Evaluation of the mastascanelite image analysis system for measuring zones of inhibition in disc diffusion susceptibility tests

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ABSTRACT

In this evaluation a mastascanelite image analysis system is compared with manual measurement of disc diffusion inhibition zones. Data for 213 diverse organisms and a total of 1679 organism/antibiotic combinations gave an overall correlation coefficient of 0.988. The mean difference between readings was +0.425 mm, with 95% confidence limits of ±2.94 mm, and the majority of scanned zones (97.51%) fell within ±3 mm of the manual measurement. The mastascanelite system forms part of a laboratory suite and was found to be objective, accurate and rapid, reading and interpreting each plate in less than a second. Interfacing to the laboratory computer system facilitated data handling and performance control.

KEY WORDS: Disc diffusion.
Image processing, computer-assisted.

Introduction

Comparative disc diffusion susceptibility tests[1] are simple and inexpensive but have two main shortfalls. Firstly, they have undergone unsupported, ad-hoc changes to such an extent that no single method remains. This means that susceptibility data from multiple centres can no longer be combined reliably for surveillance purposes. Secondly, they may permit subtle changes in overall susceptibilities to pass undetected.

To overcome these criticisms, the British Society for Antimicrobial Chemotherapy (BSAC) has published a standardised method in which zone diameters are correlated with minimum inhibitory concentration (MIC) breakpoints.[2] Zones can be interpreted with a template but it is preferred that they be measured using a ruler, callipers or an automated zone reader.

The aim of this study is to evaluate the mastascanelite (Mast Group, Bootle, UK) in comparison with manually measured zones. This is the first evaluation of this instrument, although other automated zone readers have been formally evaluated previously.[3,4]

Materials and methods

Disc testing was performed according to the recommendations of the BSAC.[5]

Iso-Sensitest agar (IST; CM471, Oxoid) was poured to a depth of 4 mm in 90 mm Petri dishes. For fastidious organisms, IST was supplemented with 5% defibrinated horse blood (E & O Laboratories, Burnhouse, Bonnybridge, Scotland) or with defibrinated horse blood and 20 mg/L nicotinamide adenine dinucleotide (Sigma Chemicals, Poole, UK) as required. Inocula giving semi-confluent growth after overnight incubation were used.

All plates were incubated at 35–37°C in air for 18–20 h, the exceptions being for streptococci, haemophili and neisseria, where the atmosphere was enriched with 4–6% CO_2. Antibiotic discs were supplied by Mast and zone diameters were determined on the mastascanelite by one person, and then measured independently (to the nearest mm) with a ruler by another.

The 213 organisms studied comprised *Escherichia coli* (n=10), *Citrobacter freundii* (n=5), *C. diversus* (n=4), *Morganella morganii* (n=5), *Proteus mirabilis* (n=5), *P. vulgaris* (n=1), *Providencia alcalifaciens* (n=3), *Klebsiella pneumoniae* (n=5) *K. oxytoca* (n=5), *Enterobacter cloacae* (n=4), *E. aerogenes* (n=6), *E. agglomerans* (n=2), *Serratia* spp. (n=3), *Shigella* spp. (n=2), *Salmonella* spp. (n=5), *Aeromonas hydrophila* (n=2), *Stenotrophomonas maltophilia* (n=5), *Acinetobacter* spp. (n=4), *Pseudomonas aeruginosa* (n=26), *Enterococcus* spp. (n=17), *Haemophilus influenzae* (n=11), *Streptococcus pneumoniae* (n=5); β-haemolytic streptococci (Lancefield group A [n=12], group B [n=10], group C [n=5], group G [n=4]), *Neisseria gonorrhoeae* (n=20), *Staphylococcus aureus* (n=21; five penicillin-sensitive and five methicillin-resistant) and coagulase-negative staphylococci (n=6; two methicillin-resistant). Antimicrobials tested against each group of organisms are shown in Table 1.

Results

Correlation of results from the mastascanelite and the manual method is shown in Figure 1. These data comprise 213 organisms and a total of 1679 organism/antibiotic combinations. The correlation for individual genera is shown in Table 2 and ranges from 0.975 to 0.992, with an overall coefficient of 0.988.

