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Validation of commercial dry-form panels (sensititre) for the susceptibility testing of AZD2563, a new long-acting oxazolidinone

R. Jones, T. Anderegg, D. Biedenbach, P. Rhomberg

North Liberty, USA

Objectives:
The escalating problems of resistant Gram-positive cocci has necessitated the rapid development of new molecular classes of antimicrobials. AZD2563 is a novel, long-acting oxazolidinone being prepared for clinical trials at once-daily dosing. This report summarizes susceptibility testing, dry-form (DF) panel validations compared to reference NCCLS methods.
Methods:
The susceptibility testing methods compared were Sensititre DF panels containing AZD2563 and linezolid (LZD; class control) vs. broth microdilution panels in reference frozen-form format (NCCLS, M7-A5) prepared by TREK Diagnostics (Westlake, OH). 462 strains were tested by M23-A2 guidelines using 111 *S. pneumoniae*, 105 staphylococci, 100 enterococci, 106 other streptococci and 40 selected Gram-negative organisms as oxazolidinone-resistant controls. Reproducibility was also determined (10 strains, 90 replicates).
Results:
The validation trial used ±100 strains of four Gram-positive organism groups specified by NCCLS M23-A2. For AZD2563 and LZD tests, 462 determinations showed 382 (82.7%) and 399 (86.4%) with identical MICs for DF and reference methods, respectively. All DF MICs were ±1 log 2 dilution of reference results for AZD2563 and LZD. Reproducibility tests used 10 strains tested 3× daily × 3 days (90 determinations). AZD2563 DF MIC reproducibility was 100% ± 1 log dilution analyzed within same day and between day results (87/90 and 76/90 results were identical, respectively). The same acceptable DF validation results were produced by a second laboratory, TREK Diagnostics.
Conclusions:
The commercial DF panels for AZD2563 produced by Sensititre were validated as producing reproducible results comparable to the NCCLS reference method. These panels appear acceptable for use by AZD2563 clinical investigators worldwide and to generate values identical to those of NCCLS M7-A5 procedures.

Poster No. P1045

Validation of commercial dry-form panels (Sensititre) for the susceptibility testing of AZD2563, a new long-acting oxazolidinone

R N Jones, T R Anderegg, D J Biedenbach, P R Rhomberg. The JONES Group/JMI Laboratories, North Liberty, Iowa, USA

Contact details: R N Jones
The Jones Group/JMI Laboratories
North Liberty, IA, USA
Tel: +1 319 665 3370
Fax: +1 319 665 3371
E-mail: ronald-jones@jmilabs.com

Introduction

- AZD2563 is a new oxazolidinone described as having activity against all clinically important Gram-positive cocci. When directly compared to linezolid (LZD), AZD2563 was more active *in vitro*[1] and has been predicted to have pharmacokinetic features allowing once-daily dosing.
- National Committee for Clinical Laboratory Standards (NCCLS) methodology specifies the use of frozen panels for broth microdilution MIC determinations but dry-form panels are preferred by many investigators. In preparation for continued clinical development and the use of broth microdilution MIC methods by investigator laboratories, the commercial products containing AZD2563 require structured validation.
- This investigation utilises guidelines published by the NCCLS in 2001[2] to determine equivalence and reproducibility of MIC results produced by dry-form panels compared to the broth microdilution method.

Methods

- Minimal criteria for the number of organisms processed were achieved by testing ≥100 strains of streptococci, staphylococci and enterococci, species against which AZD2563 was active. The actual numbers of organisms tested were: β-haemolytic streptococci (52 strains), viridans group streptococci (VgS) (54 strains), *Streptococcus pneumoniae* (111 strains), coagulase-negative *Staphylococcus* spp. (CoNS) (42 strains), *Staphylococcus aureus* (63 strains), *Enterococcus* spp. (100 strains), and 40 strains from various Gram-negative species used to challenge the method with oxazolidinone-resistant organisms. The Gram-negative bacilli included Enterobacteriaceae (25 strains; 12 species), *Pseudomonas aeruginosa* (5 strains), *Stenotrophomonas maltophilia* (5 strains), and *Acinetobacter* spp. (5 strains).
- The broth microdilution trays were produced by TREK Diagnostics/Sensititre (Westlake, OH, USA) as dry-form panels containing AZD2563 (dilution range 0.015 to 32 mg/L) and LZD (0.015 to 32 mg/L) in a final post-inoculum volume of 0.1 mL. Comparison reference trays were prepared in the same volume of cation-adjusted Mueller-Hinton broth and frozen at -70ºC or below until used. Testing utilised the same inoculum (5 x 10^5 CFU/mL) with the interpretation of endpoints conforming to the criteria of the NCCLS[3,4] read at organism-specific times ranging from 18 to 24 h.
- Quality control (QC) organisms recommended by the NCCLS were tested concurrently (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619). All recorded QC results for AZD2563 and the LZD comparison oxazolidinone were within published control ranges or those recommended by the manufacturer (AstraZeneca, data on file).
- In the reproducibility phase of this study, 10 strains of Gram-positive cocci were tested 3 times daily for 3 days. Two QC strains were included (ATCC 29212 and 29213) among the strains tested. Target levels of intermethod 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 model MIC, analysed within the same day and between days of testing.

