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A meeting of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) was held on 9-10 January 2014 at the Hyatt Regency San Antonio Riverwalk in San Antonio, Texas. 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
College of Veterinary Medicine
Kansas State University

Thomas R. Fritsche, MD, PhD
Marshfield Clinic

Cindy C. Knapp, MS
Thermo Fisher Scientific

Brian V. Lubbers, DVM, PhD, DACVCP
Kansas State Veterinary Diagnostic Lab

Markus Rose, DVM, PhD
Intervet Innovation GmbH

Stefan Schwarz, DVM
Institute of Farm Animal Genetics (FLI)

Peter Silley, PhD
Friedrich-Loeffler-Institut (FLI)

Maria M. Traczewski, BS, MT(ASCP)
Enterprise House, Ocean Village

John D. Turnidge, MD
The Clinical Microbiology Institute

**Advisors Present**

Donald J. Bade, BS
Microbial Research, Inc.

Virginia R. Fajt, DVM, PhD, DACVCP
Texas A & M University

Robert P. Hunter, MS, PhD
Elanco Animal Health

Xian-Zhi Li, PhD
Heath Canada Veterinary Drugs Directorate

Lori T. Moon, MS, MT(ASCP)
Michigan State University

Ian Morrissey, MBA, PhD, FRSM
IHMA Europe Sarl

Michael T. Sweeney, MT
Zoetis

Ching Ching Wu, DVM, PhD
National Taiwan University, School of Veterinary Medicine
Reviewers Present

Timothy S. Frana, DVM, MS, MPH, PhD  
Iowa State University

Henry S. Heine, PhD  
Institute of Therapeutic Innovation

Nicole Holliday  
Thermo Fisher Scientific

Scott B. Killian  
Thermo Fisher Scientific

Cindy Lindeman  
Zoetis

Thomas R. Shryock, PhD  
Elanco Animal Health

Susan Thomson  
Mast Group

Observers Present

Rob Eusebio, MSHA, MT(ASCP)  
Siemens Healthcare Diagnostics Inc.

Marcelo F. Galas  
National Institute of Infectious Diseases, Ministry of Health, Argentina

Rose Huang  
Merial Limited

Jennifer Lorbach  
Thermo Fisher Scientific

Maureen Mansfield  
Thermo Fisher Scientific

Sally Maysent  
Thermo Fisher Scientific

Eric Moore  
Merck Animal Health

Sharon Shinn  
Siemens Healthcare Diagnostics Inc.

Debora A. Sweeney  
Micromyx, LLC

Ronald K. Tessman, DVM, PhD, DACVIM, DACVPM  
Merial Limited

Amy Trettien  
Zoetis

Darren Trott  
School of Animal and Veterinary Science, University of Adelaide

S. Steve Yan, PhD  
FDA Center for Veterinary Medicine

Barbara L. Zimmer, PhD  
Siemens Healthcare Diagnostics Inc.

CLSI Staff Present

Tracy Dooley, BS, MT(ASCP)  
Luann Ochs, MS

Jenny Sarkisian, MLS(ASCP)℠

Opening Remarks

Dr. Papich began the meeting on Thursday, 9 January at 8:00 am. He stated that the purpose of the meeting is for the sponsors to present data and 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.