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Table 1. Antibiotic discs used in the study

Organism	Antibiotic disc content (μg unless stated)										
Enterobacteriaceae, *Acinetobacter* spp.,	TM	CIP	GM	AP	AP	CAZ	CXM	CFX	NA	NI	
Stenotrophomonas maltophilia	2.5	1	10	10	25	30	30	30	30	200	
Pseudomonas aeruginosa	MEM	AK	AZT	TOB	COL	OFL	PTZ	GM	CAZ	CIP	
	10	30	30	10	25	5	85	10	30	5	
Haemophilus influenzae	AUG	AP	T	CXM	C	CIP					
	3	2	10	5	10	1					
β-haemolytic streptococci	PEN	E	T								
	1unit	5	10								
Streptococcus pneumoniae	OX	E	T								
	1	5	10								
Neisseria gonorrhoeae	AP	AUG	DOX	E	PEN	T					
	2	3	5	5	1unit	10					
Enterococci	TEC	GM	AP	VA							
	30	120	10	5							
Staphylococci	GM	PEN	OX	E	RP	VA	TEC	FC	TM	MUP	T
	10	1unit	1	5	2	5	30	10	5	5	10

TM, trimethoprim ; CIP, ciprofloxacin ; GM, gentamicin ; AP, ampicillin ; CAZ, ceftazidime ; CXM, cefuroxime ; CFX, cephalexin ; NA, nalidixic acid ; NI, nitrofurantoin ; MEM, merepenem ; AK, amikacin ; AZT, aztreonam ; TOB, tobramycin ; COL, colistin ; OFL, ofloxacin ; PTZ, piperacillin-tazobactam ; AUG, co-amoxyclav ; T, tetracycline ; C, chloramphenicol ; PEN, penicillin ; E, erythromycin ; OX, oxacillin ; DOX, doxycycline ; TEC, teicoplanin ; VA, vancomycin ; RP, rifampicin ; FC, fucidin ; MUP, mupirocin

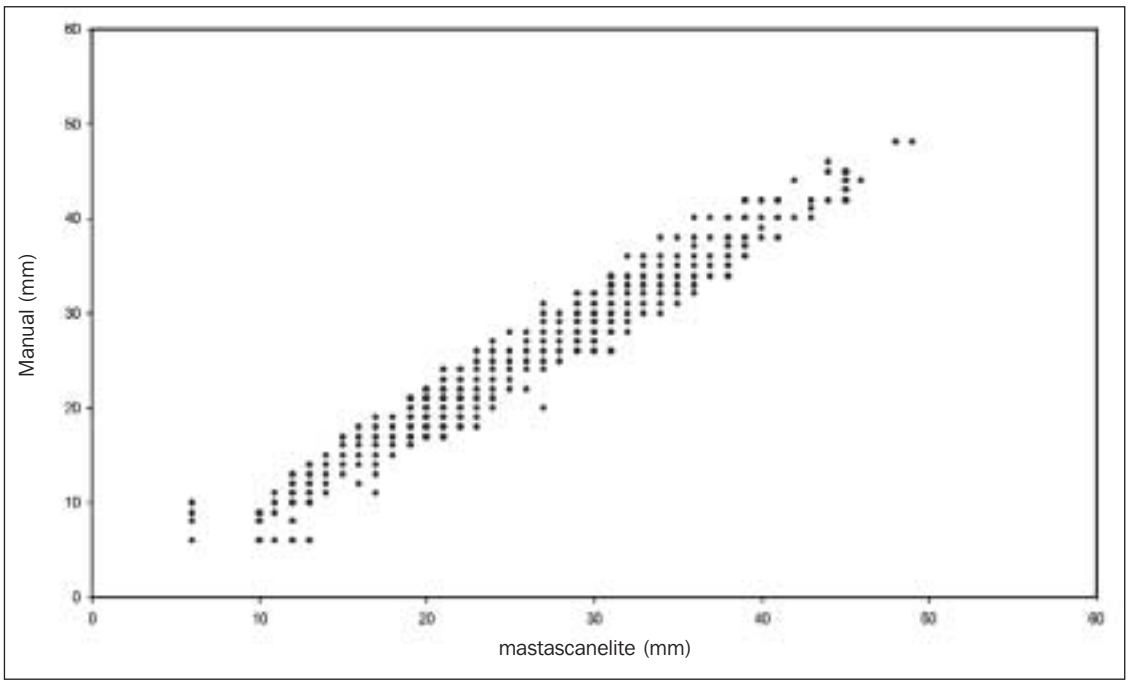

Fig. 1. Correlation of zone diameters measured by the mastascanelite and by the manual method: all organisms and all antimicrobials.

