FEEDLOT RESIDUE TESTING

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Introduction

Food safety has become one of the most visible and emotional issues confronting affluent societies. Of particular concern is the adulteration of the food supply by foreign chemicals. Even though the incidence of drug residues in foods of animal origin remains low, and the human health risks associated with these residues are small compared to other food-related hazards, the public's attention has focused on the residue issue, and it is unlikely that this will change in the near future. Growing consumer health-related concerns over residues appear to be major factors contributing to stagnant beef markets in the U.S. In a national survey conducted by the Food Marketing Institute, an independent organization representing U.S. food marketers, the number 1 concern of consumers pertained to residues in meat. Other health-related issues such as cholesterol and saturated fat content were perceived by the public as less threatening than residues (Food Marketing Institute 1988).

Representing this consumer movement for residue-free foods are well funded and organized consumer advocacy groups. These organizations direct substantial lobbying efforts at national policy makers and play an influential role in establishing food and drug regulations. Adverse public perception surrounding the drug residue issue has affected U.S. markets for red meat both domestically and abroad resulting in embargoes and other non-tariff trade barriers.

From an economic standpoint, the marketing of residue-contaminated animals can result in substantial monitary losses. Such losses result from marketing delays which are triggered by a residue violation. No further marketing of animals can take place until a representative number of animals have been tested for residues and determined to be nonviolative.

Residue Testing Methods

The most effective method of preventing drug residues in meat, milk, and eggs is by actually measuring these substances in body tissues and/or fluids prior to the marketing of animals. The major drawback to this approach has been that traditional methods for residue detection requires a high level of technical skill, sophisticated analytical equipment, time and expense. Within recent years, however, considerable progress has been made in developing economical and rapid tests for detecting specific drug residues in various species of livestock.

The earliest of the on-farm tests are bioassays which use sensitive strains of bacteria to detect the presence of antimicrobial drugs in serum, urine, and various tissues. Inhibition of bacterial growth is measured by one of two methods. The plate assay method uses either a sterile swab or disk which is placed in contact with the suspect tissue or fluid. The swab is then placed on an agar plate containing an antibiotic-sensitive strain of bacteria and the plate and swab are incubated for 8 to 24 hours depending on the specific assay. A zone of bacterial growth inhibition surrounding the swab indicates that an antimicrobial drug was present in the sample. An Example of these plate assays include the Live Animal Swab Test (LAST). The Swab Test On Premises (STOP), Calf Antibiotic Sulfa Test (CAST test), and the Sulfonamide Swab Test (SST) are plate assays that have been developed by the USDA for detecting antimicrobial drug residues in animal carcasses at abattoirs.

The colorimetric microbial inhibition test are represented by BR tests. Unlike the plate tests, bacterial inhibition is determined using a pH indicator which changes color in the presence of acid produced by *B. stearothermophilus*. If antibiotic residues are present in the sample, bacterial growth is inhibited and no color change occurs.

Although the microbial bioassays are beneficial in detecting drug residues, they suffer certain disadvantages in that they require some technical skill and equipment; the results are not immediately known; only antimicrobial drugs are detectable, and interfering substances may yield false positive results. Because they are nonspecific tests, they offer the advantage of detecting a wide variety of antimicrobial drugs. This is particularly useful when the drug treatment history of the animal is unknown.

As technologic advances have taken place in recent years, newer immunologic assays are beginning to replace the older microbiological methods of residue detection. These assays have an advantage over the older methods in that they are rapid, specific for a given drug, require little technical skill, and potentially can detect drugs other than antibacterials. A number of ELISA-based residue detection products are listed in Table 1.

