

Antibiotic Residue Avoidance Strategy Steps ... Bottom Line:

1. Identify all animals treated
2. Record all treatments: Date, ID, Dose, Route, Who treated, Withdrawal (WD)
3. Strictly follow AMDUCA guidelines which includes following FDA (drugs) - USDA (Vaccines) - EPA (Pesticides) guidelines for product use.
4. Use newer technology antibiotics when possible ...
 - a. Reduce unwanted depot effect. Select low volume products when available.
 - b. Select generic medications and vaccines with EXTREME CAUTION ... Don't step over dollars trying to pick up a pennies" ... "Cheap" products, if inferior, may cause performance loss or damage quality.
5. Select with short WD when antibiotic choice is equivalent
6. Never give more than 10 CC per IM injection site
7. Avoid using multiple antibiotics at the same time
8. Don't mix antibiotics in the same syringe ... especially if given IM or Sub-Q
9. Check ALL medication/treatment records before marketing:
 - a. Don't market cattle with less than 60 WD without examining the treatment history.
 - b. Extend the withdrawal time if the route or location of administration is altered
 - i. Example; the WD for ear route of administration ceftiofur crystalline acid will be over 120 days if given SQ in the neck
 - ii. Extend the withdrawal time for multiple medications given by summing their label recommended WD
 1. Example; if med 1 has a 10 day WD and med 2 has a 28 day WD, assign a 38 day WD
 2. Example; if med 1 has a 10 day WD and is repeated in three days, assign a 20 day WD
 - iii. Don't inject gentamycin or neomycin. The WD is estimated over 24 months and a urine test will not detect kidney residues.
 - c. Don't market cattle that have relapsed without examining the treatment history.
 - d. Don't market cattle with suspected liver or kidney damage without examining the treatment history.
 - e. Don't market cattle with antibiotic injection site knots without examining the treatment history.
10. Screen the urine for antibiotics of all cattle identified in step 9 above.
 - a. Best to use broad spectrum microbial inhibition test such as the Pre-Harvest Antibiotic Screening Test (PHAST) which uses B. megaterium as the test organism.

Live Animal Swab Test (LAST) Pre-Harvest Antibiotic Screening Test (PHAST)

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The LAST and PHAST are field tests for screening cattle for potential antibiotic residues. These tests are not FDA or USDA-FSIS approved tests nor do these carry any guarantees about detecting cattle that may contain a violative residue.

These tests mirror the antimicrobial screening tests used by the USDA-FSIS, screening for microbial inhibitors in the urine as apposed to the kidney.

The LAST mirrors the STOP (Swab Test On Premise), which uses Bacillus subtilis (Bs). The PHAST mirrors the FAST (Fast Antibiotic Screening Test) that uses Bacillus megaterium (Bm).

Another useful microbial inhibition test is the Difco Bacillus sternotheophilus disk assay. (Difco Labs, 313-462-8500). I have been disappointed in the CHARM series of test and the drug specific test kits. Test that target only beta-lactam antibiotics are not appropriate for screening cattle for marketed for meat.

Why Do We Need To Screen Selected Cattle Prior To Marketing

First, non-performing animals that have been treated with an antibiotic should be considered “High Risk for Antibiotic Residue Violation” because of the potential for their poor performance to be associated with organ (liver or kidney) dysfunction. Liver and kidney function is vital to clearing antibiotics. Second, extra-label drug use (ELDU) require, by law, requires a veterinarian to adjust the withdrawal time from the label indications to a time more appropriate to the dose used. This includes using subcutaneous (SQ) when labels indicate IV or IM and increasing the dose above the label dose. Third, doses above 10 cc/site may depot and not be eliminated as rapidly as required to meet assigned withdrawal time.

Introduction to the LAST and PHAST Tests

The LAST and PHAST are microbial inhibition tests (substances in the urine that inhibit the growth of the test microbe (Bacillus subtilis for the LAST/STOP) and (Bacillus megaterium for the PHAST/FAST). Bacillus subtilis (Bs) and Bacillus megaterium (Bm) are classified as GAAS (Generally Accepted As Safe) bacteria. The organisms will not cause disease in humans or domestic animals.

