Summary Minutes
Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Grand Hyatt Tampa Bay
Tampa, Florida
24-25 January 2008

A meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing was held on 24-25 January 2008, at the Grand Hyatt Tampa Bay in Tampa, Florida. The following were in attendance:

Jeffrey L. Watts, PhD, RM(AAM) **Chairholder**	**Pfizer Animal Health**
Mark G. Papich, DVM, MS **Vice Chairholder**	**North Carolina State University**

Members Present

Donald J. Bade	Microbial Research, Inc.
Steven D. Brown, PhD	The Clinical Microbiology Institute
Virginia R. Fajt, DVM, PhD, DACVCP	Texas A & M University
Henry Heine, PhD	USAMRIID
Rob P. Hunter, MS, PhD	Elanco Animal Health
Dik J. Mevius, DVM, PhD	Central Veterinary Institute
Peter Silley, PhD	MB Consult Limited
Robert D. Walker, PhD	Anti-Infectives Research Consultants, LLC
Ching Ching Wu, DVM, PhD	Purdue University School of Veterinary Medicine
Gary E. Zurenko, MS	Micromyx, LLC

Advisors Present

Jo Abraham, DVM, MS	Bayer HealthCare LLC
Mike Apley, DVM, PhD	Kansas State University
Melanie R. Berson, DVM	FDA Center for Veterinary Medicine
Cindy Lindeman	Pfizer Animal Health
Patrick McDermott, PhD	FDA Center for Veterinary Medicine
Stefan Schwarz, DVM	Institut Farm Animal Genetics, FLI
Thomas R. Shryock, PhD	Elanco Animal Health
Clyde Thornsberry, PhD	Eurofins Medinet
John D. Turnidge, MD	Women's and Children's Hospital

Observers Present

Dawn Merton Boothe, DVM, PhD	Auburn University
Chander Celly	Schering Plough Corporation
Thomas R. Fritsche, PhD, MD	JMI Laboratories
Joshua Hayes, PhD	FDA, Center for Veterinary Medicine
Diana Murphy Jordan, DVM, MS, PhD	Veterinary Diagnostic Laboratory
Scott B. Killian, BS	Trek Diagnostic Systems
Laura M. Koeth, MT (ASCP)	Laboratory Specialists, Inc.
Cynthia C. Knapp, MS	Trek Diagnostic Systems
Jennifer Lorbach	Trek Diangostic Systems
Brian Lubbers, DVM	Kansas State Veterinary Diagnostic Laboratory
Anthony E. Maltese, MS	Neogen
Maureen Mansfield	Trek Diagnostic Systems
Ian Morrissei	GR Micro LTD
Markus Rose	Intervet Innovation
Dr. Shabbir Simjee	Elanco Animal Health
Bernd Stephan	Bayer HealthCare AG
Michael Sweeney	Pfizer Animal Health
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute
Hans-Otto Werling	Bayer HealthCare AG
Cornelia Wilhelm	Intervet Innovation GMGH

CLSI Staff Present

Tracy A. Dooley, BS, MLT (ASCP)
Helen Gallagher
Ron Quicho

Opening Remarks

Dr. Watts began the meeting Thursday, 24 January at 8:30 a.m. In reporting changes to the subcommittee, Dr. Watts announced the appointment of Dr. Mark Papich as Vice Chairholder. He then welcomed Dr. Virginia Fajt as a new voting member of the subcommittee.

He stated that the purpose of Thursday's session was to provide an opportunity for the working groups to address their agenda topics and obtain input from the subcommittee. Informational presentations on surveillance programs and studies being conducted would also be reviewed.

Sponsor presentations and working group reports would be presented to the full subcommittee during Friday’s session.

Minutes of Prior Meeting

The subcommittee voted to approve the summary minutes from the 18-19 January 2007 meeting held in Tampa, Florida (**Approved 10-0).**

Update on CLSI Publications

Ms. Dooley provided a brief update of recent and upcoming publications within the Area Committee on Microbiology as follows:

Recently Published Documents

M50-P, *Quality Control for Commercial Microbial Identification Systems; Proposed Guideline* – December 2007

M100-S18, *Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement* – January 2008

Upcoming Publications

M31-A3, *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Third Edition*. Estimated for publication in late February 2008.

