

British Society for Antimicrobial Chemotherapy

**Standardized Disc Susceptibility Method User Day
Questions & Answers**

for

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Meeting**

held at

Freeman Hospital, Newcastle upon Tyne



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Key

AM	=	Alasdair MacGowan
JA	=	Jenny Andrews
TW	=	Trevor Winstanley
JP	=	John Perry

I Morning Session

Q *Is it possible to use surveillance rate data to predict clinical outcome?*

AM It is possible, from St Thomas's database on bacteraemia it has been done. Predictive values were generated which related to clinical outcome, however we often do not collect all co-morbidity data. For example when looking at low-level penicillin resistant pneumococci an adverse outcome was seen in Spanish data, but this was because patients had other problems as well.

Q *Earlier you mentioned that it was difficult to determine the susceptibility of Gram-negative organisms to cefuroxime because MICs were close to the BP. Would clinical response data help in this situation?*

AM Yes it would and it is possible that surveillance studies could provide some of the answers.

Q *Removal of duplicate isolates in surveillance studies – does it matter if they are included or not even if it shows increase, as long as the same proportions are used?*

AM No, except when isolates from screens are used and screening policies change.

Q *When we test the controls we find that for some combinations the results are always outside the range, for example with the E. coli and meropenem 40 out of 60 are incorrect but the other antibiotics are correct.*

JA If there is a continual problem please contact us at the SMDC and we can look at the predicament. We might even include the organism/antibiotic combination in the next QC programme if other laboratories have noticed the same problem.

TW With meropenem and Pseudomonas we know there have been manufacturing problems.

Q *With Pseudomonas and gentamicin we used to have problems with zones always being outside the limits. This has now resolved, but there was no reason why we had zones normally larger than the upper limits because our media is poured in-house from Oxoid ISA base.*

JA We know that in the past some plates supplied by central laboratories were too thin; obviously this is not the case here but again please contact the SMDC with details of any problems.

JP Could SMDC send out questionnaire asking for top six problems. Sometimes we wait for change to resolve itself, an alert may help other users.

Q *In Jersey I feel isolated when there is a problem, you need to examine what you have done, and talk to others; it is difficult a forum would be useful.*

AM The chat site on the Website can be used for this.

Q *Do you think that it is acceptable for laboratories to produce their own acceptable limits for the controls. Do you think that the method might get adapted like Stokes' with everyone using his or her own data?*

JA I agree that all laboratories should use the published ranges, but if there is a gap then calculating your own limits help until there are values available.

Q *Guidelines, Hints and Tips would be useful to help with setting up the method.*

AM A set of slides showing the method is currently being prepared for the website.

Comment I have always found a good response to problems with a rapid reply usually the next day. This was very useful when I was setting up the method in Kenya.

Q *Do you have a Rolling programme for filling the gaps in the tables?*

JA Yes and we are going to introduce controls for neisseria and a Group A streptococcus and we are also looking at anaerobes. We are hoping to provide a comprehensive table with acceptable limits for all of the antibiotic/control combinations for which recommendations are given.

Q *With regard to the discs not working. We are leaving Stokes' method behind but is it right? Are discs as reliable as we think?*

JA Currently discs are made to DIN or FDA standards, but producers of pre-poured plates use the acceptable ranges for quality control purposes. Now that laboratories are using the BSAC ranges it has been possible to identify problems with batches of discs, for example ciprofloxacin discs that were under dosed.

JP We found storage of discs in the laboratory can be a problem for example low strength co-amoxycylav discs. We switched from Mast to Oxoid and this resolved the problem. We find storage of stock discs is fine, but the in-use discs loose potency. We QC all discs weekly except for co-amoxycylav where we check the QC daily.

JA Imipenem discs give similar problems.

Q *Do you have any recommendations for Stenotrophomonas?*

JA Anna King is currently looking at recommendations for cotrimoxazole. Data should be available in about 2 months.

Q *Do BSAC have recommendations for control of Etest? We use NCTC controls weekly for penicillin against pneumococci and teicoplanin against staphylococci and we analyse the data using a Biochemistry QC programme.*

JA There are recommendations for the use of Etest by BSAC methodology on the website and if the test is read using the manufacturer's instructions MICs should be the same as those given in the tables.

Q *For Pseudomonas from cystic patients that do not grow by 24h or even 48 h how should we test these isolates? We currently do Etest, is this a better approach?*

JA For fast growing organisms disc testing should be appropriate, but for isolates that grow poorly then probably an MIC is better.

TW We use breakpoints but use discs for CF isolates and find some grow better, at 30°C, but the zone breakpoints may need changing if you incubate at 30°C.

JA Changing the conditions of testing may mean that the zone diameter BPs are not appropriate. It is also important to check that MICs incubated at 30°C agree with those where incubation has been at 37 °C.

Q *We use blood agar because some of the isolates grow better on this medium, but do not know if the MIC BP is still the same.*

JA Again check the MIC data.

JP In laboratories we test at 37°C, but the temperature may not be the same at different sites of infection. What temperature should tests be incubated.

JA For susceptibility testing the organism needs to grow well so that the antibiotic is not favoured. Thus the optimum temperature for growth should be chosen.

