Determination of Dry-Form Commercial Reagent Reproducibility and MIC Validations for LBM415, A Novel Peptide Deformylase (PDF) Inhibitor

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ABSTRACT

Background: LBM415 is a new PDF inhibitor rapidly being advanced to human clinical trials. Commercial reagent broth microdilution MIC panels will be required for investigator laboratory use, especially those products with extended shelf-lives (dry-form). This study reports the results of reagent qualifying tests. ***Methods***: The experiment was performed by NCCLS M23-A2 guidelines to assess dry-form MIC reproducibility (10 organisms x 3 tests/day x 3 days = 90 tests) and comparative MIC accuracy to the reference MIC (REF; M7-A6, 2003) using ≥ 100 strains representing the following organism groups: staphylococci, enterococci, *S. pneumoniae*, other streptococci, *H. influenzae*, and selected species refractory to PDF inhibitor action. All trays were manufactured by Sensititre (TREK Diagnostics, Cleveland, OH). ***Results***: Reproducibility results showed 80% of MICs were identical and 97.8% of MICs were within ± one $\log_2$ dilution step. Validation test results comparing dry-form to REF MICs were (% identical/± 2-fold/ ± 4-fold): for staphylococci (71/99/100%), for enterococci (55/99/100%), for *S. pneumoniae* (33/91/97%), for other streptococci (69/100/100%) and for *H. influenzae* (36/97/100%). Consistent variations were detected with SPN (49% of dry-form panel results being 1 dilution higher than REF) and HI (60% of results being 1 dilution lower than REF). LBM415 MICs were off-scale (MIC values, > 32 mg/L) for Enterobacteriaceae and non-fermentative Gram-negative bacilli (40 strains). Overall, 97% of Sensititre MIC results for were within ± one $\log_2$ dilution of REF MIC values. ***Conclusions***: LBM415 dry-form diagnostic MIC panels have been validated for accuracy and reproducibility using 520 recent clinical isolates from 5 major pathogen groups. The spectrum of activity for this PDF inhibitor compound appears focused toward Gram-positive cocci and specific fastidious respiratory tract pathogens.

INTRODUCTION

- LBM415 is the first inhibitor of the bacterial enzyme peptide deformylase (PDF) to be advanced into human clinical trials for the oral treatment of community-acquired respiratory tract and skin and skin-structure infections. PDF is required in bacteria for protein maturation and is not found in eukaryotic cells, making it a unique antibacterial target.
- LBM415 has documented activity against major pathogens including *Streptococcus pneumoniae* (penicillin-susceptible and -resistant), *Haemophilus influenzae, Moraxella catarrhalis, Chlamydia pneumoniae, Staphylococcus aureus* (oxacillin-susceptible and –resistant), β-haemolytic streptococci, enterococci (vancomycin-susceptible and -resistant); co-resistance with other classes of antimicrobials has not been detected.
- Laboratory support for continued clinical development of this compound requires the availability of validated susceptibility testing products for use by collaborating investigators. Now that MIC and disk diffusion quality control (QC) ranges have been established for reference methods, commercially prepared MIC products with extended shelf-lives need to be made available for routine susceptibility testing.
- This report summarizes the results of reagent qualifying tests utilizing guidelines (M23-A2, 2001) recommended by the National Committee for Clinical Laboratory Standards (NCCLS) to determine the equivalent performance and reproducibility of commercial dry-form MIC panels when compared to the reference broth microdilution method.

MATERIALS AND METHODS

- **ORGANISMS TESTED.** Minimal criteria for the tested number of organisms as specified by NCCLS were achieved by processing ≥ 100 strains of staphylococci, *S. pneumoniae,* other streptococci, enterococci and *H. influenzae*, and selected species refractory to PDF inhibitor action. Strains tested included: *S. aureus* (66 strains), coagulase-negative staphylococci (42 strains), *S. pneumoniae* (100 strains), β-haemolytic streptococci (52 strains), viridans group streptococci (48 strains), *Enterococcus* spp. (101 strains), *H. influenzae* (100 strains) and 42 strains of various gram-negative bacilli (Enterobacteriaceae, 26 strains; *Pseudomonas aeruginosa*, 10 strains; other non-fermentative bacilli, 6 strains) expected to produce off-scale (resistant) MIC results. Quality Control organisms included *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247. All recorded QC results for LBM415 and vancomycin (control agent) were within published limits.
- **SUSCEPTIBILITY TESTS.** The dry-form broth microdilution trays were produced by TREK Diagnostics/Sensititre (Westlake, OH, USA) and included LBM415 in a dilution range of 0.06–8 mg/L. Reference trays used for comparison were prepared using cation-adjusted Mueller-Hinton broth (Difco, Detroit, MI), and frozen at –70˚C or below until used. An inoculum of 5 x 10^5 CFU/mL was used for testing, and interpretation of endpoints conformed to NCCLS criteria.
- **DESIGN AND QC.** The reproducibility phase of the study consisted of having 10 strains of gram-positive cocci tested 3 times daily for 3 days generating a total of 90 determinations. Four were QC strains (*S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 and 25923). Target values of inter-method accuracy were selected as ≥ 95% of results within ± 1 $\log_2$ dilution of the reference test result and ≥ 50% having the same MIC value. Acceptable reproducibility was also ≥ 95% of results within ± 1 $\log_2$ dilution step of the overall modal MIC, analyzed on the same day and between days of testing.

