Summary Minutes
Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Hyatt Regency Tampa Bay
Tampa, Florida
26-27 January 2010

A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was held on 26-27 January 2010 in Tampa, Florida. The list of attendees is listed below.

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

Members Present

Donald Bade	Microbial Research, Inc.
Steven D. Brown, PhD	The Clinical Microbiology Institute
Viginia 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 (Netherlands)
Stefan Schwarz, DVM	Friedrich-Loeffler-Institute (FLI)
Peter Silley, PhD	MB Consult Limited
Gary E. Zurenko, MS	Micromyx, LLC

Members Absent (with notice)

Ching Ching Wu, DVM, PhD	Purdue University School of Veterinary Medicine

Advisors

Melanie R. Berson, DVM	FDA Center for Veterinary Medicine
Cindy Lindeman, BS	Pfizer Animal Health
Jennifer Lorbach, BS, MBA	Trek Diagnostic Systems
Marilyn N. Martinez, PhD	FDA Center for Veterinary Medicine
Patrick McDermott, PhD	FDA Center for Veterinary Medicine
Thomas R. Shryock, PhD	Elanco Animal Health
Shabbir Simjee, PhD	Elanco Animal Health
Clyde Thornsberry, PhD	Eurofins Medinet
John Turnidge, MD	Women's and Children's Hospital
S. Steve Yan, PhD	FDA Center for Veterinary Medicine

Observers Present

Brent Herrig	Intervet Innovation GmbH
Timothy Frana, DVM, MS, MPH, PhD	Iowa State University

Thomas R. Fritsche, MD, PhD	Marshfield Clinic
Joshua Hayes, PhD	FDA Center for Veterinary Medicine
Scott B. Killian	Trek Diagnostic Systems
Cynthia C. Knapp, MS	Trek Diagnostic Systems
Ron A. Miller, PhD	FDA Center for Veterinary Medicine
Lori T. Moon, MT(ASCP)	MSU Diagnostic Ctr. for Population & Animal Health
Ian Morrissey	Quotient Bioresearch Ltd.
Karen Ochonicky	Elanco Animal Health
Amy Omer	FDA Center for Veterinary Medicine
Chris Pillar	Eurofins Medinet
Marcus Rose, DVM, PhD	Intervet Innovation GmbH
Bernd Stephan, PhD	Bayer Animal Health GmbH
Michael Sweeney	Pfizer Animal Health
Karla M. Tomfohrde	Eurofins Medinet
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute

CLSI Staff Present

Marcy Hackenbrack, MCM, M (ASCP), BA
Tracy Dooley, BS, MT (ASCP)

Day One—Tuesday, January 26, 2010

- **Welcome/Introductory Remarks**

Dr. Watts opened the meeting on Tuesday, January 26, 2010 at 12:30 pm by expressing his gratitude to the participants for their time and input and requested that all participants provide a brief introduction.

Dr. Watts stated that the purpose of Tuesday's session was to provide an opportunity for the working groups to address their agenda topics and obtain input from the subcommittee. Sponsor presentations and detailed working group reports would be presented to the full subcommittee during Wednesday's session. He also indicated that any items listed on the agenda for Wednesday's meeting but are covered during this session, would be summarized at the beginning of the Wednesday session.

- **Working Group Sessions**

The Generic, Editorial, International, Education, and Veterinary Mycoplasma Working Groups briefly presented topics for discussion during the full subcommittee meeting on Day 2 and requested input from the participants. A description of the discussions and outcomes are presented in detail with the meeting review for Wednesday, January 27, 2010.

- **AST Report**

Dr. Heine reported that the FDA/CLSI interaction was discussed in great detail during the Subcommittee on Antimicrobial Susceptibility Testing (AST) meetings. He stated that transparency is critical. Volunteers must be as open as possible regarding disclosures of interest and must clearly present any conflicts when discussing or voting on specific topics. He also indicated that the goal of the interaction is to move toward regulatory guidance for antimicrobial susceptibility testing.