- Q** ***For Campylobacter species it is recommended that testing be done at 42 °C. Can testing be done at 37 °C?***
- JP** For *Campylobacter* 42°C is recommended but there is an isolation media available that may be incubated at 37°C and it would be useful if laboratories could use the same temperature.
- TW** We have compared 42 & 37°C for erythromycin and ciprofloxacin and there was no difference in result, but some organisms grow better at 42 °C. However, we recommend that nalidixic acid be used as a marker for ciprofloxacin resistance.
- JP** Which is the best temperature for testing *Stenotrophomonas* 30 °C or 37 °C.
- TW** Using different temperatures depends on whether you wish to favour the organism e.g. *Stenotrophomonas* or the antibiotic e.g. methicillin and *Staphylococcus aureus*.
- JP** A lot of organisms grow at both temperatures so testing can be done at 37°C even if growth is poorer.
- TW** I would go with the temperature that gives the most resistant result.
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- Q** ***Using a 1 mg ciprofloxacin disc we get many isolates with very small zones around 9-10 mm. Should we be using a 5 mg ciprofloxacin disc for testing?***
- JA** Personally I would use a 5 mg ciprofloxacin disc because there is an intermediate category.
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- Q** ***How does this help?***
- TW** In CF's the choice of antibiotics is limited so that with an intermediate category for ciprofloxacin the dose can be increased.
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- Q** ***You recommend Wilkins & Chalgren agar for anaerobes is this likely to change?***
- JA** Yes it may because pre-poured plates are not available commercially. Currently the Working Party and Gunnar Kahlmeter of the SRGA are looking the possibility of using ISA + 5% blood for testing.
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- Q** ***We are seeing more metronidazole resistance on primary testing. Should we repeat testing on all organisms that look resistant?***
- JA** We have seen resistant isolates in our laboratory, so laboratories should be vigilant.
- JP** Some work that is on going is the use of cefoxitin discs for detecting methicillin resistance in staphylococci. We have good evidence that cefoxitin on ISA with no salt added incubated at 37°C is as effective as oxacillin by BSAC methodology. The results are good for *S. aureus* and we hope to confirm that the conditions are equally reliable for CNS. At present there are two laboratories looking at the best strength of disc to use. It is not conclusive yet, but results so far are encouraging. We see better discrimination between MRSA and MSSA.
- JA** Hyper-penicillinase producers look sensitive by this method so there are no problems with isolates producing small zones that have to be checked for mecA.

2 Afternoon Session

Q *N. gonorrhoeae* do not grow as well on supplemented ISA as on media supplemented with blood and Isovitalex. Do you have any comments?

TW 5% do not grow well, but can be read at 24 hrs.

AM Why is not co-amoxyclav not used to treat infections instead of cefixime?

TW I do not know.

Q The zones with ceftriaxone are very large. Can the results for cefuroxime be used to infer susceptibility to ceftriaxone?

TW We do not have enough data to confirm this because there are no resistant isolates.

JA In our laboratory we test cefuroxime discs and if we get small zones of inhibition we do MIC's to cefuroxime and ceftriaxone.

AM Could you use cephaloridine to pick out reduced susceptibility?

TW Possibly yes.

Q The 5% that do not grow: what do you do?

TW Either an MIC or further supplement the media. For the organisms that grew slowly in this study we found that incubation for a few more hours improved growth

Q Are *N. gonorrhoeae* with low-level resistance to penicillin, resistant to penicillin therapy?

TW I do not know.

Q Should we call them resistant?

TW Yes I think they should be called resistant.

AM It comes down to dose. One 250mg dose may fail, but a 750mg dose may work. Early work was done with GC/dose response and it was found that up to 1g might work.

Q With nalidixic acid testing you only know that an organism is resistant to the fluoroquinolones, not whether it has high or low level resistance. What is your empiric treatment?

TW In Sheffield formally ceftriaxone, but now cefixime although there are no guidelines.

AM The kinetics for these drugs are very different. Ceftriaxone give 24 h exposure whereas cefixime has a short half-life.

Q We had an isolate with a small zone to ceftriaxone by Stokes' methodology and The Etest MIC was 0.4 mg/l .

TW David Livermore wants any isolates with reduced susceptibility to ceftriaxone. Ciprofloxacin resistance is becoming more common and no longer requires confirmation by a reference laboratory.

Answer We sent away that one.

Q We had a teicoplanin MIC on ISA for an enterococcus that did not agree with reference laboratory; they said resistant, but by Etest it was sensitive.

JP We recommend BHI agar for Etest because it gives better growth. We had a VanA *E. faecium* with a vancomycin MIC of >256 mg/L and teicoplanin MIC of 8 mg/l that grew better on BHI agar.

JA BH1 agar is recommended for VISA/GISAs; ISA for others. This issue is to be raised with David Livermore at the next Working Party meeting. This is a difficult problem when dealing with organisms with low-level resistance to the glycopeptides.

AM There are always problems with *S. aureus* tested on BHI agar because they appear resistant.

JA ISA should be used for testing CNS to teicoplanin.

- Q** **Should we be concerned?: We have had an isolate of *S. pyogenes* with an MIC creeping toward the MIC BP. We have checked the susceptibility by disc, broth MIC and Etest.**
- JA** Be concerned. Look at wild sensitive population for *S. pyogenes* on the SRGA website and see if your isolate is different. It is also worth checking the ID of this organism.
- Answer** Yes we have checked with an API. The organisms are all from one location, and we thought it was one clone but PCR did not confirm this.
- JA** E-mail David Livermore for his opinion.
- AM** You should check are they unusual, if so then the key questions are identity and are they different.
- TW** See if the MIC can be raised by sub-culturing into increasing penicillin concentrations.
- JP** Include NCTC strains with all your tests.
- AM** These strains may not be normal but resistance is not important until you reach clinical resistance. Wild type 'cut offs' are now being discussed as well as therapeutic 'cut offs'. This allows us to spot a problem before resistance appears, however it is a problem for clinicians if we report both.