

Evaluation of the BIOMIC Video Reader System for Determining Interpretive Categories of Isolates on the Basis of Disk Diffusion Susceptibility Results

E. KENT KORGENSKI¹ AND JUDY A. DALY^{1,2*}

Primary Children's Medical Center¹ and the Department of Pathology,² University of Utah, Salt Lake City, Utah 84113

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The BIOMIC System (Giles Scientific, New York, N.Y.) includes software and a video-assisted plate reader that functions with a personal computer to automate, speed read, and interpret standard antibiotic disk diffusion test plates. The video reader helps standardize endpoints, speeds quantitative measurements by 40 to 90%, and reduces fatigue and transcription and interpretation errors (H. Wei-Fang, *Am. Clin. Lab.* 13:28-29, 1994). Organisms tested were isolated from patient specimens collected at Primary Children's Medical Center and included rapidly growing gram-positive and gram-negative strains that fulfill the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing. A comparison of the plate reader-determined zones and visually measured zones for 3,339 organism-antimicrobial agent combinations was performed. The results demonstrated 0.1% (4 of 3,339) false-susceptible reads and 0.2% (6 of 3,339) false-resistant reads by the video reader compared with visual reads. Minor discrepancies (4.7% [156 of 3339]), resulting in category interpretation changes of intermediate to resistant or susceptible or changes of resistant or susceptible to intermediate, were also encountered. Of the discrepant results, 80.8% (139 of 172) resulted from a 3-mm or less zone diameter difference between the two different techniques. We conclude that the video-assisted plate reader is a reliable system for determining interpretative categories from zone diameters of standard antibiotic disk diffusion test plates.

The detection of clinically relevant antimicrobial resistance is one of the most important functions of a diagnostic clinical microbiology laboratory (4). There are many different methodologies available for detecting organism resistance to antimicrobials. Susceptibility testing methods include disk diffusion (Kirby-Bauer), broth microdilution (both manual and automated), agar dilution, and antibiotic gradient methods (3). Automated susceptibility testing has been used mainly with the microdilution method, most notably with the Vitek (Hazelwood, Mo.) and MicroScan (Sacramento, Calif.) systems. Potential advantages of automation include standardization resulting in increased accuracy, more rapid results which may positively affect patient care, technologist time savings in reading and interpreting results, improved data management, and the potential use of artificial intelligence to create an expert system for automated review and verification of the data generated (1). Accuracy, reproducibility, cost, flexibility to test large numbers of organisms, and the ease to choose different antimicrobial agents give disk diffusion methods certain advantages over automated broth microdilution systems. The BIOMIC system was developed to combine the advantages of disk diffusion methodology with the advantages of automation (8).

The BIOMIC system (Giles Scientific, New York, N.Y.) is a semiautomated data management system used to read, interpret, and report antimicrobial agent disk diffusion susceptibility results (8). The video-assisted plate reader, a component of the BIOMIC system, consists of an image capture card, a cabinet with a video camera, and software. The BIOMIC video system automatically reads and interprets disk diffusion sus-

ceptibility agar plates. The procedure followed was as outlined in the manufacturer's instructions (2). The agar plate is placed in the drawer of the video reader. A clear image appears on the video screen within 5 s with fully calculated zone diameters for review and possible adjustment by the technologist. Quantitative zone diameters (in millimeters) are calculated by digital image analysis which uses shades of gray, edge detection, and image enhancement. This analysis considers noncircular and overlapping zones, plate edges, and feathered zone edges and uses an approximately 80% growth inhibition criterion and conservative software logic to assess and draw circles on zones. The video system does not eliminate all judgements required to accurately read the disk diffusion agar plate. BIOMIC recommends that each plate be reviewed before and during video reading by an experienced microbiologist skilled in reading susceptibility tests. Zone sizes can be easily and rapidly adjusted if necessary (approximately 5% of the time according to the manufacturer). Zone sizes are interpreted by using National Committee for Clinical Laboratory Standards (NCCLS) guidelines. This evaluation compares the accuracy of zone diameters calculated by the BIOMIC video reader system to that of the NCCLS standard method of a visual read with sliding calipers to determine interpretative categories.

A total of 275 organisms, including both gram-positive cocci and gram-negative bacilli, covering 15 genera and 23 species were selected for this evaluation. The organisms were isolated from a variety of fresh clinical specimens collected at the Primary Children's Medical Center (PCMC), a pediatric tertiary-care referral center located in Salt Lake City, Utah. The organisms included in this study were consecutive isolates that met the requirements for disk diffusion susceptibility testing of organisms recovered at PCMC. Duplicate organisms recovered from the same patient were not included. The organisms tested are representative of the organism mix recovered at PCMC and included 143 strains of the *Enterobacteriaceae* (15 species),

* Corresponding author. Mailing address: Department of Pathology, University of Utah School of Medicine, Primary Children's Medical Center, 100 North Medical Dr., Salt Lake City, UT 84113-1100. Phone: (801) 588-3166. Fax: (801) 588-2435. E-mail: PCJDALY@IHC.com.

