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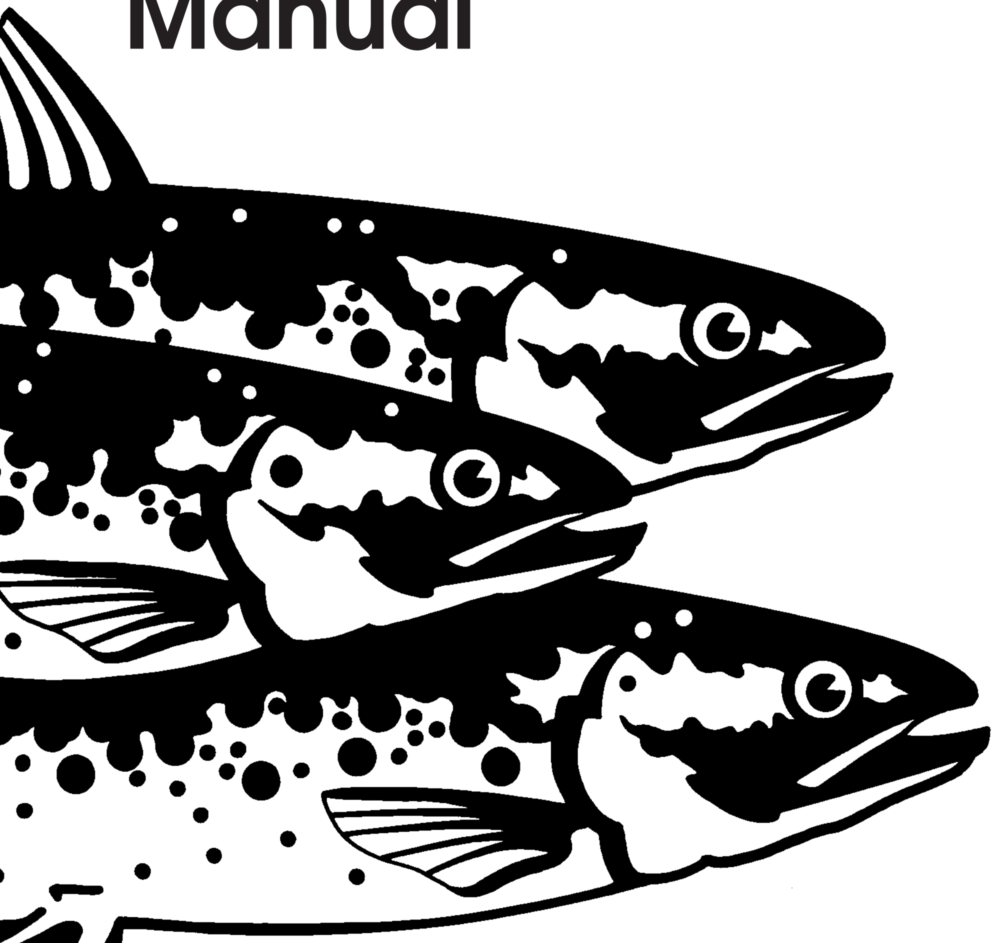
HACH®

Freshwater Aquaculture Manual



Test Kit Model FF-2

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INTRODUCTION

Aquaculture has developed into a highly sophisticated field that uses scientific techniques to maintain the water where aquatic plants and animals live. The ability to optimize parameters affecting water quality is important for efficient production, and sensitive and accurate testing methods are essential. Hach’s Model FF-2 Aquaculture Test Kit is designed to meet this need. The kit includes chemicals and apparatus for the determination of 10 important water parameters.

Acidity, alkalinity, carbon dioxide, chloride, dissolved oxygen, and hardness tests are conducted with the Digital Titrator— a compact, accurate dispensing unit that replaces a buret. Ammonia nitrogen, nitrite nitrogen, and pH determinations are colorimetric tests. Results are obtained by matching the developed color of the sample to a precalibrated color disc. The kit also contains a rugged, armored thermometer for temperature readings.

With this aquaculture kit, the analyst can obtain the data necessary for making the right management decisions. Packaged in a durable, portable case, the kit can be used for measurements in the field.

The Digital Titrator

Hach’s Digital Titrator* is a precision dispensing device that is more consistent and accurate than buret titration. A drive screw controls a plunger which forces the concentrated titrant from a cartridge in a precisely regulated flow. At the visual end point of titration, the concentration is read directly from the digital counter window as mg/L or as a conversion factor. Accuracy is rated at ± 1% or better for a titration of more than 100 digits. For titrations of less than 100 digits, the accuracy is ± 1 digit.

Titration solutions (titrants) are packaged in disposable cartridges that keep titrants pure and allow quick titrations in the lab or in the field. Each cartridge contains approximately 13 mL of solution, enough for 50–100 average titrations. More than 75 titrants are available.

Both portable and fixed-position titrations are possible with the Digital Titrator. The instrument can be clamped to a TitraStir®** Stir Plate or laboratory stand for stationary setups.

