

On-Farm Antibiotic Testing

James S. Cullor, DVM, Ph.D.
Department of Veterinary Pathology
University of California, Davis

1993
WESTERN LARGE HERD
MANAGEMENT CONFERENCE
↑
LAS VEGAS NEVADA

On-Farm Antibiotic Testing

James S. Cullor, DVM, Ph.D.

Department of Veterinary Pathology

University of California, Davis

It should be clear to all now that Food Safety Begins On The Dairy and that this premise must be the main thrust of dairy production from this time forward. The consumer will require nothing less than continued production of a safe and wholesome product, and the processing plant wants the residue status managed more closely before the milk reaches the plant. The current regulatory climate has initiated a series of programs that have resulted in an increased possibility that an antibiotic residue violation can be detected when present in the milk. Therefore, we must all begin thinking in terms of Preharvest Food Safety as we manage the dairy herd health status. This necessitates that dairy producers and their veterinarians take more calculated approaches to antibiotic use on the dairy.

The 10-point Milk and Dairy Beef Quality Assurance Program was developed in a collaborative effort between the National Milk Producers Federation and the American Veterinary Medical Association and is designed to promote and document responsible antibiotic use on the dairy. Mastitis occurs on every dairy and is one of the most common diseases that the dairy cow confronts on a daily basis. One area of emphasis in the 10-point program is individual animal testing for antibiotic residues after a specified withdrawal time has been observed post-treatment. The focus of this presentation is to examine the performance of a group of assay systems at the cowside that are currently being employed by regulatory agencies, processing plants, producers and veterinarians on bulk tanks to detect the presence of beta-lactam (β -lactam) antibiotics.

One goal of individual animal testing (cowside tests for antibiotic residues) is to serve as a beneficial aid in the production of high quality milk free of violative levels on antibiotic residues. The reliability of a antibiotic residue assay positive result is important to the dairy industry in assessing appropriate management decisions to assure a safe product is being delivered to the processing plant. Diagnostic tools employed in medical practice are regarded as a means of reducing the uncertainty in diagnosis. In this discussion, the antibiotic residue test assays are used cowside or on individual animal samples to make decisions concerning food safety issues and recommendations. False-positive test outcomes increase the level of apprehension surrounding these recommendations, and put the veterinarian at risk for litigation.

Literature Reports: Performance Of Antibiotic Residue Tests On Individual Animal Samples

The following is only a short review of several reports in the scientific literature that indicate that the antibiotic residue tests may not accurately tell the whole story concerning the presence or absence of antibiotics in the milk. The principles of false-positive or false-negative outcomes also apply to screening for pesticides or herbicides or other chemical contaminants in milk or meat.

1. Egan and Meaney (1984) used three microbial growth inhibition assays, *Bacillus stearothermophilus* var. *calidolactis*, *Bacillus subtilis*, and *Streptococcus thermophilus* Y1 to evaluate milk samples from mastitic cows and heifers, and colostrum samples from heifers. The samples assayed were not obtained from any animal treated with an antibiotic within the previous 21 days. The mammary gland secretions in this study were not heat-treated prior to performing any of the assays. The outcome of microbiological assays included isolates of *Staphylococcus aureus*, *Streptococci*, Coliforms, and no growth. This study documented the presence of natural bacterial growth inhibitors in that the false positive test outcomes ranged from 53.6% to 0.8%, depending upon the assay and the type of sample examined.

2. Seymour, Jones and McGilliard (1988) conducted a study to determine the effectiveness of on-farm screening assays (BsDA, Delvotest P, Penzyme) for the detection of antibiotics in milk and urine. Composite milk samples were obtained from 58 lactating cows that had received a single antibiotic treatment by any route of administration. Samples were obtained 72 hours post-treatment, and sampling continued every 24 hours until all residue tests indicated assay negative.

— Delvotest: Only 78% of the Delvotest results were the same as the BsDA (disc assay); 5% of the Delvo tests were negative when the BsDA was positive; and 17% were positive when the BsDA outcomes were negative.

— Penzyme: Again, only 79% of the Penzyme assay outcomes were the same as the BsDA; 4% were negative when the disc assay was positive, and 17% were positive when the BsDA (disc assay) was negative. Cows treated with cephalosporin, penicillin, and liquamycin produced those results not in agreement with the BsDA results. Although the Penzyme is reported to detect the presence of β -lactam antibiotics in milk, 19 of the 58 animals were treated with non- β -lactam antibiotics.

