

# Multisite evaluation of the daptomycin Neo-Sensitab prediffusion method against 20 *S. aureus*

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## Abstract

**Objective:** Neo-Sensitabs (NEO, Rosco Diagnostica, Copenhagen, Denmark) were developed as an alternative method to disk diffusion, providing the advantage of long-term room temperature storage. High molecular weight antimicrobial agents like daptomycin diffuse slowly in agar media, therefore, a prediffusion procedure has been established. The purpose of this study performed at 5 sites was to compare daptomycin (DAP) MIC results to NEO (prediffusion) inhibition zones sizes. **Methods:** A set of 20 *S. aureus* were tested by all sites. The strains included a range of DAP susceptible and non-susceptible strains (including 1 hGISA and 5 GISA) and 2 QC strains (*S. aureus* ATCC 29213 and ATCC 25923). DAP MIC and categorical results (S, I, R) for all strains were determined by each site by broth microdilution using dried panels (Trek Diagnostics, E. Grinstead, UK) setup and read according to EUCAST methods. All strains were tested in duplicate at each site by DAP NEO 2+18 hr prediffusion utilizing the same lot of BBL Mueller Hinton II agar. DAP NEO were placed on the non-inoculated agar for 2 hours at room temperature, subsequently removed, then held at room temperature for 18 hrs. After this prediffusion step, plates were inoculated, incubated at 35°C for 24 hrs and inhibition zones measured. Categorical results (S, I, R) were interpreted according to the manufacturer's criteria. The category agreement (CA) and error rates between the two methods (using mean MIC results from all sites) were determined. In addition, the MIC results from each of the sites were similarly compared to the mean MIC results. **Results:** The correlation of DAP NEO and DAP MIC results by site to mean DAP MIC results are shown in the table. Similar variation was seen between site DAP MICs to DAP mean MICs (%CA 60-95%, %VM 0-10.5%, %MA 0% at 4 sites and 40% at 1 site).

Table: Categorical agreement and error rates of DAP NEO compared to mean DAP MIC

Site No.	N	Error Rates			
		%CA	%VM	%MA	%MI
1	40	80.0	7.5	7.5	5.0
2	38	86.8	2.6	2.6	7.9
3	40	90.0	0	10.0	0
4	40	75.0	17.5	0	7.5
5	60	83.3	5.0	6.7	5.0

CA – category agreement, VM – very major, MA – major

**Conclusion:** Mean category agreement of DAP NEO to DAP mean MICs was 83.0%. The mean category agreement of site DAP MICs compared to DAP mean MICs was 84.9%. The result of this study are promising given the use of a single MIC breakpoint and the use of a biased small set of unique and infrequently isolated strains (80% with DAP MICs >0.5 mg/L). Additional testing with a larger set of strains with a typical DAP MIC distribution (MIC<sub>90</sub> = 0.5 mg/L) is warranted.

## Methods

**Isolates:** A set of 20 *S. aureus* were tested by all sites. The strains were a challenge set which included daptomycin susceptible and non-susceptible strains, the majority of which had MICs at 1 and 2 (near the breakpoint) and included 1 hGISA and 5 GISA. In addition, 2 quality control strains were tested (DAP MIC: *S. aureus* ATCC 29213, DAP NEO: *S. aureus* ATCC 25923).

**Testing Sites:** As listed in Figure 1

**MIC Method:** Each organism was tested one time by broth microdilution by each site according to EUCAST method (with exception of using Sensititre dried panels [Trek Diagnostics, E. Grinstead, UK]). Mean MICs were calculated and rounded to closest doubling dilution for comparison to the NeoSensitab results.

**NeoSensitab Prediffusion Method:**

Each strain was tested in duplicate by sites 1-4 and in triplicate by site 5 using a 2+18 hour prediffusion method with DAPCa Neo-Sensitabs (daptomycin 30 mcg +calcium 100 mcg, catalog number 80312N, Rosco Diagnostica, Copenhagen, Denmark). Mueller Hinton Agar (MHA) plates were made by each site using the same lot of BBL Mueller Hinton II agar (Becton Dickinson, Sparks, MD, catalog number 211438 – Lot #0130488).

- DAP NEO were placed on the non-inoculated agar for 2 hours at room temperature, subsequently removed, then held at room temperature for 18 hrs.

- MHA plates were inoculated (similar to disk diffusion procedure) by preparing a suspension equivalent in turbidity to a 0.5 McFarland standard in saline (from 3-5 morphologically similar colonies from a 18-24 h non-selective agar plate).

