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**Summary Minutes  
Subcommittee on Veterinary Antimicrobial Susceptibility Testing  
Grand Hyatt Tampa Bay  
Tampa, Florida  
18-19 January 2007**

A meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing was held on 18-19 January 2007, at the Grand Hyatt Tampa Bay in Tampa, Florida. The following were in attendance:

**Jeffrey L. Watts, PhD, RM(AAM)**  
**Chairholder**

**Pfizer Animal Health**

**Members Present**

Mike Apley, DVM, PhD  
Donald J. Bade  
Steven D. Brown, PhD  
Henry Heine, PhD  
Rob P. Hunter, MS, PhD  
Dik J. Mevius, DVM, PhD  
Mark G. Papich, DVM, MS  
Peter Silley, PhD  
Gary E. Zurenko, MS

Kansas State University  
Microbial Research, Inc.  
The Clinical Microbiology Institute  
USAMRIID  
Elanco Animal Health  
Institute for Animal Science and Health  
North Carolina State University  
MB Consult Limited  
Micromyx, LLC

**Members Absent**

Jeffrey T. Gray, PhD

University of Guelph

**Advisors Present**

Jo Abraham, DVM, MS  
Marilyn N. Martinez, PhD  
Thomas R. Shryock, PhD  
Clyde Thornsberry, PhD  
John D. Turnidge, MD  
Robert D. Walker, PhD  
Ching Ching Wu, DVM, PhD

Bayer HealthCare LLC  
FDA Center for Veterinary Medicine  
Elanco Animal Health  
Focus Bio-Inova, Inc.  
Women's and Children's Hospital  
FDA Center for Veterinary Medicine  
Purdue University

## **Observers Present**

Virginia R. Fajt, DVM, PhD, DACVCP	Texas A & M University
Thomas R. Fritsche, PhD, MD	JMI Laboratories
Jason Hammelman	Elanco Animal Health
Scott B. Killian, BS	Trek Diagnostic Systems
Cynthia C. Knapp, MS	Trek Diagnostic Systems
Jennifer Lorbach	Trek Diagnostic Systems
Maureen Mansfield	Trek Diagnostic Systems
Ron A. Miller, MS*	FDA Center for Veterinary Medicine
Ian Morrissey	GR Micro LTD
Ruby Singh, PhD	FDA Center for Veterinary Medicine
Peter R. Smith, PhD*	National University of Ireland
Bernd Stephan	Bayer healthCare AG
Leland Thompson	Bayer HealthCare
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

\*Participated by conference call on Thursday, 18 January for the Aquaculture Working Group presentation.

## **Opening Remarks**

Dr. Watts began the meeting Thursday, 18 January at 8:30 a.m. In reporting changes to the subcommittee, Dr. Watts welcomed new voting members Rob Hunter and Dik Mevius. Dr. Watts then gave an historical overview of the Veterinary Subcommittee that started its work back in 1993 using methods and interpretive criteria adapted from the human guidelines. Since that initial meeting the subcommittee has developed veterinary specific interpretive criteria for 16 different compounds and has published four veterinary susceptibility testing documents two of which are specific for aquaculture susceptibility testing. In moving forward Dr. Watts outlined the need to increase the activities of the Generic Working Group to try and have more veterinary specific interpretive criteria, increase international harmonization activities through the newly formed International Harmonization Working Group and also focus on improvement of current testing methods (e.g., develop methods for fastidious pathogens).

Dr. Watts then presented awards of his appreciation to Dr. Clyde Thornsberry, Dr. Tom Shryock, and Dr. Bob Walker for all their many contributions throughout the history of the veterinary subcommittee.

He stated that the purpose of the meeting was to review M31-A3 and try to finalize the draft as well as review the edits made in M37-A3. The subcommittee would also evaluate data for incorporation into the M31 tables.

## Minutes of Prior Meeting

The subcommittee voted to approve the summary minutes from the 9-10 June 2006 meeting held in San Diego, California (**Approved 9-0; 1 absent**).

## M31-A3 Revisions

Textual revisions for M31 to try and finalize the document were discussed with the goal of the final draft to be ready for subcommittee vote following the meeting.

