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To comply with the new e-Privacy directive, we need to ask for your consent to set the cookies. Learn more. In this study the Oxoid Aura image antibiotic sensitivity test system, used as a stand-alone device, was compared with manual zone measurement and use of a template, for the determination of sensitivities. An overall correlation coefficient of 0.99 was observed for zone diameters measured using the Aura image system and zones measured manually, when the differences between zones were within 3 mm; 5.4% of zones showed a difference in zone diameter between manual and automated measurement of >3 mm. The results obtained using the template method for interpretation were less reliable than zone measurement, with cefuroxime and ampicillin tested against Enterobacteriaceae and Acinetobacter spp. When linked to a laboratory patient database, the bar code and disc identification facilities avoided errors that were associated with manual data entry. Currently there is renewed interest in the manner in which susceptibility testing is performed in the UK and Ireland and there is a move away from the comparative method of testing that was introduced in the early 1970s.1 Although Stokes' method of testing has many qualities, it has suffered ad hoc changes by most laboratories using the method and has never been amended or supported in a formal manner, with the consequence that a uniform method of performing and interpreting the test no longer exists. A move to change from this method of testing has been initiated by the recognition that results from sensitivity testing can provide more information beyond those solely for an individual patient. For example, zone diameter data can be combined from several centres to monitor levels of bacterial resistance to antibiotics. However, to achieve this, a standardized method has to be used which relies on the measurement of zones of inhibition for interpretation.In 1998 the British Society for Antimicrobial Chemotherapy (BSAC) published in its newsletter2 details of a standardized method of disc testing which correlated zones of inhibition with its published MIC breakpoints.3 The introduction of this method has created the opportunity for measuring and recording zone diameter data which can be used to enhance the information gained from susceptibility testing. Manual measurement of zones of inhibition can take a considerable amount of time, which makes the method impractical for some diagnostic laboratories. To overcome this problem the BSAC has suggested that interpretation can be made by comparing test zones with those of a template constructed using the zone diameter breakpoints given in the recommendations (Figure 1). Although this is a helpful compromise, valuable information is lost because actual zone diameter data are not recorded. This process can be speeded up if zones are measured by an automated zone reading device and ideally, this device should provide a quick, easy to operate, reliable system that integrates with laboratory reporting and data handling systems.In this study we have evaluated the Aura image antibiotic sensitivity test system (Oxoid, Basingstoke, UK) as a stand-alone machine and compared it with manual measurement of zones of inhibition. In the first study, we compared zone measurements for organisms taken from City Hospital's laboratory collection of clinical isolates and in the second study, compared results obtained over a 4 week period by the diagnostic laboratory using a template to determine sensitivity, with interpretation based on manual and automated measurement of zones. Materials and methods Method of disc testing Disc testing was undertaken following the recommendations of the BSAC,2 using Iso-Sensitest agar (ISTA)(CM471, Oxoid) poured to a depth of 4 mm in 90 mm Petri dishes. For the growth of fastidious organisms, ISTA was supplemented with 5% defibrinated horse blood (Tissue Culture Services, Botolph Claydon, UK) or 5% defibrinated horse blood and 20 mg/L nicotinamide adenine dinucleotide (Sigma Chemicals, Poole, UK) as necessary. An inoculum equivalent to semi-confluence was used and plates were incubated at 35–37°C in air, except for fastidious organisms (haemophili, pneumococci and neisseriae) where the atmosphere was enriched with 4–6% CO_2, for 18–20 h. For manual reading, zone diameters were measured with a ruler and recorded in mm. Interpretation of sensitivity was determined by comparing the zone of inhibition with the zone diameter breakpoints recommended by the BSAC. Organisms Study 1. A total of 217 defined strains from the laboratory collection, comprising 13 Escherichia coli, 13 Klebsiella spp., nine Morganella morganii, six Acinetobacter spp., nine Serratia spp., 10 Enterobacter spp., 14 Proteus mirabilis, three Proteus vulgaris, three Providencia stuartii, 12 Pseudomonas aeruginosa, 20 enterococci, 20 Haemophilus influenzae, 22 pneumococci, 25 Staphylococcus aureus, 25 coagulase-negative staphylococci and 13 Neisseria gonorrhoeae, were studied. The antimicrobials tested against each of the genera are summarized in Table I (chosen in accordance with the BSAC guidelines). Study 