

Standardized Disc Susceptibility Testing Method User Group Meeting

21 June 2005

Ark Conference Centre, Basingstoke

QUESTIONS AND ANSWERS

Introduction Colin Booth

Q: What proportions of discs are tested as part of your QC programme?

A: The proportion is relatively small, approximately 1 % of the batch. We use the standards imposed by industry, but obviously if it is an antibiotic where there are potential problems then more testing will be undertaken.

Q: Do you use microbiological assays or HPLC to check the amount of antibiotic in discs?

A: My personal choice is to do HPLC because coming from the pharmaceutical industry where all companies use HPLC; this is what I am used to. However, most of our antibiotics discs are tested using microbiological methods using large assay plates. Where we can we do HPLC as well.

Comment from the audience: HPLC might show you that you have breakdown of the drug, but what we want to be sure of is that the microbiological activity is correct because that will affect acceptable limits for the control strain and the interpretation of susceptibility for a patient sample.

Q: What standards do you apply to disc content?

A: We use the Din standard, the titre standard 90 to 125%. Although within the specifications, we are generally at the high end of the range, so that the product is within the limits at the end of the shelf life.

Q: Are you asked by your customers to supply QCs?

A: Occasionally, but I'm not sure how often. We are supplying QCs for everything these days.

Q: The discs seem to be thinner, is that because the paper used to make the discs is different to that used in the past?

A: No the paper is exactly the same, but the way in which it is processed has changed. Before the paper fluffed up in the manufacturing process, now it doesn't. The problem we now have is getting the discs out of the cartridge one at a time and getting them to sit on the surface of the agar.

Q: Can you improve the technology of the disc dispensers?

A: You use Oxoid dispensers presumably? Are there problems?

A: Yes we use Oxoid dispensers and it is frustrating because they don't always work. Discs are dispensed edge on and drive it into the agar surface and often the dispensers get 'jammed'.

Comment from Cheryl Mooney: One thing to remember when setting up is to make sure that the skirt is at the correct height to dispense onto the plate, it is actually adjustable. Also regular cleaning is important as dust and paper can build up that causes jamming. If there are any problems please speak to your local representative and they can demonstrate that to you or replace dispensers that aren't functioning properly.

Q: When the QC camera scans the cards impregnated with antibiotic, what does it look for and what does it see?

A: If it is an antibiotic that stains the card, light from underneath the card is viewed from above so you would actually see the lanes. For antibiotics that do not stain the card, light is reflected from above onto the surface, and this is what the camera sees.

Q: Is it possible to have blank discs in the cartridge? We had an occasion where we tested a *S. aureus* to vancomycin and it was resistant. This is very unusual and so we retested and this time it was sensitive. What could have gone wrong with the first disc?

A: Are you saying that it the disc was not dosed? The potential for that to happen has been reduced dramatically with this new equipment. It used to be possible with the old equipment, but with this new equipment it is very unlikely.

Comments from the audience: We have had this happen when six discs have been tested and one disc does not give the correct result.

A: On one occasion I had to go to a hospital abroad where the scientists are recognised as 'world experts' in this technology. They also experienced these difficulties. Although the discs looked as if they were on the surface of the agar, they had not been dispensed correctly and little hairs on the surface of the discs were holding them above the agar. When the disc dispenser was adjusted the disc contacted the surface and allowed proper diffusion. So the first thing to do is press the disc onto the agar and also adjust the dispenser.

Expert rules and inexpensive identification methods Trevor Winstanley

Question from Jenny Andrews: We all have to save money in the laboratory so it would be good to have the opinion of Derek and Trevor. Is there any need to look for ESBLs in an Enterobacter because clinically you know that it will produce an AmpC enzyme? Secondly, if you have a CTX-M that you could treat with ceftazidime, the application of your expert rules in that case would be clinically incorrect – what do you think?

A: When detecting ESBLs in AmpC producing organisms like Enterobacters, Serratias, Aeromonas and Citrobacter freundii, in terms of clinical treatment it makes no difference at all because, if you identify an organism as such, the chances are you won't use a cephalosporin, any penicillin, piperacillin/tazobactam or co-amoxyclav, you would go straight to a carbapenem and that would be the antibiotic of choice for an AmpC or an ESBL. So in terms of treatment it wouldn't make any difference at all.

You need to detect ESBLs in other organisms. You may well think you are going to save money, but if you get a litigation case against you where you have deaths attributed to organisms that produce ESBLs and the BSAC have told you how to identify ESBLs, then that would take some defending in court.

Comment from Jenny Andrews: I was thinking of organisms that have a propensity for AmpC.

A: Yes the Enterobacters, it makes no difference at all clinically but is important in terms of epidemiology.

Comment from Jenny Andrews: In a way it's a waste of money.

A: If you want to be a cheap skate, the thing to do is put a cefotetan disc or ceftoxitin disc on the plate. If an organism is ceftoxitin resistant the chances are 99% of times it is going to have an AmpC. It may be impermeable some times but the majority of times if it is ceftoxitin resistant you have an AmpC. It won't affect clinical management.

Q: If you have an organism susceptible and it is an ESBL producer, is it really susceptible or should we report it resistant?