Other topics reviewed by Dr. Heine were updates on new AST working groups. He reported that the AST subcommittee has formed a fluoroquinolone working group that will be reconsidering breakpoints for enterics. A carbapenem working group has been formed to gather and compile data on carbapenem breakpoints. The Topical antimicrobial working group has recently formed and is still in the very early

stages. They will be responsible for presenting quality control guidelines for topical agents that are used for oral, skin and eye infections.

- **Presentation: Harmonization in Antimicrobial Susceptibility Testing Methodology—Reach out to China**

Dr. Yan delivered a presentation on the recent Center for Veterinary Medicine (CVM) outreach program to China. He stated that the program included a series of workshops and meetings that have been held in various locations in China in the Fall of 2009. The focus of these workshops and meetings has been on food safety of antimicrobials for use in food producing animals and Part of the technical exchanges involved antimicrobial resistance monitoring data generated from CVM's NARMS program. Thus, it was integral and necessary to include a short presentation about CLSI and VAST to the audience in China.

Antimicrobial resistance is an issue in China, and monitoring program is being established within the purview of Ministry of Agriculture where animal drug approval takes place. Dr. Yan indicated that although investigators claim to follow CLSI methodology and interpretive criteria, there needs to be continued interaction between investigators and relevant authorities governing the methodology process. China does not have a VAST equivalent agency or organization. He reported that resistance in some drug-organism combinations is rising at an alarming rate and currently, antimicrobial susceptibility is not being consistently performed in China as in U.S. He also reported that laboratory workers in China do not understand the concepts of quality control (QC), minimal inhibitory concentration (MIC) ranges for QC strains and interpretive criteria in the same way that it is in the west.

It was discussed during the presentation that M31, *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard* be considered for translation into Chinese. Ms. Dooley reported that other CLSI documents have been translated but would have to discuss the issue with the Executive Vice-President of CLSI, Glen Fine. Dr. Watts stated that he would contact Mr. Fine directly regarding the issue of translations.

Dr. Yan requested input from the subcommittee on possible opportunities for further outreach to key laboratories or organizations in China that may help in education in the area of susceptibility testing. It was also suggested that it would be beneficial to survey laboratories in China for their understanding of CLSI's testing systems/methodology, and the results of which may provide better direction for future outreach to China and perhaps to the region.

- **Letters to the Editor: Journal of Veterinary Medicine**

Dr. Papich provided an update on a letter to the editor regarding the publication of two articles on the proposed changes to CLSI interpretive criteria for methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs. He commented that these letters enhanced the awareness of the issue and provided "good press" for CLSI and VAST.

- **Reassessment of Breakpoints/Interpretive Criteria**

Dr. Martinez and Dr. Berson requested that the subcommittee discuss and reaffirm the recommendations for reassessment of breakpoints and interpretive criteria provided in M37-A3, *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition*, Section 3.6 Reassessment of Breakpoints/Interpretive Criteria and QC Parameters.

Section 3.6 states, *"The following represent situations under which a reassessment can be considered:*

- *when new dosages or formulations of an antimicrobial agent and/or new clinical usage require(s) a change;*

- *when new clinical and/or pharmacologic data suggest a need for reassessment*

When a reassessment is proposed, or later reviewed, follow the guidelines presented in this document to the fullest extent possible. The data upon which the original decision on the breakpoint was made should be considered in any reassessment."

A scenario was presented in which a new formulation or a new delivery system is developed for a drug for which breakpoints have already been published. They requested input on whether the current breakpoints and interpretive criteria are still applicable or if product specific breakpoints required.

It was agreed that a different formulations and changes in the administration of a drug will alter the pharmacodynamic and pharmacokinetic data. The subcommittee will agree to abide by M37-A3. In the event of a new dosage form, an alteration in formulation, a novel delivery system or a new clinical usage of a currently available drug, the sponsor must submit the new clinical, pharmacokinetic, and pharmacodynamic data, including the current susceptibility data, to the subcommittee to determine the appropriate breakpoints to apply to the new dosage form, new formulation, novel delivery system, or new clinical usage of the product. If data is not submitted by the sponsor, footnotes clarifying the issue may be added to the tables.