Microbial inhibition tests are indirect assays that are dependent on a residue being passed in the urine in a chemical form that inhibit the growth of the test organism. The

LAST test was developed in the late 70's by the USDA-FSIS-Science Section using the MIC for various antibiotics on Bacillus subtilis (Bs). In-vitro methodologies were the only validation of this test. The test was intended for use by dairymen as an aid in detecting antibiotic residues in milk before returning a cow to the milking line. The organism used (Bs) is the same bacteria used in the STOP test used by USDA-IICs in packing plants prior to 2000 to screen suspect cattle for violative antibiotic residues. Evaluation of the testing technology by the UCD demonstrated microbial inhibition tests are the more reliable method of detecting microbial residues.

The PHAST test was developed in 2000 by UNL-GPVEC using the MIC for various antibiotics on Bacillus megaterium (Bm). The organism used (Bm) is the same bacteria used in the FAST test used by USDA-IICs in packing plants since 2000 to screen suspect cattle for violative residues.

Because Bs and Bm are frequently more sensitive to target antibiotics than the FDA established tolerance for the target antibiotic and because there are a number of microbial inhibition substances that are not antibiotics, false positives are the most common problem with these types of tests. False negatives are thought to be rare, but are dependent on the sensitivity of the test organism to the antibiotic relative to the FDA tolerance to the target antibiotic. Bs and Bm are very sensitive to penicillin type antibiotics and intermediate to aminoglycosides. While the reliability of the LAST or PHAST for detecting violative residues in beef cattle has not been investigated, the LAST test has been used since 1980 as an aid in evaluating cattle culled from feedyards. The most common false positives have been associated with antibiotics, such as Naxcel and Oxytetracycline, which has an FDA established tolerance level above the level cleared in the urine.

THE LAST and PHAST ARE NOT A RELIABLE TEST TO EVALUATE THE RESIDUE STATUS OF AN ANIMAL WHICH HAS NOT MET THE WITHDRAWAL TIME SPECIFIED ON AN ANTIBIOTIC LABEL. Never use the LAST test to evaluate the residue status of an animal, which has not met the withdrawal time specified on the label of an antibiotic.

The LAST or PHAST is useful in evaluating cattle which have: undergone prolonged treatment, treated with multiple antibiotics, and/or failed to perform normally following therapy or have suspected organ damage which might interfere with excretion and elimination of an antibiotic.

Avoiding violative residues is dependent on: 1) using FDA approved medications, 2) following label directions when possible, 3) ELDU must have withdrawal times appropriate for the dose, medication and route of administration, 4) not exceeding dose per injection site recommendations, and 5) screening cattle which may not have cleared the antibiotics normally.

