Summary Minutes
Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Hyatt Harboside Hotel
Boston, Massachusetts
15-16 June 2011

A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was held on 15-16 June 2011 in Boston, Massachusetts. The following were in attendance:

Jeffrey L. Watts, PhD, RM (NCRM) **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
Thomas R. Fritsche, MD, PhD	Marshfield Clinic
Henry S. Heine, PhD*	Ordway Research Institute, Inc.
Robert P. Hunter, MS. PhD	Elanco Animal Health
Stefan Schwarz, DVM	Friedrich-Loeffler-Institute (FLI)
Peter Silley, PhD	MB Consult Limited
Ching Ching Wu, DVM, PhD	Purdue University School of Veterinary Medicine

Members Absent

Viginia R. Fajt, DVM, PhD, DACVCP	Texas A & M University
Gary E. Zurenko, MS	Micromyx, LLC

* Participated by Conference Call on 6/16 to Vote on Sponsor Presentations

Advisors Present

Cindy Lindeman, BS	Pfizer Animal Health
Jennifer Lorbach, BS, MBA	Trek Diagnostic Systems
Marilyn N. Martinez, 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

Reviewers Present

Cara Bastulli	Trek Diagnostic Systems
Joshua Hayes, PhD	FDA Center for Veterinary Medicine
Scott B. Killian	Trek Diagnostic Systems
Cynthia C. Knapp, MS	Trek Diagnostic Systems
Ian Morrissey	Quotient Bioresearch Ltd.
David Paisey	Trek Diagnostic Systems, Ltd.
Chris Pillar	Eurofins Medinet
Markus Rose, DVM, PhD.	Intervet Innovation GmbH
Michael T. Sweeney	Pfizer Animal Health
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute

Guests Present

Debora Sweeney	Micromyx, LLC

CLSI Staff Present

Tracy Dooley, BS, MT(ASCP)
Marcy Hackenbrack, MCM, M(ASCP), BA
Claire A. Evans

Opening Remarks

Dr. Watts began the meeting Wednesday, 14 June at 8:30 a.m. He stated that the purpose of Wednesday'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 final working group reports would be presented to the full subcommittee during Thursday's session.

Minutes of Prior Meeting

The minutes of the 6-7 January 2011 meeting held in Orlando were approved by the subcommittee with one minor editorial edit (correct spelling of enrofloxacin on page 4). The final approved version will be posted to the CLSI website on the VAST subcommittee page.

CLSI Document Status Update

M24- A2, *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes* - Published March 2011

M53-A, *Laboratory Testing and Diagnosis of HIV Infections* – Published June 2011

Upcoming Publications

X08-R, *Generation, Presentation and Application of AST Data for Bacteria of Animal Origin*; A Report – estimated for publication July 2011

New Project Proposal for Authorization

Principles and Procedures for Detection of Anaerobes in Clinical Specimens

This document will provide guidance for collecting appropriate specimens from clinical sites; methods for transport that protect anaerobes from oxygen exposure; optimal analytical methods as well as discussion

of the use and value of partial and full isolate identifications. Additional information for interpreting results, understanding the value of rapid preliminary results, and issues of quality control, quality assurance and competency will be reviewed.

This new project is estimated to be authorized for development in late June.

Working Group Reports

Generic Working Group

Working Group Participants – Co-Chairholders Mark Papich and Ching Ching Wu; Members – Shabbir Simjee, Cindy Lindeman, Virginia Fajt, John Turnidge, Stefan Schwarz, Marilyn Martinez, Tara Bidgood.

1) Proposed Interpretive Criteria for Penicillin G

Dr. Papich presented data for proposed interpretive criteria for penicillin G against bacteria isolates from horses and cattle. Based on attaining at least 90% probability of the targeted MIC value, breakpoint values of 0.5 µg/mL, 1.0 µg/mL, and 2.0 µg/mL (S, I, R) were proposed for 22,000 U/kg IM in horses. Corresponding values for the same dose in cattle were 0.25 µg/mL, 0.5 µg/mL, and 1.0 µg/mL (S, I, R). See below for the proposed for interpretive criteria as it will appear in Table 2A in M31 (**Approved 6-0; 4 absent**):

Antimicrobial Agent	Disk Content	Zone Diameter (mm)			MIC Breakpoint (µg/mL)			Comments
		S	I	R	S	I	R	
β-Lactams/Penicillins								
Penicillin G								**Breakpoint derived from microbiological, pharmacokinetic data (using accepted clinical, but extra-label doses), and pharmacodynamic data. The dose of procaine penicillin G modeled was at a dose of 22,000 U/kg, IM, q24h.**
Horses (respiratory and soft tissue) ***Staphylococcus* species** ***Streptococcus* species**	—	—	—	—	**≤ 0.5**	**1**	**≥ 2.0**	
Cattle (BRD) ***Mannheimia haemolytica*** ***Pasteurella multocida*** ***Histophilus somni***	—	—	—	—	**≤ 0.25**	**0.5**	**≥ 1.0**	**Breakpoint derived from microbiological, pharmacokinetic data (using accepted clinical, but extra-label doses), and pharmacodynamic data. The dose of procaine penicillin G modeled was at a dose of 22,000 U/kg, IM, q24h.**

