Performing the Swab Test on Premises

S T O P

For Detection of Antibiotic Residues in Livestock Kidney Tissue

A Self Instructional Guide
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United States Department of Agriculture
Food Safety and Inspection Service
Office of Policy, Program and Employee Development

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Introduction

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

- Collect and identify a kidney tissue sample from a properly identified retained carcass.
- Prepare suitable tissue swabs for testing.
- Prepare and incubate the STOP plate.
- Interpret and record the test results.
- Take proper action based on the test results.
- Submit accurate and complete STOP reports.

Primary Trainee Population:

- This guide is intended for use by FSIS Public Health Veterinarians (PHV) and designated Consumer Safety Inspectors (CSI) who have responsibility for performing the Swab Test on Premises for Antibiotic Residues.

Secondary trainee population:

- This guide may also be used by supervisors to review and evaluate STOP procedures and results.

Restrictions:

- This guide is limited to the performance of the STOP for Antibiotic Residues developed by USDA, FSIS, Science & Technology, Microbiology Division as it is performed on kidney tissue from red meat carcasses, except from Bovine species.

Use by Experienced Performers:

- Previous instructions for performing STOP are obsolete. There have been many changes to the test itself, actions to be taken, and reporting procedures. Experienced STOP performers must follow these new instructions carefully to assure accurate and uniform performance.

Use by New STOP 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 STOP 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 STOP

Definition:

- The STOP is a microbiological in-plant screening test for the detection of antibiotic residues in animal tissues. It is designed to be performed by the PHV or a designated CSI in the slaughtering plant under the PHV supervision.

Test Principle:

- STOP is based on the principle that if animal tissue contains a residue of previously administered antibiotic, 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 subtilis*. This organism is known to be sensitive to most of the commonly used antibiotics (except sulfas). The swabs and plate are incubated overnight to allow growth around the swabs. The presence of such zones of inhibition is presumptive evidence that the carcass tissues contain an antibiotic residue. Positive cases are submitted to the laboratory for confirmation. Negative cases are released provided all other inspection criteria are met and there is no reason to suspect residue from drugs that the STOP does not detect.

Time required:

- Actual work time to set up, interpret, and report the test is only a few minutes per test for an experienced performer. Waiting times built into the test are flexible and can be scheduled around other duties. Test results are obtained overnight.

Accuracy:

- Comparative studies were conducted by FSIS, Science & Technology, using 1,780 tissues. STOP and toe official bioassay were in agreement over 90% of the time. 134 tissues (9.2%) were positive to the swab test but could not be confirmed by official bioassay. Only one tissue was found to contain an identifiable antibiotic when none was detected by the swab test. This means that STOP is a very satisfactory screening test, being somewhat more sensitive than the official bioassay but with an acceptably low level of false positives.

STOP benefits:

- The honest producer and the packer both benefit from more timely release of carcasses that test negative (overnight versus 5-7 days when tissues are sent to the laboratory).
- The PHV benefits from increased confidence in their 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 antibiotic residues are being kept out of the market place.

Application:

- When the STOP program was first implemented in 1979, its use was limited. It is now expanded as the in-plant screening test of all species (except bovine).

Use of STOP:

- In most cases, STOP is used as an aid in determining the disposition of single animals. STOP can be applied any time the PHV suspects the presence of an antibiotic residue. A common reason for conduction the test is finding an injection lesion during post mortem inspection. STOP is also used for follow up testing of animals from a previously identified violative producer.

Basic Procedure:

- To perform STOP the perform needs to:
  - Take initial action
    - Retain the carcasses to be tested
    - Collect carcass traceback information
    - Collect and identify tissue samples.
    - Record initial data on the report form.
  - Prepare and incubate the plate:
    - Prepare tissue swabs
    - Streak the plant with spores of the test organism
    - Assure identification of the plate
    - Place an N5 disc and the tissue swabs on the plate
    - Incubate the plate 16-18 hours at 28-30 degrees C. (up to 24 hours incubation is acceptable).
  - Determine test results:
    - Verify growth of the test organism
    - Verify presence of an appropriate (20-26mm) zone of inhibition surrounding the N5 disc.
    - Determine the presence or absence of zones of inhibition surrounding the tissue swabs.
    - Interpret and record test results.
  - Take follow-up actions:
    - Release the carcass if negative (provided no other reason to continue retaining)
    - Submit samples to the laboratory if positive.
    - Complete and distribute the form.

Reports:

- The forms used to report actions taken in the STOP program are:
  - FSIS Form 6600-2 Antibiotic residue STOP report
  - FSIS Form 10,000-2 Domestic Laboratory Lab report

Equipment:

- The following equipment and supplies are needed to perform STOP:
  - Clean knife for collecting tissues.
  - Plastic bags and rubber bands.
  - U.S. Retained Tags
  - Ballpoint pin.
  - Sterile cotton swabs.
  - STOP agar plates.
  - *Bacillus subtilis* spore suspension.
  - Fine tipped permanent marking pen.
  - Antibiotic sensitivity discs (N5 discs).
  - Thumb forceps
  - Incubator stabilized at 28-30 degrees C.
  - Metric measuring device with millimeter graduations.