MAKING A LAST / PHAST INCUBATOR

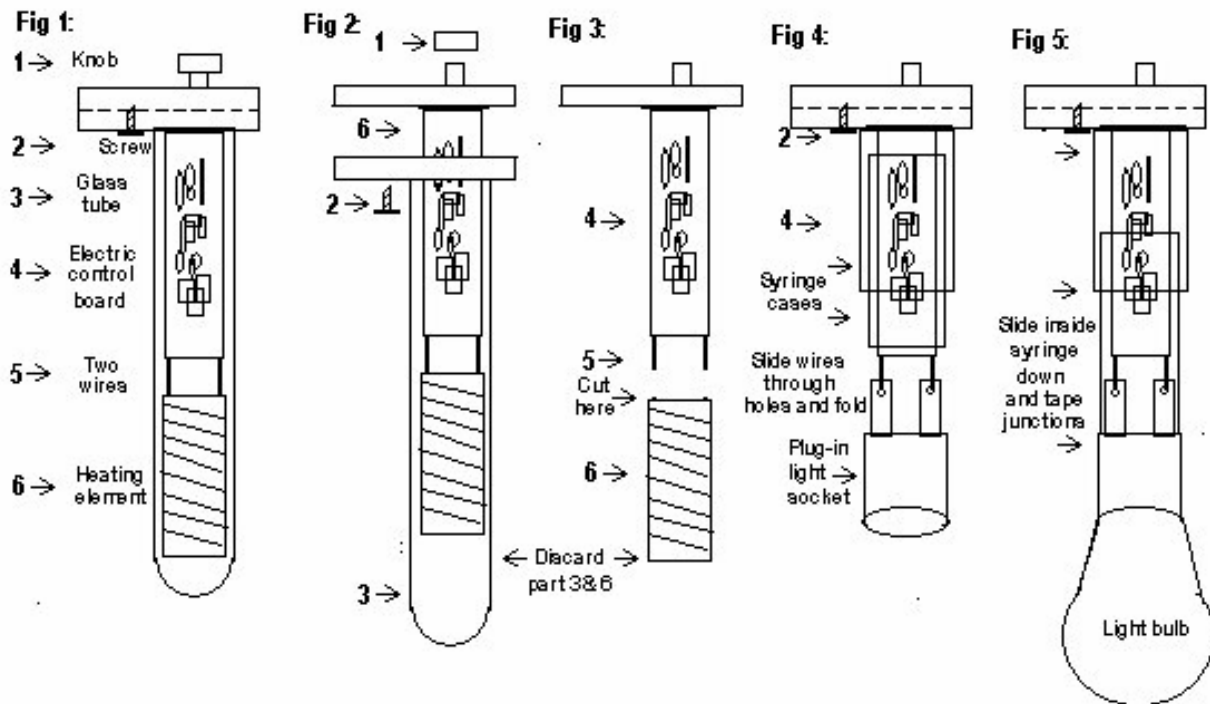
Convert an aquarium heater to a controllable heater for an ice chest bacteriology incubator. Materials required include: a constant read thermometer, Styrofoam ice chest, a 100 watt aquarium heater, 15 to 60 watt light bulb, plug-in light socket for standard light bulb, electrical tape, a 20CC BD disposable syringe, and a 30 CC BD disposable syringe. The syringes may be any two large syringe sizes that will telescope over each other and the smallest will also cover the electric board of the aquarium heater).

Steps:

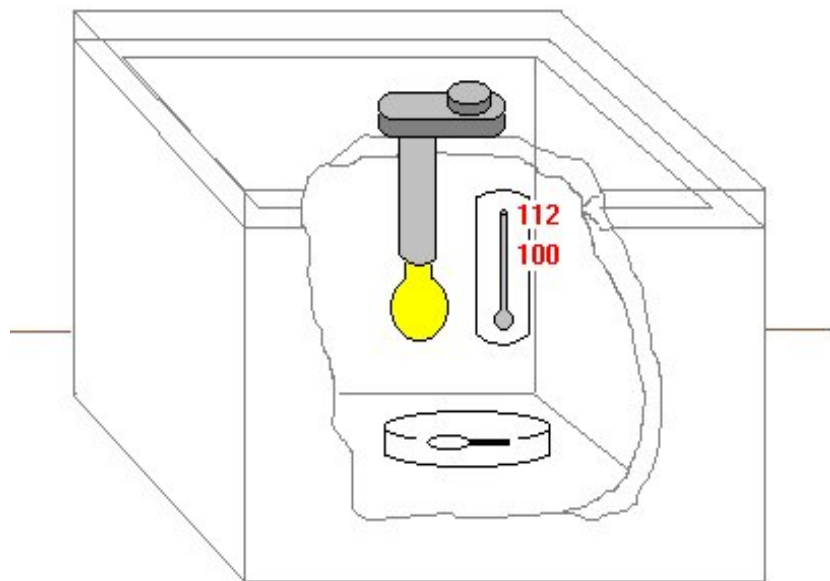
If you have any questions about the following instructions please visit with your extension educator.

1. The aquarium heater must be unplugged. Remove the temperature control knob from the top of the aquarium heater by pulling it straight up from the top of the heater. Remove the screw from the bottom of the aquarium heater housing. Gently pull the heater apart. The electric heating control board and the heating element will come out with the top of the heater. The glass tube covering the heating element and the electric heating control board will stay with the bottom part of the aquarium heater housing. Slip the glass tube out of the bottom housing and discard the glass tube.
2. Remove the white heating element from the electric board by cutting the two wires at the junction next to the white heating element. Leave the two wires as long as possible.
3. Slide the bottom aquarium housing back over the electric board and replace the screw.
4. Disassemble the two plastic syringes and discard the plungers. Carefully cut the top and the bottom off both syringes (a hacksaw works well). The two syringe cases should slide inside each other. Trim both syringes until both are slid inside each other and slipped over the electric board of the aquarium heater leaving the entire length of both wires from the electric board protruding. Slide the syringes over the aquarium heater's electric board.
5. Attach each of the two wires to each of the two prongs of the plug-in electric light socket. Make sure each wire touches it's assigned prong BUT not touching each other.
6. Securely tape the outer syringe cover to the bottom part of the aquarium housing. Extend the inter-syringe tube (the smallest) until it is snug against the light socket. Securely tape the junction of the two syringes.