In Table 1- Penicillin G will be moved from Group B to Group A for cattle and horses. The current listing for penicillin in the gray-shaded table 2B will remain for this upcoming edition of M31(A4). For the next meeting the working group will try to review data to add *Streptococcus equi* interpretive criteria.

Dr. Papich noted that Dr. Fajt will assume the role of Co-chair of the Generic Working Group when he rotates next year to Chairholder of the VAST subcomittee. He also asked for input, especially from the diagnostic laboratories for other drugs currently listed in Table 2B that the working group can look at try to set veterinary-specific breakpoints.

2) Cefoperazone Breakpoints for Bovine Mastitis Pathogens

Based on the recommendations from the January meeting to use the data from his orginal proposal (proposed clinical breakpoints in January that were not approved) to calculate epidemiological cut-off Values (ECVs), Dr. Schwarz presented the following proposed ECVs of cefoperazone for bovine mastitis pathogens:

	MIC (µg/mL)	ZD (mm) 75 µg disk	ZD (mm) 30 µg disk
	S	**S**	**S**
S. aureus	4	≥23	≥18
CoNS	4	≥22	≥20
E. coli	0.5	≥29	≥25
S. agalactiae	0.5	≥23	≥21
S. dysgalactiae	0.5	≥27	≥24
S. uberis	0.5		≥23
~~***S. uberis***~~	~~**4**~~	~~≥20~~	~~≥17~~

The subcommittee approved the proposed ECVs for the MIC and 75 µg disk (**Approved 6-0; 4 absent**) and agreed to table the ECVs proposed for the 30 µg disk until the QC data can be reviewed to see if it is acceptable, as this data is from an old QC study that possibly was only a 6 lab study.

The proposed ECVs for S. uberis at 4 µg, ≥ 20 mm and ≥ 17 mm was not approved due to failure to differentiate wild-type from non-wild-type strains.

That subcommittee agreed that since the X08-R report on *Generation, Presentation and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin* deals with ECVs that once published, a proposal can be submitted to take it to a guideline and further expand the document to include the approved ECVs above.

Editorial Working Group

Working Group Participants – Chairholder Gary Zurenko; Members – Jo Abraham, Steve Yan, Jeff Watts, Mark Papich, Henry Heine, Stefan Schwarz, Maria Traczewski, Ching Ching Wu. Note: Mr. Zurenko will no longer be able to participate on the subcommittee due to other commitments so another Chairholder will need to be selected.

The subcommittee reviewed the additional edits made to the M31 text and tables as follows:

- Sections 6.8 and 12, Detection of Resistant Staphylococci –the subcommittee agreed on the suggested revisions as shown in the meeting materials to be incorporated into the M31 text.
- Section 9.1 Reagents and Materials – a note regarding the addition of 5% sheep blood being added to MHA for testing streptococci by agar dilution will be added at the end of this section.

- Table 1 – add Pradofloxacin and Cefovecin in Group C for dogs and cats; add Cefquinome to Group C for horses and swine; add Kanamycin-cephalexin, cefoperazone, and cefquinome to Group C for bovine mastitis; add Gamithromycin and Cefquinome to Group C for cattle.
- Pull old Table 9D from M31-A3 into the A4 edition and edit. Dr. Watts will assist Ms. Dooley with the necessary edits to this table.
- Pull the cefoperazone MIC and 75 µg disk QC from M100 into M31.

All edits from the meeting will be incorporated into the M31 text and tables and then circulated to the subcommittee for a 3-week review and comment period in an effort to finalize the drafts and prepare them for consensus voting.

For January's meeting - It was suggested that the subcommittee review the antimicrobial agents listed in Table 1, Group D to see if there is human interpretive criteria available, then list in Table 2B (if this table is to remain for the next edition).

International Harmonization Working Group

Working Group Participants – Chairholder Tom Shryock; Members – Peter Silley, Bob Walker, Stefan Schwarz, Jeff Watts, Ruby Singh, Bernd Stephan.

This working group had initiated the project proposal for the development of the X08 report on *Generation, Presentation and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin* that will publish soon as well as a proposal for an M45-like document that a smaller working group has initiated gathering data in support of this project proposal. The working group has also been promoting the use of CLSI methodologies in Europe as part of their harmonization efforts.

At this time, since there are no additional items that need to be addressed by this working group they will disband for now.