Storage of plates:

- The STOP agar plates should be stored refrigerated if possible. They should NOT be frozen. Always check the condition of the plates for mold or other growth before using.

Storage of N5 discs:

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

Storage of spores:

- The *Bacillus subtilis* spores should be stored refrigerated. Make sure the cap is tightly closed after use.

Collecting Tissue Samples

Introduction: In livestock species, STOP is performed on kidney tissue. Proper collection and identification of the tissue samples is essential to assure accurate test results.

Procedure for collection:

1. Identify the carcass to be tested with a U.S. retained tag – a gang of four tags each bearing an identical number.
2. Collect and/or record all available carcass identification information (back tags, ear tags, lot number, carcass number, etc.). The establishment is required by regulation to keep and provide this information to FSIS when requested.
3. Detach one of the US retained tags from the carcass and put it in the bag with the liver that has been collected.
4. Using a clean knife, collect approximately one pound of kidney tissue from the retained carcass, place it in another clean bag (identify this with one of the gang tags from the carcass as well).
5. If you are testing more than one carcass for testing, repeat steps 1-4 to collect tissues and maintain proper identification of the tissues and carcasses.

Special Note:

- Make sure the carcass and all parts and organs are retained until STOP is completed.
- Remember to obtain all animal identification information (back tags, ear tags, etc) at the time the samples are collected. This information must be recorded on the FSIS Form 10,000-2 if you send tissues in for laboratory confirmation. Animal Identification is used to trace the animal back to the owner and is necessary for proper residue control management.

Getting Ready to Perform STOP

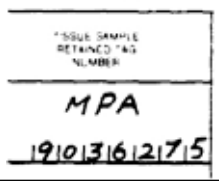
Introduction:

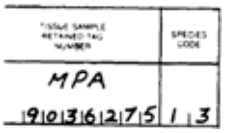
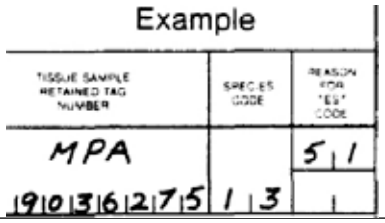
- Before running the test make sure your work area and supplies are ready and you have the correct FSIS Form 6600-2 Antibiotic Residue STOP Report to record your entries.

When to use:

- Follow this procedure after the carcass is properly retained and the tissue samples have been collected. An important consideration when performing STOP is that someone must be on duty to read the results after incubation (between 16-24 hours). If no one will be available during this time, you must hold the tissue samples under refrigeration until this condition can be met. Be sure to seal the sample bag tightly with rubber bands to prevent drying of the tissue.

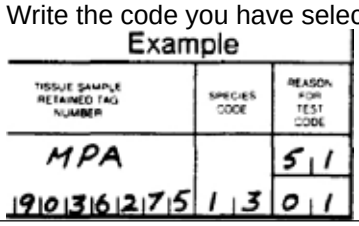
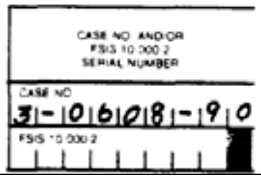
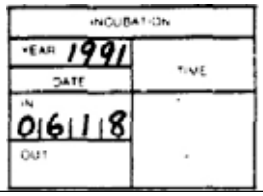
Procedure Table

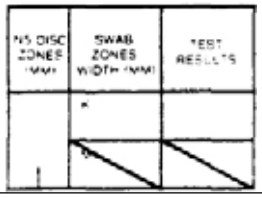
STEP	PROCEDURE
1	Verify that the incubator is stabilized at a temperature of 28-30 C. If it cannot be stabilized send samples without testing directly to the FSIS lab.
2	Clear a 2' x 2' work surface on a table or desk to set up the test.
3	Obtain the following equipment from storage: • STOP report forms (FSIS form 6600-2) • Ballpoint pen and permanent making pen. • STOP agar plates – one plate per every two carcasses being tested. • Sterile cotton swabs – three for each plate. • Vial of *Bacillus subtilis* spores. • Vial of N5 discs and thumb forceps.
4	Make sure that heading of the FSIS form 6600-2 is properly completed. • Establishment number • District/state – enter the 3 digit code or state abbrev. • Inspector's name – legibly print the name of the person responsible for conducting STOP tests in this establishment.
5	Enter the retained tag number form the tissue sample bag in the appropriate block on the first available line on the form Example 

6	From the list below, select the code that best describes the animal you are testing. Note: STOP is used for all species other than bovine and swine. The list below contains all species codes for completeness. 01 – Horse 11 – bull 12 – Steer 13 – Beef Cow 14 – Heifer 15 – Dairy cow 22 – Formula fed calf 23 – Nonformula fed calf 24 – heavy calf 31 – mature sheep 32 – lamb 40 – Goat 51 – Market Swine 52 – Boar 53 – Sow Write the code you have selected in the “species code” block next to the retained tag number. Example 
7	From the list below, select the code that describes the reason for performing this test. 51 – inspector – initiated test 52 – case follow up test [suggest delete if others agree] 53 – monitoring test [suggest delete if others agree – not doing this] Write the code you have selected in the upper half of the “reason for Test Code” block. 

