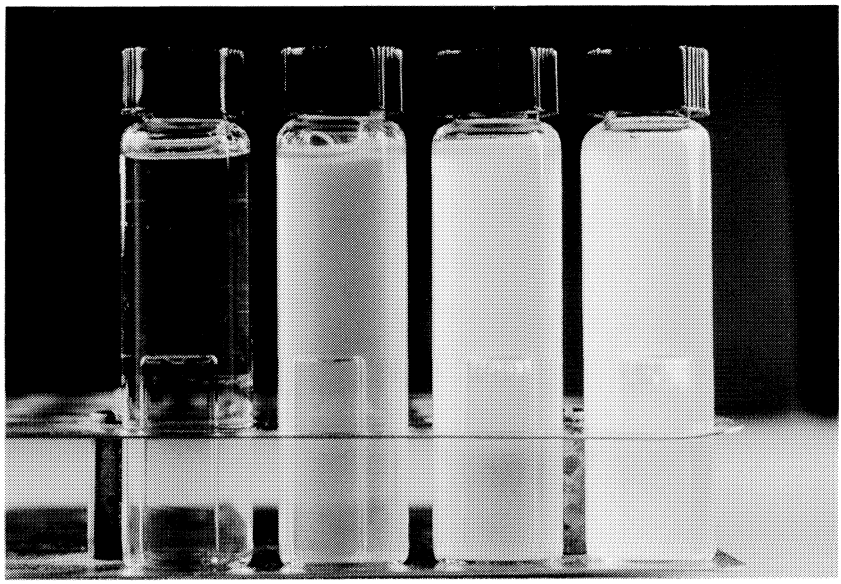
HACH

Chlorine Coliform and pH Test Kit

Model CEC
Cat. No. 22231

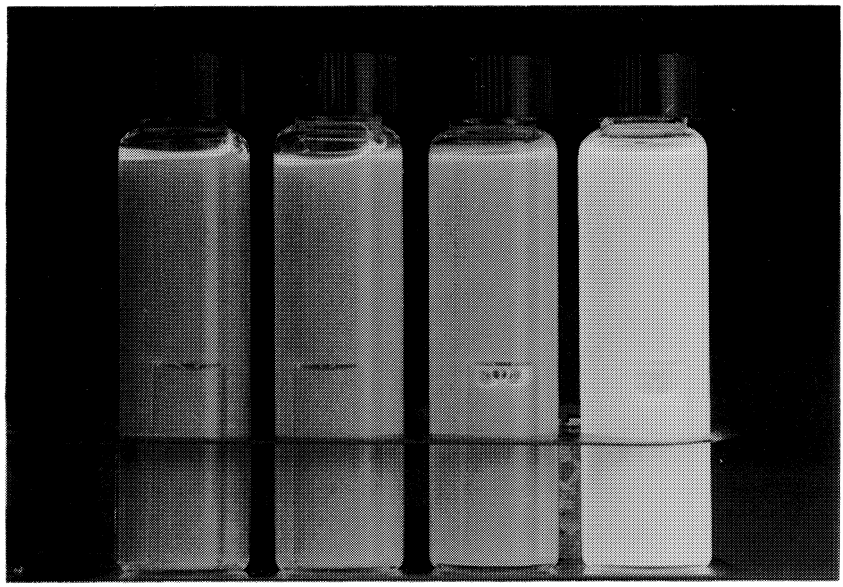
A portable unit for testing water potability

Incubated *coli*-MUG Tubes photographed under normal light.



1 2 3 4

The same *coli*-MUG Tubes photographed under UV light.



