Water for the World

Analyzing a Water Sample

Technical Note No. RWS. 3.P.3

A water supply for domestic use should be free of disease-causing organisms and substances which make the water unacceptable to its users. There are several ways to find out if a water supply is safe to drink (see "Determining the Need for Water Treatment," RWS.3.P.1). One method is analysis, the measurement of the type and level of contaminants present in a sample of the water supply. The results of water analysis can verify the findings of a sanitary survey and suggest the level of treatment a water supply needs.

There are five kinds of water quality analysis. All of them measure different characteristics of a water sample. The two most important types of analysis for small community water supplies are the bacteriological tests and physical and chemical tests. Bacteriological analysis identifies organisms associated with disease. Physical and chemical analysis identifies elements in a sample that make water turbid, offensive or poisonous to users. Of these two, bacteriological analysis is more important because bacteriological contamination is more likely to occur in small community water supplies than physical or chemical contamination is.

A physical and chemical analysis includes tests for turbidity, color, taste and odor, followed by tests for excess minerals, toxins and elements which may harm the system. Physical and chemical analysis is extensive, and is best carried out in a well-equipped laboratory because of the complexity of the testing. Field kits are available for partial physical and chemical analysis. They require especially prepared materials and equipment. These kits are costly and therefore usually are impractical for analyzing water in isolated rural areas. Physical and chemical analysis requires skill, training and experience. It is best to

Useful Definitions

CONTAMINANT - An impurity which makes water unfit for human consumption or domestic use.

FECAL COLIFORM - Bacteria found in the large intestine; a coliform count is often used as an indicator of fecal contamination in water supplies.

GROWTH MEDIUM - A liquid or gelatin which promotes the development of bacteria into colonies.

INCUBATE - To cause bacteria to develop colonies by keeping them warm.

NUTRIENT BROTH - A liquid which induces the development of bacteria colonies; a growth medium.

PATHOGENS - Disease-causing bacteria.

PETRI DISH - A shallow round transparent glass or plastic dish with an overlapping cover used for developing bacteria colonies in a growth medium.

PIPET - A slender tube into which small amounts of liquid are drawn for measuring or transferring.

POLLUTION - Contamination.

SANITARY SURVEY - An extensive field evaluation of actual and potential conditions affecting the acceptability of all available water sources.

TURBID WATER - Water that is clouded with suspended particles.

VACUUM - A pump used to pull or push water through a filter in the membrane filter technique of bacteriological analysis of water.

WATER TREATMENT - A process in which impurities such as dirt and harmful materials are removed from water.

consult an expert for details and assistance in conducting this kind of analysis. This technical note describes bacteriological analyses of drinking water.

The most serious water pollution is bacteriological contamination which can cause disease. Bacteriological analysis of a water sample finds and counts organisms whose presence indicates that disease-causing pollution has occurred in the water supply. These "indicator" organisms are members of a large group of bacteria called coliform bacteria. Most coliform bacteria do not cause disease themselves but, because they enter water supplies from the excreta of humans and animals, they indicate the possible presence of pathogens, the disease-causing organisms in excreta. Coliform bacteria are easier to identify then pathogens, so coliform levels are used to determine the bacteriological quality of water.

Testing for Bacteria

The simplest and cheapest bacteriological water analysis procedure is the Standard Plate Count (SPC). It is a method of measuring the overall bacterial content in a water sample. In the SPC, small quantities of a properly collected water sample are mixed with a growth medium in a petri dish and incubated. A portion of all the bacteria types present in the water will develop into colonies. Although the SPC does not distinguish coliform from other forms of bacteria, it is a valuable procedure for evaluating the general bacteriological quality of a water supply. It is a good method for measuring basic bacteriological content when no other means of analysis is available.

The equipment and supplies needed for the standard plate count are common to most laboratories. One milliliter of a diluted water sample is put into a sterile petri dish using a sterile pipet. Approximately 10-15ml of sterile growth media, such as glucose agar, are poured over the sample in the dish. The sample and the agar are thoroughly mixed and incubated at 35°C plus or minus (±) 0.5°C for 24 ± 2 hours. The bacteria colonies are counted after incubation using an illuminated colony counter or a reading glass. If less than 500 colonies of bacteria grow, the water is considered relatively good. Most established laboratories will be familiar with the standard plate count.

Examining a water sample specifically for the presence of coliform bacteria is called "coliform detection." A "total coliform detection" will identify many sorts of coliform bacteria in a water sample. The presence of the particular fecal coliform bacteria known as E. coli is strong evidence of the presence of pathogenic organisms. Water that is free of coliform bacteria or has a very low coliform count in a total coliform detection analysis is considered free of disease-producing bacteria. Total coliform detection is the most frequently used bacteriological analysis.

