Susceptibility Testing in Veterinary Medicine: What you can and can't conclude from antimicrobial susceptibility testing.

Michael D. Apley, DVM, PhD, DACVCP
Department of Clinical Sciences
College of Veterinary Medicine
Kansas State University

“Susceptible” and “Resistant” are thrown around in the fields of microbiology, medicine, public health, and epidemiology with great frequency. Unfortunately, these classifications are often used in a manner inconsistent with their correct application. To understand the application of veterinary susceptibility testing interpretive criteria, it is necessary to study their origin.

This presentation seeks to clarify the definition of a breakpoint, the relationship of “S, I, and R” to the clinical outcome of antimicrobial therapy, the relationship between serial dilution and disk diffusion breakpoints, and the inputs for CLSI-approved interpretive criteria.

These notes draw heavily from two CLSI publications with recognition of the efforts and contributions of the CLSI VAST Subcommittee, members of other CLSI committees that have provided valuable guidance and input, and the CLSI staff.

Clinical and Laboratory Standards Institute VET01-A4, July 2013. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria isolated from Animals; Approved Standard – Fourth Edition (Formerly M31-A4)

Clinical and Laboratory Standards Institute VET01-S2, July 2013. The document is the second informational supplement to the VET01-A4 and has the most current, updated tables.

Clinical and Laboratory Standards Institute VET02-A3, February 2008. Development of In Vitro Susceptibility testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline – Third Edition (Formerly M37-A3)

A standard consists of specific, essential requirements for materials, methods, or practices for voluntary use in unmodified form. A guideline provides criteria for a general operating practice, procedure, or material which may be used as written or modified by the user to fit specific needs.

Information from these documents are presented for purposes of general information. Only the official documents should be relied upon for guidance.

Copies of the current editions may be obtained from CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA. The CLSI office may be reached at 610-688-0100, (fax) 610-699-0700, or on the web at Exoffice@clsi.org. In this paper, quotations from CLSI are presented in boxes or in bullet point format.
These notes include:

- Definitions (page 2)
  - MIC, MBC, and breakpoint definitions
- Developing veterinary breakpoints – an overview of VET02-A3 (Page 3)
- Methods for veterinary susceptibility testing (page 6)
  - Microwell dilution
  - Disk diffusion
  - Relationship between microwell dilution and disk diffusion methods
- Approved CLSI/VAST breakpoints (page 11)
- Pathogen population distributions (page 13)
- Interpreting susceptibility testing for extralabel applications (page 14)

Veterinary breakpoints have been developed by the Veterinary Antimicrobial Susceptibility Testing (VAST) Subcommittee of the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical and Laboratory Standards (NCCLS). The VAST first met in 1993.

The CLSI process consists of tripartite participation by academia, government, and industry (pharma, manufacturers, and private labs). In the consensus process, all parties have an opportunity to review and comment on the documents. The CLSI Area Committee on Microbiology consists of the Antimicrobial Susceptibility Testing (AST) Subcommittee (human pathogens) and the VAST Subcommittee (veterinary pathogens).

What is the difference between an MIC (Minimum Inhibitory Concentration), an MBC (Minimum Bactericidal Concentration) and a breakpoint?

**MIC (from VET01-A4)** – “the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.”

**An MBC** is the lowest dilution where the culture has been completely sterilized. It is not routinely determined. Treatment decisions are made related to MICs, and more specifically, the breakpoint MICs.

**Interpretive criteria/breakpoint (from Vet01-A4)** – “Minimal inhibitory concentration (MIC) or zone diameter value used to indicate susceptible, intermediate, and resistant;

**Susceptible** – a category that implies that an infection due to the strain may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infection species, unless otherwise indicated;

**Intermediate** – a category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used; also indicates a “buffer zone” that should
prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations;

**Resistant** – resistant strains are not inhibited by the usually achievable concentration of the agent with normal dosage schedules and/or fall in the range where specific resistance mechanisms are likely (e.g., β-lactamase), and clinical outcome has not been predictable in effectiveness studies.

