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**Summary Minutes
Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Hyatt Harborside Hotel
Boston, Massachusetts
30 June-1 July 2009**

A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing was held on 30 June -1 July 2009 in **Boston, Massachusetts**. The following were in attendance:

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

Pfizer Animal Health

Mark G. Papich, DVM, MS

North Carolina State University

Members Present

Donald Bade
Steven D. Brown, PhD
Virginia R. Fajt, DVM, PhD, DACVCP
Henry Heine, PhD
Rob P. Hunter, MS, PhD
Stefan Schwarz, PhD
Peter Silley, PhD
Ching Ching Wu, DVM, PhD

Gary E. Zurenko, MS

Microbial Research, Inc.
The Clinical Microbiology Institute
Texas A & M University
USAMRIID
Elanco Animal Health
Friedrich-Loeffler-Institute (FLI)
MB Consult Limited
Purdue University School of Veterinary
Medicine
Micromyx, LLC

Members Absent (with notice)

Dik J. Mevius, DVM, PhD

Central Veterinary Institute (Netherlands)

Advisors

Cindy Lindeman, BS
Jennifer Lorbach, BS, MBA
Marilyn N. Martinez, PhD
Thomas R. Shryock, PhD
Shabbir Simjee, PhD
John Turnidge, MD
S. Steve Yan, PhD

Pfizer Animal Health
Trek Diagnostic Systems
FDA Center for Veterinary Medicine
Elanco Animal Health
Elanco Animal Health
Women's and Children's Hospital
FDA Center for Veterinary Medicine

Observers Present

Brent Herrig
Elizabeth Johnson
Scott B. Killian
Cynthia C. Knapp, MS

Intervet, Inc.
Putney, Inc.
Trek Diagnostic Systems
Trek Diagnostic Systems

Maureen Mansfield
Ron A. Miller, PhD
Ian Morrissey
Helio Sader, PhD
Jennifer J. Spokes
Maria M. Traczewski, BS, MT(ASCP)

Trek Diagnostic Systems
FDA Center for Veterinary Medicine
Quotient Bioresearch Ltd.
JMI Laboratories
Putney, Inc.
The Clinical Microbiology Institute

CLSI Staff Present

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

Welcome/Introductory Remarks

Dr. Watts opened the meeting on Tuesday, 30 June 2009 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 working group reports would be presented to the full subcommittee during Thursday's session.

Dr. Watts stated that with the absence of one voting member (Dr. Mevius), the 9 & 1 voting rules would apply. He also briefly reviewed the changes to the subcommittee membership including the resignation of Dr. Robert Walker, the addition of Dr. Stefan Schwarz as a voting member and Dr. Shabbir Simjee as an advisor.

Minutes from Previous Meeting

The subcommittee voted to approve the summary minutes from the 8-9 January 2009 meeting held in San Diego, California (**Approved 9-0; 1 absent**).

Document Status Update

1. Recently Published Documents

M39-A3, *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline*. Published February 2009

2. Upcoming Publications

M51-P, *Method for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Proposed Guideline* and corresponding M51-S1, *Informational Supplement*. Both documents will be published the end of June 2009.

M44-A2, *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition* and corresponding M44-S3, *Informational Supplement*. Both documents are estimated for publication in August/September 2009.

X7-R, Surveillance for Methicillin-Resistant *Staphylococcus aureus*: Principles, Practices, and Challenges; A Report. Document is estimated for publication in September 2009.

3. New Projects Under Development

M52-P, *User Verification 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, *Criteria for Laboratory Testing and Diagnosis of HIV-1 Infections*
Chairholder: Eric Rosenberg, MD

This document will address issues related to the sensitivity and specificity of the various methods used in HIV-1 testing, selection of appropriate testing algorithms for HIV screening and confirmatory testing, and develop interpretive criteria to assist in the diagnosis of HIV-1 infection.

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

This project was re-authorized in March. 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

This project was just re-authorized June 3rd. The document will provide standardized methods for broth microdilution and agar dilution-based susceptibility testing of human mycoplasmas.

4. Documents Currently Under Revision

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

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

M45-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-S20, *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*
Chairholder: Franklin R. Cockerill, III, MD
Text and Tables Chairholder - Jana M. Swenson, MMSc

M42-A2, *Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals* and M49-A2, *Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals*
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

6. New Project Under Consideration

Specimen Collection, Handling, Processing, Set-up and Interpretation of Fungal Cultures

The intent of this document will be the creation of a standard for 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.

The subcommittee is being selected at this time.

AST Liaison Report

Dr. Watts (for Dr. Walker) provided an update on the activities of CLSI and the AST subcommittee as it relates to the VAST subcommittee. The main points are listed below.