<table>
<tr>
<td>8</td>
<td>If you entered code “51” (inspector initiated test) in the upper “reason for Test” block, select form the list below the code that best describes the reason you suspect antibiotic residues in this animal:

Injection site 01 abdominal abscess 20 pneumonia 30
Udder infusion 02 peritonitis 21 pericarditis/endocarditis 31
History of treatment 03 pyemia/septicemia 22 lung abscess 32
Bolus 04 enteritis 23 other abscess 40
Downer/splitter 10 metritis 24 emaciation 41
Bruises/injury 11 nephritis/cystitis 25 anemia 42
Arthritis 12 acute mastitis 26 neoplasia 43
Rectal/vaginal chronic mastitis 27 misc. infection 44
Prolapse 13 traumatic reticulitis
Recent surgery 14 Complex 28 general misc. 45

Write the code you have selected in the lower half of the “reason for test code” block.
Example

</td>
</tr>
<tr>
<td>9</td>
<td>[if there is no code 52 – this block may not be needed]
if you entered code 52 in the upper “reasons for test” block, enter the case number which was assigned by the TSC in the case block number
Example

</td>
</tr>
<tr>
<td>10</td>
<td>Write today’s date in the “date in” block using the format MMDD, e.g. June 18 = 0618.

</td>
</tr>
</table>

11	Draw a slash mark through the “M swab zones” block and it’s adjacent “tst results” block to indicate that muscle was not tested. 

Preparing tissue swabs

Introduction:

- Cotton swabs saturated with tissue fluid are obtained by macerating the tissue with the sharp end of the swab shaft. Then the cotton swab is allowed to contact the macerated tissue until a maximum of tissue fluid is absorbed into the swab (at least 30 minutes but no more than 2 hours). 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 the initial data has been recorded on FSIS Form 6600-2.

Procedure Table:

STEP	PROCEDURE
1	Open the cotton swab pack, remove one swab, and jab the sharp end just through the wall of the plastic bag containing the kidney tissue sample. Then use the swab shaft to enlarge the opening in the bag to about ½" in length. NOTE: this is done to prevent squeezing fluid from the swab head when it is later removed from the tissue.
2	Push the sharp end of the shaft about ½ to ¾ inches into the tissue. Then jab it back and forth several times to macerate the tissue.
3	Reverse the swab and insert the cotton swab tip into the tissue. Twirl the swab shaft to make sure there is good contact between the swab head and the macerated tissue.
4	If you are setting up additional tests at this time, repeat steps 1-3 to prepare the other swabs.
5	Leave the swabs in place at least 30 minutes to assure maximum saturation of the swabs. If other duties prevent continuing the test after the 30-minute period, the swabs may be left in place in the tissue for up to 2 hours.

Streaking the plate with spores

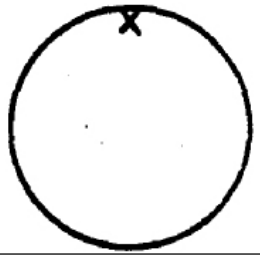
Introduction:

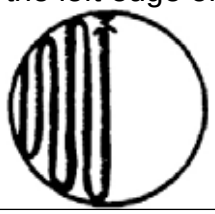
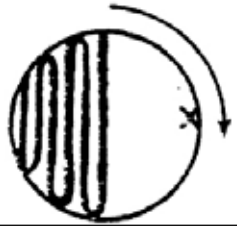
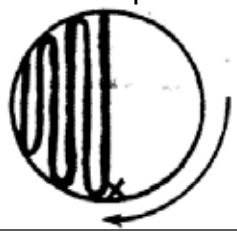
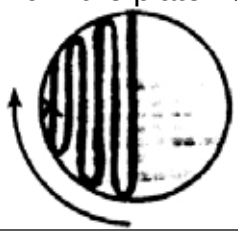
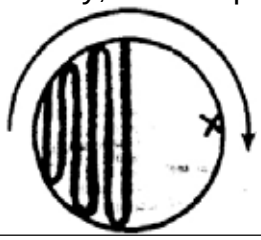
- You should streak the plate with the *Bacillus subtilis* spores just before the tissue swabs are places on the plate. A standardized technique is required to assure even distribution of the spores and consistent test results.

When to use:

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

Procedure Table:

STEP	PROCEDURE
1	Select the following items: • One STOP plate per each two carcasses being tested • Sterile cotton swabs – one per plate • Vial of *B. subtilis* spores. • Fine-tipped permanent marking pen
2	Verify that the plate is suitable for use: • Not damaged by freezing • Agar not dried out or cracked • No interfering colonies of an accidental contaminant.
3	Check to make sure the screw cap on the vial of spores is tight; then vigorously shake the vial to re-suspend the spores. NOTE: if there is particulate matter present or the fluid appears colored or off odor is detected, the spore suspension should be discarded.
4	Lift the plate cover slightly and make an “X” reference mark on the sidewall of the plate. Place the covered plated bottom-side down on the work surface with the reference mark at “12 o’clock”. 
5	Remove a sterile cotton swab from its wrapper, grasping only the shaft. Avoid touching the cotton tip to any surface.
6	Make sure the spores are thoroughly mixed; then remove the screw-cap from the spore vial. Avoid touching the inside of the cap or vial.
7	Insert the swab into the vial so that the cotton swab is completely immersed in the spore suspension.

