How to Perform the Live Animal Swab Test for Antibiotic Residues
The prevention of harmful drug and chemical residues in meat is a concern shared by farmers, consumers, food processors, and manufacturers of drugs, chemicals, and feed as well as the U.S. Department of Agriculture. Residue avoidance helps to assure the safety and wholesomeness of the Nation's meat supply and prevent disruptions in the production and marketing chain.

Violations are costly to producers because of condemnation of animals, disrupted production, and decreased sales. Every year recorded costs from settlements for insurance and other claims stemming from residue problems range from $5 to $10 million, but the toll is much higher and includes not only financial losses but the actual loss of farms where persistent contaminants have left an environment unfit for raising food animals. In addition, residues in the food supply concern consumers and potentially reduce sales. But prevention can help eliminate residue problems and increase marketability of livestock and poultry.

The Live Animal Swab Test (LAST) described in this guidebook is the first tool available for on-the-farm use in checking animals for antibiotic residues before they are slaughtered. While the testing of live animals for residues is a new concept, the use of a microbiological swab test to detect antibiotics is not. In 1979, USDA's Food Safety and Inspection Service began using a swab test on carcasses at the slaughterhouse. The Swab Test on Premises (STOP) Program has helped reduce antibiotic residue violations in cull dairy cows through the cooperative efforts of USDA and the dairy industry.

The swab test used in the STOP program has been adapted for testing live animals on the farm. It should be used on animals which have been treated with antibiotics and are ready for slaughter, and should be performed only after the antibiotics have been withdrawn for the prescribed period.

LAST was originally designed for detecting antibiotics in the urine of dairy cows, and the guidebook describes a urine test. However, in the future, producers and veterinarians will be able to use it to test the blood of cattle and other species. Because antibiotics are cleared from the blood before they are eliminated from tissues, scientists need to determine the exact time lag for each major drug and species. As soon as that information is obtained, USDA will make it available to producers so that the live animal test can be used on blood from all types of animals.

LAST is ready now for urine testing of live cows on the farm. When the urine is free of antibiotics, the tissue levels also have decreased and the animal can be safely marketed. The test can be performed by farmers as well as veterinarians.

This guide provides step-by-step instructions for each part of the simple and inexpensive test. Laboratory know-how is not necessary to perform LAST, and costly equipment is not required, but an incubator is essential. For producers who want to construct an incubator instead of purchasing one, instructions are provided for two easily made and inexpensive types.

LAST was developed by scientists in USDA's Food Safety and Inspection Service, who are working with the Extension Service to inform producers and veterinarians about the availability of this new tool for residue avoidance.

Basic to prevention are accurate records showing which animals received antibiotics and the time treatment was begun and ended. The prescribed withdrawal time for an antibiotic should also be noted and adhered to strictly. By following a manufacturer's instructions and performing LAST, producers can avoid antibiotic residues in their animals.
Equipment and Supplies

Performing the Test

Before the live animal test can be performed, it is necessary to purchase a few inexpensive items. They should be available from a veterinary supply store or a company which makes hospital or laboratory supplies. Three items have been made especially for the swab test described in this guidebook—bacterial suspensions, agar gel plates, and antibiotic discs. The manufacturers of these items are listed on page 15.

The following supplies are needed for LAST:

- Incubator—either purchased or made at home—(see pages 10-15 for instructions).
- A 1-cup container for water.
- Laboratory type cotton swabs (sterile cotton tips with wood or plastic shafts). Cosmetic swabs will do, but not ones with cardboard shafts because the urine could seep out of the cotton tip into the shaft where it would affect bacterial growth and make it difficult to interpret test results.
- A clean wide-mouthed jar with a tight-fitting lid or a self-locking plastic bag for urine collection.
- Small forceps or tweezers.
- A small, flat ruler about 6 inches (15 centimeters) long, preferably marked in millimeters.
- N5 discs (prepared paper discs containing 5 micrograms of neomycin). The purpose of these "control" discs is explained on p. 7.
- Plate containing agar gel which supports bacterial growth.
- A small bottle containing a suspension of the test bacteria, Bacillus subtilis.
- Refrigerator space—for storing urine specimens, N5 discs, plates, and bacterial suspension. (It's best to use a refrigerator out of children's reach to avoid contamination of samples or other accidents. Although the bacteria used are harmless, if a child should swallow the concentrated solution, it could cause stomach upset.)