Further information regarding FDA and CLSI process changes will be discussed during day 2 of the meeting.

- **Generation of Clinical Cut-offs**

A proposal was made for the subcommittee to review the methods for generating the clinical data needed to determine clinical cut-offs and provide recommendations for defining cut-off values. It was noted that more information is needed to properly interpret clinical trial data for the selection of clinical cut-offs and better defining the MICs associated with efficacy. It was noted that M37-A3 requires that clinical effectiveness studies be performed to develop clinical cut-offs but does not provide specific information on how to perform and interpret these studies. It was suggested that the subcommittee develop examples and add these examples to a revised version of M37.

In response to the proposal, a working group of the subcommittee was formed to review M37-A3 in preparation for revision. The working group consisted of Dr. Martinez, Dr. Papich, Dr. Hunter, Dr. Simjee, Ms. Lindeman and Dr. Silley. The group will review the document, prepare a list of recommended changes and will discuss their recommendations with the full subcommittee at the June 2010 meeting in Atlanta.

- **Adjournment**

Dr. Watts provided an overview of the agenda for Day 2. He indicated that he would provide a review of the Day 1 meeting. The meeting was adjourned at 4:45 p.m.

Day Two—Wednesday, January 27, 2010

Dr. Watts convened the meeting at 8:30 p.m. U.S. Eastern time by greeting the group and welcoming those that had not been present during the previous day's meeting. He stated that the goals for the day's meeting includes a review of the material covered during the previous day's meeting, to cover all necessary subcommittee business and to present materials for voting.

The participants introduced themselves and provided a brief description of their interests. The voting members also provided any updated disclosure of interest information.

Dr. Watts stated that with the absence of one voting member (Dr. Wu), the 9 & 0 voting rules would apply.

- **Minutes from Previous Meeting**

The subcommittee reviewed the meeting minutes from the June 2009 meeting held in Boston, Massachusetts. It was noted that there were a few spelling errors in the minutes. The spelling of Dr. Schwarz's name and the organism name for *Staphylococcus pseudintermedius* will be corrected. The subcommittee voted to approve the summary minutes from the. (**Approved 9-0; 1 absent**).

- **Document Status Update**

Ms. Dooley provided an update on the activities of the Area Committee on Microbiology.

1. Recently Published Documents

M44-A2, *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*; *Approved Guideline—Second Edition* and **M44-S3**, *Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Informational Supplement.* Both documents published August 2009.

M100-S20, *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement.* Published January 2010.

2. Upcoming Publications

X07-R, Surveillance for Methicillin-Resistant *Staphylococcus aureus:* Principles, Practices, and Challenges; A Report. Document will publish the end of February 2010.

M51-A, *Method for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Approved Guideline* and **M51-S1**, *Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Informational Supplement.*Both documents are estimated for publication in March/April 2010.

3. New Projects Under Development

M52-P, *User Verification and Validation of Microbial Identification and Antimicrobial Susceptibility Testing Systems.* Co-Chairholders: Linda M. Mann, PhD, D(ABMM) and Dee Shortridge, PhD

This document will provide general guidelines for microbiology laboratories on verification of new methods to be introduced into their laboratories with a focus on automated methods for bacterial susceptibility and identification, using commercial systems. It will not address serological assays, assays developed in-house or molecular methods.

M53-P, *Laboratory Testing and Diagnosis of HIV Infections*— Chairholder: Eric Rosenberg, MD

This document will address issues related to the sensitivity and specificity of the various methods used in HIV testing, selection of appropriate testing algorithms for HIV screening and confirmatory testing, and development of interpretive criteria to assist in the diagnosis of HIV infection.

