Summary Minutes
Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Atlanta Marriott Suites Midtown
Atlanta, Georgia
16-17 June 2010

A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was held on 16-17 June 2010 in Atlanta, Georgia. The following were in attendance:

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
Rob 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
Gary E. Zurenko, MS	Micromyx, LLC

Members Absent (with notice)

Henry Heine, PhD	Ordway Research Institute, Inc.

Advisors

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
Clyde Thornsberry, PhD	Eurofins Medinet
John Turnidge, MD	Women's and Children's Hospital

Observers Present

Maureen K. Davidson, PhD	FDA Center for Veterinary Medicine
Charles Gieseker, MS	FDA Center for Veterinary Medicine

Joshua Hayes, PhD	FDA Center for Veterinary Medicine
Daniel J. Keil, DVM, PhD, DACVM	Bayer Healthcare – Animal Health
Cynthia C. Knapp, MS	Trek Diagnostic Systems
Maureen Mansfield	Trek Diagnostic Systems
Lori T. Moon, MT(ASCP)	MSU Diagnostic Ctr. for Population & Animal Health
Ian Morrissey	Quotient Bioresearch Ltd.
Amy Omer	FDA Center for Veterinary Medicine
Chris Pillar	Eurofins Medinet
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute

CLSI Staff Present

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

Opening Remarks

Dr. Papich began the meeting Wednesday, 16 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 26-27 January 2010 meeting held in Tampa had been approved by electronic comment and vote by the subcommittee prior to the meeting. The final version was included in the meeting materials and will be posted to the CLSI website on a page specific for the VAST subcommittee that is being created at this time.

Working Group Reports

Generic Working Group

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

Dr. Mark Papich outlined the current objective of the working group which is to propose chloramphenicol interpretive criteria for dogs. Data used to determine breakpoints include microbiological data (MIC data only) obtained from Ohio State, Pfizer, German study (S. Schwarz), other published studies (Perreten et al 2010), and PK-PD data, indications, doses from sponsors label (Chloromycetin tablets, Ft. Dodge; NADA 055-051) and published literature.

Based on the obtained data the subcommittee agreed to add a comment in Table 2B (human derived interpretive criteria table) to the chloramphenicol listing for Organisms other than streptococci currently in the table as follows:

MIC distribution of canine isolates support these breakpoints for use in canine skin and soft tissue infection; however, efficacy data and pharmacokinetic-pharmacodynamic (PK-PD) targets were unavailable. **Approved 8-0; 2 absent.**

Other antimicrobial agents that the working group will look into obtaining data and setting veterinary-specific breakpoints for in the future include:

- Penicillin G for cattle and horses
- Ampicillin for cattle
- Trimethoprim- sulfamethoxazole

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.

Dr. Martinez outlined the charge of the working group to identify those aspects of the existing M37 document that needs revision, clarification, or refinement.

Some revision recommendations of the Working Groups include:

- The three-pronged approach has demonstrated weakness:

  - Rarely get CO_{CL} information, and therefore in most cases, the subcommittee's deliberations have focused primarily on CO_{WT} and CO_{PD}. Therefore, should CO_{CL} be omitted from deliberations, or should we indicate conditions under which CO_{CL} will be considered.
  - If the subcommittee keeps CO_{CL} in the algorithm, we need to improve the clarity regarding how CO_{CL} is actually established across the various kinds of studies submitted for veterinary species and when CO_{CL} estimates will not be feasible.
  - Could keeping it "optional" be used inappropriately as a mechanism for biasing the breakpoint (i.e., include when it helps, omit when it suggests higher breakpoint values).

- We need to improve our discussion of CO_{PD} – questions to consider:

  - Types of studies (or published data) that is needed to address these questions.
  - What "benchmark" values should be used when such studies are not available? Consideration should be given to all drug classes.
  - Need separate section discussing drugs where tissue concentrations differ from blood (e.g., macrolides, tetracyclines and pleuromutilins).
  - The subcommittee needs to more clearly define how much deviation we are willing to accept between the cutoff values when determining S.

