USDA APHIS
Veterinary Services
National Veterinary
Accreditation Program

National Veterinary Accreditation Program Module 35: Bovine Tuberculosis in Cattle

National Veterinary Accreditation Program

Module 35: Bovine Tuberculosis in Cattle

Completion of this module provides one unit of supplemental training for participants in USDA's National Veterinary Accreditation Program.
It familiarizes accredited veterinarians with animal health regulatory concepts and activities. Information in the module does not supersede the regulations.
For the most up-to-date regulations and standards, please refer to the Code of Federal Regulations or contact your local Area Office.

For questions about the content of this module, please contact:
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National Veterinary Accreditation Program

Module 35: Bovine Tuberculosis in Cattle

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SECTION 1: INTRODUCTION

Module 35: Bovine Tuberculosis in Cattle

Welcome to the National Veterinary Accreditation Program's Module 35: Bovine Tuberculosis in Cattle. The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), has established a national program to eradicate bovine TB in the United States. It is the National Tuberculosis Eradication Program, and it is administered by USDA APHIS, State animal health agencies, and U.S. livestock producers and uses the caudal fold test or CFT for bovine Tuberculosis. Testing cattle for TB is part of the strategy used to eliminate bovine TB in the United States.

A veterinarian must be licensed and a category II accredited veterinarian to perform a caudal fold test in cattle.

After completion of this module, you will be able to describe:

- background information on bovine tuberculosis;
- the process for diagnosing bovine tuberculosis;
- the process to perform the caudal fold test in cattle;
- interpreting the results of the caudal fold test in cattle; and
- understanding the steps once the caudal fold test is completed.

Web links for additional information on topics presented in this module can be found in the Resources and Web Links section of this document.

Completion of this module is estimated to take approximately 60 minutes but will vary depending on your familiarity with the material.

SECTION 2: BACKGROUND INFORMATION ON BOVINE TB

The National Tuberculosis Eradication Program, has nearly eradicated bovine TB from the Nation's livestock population since the program's inception in 1917. The presence of bovine TB in humans has also been reduced. Eradication efforts include surveillance methods to detect the disease including examinations made at slaughter, monitoring animals tested for exhibition, interstate movement, herd accreditation and periodic testing of dairies according to milk ordinances and

pasteurization of milk. Many consider this one of the great animal and public health achievements in the United States; however animal health officials continue to detect TB sporadically in livestock herds.

There are 3 major reasons an accredited veterinarian performs a TB CFT:
1. Movement of cattle from one area to another like from State to State, Country to Country, or in the case of the northern part of the lower peninsula of Michigan, from that area to anywhere else. Nation-wide, this is by far the most common reason.
2. Herd accreditation
Although rare, some owners choose to have a TB Accredited Free Herd. This is almost exclusively done with the purebred seedstock producers only.
3. Regular testing of dairy herds for food safety.

All questions about TB testing and other regulatory programs, should be directed to your Area Veterinary in Charge, the VS Area Office, or the State Animal Health Official in the state where you intend to do TB testing.

Bovine tuberculosis is caused by *Mycobacterium bovis*. It is a unique bacteria because it has a thick lipid capsule. It is also slow growing and multiplies once every 20 hours resulting in the chronic nature of the disease. It is difficult to culture and hides well in the body. It also has the ability to hide outside of the host and survives well in a dark, cool, moist environment. *M. bovis* can persist up to 11 months at temperatures of 54 –75 degrees Farenheit and in feces it can remain infective for up to 6 to 8 weeks.

Mycobacterium bovis infects all warm-blooded vertebrates, including humans. Approximately 1 to 5% of human isolates are *M. bovis.*

M.bovis is most commonly transmitted through aerosolization of infective droplets, which are inhaled or ingested by a susceptible host. Other routes of transmission include: shared drinking water and raw milk from infected dams to offspring or other animals. *M. bovis* can be transmitted from animals to humans and vice versa.