M46-P, *Diagnostic Microbiology for Resource Limited Laboratories* - Chairholder: Susan E. Sharp, Ph.D., D(ABMM)

The document will include basic testing methods, use of stains including how to make them and staining methods, how to use a microscope as well as include photomicrographs for gram stains, parasites, malaria smears, etc. The document will address specimen collection, handling, and processing but it will not address cultures.

M43-P, *Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas* - Chairholder: Ken Waites, MD

The document will provide standardized methods for broth microdilution and agar dilution-based susceptibility testing of human mycoplasmas.

M54-P, Specimen Collection, Handling, Processing, Set-up and Interpretation of Fungal Cultures—Chairholder: Nancy Wengenack, PhD and Vice-Chairholder: Gail L. Woods, MD

This document will provide procedures for the collection of fungal specimens, their handling, processing and interpretation of culture results. Because the relative importance of any given fungus isolated from patient specimens depends upon the pathogenic potential of the fungus and the clinical setting in which it is isolated, the issues as well as factors to consider regarding the isolate's clinical significance will also be discussed.

X08-R, Generation, Presentation and Application of AST Data for Bacteria of Animal Origin; A Report – Chairholder: Shabbir Simjee, PhD

This report will provide a review of current applications of susceptibility data generated using CLSI methodology for bacteria of animal origin as well as recommendations for summarizing, presenting, and applying the data in various ways. More specifically, the report will give an overview of the CLSI VAST approach to reference methodology, quality control, and establishment and use of clinical breakpoints. Recommendations for presentation of MIC (or zone inhibition data) in frequency histograms, use of Wild-type Cut-off Values and/or CLSI Clinical Breakpoints will also be provided.

4. Documents Currently Under Revision or in the Voting Process

M24-A to A2, *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes* Chairholder: Gail L. Woods, MD

M29-A3 to A4, *Protection of Laboratory Workers From Occupationally Acquired Infections* Chairholder: Donald R. Callihan, PhD

M45-A to A2, *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria* - Chairholder: James H. Jorgensen, PhD and Vice-Chairholder: Janet F. Hindler, MCLS, MT (ASCP).

M100-S21, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement*—Chairholder: Franklin R. Cockerill, III, MD

M42-S1 and M49-S1, Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; First Informational Supplements—Co- Chairholders: John P. Hawke, PhD and Renate Reimschuessel, PhD, VMD

5. Upcoming Revisions

M40-A to A2, *Quality Control of Microbiological Transport Systems*—Co-Chairholders: Paul Bourbeau, Ph.D. D(ABMM) and Paul Cerwinka, MSc, RM

- **Review of Agenda Items Covered during the Working Group Session and an Update on the FDA—CLSI Process**

Dr. Watts provided an overview of the agenda items covered during the Working Group session on Day 1. He also presented an update on FDA – CLSI process changes. He reported that the FDA Center for Drug Evaluation and Research (CDER) is endeavoring to become aligned with the CLSI Subcommittee on AST as the FDA Center for Veterinary Medicine (CVM) has historically been aligned with VAST.
Dr. Watts report that going forward, CLSI will change its process to better align with the regulatory process. He stated that for AST and VAST, all data submission information from the sponsor must be provided in the agenda book/agenda CD and all presentations to VAST should be equivalent to AST presentations. All working group decisions will be delayed for one cycle in order to allow for a period of review and comment. The entire process need to be documented to allow transparency to both FDA and CLSI and the sponsors. He also stated that all new breakpoints will be tentative for one year. The process may also be used as a model for interactions with other regulatory agencies.

Dr. Watts indicated that the documentation process will need to be developed. He will work with review the requirements with Dr. Rex, Chairholder of the Area Committee on Microbiology, and will work with Dr. Hunter and Dr. Berson (FDA) to develop a document that describes the process to be submitted to CVM.

