

**REPORT ON THE CDS USERS GROUP WORKSHOP
ANNUAL SCIENTIFIC MEETING
AUSTRALIAN SOCIETY FOR MICROBIOLOGY
CANBERRA 2005**

**Bell, S.M., Gatus, B.J., Pham, J.N. & Rafferty, D.L.
10 November 2005**

Table of Contents.

- i. Calculation of the Uncertainty of Measurement.
- ii. Testing clindamycin 2 µg against Gram-positive organisms.
- iii. Field test of Cefoxitin 10 µg disc and Staph. Aureus
- iv. Group B streptococci and penicillin.
- v. *Klebsiella oxytoca* and the K1 β-lactamase.
- vi. *Pseudomonas aeruginosa* producing an ESBL.
- vii. Gram-negative bacilli and carbapenemase metallo-β-lactamases.
- viii. Testing *Pasteurella* species.
- ix. Reference strains.
- x. Updated Tables 2005 (Tables 1, 2, 3 & 4).

i Calculation of Uncertainty of Measurement for the CDS Test

A discussion document on Uncertainty of Measurement was presented at the workshop. Since then the document has undergone a number of iterations and is presented here as the final document for consideration by laboratories. We thank Andrew Bowen who has expertise in this area and suggested many of the changes made to the original document

The definition of Uncertainty of Measurement (MU) extracted from ISO 15189 (1), clause 3.17, is “the uncertainty of measurement is a parameter associated with the results of a measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurement. The actual variation can be expressed in a number of ways and in the CDS test as the initial assessment expressed the variation as a range of measurements (95% confidence interval) for each antibiotic with each reference strain there are advantages for laboratories to do the same to express variations in their measurements.

There is a wealth of information on MU in the literature and on the web but the simplest and most easily understood explanation and guide to calculating MU is on the National Physical Laboratory website at http://www.npl.co.uk/publications/good_practice/uncert/

There are two ways to estimate uncertainties (see chapter 6 of the above):

Type A components of uncertainty are determined by statistical methods based on the frequency distribution of measurements results experimentally gained. The data on the CDS calibration are in this category and the details are shown below:-

In the development of the CDS Test the mean, standard deviation and 95% confidence interval of the zone sizes recorded with the appropriate reference strains for each antibiotic was calculated by the reference laboratory using over 100 measurements. This statement can be seen at the bottom of Table 3 of acceptable range of Reference strains in CDS Manual 2004 (2).

NATA Technical Circular December 2003 on ‘Uncertainty of Measurement in Biological, Forensic, Medical and Veterinary Testing’ (3) includes the following statement ‘Note 2 of clause 5.4.6.2 of ISO 17025 (4) allows the use of a well-recognized method that specifies limits to the major sources of uncertainty and specifies the form of reporting. The laboratory is considered to have satisfied the MU requirements if it can demonstrate through its initial and ongoing verification records (see 4.7 of the Manual), that it is following the verified method and reporting in accordance with the method’.

In addition to the above, laboratories should determine a type A evaluation of their application of the CDS by recording the zone sizes of the antibiotics used to test the reference strains (as per table page 20 of the Manual). Enter at least 30 measurements on to an Excel spreadsheet and record the mean, standard deviation and 95% confidence intervals of the series of measurements. If the laboratory is successfully applying the CDS tests these confidence intervals will fall within those supplied in the CDS tables. In addition the calculations will indicate in numerical terms to the laboratory what its type A uncertainty of measurement is in the application of the CDS with that particular antibiotic. It is important that in complying with NATA’s requirement to determine and record the measurement of uncertainty that laboratories do not lose sight of the primary purpose of measuring the zone sizes of the reference strains. It is to validate the results of the CDS test in real time and to correct any deficiency in methodology when this becomes apparent by application of the QANTAS checklist. Note also that the results of all tests of the antibiotic susceptibility where the zone sizes of the reference strains fall outside the acceptable range are invalid and must be repeated when the deficiency is corrected.

References.

1. **ISO/IEC 15189** Application Document Supplementary Requirements For Accreditation.
2. **Bell, S. M., Gatus, B. J., Pham, J. N and Rafferty D. L.** 2004. Antibiotic Susceptibility Testing By The CDS Method. A Manual For Medical And Veterinary Laboratories 2004. Third Edition. South Eastern Area Laboratory Services. Sydney, Australia. ISBN 0-646-43892-1.
3. **NATA Technical Circular December 2003.** National Association of Testing Authorities, Australia 2003.
4. **ISO/IEC 17025** Application Document Supplementary Requirements For Accreditation.

ii. Clindamycin 2 µg and Gram- positive organisms

Clindamycin 2 µg was calibrated for the testing of streptococci, *Corynebacterium* species and *Staphylococcus aureus*.

Previously it was not considered necessary to test Gram-positive species against clindamycin but the results of surrogate disc testing would suffice. More recently strains of streptococci group B that are sensitive to erythromycin but resistant to clindamycin have been reported in Australasia, this warrants testing of clindamycin as well as erythromycin for this species. Also the rise in prevalence of CAMRSA that may be susceptible to clindamycin in our communities probably supports the argument for direct testing of these strains to clindamycin even though less than 2% are erythromycin resistant/clindamycin susceptible. Laboratories may continue to use erythromycin as a surrogate disc for clindamycin susceptibility for other strains of Staph. aureus. An outline of the testing procedure and an explanation of the mechanisms involved are set out below.

β-Haemolytic streptococci are tested on blood Sensitest Agar, in air, at 35-37°C. Strains requiring CO₂ are tested in 5 % CO₂.

α-Haemolytic streptococci including *Streptococcus pneumoniae* and *Corynebacterium* species are tested on blood Sensitest agar, in 5 % CO₂, at 35-37°C.

Staphylococcus aureus is tested on Sensitest Agar, in air, at 35-37°C.

If the annular radius of the inhibitory zone around a clindamycin 2 µg disc is ≥ 6 mm, the organism is susceptible to clindamycin. The MIC for susceptible strains is ≤ 0.5 mg/L.

Isolates resistant to erythromycin but susceptible to clindamycin may have inducible clindamycin resistance (ICR). If there is a flattened inhibitory zone around clindamycin near the erythromycin disc, the organism is ICR+ (CDS Manual 2004, Plate 2, p. 57) and should be reported resistant to clindamycin.