- It was decided that when documents are revised, the changes will be published in the document; therefore, it will be necessary to track all document changes. Ms. Dooley will keep track of all document changes and rationale for changes must be recorded in the working group minutes.
- The AST subcommittee decided to re-define the term “non-susceptible” for compounds for which no known resistance has been detected.
- The text and tables working group will be developing therapy related comments for certain generic drugs to be included in the next version of M100. It was agreed that the VAST working group would work on similar comments for the next meeting.

Dr. Heine agreed to act as the AST liaison for the next AST subcommittee meeting.

Presentations

“Interpretive Criteria for Kanamycin and Cefalexin Interpretive Criteria for Target Mastitis Pathogens”

On the behalf of the sponsor, Boehringer Ingelheim, Dr. Chris Pillar presented data in accordance with the M37-A3 guidelines to support the establishment of interpretive criteria for determining the susceptibility of indicated target mastitis pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*) to kanamycin and cefalexin in combination (Ubrolexin). He stated that the sponsor proposed that the breakpoints be set at the following:

MIC

(kanamycin/cefalexin – ratio 10:1) S = \leq 8/0.8 μ g/mL
I = 16/1.6 μ g/mL
R = \geq 32/3.2

Disk Diffusion

(kanamycin 30 µg/cefalexin 15 µg) S = ≥ 20
I = 18-19
R = ≤ 17

The subcommittee agreed that more data was required before the interpretive criteria could be determined. The subcommittee requested that the sponsor return to the subcommittee with the following:

- information on cure rates related to MIC;
- more information on the clinical trials including the number of weeks of specimen collection and procedure for sample collection;
- time-kill data;
- tier II and III quality control data;
- pharmacodynamic data not included in the presented algorithm;
- information on the criteria and regimen for clinical cure;
- total residue data (if available);
- data showing bacterial eradication and clinical response;
- synergy reports;
- a representative of the sponsor who is close to the trial and can answer subcommittee questions; and
- all information as outlined in Section 5 of M37-A3.

It was agreed that the subcommittee will table the discussion until the January meeting at which time the data will be presented again with the above requested information.

“Antimicrobial Susceptibility Testing Conditions for Clinical Isolates of *Pasteurella multocida* and *Mannheimia haemolytica*”

Ms. Maria Traczewski presented the results of the study performed by The Clinical Microbiology Institute. The purpose of the study was to determine whether cation-adjusted Mueller Hinton broth (CAMHB) or cation-adjusted Mueller Hinton broth with 3% lysed horse blood (LHB) is the optimal media for broth microdilution testing of *Pasteurella multocida* from various animal species and *Mannheimia haemolytica* from cattle. The conclusions of the study were presented and are listed below.

- The majority of strains of *P. multocida* and *M. haemolytica* grew in both media tested.
- The majority of *P. multocida* strains that failed to grow in CAMHB were isolated from dogs. All strains were tested for confirmation of identification. It was confirmed that most were not strains of *P. multocida*. It was suggested that isolates from dogs presumed to be *P. multocida* and fail to grow in CAMHB should be fully identified or the identification should be confirmed.
- MICs tended to be slightly higher for all antimicrobials when tested in LHB as compared to CAMHB; however, agreement was generally comparable.

The subcommittee agreed on the following items:

- A comment will added to either the broth dilution section or section 11.1 in M31 which states that if *P. multocida* does not grow in CAMHB, the identification should be repeated/checked and the test repeated with LHB.
- A technical note may be published to disseminate the information. Ms. Traczewski will discuss the issue with Bob Walker.
- Ms. Traczewski will author a comment for the text and tables working group regarding “no growth” in CAMHB of presumed *P. multocida*.

- A synopsis of the study will be forwarded to Dr. James Jorgensen, the chairholder of the M45 Working Group (*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*).

Working Group Updates

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. Papich presented four issues for review and discussion by the subcommittee. The issues and the results of the discussions are listed below.

1. Proposed Breakpoints for First Generation Cephalosporins for Table 2 of M31-A3.

Dr. Papich briefly reviewed the data originally presented and voted upon during the January 2009 meeting. He reminded the participants that the subcommittee had requested a review of the statistical data prior to finalizing the breakpoints. Dr. Martinez presented the statistical data (Monte Carlo simulations). Based on the data, a motion was made to accept the breakpoints that were originally proposed (S = ≤ 2 $\mu\text{g/mL}$, I = 4 $\mu\text{g/mL}$ and R = ≥ 8 $\mu\text{g/mL}$). (**Approved 9-0; 1 absent**)

2. Oxacillin Breakpoints for *Staphylococcus pseudintermedius*

Dr. Schwarz presented a study designed to support the recommendation to evaluate methicillin resistance in *S. pseudintermedius* by using the breakpoints for *Staphylococcus* spp. instead of those for *S. aureus*. The conclusions of the study were as follows:

- Oxacillin disk diffusion (R ≤ 17 mm) and MIC breakpoints (R ≥ 0.5 $\mu\text{g/mL}$) applicable to *Staphylococcus* spp. are accurate indicators of *mecA*-mediated resistance in *S. pseudintermedius*.
- Interpretive criteria applicable to *S. aureus* failed to detect a considerable number of *mecA*-positive *S. pseudintermedius* strains.