- Plates were incubated at 35°C for 24 hrs and inhibition zones measured.

- Susceptible and intermediate categorical results were based on the manufacturer's recommendations of ≥22 mm and 21, respectively.

Figure 1 (A-E): Daptomycin Mean Broth Microdilution MIC vs. NeoSensitab 2+18hr Diffusion Zone Size

Figure 1A: Site 1, Laboratory Specialists, Inc., n=40

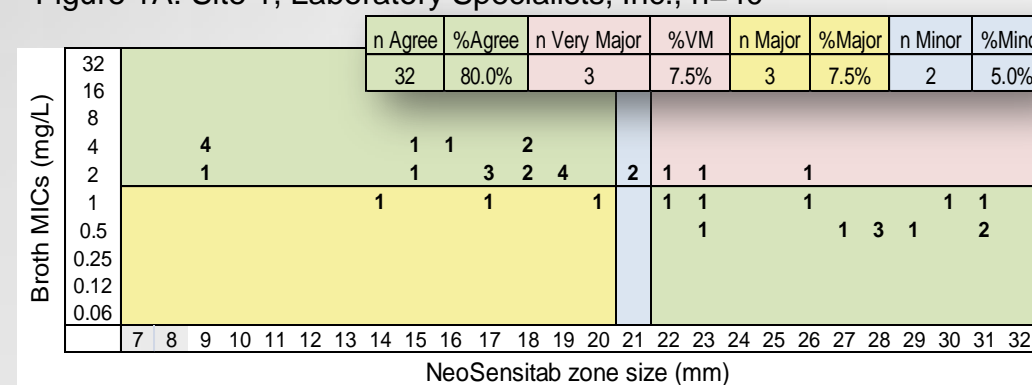


