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Intermethod agreement and preliminary quality control (QC) guidelines for susceptibility testing AZD2563 by disk diffusion and MIC methods

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Objectives:
AZD2563 is a new oxazolidinone with favorable PK/PD features enabling once-daily dosing in projected clinical trails. To support the clinical susceptibility testing, method results were compared for the reference agar and broth tests, and the QC guidelines were determined for the NCCLS broth MIC and disk diffusion methods.
Methods:
All tests or methods (M2-A7 and M7-A5, 2000) and QC study designs (M23-A2, 2001) were those published by the NCCLS. Agar dilution results for AZD2563 were compared to those of broth microdilution using 120 strains including 30 strains each of *S. pneumoniae*, other streptococci, staphylococci and enterococci. QC trials utilized eight participant laboratories and linezolid (LZD) as the drug class control. QC strains *S. aureus* (SA) ATCC 25923 and 29213, *E. faecalis* (EF) ATCC 29212 and *S. pneumoniae* (SP) ATCC 49619 were tested. Recent reports [ICAAC Abstr. D-167, 2001] of LZD disk QC range (SA) problems were also addressed.
Results:
AZD2563 broth method MICs were identical to agar dilution results for 89.2% of comparisons (LZD control = 84.2%), the remaining broth results were generally lower by one two-fold dilution step. In the multicenter QC trial 240 and 480 results were generated for MIC and disk tests, respectively. AZD2563 MIC modes were: SA at 2 mg/L, EF at 2 mg/L and SP at 1 mg/L (LZD at 4, 2, and 1 mg/L). Three or four dilution ranges (>95% of results in range) were proposed. AZD 30-µg disk zone diameters were generally 2-4 mm smaller than LZD zones, proposed ranges were 6-7 mm wide for each QC strain. SA zones for LZD required a 2-4 mm reduction in the lower range limit, pending action by NCCLS (vancomycin used as control).
Conclusions:
AZD2563 susceptibility test results for NCCLS reference methods were demonstrated to be comparable. QC ranges for AZD2563 MIC and disk diffusion methods were established in preparation for clinical laboratory use of this promising, new oxazolidinone.

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Introduction

- AZD2563 is a novel oxazolidinone from a series with O- and N-linked C-5 heterocyclic side chains. AZD2563 has an antibacterial activity similar to linezolid (LZD) that includes essentially all clinically relevant Gram-positive pathogens, including resistant subpopulations.
- Early reports described AZD2563 as having potent activity against methicillin-resistant *Staphylococcus aureus* ($MIC_{50/90}$, 1/2 mg/L) and coagulase-negative staphylococci ($MIC_{50/90}$, 0.5/1 mg/L). No staphylococcal strain had an AZD2563 MIC >4 mg/L and this potency was slightly superior to LZD. For the enterococci (including vancomycin-resistant entercocci), the MIC_{90} was either 1 or 2 mg/L for a series of six different species. *Streptococcus pneumoniae* had AZD2563 $MIC_{50/90}$ results of 0.5/1 mg/L and resistances to β-lactams, macrolides and other drugs did not affect AZD2563 activity. Other more rarely tested or unusual species such as *Corynebacterium* spp. (MIC_{90}, 0.25 mg/L), *Listeria* spp. (MIC_{90}, 2 mg/L), *Micrococcus* spp. (MIC_{90}, 1 mg/L), *Bacillus* spp. (MIC_{90}, 1 mg/L), *Stomatococcus mucilaginosus* (MIC_{90}, 1 mg/L) and some Gram-positive anaerobes were inhibited at ≤4 mg/L of AZD2563.
- With this demonstrated wide-spectrum against Gram-positive (often resistant) species, the laboratories contributing susceptibility testing to the clinical trials require accurate quality control (QC) ranges for AZD2563. At a minimum, the four principal Gram-positive QC strains should be tested by methods/procedures listed in the National Committee for Clinical Laboratory Standards (NCCLS).[1] This poster summarises a preliminary multicentre trial to establish QC ranges for the disk diffusion and MIC methods as applied to AZD2563, as well as comparing agar and broth MIC results.