NOTE: When testing streptococci and *Corynebacterium* species against clindamycin, an erythromycin 5 µg disc is placed next to a clindamycin 2 µg disc at a distance of 13 mm from edge to edge for optimum detection of ICR. When testing *Staphylococcus aureus* against clindamycin, optimum detection of ICR requires the erythromycin 5 µg disc to be placed next to a clindamycin 2 µg disc at a distance of 15 mm from edge to edge.

Short notes on the mechanisms of resistance to the macrolides (erythromycin, clarithromycin, roxithromycin) and lincosamides (clindamycin, lincomycin) in Gram-positive organisms.

1. Ribosomal modification (*erm* gene) conferring resistance to all macrolides and lincosamides constitutive or inducible (ICR+).
2. An efflux pump (*mef* gene) resistant to erythromycin but susceptible to clindamycin, ICR-)
3. Due to a still unknown mechanism recently described in group B streptococci (GBS), the organism is susceptible to erythromycin while showing a low level of resistance to the lincosamides and streptogramin A (LSA).

Summary of the phenotypes

1. S/Ery, S/Da
2. R/Ery, R/Da (constitutive)
3. R/Ery, S/Da, ICR+ (report as R/Da)
4. R/Ery, S/Da, ICR- (efflux, report as S/Da)
5. S/Ery, R/Da (unknown mechanism seen in GBS only, report S/Ery, R/Da)

(See PowerPoint presentation “Clindamycin” ASM 05 Canberra)

Acceptable range for clindamycin 2 µg discs with reference strains (annular radius in mm)

Streptococcus pneumoniae ARL 10582

(Blood Sensitest Agar, 5 % CO₂, 35-37°C)

7.1 – 9.9

Staphylococcus aureus NCTC 6571

(Sensitest Agar, air, 35-37°C)

8.5 – 12.9

iii. Field trial of cefoxitin 10 µg disc testing of Staphylococcus aureus

Cefoxitin 10 µg discs replaced methicillin 5 discs since early this year and the disc has been used extensively by many laboratories particularly the large private laboratories. The results with this disc were closely monitored and strains with zone from 5 mm to 7 mm were sent to the CDS reference laboratories for *mecA* gene detection.

There was complete agreement between the CDS test and *mecA* gene detection results. That is that all strains of Staph. aureus with zone sizes less than 6mm annular radius were *mecA* positive and strains with a zone sizes greater than 6mm were *mecA* negative.

(See PowerPoint presentation “Cefoxitin” ASM 05 Canberra)

iv. Group B streptococci and penicillin

Occasional GBS isolates may have a reduced inhibitory zone around a penicillin 0.5 u disc. The susceptibility testing of these strains can be approached in a manner similar to that used for *Streptococcus pneumoniae* (Manual 6.5), that is, GBS with a reduced inhibitory zone around a

penicillin 0.5 u disc are retested with an ampicillin 5 µg disc. Interpretation of the results are set out below:-

- If the inhibitory zone is < 6mm around a penicillin 0.5u disc but ≥ 6 mm around an ampicillin 5 µg disc, the susceptibility is reported as “There is reduced susceptibility to benzylpenicillin/ampicillin with the MIC between 0.25 and 1 mg/L.”
- Cefotaxime/ceftriaxone 0.5 µg discs are the surrogate discs for reporting the susceptibility to other cephalosporins (except ceftazidime).

v. Klebsiella oxytoca and K1 enzyme

This short note may help to clear up some misunderstanding of the role of the K1 enzyme in the susceptibility of K. oxytoca to the betalactams.

K1 enzyme is the chromosomal β-lactamase produced by *K. oxytoca* (indole-positive Klebsiella). It is the presence of basal level of K1 enzyme that confers the resistance to ampicillin seen in the majority of *K. oxytoca* isolates. However only mutants that hyper-produce K1 enzyme show resistance to cephalosporins.

Although similar to plasmid mediated ESBLs in belonging to Bush group 2be (extended broad spectrum β-lactamase), K1 enzyme is far less susceptible to the inhibition by clavulanic acid. The synergy between Augmentin/Timentin and cephalosporins in the disc approximation test is therefore modest resulting in a very small or absent key hole between the discs.

(See PowerPoint presentation “Klebsiella” ASM 05 Canberra)

vi. ESBL producing Pseudomonas aeruginosa

Although rare, ESBL producing *Pseudomonas aeruginosa* have been isolated from clinical specimens in this country. The typical synergy (key hole) between Timentin and ceftazidime in the disc approximation test is easily recognised during routine testing.

Therefore we strongly recommend the use of Timentin and ceftazidime discs in adjacent positions when testing *Pseudomonas aeruginosa*. The isolate may be forwarded to the CDS reference laboratory for confirmation by molecular techniques.

(See PowerPoint presentation “Pseudomonas” ASM 05 Canberra)

vii. Metallo-β-lactamase (MBL) producing Gram-negative bacilli

The note below may assist laboratory in identifying MBL producing strains of GNB and the Powerpoint presentation shows the use of EDTA to confirm the presence of this enzyme.

Plasmid mediated MBL's hydrolyse all β-lactams except aztreonam. They are inactivated by EDTA which binds the zinc molecule (Zn ⁺⁺). MBL's are seen in clinical isolates of *Pseudomonas aeruginosa* and *Enterobacteriaceae*.

MBL producing *P. aeruginosa* clinical isolates are resistant to ceftazidime, cefepime, Timentin, piperacillin, tazocin and the carbapenems, imipenem and meropenem. Often they are also resistant to the quinolones and the aminoglycosides.

On the other hand, MBL producing members of the *Enterobacteriaceae* are only border line resistant to imipenem and meropenem and it may be more difficult to demonstrate the presence of the enzyme. However the clue to the presence of MBL is the resistance to all other β -lactams except aztreonam. To further complicate the identification of the enzyme MBL and ESBL may be seen in the same isolate and this phenomenon is demonstrated in the Powerpoint presentation.

(See PowerPoint presentation “MBL” ASM 05 Canberra)

viii. *Pasteurella* species (ASM 04, Sydney)

The addition of Pasteurella sp. to the range organisms was presented at ASM 2004 but no report of this meeting was documented on the CDS web site. The opportunity is taken here to document the inclusion in the CDS test.