Antimicrobial Drug Residue Detection Tests Presently Available for Use in Serum and Urine[†]

Residues Detected	Test Name	Sponsor	Test Format	Specimen	Sensitivity (ppb)
Amoxicillin	Charm II Test	Charm Sciences	Receptor	Serum	32
				Urine	40
Ampicillin	Charm II Test	Charm Sciences	Receptor	Serum	20
				Urine	20
Cephalexin	Charm II Test	Charm Sciences	Receptor	Serum	80
				Urine	100
Cephapirin	Charm II Test	Charm Sciences	Receptor	Serum	40
				Urine	50
Chloramphenicol[§]	BR-Test "Blue Star"	Idetek	Microbial Inhibition	Urine	3100
	Charm II Test	Charm Sciences	Receptor	Serum	5
				Urine	5
	EZ-Screen: Chloramphenicol	Environmental Diagnostics	ELISA Card	Serum	5
				Urine	5
Chlortetracycline	Charm II Test	Charm Sciences	Receptor	Serum	100
				Urine	200
Cloxacillin	Charm II Test	Charm Sciences	Receptor	Serum	120
				Urine	150
Dihydrostreptomycin	Charm II Test	Charm Sciences	Receptor	Serum	75
				Urine	75
Erythromycin	Charm II Test	Charm Sciences	Receptor	Serum	150
				Urine	150
Gentamicin	Charm II Test	Charm Sciences	Receptor	Serum	150
				Urine	150
	EZ-Screen: Gentamicin	Environmental Diagnostics	ELISA Card	Serum	50
				Urine	50
	Signal ForeSite Gentamicin	SmithKline Beecham	ELISA Wells	Serum	30
				Urine	30
	Signal Gentamicin	SmithKline Beecham	ELISA Wells	Serum	150
				Urine	10
Hetacillin	Charm II Test	Charm Sciences	Receptor	Serum	40
				Urine	50
Kanamycin	Charm II Test	Charm Sciences	Receptor	Serum	750
				Urine	750
Neomycin	Charm II Test	Charm Sciences	Receptor	Serum	375
				Urine	375
	Signal Neomycin	SmithKline Beecham	ELISA Wells	Serum	150
				Urine	10
Oxytetracycline	BR-Test "Blue Star"	Idetek	Microbial Inhibition	Urine	60
	Charm II Test	Charm Sciences	Receptor	Serum	500
				Urine	1000
Penicillin	Charm II Test	Charm Sciences	Receptor	Serum	16
				Urine	20
	Live Animal Swab Test (LAST)	Environmental Diagnostics	Microbial Plate	Urine	Unknown

[§] The use of chloramphenicol in any food-producing animal is strictly forbidden under federal law. Consider testing for chloramphenicol in instances where the drug-treatment history is unknown.

Residues Detected	Test Name	Sponsor	Test Format	Specimen	Sensitivity (ppb)
Spiramycin	Charm II Test	Charm Sciences	Receptor	Serum	1500
				Urine	1500
Streptomycin	Charm II Test	Charm Sciences	Receptor	Serum	75
				Urine	75
Sulfadiazine	BR-Test "Blue Star"	Idetek	Microbial Inhibition	Urine	30
	Charm II Test	Charm Sciences	Receptor	Serum	40
				Urine	40
Sulfadimethoxine	Charm II Test	Charm Sciences	Receptor	Serum	16
				Urine	16
	EZ-Screen: Sulfadimethoxine	Environmental Diagnostics	ELISA Card	Serum	10
				Urine	10
Sulfamethazine	Agri-Screen Sulfamethazine Field	Neogen	ELISA Wells	Blood	400
	Agri-Screen Sulfamethazine Lab	Neogen	ELISA Wells	Blood	400
	BR-Test "Blue Star"	Idetek	Microbial Inhibition	Urine	60
	Charm II Test	Charm Sciences	Receptor	Serum	24
				Urine	24
	EZ-Screen: Sulfamethazine	Environmental Diagnostics	ELISA Card	Serum	10
				Urine	10
	Signal ForeSite Sulfamethazine	SmithKline Beecham	ELISA Wells	Serum	10
				Urine	10
	Signal Sulfamethazine	SmithKline Beecham	ELISA Wells	Serum	150
				Urine	10
	Sulfamethazine Serum/Plasma	Idetek	ELISA Microtier Plate	Serum	100
Sulfamethizole	Charm II Test	Charm Sciences	Receptor	Serum	16
				Urine	24
Sulfamethoxazole	Charm II Test	Charm Sciences	Receptor	Serum	8
				Urine	8
Sulfanilamide	Charm II Test	Charm Sciences	Receptor	Serum	80
				Urine	80
Sulfapyridine	Charm II Test	Charm Sciences	Receptor	Serum	40
				Urine	40
Sulfathiazole	BR-Test "Blue Star"	Idetek	Microbial Inhibition	Urine	10
	Charm II Test	Charm Sciences	Receptor	Serum	16
				Urine	16
Sulfisoxazole	Charm II Test	Charm Sciences	Receptor	Serum	24
				Urine	24
Tetracycline	Charm II Test	Charm Sciences	Receptor	Serum	50
				Urine	100
Tylosin	BR-Test "Blue Star"	Idetek	Microbial Inhibition	Urine	60
	Charm II Test	Charm Sciences	Receptor	Serum	150
				Urine	150
	EZ-Screen: Tylosin	Environmental Diagnostics	ELISA Card	Serum	100
				Urine	100