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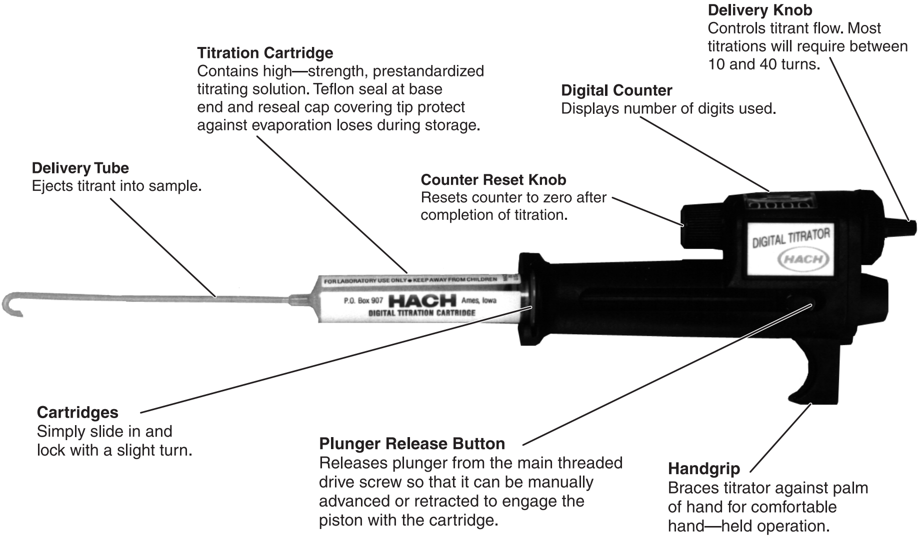
* U.S. patent 4,086,062.

** TitraStir is a registered trademark of Hach Company.



Basic Operation

1. Slide the appropriate titration cartridge all the way onto the titrator body and lock in position with a slight turn.
2. Remove the cap and insert a delivery tube into the end of the cartridge. Do not insert the tube past the cartridge extension.
3. Flush out the delivery tube by turning the dispensing knob until titrant begins flowing from the end of the tube.
4. Wipe the tip and *reset the counter to zero* with the counter reset knob.
5. Immerse the end of the delivery tube into the sample and titrate by turning the dispensing knob while swirling the flask until the end point (a color change) is reached.
6. At the end point, read the number in the digital counter window to determine the sample concentration.
7. Press the plunger release button and manually retract the plunger from the titrator cartridge. Remove the cartridge.
8. Remove the delivery tube and reseal the cartridge with the cap.
9. Always flush the delivery tube with water immediately after use so the titrant does not dry and clog the tube.

ACIDITY

Acidity refers to the capacity of water to donate hydrogen ions. The acidity of natural waters is normally very low and is primarily due to dissolved carbon dioxide which can be determined by titrating to its neutralization point at pH 8.3. This value corresponds to the color change of phenolphthalein indicator and is commonly called the phenolphthalein acidity. Another source of acidity in natural waters is the presence of mineral acids. Sulfuric acid may be present in rainfall in urban and industrialized areas because burning fuels release oxidized sulfur compounds into the atmosphere. A pH of 3.7 has been arbitrarily chosen to give an estimate of the strong mineral acids present.

Methyl orange indicator undergoes a color change at pH 3.7 and results are commonly referred to as the methyl orange acidity. However, the methyl orange end point is difficult to see. Bromphenol blue is used as a substitute in the titration procedure that follows. Bromphenol blue indicator gives a sharp yellow to pure green end point in fresh water at a pH of 3.7**.**

Because acidity is caused in large part by dissolved gases such as carbon dioxide, care must be taken to avoid aerating or shaking the sample or the gases may be lost.

Procedure

1. Attach a clean delivery tube to a 1.600 N Sodium Hydroxide Titration Cartridge. Slide the cartridge all the way onto the titrator body and lock in position with a slight turn.

2. Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3. Take a water sample by filling a clean 100-mL graduated cylinder to the 100 mL-mark. Pour the sample into a clean 250-mL Erlenmeyer flask.

4. Add the contents of one Bromphenol Blue Powder Pillow and swirl to mix.

5. While swirling the flask, turn the delivery knob to titrate the sample with sodium hydroxide until the color changes from yellow to pure green.

6. Read the concentration from the digital counter window. This is methyl orange acidity (as mg/L $CaCO_3$) — an indicator of mineral acidity.
7. Reset the counter to zero.
8. Take another water sample by filling the 100-mL graduated cylinder to the 100-mL mark. Pour the sample into a clean 250-mL Erlenmeyer flask.
9. Add the contents of one Phenolphthalein Powder Pillow and swirl to mix.
10. Titrate with sodium hydroxide until a light pink color forms and *persists for 30 seconds*.
11. Read the concentration in the digital counter window. This is total acidity (as mg/L $CaCO_3$).

REAGENTS AND APPARATUS

Description	Cat. No.
Bromphenol Blue Powder Pillows (100)	14550-99
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 1.600 N Sodium Hydroxide	14379-01
Flask, Erlenmeyer, 250-mL	505-46
Phenolphthalein Powder Pillows (100)	942-99

ALKALINITY

Alkalinity refers to the amount of titratable bases in water expressed as milligrams per liter of equivalent calcium carbonate. The presence of carbonates, bicarbonates, and hydroxides is the most common cause of alkalinity in natural waters. Alkalinity is an important indicator of the need for water treatments such as the addition of lime.

