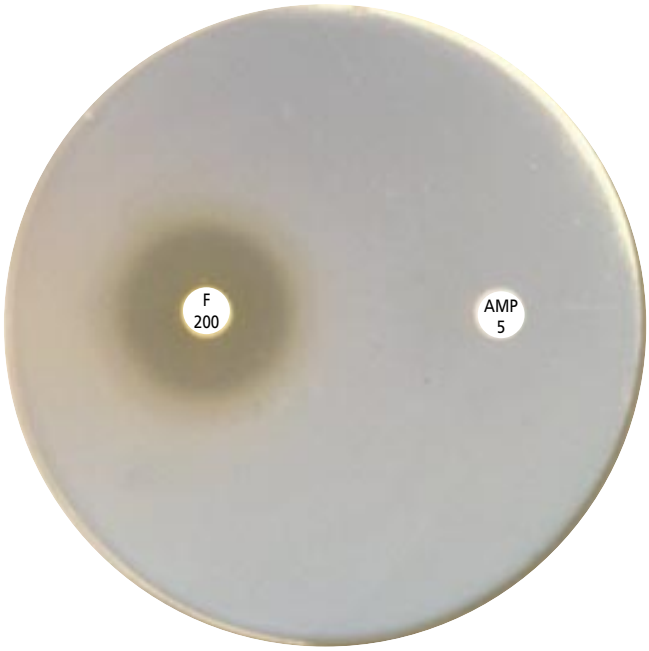


Plate 10a:

Typical *E. faecium* with a hazy edge of the nitrofurantoin inhibitory zone (F 200) and no zone around the ampicillin (AMP 5) disc.

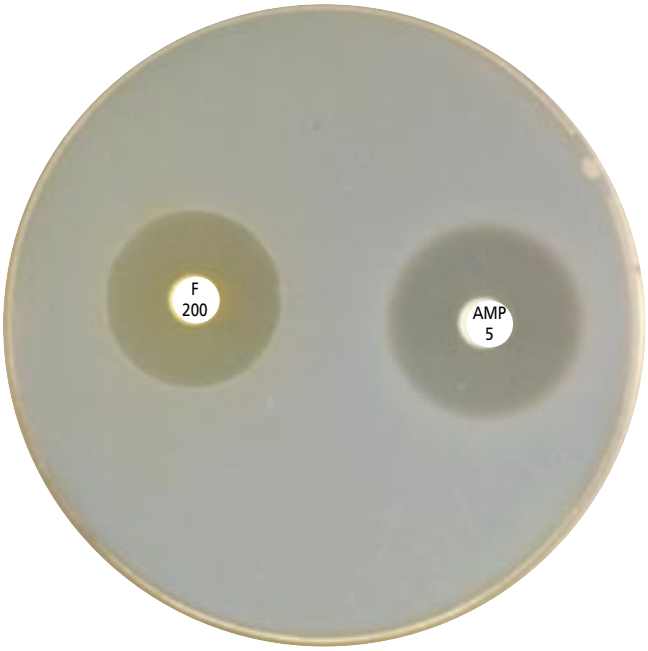


Plate 10b:

E. faecalis susceptible to ampicillin (AMP 5). Note the sharp edge of the nitrofurantoin (F 200) inhibitory zone.

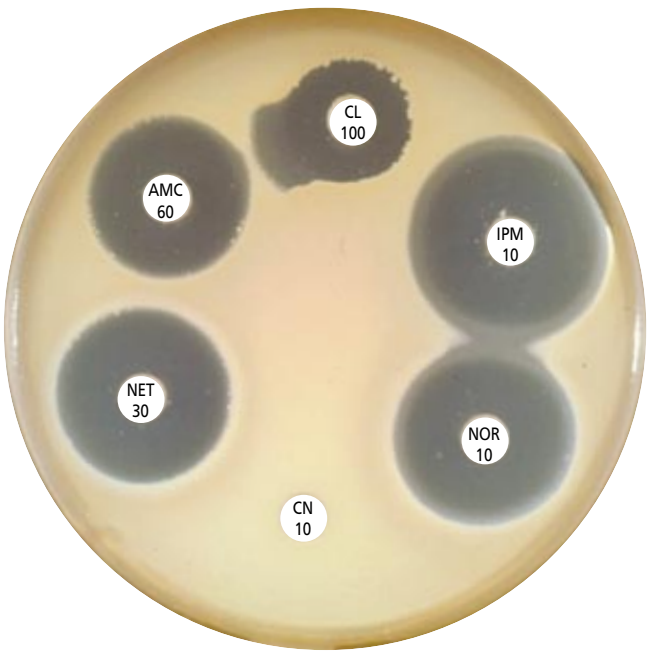


Plate 11a:

A “keyhole” effect between Augmentin (AMC 60) and cephalexin (CL 100) indicates the presence of an extended-spectrum β-lactamase (ESBL).