M37-A3, Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents*;* Approved Guideline – Third Edition. Estimated for publication in late February 2008.

M48-A, *Laboratory Diagnosis of Mycobacterial Infections; Approved Guideline.* Estimated for publication in late March 2008

Overview of GermVet Surveillance Program

Dr. Stefan Schwarz provided an overview of the results of the BfT-GermVet study conducted by the Federal Research Centre for Agriculture (FAL), the Free University of Berlin (FU), and the Ludwig-Maximillians Univeristy Munich (LMU) and was logistically supported by the Federal Office of Consumer Protection and Food Saftey (BVL). Prior to 2001 there had been no national resistance monitoring program in place in Germany for bacteria of animal origin. In 2001, the GE*RM*-Vet study, conducted by the BVL, was initiated to obtain representative and valid data on the susceptibility of bacterial pathogens of animals against antimicrobial agents approved for animal use. GE*RM*-Vet represents the first national monitoring program in the veterinary field in Germany. This program focuse on the most relevant bacteria from food-producing animals.

The Bft-Germ Vet study represents a complementary program to GE*RM*-Vet and monitored during a 27-month sampling period 31 pathogen/disease combinations mainly from horses, dogs and cats but also certain combinations from cattle and swine not covered by GERM-Vet. The quality criteria for the study included:

- Isolate must originate from acutely ill animals.
- Animals must not have been treated with antimicrobial agents in the 4 weeks prior to probe sampling.
- Maximum 2 isolates per animal herd.
- Well–balanced geographical distribution.

Susceptibility testing was performed by broth microdilution following CLSI document M31-A2. A total of 1632 bacterial isolates were tested with <80-100 isolates from various pathogen/disease combinations.

Results for Staphylococci:

Swine:	Urinary/genital tract (incl. MMA) (n = 46)
	Skin (n = 44)
Dog / Cat:	Respiratory tract (n = 57)
	Skin/ear/mouth (n = 101)

Most frequent resistances:

Penicillin G	(53 – 77%)
Tetracycline	(33 – 52%)
Erythromycin	(13 – 27%)
Sulfamethoxazole	(2 – 30%)
Chloramphenicol	(4 – 22%)
Trimethoprim/Sulfa	(2 – 13%)

In total: 7 Oxacillin-resistant staphylococci (2.8%)

Methicillin-resistant Staphylococci:

S. intermedius: 2 Strains of canine origin
[skin – 1; respiratory tract – 1]

↓

S. pseudintermedius

S. aureus: 5 Strains of porcine origin
[skin – 2; urinary/genital tract – 2, MMA syndrome – 1]

Methicillin-resistant *S. aureus* from swine:

- *spa*-Types t011 and t034 are among those *spa*-types most frequently associated with MLST-type 398
- None of the 5 MRSA strains was typeable by *Sma*I macrorestriction analysis (PFGE)

Hints towards (a) the presence of ST398 strains from swine in Germany and (b) the involvement of these strains in acute disease conditions of swine.

Results for Streptococci:

Swine:	Urinary/genital tract incl. MMA (n = 54)
	CNS / joints (n = 77)
Horse:	Respiratory tract (n = 77)
	Genital tract (n = 102)
Dog / Cat:	Respiratory tract (n = 21)

Urinary/genital tract (n = 90)
Skin/ear/mouth (n = 79)

Most frequent resistances:
Sulfamethoxazole (20 – 78%)
Tetracycline (17 – 93%)
Gentamicin (14 – 79%)
Erythromycin (0 – 33%)

In total: 1 Borderline penicillin-resistant (0.2%) and 2 borderline ceftiofur-resistant (0.4%) streptococci.

Results for *P. multocida:*

Dog / Cat: Respiratory tract (n = 72)
Skin / ear / mouth (n = 20)
Sole resistance: Sulfamethoxazole (43 – 45%)

Results for *B. bronchiseptica*:

Dog / Cat:Respiratory tract (n = 42)
Most frequent resistances: Cefazolin (100%)
Sulfamethoxazole (81%)
Trimethoprim/Sulfa (81%)

In conclusion, representative and valid data on the *in-vitro* susceptibility of bacterial pathogens of animal origin obtained from various disease conditions in Germany were determined as a joint effort. All data from Bft-GermVet and part of the data from the GE*R*M-Vet studies have been published in a special issue of the Berliner Und Műnchener Tierärztliche Wochenschrift in September/October 2007. Dr. Schwarz has a few of these issues available for those interested in a copy please e-mail him.

