How do I collect a *Bac-T* sample?

*Bac-T* is our shorthand term for the total coliform bacteria presence/absence test.

Accurate testing of any sample in the laboratory begins with proper sample collection. In the case of *Bac-T* testing this means thorough disinfection of the sample collection point prior to sampling. Inadequate disinfection often leads to a positive test result when in fact the water itself is fine.

- **Sample from an inside location.**
  Coliform bacteria are very common and live on many surfaces. Sampling outside (such as a hose faucet) exposes the collection process to a random (usually high and uncontrolled) level of bacteria. Sampling inside, by its very nature, tends to have lower levels of bacteria.

  That said, some inside locations tend to have higher bacteria possibilities than other locations. Bathrooms (due to the activity in that location) may have higher levels of bacteria. The same for kitchens and mudroom sinks.

  Also, select a cold water, non-swivel faucet. Kitchen faucets often have swivel outlets. Bathrooms tend to have fixed outlets. Everything considered, it’s best to choose a fixed outlet, cold water faucet.

- **Prepare a bleach solution.**
  Bleach (sodium hypochlorite solution) is a great disinfectant, and is effective against many bacteria and some viruses. Regular bleach from the store is about 5.25% concentration. More recently, bleach can be purchased in the 8-10% concentration range. For you awareness, the higher the concentration the less stable the solution and the faster it tends to degrade while it’s setting on the shelf or in a cabinet. More isn’t always better.

  Make a 1:10 solution by mixing ¼ cup bleach with 2¼ cups water. This doesn’t have to be perfectly accurate. The solution can be applied using a spray bottle, cloth swab or brush.

- **Prepare the location.**
  Wash your hands before beginning. This will reduce inadvertent contamination of the sample.

  Remove the aerator (if there is one). Like the rotating joint in a swivel faucet, aerators can harbor bacteria.

  Apply a liberal amount of bleach solution to both the outside and inside of the faucet. It’s also a good idea to disinfect handles on the water control valves as you have to touch these during the sampling process. And it doesn’t hurt to disinfect the sink area around the faucet.
Allow the bleach to disinfect for about 3-5 minutes. Adequate contact time is necessary to achieve proper bacterial disinfection.

- Inspect the bottle

The testing regulation specifies that a **collected sample must be a minimum 100 mL volume and must have minimum 1 inch headspace.** In older style bottles there is a line embossed on the bottle showing the 100 mL line, but no indication of where to stop for the 1 inch headspace. You will have to guess. In other words, fill the bottle to just barely over the 100 mL line. Newer bottles have 2 embossed lines: one line for the 100 mL minimum volume, and a second line at 120 mL to indicate the 1 inch headspace. Fill newer bottles between the 2 lines. The volume must be collected in the initial grab. You are not allowed to pour water out if you overfill because you will also pour out the preservative. If you accidently overfill, discard the bottle and try again.

Remove the seal from the sample bottle. **Caution** … do not use a bottle without a seal, or has been previously opened, or does not contain preservative chemical. That chemical is sodium thiosulfate and may be present in either powder or tablet form, or if it has been exposed to elevated temperature may have melted and appear as a drop of liquid. Also, bottles have expiration dates. If you have been storing bottles for more than one year, it would be best to contact the laboratory to confirm the bottle is still good to use.

- Collect the sample.

The objective of sampling is to test ability of the distribution system to deliver sanitary water from the source.

Turn on the faucet and flow water fast for about 5 minutes. This will flush residual bleach from the faucet and draw water from the source to the faucet.

Slow the flow to about a pencil size stream.

Remove the cap from the bottle and hold the cap right side up with one hand (inside of the cap facing downward). Don’t lay the cap down or invert it as this may lead to contamination of the sample. Also take care not to touch the inside of the cap or of the bottle.

Insert the bottle into the flowing water and collect the proper volume.