Alkalinity is expressed as phenolphthalein alkalinity or as total alkalinity. The phenolphthalein alkalinity is determined by titration with sulfuric acid to a pH of 8.3 (the phenolphthalein end point) and registers the total hydroxide and one half the carbonate present. The total alkalinity is determined by titration to a pH of 5.1, 4.8, 4.5, or 3.7 depending on the various compositions and alkalinities of the water sample as described in *Note A*. The total alkalinity includes all carbonate, bicarbonate, and hydroxide alkalinity.

Procedure

1. Attach a clean, straight-stem delivery tube to a 1.600 N Sulfuric Acid Titration Cartridge. Slide the cartridge all the way onto the titrator body and lock in position with a slight turn.

2. Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3. Take a water sample by filling a clean 100-mL graduated cylinder to the 100-mL mark. Pour the sample into a clean 250-mL Erlenmeyer flask.

4. Add the contents of one Phenolphthalein Powder Pillow and swirl to mix. If a pink color does not develop, proceed with *step 7*.

5. If the sample turns pink, titrate the sample with sulfuric acid while swirling the flask. The end point is a change from pink to colorless.

6. Read the concentration of phenolphthalein alkalinity (as mg/L $CaCO_3$) from the digital counter window.

7. Add the contents of one Bromcresol Green-Methyl Red, or a Bromphenol Blue Powder Pillow as appropriate, to the same sample and swirl to mix. See *Notes A* and *B*.

8. Continue to titrate to a light greenish blue-gray (pH 5.1), a light bluish pink-gray (pH 4.8) or a light pink (pH 4.5).

9. Read the total alkalinity (as mg/L $CaCO_3$) from the digital counter window.

***Note A** The following end points are recommended for determination of the total alkalinity in water samples of various compositions and alkalinities.*

Sample Type	End Point
Alkalinity about 30 mg/L	pH 5.1
Alkalinity about 150 mg/L	pH 4.8
Alkalinity about 500 mg/L	pH 4.5
Silicates or phosphates known to be present	pH 4.5
Industrial wastes or complex mixture	pH 3.7

To determine the indicator color at the total alkalinity end point, mix the contents of one pH Buffer Powder Pillow of the desired pH with 50 mL of deionized water in a 250-mL Erlenmeyer flask and add one Bromcresol Green-Methyl Red Powder Pillow. Use the color of the buffer indicator solution as a reference when titrating samples.

***Note B** When titrating to pH 3.7, use a Bromphenol Blue Powder Pillow in place of a Bromcresol Green-Methyl Red Powder Pillow, both in the reference solution and in the sample. The end point is a color change from purple, to blue, to green.*

REAGENTS AND APPARATUS

Description	Cat No.
Bromcresol Green-Methyl Red Powder Pillows (100)	943-99
Bromphenol Blue Powder Pillows (100)	14550-99
Buffer Powder Pillows, pH 3.7 (25)*	14551-68
Buffer Powder Pillows, pH 4.50 (25)*	895-68
Buffer Powder Pillows, pH 4.80 (25)*	896-68
Buffer Powder Pillows, pH 8.30 (25)*	898-68
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 1.600 N Sulfuric Acid	14389-01
Flask, Erlenmeyer, 250-mL	505-46
Phenolphthalein Powder Pillows (100)	924-99
Water, deionized, 100 mL	272-42

* Not included in kit.

AMMONIA

The presence of ammonia in fish waters is normal due to natural fish metabolism and microbiological decay of organic matter. In water, ammonia nitrogen can exist in two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+). Un-ionized ammonia is toxic to fish, while the ammonium ion is non-toxic except at extremely high levels. The pH and temperature of water regulate the proportion of each form. *Table 1* on page *9* lists the percentages of un-ionized ammonia at various pH and temperature levels.

The Nessler Method for ammonia nitrogen testing is a sensitive, single reagent test. Interference due to high water hardness is eliminated by adding Rochelle Salt Solution to the sample.

Procedure

1. Fill one viewing tube to the 5-mL mark with deionized water. This will be the reagent blank.

2. Fill the second 5-mL viewing tube to the 5-mL mark with sample. This will be the prepared sample.

3. Add 1 drop of Rochelle Salt Solution to each viewing tube and swirl to mix.

4. Add 3 drops of Nessler Reagent to each viewing tube and swirl to mix. Stopper both tubes.

5. Allow 10 minutes for color development.

6. Place the Ammonia Color Disc into the Color Comparator if it is not already in place.

7. Insert the prepared sample in the right-hand opening of the Color Comparator. (See Prepared Sample position in *Figure 1* on page *8*.)

8. Insert the reagent blank into the left-hand opening of the Color Comparator. (See Untreated Sample position in *Figure 1* on page *8*.)

9. Hold the Comparator up to a light source such as the sky, a window, or a lamp and view through the two openings in the front. Rotate the disc to obtain a color match.