Presentation of Amoxicillin Breakpoints Developed by the German Antibiotic Working Group

Dr. Stefan Schwarz provided an overview of the results of a study conducted by the Working Group "Antibiotic Resistance" of the German Veterinary Medical Society to propose amoxicillin breakpoints for porcine respiratory pagthogens.

The objectives of the Working Group "Antibiotic Resistance" were:

- To harmonize and standardize susceptibility testing in routine diagnostic laboratories by: 1) advocating use of broth microdilution testing; 2) develop M31-A2 based SOP's in German; 3) conducting workshops for laboratory staff to learn broth microdilution testing; 4) conducting ring trials on an almost yearly basis; and 5) developing science-based panels for microtiter plates (mastitis layout for dairy cattle, food-producing animal layout for cattle and swine, and a pet and companion animal layout for dogs and cats).

- Derive veterinary-specific clinical breakpoints: A first attempt was made for amoxicillin and porcine respiratory pathogens based on:

– Known pharmacological data of amoxicillin in swine – there are several studies available in the published literature, but there are differences in the dosages used, in the routes of administration, in the health status of the animals, and not all studies determined the same parameters by the same methodology

– PK/PD considerations:

  • Most respiratory tract pathogens of clinical interest are located extracellularly / in the interstitial fluid
  • If there is no barrier to impede drug diffusion, plasma concentration of free (unbound) antibiotic approximates its free concentration in the interstitial fluid (Craig, 1995)
  • Amoxicillin has low protein binding
  • Penetration of amoxicillin from plasma into bronchial fluid and lung tissue were investigated by Agerso and Friis (1997,1998) - Amoxicillin plasma concentrations are predictive of the concentration in the lung (interstitial fluid)

  Conclusions of the pharmacology of amoxicillin in swine:

    • The predicted tissue concentration in the lung after parenteral administration of amoxicillin at the recommended dose of 10 mg/kg (7.5 – 15 mg/kg) amounts to 0.5 – 1.0 µg/mL for up to 12 hours, depending on the formulation
    • To reach therapeutically relevant serum levels of 0.5 – 1 µg/mL, oral doses of at least 10 mg/kg every 12 hours are required

– Published results of clinical efficacy studies

  • Very few studies available, with most of them giving no information on the MIC of the infecting strain (Tanigawa & Sawada 2003 - at a dose of 15 mg/kg i.m.: clinical cure of an infecting *A. pleuropneumoniae* strain with an MIC of 0.39 µg/mL).

  • Assuming that an amoxicillin concentration of 0.5 – 1.0 µg/mL in the lungs can be achieved by regular dosing, this observation suggests that bacteria with an MIC of 0.4 µg/mL are reliably killed and eliminated from the lungs.

– Available susceptibility data of porcine respiratory tract pathogens

    • Most *A. pleuropneumoniae*, *S. suis*, and *P. multocida* strains (> 93%) exhibited low MIC values of amoxicillin of ≤ 0.5 µg/ml
    • For *H. parasuis*, striking differences in the MICs were seen with regard to the geographic origin of the strains - more data are needed
    • Amoxicillin is largely ineffective against *B. bronchiseptica*
    • *Mycoplasma hyopneumoniae* and other mycoplasmas are intrinsically resistant to β-lactams.

Overall conclusions:

- The predicted tissue concentration in the lung after parenteral administration of amoxicillin at the recommended dose of 10 mg/kg (7.5 – 15 mg/kg) amounts to 0.5 – 1.0 µg/mL
- An oral dose of 10 mg/kg every 12 h is required to achieve the same plasma concentrations of 0.5 – 1.0 µg/mL
- Bacterial cure was observed when pigs were treated with the recommended dose in an experimental infection with *A. pleuropneumoniae*
- The majority of pathogens associated with porcine respiratory disease are susceptible at MICs of ≤ 0.5 µg/mL

Suggested amoxicillin breakpoints for SRD pathogens – S ≤0.5, I 1, R ≥2 with comment - Breakpoint derived from microbiological, pharmacokinetic (using accepted clinical doses) and pharmacodynamic data. For swine, the dose of ampicillin modeled was 10 mg/kg IM q24 or 10 mg/kg every 12 h orally.