2. Fifty-one Enterobacteriaceae and six Acinetobacter spp. isolated from systemic infections, 198 organisms isolated from urinary tract infections (including six staphylococci, two enterococci and 190 Enterobacteriaceae), 187 staphylococci and 10 H. influenzae were studied against the antimicrobials shown in Table I (chosen in accordance with the BSAC guidelines). Aura image system The Aura image system provides a fully automated zone measuring device which is also combined with a comprehensive epidemiological database (Figure 2). It has two unique features, the first being that a bar code can be placed on the edge of the test Petri dish on the day of inoculation and using a hand-held bar code reader this information is automatically entered into the corresponding patient/ organism database. On the second day, when zones are analysed, the bar code is automatically interpreted together with the plate image using a mirror to reflect the bar code label alongside the plate image. Secondly, the scanner has a specially written program (optical character recognition, OCR), which recognizes Oxoid antibiotic disc codes and eliminates the need for the operator to present the Petri dish to the machine in a given orientation. This facility can be disabled if discs from other suppliers are used.For this study, using the scanner as a stand-alone system unconnected to the laboratory computing system, plates were scanned to measure zones of inhibition. The system identified the pattern of discs being studied and automatically recorded a zone diameter (in mm) next to the appropriate antibiotic disc. If the automatic zone recognition facility failed to identify the correct zone diameter for measurement, the mouse was used to ‘drag and drop’ the zone edge marker to the desired position. The automatic measurement and manually adjusted measurements were recorded on the results screen. Analysis of data For study 1, the correlation of zone diameters obtained using the Aura image system (automatic reading or reading after manual adjustment of zone fit) and those measured manually was determined using a standard spreadsheet package (Excel, Microsoft). The mean difference in zone diameter between manual measurement and the Aura image system measurement was calculated and for Enterobacteriaceae only, plotted against the mean zone diameter (mean of manual and scanner measurements) to see if there was evidence of a relationship between measurement and bias.4 Having established the mean zone difference, this figure was used to calculate the rate of manual correction.For Study 2, interpretation of sensitivity was determined using BSAC zone diameter breakpoints. These break-point values were then compared with zones of inhibition obtained by manual and automated measurement and the resulting interpretation was compared with the findings obtained by the diagnostic laboratory using templates (Figure 1). The number of times there was a need to adjust zones manually when using the Aura image system was also assessed. Results Study 1 Correlation of manual and Aura image measurements for the combined data for Enterobacteriaceae and Acinetobacter spp. tested against all of the antimicrobials, is shown in Figure 3 and a summary of correlation coefficients (r) for all of the genera tested is given in Table II; r values ranged from 0.986 to 0.998. Although r values were acceptable for all genera, in the case of P. aeruginosa and particularly for strains producing pigment, there was need to adjust 38.9% of zones manually, compared with c.1 zone in 20 for all of the other genera. When differences in zone diameter between the Aura image and manual measurement were compared, the overall mean difference was –0.18 mm with 95% confidence limits of ±2.84 mm. Although most of the scanner results for Enterobacteriaceae fell within ±3 mm of manual measurements, scanned zones for trimethoprim and ciprofloxacin were generally smaller than those measured manually. While there was no evidence of a relationship between measurement and bias for trimethoprim, there was a suggestion that variability increased with zone size for ciprofloxacin. The variation in zone size was generally greater with the larger zone sizes (>25 mm), but this may be a reflection of the difficulty in the accurate manual measurement of large zones when only one radius can be measured or when large adjacent zones overlapped. For gentamicin, scanned zone sizes tended to be larger than those measured manually (Figure 4), while observations for ceftazidime, ampicillin and cefuroxime revealed a suggestion of bias changing from negative to positive for measurements above 25 mm. Study 2 The time periods needed to read a Petri dish containing six antibiotic discs using the template, manual measurement and the Aura scanning device were 20, 40 and 15 s, respectively. The percentage of zones measured by the Aura scanning device that required manual adjustment (as a proportion of the total number of observations for all organisms against all antibiotics studied), was 2.8% for Enterobacteriaceae, 7.1% for staphylococci, 6.7% for H. influenzae and 4.6% for urinary tract isolates, with an overall adjustment