Contents

INTRODUCTION

Objectives

By using this guide, the performer will be able to:

- Collect and identify a tissue sample from a properly identified retained carcass,
- Prepare suitable tissue swabs for testing,
- Prepare and incubate the FAST plate,
- Interpret and record the test results,
- Take proper action based on the test results,
- Submit accurate and complete FAST reports, and
- Receive, check, modify, and/or stabilize the FAST incubator.

Primary Trainee Population

This guide is intended for use by FSIS veterinarians and designated food inspectors who have responsibility for performing the Fast Antimicrobial Screen Test.

Secondary Trainee Population

This guide may also be used by supervisors to review FAST procedures and results.

Restrictions

This guide is limited to the performance of the Fast Antimicrobial Screen Test for antibiotic and sulfonamide residues developed by USDA, FSIS, Science & Technology, Microbiology Division as it is performed on tissues from red meat carcasses.

Use by FAST Performers

This guide is designed to lead the new performer through each step in performing the test. Careful attention to detail is necessary. The guide must be used each time FAST is performed until the performer is able, without guidance, to accurately and completely perform all behaviors described. The guide can then be used as a reference when future tests are performed.

OVERVIEW OF FAST

Definition

The Fast Antimicrobial Screen Test (FAST) is a biological screening test for the detection of antimicrobial residues in animal tissues. It is designed to be performed by a veterinarian or a designated food inspector in a slaughtering plant. FAST is an adaptation of the antimicrobial screening test that has been used in FSIS laboratories for many years.

Test Principle

FAST is based on the principle that if animal tissue contains a residue of previously administered antimicrobial, fluid from the tissue will inhibit the growth of a sensitive organism on a bacterial culture plate. In this test, cotton swabs saturated with tissue fluid from a suspected carcass are placed on a culture plate whose surface has been seeded with spores of a harmless organism (*Bacillus megaterium).* This organism is known to be sensitive to most of the commonly used antimicrobials. The swabs and plate are incubated to allow growth of the organisms; then, the plates are examined for zones of inhibited growth around the swabs. The presence of such zones of inhibition is presumptive evidence that the carcass tissues contain an antimicrobial residue. Positive cases are submitted to the laboratory. Negative cases may be released *within about 6 hours* provided all other inspection criteria are met.

Time Required

Actual work time to set up, interpret, and report the test is only 5–10 minutes per test for an experienced performer. Waiting times built into the test are flexible and can be scheduled around other duties. Test results may be obtained after a minimum of 6 hours incubation *or* after overnight incubation up to a maximum of 24 hours from the time the test is initially started (i.e., from the time the plate is incubated).

FAST Benefits

FAST is one of those uncommon programs in which nearly everyone involved benefits.

- The producer and the packer both benefit from more timely release of carcasses that test negative.
- The FSIS veterinarian benefits from increased capability in making disposition of retained carcasses.
- The agency and the taxpayer benefit from reduced laboratory and mailing costs.
- The consumer benefits from the assurance that meat products containing antimicrobial residues are being kept out of the marketplace.

Use of FAST

In most cases, FAST is used as an aid in determining the disposition of single animals. FAST can be applied any time the veterinarian suspects the presence of an antimicrobial residue. A common reason for conducting the test is finding an injection lesion during postmortem inspection. FAST is also used for follow-up testing of animals from a previously identified violative producer and will probably be used in the monitoring phase of residue testing in the future.

OVERVIEW OF FAST

Basic Procedures

To perform FAST, the performer needs to:

Take initial action—

- Retain the carcasses to be tested,
- Collect carcass trace-back information,
- Collect and identify tissue samples, and
- Record initial data on the report form.

Prepare and incubate the plate—

- Prepare tissue swabs,
- Streak the plate with spores of the test organism,
- Ensure identification of the plate,
- Place an N5 (5 mcg neomycin) disc and the tissue swabs on the plate, and
- Incubate the plate 6-24 hours at 44° ± 0.5° Celsius.

Determine test results—

- Verify growth of the test organism,
- Verify the presence of the N5 zone of inhibition,
- Determine the presence or absence of zones of inhibition surrounding the tissue swabs, and
- Interpret and record test results.

Take follow-up actions—

- Release the carcass if the results are negative,
- Submit samples to the laboratory if results are positive, and
- Complete and distribute the report form.

Equipment

The following equipment and supplies are required:

- Clean knife, plastic bags, fine-tipped permanent marker, and rubber bands;
- U.S. Retained Tags;
- Sterile cotton swabs;
- FAST agar plates;
- *Bacillus megaterium* spore suspension;
- Antibiotic sensitivity discs (N5 discs);
- Thumb forceps;
- Incubator stabilized at 44° ± 0.5° Celsius; and
- Metric measuring device with millimeter graduations.

Reports

The report forms used in the FAST program are:

- FSIS Form 6600-7 (4/94)—FAST Antimicrobial Screen Test Worksheet and
- FSIS Form 10,000-2 (4/92)—Domestic Laboratory Report.

Storage of Plates

The FAST agar plates are considered shelf-stable and should be stored at room temperature, protected from extremes of heat, cold, and moisture. Refrigeration may prolong shelf life, but freezing will ruin the plate because it separates water from the agar.

Storage of N5 Discs

The N5 discs are perishable and must be refrigerated. The protective container for the dispenser of N5 discs should not be opened until the discs are first used. After each use, the dispenser and the desiccant pellet should be placed in a sealed plastic bag and returned to refrigerated storage.

Storage of Spores

The *Bacillus megaterium* spores are considered shelf-stable, but their useful shelf life will be prolonged if they are kept refrigerated. Be sure the screw-cap is tight to prevent evaporation of the ethanol carrier.

COLLECTING TISSUE SAMPLES

Introduction	In livestock species, FAST is performed on kidney tissue. Proper collection and identification of the tissue samples is essential to ensure accurate test results.
STEP 1	Identify the carcass to be tested with a U.S. Retained Tag, FSIS Form 6502-2 (4/89), a gang of four tags each bearing an identical number. Make sure the carcass and all its parts and organs are retained until FAST is completed.
STEP 2	Collect and record all the available carcass identification information (back tags, ear tags, carcass number, etc.).
STEP 3	Detach one of the U.S. Retained Tags from the carcass and attach it to the kidney.
STEP 4	Using a clean knife, collect approximately one pound of kidney tissue from the retained carcass and place the tissue in a plastic bag. Separate a U.S. Retained Tag from the tags on the carcass and attach it to the kidney tissue sample bag.
STEP 5	If you are testing more than one carcass, repeat STEPS 1-4 to collect tissues and identification from the other carcasses.
STEP 6	Go on to GETTING READY TO PERFORM FAST, STEP 1, page 7.

GETTING READY TO PERFORM FAST

Introduction

Before running the test, you must obtain supplies and equipment, prepare the work area, and make several entries on FAST Antimicrobial Screen Test Worksheet, FSIS Form 6600-7 (4/94).

When To Use

Follow this procedure after the carcass is properly retained and the tissue samples have been collected. An important consideration when performing FAST is that someone must be on duty to read the results after incubation (between 6 and 24 hours after beginning the procedure). If no one will be available during this time, you must hold the tissue samples under refrigeration until this condition can be met.

STEP 1

If the incubator is stabilized at a temperature of 44° ± 0.5° Celsius, go on to STEP 2.

Otherwise, go to RECEIVING AND STABILIZING THE FAST INCUBATOR, STEP 1, page 38.

If the incubator temperature cannot be stabilized, you must retain the tissue samples in refrigerated storage until a stabilized incubator is available or submit the samples to the laboratory for testing.

STEP 2

Clear a 2' × 2' work surface on a table or desk for conducting the test.

