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Instrumentation in antimicrobial susceptibility testing

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Studies in the 1960s demonstrated the problems of variability in susceptibility testing methods, especially those affecting the performance of disc diffusion procedures. These studies made apparent the need for standardization and resulted in more clearly defined performance limits for growth medium, incubation conditions, inoculum concentration, disc content for diffusion methods, the setting of interpretative MIC breakpoints and the establishment of quality control parameters. More recently, there has been a growing interest in the use of instrumentation for reading disc diffusion tests and the endpoints of agar or broth dilution MIC determinations. Instrumentation ranges in complexity from the simple optical reading of zones of inhibition or growth endpoints, requiring operator interpretation, to more sophisticated devices for reading, recording and 'expert system' analysis of results with interfacing of instruments to laboratory information management systems. Some of the more developed systems are fully automated and can also identify the organisms tested. The pressure to reduce labour costs and provide results earlier favours the use of more automated systems whilst the requirement for resistance surveillance provides impetus for the use of systems that provide quantitative results and electronic data handling.

Introduction

Although there is a variety of methods available to clinical microbiology laboratories for antimicrobial susceptibility testing, disc diffusion techniques remain the most widely used for routine tests. In the past, instrumentation has had little impact on disc diffusion tests, but because of increased interest in using routinely derived susceptibility data for resistance surveillance, the potential of automated zone readers has recently attracted attention. The requirements of surveillance and, particularly in large busy laboratories, consideration of the potential for labour saving has also brought the use of automated systems under review. The manual determination of full MICs by agar or macro/microbroth dilution methods is infrequently undertaken in most clinical laboratories but instrument systems for setting up and reading such tests are available. Agar incorporation breakpoint methods are used for routine testing in several clinical laboratories in the UK and instrument systems for inoculating and reading plates are widely used.

Instrumentation for disc diffusion susceptibility testing

Studies in the 1960s, particularly the international collaborative study of Ericsson & Sherris,[1] brought into focus the problems of variables in susceptibility testing methods, especially those affecting the performance of disc diffusion methods. These studies indicated the need for standardization. Standardization has centred around reducing variability by defining performance limits for growth medium (including removal of antagonists and appropriate supplementation for bacteria with fastidious growth requirements), incubation conditions, inoculum concentration, disc content (in the case of disc diffusion), setting of interpretative criteria (breakpoint MICs and inhibition zone diameters) and establishment of quality control parameters. Measurement of zone sizes is a significant variable that has received less attention despite being subjective and markedly affected by lighting conditions.

More recently, there has been a growing interest in the use of instrumentation for reading disc diffusion tests.

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Several systems are available that use a camera or scanner to capture an image of the plate and then use image analysis software to measure zone sizes. Automated zone readers should reduce operator variability in reading plates and error in transcription of results, and provide automatic interpretation of zone diameters. Zone readers interfaced to laboratory information management systems (LIMS) would enable efficient data handling, including the storage and retrieval of epidemiological information in that accumulated zone diameters could be used locally for detailed examination of resistance and transferred electronically for regional or national resistance surveillance. Some systems also include so-called 'expert' software to improve the quality of interpretation by the filtering of results according to a set of rules.[2] Systems known to us include Accuzone (AccuMed International Inc., West Lake, OH, USA),[3] Aura Image (Oxoid, Basingstoke, UK),[4] BIOMIC (Giles Scientific, New York, NY, USA),[5] BioVideobact (Launch, Longfield, UK), Mastascan Elite (Mast, Bootle, UK), Osiris (Bio-Rad, Hemel Hempsead, UK),[6] ProtoZONE (Don Whitley Scientific, Shipley, UK) and SIRSCAN (Becton Dickinson, Oxford, UK).[7,8] They differ in the way data are input to identify specimens and select appropriate tests, in how the results are presented and in the flexibility and sophistication of the software. The systems appear to perform reasonably well with organisms that grow well on plain agar, but less well with tests on media containing blood. With these systems it is advisable for an operator to check the on-screen image of each plate to ensure that zones have been read correctly. All systems allow rapid manual adjustment of zone sizes if necessary.

If plates are set up and read with reproducibility, accuracy and close attention to quality control performance, it is suggested that results from routine qualitative disc diffusion techniques may be used to estimate the MIC by the application of appropriate computer algorithms.[9] The attraction of this approach is the possibility of combining routine disc diffusion susceptibility data with MIC data generated by other systems as part of a 'real-time' national or international surveillance programme as currently exemplified by the MRL TSN system.[10]

Instrumentation in other areas of disc diffusion tests has been limited to simple mechanical disc dispensers (available from disc manufacturers) for application of discs to inoculated plates, and simple turbidimetric devices (available from disc manufacturers) or spectrophotometers for reading the density of suspensions of organisms when standardizing inocula.[11]

Instrumentation for agar dilution methods

One of the earliest applications of instrumentation in susceptibility testing was the use of multipoint inoculum replicating systems for the application of multiple bacterial strains to a series of agar plates in MIC or breakpoint susceptibility tests. These devices deliver approximately 1 μL spots of inoculum, depending on the size of the inoculator pins, and up to 96 test organisms per plate, depending on the plate size and pin arrangement. The device originally described in 1959 was simply mechanical,[12] and although systems widely used today are motorized, plates are still fed manually.