Methods

- A total of eight participants contributed study results to this investigation. The responsible investigator and laboratory names follow: R N Jones, MD, JMI Laboratories; C Knapp, MS, TREK Diagnostic Systems Inc.; R Rennie, PhD, University of Alberta; G Hall, PhD, The Cleveland Clinic Foundation; A Wanger, PhD, University of Texas Medical Center (Houston); T H Haugen, MD, PhD, VA Medical Center (Iowa City); D Hardy, PhD, University of Rochester (Strong Memorial Hospital).
- As specified by the NCCLS,[1] several lots of broth were used in the MIC (4) and disk (3) phases of the QC study. The organisms processed were *S. aureus* ATCC 25923 and 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. All tests were performed by standardised NCCLS methods[2,3] with control agents LZD and vancomycin. Two controls were utilised to allow a re-evaluation of the LZD disk diffusion ranges, against *S. aureus* ATCC 25923. The control MIC results, for example, had 99.2% of values within current NCCLS 2002 ranges.[4]
- Additional QC for the protocol included the determination of inoculum colony counts that were near target (5.0×10^5 CFU/mL) at 2.7×10^5 CFU/mL with a range of 1.0×10^5 to 4.8×10^5 CFU/mL.
- All replicate testing conformed to NCCLS 2001 guidelines[1] producing 320 MIC values for each organism and 420 disk diffusion zone diameters per QC strain.

Results

- The agar dilution MIC results for AZD2563 correlated with broth microdilution MIC values at 100.0% ± 1 $\log_2$ dilution (89.2% of results were identical; Table 1).

Table 1. Comparisons of broth microdilution MICs to those of the agar dilution method (NCCLS)[a] for 2 oxazolidinones tested against 120 isolates of Gram-positive cocci.

Antimicrobial/organisms	Broth/agar dilution MIC ratio ≤0.25	0.5	1	2	≥4
AZD2563					
S. pneumoniae (30)	0	0	30	0	0
Other streptococci (30)	0	13	17	0	0
Staphylococci (30)	0	0	30	0	0
Enterococci (30)	0	0	30	0	0
Total (120)	0	13[b]	107[b]	0[b]	0
LZD					
S. pneumoniae (30)	0	0	29	1	0
Other streptococci (30)	0	15	11	3	0
Staphylcocci (30)	0	0	30	0	0
Enterococci (30)	0	0	30	0	0
Total (120)	0	15[c]	101[c]	4[c]	0

[a]Testing was by reference NCCLS methods
[b]All of the AZD2563 results were within the ± 1 $\log_2$ dilution range and 89.2% were at a ratio of 1
[c]All of the control LZD results were within the ± 1 $\log_2$ dilution range and 84.2% were at a ratio of 1

- No significant variations in the AZD2563 MIC values were observed between the four lots of Mueller-Hinton broth or three lots of agar used in the QC trials (see Table 2).

Table 2. Comparison of the AZD2563 MIC distributions among four lots of Mueller-Hinton broth when testing *E. faecalis* ATCC 29212.

MIC (mg/L)	Occurrences by lot[a] A	B	C	D	Total
0.5					
1	7	29	4	8	48[b]
2	65	51	66	67	249[b]
4	8		10	5	23[b]
8					
Total	80	80	80	80	320
Mode	2	2	2	2	2
Range	3	2	3	3	3

[a]A = Difco lot #138582; B = Criterion lot #0264; C = BD lot #0174002; D = Difco lot #0325004
[b]Proposed QC range of 1 to 4 mg/L, that includes 100% of participant results

Table 3. Inter- and intralaboratory comparisons of AZD2563 MIC results versus *E. faecalis* ATCC 29212 for an eight medical centre protocol meeting (study design guidelines found in NCCLS M23-A2).[1]

MIC (mg/L)	Laboratory code (occurrences) A	B	C	D	E	F	G	H	Total
0.5									
1	22		3		3		12	8	48 (15.0)[a]
2	18	40	37	40	37	17	28	32	249 (77.8)[a]
4						23			23 (7.2)[a]
8									
Total	40	40	40	40	40	40	40	40	320
Mode	1	2	2	2	2	4	2	2	2
Range	2	1	2	1	2	2	2	2	3

[a]Proposed range (%) includes 1 to 4 mg/L (100% of reported values)

Table 4. Inter- and intralaboratory comparisons of AZD2563 MIC results versus *S. aureus* ATCC 29213 for an eight medical centre protocol meeting (study design guidelines found in NCCLS M23-A2).[1]

MIC (mg/L)	Laboratory code (occurrences) A	B	C	D	E	F	G	H	Total
0.5									
1	21		1		7		11	14	54 (16.9)[a]
2	19	40	28	38	33	21	27	23	229 (71.6)[a]
4			11	2		19	2	3	37 (11.5)[a]
8									
Total	40	40	40	40	40	40	40	40	320
Mode	1	2	2	2	2	2	2	2	2
Range	2	1	3	2	2	2	3	3	3

[a]Proposed range (%) includes 1 to 4 mg/L (100% of reported values)

Table 5. Inter- and intralaboratory comparisons of AZD2563 MIC results versus *S. pneumoniae* ATCC 49619 for an eight medical centre protocol meeting (study design guidelines found in NCCLS M23-A2).[1]