After collecting the sample, immediately re-cap the bottle and assure a firm seal. Then turn off the water. Label the sample and complete the chain-of-custody form taking special note of the date, time, sampling location, and who collected the sample.

- Handling and delivery

Testing for *Bac-T* must begin within 30 hours of collection. Try to get the sample to the lab as soon as reasonable. There are no additional specific handling requirements, but to help assure accurate test results here are a few guidelines.

- Don’t put the sample bottle in a hot place, such as on the dashboard of your car.
- Don’t leave the bottle out in direct sunlight.
- Don’t freeze the bottle.
- If you have to wait overnight before bringing the sample to the laboratory, store the bottle in a refrigerator. Don’t allow to freeze!

Remember to bring the chain-of-custody form with the sample.

If you are shipping or mailing the sample, that is going to have to be an overnight process in order for the laboratory to receive the sample and start the test within the 30 hour limit.
Finally, the sample must arrive at the laboratory at a temperature less than 95°F (35°C). Samples will be rejected for testing if they arrive at a temperature greater than 35°C. This may occur for example in the summer if the sample is sent via an express shipping service or the mail. High temperature may kill bacteria and render the sample inappropriate for testing. Consider hand delivering samples or shipping on ice.

**NOTES**
- For regulated samples complying with the groundwater rule (GWR), *Bac-T* samples must be stored, transported, and received at the laboratory on solid ice.
- All samples specifically for fecal coliform or *E. coli* testing must be received at the laboratory on solid ice.

**My BacT sample tested positive (total coliform presence/absence test is TC+). I'm pretty sure the water is good. What happened? What do I do?**

First of all, if the sample is from a regulated facility (i.e. the facility has a PWS (public water system) number), then the facility is now under the official TCR REPEAT process. Also see the FAQ item above for guidance in disinfection prior to sampling.

Most TC+ *BacT* test results are due to sampler error. See a separate FAQ item for discussion about sample collection and handling.

The following discussion is our opinion based on testing 1000s of *BacT* samples, from our own experience, and from talking to clients, regulators and well installation companies. Other sources will likely have other opinions.

1) **Evaluate the situation**
- Understand that most positive *BacT* results are due to error in the sample collection process. If you think the TC+ result may have been due to a collection error, then adjusting the collection technique and resampling is probably the best way forward.
- On the other hand, if you are confident that the sample was collected properly then there may be a bacterial contamination in your water system. Here are some issues to ponder.
  - Has the system been opened at any time? Have you worked on the piping? Installed a new valve or pressure tank? Pulled and reinstalled the pump? Any opening or handling of components gives bacteria access to the system. The last action when putting a new or repaired system into operation is to disinfect.
  - Do you have an older water system? Older wells may lack features used on more recent wells. These more modern construction features are intended to protect both the physical structure of the well and to minimize contamination of the water.
    - In the old days wells were often installed with casing that ended below grade (below natural ground level). The top of the casing was often in a sump. In situations like this, rain water can flow down into the sump and then into the well, carrying all manner of material from the ground into your drinking water source.
    - Well casings were often uncapped. It was not uncommon to put a board or bucket over the casing. However insects and mice can still get into the well.
    - Casings often lack protective skirts. A sloped, concrete pad around the casing helps to stabilize the pipe and shed water away. Also the casing is not a perfect seal against the ground that it penetrates. Over time the ground can separate away from the casing creating gaps down into the ground on the outside of the casing. This allows surface water (and all that it carries) to penetrate directly down to the aquifer.
  - What about maintenance?
    - Has the casing cracked or shifted creating an opening?
    - Is the cap secure and sealed to the casing?
    - Are the necessary well components sealed to the cap? Passing in and out of the casing are the pump electric cable, the riser pipe bringing water from the pump, and
a breather (pressure equalizer) necessary to allow air in as water is pumped out. Those components need to be sealed to the cap. Also the breather needs a screen to prevent insects, mice and birds from getting in.

