Implementation of EUCAST using the Inoclic™ system

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Introduction

The BSAC announced that from January 2016, the BSAC would no longer maintain and develop the BSAC disk susceptibility testing method and recommended for testing to be performed using the EUCAST disk diffusion method developed in 2013. The department of Microbiology at Bedford Hospital employed the BSAC method and therefore was required to switch methodologies to ensure results are accurate, valid and equate to a determined MIC.

The EUCAST methodology requires the use of an inoculum with a density of 0.5 McFarland in sterile saline, which may be measured by a calibrated photometric device or by visual comparison to a 0.5 McFarland; visual determination allows for inter operator variation and possibilities for deviation from the standardised method.

The Inoclic™ system (Figure 1) from i2a Diagnostics and Pro-Lab Diagnostics has been proposed as an alternative to creating a 0.5 McFarland suspension, by using a calibrated rod to create an inoculum for AST, therefore removing any possibility for inter operator variation

Figure 1: Inoclic™ calibated rod

The Microbiology department at Bedford Hospital evaluated the performance of the Inoclic™ system against the 0.5 McFarland method using 4 NCTC control organisms and antibiotic panels routinely used in the laboratory.

Method

4 NCTC control strains were tested against a range of antibiotics 50 times using the Inoclic™ system and a 0.5 McFarland to create the inoculum, followed by the EUCAST standardised disk diffusion for agar requirements, incubation times and conditions. The following organisms and antibiotic combinations were tested:

Control strain	Antibiotics tested
S. aureus NCTC 12973	Cefoxitin, Erythromycin, Rifampicin, Fusidic acid, Tetracycline
E. coli NCTC 12241	Augmentin, Nitrofurantoin, Gentamicin, Cefpodoxime, Cephalexin, Trimethoprim
S. pneumoniae NCTC 12977	Oxacillin, Tetracycline, Erythromycin, Vancomycin, Linezolid
H. influenzae NCTC 12975	Ampicillin, Augmentin, Tetracycline, Erythromycin, Cefuroxime, Naladixic acid

The zones of inhibition were measured after 18h incubation and compared to the quality control ranges provided by EUCAST. The standard deviations for each control organism and antibiotics were calculated and the data plotted onto graphs.

Results

Using the method stated by the i2a Diagnostics for the *S. pneumoniae* NTC 12977 failed to produce a semi-confluent growth as shown in figure 2. The method was therefore adapted to create a heavier inoculum enabling the comparison to be performed

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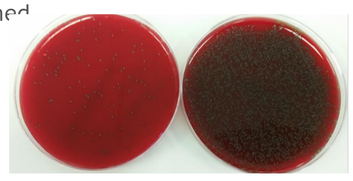

Figure 2 : *S. pneumonira* NCTC 12977 growth using the stated method (left) and modified method (right)

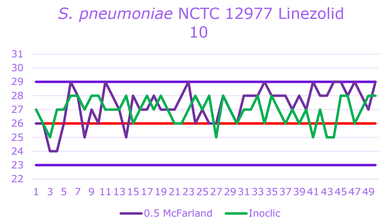

Figure 3 : Graphs plotting zone diameters against the target and upper and lower range as stated in the EUCAST QC tables.

1040 zone diameter measurements were recorded for each method with the different organism and antibiotic combinations; of these 7 were outside of the acceptable range when tested using a 0.5 McFarland inoculum and 3 were outside of the acceptable range when using the Inoclic™ system.

Plotting all zone diameters indicating a strong positive correlation between the results of the 2 methods as shown in figure 4.

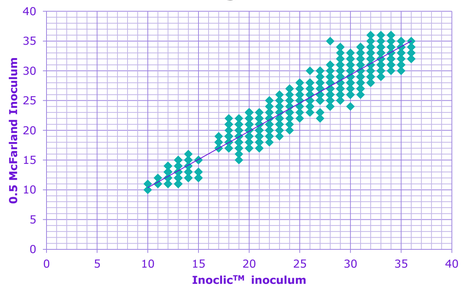

Figure 3 : Zone diameters for the Inoclic system plotted against the 0.5 McFarland

The correlation coefficients for all antibiotics tested against specific organisms were determined to be 0.9043 for *S. aureus* NCTC 12973, 0.8974 for *E. coli* NCTC 12241, 0.9701 for *H. influenzae* NCTC 12975 and 0.9765 for *S. pneumoniae* NCTC 12977.

The zone diameters for both methods were plotted against the QC ranges provided by EUCAST – the graphs indicated that the Inoclic™ system resulted in values closer to the mean and values which fluctuated above and below the mean. Examples are shown in Figure 3.

Conclusion

The Inoclic™ system performed better than the 0.5 McFarland standard for the control strains tested and is a suitable alternative for use in microbiology laboratories for routine AST.