7. Visually examine the wires as they attach to the prongs of the light socket to ensure each only touches its assigned light socket prong. Securely tape the junction between the syringe and the plug-in light socket.
8. Install light bulb, plug in and see if light comes on. IT SHOULD.



9. "Poor-Boy" Incubator:
Cut a hole in the ice chest to allow the light socket to slide inside the chest. Install the thermometer. And adjust the control knob on the top of the heater until the light stay on often enough to keep the temperature in the incubator at 85 to 112 degrees F (30 to 44 degrees C).



COLLECTING URINE

Veterinarians are taught to collect urine from female cattle in veterinary school, but few are taught to collect urine from male cattle.

Collecting urine from male cattle requires a SCREW TOP TUBE (plastic tubes are safer). While an animal is restrained in a chute, grasp the hairs on one side of the prepuce and slide the screw top test tube into the prepuce. The rough rings of the test tube sliding into the prepuce will stimulate most male cattle to urinate. Sliding the test tube back and forth inside the prepuce may stimulate urination in cattle that did not immediately urinate when the tube was first slid in the prepuce. If the animal does not void at least a few milliliters of urine, leaving at least one half of the tube in the prepuce; pull the hair from the tip of the sheath around the test tube and secure with a rubber band. Turn the animal out and wait for him to urinate. You usually do not have to wait long before you can bring him back through the chute and collect a urine filled tube.

One caution about collecting urine from females. Get a mid-stream sample. Fecal material or a significant quantity of cell debris may cause a false positive test.

LAST TEST SUPPLIES

The University of Nebraska Great Plains Veterinary Educational Center will make Bacillus subtilis (Bs) spores (ATCC 6633) and/or Bacillus megaterium (Bm) spores (ATCC 9885) available to Nebraska veterinarians. The Bs spores (Difco #0453-36) and/or Bm spores (Med-Tox Lab) will come diluted to 1×10^6 spores per milliliter. The charge will be approximately \$10 per vial of spores. For LAST (Bs) or PHAST (Bm) spores, call 402-762-4500 or e-mail Dee Griffin (dgriffin@gpvec.unl.edu).

Additional sources of PHAST spores (*B. megaterium*) are Raven Biological Laboratories 8607 Park Drive, P.O. Box 27261, Omaha, Nebraska 68127 (800.728.5702), info@ravenlabs.com, <http://www.ravenlabs.com/>. Additionally, MedTox Diagnostics, Dr. Robert Schmidt, 1238 Anthony Rd, Burlington, NC 27215, 800-334-1116, Catalog/Part # 600181 (Fast Kit) ... 10 plates ... \$48.30)

The N5 (5 mcg neomycin) antibiotic sensitivity disk used for the control (item # 617189) and Muller-Hinton agar plates (item # 1008) can be obtained from Physicians Lab Supply, P.O. Box 853, Rochester, MI 48308 (1-800-445-6507). The approximate cost for the N5 disks is \$5 per cartridge of 50 disks. The approximate cost for the 100cm Muller-Hinton agar plates are \$5 per package of 10 plates.

NOTE: WRAPPING AGAR PLATES IN PLASTIC WRAP (HANDI / GLAD) WILL DRAMATICALLY EXTEND THE SHELF LIFE.

The best tube I have used for collecting urine is the Becton-Dickenson FALCOM 2027 tube. The cost is approximately \$25 per package of 125 tubes. Any sterile rough edge tube smaller than 10 ml will work.

Any sterile cotton tipped applicator will work as long as the stick behind the cotton tip breaks easily.

STREAK THE PLATE

Soak a cotton swab soaked in LAST spores is used to completely streak the plate.