Almost all rural water supplies show coliform bacteria when analyzed. Not all water supplies can be condemned, so it is the level of coliform content in water tested, not simply the presence or absence of coliform bacteria that determines the safety of a drinking supply. The World Health Organization (WHO) International Standards for the bacteriological quality of drinking water vary depending on whether the water supply is unimproved or is disinfected or piped. Much higher standards are expected for improved supplies. Ideally, no E. coli are acceptable in any drinking water sample, and only low levels of other coliform bacteria are tolerated in unimproved supplies. The basic WHO standards are explained in the section on "Interpreting Results of Coliform Bacteria Detection."

Water analysis can be conducted in a laboratory or in the field using special kits. Laboratories provide a controlled environment for analysis and often have extensive facilities for testing. Field kits decrease the problems of storing and transporting samples and are convenient, but field kit equipment is not always as complete as a laboratory. Lack of laboratory facilities is one of the greatest difficulties of conducting bacteriological examinations of water in remote areas. Analysis should be begun as soon as possible after samples are collected to minimize changes in the bacteriological character of the water. Analysis can be done properly in a laboratory when testing can begin within six hours. Field kits start the analysis procedure on the collection site, so they are highly recommended for circumstances where the delay between collection and laboratory analysis will be over six hours. When neither laboratory or field analysis can begin within six hours, the samples must be properly stored and transported to the nearest testing facility (see "Taking a Water Sample," RWS.3.P.2). Some field kits are designed to hold samples under proper conditions until they arrive at a laboratory for analysis. Others contain complete portable analysis facilities.

Testing for Coliform Bacteria

There are two basic methods of detecting coliform bacteria. First, there is the "multiple tube method," in which small measured volumes of sample water are added to a nutrient broth in one or more sets of five test tubes. The sample tubes are incubated and the nutrient broth supports the multiplication of the bacteria. After 48 hours in incubation, the most probable number (MPN) of bacteria in the water sample is estimated based on the number of tubes which produce gas, the sign of baterial growth. Field kits are available for the multiple tube method but this test is most effectively performed in a well-equipped laboratory.

In the second method, the "membrane filter technique," a measured volume of sample water is drawn or pushed through a flat filter which retains any bacteria present in the water. The filter is then placed on a growth medium and incubated. The bacteria multiply, forming visible colonies. The colonies are counted directly by eye or with the aid of a binocular widefield microscope.

The accuracy of both these methods of coliform detection is highly dependent on a water sample that is properly collected from the water source being evaluated. Refer to "Taking a Water Sample," RWS.3.P.2.

Multiple Tube Method of Coliform Bacteria Detection. In the multiple tube method, the number of coliform bacteria in a water sample is calculated based on a statistical estimate of bacteria growing in a set of five test tubes containing a mixture of nutrient broth and water sample. Any coliform bacteria present will ferment lactose in the form of gas when this mixture is incubated. Formation of a gas bubble in the test tube indicates the presence of coliform bacteria. An inverted vial is placed in the test tube to trap any gas bubbles that form. See Figure 1.

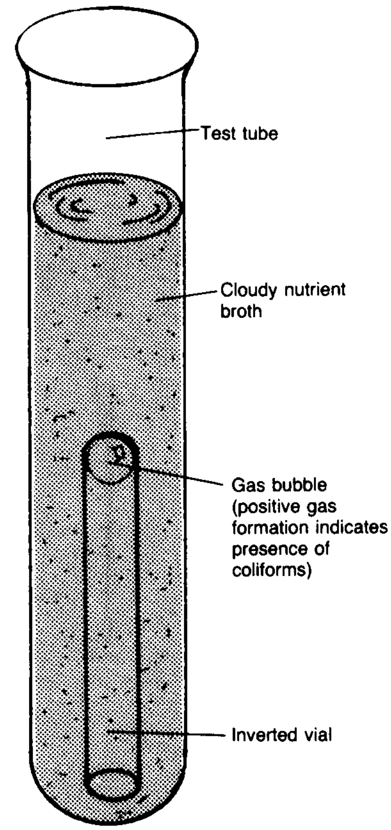

Figure 1. Multiple Tube Method of Coliform Detection

There are three different types of multiple tube tests. They are the Presumptive Test, the Confirmed Test, and the Complete Test. The Presumptive Test is for general coliform bacteria detection. The Confirmed Test is more thorough than the Presumptive Test, and the Complete Test is even more accurate. However, the Confirmed and Complete Tests require more supplies, skills and experience to perform. The Presumptive Test is sufficient for testing most rural water supplies.