**Nonsusceptible** – a category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have minimal inhibitory concentrations (MICs) above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible; **Note 1**: An isolate that is interpreted as nonsusceptible does not necessarily mean that the organism has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wildtype distribution subsequent to the time the susceptible-only breakpoint is set; **Note 2**: For strains yielding results in the “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed.”

How are interpretive criteria developed for Veterinary antimicrobial susceptibility testing?

CLSI document VET02-A3 (formerly M37-A3) “offers guidance for the development of quality control (QC) limits and interpretive criteria for antimicrobial susceptibility testing, performed by disk diffusion and dilution testing with bacteria isolated from animals…”

Proposed QC limits and interpretive criteria are reviewed by the CLSI VAST Subcommittee. When approved, they are included in the VET02 document. These notes present an overview of the contents of VET02-A3 with emphasis on overall procedure. The bulleted text below presents an outline of the VET02-A3 guideline for developing susceptibility testing criteria and quality control parameters for veterinary antimicrobial agents.

- It is important to note that the susceptibility testing development and testing guidelines apply only to antimicrobial agents intended for treatment or control of infectious disease in domestic animals. The guidelines do not address antimicrobials used for growth promotion or prophylaxis, nor do they apply to directly applied antimicrobials (e.g., lotions, eye drops).
- **There are 3 main methods for evaluating data contributing to establishment of a breakpoint.** These methods are incorporated into establishment of interpretive criteria by the VAST Subcommittee. None of them are reported to clinical laboratories and they are not published. Rather, they may each be considered in the process of developing the final interpretive criteria.
  - The “clinical cutoff” (CO<sub>cl</sub>) is derived by inspecting clinical and/or microbiological treatment outcome data in relation to associated pathogen MICs.
  - The “wild-type” cutoff (CO<sub>wt</sub>) has also been referred to as a “microbiological breakpoint”. This cutoff is derived by separating populations of wild-type isolates from diagnostic laboratory surveys based on MIC distributions. For this reason, it
has also been referred to as an “epidemiological breakpoint”. (See Pathogen population distributions below).

- The “pharmacodynamic cutoff” (CO_{pd}) is calculated using a combination of pharmacodynamic and pharmacokinetic parameters related through Monte Carlo simulation.

- Two unique cases are addressed in the early part of the document
  - **Reassessment** of breakpoints/interpretive criteria and QC parameters, including examples of when reassessment would be appropriate
  - **Development of interpretive criteria for generic or older compounds**

- **Emphasis is placed on quality control (QC) testing**, consisting of preliminary QC testing (Tier 1), establishing acceptable QC ranges (Tier 2), and confirmation and reassessment of QC ranges (Tier 3). This is a critical initial step.

- **Data to be provided to establish breakpoints and interpretive criteria**
  - **Clinical effectiveness studies**: VET02-A3 contains specific requests for inclusions in the efficacy study reports presented to the committee. Some of the data required for establishment of CLSI breakpoints are not generally required for drug approval by regulatory agencies, such as testing media components.
  - **Isolate collections and susceptibility test data presentation**:
    - **Wildtype cutoff (CO_{wt})**: proposed based on susceptibility results from a non-clinical trial collection. Three types of culture collections are described in the document.
    - **Clinical cutoff (CO_{cl})**: proposed based on the collection of isolates obtained during clinical effectiveness studies.
  - **Pharmacodynamic cutoff (CO_{pd})**: proposed based on the relationship of pharmacokinetics and bacterial inhibition or killing.
    - Appendix C of VET02-A3 contains detailed information on the pharmacokinetic/pharmacodynamic approach.
  - **Cross-resistance studies** indicate the relationship of new chemical entities to existing antimicrobial classes. These studies are not necessary for well-characterized classes or for adding new pathogens to existing claims.
  - **Guidance for comparison of dilution test methods and use of commercially prepared microdilution panels is provided.**
  - **Pharmacokinetic studies**:
    - The exposure parameters include maximum achieved concentration (Cmax), area under the serum or plasma concentration curve (AUC), and time above a set concentration. These are typically expressed in relation to the *in vitro* susceptibility of the target pathogen.
    - The most common correlations examined, which may vary by antimicrobial class, are time above the MIC of the organism (T > MIC), AUC/MIC, and Cmax/MIC.
    - A very important point is that these metrics are generally estimated in human medicine based on steady state antimicrobial concentrations achieved over 24 hour intervals. Veterinary medicine has more diverse methods of drug administration (e.g. long-acting, single injection antimicrobials) that may require some different definitions of these parameter relationships.
While serum or plasma concentrations are typically used as the basis for these PK/PD evaluations, drugs with very high tissue to serum/plasma ratios, or infections in locations such as the central nervous system may require different models for evaluation.