1 2 3 4

TUBE 1 No growth, no fluorescence (sterile water)
TUBE 2 Growth, no gas, no fluorescence (noncoliform)
TUBE 3 Growth, gas, no fluorescence (coliform)
TUBE 4 Growth, gas, fluorescence *(Escherichia coli)*

Many of the microorganisms causing serious disease, such as typhoid fever and dysentery, can be traced directly to polluted drinking water. These disease-producing organisms are discharged along with fecal wastes and are difficult to detect in water supplies. Fortunately, less harmful, easily isolated bacteria called indicator organisms travel with the disease-producing microbes. Among these indicators are coliform bacteria. They live in the intestines of man and other animals and are almost always present, even in healthy persons. The presence of coliforms in water is a warning signal that more dangerous bacteria may be present.

A number of different methods are used to detect coliforms. The coli-MUG method is a modification of the Most Probable Number (fermentation tube) technique. It is a simple and accurate method for simultaneously detecting total coliforms and *Escherichia coli*, a fecal coliform.

Chlorine is added to drinking water supplies to destroy harmful bacteria. A constant level of 1mg/L free chlorine generally is adequate to control bacteria without causing a noxious odor or taste. Chlorine can be present in water as free available chlorine and as combined available chlorine. Both forms can exist in the same water and be determined together as the total available chlorine. Free chlorine is present as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, nitrogen trichloride and other chloro derivatives. A stable reagent in powder form for analyzing both free and total chlorine is DPD (N,N-Diethyl-p-phenylenediamine). It offers high sensitivity, rapid color development and minimal fading.

COLI-MUG PROCEDURE

Handling Techniques

Sterilization: Care must be taken to prevent contamination when conducting coliform tests. All materials used for containing or transferring samples must be sterile.

Sample Collection: Use a sterile plastic bag (Cat. No. 14720) to collect unchlorinated samples. For chlorinated samples, sterile plastic bags (Cat. No. 20753) containing a dechlorinating agent should be used. Sample containers should not be filled completely. Maintain an air space of at least one-fourth the total volume. If samples are collected from a faucet, hydrant or pump, allow the water to run for three to five minutes before sampling to minimize outside contamination. If sample collection is from a lake , stream or reservoir, fill a sterile sample container below the surface of the water. Use a sweeping motion and keep the open end of the container in the direction of the sweep.

Procedure

1. Wash hands thoroughly with soap and water, open the sealed plastic package and remove the *coli*-MUG tubes.
2. After shipment, inner fermentation vials usually will contain only air. If these inner vials contain any liquid, be sure to invert the assemblies and allow the inner vials to fill with air.
3. Remove cap, being careful not to touch the open end of the tube or the inside of the cap.
4. Dispense 10 mL of sample into each of the five tubes. Approximately 10 mL are obtained when the tubes are filled level full with sample.
5. Replace the screw cap and invert the tube assembly a few times to mix the water sample with *coli*-MUG broth. Be sure the inner fermentation vial is full of liquid and no air bubbles remain.
6. Place the tubes in the dri-bath incubator at a temperature of 35 ± 0.5°C or 95 ± 1°F. After one hour, examine the inner fermentation vials for trapped air. If air bubbles are present again invert the assemblies briefly to allow the bubbles to escape from the inverted fermentation vials.Return all tubes to the incubator. Tubes must be kept in an upright position (with the fermentation vials upside down) at all times for the rest of the procedure.
7. At the end of 24 ± 2 hours, tap each tube gently and examine. (If gas bubbles appear before 24 hours have elapsed, proceed to Step 8 without waiting the entire 24 hours.) If gas has collected in the inner fermentation vial and the broth appears cloudy, coliform bacteria are presumed to be present. If gas has not formed, continue incubation and examine again after a total of 48 ± 3 hours. Formation of even a small amount of gas within 48 hours constitutes a positive test.
8. After observing the tubes for gas production, check them for fluorescence. Fluorescence may be detected before gas production (as early as four hours in some cases) and usually is seen within 24 hours. To check for fluorescence, cover the tubes with a box or check them in a darkened area under diffuse lighting. (Too much visible light masks the presence of fluorescence.) Hold the tubes under a long-wave ultraviolet (UV) light, or hold the UV

light so that it shines on the tubes. Germicidal lamps (short-wave UV lights) are not suitable for this purpose. The presence of fluorescence in a tube indicates the presence of *Escherichia coli*. If fluorescence is not detected after 24 hours, continue incubation and examine again after a total of 48 ± 3 hours.