M37 Revision Working Group

Working Group Participants – Chairholder Marilyn Martinez; Members - Josh Hayes, Rob Hunter, Cindy Lindeman, Mark Papich, Peter Silley, Shabbir Simjee, Steve Yan.

In continuing the discussions from January's meeting regarding the necessary revisions to M37, Dr. Martinez focused on coming up with a method for estimating CO_{CL}. In the current M37, there is only a single line about how to derive the CO_{CL}. It states:

"CO_{CL} – the "clinical cutoff" value selected by inspecting clinical/microbiological outcome vs MIC from prospective clinical studies."

The subcommittee has found this description is too vague to guide either sponsors or the VAST on appropriate considerations/methods to estimate CO_{CL} so it is important to give guidance in the next edition of M37 for determining (or not) the CO_{CL}.

Discussion:

There are three potential cut off values (COVs) that can be used when setting "S":

- Clinical Cutoff (CO_{CL})

- Pharmacodynamic Cutoff (CO_{PD})
- Epidemiological Cutoff (CO_{WT})

- In January, the working group will provide an updated M37 document (A4) containing the revised discussion of the CO_{PD}.

  With regard to establishing a CO_{CL}, the following data should be submitted to the VAST.

  1. Pre-treatment MIC data for study subjects.

  2. Placebo control data (where overall failure and success rate is recorded). Historical data for untreated animals may not accurately reflect the severity, virulence or general condition of the animals used in the clinical field trial. However, they may be some situations where very strong, repeatable evidence provide the information needed to generate a statistical criterion for estimating CO_{CL}.

  3. Outcomes from the individual clinical trial locations should be provided to the subcommittee to determine if the data can be pooled for statistical analysis (i.e., the success rate should be similar across the MIC values to be pooled across locations. If there are differences, potential reasons for these discrepancies may need to be discussed prior to pooling).

  4. With regard to pooling data across MIC values to increase numbers, pooling should only be done if:
     - Each pooled MIC value has an N value of 4 or less.
     - The upper MIC value with which the samples are to be pooled achieves significant clinical response without pooling.

- In a perfect world where there are clinical data generated on thousands of animals, the CO_{CL} and CO_{PD} should be similar if the CO_{PD} is accurately estimated (i.e., based upon the appropriate therapeutic goals and accommodates population variability in drug exposure). Therefore, it is important to carefully consider the factors influencing the PD target which will be used for target attainment.

  However, in some situations, the estimation of a CO_{CL} may not be feasible. When this occurs, the distribution of MIC values obtained during the clinical trial should nevertheless be submitted to facilitate the VAST deliberation of "S". However, this demonstration of a relationship between MIC and clinical outcome should not be confused with CO_{CL} because:

  1. Without information on the spontaneous cure rate, the VAST cannot statistically evaluate the relationship between MIC-associated clinical outcomes versus spontaneous cures (based upon data generated with the untreated (placebo) control).

  2. In cases where only post-treatment MIC values are available, we cannot ascertain if a patient was originally infected with a pathogen presenting with a lower MIC but experience an increase in pathogen MIC value during the course of therapy. However, knowing the relationship between cure and MIC can be valuable information during the assessment of "S" based upon CO_{WT} and CO_{PD}. For example, pre-treatment MIC data are generally not available for pigs and poultry (but are available for bovine foot rot, mastitis, and BRD, dog and cats (horse?).

BETWEEN 1 AND 2:	If > 1 DD diff	If 1 DD diff		If > 1 DD diff	If 1 DD diff
WT>PD>CL	PD	WT	WT>PD	PD	WT
WT>CL>PD	CL	WT	WT>PD		
PD>WT>CL	WT	WT	PD>WT	WT	PD
PD>CL>WT	CL	CL	PD>WT		
CL>WT>PD	WT	CL	WT>PD	SEE ABOVE	SEE ABOVE
CL>PD>WT	PD	PD	PD>WT	SEE ABOVE	SEE ABOVE
WT=PD>CL	WT	WT	WT=PD	WT	WT
PD=CL>WT	PD = CL	PD = CL	PD>WT	SEE ABOVE	SEE ABOVE
WT>PD=CL	PD = CL	WT	WT>PD	SEE ABOVE	SEE ABOVE
PD>WT=CL	WT=CL	WT=CL	PD>WT	SEE ABOVE	SEE ABOVE
CL>WT=PD	WT=PD	CL	WT=PD	SEE ABOVE	SEE ABOVE
WT=PD=CL	WT	WT	WT=PD		
we need examples					

For January the working group will also update the current flowcharts in M37and add examples that can be put in an appendix.