Path Forward – The Generic Working Group will review the data and references in greater detail and come back to the subcommittee with a comprehensive breakpoint presentation to include histograms and Monte Carlo simulations.

EUCAST Update

Dr. Shabbir Simjee provided an overview on setting clinical breakpoints in the EU. EUCAST, formed in 1996 and restructured in 2002. Professor Gunnar Kahlmeter is the current Chairman for EUCAST.

EUCAST's approach to setting clinical breakpoints and epidemiological cut-off values - EUCAST has re-defined susceptible, intermediate and resistant and defined the terms 'wild type' and 'non-wild type' organisms. The national breakpoint committees have also agreed on a common format for susceptible (S≤) and resistant (R>).

EUCAST definition of clinical breakpoints:

Clinically Susceptible (S)
- a microorganism is defined as susceptible if inhibited *in-vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic success
- a microorganism is categorized as susceptible (S) by applying the appropriate breakpoint in a defined phenotypic test system.

Clinically Intermediate (I)
- a microorganism is defined as intermediate by a level of antimicrobial agent activity associated with uncertain therapeutic effect.

  - It implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physically concentrated or when a high dosage of drug can be used; it also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.

- a microorganism is **categorized** as intermediate (I) by applying the appropriate breakpoints in a defined phenotypic test system

Clinically Resistant (R)

- a microorganism is defined as resistant if inhibited *in-vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure.
- a microorganism is categorized as resistant (R) by applying the appropriate breakpoint in a defined phenotypic test system.

Clinical breakpoints may be altered with legitimate changes in circumstances.
Clinical breakpoints are presented as S≤x mg/L; I>x, ≤y mg/L; R>y mg/L.

EUCAST definition of epidemiological cut-off values:

Wild type (WT)

- a microorganism is defined as wild type (WT) for a species by the absence of acquired and mutational resistance mechanisms to the drug in question.
- a microorganism is categorized as wild type (WT) for a species by applying the appropriate cut-off value in a defined phenotypic test system.
- wild type microorganisms may or may not respond clinically to antimicrobial treatment.

Microbiological resistance - non-wild type (NWT)

- a microorganism is defined as non-wild type (NWT) for a species by the presence of an acquired or mutational resistance mechanism to the drug in question.
- a microorganism is categorized as non-wild type (NWT) for a species by applying the appropriate cut-off value in a defined phenotypic test system.
- non-wild type microorganisms may or may not respond clinically to antimicrobial treatment. Epidemiological cut-off values will not be altered by changing circumstances. The wild type is presented as WT≤z mg/L and non-wild type as NWT >z mg/L.

The committees under EUCAST are proceeding in setting breakpoints by:

- Collecting and tabulating information from all national breakpoint committees on dosing, current breakpoints, available formulations, and clinical indications.
- Collecting MIC distributions to define epidemiological cut-off values.
- Comparing existing national clinical breakpoints.
- Reviewing PK/PD data and Monte Carlo simulations, then define a PK/PD breakpoint.
- Checking PK/PD breakpoints against target species wild type MIC distributions to avoid splitting the wild type to obtain tentative breakpoints.

Once tentative breakpoints are agreed upon, a rationale document is prepared and published on the EUCAST website.

EMEA SOP for setting breakpoints through EUCAST - between 2004 and 2005, a formal relationship was developed between EUCAST and EMEA/CHMP for setting breakpoints. EUCAST reviews submitted data and makes recommendations, publishing breakpoints independently. The Committee for Medicinal Products for Human Use (CHMP) reserves the right to disagree with EUCAST and has final say as to indications.

Small Animal Antimicrobial *E. coli* Surveillance Study

Dr. Dawn Boothe from Auburn College of Veterinary Medicine gave an overview of a surveillance study to be conducted that will evaluate samples of *Escherichia coli* collected from dogs and cats and document patterns of antimicrobial resistance and susceptibility over a three-year period. Patterns of resistance will be correlated with antimicrobial use with the data used to improve guidelines for antimicrobial drug use to help inhibit resistance to these drugs. Testing will be done by Etest.