The use of image analysis systems to read agar incorporation breakpoint plates[13] pre-dates the use of image analysis for reading zone diameters, although there are no published data on performance of the earlier readers for breakpoint plates. One of the recently developed zone readers, the Mastascan Elite, can also read breakpoint plates, but there are no published data on performance at present.

Instrumentation for broth dilution systems

Broth methods of susceptibility testing have been adapted to instrument-based systems with various degrees of mechanization and automation. At the lower end of sophistication endpoints are read visually and results are manually entered, and computer programs are simply used as an aid in recording, storage and analysis of data and in the generation of hard-copy reports. Interfacing with LIMS is possible and 'expert' quality filters may be applied to the results before reporting. At the upper end of development, a small number of automated systems is available commercially and these incorporate microprocessor-controlled robotics for the manipulation of test cultures during incubation, and instrument-based growth detection by turbidometric or fluorometric methods before data handling and reporting. Some of these instruments have been adapted and validated as short-incubation systems (4–10 h depending upon the species and antimicrobial agent) and most can be used for identification as well as susceptibility testing.

Manual recording of broth microdilution systems

Broth microdilution trays or strip galleries contain dried antimicrobials which, when rehydrated with inoculated growth medium, provide dilution series for full MIC determination or susceptibility based on breakpoints. They are available from a number of producers and incorporate either individual compounds or groups of antimicrobials specified by the manufacturer or by the customer. Alternatively, automated dispensers may be used for in-house preparation of microdilution trays from antimicrobial stock solutions made up by the user.

Most manufacturers of these products also offer some sort of reading device for recording visually determined growth endpoints, e.g. the Microscan Touch SCAN-SR (Dade Behring Inc., West Sacramento, CA, USA), the Sceptor (Becton Dickinson Diagnostic Instrument Sys-

tems, Sparks, MD, USA) and the Sensititre Sensitouch (Trek Diagnostic Systems, East Grinstead, UK). Inoculation systems are available for use with some of these instruments, e.g. the Sensititre AutoInoculator. The reading devices include computerized data management systems, which are able to apply MIC breakpoints for qualitative interpretation of results, store and retrieve cumulative information and generate printed reports. If preferred, they may be used with trays containing antimicrobials in broth provided by some commercial manufacturers (e.g. Sensititre) or those prepared in-house. They are, therefore, highly versatile, since all procedures up to and including reading can be modified to suit the requirements of a particular laboratory.

Automated reading of broth microdilution systems

Automated reading devices, which interpret characteristics of bacterial growth in microtitre trays or strip galleries which have been set up manually or mechanically and incubated for short periods or overnight in standard incubators, are available from a number of manufacturers. These include the Microscan autoSCAN-4 (Dade Behring Inc.), the AutoSceptor (Becton Dickinson Diagnostic Instrument Systems) the mini API (bioMérieux, Marcy l'Etoile, France) and the Sensititre AutoReader (Trek Diagnostic Systems). Detection is photometric, turbidometric or fluorometric depending upon the system. These instruments are designed for use in a similar role to inhibition zone diameter readers, minimizing operator and transcription error but offering little, if any, advance in process automation.

Instrumentation for automated broth-based susceptibility testing

Development of automated susceptibility testing systems

Attempts to design and validate automated systems for the identification and susceptibility testing of clinically important bacteria have been ongoing for the last 30 years. The earliest automated system was the TAAS (Technicon Automated Antimicrobial Susceptibility) system developed in the early 1970s by the Technicon Instrument Corporation (Tarrytown, NY, USA). The basis of the system was the elution of antimicrobials from discs and comparison of growth of test and antimicrobial-free control cultures to provide a growth index from which susceptibility could be predicted. This system was never marketed. However, in 1974, the Autobac I disc elution system became available commercially from Pfizer Diagnostics. This system claimed to provide reliable results, at least for some species–antimicrobial combinations, in as little as 3 h. The Abbott MS-2 system, a 4 h disc elution method followed in 1977, the same year in which the McDonnell Douglas Corporation introduced the AutoMicrobic System (AMS), the forerunner of the Vitek system (bioMérieux). The AMS was developed as a by-product of US space exploration in the 1960s. It was highly automated and used dehydrated reagents/antimicrobials in sealed plastic cards for organism identification and susceptibility testing. This time also saw the introduction of standardized antimicrobial-containing microtitre trays. This technological development formed the basis of such commercial products as Micromedia Systems, Sensititre, BBL Sceptor and a range of systems from Microscan that evolved into the TouchScan, AutoScan and, ultimately, the Microscan WalkAway System. Recently there have been preliminary reports on another highly automated identification and susceptibility testing system, Phoenix (Becton-Dickinson), which is based on broth dilution with a redox indicator to enhance detection of microbial growth.[14,15]