10. Read the concentration of ammonia nitrogen in mg/L (N) through the scale window. See *step 11*.

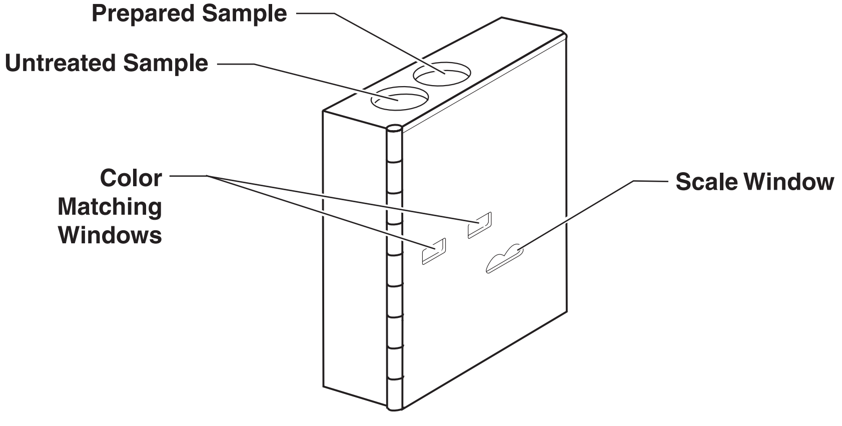
11. To express test results as toxic ammonia (NH_3), use the following equation:

$$\frac{\text{mg/L } NH_3 \text{ as N} \times \text{value from Table 1}}{100} \times 1.2 = \text{mg/L } NH_3$$

To express results as ammonium ion (NH_4^+), use the following equation:

$$\frac{\text{mg/L } NH_3 \text{ as N} \times (100 - \text{value from Table 1})}{100} \times 1.3 = \text{mg/L } NH_4^+$$

Figure 1 Color Comparator



REAGENTS AND APPARATUS

Description	Cat No.
Color Comparator Box	1732-00
Color Disc, ammonia nitrogen	1854-00
Color Viewing Tubes, plastic (4)	46600-04
Nessler Reagent, 100-mL MDB	21194-32
Rochelle Salt Solution, 29-mL DB	1725-33
Water, deionized, 100 mL	272-42

Table 1 Percentage Un-ionized Ammonia in Aqueous Solution by pH Value and Temperature Calculated from data in Emerson, et. al*

	Temperature (°C)														
pH	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**
7.0	0.11	0.13	0.16	0.18	0.22	0.25	0.29	0.34	0.39	0.46	0.52	0.60	0.69	0.80	0.91
7.2	0.18	0.21	0.25	0.29	0.34	0.40	0.46	0.54	0.62	0.82	0.83	0.96	1.10	1.26	1.44
7.4	0.29	0.34	0.40	0.46	0.54	0.63	0.73	0.85	0.98	1.14	1.31	1.50	1.73	1.98	2.26
7.6	0.45	0.53	0.63	0.73	0.86	1.00	1.16	1.34	1.55	1.79	2.06	2.36	2.71	3.10	3.53
7.8	0.72	0.84	0.99	1.16	1.35	1.57	1.82	2.11	2.44	2.81	3.22	3.70	4.23	4.82	5.48
8.0	1.13	1.33	1.56	1.82	2.12	2.47	2.86	3.30	3.81	4.38	5.02	5.74	6.54	7.43	8.42
8.2	1.79	2.10	2.45	2.86	3.32	3.85	4.45	5.14	5.90	6.76	7.72	8.80	9.98	11.29	12.72
8.4	2.80	3.28	3.83	4.45	5.17	5.97	6.88	7.90	9.04	10.31	11.71	13.26	14.95	16.78	18.77
8.6	4.37	5.10	5.93	6.88	7.95	9.14	10.48	11.97	13.61	15.41	17.37	19.50	21.78	24.22	26.80
8.8	6.75	7.85	9.09	10.48	12.04	13.76	15.66	17.73	19.98	22.41	25.00	27.74	30.62	33.62	36.72
9.0	10.30	11.90	13.68	15.65	17.82	20.18	22.73	25.46	28.36	31.40	34.56	37.83	41.16	44.53	47.91
9.2	15.39	17.63	20.08	22.73	25.58	28.61	31.80	35.12	38.55	42.04	45.57	49.09	52.58	55.99	59.31
9.4	22.38	25.33	28.47	31.80	35.26	38.84	42.49	46.18	49.85	53.48	57.02	60.45	63.73	66.85	69.79
9.6	31.36	34.96	38.38	42.49	46.33	50.16	53.94	57.62	61.17	64.56	67.77	70.78	73.58	76.17	78.55
9.8	42.00	46.00	50.00	53.94	57.78	61.47	64.99	68.31	71.40	74.28	76.92	79.33	81.53	83.51	85.30
10.0	53.44	57.45	61.31	64.98	68.44	71.66	74.63	77.35	79.83	82.07	84.08	85.88	87.49	88.92	90.19
10.2	64.53	68.15	71.52	74.63	77.46	80.03	82.34	84.41	86.25	87.88	89.33	90.60	91.73	92.71	93.58

* Emerson, K., R. C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can.*, 32:2379-2383.

CARBON DIOXIDE

Carbon dioxide (CO_2) is present in all surface waters in amounts generally less than 10 mg/L, although higher concentrations in ground waters are not uncommon. High concentrations of carbon dioxide may be tolerated by fish if dissolved oxygen concentrations are also high. Fish are known to avoid areas of high carbon dioxide levels. The relationship of carbon dioxide to fish respiration and photosynthesis creates daily fluctuations in CO_2 concentrations. Levels usually increase during the night and decrease during the day. High levels of carbon dioxide, such as those that occur after plankton die-offs, will suppress absorption by fish and may become toxic when dissolved oxygen levels are critically low.