Input from the subcommittee regarding the study to be conducted included:

- Test all QC organisms to make sure in range with CLSI ranges since using Etest (drop *E. faecalis* and test *E. coli* ATCC 35218 and K. pneumoniae ATCC 700603 for ESBL detection).
- Suggest including cefoxitin.
- Demonstrate correlation of Etest with microdilution method (possibly obtain data from AB Biodisk)

Dr. Boothe will provide updates to the subcommittee each year on the progress of the study.

AST Liaison Report

Dr. Bob Walker provided an update on the activities of the AST subcommittee as it may relates to the VAST subcommittee as follows:

- The CLSI Board of Directors recently approved the 2008 budget which includes a significant investment in education. The VAST subcommittee may want to try and take advantage of this in their efforts to educate users of the veterinary documents.
- In outlining the outcome of the AST strategic direction of how the AST subcommittee will set initial breakpoints (AST will review breakpoints set by FDA and determine whether to accept these), Dr. Walker noted that this approach differs from what is done in veterinary medicine. In veterinary medicine the FDA/CVM does not set breakpoints when they approve a drug for use in animals.
- Recommendations for reading the zone of inhibition for D-zone testing were approved and published in the new M100.
- New breakpoints for ciprofloxacin and levofloxacin when testing against *B. anthracis* and *Y. pestis* were approved.
- Non-meningitis penicillin breakpoints for *S. pneumoniae* were approved.

Working Group Updates

<u>Education Working Group</u>

Dr. Jeff Watts outlined the need to generate slide decks for presentations. Various presentations could be put together for use as educational tools geared towards specific audiences (eg, teachers vet students, outline clinical relevance of susceptibility testing to clinicians, vet diagnostic labs for accreditation). Dr. Watts asked that people submit any slides that they may have so that the working group may put together slide decks for use by anyone that may be giving presentations in the future.

Campylobacter Working Group

Drs. Bob Walker, Pat McDermott, and Tom, Fritsche provided an overview of the activities of the working group. Currently there are methodology and QC recommendations for agar dilution and broth microdilution testing, but due to the difficulty in determining accurate and reproducible zone sizes, disk diffusion testing has not yet been validated. The working group is currently working on this and has initiated a 9-lab study. Results of this study will be presented to the subcommittee at the next meeting.

Dr. Walker also outlined a separate study that they are looking to conduct to define the optimal Conditions for the *in vitro* antimicrobial susceptibility testing of clinical isolates of *Pasteurella multocida* and *Mannheimia haemolytica*.

Generic Working Group

Dr. Mark Papich outlined the current objective of the working group which is to determine the interpretive criteria for 1st generation cephalosporins (cefazolin, cephalexin, and cephalothin) against bacterial isolates from canine and equine animals. Data to be used to help determine breakpoints include microbiological data obtained by Dr. Ching Ching Wu from AVMA survey (MIC data only) and PK-PD data, indications, doses from U.S. Pharmacopeia and consensus published literature.

He gave an overview of the data that the working group has to date and will bring a full presentation with proposed interpretive criteria for review at the next subcommittee meeting.

Dr. Jeff Watts reviewed mastitis data for Cephalothin for MIC and disk values and will provide this data to the working group for review.

International Harmonization Working Group

Dr. Tom Shryock outlined the charge of the working group to try and expand the M31 document to include:

- Additional testing methods considered acceptable to the Vet subcommittee (e.g., ISO).
- QC values for these methods as well as other methods of generating QC that is comparable to CLSI methods.
- Breakpoints and interpretive criteria for non-US approved therapeutic products.

In an effort to do this, one suggestion was for the working group to put together some mock-up tables to include VAST breakpoints and possibly non-U.S. approved breakpoints with the same indications, dose, and route as for the U.S. The working group will also try to reach out and encourage sponsors to participate in the process.

Editorial Working Group

Mr. Gary Zurenko outlined the goals of the working group for the next edition of M31 to include:

- Make the documents more useful internationally

- Update Glossary 1
- Confirm availability and culture strain numbers that are currently in Table 3
- Create a mock-up animal specific breakpoint table for subcommittee input
- Consider publication of an M100-type supplement during interim years when the text is not updated.
- Incorporate changes from the International Harmonization working group.