### M31 Text Revisions Made:

- **Section 4.1 Limitations of Disk Diffusion and Dilution Methods** - 3<sup>rd</sup> paragraph, **delete** last sentence regarding initially susceptible organisms that become resistant within 3 to 4 days occurring most frequently with *Enterobacter* spp., *Citrobacter* spp., etc.
- **Section 5.3.1.1 Penicillins** – **delete** sentence in 3<sup>rd</sup> paragraph referring to testing of one PRP.
- **Section 5.3.6 Quinolones** – add ibafloxacin a drug approved in Europe for cats and dogs.
- **5.3.8 Phenicol**s – 2<sup>nd</sup> line replace text "... blocking the transfer of soluble ribonucleic acid to ribosomes" with "binding to the peptidyltransferase center of the ribosomes and prevention of peptide chain elongation"
- **Section 5.5.1 Examples** – change reference to enteric bacillus to a member of the Enterobacteriaceae.
- **Section 7.3.2.4 Growth Method Inoculum Preparation** – in # 3 note added text "if a 1-mm pin is used, the adjusted suspension is used without dilution."
- **Section 7.3.2.6 (2) Incubating Agar Plates** – Add *A. pleuropneumoniae*
- **Section 7.3.3.2 Test Procedure** – added text regarding the incubation of plates in a microaerobic atmosphere.
- **Section 7.3.4.1 Broth Medium** - last paragraph, added examples of drugs that rapidly degrade.
- **Section 7.3.4.1.5 Interpreting Results** – replaced text with appropriate text for broth dilution.
- Added new **Section 7.3.4.1.9 Broth Microdilution Testing of *Campylobacter* species**
- **Section 7.4.1 Methicillin/Oxacillin Resistance** – replace the current text with the text from M7-A7.

### M31 Tables

#### Table 1

- **Group A**
  - Under Dogs and Cats add (dogs only) for Ampicillin
- **Group B**
  - Under Poultry delete Clindamycin
  - Under Dogs and Cats add Ampicillin (cats only)
- **Group C**
  - For Group C designation add underlined – No Veterinary Specie-Specific
  - Under Swine delete Sulfonamides
  - Under Cattle delete Neomycin

- Under Poultry add Clindamycin

- **Group D**

- Under Swine delete Sulfonamides

## Table 2

- Delete (formerly *Haemophilus somnus*) through the document.
- Add a comment for Gentamicin – "These breakpoints should not be used for *H. somni* and *A. pleuropneumoniae*."
- Under Lincosamides delete the gray shaded interpretive criteria for *C. perfringens* for chickens
- Under Phenicol correct the organism listed as *Salmonella enterica* subsp. *enterica* serovar *choleraesuis* to *Salmonella Choleraesuis*
- **Table 5C** – Add to the title of this table "using Agar Dilution"
- Create a new **Table 5D** - Name this table "Proposed Quality Control Ranges of MICs (µg/mL) for Anaerobic Reference Strains Using Broth Microdilution" and list QC ranges for Pradofloxacin from Table 5C.
- Add as a new **Table 9D** the Disk Diffusion Test for Prediction of *mec-A*- mediated Resistance in Staphylococci found in M100-S17

All changes will be incorporated into the M31 document and circulated to the members and advisors for a short review period. The document will then be sent to the full subcommittee for a 20-day vote.

## M37-A3 Revisions

Revisions made to the M37 document as well as inserted comments shown in the agenda book were reviewed and discussed with additional changes made as follows:

- **Definitions** – This section was rearranged with some of the definitions revised.
- **Section 5.2.1** – Number of isolates from susceptibility test results from a non-clinical trial collection changed from 500 to 50 isolates per target species.
- **Section 5.2.2** – Text added noting that the isolates obtained during the clinical effectiveness studies will be used to generate the Micro<sub>clin</sub> cutoff will consider each target species individually.
- **Appendix C and elsewhere in the document** – revised to make text less U.S. centric.
- **Appendix C** – Make definitions in Appendix C the same as those in the Definitions section.
- Incorporate the terminology used to develop and describe breakpoints as outlined by Dr. Turnidge (**Accepted 9-0; 1 absent**). The new terms proposed are as follows:
  - The term “breakpoint” (BP) would be used to describe the final product of subcommittee deliberations. It would mean the “interpretive standard” and “interpretive criteria”, terms which are also used in CLSI documents such as M100 and M31.

- Values used to assist in the setting of breakpoints would be called “cutoffs”.

- a. the “microbiological breakpoint” that separates populations on MIC distributions be called “wild-type cutoff” and be abbreviated CO<sub>WT</sub>
- b. the “breakpoint” that can be calculated using PK/PD parameters and Monte Carlo simulation be called the “pharmacodynamic cutoff” and be abbreviated CO<sub>PD</sub>.
- c. the value selected by inspecting clinical/microbiological outcome versus MIC from prospective clinical studies be called the “clinical” cutoff” and be abbreviated CO<sub>CL</sub>.