- **Disk Diffusion QC Ranges for Ciprofloxacin, Nalidixic acid, Erythromycin and Tetracycline Against *Campylobacter jejuni***

Dr. McDermott presented data on a study performed to determine quality control ranges for *Campylobacter jejuni* ATCC 33560 against nalidixic acid, ciprofloxacin, erythromycin and tetracycline. As a result of the study, the following QC ranges were proposed:

- Nalidixic acid 25-34 mm
- Ciprofloxacin 33-44 mm
- Erythromycin 26-37 mm
- Tetracycline 15 mm

Dr. McDermott stated that the proposed zone diameters are for 24 hour readings only. He reported that 48 hour reading showed inconsistent results.

It was recommended that a formal statistical analysis be performed to re-evaluate the 48 hour reading data. Dr. Brown volunteered to perform the analysis on the raw data for presentation at the meeting scheduled in June 2010.

Note: Dr. Turnidge prepared the statistical analysis of the QC ranges and presented the results during the meeting on Day 2. The statistical analysis showed QC ranges for nalidixic acid, ciprofloxacin and erythromycin as shown below:

- Nalidixic acid 25-34 mm (same as originally proposed by Dr. McDermott)
- Ciprofloxacin 32-45 mm
- Erythromycin 26-38 mm
- Tetracycline No range

A motion to accept the statistically derived QC ranges for nalidixic acid, ciprofloxacin and erythromycin but not tetracycline was made and accepted. (**Approved 8-0; 2 absent**)

- **Quality Control Study—Gamithromycin**

Dr. Pillar presented data intended to be utilized in the establishment of quality control ranges for gamithromycin using the broth microdilution method. Based on the available data from the Tier 2 QC study performed in accordance with M37-A3, *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition*, the following QC ranges were proposed:

Organism	Proposed QC range (µg/mL)
***S. aureus* ATCC 29213**	**0.5 - 4**
***E. faecalis* ATCC 29212**	**4 - 32**
***S. pneumoniae* ATCC 49619**	**0.03 – 0.12**
***H. somni* ATCC 700025**	**0.25 – 1**
***A. pleuropneumoniae* ATCC 27090**	**2 - 8**

A motion to approve the proposed QC ranges was made and seconded. (**Approved 9-0; 1 absent**)

- **Kanamycin/Cephalexin (10:1) - CLSI M37-A3 Broth Microdilution MIC Quality Control Study**

Dr. Pillar presented Tier 2 quality control study data for a 10:1 combination of kanamycin/cephalexin against *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *S. pneumoniae* ATCC 49619. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (µg/mL)
***S. aureus* ATCC 29213**	**1/0.1 – 4/0.4**
***E. coli* ATCC 25922**	**2/0.2 – 8/0.8**
***S. pneumoniae* ATCC 49619**	**8/0.8 – 32/3.2**

Dr. Brown suggested that based on the presented data, the ranges for *S. pneumonia* ATCC 49619 be amended to adhere to the 60% rule as stated in M23-A3, *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition.* The amended ranges for *S. pneumoniae* ATCC 49619 would be 8/0.8-64-6.4.

A motion was made and seconded to approve the proposed ranges with the amended ranges for *S. pneumoniae* ATCC 49619. (**Approved 9-0; 1 absent**)

- ***In vitro* Testing of Cephalexin in Combination with Kanamycin (Ubrolexin) Against Target Bovine Mastitis Pathogens**

Dr. Pillar presented data on clinical efficacy and microbiology efficacy in support of a request by the sponsor to set epidemiological cutoff values (ECVs) for *in vitro* testing of a combination of

cephalexin/kanamycin (Ubrolexin) against target bovine mastitis pathogens including *E. coli*, *S. aureus*, *S. uberis*, and *S. dysgalactiae.*

The subcommittee discussed issues associated with substantiating clinical efficacy in treatment of clinical bovine mastitis and how to utilize the data presented by Dr. Pillar. The main points of discussion are listed below.

- It was questioned as to whether or not the data can be utilized. Also, the question was posed as to where the data would be published, either in a new, separate document or as a new table in M31.
- It was suggested that the subcommittee might wish to add a separate table (possible 2C) for Wild type (WT) vs Non WT be added with associated text to M31.
- Other concerns raised involved the need for additional data sets in order to set species-specific ECVs. If so, what guidance can be provided to the sponsor for collection of additional data?