LAST can be performed quickly (inside cover and figure 1). Cotton swabs are dipped in urine and placed on a specially prepared test plate. During a period of incubation, bacterial growth will form on the plate. The extent of this growth indicates whether antibiotics are present in the animal.

Antibiotics in the urine will kill the bacterial growth on the test plate. Therefore, the presence of an easy-to-see clear zone around the swab is evidence of antibiotics. In this case, the animal should not be marketed. After a few days, it should be re-tested, and if the test produces negative results, the animal can then be marketed.

Conversely, when antibiotics are not present in the animal, the bacterial growth will not be stopped and will completely surround the cotton swab on the test plate. This negative result indicates it is safe to slaughter the animal.

COLLECT THE URINE SPECIMEN after the animal has been off medication for the prescribed withdrawal period.

Urine can be collected in a new plastic bag (self-locking bags are easy to use) or a clean jar. If a jar is used, it should be washed and thoroughly rinsed to completely remove soap or detergents which can interfere with the test. Allow the jar to air dry and cover it until it is used.

The best specimen to use for this test is from the animal's first urination in the morning.

Steps:
1. To induce urination, rhythmically and lightly massage below the cow's vaginal area (figure 2).
2. Catch the sample in a self-locking plastic bag or a jar marked with the cow's identification number and the date.
3. Begin the test within 4 hours; refrigerate the sample until the test is performed.

Figure 1.
Supplies needed to perform the test.
WARM UP THE INCUBATOR about 1 hour before using it. For the test to work properly, the incubator must be between 82° and 86° F. (29° and 31°C.). To assure proper humidity, place a cup or glass of water in the incubator.

PREPARE THE PLATE. The test plates for LAST are 50 millimeters in diameter, which is smaller than those normally used in the laboratory. They contain agar gel, which supports bacterial growth.

Steps:
1. Remove the plate from its plastic wrap and place it on a level surface.
2. Mark an "X" on the side of the plate (figure 3). Use the mark as a guidepost to show where you begin streaking the plate. Consider the mark as the 12 on a clockface.
3. Shake the tightly capped bottle of bacterial suspension to mix uniformly.
4. Remove one of the swabs from its wrapper. To prevent accidental contamination with any substance which kills bacteria (soap, detergents, antibiotics), do not let the cotton tips touch anything.
5. Open the small bottle of bacteria, keeping the top in your hand (See figure 4).
6. Insert the swab into the bacterial suspension, completely immersing the cotton tip in the suspension. Before taking the swab out of the bottle, raise it out of the suspension and gently shake off excess moisture.
7. Lift the swab out of the bottle and replace the bottle cap tightly.
STREAK THE PLATE. The soaked cotton swab is then used to completely streak the plate.

Steps:

1. Remove the lid. Beginning at the X, streak half the plate. Streak back and forth, progressing to the left. Use light, gentle strokes. Excessive pressure can break the gel and interfere with bacterial growth and test results.

2. Using figure 5 as a guide, turn the plate counter-clockwise one-quarter turn. Repeat the streaking process—always streaking from top to bottom and back again, moving toward the left side. Repeat for a total of three turns. Finally, turn the plate one-half turn, and streak again. By turning the plate in this way, each portion is streaked more than once, and complete coverage is assured.

3. Discard the swab. Do not reuse a swab.
Again turn the plate 1/4 turn and repeat the streaking pattern.

Finally, turn the plate 1/2 turn and repeat the streaking pattern.

POSITION THE N5 DISC. The N5 disc contains 5 micrograms of neomycin, an antibiotic that stops *Bacillus subtilis* from growing. It serves as the "control" to show the test is working normally. After the plate is streaked and incubated, the bacteria should grow on the gel but not around the N5 disc.

Steps:

1. Remove the N5 disc dispenser from its plastic tube. Open the bacterial plate and dispense one disc into the cover of the streaked plate (figure 6). **DO NOT** touch the disc with your fingers because the antibiotic could contaminate the swabs and cause the test to give incorrect results. If you accidentally touch the disc, wash and rinse your hands well before continuing.

2. Using clean tweezers or forceps, pick the N5 disc from the cover and gently place it on the surface of the gel (figure 7). **Gently press** the disc in place with the forceps. **DO NOT** break the surface of the gel; **DO NOT** reposition the disc: growth will be inhibited where the disc touches the gel. If you do, begin again with a new plate.