rate of 5.4%.A summary of the agreement between the three methods of interpretation is shown in Table III. In the case of the Enterobacteriaceae, there was agreement between the three methods ranging from 68.2% to 96.5%. In the cases of disagreement between the three methods, there was generally agreement between the Aura image and manual measurement of zones. This was particularly true for tests with cefuroxime, which showed the poorest agreement (68.2%). Greatest disagreement between methods was seen with manual and template interpretation.For staphylococci, agreement between the three methods was greater than or equal to 89.5% for all of the antimicrobials except trimethoprim, where agreement was 57.9% for the 19 strains studied. For the observations not in agreement, there was an almost equal split of agreement between interpretation by template and manual measurement or Aura and manual measurement.For the 10 strains of H. influenzae, agreement was equal to or greater than 90% and for the urinary tract isolates, agreement ranged from 88.1% to 100%. Discussion Zone diameter data gained from disc sensitivity testing has been utilized for many purposes including determination of the normal distribution of ‘wild sensitive’ populations (these data being necessary to identify those strains with possible mechanisms of resistance), surveillance of resistance, daily monitoring of method performance with reference to control strains and interpretation of sensitivity by comparing test zones with zone diameter breakpoints. All these procedures rely on a standardized method of testing and the accurate measurement of zones of inhibition.If data are to be combined from both manual and automated measurements, it is necessary to demonstrate that there is good correlation and agreement within acceptable limits between methods. For all genera studied in the present investigation, the correlation of zone diameter data obtained by manual and automated measurement was c.0.994 and 94.6% of zones measurements were within 3 mm. A comparison of automated reading using the OSIRIS Video Reader System (Sanofi Diagnostic Pasteur, Guildford, UK) and manual zone reading,5 gave a 10% difference using a criteria of 3 mm with a wide range of clinical isolates. It should also be demonstrated that there is no evidence of bias between methods of measurement. In the present study it was sometimes only possible to measure large zones of inhibition (c.25 mm) manually by measuring the radius and doubling it to obtain a zone diameter. It is possible that this exercise with manual measurement introduced an error which might explain the variability observed between the two methods of measurement for zones above 25 mm.Although the use of templates is useful for laboratories who do not have an automated measuring device, this study has shown that for some antibiotic–organism combinations, interpretation of sensitivity using a template may give less reliable results than methods using zone measurement. This was observed particularly with cefuroxime when testing Enterobacteriaceae and Acinetobacter spp. and, to a lesser extent, with ampicillin when testing the aforementioned genera and isolates from urinary tract infections. Of the eight Enterobacter spp. and five E. coli interpreted as sensitive to cefuroxime using the template, both the manual and Aura image system interpreted these strains as having intermediate sensitivity. This may not be considered a major error in reporting, however, it could have clinical significance because reporting intermediate sensitivity might influence the route of antibiotic administration or might stimulate an alternative antibiotic to be chosen for therapy. Of more importance were two strains of staphylococci reported as resistant to vancomycin using the template for interpretation. When zone diameters were measured, both organisms were found to be sensitive to vancomycin, and it was presumed that there was a mistake in manual data entry.The speed with which the Aura image automated system measured zones, the infrequency of zone amendment (c.1 zone per 20 measurements), the facility for random plate orientation and disc recognition, the ability to differentiate between zones of haemolysis and zones of growth inhibition and the ease with which the system can be integrated with laboratory data handling systems endorse the use of this machine for diagnostic and research purposes. The automated system, if programmed suitably6,7 also offers the facility for automatic monitoring of the daily performance of testing procedures (where discrepant results for control strains can be flagged) and automatic reporting of susceptibility based on ‘rules of interpretation’ such as reporting all Proteus as resistant to nitrofurantoin irrespective of zone size. Table I.Antibiotic discs used for testing Organism . . . . Antibiotic disc content (μg) unless stated . aDisc not tested in Study 2. bDisc not tested in Study 1. GEN, gentamicin; AMP, ampicillin; CAZ, ceftazidime; CXM, cefuroxime; TMP, trimethoprim; CIP, ciprofloxacin; AMK, amikacin; IPM, imipenem; PIP, piperacillin; TZP, piperacillin–tazobactam; TEC, teicoplanin; VAN, vancomycin; CTX, cefotaxime; TET, tetracycline; CHL, chloramphenicol; PEN, penicillin; ERY, erythromycin; OXA, oxacillin; MET, methicillin; RD, rifampicillin; FD, fusidic