A motion was to accept the epidemiological data and publish it in a new table in the next revision of M31 made and seconded. **(Rejected 3-5; 2 absent)**

A new motion to table the discussion on how to handle the ECV data until the June 2010 meeting was made and seconded. (**Approved 9-0; 1 absent**)

- **Working Group Updates**

1. Generic Working Group

Amoxicillin/Clavulanic acid Breakpoints for Cats

Dr. Papich provided an update on the activities of the generic working group. He stated that the goal of the working group is to eliminate the gray areas (human interpretive criteria) from Table 2 of the next version of M31, *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard* and to update the information in the table to include breakpoints specific for animals.

Dr. Papich presented data intended to be used to support the development of breakpoints for testing of amoxacillin/clavulanic acid against organisms isolated from cats. He reminded the group that breakpoints for testing against organisms isolated from dogs were approved during the January 2010 meeting. He presented PK/PD data a statistical analysis for cats. Based on the presented data, he proposed the following breakpoints:

- S = 0.25/0.12 μg/ml
- I = 0.5/0.25 μg/ml
- R = 1.0/0/5 μg/ml

A motion to approve the proposed breakpoints was made and seconded. (**Approved 8-0; 2 absent**)

It was suggested that an exception comment regarding the breakpoints for amoxicillin when testing organisms isolated from urine be added to Table 2. A motion to add the following comment to Table 2 for testing with amoxicillin was made and seconded.

"Breakpoints of ≤ 8 should be used for urinary tract infections." (**Approved 8-0; 2 absent**)

A motion to remove human breakpoint data for amoxicillin/clavulanic acid from Table 2A and to move it to a new table designated 2B was also made and seconded. (**Approved 8-0; 2 absent**)

It was agreed that the working group will review the possibility of listing more specific organisms for dogs as has been done for cats.

Chloramphenicol and Rifampin Breakpoints for Resistant *S. pseudintermedius*

Dr. Papich reported that the next generic drugs for which animal specific breakpoints will be added will be chloramphenicol and rifampin for methicillin-resistant *S. pseudintermedius*. He indicated that currently, there are no breakpoints for *S. pseudintermedius* against chloramphenicol or rifampin and that the breakpoints for methicillin-resistant coagulase negative staphylococcus do not fit with *mecA* carriers such as *S. pseudintermedius.* He requested that data on *S. pseudintermedius* against chloramphenicol and rifampin be submitted to him.

Identification of *Staphylococcus pseudintermedius*

Dr. Schwarz provided an overview of the issues regarding phenotypic identification of *S. pseudintermedius.* He reported that *S.pseudintermedius* (and *S. delphini*) is not included in commercially available identification systems; therefore, biochemical differentiation from related species (eg, *S. intermedius*, *S. delphini*) is not very reliable and not suitable for routine diagnostics. He indicated that molecular tools for identification are available but are very labor intensive. He suggested that a simplified system for routine diagnostics be recommended: **All phenotypic *S. intermedius group* (SIG) strains from dogs should be identified as *S. pseudintermedius.***

It was agreed that a letter to the editor regarding the phenotypic identification of *S. intermedius group* (SIG) strains from dogs be submitted of the "Journal of Veterinary Medicine".

Correlation between oxacillin resistance and the carriage of *mecA* among coagulase-negative staphylococci

Dr. Schwarz presented data regarding the correlation between oxacillin resistance and *mecA* carriage among coagulase-negative staphylococci. He reported that the data indicates that MICs and zone diameters may not correctly reflect the carriage of the *mecA* gene and resistant coagulase-negative staphylococci may be categorized as methicillin sensitive when they are *mecA* carriers.

