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# Proposed Quality Control Guidelines for Antimicrobial Susceptibility Tests Using Tilmicosin

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**Quality control guidelines for tilmicosin, a novel veterinary-use-only macrolide, were developed in a multilaboratory study according to established National Committee for Clinical Laboratory Standards (NCCLS) procedures (M23-T2). Tilmicosin was incorporated into Sensititre plates for broth microdilution endpoint testing and into two lots of 15-μg disks for Kirby-Bauer agar disk diffusion testing. One common lot and five unique lots of Mueller-Hinton media were used. (Broth was cation adjusted, and agar was supplemented with 5% defibrinated sheep blood.) Bacteria used for reference strains included *Pasteurella haemolytica* 128K, *Pasteurella multocida* ATCC 43137, and *Staphylococcus aureus* ATCC 29213 (microdilution) and ATCC 25923 (disk). Replicate tests were conducted. Disk diffusion and broth microdilution quality control ranges are proposed.**

Tilmicosin is a novel macrolide antibiotic developed exclusively for veterinary use (12). As Micotil (Elanco Animal Health, Indianapolis, Ind.), it is used to treat bovine respiratory disease associated with pasteurellae (5–7, 13). Because antimicrobial susceptibility testing is performed with isolates from diseased calves, it is critical for the laboratory to adhere to quality control guidelines for test validation. To date, these guidelines have not been developed for veterinary-use antibiotics, but with the recent formation of the NCCLS Subcommittee on Veterinary Antimicrobial Susceptibility Testing, the impetus to develop such information is emerging (8, 14). The objective of this study was to evaluate four bacterial isolates for use as reference strains in the development of quality control guidelines for both the broth microdilution and the agar disk diffusion techniques.

## MATERIALS AND METHODS

**Bacteria.** *Staphylococcus aureus* ATCC 29213 and ATCC 25923 were chosen because they are susceptible, commercially available standard NCCLS reference

TABLE 1. Broth microdilution quality control results from five laboratories using five unique and one common lot of Mueller-Hinton broth[a]

| Antimicrobial agent | Quality control organism | Medium | No. of occurrences at MIC (μg/ml) of: | | | | | | % of observations within range |
|---|---|---|---|---|---|---|---|---|---|
| | | | ≤0.5 | 1 | 2 | 4 | 8 | 16 | |
| Tilmicosin | *P. haemolytica* | Common | | 4 | 20 | 1 | | | |
| | | Unique | | 9 | 56 | 34 | | 1 | |
| | | Total | | [13 | 76 | 35] | | 1 | 99 |
| Tilmicosin | *P. multocida* | Common | | 22 | 3 | | | | |
| | | Unique | 1 | 45 | 39 | 13 | 1 | 1 | |
| | | Total | [1 | 67 | 42 | 13] | 1 | 1 | 98 |
| Tilmicosin | *S. aureus* | Common | 6 | 17 | 2 | | | | |
| | | Unique | 3 | 82 | 10 | 1 | 3 | 1 | |
| | | Total | [9 | 99 | 12] | 1 | 3 | 1 | 96 |
| | | | ≤0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | |
| Erythromycin | *S. aureus* | Common | 14 | 8 | 2 | 1 | | | |
| | | Unique | 58 | 27 | 11 | 1 | 1 | 2 | |
| | | Total | ([72 | 35] | 13)[b] | 2 | 1 | 2 | 86 (94)[b] |

[a] Brackets indicate the proposed quality control range.
[b] The NCCLS control limit is 0.125 to 0.5 μg/ml, with 86% of the observations within the approved range (there were 11 observations outside the limit at laboratory B and 5 at laboratory C). If the 1-μg/ml observations are considered acceptable, then 94% of the observations were within the range.

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TABLE 2. Zone distribution in five laboratories using both lots of tilmicosin disks, both unique and common lots of MHA, and *P. haemolytica*[a]