Steps:

1. Soak a cotton swab in LAST spore (Bacillus subtilis). Even though LAST spore will not cause disease in humans or animals, handle with care.
2. Remove the lid from Muller-Hinton agar. Beginning at one side, completely streak the entire surface of the agar plate. Rotate the plate 45 degrees and repeat. The objective is to cover the plate with a carpet of B. subtilis. Use light, gentle strokes. Excessive pressure can break the gel and interfere with bacterial growth and test results.
3. Discard the swab. *Do not reuse a swab.*

POSITION THE N5 DISC

The N5 disc contains 5 micrograms of neomycin; an antibiotic that stops Bs and/or BM spores from germinating and growing. The disc 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. The clear zone around the control disc should be at least 5/8 inch (16 mm) for the zones around the LAST or PHAST screening swabs to be considered valid.

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. Returns 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 wrapper, and dip it into the bag or jar of urine (fig 8).
2. Gently shake off excess urine, keeping the swab in the bag, tube, 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 (fig 9).
5. Gently press the shaft with your fingertip to firmly seat the swab tip (fig 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 (fig 10).
9. Incubate the plate for 12 to 24 hours at 100° F (37° C). The Bs and Bm spores grow well between 85° F (29° C). At the lower temperature, the spores are slower to germinate and the test will require longer than 12 hours incubation to obtain an interpretable result. At temperatures above 110° F, the spores may not germinate.

INTERPRET THE RESULTS

The warmth of the incubator allows the bacteria to grow. The bacterial growth will make the gel appear opaque and cream colored or grayish instead of clear. But the presence of antibiotics interferes with bacterial growth, creating transparent areas.

Steps:

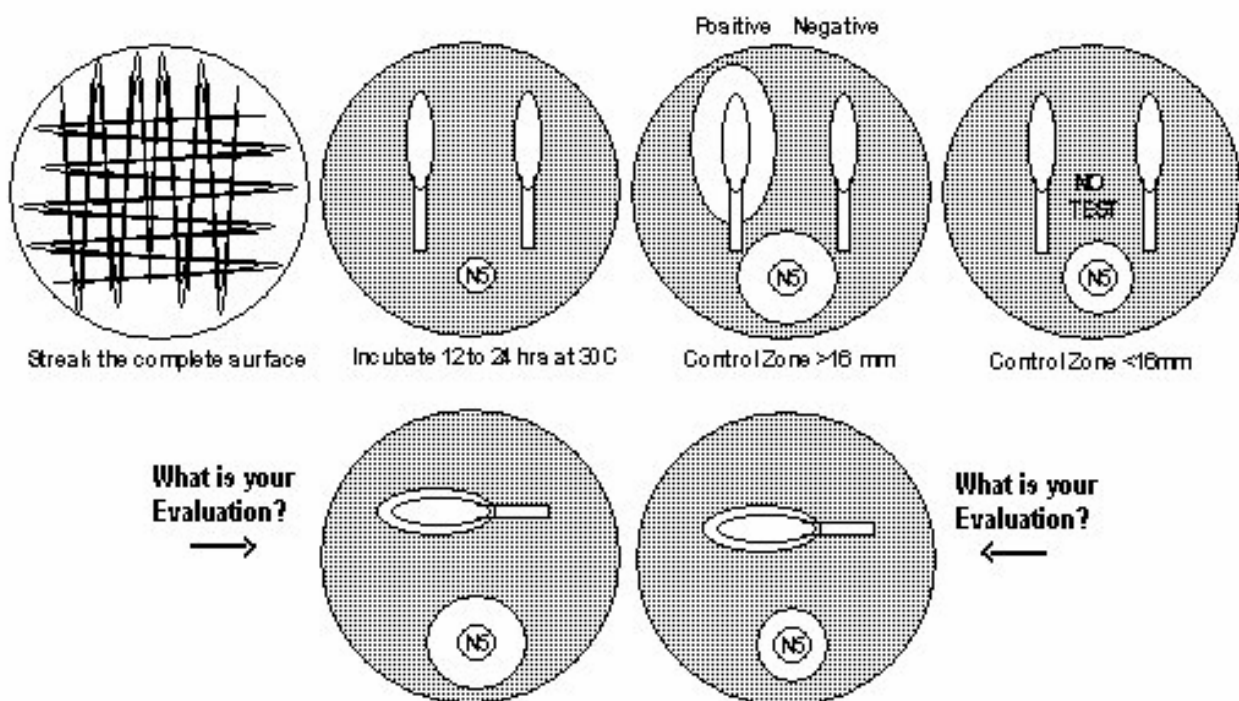
1. Remove the plate from the incubator. Take off the lid and examine the gel. Where bacteria have grown, the gel will have turned creamy or grayish and opaque.
2. CHECK N5 DISC. Examine the areas around the cotton tips and N5 disc, using figure 11 A-F as a guide. The area around the N5 disc should be 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 N5 disc that is at least 5/8" (16mm) in diameter.