RESULTS

- For those organisms falling within the LBM415 spectrum of activity, 54% of MIC values for the 2 methods were identical (range, 35% for *S. pneumoniae* to 70% for both staphylococci and streptococci other than *S. pneumoniae*) and 99.2% were within ± 1 $\log_2$ dilution of the reference test result (Table 1).
- Consistent variations between methods were detected only with *S. pneumoniae* (58% of dry-form panel MIC results being 2-fold or greater above the reference panel results) and *H. influenzae* (61% of MIC results being 1 $\log_2$ dilution lower than the reference panel MIC).
- While isolates were selected to minimize off-scale results, 5 viridans group streptococci (< 1% of the total) gave off-scale (≤ 0.06 mg/L) results.
- MIC results were off-scale (> 32 mg/L) for all Enterobacteriaceae and 13 of 16 tested non-fermentative gram-negative bacilli (40 strains); for analysis purposes these results were considered identical (no variation).
- For all organisms (n=551) tested, 57.0% of results were identical and 99.3% of inter-method results were within ± 1 $\log_2$ dilution.
- Performance of same-day and between-day exact reproducibility results were 84.4 and 75.6%, respectively (Table 2), with 98.9 and 96.7% of MIC values being within ± 1 $\log_2$ dilution step. These reported results exceed the predefined criteria established for acceptable performance.

CONCLUSIONS

- This study has examined the ability of commercial (TREK Diagnostics) broth microdilution dry-form panels to produce accurate MIC results for LBM415, a novel inhibitor of bacterial PDF, when compared with an established reference method.
- Accuracy and reproducibility of the dry-form panels were observed to exceed acceptable limits.
- The commercial dry-form (Sensititre) product can be used to produce accurate and reproducible results in support of further LBM415 clinical trials when used according to manufacturer's directions and when controlled for by using appropriate organisms and methods as published in contemporary NCCLS documents.

SELECTED REFERENCES

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TABLE 1. Validation experiments comparing results from dry-form LBM415 broth microdilution trays with reference frozen-form LBM415 tray MICs

	Dry-form MIC variations in $\log_2$ dilutions:					
Organism collection (no. tested)	-2	-1	Same	+1	+2	+3
Organism within the spectrum of the compound						
***Staphylococcus* spp. (108)**	0	19	76	13	0	0
***Enterococcus* spp. (101)**	0	33	55	13	0	0
***S. pneumoniae* (100)**	0	7	35	54	3	1
Other *Streptococcus* spp. (100)	0	22	70	8	0	0
***H. influenzae* (100)**	0	61	38	1	0	0
Subtotal (509)	0	142	274	89	3	1
(%)	(0.0)	(27.9)	(53.8)	(17.5)	(0.6)	(0.2)
Other gram-negative bacilli[a] (42)	0	1	40	1	0	0
All strains (551)	0	143	314	90	3	1
(%)	(0.0)	(26.0)[b]	(57.0)[b]	(16.3)[b]	(0.5)	(0.2)

a. Includes: Enterobacteriaceae (26 strains), *P. aeruginosa* (10 strains), and non-fermentative bacilli (6 strains); 39 of 42 results had MICs > 32 mg/L.
b. 99.3% of all LBM415 results and 99.2% of all results for those organisms falling within the spectrum of activity were within ± 1 $\log_2$ dilution; 57.0% had the same MIC value by both tested methods, meeting target objectives for validation (≥ 50%).

TABLE 2. LBM415 dry-form commercial (Sensititre, TREK Diagnostics) MIC panel reproducibility results testing 3 replicates daily for 3 separate days (90 total results)[a]

	LBM415 MIC variation in $\log_2$ dilutions									
	Replicates on same day[b]					Replicates between days[c]				
Organism	-2	-1	Same	+1	+2	-2	-1	Same	+1	+2
***E. faecalis* ATCC 29212**	0	0	7	2	0	0	0	7	2	0
***E. faecalis* 95-16130**	0	0	9	0	0	0	0	9	0	0
***E. faecium* 102-15947A**	0	1	8	0	0	0	1	8	0	0
***S. pneumoniae* ATCC 49619**	0	1	8	0	0	0	1	8	0	0
***S. pneumoniae* 102-16000A**	1	1	6	1	0	0	2	4	3	0
***S. pyogenes* 7-16229A**	0	1	8	0	0	0	1	8	0	0
***S. aureus* ATCC 25923**	0	0	7	2	0	0	4	5	0	0
***S. aureus* ATCC 29213**	0	0	8	1	0	0	0	8	1	0
***S. epidermidis* 102-15955**	0	2	7	0	0	0	2	7	0	0
***S. epidermidis* 96-16267**	0	0	8	1	0	3	2	4	0	0
Total	1	6	76	7	0	3	13	68	6	0
(%)	(1.1)	(6.7)	(84.4)[b]	(7.8)	(0.0)	(3.3)	(14.4)	(75.6)[c]	(6.7)	(0.0)

a. Note that all MIC results were on-scale values (0.25 to 4 mg/L).
b. Exact replicate-to-replicate reproducibility was 84.4%. Nearly all MIC results (98.9%) were ± 1 $\log_2$ dilution step.
c. The exact MIC was achieved between days for 75.6% of tests. Again, almost all (96.7%) were ± 1 $\log_2$ dilution step.