When determining carbon dioxide in water, the sample is titrated with sodium hydroxide to the phenolphthalein end point.

Procedure

1. Attach a clean delivery tube to a 0.3636 N Sodium Hydroxide Titration Cartridge. Slide the cartridge all the way onto the titrator body and lock in position with a slight turn. See *Note A*.

2. Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3. Take a water sample by filling a clean 100-mL graduated cylinder to the 100-mL mark. If possible, allow the water to overflow the cylinder several times, then pour off the excess until the 100-mL mark is reached. See *Notes B* and *C*.

4. Pour sample into a clean 250-mL Erlenmeyer flask. Add the contents of one Phenolphthalein Powder Pillow and swirl to mix.

5. Titrate the sample while swirling the flask gently until a light pink color forms and persists for 30 seconds.

6. Record the reading in the digital counter window. Divide the number by 5 to determine mg/L carbon dioxide (CO_2).

Note A *Sodium hydroxide solutions absorb carbon dioxide slowly when exposed to air, causing a partial loss of strength. The sodium hydroxide cartridge should be capped when not in use.*

Note B *Avoid shaking or excessive swirling of the samples; aerating will cause a loss of carbon dioxide. Analysis should be performed in the field as soon as possible after taking the sample.*

Note C *Strong mineral acids cause high results in the carbon dioxide test; they must be absent or in quantities low enough to be negligible.*

REAGENTS AND APPARATUS

Description	Cat. No.
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 0.3636 N Sodium Hydroxide	14378-01
Flask, Erlenmeyer, 250-mL	505-46
Phenolphthalein Powder Pillows (100)	942-99

CHLORIDE

Chlorides are present in all fish waters at highly variable levels. Concentrations of chlorides are usually higher in waters near coastal regions. Measurement of chloride values before and after the addition of salt to fish waters can be used to estimate pond levels.

The mercuric nitrate method of chloride analysis has become popular due to the sharp yellow to pinkish-purple end point of diphenylcarbazone. A single, stable powder has been developed that combines the color indicator with an appropriate buffer to establish the correct pH.

Procedure

(For chloride concentrations below 100 mg/L as Cl^-.)

1. Attach a clean delivery tube to a 0.2256 N Mercuric Nitrate Titration Cartridge. Slide the cartridge all the way onto the titrator body and lock in position with a slight turn.

2. Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3. Take a water sample by filling the 100-mL graduated cylinder to the 100-mL mark. Pour the sample into a clean 250-mL Erlenmeyer flask. See *Note A*.

4. Add the contents of one Diphenylcarbazone Reagent Powder Pillow and swirl to mix. See *Note B*.

5. Titrate the sample with mercuric nitrate until the color changes from yellow to light pink.

6. Determine the concentration in mg/L of chloride (Cl^-) in the sample by dividing the reading in the digital counter window by 10. See *Note C*.

Note A *For samples with chloride concentrations greater than 100 mg/L, a dilution with chloride-free water or deionized water is necessary. For example, a 1 to 2 dilution could be made by measuring 50 mL of the water sample in a 100-mL graduated cylinder and adding deionized water to the 100-mL mark. Then multiply the test result by two to obtain the actual concentration.*

Note B *Results will not be affected if a small portion of the diphenylcarbazone reagent powder does not dissolve.*

Note C *Results may be expressed as mg/L sodium chloride (NaCl) by multiplying the mg/L chloride (Cl^-) by 1.65.*

REAGENTS AND APPARATUS

Description	Cat. No.
Cylinder, Graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 0.2256 N Mercuric Nitrate	14393-01
Diphenylcarbazone Reagent Powder Pillows (100)	836-99
Flask, Erlenmeyer, 250-mL	505-46
Water, deionized, 100 mL	272-42

DISSOLVED OXYGEN

Probably the single most important water quality parameter in aquaculture is the dissolved oxygen (DO) content. Suitability of water for fish and other organisms can be measured or estimated from DO values. DO concentrations in water depend on many variables including temperature, sunlight, atmospheric pressure, salinity, plant life, and water turbulence. See *Table 2*. Oxygen from the air will slowly diffuse into natural waters. However, the primary source of oxygen in fish waters originates from photosynthesis by phytoplankton. Prolonged exposure to low concentrations of DO can be harmful to aquatic life. Frequent DO measurements are essential for adequate control.

In the Winkler Method for DO testing, samples are treated with manganous sulfate and alkaline iodide-azide reagents to form an orange-brown precipitate. Sulfamic acid is added, which reacts with the iodide to release iodine. The free iodine is then titrated with standard Sodium Thiosulfate, Stabilized. The concentration of DO is directly proportional to the amount of titrant used.

Because DO concentrations vary with water depth, temperature, and other factors, several samplings at different sites and depths may be required for best results.

Procedure

1. Collect sample in a clean 60-mL glass-stoppered BOD bottle. Do not allow air bubbles to be trapped in the bottle. See *Note A*.

2. Add the contents of one Dissolved Oxygen 1 Powder Pillow and one Dissolved Oxygen 2 Powder Pillow. Carefully insert the stopper so that air will not be trapped in the bottle.