Figure 1B: Site 2, Statens Serum Institute, n=38

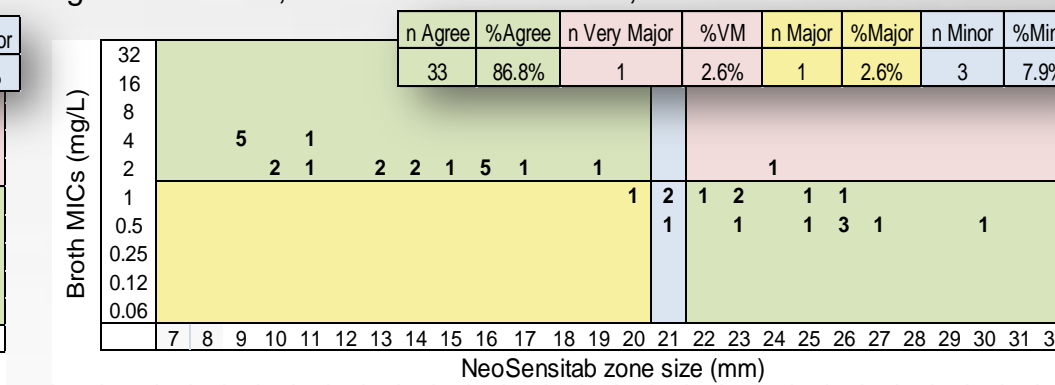


Figure 1C: Site 3, Cubist Pharmaceuticals, n=40

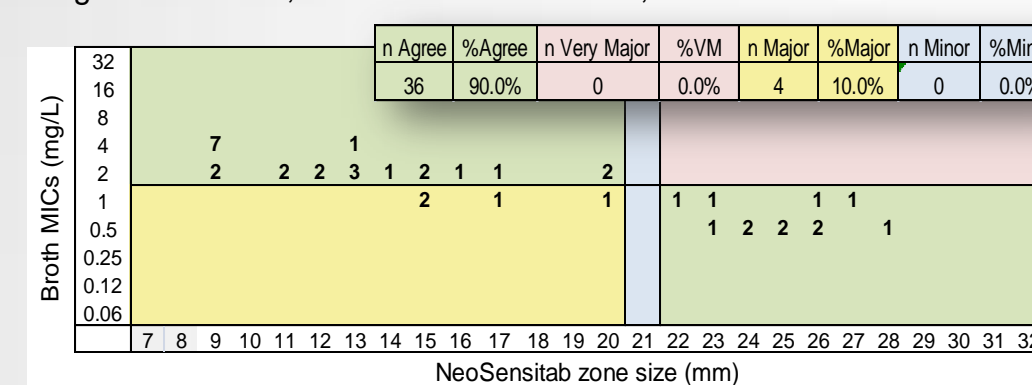


Figure 1D: Site 4, Quotient Bioresearch, n=40

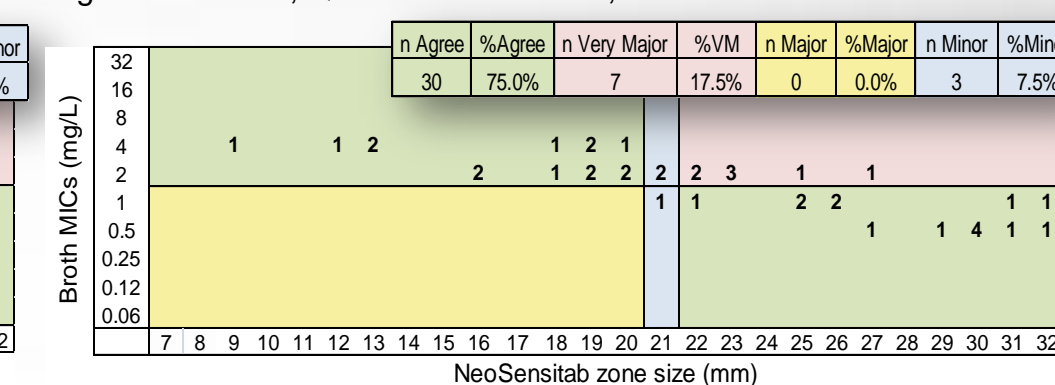


Figure 1E: Site 5, Novartis, n=60

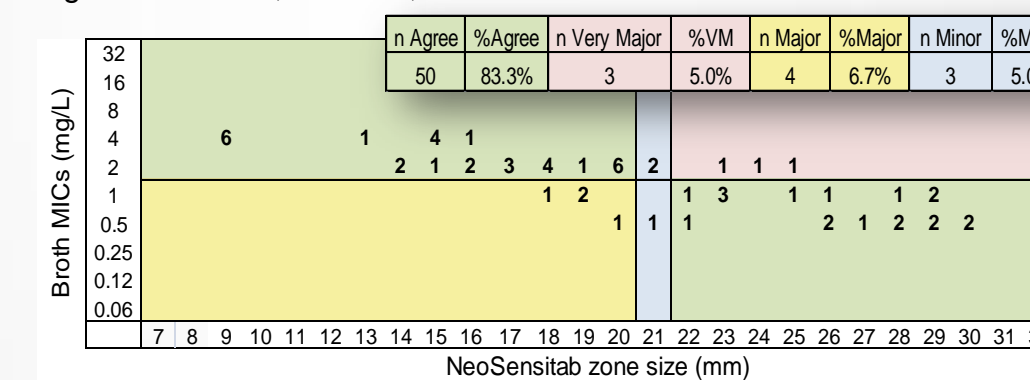


Table 1: Daptomycin MIC Results (mg/L) and NeoSensitab Results (mm) by Site and Mean