All changes will be incorporated into the M37 document and circulated to the members and advisors for a short review period. The document will then be sent to the full subcommittee for a 20-day vote.

### Aquaculture Working Group

Mr. Miller and Dr. Smith presented data in support of QC ranges for amoxicillin against *E. coli* ATCC<sup>®</sup> 25922 for inclusion in the next edition of M42, *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animal* as follows:

QC Organism	Proposed Range Zone Diameter (mm) Disk Content 25 µg	Vote
<i>E. coli</i> ATCC <sup>®</sup> 25922	18-27	Accept 9-0; 1 absent

### Cefovecin Quality Control Presentation

Ms. Traczewski presented data in support of QC ranges for cefovecin for inclusion in M31 as follows:

**Table 4. (Approved 8-0; 1 abstain, 1 absent)**

Antimicrobial Agent	Disk Content	<i>Staphylococcus aureus</i> ATCC <sup>®</sup> 25923	<i>Escherichia coli</i> ATCC <sup>®</sup> 25922	<i>Streptococcus pneumoniae</i> ATCC <sup>®</sup> 49619	<i>Pseudomonas aeruginosa</i> ATCC <sup>®</sup> 27853
Cefovecin	30 µg	25 – 32 mm	25 – 30 mm	25 – 31 mm	–

**Table 5. (Approved 8-0; 1 abstain, 1 absent)**

Antimicrobial Agent	<i>Staphylococcus aureus</i> ATCC <sup>®</sup> 29213	<i>Escherichia coli</i> ATCC <sup>®</sup> 25922	<i>Streptococcus pneumoniae</i> ATCC <sup>®</sup> 49619	<i>Pseudomonas aeruginosa</i> ATCC <sup>®</sup> 27853
Cefovecin	0.5 - 2	0.5 - 2	0.12 – 0.5	512 - 2048

**Table 5C. Agar Dilution for Anaerobic Reference Strains (Approved 8-0; 1 abstain, 1 absent)**

Antimicrobial Agent	<i>Bacteroides fragilis</i> ATCC® 25285	<i>Bacteroides thetaiotaomicron</i> ATCC® 29741	<i>Eubacterium lentum</i> ATCC® 43055
Cefovecin	8 - 32	16 - 128	–

**New Table 5D. Broth Microdilution for Anaerobic Reference Strains (Approved 8-0; 1 abstain, 1 absent)**

Antimicrobial Agent	<i>Bacteroides fragilis</i> ATCC® 25285	<i>Bacteroides thetaiotaomicron</i> ATCC® 29741	<i>Eubacterium lentum</i> ATCC® 43055
Cefovecin	8 - 32	16 - 64	–

**Table 6. (Approved 8-0; 1 abstain, 1 absent)**

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		MIC (µg/mL)	
		<i>Histophilus somni</i> ATCC®700025	<i>Actinobacillus pleuropneumoniae</i> ATCC®27090	<i>Histophilus somni</i> ATCC® 700025	<i>Actinobacillus pleuropneumoniae</i> ATCC® 27090
Cefovecin	30 µg	–	34 – 43*	0.001 – 0.008	0.008 – 0.03

\* Add a footnote stating: If out of range values are observed check media lot.

**Re-evaluation of Tilmicosin Breakpoints for Bovine Respiratory Disease Associated with *Mannheimia haemolytica***

Drs. Hunter, Hammelman, and Shryock presented data to propose revised MIC breakpoints for tilmicosin (current breakpoints in Table 2: ≤ 8 µg/mL susceptible, 16 µg/mL intermediate, and ≥ 32 µg/mL resistant) for the treatment of BRD associated with *M. haemolytica*.

The proposed new breakpoints for tilmicosin for the treatment of BRD associated with *M. haemolytica* are: ≤ 16 µg/mL susceptible, 32 µg/mL intermediate, and ≥ 64 µg/mL resistant.

The subcommittee agreed to table this proposal till the next meeting and requested that the sponsor bring back additional data. The subcommittee requested to see:

Pharmacology:

- Bronchial secretion data was requested; induced disease model deemed acceptable, if needed.
- AUC 0-infinity was not perceived as appropriate. Other AUC measures (0-24, 0-48, 0-72) were requested.
- Comparison of tilmicosin AUC:MIC ratios for other macrolides

Pharmacodynamics:

- Kill kinetics, PAE data, etc., were requested. AUC/MIC matched to successes and failures.
- Clinical data: Stratification of MIC-clinical outcome (e.g. what is success/failure at 2, 4, 8, 16, 32 ug/ml?)
- Pre- and Post-treatment MICs for the same animal (nasal vs. lung isolate comparison).
- Microbiology data: Surveillance data to address whether an MIC shift has been observed since launch of the product.