- Does the system have an overhead water tank for volume reserve and to create pressure in the distribution system? All of the issues of sealing and pressure equalization apply as with the well cap. For your awareness, each foot of elevation of the water above ground creates about 0.43 pounds per square inch of pressure (psi). Systems usually operate in 30-70 psi range. 40-45 psi is typical. Creating 40 psi needs a tank over 90 feet high.
- Older wells, especially cased with iron pipe or drilled into certain soil types, can eventually grow bacteria that get nutrients from the soil. Iron-reducing or sulfur-reducing bacteria can grow, leading to odor and taste issues, and can even block off the pump intake screen.

2) Take action

- Did you address the issues above? There’s little advantage to disinfecting the system if problems are uncorrected. You may eliminate the current bacterial population, but it will return if the root cause is not fixed.
- This is a reminder that anytime the system is opened or repaired, it’s best to disinfect as the final action prior to putting the system into operation.
- Over years of time we have observed that people tend to want to disinfect in a piecemeal fashion to get it done quickly. Disinfecting a single faucet or valve or section of pipe may be relatively more convenient, but it often doesn’t go well. The bacteria tend to hang on and the TC+ results continue or resurface soon.
- We recommend taking the “nuclear option”. That is “shock disinfect” the entire system from the well to the faucets. It takes longer to do the job, but you’re likely to achieve the end result in one pass. Here’s a way to do that.
  - Calculate how much water is in the entire system. That includes the well, pressure or storage tank, hot water heater, and all the piping. There is a calculation spreadsheet elsewhere on our website to help you calculate water in your system and bleach needed for disinfection.
    - For example:
      - Water in well (500 gallons) + Pressure tank (50 gallons) + Hot water heater (40 gallons) + Piping (75 gallons) = 665 gallons
    - Calculate the amount of bleach needed to achieve about 200 mg/L (ppm) in all that water. For example (assuming 5.25% available chlorine in regular bleach) you need about 3 pints of bleach per every 100 gallons of water:
      - 665 gallons/100 X 3 = about 20 pints = about 2½ gallons of bleach
    - Using a clean, plastic bucket, mix the bleach into some water and pour the mixture into the well.
    - Find the closest valve to the well head, attach a hose and direct the hose back into the top of the well. This requires removing the breather or in some other way opening the cap. Even better is to attach a spray head onto the hose.
    - Turn on the pump. This will pull heavily chlorinated water up through the riser pipe, through the hose, and back down into the well, wetting/spraying chlorinated water onto the inside surfaces of the well. Run this for at least 20 minutes.
    - Close the hose valve. Reseal the well cap.
    - One by one, go to every faucet outlet of the distribution system, open the faucet and flow water until you smell chlorine, then close the faucet. Do every faucet.
    - Let the system set at least overnight. A couple of days is better.
    - Go to several faucets in the system, open these and let run until the chlorinated water is purged from the system. Generally we say 3 volumes is necessary to achieve purging. In the above example that would be 665 gallons X 3 = about 2000 gallons total from the well.
  - Resample for BacT.
  - Do not open the system for any reason unless you are prepared to disinfect again.
**Do Bac-T samples have to arrive at the lab on ice?**

The short answer is “it depends”. Routine monitoring samples and repeat samples (due to a previous TCR+ result) from the distribution system can arrive at the lab not on ice. Triggered source water samples (from the well) as part of the repeat process must arrive on ice.

Keep in mind that any sample for microbiology testing is better held and transported in a chilled condition. This will slow down any biological process and better maintain the microbes prior to testing. However, don’t freeze the sample as that might kill microbes that might otherwise be measured in the testing process.

In that same line of reasoning, don’t allow Bac-T samples to become hot, as for example in a vehicle in the summer. The heat and UV light will both work to kill microbes.

Fecal coliform test samples (like from wastewater treatment plants and animal waste lagoons) must arrive on ice. These are not Bac-T samples, so generally don’t worry about this.