| Zone diam and characteristic | Zone distribution in laboratory(ies): | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | All |
| Diam (mm) | | | | | | |
| 11 | | | | | | |
| 12 | | | | | | |
| 13 | 6 | | | | | 6 |
| 14 | 18 | | | | 7 | 25 |
| 15 | 22 | | | | 35 | 57 |
| 16 | 10 | 6 | 1 | 1 | 18 | 36 |
| 17 | 4 | 23 | 5 | 47 | | 79 |
| 18 | | 25 | 36 | 12 | | 73 |
| 19 | | 6 | 17 | | | 23 |
| 20 | | | 1 | | | 1 |
| 21 | | | | | | |
| 22 | | | | | | |
| 23 | | | | | | |
| Characteristics | | | | | | |
| *n* | 60 | 60 | 60 | 60 | 60 | 300 |
| Mean | 14.8 | 17.5 | 18.2 | 17.2 | 15.2 | 16.6 |
| Variance | 1.11 | 0.66 | 0.47 | 0.19 | 0.40 | 2.36 |
| SD | 1.06 | 0.81 | 0.68 | 0.43 | 0.62 | 1.54 |
| Minimum | 13 | 16 | 16 | 16 | 14 | 13 |
| Maximum | 17 | 19 | 20 | 18 | 16 | 20 |
| Range | 4 | 3 | 4 | 2 | 2 | 7 |
| Median | 15 | 18 | 18 | 17 | 15 | 17 |
| Mode | 15 | 18 | 18 | 17 | 15 | 17 |

[a] Dashed lines indicate the calculated quality control limits (4), and solid lines indicate the proposed limits which allow for an inclusion rate of >95% of the observations (1).

strains. *Pasteurella haemolytica* 128K, a Lilly Research Laboratories reference strain for tilmicosin, was used for comparison. *Pasteurella multocida* ATCC 43137 was evaluated because it is available from the American Type Culture Collection (Rockville, Md.) and represents the genus for which tilmicosin is used as therapy. Because tilmicosin has little or no activity against gram-negative enteric bacteria, none were included.

**Media.** Mueller-Hinton broth (powder) from three manufacturers (Difco, Detroit, Mich.; Becton Dickinson Microbiology Systems, Cockeysville, Md.; and Accumedia, Cockeysville, Md.), representing five different lots, was used for the broth microdilution study. The broth was cation adjusted to 20 mg/liter for calcium and 10 mg/liter for magnesium, either by the manufacturer or by the participating laboratory after consultation with the manufacturer as to the exact preexisting cation concentration. Mueller-Hinton agar (MHA) from three manufacturers (listed above), representing five different lots, was used for the disk diffusion studies. The agar medium was supplemented with 5% defibrinated sheep blood.

**Antibiotics.** Tilmicosin and erythromycin were incorporated into a custom-made 96-well plate by Sensititre (Westlake, Ohio) in predefined dilution ranges of 0.5 to 64 and 0.25 to 32 μg/ml, respectively. A standard well volume of 50 μl, recommended by Sensititre, was used. A single lot of plates was used by all laboratories. Two lots of 15-μg tilmicosin disks were obtained from Difco, and five lots of 15-μg erythromycin disks (one lot per laboratory) were obtained from Becton Dickinson Microbiology Systems.

**Study design.** NCCLS document M23-T2 (9) served as the protocol for this study. There were five separate laboratories (Central Arizona Veterinary Laboratories, Inc., with four satellite laboratories in Kansas, Texas, Arizona, and Nebraska; and Lilly Research Laboratories, Greenfield, Ind.) that participated in the study. Each laboratory performed daily tests for 20 days by the NCCLS broth microdilution method (10) with its unique lot of medium with each of the three organisms and performed five tests with the common lot of medium. Thus, for each laboratory, there were a total of 25 observations for the broth microdilution test. For the agar disk diffusion test (11), each laboratory performed 20 daily tests with its unique lot of medium with each of the three organisms and two lots of tilmicosin disks; the common lot of medium was used to similarly perform 10 tests. Each laboratory thus had a total of 60 observations for the agar disk diffusion test.

The inoculum was standardized to $5 \times 10^5$ CFU/ml for microdilution testing; for disk diffusion testing, the inoculum was equivalent to a 0.5 McFarland standard. Zones of growth inhibition were measured as recommended by the NCCLS (10, 11).