Dr. Schwarz proposed that, at minimum, an intermediate (I) category be included in Table 2A for the MIC interpretive criteria and a comment regarding *mecA* carriage be added. This would cover MICs of 0.5 and 1.0 mg/L, would provide a "buffer zone" for isolates that may or may not carry *mec A,* would provide better correlation with *mecA* carriage, and would not cause a dramatic change in the existing resistance interpretive criteria. The comment would specify how to utilize the information for mec A carriage prior to using a drug that exhibits intermediate results.

It was noted that, currently, human breakpoints are being utilized for reporting methicillin resistance. As per the last meeting, all human breakpoints are being moved to Table 2B and there is no I category for the human breakpoints. It was suggested that species specific breakpoints will be necessary before the Intermediate category can be added to Table 2A in M31.

MecA vs MIC data will be analyzed further. A comment regarding the MIC/*mecA* relationship will be written and will be reviewed and voted upon during the June 2010 meeting.

2. Aquaculture Working Group

Dr. Miller reviewed the proposed changes to the informational supplement to documents M42-A, *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline* and M49-A, *Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline.* These included the addition of clinical breakpoints for oxolinic acid and oxytetracycline and wild type cutoff values (CO_{WT}) for gentamicin, erythromycin, florfenicol, ormethoprim-sulfadimethoxine, and trimethoprim-sulfmethoxazole for *Aeromonas salmonicida.*

The working group proposed the following MICs and zone diameters (ZD) for *A. salmonicida* and oxytetracycline.

MICs: S ≤ 1 µg/ml
I = 2 – 4 µg/ml
R ≥ 8 µg/ml

ZDs: S ≥ 28 mm
I = 22 – 27 mm
R ≤ 21 mm

A motion to accept the proposed MICs and ZDs for *A. salmonicida* and oxytetracycline was made and accepted. (**Approved 8-0; 2 absent**)

The working group proposed the following MICs and zone diameters (ZD) for *A. salmonicida* and oxolinic acid.

MICs: S ≤ 0.12 µg/ml
I = 0.25 – 0.5 µg/ml
R ≥ 1 µg/ml

ZDs: S ≥ 30 mm
I = 25 - 29 mm
R ≤ 24 mm

A motion to accept the proposed MICs and ZDs for *A. salmonicida* and oxolinic acid was made and accepted. (**Approved 8-0; 2 absent**)

The working group proposed wild-type cut-off values for *A. salmonicida* and gentamicin, erythromycin, florfenicol, ormethoprim-sulfadimethoxine, and trimethoprim-sulfmethoxazole.

Antimicrobial Agent	**Zone Diameter Cut-off (mm)**		**MIC cut-off (µg/ml)**	
	WT	**Non-WT**	**WT**	**Non-WT**
gentamicin	≥ 18	≤ 17	N/A	N/A
erythromycin	≥ 14	≤ 13	N/A	N/A
florfenicol	≥ 27	≤ 26	≤ 4	≥8
ormethoprim-sulfadimethoxine	≥ 20	≤ 19	≤0.5/9.5	≥ 1/19
trimethoprim-sulfmethoxazole	≥ 20	≤ 19	N/A	N/A

A motion to accept all CO_{WT} except with the gentamicin CO_{WT} as ≥ 18 mm was made and seconded. (**Approved 8-0; 2 absent**)

A table (Table 1) with the clinical breakpoints and zone diameters for *A. salmonicida* and oxolinic acid will be added to the M42/M49-S1 Supplement along with a separate table (Table 2) with epidemiologic cut-off values (ECVs). Refer to Supplement mock-up provided with the agenda materials. When M42-A and M49-A is next updated, these tables will then be incorporated into the main documents. A comment regarding ECVs similar to that used in M51-S1, *Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Informational Supplement*will be added to Table 2 of the Supplement. . The final version of the M42/M49-S1 Supplement showing all changes as approved at this meeting (Approved 8-0; 2 absent), will be circulated to the subcommittee prior to being submitted for area committee voting.