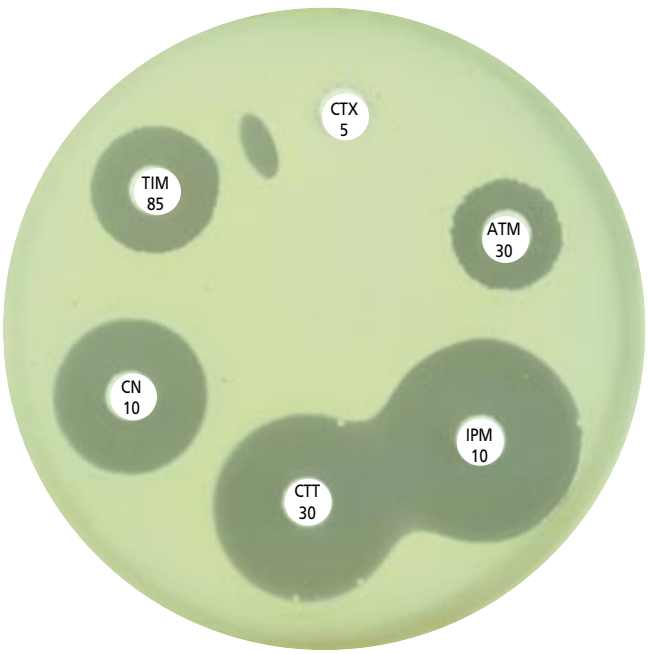


Plate 11b:

A clear elliptical area between Timentin (TIM 85) and cefotaxime (CTX 5) indicates the presence of an extended-spectrum β-lactamase (ESBL).

Note: The susceptibility to cefotetan (CTT 30).

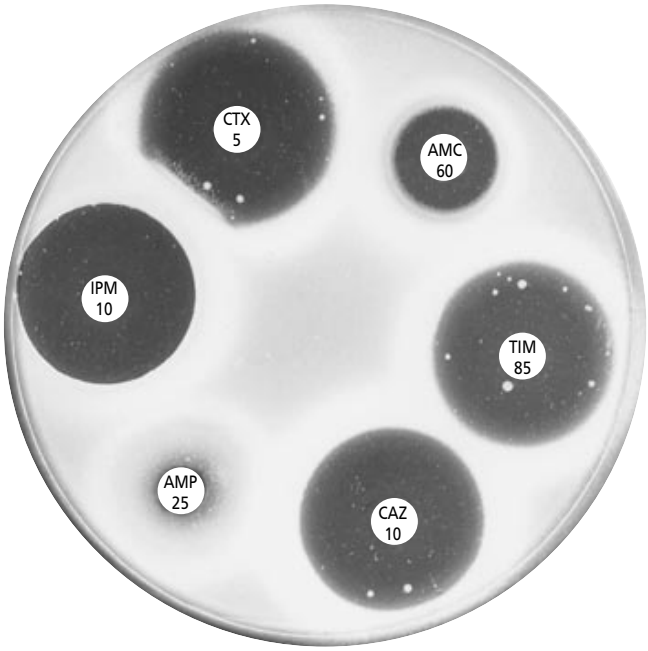


Plate 12a:

The flattened zone between imipenem (IPM 10) and cefotaxime (CTX 5) indicates the presence of an inducible cephalosporinase.

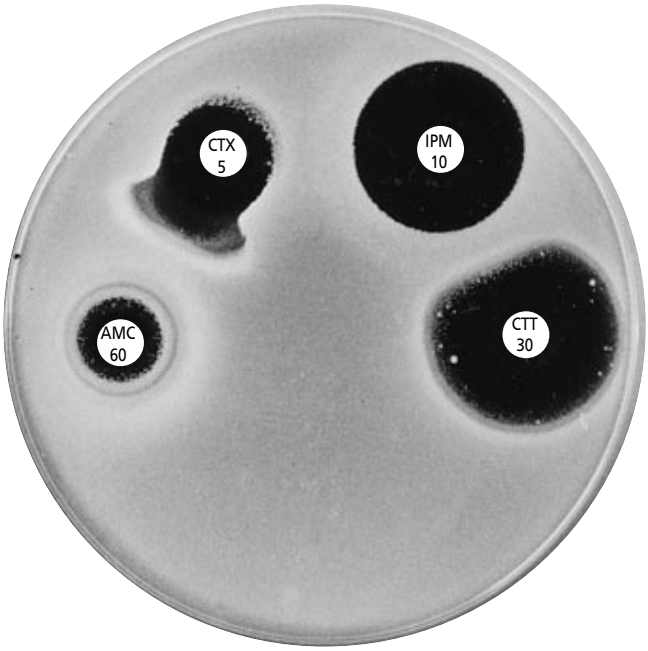


Plate 12b:

The flattened zone between imipenem (IPM 10) and cefotetan (CTT 30) suggests the presence of an inducible cephalosporinase.

Also, the “keyhole” between Augmentin (AMC 60) and cefotaxime (CTX 5) indicates the presence of an extended spectrum β-lactamase (ESBL).