Cattle and bison usually show no clinical signs to TB unless the disease has affected multiple organ systems and is very advanced. Typically, infected cattle are asymptomatic and are only detected by skin testing or at slaughter during our national slaughter surveillance program.
If cattle do show signs they include:
- progressive weight loss;

- weakness;
- anorexia;
- fluctuating fever;
- chronic, moist cough; and
- difficulty breathing.

The prevalence of TB in cattle is extremely low in the US occurring roughly 13 times per 1 million cattle herds on an annual basis.

Producers can reduce their risk of getting the disease by adding only test negative livestock from known negative herds to their own herds. When TB is found in a herd, it is eliminated by either depopulating the affected herds or by testing and then removing reactor animals from affected herds.

M. bovis can be inactivated by a solution of common household bleach (1:9 dilution) and other commercial disinfectants.

However, successful disinfection can only be accomplished after adequate cleaning. The presence of organic matter greatly inhibits most methods of disinfection.

SECTION 3: DIAGNOSING BOVINE TB

Diagnosing TB in live animals depends on using an effective testing technique with an intradermal injection of tuberculin obtained through your State animal health official or VS Area Office. Several varieties of tuberculin are produced. However, use only bovine purified protein derivative tuberculin, PPD bovis licensed by USDA for official testing. See Table 1 for tuberculin test requirements for different species of animals.

All species infected with Mycobacteria develop an immune response to the bacteria, which can be detected by a corresponding tuberculin skin test. Tuberculin is a sterile product made by growing specific Mycobacteria species, killing them with heat, and properly diluting and preserving the material, which is then called Purified Protein Derivative (PPD) tuberculin.

A small amount of this PPD tuberculin is then injected intradermally in the animal(s) being tested to determine infection with or exposure to Mycobacterium. Veterinarians are legally responsible for properly conducting and evaluating the results of tuberculin tests. Therefore, perform the test yourself; do not delegate the

responsibility to a technician. For TB testing in species other than cattle or bison (e.g., cervids), contact your State animal health official or VS Area Office for additional guidance. All accredited veterinarians must immediately report every suspected or diagnosed bovine TB cases promptly to both the Area Veterinarian in Charge (AVIC) and their State Animal Health Official.

Remember, official TB testing can only be performed by licensed and category II accredited veterinarians.

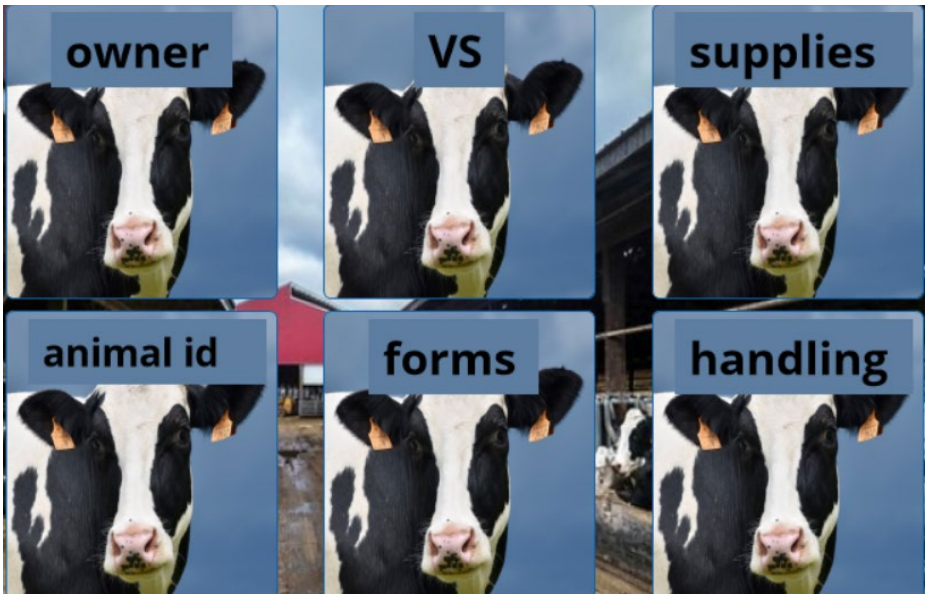
To differentiate animals infected with *M. bovis* from those sensitized by exposure to other Mycobacteria (e.g. *M. avium*), a second test, called a Comparative Cervical Test (CCT) that involves the intradermal injection of bovine and avian tuberculin PPD into different sites on the same side of the neck of the animal is done.
Only Federal or State regulatory veterinarians who have had specialized training may conduct CCT testing and it must be performed within 10 days of the initial caudal-fold injection in cattle and bison, or the herd owner must wait 60 days, after the injection of the CFT before a follow-up CCT can be administered.

