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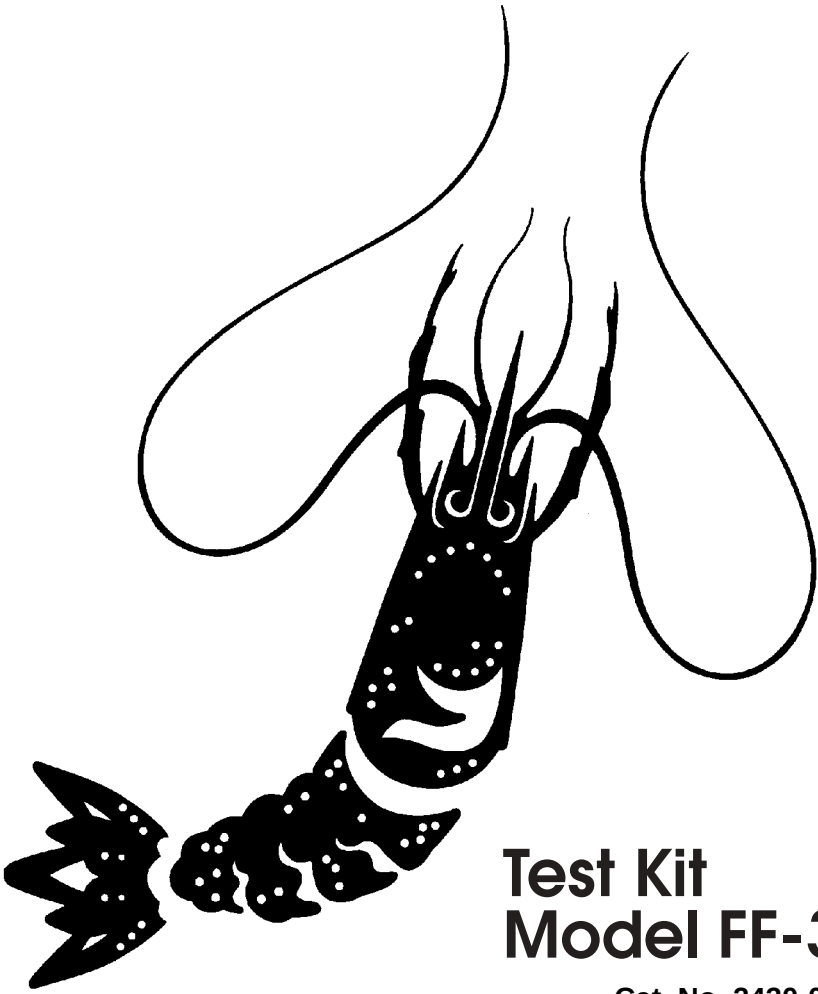
HACH®

Saltwater Aquaculture Manual



Test Kit Model FF-3

Cat. No. 2430-03

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TABLE OF CONTENTS

INTRODUCTION

Aquaculture has developed into a highly sophisticated field that uses scientific techniques to maintain the water where marine 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–3 Saltwater Aquaculture Test Kit is designed to meet this need. The kit includes chemicals and apparatus for the determination of 10 important water parameters and a rugged, armored thermometer for temperature readings. Packaged in a durable, portable case, the kit is well-suited for measurements in the field.

Acidity, alkalinity, carbon dioxide, dissolved oxygen, hardness, and salinity 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 pre-calibrated color disc.

With this aquaculture kit, the analyst can obtain the data necessary for making the right management decisions.

