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Subcommittee on Veterinary Antimicrobial Susceptibility Testing
Grand Hyatt Tampa Bay
Tampa, Florida, USA
10-11 January 2013

Summary Minutes

A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was held on 10-11 January 2013 at the Grand Hyatt Tampa Bay Hotel in Tampa Florida. The following were in attendance:

Mark G. Papich, DVM, MS **Chairholder**	**North Carolina State University**
Shabbir Simjee, PhD **Vice Chairholder**	**Elanco Animal Health**

Members Present

Mike Apley, DVM, PhD	Kansas State University
Viginia R. Fajt, DVM, PhD, DACVCP	Texas A & M University
Thomas R. Fritsche, MD, PhD	Marshfield Clinic
Cynthia C. Knapp, MS	Thermo Fisher Scientific
Markus Rose, DVM, PhD	Intervet Innovation GmbH
Stefan Schwarz, DVM	Friedrich-Loeffler-Institute (FLI)
Maria M. Traczewski, BS, MT(ASCP)	The Clinical Microbiology Institute
John Turnidge, MD	SA Pathology
Jeffrey L. Watts, PhD, RM (NCRM)	Pfizer Animal Health
Ching Ching Wu, DVM, PhD	National Taiwan University School of VET Medicine

Advisors Present

Donald J. Bade, BS	Microbial Research, Inc.
Tara Bidgood, DVM, PhD, DACVCP	Pfizer Animal Health
Steven D. Brown, PhD, ABMM	The Clinical Microbiology Institute
Luca Guardabassi, DVM, PhD	University of Copenhagen
Henry S. Heine, PhD	Institute of Therapeutic Innovation
Robert P. Hunter, MS, PhD	Elanco Animal Health
Cindy Lindeman	Pfizer Animal Health
Brian V. Lubbers, DVM, PhD, DACVCP	Kansas State Veterinary Diagnostic Laboratory
Marilyn N. Martinez, PhD	FDA Center for Veterinary Medicine
Lori T. Moon, MT(ASCP)	MSU Diagnostic Center for Population & Animal Health
Ian Morrissey, MBA, PhD, FRSM	IHMA Europe Sàrl
Thomas R. Shryock, PhD	Elanco Animal Health

Peter Silley, PhD	MB Consult Limited
Michael T. Sweeney	Pfizer Animal Health

Reviewers Present

Timothy S. Frana, DVM, MS, MPH, PhD	Iowa State University
Scott B. Killian	Thermo Fisher Scientific
Xian-Zhi Li	Heath Canada Veterinary Drugs Directorate
Maureen Mansfield	Thermo Fisher Scientific

Observers Present

Aaron Boswsell	TOKU-E Company
John Dallow	Quotient Bioresearch
Andy DeRose	Bayer Health Care
Jennifer M. Lorbach	Thermo Fisher Scientific
Jeremy Newman	TOKU-E Company
Bernd Stephan, PhD	Bayer Animal Health GmbH
Debora A. Sweeney	Micromyx, LLC

CLSI Staff Present

Tracy Dooley, BS, MT(ASCP)	CLSI
Jenny Sarkisian, MLS(ASCP)^{CM}	CLSI

Opening Remarks

Dr. Papich began the meeting on Thursday, 10 January at 8:00 am. He stated that the purpose of the meeting is for the working groups to address their agenda item topics and obtain input from the subcommittee. During this time, the subcommittee will make motions and vote on the agenda topics. Ms. Dooley reminded the committee recording secretaries, that it is their responsibility to draft the summary minutes of their discussion items.

