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Can Antibiotic Test Kits Be Useful?

Bart Weimer
Utah State University

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Can Antibiotic Test Kits Be Useful?

Cow-side antibiotic test kits are currently being evaluated by the Food and Drug Administration for their effectiveness. The outcome of this evaluation will produce a list of AOAC approved test kits for use on the farm or by milk processors. This evaluation procedure was initially scheduled for completion by July 1. It is now at least nine months behind schedule.

Many tests are available and each has its own special value and concerns. The key is to select the test which fits your needs and testing situation. An alternative is to find what test your processor is using and use the same kit if it fits your situation and needs.

If you have any questions, comments, or concerns please contact Dr. Bart Weimer (750-3356), Dr. Clell Bagley (750-1882), or Dr. Wallace Taylor (750-2164).

So, can antibiotic test kits be useful? Yes, testing kits have a place on the farm for use with individual cows. Use caution when performing the test and interpreting the results. Many processors will check individual cow samples for producers, at minimal charges, if the dairymen brings the samples to the processing plant. One use for on-farm antibiotic kits is to determine if a sample is ready to be checked by the processor. In other words, test kits are used to reject cows from the milking string and processor tests are used to accept cows for milking. The value of this approach is a false positive from an individual cow would cause that individual cow's milk to be dumped, but a false positive at the processor would cause the entire load to be dumped - a $500 to $10,000 savings to you depending on your situation.

The Milk and Dairy Beef Quality Assurance Program (MDBQAP) is designed to eliminate antibiotic residues in consumers milk. It requires milk processors to screen all bulk milk at the processing plant before it is processed. This practice is not new to many Utah processors, but now the names of dairymen whose milk tests are positive will be relayed to the Utah Department of Agriculture for regulatory action. The MDBQAP producer manual suggests cow-side antibiotic testing become routine to protect a producer from accidentally contaminating milk with antibiotics. Unfortunately, research conducted in California has raised questions about the usefulness of antibiotic test kits on the farm.

### Table 1. Percentage of false positive samples for selected antibiotic test kit evaluation of milk from individual cows (Data adapted from Cullor).

<table>
<thead>
<tr>
<th>ANTIBIOTIC TEST KIT</th>
<th>Charm Farm</th>
<th>CITE</th>
<th>Delvo-P</th>
<th>LacTek</th>
<th>Disc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment (q)*</td>
<td>87.8</td>
<td>85.4</td>
<td>78.0</td>
<td>0.0</td>
<td>47.5</td>
</tr>
<tr>
<td>Amoxicillin (q)**</td>
<td>100</td>
<td>66.7</td>
<td>62.5</td>
<td>0.0</td>
<td>37.5</td>
</tr>
<tr>
<td>Oxytocin (q)**</td>
<td>77.8</td>
<td>33.3</td>
<td>44.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Composite</td>
<td>55.9</td>
<td>2.9</td>
<td>0.0</td>
<td>0.0</td>
<td>NR***</td>
</tr>
</tbody>
</table>

- *Individual quarter samples, **Samples were tested 21 days after treatment, ***NR=not reported.
James S. Cullor, D.V.M., Ph.D., a researcher at the University of California School of Veterinary Medicine, Davis, recently found that four out of five commercially available β-lactam (penicillin and its derivatives) antibiotic detection kits yielded false positive results in milk from individual cows. A false positive test occurs when a sample shows positive but no antibiotic is actually present. A false negative test occurs when a test fails to detect an antibiotic residue even though it is present. One testing kit showed almost 56% of individual cow samples were positive after 30 days even though no antibiotic treatment had been given (Table 1).

Specific details should be noted from the results listed in Table 1; First, most of the samples were taken from individual quarters; 2) many cows tested positive before antibiotic treatment; 3) cows tested positive for much longer than the designated withdrawal time; and 4) composite samples were much less prone to false positives than individual quarter or cow samples. To avoid false positive results, antibiotic testing on the farm should be done exactly as directed by the kit manufacturer. Some particular care should be given to the following concerns:

1. Know the specific antibiotic given to the animal and use the correct test kit (see page 44 of your MDBQAP producer manual).

2. Pay special attention to incubation times and temperatures outlined in the kit—incubate the test at the specified temperature. This may require buying an incubator or performing the test in the house during the winter.

3. Collect the sample as directed by the test kit instructions. Avoid quarter samples.

4. Mix quarter samples in the same ratio - take an equal amount from each quarter and mix them in a clean, antibiotic-free container before testing.