Isolate No.	MIC (mcg/mL)					No. of 2-fold Dilutions in Range	Mean MIC	Mean Zone	NeoSensitab Zone (mm)					No. of mm in Range		
	Site 1	Site 2	Site 3	Site 4	Site 5				Site 1	Site 2	Site 3	Site 4	Site 5			
12	2	4	4	4	4	2-4	2	4	13.26	15,18	9,11	9,9	18,19	15,15,13	9-19	11
13	4	NA	2	4	8	2-8	3	4	9.77	9,9	NT	9,9	12,13	9,9,9	9-13	5
18	2	4	4	4	8	4-8	2	4	9.34	9,9	9,9	9,9	9,13	9,9,9	9-13	5
19	2	4	2	4	4	2-4	2	4	13.84	16,18	9,9	9,13	19,20	15,16,15	9-20	12
4	1	2	2	1	2	1-2	2	2	22.07	22,26	16,19	20,20	25,27	24,25,23	16-27	12
6	1	2	2	2	2	1-2	2	2	19.07	21,23	16,24	12,16	22,23	18,20,18	12-24	13
8	1	2	2	2	4	1-4	3	2	16.31	17,18	16,16	11,12	21,22	16,18,16	11-22	12
9	2	2	2	2	4	2-4	3	2	17.40	18,19	15,16	15,15	18,20	18,21,20	15-21	7
10	1	2	2	2	4	1-4	3	2	16.22	19,19	11,17	9,11	19,23	20,21,20	9-23	15
11	1	4	2	2	4	1-4	3	2	17.30	17,21	13,14	13,17	19,23	19,20,20	13-23	11
17	2	2	2	4	4	2-4	2	2	15.11	9,19	13,14	13,14	20,21	15,17,17	9-21	13
20	1	2	2	2	4	1-4	3	2	13.10	15,17	10,10	9,13	16,16	14,14,16	9-17	9
1	0.5	1	2	0.5	2, 1	0.5-2	3	1	28.40	30,31	25,26	26,27	31,32	28,29,29	25-32	8
5	1	2	1	1	2	1-2	2	1	22.83	20,23	20,21	22,23	26,26	23,25,22	20-26	7
7	0.5	2	1	1	2	0.5-2	3	1	22.34	22,26	21,23	15,20	25,25	23,26,23	15-26	12
16	1	1	0.5	1	2	0.5-2	3	1	18.54	14,17	22,23	15,17	21,22	18,19,19	14-23	10
2	0.5	1	0.5	0.5	1, 1	0.5-1	2	0.5	28.34	28,31	23,30	25,26	30,32	29,30,28	23-32	10
3	0.5	0.5	0.5	0.5	1, 1	0.5-1	2	0.5	27.98	28,31	26,26	25,28	30,31	26,30,29	25-31	7
14	0.5	1	0.5	1	2	0.5-2	3	0.5	24.20	23,29	21,25	23,26	27,29	20,21,22	20-29	10
15	0.5	0.5	0.5	0.5	2	0.5-2	2	0.5	26.98	27,28	26,27	24,24	30,30	27,28,26	24-30	7
QC*	0.5	0.5	0.5	0.5	1	0.5-1	2	0.50	26.56	30, 31	22, 24	22,25,27	25, 29	27x4,28x3	22-31	10

## Results

All daptomycin MIC and Neo-Sensitab (NEO) results by site and mean values are shown in Table 1.

Scatter plots of replicate NEO zone results (mm) versus mean MIC results (mg/L) are shown in Figures 1A-1E.

**MIC Results:**

- The average category agreement of site MICs compared to the mean MIC was 84.9% (range of 70-95%). The essential agreement of site MICs to mean MICs (within +/- 1 dilution) was 100% for all sites.
- 4 strains were non-susceptible with mean MICs of 4 mg/L and all MICs were considered non-susceptible (within a 2-8 mg/L range).
- Borderline mean MICs (1 or 2 mg/L) were obtained for 12 strains. The mean MIC for 4 strains was 1 mg/L (susceptible) and 35% of all MICs (7/20) were 2 mg/L (non-susceptible). The mean MIC for 8 strains was 2 mg/L (non-susceptible) and 15% of all MICs (6/40) were 1 mg/L (susceptible).

**Mean MIC results compared to NEO results:**

- Category agreement of mean MIC to NEO ranged from 75 to 90% based on site specific plots. The average category agreement rates was 83.0%.
- Among 4 strains with mean MICs of 4 mg/L, all tested as resistant by NEO (no very major errors). Among 8 strains with mean MICs of 2 mg/L, 15.9% (14/88) had zones of >21 mm (susceptible, very major error) and 6.8% (6/88) had zones of 21 mm (intermediate, minor error).
- Among 4 strains with mean MICs of 1 mg/L, 33.3% (12/36) had zones of <21 (resistant, major error) and 8.3% (3/36) had zones of 21 mm (intermediate, minor error). Among 4 strains with mean MICs of 0.5 mg/L, 2.8% (1/36) had MICs of <21 (resistant, major error) and 5.6% (2/36) had MICs of 21 mm (intermediate, minor error).

## Conclusions

- A variation in replicate MIC values of ± doubling dilution is typical in broth microdilution testing. The strains in this study were selected to include a large percentage with borderline susceptible/non-susceptible MICs and without an intermediate category, the lower than optimal (<90%) category agreement rates (both for the intra-laboratory MIC comparison and for the MIC/NEO comparison) are not surprising.
- Overall, the DAPCa Neo-Sensitab pre-diffusion method performed well against this challenge set, although difference between laboratories were noted. Additional multi-site testing to evaluate potential source of lab to lab variation and testing with a larger and typical set of strains is warranted.
- A routine disk diffusion method is not available for daptomycin and the pre-diffusion Neo-Sensitab method provides a cost effective way to test for daptomycin susceptibility against *S. aureus*

## References

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## Acknowledgement

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