TABLE 3. Zone distribution in five laboratories using both lots of tilmicosin disks, both unique and common lots of MHA, and *P. multocida*[a]

| Zone diam and characteristic | Zone distribution in laboratory(ies): | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | All |
| Diam (mm) | | | | | | |
| 11 | 1 | | | | | 1 |
| 12 | 2 | | | | | 2 |
| 13 | 13 | | | | 2 | 15 |
| 14 | 9 | | | | 9 | 18 |
| 15 | 23 | 1 | | | 18 | 42 |
| 16 | 10 | 22 | | | 22 | 54 |
| 17 | 2 | 24 | 5 | 1 | 9 | 41 |
| 18 | | 10 | 17 | 43 | | 70 |
| 19 | | 3 | 24 | 15 | | 42 |
| 20 | | | 9 | 1 | | 10 |
| 21 | | | 2 | | | 2 |
| 22 | | | 2 | | | 2 |
| 23 | | | 1 | | | 1 |
| Characteristics | | | | | | |
| *n* | 60 | 60 | 60 | 60 | 60 | 300 |
| Mean | 14.5 | 16.9 | 18.9 | 18.3 | 15.5 | 16.8 |
| Variance | 1.64 | 0.80 | 1.49 | 0.27 | 1.07 | 3.83 |
| SD | 1.28 | 0.89 | 1.22 | 0.52 | 1.03 | 1.96 |
| Minimum | 11 | 15 | 17 | 17 | 13 | 11 |
| Maximum | 17 | 19 | 23 | 20 | 17 | 23 |
| Range | 6 | 4 | 6 | 3 | 4 | 12 |
| Median | 15 | 17 | 19 | 18 | 16 | 17 |
| Mode | 15 | 17 | 19 | 18 | 16 | 18 |

[a] Dashed lines indicate the calculated quality control limits (4), and solid lines indicate the proposed limits which allow for an inclusion rate of >95% of the observations (1).

**Analysis of data.** The statistical methods of Barry et al. (2), Gavan et al. (4), and Bale et al. (1) were used to set the proposed broth microdilution quality control ranges and zone diameter ranges in accordance with document M23-T2 (9).

TABLE 4. Zone distribution in five laboratories using both lots of tilmicosin disks, both unique and common lots of MHA, and *S. aureus*[a]

| Zone diam and characteristic | Zone distribution in laboratory(ies): | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | All |
| Diam (mm) | | | | | | |
| 16 | 4 | | | | 2 | 6 |
| 17 | 15 | 3 | | 1 | 24 | 43 |
| 18 | 28 | 20 | 1 | 23 | 20 | 92 |
| 19 | 12 | 21 | 14 | 34 | 10 | 91 |
| 20 | 1 | 11 | 34 | 2 | 4 | 52 |
| 21 | | 5 | 9 | | | 14 |
| 22 | | | 1 | | | 1 |
| 23 | | | 1 | | | 1 |
| Characteristics | | | | | | |
| *n* | 60 | 60 | 60 | 60 | 60 | 300 |
| Mean | 17.9 | 18.9 | 20.0 | 18.6 | 17.8 | 18.6 |
| Variance | 0.77 | 1.06 | 0.68 | 0.34 | 0.96 | 1.38 |
| SD | 0.88 | 1.03 | 0.82 | 0.59 | 0.98 | 1.17 |
| Minimum | 16 | 17 | 18 | 17 | 16 | 16 |
| Maximum | 20 | 21 | 23 | 20 | 20 | 23 |
| Range | 4 | 4 | 5 | 3 | 4 | 7 |
| Median | 18 | 19 | 20 | 19 | 18 | 19 |
| Mode | 18 | 19 | 20 | 19 | 17 | 18 |

[a] Solid lines indicate the proposed limits which allow for an inclusion rate of >95% of the observations (1, 4).

TABLE 5. Performance of two different lots of tilmicosin disks tested by all laboratories using both unique lots and one common lot of MHA

| Disk manufacturer and lot no. | Zone diam (mm [mean ± SD]) of[a]: | | |
|---|---|---|---|
| | *P. haemolytica* | *P. multocida* | *S. aureus* |
| Difco 10496 | 16.5 ± 1.5 | 16.7 ± 2.0 | 18.6 ± 1.2 |
| Difco 691404 | 16.6 ± 1.5 | 16.8 ± 1.9 | 18.6 ± 1.2 |
| Combined data | 16.6 ± 1.5 | 16.8 ± 2.0 | 18.6 ± 1.2 |

[a] Values represent mean zone diameters and standard deviations (SD) among 30 zones recorded in all laboratories for each disk lot.