Meeting Discussion

Following are the substantive discussion points of the meeting (See Table)
<table>
<thead>
<tr>
<th>Committee Discussion Points</th>
<th>Agenda Topic</th>
<th>Rationale for Decisions Made and/or path Forward</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CLSI Document Status Updates</td>
<td><strong>Recently Published CLSI Documents</strong></td>
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<tr>
<td></td>
<td><strong>Upcoming Publications</strong></td>
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<tr>
<td></td>
<td>M39-A4, <em>Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data</em>; - Estimated for publication the end of January.</td>
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<tr>
<td></td>
<td>VET04-A2, <em>Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals</em> - Estimated for publication in February</td>
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<td></td>
<td>M40-A2, <em>Quality Control of Microbiological Transport Systems</em>; - Estimated for publication in April.</td>
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<td></td>
<td>M29-A4, <em>Protection of Laboratory Workers from Occupationally Acquired Infections</em> - Estimated for publication in April.</td>
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<tr>
<td></td>
<td>M56-A, <em>Principles and Procedures for Detection of Anaerobes in Clinical Specimens</em>; Approved Guideline - Estimated for publication in May</td>
<td></td>
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<tr>
<td>2. Interpretive Criteria for Gamithromycin for Bovine Respiratory Disease</td>
<td>Drs. Tessman and Widener presented data for MIC and disk diffusion breakpoints of Gamithromycin for cattle for <em>Mannheimia haemolytica</em>, <em>Pasteurella multocida</em>, and <em>Histophilus somni</em>. Based on the data presented, the following interpretive criteria were proposed:</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Zone Diameter (mm)</th>
<th>MIC Breakpoint (µg/mL)</th>
<th>Comments</th>
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<tr>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
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<tr>
<td><strong>Macrolides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cattle (BRD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamithromycin</td>
<td>15 µg</td>
<td>≥ 15</td>
<td>12-14</td>
<td>≤ 11</td>
</tr>
</tbody>
</table>
Motion: Accept proposal as presented
Vote: Passed 8-0; 2 absent

3. Interpretive Criteria for Tildipirosin for Bovine and Swine Respiratory Disease

Presenter: Dr. Rose

Dr. Rose presented data for MIC and disk diffusion breakpoints of Tildipirosin for cattle (BRD) and swine (SRD) for *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. Based on the data presented, the following interpretive criteria were proposed:

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Zone Diameter (mm)</th>
<th>MIC Breakpoint (µg/mL)</th>
<th>Comments</th>
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<tr>
<td></td>
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<td>S  I  R</td>
<td>S  I  R</td>
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<tr>
<td><strong>Macrolides</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Cattle (BRD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tildipirosin</td>
<td>60 µg</td>
<td>≥ 20 17-19</td>
<td>≤16 8 ≥ 16</td>
<td></td>
</tr>
<tr>
<td><em>Mannheimia haemolytica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pasteurella multocida</em></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Histophilus somni</em></td>
<td></td>
<td>≥ 17 14-16</td>
<td>≤8 16 ≥ 32</td>
<td></td>
</tr>
<tr>
<td><strong>Swine (SRD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pleuropneumoniae</em></td>
<td>60 µg</td>
<td>≤16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pasteurella multocida</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. bronchiseptica</em></td>
<td></td>
<td>8 ≥ 8</td>
<td></td>
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</tbody>
</table>

Disk diffusion interpretive criteria have not been established. It is recommended to test *A. pleuropneumoniae* by MIC.

The susceptible only category is used for populations of organisms (usually one species) for which regression analysis (disk vs. MIC) cannot be performed. This breakpoint will permit detection of strains with decreased susceptibility as compared to the original population.
Add Tildipirosin in Table 1, Group A, Cattle and Swine

**Motion:** Accept proposal as presented

**Vote:** Passed 8-0; 2 absent

### 4. Interpretive Criteria for Amikacin for Horses and Dogs

Presenter: Dr. Papich

Dr. Papich presented data for MIC breakpoints of Amikacin for horses and dogs. Based on the data presented, the following interpretive criteria were proposed:

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Zone Diameter (mm)</th>
<th>MIC Breakpoint (µg/mL)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S I R</td>
<td>S I R</td>
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<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
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<tr>
<td><strong>Dogs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>≤ 4</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td></td>
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<td></td>
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<tr>
<td><em>spp.</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td></td>
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<tr>
<td><em>aureus</em></td>
<td></td>
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<tr>
<td><em>Streptococcus</em></td>
<td></td>
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<tr>
<td><em>equi</em></td>
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<tr>
<td><em>Streptococcus</em></td>
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<tr>
<td><em>equi</em></td>
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<tr>
<td><em>Pseudomonas</em></td>
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<td><em>spp.</em></td>
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<tr>
<td><strong>Horses (Foals)</strong></td>
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<tr>
<td><em>Escherichia coli</em></td>
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<tr>
<td><em>Staphylococcus</em></td>
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<tr>
<td><em>aureus</em></td>
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<tr>
<td><em>Streptococcus</em></td>
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</tr>
<tr>
<td><em>equi</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
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<tr>
<td><em>equi</em></td>
<td></td>
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<tr>
<td><em>Pseudomonas</em></td>
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<tr>
<td><em>spp.</em></td>
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<tr>
<td><strong>Horses (Adult)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
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<tr>
<td><em>Staphylococcus</em></td>
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</tr>
<tr>
<td><em>aureus</em></td>
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<tr>
<td><em>Streptococcus</em></td>
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<tr>
<td><em>equi</em></td>
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<td><em>Streptococcus</em></td>
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<td><em>equi</em></td>
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<tr>
<td><em>Pseudomonas</em></td>
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<td><em>spp.</em></td>
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</table>

Breakpoint derived from microbiological, pharmacokinetic (PK) (using accepted clinical doses), and pharmacodynamic (PD) data. For dogs, the dose of amikacin modeled was 15 mg/kg, q24hr.

Breakpoint derived from microbiological, PK (using accepted clinical doses), and PD data. For foals less than 11 days of age, the dose of amikacin modeled was 20 mg/kg, q24hr, IV.

Breakpoint derived from microbiological, PK (using accepted clinical doses), and PD data. For adult horses, the dose of amikacin modeled was 10 mg/kg, q24hr, IV.
### 5. Working Group on Analysis of Antimicrobial Resistance Monitoring Data

**Chairholder:** Shabbir Simjee  
**Recording Secretary:** Nicole Holliday  
**Members:** Mike Apley, John Dallow, Tim Frana, Megan Jacob, Cindy Knapp, Brian Lubbers, Ron Miller, Ian Morrissey, Stefan Schwarz, Peter Silley, Michael Sweeney, John Turnidge

### Presentation: Aim of Vet05-ECV cutoff values

This new Working Group would use the currently published Report Vet05-R (previously X08-R – will now be designated as VET07), and take it to a Guideline that would prescribe epidemiological cut-off values for bacteria of animal origin which in turn would be used for observing trends in MIC distribution over time. The prescribed ECVs are intended to be used in antimicrobial resistance monitoring programs.

**Note:** The ECVs will not replace current clinical breakpoints

#### Questions

**What data do we use and what is already available for 3 main animal groups?**
- Cattle, Swine and Poultry - there were discussions in wanting to break up the data into host animal species or not. Was suggested that Shabbir will collect a small amount of data for analysis and then a decision made on pooling data or keeping host species separate
- Existing surveillance data-NARMS US & Canada, National EU (Europe) and Industry programs
- To set ECVs you only need distributions. Need to review existing surveillance e.g. NARMS
- Need to have on scale results
- Do or do not break MIC distributions down by production types i.e. broilers vs. layers vs. breeders? This remains to be decided

Put in as much surveillance data as possible- do not limit the data (methods utilized for obtaining MIC s?). Make sure each source of information is separated. **Group agreed.**

### Issues/ Concerns?

Add Amikacin in Table 1, Group A for Dogs and Horses

**Motion:** Accept proposal as presented  
**Vote:** Passed 8-0; 2 absent
Concern is for on scale results and incomplete data sets
Worried about the integrity of the data if you specify i.e. dairy, beef
John Dallow to circulate a standardised data capture sheet or that each team is capturing the same level of detail

