

# USE OF ANTIBIOTIC RESIDUE TEST KITS FOR GOAT MILK

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## Background and Purpose

Mastitis is known as the most common disease syndrome in the dairy industry. Treating mastitic lactating animals with antibiotics is a veterinary practice to cure the disease. However, the antibiotic used may persist in the milk for a period of time depending on drug selected, dosage applied, route administered, body weight of the animal treated, etc. Antibiotic residues in milk are of great concern to dairy farmers, milk processors, consumers and regulatory agencies. Therefore, the Food and Drug Administration (FDA) established tolerance (safe) levels of antibiotic residues in milk for consumer protection (Table 1). Antibiotic residues in goat milk exceeding tolerance levels not only present potential health risks to the consumer but also interfere with milk product processing such as cheese manufacturing.

Table 1. Tolerance levels of antibiotic residues in milk established by FDA and detection levels of antibiotic residue test kits claimed by test manufacturers.

<i>Antibiotic</i>	Tolerance level (ppb)	Detection level (ppb)				
		<b>SNAP Reader</b>	<b>LacTek (CEF)</b>	<b>LacTek (BL)</b>	<b>Penzyme Milk</b>	<b>Delvotest P</b>
Amoxicillin	10	8		7.1	6	7.7
Ampicillin	10	8.2		4.7	7	5.1
Ceftiofur	50	10.5	44.2			
Cephapirin	20	3.5		18.7	11.6	7
Cloxacillin	10	N/A		7.7	N/A	N/A
Penicillin G	5	3.5		4.1	5	3.1

There are approximately 1.5 million dairy goats in the United States which generate almost half a billion dollars income annually from goat milk production alone (Haenlein and Hinckley, 1995). Thus producing safe, high quality milk for consumers is a top priority for dairy goat producers. Violations of the antibiotic residue regulations may damage the image of this growing dairy goat industry. Therefore, use of antibiotics in dairy goats should be strictly monitored.

Screening tests are needed to provide high sensitivity and specificity for testing antibiotic residues in goat milk on the farm, in processing plants and in regulatory laboratories.

Screening tests listed under the Appendix N of the Grade A Pasteurized Milk Ordinance (PMO, 1995) for antibiotic residues in cow milk have been validated and accepted by the Laboratory Committee of the National Conference on Interstate Milk Shipments (NCIMS). The Milk and Dairy Beef Residue Prevention Protocol (MDBRPP) developed jointly by the American Veterinary Medical Association (AVMA) and the National Milk Producers Federation (NMPF) lists all screening tests available for detection of antibiotic residues in cow milk (Boeckman and Carlson, 1994). This protocol also specifies sensitivity levels of each test and tolerance levels of approved drugs. However, these test kits were not verified using milk from individual cows and false-positive results have been reported by many researchers (Jones and Seymour, 1988; Seymour et al., 1988a and 1988b; Cullor, 1992; Cullor et al., 1992; Tyler et al., 1992; Sischo and Burns, 1993; Van Eenennaam et al, 1993; Sischo, 1996). *False-positive* results erroneously call for withholding of individual milk not to be used for food and lead to economic loss for the producer while *false-negative* results allows antibiotic-contaminated milk to enter the food chain for human consumption. Therefore, regulatory agencies, the dairy industry and test manufacturers have been searching for accurate yet quick tests to screen antibiotic residues in milk. Due to the significant differences in milk secretory mechanisms and milk composition between cows and goats, the adequacy of these test kits for dairy goats must be evaluated using goat milk (Zeng et al., 1996). In identifying urgent issues of goat milk to be resolved, Hinckley et al. (1994) stated that "valid antibiotic withdrawal times and residue testing methodology specific for goat milk must be developed and approved". Goat producers as well as regulatory agencies demand scientific evidence regarding the effectiveness of these test kits for screening antibiotic residues in goat milk. Validation of antibiotic residue tests is important for monitoring antibiotic use on the farm as well as for preventing antibiotic-contaminated milk from entering bulk tank milk and milk products. Therefore, extension specialists and researchers of the E. (Kika) de la Garza Institute for Goat Research. Langston, OK, conducted field studies to validate the claimed sensitivity and specificity of antibiotic residue test kits using antibiotic-fortified goat milk and to evaluate the effectiveness and accuracy of the test kits for detection of antibiotic residues in drug-incurred milk from individual goats.

Antibiotic residue tests kits validated in the study were:

- ◆ Penzyme Milk Test (Cultor Food Science, Inc., Milwaukee, WI)
- ◆ Delvotest P (Gist-Brocades Food Ingredients, Inc., Menomonee Falls, WI)
- ◆ SNAP Test (IDEXX Laboratories, Inc., Westbrook, ME) and,
- ◆ LacTek Test (B-L and CEF, IDEXX Laboratories, Inc., Westbrook, ME).

**Penzyme Milk Test** is an enzyme assay. Carboxypeptidase causes a color change in the content of the test vial in the absence of antibiotics as an orange/pink color appears. With the presence of sufficient beta-lactam antibiotics in milk, the enzyme forms a stable and inactive complex and the yellow color of the vial content remains. Results are obtained in 20 min.

**Delvotest P** is a culture medium screening test, using *Bacillus sterothermophilus var. calidolactis*. This bacterium produces acids from glucose and turns bromcresol purple to yellow. The presence of antibiotics in milk inhibits growth of the culture and prohibits the above reaction.

Therefore, an unchanged purple color in the medium is interpreted as positive while a yellow or yellow/purple color as negative for antibiotic residues. This test takes 2.5 to 3 h to obtain results.

**SNAP Test** is an Enzyme-Linked Receptor Binding Assay. An enzyme-labeled conjugate (A) binds with drugs present in milk during incubation in the test tube included in the test kit. When the mixture is added to the SNAP device, the unbound conjugate "A" binds to the sample spot while the enzyme-labeled conjugate "B" binds to the control spot. A blue color is developed when the enzyme portion of conjugates on the spots cleaves the substrate after the activator is "snapped" down. The higher the concentration of drugs in milk, the lighter blue the color is in the sample spot. Results can be obtained visually or using a SNAP image reader. This test screens antibiotic residues in milk in approximately 10 min.

**LacTek Test** is an Enzyme Linked Immunosorbant Assay. Antibiotic residues in milk compete with an enzyme tracer for antibiotic binding sites on the tube wall. The presence of antibiotic residues in milk sample will reduce the amount of tracer which binds to the tube resulting in decreased color development. Qualitative results may then be obtained by running a standard and sample tubes with a spectrophotometer. There are two separate test kits, LacTek CEF for ceftiofur and LacTek B-L for other beta lactam drugs. Results can be obtained in approximately 10 min. Based on the results of drug-fortified goat milk and drug-incurred goat milk studies, SNAP, Penzyme Milk, Delvotest P and LacTek CEF tests were sensitive and reliable in detecting antibiotic residues in goat milk. They all showed over 90% specificity and 90% sensitivity at tolerance and detection levels. These test kits should be approved for screening antibiotic residues in goat milk. Only LacTek B-L failed to meet the 90% specificity requirement. Therefore, its use for screening antibiotic residues in goat milk needs further investigation.

The objective of this workshop is to familiarize dairy goat producers with two milk residue detection test kits, Penzyme and SNAP. The E. (Kika) de la Garza Institute for Goat Research, Langston, OK, does not endorse these kits and the mention of proprietary names is for educational purposes only.

The procedures and methods to conduct the tests follow.

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