Meeting Discussion

Following are the substantive discussion points of the meeting (See Table)

		Agenda Topic
Committee Discussion Points		**Rationale for Decisions Made and/or path Forward**
1.	**Generic Working Group** Chairholder: Ching Ching Wu Recording Secretary: Stefan Schwarz Members: Shabbir Simjee, Cindy Lindeman, Virginia Fajt, Mark Papich, John Turnidge, Marilyn Martinez, Rob Hunter, Tim Frana, Vijay Singu, Tara Bidgood , and Luca Guardabassi (new member)	**1. Clindamycin** - This was a follow-up to the discussion at the January 2012 meeting regarding breakpoints for dogs and cats for clindamycin. At that time, we lacked PK-PD data for dogs or cats, and lacked important pharmacokinetic data (protein binding) for cats. The status has not changed much. Dr. Mark Papich presented the basic facts, i.e. that (i) the only CLSI-approved breakpoints are only for staphylococci and skin/soft tissue infections in dogs, (ii) there are no breakpoints for cats, and (iii) there are no breakpoints for other organisms. Dr. Papich provided a brief overview of the approved doses and indications for clindamycin in dogs and cats. Protein binding of clindamycin is about 80% in humans, but there are no data available for cats. Dr. Jeff Watts mentioned that Pfizer will provide the respective data, possibly for the next CLSI-VAST meeting. Dr. Watts reviewed a presentation that was originally presented to VAST in 1999 regarding proposed breakpoints, activity, and MIC information. There was sufficient data during that presentation to include staphylococci and β-hemolytic streptococci in 1999 which formed the basis for the approval of the current clindamycin breakpoints. However, only staphylococci appeared in Table 2. Motion 1: Add β-hemolytic streptococci to the currently approved breakpoints Vote: approved: 9 – 0 (1 absent) **2. Penicillin G** - This is also a follow-up to the presentation at the January 2012 meeting. At that time data was presented to generate interpretive criteria for penicillin use in pigs. (At previous meetings the VAST established these values for cattle and horses.) Dr. Papich reviewed the presentation given at the last meeting for penicillin G use in pigs. The currently available breakpoints in M31-A3 are adopted from human medicine. There were MIC distributions of porcine pathogens mainly from the USA from different laboratories. At the January 2012 meeting, there was data from a study in pigs at two different doses (15,000 U/kg and 66,000 U/kg). Since January 2012, Dr. Mike Apley was able to provide additional PK data, but it was a different injection site (rear) and larger pig size (sows) than earlier studies; therefore, the data seemed at variance with the other studies. Dr. Marilyn Martinez states that variation in the injection sites as well as in the mode of application (needle vs. needle-free) has an influence on the absorption of the drug and as a consequence, also on the PK data. Protein binding in pigs is ca. 37%. Dr. Papich proceeded with an analysis showing Monte Carlo-Simulations using a dose of 33,000 U/kg (interpolated between the two other doses studies), a dose interval of 24h, and 50% time > MIC. This allowed a target attainment for a MIC of 0.25 µg/mL. This value agrees with MIC distributions of porcine *Pasteurella multocida* and *Streptococcus suis*. Motion 2: Approve breakpoints for *Pasteurella multocida* and *Streptococcus suis* from pigs: S: ≤ 0.25 µg/mL, I: 0.5 µg/mL, R: ≥ 1.0 µg/mL; In the comments box: 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 33,000 U/kg, IM by needle in the neck, q24h. Vote: approved: 10 – 0 Motion 3: Move Penicillin G for pigs from Table 2B to Table 2A; add a footnote to Table 1 in the M31 document and comment on extra-label use Vote: approved: 10 – 0 **3. Doxycycline** - At the request of diagnostic laboratories and veterinary clinicians, interpretive criteria for doxycycline was sought for applications in dogs. Dr. Papich provided an overview of doxycycline properties and use and states that there are no breakpoints available for bacteria from dogs. The current human breakpoint (S) is ≤ 4 µg/mL. Moreover, he states that there is no approved compound in the USA – except an implant form for periodontal diseases. In contrast, there are approved compounds in Europe and in Australia. Dr. Papich also showed MIC distributions for canine Staphylococcal bacteria from both, the USA and Europe (Denmark). Doxycycline has a high protein binding of 91.4 – 91.8%. As a consequence, there are dramatic differences between the total concentration and the concentration of the unbound fraction. For activity measurements, focus should be put on the unbound fraction. Dr. Martinez asked for the mutant selection window. Several people in the audience commented that doxycycline resistance is usually not mutation-based and that as such doxycycline-resistant mutants play a minor role- if at all. Doxycycline pharmacokinetics were available from three studies. Using these pharmacokinetic results, and considering a dose of 5 mg/kg q12h, oral in dogs (the most common oral dose cited), Dr. Papich presented results from Monte Carlo Simulations which were followed by extensive discussions. Dr. Papich suggested to approve the following breakpoints: Motion 4: Approve breakpoints for *Staphylococcus* spp. from skin and soft tissue infections of dogs: S: ≤ 0.12 µg/mL, I: 0.25-0.5 µg/mL, R: ≥ 1 µg/mL; in the comments box: Breakpoint derived from microbiological doxycycline testing, pharmacokinetic data using a clinical dose of 5 mg/kg of doxycycline orally twice daily, and pharmacodynamics data. Second failed → Motion failed After extensive discussions a new motion was attempted using slightly modified breakpoints. Motion 5: Approve breakpoints for *Staphylococcus* spp. from skin and soft tissue infections of dogs: S: ≤