8	Gently shake off any excess fluid and remove the swab from the vial (avoid touching the swab).
9	Replace the screw-cap and set the vial of spores aside
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, while moving the swab to the left edge of the plate. 
11	Turn the plate ¼ turn clockwise and repeat the streaking pattern from the center to the left edge. 
12	Turn the plate ¼ turn and repeat the pattern. 
13	Turn the plate ¼ turn and repeat the pattern. 
14	Finally, turn the plate ½ turn and repeat the pattern. 
15	Replace the cover on the plate and discard the used swab. Use a fresh swab for each plate prepared.

Comments:

- Dip the swab into the spore suspension only one time.
- Do not reuse the swab to streak additional plates.
- If your work area has a dark surface, placing a white sheet of paper under the plate will help you see the reference mark on the plate.

Special note: in steps 10-14, a left-handed performer should swab the plate from the center to the right edge and turn the plate counter clockwise.

Identifying the STOP plate

Introduction:

- Accurate identification of the plate is essential to assure 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(s) with spores and you are ready to set up the test.

Procedure Table:

STEP	PROCEDURE
1	If you are testing: • One carcass only…….. Go to step 2. • More than one carcass……skip step 2 and **Go to step 3.**
2	Locate the area of the plate directly opposite the "X" reference mark. Lift the plate cover slightly and use the fine-tip marking pen to write the last three digits of the tissue sample retained tag number on the vertical edge of the plate itself. **Skip steps 3,4,5 and Go to step 6.**
3	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.
4	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 first tissue sample retained tag number on the vertical edge of the plate itself.
5	Locate the area of the plate directly opposite the first three-digit number. Then, lift the plate cover slightly and write the last three digists of the second tissue sample retained tag number on the vertical edge of the plate itself.
6	Check to make sure that the plate number(s) match the last three digits of the retained tag number(s) recorded on the FSIS Form 6600-2.

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 such factors as incubation temperature and humidity. 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.

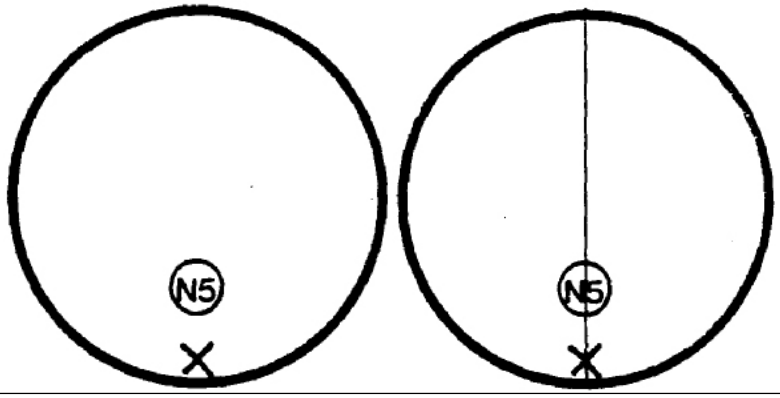
Disc Storage:

- To maintain the potency of the N5 discs, they must be kept cold and dry. Replace the disc vial in its protective container (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 the procedure below after you have identified the plate for use.

Procedure Table:

STEP	PROCEDURE
1	Remove the cap from the N5 dispenser vial and set the cap aside.
2	Remove the cover from the plate and place it open-side up beside the plate
3	Dispense one N5 disc into the open cover. Then pick up the disc by its edges with the thumb forceps.
4	Select a point about ½ inch in from the “X” reference mark and carefully drop the disc flat onto the agar. 

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.
6	Replace the cover on the plate and replace the cap on the N5 disc vial.

Comments:

- 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.
- Remember to return the vial of N5 discs to refrigerated storage after each use.
- If you are testing one carcass **go to section titled "positioning the swab on the plate (single test)".**
- If you are testing more than one carcass **go to section titled "positioning the swabs on the plate (two tests per plate)".**

Positioning the swab on the plate (Single Test)

Introduction:

- The cotton swab saturated with tissue fluids must be places on the plate so that:
  - The swab and the N5 disc are well separated, and
  - There is good contact between the swab and agar.

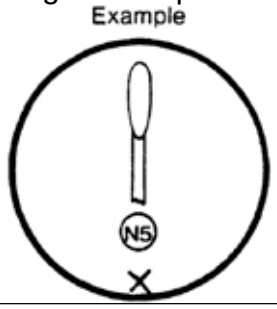
Comment:

- Sterile technique is not required, but your hands should be washed, rinsed, and dried to prevent contaminating the swabs with any substance that might interfere with the test results. Handle the swab shaft only with your clean fingers. Do NO 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.

