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Determination of Tier I Quality Control Ranges for WCK 771

M. Hackel [1], Sachin Bhagwat[2], Jaikumar Satav[2], Kushal Umarkar[2], Amol Kulkarni[2], Aniruddha Udaykar[2], Mahesh Patel[2], D. Sahm[1]
[1]International Health Management Associates, Inc., Schaumburg, IL, USA,
[2]Wockhardt Research Center, Aurangabad, India

IHMA, Inc.
2122 Palmer Drive
Schaumburg, IL 60173 USA
Phone: +1.847.303.5003
Fax: +1.847.303.5601
www.ihmainc.com

Revised Abstract

Background: : WCK 771 is a new broad spectrum anti-MRSA benzoquinolizine quinolone being developed by Wockhardt for the treatment of ABSSSI and HABP caused by MRSA. As part of this drug's development it is important to establish quality control ranges for disk diffusion and minimal inhibitory concentration to ensure precision in *in vitro* testing of the product. This Tier 1 study was done to establish initial quality control ranges for the commonly used CLSI control strains for both disk diffusion and broth microdilution testing. **Methods:** The strains tested included *S. aureus* ATCC 25923 (disk only) *S. aureus* ATCC 29213 (broth dilution testing only), *H. influenzae* ATCC 49247 (broth dilution and disk diffusion testing), *S. pneumoniae* ATCC 49619 (broth dilution and disk diffusion testing), *E. coli* ATCC 25922 (broth dilution and disk diffusion testing), and *P. aeruginosa* ATCC 27853 (broth dilution and disk diffusion testing). WCK 771 disk masses included WCK 771 5 µg, 7.5 µg, 10 µg and 15 µg and MIC dilution range was 0.004 – 8 µg/ml. Levofloxacin disk and broth testing served as the control. Each strain was tested over 20 replicates according to CLSI guidelines for disk diffusion and broth microdilution testing. **Results:** The recommended Tier 1 disk (10 µg only) and broth microdilution ranges are provided in the following table:

Recommended Tier 1 QC Ranges		
ATCC Strain	10 µg Disk (mm)	Broth (µg/ml)
S. aureus ATCC 25923	35 - 38	NA
S. aureus ATCC 29213	NA	0.015 - 0.12
E. coli ATCC 25922	29 - 33	0.06 - 0.25
P. aeruginosa ATCC 27853	17 - 21	1 - 4
H. influenzae ATCC 49247	34 - 39	0.015 - 0.06
S. pneumoniae ATCC 49619	25 - 30	0.12 -0.5

Conclusions: Based on the replicate testing results the Tier 1 QC ranges for disk diffusion and broth microdilution testing have been established. All disk masses gave comparable results (data not shown). A 10 µg mass was chosen since this disk mass consistently provides a zones of ≥15 mm for the susceptible target pathogens including *Pseudomonas* and provides a good correlation with proposed PK-PD MIC breakpoints based on its high clinical exposures at the selected doses. These ranges provide reliable QC guidelines until such time as a CLSI M23 Tier 2 study is completed.

Introduction

WCK 771 (levonadifloxacin) is a new broad spectrum anti-MRSA benzoquinolizine quinolone under development by Wockhardt. As part of this drug's development it is important to establish quality control ranges for disk diffusion and minimal inhibitory concentration to ensure precision in *in vitro* testing of the product. This Tier 1 study was done to establish initial quality control ranges for the commonly used CLSI control strains for both disk diffusion and broth microdilution testing.

Materials & Methods

Quality control (QC) strains tested included:

- *S. aureus* ATCC 25923 (disk only)
- *S. aureus* ATCC 29213(broth dilution testing only)
- *H. influenzae* ATCC 49247 (broth dilution and disk diffusion testing)
- *S. pneumoniae* ATCC 49619 (broth dilution and disk diffusion testing)
- *E. coli* ATCC 25922 (broth dilution and disk diffusion testing)
- *P. aeruginosa* ATCC 27853 (broth dilution and disk diffusion testing).

WCK 771 disk masses included:

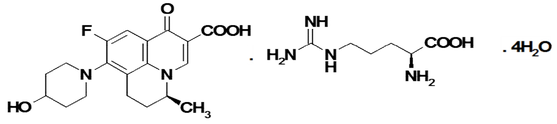
- 5 µg
- 7.5 µg
- 10 µg
- 15 µg

MIC dilution range was 0.004 – 8 µg/mL and levofloxacin disk and broth testing served as the control.

All testing followed CLSI guidelines [1, 2, 3]. Each quality control strain was tested over 20 replicates, each involving fresh inoculum preparation and inoculation of agar medium for disk diffusion testing or broth medium for microdilution testing (i.e. MIC determinations). All levofloxacin results were within CLSI QC ranges [3].

Results

Figure 1.Structure of WCK 771.



WCK 771

Table 1. Replicate Broth Microdilution MIC Results for WCK 771.