**What’s the big deal about hours and days of sample receipt at the lab?**

Some sample types (ex: Bac-T and nitrate) have specific maximum holding times from collection to beginning of testing, AND us lab workers like to go home now and then. When these types of samples are received at the lab, we have to begin the testing within whatever time is left since collection. For those samples, that arrive on a Friday or day before a holiday, this means that someone has to come to the lab the next day (Saturday or the holiday) to complete the testing. That’s not fun, and we charge extra for it.

Also for cost and labor efficiency, when running a lab test we try to group together all the samples of a given type into one batch. This means that that testing type is started at a certain time of the day. Samples that arrive after that time often have to be held until the next testing cycle.

**When can I find out a Bac-T result?**

The Bac-T test takes 24 hours after starting the incubation (the culture growth). On any given day, except for line tests and other rush samples, we start the testing of that day’s batch of samples in the mid-to-late afternoon. This means a test result will be available mid-to-late afternoon the next day. Our practice is to notify customers only when a test result is positive, that is coliform bacteria are present in the sample. We figure a customer would want to know their water is contaminated with bacteria. On the other hand, we don’t generally call customers when a sample tests negative for bacteria. In other words, no news is good news. If you specifically want a verbal report of the results, let us know when you deliver the sample for testing. Otherwise, if don’t hear from us, the test report and invoice will be sent (Dropbox, email, or snail mail) soon after the testing is complete.

**Why the push to use Dropbox?**

The short answer is “cost control”.

Regular mail through the post office (i.e. “snail mail”) has obvious cost issues: printing, envelopes, stamps, handling labor, and carrying to the P.O.

Using email is better because those costs are eliminated. However email has its own issues: email addresses, electronic delivery problems, and misplacement after receipt.

We see Dropbox as an improvement over email in the following ways:

- All reports, invoices and other documents always go to the same internet web location. There are no issues of email addressing, or non-receipt due to spam/filter problems.
- All documents are maintained at the website. They can be viewed, copied and printed from the Dropbox, but never deleted. If you misplace a document in your files, just go back to the Dropbox and make another copy.
As with any business, controlling costs is critical to success. When we control our operating costs, then testing fees can be kept low and stable.

**Why is the lead and copper sampling schedule so complicated?**

- **Responsibilities**
  - EPA defines the monitoring schedule.
  - Oklahoma DEQ manages the lead and copper sample collection activity and manually sets the Effective Begin Date entries in SDWIS.
  - Questions regarding the need to sample for lead and copper or the schedule for those samples should be directed to the DEQ coordinator for Lead and Copper in Public Water Supplies.
  - ODEQ sends out notifications of sampling needs as they occur. Or the PWS can call ODEQ to learn of their schedule.
  - Those notifications direct the PWS to contact an Oklahoma-accredited laboratory for sample collection bottles and testing service.
  - The OPSU water quality laboratory maintains that accreditation and is ready to supply sample bottles and the testing as requested by the PWS.

- When looking at the lead and copper schedule in SDWIS, the begin date of the sampling schedule is shown as the Effective Begin Date. This means that for a PWS that samples every 3 years and never has a violation, the Effective Begin Date will always display the start date of the original 3-year sampling periods. For example:

<table>
<thead>
<tr>
<th>Facility State Asgn ID</th>
<th>Water System Facility Name</th>
<th>Analyte Group Code</th>
<th>Analyte Group Name</th>
<th>Sample Count</th>
<th>Sample Type</th>
<th>Sample Frequency</th>
<th>Effective Begin Date</th>
<th>Effective End Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS001</td>
<td>DISTRIBUTION SYSTEM</td>
<td>PBCU</td>
<td>LEAD AND COPPER</td>
<td>5</td>
<td>RT</td>
<td>3Y</td>
<td>01-01-2002</td>
<td></td>
</tr>
</tbody>
</table>