Procedure Table:

STEP	PROCEDURE
1	Remove the swab from the tissue and hold it with the cotton tip down to prevent fluid from flowing down the plastic shaft.
2	Being careful not to touch the cotton tip itself, break the shaft off as short as possible.
3	Remove the cover from the plate and gently place the swab on the surface of the agar so the broken end of the shaft is near the N5 disc and the cotton tip is as far away from the disc as possible but not touching the edge of the plate. 
4	Being careful not to break the agar surface, lightly press the swab shaft with your finger tip to assure proper contact of the swab head with the agar.

Go to "Incubating the STOP plate".

Positioning the swabs on the plate (Two Tests per Plate)

Introduction:

- The cotton swabs saturated with tissue fluids must be placed on the plate so that:
    - Each swab is in its respective marked section,
    - The swabs and the N5 disc are well separated, and
    - There is good contact between the swabs and the agar.

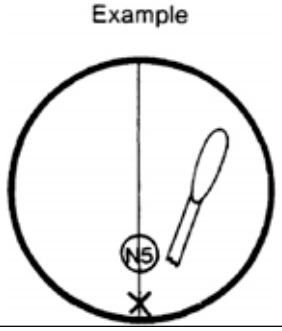
Comment:

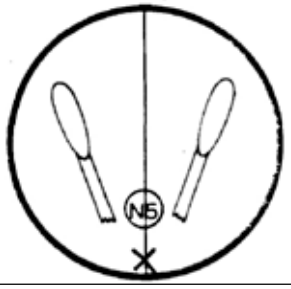
- Sterile technique is not required, but your hands should be washed, rinsed, and dried to prevent contaminating the swabs with any substance that might interfere with the testing results. Handle the swab shaft only with your clean fingers. Do NOT use the forceps! They may be contaminated with neomycin.

When to use:

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

Procedure Table:

STEP	PROCEDURE
1	Remove the swab from the tissue and hold it with the cotton tip down to prevent fluid from flowing down the plastic shaft.
2	Being careful not to touch the cotton tip itself, break the shaft off as short as possible.
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. 
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.
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.

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

Go to "incubating the STOP plate".

Incubating the STOP Plate

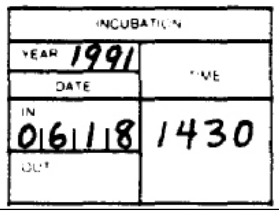
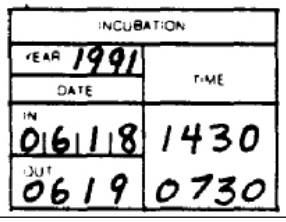
Introduction:

- For optimum growth of the test organism, creating a highly visible lawn of colonies and clear zones of inhibition, the plate should be incubated at 28-30 degrees C before using it for STOP.

Caution:

- Incubation temperatures for FAST are different than for STOP. You must use separate incubators; one for FAST tests and one for STOP tests.

Procedure Table:

STEP	PROCEDURE
1	Make sure the incubator is properly stabilized at 28-30 degrees C.
2	Make sure that an open container of water is inside the chamber to provide proper humidity
3	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.
4	Record the "time in" on the FSIS Form 6600-2. Example 
5	Secure the incubator in a manner that will preclude tampering during incubation.
6	Remove the plate from the incubator after 16-18 hours of incubation and record the "time out" on FSIS form 6600-2. Example 

Comments:

- 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 STOP or submitting them to the laboratory.

- You should read the results of the test immediately after incubation, if possible. If you need to delay reading the results or wish to show the plate to someone else, place the incubated plate in a refrigerator to prevent further growth of the organisms.

Go to "verifying function of the STOP system".

Verifying function of the STOP System

Introduction:

- Growth of the test organism during incubation of the plate results in millions of tiny white colonies on the surface of the agar. These colonies are so closely spaced that their presence is commonly called a bacterial lawn. This "lawn" should develop on all areas of the plate except where growth was inhibited by an antibiotic. To verify that the STOP system has functioned properly during incubation, you must:
    - Determine that there has been growth of the test organism, and
    - Determine that the diameter of the zone of inhibition (ZI) surrounding the N5 disc is within the acceptable range of 20-26 millimeters.

Definition:

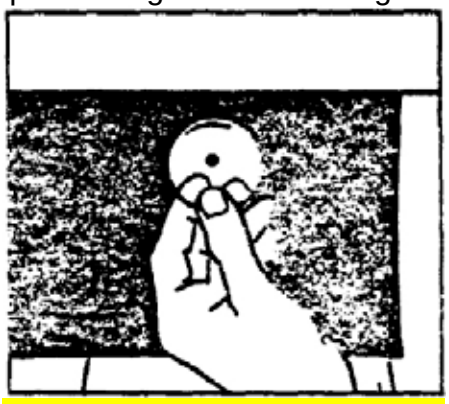
- A zone of inhibition (ZI) is an area free of colonies of the test organism caused by the presence of some growth-inhibiting substance, e.g., an antibiotic.