† Inclusion of product names and associated information does not constitue an endorsement by the author. Unless otherwise noted, all information contained herein was provided by the product's sponsor and no further attempts were made to validate or corroborate the sponsor's information. The author assumes no responsibility for penalties which may result from the use of this table or any of the products listed herein.

The competitive receptor binding assay are represented by the Charm Test II and the Charm Cowside Test. Although these tests were developed to detect drugs in milk, they can be adapted for use with other matrixes including serum and urine. Unlike the immunoassays which utilize antibodies specific for the particular drug being detected, the competitive receptor binding assays use bacterial cell receptors which are specific for various classes of antimicrobial drugs. The bacterial receptors are added to the sample being tested, along with a radiolabeled antimicrobial drug of the class being tested (e.g. ^{14}C-penicillin for the beta-lactams, ^{3}H-sulfamethazine for the sulfonamides, etc.). When the sample contains no antibiotic residues, all of the bacterial receptor sites will be occupied by the radiolabeled drug, but when the sample contains antibiotic drugs, some of the receptor sites will be occupied by the unlabeled drug. The number of receptor sites occupied by unlabeled drug is directly proportional to the concentration of antibiotic in the sample thus allowing a quantitative measurement of residue contamination. The relative proportion of receptor sites occupied by radiolabeled vs. unlabeled antibiotic is determined by measuring the amount of radioactivity in the sample compared to a control (noncontaminated) sample. The more radioactivity detected in the sample, the less antibiotic there is in that sample.

Indications for Testing

In developing a residue prevention program, testing should be considered for cattle that have been treated with drugs used in an extra-label manner as administered and/or prescribed by a licensed veterinarian. Because official withholding times do not exist for drugs used in an extra-label manner, testing offers the best protection against violative drug residues. Consider testing any sick or dehydrated cattle which have received medication even if in accordance with label directions and the label-recommended withholding time was observed. Withholding times on drug labels are based on drug clearance times for healthy animals. Sick animals may require longer withholding times. Consider testing animals intended for slaughter when drug-treatment history of the animal is in question or when there is any concern that violative residues may be present.

Test Selection

Use only those tests which are specifically intended to detect drugs in tissue or fluid. For example, some tests are valid for urine only while others are specifically designed to detect drugs in milk. Testing urine with a product intended for use with milk may yield unpredictable results. If it is necessary to use a test soley intended for milk, check with test manufacturer to determine if it can be adapted for use with serum or urine.

The test must be capable of detecting the drug in question. Table 1 lists several antimicrobial drugs and the tests which are capable of detecting them in urine and serum. When selecting a test from Table 1, it is advisable to choose the most sensitive method to minimize the possibility of violative residues at slaughter.

When testing for a specific drug, it is generally better to use a specific test that detects only that drug rather than use a more general test which screens for multiple drugs. When testing animals for which the drug-treatment history is unknown, it is best to use a general test that detects a number of drugs.

