

# Practical Antibiotic/Sulfonamide Residue Detection

Charles N. Dobbins, Jr., D.V.M.  
 Head, Extension Veterinary Department  
 The University of Georgia  
 Athens, Georgia

Antibiotic/sulfonamide residue detection is a serious matter for producers and veterinarians. With the increased emphasis by the Food Safety and Inspection Service (FSIS), antibiotic/sulfonamide residues in swine and calves are in the spotlight. Antibiotic residues in milk, a responsibility of the Food and Drug Administration, have been a continuing problem.

The solution for producers and veterinarians in the past has been primarily based on withholding the marketing of animals or milk until the prescribed withholding period has expired. I don't believe anyone purposely sets out to contaminate meat or milk with antibiotic/sulfonamide residues. However, due to the logistics of animal handling, feed mixing, feed distribution, therapeutic treatments, hired labor, inadequate records, and occasional unconcern, antibiotic/sulfonamide residues do frequently occur.

Over the past few years, several antibiotic/sulfonamide tests have been devised and are commercially available. All have outstanding beneficial characteristics. Most have some drawbacks. Most of the commercially available tests require a substantial sum to obtain the basic equipment to conduct the test. Thereafter the cost per test is relatively high. However, the time required to conduct the test is normally short.

I would like to discuss three tests that will give the average practitioner the capability of determining a presence or absence of antibiotic/sulfonamide residues in meat, milk and feed. These three tests are the Live Animal Swab Test (LAST), the Sulfa Swab Test (SST or CAST) and the Sulfa Column Test. These three tests give the veterinarian the easy and economical capability to cover almost any situation related to antibiotic/sulfonamide residues. The initial cost of the LAST and SST tests is less than \$15 and can be conducted by any teenage person with only a few minutes instruction. The Sulfa Column Test is a bit more complicated but with a little experience, can be handled by the average member of your hospital staff. The initial cost of the Sulfa Column Test would be approximately \$50.

The selection and use of the three tests would depend on the type residues suspected. The LAST test has more information available as to the minimal detectable levels of antibiotics than do the other tests. A table of minimal detectable levels of antibiotics by the LAST test is attached. The primary shortcoming of the last test is that it will not detect sulfonamide residues. The SST, on the other hand,

will detect sulfonamide and antibiotic residues. We do not have a list of the antibiotics or the minimal detectable levels, however, it is my understanding that FSIS has this information available.

The Sulfa Column Test is needed to determine if contaminated feed samples contain sulfonamides. For example, a positive feed sample on the LAST or SST test would tell you that there is some inhibitory factor in the feed but will not identify it. The Sulfa Column Test would identify any sulfonamides that might be present in the feed. A negative Sulfa Column Test would indicate that the inhibitory factor is probably an antibiotic rather than a sulfonamide.

**The beauty of the LAST and SST test is that they are quite economical and have a long shelf life. Technical training is not required to conduct the test.**

The LAST test utilizes an alcohol suspension of *B. subtilis* spores that are spread over antibiotic #5 media. Since the

TABLE 1. Minimal Detectable Levels (MDL) of Antibiotics By Bioassay and Stop.

Antibiotics	MDL (MCG/ML)	
	Bioassay	Stop
Ampicillin	0.0063	0.05
Bacitracin	0.1	5.0
Chloramphenicol	2.0	8.0
Chlortetracycline	0.01	0.01
Cloxacillin	0.16	0.16
Erythromycin	0.025	0.025
Flavomycin	0.025	20.0
Gentamycin	0.04	0.04
Hydromycin	5.0	5.0
Lincomycin	0.15	5.0
Neomycin	0.25	0.25
Novobiocin	0.125	15.0
Oleandomycin	0.25	0.25
Oxytetracycline	0.08	0.08
Penicillin	0.0125	0.0125
Polymyxin	Resistant to Gram Positive Organisms At 1000 MCG/ML	
Spectinomycin	10.0	10.0
Streptomycin	0.25	0.25
Sulfamethazine	31.0	500.0
Tetracycline	0.08	0.08
Tylosin	0.2	0.2
Virginiamycin	1.28	5.0

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spores remain in the alcohol suspension until one is ready to conduct the test, shelf life problems are considerably reduced. The SST is a similar test in which *Bacillus megaterium* spores are suspended in alcohol and are spread over the surface of Mueller Hinton media. Both of these tests have been known for many years. The innovative portion that makes this test practical is the alcohol suspension of spores, rather than the introduction of the spores into the media as the plate is being poured.