STEP 3

Obtain the following equipment from storage:

- FAST report forms (FSIS Form 6600-7 (4/94)],
- Ballpoint pen and permanent marking pen,
- FAST agar plates—one for every two tests,
- Sterile cotton swabs—three for each plate,
- Vial of *Bacillus megaterium* spores,
- Dispenser of N5 discs and thumb forceps.

GETTING READY TO PERFORM FAST

STEP 4

Make sure that the heading of FSIS Form 6600-7 (4/94) is properly completed:

- Establishment number,
- Establishment name,
- Region, area, circuit, and state—Enter the appropriate numerical codes. Leave blank if unknown.

STEP 5

Enter the Retained Tag number from the tissue sample bag in the "Retain Tag Number" column on the first available line on the form. If the tissue sample came from a carcass that had a Back Tag or Trace Back ID number on it, enter the number in the "Back Tag or Trace Back ID" column.

STEP 6

From the list of "Species Codes" shown on the reverse of the inspection office copy of FSIS Form 6600-7 (4/94), select the code that best describes the animal you are testing.

STEP 7

Write the code you have selected in the "Species Codes" column to the right of the "Back Tag or Trace Back ID" column.

STEP 8

From the list of "Reason Codes" on the reverse of the inspection office copy of FSIS Form 6600-7 (4/94), select the code that describes the primary reason for performing this test.

STEP 9

Write the code you have selected in column "P" (Primary) of the "Reason Codes" columns.

STEP 10

If there is a secondary reason for performing this test, select the code that describes the secondary reason.

STEP 11

Write the code you have selected in the "S" (Secondary) column of the "Reason Codes" column. Go to PREPARING TISSUE SWABS, STEP 1, page 10.

STEP 12

If you entered code "05" (Case follow-up) in column "P" of the "Reason Codes" columns, enter the case number that was assigned by the regional office in the "Case #" column on FSIS Form 6600-7 (4/94).

Examples

For sampling healthy-appearing bob veal under CFR 310.21(c)(4):

P = 49 S = 47

For case follow-up on a normal-appearing animal:

P = 05 S = 47

For a downer with an injection site found on postmortem examination from a producer who has no past residue history:

P = 10 S = 01

STEP 13

Write today's date in the "Date Test Started" column using the format MM/DD/YY, e.g., June 18, 1994 = 06/18/94.

STEP 14

Go to PREPARING TISSUE SWABS, STEP 1, page 10.

PREPARING TISSUE SWABS

Introduction	Cotton swabs saturated with tissue fluid are obtained by macerating the tissue with the sharp end of the swab shaft and then allowing the cotton to contact the macerated tissue until a maximum of tissue fluid is absorbed into the swab. Sterile technique is *not* required because the relatively short incubation time rarely allows interfering growth of contaminating organisms. However, your hands should be clean and dry whenever the swabs are handled to avoid contaminating the swabs with substances that might interfere with growth of the test organism. The cotton swab itself should **never** contact anything except the sample tissue and the plate.
When To Use	Follow this procedure after the tissue samples are collected and data has been recorded on FSIS Form 6600-7 (4/94).
STEP 1	Open the swab pack, remove one swab, and jab the sharp end just through the wall of the plastic bag containing the kidney tissue sample. To prevent squeezing fluid from the swab head when it is later removed from the tissue, use the swab shaft to enlarge the opening in the bag to about ½ inch in length.
STEP 2	Push the sharp end of the shaft about ½ to ¾ inch into the tissue. Then jab it back and forth several times to macerate the tissue.
STEP 3	Reverse the swab and insert the cotton swab tip into the tissue. Twirl the swab shaft to make sure that there is good contact between the swab head and the macerated tissue.
STEP 4	If you are setting up additional tests at this time, repeat STEPS 1-3 to prepare the other swabs.

STEP 5	Leave the swabs in place at least 30 minutes to ensure maximum saturation of the swabs. If other duties prevent continuing with the test after the 30-minute period, the swabs may be left in place in the tissue for up to 2 hours.
STEP 6	Go to STREAKING THE PLATE WITH SPORES, STEP 1, page 12.

STREAKING THE PLATE WITH SPORES

Introduction

Streak the plate with the *Bacillus megaterium* spores just before the tissue swabs are placed on the plate. A standardized technique is required to ensure even distribution of the spores and consistent test results.

When To Use

Follow this procedure while waiting for the tissue swabs to become fully saturated.

STEP 1

Select the following items:

- FAST agar plates,
- Sterile cotton swabs—one per plate,
- Vial of *Bacillus megaterium* spores, and
- Fine-tipped permanent marking pen.

STEP 2

Verify that the plate is suitable for use:

- Not damaged by freezing,
- Has equilibrated to room temperature,
- Agar not dried out or cracked, and
- No interfering colonies of an accidental contaminant.
- Check that the expiration date is not exceeded.

STEP 3

Check to make sure the screw cap on the vial of spores is tight; then *vigorously* shake the vial to resuspend the spores.

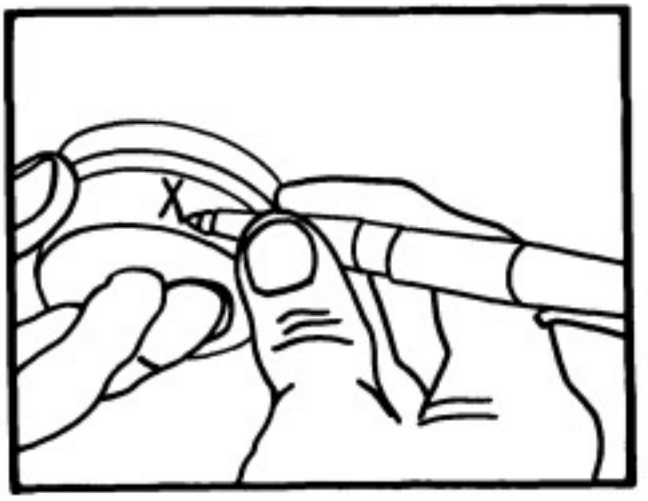
Special Note

If there is particulate matter present or the fluid appears colored, the spore suspension should be discarded.

STREAKING THE PLATE WITH SPORES

STEP 4

Lift the plate cover slightly and make an "X" reference mark on the outer sidewall of the plate. Place the covered plate bottom-side down on the work surface with the reference mark at "12 o'clock."



STEP 5

Make sure the spores are thoroughly mixed—**SHAKE HARD!**; then remove the screw-cap from the spore vial. Avoid touching the inside of the cap or vial with hands.

STEP 6

Remove a sterile cotton swab from its wrapper, grasping only the shaft. Avoid touching the cotton tip to any suface.

STEP 7

Insert the swab into the vial so that the cotton swab is completely immersed in the spore suspension.

Special Note

- Dip the swab into the spore suspension one time only.
- Do not reuse the swab to streak additional plates.

STEP 8

Gently touch the swab to the side of the vial to remove any excess fluid. (Avoid touching the swab with hands.)

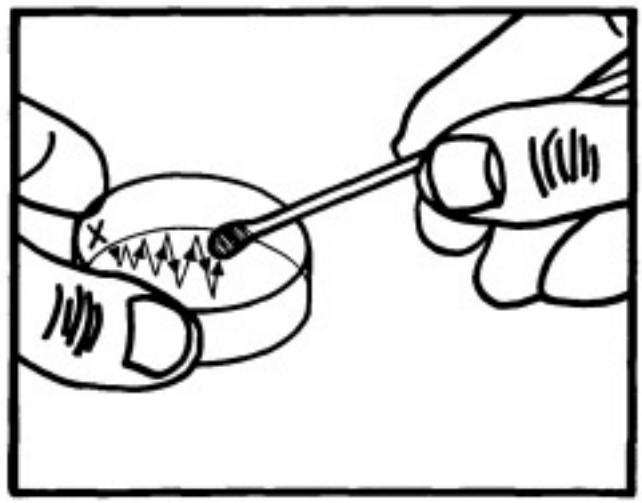
STEP 9

Replace the screw-cap and set the vial of spores aside.