When to use:

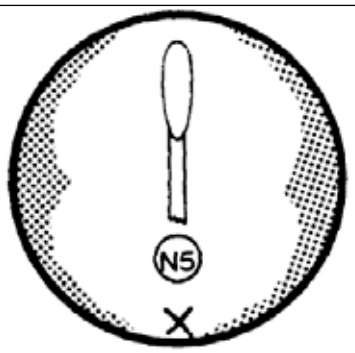
- Follow this procedure immediately after removing the plate from the incubator

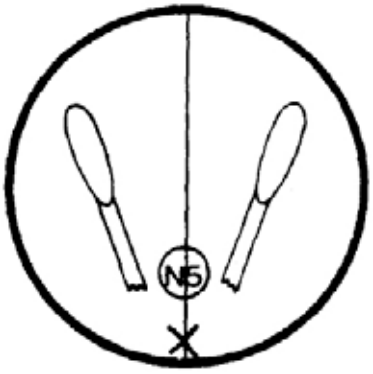
Procedure Table:

STEP	PROCEDURE
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.

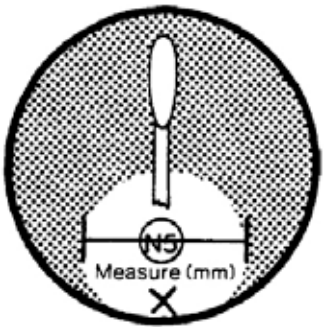
2	Lift the plate out of the cover and hold it with the bottom toward you so you can look through the plate to observe for growth of the test organism. NOTE: lighting conditions vary so you will have to experiment to get the best possible view. One good method is to hold the plate up to a shaded desk lamp so the light shines through the plate but not directly into your eyes. 

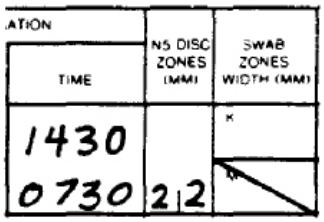
<table>
<tr><td>3</td><td>Growth of the test organism as evidenced by a bacterial lawn should be readily visible in at least the areas of the plate farthest away from the N5 disc and the swab(s).

<table>
<tr><td>If growth of the test organism is:</td><td>THEN..</td></tr>
<tr><td>Evident….</td><td> 

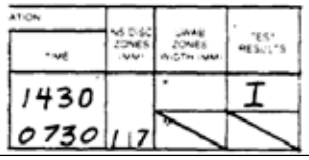
go to “step 4”</td></tr>
<tr><td>NOT evident..</td><td> 

go to “handling culture failures”</td></tr>
</table></td></tr>
<tr><td>4</td><td>Examine the zone of inhibition (the zone free of colonies of the test organism around the N5 disc).</td></tr>
</table>

<table>
<tr><td>5</td><td>Measure the diameter of the N5 disc zone with a metric ruler, and record the diameter in millimeters (mm) in the appropriate block on FSIS form 6600-2.
 



Note: be careful not to confuse millimeters with centimeters. The labeled graduations on most metric rulers are centimeters. Remember, there are approximately 25 millimeters in one inch.</td></tr>
<tr><td>6</td><td><table>
<tr><td>If the diameter of the N5 disc zone was…….</td><td>THEN…….</td></tr>
<tr><td>20 – 26 millimeters…….</td><td>Go to section: “Interpreting and recording test results”</td></tr>
<tr><td>Less than 20 or more than 26 millimeters……..</td><td>The results of this test may not be reliable. You must either rerun the test or submit tissues to the laboratory. CONTINUE with STEP 7</td></tr>
</table></td></tr>
<tr><td>7</td><td>Write “I” (inconclusive) in the test results block for this test on FSIS Form 6600-2.

Example
 
</td></tr>
</table>

<table>
<tr><td>8</td><td>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 or over saturation of the swab used to apply the spores.
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 26mm)
Not enough spores on the plate – possibly caused by inadequate mixing of the spores or under saturation of the swab used to apply the spores.
Incubator humidity too low.
Incubator temperature too high or too low.
Your decision on whether or not to rerun this test or submit tissues to the laboratory immediately depends on several factors:
Will you have to wait for new supplies?
Will you be able to make changes that will likely result in a N5 disc zone within range?
What is the impact of waiting another day for STOP results versus submitting tissues now?</td></tr>
<tr><td>9</td><td><table>
<tr><td>If you have decided to……..</td><td>THEN…….</td></tr>
<tr><td>Submit tissues from this carcass to the laboratory…..</td><td>Treat this test as though it were “positive” and go to “follow up action – positive STOP”.</td></tr>
<tr><td>Rerun this test…….</td><td>Go to section “getting ready to perform STOP”.</td></tr>
</table></td></tr>
</table>

Special note:

- If you continue to experience out-of-range N5 disc zones, you should contact the Technical Service Center for guidance. If the problem is caused by faulty manufacture of the spores or plates, it is probably affecting other STOP performers as well.

Interpreting and recording test results

Introduction:

- STOP 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.

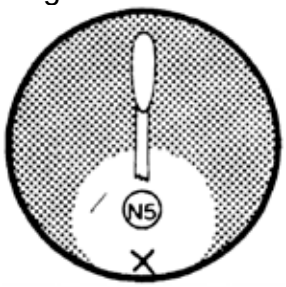
When to use:

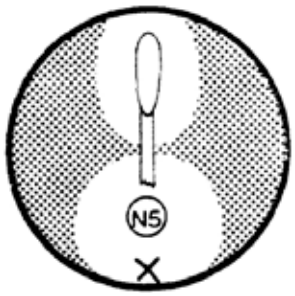
- Follow this procedure to read and interpret the test results only after you have verified that the STOP system functioned properly during incubation.