3. Editorial Working Group

Mr. Zurenko reviewed the editorial working group items for consideration by the subcommittee. The editorial working group proposed the following changes to M31:

a. Inducible clindamycin resistance (Refer to agenda CD, Tab D, Item 1)

- addition of text to Section 15.5
- addition of footnote to Table 4
- addition of Table 9G

A motion to accept the proposed revisions regarding inducible clindamycin resistance was made and seconded. (**Approved 8-0; 2 absent**)

b. Re-organization of Table 2 as Tables 2A and 2B

- Table 2A would include animal species-specific zone diameter interpretive standards, MIC breakpoints, and general comments for veterinary pathogens.

- Table 2B would include human-derived zone diameter interpretive standards, MIC breakpoints and general comments for veterinary pathogens.

A motion to accept the re-organization of Table 2 into Tables 2A and 2B was made and accepted. (**Approved 8-0; 2 absent**)

It was agreed that a final review of the text and tables was required prior to revision of M31. The working group will produce a mock-up of the tables and other proposed changes. The mock-up will be completed in March and circulated to the subcommittee for a formal vote after the June meeting.

4. International Harmonization Working Group

Dr. Shryock provided an overview of the goals for the International Harmonization working group. He proposed that the new role of the International Harmonization working group to serve as an advisory link between VAST and non–US drug sponsors to help create the best data package possible prior to presentation at subcommittee meetings. He indicated that it may be necessary to add entries to M31 and may require consideration of a new approval category to denote non-US approval or abbreviated data package review. If data is generated for an organism or drug that meets the criteria for M31, then that organism/drug information would move to main table in M31.

It was suggested that a separate document for unusual organisms and/or non-US approved drugs be proposed. This document would be similar to M45, *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline* and would include data published outside the U.S.

Dr. Shryock volunteered to prepare a project proposal that will be reviewed by the working group and will be available for review by the subcommittee at the June 2010 meeting.

5. Education Working Group

Dr. Fajt reported the working group is preparing two articles for publication that should be ready for review at the June 2010 meeting. She requested volunteers to provide input on the content of the articles. Mr. Sweeney, Dr. Miller, Dr. Schwarz, and Dr. Papich agreed to assist in developing the articles. Dr. Fajt indicated that the goal is for the articles to be submitted and publish during 2010.

6. Veterinary *Mycoplasma* Working Group

Mr. Bade reported on an ongoing study to evaluate a broth microdilution method of susceptibility testing of florfenicol with *Mycoplasma bovis* for intra- and interlaboratory reproducibility. He presented results for testing using three different lots of media at three different sites.

Based on the study data, the method was shown to be reproducible from lot-to-lot of media and from site-to-site. He reported that the next steps for the evaluation would be to compare the method to the phenol red method at one or more sites, identify a manufacturer of the specialized media used for the method, identify an additional manufacturer of the base media, to define QC organisms, and perform a seven laboratory study to determine QC ranges for multiple compounds. Mr. Bade indicated that the goal is to complete the seven laboratory study by June 2010.

Once completed, the method will formally be presented to the Subcommittee on Veterinary Antimicrobial Susceptibility Testing and request QC range approval for *M. bovis*. Subsequently, the working group will work on the method and QC approval for *M. hyopneumoniae*.

- **Other Business**

There was no other business to discuss.

- **Plans for Next Meeting**

The next meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing will be scheduled as a two-day meeting on Wednesday, 16 June and Thursday, 17 June 2010 in Atlanta, Georgia.

The submission deadline for the June meeting will be **Friday, 7 May 2010**. Materials for the June meeting will be distributed to the subcommittee on a CD prior to the meeting. The meeting rooms will be equipped with power strips for those who prefer to view the material on their computer instead of printing the material.

- **Adjournment**

Dr. Watts thanked the participants for their attendance and input. The meeting was adjourned at 4:15 pm.

Respectfully submitted,

Marcy Hackenbrack, MCM, M(ASCP), BA
Standards Administrator

cc: John Rex, PhD, FACP
Tracy A. Dooley, BS, MLT (ASCP)