acid; MUP, mupirocin; SPT, spectinomycin; NAL, nalidixic acid; LEX, cephalexin; NIT, nitrofurantoin. Enterobacteriaceae and Acinetobacter spp. GEN 10 AMP 10 CAZ 30 CXM 30 TMPa 5 CIP 1 Pseudomonas aeruginosa CIP 5 CIP 1 AMK 30 GEN 10 CAZ 30 IPM 10 PIP 75 TZB 75/10 Enterococci GEN 200 GEN 120 AMP 10 TEC 30 VAN 5 Haemophilus influenzae AMP 2 CXM 5 CTX 5 TET 10 CHL 10 Haemolytic streptococci TET 10 PEN 1 unit ERY 5 Streptococcus pneumoniae TET 10 CHL 10 OXA 1 ERY 5 CIP 1 Staphylococci GEN 10 PEN 1 unit METa 5 ERY 5 RD 2 OXAa 1 VAN 5 Staphylococci TECa 30 FDb 10 TMPb 5 MUPb 5 TETb 10 Neisseria gonorrhoeae SPT 25 NAL 30 PEN 1 unit CXM 5 TET 10 Urinary tract isolates LEX 30 TMP 2.5 NAL 30 NIT 200 GEN 10 AMP 25 Table II.Correlation coefficients (r) of zone diameters measured manually and using the Aura image for bacteria tested against all antimicrobials tested Genera . No. of observations . r . Enterobacteriaceae and Acinetobacter spp. 528 0.994 Pseudomonas aeruginosa 96 0.998 Staphylococcus aureus 199 0.993 Coagulase-negative staphylococci 190 0.994 Enterococci 100 0.986 Pneumococci 110 0.992 Haemolytic streptococci 51 0.993 Haemophilus influenzae 100 0.989 Neisseria gonorrhoeae 65 0.993 Table III.Comparison of the interpretation of sensitivity by manual measurement, use of the Aura image and use of a template . . Tests with same sensitivity result for all three methodsa . Results summary of tests where there was not agreement . . Genera . Antibiotic disc + Content (μg) . number . % . number . manual + template agree . manual + Aura image agree . No. of Aura image zones adjustedb . aColumns indicate where manual zone measurement, Aura image zone measurement (adjusted if necessary) and template methods all gave the same category of sensitivity result i.e. sensitive, intermediate or resistant. bColumn shows number and percentage of Aura image zones adjusted by the operator. Enterobacteriaceae ampicillin (10) 50/57 87.7 7 0 6/7 1 (1.7%) and Acinetobacter spp. gentamicin (10) 55/57 96.5 2 0 1/2 1 (1.7%) ceftazidime (30) 51/57 89.4 6 1/6 5/6 2 (3.5%) ciprofloxacin (1) 55/57 96.5 2 0 2/2 2 (3.5%) cefuroxime (30) 39/57 68.2 18 3/18 13/18 2 (3.5%) Staphylococci vancomycin (5) 183/187 97.9 4 2/4 2/4 16 (8.5%) fusidic acid (10) 171/187 91.4 16 12/16 3/16 24 (12.8%) erythromycin (5) 187/187 100 0 11 (5.9%) gentamicin (10) 183/187 97.9 4 1/4 1/4 8 (4.2%) penicillin (1 unit) 185/187 98.9 2 0/2 2/2 8 (4.2%) rifampicin (2) 19/19 100 0 1 (5.2%) trimethoprim (5) 11/19 57.9 8 5/8 3/8 1 (5.2%) mupirocin (5) 17/19 89.5 2 2/2 0/2 2 (10.4%) tetracycline (10) 19/19 100 0 1 (5.2%) H. influenzae ampicillin (2) 10/10 100 0 0 tetracycline (10) 10/10 100 0 0 cefuroxime (5) 9/10 90.0 1 0/1 1/1 1 (10%) Urinary tract isolates cephalexin (30) 197/202 97.5 5 2/5 1/5 7 (4.3%) trimethoprim (2.5) 198/202 98.0 4 1/4 2/4 6 (2.9%) nalidixic acid (30) 202/202 100 0 11 (5.4%) nitrofurantoin (200) 190/202 94.1 12 3/12 7/12 8 (3.9%) gentamicin (10) 198/202 98.0 4 3/4 0/4 10 (4.9%) ampicillin (25) 178/202 88.1 24 12/24 10/24 14 (6.9%) Overall 95.2% 5.4% Open in new tabDownload slideTemplate for determining sensitivity. The test plate is placed over the template and zones of inhibition are examined in relation to the template zones. If the zone of inhibition is equal to or larger than the template zone it is considered sensitive. If the zone of inhibition falls within the area marked R it is interpreted as resistant. Open in new tabDownload slideAnalysis screen showing plate image, fitted zones, patient and organism details, and interpretative zone criteria. Open in new tabDownload slideComparison of Aura image and manual measurement of zones of inhibition (mm): combined data for Enterobacteriaceae and Acinetobacter spp. tested against all antimicrobials. Open in new tabDownload slidePlot of mean zone diameter (mean of manual and scanner measurement) versus zone difference (difference between manual and scanner measurement) for gentamicin against Enterobacteriaceae.We would like to thank Dr T. Marshall for advice on statistical analysis and Dr J. Broughall of Oxoid for his financial support and advice on this project. References 1Stokes, E. J. & Waterworth, P. M. (1). Antibiotic sensitivity tests by diffusion methods. Association of Clinical Pathologists Broadsheet (revised December 1972).2British Society for Antimicrobial Chemotherapy. (1998). Standardized Disc Sensitivity Testing Method. Newsletter of the British Society for Antimicrobial Chemotherapy, Summer 1998.3Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy. (). A guide to sensitivity testing. Journal of Antimicrobial Chemotherapy , Suppl. D, –50.4Bland, J. M. & Altman, D. G. (). Statistical methods for assessing agreement between two methods of clinical measurement. , –10.5Haddod-Prots, L., Assayag, D., Jarlier, V. & Nguyen, J. (). Evaluation of the OSIRIS Video Reader System for interpretation of disk diffusion susceptibility tests. Clinical Microbiology and Infection , Suppl. 3, .6Westgard, J. 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