		0.12 µg/mL, I: 0.25 µg/mL, R: ≥ 0.5 µg/mL; in the comments box: Breakpoint derived from microbiological doxycycline testing, pharmacokinetic data using a clinical dose of 5 mg/kg of doxycycline orally twice daily, and pharmacodynamics data. Vote: failed: 2 – 7 (1 absent) The reason for the failed vote was a lack of efficacy data, and lack of a comparison between doxycycline testing and tetracycline testing results. (Tetracycline is used as the test class drug for all other tetracyclines.) In a follow-up discussion, it was agreed that the Generic Working Group would seek additional data. Dr. Luca Guardabassi agreed to supply the committee with scattegrams comparing doxycycline to tetracycline against these strains. This will be presented at the next VAST meeting. Therefore there was a consensus to postpone the doxycycline breakpoints to the next CLSI-VAST meeting and present more MIC data (also comparing tetracycline and doxycycline MICs and information on the presence of tet genes). **Action: Presentation of Doxycycline for dogs is postponed to the next meeting in June for more MIC data.**
2.	**VFM Working Group** Members: Donald Bade, Mark Papich, Shabs Simjee, Jeff Watts, Cynthia Knapp, Scott Killian, Cindy Lindeman, Maria Traczewski, Tom Shryock, Ching Ching Wu, Lori Moon	Mr. Bade presented data obtained for evaluating media for the testing of fastidious Gram-Negative veterinary pathogens that do not rely upon Supplement C. Testing was performed at four different laboratories using 10 clinical isolates and one ATCC® isolate from each of the following: *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Histophilus somni*. Twenty-four different formulations of media were tested in both aerobic and 5% CO_2 atmospheres. The medium identified as MFN may be a candidate when considering the growth of *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Histophilus somni*. The use of fetal calf serum in MFN poses some issues with international shipping as well as leads to variation among sources that may be available to individual laboratories and manufacturers. Considering a replacement medium that is capable of supporting the growth of *Actinobacillus pleuropneumoniae* and *Histophilus somni* only, the medium identified as MHF-Y appeared to be a good choice. VFM could not be recommended to test HP as there was insufficient growth with CO_2. Aerobic growth of HP in VFM was promising, but would require bridging study. Below a list of a small scale of additional media formulations that will be performed to help finalize the formulation. Once the formulation is determined, funding will be needed to test QC performance of the various antimicrobials. Some follow-up growth tests were identified to be performed on a small scale basis at the multiple labs: • Test MHF-Y with Fetal bovine Serum. Test in a manner that would allow the laboratories to add the FBS from their own source. • Test MHF-Y with BHI as a base – some problems with the BHI consistency.