Results

- For each group of Gram-positive organisms (*S. pneumoniae*, other streptococci, staphylococci, enterococci at ≥100 strains each) identical MIC values were observed by both methods for 65.0% (enterococci) to 93.7% (*S. pneumoniae*) of results (Table 1).

Table 1. Validation experiments comparing results from dry-form AZD2563 trays[a] to reference frozen-form AZD2563 tray MICs.

Organisms (no. tested)	Dry-form MIC variations in $\log_2$ dilutions				
	-2	-1	Same	+1	+2
Streprococci					
β-haemolytic (52)	0	0	50	2	0
VgS (54)	0	0	47	7	0
S. pneumoniae (111)	0	2	104	5	0
Staphylococci					
CoNS (42)	0	0	24	18	0
S. aureus (63)	0	9	52	2	0
Enterococci (100)	0	1	65	34	0
Other species (40)	0	0	40	0	0
Totals (n=462)					
AZD2563 (LZD[b])	0 (0)	12[c] (45[c])	382[c] (399[c])	68[c] (18[c])	0 (0)

[a]Sensititre trays produced by TREK Diagnostics (Westlake, OH, USA)
[b]LZD test performed as a control drug in the same class
[c]All AZD2563 results were within ± 1 log, dilution, 82.7% having the same MIC value by both tested methods, and for LZD 86.4% of results were the same

- Among the enterococci and CoNS strains, a trend toward slightly higher MIC results was noted for the dry-form method.
- Overall (462 tests), the dry-form MIC values for AZD2563 were <u>all</u> within ± 1 $\log_2$ dilution step of the reference method MIC; 82.7% of comparison MICs were identical. LZD MIC comparisons between methods (Table 1) revealed a similar level of acceptable accuracy.
- Results of the reproducibility testing are depicted in Table 2 for the AZD2563 MICs performed in dry-form commercial panels. The same AZD2563 MIC result was observed for these variation analyses from results performed on the 'same day' (96.7%) and 'between days' (84.4%).
- The LZD reproducibility results were similar (97.8 and 81.1%) and both oxazolidinones showed all reproducibility MIC values recorded within ± 1 $\log_2$ dilution of the established mode for each strain, eg acceptable by predefined study criteria.

Table 2. AZD2563 dry-form panel reproducibility results when testing 10 Gram-positive organisms (7 species) at 3 replicates daily for 3 days, or 90 total results.

Organisms (no. tested)	AZD2563 MIC variation in $\log_2$ dilutions (LZD results)					
	Within replicates on same day			Replicates between days		
	-1	Same	+1	-1	Same	+1
S. pneumoniae F377	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
S. pneumoniae F477	1 (1)	8 (8)	0 (0)	1 (1)	8 (8)	0 (0)
VgS 11-8649A	0 (0)	9 (9)	0 (0)	0 (0)	6 (6)	3 (3)
S. pyogenes 35-356A	0 (0)	9 (9)	0 (0)	3 (3)	6 (6)	0 (0)
CoNS 15-2091A	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
S. aureus 1-253A	0 (0)	9 (9)	0 (0)	3 (0)	6 (6)	0 (3)
S. aureus ATCC 29213	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
E. faecium A5138	0 (0)	9 (9)	0 (0)	0 (3)	9 (6)	0 (0)
E. faecalis A2477	2 (1)	7 (8)	0 (0)	0 (4)	5 (5)	4 (0)
E. faecalis ATCC 29212	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
Total	3 (2)	87 (88)[a]	0 (0)	7 (11)	76 (73)[b]	7 (6)

[a]Exact replicate-to-replicate reproducibility was 96.7 and 97.8% for AZD2563 and LZD, respectively. All MIC results (100.0%) were ± 1 $\log_2$ dilution step for both oxazolidinones
[b]The exact MIC was achieved between days in 84.4 and 81.1% for AZD2563 and LZD, respectively. All MIC results (100.0%) were ± 1 $\log_2$ dilution step for both oxazolidinones

Conclusions

- Accuracy and reproducibility results showed all AZD2563 MICs (100.0%) within acceptable limits, and the same result as the reference method was produced in 82.4% of intermethod test validation comparisons and 96.7% of reproducibility tests. These results confirm those reported by the manufacturer (TREK Diagnostics, Westlake, OH, USA; data on file).
- With these validation results for AZD2563 and the LZD control, clinical laboratories using this commercial product should be able to accurately and reproducibly detect oxazolidinone-resistant isolates (MIC, ≥8 mg/L), and AZD2563 can be adequately followed by *in vitro* susceptibility tests in the clinical trial phases of development.

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