- When possible, drug concentrations should be presented as both total and free drug.
  - Pharmacodynamic studies consisting of time-kill kinetic data with standard organisms. Other possible data include minimal bactericidal concentrations, post-antibiotic effects, and sub-MIC effects.

**Process of establishing breakpoints and interpretive criteria**
- The establishment of an “S” breakpoint, and “I” and “R” breakpoints if possible, is a highly complex decision. Specific guidelines on the consideration of CO_{wt}, CO_{cl}, and CO_{pd} inputs are included in VET02-A3. A decision tree has been developed that guides the committee through the process.

**Evaluation of Dilution MIC and Disk Diffusion Data**
- After the “S”, “I”, and “R” breakpoints have been established, error rate bounding is applied to set zone of inhibition diameters in relation to the serial dilution breakpoints.
- Guidance for linear regression and error rate-bounding are provided, with error rate-bounding being the preferred method.

**Final determination of breakpoints and interpretive criteria is based on subcommittee evaluation of...**
- PK/PD parameters
- Scattergram data from relevant isolate collections
- Clinical effectiveness study data

In addition to sponsor-related interpretive criteria proposals brought to the committee, members of the generic breakpoint working group have brought forward data to support development of interpretive criteria for additional applications. These interpretive criteria have been approved by the committee based on pharmacokinetic/pharmacodynamics (CO_{pd}), and microbiological data (CO_{wt}). The process for a generic breakpoint is initiated by the VAST generic breakpoint working group.

What is the take message from this section for a veterinarian?

1. Interpretive criteria are only applicable when the susceptibility testing methods are conducted according to CLSI standards

2. The interpretive breakpoints are set from a variety of data related to the interaction of a specific drug regimen (the antimicrobial, dose, route, duration, frequency) and a disease (animal species, possibly animal use class, disease, specific pathogens) and these interpretive breakpoints apply to this specific situation. We use breakpoints in other applications also, but the confidence in our interpretation decays dramatically as we move away from the specific situation for which the interpretive breakpoints were set.
3. The generic breakpoints are set off of PK/PD and wildtype distribution data without contribution of MIC distributions for treatment successes and failures. They are much better than just using a non-approved breakpoint which has not been specifically validated for an application.

Questions related to bringing proposed interpretive criteria before the CLSI VAST Subcommittee should be directed to the Committee Chair holder. The Chair holder and committee members are listed in each CLSI document.

**How is antimicrobial susceptibility testing conducted (properly)?**

**Methods for bacterial susceptibility testing - Microwell dilution method:**

This system uses a plate with wells that contain different concentrations of the selected antimicrobials (or a series of tubes). Ideally we would have a well for each antimicrobial at 1:2 dilution intervals to accurately evaluate the minimal inhibitory concentration (MIC) of the compound for each pathogen. However, practical consideration of cost often mandates focusing on the susceptible and intermediate breakpoint dilutions.