If an animal falls into the suspect zone after performing the CCT, it is considered a bovine tuberculosis suspect. The animal can be retested with the CCT in 60 days or removed from the herd and euthanized at the owner's expense.

If the results of an animal that was retested remain in the suspect zone, the animal will be reclassified as a reactor and it will be euthanized. This can be done at slaughter with certain provisions. Tuberculosis lesions include internal lesions or lymph nodes that have firm, round tubercles and granulomas. Classification of the animal or herd depends on the results of a necropsy, tissue culturing, polymerase chain reaction (PCR) testing or histopathology.

There is a blood test that can be used as a secondary test, although it is not used in certain states. For more information check with your VS Area Office for details.

Before caudal fold testing (CFT), you must first complete a list of preparatory tasks.



Step one is to contact the owner and make sure you have answers to the following questions before testing is initiated.

What is the purpose for the testing?
Does the owner have appropriate animal restraint?
Will they be available to help?
How many animals will you be testing?
Are the animals officially identified?
What are the tentative dates they are available for testing?

If the test is for movement purposes, the group of animals may not move until all testing is completed, including the secondary tests. Make sure the owner understands they need to schedule the testing at least 2 weeks prior to the animals' movement date. That will give you enough time to finish the caudal fold testing and give Veterinary Services enough time to finish the secondary testing if responders are found.

Next, contact your local field Veterinary Medical Officer or State Animal Health Official (SAHO) for how they would like you to notify them of planned TB testing so that they may schedule their resources appropriately. Follow up testing by a federal or state official who has been trained to perform the comparative cervical test (CCT), is required within 7 days from the day the accredited veterinarian has read the caudal fold test (CFT) or 10 days from the date of injection. If this window is missed, the entire herd must be quarantined for 60 days before secondary testing can be performed to determine the disposition of any responders (suspects) found.

If the VS Area Office knows about the caudal fold testing ahead of time, they can make sure to have someone available for comparative cervical testing if needed.

Assemble the needed supplies.

Ensure you have personal protective equipment like gloves, coveralls, boots, knee and shin guards, and ear and eye protection. You will need marking paint or chalk to identify which side of each cow the PPD was injected. Bring a sharps container for needles and trash bags for used syringes. It is also recommended that you have a cooler and ice packs to keep the PPD cold.

You will need a method to restrain each animal. Additionally, each animal must be official identified and if they are identified with RFID an RFID reader will allow you to instantly read and record the numbers. You must have one disposable 1.0 cc plastic tuberculin syringe and a 26 gauge 3//8" needle for each animal being tested. A larger gauge and longer or shorter needle might allow the tuberculin to leak from the injection site.