STREAKING THE PLATE WITH SPORES

STEP 10

Remove the cover from the plate and streak the spores over the surface of the agar by starting at the reference mark and gently streaking from top to bottom, back and forth, moving the swab to the right edge of the plate.



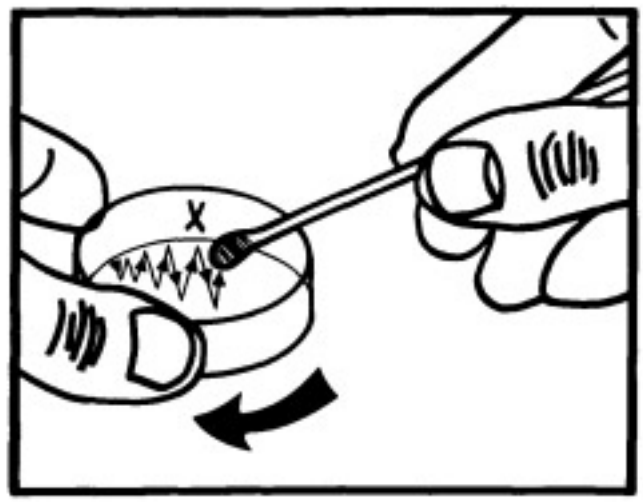
Special Notes

Be careful not to dig or plow the surface of the agar.

In STEPS 10-14, a left-handed performer may wish to swab the plate from the reference mark to the left edge and turn the plate counterclockwise.

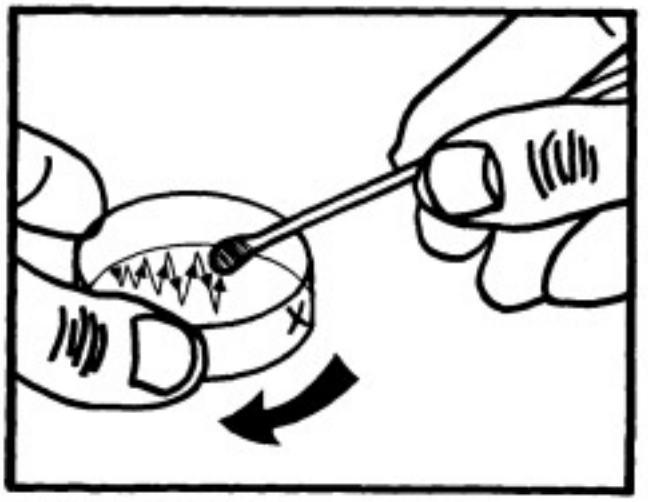
STEP 11

Turn the plate ¼-turn clockwise and repeat the streaking pattern, gently streaking from top to bottom, back and forth, moving the swab to the right edge of the plate.



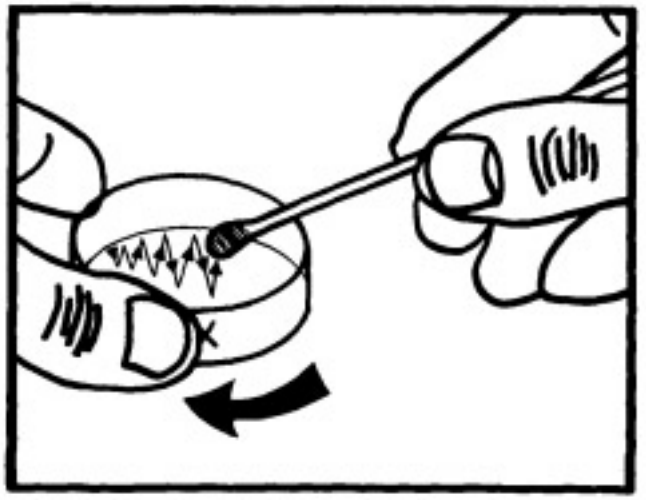
STEP 12

Turn the plate ¼-turn and repeat the pattern.



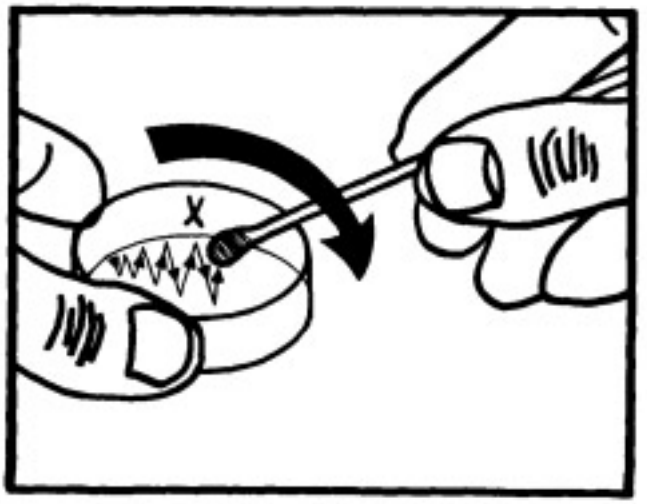
STEP 13

Turn the plate ¼-turn and repeat the pattern.



STEP 14

Finally, turn the plate ½-turn and repeat the pattern.



STREAKING THE PLATE WITH SPORES

STEP 15	Replace the cover on the plate and *discard the used swab.* Use a fresh swab for each plate prepared.
STEP 16	Go to IDENTIFYING THE FAST PLATE, STEP 1, page 17.

IDENTIFYING THE FAST PLATE

Introduction

Accurate identification of the plate is essential to ensure proper reporting of the test results. The plate is identified with the last three digits of the tissue sample's retained tag number. If more than one test is being performed at the same time, each plate may be used for up to two tests.

When To Use

Follow this procedure after you have streaked the plate with spores.

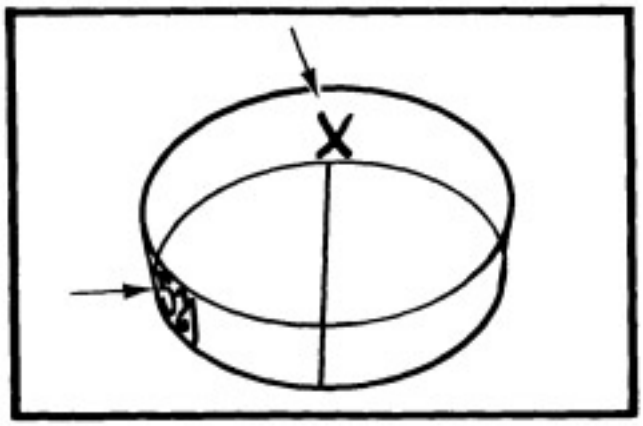
STEP 1

Using the fine-tip permanent marking pen, start at the "X" and draw a line across the bottom of the plate to divide it into two equal sections.

STEP 2

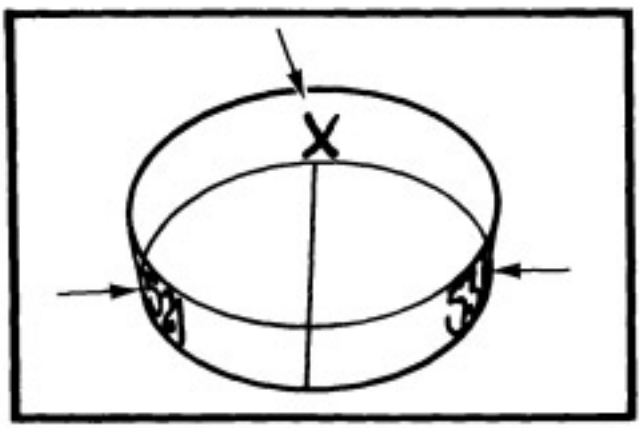
Locate the area of the plate 90 degrees from the "X" reference mark. Then lift the plate cover slightly and write the last three digits of the tissue sample retained tag number on the vertical edge of the plate itself.