## RESULTS

**Broth microdilution testing.** Table 1 summarizes the results of the multilaboratory study with the broth microdilution technique and Sensititre plates. The frequency of distribution of the MIC endpoints was recorded for each organism. The modal MIC ± 1 $\log_2$ dilution was calculated to determine the quality control range (indicated by bracketed values), and the percentage of observations included within that range was determined. At least 95% of the MIC determinations should be within the proposed control limits (2), and this criterion was met for all three organisms evaluated. In the case of tilmicosin and *P. multocida*, the data appeared to be skewed such that the modal value was most likely between 1 and 2 μg/ml; thus the range included four doubling dilutions (two on either side of the midpoint value). Erythromycin was tested against *S. aureus* as a macrolide class control, because NCCLS data were available for quality control comparison (10). These limits are 0.125 to 0.5 μg/ml for *S. aureus*. In the present study, because of space limitations, the custom-made plates had the lowest dilution at 0.25 μg/ml; thus, endpoints below that level could not be positively ascertained. Assuming that the values recorded as ≤0.25 μg/ml were within the control range, then 86% of the observations were in the control range. It was noted that there were 6 observations only 1 dilution outside the high limit at laboratory B and 5 at laboratory C (out of a total of 13 observations at 1 μg/ml). If these values were to be included, then 94% of the observations were within the control range.

**Agar disk diffusion testing.** Tables 2 through 4 summarize the disk diffusion testing results for *P. haemolytica*, *P. multocida*, and *S. aureus*, respectively, with both lots of tilmicosin disks (six lots of erythromycin disks with *S. aureus* only were used as controls) and with six lots of Mueller-Hinton agar. Sixty observations from each laboratory were analyzed on an individual laboratory basis and cumulatively. The median and mode were identical in nearly all cases for all three test organisms in each laboratory. The median value for the population was used to establish the measure of central tendency. The upper and lower control limits were determined on the basis of the range of inhibitory zone diameters obtained in the individual laboratories and with ± 0.5 the median range applied to the population median (4). Thus, for *P. haemolytica* the calculated control limit was 17 ± 2 mm, for *P. multocida* it was 17 ± 2 mm, and for *S. aureus* it was 19 ± 2 mm. However, because an interval containing 95% of the test values is preferred by the NCCLS (1), a widening of some of the quality control zone ranges was required. By this approach, the proposed control limits for disk diffusion testing are 17 ± 3 mm for *P. haemolytica*, and 17 ± 4 mm for *P. multocida*; for *S. aureus*, the control limit remained 19 ± 2 mm. Erythromycin disks (15 μg) were used as controls throughout the agar disk diffusion study. The NCCLS range of acceptable zone diameters is 22 to 30 mm for *S. aureus* tested on unsupplemented MHA. This particular study revealed that 82% of the data were within these bounds, but 94% could be included if zones measuring 21 mm were also included. These data reflect the percentage of 300 zones on six different medium lots and six disk lots. It is unknown whether the supplementation of the MHA with 5% defibrinated sheep blood could account for this minor discrepancy, because unsupplemented MHA was not used as a control. Nevertheless, on the basis of these findings, the performance of the test was considered valid.

There was no difference between the mean zone diameters of two different lots of tilmicosin disks as tested by all five laboratories on six lots of MHA with the three organisms (Table 5). The interlaboratory reproducibility was assessed by the use of a common lot of MHA and two lots of tilmicosin disks (Table 6). For all three organisms, the median ranged only 3 to 4 mm between laboratories. Across laboratories, the ranges of zone diameters extended from 13 to 20 mm for *P. haemolytica*, 12 to 23 mm for *P. multocida*, and 16 to 21 mm for *S. aureus*. The effects of unique lots of MHA were assessed with the two lots of tilmicosin disks (Table 7). For all three organisms, the mean and median values were essentially the same, indicating similar levels of performance between the different lots of MHA. The comparison of the medians obtained with the common lot of MHA with those obtained with the unique lots revealed identical values, again demonstrating the uniformity of the test. The intralaboratory variability in zone diameters with both lots of tilmicosin disks and the unique lots of MHA was examined (Table 8). The range between the minimum and maximum zone diameters for a particular laboratory were 2 to 4 mm for *P. haemolytica*, 1 to 5 mm for *P. multocida*, and 2 to 4 mm for *S. aureus*, although the