Note: Frequently the bacterial growth adjacent to the swab will appear thicker as if a white halo has developed. It appears to be associated with estrogenic compounds voided in the urine of some cattle and does not appear to interfere with the microbial inhibition test.

Figure 5. Interpreting Test Results

| | Antibiotic Negative | Antibiotic Positive | Test Inconclusive | Antibiotic Positive | Antibiotic Negative | Antibiotic Positive |
|--------------------------|--|--|---|--|--|--|
| IF YOU HAVE... | Opaque bacterial growth right up to the swab tips | Clear zones around swab tips | Opaque bacterial growth right up to swab tips | Clear zones around swab tips | Opaque bacterial growth right up to the swab tips | Clear zones around swab tips |
| AND YOU HAVE... | A clear zone around the N5 disc 10/16" - 15/16" (16-24 cm) | Clear zone around N5 disc between 10/16" - 15/16" (16-24 cm) | Clear zone around N5 disc that is less than 10/16" (16mm) | Clear zone around N5 disc is less than 10/16" (16mm) | A clear zone around the N5 disc greater than 15/16" (24mm) | Clear zone around N5 disc greater than 15/16" (24mm) |
| THEN TAKE THIS ACTION... | Animal is ready for market. | Animal has antibiotic residues. Retest in 2 to 3 days. | Rerun test. | Animal has antibiotic residues. Retest in 2 to 3 days. | Animal is ready to market. | Animal has antibiotic residues. Retest in 2 to 3 days. |



Residue Detection Limits for Antibiotic Commonly Used in Cattle

| Generic Name | Trade Name | FDA NADA | Tolerance ^c | STOP ^{ae} Detect | FAST ^{be} Detect | WD |
|--------------------------|-------------------------|-------------------|---------------------------------------|---------------------------|---------------------------|--------|
| Ampicillin / Amoxicillin | Polyflex / Amoxi-Inject | 055-030 / 055-089 | 0.1ppm edible | >0.1 ^f | 0.2 ^d | 8 |
| Ceftiofur | Naxcel / Excenel | 140-338 / 140-890 | 8 ppm kidney, 3 ppm muscle | >0.1 ^f | ~ 0.1 ^f | 0-2 |
| Danofloxacin | A180 | 141-207 | 4 ppm liver, | >0.1 ^f | >0.1 ^f | 4 |
| Enrofloxacin | Baytril | 141-068 | 0.3ppm muscle, | >0.1 ^f | 0.1 to 1 ^f | 28 |
| Erythromycin | Gallimycin | 012-123 | 0.1ppm edible | >0.4 ^d | >0.05 ^d | 8 / 28 |
| Florfenicol | Nuflor | 141-063 | 12ppm kidney, 3.7 ppm liver | >1 ^f | 1 to 10 ^f | 28 |
| Gentamicin | Garasol | 101-862 | No tolerance-cattle, 0.4 kidney swine | >1 ^f | 0.13 ^d | 365+ |
| Lincomycin | Lincocin | 200-189 | No tolerance-cattle, 0.1 edible swine | >10 ^f | 8 ^d | 60 |
| Neomycin | Biosol | 200-113 | 7.2ppm kidney, 1.2ppm muscle | ~10 ^f | 0.06 ^d | 365+ |
| Oxytetracycline (depo) | TNTC | 097-452 | 12ppm kidney, 2ppm muscle | >10 ^f | 0.8 ^d | 21 -36 |
| Pen G (300k = 200mg*180) | TNTC | 065-505 | 0.05ppm edible | >0.1 ^f | <0.01 ^d | 60 |
| Spectinomycin | Adspec | 141-077 | 4ppm kidney, 0.25ppm edible | >10 ^f | 6.2 ^d | 11 |
| Sulfa-chlorpyridazine | Vetisulid-215 | 033-318 | 0.1ppm edible | >100 ^f | ~10 ^f | 8-21 |
| Sulfa-diamethoxine | Albon40 / AlbonSR | 041-245 / 093-107 | 0.1ppm edible | >100 ^f | ~1 ^f | 8 -21 |
| Sulfa-methazine | TNTC | 140-270 | 0.1ppm edible | >100 ^f | ??? | 8-21 |
| Tilmicosin | Micotil | 140-929 | 14.4ppm kidney, 1.2ppm liver cattle | >10 ^f | 1 to 10 ^f | 28 |
| Tulathromycin | Draxxin | 141-244 | 5.5 ppm liver (~=1.6 ppm kid?) | >0.1 ^f | >0.1 ^f | 18 |
| Tylosin | Tylan | 012-965 | 0.2ppm kidney, & liver cattle | >1 ^f | 1 to 10 ^f | 21 |