Microwell dilution testing example: The figure below illustrates serial 1:2 dilutions of an antimicrobial being tested against a bacterial isolate. The isolate was first cultured by streaking a swab from the tissue sample on an agar plate. Then, 3-5 colonies of the isolate were collected with a loop and inoculated in a broth culture. The next day, the culture must be within a standardized turbidity range prior to inoculating a standard volume into each well of the plate (or in each tube).

*It is important to realize that the amount of bacteria per well is the same across all drug concentrations. Only the drug concentration changes.*

The numbers below indicate the concentration of antimicrobial in each tube (µg/ml). The greater the number, the higher the concentration of drug in the well or tube. As the MIC and MBC values (defined below) move to the right, this means that a greater concentration of the drug is necessary to inhibit (MIC) or sterilize the culture (MBC). The higher the concentration required for the MIC, the less susceptible the isolate is to the antimicrobial being tested (it takes more drug to inhibit growth).

**The MBC is shown for conceptual purposes only. Only the MIC is used in susceptibility testing.**
As growth moves to the right it means that a higher concentration of the antimicrobial is necessary to inhibit growth of the organism. Once it gets to a certain point (the resistant breakpoint) we predict that the drug will not be able to inhibit the pathogen in the animal.

In the above example, the dilution of 2 µg/ml is the lowest concentration that inhibited visible growth for the 24 hour testing period. Therefore, it is reported as the MIC for this organism. However, the culture is not sterilized at the MIC. In this example, the lowest concentration that sterilized the culture was 8.0. This is called the minimum bactericidal concentration (MBC).

Cost typically prohibits this full-range testing technique for routine use, although more diagnostic laboratories are using an extended-range microwell plate. In many labs testing focuses on “breakpoints” (defined above) that are selected based on reported serum/plasma pharmacokinetic properties, pharmacodynamic characteristics of the drug, clinical data, and microbiological data for antimicrobials in the species of interest. For example, the CLSI/VAST approved breakpoints for florfenicol and bovine respiratory disease are 2, 4, and 8 µg/ml (for S, I, and R, respectively). Breakpoint testing would only test against the 2 and 4 µg/ml concentrations and are interpreted in this manner.

- A pathogen growing in neither of the wells would be considered susceptible because growth is inhibited by a concentration of ≤ 2 µg/ml
- A pathogen growing only in the 2 µg/ml well would be considered intermediate because it is not inhibited by the susceptible breakpoint concentration of 2 µg/ml, but is inhibited by the intermediate concentration of 4 µg/ml
- A pathogen growing in both wells would be considered resistant because it is not inhibited by the intermediate breakpoint concentration of 4 µg/ml, and the next concentration that would be tested in a serial dilution is 8 µg/ml, which is the resistant
breakpoint. Even if the pathogen growth would be inhibited in the 8 µg/ml well, the resistant breakpoint is ≥ 8 µg/ml, so the finding would be resistant. If growth were not inhibited in the 8 µg/ml well, the finding is still resistant because the concentration required to inhibit growth is > 8 µg/ml.

Methods for bacterial susceptibility testing - Kirby-Bauer (“disk diffusion”):

A paper disk containing the antimicrobial is placed on an agar plate that has been inoculated with the pathogen. The plate is incubated and the zones of inhibition (absence of any visible bacterial growth) are measured surrounding the disks. The diameter of the zone is correlated back to serial-dilution concentrations used to set “susceptible”, “intermediate susceptibility”, and “resistant” classifications for pathogens. This technique is obviously heavily dependent on quality control. Depth and contents of the agar, inoculum concentration, growth conditions, and antimicrobial contents of the disks must be closely controlled.

The size of the zone of inhibition is dependent on the MIC of the organism, the rate of diffusion of the antimicrobial in the agar (related to depth and composition), duration of incubation, inoculum concentration, and temperature of incubation. To assure that a laboratory is producing valid results, they must periodically test QC (quality control) organisms with known zone diameters to confirm they are adhering to standardized methods.

