Antimicrobial Susceptibility Testing

In keeping up with the most current testing methodologies, the WVDL primarily uses the broth microdilution method for antimicrobial susceptibility testing, although the Kirby Bauer method is still employed when needed. In this document, we attempt to answer many of the frequently asked questions regarding antimicrobial susceptibility testing. Of particular importance is the concept of efficacy ratios. Efficacy ratios can be used to calculate which antimicrobial drug has the highest predicted efficacy or activity against a given bacterial isolate. It is our hope that this document will be useful to our clients, the practicing veterinarians.

- **What is the broth microdilution method?** The broth microdilution method is a liquid culture method whereby a standard amount of bacteria are inoculated into the wells of a 96 well micro-titer plate that contain different dilutions of antimicrobial drugs. For example in the standard bovine/porcine panel (see image below of BOPO7F layout; Trek Diagnostic Systems), four wells contain the antibiotic ceftiofur (XNL) with dilutions of 8, 4, 2, and 1 µg/ml and four wells contain spectinomycin (SPE) with dilutions of 64, 32, 16 and 8 µg/ml, respectively. After 18-24 hours, the plates are examined either visually for evidence of bacterial growth. Results are recorded as minimum inhibitory concentrations (MIC) and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs.

- **What is the Kirby Bauer disk diffusion test?** The Kirby Bauer test is a qualitative assay whereby disks of paper are impregnated with a single concentration of different antibiotics. The disks are placed on the surface of an agar plate that has been inoculated with test bacteria. During incubation, the antibiotics diffuse outward from the disks creating a concentration gradient. After 18-24 hours, the zone diameter (zone of inhibition) is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs.

- **What is the MIC?** The MIC is the highest dilution (lowest concentration) of antimicrobial drug that completely inhibits bacterial growth. If available, the MIC value is reported with interpretation guidelines (S, I, R) that have been established by the Clinical and Laboratory Standards Institute (CLSI). Occasionally, other interpretation guidelines are reported. For example, when no interpretive guidelines have been established for the specific bacteria/drug/animal species combination being tested, the MIC result may be reported with 'No Interpretation' (typically abbreviated as NI, or NA or NM). CLSI guidelines change periodically in response to growing research, and this may result in new interpretations. The WVDL works diligently to stay current with those changes.

- **What are break-points?** Breakpoints are the MIC values used as cutoffs for each interpretation category established by CLSI. These breakpoints, and their associated interpretations, are specific to each organism-drug-animal species combination. For example, in a case of bovine pneumonia involving *Pasteurella multocida,* the break-points for ceftiofur are ≤ 2 (S), 4 (I) and ≥ 8 (R) µg/ml.

- **What are efficacy ratios?** Efficacy ratios (ER) are calculated by taking the resistant break-point MIC value and dividing it by the MIC value obtained as a result of susceptibility testing using the broth microdilution method. It is a tool that can be used to evaluate the relative efficacy of different antimicrobial drugs. For example, recently the WVDL obtained the following MIC results from an isolate of *Mannheimia haemolytica* from a bovine sample.

Drug	Test Result MIC (µg/ml)	Interpretation	Resistant Break-point MIC (µg/ml)	Efficacy Ratio (ER)
Ceftiofur	0.5	S	≥ 8	16
Florfenicol	0.25	S	≥ 8	32
Spectinomycin	16	S	≥ 128	8
Gamithromycin	2	S	≥ 16	8
Tulathromycin	8	S	≥ 64	8

In this example, the ER for ceftiofur is 16. This was calculated by taking the resistant break-point MIC for ceftiofur (8 µg/ml) and dividing it by the measured MIC of 0.5 µg/ml. Efficacy ratios for all other drugs were calculated in the same way.

Given these results, even though this isolate is "Susceptible" to all the drugs listed above, florfenicol has the greatest predicted efficacy, based on an ER of 32. It is important to remember that several factors influence the decision of which antimicrobial drug should be used for any particular case, and the final decision ultimately lies with the practicing veterinarian.

*Data from CLSI (formally NCCLS) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals

thermoscientific

SENSITITRE™ BOVINE/PORCINE PLATE FORMAT

Plate Code: **BOPO7F**   Plate Type: **MIC**

	1	2	3	4	5	6	7	8	9	10	11	12
A	PEN 0.12	PEN 0.25	PEN 0.5	PEN 1	PEN 2	PEN 4	PEN 8	TET 0.5	TET 1	TET 2	TET 4	TET 8
B	AMP 0.25	AMP 0.5	AMP 1	AMP 2	AMP 4	AMP 8	AMP 16	GEN 1	GEN 2	GEN 4	GEN 8	GEN 16
C	TIA 0.5	TIA 1	TIA 2	TIA 4	TIA 8	TIA 16	TIA 32	TIP 1	TIP 2	TIP 4	TIP 8	TIP 16
D	TYLT 0.5	TYLT 1	TYLT 2	TYLT 4	TYLT 8	TYLT 16	TYLT 32	TIL 2	TIL 4	TIL 8	TIL 16	NEO 4
E	NEO 8	NEO 16	NEO 32	TUL 8	TUL 16	TUL 32	TUL 64	ENRO 0.12	ENRO 0.25	ENRO 0.5	ENRO 1	ENRO 2
F	CLI 0.25	CLI 0.5	CLI 1	CLI 2	CLI 4	CLI 8	CLI 16	DANO 0.12	DANO 0.25	DANO 0.5	DANO 1	POS
G	XNL 0.25	XNL 0.5	XNL 1	XNL 2	XNL 4	XNL 8	GAM 1	GAM 2	GAM 4	GAM 8	SDM 256	POS
H	FFN 0.25	FFN 0.5	FFN 1	FFN 2	FFN 4	FFN 8	SPE 8	SPE 16	SPE 32	SPE 64	SXT 2/38	POS

ANTIMICROBICS

AMP	Ampicillin
CLI	Clindamycin
DANO	Danofloxacin
ENRO	Enrofloxacin
FFN	Florfenicol
GAM	Gamithromycin
GEN	Gentamicin
NEO	Neomycin
PEN	Penicillin
POS	Positive Control
SDM	Sulphadimethoxine
SPE	Spectinomycin
SXT	Trimethoprim / sulfamethoxazole
TET	Tetracycline
TIA	Tiamulin
TIL	Tilmicosin
TIP	Tildipirosin
TUL	Tulathromycin
TYLT	Tylosin tartrate
XNL	Ceftiofur