Procedure Table:

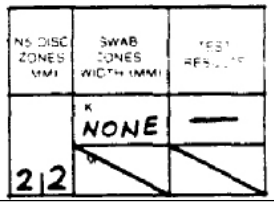
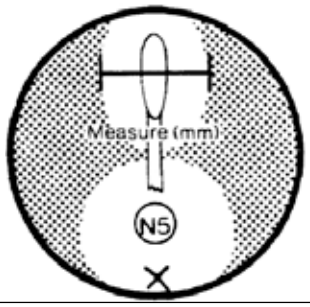
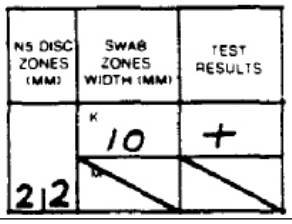
<table>
<tr><td>STEP</td><td>PROCEDURE</td></tr>
<tr><td>1</td><td>Examine the plate in the area of the swab impression to determine if a clear zone of inhibition (ZI) is present.

Special note: diffusion of tissue pigment may discolor the agar, so look carefully for growth. Also, 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.

<table>
<tr><td>IF a clear zone of inhibition is…</td><td>THEN consider the test result for this swab as…..</td></tr>
<tr><td>NOT present</td><td>Negative
 

go to step 2</td></tr>
<tr><td>Present</td><td>Positive
 

SKIP step 2, go to step 3</td></tr>
</table>
</td></tr>
</table>

2	You have determined that the test result for this swab is Negative (-). Read the 3-digit number on the plate that identifies this swab. Then locate the line on FSIS Form 6600-2 where the original entries for this case were made and written “NONE” in the “K” swab zones block and write “-“in the “test results” block. **Go to step 5** Example 
3	You have determined the test results to be Positive (+). Measure the width of the swab ZI with a metric ruler. Be sure to measure perpendicular to the swab shaft. (The lengths of the swab zone are no longer measured). 
4	Read the 3-digit number on the plate that identifies this swab. Then, locate the line on FSIS Form 6600-2 where the original entries for this case were made and write the width of the swab ZI (in mm) in the “K” swab zones block and write “+” in the Test results block. Example 
5	If there is another swab on this plate, GO BACK TO STEP 1 to interpret and record the results of the second swab.
6	After STOP 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.

If the result of any test was negative – **go to "follow up action – negative STOP".**

If the result of any test was positive – **go to "follow up action – positive STOP".**

Follow up action – Negative STOP

Introduction:

- If the STOP 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 STOP result for kidney tissue is negative.

Procedure Table:

STEP	PROCEDURE
1	Release the carcass based on the negative STOP results after appropriate trimming, e.g., removal of injection lesions, provided other reasons for retaining the carcass are resolved. No tissues need be sent to the laboratory. (Note: In some cases of a negative STOP result, if in your professional judgment you feel there is still reason to suspect a residue from use of antimicrobials, sulfa, or pesticides you would continue to retain the carcass, and send the collected tissues for confirmation at the appropriate FSIS lab.)
2	• Inventory your STOP supplies and follow the instructions you have received from your District to order any additional supplies needed. • Determine whether FSIS Form 6600-2 (STOP report) should be submitted at this time.

Follow up action – Positive STOP

Introduction:

- STOP is an in-plant screening test and is quite accurate in determining if antibiotic residues are present, but is does not provide quantitative results. If the STOP result for the kidney swab is positive, tissue samples must be submitted to the laboratory for a bioassay testing. The purpose of the laboratory testing is to identify the antimicrobial residue and to determine if the residue is present at violative levels. The carcass must be retained pending the laboratory results.

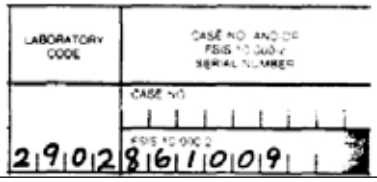
When to use:

- Follow this procedure if the result for kidney tissue is positive.

Procedure Table:

STEP	PROCEDURE
1	Continue to retain the carcass pending laboratory results.
2	Collect approximately one pound each of muscle, kidney and liver from the retained carcass. Place each tissue sample in a separate double bag, label it and freeze it. NOTE: Do NOT send tissues from injection sites to the laboratory unless you are requested to do so.
3	Follow existing instructions for completing the FSIS Form 10,000-2 Domestic Laboratory Report. Data from the Domestic Laboratory Form (FSIS Form 10000.2) is stored in an electronic format. Therefore the form must be filled out correctly if the data is to be available to the end user. Accuracy and attention to detail are important. If an entry is not required, leave the field blank • Block #1: Leave blank. • Block #2: Leave blank • Block #3: Enter the numeric State Code as it appears in the Meat and Poultry Inspection Directory. State codes require two (2) characters. • Block #4: Enter the District Code as it appears in the Meat and Poultry Inspection Directory. District codes require four (4) characters; two alpha-characters, a "D" and an "O" followed by two numbers. • Block #5: Leave blank • Block #6: Not applicable. This field is automatically filled. • Block #7: Enter the official establishment number. Official establishment numbers require eight (8) characters. The proper format is five (5) numbers, followed by two alpha-character modifiers IF USED. or two dashes (--) if the alpha-character modifiers are not used, followed by an "M" if the establishment is a red meat establishment or a "P" if the establishment is a poultry establishment.