- Consider replacing the current flow chart with the following table that was recommended by Dr.Turnidge:

					Interpretation
Wild-type	>	PD	>	Clinical	(COwt)* (COcl) - how far is too far apart?
Wild-type	>	Clinical	>	PD	?
PD	>	Wild-type	>	Clinical	?
PD	>	Clinical	>	Wild-type	?
Clinical	>	Wild-type	>	PD	?
Clinical	>	PD	>	Wild-type	?
Wild-type	=	PD	>	Clinical	?
Wild-type	=	Clinical	>	PD	(?
PD	=	Clinical	>	Wild-type	(COcl)**
Wild-type	>	PD	=	Clinical	?
PD	>	Wild-type	=	Clinical	?
Clinical	>	Wild-type	=	PD	?
Wild-type	=	PD	=	Clinical	any of them!

- Need to clarify claims: clearly state that there is no difference in breakpoints for treatment and control claims. All breakpoints are based upon "treatment", which includes both therapy (administration of animals with frank clinical disease) and control.

- Need to provide a definition of treatment or therapy vs. control (metaphylaxis) vs. prevention (prophylaxis). Possibly describe differences in how these terms may be applied to herd vs. individual patient therapy, recognizing the difference in the management of companion and food-producing animals.

- Suggested revisions to Section 4.2.2 (from Dr. Hayes):

  - "When using broth dilution, 10 replicates of each QC strain should be tested over a minimum of 3 days with a maximum of 4 replicates per day. Each replicate should use individually-prepared inoculum suspensions. This results in 70 data points for each individual media lot and 210 total data points for each QC strain. The same principles should be used when other media are required (e.g., fastidious or anaerobic organisms)."
  - "When using agar dilution, 10 replicates of each QC strain should be tested each day for a minimum of 2 days. Each replicate should use individually-prepared inoculum suspensions. This results in 140 data points for each individual media lot and 420 total data points for each QC strain. All 10 replicates of each strain can be inoculated onto the same set of agar dilution plates."

- Clarify Section 5.2 – in discussing development of disk breakpoints the Working Group questioned the need to retest isolates by MIC since this was already done previously. Also how many isolates need to be tested or can a subset be tested. M23 says 500 isolates – how are these isolates selected – randomly or by MIC? The Working plans to review this section and see if clarification is needed.

Path Forward – the working group plans to develop an initial draft of the proposed modifications for review in 2011. The working group will also review current definitions in M31 to see if any changes are needed and will contact industry and regulatory authorities to insure acceptance of proposed revised definitions.

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.

Mr. Zurenko reviewed the changes made and/or to be done to the M31 text and tables including:

M31 text:

- Foreword will be updated by Dr. Watts
- Section 6.8.1.3 – added text regarding *S. aureus* interpretive criteria should only be used for strains of *S. aureus* and not for other coagulase-positive staphylococci isolated from veterinary sources such as *S. pseudintermedius.*

M31 tables:

- Change current comment – "Information in boldface type is considered tentative for one year" to read "Information in boldface type is new or modified since the previous edition". Review all tables to ensure comment appears where appropriate.
- Table 2B – subcommittee agreed to retain gray shading and draft a comment to be added under General Comments stating that the user should refer to Table 2A first and if veterinary specific interpretive criteria is not available then refer to Table 2B. Also note that the laboratory should inform the clinician of which species the interpretive criteria were derived from (eg, dog, cat, human).
- Table 4A – add the temperature and time for *Campylobacter* (36 °C for 24 hours).

All changes will be incorporated into the documents and circulated to the Editorial Working Group to review in preparation to finalize the documents and submit them for vote.

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

Dr. Wu outlined preliminary data showing the comparison of 2 methodologies – phenol red method and HBAN method. Both methods outlined below are reproducible but different MICs are obtained with the 2 methods.