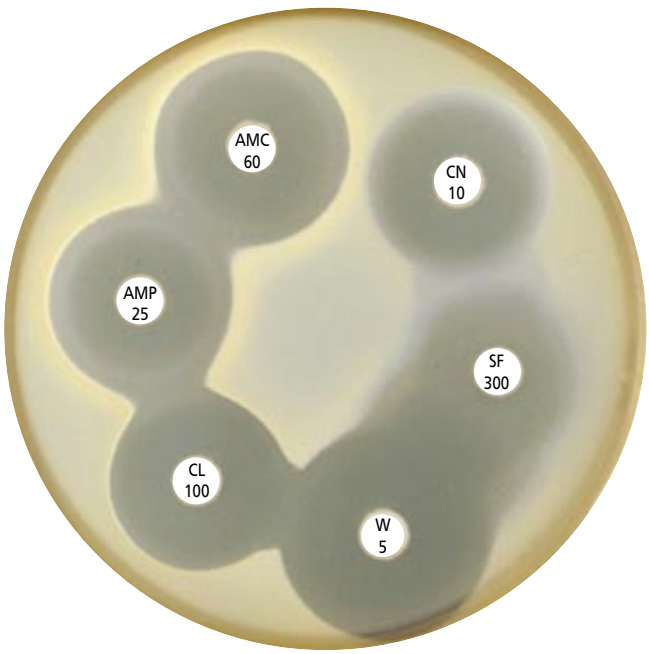


Plate 13a:

QA reference strain *E. fcoli* NCTC 10418, susceptible to ampicillin (AMP 25), Augmentin (AMC 60), cephalexin (CL 100), gentamicin (CN 10), sulphafurazole (SF 300) and trimethoprim (W 5).

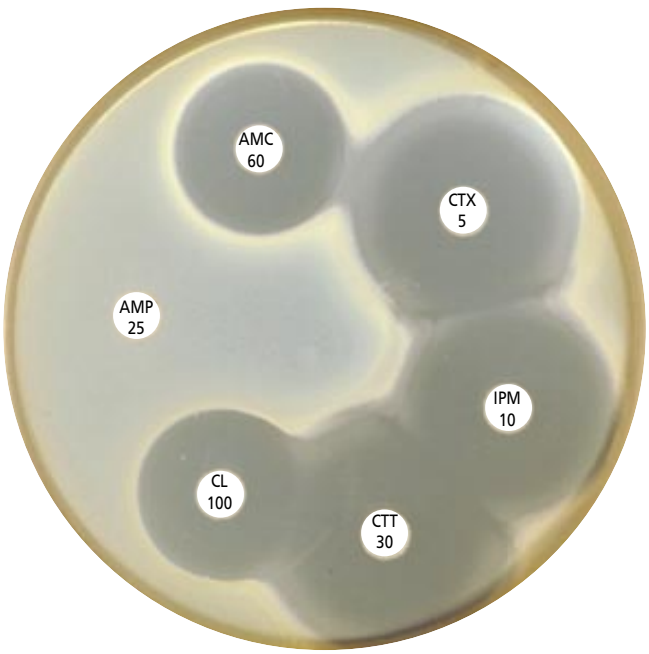


Plate 13b:

QA reference strain *E. coli* NCTC 11560 producing TEM β-lactamase, resistant to ampicillin (AMP 25) and susceptible to Augmentin (AMC 60), cefotaxime (CTX 5), imipenem (IPM 10), cefotetan (CTT 30) and cephalexin (CL 100).

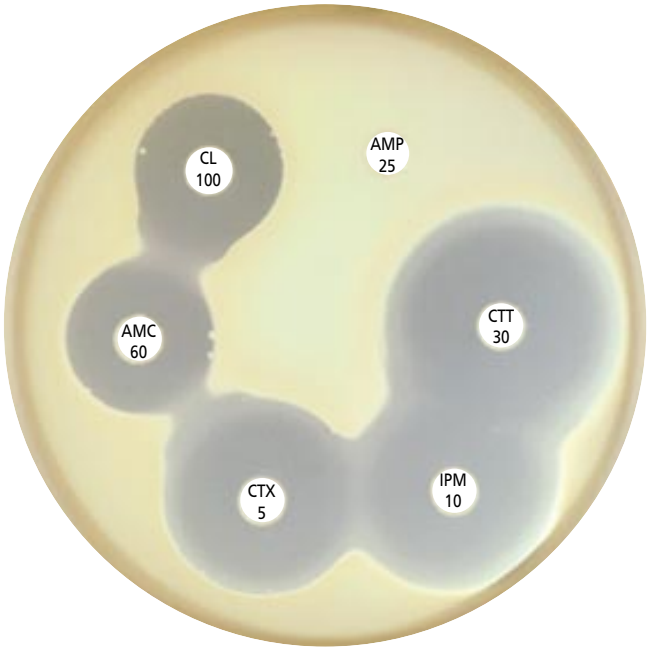


Plate 14a:

Klebsiella oxytoca producing low level K1 β-lactamase.

Note the synergy between cephalexin (CL 100), cefotaxime (CTX 5) and Augmentin (AMC 60).

Plate 14b:

Klebsiella oxytoca producing high level K1 β-lactamase.

Note the tiny "keyhole" effect between cefotaxime (CTX 5) and Augmentin (AMC 60) and the small zone around cephalexin (CL 100).