Pasteurella species including *P. multocida*, *P. gallinarum*, *P. pneumotropica* and *P. haemolytica* (*Mannheimia haemolytica*) are now calibrated for testing by the CDS method. Since a number of strains of *P. gallinarum*, *P. pneumotropica* and *P. haemolytica* (*Mannheimia haemolytica*) were found to require CO₂, it is recommended that the testing of isolates of the genus *Pasteurella* be performed on Sensitest Agar supplemented with 5% horse blood, in 5 % CO₂ atmosphere at 35-37°C.

For testing and reporting benzylpenicillin, ampicillin, amoxycillin and ceftiofur, two antibiotic discs are used, benzylpenicillin 0.5 u and ampicillin 5 μ g.

1. If the annular radius of the inhibitory zone is ≥ 4 mm with a benzylpenicillin 0.5 u disc, the organism is susceptible to benzylpenicillin, ampicillin, amoxycillin and ceftiofur (See Table 1b).
2. If the annular radius of the inhibitory zone is < 4 mm with a benzylpenicillin 0.5 u disc and ≥ 6 mm with an ampicillin 5 μ g disc, report the susceptibility as follows: “There is reduced susceptibility to benzylpenicillin, ampicillin, amoxycillin and ceftiofur with the MIC between 0.25 and 2.0 mg/L” (See Table 2b).

If the annular radius of the inhibitory zone is < 6 mm with an ampicillin 5 μ g disc, the organism is resistant to benzylpenicillin, ampicillin, amoxycillin and ceftiofur (See Table 1b).

Note: *Pasteurella multocida* is tested against ampicillin 5 μ g not benzylpenicillin 0.5 u. If the annular radius of the inhibitory zone is ≥ 6 mm with an ampicillin 5 μ g disc, report as susceptible.

ix. Reference strains

In future descriptions of the CDS we will refer to the reference strains by their Australian Collection of Micro-organisms accession numbers. These are shown below together with previously used collection numbers.

| Accession number | Reference Strain | |
|------------------|------------------------------|------------|
| ACM 5196 | <i>Bacteroides fragilis</i> | ATCC 25285 |
| ACM 5183 | <i>Campylobacter jejuni</i> | NCTC 11168 |
| ACM 5184 | <i>Enterococcus faecalis</i> | POW 1994 |

| | | |
|----------|---------------------------------|---|
| ACM 5185 | <i>Escherichia coli</i> | NCTC 10418 |
| ACM 5186 | <i>Escherichia coli</i> | NCTC 11560 (β -lactamase positive) |
| ACM 5187 | <i>Haemophilus influenzae</i> | NCTC 4560 |
| ACM 5188 | <i>Haemophilus influenzae</i> | NCTC 11315 (β -lactamase positive) |
| ACM 5189 | <i>Pseudomonas aeruginosa</i> | NCTC 10662 |
| ACM 5190 | <i>Staphylococcus aureus</i> | NCTC 6571 |
| ACM 5191 | <i>Streptococcus pneumoniae</i> | ARL 10582 |

The reference strains may be obtained 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
jeanette.pham@sesiahs.health.nsw.gov.au

OR

Lucy Rivas
 The Australian Collection of Micro-organisms (ACM)
 Department of Microbiology and Parasitology
 Building 76 – Molecular and Microbial Sciences
 University of Queensland
 St Lucia QLD 4072

Tel: (07) 3365 3211
 Fax: (07) 3365 1566
 Email: l.rivas@mailbox.uq.edu.au

x. Updated Tables 2005

Table 1a. Calibrations 2005. 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) |
|---|-------------------|--------------------------------------|------------------------------------|
| <i>Corynebacterium spp.</i> | | | |
| (Blood Sensitest, CO₂, 35-37°C) * | | | |
| Ampicillin • | 5 | | ≤ 2 |
| Benzylpenicillin | 0.5 u | | ≤ 0.125 |
| Chloramphenicol | 30 | | ≤ 8 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Clindamycin | 2 | | ≤ 0.5 |
| Erythromycin | 5 | | ≤ 0.5 |
| Fusidic acid | 2.5 | | ≤ 0.5 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Quinupristin/dalfopristin | 15 | | ≤ 2 |
| Rifampicin | 1 | | ≤ 0.5 |
| Teicoplanin | 15 | 2 mm | ≤ 8 |
| Tetracycline | 30 | | ≤ 4 |
| Vancomycin | 5 | 2 mm | ≤ 4 |
| <i>Enterococci</i> | | | |
| (Blood Sensitest, air, 35-37°C) | | | |
| Ampicillin | 5 ^Φ | 4 mm ^Φ | ≤ 4 |
| Chloramphenicol | 30 | 4 mm | ≤ 8 |
| Gentamicin | 200 | 4 mm | ≤ 512 |
| Linezolid | 10 | | ≤ 4 |
| Nitrofurantoin ⁺ | 200 | 4 mm | ≤ 64 |
| Quinupristin/dalfopristin [§] | 15 | | ≤ 2 |
| Teicoplanin | 15 | 2 mm | ≤ 8 |
| Vancomycin | 5 | (See foot note) [%] | ≤ 4 |
| <i>Listeria spp.</i> | | | |
| (Blood Sensitest, air, 35-37°C) | | | |
| Ampicillin | 5 | | ≤ 1 |
| Gentamicin | 10 | | ≤ 1 |

^Φ 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 benzylpenicillin 0.5 u, test ampicillin 5 µg.

+ For testing urine isolates only.

[§] Quinupristin/dalfopristin are inactive against *Enterococcus faecalis*.