- Phenol red method
  - Requires three to five days incubation
  - Used for multiple *Mycoplasma* spp.
  - End point inoculum determination/multiple test sets—can be improved overtime
  - Cost/time/training required

- HBAN method
  - Overnight incubation
  - Ease of use/minimum training
  - Not for multiple species (*M. bovis* only)

Recommendations going forward:

- Important to have one consistent method.
  - Endpoint should be consistent
  - Better to get the method right rather than having a method out there.
- Test combinations of media
  - Phenol Red Broth base with AlamarBlue
  - HBAN and growth of other *Mycoplasma* spp
  - Other possible ingredients from published papers
- Actual MIC value is not of great concern
  - May be perception by some groups that high MIC correlates with resistance, regardless of breakpoints.
- Stability of compounds needs addressed
  - Incubation time should be minimized
  - pH change may be problematic for some drugs.

Dr. Wu will provide further updates of the Working Groups progress at the next meeting of the subcommittee.

International Harmonization Working Group

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

Dr. Shryock presented a proposal to develop a new guideline that would address additional pathogens and antibiotics not currently addressed in M31. This document would be similar to the M45 document - *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently-Isolated or Fastidious Bacteria* developed by the human AST subcommittee.

Currently, veterinary clinical breakpoints are established by VAST subcommittee approval, following the M37 guideline. Many additional pathogens, antibiotics and susceptibility test methodologies or interpretations cannot be included in M31 because they do not conform to the M37 guideline. This new guideline would address this gap.

The subcommittee reviewed the proposal and agreed that this should be developed by a formal Working Group under the VAST subcommittee (**Approved 8-0; 2 absent**).

Education Working Group

Working Group Participants – Chairholder Virginia Fajt; Members – Mike Apley, Bob Badel, Jennifer Lorbach, Tom Shryock, Ching Ching Wu.

Dr. Fajt provided an overview of the ongoing efforts of the working group. Currently they are working on 2 articles that will be targeted to well read journals (eg, JVDI):

Article 1 – Recommendations for Researchers detailing the use of CLSI Veterinary standards.

Dr. Fajt went through the outline of the sections for this article (*attached at the end of these minutes as Appendix A*) requesting that **anyone interested in writing a section that has not been assigned to an author yet to please contact her (see Appendix A for highlighted Sections that still need authors)**.

Article 2 – Guidelines for Clinical Use detailing how to use and implement the VAST documents.

Presentations

QC Ranges for Disk Diffusion Testing of Kanamycin-cephalexin for the Treatment of Bovine Mastitis

Dr. Pillar presented Tier 2 quality control study data for disk diffusion testing of kanamycin/cephalexin (30 µg/15 µg) against *S. aureus* ATCC® 25923, *E. coli* ATC® 25922 and *S. pneumoniae* ATCC® 49619. Based on the data presented, the following QC ranges were proposed:

Organism	Proposed QC range (mm)	Vote
S. aureus ATCC® 25923	19-25	**Approved 8-0; 2 absent**
E. coli ATCC® 25922	19-25	**Approved 8-0; 2 absent**
S. pneumoniae ATCC® 49619	13-20	**Approved 8-0; 2 absent**

Information Generated Regarding MIC Testing of *Haemophilus parasuis*

Mr. Bade summarized testing conducted to find appropriate media for broth microdilution testing of *H. parasuis.* In the past, there has been difficulty with growth of *H. parasuis* in broth microdilution MIC testing with VFM and HTM.

EUCAST has developed a media designated MH-F for use in testing human isolates of fastidious organisms, such as *Haemophilus* species and streptococci and is currently validating MH-F suitability in testing *H. influenzae.*

Preliminary testing was conducted to see if MH-F could potentially be suitable for *H. parasuis.* VFM media was also used. The testing procedure outlined in M31-A3 for *H. somni* and *A. pleuropneumoniae* was followed. Testing using 10 clinical isolates of each organism - *H. parasuis, A. pleuropneumoniae,* and *H. somni* against various antimicrobial agents was conducted. Results showed the following:

- Of the 10 isolates tested, only two grew in VFM but all grew very well in MH-F.
- There were some unusual growth patterns with ceftiofur and cefquinomein MH-F. Problem with cephalosporins?
- Four isolates were tested comparing results (growth and MIC) in an aerobic and CO_2 environment.There was no visual difference of growth aerobically versus CO_2
- One isolate showed unusual growth patterns in ceftiofur, cefquinome and penicillin.
- MIC results comparable (±1 DD) for all except TIL (CO2 increase by 2 DD) and TUL (CO_2 increased by 3-4 DD).