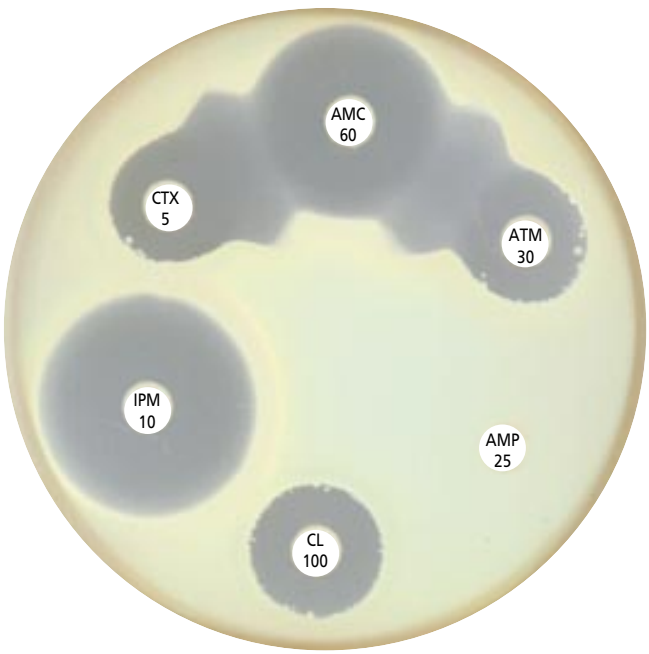


Plate 15a:

Klebsiella pneumoniae producing an ESBL indicated by the "keyhole" effect between cefotaxime (CTX 5), aztreonam (ATM 30) and Augmentin (AMC 60).

Plate 15b:

Klebsiella pneumoniae producing an ESBL indicated by the elliptical clear zone between cefotaxime (CTX 5) and Augmentin (AMC 60).

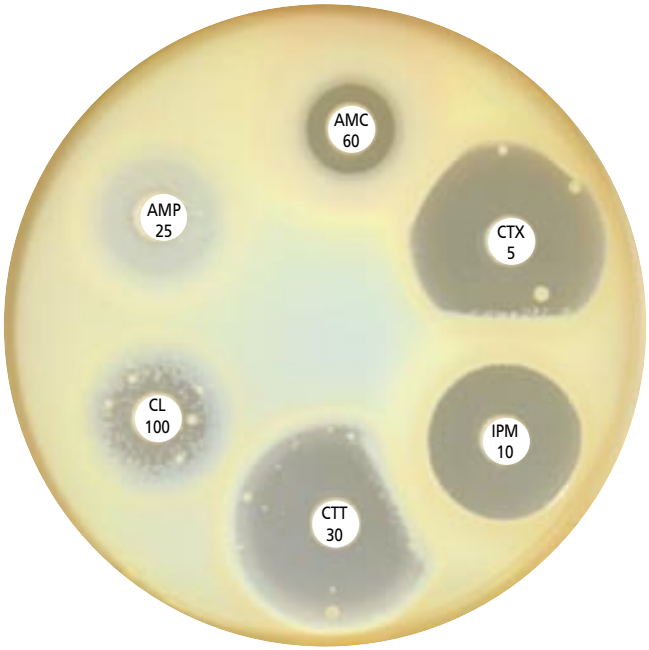


Plate 16a:

Enterobacter cloacae with an inducible group 1 β-lactamase. The organism is resistant to Augmentin (AMC 60).

Note the flattened edges of the cefotaxime (CTX 5) and cefotetan (CTT 30) zones adjacent to the imipenem (IPM 10) disc.

Plate 16b:

Enterobacter cloacae producing high level group 1 β-lactamase. The organism is resistant to Timentin (TIM 85), cefotaxime (CTX 5), imipenem (IPM 10), cefotetan (CTT 30), ceftazidime (CAZ 10) and ampicillin (AMP 25) discs.

Plate 17a:

Proteus penneri suceptible to Augmentin (AMC 60) but with a flattened zone around cefotaxime (CTX 5) adjacent to the imipenem (IPM 10) disc indicating the presence of an inducible group 2e β-lactamase.

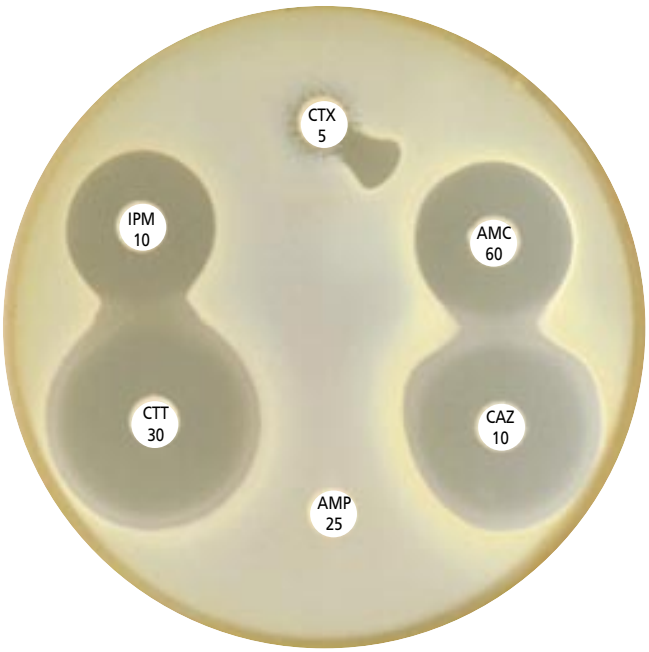


Plate 17b

Proteus penneri producing high levels of group 2e β-lactamase.