		• Test MHF-Y with bovine serum albumin from an approved source. • Test VFM with additional Yeast extract (no Supplement C). • Test additional sources of Yeast extract to assure consistency. • Test Log growth of HP (24-48 hours) to see if possible to obtain MIC's from a log growth culture rather than from direct suspension. • Possibly, a potential source for supplement C has been identified, so it would not be limited to one source.
3.	**Education Working Group** Chairholder: Virginia Fajt Recording Secretary: Mike Apley Members: Bob Badel, Rob Hunter, Jennifer Lorbach, Mark Papich, Tom Shryock, Stefan Schwarz, Ching Ching Wu	During the regularly scheduled VAST meeting, the Education Working Group discussed the following projects for the WG during 2013: 1. Create rationale documents for newly set breakpoints, with special emphasis on explaining the approaches used for generic drugs 2. Possibility of having Table 2 as a stand-alone item for purchase, which might be useful and marketable to veterinarians and educators 3. Complete the work on a manuscript that is designed to give advice to reviewers and researchers on performing and interpreting antimicrobial susceptibility testing (this manuscript is about 80% completed) 4. Begin work on a review article that would provide advice to clinicians about how to use and interpret antimicrobial susceptibility testing 5. Provide assistance with getting letters to editors and listservs when the larger committee comes up with a summary of the gaps in the research data that would assist us in setting breakpoints
4.	**X08 Update** Presenter: Shabir Simjee	Mr. Simjee, chairholder of the X08 Report published in September 2011 gave a status update on the next steps for X08. Since the January 2012 VAST meeting, there has been no progress. He again, proposed that the X08 report be expanded and be moved to a guideline under the VAST subcommittee. The expansion will address ECVs for difficult indications (eg mastitis), for those antibiotics that do not currently have clinical breakpoints, as well as for antibiotics that do have clinical breakpoints and are routinely used in veterinary resistance monitoring programs. The committee agreed to leave X08 as is and bring it up again at the next VAST meeting in June.
5.	**Editorial Working Group** Chairholder: Mike Sweeney Recording Secretary: Maria Traczewski	Marilyn Martinez provided the following list of suggested changes for Table 1 based on FDA suggestions for the most recent draft of M31-S2: 1. CVM proposes that a sentence be added to the forward, as provided below, to clarify the change in footnotes provided in this version of the M31 document. **Foreword**

	Members: Steve Yan, Jeff Watts, Mark Papich, Henry Heine, Markus Rose, Stefan Schwarz, Lori Moon, Ching Ching Wu	This version of M31 represents the continued efforts of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) to produce a globally useful, clinically relevant document for the standardized *in vitro* susceptibility testing of veterinary pathogens. Due to potential international differences in illegal or prohibited uses, jurisdiction-specific restrictions are described in the accompanying footnotes. The subcommittee has worked diligently to improve the fourth edition of M31 by incorporating relevant updates derived from CLSI documents M02 and M07, developing new recommendations for emerging resistant veterinary pathogens, and by restructuring the M31-S2 tables to provide easier access to veterinary-specific interpretive criteria. The subcommittee expresses its appreciation to the users of M31 for their continued support and application of the standard in their daily work routine, and encourages the user community to provide feedback so that M31 can be updated frequently to maintain its clinical relevance. **Committee action: wording added as shown** 2. There are two changes in the drugs listed in Table 1 that need to be modified: a. Group B: Please include a footnote (n) to Cefquinome in cattle. b. Group D: Tylvalosin is now approved for use in swine. Therefore, the footnote can be removed. 3. Footnote (**n)**: please modify text as follows: Not approved in the US and Canada, or prohibited from extralabel use in the US **Committee Action: Wording in footnote (n) was combined with footnote (d) and footnote (n) was removed. Footnote (d) was added to Cefquinome in cattle and removed for tylvalosin under swine.** **New footnote (d):** **d. This drug is not approved in the US and Canada, or this drug is prohibited from certain extra-label uses in US, but may be approved in other countries (check country and local regulations – also see Note 4 for website references).** 4. Note 4: Remove Canada from this footnote as the site provided is specific to the US. Also, please revise the site to: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/default.htm **Committee Action: Changes approved** 5. Note that this alternative website for the US should also be used in the text document, Page 8: The laboratory client is responsible for using the agent appropriately for the various animal types or categories (eg, calves, lactating dairy cattle). **The laboratory client assumes all responsibility for efficacy, safety**