When differences in zone diameter measured by the mastascanelite were compared with manual readings, the difference was found to be +0.425 mm, with 95% confidence limits of ±2.94 mm (± 2SD for a normal distribution). The majority of scanned zones (97.51%) fell within ±3 mm of the manual measurement, 90.12% within ±2 mm and 68.76% within ±1 mm.

When BSAC interpretative criteria were used to compare the two data sets for the Enterobacteriaceae, 23/760 individual test results differed. Overall, results for ciprofloxacin, gentamicin and ceftazidime were unaltered, but results for trimethoprim (n=7, mainly intermediate to susceptible), ampicillin (n=7), cefuroxime (n=1), cephalexin (n=4), nalidixic acid (n=1) and nitrofurantoin (n=3) differed.

The mastascanelite was able to determine the zone diameter unaided for the majority of organisms. With some (notably Lancefield group B streptococci and *Streptococcus*

Table 2 Correlation coefficient (*r*) of zone diameters measured by ruler and using the mastascanelite for all antimicrobials

Organism	Observations	*r*
Enterobacteriaceae, *Acinetobacter* spp., *Stenotrophomonas maltophilia*	760	0.990
Pseudomonas aeruginosa	260	0.985
Haemophilus influenzae	66	0.975
Streptococci	108	0.979
Neisseria gonorrhoeae	120	0.988
Enterococci	68	0.992
Staphylococci	297	0.990
All organisms	1679	0.988

pneumoniae), however, manual adjustment of the zone edge by the operator was necessary. The instrument coped well with pigmented pseudomonads and could differentiate between growth and haemolysis caused by streptococci.

Discussion

Irrespective of the overall disc diffusion method employed, it is essential that results generated by automated zone readers are in line with manual results. Andrews *et al*.[3] evaluated the Oxoid Aura image system and found the correlation with manual measurement to be approximately 0.994, with 94.6% of zones falling within 3 mm.

Sanchez *et al*.[4] evaluated the Osiris Video Reader System (Sanofi Diagnostic Pasteur, Guildford, UK) and reported essential agreement (3 mm discrepancy with manual reading), with 91.6% of routine isolates and 94.8% of those with well-characterised resistance mechanisms.

In the present evaluation of the mastascanelite, correlation with a manual measurement was found to be 0.988, with 97.51% of zones falling within 3 mm.

Clearly, the performance of automated zone readers depends upon the combination of organism and antimicrobial studied. Overall, enterococci and staphylococci gave higher correlation coefficients than did haemophili or streptococci, and antimicrobials resulting in smaller zone sizes (e.g., vancomycin and staphylococci or teicoplanin and enterococci) tended to give lower correlation coefficients. The difference in susceptibility results with the two data sets was considered acceptable and most differences occurred with urinary antibiotics.

Andrews *et al*.[3] remarked that the variation in zone reading increased as the zone diameter increased (>25 mm). The results of the present study support this, as we were unable to evaluate the mastascanelite with *N. gonorrhoeae* and the more potent antibiotics such as ceftriaxone, azithromycin, ciprofloxacin and cefuroxime at their recommended disc strengths.

Automated zone readers are objective, accurate and rapid. When interfaced to laboratory computer systems, as in this case, they eliminate transcription errors and facilitate data handling and performance control. Overall, the mastascanelite compared favourably with other automated zone readers in terms of the ability to read zones.[3,4] Additionally, it proved highly configurable and could read and interpret results in less than a second, taking 60 measurements for each zone and using image enhancement.

The mastascanelite has a data analysis module (an epidemiology package and an expert system[6]) plus the additional functionality of modules capable of reading agar-incorporation plates (breakpoint, MIC or identification). In addition, a cost-effective microtitre urine-screening module has also been developed.

Overall, the flexibility of mastascanelite makes it an attractive, multifunctional proposition for the busy clinical laboratory. □

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