- If a PWS has a monitoring violation, and EPA puts it on a 1-year cycle (for example), the SDWIS display of the Effective Begin Date is changed to show the start date of the new monitoring period. The end date of the monitoring period (the violation period) will be one year after its start date.
  - When the PWS comes back into compliance, EPA inserts it back into the original cycle, not at the beginning of a new cycle. However, the SDWIS display does not return to the original start date. Instead the date the PWS got back on the 3-year cycle will be displayed as the Effective Begin Date. This means the displayed start date for the monitoring period and the actual time position within the monitoring period are disconnected. From that point going forward, there is no convenient way to look at the Sample Frequency and Effective Begin Date and know where the PWS is within the actual sample monitoring cycle.
  - Here is an example. A PWS has been on a 3-year cycle since 2002. Without a violation, the 3-year periods would be as shown above. Then the PWS has a 1-year violation in 2009. The SDWIS display will be changed to show the beginning of the 1-year violation. The Effective Begin Date will be shown as 01/01/2009. After the violation period, EPA moves the PWS back into the current 3-year cycle (which had an original Effective Begin Date of 01/01/2002). However, ODEQ sets the begin date as 01/01/2010, which is the date that the PWS got back onto a 3-year cycle. This gives the impression that the 3-year cycle is running 2010-2012, which is not true. As far as EPA is concerned, the cycle is still 2008-2010 and then 2011-2013.
For a PWS that had a violation and was thrown into this frequency, cycle and begin date confusion, it is
difficult, very time consuming, and in some cases impossible for a laboratory to accurately monitor PWS
lead and copper sampling schedules. Our laboratory does not do this, and we will not notify a PWS about
their lead and copper sampling needs. Those notifications will come from ODEQ. The PWS may then
contact our laboratory for the testing service.

What are the analytical issues for sampling and testing waste control operations at animal management
facilities?

Animal waste management generally consists of operating and testing the following areas.

- Waste storage facility (i.e. lagoon)
- Groundwater monitoring wells around the area of the storage facility
- Discharge from the waste facility (for example when the water is used for irrigation)
- Actions should a spill occur

The requirements to test these areas are defined in Oklahoma Administrative Code, in Oklahoma Statues,
and/or by USEPA NPDES permits. Overall regulatory control is by the Oklahoma Department of Agriculture
(ODA).

There are generally 2 primary issues for an animal management facilities operator to consider when selecting a
testing laboratory and when planning the sampling activity.

- ODA requires that the laboratory conducting the required testing be approved by the Oklahoma
  Department of Environmental Quality (ODEQ). That is the lab must be officially accredited by DEQ in the
  field of non-potable water testing for the required analytes.
- Certain of the required analytes have very specific sample collection and testing limitations as described in
  the following.
  - **E. coli and general fecal coliform bacteria**
    - Must arrive at the laboratory on solid ice within 6 hours of collection, and the lab must start
      the testing within 8 hours of collection
  - **Nitrate, nitrite, o-phosphate and/or BOD**
    - Must arrive at the laboratory on solid ice, and the lab must start testing within 48 hours of
      collection
  - **pH**
    - Must arrive at the laboratory **NOT on ice**, and testing should be conducted immediately
      [On a practical note, our laboratory policy is to test pH within 2 hours of sample log-in.]

To achieve regulatory compliance and data reliability, both the waste facility operator and the laboratory are
under very tight time and sample handling limits.

Because of the time, effort and cost involved in complying with this required activity, the operator should
consider the following.

- Select a laboratory
  - Accredited by DEQ for wastewater testing
  - Near the area of the samples, to minimize the transportation and logistical issues
- Coordinate with the laboratory
  - Schedule sampling and delivery to the lab for days and times that keep the samples within testing
    requirements
  - Obtain the proper collection bottles, and coolers if needed
- Sampling
  - Use the proper containers and collect the proper volumes
  - Assure samples are labeled and chain-of-custody forms are completed