3. Replace the cover on the plate.

4. Return the N5 dispenser to the refrigerator.

TEST THE URINE. Now that the test plate is prepared, you are ready to test the urine.

Steps:

1. Remove a fresh swab from its paper wrapper, and dip it into the bag or jar of urine (figure 8).

2. Gently shake off excess urine, keeping the swab in the bag or jar.

3. Break the shaft as close to the cotton tip as possible **without touching the tip**, and discard the shaft.

4. Uncover the test plate. Hold the shaft with your fingers, and carefully put the swab next to the N5 disc in a rabbit-ear configuration (figure 9). **DO NOT** touch the cotton tip or break the gel.

5. Gently press the shaft with your finger tip to firmly seat the swab tip (figure 9).

**CAUTION:** Discard the plate and prepare another one if (a) the swab should roll across the gel or if (b) the gel cracks. Both events could invalidate the test, giving incorrect results.

6. Replace the cover on the plate.

7. Take out another swab and repeat steps 1 through 5, placing the second swab tip on the other side of the N5 disc in a rabbit-ear configuration.

8. Replace the cover, and put the plate into the preheated incubator (figure 10).

9. Incubate the plate for 18 to 24 hours at 84°F. (29°C.).
### Figure 11.
Interpreting Test Results.

<table>
<thead>
<tr>
<th><strong>A. ANTIBIOTIC NEGATIVE</strong></th>
<th><strong>B. ANTIBIOTIC POSITIVE</strong></th>
<th><strong>C. TEST INCONCLUSIVE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IF YOU HAVE...</strong></td>
<td><strong>AND YOU HAVE...</strong></td>
<td><strong>THEN TAKE THIS ACTION...</strong></td>
</tr>
<tr>
<td>Opaque bacterial growth right up to the swab tips</td>
<td>A clear zone around the N5 disc 10/16&quot;-15/16&quot; (16-24mm)</td>
<td>Animal is ready for market.</td>
</tr>
<tr>
<td><strong>Clear zones around swab tips</strong></td>
<td>Clear zone around N5 disc between 10/16&quot;-15/16&quot; (16-24mm)</td>
<td>Animal has antibiotic residues. Retest in 2 to 3 days.</td>
</tr>
<tr>
<td><strong>Opaque bacterial growth right up to swab tips</strong></td>
<td>Clear zone around N5 disc that is less than 10/16&quot; (16mm)</td>
<td>Rerun test.</td>
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**INTERPRET THE TEST RESULTS.** The warmth of the incubator allows the bacteria to grow. The bacterial growth will make the gel appear opaque and cream colored or greyish instead of clear. But the presence of antibiotics interferes with bacterial growth, creating transparent areas.

**Steps:**

1. Remove the plate from the incubator. Take off the lid and examine the gel. Where bacteria have grown, the gel will have turned creamy or greyish and opaque.

2. **CHECK N5 DISC.** Examine the areas around the cotton tips and N5 disc, using figure 11 A-F as a guide. The area around the N5 disc should be transparent. This clear area is called a "zone of inhibition" because bacterial growth has been stopped or inhibited by antibiotics.

3. **POSITIVE TEST.** If the cotton tips are surrounded by clear "zones of inhibition," the test is positive (Figure 11 B, D, and F). This means antibiotics are present in the animal's urine and tissues. Therefore, the animal should be re-tested in two or three days. Animals should not be marketed until the test produces negative results (no antibiotics).

4. If the cotton tips are surrounded by bacterial growth, no antibiotic residues are probably present in the animal (Figure 11 A and E). It is necessary, however, to verify the accuracy of the test before marketing the animal. To do this, measure the diameter of the clear area around the N5 disc by placing the ruler under the plate (Figure 12).

5. **MEASURE THE ZONE AROUND THE N5 DISC.** A clear zone less than 5/8 of an inch (16mm) in diameter indicates a problem with the test. It is unreliable, and must be rerun. DO NOT market the animal until you have a negative test with a zone of inhibition around the N5 disc that is at least 5/8" (16mm) in diameter.
<table>
<thead>
<tr>
<th>D. ANTIBIOTIC</th>
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<tbody>
<tr>
<td><strong>POSITIVE</strong></td>
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Figure 12.
For a negative test, measure the diameter of the zone of inhibition around the N5 disc.
Building an Incubator

If the expense of an incubator is not warranted by the number of animals you expect to market, you can build your own. Instructions are provided for constructing two types of incubators with readily available, inexpensive components. One is heated by light bulbs and another by bottles filled with hot water. Both provide the warmth needed for bacterial growth.