**Action Items**

*Action items*
- Shabbir- to tabulate MIC distributions for past five years from 4-5 AMR monitoring programs. Suggest E. faecium and E. faecalis from cattle and poultry vs. erythromycin and tetracycline. The data will be sent to John Turnidge for analysis through his stats package to determine the ECVs and to see if there are host differences.
- John Dallow to send standardized spreadsheets to Shabbir
- Shabbir to send data to John Turnidge in 2-3 weeks.
- Shabbir to have teleconference with working group after data set is analyzed one month from now

**Discussion**
Once data is tabulated and analyzed, then the group will decide if we pool data or keep it separate. The group was split into three teams to streamline the data collection process, the three teams are:

1. Cattle-Mike, Brian, Ron, and John
2. Swine-Mike, Ching Ching, Tim
3. Poultry-Ian, Shabbir, Cindy, and Nikki

**Project Timeline**
15 mos. for first draft of report

### 6. VFM Working Group
**Chairholder:** Don Bade
**Recording Secretary:** Cynthia Knapp

**Presentation:**
Don Bade presented the next set of testing data that was performed at 4 different testing labs:
1. Donald J. Bade/ Chandra Machin, Microbial Research, Inc. (MRI)
2. Cynthia C. Knapp/ Scott Killian, Thermo Fisher Scientific
3. Timothy S. Frana/ Joann M. Kinyon, Iowa State University
4. Maria M. Traczewski, The Clinical Microbiology Institute (CMI)
Members: Mark Papich, Shabs Sinjee, Jeff Watts, Scott Killian, Cindy Lindeman, Maria Traczewski, Tom Shryock, Ching Ching Wu, Lori Moon

Objective:
To evaluate the performance of MHF-Y broth, as an alternative broth for VFM for performing MIC’s for: *Actinobacillus pleuropneumoniae* and *Histophilus somni*.

Specifically for this testing period, the following

Objectives were:
1. Can MHF-Y be prepared from multiple lots of MHB media and multiple lots of yeast extract?
2. Can multiple labs prepare it and still produce good growth with no precipitation for HS and APP
3. Do these organisms grow as well in Air vs. CO2 using these media?

Media formulation utilized:
MHF-Y was prepared by multiple investigators. A total of four lots of media were tested. Each lab prepared a lot and approximately 300 mL of the media was shipped to each of the other investigators under refrigeration conditions. The media was held at 2-8°C until used.

Testing:
Microtitre plates containing the 4 lots of MHF-Y plates were tested with fresh (unfrozen) media (3 labs) and after being frozen at ≤ -65°C and thawed (2 labs).

One of the plates, or set of plates, was incubated under CO2. The other plate, or set of plates, incubated aerobically (ambient air) to assess the difference in growth for both atmospheres. Incubation temperature was 36±2°C.

Reading plates:

Score Interpretation
0 = No visible growth
1 = Very little growth-unacceptable for MIC interpretation
2 = Weak growth for the organism – difficult to interpret MIC but possible
3 = Good growth for the organism – MIC evaluation is acceptable
## Results/Conclusion:

There was good growth for all the *H. somni* and *A. pleuropneumoniae* with over 90% of the isolates grew equal to or greater than 2 (growth score). There was little difference in the observed growth for aerobic versus CO2 incubation.

There was a definite difference in media observed with regards to observed precipitation*

- **Lot A** produced turbidity equivalent to growth of a score of 2 for over 80% of the 80 observations when incubated aerobically and for over 60% of the wells when incubated with CO2.
- **Lot B** had wells with scores of 1.
- **Lot C** showed 36% of the aerobic wells with precipitation similar to a growth score of 2 and none with CO2.
- **Lot D** had no precipitation observed in any laboratory, aerobically or in CO2

* The use of raw materials, specifically the yeast extracts and lysed horse blood, does impact the amount of precipitation observed.

A quick screen for performance of MICs was done using the Sensititre BOPO6F with all 4 lots and VFM using the QC isolates and results were presented. Correlation of MHF-Y to VFM was good.

