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# Initial Quality Control Guidelines for MIC Susceptibility Testing of NVP PDF-713, A Novel Peptide Deformylase Inhibitor

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## AMENDED ABSTRACT

**Background:** Compound NVP PDF-713 is a peptide deformylase (PDF) inhibitor which has emerged as a viable clinical candidate combining a complete spectrum of activity against important multi-resistant Gram-positive organisms and the two Gram-negative species most frequently associated with community-acquired respiratory tract infections (*Haemophilus influenzae, Moraxella catarrhalis*). This report summarizes the results of broth microdilution MIC quality control (QC) investigations for NVP PDF-713.

**Methods:** This study followed the M23-A2 and M7-A6 guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS). The investigation used eight laboratories, four broth media (three manufacturers) and four QC strains. QC strains tested against NVP PDF-713 included: *Enterococcus faecalis* (EF) ATCC 29212, *Staphylococcus aureus* (SA) ATCC 29213, *Streptococcus pneumoniae* (SPN) ATCC 49619 and *H. influenzae* (HI) ATCC 49247. A total of 320 values were generated per QC organism for NVP PDF-713 and 320 values for each of two control agents (clarithromycin and vancomycin).

**Results:** Proposed three $\log_2$ dilution MIC ranges contained 95.6-99.4% of the reported values by all participating centers. The ranges for NVP PDF-713 calculated for routine QC in clinical laboratories were: 2-8 μg/ml for EF, 0.5-2 μg/ml for SA, 0.25-1 μg/ml for SPN and 1-4 μg/ml for HI. Only two laboratories (F, H) produced all MIC values beyond selected QC ranges. Concurrent testing of clarithromycin (HI) and linezolid (EF, SA, SPN) as internal control agents resulted in all MIC results within NCCLS published QC guidelines. The average inoculum concentrations were 3.5 x $10^5$ CFU/ml (range: 3.0 x $10^4$-1.0 x $10^6$ CFU/ml) for all participants.

**Conclusions:** QC ranges for the NVP PDF-713 test using NCCLS methods were established and appear acceptable in preparation for the early phases of clinical trials. These ranges will contribute to overall test accuracy for this first clinically applied agent in the PDF class.

## INTRODUCTION

Over the past several decades, the development of antimicrobial agents has focused on a limited number of targets including bacterial cell wall synthesis and ribosomal proteins. With a paucity of targets, and the rapidly emerging bacterial resistance, cross-resistance has become common among several antimicrobial classes. To combat the contemporary problems encountered by cross-resistance, antimicrobial agents with novel mechanisms of action must be developed. One such target candidate is peptide deformylase (PDF), which is a required enzyme for prokaryote protein synthesis, but not essential for eukaryote cytoplasmic protein production, thus making an inhibitor of PDF a desirable mechanism of action. Several PDF inhibitors have been described and found to possess potent activity against many antimicrobial-resistant Gram-positive cocci, as well as having reasonable activity against Gram-negative bacilli, including *Haemophilus* spp. and *Moraxella catarrhalis*. This development has become especially important during the last decade with increasing antimicrobial resistance documented among many clinically significant Gram-positive organisms and community-acquired respiratory tract pathogens.

Among numerous PDF inhibitor clinical candidates studied, compound NVP PDF-713 (Novartis, Basel, Switzerland) has emerged as a viable agent combining satisfactory potency and spectrum against key Gram-positive organisms, as well as *H. influenzae* and *M. catarrhalis*. To determine the accurate assessment of the susceptibility testing profiles among clinical isolates, quality control (QC) guidelines for NVP PDF-713 are required.

## MATERIALS & METHODS

A multi-center study group was recruited that consisted of the following laboratories (directors): The Cleveland Clinic Foundation (G. Hall), Cleveland, OH; The Jones Group/JMI Laboratories (A. Fuhrmeister), North Liberty, IA; Michigan State University (G. Stein), East Lansing, MI; TREK Diagnostics (C. Knapp), Cleveland, OH; University of Alberta (R. Rennie), Edmonton, AB, Canada; University of Texas (A. Wanger), Houston, TX; University of Washington (T. Fritsche), Seattle, WA; and Strong Memorial Hospital (D. Hardy), Rochester, NY. Each site participated in a study for the development of MIC QC guidelines for NVP PDF-713 using common ATCC control strains. The QC study followed the M23-A2 guidelines and the M7-A6 broth microdilution test method recommended by the National Committee for Clinical Laboratory Standards(NCCLS).