	• Block #8: Enter the retain tag number for the carcass. The Retain tag number requires eight (8) numerical characters. Do not enter the "MPD". Enter only the eight (8) numbers. • Block #9: Enter the Condemn tag number if applicable • Block #10: Enter the word “STOP”. • Block #11: If the sample submitted is for a "follow-up" case, enter the case number as recorded on the corresponding FSIS Form 6600-7. • Block #12: If two or more samples are submitted for the same producer, herd, or flock owner, enter the serial numbers from block 6 of FSIS Form 10000-2 for the other samples collected from the same producer, herd, or flock owner. • Block #13: Enter the date the sample was collected. Dates require six (6) characters in a mm/dd/yy format. • Block #14: Enter the date the sample was mailed to the Midwestern Lab. Dates require six (6) characters in a mm/dd/yy format. • Block #15: Check the box “Midwestern Lab”. • Block #16: Enter the name and address of the producer, herd, or flock owner. If the livestock came from a sale barn and the identity of the producer, herd, or flock owner is unknown, enter the name and address of the sale barn. If the producer, herd, or flock owner's name is not available, enter the name and address of the slaughter establishment. Enter the alpha-state code and five digit ZIP CODE in the appropriate boxes. • Block #17: Enter the species as listed on the reverse of FSIS Form 10000-2 • Block #18: Enter the species code as listed on the reverse of FSIS Form 10000-2 • Block #19: Leave blank • Block #20: Leave blank • Block #21: Check "Residue" and enter "200" as the "Residue Class Code" and "800" as the "Specific Residue" • Block #22: Check "LIVER", "MUSCLE", and "KIDNEY" • Block #23: Enter all available tag numbers in the appropriate field and identify the tag types in the appropriate field. No entry is required for the "F/U Form No".; and "F/U Source" fields. • Block #24: For a positive STOP test, enter "STOP Positive" and the width of the swab zone of inhibition. If samples are submitted for an inconclusive STOP test, enter "STOP Inconclusive" and the width of the swab zone of inhibition. If samples are submitted for a system failure, enter "STOP culture Failure". • Block #25: Enter the name of the person preparing the sample and completing FSIS Form 10000.2. • Block #26: Enter the badge number of the person preparing the sample and completing FSIS Form 10000.2. • Block #27: Enter the telephone number of the person preparing the sample and completing FSIS Form 10000.2. • Block #28 through 39: Leave blank
4	Determine which technical support laboratory is designated to receive the sample form. LEARN has an updated list of tests and the labs which perform the tests.
5	From the list below, select the code for the laboratory. • 1302 – Eastern Lab, Athens, GA • 2902 – Midwestern Lab, St. Louis MO • 0602 – Western Lab, Alameda, CA

6	Record the laboratory code and the serial number from the FSIS Form 10,000-2 in the appropriate blocks on the STOP report FSIS Form 6600-2. Example 
7	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 sealed sample shipping containers to the appropriate laboratory. Refer to FSIS Directive 7355.1, FSIS Directive 10,210.1 for additional information.
8	• Inventory your STOP supplies and follow the instructions you have received from your district to order any additional supplies needed. • Determine whether FSIS Form 6600-2 (STOP Report) should be submitted at this time. (See “submitting the FSIS Form 6600-2”).

Submitting the FSIS Form 6600-2

Introduction:

- Data from the FSIS Form 6600-2 is entered in an FSIS computer database. From this database, various reports can be generated. Timely submittal of the FSIS Form 6600-2 is essential to assure implementation of residue control programs.

Procedure:

- As soon as the FSIS Form 6600-2 is full OR at the end of each calendar month, *whichever occurs first*, mail the original to:
    - STOP reports
    - USDA, FSIS, Data Services Center
    - 210 Walnut street, Room 791
    - Des Moines, IA 50309
- File the other copies in the USDA inspection office.

Handling culture failures

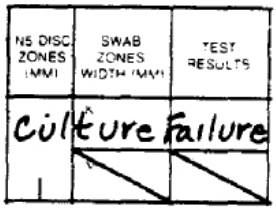
Introduction:

- 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 subtilis* 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 anytime the test organism fails to grow into a visible “lawn”.

Procedure Table:

STEP	PROCEDURE
1	Locate the line on FSIS Form 6600-2 where the original entries were made and write “culture failure” across the “N5 disc zones” and “swab zones” columns. Example 
2	If you have determined the cause of the culture failure and can rerun the STOP on these tissues, you should do so now.
3	If you have NOT determined the cause of the culture failure you should seek guidance from the Technical Service Center, Omaha, NE. Unless you are told otherwise, you must submit tissues to the laboratory. Follow instructions from the “follow up action – Positive STOP”, except write “STOP culture failure” in block 24 of FSIS form 10,000-2 Domestic laboratory report.