TABLE 6. Tilmicosin zone diameters recorded by five laboratories using a common lot of MHA[a]

| Laboratory | Zone diam (mm) of[b]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *P. haemolytica* | | | *P. multocida* | | | *S. aureus* | | |
| | Median | Minimum | Maximum | Median | Minimum | Maximum | Median | Minimum | Maximum |
| A | 15 | 13 | 17 | 15 | 12 | 17 | 18 | 17 | 20 |
| B | 17 | 16 | 18 | 16 | 16 | 17 | 19 | 17 | 20 |
| C | 18 | 16 | 20 | 19 | 17 | 23 | 20 | 18 | 21 |
| D | 17 | 17 | 17 | 18 | 17 | 20 | 18 | 17 | 19 |
| E | 15 | 14 | 16 | 15 | 13 | 16 | 17 | 16 | 19 |
| Combined data | | 13 | 20 | | 12 | 23 | | 16 | 21 |

[a] Each strain was tested by each laboratory against two lots of disks, giving 20 zone diameters per strain per laboratory.
[b] Values reflect minimum, maximum and median zone diameters among 20 zones recorded in each laboratory.

TABLE 7. Mean and median zone diameters with two lots of tilmicosin disks tested in all laboratories, each with a unique lot of MHA

| Laboratory and characteristic | MHA manufacturer and lot no. | Zone diam (mm) of[a]: | | | | | |
|---|---|---|---|---|---|---|---|
| | | *P. haemolytica* | | *P. multocida* | | *S. aureus* | |
| | | Mean | Median | Mean | Median | Mean | Median |
| Laboratory | | | | | | | |
| A | BBL C5DAJA | 14.7 | 15 | 14.4 | 15 | 17.7 | 18 |
| B | Difco 27480 | 17.8 | 18 | 17.2 | 17 | 19.1 | 19 |
| C | BBL K5DBKH | 18.3 | 18 | 19.0 | 19 | 20.1 | 20 |
| D | Difco 18014 | 17.3 | 17 | 18.3 | 18 | 18.8 | 19 |
| E | Accumedia 9305-155 | 15.2 | 15 | 15.7 | 16 | 18.0 | 18 |
| Characteristics | | | | | | | |
| Minimum mean | | 14.7 | 15 | 14.4 | 15 | 17.7 | 18 |
| Maximum mean | | 18.3 | 18 | 19.0 | 19 | 20.1 | 20 |
| Average of means | | 16.6 | | 16.9 | | 18.7 | |
| Median of Laboratories A–E | | | 17 | | 17 | | 19 |

[a] Each strain was tested by each laboratory against two lots of disks, giving 40 zone diameters per strain per laboratory.

combined range suggested larger variation (e.g., 6 mm for *P. haemolytica*, 11 mm for *P. multocida*, and 7 mm for *S. aureus*). The individual ranges and the combined ranges obtained for the unique lots of MHA are within 2 mm in all cases compared with the ranges obtained from the five laboratories with the common lot of MHA, illustrating the uniformity of the test performance.

## DISCUSSION

Tilmicosin is currently used in the treatment of bovine respiratory disease associated with *P. haemolytica* and *P. multocida*. Veterinary diagnostic laboratories frequently conduct antimicrobial susceptibility tests with tilmicosin (and other agents) against these microorganisms because they have been observed to exhibit acceptable growth in cation-adjusted Mueller-Hinton broth or on MHA supplemented with 5% sheep blood. However, to properly validate the susceptibility test data, the clinical laboratories require quality control guidelines for the antibiotics tested. The objective of this study was to develop such guidelines for tilmicosin by using NCCLS document M23-T2 (9) as a basis, with erythromycin as a positive control. Although this document was written for clinical microbiology laboratories dealing with human pathogens and antimicrobial agents, the same basic principles apply to the development of veterinary-use antibiotic quality control, because the in vitro testing methods are essentially identical. Four bacterial isolates were chosen for evaluation as reference strains. *P. haemolytica* 128K is a Lilly Research Laboratories reference strain that has been supplied to several diagnostic laboratories for use as an interim quality control strain; thus, it was used only to enable comparison of the results from previous studies with those from the present study. *P. multocida* ATCC 43137 was evaluated because it is available from the American Type Culture Collection and represents the genus for which tilmicosin is used as therapy. However, it is not included as a member of the standard battery of NCCLS reference bacteria. To meet this need, *S. aureus* ATCC 29213 (microdilution) and ATCC 25923 (disk) were chosen, because they are commercially available standard NCCLS reference strains that are susceptible to tilmicosin, unlike the gram-negative bacteria commonly used as quality control reference strains.