Veterinary Mycoplasma Working Group

Dr. Ching Ching Wu discussed the need to standardize mycoplasma susceptibility testing. In an effort to address this, the working group will begin by developing a protocol and initiate testing to establish QC for bovine mycoplasma (*M. bovis*) as this mycoplasma grows well and there are currently two methods that have been used with fairly good reproducibility. Testing will be conducted initially conducted in 2 labs that have been performing this testing routinely, using the existing lab QC strains to establish QC ranges for *M. bovis.* Testing will be done using 2 lots of media, 2 techs, 15 replicates, 2 drugs. Based on the results and the recommended methods, testing will be expanded to 5 labs.

Some challenges that the working group faces in trying to standardize mycoplasma susceptibility testing include:

- Multiple species with different media needs
- A few of the drugs are inactivated in the testing system
- This is not a test that will be done in all labs

Dr. Wu will update the subcommittee as testing progresses.

Breakpoint Presentations

Tulathromycin for Swine Respiratory Disease

Ms. Cindy Lindeman presented data in support of placement of Tulathromycin in Table 1, Group A, Swine as well as proposed interpretive criteria when testing swine respiratory disease pathogens as follows:

Table 1:

Group A — Veterinary-Specific Interpretive Criteria Primary Test and Report	Swine
	Ceftiofur
	Florfenicol
	Tiamulin
	Tilmicosin
	Tulathromycin

Table 2:

Antimicrobial Agent	Disk Content	Zone Diameter (mm)			MIC Breakpoint (μg/mL)		
		S	I	R	S	I	R
Tulathromycin **Swine (Respiratory Disease)** ***Pasteurella multocida*** ***Bordetella bronchiseptica*** ***Actinobacillus pleuropneumoniae***	**30 μg**	**≥ 18** **≥ 10**	**15-17**	**≤ 14**	**≤ 16** **≤ 64**	**32** **-**	**≥ 64** **-**

The proposed Table 1 placement and interpretive criteria were approved with the addition of the below comment to be added for A. pleuropneumoniae (**Approved 10-0; 1 absent**).

APP Comment:

Hazy growth or double zones should be ignored. The outer, discrete zone of inhibition should be read. To detect isolates non-susceptible to Tulathromycin, broth microdilution testing is required.

Tulathromycin and Ceftiofur QC for Anaerobic MIC Testing

Ms. Laura Koeth presented data for Tulathromycin and Ceftiofur MIC QC ranges for testing anaerobic bacteria as follows:

The following QC ranges were proposed for ceftiofur and approved as shown below

Strain	Broth	Agar	Vote Results
B. fragilis ATCC 25285	8-64	32-256	**Approved 11-0**
B. thetaiotaomicron ATCC 29741	32-128	64-256	**Approved 11-0**
E. lentum ATCC 43055	16-128	none	**Not approved**
C. difficile ATCC 700057	64-512	none	**Approved 11-0**

A QC range was proposed for Tulathromycin for testing *E. lentum* ATCC 430055 as follows:

Strain	**Broth**	**Agar**

E. lentum ATCC 43055	0.5-4	2-16

The subcommittee approved these ranges **(9-2)** contingent on the approval of the new QC range for clindamycin vs. E. lentum by the AST subcommittee. Both the Anaerobe and QC Working Groups of the AST subcommittee did not approve and no recommendations were presented to the full AST subcommittee. Based on this the QC ranges for Tulathromycin are considered not approved by the VAST subcommittee.

Ceftiofur QC – *S. pneumoniae*

Ms. Maria Traczewski presented data for Ceftiofur QC limits for testing *S. pneumoniae* ATCC 49619 (MICs in CAMHB + LHB and 30 µg disks on either MHA + SB agar) as follows:

Control Strain	MIC (ug/mL)	Zone Diameter (mm)	Voting Result
S. pneumoniae ATCC 49619	0.12 – 0.5	32 – 34	**Approved 9-0; 1 abstain, 1 absent**

Danofloxacin QC – *A. pleuropneumoniae*

Ms. Maria Traczewski presented data for Danofloxacin MIC QC limits for testing *A. pleuropneumoniae* ATCC 27090 (MICs in VFM) as follows:

Control Strain	Proposed QC Limits MIC (ug/mL)	Voting Result
A. pleuropneumoniae ATCC 27090	0.03 – 0.12	**Approved 9-0; 1 abstain, 1 absent**

Workshop Announcement

Dr. Melanie Berson gave an update on a workshop sponsored by the American Academy of Veterinary Pharmacology and Therapeutics.