5. Know the limitations of the test kit you are using. All kits available for testing β-lactams, for example, will not detect every β-lactam. See page 44 of your MDBQAP producer manual for a listing of specific antibiotics and the kits to use in testing for them.

Dr. Cullor used two values, statistical specificity and statistical sensitivity, for each test kit to indicate how well each kit detects what it claims. Statistical specificity is the probability of getting a true result when an animal has not been treated with antibiotics (Table 2).

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<th>LacTek</th>
<th>Disc.</th>
</tr>
</thead>
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<tr>
<td>Specificity</td>
<td>.12</td>
<td>.15</td>
<td>.22</td>
<td>1.00</td>
<td>.54</td>
</tr>
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Table 2. Statistical specificity for some β-lactam test kits.
James S. Cullor, D.V.M., Ph.D., a researcher at the University of California School of Veterinary Medicine, Davis, recently found that four out of five commercially available β-lactam (penicillin and its derivatives) antibiotic detection kits yielded false positive results in milk from individual cows. A false positive test occurs when a sample shows positive but no antibiotic is actually present. A false negative test occurs when a test fails to detect an antibiotic residue even though it is present. One testing kit showed almost 56% of individual cow samples were positive after 30 days even though no antibiotic treatment had been given (Table 1).

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Dr. Cullor used two values, statistical specificity and statistical sensitivity, for each test kit to indicate how well each kit detects what it claims. Statistical specificity is the probability of getting a true result when an animal has not been treated with antibiotics (Table 2). Alternatively, statistical sensitivity is the probability of getting a true result when a cow has been treated with the antibiotic in mind that these values are for individual cow samples and not bulk milk. The values listed in Table 2 can be used as a percentage to determine the number of samples giving a correct result. A value of 1.0 (100%) means that the kit did not give any false positive on individual cow samples.

Specificity values can be multiplied when more than one testing kit is used on the same milk sample. This indicates the statistical probability of arriving at the correct test result when using more than one testing kit. For example, if the LaCTek, Delvo-P, and the Disc assay are used on the same sample, the statistical specificity would be 11.9% (1.00 x .22 x .54 = .119). This means that the chance of arriving at the correct result after using three different testing kits would be 11.9%. But if the LaCTek and Disc assay kits were used, the statistical specificity would be 54% (1.00 x .54 = .54). This highlights the need to carefully select the test kit that fits your needs and fits into the processors testing scheme that will give you the maximum chance of arriving at correct results.

Other considerations are testing time and cost. Prices vary from $1.00 to $5.00 per test and testing times range from 30 minutes to 3 hours. Kits that are faster are generally antibody-based and require very careful attention to the instructions, especially the incubation temperature. Each manufacturer has additional equipment available for use with its testing kit (Table 3).

Dr. Cullor’s data raises many questions concerning antibiotic testing for individual cows because he used cows with mastitis to evaluate commercial kits, but the kits were developed using normal bulk milk spiked with antibiotics. Drs. Bagley and Weimer from Utah State University (and many others around the country) are conducting trials on individual cows to confirm the data found in California. Tests done on milk from bulk storage tanks are much more likely to arrive at the correct result. Data from individual cows cannot be extrapolated to bulk milk data. Antibiotic testing is more accurate in bulk tanks because the milk has been combined, which dilutes any of the natural antibiotics normally found in animals with subclinical or clinical mastitis. Most Utah processors use a multilevel evaluation testing scheme when testing bulk tankers for antibiotics to reduce false results. For example, the processor may screen each load with a rapid test. If the screening test is positive, a different test is done to check or confirm the results of the rapid screening test. Some processors are using a third test as final confirmation. The final confirmation is usually the Disc assay because it is an AOAC final action test and is officially recognized by the Utah Department of Agriculture.

Table 2. Statistical specificity for some β-lactam test kits.

<table>
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Table 3. Commerciy available β-lactam antibiotic test kit prices.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Price/Test ($)</th>
<th>Optional Equipment ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDEXX - CITE (30 min)</td>
<td>3.00</td>
<td>1,000 - 2,000</td>
</tr>
<tr>
<td>Delvotest SP (3 h)</td>
<td>1.08</td>
<td>100 - 200</td>
</tr>
<tr>
<td>Penzyme (30 min)</td>
<td>2.28</td>
<td>100 - 200</td>
</tr>
<tr>
<td>LaCTek (30 min)</td>
<td>1.30</td>
<td>1,000</td>
</tr>
<tr>
<td>Charm (3 h)</td>
<td>1.25</td>
<td>595</td>
</tr>
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*prices as of January 1, 1992; *AOAC approved for farm use
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