Table 1a. Calibrations 2005. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS CONTINUED

| Antibiotic | Disc potency (µg) | Exception to standard interpretation | MIC for susceptible strains (mg/L) |
|----------------------------------|-------------------|--------------------------------------|------------------------------------|
| <i>Staphylococci</i> | | | |
| (Sensitest, air, 35-37°C) | | | |
| Ampicillin • | 5 | | ≤ 0.5 |
| Benzylpenicillin § | 0.5 u | | ≤ 0.06 |
| Cefoxitin % | 10 | | ≤ 4 |
| Cephalexin • | 100 | | ≤ 16 |
| Chloramphenicol | 30 | | ≤ 8 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Clindamycin | 2 | | ≤ 0.5 |
| Co-trimoxazole | 25 | | ≤ 1/19 |
| Erythromycin | 5 | | ≤ 0.5 |
| Fusidic acid | 2.5 | | ≤ 0.5 |
| Gentamicin | 10 | | ≤ 1 |
| Kanamycin | 50 | | ≤ 8 |
| Linezolid | 10 | | ≤ 4 |
| Methicillin § | 5 | | ≤ 4 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Mupirocin | 5 | | ≤ 2 |
| Neomycin # | 30 | | ≤ 4 |
| Nitrofurantoin + | 200 | | ≤ 32 |
| Novobiocin # | 5 | | ≤ 1 |
| Oxacillin * | 1 | | ≤ 0.25 |
| Quinupristin/dalfopristin | 15 | | ≤ 2 |
| Rifampicin | 1 | | ≤ 0.5 |
| Sulphafurazole | 300 | | ≤ 64 |
| Teicoplanin | 15 | 2 mm | ≤ 8 |
| Tetracycline | 30 | | ≤ 4 |
| Trimethoprim | 5 | | ≤ 4 |
| Vancomycin | 5 | 2 mm | ≤ 4 |

+ For testing urine isolates only

§ NOT for testing *Staphylococcus saprophyticus*.

% For testing *Staphylococcus aureus* ONLY.

• For testing *Staphylococcus saprophyticus* ONLY.

* For testing coagulase-negative staphylococci (except *Staphylococcus saprophyticus*).

Antibiotic calibrated for veterinary medicine.

Table 1a. Calibrations 2005. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-POSITIVE ORGANISMS CONTINUED

| Antibiotic | Disc potency (µg) | Exception to standard interpretation | MIC for susceptible strains (mg/L) |
|---|-------------------|--------------------------------------|------------------------------------|
| Streptococci & Erysipelothrix spp. | | | |
| (Blood Sensitest, air, 35-37°C) @ | | | |
| Ampicillin ↓ | 5 | 4 mm | ≤ 2 |
| Benzylpenicillin | 0.5 u | | ≤ 0.125 |
| Cefotaxime | 0.5 | | ≤ 0.25 |
| Ceftriaxone | 0.5 | | ≤ 0.25 |
| Cefotaxime ↓ | 5 | | ≤ 2 |
| Ceftriaxone ↓ | 5 | | ≤ 2 |
| Chloramphenicol | 30 | | ≤ 8 |
| Clindamycin | 2 | | ≤ 0.5 |
| Co-trimoxazole | 25 | | ≤ 0.5/9.5 |
| Erythromycin | 5 | | ≤ 0.5 |
| Moxifloxacin/Gatifloxacin | 2.5 | 4 mm | ≤ 1 |
| Nitrofurantoin + | 200 | | ≤ 32 |
| Quinupristin/dalfopristin | 15 | | ≤ 2 |
| Rifampicin | 1 | | ≤ 0.5 |
| Teicoplanin | 15 | 2 mm | ≤ 8 |
| Tetracycline | 30 | | ≤ 4 |
| Vancomycin | 5 | 2 mm | ≤ 4 |

+ For testing urine isolates only

@ *Streptococcus pneumoniae*, *Streptococcus anginosus (milleri)* and *Erysipelothrix* spp. are incubated in 5% CO₂.

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

Table 1b. Calibrations 2005. 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) |
|--|----------------------|---|---------------------------------------|
| <i>Enterobacteriaceae, Vibrionaceae, & Acinetobacter spp.</i> | | | |
| (Sensitest, air, 35-37°C) * | | | |
| Amikacin | 30 | | ≤ 4 |
| Ampicillin | 25 | | ≤ 8 |
| Apramicin # | 15 | | ≤ 8 |
| Augmentin • | 60 | | ≤16/8 |
| Aztreonam | 30 | | ≤ 8 |
| Cefazolin | 30 | | ≤16 |
| Cefepime | 10 | | ≤ 2 |
| Cefotaxime | 5 | | ≤ 1 |
| Cefotetan | 30 | | ≤ 8 |
| Cefoxitin | 30 | | ≤ 8 |
| Cefpirome | 10 | | ≤ 2 |
| Cefpodoxime | 10 | | ≤ 2 |
| Ceftazidime | 10 | | ≤ 4 |
| Ceftriaxone | 5 | | ≤ 1 |
| Cefuroxime | 30 | | ≤ 8 |
| Cephalexin | 100 | | ≤ 16 |
| Chloramphenicol | 30 | | ≤ 8 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Enoxacin | 10 | | ≤ 4 |
| Ertapenem @ | 10 | | ≤ 4 |
| Gentamicin | 10 | 4 mm & | ≤ 2 |
| Imipenem | 10 | | ≤ 4 |
| Kanamycin | 50 | | ≤ 8 |
| Meropenem | 5 | | ≤ 2 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Nalidixic acid + | 30 | | ≤ 4 |
| Neomycin # | 30 | | ≤ 4 |
| Netilmicin | 30 | | ≤ 2 |
| Nitrofurantoin + | 200 | | ≤ 32 |
| Norfloxacin + | 10 | | ≤ 4 |
| Spectinomycin # | 25 | | ≤ 32 |
| Streptomycin # | 25 | | ≤ 16 |
| Sulphafurazole | 300 | | ≤ 64 |
| Tazocin • | 55 | | ≤ 16/2 |
| Tetracycline | 30 | | ≤ 4.0 |
| Timentin • | 85 | | ≤ 32/2 |
| Tobramycin | 10 | 4 mm & | ≤ 2 |
| Trimethoprim | 5 | | ≤ 4 |

* *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.

@ *Acinetobacter* spp. are considered resistant to ertapenem.

& New annular radius of the inhibitory zone for susceptible strains following recalibration.

Antibiotic calibrated for veterinary medicine.