A: There is a school of thought that says it is the MIC stupid! And you should go by the MIC and if these organisms have low MICs you can treat them. The difficulty is that there aren't vast amounts of clinical data and that's what we really need. People who have accumulated clinical data relating treatment outcome to MIC so that the weight of evidence is that if you have a low MIC you can

actually treat some of these strains. It is an argument that is going on at the moment and certainly CLSI are going towards the idea that if you have a low MIC even if it is an ESBL producer; you may be able to treat. The weight of evidence at the moment supports that. Some organisms such as the CTX-M producers where you can have the same enzyme in a variety of different strains of *E. coli* and if you look at the MIC distribution it actually straddles the breakpoint. So you would have the same CTX-M enzyme in different strains of *E. coli* and you might be calling one resistant and one susceptible. A lot of them are going to be very close to the breakpoint. If you bump up the inoculum a bit you can push the MICs up so it would slightly concern me, that with organisms like that you would be treading on thin ice in terms of treatment.

Q: I notice you didn't have your expert system converting the ESBL producers to Piperacillin/tazobactam resistant – you do now – OK.

A: That is an example of where *in-vitro* they often look susceptible and yet there is clinical evidence that treatment is more likely to fail if the organism is an ESBL producer even if it looks susceptible *in-vitro*. You can push up the MICs with piperacillin/tazobactam by increasing the inoculum.

Q: Where do you see the future going, being content with calling Gram negatives Coliforms or identifying organisms to species level? Also, do you think it is going to progress to a level where we identify enzymes?

A: Certainly the ESBLs I would test with at least cefpodoxime and cefoxitin on a plate. Cefoxitin will tell you if it has an AmpC. If it is cefpodoxime resistant then you may wish to determine whether it has got an ESBL.

Q: How do you distinguish between the Klebsiellas and the Enterobacters and Citrobacters?

A: We use agar incorporation biochemical test at the moment. We are trying to incorporate a chromogenic plate and cut down the number of tests we are looking at so we are down to four tests at the moment. On the slide you saw they were using a chromogenic agar, lysine, ornithine and indole.

Comment from Derek Brown: We are using a chromogenic agar and then an API for those that are not identified on this medium.

Introduction of the BSAC slide show Derek Brown

Q: Should we bother having two media for fastidious organisms, one of which has 5% defibrinated horse blood and another one, which has horse blood and NAD. Do you actually use these two media? It has been suggested that we have the media with NAD for all fastidious organisms – it saves having extra media and saves confusion over similar looking media. Would people who use separate media have a problem with using the same medium for everything?

A: As long as it isn't more expensive.

Q: Using media containing NAD with automated zone readers?

A: There is no difference in the appearance of the media. The organisms that don't need the NAD are not in any way affected by its presence. Reading any plates containing blood in an automated zone reader is difficult in any case.

Q: Is the shelf life with NAD shorter?

A: I don't know – I will have to check. As the pre-poured media with NAD get to the end of their shelf life the organisms don't grow so well.

Comment Jenny Andrews: We chose 20 mg for that reason. The literature says 5 mg/L but you can't get away with 5 mg if you are storing plates for 2-3 weeks.

Comment from the audience: Problem with NAD going off due to overheating the medium. Important to have the medium preparator at the correct temperature.

Comment from the audience: About plastic loops, someone was a trainer and told not to use plastic loops. Straight wire or platinum loops are better to pick up colonies.

Comment from the audience: Some people can't get *N. gonorrhoeae* to grow on plates and I think it is because the inoculum is too light. We have said try making up your own Standard or we have supplied them with a standard and then they have not had such a problem.

Reading zones

Q: Who reads their plates from the back and who from the front?

A: The vast majority read from the front. – Because they are using templates. If I use a template I read from the front, but if I am measuring with callipers it is much easier to read from the back. Upon questioning nobody routinely measured all zones.

Q: Do people think it would be useful to produce a series of photos actually defining how zone edges should be read?

A: Yes, especially when zone edges aren't complete and when another zone is very near.

Q: Does everyone put 6 discs on plates for their controls?

A: Yes

Q: What do you do with something like ciprofloxacin, where the zone is huge?

A: Measure the radius. It is difficult because of interactions with the next door zone.

Comment from the audience: Pictures are what we need.

Q: Do people like a light or dark background when reading zones?

A: Most use dark background.

Q: When we started setting us this method there was a lot of discussion as to whether we should stick with the traditional UK controls which no one else in the world used or should we switch to the controls that were defined by the NCCLS method originally but are in fact used in several other methods as well? There was no consensus on that and we have gone down the road of both, although we don't have complete control ranges for both organisms. Do people have any feelings about the control organisms we use? Are you happy with the Oxfron Staphylococcus?

A: I think the whole point of the BSAC method is to make British susceptibility testing comparable with the rest of the world. So if you do that for standardisation you use the same controls.

Comment from Jenny Andrews: The aim was to have a standardised method related to MIC breakpoints in the UK, not a method comparable with the world,.

Comment from Derek Brown: I think that the NCCLS have got a lot of their BPs wrong. There is an organisation in Europe, EUCAST, which includes all BP Committees in Europe other than NCCLS (although we do have representatives on it who use NCCLS) and we have agreed to harmonise all our breakpoints. We are been through a process of agreeing breakpoints for quinolones, aminoglycosides, glycopeptides. We are working on cephalosporins at the moment. CLSI (Clinical Laboratory Standards Institute) is the new name for NCCLS.

Q: Why don't you recommend passaging control organism more than five times?

A: Simply because if you do serial passage and you will be likely to end up with an organism very different from what you started with.

Q: Does that really happen?

A: I don't know how often it does. Some studies were done where the Oxford Staphylococcus was collected from 30 different laboratories and tests done in parallel with all the sub-cultures of and significant differences were found between them.

Some recommendations say not to subculture organisms even 6 times.