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Commercial Broth Microdilution Panel Validation and Reproducibility Trials for Garenoxacin (BMS-284756), A New Desfluoroquinolone

P. Rhomberg[1], K. Gordon[1], RN Jones[1,2]
[1]The The JONES Group/JMI Laboratories, North Liberty, Iowa [www.jmilabs.com]; [2]Tufts University School of Medicine, Boston, Massachusetts

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ABSTRACT

Background: Garenoxacin (GNX), formerly BMS-284756, has demonstrated potent activity against a wide range of pathogens and has been advanced into Phase IV clinical trials. To clarify the GNX results derived from commercial long shelf-life products, a comparison conforming to NCCLS M23-A2 guidelines was performed.
Methods: Susceptibilities were performed using dry-form (DF) panels produced SensiTitre (TREK Diagnostics, Westlake, OH) and reference (R) NCCLS trays for > 700 strains (≥ 100 strains x 7 organism groups). The groups included staphylococci, *S. pneumoniae*, other streptococci (ß-haemolytic, viridans group), enterococci, *H. influenzae*, Enterobacteriaceae, and non-fermentative Gram-negative bacilli. Resistant strains to fluoroquinolones were over represented. Strains were tested by both methods with target results being ≥ 95% ± 1 $\log_2$ dilution. Reproducibility of commercial product MICs was determined for 14 strains tested 3x daily x 3 days (target ≥ 95% ± 1 $\log_2$ dilution). QC results conformed to previously established limits (Biedenbach et al., 2002).
Results: Validation MICs from DF panels produced 71.4% of results the same as the R MIC and 97.0% of results were within ± 1 $\log_2$ dilution on initial testing. Repeated tests with the 3.0% discordant strains resulted in resolution to within acceptable, target limits. Reproducibility tests showed 92.1 and 90.5% of GXN results were identical within the same day and between days, respectively. Control results for ciprofloxacin (CIPRO) and gatifloxacin demonstrated similar values (example: CIPRO reproducibility at 88.4 - 90.5%).
Conclusions: GXN MIC results derived from commercial DF products very closely approximate those of the NCCLS R test (≥ 97.0%) and were highly reproducible (90.5 - 92.1%). Results from GXN clinical trial using these products can be applied with confidence to the regulatory processes in the US and European Union, as well as leading to appropriate NCCLS breakpoints for susceptibility.

INTRODUCTION

Garenoxacin (formerly BMS-284756), a novel des-fluoro(6)quinolone, has demonstrated potent activity against a wide variety of bacterial pathogens and is near completion of Phase III clinical trials. As garenoxacin moves into clinical use, routine susceptibility testing using commercial dry-form broth microdilution products will be needed. These products must be validated by utilizing a structured study design (NCCLS) that compares the results to reference MIC results.

The reported protocol was developed to comply with NCCLS guidelines published in the M23-A2 (2001) document to validate commercial products. The spectrum of garenoxacin activity requires validation with seven organism groups: staphylococci, streptococci, pneumococci, enterococci, Enterobacteriaceae, other Gram-negative bacilli, and *Haemophilus influenzae*. Reproducibility was assessed by replicate testing a selected group of isolates.

MATERIALS AND METHODS

Garenoxacin susceptibility was determined using both commercial dry-form panels (SensiTitre/TREK) processed by package insert procedures, and frozen broth microdilution reference panels utilizing NCCLS M7-A5 methods. Over 700 recent clinical isolates from the SENTRY Antimicrobial Surveillance Program (2001 - 2002) were tested in accordance with NCCLS M23-A2 [2001] guidelines which require at least 100 isolates be tested for each organism group listed in the M100-S12 [2002] document tables against which garenoxacin has proven activity. Gatifloxacin was used as the comparison agent for this MIC validation study.

Garenoxacin and ciprofloxacin (control) commercial MIC reproducibility was assessed by selecting 14 strains including 10 ATCC isolates often used for quality assurance. Susceptibility testing was performed three times daily for three days generating a total of 126 determinations. The target for acceptable reproducibility testing studies was ≥ 95% of MIC results within ± one $\log_2$ dilution between methods.

RESULTS

- Commercial garenoxacin MIC results on dry-form panels were all within ± one $\log_2$ dilution step of the reference method results (Table 1).

Table 1. Variations between garenoxacin MIC results performed on dry-form panels (SensiTitre/TREK) versus reference frozen broth microdilution trays (1,078 strains).

	Variations, all tests (n=1,078)					Variations, on-scale tests (n=497)				
Organism collection (no. tested)	-2	-1	0	+1	+2	-2	-1	0	+1	+2
Gram-positive cocci										
Enterococci (102)	0	6	60	36	0	0	6	48	30	0
Staphylococci (153)	0	3	129	21	0	0	3	55	16	0
S. pneumoniae (167)	0	0	71	96	0	0	0	4	96	0
Streptococci (141)[a]	0	2	102	37	0	0	1	63	37	0
Subtotal (563)	0	11	362	190	0	0	10	170	179	0
Gram-negative bacilli										
H. influenzae (308)	0	3	291	14	0	0	2	9	2	0
Enterobacteriaceae (105)	0	4	64	37	0	0	3	30	34	0
Non-fermenters (102)	0	2	73	27	0	0	2	31	25	0
Subtotal (515)	0	9	428	78	0	0	7	70	61	0
Total (1,078)	0	20	790	268	0	0	17	240	240	0
(%)	(0)	(2)	(73)	(25)	(0)	(0)	(3)	(48)	(48)	(0)

a. Includes viridans group (80 strains) and ß-haemolytic species (61 strains).