Table 1b. Calibrations 2005. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-NEGATIVE ORGANISMS CONTINUED

| Antibiotic | Disc potency (µg) | Exception to standard interpretation | MIC for susceptible strains (mg/L) |
|---|----------------------|---|---------------------------------------|
| <i>Pseudomonas</i> spp., <i>Burkholderia</i> spp. & <i>Chryseobacterium</i> spp. | | | |
| (Sensitest, air, 35-37°C) | | | |
| Amikacin | 30 | 4 mm | ≤ 16 |
| Aztreonam | 30 | | ≤ 8 |
| Cefepime | 10 | | ≤ 2 |
| Cefpirome | 10 | | ≤ 2 |
| Ceftazidime | 10 | | ≤ 4 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Ertapenem | 10 | | ≤ 4 |
| Gentamicin | 10 | 4 mm | ≤ 4 |
| Imipenem | 10 | | ≤ 4 |
| Meropenem | 5 | | ≤ 2 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Netilmicin | 30 | 4 mm | ≤ 8 |
| Norfloxacin ⁺ | 10 | | ≤ 4 |
| Piperacillin | 50 | | ≤ 16 |
| Polymyxin | 300 u | 4 mm | ≤ 1 |
| Sulphafurazole | 300 | | ≤ 64 |
| Tazocin | 55 | | ≤ 16/2 |
| Ticarcillin | 75 | | ≤ 32 |
| Timentin | 85 | | ≤ 32/2 |
| Tobramycin | 10 | 4 mm | ≤ 4 |
| Trimethoprim | 5 | | ≤ 4 |
| <i>Campylobacter</i> spp. | | | |
| (Blood Sensitest, microaerophilic, 42°C) | | | |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Erythromycin | 5 | 4 mm | ≤ 0.5 |
| Gentamicin | 10 | | ≤ 1 |
| Tetracycline | 30 | | ≤ 4 |
| <i>Haemophilus influenzae</i>/<i>Haemophilus</i> spp. | | | |
| (HTM[®] agar, 5% CO₂, 35-37°C) | | | |
| Ampicillin | 5 | | ≤ 1 |
| Augmentin | 15 | | ≤ 2/1 |
| Cefaclor | 30 | | ≤ 4 |
| Cefotaxime | 0.5 | | ≤ 0.25 |
| Cefpodoxime | 10 | | ≤ 2 |
| Ceftriaxone | 0.5 | | ≤ 0.25 |
| Cefuroxime | 30 | | ≤ 4 |
| Chloramphenicol | 10 | | ≤ 2 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Co-trimoxazole | 25 | | ≤ 1/19 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Tetracycline | 30 | | ≤ 4 |

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

Table 1b. Calibrations 2005. Antibiotics, disc potencies, the MIC breakpoint for susceptible strains, the media and incubation conditions used.

GRAM-NEGATIVE ORGANISMS CONTINUED

| Antibiotic | Disc potency (µg) | Exception to standard interpretation | MIC for susceptible strains (mg/L) |
|--|----------------------|---|---------------------------------------|
| <i>Helicobacter pylori</i> | | | |
| (Chocolate Columbia Blood Agar, microaerophilic, 35-37°C) | | | |
| Amoxicillin | 2 | | ≤ 1 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Erythromycin * | 5 | | ≤ 0.5 |
| Metronidazole | 5 | | ≤ 4 |
| Rifampicin % | 5 | | ≤ 2 |
| Tetracycline | 30 | | ≤ 4 |
| <i>Branhamella catarrhalis</i> | | | |
| (Blood Sensitest, 5% CO₂, 35-37°C) | | | |
| Benzylpenicillin | 0.5 u | | ≤ 0.25 |
| Cefaclor | 30 | | ≤ 4 |
| Cefpodoxime | 10 | | ≤ 2 |
| Cefuroxime | 30 | | ≤ 4 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Co-trimoxazole | 25 | | ≤ 1/19 |
| Erythromycin | 5 | | ≤ 0.5 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Tetracycline | 30 | | ≤ 4 |
| <i>Neisseria meningitidis</i> | | | |
| (Blood Sensitest, 5% CO₂, 35-37°C) | | | |
| Benzylpenicillin | 0.5 u | 4 mm | ≤ 0.25 |
| Cefotaxime | 0.5 | | ≤ 0.25 |
| Ceftriaxone | 0.5 | | ≤ 0.25 |
| Chloramphenicol | 10 | | ≤ 2 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Rifampicin | 1 | | ≤ 0.5 |
| <i>Pasteurella species</i> | | | |
| (Blood Sensitest, 5% CO₂, 35-37°C) | | | |
| Benzylpenicillin | 0.5 u | 4 mm | ≤ 0.25 |
| Ampicillin [@] | 5 | | ≤ 2 |
| Ciprofloxacin | 2.5 | | ≤ 1 |
| Moxifloxacin/Gatifloxacin | 2.5 | | ≤ 1 |
| Tetracycline | 30 | | ≤ 4 |
| <i>Stenotrophomonas maltophilia</i> | | | |
| (Sensitest, air, 35-37°C) | | | |
| Sulphafurazole | 300 | | ≤ 64 |

* Erythromycin 5 µg is the surrogate disc for reporting the susceptibility to clarithromycin. The MIC of clarithromycin for susceptible strains is ≤ 0.5 mg/L.

% Rifampicin 5 µg is the surrogate disc for reporting the susceptibility to rifabutin.

@ *Pasteurella multocida* is tested against ampicillin 5 µg not benzylpenicillin 0.5 u.

Table 2a. Surrogate disc testing 2006. 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 <i>S. saprophyticus</i> from urine) | | |
| Amoxicillin/ Ampicillin/ Penicillin V | Benzylpenicillin | 0.5 u |
| Augmentin | Methicillin/Oxacillin [•] /Cefoxitin [@] | 5 |
| Azithromycin/ Clarithromycin/ Roxithromycin | Erythromycin | 5 |
| <i>Ceftiofur</i> [#] / other cephalosporins ^{&} | Methicillin/Oxacillin [•] /Cefoxitin [@] | 5 |
| Clindamycin/lincomycin | Erythromycin | 5 |
| Cloxacillin/ Dicloxacillin/ Flucloxacillin | Methicillin/Oxacillin [•] /Cefoxitin [@] | 5 |
| Co-trimoxazole ⁺ | Sulphafurazole | 300 |
| Co-trimoxazole ⁺ | Trimethoprim | 5 |
| <i>Enrofloxacin</i> [#] / <i>Orbifloxacin</i> [#] | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> [#] | Moxifloxacin | 2.5 |
| Norfloxacin [§] | Ciprofloxacin | 2.5 |
| Sulphonamides | Sulphafurazole | 300 |
| Tetracyclines | Tetracycline | 30 |
| <i>Tylosin</i> [#] | Erythromycin | 5 |
| <i>Staphylococcus saprophyticus</i> from urine | | |
| Amoxicillin/ Benzylpenicillin/ Penicillin V | Ampicillin | 5 |
| Augmentin | Cephalexin | 100 |
| <i>Ceftiofur</i> [#] / other cephalosporins ^{&} | Cephalexin | 100 |
| Cloxacillin/ Dicloxacillin/ Flucloxacillin | Cephalexin | 100 |
| Co-trimoxazole ⁺ | Sulphafurazole | 300 |
| Co-trimoxazole ⁺ | Trimethoprim | 5 |
| <i>Enrofloxacin</i> [#] / <i>Orbifloxacin</i> [#] | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> [#] | Moxifloxacin | 2.5 |
| Norfloxacin [§] | Ciprofloxacin | 2.5 |
| Sulphonamides | Sulphafurazole | 300 |
| Tetracyclines | Tetracycline | 30 |
| <i>Streptococci</i>[*] | | |
| Amoxicillin/ Ampicillin/ Penicillin V | Benzylpenicillin | 0.5 u |
| Amoxicillin/ Benzylpenicillin | Ampicillin [↓] | 5 |
| Azithromycin/ Clarithromycin/ Roxithromycin | Erythromycin | 5 |
| <i>Ceftiofur</i> [#] | Benzylpenicillin | 0.5 u |
| Cephalosporins (except <i>ceftiofur</i>) [#] | Cefotaxime/Ceftriaxone | 0.5 |
| Lincomycin | Clindamycin | 2 |
| <i>Marbofloxacin</i> [#] | Moxifloxacin | 2.5 |
| Tetracyclines | Tetracycline | 30 |
| <i>Tylosin</i> [#] | Erythromycin | 5 |