More than 20 identification and susceptibility testing instruments/systems have been developed. However, only three are currently in widespread use, the Vitek, the Microscan WalkAway and the Sensititre systems. Although only installed in a small minority of laboratories in the UK, the Vitek is the most widely used of these methods in this country.

Microscan WalkAway system

This system was developed in the late 1980s and is capable of automating either overnight or, with the use of Microscan Rapid Panels, short-incubation identification and susceptibility tests. The system uses standard-size microtitre trays in which growth is detected photometrically (after overnight incubation) or fluorometrically (after short incubation). Preparation of plates before incubation is manual, with inoculation using a multipoint inoculator, after which plates are placed in the incubator component of the system. Detection of growth in the inoculated plates is robotically automated and the data managed using computer-based algorithms.

Sensititre ARIS (Automatic Reading and Incubation System)

Like the Microscan WalkAway System, the Sensititre ARIS uses standard-size microtitre trays which, after inoculation using the AutoInoculator, are placed in the system incubator and automatically positioned for fluorometric monitoring of growth following hydrolysis of fluorogenic substrates. The whole process is controlled with a microcomputer. This system was developed during the 1980s and is marketed in the USA for overnight susceptibility testing only. However, it is available in some countries for short-incubation testing.

The Vitek system

The Vitek system differs in design from the standard-size microtitre tray concept of the Microscan WalkAway and Sensititre ARIS. The culture system for the Vitek is a thin plastic card, comprising about 30 wells or microcuvettes linked by capillaries through which the bacterial test suspension passes to inoculate wells and rehydrate reagents in the wells. The cards are available as a variety of configurations of antimicrobials and identification media. Custom cards can be ordered if sufficient numbers are used by a laboratory. The integrated system comprises a filler/sealer, a reader/incubator and a computer control module. Growth is determined turbidometrically at hourly intervals for up to 15 h. Normalized linear regression analysis of the growth is used to determine computer algorithm-derived MIC which may be reported directly or interpreted qualitatively.

A more automated version of the Vitek system, the Vitek 2 has been developed. This system automates sample processing, including initial inoculum preparation, density verification, card filling and card sealing. The instrument automatically transfers cards to the reader/incubator and ejects them into a disposal bin at the end of testing. The Vitek 2 system will allow the testing of up to 20 antimicrobials, depending upon the species tested.

Advantages and limitations of automated systems

These have been reviewed in detail by Ferraro & Jorgensen.[16] Automated systems offer improved reproducibility and are labour saving compared with manual systems although consumable items are more expensive, and instrument costs must be met whether they are purchased, leased or included in a reagent rental contract. Some systems have the potential to provide results on the same day that tests are set up, but this may require changes to working practice to deal with results available in the evening. The results need to be reported promptly and clinicians need to be able to respond promptly to reports or the potential advantages to the patient of rapid testing may be lost. Interfacing of systems to LIMS and the application of expert systems provide benefits similar to those described above for automated zone readers in disc diffusion tests.

The panels of agents included in automated systems tend to be extensive and likely to cover the requirements of most laboratories, but flexibility to change panels is limited and additional, less frequently tested agents cannot be added as required. Automated systems cannot be used for all clinically important bacteria so alternative methods must be used for such tests. Overall, the performance of automated systems correlates reasonably well with reference methods but problems have been reported for some organism–antimicrobial combinations, particularly with the rapid test systems. Problems with inducible β-lactamase-mediated resistance in Gram-negative bacilli, low-level glycopeptide resistance in enterococci and staphylococci and methicillin resistance in staphylococci have been reported. Preliminary reports suggest that some of these problems have been largely resolved in more recent systems.[17–18]

Conclusions

The instrumentation available for antimicrobial susceptibility testing offers various degrees of automation, ranging from simple mechanization of traditional methods to fully automated systems that can also identify the organisms. The pressure to reduce labour costs and provide earlier results favours use of the more automated systems. The requirements of resistance surveillance provide impetus for use of systems that provide quantitative results and electronic data handling, which can be achieved by the use of image analysis-based zone readers and by automated broth dilution systems.

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