Based on the presented data, a motion was made to leave the breakpoints in Table 2 of M31-A3 as they are but re-word the comment to read, “*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*.” (**Approved 9-0; 1 absent**)

A motion was also made to remove Table 9D and add a footnote to Table 2 stating that cefoxitin breakpoints are not predictive of *mecA* resistance to oxacillin in *S. pseudintermedius*. (**Approved 9-0; 1 absent**)

3. Interpretive Criteria for Amoxicillin/Clavulate with Canine Isolates

Dr. Papich presented data to justify setting MIC interpretive criteria for use of amoxicillin/clavulate against bacteria in dogs for skin and soft tissue. These criteria will be added to Table 2 of M31-A3.

Based on the data presented, a motion was made to set the breakpoints as (amoxicillin/clavulate):

S = $\leq 0.25/0.12$ $\mu\text{g/mL}$

I = $0.5/0.25$ $\mu\text{g/mL}$

R = $\geq 1/0.5$ $\mu\text{g/mL}$

The comment will change to read “Amoxicillin-Clavulanate breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and pharmacokinetic-pharmacodynamic (PK-PD) analysis of amoxicillin **in dogs**. The dosage regimen used for PK-PD analysis of amoxicillin was 11 mg/kg administered every 12 hours orally.” The grayed area will stay the same. **(Approved 9-0; 1 absent)**

4. Interpretive Criteria for Amoxicillin with Porcine Isolates

Dr. Papich and Dr. Schwarz presented data to determine breakpoints for amoxicillin against bacteria in pigs. These breakpoints would apply to intra-muscular (IM) injection only.

Based on the data provided, the following was proposed:

Breakpoints (IM only)

S = $\leq 0.5 \mu\text{g/mL}$

I = $1 \mu\text{g/mL}$

R = $2 \mu\text{g/mL}$

The following comment will be added to Table 2 of M31-A3, “Ampicillin is used to test for susceptibility to amoxicillin and hetacillin.” For swine breakpoints, the comment will be changed to read, “Breakpoint derived from microbiological data using ampicillin, pharmacokinetic data from a dose of 15 mg/kg IM of amoxicillin once daily, and pharmacodynamic data.” **(Approved 9-0; 1 absent)**

Dr. Papich will provide the appropriate changes in the comments to the Editorial working group.

5. Revision of Breakpoints for Tetracycline

It was decided that the breakpoints for tetracycline will not change; therefore, there was no further discussion.

International Harmonization Working Group

Working Group participants—Chairholder Tom Shryock; Members—Peter Silley, Dik Mevius, Stefan Schwarz, Jeff Watts, Ruby Singh, Bernd Stephan

Note: Dr. Brown left the meeting to attend to other obligations. Voting rules for 8-0; 2 absent in place.

Dr. Shryock presented a project proposal for review. The project, a report tentatively titled, “CLSI Perspectives on the Generation, Presentation and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin” would provide guidance on generating antimicrobial resistance surveillance reports for antimicrobial activity against bacteria of animal origin. The report would be globally applicable and would provide an authoritative source for information on breakpoints, epidemiologic cut-off values, various applications and regulatory documents.

A motion was made to approve the development of the project as proposed. **(Approved 8-0; 2 absent)**

Editorial Working Group

Working Group participants—Gary Zurenko, Chair, Members—Jo Abraham, Melanie Berson, Henry Heine, Mark Papich, Stefan Schwarz, Maria Traczewski, Steve Yan, Bob Walker, Jeff Watts, Ching Ching Wu

Dr. Zurenko presented changes to be proposed for changes to M3-A3. The group discussed the changes as distributed electronically to the group. He indicated that the deadline for all final changes is June 2011.

- **Update of M31, Glossary 1 (resistance mechanisms)**

Dr. Zurenko asked the group to review the circulated glossary with the yellow highlighted areas. He asked the group to submit any additional changes by June 2011 for inclusion in the next edition.

- **Update of M31, Table 3 (QC cultures)**

The draft incorporating changes including the addition of *S. aureus* ATCC® BAA977 and ATCC® BAA976 has been circulated. It was suggested that the table be reformatted to include more recent QC data and that other commercial sources of QC organism suppliers be added. The protocols for how the data was collected will be reviewed.

- **Addition of text from M7-A8 regarding detection of inducible clindamycin resistance**

The draft pages relevant to M31 have been distributed. Dr. Zurenko asked the group to review the changes to the comment section and submit any additional changes. Dr. Yan will follow-up on the original work and applications and Ms. Knapp will review the strains. The outcome of the Working Group session will be a final version to be proposed for Subcommittee approval.