| | |
|----|--|
| .a | <i>Bacillus subtilis</i> (ATCC: 6633) the test bacterium for the STOP antibiotic residue screening test |
| .b | <i>Bacillus megaterium</i> (ATCC: 9885) the test bacterium for the FAST/CAST antibiotic residue screening test |
| .c | FDA published tolerance for levels found in listed edible tissue/food ... http://www.cvm.gov ...FOI January 2000 |
| .d | Korsrud, G.O., Evaluation of the swab test on premises, the calf antibiotic and sulfa test, and a microbial inhibitor test with standard solution of 22 antibiotics. <i>Journal of Food Protection</i> . 51:1 43-46, 1988. |
| .e | Test antibiotic diluted to ppm (microgram/ML) listed. |
| .f | FAST: Test conducted at NU-GPVEC, USDA-FSIS Approved Test Source: MedTox Diagnostics, Kit # 600181 Burlington, NC, 800-334-1116 |

USDA-FSIS Residue Testing Program Information: <http://www.fsis.usda.gov/OA/background/mircrotest.htm> ... <http://www.fsis.usda.gov/OA/background/residue.htm> ... <http://www.fsis.usda.gov/ophs/nrp2000/index.htm>

| Antimicrobial groups approved for cattle: | | | |
|---|-------------------------------------|------------------|--|
| Aminocyclitols | spectinomycin | fluoroquinolones | enrofloxacin, danofloxacin |
| Aminoglycosides | gentamicin, neomycin | lincosamides | lincomycin |
| beta-lactams | penicillin G, ampicillin, ceftiofur | macrolides | tilmicosin, tulathromycin, tylosin |
| chloramphenicol derivatives | florfenicol | sulfonamides | sulfadimethoxine, sulfamethazine, sulfachlorpyridazine |
| Flavophospholipol | bambermycin | tetracyclines | oxytetracycline, chlortetracycline |

Safety evaluation of Bacillus subtilis and Bacillus megaterium

The Bacterium

Bacillus subtilis, American Type Culture Collection (ATCC) 6655 (Difco #0453), and Bacillus megaterium (ATCC 9885) are non-pathogenic bacterium commonly used in quality control testing by the United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) (Sneath 1990, USDA-FSIS 1983). Genet referred to Bacillus subtilis as "a well-characterized, gram-positive, non-pathogenic, spore-forming soil bacterium (Kreft 1982). De Boer in an article entitled "On the safety of Bacillus subtilis and Bacillus amyloliquefaciens: A review" said an overwhelming majority of recombinant microorganisms to be used by industry are expected to be based on harmless hosts. He went on to say, "Many of these (including Bacillus subtilis) have been proven safe over many years of experience in industrial settings." "Furthermore, extensive information on the incapacity to cause disease, i.e. non-pathogenic and non-toxicogenic, potential, of some of these organisms (including Bacillus subtilis) can be found in the literature" (de Boer 1991). Other papers also listed Bacillus subtilis as non-pathogenic, especially to humans. (Ghiani 1993, Raza 1993, Harwood 1992, and Zazzerini 1985). The best reference to the safety of Bacillus subtilis is found in Food and Drug Administration (FDA) regulations in which the bacterium has a "Generally Recognized As Safe" (GRAS) classification (Wasserman 1984).

