MICs of Nontuberculous Mycobacteria Using the Sensititre® Vizion™ System Compared to Manual Readings

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Objectives: To compare MIC readings of nontuberculous mycobacteria using the Sensititre® Vizion™ System (TREK Diagnostic Systems) with visual readings.

Methods: 189 isolates of nontuberculous mycobacteria (30 *Mycobacterium abscessus*, 12 *M. chelonae*, 22 *M. fortuitum* group, 2 *M. immunogenum*, 1 undescribed rapidly growing *Mycobacterium* sp., 4 *M. mucogenicum,* 1 *M. smegmatis* group, 1 *M. neoaurum,* 67 *M. avium* complex, 12 *M. marinum*, 4 *M. simiae*, 17 *M. kansasii*, 2 *M. parascrofulaceum*, 2 *M. lentiflavum,* 1 *M. arupense*, 3 *M. terrae* complex, 1 *M. szulgai*, 2 *M. gordonae, 1 M. triplex,* 1 *M. avium-intracellulare-scrofulaceum* complex (MAIS); 3 *Mycobacterium* sp.) were tested using Sensititre 96-well MIC plates following the Clinical and Laboratory Institute (M24-A) including guidelines for antimicrobials tested. After incubation according to species, all plates were read visually using a Sensititre mirrored light box. Subsequently, the same 96-well plates were read in a blinded fashion using the Vizion System. The Vizion instrument projects a digital image of the growth in each well onto a touch screen monitor with the plate's antimicrobial template overlaid on the image. Used in conjunction with Sensititre SWIN® Software, touching or clicking the MIC well provided instant feedback of interpretations and the images were stored for later review. The results were automatically recorded and printed with interpretations. Comparisons were made between the readings using current recommended CLSI antimicrobial susceptibility criteria for interpretation of susceptible (S), intermediate (I) and resistant (R) isolates. Those with susceptibility interpretive category change between the two methods from S to R or R to S had major errors. Minor errors were those with susceptibility interpretative category change from S to I, I to S, I to R, or R to I.

Results: 99% of the MICs were the same interpretative category for visual and Vizion readings. Only 1% of the MIC comparisons had minor errors and there were no major errors.

Conclusion: The Vizion System demonstrated excellent correlation to visual MIC reads of nontuberculous mycobacteria. Additionally, the Vizion System allows more rapid MIC reads, thus streamlining laboratory workflow, providing a teaching and training tool for new laboratorians and enhancing collaborations between laboratories using the stored images. The Sensititre SWIN Software also provides a built-in comprehensive epidemiology program which allows inter and intra laboratory comparison of MICs and generation of antibiograms.

Introduction

More than 120 mycobacterial species are currently recognized and the numbers of nontuberculous species are steadily increasing. The majority of species encountered so far have been found to be pathogenic for humans or animals.

Antimicrobial susceptibility testing is important for species that are considered clinically significant. The Clinical and Laboratory Standards Institute (CLSI) recently published guidelines and recommendations for testing of nontuberculous mycobacteria (CLSI, M24-A, 2003). Broth microdilution was recommended for isolates of rapidly growing mycobacteria (RGM) and is also the method we use in our laboratory for other nontuberculous mycobacteria. Our laboratory undertook a study to compare MICs by manual broth microdilution with the Vizion System.

Materials and Methods

MICs for 189 clinical isolates of nontuberculous mycobacteria were performed in cation adjusted Mueller-Hinton broth using susceptibility testing procedures published by the Clinical and Laboratory Standards Institute (CLSI) in the M-24A document. Briefly, the organisms were suspended in sterile distilled water to an inoculum turbidity which matched the 0.5 McFarland standard using a nephelometer. Appropriate dilutions were prepared and the 96-well panels were inoculated using a multichannel pipettor. Rapidly growing mycobacteria (RGM) panels were incubated 72 hours and slowly growing mycobacteria (SGM) panels were incubated 7 days. Both panel types were then read using a Sensititre mirrored light box and overhead lighting to facilitate reading of the wells. The same panels were then read in a blinded fashion using the Vizion and adjustments to lighting were made using the Vizion controls. Once the plate was loaded and the panel type identified on the computer, the appropriate template appeared on the monitor screen. The well which was determined to be the MIC was then "touched" on the screen and results were automatically recorded and printed with interpretive categories printed on the report (susceptible S; intermediate I, resistant R).

Interpretive categories used were those described in the M-24A document for the following agents: amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, sulfamethoxazole and tobramycin for the RGM. For the SGM, the category interpretations used were rifampin, minocycline, rifabutin, ethambutol, streptomycin, clarithromycin, amikacin, ciprofloxacin, sulfamethoxazole and TMP-SMX. For gatifloxacin and moxifloxacin, interpretive categories used were those outlined in the CLSI documents for susceptibility testing of bacteria (M100-S16).