It is noteworthy that this study did not test mammary gland secretions prior to antibiotic therapy. Therefore, it is unknown what influence the natural inhibitory host defense substances might have had on this study. The pretreatment assay outcomes are necessary in providing the appropriate medical @negative control@ for evaluating the true assay specificity.

Their study also included an initial investigation into the accuracy of the Live Animal Swab Test (LAST). This on-farm screening assay is used to detect potential antibiotic residues in meat before the animal is processed. Urine was obtained from 39 cull dairy cows prior to slaughter and the LAST assay was performed on this set of biological samples. Treatment records from these study subjects were studied to determine their treatment status and if appropriate withdrawal times had been observed. The assay results indicated that 27 of the 39 cows (69%) contained violative residues in their urine, despite the fact that all animals had completed the recommended withholding period specified for each antibiotic administered. It is clear that this test is not specific enough for detecting the presence of antibiotics, as 75% (15 of 20) of the untreated animals in the study were assay positive for antibiotic residues.

3. Tyler et al. (1992) employed several antibiotic residue assay formats in examining the mammary gland secretions from 8 lactating cows with experimentally-induced endotoxin mastitis. The intramammary endotoxin challenge produced a systemic mediator shock and intramammary inflammation. Mammary gland secretions were collected prechallenge and on a scheduled basis for 288 hours after the endotoxin infusion. The proportion of false-positive assay results varied from 0 to 1.00 among combinations of sampling time and mammary secretion evaluated (endotoxin-infused quarter vs a composite sample from the noninfused quarters). The LacTek β -lactam

had no false positive assay outcomes in this investigation, while the Charm Farm assay yielded the highest proportion of false-positive results (0.86). The other two commonly-used residue assays, the Delvotest P and the CITE Probe β -lactam also yielded a high proportion of false positive assay outcomes at 0.45 and 0.48 respectively. The authors concluded that the ability of some of these assays to correctly identify a patient that has not received antibiotics (test specificity) varies greatly among assay kits, and that intramammary inflammation may increase the proportion of false positive assay outcomes.

4) Cullor et al. (1992) performed milk antibiotic residue assays on mammary gland secretions from individual cows. The assays were performed on: a) mammary gland secretions, AM/PM for 14 days, from three cows with experimentally-induced coliform mastitis, b) mammary gland secretions from seven cows with naturally-occurring coliform mastitis, and c) bulk tank milk that was fortified with bovine serum or plasma from antibiotic-free donors. BsDA= disc assay

* Experimentally-induced coliform mastitis: All but one of the assays identified the normal mammary gland defense as antibiotic positive. The patients were not treated with antibiotics. The number of correct assay outcomes are as follows: Charm Farm (10/72), CITE Probe β -lactam (11/72), Delvotest P (10/72), BsDA (50/72), and the LacTek β -lactam (72/72). The data sets from the challenge and control quarters document similar poor performance from all assays. However, the LacTek β -lactam assay correctly identified these samples as not containing antibiotic residues.

— Naturally-occurring coliform mastitis: The LacTek was the only assay that correctly identified the pretreatment quarter samples as not containing β -lactam antibiotics. The percent false-positive test results for the other assays are as follows: Charm Farm (100%), CITE Probe (100%), Delvotest P (83%), and the BsDA (33%).

— Bulk tank milk fortified with bovine plasma: Both the Charm Farm and the CITE Probe assay incorrectly identified the serum/plasma fortified bulk tank milk as being contaminated with β -lactam antibiotic.

5) Van Eenennaam et al. (submitted 1993) performed antibiotic residue assays mammary gland secretions from 172 commercial dairy cows and heifers with cases of mild to moderate clinical mastitis. False-positive assay results were recorded on pretreatment samples, non-treated animals, and samples obtained 21 days after the first treatments had been administered. The percentage of false-positive results was 43.6% (n=839) for the β -lactam CITE Probe, 37.7% (n=839) for the Delvotest P, 81.7% (n=387) for the Charm Farm assay, 2.6% (n=836) for the LacTek β -lactam test, and 18.8% (n=819) for the disc assay (BsDA). The study also documented apparent problems with false negative outcomes for some of the test kits. One example of mention is at milking quarter sample 4, the CITE Probe β -lactam had a false-negative rate of 15.3%.