The broth microdilution MIC test is frequently conducted in the veterinary diagnostic laboratory by using a commercially manufactured panel containing prediluted antibiotics within the wells. It has previously been shown that the Sensititre system is essentially equivalent to a standardized microdilution method (3). To reflect this trend, Sensititre custom-made plates were obtained and used throughout the study. The use of a single lot of plates eliminated the potential for errors that could have occurred because of preparation and dilution of the antibiotic in the collaborating laboratories. Acceptable growth

TABLE 8. Intralaboratory variability in zone diameters with both lots of tilmicosin disks tested in all laboratories, each using a unique lot of MHA

| Laboratory | Zone diam (mm) of[a]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *P. haemolytica* | | | *P. multocida* | | | *S. aureus* | | |
| | Minimum | Maximum | Range (4 SD)[b] | Minimum | Maximum | Range (4 SD) | Minimum | Maximum | Range (4 SD) |
| A | 13 | 17 | 12.6–16.8 | 11 | 16 | 11.9–16.9 | 16 | 19 | 15.9–19.4 |
| B | 17 | 19 | 16.3–19.2 | 15 | 19 | 15.3–19.0 | 17 | 21 | 16.8–21.4 |
| C | 17 | 19 | 17.2–19.4 | 17 | 22 | 16.7–21.2 | 19 | 23 | 18.4–21.8 |
| D | 16 | 18 | 16.3–16.3 | 18 | 19 | 17.4–19.2 | 18 | 20 | 17.8–19.8 |
| E | 14 | 16 | 14.0–16.4 | 14 | 17 | 13.7–17.6 | 16 | 20 | 15.9–20.0 |
| Combined | 13 | 19 | 13.4–19.9 | 11 | 22 | 13.0–20.8 | 16 | 23 | 16.2–21.2 |

[a] Each strain was tested by each laboratory against two lots of disks, giving 40 zone diameters per strain per laboratory.
[b] 95% confidence intervals expressed as four standard deviations (4 SD [i.e., mean ± 2 standard deviations]).

TABLE 9. Summary of proposed MIC and zone ranges for tilmicosin

| Organism | MIC range (μg/ml) | % of observations in range | Zone diam range (mm) | % of observations in range |
|---|---|---|---|---|
| *P. haemolytica* 128K | 1–4 | 99 | 14–20 | 98 |
| *P. multocida* ATCC 43137 | 0.5–4 | 98 | 13–21 | 98 |
| *S. aureus* ATCC 29213 | 0.5–2 | 96 | | |
| *S. aureus* ATCC 25923 | | | 17–21 | 97 |

of the pasteurellae in Mueller-Hinton broth eliminated the need for additional medium supplementation.

The proposed quality control ranges for the three organisms used in the broth microdilution test were based on a total of 125 observations, and the limits encompass >95% of the datum points (Table 9). The proposed quality control limit for *P. haemolytica* 128K is 2 μg/ml ± 1 dilution (range, 1 to 4 μg/ml), that for *P. multocida* ATCC 43137 is 1 to 2 μg/ml ± 1 dilution (range, 0.5 to 4 μg/ml), and that for *S. aureus* ATCC 29213 is 1 μg/ml ± 1 dilution (range, 0.5 to 2 μg/ml).

The agar disk diffusion test (also known as the Kirby-Bauer test) is also frequently performed in the veterinary diagnostic laboratory. Although MHA is acceptable for the growth of many strains of pasteurellae, it was observed that more consistent growth with a more distinct zone of inhibition could be obtained when the medium was supplemented with 5% defibrinated sheep blood. Thus, the present study used this medium to develop the quality control limits for the three strains tested. The exact physiological reason for this phenomenon is unknown, because MHB without supplementation supported acceptable growth. It is possible that the somewhat fastidious nature of the pasteurellae was more evident when grown on a solid surface lawn than on a nutrient bath in broth medium.

The inter- and intralaboratory variation of results from comparisons among tilmicosin disk lots, medium lots, and the three reference strain candidates was very low. This uniformity of results strengthens the argument for the proposed control limits.

The proposed quality control limits for agar disk diffusion with 15-μg tilmicosin disks are as follows: *P. haemolytica* 128K, 14 to 20 mm, which includes 98% of the observed zone diameters; *P. multocida* ATCC 43137, 13 to 21 mm (98%); and *S. aureus* ATCC 25923, 17 to 21 mm (97%) (Table 9).

Through the use of these proposed quality control limits for MIC and disk diffusion testing, veterinary clinical microbiology laboratories will be able to confirm the validity of their antimicrobial susceptibility test procedures for tilmicosin.

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