Quality control performed were those recommended by the CLSI M24A document with the addition of in-house controls. *Mycobacterium peregrinum* ATCC 700686 and *Enterococcus faecalis* ATCC 29212 were tested on the RGM plate. For the slowly growing mycobacteria *M. avium* ATCC 700898, *M. marinum* ATCC 927 and *Enterococcus faecalis* ATCC 29212 , *M. smegmatis* ATCC 19420 and *Pseudomonas aeruginosa* ATCC 27853 were also utilized.

Results

Overall, there was 99% MIC categorical agreement with the reads performed visually as compared to the Vizion reads (see Table 1). There were only two minor discrepancies (I → R / R → I, or I → S / S → I) among the total 189 isolates. Both of these discrepancies were seen with two different species of SGM and two different antimicrobials. One discrepancy was noted with streptomycin in only one isolate among 17 isolates of *M. kansasii*. As defined by the CLSI, the error was minor (one dilution difference but a category change from I to R). The second minor error was seen in one of the two isolates of *M. simiae* with a category change of R to I and two dilutions difference (see Table 2). There were no major errors (S → R, R → S) noted in any of the results.

Quality controls were all within acceptable limitations and no discrepancies (errors) were noted (see Table 3).

Conclusion

Although this was a small single center study, the Vizion System demonstrated excellent correlation to visual reads of MICs for the nontuberculous mycobacteria. The Vizion's built-in lighting adjustment was advantageous for facilitating the readings of MICs especially for isolates which had less than optimal growth. The ability to store MIC readings for further review was an added convenience and also enabled the supervisor to check any questionable readings and also allowed for the generation of antibiograms. Laboratories must consider the cost of the system and training requirements prior to its use. However, specific training programs can be implemented in a relatively short time for most laboratories, Additional larger multicenter studies are recommended to further establish utility of the system in the clinical laboratory.

Table 1. Comparison of standard MIC interpretive readings (categories) of the nontuberculous mycobacteria with interpretive readings performed using the Vizion System.

NTM Species	Interpretive Match Standard: Read to Vizion	Interpretive Match Standard: % Match	Interpretive Discrepant Standard: Read to Vizion	Interpretive Discrepant Standard: % Error
ALL DRUGS			ALL DRUGS	
Rapidly Growing Mycobacterium (RGM)				
M. abscessus	30 / 30	100	0 / 30	0
M. chelonae	12 / 12	100	0 / 12	0
M. fortuitum group	22 / 22	100	0 / 22	0
M. immunogenum	2 / 2	100	0 / 2	0
M. mucogenicum	4 / 4	100	0 / 4	0
Other RGM	3 / 3	100	0 / 3	0
Slowly Growing Mycobacterium (SGM)				
M. arupense	1 / 1	100	0 / 1	0
M. avium complex	67 / 67	100	0 / 67	0
M. avium-intracellulare scrofulaceum complex	1 / 1	100	0 / 1	0
M. gordonae	2 / 2	100	0 / 2	0
M. kansasii	16 / 17	94	1 / 17	6
M. lentiflavum	2 / 2	100	0 / 2	0
M. marinum	12 / 12	100	0 / 12	0
M. parascrofulaceum	5 / 5	100	0 / 5	0
M. simiae	3 / 4	75	1 / 4	25
M. szulgai	1 / 1	100	0 / 1	0
M. terrae complex	3 / 3	100	0 / 3	0
M. triplex	1 / 1	100	0 / 1	0
Mycobacterium sp.	3 / 3	100	0 / 3	0

Table 2. Comparison of category matches for the two isolates of slowly growing non- tuberculous mycobacteria with discrepancies between standard and Vizion readings.

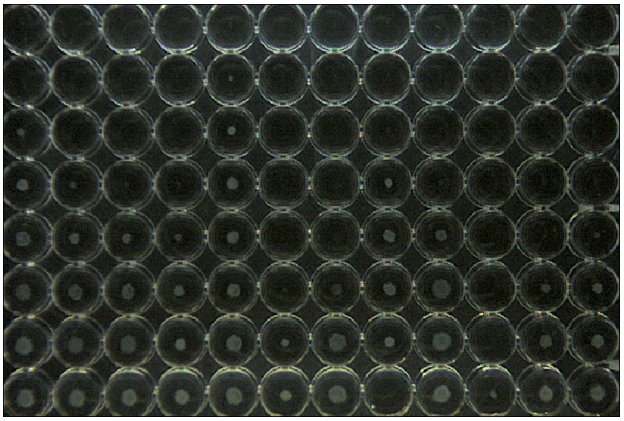
Slowly Growing NTM Species/ Antimicrobial	Interpretive Match	%	No. Discrepancies	%	Type of Discrepancy (Error)
M. kansasii					
Amikacin	17 / 17	100	0 / 17	0	-
Ciprofloxacin	17 / 17	100	0 / 17	0	-
Clarithromycin	17 / 17	100	0 / 17	0	-
Ethambutol	17 / 17	100	0 / 17	0	-
Gatifloxacin	17 / 17	100	0 / 17	0	-
Linezolid	17 / 17	100	0 / 17	0	-
Minocycline	17 / 17	100	0 / 17	0	-
Moxifloxacin	17 / 17	100	0 / 17	0	-
Rifabutin	17 / 17	100	0 / 17	0	-
Rifampin	17 / 17	100	0 / 17	0	-
Streptomycin	16 / 17	94	1 / 17	6	Minor (1 dilution)
TMP-SMX	17 / 17	100	0 / 17	0	-
M. simiae					
Amikacin	1 / 4	75	1 / 4	25	Minor (2 dilutions)
Ciprofloxacin	4 / 4	100	0 / 4	0	-
Clarithromycin	4 / 4	100	0 / 4	0	-
Ethambutol	4 / 4	100	0 / 4	0	-
Gatifloxacin	4 / 4	100	0 / 4	0	-
Linezolid	4 / 4	100	0 / 4	0	-
Minocycline	4 / 4	100	0 / 4	0	-
Moxifloxacin	4 / 4	100	0 / 4	0	-
Rifabutin	4 / 4	100	0 / 4	0	-
Rifampin	4 / 4	100	0 / 4	0	-
Streptomycin	4 / 4	100	0 / 4	0	-
TMP-SMX	4 / 4	100	0 / 4	0	-