Identification and Characterization of Methicillin-resistant Coagulase-negative staphylococci from Bovine Mastitis

Dr. Schwarz provided an overview of testing conducted over the last year to evaluate the correlation between phenotypic and genotypic tests for the correct assessment of methicillin resistance among coagulase-neative staphylococci (CoNS) obtained from dairy cattle. A total of 121 CoNS from cases of bovine mastits were tested for oxacillin susceptibility by disk diffusion and broth microdilution. Isolates classified as methicillin-resistant by either method were then tested by PCR for the *mecA* gene and the SCC*mec* type. The cefoxitin disk test was also applied. Pulsed-field gel electrophoresis was used to determine the genetic relationships of the resistant isolates.

Final results of the study indicated that isolates of CoNS with oxacillin MICs of 0.5 and 1 mg/L should be confirmed for the presence of *mecA* before reporting them as methicillin-resistant. To address this in M31 **the following was proposed to be added to Table 2B for Oxacillin/*Staphylococcus* spp.:**

Oxacillin interpretive criteria may overcall resistance for some coagulase-negative staphylococci from bovine mastitis because some strains for which the oxacillin MICs are 0.5 to 1 µg/mL lack *mecA*. Testing for *mecA* or for PBP 2a is recommended for strains for which the oxacillin MICs are 0.5 to 1 µg/mL before reporting complete beta-lactam resistance. (**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, 6 January and Friday, 7 January 2011 in Orlando, Florida.

The submission deadline for the January meeting will be **Wednesday, 1 December 2010**. 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 12:00 p.m.

Respectfully submitted,

Tracy Dooley, BS, MT (ASCP)
Standards Administrator

APPENDIX A. Education Working Group Article 1 Section Outline

Authors needed for the Sections highlighted below.

Section 1: Recommendations for Researchers - PICK ONE OR MORE SECTIONS TO REVIEW AND ADD TO/FLESH OUT (more than one author can pick a section):

B. Recommendations for selecting antimicrobials to test: 1. Selecting drugs used in animals vs. drugs used in humans (**MIKE SWEENEY, CHING CHING WU, MARK PAPICH**)

B. Recommendations for selecting antimicrobials to test: 2. Selecting class representatives (explanation of what that means and then how to decide to select one or more within a class) (**MIKE SWEENEY, CHING CHING WU, MARK PAPICH**)

C. Recommendations for selecting human or veterinary breakpoints (differences between them, full-range vs. concentrations close to breakpoints) (**MARK PAPICH**)

D. Recommendations for selecting testing methodology: 1. Importance of standardized methods (**tentatively RON MILLER**)

D. Recommendations for selecting testing methodology: 2. Options for methods (see text for outline here) (**tentatively RON MILLER**)

D. Recommendations for selecting testing methodology: 3. Importance of using updated standards (**tentatively RON MILLER**)

E. Recommendations for summarizing results: 1. Using percent susceptible or number of drugs susceptible

E. Recommendations for summarizing results: 2. Including actual breakpoints in manuscript

E. Recommendations for summarizing results: 3. Pros and cons of combining intermediate with S or R

F. Recommendations for interpreting results: 1. Comparing results with other studies (**tentatively ROB HUNTER**)

F. Recommendations for interpreting results: 2. Making predictions about clinical success (**tentatively ROB HUNTER**)

F. Recommendations for interpreting results: 3. MORE IDEAS NEEDED HERE ON INTERPRETATION