If you are testing two carcasses, go to STEP 3. If not, go to STEP 4.



STEP 3

Locate the area of the plate directly opposite the first three-digit number. Then lift the plate cover slightly and write the last three digits of the second tissue sample retained tag number on the vertical edge of the plate itself.



STEP 4

Check to be sure that the plate numbers match the last three digits of the retained tag numbers recorded on the FSIS Form 6600-7 (4/94).

STEP 5

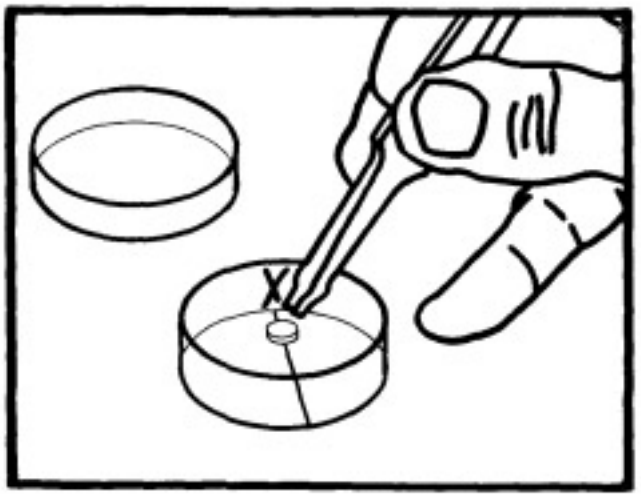
Go to POSITIONING THE N5 DISC, STEP 1, page 18.

POSITIONING THE N5 DISC

Introduction	A paper disc containing 5 mcg of the antibiotic neomycin (N5) is placed on the plate prior to incubation. Its purpose is to act as a control to verify that the test organism is in fact sensitive to antibiotics. Growth of the organism will be inhibited by the neomycin that diffuses from the disc into the agar. The size of the zone of inhibition surrounding the N5 disc is influenced by the antibiotic concentration, by the viability and sensitivity of the test organism, and by incubation temperature. Thus the diameter of the zone of inhibition provides a measure of the quality control exercised by the manufacturer of the spores and plates, and is used to verify the proper function of the system during incubation.
Disc Storage	To maintain the potency of the N5 discs, they must be kept cold and dry. Replace the disc vial in its sealed plastic bag with desiccant and return it to refrigerated storage after each use. **Discs should not be used beyond the expiration date printed on the vial.**
When To Use	Follow this procedure after you have identified the plate for use.
STEP 1	Remove the cap from the N5 dispenser vial and set the cap aside.
STEP 2	Remove the cover from the plate and place it open-side up beside the plate.
STEP 3	Dispense one N5 disc into the open cover. Then pick up the disc by its edges with the thumb forceps.

STEP 4

Select a point about one-half inch in from the "X" reference mark and directly on the line dividing the plate. Carefully place the disc flat onto the agar.



STEP 5

Being careful not to press hard enough to break the surface of the agar, lightly touch the disc with the forceps tip to assure uniform contact. If you accidentally touch the N5 disc with your fingers, wash your hands thoroughly before continuing.

DO NOT try to reposition the disc if it is not exactly over the line. Use another plate if it is badly out of position.

STEP 6

Replace the cover on the plate, replace the cap on the N5 disc vial, and return the vial of N5 discs to refrigerated storage.

STEP 7

Go to POSITIONING SWAB ON THE PLATE, STEP 1, page 20.

POSITIONING SWAB ON THE PLATE

Introduction

The cotton swabs saturated with tissue fluids must be placed on the plate so that

- Each swab is in the proper section of the plate.
- The swabs and the N5 disc are well separated.
- There is good contact between the swabs and the agar.

Comment

Sterile technique is not required, but the hands should be washed, rinsed, and dried to prevent contaminating the swabs with any substance that might interfere with test results. Handle the swab shaft only with your clean fingers. **DO NOT** use the forceps! They may be contaminated with neomycin.

When To Use

Follow this procedure after you have placed the N5 disc on the plate.

STEP 1

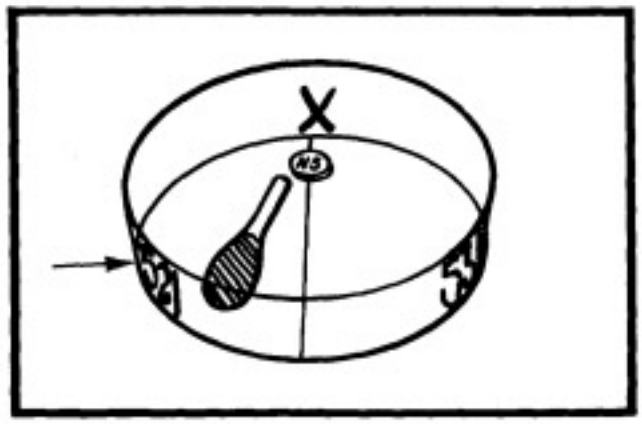
Remove the swab from the tissue and hold it with the cotton tip down to prevent fluid from flowing down the plastic shaft.

STEP 2

Being careful not to touch the cotton tip itself, break the shaft off as short as possible.

STEP 3

Locate the side of the plate marked with the last three digits of the retained tag number for this swab. Then remove the cover from the plate and gently place the swab on the surface of the agar so the broken edge of the shaft is near the N5 disc and the cotton tip is in the center of its section.



STEP 4

Being careful not to break the agar surface, lightly press the swab shaft with your fingertip to assure proper contact of the swab head with the agar.

If you are testing two carcasses, continue to STEP 5. If not, replace the cover on the plate and go to INCUBATING THE FAST PLATE, STEP 1, page 23.

STEP 5

Remove the other swab from the tissue and hold it with the cotton tip down. Check the number on the retained tag attached to the tissue sample bag.

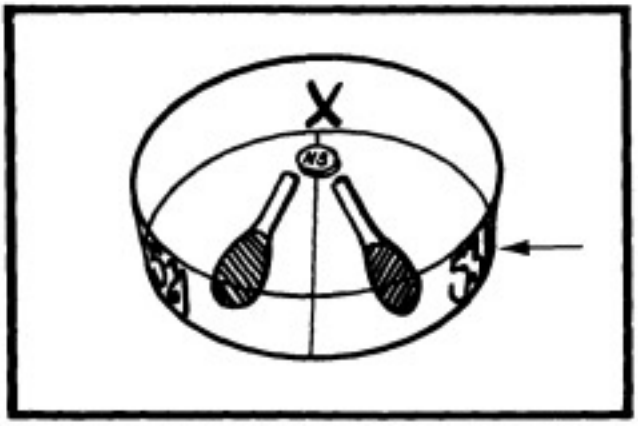
STEP 6

Being careful not to touch the cotton tip itself, break the shaft off as short as possible.

POSITIONING SWAB ON THE PLATE

STEP 7

Locate the side of the plate marked with the last three digits of the retained tag number for this swab. Then remove the cover from the plate and gently place the swab on the surface of the agar so the broken edge of the shaft is near the N5 disc and the cotton tip is in the center of its section.



STEP 8

Being careful not to break the agar surface, lightly press the swab shaft with your fingertip to assure proper contact of the swab head with the agar. Replace the cover on the plate.

STEP 9

Go to INCUBATING THE FAST PLATE, STEP 1, page 23.

INCUBATING THE FAST PLATE

Introduction

For optimum growth of the test organism, creating a highly visible lawn of colonies and clear zones of inhibition, the plate must be incubated at 44° ± 0.5° Celsius for a minimum of 6 hours. Incubation may proceed up to a maximum of 24 hours without jeopardizing the test results.

STEP 1

Make sure the incubator is properly stabilized at 44° ± 0.5° Celsius. Record the temperature in the "Incub. Temp (°C)" column of the FSIS Form 6600-7 (4/94).