Use USDA Veterinary Services approved PPD Bovis tuberculin. If you have some on hand, check the expiration date to be certain that the tuberculin is still valid. If you don't have PPD on hand you can order it from the National Veterinary Services Laboratory or the entity that supplies it in your state. This can be determined by contacting your VS Area Office or State Animal Health Official's Office. For NVSL orders use the VS Form 4-9, Reagents Request order form. Processing of orders can take up to ten business days. Once you know how many cattle you will be testing, you can determine how much tuberculin to order. It comes in 10 ml (75 to 80 doses*) , 5 ml, and 1 ml vials (5-6 doses*), but some is lost in the hub of the needle and syringe, so always order approximately 10% more than you will need. It's free. Store it appropriately by keeping it cold and out of direct sunlight until use.
* Numbers represent average estimates of doses in each bottle. Always error on the side of caution so you have plenty of doses of PPD available.

Make sure you have the VS Form 6-22, Tuberculosis Test Record on hand when you perform the CFT.
Complete VS Form 6–22, Tuberculosis Test Record.

- Identify the animal on the form by its official identification as outlined in the section entitled "Animal Identification."

- All cattle and bison tested shall be individually identified by official eartags. Such identification must be recorded in its entirety on the test record at the time of injection and must be confirmed at the time of observation.
- Additional identification, such as bangle tags, non-official metal ear tags, neck chain numbers, tags, brands, horn numbers, and names, should also be recorded on the test record as supplemental information, but must never be used as the sole method of identification.
- When cattle and bison have been tagged with more than one official ear tag, all ear tag numbers must be recorded in their entirety.
- The breed, sex, and approximate age, in years or months if less than one year, of each animal tested must be recorded in their entirety on the test record.
- Inform the owner of the number of animals injected, and not to move or sell any tested animals until both the CFT and CCT is complete or a regulatory veterinarian gives them permission.

The VS 6-22 should be submitted to your regulatory veterinary officials within 7 days of reading the test. Different States have different policies. VS Officials need the form before they can inject the secondary test.

To record the results of the CFT on the VS Form 6-22, enter "N" for negative, when you observe no change in the tissue at the site of injection or enter "S" for suspect, when you observe or palpate any increase in caudal fold thickness, size, or sensitivity, at the injection site. Any increase or change must be recorded as a suspect.

For details on how to complete the VS Form 6-22 see Appendix D pages 16-16 through 16-19 on the Veterinary Services Website.

National Uniform Eartagging System (NUES) Tags or "Brite" Tags have nine characters, two for the state or tribal code, three in an alpha series followed by four digits in a sequential series. They are tamper evident and are imprinted with the official US shield.

Because the NUES or "Brite Tag" is hard to read, it is not uncommon for other official identification tags to be applied to the animal in a sale barn, when completing a CVI for movement, or conducting a previous TB test.

Official Animal Identification Number (AIN), are devices with an "840" prefix. When using the 840 AIN for USA origin cattle, the minimum standard for official

identification is a visual eartag. Eartags with the AIN as a USDA official tag are designed for a one-time use or are tamper evident.

They are imprinted with a 15-digit number starting with 840. An example is 840 003 123 456 789 and the Official Eartag Shield. They are unlawful to remove and are marked as such. The devices are also imprinted with the manufacturer's logo or trademark. By regulation, 840 tags may only be placed in cattle born in the USA.

840 Animal Identification Number Tags may be paired with radio frequency (RF) devices. For low frequency tags the reader needs to be close to the tag and only one tag can be read at a time. When you use high frequency tags the reader can read tags from several feet away and you can read multiple tags at one time.

Using electronic ID can significantly reduce the time spent reading the official ID of each animal and can be done without having to fully restrain the head.

All cattle tested must be sufficiently restrained to permit careful application and reading of the TB injection.
No test should be applied or observed without having the animal restrained in a satisfactory manner.

The best method of restraint is the cattle chute with head restraint. This method is highly preferred above using a lariat and halter. Lariat and halter restraint depends on having something to which the animal can be secured. Although this is a common method of restraint it is not sufficient for TB testing.

Cows kick in an arch. Avoid this kicking region. The proper approach to a cow is critical to working with them safely. Any movement in their blind spot will make them uneasy and nervous. Always "announce" your presence when approaching a cow and gently touch the cow rather than let the first contact be a bump.