3. Pour any excess water off the bottle rim and invert several times to mix. A delay in mixing may prevent the powders from dissolving properly. A brownish-orange floc precipitate may remain in the bottom of the bottle. This will not affect the results of the test.

4. Allow the sample to stand until the floc has settled, leaving the top half of the solution clear. Invert the bottle several times again, then let it stand until the upper half has cleared again. See *Note B*.

5. Remove the stopper and add the contents of one Dissolved Oxygen 3 Powder Pillow. Replace the stopper, being careful not to trap any air bubbles in the bottle, and invert several times to mix. The floc will dissolve, leaving a yellow color if dissolved oxygen is present.

6. Pour off exactly 50 mL of the prepared sample by filling the 100-mL graduated cylinder to the 50-mL mark.
7. Attach a clean delivery tube to a 0.0250 N Sodium Thiosulfate, Stabilized Titration Cartridge. Slide the cartridge all the way onto the titrator body and lock in position with a slight turn.
8. Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.
9. Titrate the 10 mL of prepared sample remaining in the BOD bottle with the sodium thiosulfate to a pale yellow color.
10. Add two drops of Starch Indicator Solution and swirl to mix. A blue color will develop.
11. Continue the titration until the solution changes from dark blue to colorless.
12. Read the number in the digital counter window and divide by 40 to determine the concentration of dissolved oxygen (in mg/L). See *Note C*.

Note A *If testing running water, allow the sample to overflow the bottle for 2 to 3 minutes. If testing standing water, merely allow the bottle to fill completely, rinse, and refill. For subsurface sampling, the optional Dissolved Oxygen Sampler is recommended.*

Note B *Allowing the floc to settle twice ensures reaction of the chemicals with all the dissolved oxygen present. Floc settles very slowly in salt water and usually will require an additional five minutes before proceeding with step 5. Results will not be affected if the floc will not settle.*

Note C *For a more sensitive test, titrate 25 mL of sample (pour off 35 mL into the 100-mL graduated cylinder) and divide the digital counter reading by 100 for concentration of dissolved oxygen (in mg/L).*

The sension™6 Dissolved Oxygen Meter

Those wanting faster, more precise testing should consider Hach's new ***sension6*** Dissolved Oxygen Meter and Electrode as an alternative to the Winkler DO method with the Digital Titrator. The state-of-the-art ***sension6*** DO Meter not only auto-calibrates but compensates for sample temperature automatically and corrects for altitude, barometric pressure, and salinity. The meter reads % saturation as well as mg/L and can be calibrated to a Winkler titration value. A 50-point datalogging capability permits downloading to a text or spreadsheet file as well as uploading of stored data.

REAGENTS AND APPARATUS

Description	Cat. No.
BOD Bottle, 60-mL, glass	1909-00
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 0.0250 N Sodium Thiosulfate	24093-01
Dissolved Oxygen 1 Powder Pillows (100)	981-99
Dissolved Oxygen 2 Powder Pillows (100)	982-99
Dissolved Oxygen 3 Powder Pillows (25)	987-68
Dissolved Oxygen Sampler*	1962-00
sension™6 Dissolved Oxygen Meter with Probe*	51850-10
Starch Indicator Solution, 100-mL MDB	349-32

* Not included in kit.

Table 2 Solubility of Oxygen in Water vs. Temperature and Barometric Pressure

	Pressure							
mm Hg	**600**	**625**	**650**	**675**	**700**	**725**	**750**	**760**
inches Hg	**23.6**	**24.6**	**25.6**	**26.6**	**27.6**	**28.5**	**29.5**	**29.9**
Temp. °C	**Oxygen Solubility (mg/L)**							
10	8.88	9.26	9.64	10.01	10.39	10.76	11.14	11.29
11	8.68	9.04	9.41	9.78	10.15	10.51	10.88	11.03
12	8.48	8.84	9.20	9.56	9.92	10.27	10.63	10.78
13	8.29	8.64	8.99	9.34	9.69	10.04	10.40	10.54
14	8.10	8.45	8.79	9.14	9.48	9.82	10.17	10.31
15	7.93	8.26	8.60	8.94	9.28	9.61	9.95	10.08
16	7.76	8.09	8.42	8.75	9.08	9.41	9.74	9.87
17	7.59	7.92	8.24	8.56	8.89	9.21	9.54	9.67
18	7.43	7.75	8.07	8.39	8.70	9.02	9.34	9.47
19	7.28	7.59	7.91	8.22	8.53	8.84	9.15	9.28
20	7.13	7.44	7.75	8.05	8.36	8.66	8.97	9.09
21	6.99	7.29	7.59	7.89	8.19	8.49	8.79	8.92
22	6.85	7.15	7.45	7.74	8.04	8.33	8.63	8.74
23	6.72	7.01	7.30	7.59	7.88	8.17	8.46	8.58
24	6.59	6.88	7.16	7.45	7.73	8.02	8.30	8.42
25	6.47	6.75	7.03	7.31	7.59	7.87	8.15	8.26
26	6.35	6.62	6.90	7.18	7.45	7.73	8.00	8.11
27	6.23	6.50	6.77	7.05	7.32	7.59	7.86	7.97
28	6.12	6.38	6.65	6.92	7.19	7.45	7.72	7.83
29	6.01	6.27	6.53	6.80	7.06	7.32	7.59	7.69
30	5.90	6.16	6.42	6.68	6.94	7.20	7.46	7.56

HARDNESS

Calcium and magnesium are the most abundant alkaline earth metals found in natural waters. Hardness is defined as the characteristic of water that represents the total concentration of calcium and magnesium expressed as their calcium carbonate equivalent. Other divalent ions also contribute to hardness, but their effects are usually negligible in natural waters. Hardness measurements are used by fish culturists because of the importance of hardness in attaining productive waters. Treatment plans may be necessary when levels of total hardness are too low or differ significantly from total alkalinity.