The frozen-form, reference broth microdilution panels were prepared by TREK Diagnostics (Cleveland, OH) and contained either four Mueller-Hinton broth lots (Difco, Detroit, MI [two lots]; Hardy, Santa Maria, CA; BBL, Sparks, MD) supplemented with or without 5% lysed horse blood or four lots of Haemophilus Test Medium (Difco [two lots]; Hardy; BBL). All panels were stored at -80ºC until used. The antimicrobial agents in this study were obtained as follows: NVP PDF-713 from Novartis, linezolid from Pharmacia & Upjohn (Kalamazoo, MI) and clarithromycin from Abbott Laboratories (North Chicago, IL), the latter two compounds served as internal control agents. Each laboratory tested four QC strains that included: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247 each tested daily for 10 days, generating 320 MIC values per QC organism.

Concurrent testing using linezolid as the internal control for *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619, and clarithromycin as the internal control for *H. influenzae* ATCC 49247 showed that 100.0% of the MIC results (640 values) were within the published NCCLS guidelines. Colony counts were performed from the broth microdilution panels by subculturing in a quantitative manner onto drug-free plates. The inoculum counts ranged from 3.0 x $10^4$ to 1.0 x $10^6$ CFU/ml with an average for all laboratories at 3.5 x $10^5$ CFU/ml (target inoculum, 5.0 x $10^5$ CFU/ml).

**Table 1.** Distributions of NVP PDF-713 MIC values for all qualifying results from an eight laboratory study using four control organisms.

| | MIC occurrences by control strain: | | | |
|---|---|---|---|---|
| MIC (μg/ml) | *H. influenzae* ATCC 49247 | S. aureus ATCC 29213 | *E. faecalis* ATCC 29212 | *S. pneumoniae* ATCC 49619 |
| 0.12 | | | | 8 |
| 0.25 | | 2 | | 69[a] |
| 0.5 | 8 | 38[a] | | 178[a] |
| 1 | 70[a] | 230[a] | | 65[a] |
| 2 | 220[a] | 50[a] | 7[a] | |
| 4 | 22[a] | | 287[a] | |
| 8 | | | 12[a] | |
| >8 | | | 14 | |

a. Proposed ranges that included 95.6 - 99.4% of all reported MIC results.

## RESULTS

- Proposed QC ranges were optimized to encompass ≥ 95% of all results as recommended by NCCLS M23-A2 guideline (Table 1).
- The MIC results for each tested antimicrobial agent were tabulated and compared by intra- and inter-laboratory analysis to determine potential, unacceptable technical variations. Broth media lots were also compared to determine any manufacturer variation. No significant variations among laboratories or media lots were observed.
- Table 2 shows an example of NVP PDF-713 MIC distributions among eight participant laboratories when testing *S. pneumoniae* ATCC 49619. For the eight sites, the modal MIC value was 0.5 μg/ml (55.6% of the total results) with a MIC range for each laboratory varying from two to three $\log_2$ dilutions. The proposed MIC range for *S. pneumoniae* ATCC 49619 was 0.25 - 1 μg/ml and these QC criteria would encompass 97.5% of all reported results.
- Similar results were obtained for *H. influenzae* ATCC 49247 with nearly 69% of the total QC results at the modal value of 2 μg/ml (Table 1). The MIC range for each laboratory was only one to three $\log_2$ dilution steps, and a MIC QC range of 1 - 4 μg/ml was proposed which would contain 97.5% of all reported results.
- The results for *S. aureus* ATCC 29213 showed a modal value at 1 μg/ml (71.9% of the total results) and a proposed MIC range of 0.5 - 2 μg/ml that would include 99.4% of all reported results (Table 1).
- *E. faecalis* ATCC 29212 had the highest percent of the total results (89.7%) at the MIC modal value of 4 μg/ml. The proposed MIC QC range was 2 - 8 μg/ml for *E. faecalis* ATCC 29212 (Table 1).

**Table 2.** Inter- and intra-laboratory comparisons of NVP PDF-713 MIC results for *S. pneumoniae* ATCC 49619.

| | Occurrences by laboratory: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| NVP PDF-713 MIC (μg/ml) | A | B | C | D | E | F | G | H | Total |
| 0.12 | | | | | | 8 | | | 8 |
| 0.25 | 4 | | | 20 | 14 | 31 | | | 69[a] |
| 0.5 | 36 | 26 | 22 | 20 | 26 | 1 | 29 | 18 | 178[a] |
| 1 | | 14 | 18 | | | | 11 | 22 | 65[a] |

a. Proposed MIC range that includes 97.5% of reported results.

## CONCLUSIONS

- Table 1 summarizes all of the proposed MIC ranges for the four QC strains tested. The proposed three $\log_2$ dilution MIC ranges for broth microdilution tests with NVP PDF-713 would include 95.6 - 99.4% of all reported results in this initial study.
- NVP PDF-713, a novel clinical candidate PDF inhibitor, has demonstrated excellent in vitro activity against the Gram-positive and Gram-negative organisms most frequently recovered from respiratory tract infections. The QC MIC ranges for NPV PDF-713 proposed by these study findings will be important for the accurate development of this compound and other PDF inhibitors as they advance through initial clinical trials worldwide.

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