Relationship of serial dilution and disk diffusion testing:
Breakpoints approved by the CLSI/VAST Subcommittee have both serial dilution interpretive breakpoints and disk diffusion interpretive zone diameters that are correlated back to the serial dilution breakpoints. Below is an example of the interpretive criteria for tilmicosin for bovine respiratory disease due to *Mannheimia haemolytica* when used according to the label regimen.

<table>
<thead>
<tr>
<th>Disc Content</th>
<th>Zone Diameter (mm)</th>
<th>MIC Breakpoint (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 µg</td>
<td>S 11-13 ≤10</td>
<td>S ≤8 R 16 ≥32</td>
</tr>
</tbody>
</table>

The disk diffusion zones are correlated to serial dilution MICs based on a process called “error rate bounding” where the MIC distribution of the bacterial population of interest is plotted and used to correlate diffusion zone sizes to dilution MICs to minimize different types of errors. The figure below illustrates the error rate bounding process for the tilmicosin bovine respiratory disease breakpoints as published by Shryock, et al. The lines for zone diameter criteria (the vertical lines) are moved to minimize very major errors (resistant by serial dilution but susceptible by disk diffusion), major errors (susceptible by serial dilution but resistant by disk diffusion), and minor errors (intermediate by serial dilution but resistant or susceptible by disk diffusion).

Scattergram of MIC and corresponding zone diameters for 380 isolates of *Pasteurella haemolytica* (n=242) and *P. multocida* (n=138) tested against tilmicosin


It is very important to note that the relationship between serial dilution breakpoints and zone interpretive criteria are based on the distribution of a specific isolate or isolates, and may change for other bacterial species.

Practitioners commonly receive susceptibility information from both standard dilution tests and disk diffusion tests. How do MICs and zone diameters relate according to VET01-A4?
Equivalent Minimal Inhibitory Concentration Breakpoints (from VET01-A4)

“Disk diffusion zone diameters correlate inversely with MICs from standard dilution tests, usually broth microdilution. VET01-S2 Tables 2A and 2B list the zone diameters and MIC breakpoints used for the interpretive guidelines. Zone diameters and MIC breakpoints are correlated based upon zone-diameter versus MIC regression, population distributions, pharmacokinetics, and clinical efficacy studies (also see CLSI document M23). However, the zone diameters may not correspond precisely to the listed MIC breakpoints due to differences in the methodologies and the original databases. Regression line analysis should not be used to extrapolate MIC values from measurements of zones of inhibition because, in many cases, the relationship, while mathematically correct, cannot be considered comparable to an MIC derived by actual dilution testing for a given isolate (see CLSI document VET02). Thus, the information provided in VET01-S@ Tables 2A and 2B cannot be used to convert zone diameters to absolute MIC values.”

Approved CLSI/VAST breakpoints

Status as a CLSI/VAST approved veterinary breakpoint means that the breakpoint has been evaluated in light of the data mentioned above and designed to give a reasonable projection of clinical outcome. Remember, that an approved breakpoint is specific for the following factors.

- Animal species
- Disease
- Pathogen
- Drug
- Regimen: Dose, Route, Duration, Frequency

When any of these factors are changed, the approved breakpoint may no longer be valid for placing the above combination of factors in a population of animal/disease/pathogen/drug where clinical success is likely or unlikely. For example, none of the breakpoints are approved for predicting clinical efficacy of therapy of enteric diseases. It is very important to know when you are, and when you are not using approved interpretive criteria for susceptibility testing results interpretation.

The Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS), Veterinary Antimicrobial Susceptibility Testing (VAST) Subcommittee has approved the following veterinary specific breakpoints. The CLSI/VAST Subcommittee periodically updates guidance publications for veterinary susceptibility testing. These documents are produced through a consensus process. These breakpoints are detailed in CLSI VET01-S2, including the specific pathogens associated with these breakpoints.
**B-Lactams**
- Ceftiofur - bovine: respiratory disease, mastitis
  - swine: respiratory disease
  - equine: respiratory disease
- Cefpodoxime - canine: wounds and abscesses
- Penicillin/novobiocin - bovine: mastitis