In the total hardness test, the water sample is buffered to a pH of 10.1 where the test functions best. An organic dye is added which reacts with calcium and magnesium ions to give a red-colored complex. The solution is then titrated with standard EDTA to a blue end point. The amount of EDTA titrant added is directly proportional to the concentration of total hardness (as $CaCO_3$).

Procedure

1. Attach a clean delivery tube to a 0.800 M EDTA Titration Cartridge. Slide the cartridge all the way onto the titrator body and lock in position with a slight turn.

2. Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3. Take a water sample by filling a clean 100-mL graduated cylinder to the 100-mL mark. Pour the sample into a clean 250-mL Erlenmeyer flask.

4. Using the 1-mL calibrated dropper, add 2 mL of Hardness 1 Buffer Solution and swirl to mix.

5. Add the contents of one ManVer®* 2 Powder Pillow and swirl to mix.

6. Titrate the sample with the EDTA solution while swirling the flask until the color changes from red to pure blue. Titrate slowly toward the end point to allow time for the reaction and color change to take place, especially for samples below 20 °C (68 °F).

* ManVer is a registered trademark of Hach Company.

REAGENTS AND APPARATUS

Description	Cat. No.
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 0.800 M EDTA	14399-01
Flask, Erlenmeyer, 250-mL	505-46
Hardness 1 Buffer Solution, 100-mL MDB	424-32
ManVer® 2 Powder Pillows (100)	851-99

NITRITE

Nitrite nitrogen occurs as an intermediate stage in the biological decomposition of compounds containing organic nitrogen. Nitrites are not often found in surface waters because in aerobic conditions they are readily oxidized to nitrates. Levels of nitrites greater than natural residual amounts can be acutely toxic to fish. The test shown below is very sensitive to low nitrite concentrations.

Procedure

1. Rinse a sample tube several times with the sample, then fill to the 5-mL mark. Add the contents of one NitriVer®* 3 Powder Pillow for 5-mL Sample.

2. Stopper the tube and shake vigorously for exactly one minute. A red color will develop if nitrite is present.

3. Place the Nitrite Nitrogen Color Disc into the Color Comparator if it is not already in place.

4. Allow the prepared sample to sit undisturbed for 10 minutes, then place the tube into the right-hand opening of the comparator. (See *Figure 1* on page *8*.)

5. Fill the other viewing tube to the 5-mL mark with the original sample water. Stopper and place it into the left-hand opening.

6. Hold the Comparator up to a window, the sky, or a lamp and view through the openings in front. Rotate the disc until a color match is obtained.

7. Read the mg/L nitrite nitrogen (N) through the scale window.

Note: *The test results can be converted from mg/L nitrite nitrogen (N) to mg/L nitrite (NO_2^-) by multiplying the final reading by 3.3.*

REAGENTS AND APPARATUS

Description	**Cat. No.**
Color Comparator Box	1732-00
Color Disc, nitrite nitrogen	14084-00
Color Viewing Tubes, plastic (4)	46600-04
NitriVer® 3 Powder Pillows (100)	14078-99

* NitriVer is a registered trademark of Hach Company.

pH

The pH of water is a measure of the hydrogen ion concentration on a scale of 0 (very acidic) to 14 (very basic), with pH 7 being the neutral point. The pH value represents the instantaneous hydrogen ion activity rather than the buffering capacity or total reserve as in acidity or alkalinity tests. The pH of most natural water ranges from 4 to 9 and is greatly influenced by the presence of carbon dioxide, carbonates, bicarbonates, and acid rain. Phytoplankton and other aquatic plant life will remove carbon dioxide from the water during photosynthesis, causing the pH to rise during the day. In order to assess the pH cycle of a body of water, pH measurements should be made at different times. Waters with pH values of about 6.5 to 9 at daybreak are considered best for fish production. The acid and alkaline death points for most fish are approximately pH 4 and pH 11.

Procedure

1. Fill two viewing tubes to the 5-mL mark with sample. It is imperative that the tubes be rinsed completely free of any solutions that may have been used previously. Place the Wide Range pH Color Disc into the Color Comparator if it is not already in place.
2. Add 6 drops of Wide Range 4 pH Indicator Solution to one of the tubes and swirl to mix. Stopper both tubes.
3. Insert the tube containing indicator into the right-hand opening of the Color Comparator.
4. Insert the tube of untreated sample into the left-hand opening of the Color Comparator.
5. Hold the Color Comparator up to light such as the sky, a window, or a lamp and view through the two openings in the front. Rotate the color disc until a color match is obtained.
6. Read the pH through the scale window.