The workshop entitled " Exploration of Developmental Approaches to Companion Animal Antimicrobials: Providing for the Unmet Therapeutic Needs of Dogs and Cats will be held on 23-24 October 2008 in Rockville, MD. Complete information regarding the agenda and registration fees will be posted on the AAVPT website (www.aavpt.org) on the Events page.

QC Range Statistics

Dr. John Turnidge discussed with the subcommittee alternative methods for examining and analyzing the different types of data that the VAST and AST subcommittees are asked to review and vote on. In the background provided in the agenda book titled *What is the "error" of an MIC Estimation*, Dr. Turnidge outlines how the concept of the "error" or variation on an MIC measurement being "plus or minus one dilution", could lead to misinterpretation of results and

incorrect setting of MIC and zone diameter breakpoints or QC ranges. To address susceptibility data analysis issues Dr. Turnidge is seeking to set up a working group under the AST subcommittee. Anyone interested in participating on joining the efforts of this working group if approved please contact Dr. Turnidge.

***In-vitro* Pharmacodynamics of Oxytetracycline Utilizing a Swine Isolate of *Pasteurella multocida* in a Hollow Fiber System**

Dr. Brian Lubber, in follow-up to questions asked at a previous meeting regarding:

- What are the mathematical relationships between AUC, Time and Concentration?
- Do the relationships change as magnitudes change?
- Is 24 hour AUC taken out to 72 hours equivalent?
- Is the AUC:MIC ratio the appropriate PK/PD parameter for oxytetracycline? And if so, is 40 the correct magnitude of this parameter?

To try and answer these questions Dr. Lubber outlined a research study that he initiated using a hollow fiber infection model to test oxytetracycline. The hypothesis of the research study design:

– Two oxytetracycline dosing regimens resulting in AUC:MIC of 40 would not have different effects on a bacterial population
  - High C_{max} – Short $T_{1/2}$
  - Low C_{max} – Long $T_{1/2}$

Experimental Outcomes:

	Target Values		Actual Values	
	$T_{1/2}$	C_{max}	$T_{1/2}$	C_{max}
High Concentration	8 hr	7 µg/mL	9 hr	3.3 ug/mL
Low Concentration	24 hr	2.5 µg/mL	11 hr	2.3 ug/mL

	Target Values		Actual Values	
	$T_{1/2}$	C_{max}	$T_{1/2}$	C_{max}
High Concentration	8 hr	7 µg/mL	7.2 hr	6.6 ug/mL
Low Concentration	24 hr	2.5 µg/mL	22.4 hr	2.8 ug/mL

Future Experimental direction:

- Continued refinement of ability to achieve PK targets and management of bacterial growth
  - Replicated comparison of same AUC/MIC ratios over 24 and 72 hours.
  - How does this comparison change when the Q24H dosing regimen is repeated 3 times as would be done in field application?
- Dose fractionation
  - Does dose fractionation over 24 and 72 hour dosing interval provide the same results?

- How do pharmacodynamic parameters change in relation to immunosuppression and/or disease pressure?
  - Neutropenia
  - Swine specific: PRRS, PCV2
- What price do we pay for the convenience of murine models?
  - Individual animal PK vs. group average PK values for determination of PK/PD parameters

- Live animal study
  - Swine model
    - Respiratory?
    - Thigh infection?
    - Peritonitis?
  - Individual animal PK correlated with outcome

In asking for study design input, the subcommittee indicated that reported bacterial concentrations (10^{18}) were much too high and likely resulted from a methodology error. Dr. Lubbers indicated that he will follow-up with his microbiology laboratory on this issue and will update the subcommittee as the study progresses.

Next Meeting

The next meeting of the Subcommittee is tentatively scheduled for June 13-14 in Boston, MA. If sufficient agenda items are not received by the end of February, this meeting will be postponed.

Adjournment

The meeting adjourned at approximately 4:05 p.m. on 25 January 2008.

Respectfully submitted,

Tracy A. Dooley, BS, MLT (ASCP)
Standards Administrator