70 staphylococcal strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and coagulase-negative *Staphylococcus* species), 49 *Pseudomonas aeruginosa* strains, 7 non-*Enterobacteriaceae* gram-negative bacilli (*Acinetobacter* species and *Stenotrophomonas maltophilia*), and 6 *Haemophilus influenzae* strains. The antimicrobial agents tested were from panels normally tested at PCMC and were dependent upon both the identification and source of the organism. *Enterobacteriaceae* isolated from sites other than urine were tested against amikacin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cephalothin, gentamicin, imipenem, piperacillin, trimethoprim-sulfamethoxazole, and ticarcillin-clavulanic acid. *Enterobacteriaceae* recovered from urine were tested against amoxicillin-clavulanic acid, ampicillin, carbenicillin, cefixime, cefotaxime, ceftriaxone, cefuroxime, cephalothin, gentamicin, nitrofurantoin, sulfonamides, and trimethoprim-sulfamethoxazole. *P. aeruginosa* strains and non-*Enterobacteriaceae* gram-negative bacilli were tested against amikacin, aztreonam, carbenicillin, ceftazidime, ciprofloxacin, colistin sulfate, gentamicin, imipenem, piperacillin, ticarcillin-clavulanic acid, tobramycin, and trimethoprim-sulfamethoxazole. Staphylococci were tested against ceftazidime, cefuroxime, cephalothin, clindamycin, erythromycin, gentamicin, imipenem, oxacillin, penicillin, ticarcillin-clavulanic acid, trimethoprim-sulfamethoxazole, and vancomycin. Each organism tested met the criteria and guidelines for susceptibility testing established by the NCCLS, and category interpretations were made following NCCLS guidelines (6).

The disk diffusion susceptibility method as described by the NCCLS was followed (7). Briefly, all inocula were prepared from pure culture growth isolates that were 18 to 24 h old. Organisms were prepared in 0.85% saline or brain heart infusion broth and adjusted to a 0.5 McFarland standard with a photometer. All organisms were tested on Mueller-Hinton agar, except those of the *Haemophilus* species, which were tested on *Haemophilus* Test Medium. Media with blood supplement were not included in this study; these plates can be read by the video system but require more on-screen user adjustment with a mouse. Susceptibility plates were incubated in ambient air at 35°C except those for the *Haemophilus* species, which were incubated at 35°C in 5% CO₂. Zones were measured after 16 to 18 h of incubation by all staff microbiologists during routine bench rotation assignments. Video and caliper readings for each susceptibility test were made within 15 min of each other.

Interpretative categories (susceptible, intermediate, and resistant) were calculated for each zone measurement for each organism-antimicrobial agent combination tested (6). The visual caliper read was considered the "gold standard." Discrepancies in interpretative categories were noted along with differences in zone measurements in millimeters. A false-susceptible read was defined as a caliper read interpretation of resistant and a video read interpretation of susceptible. A false-resistant read was defined as a caliper read interpretation of susceptible and a video read interpretation of resistant. The following discrepancies were defined as minor: the caliper read was resistant and the video read was intermediate, the caliper read was intermediate and the video read was susceptible, the caliper read was intermediate and the video read was susceptible, the caliper read was intermediate and the video read was resistant, and the caliper read was susceptible and the video read was intermediate.

A total of 3,339 organism-antimicrobial agent combinations were tested. Interpretative category discrepancies were classified as either false susceptible, false resistant, or minor (Table 1). Four of 3,339 (0.12%) organism-antimicrobial agent combinations tested were found to be resistant by caliper read and

TABLE 1. Interpretative category discrepancies

Parameter	Value
Total organisms tested	275
Total organism-antimicrobial agent combinations tested	3,339
False reads by video system	
Caliper resistant vs video susceptible	4/3,339 (0.12%)
Caliper susceptible vs video resistant	6/3,339 (0.18%)
Minor discrepancies by video system	
Caliper resistant vs video intermediate	23/3,339 (0.69%)
Caliper intermediate vs video susceptible	23/3,339 (0.69%)
Caliper intermediate vs video resistant	27/3,339 (0.81%)
Caliper susceptible vs video intermediate	83/3,339 (2.49%)
Total minor discrepancies	156/3,339 (4.67%)

susceptible by video read and were classified as false-susceptible. Six of 3,339 (0.18%) organism-antimicrobial agent combinations tested were found to be susceptible by caliper read and resistant by video read and were classified as false-resistant. The results for 156 of 3,339 (4.67%) organism-antimicrobial agent combinations tested were classified as minor discrepancies. Discrepancies appeared to be random, with no particular organism-antimicrobial agent combination being noted as a problem.

Differences in zone measurements in millimeters between reading methods were also determined for interpretative category discrepancies. The following results were obtained for 172 discrepant results: for 49 (28.5%), the difference was 1 mm; for 50 (29.1%), the difference was 2 mm; for 40 (23.3%) the difference was 3 mm; and for 33 (19.2%), the difference was >3 mm. In all, 139 (80.8%) of the discrepant results were 3 mm or less in their differences in measurement between the caliper read and the video read. Organism-antimicrobial combinations that are clustered near the breakpoints are more likely to show changes in interpretative categories (5).

Reproducibility studies using 19 isolates, including both American Type Culture Collection strains and patient isolates, were performed by disk diffusion susceptibility tests on 3 consecutive days. A total of 228 zone measurements, with an organism-antimicrobial agent combination test mix similar to that used in the comparison study, were made by both the caliper read and video read and were recorded on each of the 3 days. The mean variance for the 3-consecutive-day test results for the visual caliper read was 1.8 mm, and the corresponding mean variance for the video read was 2.0 mm.

In summary, the video system is a component of the BIOMIC system. It rapidly reads zone measurements in millimeters and eliminates the tedious task of individual antibiotic disk zone measurement. Our study compared zone measurements determined by the video reader with zone measurements determined by the NCCLS standard method of a visual read with sliding calipers. Our results show that there were 0.1% false-susceptible reads, 0.2% false-resistant reads, and 4.9% minor discrepancies by the video read compared with the caliper read. In conclusion, the BIOMIC video reader is an acceptable alternative to sliding calipers for the determination of interpretative categories based upon the zone measurements of disk diffusion susceptibility tests.

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