

Newsletter 34 (August 2013)

Calibration of co-trimoxazole (SXT 25 µg discs) for the testing of Gram negative organisms by the CDS method

When co-trimoxazole was introduced the CDS tested and reported the susceptibility of each of the components separately, in line with the then accepted practice. However as the number of antibiotics used in Gram negative infections increased it became necessary to create space on the susceptibility plate by testing the combination of trimethoprim/sulphamethoxazole in a single disc. However an inhibitory zone will occur where there is susceptibility to one or other of the components as well as where there is susceptibility to the combination. In the situation where the laboratory is requested to issue a predictor of possible clinical synergy of the two components in co-trimoxazole this can only be assumed (with the exception of *Stenotrophomonas*) if the organism is susceptible to both trimethoprim and sulphonamide. In these circumstances the laboratory would need to test both trimethoprim and sulphonamide (sulphafurazole) separately as previously described.

The results of calibration of using the co-trimoxazole 25 µg disc are shown below:

SXT 25 µg versus MIC (1 part of trimethoprim to 19 parts of sulphamethoxazole):

A. *Acinetobacter* species, members of Enterobacteriaceae and Vibrionaceae.

MIC of susceptible strains $\leq 1/19$ mg/L
Annular radius of susceptible strains ≥ 6 mm

B. *Burkholderia* species, *Chryseobacterium* species, *Flavobacterium* species, *Stenotrophomonas maltophilia* and other non-Pseudomonas non-fermenters.

MIC of susceptible strains $\leq 2/38$ mg/L
Annular radius of susceptible strains ≥ 6 mm

Note: *S. maltophilia* usually have a hazy or light growth visible within the STX 25 inhibitory zone as is also observed with the SF 300 zone. This does not necessarily indicate resistance to co-trimoxazole or sulphamethoxazole. If there is any doubt it is suggested that the test

should be repeated, using a 1/10 dilution of the CDS inoculum. The inhibitory zone will then be more distinctive and easier to read.

Acceptable range for quality control (annular radius in mm):
Escherichia coli ACM 5185: 10.2 – 13.0 mm

Antibiotic Susceptibility Testing of anaerobes

The Supplemented Brucella Medium Base agar used in the calibration of anaerobes and recommended for the CDS testing is the medium described in the Manual of Clinical Microbiology 8th Ed; Susceptibility Test method: Anaerobic Bacteria, p. 1141-1148. Recently, as requested by CDS Users, a comparison has been done between the agar recommended in the CDS Manual and pre-poured plates containing Brucella supplemented agar (PP2459, Oxoid). The two media gave similar results i.e. S or R with 0 to +/- 1 mm in annular radius. CDS Users who do not have the facility to prepare their own media can use Brucella supplemented agar plates (PP2459, Oxoid) to test anaerobes.

Antibiotic Susceptibility Testing (AST) of infrequently isolated organisms not listed in the calibration Table 10.1

It is simply not possible to calibrate formally the CDS for every organism that could be involved in an infective process. In the first place gathering a sufficient number of unusual isolates can be extremely difficult and secondly we do not have the resources to carry out the procedure on endless number of species. The advice given below is based on less formal testing in the CDS laboratory of these species. When requested, the interpretation of results of testing of organisms not calibrated by the CDS may be extrapolated, with caution, from that of organisms with similar characteristics and growth requirements. The report should include a comment to indicate it is a provisional result and if necessary the susceptibilities may need to be confirmed by a quantitative technique. The species where this applies are grouped below:

Aerococcus sp., *Gemella* sp., *Granulicatella* sp., *Abiotrophia*: For these catalase negative, Gram positive cocci, the interpretation of AST results is extrapolated from those of streptococci. As with streptococci they are tested on Blood Sensitest Agar in an atmosphere of 5% CO₂ and with the

antibiotic discs calibrated for *Streptococcus* sp. If the organism has an inhibitory zone < 6mm with P 0.5 u and an inhibitory zone \geq 6mm with AMP 5 μ g, the organism is reported as having a decreased susceptibility to penicillin, amoxycillin, ampicillin with an MIC between 0.25 and 2mg/L. *Aerococcus* sp. are generally susceptible to penicillin and resistant to aminoglycosides. *Aerococcus* sp. can also be tested against ciprofloxacin 2.5 μ g disc calibrated for *Staphylococcus* sp. *Granulicatella* sp. and *Abiotrophia defectiva* can be tested by adding 5 drops of a sterile aqueous solution containing pyridoxal (1000 mg/L) to 2.5 ml of saline in preparation of the inoculum.

Pediococcus, *Leuconostoc* (catalase negative, Gram positive cocci), These species are inherently resistant to vancomycin and teicoplanin. Interpretation of testing of these species is the same as that for streptococci. They are tested on Blood Sensitest Agar, in an atmosphere of 5% CO₂ and with the antibiotic discs calibrated for *Streptococcus* sp. As with streptococci, if the organism has an inhibitory zone < 6mm with P 0.5 u and an inhibitory zone \geq 6mm with AMP 5 μ g, the organism is reported as having a decreased susceptibility to penicillin, amoxycillin, ampicillin with an MIC between 0.25 and 2mg/L.

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Coryneform group: (*Arcanobacterium*, *Dermabacter*, *Rothia* spp). These species are tested on Blood Sensitest Agar, in an atmosphere of 5% CO₂ and with the antibiotic discs calibrated for *Corynebacterium* sp. and interpretation of the results is the same as that with *Corynebacterium* sp.. Interpretation of AST results is extrapolated from those of *Corynebacterium* sp. if the organism has an inhibitory zone of < 6mm with P 0.5u and an inhibitory zone of \geq 6mm with AMP 5 μ g, the organism is reported as having a decreased susceptibility to penicillin, amoxycillin, ampicillin with an MIC between 0.25 and 2mg/L.

Bacillus sp: The interpretation of results may be extrapolated from those of *Corynebacterium* sp. However, unlike *Corynebacterium* spp., some members of *Bacillus* species may produce a β -lactamase. Therefore, we recommend only the use of a P 0.5 u disc for the testing and reporting of the susceptibility to penicillin, amoxycillin and ampicillin. Do not test *Bacillus* sp. against AMP 5 μ g disc as this disc may give false susceptibility with β -lactamase positive isolates.

Actinobacillus sp., Cardiobacterium, Capnocytophaga, Eikenella, Kingella and other fastidious Gram negative organisms: The

interpretation of AST results is extrapolated from those of *Pasteurella* spp.. They are tested on Blood Sensitest Agar, in an atmosphere of 5% CO₂ using the discs calibrated for *Pasteurella* spp. Slow growing organisms may require a suspension adjusted to an equivalent 0.5 McFarland standard instead of the standard inoculum of 10⁷ cfu/ml.