Table 2. Variations between gatifloxacin MIC results performed on dry-form panels (SensiTitre/TREK) versus reference frozen broth microdilution trays (1,078 strains)/control agent.

	Variations, all tests (n=1,078)					Variations, on-scale tests (n=589)				
Organism collection (no. tested)	-2	-1	0	+1	+2	-2	-1	0	+1	+2
Gram-positive cocci										
Enterococci (102)	0	7	79	15	1	0	7	27	13	1
Staphylococci (153)	0	9	98	46	0	0	4	72	46	0
S. pneumoniae (167)	0	1	68	98	1	0	0	69	98	1
Streptococci (141)[a]	1	4	84	52	0	0	4	81	51	0
Subtotal (563)	1	20	329	211	2	0	15	248	208	2
Gram-negative bacilli										
H. influenzae (308)	0	1	304	1	2	0	1	5	1	2
Enterobacteriaceae (105)	0	6	79	20	0	0	4	21	20	0
Non-fermentors (102)	1	4	80	17	0	1	4	40	17	0
Subtotal (515)	1	11	463	38	2	1	9	66	38	2
Total (1,078)	2	31	792	249	4	1	24	314	246	4
(%)	(<1)	(3)	(73)	(23)	(<1)	(<1)	(4)	(53)	(42)	(<1)

a. Includes viridans group streptococci (80 strains) and ß-haemolytic species (61 strains).

- Analyzing only the on-scale MIC values for garenoxacin, a trend toward higher (0.5 x one $\log_2$ dilution step) MICs was observed (48% identical, 48% at two-fold higher) with the commercial dry-form panels. Higher MIC values were most often observed for *S. pneumoniae* where 96.0% of strains had a two-fold greater MIC.
- The comparison quinolone (gatifloxacin) had very similar validation results with > 99% of MIC values within ± one $\log_2$ dilution and a similar trend when analyzing only on-scale values was observed for higher commercial MICs (Table 2).
- Both garenoxacin and ciprofloxacin (control) had excellent MIC reproducibility results with all values within ± one $\log_2$ dilution on same day results and between-days testing (Table 3).
- Greater than 90% of all garenoxacin MIC values were identical for all reproducibility organisms tested.

Table 3. Garenoxacin dry-form panel reproducibility results testing at three replicates daily for three days or 126 total results.

	Garenoxacin MIC variation in $\log_2$ dilutions (ciprofloxacin results):					
	Within replicates on same day[a]			Replicates between days[b]		
Organism[c]	-1	Same	+1	-1	Same	+1
E. coli ATCC 25922	0(0)	8(9)	1(0)	0(0)	8(9)	1(0)
E. coli ATCC 35218	1(0)	8(9)	0(0)	1(0)	8(9)	0(0)
K. pneumoniae ATCC 13883	2(0)	7(8)	0(1)	2(0)	7(8)	0(1)
P. aeruginosa 2-5668	0(0)	8(9)	1(0)	0(0)	8(9)	1(0)
P. aeruginosa ATCC 27853	0(1)	9(7)	0(1)	0(0)	9(6)	0(3)
S. aureus 9144	0(2)	8(7)	1(0)	0(2)	8(7)	1(0)
S. aureus ATCC 29213	0(0)	8(8)	1(1)	0(0)	8(8)	1(1)
S. aureus ATCC 25923	0(0)	8(9)	1(0)	2(0)	7(9)	0(0)
Enterococcus spp. 96-10852A	0(0)	9(9)	0(0)	0(0)	9(9)	0(0)
E. faecalis ATCC 29212	0(2)	8(6)	1(1)	0(4)	8(5)	1(0)
S. pneumoniae ATCC 49619	1(1)	8(7)	0(1)	0(1)	7(7)	2(1)
S. pneumoniae 2666B	0(0)	9(8)	0(1)	0(0)	9(8)	0(1)
H. influenzae ATCC 49247	0(0)	9(9)	0(0)	0(0)	9(9)	0(0)
H. influenzae ATCC 49766	0(0)	9(9)	0(0)	0(0)	9(9)	0(0)
Total	4(6)	116(114)	6(6)	5(7)	114(112)	7(7)

a. Exact replicate-to-replicate reproducibility was 92.1 and 90.5% for garenoxacin and ciprofloxacin respectively. All MIC results (100.0%) were ± one $\log_2$ dilution step for both.
b. The exact MIC was achieved between days in 90.5 and 88.9% for garenoxacin and ciprofloxacin respectively. All MIC results (100.0%) were ± one $\log_2$ dilution step for both.
c. Note that garenoxacin had 12 of 14 organisms with on-scale results and ciprofloxacin had 8 of 14 organisms with on-scale MICs.

CONCLUSIONS

- All MIC comparisons for garenoxacin between dry-form and frozen reference broth microdilution results showed equality within ± one $\log_2$ dilution.
- A slight trend toward higher MIC values (0.5 $\log_2$ dilution step) for the commercial dry-form method was observed for both garenoxacin and gatifloxacin that was most pronounced for *S. pneumoniae*.
- Garenoxacin commercial dry-form MIC reagents appear qualified for routine clinical laboratory use, following the FDA release of this des-fluoro(6)quinolone product.

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