What are the consequences of false-positive antibiotic residue test results?

A). They lead to unwarranted waste of milk and economic loss.

B). The socioeconomic impact can harm the dairy industry if antibiotic tests with inadequate biomedical specificity, the ability to correctly identify an untreated cow, are indiscriminately used to test individual cow samples. The false-positive outcomes create a mis-

trust between the consumer and the producer, veterinarian, and regulatory personnel, because they are interpreted that the safety of the milk is not being adequately monitored at the level of the bulk tank.

C). False-positive residue test results can lead to the inaccurate conclusion that a significant proportion of normal dairy cows are delivering residues into our milk supply each day.

D). In the face of genuine efforts made by the dairy and medical industries to produce a safe and wholesome dairy product, widely publicized negative reports of residues in milk that are based upon inappropriately validated and applied technologies will be the reports that the consuming public remember.

E). The welfare of the individual dairy cow is at risk because too many positive assay outcomes after recommended withdrawal times have been followed will result in her being sent to the slaughterhouse. In this case, the false positive assay outcomes result in the untimely death of the dairy cow.

F). Eventually, this problem will have a negative impact on international trade because of the misconception that too many antibiotics are being administered to individual animals and are not being detected at the meat processing plant.

Other adverse consequences could be listed. However, these should be sufficient to raise concern that the approach of erring on the safe side sounds good at first, but doesn't really serve the purpose of addressing all aspects of producing dairy products free of violative residues.

Recommendations: Producer, Practitioner...Test the Tests

Some Practical Ways to Test the Tests: The following is a modified version of the four phase validation program suggested by me in other publications (6). We'll call them Phase I-P, and Phase II-P to designate the practitioner/producer or practical phase of the antibiotic residue test kit evaluation.

Test the Tests!

Phase I-P of the suggestions could be easily accomplished by the practitioner in the following manner:

* Obtain 25 ml of plasma from each of 5 cows that they can certify: 1) are in normal physical condition, and 2) have not received any therapy for at least 30 days prior to collection time. * Pool the plasma from these animals and use it to spike the bulk tank milk. * Bulk tank milk: must have a SCC below 1 million/ml and the veterinarian can document that no treated animals went into the bulk tank that day. This sample must be fresh each day that they use it to test the tests, because some assays cannot be used on frozen milk samples. * Make up the following sample sets to test β -lactam residue assays:

- 1) Zero control (100% v/v bulk tank milk): [v/v= volume/volume]
- 2) 10% v/v plasma and 90% v/v bulk tank milk
- 3) 20% v/v plasma and 80% v/v bulk tank milk
- 4) 40 % v/v plasma and 60 % v/v bulk tank milk
- 5) Positive control: mix 1.0 ml of a β -lactam antibiotic in 3 ml of bulk tank milk

* Test the test kit by running it in triplicate on each sample set according to manufacturer recommendations.

* The residue kit should yield a assay negative outcome on the zero control milk and an @assay positive outcome on the positive control sample. * An assay positive outcome on any one of the other sample sets is suggestive that the test kit possesses an inappropriate assay specificity, and it may be unable to correctly identify that a sample does not contain β -lactam antibiotics.

Phase II-P of the test kit evaluation may be accomplished as follows:

* Collect pretreatment mammary gland secretions from 30 individual animals that have been diagnosed as having clinical mastitis in one quarter. The procedure for the sample collection is provided below. * Sample 1: Is composed of premilking mammary gland secretions from the mastitic quarter (5 ml) * Sample 2: Is made from 5.0 ml aliquots of premilking mammary gland secretions from each of the 3 remaining normal quarters. * Test the test by running it in triplicate on each sample set according to manufacturer@s recommendations on the following sample sets:

- 1) Zero control (100% v/v bulk tank milk)
- 2) Positive control: mix 1.0 ml of a β -lactam antibiotic in 3 ml of bulk tank milk
- 3) Sample 1: pre-treatment milk from the infected quarter
- 4) Sample 2: pre-treatment milk from the composite sample of the 3 normal quarters

* The residue kit should yield a assay negative outcome on the zero control milk and an assay positive outcome on the positive control sample. * An assay positive outcome on any one of the other sample sets is suggestive that the test kit possesses an inappropriate assay specificity, and it is unable to correctly identify that this clinical case of mastitis has not been treated with β -lactam antibiotics.