Plan ahead when preparing to work with cattle. Have proper equipment available and make sure it works before you use it. Check lanes, fences and chutes before moving cattle and try for minimum restraint time and use minimum force to move the animals. You should always be confident and in control and remember that most injuries occur from improper handling.

Move slowly and deliberately around cattle. Slow is smooth, smooth is fast. Establish a routine.

Unexpected loud or unusual noise can be highly stressful to cattle especially metal clanging or banging, high pitched noises or noisy machinery.
TB testing tips
Select each box to learn more.
1. Avoid exposing PPD to light, air, high temperatures, and freezing
   - Tuberculin oxidizes and loses potency
   - Do not draw and store in a syringe longer than 12 hours
   - Discard partially filled vials after 2 weeks

2. No meat or milk restrictions after PPD injection

3. Do not test obviously sick cattle!

4. Do not treat while testing
   - Drugs, vaccines or hormones can alter the animal's immune response.
   - Treat CFT negative animals after reading.
   - Do not treat CFT responders. Treatment can interfere with secondary testing. Wait until the test is read before beginning treatment.

5. Contact the VS Area Office or State Animal Health Official's Office for information on obtaining the tuberculin (PPD – Purified Protein Derivative) and the tuberculosis testing forms, VS Form 6-22 or State equivalent.

SECTION 4: PERFORMING THE CAUDAL FOLD TEST

First, what is the caudal fold? It's the little fold of skin that hangs down on either side of the ruminant's tail from the attachment above the anus to a point about 3 - 4 inches down the tail.

There are three reasons we use this area for the test. Select each box to learn more.

1. It's easy to see and reach.
2. It's some of the thinnest skin on a cow.
3. It doesn't have any hair to disguise a response.

Fill a 1 ml syringe with a 26 gauge 3/8" needle with 0.1ml of tuberculin.

Select the arrow to watch a video by Dr. Richanne Lomkin, who is a USDA Veterinary Services Veterinary Medical Officer. She will illustrate the process of how to inject the PPD tuberculin.

If you are approaching the cow to do the caudal fold test there are three different ways that you can get to the caudal fold. My favorite way is to take my non-dominant hand, push that caudal fold out. So, run my hand underneath the tail, use my fingers to push that caudal fold out, then I've got some control of the tail. Then I bring my dominant hand in here and then it is a .1 ml injection. You want to hold that needle as parallel to the skin as I can go intradermal when I do the injection and then pull it out. You should see a bleb form right there on the skin where you injected. That is my favorite way. The next way is to take your non-dominant hand, roll the skin out on your dominant side, and then inject this way. That's actually a lot of people's favorite way to do it. I don't like this way as much, but it will work. Finally, you can roll it out on your non-dominant side, come over with your dominant hand, and side rest your hand on the tail head itself, and that way your hand is stabilized so if the cow is dancing a little bit it gives you a little more stability. So those are the three ways. You can put your hand underneath and poke it out that way and inject, you can also come in this way and inject if she's dancing a lot, you can roll it out and inject like this or you can roll it out and inject like that and I'm basically ambidextrous which is why I am able to do it without switching back and forth but you will need to figure out which hand will hold the syringe and which hand you will use to manipulate the caudal fold.

Please watch the video in Module 35 online.

Inject the Bovine PPD **intradermally** on one side of the caudal fold.

A good injection produces a bleb. Re-inject if you don't make a good **intradermal** injection.

Mark the hip of the cow with marking paint on the side that you injected.

Record all of the cow's identification including the official ID number, farm ID number, herd tags, tattoos, brands etc. Also record the cows age, breed, and sex on the VS 6-22, TB Test Chart form.