REAGENTS AND APPARATUS

Description	Cat. No.
Color Comparator Box	1732-00
Color Disc, wide range pH	1919-00
Color Viewing Tubes, plastic (4)	46600-04
Wide Range 4 pH Indicator Solution, 100-mL MDB	23293-32

TEMPERATURE

Temperature has a great effect on the chemical and biological systems in water. Lower temperatures will lower growth rates of fish and fish food organisms. At higher temperatures, processes such as dissolved oxygen uptake by aquatic life will increase. Fish generally do not tolerate sudden changes in water temperature. They will, however, tolerate different temperature zones if the changes are gradual. Different temperature zones can occur in natural waters due to the changing density of water with temperature. Because of this, one should record the depth at which the thermometer reading was taken.

See *Table 3* for Celsius/Fahrenheit conversion.

Table 3 Celsius to Fahrenheit Degrees

°C	°F	°C	°F	°C	°F
0	32.0	14	57.2	28	82.4
1	33.8	15	59.0	29	84.2
2	35.6	16	60.8	30	86.0
3	37.4	17	62.6	31	87.8
4	39.2	18	64.4	32	89.6
5	41.0	19	66.2	33	91.4
6	42.8	20	68.0	34	93.2
7	44.6	21	69.8	35	95.0
8	46.4	22	71.6	36	96.8
9	48.2	23	73.4	37	98.6
10	50.0	24	75.2	38	100.4
11	51.8	25	77.0	39	102.2
12	53.6	26	78.8	40	104.0
13	55.4	27	80.6		

APPARATUS

Description	**Cat. No.**
Thermometer, pocket, –30° to 120 °F	1895-00

PARTS PER MILLION CONVERSION

Conversion for Parts Per Million*, Proportion, and Percent

Parts Per Million	Proportion	Percent
0.1	1:10,000,000	0.00001
0.5	1:2,000,000	0.00005
1.0	1:1,000,000	0.0001
2.0	1:500,000	0.0002
3.0	1:333,333	0.0003
4.0	1:250,000	0.0004
5.0	1:200,000	0.0005
10.0	1:100,000	0.001
15.0	1:66,667	0.0015
20.0	1:50,000	0.002
25.0	1:40,000	0.0025
50.0	1:20,000	0.005
100.0	1:10,000	0.01
150.0	1:6,667	0.015
200.0	1:5,000	0.02
250.0	1:4,000	0.025
500.0	1:2,000	0.05
1,000.0	1:1000	0.1
5,000.0	1:200	0.5
10,000.0	1:100	1.0
50,000.0	1:20	5.0

* In water, ppm is essentially equivalent to mg/L.

REAGENTS AND APPARATUS

Description	Cat. No.
BOD Bottle, 60-mL, glass	1909-00
Bromcresol Green-Methyl Red Indicator Pillows (100)	943-99
Bromphenol Blue Powder Pillows (100)	14550-99
Buffer Powder Pillows, pH 3.7 (25)*	14551-68
Buffer Powder Pillows, pH 4.50 (25)*	895-68
Buffer Powder Pillows, pH 4.80 (25)*	896-68
Buffer Powder Pillows, pH 8.30 (25)*	898-68
Color Comparator Box	1732-00
Color Disc, ammonia nitrogen	1854-00
Color Disc, nitrite nitrogen	14084-00
Color Disc, wide range pH	1919-00
Color Viewing Tubes, plastic (4)	46600-04
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-01
Digital Titrator Cartridge, 0.800 M EDTA	14399-01
Digital Titrator Cartridge, 0.2256 N Mercuric Nitrate	14393-01
Digital Titrator Cartridge, 0.3636 N Sodium Hydroxide	14378-01
Digital Titrator Cartridge, 1.600 N Sodium Hydroxide	14379-01
Digital Titrator Cartridge, 0.0250 N Sodium Thiosulfate	24093-01
Digital Titrator Cartridge, 1.600 N Sulfuric Acid	14389-01
Digital Titrator manual	16900-08
Diphenylcarbazone Reagent Powder Pillows (100)	836-99
Dissolved Oxygen 1 Powder Pillows (100)	981-99
Dissolved Oxygen 2 Powder Pillows (100)	982-99
Dissolved Oxygen 3 Powder Pillows (25)	987-68
Dissolved Oxygen Sampler*	1962-00
Flask, Erlenmeyer, 250-mL	505-46
Freshwater Aquaculture Manual, Test Kit Model FF-2	2430-89
Hardness 1 Buffer Solution, 100-mL MDB	424-32
ManVer® 2 Powder Pillows (100)	851-99
Nessler Reagent, 100-mL MDB	21194-32
NitriVer® 3 Powder Pillows (100)	14078-99
Phenolphthalein Powder Pillows (100)	942-99
Rochelle Salt Solution, 29-mL DB	1725-33
Sampling Bottle, wide mouth, 500-mL*	14724-11
sension™***6*** Dissolved Oxygen Meter with Probe*	51850-10
Starch Indicator Solution, 100-mL MDB	349-32
Stopper, color viewing tube*	1731-00
Thermometer, pocket, –30° to 120 °F	1895-00
TitraStir® Stir Plate, 115 Vac*	19400-00
TitraStir® Stir Plate, 230 Vac*	19400-10
Water, deionized, 100 mL	272-42
Wide Range 4 pH Indicator Solution, 100-mL MDB	23293-32

* Not included in kit.

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