Interpretation of Results

If all five of the *Coli*-MUG tubes for a given sample do not fluoresce or do not contain gas bubbles, the water is acceptable for drinking. Marginal water is indicated if one or two fermentation tubes of a standard five-tube test show the presence of gas and/or fluorescence. See Table 1.

Table 1 - Interpretation of Results

Negative	Positive	Water Quality	MPN/100ml
0	5	Not acceptable, treatment is needed.	>16
1	4	Not acceptable, treatment is needed.	16
2	3	Not acceptable, treatment is needed.	9.2
3	2	Marginal, treatment may be needed.	5.2
4	1	Marginal, treatment may be needed.	2.2
5	0	Acceptable, no treatment is needed.	<2.2

The following table show all possible results of this test.

Possible Results of *Coli*-MUG Method

Gas	Fluorescence	Results
+	+	positive
+	-	positive
-	+	positive
-	-	negative

Positive tubes showing fluorescence are an indication of fecal pollution. If results indicate water of marginal quality, treatment may be required, especially if the positive tubes show fluorescence.

Sample should be examined daily until all tubes give negative results for two consecutive days. If three or more tubes give positive results, corrective measures should be taken immediately and additional samples should be tested to determine effectiveness of treatment.

Treatment

If results indicate treatment is needed, chlorine may be added to destroy harmful bacteria. A constant level of 1 mg/L free chlorine generally is adequate to control bacteria without causing a noxious odor or taste. Consult health authorities in your area to determine the method of chlorine treatment to be used.

Reagents and Apparatus

Cat. No.	Description	Unit
21821-15	*Coli*-MUG tubes, sterile	pkg/15
22814-00	Incubator, dri-bath, hold 12 tubes, 120V, 60Hz, ± 0.5°C variable temperature setting (240 V also available)	each
24152-00	Light, UV, Longwave, Portable	each
22822-00	Thermometer	each
20753-25	Sterile Whirl-Pak bags, Contains dechlorinating agent	pkg/25

Optional Apparatus

Cat. No.	Description	Unit
2097-98	Pipet, sterile, disposable (delivers 10 or 11 mL)	25
14720-00	Sterile Whirl-Pak bags, 170mL (6oz)	500
20753-33	Sterile Whirl-Pak bags, 170mL (60z), Contains dechlorinating agent	100
45900-00	25 - Well Dri Bath Incubater, 115/230 V, 50/60Hz	each
45900-02	25 - Well Dri Bath Incubator, 115/230V, 50/60Hz with European power cord and fuses	each
21843-00	UV light, long-wave, 6 watt	each

Disposal Procedure

Because used tubes may contain disease-causing organisms, the tubes should be sterilized before discarding. After results have been recorded, dispose of the tubes as follows:

1. Put in a pan.
2. Fill pan with water and heat to boiling.
3. Boil tubes for five minutes.
4. Put tubes in plastic bag and dispose of in garbage can.

FREE CHLORINE (LOW RANGE) TEST INSTRUCTIONS
0-0.7mg/L

To ensure accurate results read carefully before proceeding:

High concentrations of monochloramine will interfere with the results in the free chlorine test. This interference does not apply to the total chlorine test. The free chlorine concentration must be read within one minute of adding the DPD Free Chlorine Reagent.