**Macrolides**
- Tilmicosin - bovine: respiratory disease
  - swine: respiratory disease
- Tulathromycin - bovine respiratory disease
  - swine respiratory disease

**Phenicols**
- Florfenicol - bovine: respiratory disease
  - swine: respiratory disease

**Aminocyclitols**
- Spectinomycin sulfate - bovine: bovine respiratory disease

**Lincosamides**
- Pirlimycin - bovine: mastitis
- Clindamycin - canine: skin, soft tissue

**Pleuromutilins**
- Tiamulin - swine respiratory disease

**Fluoroquinolones**
- Danofloxacin - bovine: respiratory disease
- Difloxacin - canine: skin, soft tissue, UTI
- Enrofloxacin - bovine: respiratory disease
  - swine: respiratory disease
  - canine: skin, soft tissue, respiratory, UTI
  - feline: skin, soft tissue
- Marbofloxacin - feline: skin, soft tissue
  - canine: skin, soft tissue, UTI
- Orbifloxacin - canine: skin, soft tissue, UTI

The following breakpoints are included in CLSI VET01-A3 as “generic” breakpoints, where the breakpoint was determined on the basis of published pharmacokinetic parameters in the designated species in combination with available target pathogen susceptibility data.

**Amoxicillin/clavulanic acid** – Dogs (skin and soft tissue), Cats (skin, soft tissue, UTI)
**Ampicillin** - **Horses** (respiratory disease), **Dogs** (skin and soft tissue infections, UTI for E. coli), Swine (respiratory disease)

**Penicillin G** – Horses (respiratory disease, soft tissue), Cattle (respiratory disease)

**Cephalothin** – Dogs (skin and soft tissue), Horses (respiratory, genital tract)

**Cefazolin** – Dogs (skin and soft tissue, respiratory, urinary/genital), Horses (respiratory, genital tract)

**Gentamicin** - **Horses** (enterobacteriaceae, *Pseudomonas aeruginosa*, *Actinobacillus* spp.) and **Dogs** (enterobacteriaceae, *Pseudomonas aeruginosa*, *actinobacillus* spp.)

**Oxytracycline** – **Cattle** (respiratory disease) and **swine** (respiratory disease)

For some antimicrobials used in veterinary medicine, the CLSI/VAST Subcommittee has found it necessary to use human-derived breakpoints since no sponsor has brought the information to the subcommittee to develop approved breakpoints. The VAST Subcommittee is working on developing “generic” breakpoints for veterinary labels without approved breakpoints and for extralabel uses. The following antimicrobials have human-derived breakpoint criteria adapted by the VAST Subcommittee.

- **Aminoglycosides**
  - amikacin, gentamicin, kanamycin

- **β-lactams**
  - amoxicillin-clavulanic acid, ticarcillin-clavulanic acid
  - ampicillin, oxacillin, penicillin, ticarcillin, imipenem, cefazolin

- **Others**
  - erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole
  - rifampin, sulfisoxazole, tetracyclines, vancomycin

For these antimicrobials, and for extralabel use of antimicrobials with approved veterinary breakpoints, it is necessary to evaluate the susceptibility testing results in light of the MIC breakpoint used and the pharmacokinetics/pharmacodynamics of the animal and pathogen being treated.

**Interpreting susceptibility testing for extralabel applications**

When the disk diffusion method is used for extralabel applications, not only are the dilution MICs suspect as to clinical application, but there is also the question of if the zone diameter criteria still correlate to the MICs. The take-home message is to know what susceptibility testing situations have veterinary approved breakpoints. For unapproved breakpoints, “susceptible” is probably better than “resistant”, as this may place the “S” pathogen in a defined population of zone diameters or MICs, but the “S” result does not necessarily mean that there is an increased chance for clinical success.
Pathogen population descriptions

When a population of isolates is tested, you may see the data reported as an MIC50, where inhibition of growth of 50% of the isolates has occurred, and an MIC90, where inhibition of growth of 90% of the isolates has occurred.