Another innovation by FSIS scientists is the simple, inexpensive, but effective incubator that is constructed utilizing a styrofoam box and a fish tank heater. The inexpensive fish tank heater is capable of maintaining the proper temperatures for the LAST and SST tests provided it is not placed on a cold floor or in a drafty location.

To conduct the LAST and SST tests, one merely takes the proper media and makes a uniform distribution of the appropriate spores over the surface of the media. Samples of body fluids, such as whole blood, saliva, serum, urine, milk or feed and body tissue may be tested by applying the appropriate solution to a cotton swab or a half-inch analytical paper disk. (Schleicher and Schuell #740-E) These swabs or disks are then placed on the surface of the media. A 5 microgram neomycin disk is placed on the surface to serve as a quality control factor. The dish is placed in an incubator at the appropriate temperature for a minimum of 12 hours. The test is read by observing for the presence or absence of a zone of inhibition around the cotton swab or paper disk. The size of the zone around the neomycin disk is measured to determine that proper conditions have been met.

While the time of the SST and LAST test is 12-18 hours, there is no real disadvantage in most cases. One merely plans appropriate time to allow results to occur before the information is needed. For example, if a milk sample is being tested and placed on the media at the night's milking, the information is available by the next morning. If a shorter time for test results is necessary, then the LAST or SST test would not be suitable.

FSIS has developed and approved the LAST and SST tests for use with urine and other body fluids. However, if whole blood, serum or saliva is utilized as the test body fluid, there must be a period of 4 days lapse between a negative test and the time the animals are marketed. In the case of milk,

the Food and Drug Administration has not approved the LAST test, however, the *B. subtilis* organism was the organism used by laboratory officials before the adoption of *Bacillus stearothermophilus*. *Bacillus stearothermophilus* is considered to be at least 10 times more sensitive to antibiotics than *B. subtilis*. However, if the LAST or *B. subtilis* test is used on an individual cow, we have found no problems in over 6 months of intensive testing. We would not recommend using *B. subtilis* or the LAST test on a bulk milk sample. When used on an individual cow basis, we have found it completely reliable. In fact, our testing has indicated that most antibiotics clear from the milk before 72 hours. In many cases, the withdrawal time is 96 hours. When we have milk samples that show no zones of inhibition on the LAST test, we approve the milk from the next milking to be added to the bulk tank at that point and have had no problems with antibiotic residues.

The Sulfa Column Test is needed in checking animal feed for antibiotic/sulfonamide residues. Screening is normally done utilizing the SST test then doing additional testing on positive samples to indicate the presence or absence of sulfonamides. We have found that levels of antibiotics such as neomycin, are approved up to 50 grams per ton without requiring any withdrawal time. Apparently these low levels do not spill over into the body fluids and tissues. They will however give a positive LAST or SST test. Therefore, additional testing must be done on positive samples.

We have found that occasionally we get false positive tests on the LAST test with milk and serum. Apparently there is some inhibitory factor present. We are able to eliminate the false positive tests by preheating the positive samples of milk or serum in a water bath at 55° C for 30 minutes.

### Summary

With the availability of the Live Animal Swab Test (LAST), the Sulfa Swab Test (SST) and the Sulfa Column Test, a practitioner can have the capability of determining antibiotic/sulfonamides in meat and milk prior to marketing.

**You may purchase supplies needed for the LAST and SST tests from the following: Granite Diagnostics, Box 908, 1308 Rainey Street, Burlington, NC 27215.**

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