The Light-Bulb Incubator

This incubator (figure 13) is constructed from a picnic cooler and heated with light bulbs.

Components and Materials Needed

- Automatic aquarium heater
- Foam or other picnic chest (minimum about 14" long, 10" wide and 8" deep)
- Two wired porcelain lamp sockets (with standard sized base)
- Two 7-1/2 watt "night light" bulbs (standard sized base—not small ornamental bulbs)
- Thermometer
- Sharp knife
- Screwdriver
- Electrical tape
- Small container for water (6-ounce cup or glass)
- Jar lids for leveling
- Silicone glue
- Insulated wire fasteners (wirenuts)

Steps:

1. Lay the cooler on a long side so that the opening is in front. One long side of the cooler is the incubator top and the other side is the bottom. If the incubator does not set level, level it by gluing jar lids or other suitable objects on the outside.

2. Using a sharp instrument, cut an opening about 1-1/2 by 1-1/4 inches near the top of the back panel of the incubator to house the thermostat.
Installing the Thermostat. To obtain an inexpensive thermostat, you can remove one from most automatic aquarium heaters. An inexpensive one with low wattage works well; the heating element is not needed (figure 14).

Steps:

1. Separate the thermostat from the heating element. On most types, the unit separates easily when the retaining screw next to the glass tube is removed (figure 15A-B). Then, you can slip off the glass tube to expose the heating element.

2. Clip or untwist the two wires, leaving enough wire to make the electrical connection to the lamp sockets. (The disconnected heating element and the glass tube are not needed for the incubator.) (figure 16.)

3. Reassemble the thermostat, keeping the wire and plug intact because they become the power source for the incubator (figures 17A-17B).

4. Insert the thermostat into the opening in the back of the incubator.

Figure 14.
An aquarium heater and thermostat.

Figure 15-A and 15-B.
Most units separate easily when the retaining screw next to the glass tube is removed.
Disconnect the heating element, leaving enough wire to make the connection to the lamp sockets.

Installing the lamp sockets.
Steps:
1. In the top of the incubator, make 4 small holes for the wiring for the lamps (two small holes for each lamp socket), so the lamps will be centered in each half of the incubator.
2. Feed the wires from each lamp socket up through one hole in the top of the incubator and back into the adjacent hole.
3. Let the sockets hang so the bulbs will be about half way between the top and bottom of the incubator. For added stability, tape the wires to the top of the incubator.

Connecting the lamps and the thermostat. The lamps are connected to the thermostat in a "parallel" circuit.
Steps:
1. Most wired sockets have two color-coded wires. Inside the incubator, twist together the wires of the same color from the two sockets (figures 18A and 18B).
2. Take these joined wires and twist to one of the wires on the thermostat. Use insulated fasteners (wirenuts) on the joined wire and always cover securely with electrical tape.
3. Twist together the other two socket wires of the same color, and join these to the remaining wire on the thermostat. Again, fasten wires together and secure with electrical tape.
4. Insert a 7-1/2-watt "night light" bulb in each socket.
5. Take all precautions needed when working with electricity. To check the installation, plug the incubator into any 110-volt outlet. Turn the adjustment knob until the bulbs light.

Reassemble the thermostat and install it in the incubator.
Calibrating the heater and thermostat. Thermostats operate by opening and closing electrical contacts in response to the surrounding temperature. They can be adjusted with the connected knob.

Steps:

1. To calibrate, put the incubator in an area as free from draft as possible, and attach the front cover securely.
2. Insert the thermometer through the front cover. Position the thermometer to measure the temperature of the air around the plates.
3. If the temperature is below 84°F (29°C), adjust the thermostat so that the lights go on. If the lights go out before the temperature reaches 84°F, readjust the thermostat until the lights go back on.
4. Check the incubator every 5 to 10 minutes for an hour, adjusting the thermostat knob as necessary. In this way, you will calibrate the thermostat to maintain the proper temperature. The incubator now is ready for use (figure 19).