Interpretation of Test Results

Because all of the on-farm tests are designed to dectect residues in serum or urine, their value as predictive indicators of tissue residues is based on the assumption that drug concentrations in urine, serum, and tissue are in some way correlated. Although pharmcokinetic theory would support such an assumption, in reality, this hypothesis has not been substantiated for any drug in cattle. It is not surprising, therefore, that reports abound in the literature indicating incorrect (false positive and false negative) results when urine was analyzed in an attempt to predict tissue residues. The LAST test generally continues to detect penicillin residues in the urine after tissue residues deplete to nondetectable concentrations. This results in a low number of false negative results and virtually assures that a negative LAST test will prevent penicillin-adulterated animals from entering market channels [1, 2]. Conversely, the LAST test yielded frequent negative results in cattle when oxytetracycline was present in the kidneys at concentrations in the range of 0.1 to 0.4 ppm [4]. The tolerance for oxytetracycline in kidneys of cattle is 0.1 ppm. A significant incidence of false negative results also would be expected for the aminoglycosides and possibly other classes of antimicrobial drugs. The LAST test has been reported to yield a false positive result rate as high as 69% and factors such as urine pH and osmolality appear to affect substantially the test results [3].

Despite the inherent inaccuracies of residue tests, a positive test result should be interpreted as indicating a high probability that the animal contains violative tissue residues. In such cases, the animal should be retained and re-tested at regular intervals until negative results are obtained, or it becomes apparent that false positive results are occurring.

Limitations of Tests and Testing

Incorrect results (false positives or false negatives) may occur for several reasons. Carelessness, improper handling of test materials, contamination by foreign substances in the environment or on the hands of the operator, and failure to read or follow instructions are some frequent causes for testing errors. Residue tests detect only specific drugs or specific classes of drugs. If the animal has received more than one drug, a single test may not be adequate to ensure that edible tissues are free from all drug residues. Furthermore, on-farm residue tests do not detect all drugs and other deleterious substances. Nonspecific residue tests which screen for multiple drugs may not be sensitive enough to detect some of the drugs at the tolerance or established safe level. Therefore, it is possible that violative residues may be present even though the test results are negative. As discussed in the previous section, testing serum or urine of animals destined for market can occasionally yield incorrect results because the relationship between blood or urine concentrations and tissues levels has not been established for most drugs. Some drugs may not be detected in blood and urine but remain in the liver or kidneys. At the time of slaughter, drug residues may be detected and the carcass condemned. Most residue tests have a limited shelf-life. Use of these tests after the expiration date will yield unpredictable results. Tests should be stored in the manner indicated by the product's manufacturer. Proper storage temperature is essential for some tests as well as protection from light and/or moisture.

Liability

Screening tests are useful and powerful tools for use on the farm in preventing residues from being present in human food. However, they are not foolproof and in some instances, residues may be present even though the test results were negative. Testing for residues does not excuse the producer from penalties associated with the marketing of residue-adulterated products. At present there is no authority for any regulatory agency to require pre-market approval of residue detection products making it possible for untested products to enter the marketplace. Presently, the FDA does not sanction or approve any tests. There exists an urgent need to validate these live animal tests and to initiate research aimed at improved methods of residue detection. Until these goals are met, the responsibility for producing residue-free products still lies with the producer and the veterinarian.

SUMMARY

On-farm testing for residues has become an integral component of dairy quality assurance programs, but despite recent technologic advances, little progress has been made to adapt these methods to feedlot residue prevention programs. This paper addresses the conditions under which residue testing should be considered; the proper selection and interpretation of tests; the inherent limitations and potential misuses of residue tests; and the liabilities which may result when the tests fail to detect violative residues. Included is a list of commercially available residue detection tests, the drugs which they detect, and the sensitivity of each test for the particular drug of concern. By knowing which tests are available and understanding the limitations of various tests, it is hoped that residue testing will become an accepted practice in feedlots.

Table 2. Address and Telephone Numbers of Companies Marketing Drug Residue Detection Tests

Charm Sciences Inc.
36 Franklin Street
Malden, MA 02148
Phone 617-322-1523

Environmental Diagnostics, Inc.
Box 908
1238 Anthony Road
Burlington, NC 27215
Phone 800-334-1116

Idetek, Inc.
1057 Sneath Lane
San Bruno, CA 94066
Phone 800-433-8351

Neogen Corp.
620 Lesher Place
Lansing, MI 48912
Phone 800-234-5333

SmithKline Beecham Animal Health
1600 Paoli Pike
P.O. Box 2650
West Chester, PA 19380
Phone 215-251-7400

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3. Seymour, E.H., Jones., G.M., McGilliard, M.L. 1987. Comparisons of on- farm screening tests for detection of antibiotic residues. *J. Dairy Sci.* 71:539-544.

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