M45-Like Document Update

Working Group Participants: Chairholder, Maria Traczewski, Co- Chairholder, Michael Sweeney: Members- Donald Bade, Tom Fritsche, Brian Lubbers, Patrick McDonough

In follow-up to the recommendation from the Microbiology Consensus Committee, an informal working group was formed in January and they initiated some of the upfront work (eg, determine the pathogens to be addressed, review the methods from literature) that could be included in an M45-like document. Based on the list of pathogens the subcommittee recommended to start with those organisms that have existing methodologies and data for the initial document to be developed. Additional organisms that would take more time to do literature searches or studies can be added as supplemental tables at a later time.

Ms. Traczewski will work with Ms. Dooley to provide a summary of the path forward for the development of a document including a list of the pathogens that will be focused on. The original project proposal will be updated to reflect this and will be re-submitted to the Consensus Committee to request approval to move forward as a new project.

Veterinary Mycoplasma Working Group

Working Group Participants –Chairholder Ching Ching Wu; Members – Joann Kinyon, Cecile Bebear, Mary Brown, Don Bade, Lynn Duffy, Roger Ayling, Ken Waites

The subcommittee agreed that the work of this group will fall under the new M45-like document once the project is approved.

There were no updates of the mycoplasma working group at this time.

Presentations

QC Ranges for Microdilution Susceptibility Tests for Tildipirosin (formerly PMT-Macrolide)

Dr. Brown presented quality control study data for MIC testing of Tildipirosin against *E. coli* ATCC® 25922, *S. aureus* ATCC® 29213, *A. pleuropneumoniae*, ATCC® 27090, and *H. somni* ATCC® 700025. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (µg/mL)	Vote
E. coli ATCC® 25922	1-8 (100%)	**Approved 7-0; 1 abstain, 2 absent**
S. aureus ATCC® 29213	2-16 (99.5%)	**Approved 7-0; 1 abstain, 2 absent**
A. pleuropneumoniae, ATCC® 27090	2-16 (87.8%)	**Approved 7-0; 1 abstain, 2 absent**
H. somni ATCC® 700025	2-8 (96.0%)	**Approved 7-0; 1 abstain, 2 absent**

Numbers in parenthesis denote the % of MICs within the proposed control limits

Add in Glossary 1: Antimicrobial class – Macrolides: 16-membered rings
Add in Glossary 2: Abbreviation – TIP
Add in Table 8, Solvents and Diluents- Solvent- 0.1 mol/L phosphate buffer pH 6.0 (33% v/v)
Diluent - 0.1 mol/L phosphate buffer pH 8.0 (67% v/v)

QC Ranges for Microdilution Susceptibility Tests for Narasin

Dr. Brown presented quality control study data for MIC testing of Narasin against *S. aureus* ATCC® 29213 and *E. faecalis* ATCC® 29212. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (µg/mL)	Vote
S. aureus ATCC® 29213	0.25-2 (99.5%)	**Approved 6-0; 2 abstain, 2 absent**
E. faecalis ATCC® 29212	0.25-1 (100%)	**Approved 6-0; 2 abstain, 2 absent**

Numbers in parenthesis denote the % of MICs within the proposed control limits

Narasin is currently listed in Glossary 1 and 2 as well as Table 8.

Note: Narasin is currently approved for use as an anti-coccidial agent in animals. Because of the antibacterial activity for this drug, the sponsor is anticipating future studies along these lines and wanted to have QC controls for this purpose.

QC Ranges for Microdilution Susceptibility Tests for Monensin

Dr. Brown presented quality control study data for MIC testing of Monensin against *S. aureus* ATCC® 29213 and *E. faecalis* ATCC® 29212. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (µg/mL)	Vote
S. aureus ATCC® 29213	2-16 (100%)	**Approved 6-0; 2 abstain, 2 absent**
E. faecalis ATCC® 29212	4-16 (98.75%)	**Approved 6-0; 2 abstain, 2 absent**

Numbers in parenthesis denote the % of MICs within the proposed control limits

Monensin is currently listed in Glossary 1 and 2 as well as Table 8.

QC Ranges for Disk Diffusion Testing of Gamithromycin for the Treatment of Bovine Respiratory Disease

Dr. Pillar presented quality control study data for disk diffusion testing of gamithromycin (15 µg) against *A. pleuropneumoniae* ATCC® 27090 and *H. somni* ATCC® 700025. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (mm)	Vote
A. pleuropneumoniae ATCC® 27090	14-19	**Approved 8-0; 2 absent**
H. somni ATCC® 700025	18-29	**Approved 8-0; 2 absent**

Plans for Next Meeting

The next meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing will be scheduled as a two-day meeting on Thursday, 19 January and Friday, 20 January 2012 in Tempe, Arizona.

The submission deadline for the January meeting will be **Friday, 2 December 2011**. Materials for the January 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 11:25 a.m.

Respectfully submitted,

Tracy Dooley, BS, MT (ASCP)
Standards Administrator