STEP 2

Place the plate on the incubator shelf with the cover on top. **DO NOT** invert the plate because the swabs may be dislodged during incubation.

STEP 3

Using military time, record the "Time In" on the FSIS Form 6600-7 (4/94) in the "Military Time IN" column using the format HHMM.

STEP 4

Secure the incubator in a manner that will preclude tampering during incubation.

STEP 5

If possible, remove the plate from the incubator after 6 hours of incubation. Using military time, record the "Time Out" on FSIS Form 6600-7 (4/94) in the "Military Time Out" column using the format HHMM.

Otherwise, remove the plate from the incubator within 24 hours. Using military time, record the "Time Out" on FSIS Form 6600-7 (4/94) in the "Military Time Out" column.

Special Note

FAST plates can be read after incubating them for a minimum period of 6 hours *or* up to a maximum of 24 hours from the time that the plates are initially placed in the incubator ("Military Time In"). If you receive samples and can perform FAST early enough so that 6 hours of incubation time remains during your standard work shift, you can make readings by the end of your work shift. If you receive samples too late during the work shift to allow the plates to be read during your present shift, allow the plates to incubate overnight and make readings at any time after the start of the next day's work shift up to a maximum period of 24 hours plate incubation.

Prolonged incubation (more than 24 hours) may allow the growth of interfering organisms, or the antibiotic activity present may dissipate. This may allow the test organism to grow in the zone of inhibition, thus creating false negative test results. The tissues should be retested either by redoing FAST or submitting them to the laboratory.

INCUBATING THE FAST PLATE

Special Note	Read the results of the test immediately after the appropriate incubation period, if possible. If you need to delay reading the results or wish to show the plate to someone else, you may place the incubated plate in a refrigerator to prevent further growth of the organism.
STEP 6	Go to VERIFYING FUNCTION OF THE FAST SYSTEM, STEP 1, page 25.

VERIFYING FUNCTION OF THE FAST SYSTEM

Introduction

Growth of the test organism during incubation of the plate results in a distinct yellow color within 6 hours. A purple zone of inhibition surrounding the N5 disc should be obvious within 6 hours. To verify that the FAST system has functioned properly during incubation, you must:

- Determine that there has been growth of the test organism.
- Determine that the diameter of the zone of inhibition surrounding the N5 disc is within the acceptable range of 20-26 millimeters.

After overnight incubation, the plate color is usually yellow or amber. Growth is indicated by an opaque cream-colored or grayish appearance instead of clear gel. A clear yellow or amber zone around the N5 disc should be apparent.

Definition

A zone of inhibition is an area free of colonies of the test organism caused by the presence of some growth-inhibiting substance, e.g., an antibiotic. Unless incubated more than 6 hours, the color in the zone of inhibition should be purple; otherwise it will be yellow or amber.

When To Use

Follow this procedure immediately after removing the plate from the incubator.

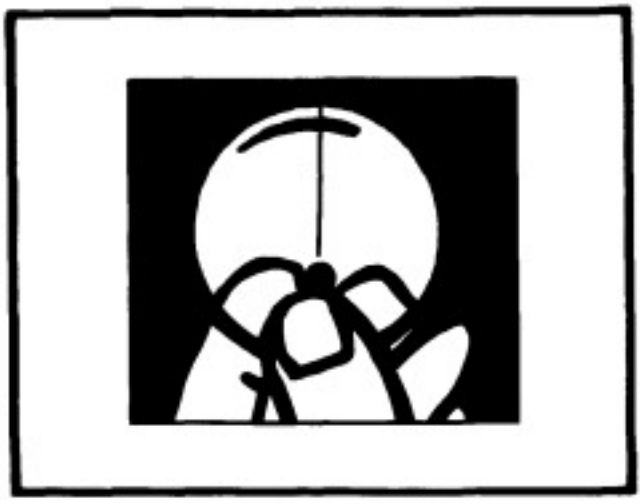
STEP 1

Turn the plate upside down on a flat surface and lightly tap the bottom of the plate with your fingertip until the swabs are dislodged from the agar surface and drop into the inverted cover.

VERIFYING FUNCTION OF THE FAST SYSTEM

STEP 2

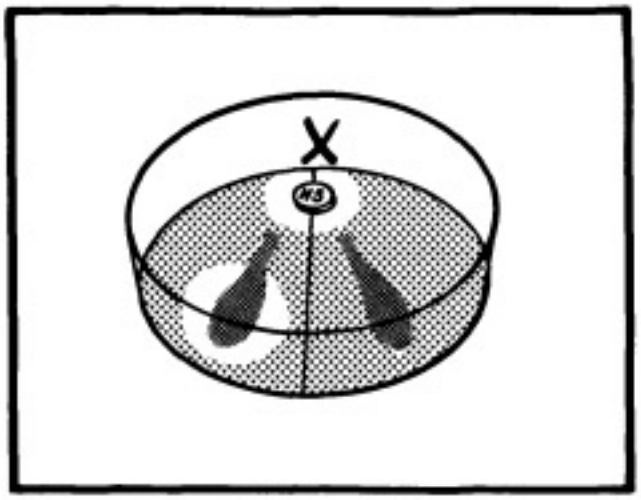
Lift the plate out of the cover and hold it with the bottom toward you so you can look through the plate to observe for growth of the test organism. Growth of the test organism should be readily visible in at least the areas of the plate farthest away from the N5 disc and the swab(s).



STEP 3

IF growth of the test organism is evident, go on to STEP 4.

Otherwise, go to HANDLING CULTURE FAILURES, STEP 1, page 36.



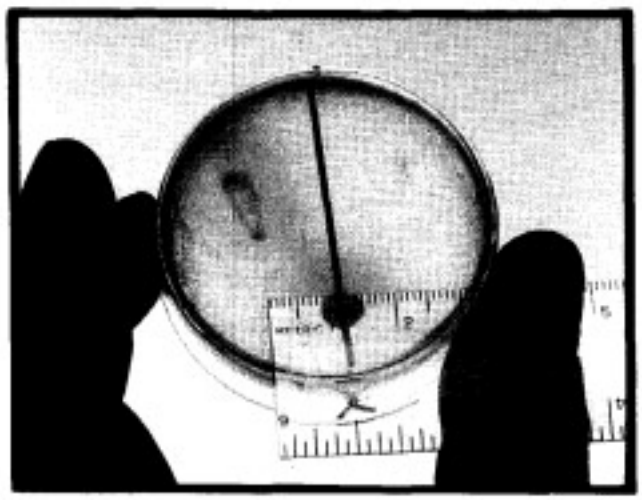
STEP 4

Observe the zone of inhibition

- At 6 hours incubation: purple zone free of colonies of the test organism around the N5 disc.
- After incubation more than 6 hours: clear yellow or amber zone of inhibition free of growth.

STEP 5

Measure the diameter of the N5 disc zone with a metric ruler, and record the diameter in millimeters (mm) in the "Zone of Inhibition (mm) N5" column on FSIS Form 6600-7 (4/94).



STEP 6

IF the diameter of the N5 disc zone is 20 to 26 millimeters, go to INTERPRETING AND RECORDING FAST DATA, STEP 1, page 29.

Otherwise, the results of this test may not be reliable. You must either rerun the test or submit tissues to the laboratory. Go to STEP 7.

STEP 7

Write "I" (inconclusive) in the "Test Results (+ or −) 6 hrs" column *or* the "Test Results (+ or −) 18 hrs" column for this test on FSIS Form 6600-7 (4/94).

If your plate reading was made at the 6-hour incubation period, place your results only in the 6 hours column; if you read the plate after an overnight incubation (but no more than 24 hours), place your results only in the 18 hours column. **DO NOT** place entries in both time columns.