		(including public health issues of antimicrobial-resistant, foodborne bacteria), and residue avoidance with extra-label uses of antimicrobial agents. The laboratory should be prepared to offer advice to the veterinarian to enable appropriate decision making. Although the laboratory may choose to modify the list of antimicrobial agents it tests and reports, on the basis of public health concerns, it needs to be done in consultation with appropriate experts, based on good clinical judgment, and in accordance with recognized principles of judicious use. Updates on illegal or prohibited use of agents in the US and Canada can be found at http://www.fda.gov/AnimalVeterinary/default.htm. Updates on prohibited agents in the EU can be found at http://www.ema.europa.eu/ema/index.jsp?curl=pages/audience/alp_audiencetype_000003.jsp&mid **Committee Action: Changes approved** **Items for Discussion:** Some possible revisions to tables in the next version of M31-S3 were proposed for discussion. 1. Consider having separate S3's with US and EU breakpoints. 2. Consider grouping Tables 2 by organism group rather than by drugs. a. This would make the VAST tables look more like M100 b. Would also allow for putting the methods for testing at the start of each table. OR: 3. Consider grouping Tables 2 by animal species. There was discussion of merits of each of above. **Action: Editorial working group will make mockups of each scenario to present at the June meeting.**
7.	**M56 Working Group** Co-Chairholder: Maria Traczewski Co-Chairholder: Mike Sweeney Members: Donald Bade, Tom Fritsche, Rob Hunter, Brian Lubbers, Patrick McDonough, Stefan Schwarz,	1. The working group held a 2 hour meeting on Thursday from 4:30-6:30 pm. The working group went through the current document in order to review the drug information listings. Drugs that are not used for treatment of animals were removed from several tables. 2. Ms. Traczewski updated the VAST group on the status of the M56 draft document. The WG has added MIC data for a number of fastidious and hard to grow organisms that are encountered in veterinary medicine. As the group continues to finish this activity, there are some references that do not contain CLSI-accepted methodologies such as *Brachyspira hyodysenteriae* MIC testing. Some suggestions were to share lab methods

	Shabs Simjee, Vijay Singu, Ching Ching Wu	or even include MIC data for organisms that have more than one reference. Ms. Traczewski asked the group if organisms that don't have a CLSI-recommended procedure should be captured in an appendix. The suggestion was to search for references that have similar methods for MIC testing and include in the tables. Additionally, any unreferenced organisms (such as *Nicoletella semolina*) could be included in a list. **Action: Dr. Frana will share his MIC methodology for MIC testing of *B. hyodysenteriae* with the WG. Peter Silley will share some recent publications with a method for testing *Brachyspira*.** 3. We may put organisms with too few references or data in the index with list of references considered best choices instead of in a table. 4. With regards to *C. jejuni* MIC testing, it's already included in M31 and M45 (with QC and BP data) and Ms. Traczewski asked if it should also be included in M56. The consensus was to include it as it would have all information from M31 and M45 in one place. **Action: WG will keep *C. jejuni* in the Draft M56 document.** 5. It was suggested to the WG members that references that are found or used by members other than what has been provided by the Chairholder should be sent to M. Traczewski and also added to the CLSI website. 6. The M56 first draft is on schedule to be presented to the VAST committee at the June 2013 meeting.
8.	**AST Liaison Report** Presenter: Dr. Henry Heine	Dr. Heine 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: - The potential options for process improvement have been broken down into two parts that are being considered – 1) tactical – how do you review a drug where there is no sponsor; and 2) strategic – how to determine which drug or class of drugs should be revised. - The QC committee has outlined a revised QC plan that reduces the QC testing from daily to weekly. The new plan: 15 replicate (3 x 5 day) plan which can be performed in 2 phases. After the QC is performed if 2-3 out of range for all 30 replicates than QC is accepted, it fails if ≥4 out of range.
6.	**CLSI Document Status Update**	**Recently Published CLSI Documents** <u>Published December 2012</u> M54-A, *Principles and Procedures for Detection of Fungi in Clinical Specimens – Direct Examination and*