[&] 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 *Streptococcus anginosus*, the susceptibility to benzylpenicillin, ampicillin, amoxicillin, cloxacillin and cephalosporin antibiotics (except ceftazidime) is extrapolated from the testing of benzylpenicillin 0.5 u.

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

[•] For testing coagulase-negative staphylococci (except *Staphylococcus saprophyticus*) ONLY.

[@] For testing *Staphylococcus aureus* ONLY.

[#] Antibiotic used in veterinary medicine only.

[%] Clindamycin 2 µg has been calibrated for the detection of ICR-negative staphylococci (see ASM 2005 handout).

Table 2a. Surrogate disc testing 2005. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-POSITIVE ORGANISMS CONTINUED

| Antibiotic reported | Surrogate disc used | Disc potency (µg) |
|--|---------------------|-------------------|
| <i>Corynebacterium</i> spp. | | |
| Amoxicillin/ Ampicillin/ Penicillin V | Benzylpenicillin | 0.5 u |
| Azithromycin/ Clarithromycin/ Roxithromycin | Erythromycin | 5 |
| Ceftiofur [#] / other cephalosporins ^{&} | Benzylpenicillin | 0.5 u |
| Enrofloxacin [#] /Orbifloxacin [#] | Ciprofloxacin | 2.5 |
| Lincomycin | Clindamycin | 2 |
| Marbofloxacin [#] | Moxifloxacin | 2.5 |
| Norfloxacin [§] | Ciprofloxacin | 2.5 |
| Tetracyclines | Tetracycline | 30 |
| Tylosin [#] | Erythromycin | 5 |
| <i>Enterococci</i> | | |
| Amoxicillin/ Benzylpenicillin | Ampicillin | 5 |
| <i>Listeria</i> spp. | | |
| Amoxicillin/ Benzylpenicillin | Ampicillin | 5 |

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

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

[#] Antibiotic used in veterinary medicine only.

Table 2b. Surrogate disc testing 2005. 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) |
|---|------------------------|-------------------|
| <i>Branhamella catarrhalis</i> | | |
| Azithromycin/ Clarithromycin/ Roxithromycin | Erythromycin | 5 |
| Amoxycillin/ Ampicillin/ Penicillin V | Benzylpenicillin | 0.5 u |
| Augmentin | Cefuroxime/Cefaclor | 30 |
| <i>Ceftiofur</i> # | Cefuroxime/Cefaclor | 30 |
| Cephalosporins | Cefuroxime/Cefaclor | 30 |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| Tetracyclines | Tetracycline | 30 |
| <i>Campylobacter spp.</i> | | |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| Tetracyclines | Tetracycline | 30 |
| <i>Enterobacteriaceae/ Vibronaceae/ Acinetobacter spp.</i> | | |
| Amoxycillin | Ampicillin | 25 |
| <i>Ceftiofur</i> # | Cefazolin | 30 |
| Cephalothin @ | Ampicillin | 25 |
| Ceftriaxone | Cefotaxime | 5 |
| Cefotaxime | Ceftriaxone | 5 |
| Co-trimoxazole + | Sulphafurazole | 300 |
| Co-trimoxazole + | Trimethoprim | 5 |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| Piperacillin | Ampicillin | 25 |
| Sulphonamides | Sulphafurazole | 300 |
| Tetracyclines | Tetracycline | 30 |
| Ticarcillin | Ampicillin | 25 |
| <i>Haemophilus influenzae/Haemophilus spp.</i> | | |
| Amoxycillin | Ampicillin | 5 |
| Cefepime | Cefotaxime/Ceftriaxone | 0.5 |
| Cefotaxime | Ceftriaxone | 0.5 |
| Cefpirome | Cefotaxime/Ceftriaxone | 0.5 |
| Ceftazidime | Cefotaxime/Ceftriaxone | 0.5 |
| <i>Ceftiofur</i> # | Cefuroxime/Cefaclor | 30 |
| Ceftriaxone | Cefotaxime | 0.5 |
| Cephalexin | Cefuroxime/Cefaclor | 30 |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| Tetracyclines | Tetracycline | 30 |
| <i>Helicobacter pylori</i> | | |
| Clarithromycin | Erythromycin | 5 |
| Rifabutin | Rifampicin | 5 |

@ Not for *Acinetobacter* spp.

Antibiotic used in veterinary medicine only.

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

Table 2b. Surrogate disc testing 2005. Antibiotics that can be reported based on susceptibility results obtained with a surrogate disc.

