

TESTING FOR BACTERIA

This article focuses on basic information regarding the types of testing available for bacteria and interpretation of results. It specifically details methods currently available at National Testing Laboratories (NTL). Hopefully this article will enable the water professionals to determine the type of test that would be best suited when bacterial problems are suspected. Standard quality control plates although not discussed are used with all methods.

NTL does offer several methods for the testing of coliform, E.coli and heterotrophic bacteria. The method currently used and the most familiar to our well drillers is the Chromogenic Substrate Test. This method is also known affectionately as the "Presence/Absence Method" because of the type of result that is generated. This method is very accurate in determining the presence or absence of both coliform and E.coli bacteria. Special enzymatic substrates are added to the samples that are received and the bottles are incubated for twenty-four (24) hours. After twenty-four (24) hours, the samples are examined for any color change in the sampling bottle. Coliform bacteria in this technique are defined as the bacteria possessing the enzyme β -D-galactosidase. This enzyme cleaves the chromogenic substrate used and results in release of the chromogen, causing a distinct color change in the sample. E.coli bacteria are identified because they have an enzyme that results in the release of a fluorogen in the presence of the fluorogenic substrate. The fluorogen can be viewed when observed under long-wavelength ultraviolet light. One advantage to running this type of test is that there is no interference from other types of non-coliform bacteria. There is often interference observed in some other analytical methods that yield a *count* of bacteria. One of these "count methods" is known as the Standard Total Coliform Membrane Filter Procedure. This process is slightly more complex and can take additional time for processing if confirmations of presence results are necessary. A sample is initially filtered through a semi-permeable membrane, which has a pore size small enough, that coliforms, if present, will not filter through. Once the sample is filtered, the membrane is placed in a special nutrient dish, inverted and incubated for twenty-two (22) to twenty-four (24) hours. The definition of coliforms that pertains to this test is those bacteria that produce a metallic (golden) sheen within the incubation period. To determine specific colony counts, the analyst uses a specialized microscope. The typical coliform colony has a pink to dark-red color with a metallic surface sheen. Colonies that lack sheen may be pink, red, white or colorless and are considered non-coliform colonies. If there is a colony in question it goes through the "confirmation" process. To describe the confirmation process in simple terms, colonies are taken from the sample and transferred to a different broth or medium that after an additional incubation period causes a color change in the presence of coliforms. The main problem with this method is that if there is a large presence of non-coliform bacterial colonies only a "too numerous to count" result can be reported. Sometimes it may not be known if there are coliforms on the plate that were "masked out" or unable to be seen. We only recommend this test if an actual count of bacteria must be obtained. For example, to meet regulatory requirements this is the test that is often necessary to perform.

One of the final methods that we will touch on slightly is the Standard Plate Count test. Essentially there are three different methods and four different media that are approved for use. The end result of all these methods is that an estimate of the number of live heterotrophic bacteria in the water can be determined. An approved counting aid specified by the method should be used to determine the results and counts should be done promptly after incubation. We have found this test to be extremely useful in situations when an odor is detected at a site but coliforms are absent. This test is very simple to order as an "add-on" to a WATERCHECK or WATERCHECK WITH PESTICIDES OPTION test package if desired. Please contact Tami or Marianne for details.

In summary, a bacteria test is not as simple as it may seem. Different tests are used to achieve different results and are based on the need of the analysis. The presence/absence test is the best in determining in general if there may be a coliform or E.coli problem that could indicate a health concern. If a count of bacteria is necessary for meeting regulatory requirements or treatment clarification, then a membrane filtration or standard plate count test may be the ticket. NTL can also perform testing for Iron or Sulfur bacteria when requested. Give us a call to discuss your needs or problems.