## Discussion on Next Steps and Action Items:

- **Action**: Don will have a conference call with the team members to discuss the teams next steps based on the discussions below from the CLSI VAST meeting January 2014.

1. Name change for the MHF-Y?
   - a. Tom Fritsche mentioned, Eucast uses MHF so stay with MHF-Y,
   - b. Ching Ching likes VFM2
   - c. No formal decision made. Will be left to the Working Group.

2. Can we use the dried plate BOPO6F provided by Sensititre for preliminary screen?
   - a. Set up in O2 and CO2 (need CO2 based on Macrolides QC has been established with CO2 already)
   - b. Use VFM and MHF-Y (3 lots of MHF-Y and one control lot of VFM)
c. Use 10 isolates of HS and AP previously tested with QC isolates
d. 4 labs?

3. Next studies needed if the Screen testing is ok will be:
   
a. 100 wild type isolates tested for performance.
b. A bridging study for QC with 7-8 labs and 3 lots of broth
c. Need to work out a budget for these studies.
   - **Action:** Don and Mark will work on this together
   - **Action:** Don and Mark will work on a letter for the Pharma companies.

<table>
<thead>
<tr>
<th>7. Editorial Working Group</th>
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<tbody>
<tr>
<td><strong>Chairholder:</strong> Mike Sweeney</td>
</tr>
<tr>
<td><strong>Recording Secretary:</strong> Maria Traczewski</td>
</tr>
<tr>
<td><strong>Members:</strong> Steve Yan, Jeff Watts, Mark Papich, Henry Heine, Markus Rose, Stefan Schwarz, Lori Moon, Ching Ching Wu</td>
</tr>
</tbody>
</table>

1. The WG is completing the new formatted By Organism tables with a target completion date of March 2014. Actions **(in bold)** that still need to be done by March include:
   
   - Bordetella: add new tildipirosin breakpoints based on acceptance of proposed BPs by sponsor (**This table has been updated by Mike**)
   - Enterobacteriaceae: Re-list by animal species and repeat drugs for each species (**Mike to do, Tom will proof**). Also, enter new amikacin BP values for horses and dogs (**This has been updated by Mike**)
   - *Pasteurellaceae*: Since this is a very lengthy table, the WG agreed to break this table into 4 smaller tables and will include a table each for *Pasteurella*, *Mannheimia*, APP, and *Histophilus* (**Stefan to do**)
   - Pseudomonas: This table has not been started yet (**Maria to do; need to include new amikacin BPs for horses/dogs based on generic WG presentation**)
   - Staphylococcus: Enter new amikacin BP values for horses and dogs (**This has been updated by Mike**)
   - *Enterococcus*: This table looks completed
   - Move *Listeria* table to Vet06
   - Delete *Haemophilus* table
   - Make 2nd option By Species using table species lists, list drugs by test and report group (**Maria to do**)

   The overall goal is to have these actions completed by the next Editorial WG teleconference which will be scheduled for sometime in March. Once the WG proofs and agrees on all tables, then the tables will be submitted to VAST for review and a vote at the June meeting (or via email if meeting is not held) for inclusion into new version of Supplement.
2. The WG also discussed and presented the idea of additional new information in future supplements:

- E-version of Vet01/Supplement
- Discrepant results table
- Intrinsic resistance table
- Page that lists summary of changes from last version of Standard/Supplement
- The WG has asked that VAST members who find errors in Vet01-A4 and S2 to contact MSweeney who will keep a record of needed changes and communicate these changes to Jenny for incorporation of next versions
- The WG will discuss these ideas further once the above actions in (1) are completed
**Next Meeting Reminder:**

The next meeting of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing will be scheduled as a two-day meeting on 8-9 January 2015, in Ft. Lauderdale, Florida.

**Adjournment**

Dr. Papich thanked the participants for their attendance and input. The meeting was adjourned at 11:57AM.

Respectfully submitted,

Tracy Dooley, BS, MLT(ASCP)
Standards Project Manager