VERIFYING FUNCTION OF THE FAST SYSTEM

STEP 8

Consider the following causes for out-of-range N5 disc zones:

Zone too small (less than 20 mm)—

- Too many spores on the plate, possibly caused by evaporation or inadequate mixing of the spore suspension, oversaturation of the swab used to apply the spores, or manufacturing error.
- N5 discs outdated or temperature-abused.
- Too much moisture on the surface of the plate.
- Incubator temperature too high or too low.

Zone too large (greater than 26 mm)—

- Not enough spores on the plate—possibly caused by inadequate mixing of the spores, undersaturation of the swab used to apply the spores, or manufacturing error.
- Incubator temperature too high or too low.

Your decision on whether to rerun this test or submit tissues to the laboratory immediately depends on several factors:

- Will you be able to make changes that will likely result in an N5 disc zone within range?
- What is the impact of waiting for FAST results versus submitting tissues now?

STEP 9

IF you have decided to submit tissues to the laboratory, treat this test as though it were "positive" and go to FOLLOW-UP ACTION—POSITIVE FAST, STEP 1, page 32.

Special Note

If you continue to experience out-of-range N5 disc zones, you should contact the Residue Staff Officer in your region for guidance.

INTERPRETING AND RECORDING FAST DATA

Introduction

FAST results are based on the presence or absence of zones of inhibition surrounding the area where the tissue swabs were in contact with the agar during incubation.

When To Use

Follow this procedure to read and interpret the test results only after you have verified that the FAST system functioned properly during incubation.

STEP 1

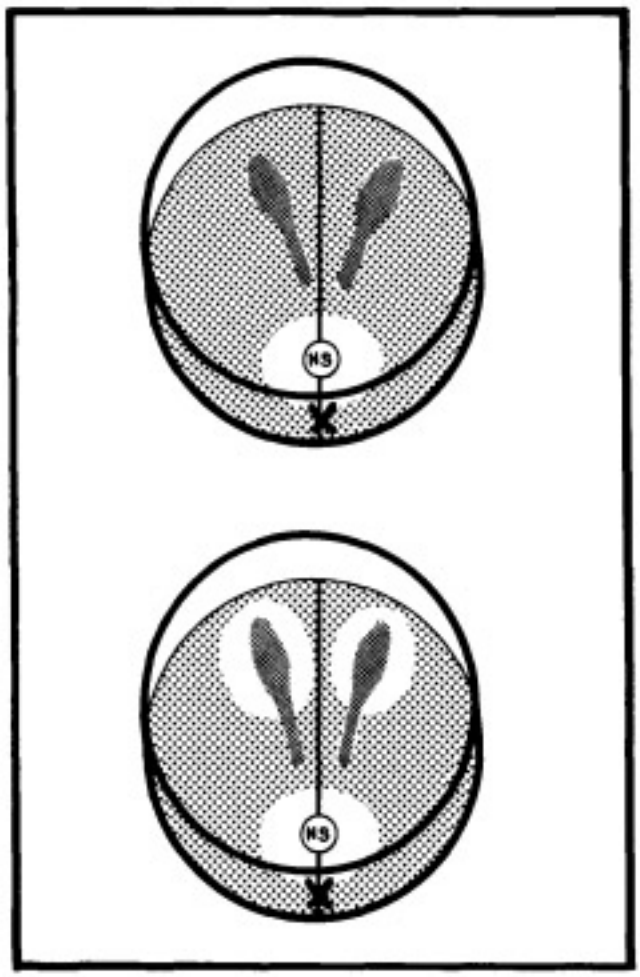
Examine the plate in the area of the swab impression to determine if a clear zone of inhibition is present.

Special Note:
Pressure from the swab may prevent colonies from developing normally. DO NOT consider lack of growth where the swab actually contacted the agar as a zone of inhibition.

Use the "Interpretation of Results" shown on the reverse of the inspection office copy of FSIS Form 6600-7 (4/94) for guidance.

If a zone of inhibition is present, go to STEP 3.

Otherwise, the test result is negative. Go to STEP 2.



STEP 2

You have determined that the test result for this swab is negative. Read the three-digit number on the plate that identifies this swab. Then locate the line on Form 6600-7 (4/94) where the original entries for this case were made and write "NONE" in the "Zone of Inhibition (mm) Swab" column and write " – " in the appropriate "Test Results" column (6 hrs or 18 hrs) depending on whether you are reading the plate after a 6 hr or overnight plate incubation. **DO NOT** place entries in both time columns. Go to STEP 5.

INTERPRETING AND RECORDING FAST DATA

STEP 3

You have determined the test result to be positive. Measure the width of the swab zone of inhibition with a metric ruler. Be sure to measure perpendicular to the swab shaft.

STEP 4

Read the three-digit number on the plate that identifies this swab. Then locate the line on FSIS Form 6600-7 (4/94) where the original entries for this case were made and write the width of the swab zone of inhibition (in mm) in the "Zone of Inhibition (mm) Swab" column and write "+" in the appropriate "Test Results" column (6 hrs or 18 hrs) depending on whether you are reading the plate after a 6 hr or overnight plate incubation. **DO NOT** place entries in both time columns.

STEP 5

If there is another swab on this plate, go back to STEP 1 to interpret and record the results of the second swab.

After the FAST results have been interpreted and recorded, the used test plate may be discarded. If you are going to keep the plate for someone else to see, it should be refrigerated.

STEP 6

If the result of any test was negative, go to FOLLOW-UP ACTION—NEGATIVE FAST, STEP 1, page 31.

If the result of any test was positive go to FOLLOW-UP ACTION—POSITIVE FAST, STEP 1, page 32.

FOLLOW-UP ACTION, NEGATIVE FAST

Introduction	If the FAST result for the kidney swabs is negative, there is high assurance that the carcass and visceral organs do not contain violative levels of antibiotic residues. The carcass disposition and follow-up actions are based on the reason the carcass was retained.
When To Use	Follow this procedure only if the FAST result for kidney tissue is *negative*.
STEP 1	Release the carcass based on the negative FAST results after appropriate trimming, e.g., removal of injection lesions, **provided** any other reasons for retaining the carcass are resolved. No tissues need to be sent to the laboratory.
STEP 2	Locate the column headed "DISP. CODE" on the FSIS Form 6600-7 (4/94). Write "40" (negative) in the "DISP. CODE" column, selected from the list of "Disposition Codes" shown on the reverse of the inspection office copy of the same form.
STEP 3	If this was a "Case Follow-Up" test (previous violation on the producer's premises), go to STEP 4. Otherwise go to STEP 6.
STEP 4	Make sure you have recorded the case number (provided by the regional office) on FSIS Form 6600-7 (4/94) in the "Case #" column.
STEP 5	Notify the Regional Residue Officer (through your Area Supervisor) of the negative FAST retest.
STEP 6	If you have other follow-up actions to take, return to INTERPRETING FAST DATA, STEP 6, page 30. Otherwise, go to SUBMITTING FSIS FORM 6600-7 (4/94), STEP 1, page 35.

FOLLOW-UP ACTION, POSITIVE FAST

Introduction	FAST is a screening test and is quite accurate in determining if antibiotic or sulfonamide residues are present. If the FAST result for the kidney swab is at least 15 mm for bob calves or positive at any zone size for other slaughter classes, the carcass must be retained and tissue samples submitted to the laboratory for bioassay testing. The purpose of the laboratory testing is to confirm whether violative levels of antibiotics or sulfonamides are present.
When To Use	Follow this procedure if the result for kidney tissue is *positive.*
STEP 1	If the species code is 21 (bob calf), go to STEP 2. For all other species codes, go to STEP 3.
STEP 2	If the zone of inhibition for kidney is 15 mm or greater and the species code is 21 (bob calf), **continue to retain the carcass** and go to STEP 3. If a zone of inhibition is less than 15 mm for bob calves, the test is considered negative. Go to FOLLOW-UP ACTION, NEGATIVE FAST, STEP 1, page 31.
STEP 3	Collect approximately one pound each of muscle, kidney, and liver from the retained carcass. Place each tissue sample in a separate double bag and freeze them. NOTE: **DO NOT** send tissues from injection sites to the laboratory unless you are requested to do so.
STEP 4	Follow existing instructions for completing the FSIS Form 10,000-2 (4/92), Domestic Laboratory Report.
STEP 5	If this was a "case follow-up" animal, enter the case number (provided by the regional office) in block 11.