MIC (mg/L)	Laboratory code (occurrences) A	B	C	D	E	F	G	H	Total
0.12									
0.25							2		2 (0.6)[a]
0.5	14	29	19		3	25	32	18	140 (43.8)[a]
1	26	11	20	39	37	15	6	22	176 (55.0)[a]
2			1	1					2 (0.6)[a]
4									
Total	40	40	40	40	40	40	40	40	320
Mode	1	0.5	1	1	1	0.5	0.5	1	1
Range	2	2	3	2	2	2	3	2	4

[a]Proposed range (%) includes 0.25 to 2 mg/L (100% of reported values)

- MIC QC experiments established ranges for three control organisms with all participant-reported results within the proposed ranges (Tables 3 to 5).
- A four-dilution MIC range (0.25 to 2 mg/L) was required for the *S. pneumoniae* ATCC 49619 strain because of MIC occurrences of 43.8 and 55.0% at 0.5 and 1 mg/L, respectively.
- Disk diffusion QC experiments established AZD2563 zone diameter ranges (7 mm wide) for the two appropriate control organisms (Tables 6 and 7).

Table 6. Inter- and intralaboratory comparisons of AZD2563 zone diameter results versus *S. aureus* ATCC 25923 for a seven medical centre protocol meeting that met the study guidelines of the NCCLS M23-A2 document.[1]

Zone diameter (mm)	Laboratory code (occurrences) A	B	C	D	E	F	G	Total
20								0
21							1	1
22							5	5
23							5	5 (1.2)[a]
24				2	11	6	19	38 (9.1)[a]
25		9	8	2	27	25	10	81 (19.3)[a]
26	12	19	18	12	21	25	15	122 (29.0)[a]
27	26	15	16	21	1	4	3	86 (20.5)[a]
28	11	14	13	15			2	55 (13.1)[a]
29	11	3	5	8				27 (6.4)[a]
30								0
Total	60	60	60	60	60	60	60	420
Median	27	27	27	27	25	25	24.5	26
Range	4	5	5	6	4	4	8	9

[a]Proposed range (%) (median ± 0.5 average range or 26 ± 3mm = 23 to 29mm) that includes 98.6% of reported zone diameters

Table 7. Inter- and intralaboratory comparisons of AZD2563 zone diameter results versus *S. pneumoniae* ATCC 49619 for a seven medical centre protocol meeting that met the study guidelines of the NCCLS M23-A2 document.[1]

Zone diameter (mm)	Laboratory code (occurrences) A	B	C	D	E	F	G	Total
24								0
25					2			2 (0.5)[a]
26			2	1	7	11	5	26 (6.2)[a]
27		3	8	17	17	19	35	99 (23.6)[a]
28	6	16	19	27	28	23	20	139 (33.1)[a]
29	9	20	13	11	8	3		64 (15.2)[a]
30	28	20	13	4		2		67 (15.9)[a]
31	16	1	4					21 (5.0)[a]
32	1		1					2
33								0
Total	60	60	60	60	60	60	60	420
Median	30	29	29	28	28	27	27	28
Range	5	5	7	5	4	6	3	8

[a]Proposed range (%) (median ± 0.5 average range or 28 ± 3mm = 25 to 31mm) that includes 99.5% of reported zone diameters

Conclusions

- AZD2563 agar and broth dilution tests performed by the NCCLS methods produce comparable results.
- QC ranges for AZD2563 using NCCLS broth microdilution and disk diffusion methods were established (Table 8) by a seven laboratory protocol.

Table 8. Proposed QC ranges for the broth microdilution and disk diffusion susceptibility testing of AZD2563 by NCCLS methods.

Organism (ATCC number)	MIC tests (mg/L) Proposed range	% in range	Disk diffusion tests (mm) Proposed range	% in range
E. faecalis (29212)	1-4	100.0		
S. aureus (25923)[a]			23-29	98.6
S. aureus (29213)	1-4	100.0		
S. pneumoniae (49619)	0.25-2	100.0	25-31	99.5

[a]Note that re-evaluation of the LZD QC range using vancomycin as the control agent (M23-A2) indicates an adjustment to 25 to 31 mm (includes 95.7% of reported zone diameters) or 24 to 32 mm (includes 99.0% of zone diameter; data not shown)

- These AZD2563 guidelines should assist the quality assurance of susceptibility tests performed during the AZD2563 clinical trials.

References

1. National Committee for Clinical Laboratory Standards (NCCLS). Development of in vitro susceptibility testing criteria and quality control parameters, 2nd ed. Approved guideline M23-A2. Wayne, PA: NCCLS, 2001.
2. National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A5. Wayne, PA: NCCLS, 2000.
3. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard M2-A7. Wayne, PA: NCCLS, 2000.
4. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing. Document M100-S12. Wayne, PA: NCCLS, 2002.

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