Figures 18-A and 18-B.
Twist together wires of the same color from the two sockets.

Figure 19.
The lightbulb incubator is now ready for use.
The Water Bottle Incubator

With this type of incubator (figures 20 and 21), the warmth needed for bacterial growth comes from hot water in bottles. Therefore, it is important to have all materials ready and to move quickly to avoid heat loss. You can use a metal or plastic picnic cooler or even a cardboard box. If you use a box, the bottles need to be surrounded by crumpled newspaper for insulation. The plates are placed on a piece of cardboard resting on bottles or newspaper.

- **Equipment needed:**
  - Picnic cooler or a cardboard box (preferably with a fitted lid) at least 18" long x 14" wide x 18" deep. (If the box has no fitted lid, tape the top closed during the incubation period.)
  - Newspaper
  - Two 1-gallon bottles (such as plastic antifreeze or milk bottles)
  - Thermometer
  - Duct or masking tape

Steps:

1. When you are ready to incubate the test plates, fill the two bottles with hot water.
2. Set the bottles upright on each side of the cooler or cardboard box. When using a box, quickly pack crumpled newspaper around each bottle.
3. When using the box-type incubator, check the temperature of the water before closing the box. It
Sources for Supplies and Equipment*

should reach about 88° F. (31° C.). If it is lower, put warmer water in the bottles.

4. To make a shelf, put a piece of cardboard on top of the bottles (and newspaper).

5. Put the test plate on the shelf.

6. For the box-type, cover everything with folded newspapers (figure 22). Pierce a hole in the top of the box, and insert the thermometer. Be sure the tip of the thermometer is near the test plate so that the temperature of the air around the plate will be accurately recorded (figure 23).

7. If the box does not have a fitted top, tape it closed. Put it on a table (not on the floor) in a warm place protected from drafts.

8. A few minutes after closing the box-type incubator, check the temperature on the thermometer. If it is lower than about 84° F. (29° C.), put warmer water in the bottles or put the incubator in a warmer room.

Incubators

Incubators are available from several manufacturers. While this list is not all inclusive, it is provided as a convenience. Other brands are also suitable for the LAST test.

- American Scientific Products
  8855 McGaw Road
  Columbia, MD 21045
  Phone: (301) 992-0800

- Lab-Line Instruments, Inc.
  Lab-Line Plaza
  Melrose Park, IL 60160
  Phone: (312) 345-7400

- SGA Scientific Inc. (Company has 7 outlets)
  735 Broad Street
  Bloomfield, NJ 07003
  Phone: (201) 748-6600
  Cat. No. 1-4019

- Sargent-Welch (Company has 10 outlets)
  5915 Peeler Street
  P.O. Box 35445
  Dallas, TX 75235
  Phone: (214) 357-9381
  Cat. No. S-43654

- VWR Scientific (Company has 31 outlets)
  P.O. Box 1004
  Norwalk, CA 90650
  Phone: (213) 921-0821
  Cat. No. 35923-014

- Arthur H. Thomas Co.
  P.O. Box 779
  Philadelphia, PA 19105
  Phone: (215) 574-4555
  Cat. No. 6127-AID

Supplies

The companies manufacturing incubators also supply cotton-tipped swabs, plastic self-locking bags, and small forceps.

N5 Disc (Neomycin antibiotic sensitivity disc)

- BBL
  P.O. Box 243
  Cockeysville, MD 21030
  Phone: (301) 628-7601

- Difco
  Box 1058A
  Detroit, MI 48232
  Phone: (313) 961-0800

Plates and bacterial suspension

Some of the firms listed below also may be able to supply a kit containing items needed to perform the test.

- J & L Analytical Services
  (2068 Laphan Drive)
  Mail: P.O. Box 6185
  Modesto, CA 95355
  Attention: Dr. R. Jacobs
  Phone: (209) 524-9746

- Northeast Laboratory Services
  (China Road). Attn: William Colby
  Mail: P.O. Box 788
  Waterville, ME 04901
  Phone: (207) 873-2068

- Flow Laboratories, Inc.
  7655 Old Spring House Road
  McLean, VA 22102
  Phone: (703) 893-5925

*This listing of company names is provided as a convenience because some of the items needed for LAST are available from only a few sources. It is not intended as a warranty or an endorsement by the U.S. Department of Agriculture to the exclusion of other manufacturers not mentioned.