In the Presumptive Test, the five test tubes filled with a mixture of nutrient broth and water sample are incubated at 35°C ± 0.5C for 24 ± 2 hours. If gas bubbles have formed in the nutrient broth surrounding the vial and if there is a small gas bubble in the inverted vial after this period of time, the test is considered positive, meaning coliform bacteria are probably present in the water supply. If no gas has formed, the test tubes are incubated for another 24 hours. If gas bubbles form during the second incubation period, the test is also considered positive. Absence of gas at the end of the second incubation period constitutes a negative test, meaning coliform bacteria are definitely not present. Refer to Figure 2.

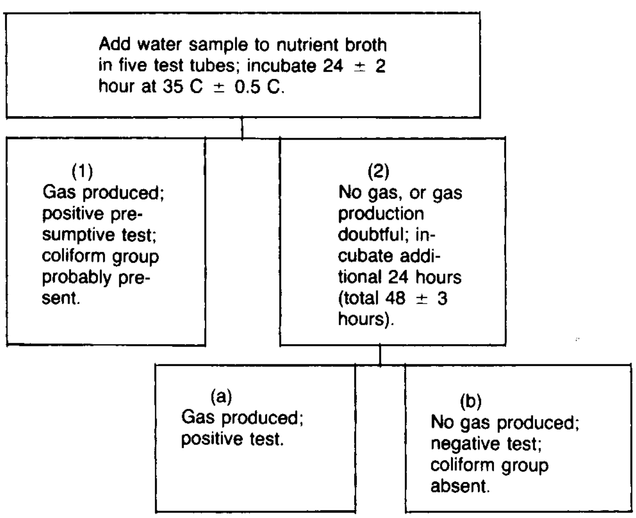

Figure 2. Multiple Tube Method of Coliform Detection, Presumptive Test

A confirmed or Complete Test can be performed on the same water samples following a postive Presumptive Test. However, the most probable number (MPN) of coliform bacteria per 100ml of water can be estimated after a positive Presumptive Test using a standard MPN table. See Table 1. It is important to remember that MPN values are not absolute numbers of coliform bacteria present. They are good estimates for judging water quality.

Table 1. Most Probable Number Index for Coliform Bacteria (When Five 10ml Portions Are Used)

Number of Tubes Giving Positive Reaction Out of Five 10ml Water Samples	Most Probable Number (MPN) of Coliform Bacteria per 100ml of Water
0	2.2
1	2.2
2	5.1
3	9.2
4	16.0
5	16.0

Membrane Filter Technique of Coliform Bacteria Detection. In the membrane filter method, a water sample is drawn from a funnel by a vacuum through a flat filter. The wet filter is removed from the filter holder and placed in a petri dish over a pad saturated with a growth medium. Refer to Figure 3. The petri dish is placed in an incubator. Any coliform bacteria present will grow in distinctly colored colonies on the filter. After the proper incubation period, the filter is examined with the naked eye or under a microscope and the colonies are counted.

Membrane filters must be examined within 30 minutes of their removal from the incubator so that the difference between the colored colonies is easier to see. The colors will stabilize after the filters dry, and the results

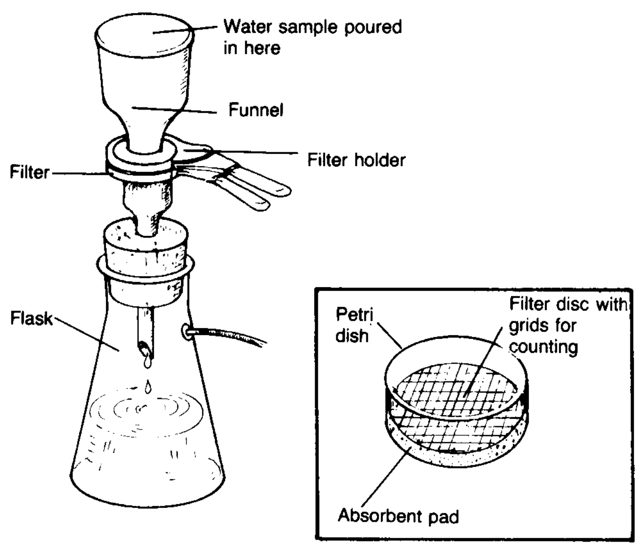

Figure 3. Membrane Filter Method of Coliform Detection

can be preserved for future reference by storing the filter between two layers of plastic film.