This set of experiments is not difficult to perform. Collaborate with your veterinarian or Extension Specialist on this important issue. If the test(s) you're currently using doesn't do a good job and incorrectly identifies the cow as having antibiotics in her milk before any treatment has been administered, ask yourself "Is this really a good tool to make accurate management decisions?" Afterall, it's your dollars that may be going down the drain, and your cow that's going to be culled.

Conclusions

Mastitis is the single most common disease syndrome in dairy cows. Any residue test that does not account for mammary gland inflammation and other host defense mechanisms in its assay format contains a serious scientific and practical defect. If the test cannot differentiate between normal host defense and the presence of antibiotics in the milk, it is indefensible as either a screening or diagnostic assay under any circumstances. Remember, the correct definitions for test kit sensitivity and specificity under medical diagnostic field conditions are as follows:

Specificity (Biomedical): The probability of correctly identifying true-negative (non-treated) animals (Laboratory definition of specificity: the ability to differentiate between penicillin and tetracycline).

Sensitivity (Biomedical): The probability of correctly identifying true-positive (antibiotic treated) animals (Laboratory definition of sensitivity: the ability to detect parts per million (ppm) or parts per billion (ppb), etc.)

The laboratory definitions can not appropriately be applied to cowside tests, or any other biological sample (e.g. bulk tank milk, etc.)

It has been previously documented in this presentation that false-positive antibiotic residue assay outcomes are a serious problem. These data sets clearly demonstrate that several antibiotic residue assays that yield false-positive outcomes are on the market today, and can create unwarranted concerns for regulatory personnel, veterinarians, consumers, and dairy producers.

Additionally, when some of the test kits with various assay formats are employed on individual cow mammary gland secretions, they cause milk to be discarded unjustifiably far beyond current regulatory withdrawal times and adversely affect the way the producer and veterinarian may employ necessary medications for the welfare of their patient. It is clear that under these circumstances both the producer and the veterinarian could be falsely accused of not following regulatory guidelines and suffer profound adverse consequences due to this critical defect in the residue assay.

A thorough evaluation of all data available on appropriate research and development of the @residue test@ must be sought before recommendations are put forth. Remember, the test kit validation procedures previously used to allow these kits on the market is probably still in place, and is not likely to be appropriately modified in any substantial manner. Performance of an assay in spiked samples of normal milk with parent compound of an antibiotic is not the same as treating an active case of mastitis or other form of systemic disease and then determining when the patient may safely go back into production. This method of assay validation has little, if any biological relevance.

The 10-point Milk and Dairy Beef Quality Assurance Program is a valuable tool to aid in assuring the consumer of maintaining a safe and wholesome product. The producer and veterinarian can maintain appropriate on-the-farm controls over the use of medications by employing current regulatory guidelines and by supplementing them with the other portions of the 10-point plan. Dairymen, milk processors, and those who advise them need more specific tests to help them assure consumers of a safe, residue-free milk supply.

References:

1. Egan, J. and W.J. Meaney. 1984. The inhibitory effect of mastitic milk and colostrum on test methods used for antibiotic detection. *Ir. J Food Sci Technol* 8:115-120.
2. Seymour, E.H., G.M. Jones, M.L. McGilliard. 1988. Comparisons of on-farm screening tests for detection of antibiotic residues. *J Dairy Sci* 71:539-544.
3. Tyler, J.W., J.S. Cullor, R.J. Erskine, W.L. Smith, J. Dellinger, K. McClure. 1992. Milk antimicrobial residue assay results in cattle with experimental, endotoxin-induced mastitis. *J Am Vet Med Assoc* 201(9):1378-1384.
4. Cullor, J.S., A.L. Van Eenennaam, J. Dellinger, L. Perani, W.L. Smith, L. Jensen. 1992. Antibiotic residue assays: Can they be used to test milk from individual cows? *Vet Med*, (May) pgs. 477-494.
5. Van Eenennaam, A.L., J.S. Cullor, L. Perani, I. Gardner, W.L. Smith, J. Dellinger, W. Guterbock. 1993. Performance of milk antibiotic residue screening tests in cattle with naturally-occurring clinical mastitis. (Submitted to *J Dairy Sci*)
6. Cullor, J.S. 1992. Tests for identifying antibiotic residues in milk: How well do they work? *Veterinary Medicine*, December pp. 1235-1241.

1993 WESTERN LARGE HERD MANAGEMENT CONFERENCE
NOTES