GRAM-NEGATIVE ORGANISMS CONTINUED

| Antibiotic reported | Surrogate disc used | Disc potency (µg) |
|---|---------------------|-------------------|
| N. meningitidis | | |
| Ampicillin / Amoxicillin | Benzylpenicillin | 0.5 u |
| Cefotaxime | Ceftriaxone | 0.5 |
| <i>Ceftiofur</i> # | Benzylpenicillin | 0.5 u |
| Ceftriaxone | Cefotaxime | 0.5 |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| <i>Pasteurella</i> species | | |
| Ampicillin / Amoxicillin | Benzylpenicillin® | 0.5 u |
| Amoxicillin / Benzylpenicillin | Ampicillin | 5 |
| <i>Ceftiofur</i> # | Ampicillin | 5 |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| Tetracyclines (eg. Doxycycline) | Tetracycline | 30 |
| <i>Pseudomonas</i> spp., <i>Burkholderia</i> spp. & <i>Chryseobacterium</i> spp. | | |
| Azlocillin | Piperacillin | 50 |
| Colistin | Polymyxin | 300 u |
| Co-trimoxazole + | Trimethoprim | 5 |
| Co-trimoxazole + | Sulphafurazole | 300 |
| <i>Enrofloxacin</i> #/ <i>Orbifloxacin</i> # | Ciprofloxacin | 2.5 |
| <i>Marbofloxacin</i> # | Moxifloxacin | 2.5 |
| <i>Stenotrophomonas maltophilia</i> | | |
| Co-trimoxazole | Sulphafurazole | 300 |

Antibiotic used in veterinary medicine only.

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

® Not applicable to *Pasteurella multocida*.

Table 3a. Reference strains 2005. 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) |
|---|----------------------|-----------------------------|
| <i>Enterococcus faecalis</i> POW 1994 | | |
| (Blood Sensitest, air, 35°C) | | |
| Ampicillin | 5 | 5.9 - 9.2 |
| Chloramphenicol | 30 | 6.3 - 8.7 |
| Gentamicin | 200 | 6.6 - 9.9 |
| Linezolid | 10 | 6.6 - 9.0 |
| Nitrofurantoin | 200 | 6.1 - 8.7 |
| Teicoplanin | 15 | 3.1 - 5.3 |
| Vancomycin | 5 | 2.0 - 3.7 |
| <i>Staphylococcus aureus</i> NCTC 6571 | | |
| (Sensitest, air, 35°C) | | |
| Amoxicillin • | 2 | 9.1 - 11.9 |
| Ampicillin | 5 | 12.1 - 18.1 |
| Benzylpenicillin | 0.5 u | 8.7 - 13.5 |
| Cefoxitin | 10 | 7.1 - 10.1 |
| Cephalexin | 100 | 10.7 - 15.5 |
| Chloramphenicol | 30 | 7.8 - 11.4 |
| Ciprofloxacin | 2.5 | 9.2 - 12.4 |
| Clindamycin | 2 | 8.5 - 12.9 ^{&} |
| Co-trimoxazole | 25 | 10.1 - 13.3 |
| Erythromycin | 5 | 8.0 - 10.8 |
| 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 |
| <i>Neomycin</i> | 30 | 8.1 - 12.9 |
| <i>Novobiocin</i> | 5 | 6.1 - 12.5 |
| Oxacillin | 1 | 7.4 - 10.4 |
| Quinupristin/dalfopristin | 15 | 9.2 - 12.4 |
| 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 |

* The acceptable range (95% confidence limits) is the mean \pm 2 standard deviations. The mean was derived from > 120 measurements with different operators using different batches of both agar and discs. It is statistically acceptable to use one hundred measurements to represent the "normal distribution" and this gives a confidence limit of 95%, meaning an in-built MU of 5% for the test.

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.

• For *Helicobacter pylori* ONLY.

& New/adjusted acceptable range.

Table 3a. Reference strains 2005. 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 CONTINUED

| Antibiotic | Disc content (µg) | Acceptable range* (mm) |
|--|----------------------|----------------------------|
| <i>Streptococcus pneumoniae</i> ARL 10582 | | |
| (Blood Sensitest, 5% CO₂, 35-37°C) | | |
| Ampicillin | 5 | 10.8 - 15.2 |
| Benzylpenicillin | 0.5u | 8.3 - 14.8 |
| Cefotaxime | 0.5 | 9.3 - 14.8 |
| Cefotaxime | 5 | 10.9 - 15.3 |
| Ceftriaxone | 0.5 | 9.1 - 14.3 |
| Ceftriaxone | 5 | 11.5 - 15.2 |
| Chloramphenicol | 30 | 8.0 - 13.2 |
| Clindamycin | 2 | 7.1 - 9.9 ^{&} |
| 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 |
| Quinupristin/dalfopristin | 15 | 6.4 - 9.2 |
| 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 \pm 2 standard deviations. The mean was derived from > 120 measurements with different operators using different batches of both agar and discs. It is statistically acceptable to use one hundred measurements to represent the "normal distribution" and this gives a confidence limit of 95%, meaning an in-built MU of 5% for the test.

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.

[&] New/adjusted acceptable range.

Table 3b. Reference strains 2005. 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) |
|--|----------------------|-----------------------------|
| <i>Escherichia coli</i> NCTC 10418 [@] (Sensitest, air, 35-37°C) | | |
| Amikacin | 30 | 6.7 - 10.3 |
| Ampicillin | 25 | 7.5 - 10.7 |
| Apramycin | 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 | 8.3 - 11.1 |
| 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 |
| Ertapenem | 10 | 12.1 - 16.1 |
| 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 | 6.7 - 10.7 ^{&} |
| Tetracycline | 30 | 5.8 - 11.0 |
| Tobramycin | 10 | 6.4 - 8.4 |
| Trimethoprim | 5 | 8.8 - 13.6 |

* The acceptable range (95% confidence limits) is the mean \pm 2 standard deviations. The mean was derived from > 120 measurements with different operators using different batches of both agar and discs. It is statistically acceptable to use one hundred measurements to represent the "normal distribution" and this gives a confidence limit of 95%, meaning an in-built MU of 5% for the test.

[@] If antibiotic discs 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.

[&] New/adjusted acceptable range.