Note the susceptibility to ceftazidime (CAZ 10) / cefotetan (CTT 30) and the "keyhole effect" between Augmentin (AMC 60) and cefotaxime (CTX 5).

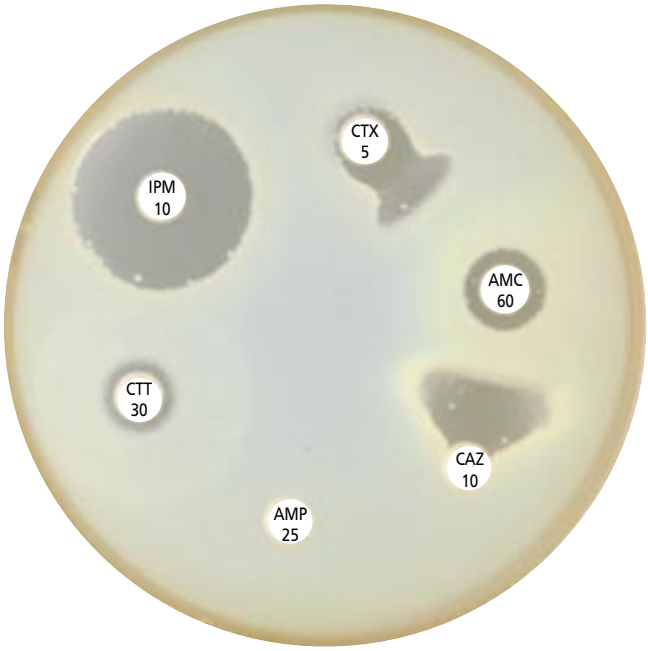


Plate 18a:

Enterobacter cloacae with an inducible group 1 β-lactamase and an ESBL.

Note the "keyhole" between Augmentin (AMC 60) and cefotaxime (CTX 5), ceftazidime (CAZ 10) and resistance to Augmentin (AMC 60) and cefotetan (CTT 30)

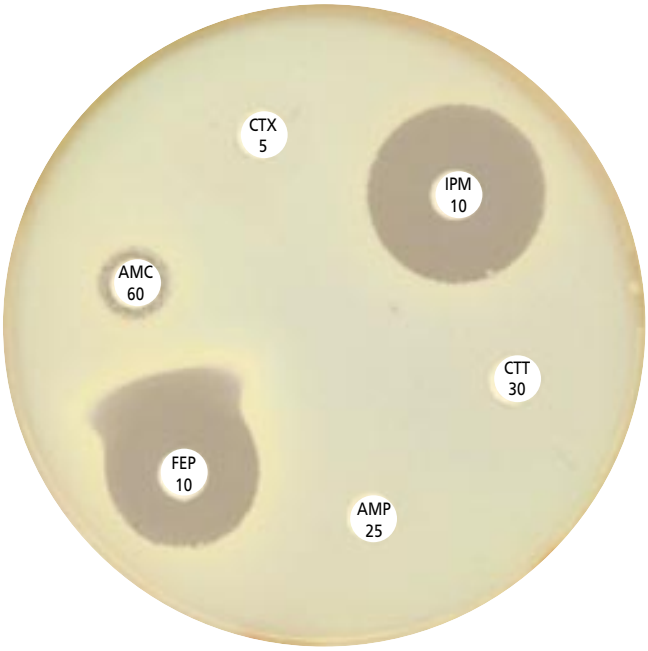


Plate 18b:

Enterobacter cloacae producing high levels of group 1 β-lactamase and an ESBL.

Note the "keyhole" between Augmentin (AMC 60) and cefepime (FEP 10) and the absence of the "keyhole" between Augmentin (AMC 60) and cefotaxime (CTX 5).

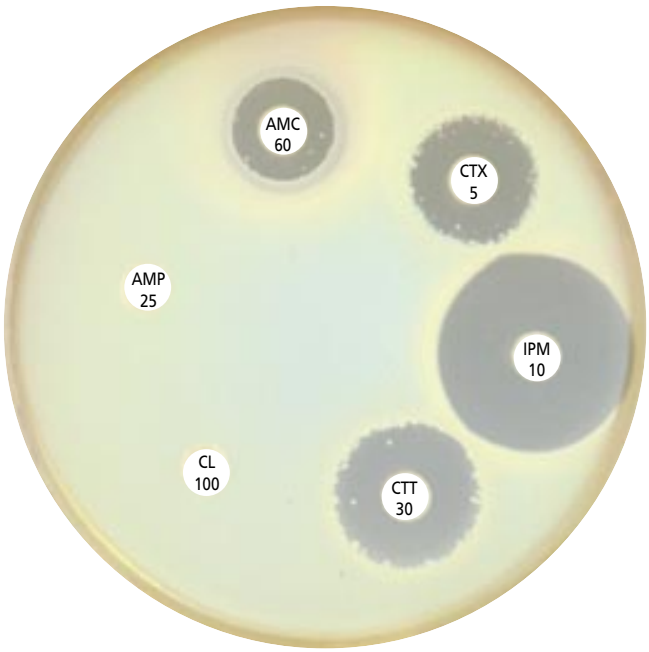


Plate 19a:

E. coli with low activity of the plasmid mediated AmpC β-lactamase.

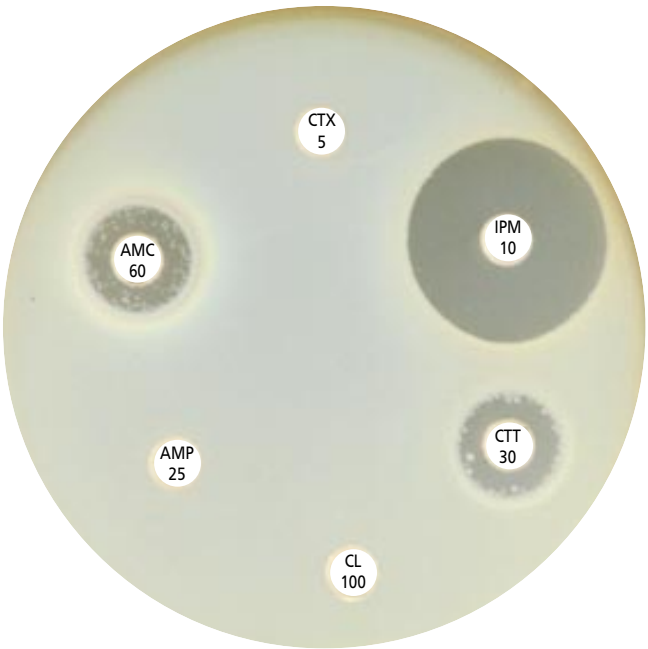


Plate 19b:

E. coli with high activity of the plasmid mediated AmpC β-lactamase.

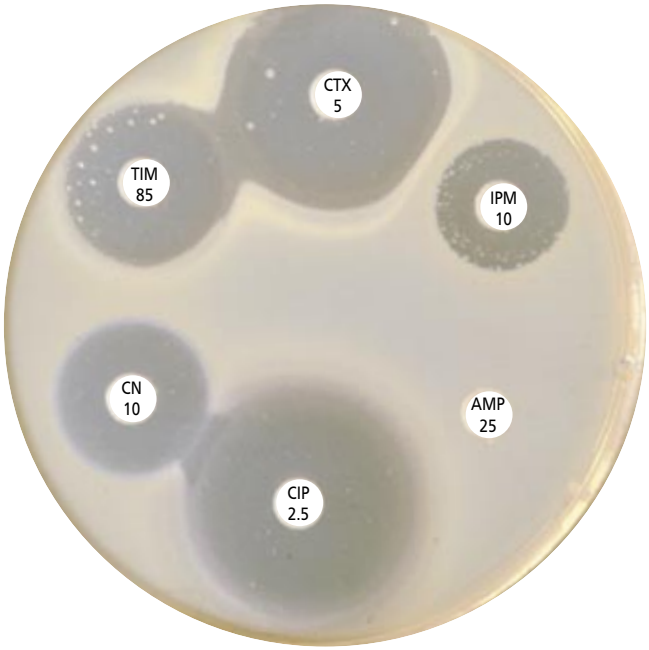


Plate 20a:

A. hydrophila producing the inducible cephalosporinase A1 (flattening of the zone of inhibition around cefotaxime, CTX 5) and the carbapenemase A2 (reduced zone and presence of resistant colonies in the zone around imipenem, IPM 10).

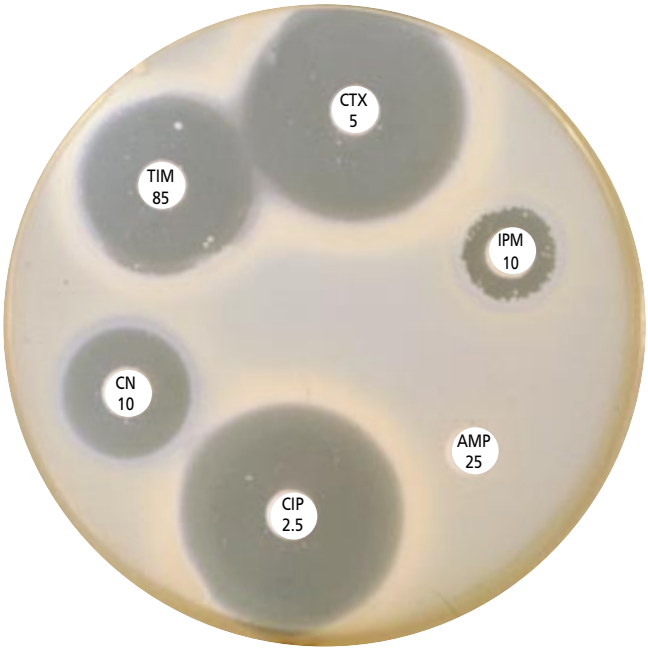


Plate 20b:

A. sobria producing the carbapenemase A2 but lacking the cephalosporinase A1.

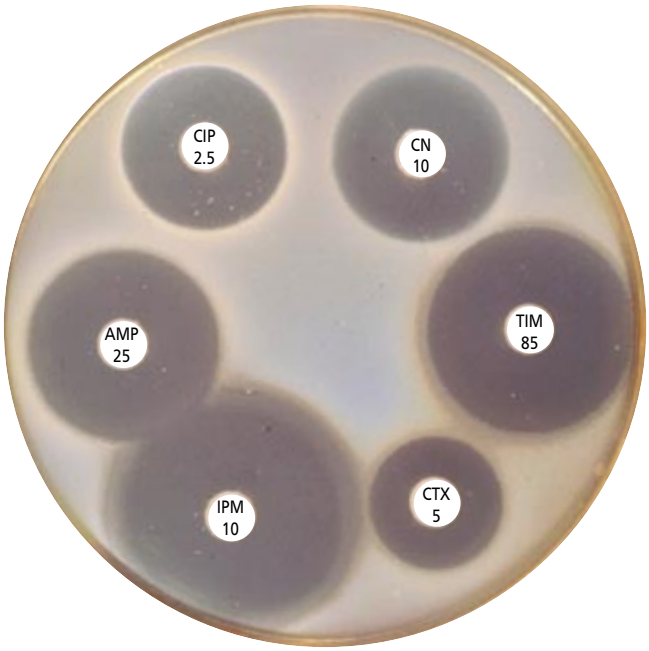


Plate 21a:

A typical blood culture isolate of *A. lwoffi* with a large zone around ampicillin (AMP 25) and a reduced zone around cefotaxime (CTX 5).

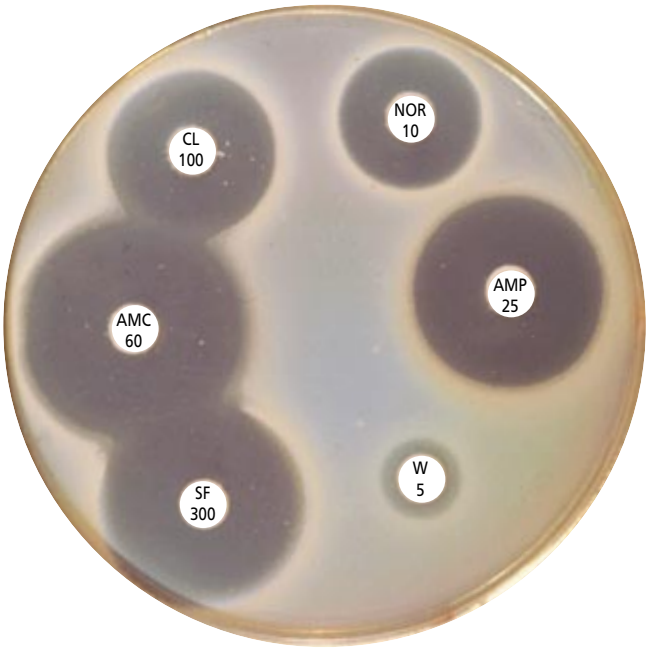


Plate 21b:

A urine isolate of *A. lwoffi* with a large zone around ampicillin (AMP 25), a smaller zone around cephalexin (CL 100), the typical resistance to trimethoprim and the lack of synergy with sulphafurazole.

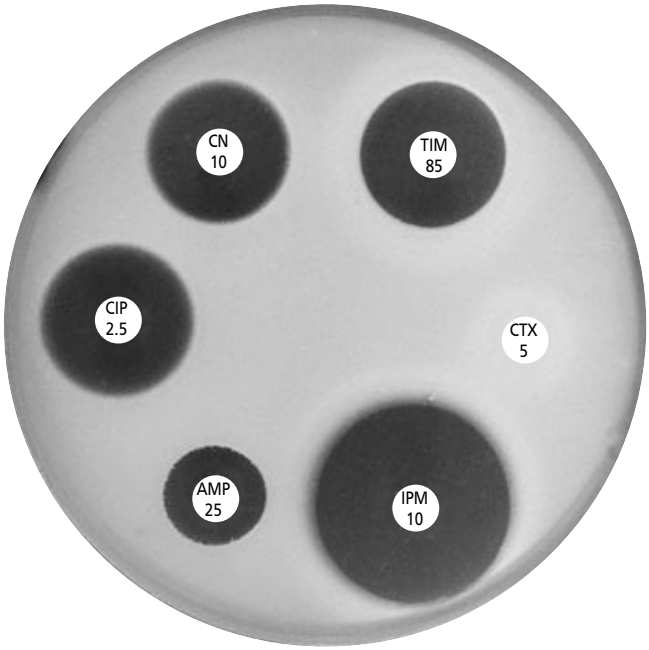


Plate 22a:

A typical isolate of *A. baumannii* with a small zone around ampicillin (AMP 25) and no zone around cefotaxime (CTX 5).