SECTION 5: READING THE CAUDAL FOLD TEST

72 hours later, three days, you are going to come back and actually read these tests. To say reading is a misnomer because I actually think my eyes mess me up more than anything, so I usually just feel and if I feel something weird, I come back and take a look at it. I'll come along and I raise up the tail, not very far, and then you want to run your fingers over the side that you injected. So, on the side of the caudal fold that you injected, and then you run your fingers over the caudal fold that you did not inject, and then you come back and palpate the side that you did inject again. If you don't feel any changes then you move on to the next cow. If you feel a change then you need to stop and think about it a little harder. So, this cow actually has two really good examples of a response. This response down here is about the size of a large bean and that response is more your normal size of what you are going to see in most cows; it may even be smaller than that. My average is about pea sized that I feel but this is common as well. It is a firm nodule. Now this one up here is a pretty impressive sized response. I will see a response like this occasionally but that is not the most common response. Maybe like one in fifty cows that respond is going to have one that big. Let me go ahead and jack this tail a little bit so you can appreciate how big that response is. Also, you can see there is a little bit of necrosis at the injection site. That is pretty common too. So that delayed type hypersensitivity, all of those cells are flooding into that area and that is what is causing that response.

SECTION 6: STEPS ONCE THE CAUDAL FOLD TEST IS COMPLETED

If there is no detected immune response the animal is classified as negative and may be returned to the herd.

Any visible or palpable response at the site of the injection is considered a response and the animal will be subjected to further testing. A caudal fold response does not mean the animal has bovine TB. The response can also be from exposure to other forms of *Mycobacterium*, that are closely related to *Mycobacterium bovis*. In fact, between 1 and 5 percent of all animals tested will exhibit a response.

All responses must be reported immediately to your VS Area Office or your State Animal Health Official.

The VS Form 6-22 must be submitted to your regulatory veterinary official within 7 days of reading the test but different States have different policies so confirm the timeline with your VS Area Office.

If this test was for movement purposes, the animals may not move until all testing is completed. This includes the secondary test. Make sure the owner understands they need to schedule the testing at least 2 weeks prior to the animals' movement date. That will give you enough time for both the caudal fold testing and the secondary testing if responders are found.
Because performing this test correctly is important, Veterinary Services will work with you to ensure you are performing this test correctly. Also, because this test is part of your obligations as an accredited veterinarian if you perform it incorrectly VS may take actions against your accreditation. VS monitors your CFT responder rate to ensure it falls within the expected response rate range. There are options to complete this and many other official forms electronically. Please contact your VS Area Office for details.

Don't commit these common errors when completing the caudal fold test for tuberculosis.
•Not recording or incorrectly recording official ID on the VS Form 6-22
•Using tuberculin that is expired
•The date of the caudal fold test is not listed on the VS Form 6-22
•The owner of the animals is not listed on the VS Form 6-22
•No address listed on the VS Form 6-22 (what piece of dirt the test was done on)
•No test results listed on the VS Form 6-22
•The accredited veterinarian did not sign the VS Form 6-22
- Allowing another accredited veterinarian to read the test that you injected
- The form is not legible
- Failure to ensure that each animal tested has official ID and listing that official ID on the form
- Failure to report the caudal fold test results to Regulatory Officials in a timely manner.

SECTION 7: CONCLUSION

Thanks to you, the accredited veterinarian, cattle producers, researchers, and regulatory officials, the prevalence of TB in the United States decreased from 5% to <0.001% over the past 100 years. Working together we have saved millions of dollars and countless lives.

Now that you have completed this module you will be able to explain:
background information on bovine tuberculosis;
the process for diagnosing bovine tuberculosis;
the process to perform the caudal fold test in cattle;
interpreting the results of the caudal fold test in cattle; and
the steps you must perform once the caudal fold test is completed.

SECTION 10: ACKNOWLEDGEMENTS

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If you suspect a FAD IMMEDIATELY

contact your

VS Area Veterinarian in Charge (AVIC)

at 1-866-536-7593

and

your State Animal Health Official (SAHO)