**Generic Working Group**

Dr. Papich outlined the working group's objective to determine the interpretive criteria for tetracycline against bacteria isolates from Bovine and Porcine, using oxytetracycline. He presented various data (PK-PD, microbiologic, scattergrams, Monte Carlo simulations) and proposed the following:

Antibacterial Agent	Disk Content	Zone diameter (mm)			MIC breakpoint (µg/mL)			Comment
		S	I	R	S	I	R	
Gentamicin								Breakpoint derived from pharmacokinetic data of oxytetracycline at 20 mg/kg IM, once, and pharmacodynamic data.
Cattle (All bacteria)		–	–	–	≤ 2	4	≥ 8	These interpretive criteria are applicable only for the injectable formulations. Tetracycline is the class representative.
<b>(Approved 7-1; 2 absent)</b>								
Swine (All bacteria)		–	–	–	≤ 0.5	1	≥ 2	Breakpoint derived from pharmacokinetic data of oxytetracycline at 20 mg/kg IM, once, and pharmacodynamic data. These interpretive criteria are applicable only for the injectable formulations. Tetracycline is the class representative.
<b>(Approved 5-3; 2 absent)</b>								

The Working Group plans to look at dosing data for oxytetracycline – 1 dose at 72 hours and look at clinical response. Dr. Craig's mouse model data will also be reviewed to see if it is based on a single dose regimen. The working group will then design a study protocol in animal models to look at the AUC<sub>72</sub> and compare this to AUC<sub>24</sub>. The protocol will be circulated for input.

**International Harmonization Working Group**

Dr. Shryock, chairholder for the newly formed working group whose members include: J. Watts, B. Walker, D. Mevius, S. Schwarz, and P. Silley are charged with:



- Looking to expand the current CLSI M31 document to include additional testing methods considered acceptable to VAST as well as QC values for these testing methods;
- Look at the testing method and QC for *Brachyspira*;
- Expand the M31 tables to include non-U.S. approved drugs with same indications, dose, and route as the U.S.;
- Look at the acceptability of QC for non-U.S. approved drugs generated by non-CLSI methods (e.g., ring test);
- Encourage sponsors and other organizations to participate in the process; and
- Explore harmonization opportunities (e.g., ISO, VICH, VETCAST).

### **Veterinary Mycoplasma Working Group**

This newly formed working group is Co-chaired by Drs. Wu and Waites. Members of the working group include: J. Kinyon, C. Bebear, M. Brown, D. Bade, L. Duffy, and R. Ayling. This working group will look to standardize susceptibility testing methods and will develop protocols to initiate QC studies and begin testing with *M. bovis*. Sponsor support will be necessary for drugs to be tested.

### **Informational Presentation – Testing for *Pasteurella***

Dr. Walker gave an overview of testing done for *Pasteurella multocida* using 116 isolates from dogs and cats. Testing was done using blood Mueller-Hinton (BMH) in CO<sub>2</sub> or BMH in ambient air. When reading zone size, if the measured zone and MIC were not in close agreement then the isolates were then tested using plain MH in CO<sub>2</sub> or MH in ambient air. It was concluded that testing of *P. multocida* can be done by disk diffusion; if zone sizes are too large or no growth then testing should be performed by broth microdilution with 5% lysed horse blood in ambient air.

In proposing to have additional data for inclusion in Table 7 for *Pasteurella* and possibly *Mannheimia*, Dr. Walker proposed to do a study testing 100 isolates (bovine and porcine) by disk diffusion, broth microdilution and possibly agar dilution to see if CO<sub>2</sub> is necessary and if a supplement is needed. Other participants for this study include: C. Wu, D. Mevius, CMI, and T. Fritsche.

### **Next Meeting**

The next meeting of the subcommittee will be determined based on whether there will be enough material to warrant a meeting. **Sponsors are pleased asked to notify the chairholder by the end of February if they intend to present QC or interpretive criteria data in June so that plans can be made.**

If the meeting is to be held, the deadline to submit material would be Friday, 11 May. The subcommittee will be notified of the meeting status.

### **Adjournment**

The meeting adjourned at approximately 3:50 p.m. on 19 January 2007.

Respectfully submitted,

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