1. Rinse the square mixing bottle thoroughly with the water to be tested. Fill the bottle to the 25-mL mark with the sample.
2. Open one DPD Free Chlorine Reagent Powder Pillow. Add the contents of the pillow to the mixing bottle. Swirl to mix as shown in Figure 1. The powder does not have to dissolve completely to obtain accurate results.
3. Place the lengthwise viewing adapter in the color comparator as shown in Figure 2a.
4. Fill one sample tube to the line underlining "Cat. 1730-00" with the prepared sample. This will be approximately 3 inches up from the bottom of the tube.
5. Place the tube containing the prepared water sample into the comparator opening labeled Prepared Sample Position in Figure 2a.
6. Fill the other sample tube with untreated water or a reagent blank to the line underlining "Cat. 1730-00." Insert this tube into the comparator opening labeled Clear Sample Position in Figure 2a.
7. Hold the comparator with the tube tops pointing to a window or light source as in Figure 2b. View through the openings in the front of the comparator. When viewing, use care to not spill samples from unstopped tubes.
8. Rotate the disc to obtain a color match. Read the chlorine concentration from the scale window within one minute of the addition of the powder. Divide the value by 5 to obtain the mg/L free chlorine.

TOTAL CHLORINE (LOW RANGE) TEST INSTRUCTIONS
0-0.7mg/L

1. Rinse the square mixing bottle thoroughly with the water to be tested. Fill the bottle to the 25-mL mark with the sample.
2. Open one DPD Total Chlorine Reagent Powder Pillow. Add the contents of the pillow to the mixing bottle. Swirl to mix as shown in Figure 1. Allow the sample to stand for three minutes before proceeding to Step 3.
3. Place the lengthwise viewing adapter in the color comparator as shown in Figure 2a.
4. Fill one sample tube to the line underlining "Cat. 1730-00." with the prepared sample. This will be approximately 15 mL. If not using 1730-00 tubes, fill to the line found at approximately 3 inches up from the bottom of the tube.
5. PLace the tube containing the prepared water sample into the comparator opening labeled Prepared Sample Position in Figure 2a.
6. Fill the other sample tube with untreated water or a reagent blank to the line underlining "Cat. 1730-00." Insert this tube into the comparator opening labeled Clear Sample Position in Figure 2a.
7. Hold the comparator with the tube tops pointing to a window or light source as in Figure 2b. View through the openings in the front of the comparator. When viewing, use care not to spill samples from unstopped tubes.
8. Rotate the disc to obtain a color match. Read the chlorine concentration from the scale window. Divide the value by 5 to obtain the mg/L total chlorine.

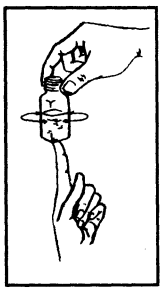


Figure 1

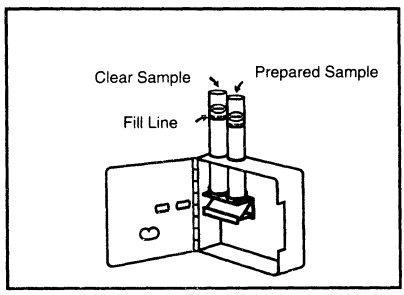


Figure 2a

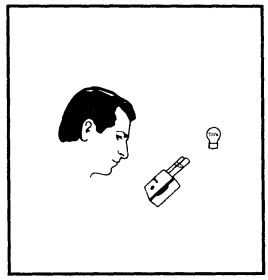


Figure 2b

FREE CHLORINE (HIGH RANGE) TEST INSTRUCTIONS
0-3.5 mg/L

To ensure accurate results read carefully before proceeding:

High concentrations of monochloramine will interfere with the results of this test. The free chlorine concentration must be read within one minute of adding the DPD Free Chlorine Reagent to the mixing bottle.