The data below show the difference in the MIC50 and MIC90 for chlortetracycline and oxytetracycline tested against the same 96 isolates of *Mannheimia haemolytica*. The testing was done using the Sensititre® extended-range microwell dilution plate. Each isolate was inoculated into wells containing 0.5, 1, 2, 4, 8, and 16 µg/ml. The reported MIC for an isolate was the first dilution that inhibited visible growth during the 24 hour testing period. The 16 µg/ml well was not actually tested, but would have been the next dilution tested. Organisms not inhibited by 8 µg/ml were reported at ≥ 16 µg/ml as that would have been the next dilution tested. This is a common way to report organisms that were not inhibited by the highest dilution tested.

**Number of isolates of *Mannheimia haemolytica* inhibited at each concentration in-vitro. Iowa State University Diagnostic Laboratory Data, 2002, N = 96.**

<table>
<thead>
<tr>
<th></th>
<th>≤0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>≥16</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative %</td>
<td>38%</td>
<td>58%</td>
<td>90%</td>
<td>95%</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative %</td>
<td>44%</td>
<td>47%</td>
<td>49%</td>
<td>51%</td>
<td>69%</td>
<td>100%</td>
</tr>
</tbody>
</table>

CTC: MIC50 is 1, MIC90 is 2  
OTC: MIC50 is 4, MIC 90 is ≥16.

Monophasic and biphasic populations

A monophasic population is one where the population of bacteria is described by one contiguous group, with the majority of isolates included in that group. A biphasic population has two distinct groups related to MIC status. In the figure below, The MIC data for OTC and CTC described above has been graphed. Note that there is one contiguous group for CTC (a monophasic population) and two distinct groupings for OTC with few isolates between the groups (a biphasic population).
What about susceptibility testing where CLSI veterinary-specific interpretive criteria are not available? A distinction between veterinary-specific interpretive criteria and criteria adapted from human medicine is made in VET01-A4.

“Minimal Inhibitory Concentration and Zone-Size Interpretive Criteria

VET01-S2 Table 2B shows lists the interpretive criteria for which there are no animal species-specific breakpoints, but for which criteria for infections in humans are available. For those agents for which veterinary-specific interpretive criteria are not available, use caution when using these values in relation to veterinary bacterial isolates for three reasons. First, the value listed in the gray shaded areas listed in VET01-S2 Table 2B were developed in human medicine by comparing zone diameters to MICs in broth or agar dilution tests and from population distributions of zones and/or MICs of known susceptible and resistant strains. Second, the MICs and correlated zone-size distributions were analyzed in relation to the clinical pharmacokinetics of the drug from normal dose-ranges and regimens in humans. Third, the in vitro and pharmacologic data have been analyzed in relation to studies of clinical outcome of treatment of specific human pathogens.

Additionally, caution should be exercised in using the interpretive criteria listed in VET01-S2 Table 2A. These criteria apply to particular uses of the antimicrobial drugs in specific animal species. Extension of these data to other disease indications or other animal species may lead to an incorrect prediction of clinical outcome. Antimicrobial concentrations differ across regions of the body depending on the specific drug, route of administration, drug formulation, and the animal's metabolism. These differences can profoundly affect clinical performance of the drug. Therefore, the subcommittee has listed only approved animal species and pathogens in VET01-S2 Table 2A to define those conditions where interpretive criteria are known to be applicable.”
How do susceptibility testing results apply to clinical outcomes in the field?

A susceptibility testing result does not guarantee the treatment outcome of the specific animal from which the isolate was collected. The result indicates that the animal is in a population of an animal-drug regimen-pathogen relationship with a characteristic relationship between the probability of the different possible clinical outcomes. There may be failures with “S” isolates and there may be successes with “R” isolates. When these susceptibility testing criteria are applied to situations where clinical and/or pharmacokinetic data have not been correlated to clinical outcome, then this relationship of “S”, “I”, and “R” to clinical outcome may or may not exist.