Table 3b. Reference strains 2005. 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) |
|--|----------------------|---------------------------|
| <i>Escherichia coli</i> NCTC 11560 (Sensitest, air, 35-37°C) | | |
| Augmentin | 60 | 6.4 - 9.6 |
| Timentin | 85 | 6.0 - 8.4 |
| Tazocin | 55 | 7.4 - 9.2 |
| <i>Bacteroides fragilis</i> ATCC 25285 (Blood Sensitest, anaerobic, 35-37°C) | | |
| Metronidazole | 5 | 7.1 - 13.5 |
| <i>Campylobacter jejuni</i> 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 |
| <i>Haemophilus influenzae</i> 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 |
| <i>Haemophilus influenzae</i> NCTC 11315 (HTM [®] agar, 5% CO ₂ , 35-37°C) | | |
| Augmentin | 15 | 7.7 - 10.1 |

* The acceptable range (95% confidence limits) is the mean \pm 2 standard deviations. The mean was derived from > 120 measurements with different operators using different batches of both agar and discs. It is statistically acceptable to use one hundred measurements to represent the "normal distribution" and this gives a confidence limit of 95%, meaning an in-built MU of 5% for the test.

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

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.

Table 3b. Reference strains 2005. 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) |
|---|----------------------|---------------------------|
| <i>Pseudomonas aeruginosa</i> NCTC 10662[@] | | |
| (Sensitest, air, 35-37°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 |
| Ertapenem [§] | 10 | -- |
| 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 \pm 2 standard deviations. The mean was derived from > 120 measurements with different operators using different batches of both agar and discs. It is statistically acceptable to use one hundred measurements to represent the "normal distribution" and this gives a confidence limit of 95%, meaning an in-built MU of 5% for the test.

[@] If antibiotic discs 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.

[§] Test *Escherichia coli* NCTC 10418 instead.

Table 4, 2005. A guide for the testing/reporting of β -lactam antibiotics for *Enterobacteriaceae*/*Aeromonas* spp., *Pseudomonas*/*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 |
| <i>Ent. cloacae</i> / <i>Ent. aerogenes</i> | R | R | R | R | R | R | T | R | R |
| <i>Cit. freundii</i> | R | R | R | R | R | R | T | R | R |
| <i>Ser. marcescens</i> | R | R | R | R | R | R | T | R | R |
| <i>Prov. stuartii</i> / <i>Prov. rettgeri</i> | R | R | T | R | R | R | T | R | R |
| <i>Morg. morgani</i> | R | R | T | R | R | R | T | R | R |
| <i>Proteus vulgaris</i> / <i>Proteus penneri</i> ¹ | R | T | R | T | R | R | T | R | T |
| <i>Klebsiella oxytoca</i> ² | R | T | R | T | R | R | T | R | T |
| <i>Aeromonas</i> /A2 (most <i>A. sobria</i>) ³ | R | R | T | T | T | T | T | T | T |
| <i>Aeromonas</i> /A1 & A2 | R | R | T | R | R | R | T | R | R |
| <i>Hafnia alvei</i> | R | R | R | R | R | R | T | R | R |
| <i>Enterobacteriaceae</i> with ESBL | R | T ⁴ | R | R | R | R | R | R | T |
| <i>Enterobacteriaceae</i> with inducible β -ses and ESBL | R | R | R | R | R | R | R | R | R |
| <i>Pseudomonas</i> / <i>Burkholderia</i> spp. | R | R ⁵ | T | T | R | R | T | R | R |
| <i>Steno. maltophilia</i> | R | R | R | R | R | R | R | R | R |

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

1. Isolates with high β -lactamase activity may give no zone around CTX 5 μ g but show a "key-hole" effect that may be mistaken as an indication of the presence of an ESBL. However, they may be susceptible to ceftazidime that can be tested.
2. *Klebsiella oxytoca* hyper-producer produces increased quantities of the K1 β -lactamase and there may be enhancement of the inhibitory zones between a cephalosporin disc and a disc containing clavulanate that may be mistaken as indicating an ESBL is present. However, the organism is usually susceptible to ceftazidime that can be tested.
3. *Aeromonas caviae* does not possess a carbapenemase (A2) and can be tested against imipenem, meropenem and ertapenem.
4. Test isolates from urine ONLY. Isolates from other sites are considered RESISTANT.
5. *Burkholderia pseudomallei* may be susceptible to Augmentin and therefore can be tested.

Table 4, 2005 (continued). A guide for the testing/reporting of β -lactam antibiotics for *Enterobacteriaceae*/*Aeromonas* spp., *Pseudomonas*/*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/MEM ETP | KZ | PRL | TIM | TZP |
| <i>Ent. cloacae</i> / <i>Ent. aerogenes</i> | R | T | R | T | R | R | R | R |
| <i>Cit. freundii</i> | R | T | R | T | R | R | R | R |
| <i>Ser. marcescens</i> | R | T | R | T | R | R | R | R |
| <i>Prov. stuartii</i> / <i>Prov. rettgeri</i> | R | T | R | T | R | R | R | T |
| <i>Morg. morgani</i> | R | T | R | T | R | R | R | T |
| <i>Proteus vulgaris</i> / <i>Proteus penneri</i> ¹ | R | T | T | T | R | R | T | T |
| <i>Klebsiella oxytoca</i> ² | R | T | T | T | R | R | T | T |
| <i>Aeromonas</i> /A2 (most <i>A. sobria</i>) ³ | T | T | T | R | T | R | R | R |
| <i>Aeromonas</i> /A1 & A2 | R | T | R | R | R | R | R | R |
| <i>Hafnia alvei</i> | R | T | R | T | R | R | R | R |
| <i>Enterobacteriaceae</i> with ESBL | R | R | R | T | R | R | T ⁴ | T ⁴ |
| <i>Enterobacteriaceae</i> with inducible β -ses and ESBL | R | R | R | T | R | R | R | R |
| <i>Pseudomonas</i> / <i>Burkholderia</i> spp. | R | T | R | T | R | T ⁵ | T | T |
| <i>Steno. maltophilia</i> | R | R | R | R | R | R | R | R |

CTX=cefotaxime, ETP=ertapenem, 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 μ g but show a "key-hole" effect that may be mistaken as an indication of the presence of an ESBL. However, they may be susceptible to ceftazidime that can be tested.
2. *Klebsiella oxytoca* hyper-producer produces increased quantities of the K1 β -lactamase and there may be enhancement of the inhibitory zones between a cephalosporin disc and a disc containing clavulanate that may be mistaken as indicating an ESBL is present. However, the organism is usually susceptible to ceftazidime that can be tested.
3. *Aeromonas caviae* does not possess a carbapenemase (A 2) and can be tested against imipenem, meropenem and ertapenem.
4. Test isolates from urine ONLY. Isolates from other sites are considered RESISTANT.
5. *Burkholderia pseudomallei* is usually resistant to piperacillin.