Bacterium Culture

Vials of Bacillus subtilis spores are available through the University of Nebraska Great Plains Veterinary Educational Center (402-762-4500), Difco (201) 847-6800, Dairy Herd Improvement Association and the USDA-FSIS. Vials of and Bacillus megaterium spores are available from University of Nebraska Great Plains Veterinary Educational Center (402-762-4500), Med-Tox Diagnostics (800-334-1116). Each vial contains 1.0×10^6 spores. This bacterium will not cause disease in plants or vertebrates and grows well in nutrient agar containing five percent sheep red blood cells incubated at 29°C (Sneath 1990).

Standard Microbiological Safety

A 0.525 percent sodium hypochlorite (NaOCl) solution will be prepared for cleaning the work area in my kitchen after completion of each days experimental work. Control cultures of the work area will be preformed following each days activities to insure sterilization of the work area was accomplished.

References

De Boer, A.S. 1991. On the safety of *Bacillus subtilis* and *Bacillus amyloliquefaciens*: A review. *Applied Microbiology and Biotechnology* vol 36, no 1, pp. 1-4.

FDA published tolerance for levels found in listed eatable tissue/food ...

<http://www.cvm.gov> ...FOI January 2000

Ghiani, M. 1993. Microbial purification techniques of mineral dressing plants reject waters. *Advances On Biohydrometallurgy - Microbiology and Applications*. Vol 11, no 1-3 pp 153-158.

Griffin, D. D. 2000. Pre-Harvest Antibiotic Screening Test (PHAST) using *Bacillus megaterium* to screen cattle urine for microbial growth inhibition. University of Nebraska Great Plains Veterinary Educational Center, Teaching Notes, July 2000.

Harwood, C. R. 1992. *Bacillus subtilis* and its relatives: Molecular biological and industrial workhorses. *Trends Biotechnology*. vol 10, no 7, pp 247-256.

Korsrud, G.O., Evaluation of the swab test on premises, the calf antibiotic and sulfa test, and a microbial inhibitor test with standard solution of 22 antibiotics. *Journal of Food Protection*. 51:1 43-46, 1988.

Kreft, J. 1982. Cloning vectors derived from plasmids and phage of *Bacillus*. *Gene Cloning in Organisms Other Than E. coli*. ISBN: 3-540-11117-4 pp 1-17.

Raza, T. A. 1993. Comparison of vaginal bacterial flora in teddy goats with and without reproductive disorders. *Industrial Journal of Dairy Science*. vol 46, no 1, pp 1-5.

Schmidt, R. FAST Instructions for Kit # 600181 (*Bacillus megaterium* to screen cattle kidney for antibiotic residues). Med-Tox Diagnostics, Burlington, NC 27215 (800-334-1116)

Sneath, P. H. 1990. *Bergey's Manual of Systematic Bacteriology*. Baltimore, MD: Williams & Wilkins.

USDA-FSIS 1983. *Agriculture Handbook* no 601.

Wasserman, B.P. 1984. Thermostable enzyme production. *Food Technology*. vol 38, no 2, pp78-89.

Zazzerini, A. 1985. Morphological and biological features of *Sclerotium bataticola* Taub. on sunflower. *Riv. Patol. Veg.* vol 21, no 3, pp 129-140.

INFORMATION ABOUT DRUG USAGE CAN BE FOUND FROM:

FDA: 301-594-1737 for AMDUCA Information ... AMDUCA Info also available from the FARAD Internet site and at the AVMA Members Only Internet site: (requires ID number)
<http://www.avma.org/scienact/amduca/amduca1.asp>

FDA web site: <http://www.fda.gov/cvm/default.html>

US Pharmacopeia: <http://www.usp.org/>

Food Animal Residue Avoidance Database (FARAD) developed to provide drug information to veterinarians and producers to help them evaluate the potential for a drug residue. Presently, funding has been eliminated from the government budget and the service is not available. Should funding be reestablished FARAD may be contacted from the phone numbers or Internet site below.

FARAD Regional Access Center: 1-888-USFARAD (1-888-873-2723)

FARAD web site: <http://www.farad.org/>