		Culture; Approved Guideline M27-S4, *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*; Fourth Informational Supplement M100-S23, *Performance Standards for Antimicrobial Susceptibility Testing*; Twenty Third Informational Supplement **Upcoming Publications** <u>Estimated Publication in May 2013</u> M31-A4 and S2 Supplement
7.	**Formation of M37 Working Group** Presenter: Dr. Marilyn Martinez	Dr. Martinez presented changes that are needed for M37, and the committee agreed that the document will be moved to the next level. The following changes were discussed: • Updates on concepts such as the importance of day 1 exposure (versus SS): See Martinez, Papich, Drusano, 2013. • Include recent citations (e.g., Sarah Wagner, JVPT, describing lack of relationship between drug concentrations in milk versus drug at infection site during intramammary infusion. • Improved readability (elimination of redundant text). • PK-PD targets: Updated to better reflect current understanding (previous benchmarks were overly conservative. Propose we use benchmark examples in a recent chapter by Martinez, Toutain and Turnidge). • Completion of work on CO_{CL} discussed several meetings ago by Martinez and Turnidge (propose that we present in June) • Proposed alternative method for estimating CO_{CL}. • Its implications on the current paradigm for establishing "S" – i.e., should we work in a manner similar to the AST where CO_{WT} and CO_{PD} are the primary determinants of "S" unless the data are available to define CO_{CL}? • Use of day 1 versus ss concentrations for CO_{PD}. The following are the new M37 working group members: Marilyn Martinez –Chair Rob Hunter – Vice Chair John Turnidge

		Mark Papich Peter Silley Jeff Watts Xian-Zhi Li Markus Rose The committee also decided that a Benchmarks PK/PD paper is needed and the following individuals will draft the paper: Marilyn Martinez - Chair Peter Silley Rob Hunter Markus Rose Virginia Fajt Also, Dr. Watts will draft a proposal for a mastitis guideline before the June VAST meeting.
8.	**Dr. Shryock's Proposal**	Dr. Shryock presented the current state of VAST committee and what some options are to move it to a Future State. The current state of the committee is to create and use guidelines with recommendations for culture and susceptibility testing to guide veterinarians in selection of appropriate antibiotics. However, not all antibiotics have breakpoints in M31; fewer new antibiotics are coming to VAST; M56 initiative is limited to available data; and antimicrobial resistance monitoring program reports need harmonization. He challenged the committee with the current gaps, such as the need to "M37A3 like" data, and types and quality of data. He also challenged the committee with proposals for a VAST Path Forward to address the issues. A new working group has been created to find the gaps and suggest considerations and how to affectively implement them. The following are the new working group members: Tom Shryock – chair Henry Heine – secretary Stefan Schwarz Mark Pappich Shabs Simjee Ian Morrisey Luca Guardabassi

9.	**Rationale Documents** Presenter: Mike Apley	Dr. Apley presented how the committee should move forward with creating rationale documents using the Swine penicillin G breakpoint that was approved from the first day of the meetings. The rationale documents will be used in the future by the committee to reference as to why there were changes/additions made to the documents. **Action: Drs. Apley and Papich volunteered to go backwards and create the rationale documents for all the drugs. Going forward, new drugs will have rationale documents as well that will be posted on the CLSI website.**
10	**Presentation** Presenter – Stefan Schwarz	Dr. Schwarz presented data on in-vitro susceptibility testing by broth microdilution of *Rhodococcus equi* by comparatively using two different media (MH medium with/without 2% (v/v) lysed horse blood) and two different incubation times (24h, 48 h). AST by using MH medium with 2% lysed horse blood and reading the results after 24 h yielded the most stable results. This method has been published in the meantime (Riesenberg et al. J. Antimicrob. Chemother. 2013, Apr 19. [Epub ahead of print] doi: 10.1093/jac/dkt134) and has been included in the respective document intended for inclusion in M56.

Next Meeting Reminder:

The next meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing will be scheduled as a two-day meeting on 21-22 June 2013, in Baltimore, Maryland.

Adjournment

Dr. Papich thanked the participants for their attendance and input. The meeting was adjourned at 3:50PM.

Respectfully submitted,

Jenny Sarkisian, MLS(ASCP)^{CM}
Standards Project Manager