Table 3. Comparison of standard (visual) MIC interpretive readings (categories) of the quality control organisms with interpretive readings using the Vizion.

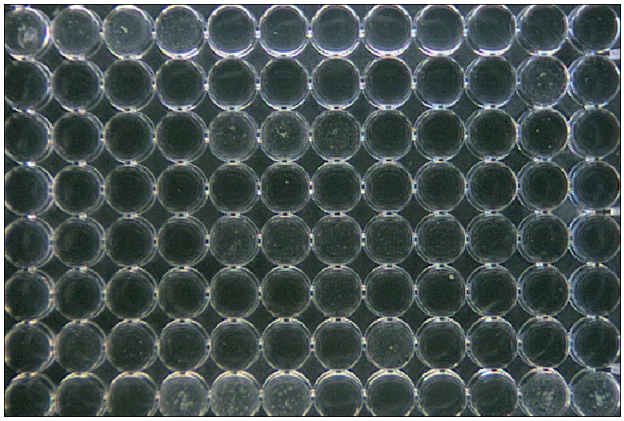
QC Strains	Interpretive Match Standard Read to Vizion % ALL DRUGS	Interpretive Discrepant Standard Read to Vizion % ALL DRUGS
Enterococcus faecalis (RGM) ATCC 29212	100	100
Mycobacterium peregrinum (RGM) ATCC 700686	100	100
Enterococcus faecalis (SGM*) ATCC 29212	100	100
Pseudomonas aeruginosa (SGM) ATCC 27853	100	100
Mycobacterium avium (SGM-MAC) ATCC 700898	100	100
Mycobacterium marinum (SGM) ATCC 927	100	100
Mycobacterium smegmatis (SGM) ATCC 19420	100	100

*SGM = Slowly growing mycobacteria

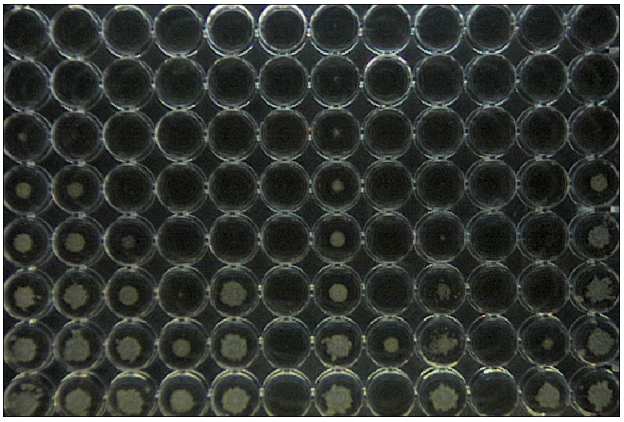
Figures 1-4 Screen Shots Taken Using the Vizion System



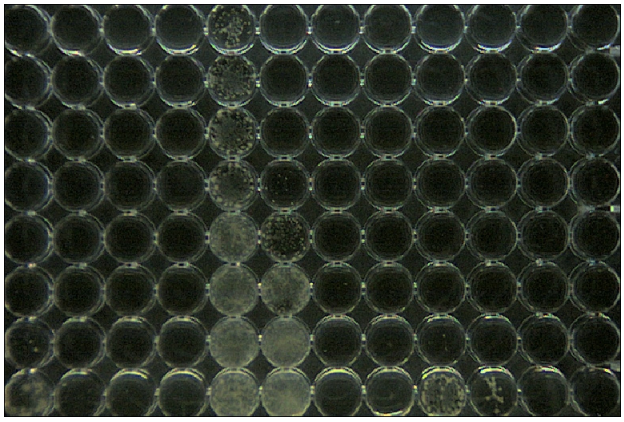
M. avium complex



M. fortuitum group



M. marinum



M. smegmatis

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