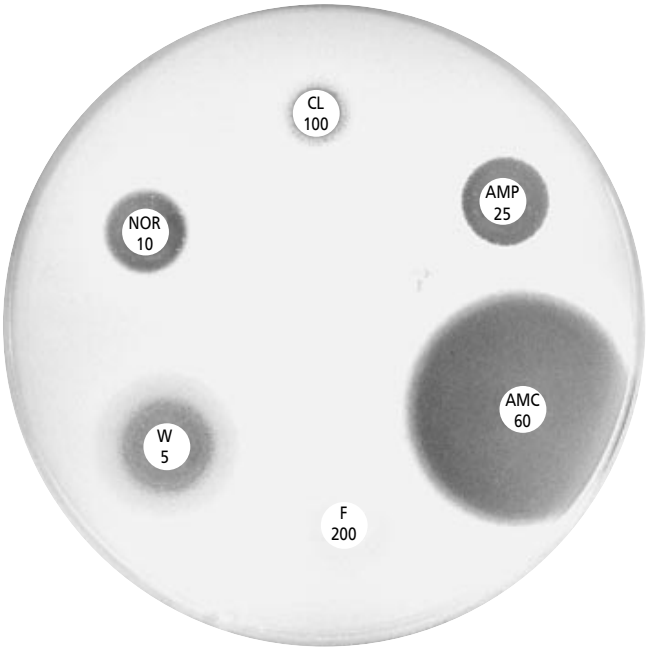


Plate 22b:

A urine isolate of *A. baumannii* with a large zone around Augmentin (AMC 60), a small zone around ampicillin (AMP 25) and none around cephalexin (CL 100).

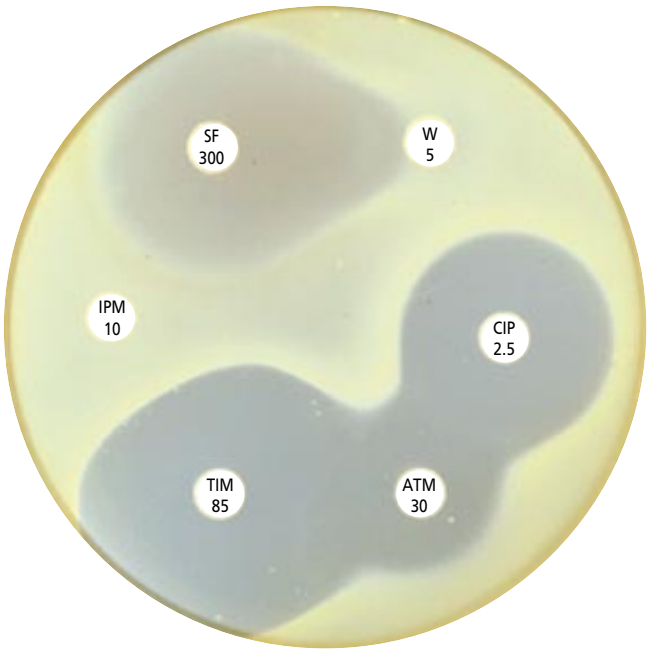


Plate 23a:

The typical susceptibility pattern of *Stenotrophomonas maltophilia* with no zone around imipenem (IPM 10) and the pear shaped zone of inhibition reflecting the synergy between sulphafurazole (SF 300) and trimethoprim (W 5).

Note the presence of numerous resistant colonies in the zones of inhibition around Timentin (TIM 85) and aztreonam (ATM 30).

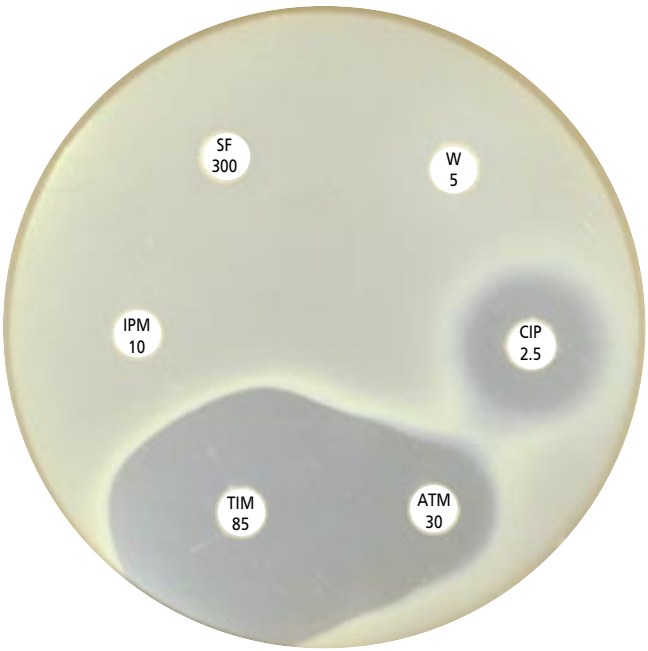


Plate 23b:

Stenotrophomonas maltophilia resistant to sulphafurazole (SF 300).

9. CDS Representatives.

The CDS Representatives who are able to supply reference strains for Quality Control of the CDS method are:

Australian Collection of Microorganisms
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St. Lucia Queensland 4072
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Fax: (07) 33651566
Email: l.rivas@mailbox.uq.edu.au

Australian Capital Territory
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S. M. Bell, B. J. Gatus, J. N. Pham & D. L. Rafferty

The Antibiotic Reference Laboratory

ISBN 0-9581853-0-1