Field kits for the membrane filter test are available as units from several manufacturers. Consult the regional or national water authority for information on acquiring kits. If one brand of equipment is already being used by an agency, additional equipment should be from the same manufacturer to assure compatibility. Parts of field kits are usually not interchangable. Be sure a complete instruction manual is included with the kit.

Facilities for incubation are the main constraint for coliform bacteria detection. Temperatures must be controlled very carefully. It is not always possible to maintain the exact temperature ranges in an incubator under field conditions unless portable incubators are used. Portable incubators are relatively expensive and require a power source such as a car battery for operation. If incubation with accurate temperature control is not possible on site, the filter must be transported to an incubator immediately. If no incubator with accurate temperature control is available at all, the incubation temperature for the total coliform test can be provided by the human body. Vests with pockets for membrane filter plates can be made or purchased.

Comparison of the Multiple Tube and Membrane Filter Methods. The advantages of the multiple tube method over the membrane filter method are as follows:

1. The equipment and supplies necessary for the multiple tube test are common to well-equipped laboratories. They are more readily available in most countries than equip ment and supplies for the membrane filter method. Membrane filter portable kits must be imported from the manufacturer which involves high expenditure of foreign exchange for aquisition and replacement parts. Because costs are high, availability and distribution may also be problems.

2. The equipment for the multiple tube method can be re-used. The membrane filter portable kits are disposable and cannot be used more than once.

The multiple tube method of bacteriological analysis is appropriate for established laboratories near the water supply to be tested but not for isolated rural areas without reliable laboratory service.

Advantages of the membrane filter method over the multiple tube method for field analysis are as follows:

1. The number of coliform bacteria colonies grown in a filter can be visually identified and counted. The multiple tube method estimates the number of coliform bacteria statistically. The membrane filter results can be preserved for future reference unlike the multiple tube results.

2. The membrane filter method requires less equipment preparation and clean-up. Disposable equipment and pre-prepared supplies are standard parts of membrane filter field kits. The membrane filter test also takes less time to perform than the multiple tube method. The membrane filter

method requires 24 hours from sample collection to interpretation of results. The multiple tube method requires 48 hours for incubation alone, and can take up to 96 hours for complete procedures. Therefore, where labor costs are high, the membrane filter method may be less expensive to use.

3. The membrane filter technique is better adapted to field work and emergencies than the multiple tube method. Preparation for, performance of, and clean-up after the membrane filter test are less complicated and quicker than for the multiple tube method. Membrane filter test equipment and supplies take up less space than those for multiple tube tests. More equipment especially adapted for field conditions is available for the membrane filter tests than for the multiple tube tests.

The membrane filter method is appropriate when reliable laboratory service is not available within six hours of water sample collection and when the kits are available and not too high priced.

Interpreting Results of Coliform Bacteria Detection. Ideally, all samples should be completely free of coliform bacteria. It is not always possible to attain such a high standard in rural areas. Local standards should be used if they exist. The World Health Organization recommends the following standards:

1. Throughout any year, 95 percent of the drinking water samples should not contain any coliform organisms in 100ml of water.

2. No sample should contain E. coli in 100ml of water.

3. No sample should contain more than 10 coliform organisms of other types per 100ml.

4. Coliform organisms should not be detectable in any two consecutive samples of 100ml of water.

If the supply does not meet these standards or local standards, it should be considered unsuitable for use without treatment. If any coliform organisms are found, further investigation is needed to determine their source. The first step is immediate re-sampling and analysis (see "Taking a Water Sample," RWS.3.P.2). If subsequent water samples do not meet the standards summarized here, refer to "Planning a Water Treatment System," RWS.3.P.4, for recommendations on improving the water quality. Disinfected water supplies should be completely free of any coliforms, however polluted the raw water may have been. See "Operating and Maintaining a Chemical Disinfection Unit," RWS.3.O.4, for information on interpreting results of analysis of disinfected water supplies.

Summary

Water sample collection and analysis should be repeated under varying conditions. A number of samples should be collected over a period of days for each analysis period. Reliance cannot be placed on the results of analysis of a single sample from a water supply.

When an analysis is completed, results need to be very carefully interpreted in conjunction with the observations of a sanitary survey. An analysis determines the type and level of contamination present in a sample of a water supply. A sanitary survey identifies the probable sources of that contamination. Conclusions from both processes need to be balanced to judge the safety of existing water systems and plan appropriate water quality improvements.