	WCK 771 MIC (µg/mL)							
	0.015	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**
S. aureus ATCC 29213		4	16					
E. coli ATCC 25922				20				
H. influenzae ATCC 49247		20						
P. aeruginosa ATCC 27853								20
S. pneumoniae ATCC 49619					20			

Figure 2. Replicate Disk Diffusion Testing Results (mm) with *S. aureus* ATCC 25923

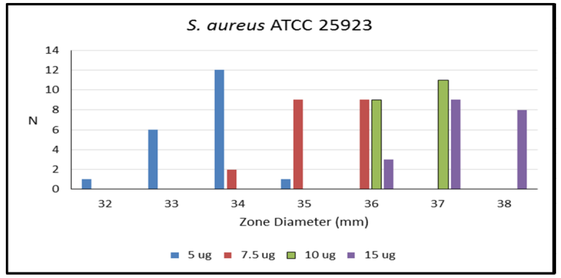


Figure 3. Replicate Disk Diffusion Testing Results (mm) with *E. coli* ATCC 25922

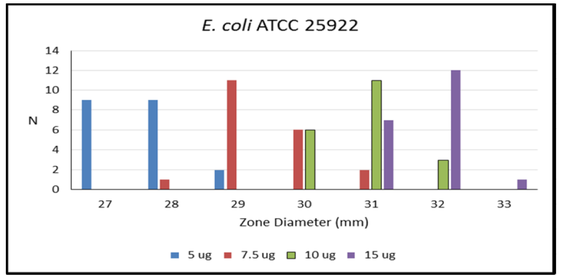


Figure 4. Replicate Disk Diffusion Testing Results (mm) with *P. aeruginosa* ATCC 27853

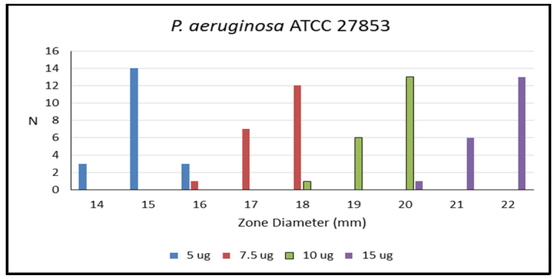


Figure 5. Replicate Disk Diffusion Testing Results (mm) with *H. influenzae* ATCC 49247

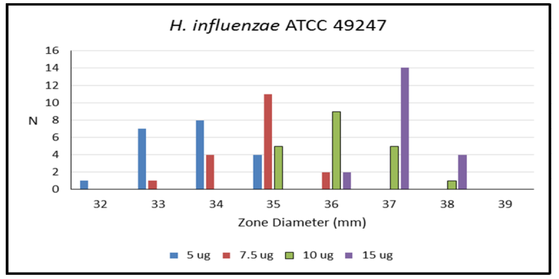
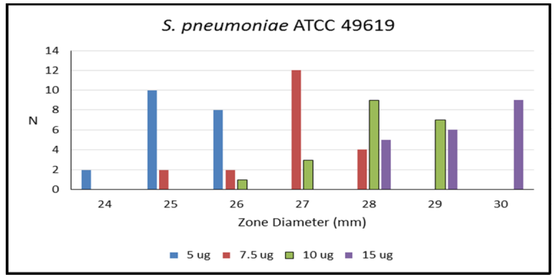


Figure 6. Replicate Disk Diffusion Testing Results (mm) with *S. pneumoniae* ATCC 49619



Conclusions

- Although the disk and MIC ranges for WCK 771 were narrow, the recommended QC ranges were expanded to include one mm or µg /mL dilution above and below the highest and lowest values observed, respectively.
- Based on the replicate testing results with the 10 µg disk, the following Tier 1 QC ranges for disk diffusion were recommended for use up until such time as the M23 Tier 2 QC study was completed:
  - *S. aureus* ATCC 25923: **35 - 38 mm**
  - *E. coli* ATCC 25922: **29 – 33 mm**
  - *P. aeruginosa* ATCC 27853: **17 - 21 mm**
  - *H. influenzae* ATCC 49247: **34 - 39 mm**
  - *S. pneumoniae* ATCC 49619: **25 - 30 mm**
- Based on the replicate testing results, the following Tier 1 QC ranges for broth microdilution were recommended for use up until such time as the M23 Tier 2 QC study was completed:
  - *S. aureus* ATCC 29213: **0.015 – 0.12 µg /ml**
  - *E. coli* ATCC 25922: **0.06 – 0.25 µg /ml**
  - *P. aeruginosa* ATCC 27853: **1 – 4 µg /ml**
  - *H. influenzae* ATCC 49247: **0.015 – 0.06 µg /ml**
  - *S. pneumoniae* ATCC 49619: **0.12 – 0.5 µg /ml**

References:

1. Clinical Laboratory Standards Institute (CLSI), 2012. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard -- Eleventh Edition.* CLSI document M02-A11. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
2. Clinical Laboratory Standards Institute (CLSI), 2015. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards -- Tenth Edition.* CLSI document M07-A10. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
3. Clinical and Laboratory Standards Institute (CLSI), 2015. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement.* CLSI Document M100-S25. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.