- **Clarification of text in M31 regarding reporting oxacillin resistant results**

It was agreed that the text will be altered to match the text proposed by Dr. Schwarz during his presentation. Table D will be eliminated. The table will be voted on once it has been finalized.

- **Addition of Appendix table to outline inducible clindamycin resistance assays**

It was agreed that Table 9G- Screening Tests for Inducible Resistance to Clindamycin will be added to M31-A3. Appropriate text regarding the growth of *P. multocida* in CAMHB will be provided by Ms. Traczewski.

- **Consideration for animal specific versions (companion vs food animals) of Table 2 including the removal of the “gray” breakpoint information where appropriate**

Dr. Zurenko presented a “mock-up” of tables separated by type of animal. This will allow for the removal of the “gray” breakpoints associated with humans. It was agreed that this approach will create new issues and confusion for the reader.

The working group recommended that Table 2 be split into Table 2a with vet species-specific breakpoint data and Table 2b with human breakpoint data for situations where species specific data is not available. New text explaining the new table organization will be added.

A motion was made to separate the tables into one for human breakpoints (gray) and one providing vet species specific breakpoints. The tables will be reviewed at the January 2010 meeting with a first draft available at the June 2010 meeting. **(Approved 8-0; 2 absent)**

Aquaculture Working Group

Working group participants—Renate Reimschuessel and John Hawke, Co-chairholders; Members—Guillaume Blanc, Jeremy Carson, Mauro Giacomini, Charles Giesecker, Ron Miller, Peter Smith, Temdoug Somsiri, Ching Ching Wu

Dr. Miller presented a report for the Aquaculture working group.

The first part of the report was to provide a presentation on research proposed to develop and standardize both dilution testing methods for *Flavobacterium columnare* and *Flavobacterium psychophilum*. He requested volunteers from expert laboratories to perform the testing.

Dr. Miller also outlined the proposed changes to M42-A and M49-A.

Proposal #1: A motion was made to change the text regarding the frequency of QC testing to the following, “The weekly QC testing option outlined below is not applicable when MIC tests are performed less than once a week. Quality control testing should be performed each test day for MIC test performed less than once a week.” **(Approved 8-0; 2 absent)**

Proposal #2: The aquaculture working group needs to address how clinical breakpoints should be established in aquatic animals. It was agreed that the data should be scored on treatment outcomes related to MICs. Wild Type cut-off (WT_{co}) values for *Aeromonas salmonicida* for oxytetracycline, florfenicol, ormetoprim-sulfadimethoxine, trimethoprim-sulfamethoxazole, erythromycin, oxolonic acid, and gentamicin were proposed. Enrofloxacin and flumequine zone diameter data for *A. salmonicida* were also presented, but not WT_{co} values were proposed. It was agreed that further PK data and/or clinical outcome data is required to generate clinical breakpoints. A motion was made to postpone the vote on the change until January 2010. The Subcommittee agreed in principle with the proposed WT_{co} values but requested additional supportive data, a modified table and appropriate text. The proposed tables and text will be distributed and reviewed via email prior to the January 2010 Subcommittee meeting.

Education Working Group

Working group participants—Virginia Fajt, Chairholder; Members—Mike Apley, Bob Badel, Jennifer Lorbach, Tom Shryock, Ching Ching Wu

Dr. Fajt presented ideas to the group for ways of disseminating information regarding interpretation of data and clinical implications compiled by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing. The suggestions are listed below.

- Publish articles with references to the appropriate guidelines
- Schedule workshops or teleconferences through APHL
- Have individuals on the subcommittee send letters to the editor of appropriate journals
- Author and publish papers in appropriate journals

Dr. Fajt asked the group to submit other ideas to the education working group for discussion.

Other Business

Dr. Wu presented an update on research being conducted to evaluate the reproducibility of florfenicol broth microdilution susceptibility tests for *Mycoplasma bovis*. She indicated that the method has been giving reproducible results; however, additional steps will be required prior to method approval. These include:

- a comparison of this method to the Wu-Phenol red method at one or more sites;
- defining a manufacturer of the HBAN;
- defining an additional manufacturer of the base media;
- completion of a seven lab QC study to determine QC ranges;
- formal presentation of the results to the CLSI VAST for method and QC range approval and;
- the inclusion of additional compounds.

Dr. Wu indicated that the timeline for completion of the QC study is 2010.

Plans for Next Meeting

The next meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing will be scheduled as a day and a half meeting on Tuesday afternoon, 26 January and a full day Wednesday, 27 January 2010 in Tampa, Florida.

Ms. Dooley stated that the submission deadline for the January meeting will be **Wednesday, 9 December 2009**. 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 4:20 pm.

Respectfully submitted,

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

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