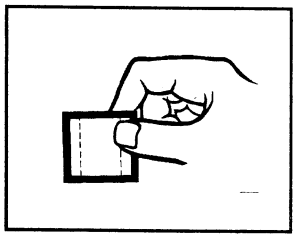
1. If the comparator has the lengthwise viewing adapter in place, remove it.
2. Rinse the square mixing bottle thoroughly with the water to be tested. Fill the bottle to the 25-mL mark with the water sample.
3. Open one DPD Free Chlorine Reagent Powder Pillow. Add the contents of the pillow to the mixing bottle. Swirl to mix as shown in Figure 1. The powder does not have to dissolve to obtain accurate results.
4. Fill one viewing tube to the 5-mL mark with the prepared sample.
5. Hold the color comparator with the front facing you. The front of the comparator has two viewing windows and the Hach logo. Insert the tube of prepared sample into the right top opening of the comparator.
6. Fill the second viewing with an untreated water sample. Insert this tube into the left top opening of the comparator.
7. Hold the comparator up to a light source such as the sky, a window or lamp and view through the openings in front. Rotate the disc to obtain a color match.
8. Read the mg/L free chlorine through the scale window within one minute of the addition of the powder.

TOTAL CHLORINE (HIGH RANGE) TEST INSTRUCTIONS
0-3.5 mg/L

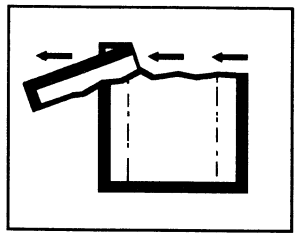
1. If the comparator has the lengthwise viewing adapter in place, remove it.
2. Rinse the square mixing bottle thoroughly with the water to be tested. Fill the bottle to the 25-mL mark with the water sample.
3. Open one DPD Total Chlorine Reagent Powder Pillow. Add the contents of the pillow to the mixing bottle. Swirl to mix as shown in Figure 1. Allow the sample to stand for three minutes before proceeding to Step 4.
4. Fill one viewing tube to the 5-mL mark with the prepared sample.
5. Hold the color comparator with the front facing you. The front of the comparator has two viewing windows and the Hach logo. Insert the tube of prepared sample into the right top opening of the comparator.
6. Fill the second viewing tube with an untreated water sample. Insert this tube into the left top opening of the comparator.
7. Hold the comparator up to a light source such as the sky, a window or a lamp, and view through the openings in front. Rotate the disc to obtain a color match.
8. Read the mg/L total chlorine through the scale window.

Pocket Pal pH Test Instructions

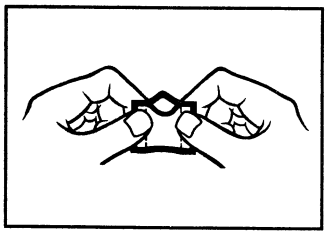
1. Fill the 100 mL beaker with approximately 50 mL of sample.
2. Place the pocket pal pH Tester in the sample and read the pH when the instrument has stabilized.



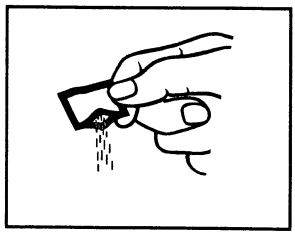
1. Tap



2. Tear



3. Push



4. Pour

Replacements

Cat. No.	Description	Unit
14070-99	DPD Free Chlorine Reagent Powder Pillows	pkg/100
14064-99	DPD Total Chlorine Reagent Powder Pillows	pkg/100
1732-00	Color Comparator	each
1730-00	Color Viewing Tube	each
21988-00	DPD Chlorine Color Disc, 0-3.5 mg/L	each
14480-00	Stoppers for viewing tube	pkg/6
24122-00	Lengthwise Viewing Adapter	each
12222-11	Buffer Solution, pH 7.00 (not included in kit)	473 mL
323-37	Sodium Thiosulfate Solution 0.1N (not included in kit)	118 mL MDB*
1080-42	Beaker	each
44350-00	Pocket Pal pH Tester	each
23510-00	Bottle, mixing	each

It is suggested that the pH Tester accuracy be checked from time to time using Buffer Solution, pH 7.00, Cat. No. 12222-11. *See Replacements.* To use, substitute for the water sample, following the procedure for the pH test.

10/92

MADE IN U.S.A.

WARNING: The chemical in this kit may be hazardous to the health and safety of the user if inappropriately handled. Please read all warnings before performing the tests and use appropriate safety equipment.

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