STEP 6	If the producer (owner) is known, enter the name and address in block 16.
STEP 7	Enter "200" as the "Residue Class Code" in block 21 and enter "800" as the "Specific Residue" in block 21.
STEP 8	Write "FAST Positive" and the width of the swab zone of inhibition in block 24 for kidney.
STEP 9	Record all available trace-back information such as back tag and ear tag numbers, brands, tattoos, etc., in blocks 23 and 24 as necessary.
STEP 10	Determine which Field Services Laboratory is designated to receive FAST samples from your region. (If you are unsure, refer to the current revision of FSIS Directive 10,620.1). Select the code for your designated laboratory from the following list: 1302-Eastern Laboratory, Athens, GA; 2902-Midwestern Laboratory, St. Louis, MO; or 0602-Western Laboratory, Almeda, CA
STEP 11	Record the serial number from the FSIS Form 10,000-2 in the "FSIS 10,000-2 LAB REPORT SERIAL NO." column on the FSIS Form 6600-7 (4/94).
STEP 12	Seal the FSIS Form 10,000-2 in a clear plastic bag. Pack the completed form and the frozen tissue samples in a sample shipping container with sufficient coolant to ensure arrival at the laboratory in good condition. Mail the sample shipping containers to the laboratory.

FOLLOW-UP ACTION, POSITIVE FAST

STEP 13

Upon receipt of laboratory results, enter one of the following codes in the "DISP. CODE" block of FSIS Form 6600-7 (4/94):

CODE	LABORATORY TEST RESULT
51	Exceeds residue limit in one or more tissues.
55	Does not exceed residue limits in tissues (all tissues in compliance).

STEP 14

If you have other follow-up actions to take, return to INTERPRETING FAST DATA, STEP 6, page 30.

Otherwise, go to SUBMITTING FSIS FORM 6600-7 (4/94), STEP 1, page 35.

SUBMITTING FSIS FORM 6600-7 (4/94)

Introduction

Data from the FSIS Form 6600-7 (4/94) is entered into an FSIS computer database. From this database, various reports can be generated. Timely submittal of the FSIS Form 6600-7 (4/94) is essential to assure that FSIS staffs can perform proper case follow-up and make valid planning decisions.

When To Use

Submit the FSIS Form 6600-7 (4/94) as soon as it is full *or* at the end of each calendar month, *whichever occurs first.*

STEP 1

Review the FSIS Form 6600-7 (4/94) to be sure that *all* appropriate data columns have been properly and completely filled out for each test sample, including the inspector's initials and badge ID number, before continuing on to STEP 2.

STEP 2

If the FSIS Form 6600-7 (4/94) is full *or* the end of a calendar month has occurred, mail the original to:

FAST Reports
USDA, FSIS, Data Services Center
210 Walnut Street, Room 791
Des Moines, IA 50309

Otherwise, go to FINISHING UP FAST, STEP 1, page 37.

STEP 3

Mail the first copy through your Area Supervisor to the Regional Office.

STEP 4

File the other copy in the USDA inspection office.

HANDLING CULTURE FAILURES

Introduction

Failure of the test organism to grow into a visible "lawn" is a rare occurrence. When it happens, it could be due to any of several causes:

- The *Bacillus megaterium* spores may not be viable.
- You may have forgotten to swab the plate with spores.
- The plate may have been too old or dried out.
- The incubator temperature may have been incorrect.
- The tissues you tested may have contained an extremely high concentration of antibiotic.

When To Use

Follow this procedure any time the test organism fails to grow.

STEP 1

Locate the line on FSIS Form 6600-7 (4/94) where the original entries were made and write "F" (failure) in the appropriate "Test Results" column (6 hrs or 18 hrs) depending on whether you are reading the plate after a 6 hr. or overnight plate incubation. **DO NOT** place entries in both time columns.

STEP 2

If you have determined the cause of the culture failure and can rerun the FAST test on these tissues, you should do so now.

STEP 3

If you have NOT determined the cause of the culture failure, you should seek guidance through channels from the Residue Staff Officer in your region.

Unless you are told otherwise, you must submit tissues to the laboratory. Follow instructions in FOLLOW-UP ACTIONS—POSITIVE FAST, page 32, STEPS 3-15, except write "FAST culture failure" in block 24 on the FSIS Form 10,000-2, Domestic Laboratory Report.

FINISHING UP FAST

Introduction	Maintain your inventory of FAST supplies each time you use the test.
STEP 1	Inventory your FAST supplies and follow the instructions you have received from your region to order any additional supplies needed.
STEP 2	This completes the FAST test. Stop.

RECEIVING AND STABILIZING THE FAST INCUBATOR

Introduction

A stabilized, properly operating incubator is essential to the test. There are two steps in preparing the incubator for use:

- Receiving the incubator.
- Stabilizing the operating temperature.

When to Use

This procedure is to be used when you are relocating the incubator or placing it in service after a period of nonuse, when the temperature needs to be re-established, or upon receipt of a new incubator.

STEP 1

When you are relocating the incubator or placing it in service after a period of nonuse, go to STEP 3.

Otherwise, go to STEP 2.

STEP 2

Open the shipping container carefully. Check the incubator for shipping damage. Check for the following items:

- 600 Series Isotemp Incubator
- Chamber shelf
- Manufacturer's instructions

If you note any damages or shortages, contact the FAST equipment supply in your region for instructions.

STEP 3

Position the incubator at the desired operating location near an AC outlet.

Note: To ensure proper air circulation, a minimum clearance of 2 inches should be provided around all sides of the incubator, including the top.

STEP 4

Connect the line cord to a suitable AC outlet.

Note: Check the data plate on the rear of the incubator for voltage, current, and line frequency specifications. Be certain that the power requirements will not overload the AC circuit to which it will be connected.

STEP 5

Set the power switch to the ON position. Set the heater control knob to 44° Celsius.

STEP 6

Locate the thermometer received with your FAST supplies. Place the thermometer inside the incubator in such a way that it can be read without opening the glass door.

STEP 7

Allow the incubator temperature at least 24 hours to stabilize.

STEP 8

If the thermometer reading does not agree with the digital display, use a small screwdriver to adjust the trimpot screw (accessible through the hole in the control panel just below the display) until the display agrees with the reading from the thermometer.

Note: Turning the trimpot screw clockwise increases the reading and turning it counterclockwise decreases the reading.

STEP 9

Adjust the heater control until a stable reading of 44° Celsius is obtained.

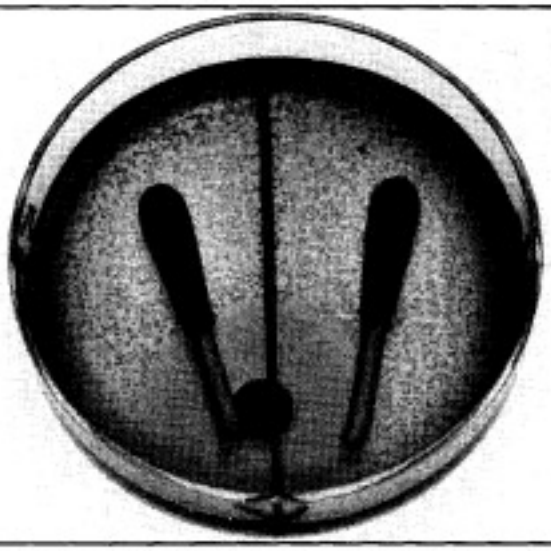
STEP 10

Use a sharp-pointed marker to mark a reference on the heater control.

Check the temperature daily. Slight adjustments of the temperature control knob may be necessary.

Remember to use this guide each time you move the incubator from one location to another, or place it in service after a period of nonuse.

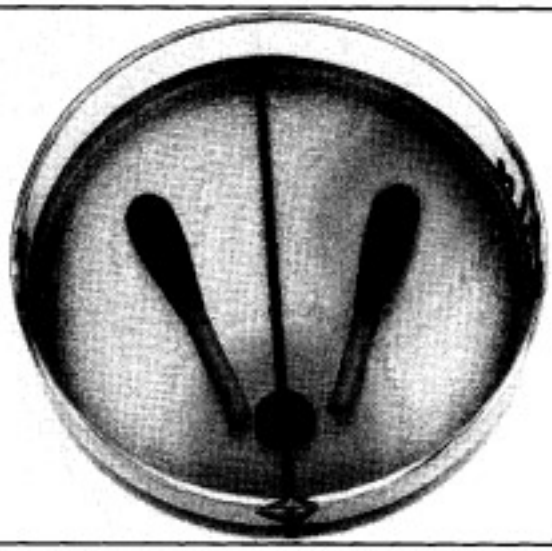
6-HOUR INCUBATION TEST RESULTS



If you have a clear purple zone around the N5 disc measuring between 20 and 26 mm,

And you have cloudy yellow bacterial growth right up to the swab tips,

Then the test is negative.

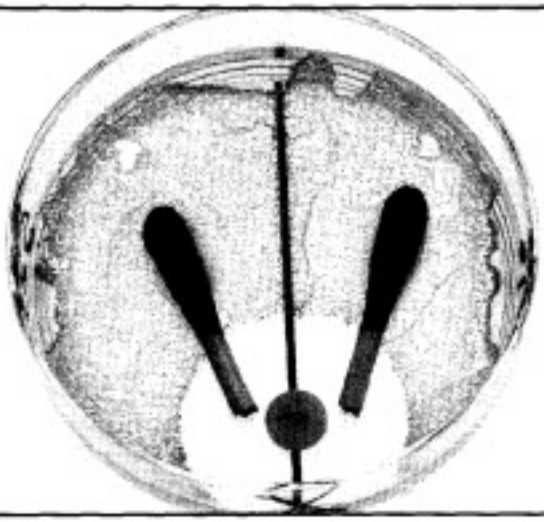


If you have a clear purple zone around the N5 disc measuring between 20 and 26 mm,

And you have a clear purple zone around a swab tip,

Then the test is positive.

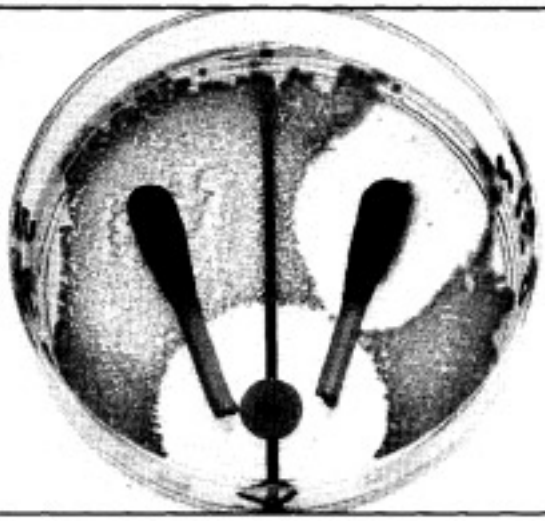
OVERNIGHT INCUBATION TEST RESULTS



If you have a clear yellow or amber zone around the N5 disc measuring between 20 and 26 mm,

And you have cloudy yellow or amber bacterial growth right up to the swab tips,

Then the test is negative.



If you have a clear yellow or amber zone around the N5 disc measuring between 20 and 26 mm,

And you have a clear yellow or amber zone around a swab tip,

Then the test is positive.

Revised June 1996

FAST ANTIMICROBIAL SCREEN TEST WORKSHEET

SERIAL NO.

ESTABLISHMENT NO.: 38 | ESTABLISHMENT NAME: Best Meats | REGION: 02 | AREA: 03 | CIRCUIT: 06 | STATE: AZ

DATE TEST STARTED (mm/dd/yy)	RETAIN TAG NUMBER	BACK TAG OR TRACE BACK ID	SPECIES CODES	REASON CODES P	REASON CODES S	INCUB. TEMP (°C)	MILITARY TIME IN	MILITARY TIME OUT	ZONE OF INHIBITION (mm) NS	ZONE OF INHIBITION (mm) SWAB	TEST RESULTS (+ or −) 6 Hrs	TEST RESULTS (+ or −) 18 Hrs	DISP. CODE	FSIS 10,000-2 LAB REPORT SERIAL NO.	CASE #	INSPECTOR'S BADGE ID NO.	INSPECTOR'S INITIAL
04/09/96	38740521	649	21	99	47	44	1600	0730	22	17		+	51	314305		1387	JRW
04/11/96	38740551	718	21	99	47	44.5	0800	1400	20	none	−		40			1387	JRW
04/23/96	69563693	63	13	10	27	44	0930	1530	25	13	+		55	314316		1387	JRW
04/24/96	69563711	—	13	05	47	43.5	0700	1300	24	none	−		40		40096-96	1387	JRW
04/27/96	73842911	—	13	10	01	44	1930	0700	22	none		−	40			1387	JRW
04/27/96	74800244	121	21	99	47	44	1500	0730	22	14		−	40			1387	JRW

FSIS FORM 6600-7 (4/96) REPLACES FSIS FORM 6600-7 (4/94), WHICH MAY BE USED UNTIL EXHAUSTED. ORIGINAL - DES MOINES

FSIS FORM 6600-7 *(REVERSE)*

INTERPRETATION OF RESULTS		SPECIES CODES	REASON CODES: P = Primary S = Secondary	DISPOSITION CODES
6 HOURS	18 HOURS	01 Horse 11 Bull 12 Steer 13 Beef Cow 14 Heifer 15 Dairy Cow 21 Bob Veal 22 Formula 23 Non Formula 24 Heavy Calf 31 Mature Sheep 32 Lamb 40 Goat 51 Market Swine 52 Boar 53 Sow 54 Roaster Pig	01 Injection Site 02 Udder Infusion 03 History of Treatment 04 Bolus 05 Case Follow up 10 Downer/Splitter 11 Bruises/Injuries 12 Arthritis 13 Rectal/Vaginal Prolapse 14 Recent Surgery 20 Abdominal Abscess 21 Peritonitis 22 Pyemia/Septicemia 23 Enteritis 24 Metritis 25 Nephritis/Cystitis 26 Acute Mastitis 27 Chronic Mastitis 28 Traumatic Reticulitis Complex 30 Pneumonia 31 Pericarditis, Endocarditis 32 Lung Abscess 40 Other Abscess 41 Emaciation 42 Anemia 43 Neoplasia 44 Misc. Infection 45 General Misc. 47 Normal 48 Show Animals 49 Statistically Selected	40 Negative 51 FAST + Lab Conf. (Violative) 54 Other 55 FAST + Lab Conf. (in Compliance)
+ POSITIVE: Purple zone of inhibition around swab, surrounded by yellow area of bacterial growth — NEGATIVE: Entire plate is yellow, plate covered by bacterial growth	*NOTE:* *After overnight incubation, record zone of inhibition around swab, regardless of color of plate*			

DISTRIBUTION:

When FSIS Form 6600-7 if full or at the end of each Calendar month, whichever occurs first

Mail original to:
DATA SERVICES CENTER
USDA, FSIS, MPI
210 Walnut Street, Rm. 791
Des Moines, IA 50309

Mail first copy to Region/Area Office

File other copy in the inspection office.