The Digital Titrator

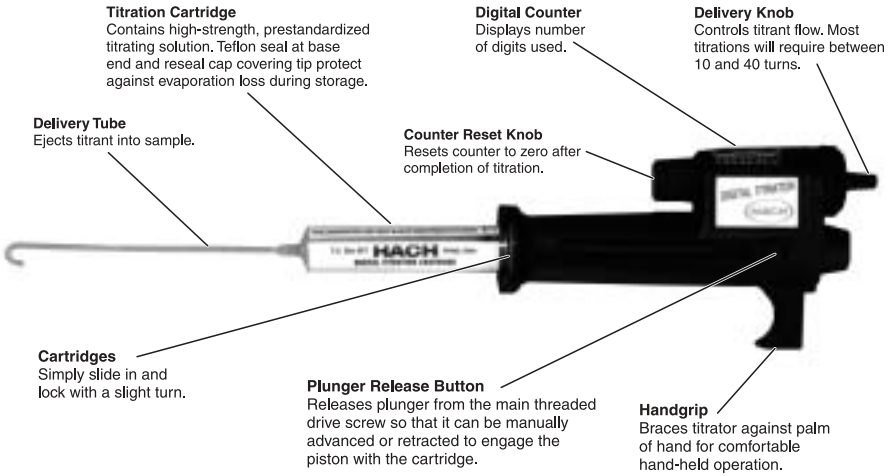
Hach's Digital Titrator* is a precision dispensing device that is more consistent and accurate than a buret. A drive screw controls a plunger which forces the concentrated titrant from a cartridge in a precisely regulated flow. At the visual end point of titration, in most cases the concentration is read directly from the digital counter as mg/L. 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.

* 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 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 or affect subsequent tests.

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 orginating from the oxidation of sulfides in iron pyrite and clay. 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 blue-violet end point in seawater 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 blue-

violet. The digital counter reading is methyl orange acidity (as mg/L $CaCO_3$) — an indicator of mineral acidity.

6. Reset the counter to zero.

7. 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.

8. Add the contents of one Phenolphthalein Powder Pillow and swirl to mix.

9. Titrate with sodium hydroxide until a light pink color forms and *persists for 30 seconds*.

10. The digital counter reading 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	16900-02
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 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, the phenolphthalein alkalinity is zero. 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. The digital counter reading is phenolphthalein alkalinity (as mg/L $CaCO_3$).

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. The digital counter reading is total alkalinity (as mg/L $CaCO_3$).

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	16900-02
Digital Titrator Cartridge, 1.600 N Sulfuric Acid	14389-01
Flask, Erlenmeyer, 250-mL	505-46
Phenolphthalein Powder Pillows (100)	942-99
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 *11* lists the percentages of un-ionized ammonia at various pH and temperature levels.

This procedure uses the Ammonia Salicylate Method which is formulated for saltwater.

Procedure

1. Rinse two viewing tubes with the water to be tested and fill both to the 5-mL mark.

2. Add the contents of one Ammonia Salicylate Reagent Powder Pillow to one of the tubes. Stopper and shake until all the powder is dissolved. Wait three minutes.

3. Add the contents of one Ammonia Cyanurate Reagent Powder Pillow to this same tube. Stopper the tube and shake until all powder is dissolved. Allow at least 15 minutes for the color to develop fully. The color is stable for several hours if the tube is kept stoppered. See *Note A*.

4. Wipe both tubes.

5. Insert the tube containing the reagents into the right-hand opening of the Color Comparator.

6. Insert the other tube into the left-hand opening of the Color Comparator.

7. Place the Ammonia Salicylate Color Disc into the Color Comparator if it is not already in place and close the Comparator door.

8. Hold the Color -Comparator up to light and view through the two openings in the front. Rotate the disc to obtain a color match.

9. Read the concentration of ammonia nitrogen in mg/L (N) through the scale window. See *Note B*.

Note A *If the test yields unexpectedly high readings, the glassware may be contaminated. Because of the sensitivity of this test, the glassware must be kept extremely clean. If contamination is suspected, continue the test procedure then rinse the tubes with the water to be analyzed and run the test again. This will allow the test reagents to clean the tubes and eliminate any contamination. Comparing the results of the two analyses should indicate if any interference was present.*

Note B *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:

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

Note C *This test is calibrated for seawater. When this test is used for brackish or fresh water, the NH_3–N reading indicated on the wheel may be somewhat higher than the actual value. In most cases, this error will be less than 10% although in very low salinity distilled or fresh water, errors can be as high as +16%.*

Note D *The limits of this test can be increased to 5 mg NH_3–N by diluting the sample. Using the 3-mL syringe, deliver a 2.5-mL sample into the tube, then add 2.5 mL deionized water. Multiply the color wheel reading by two to compensate for the dilution.*

REAGENTS AND APPARATUS

Description	Cat No.
Ammonia Salicylate Reagent Powder Pillows (50)	23952-66
Ammonia Cyanurate Reagent Powder Pillows (50)	23954-66
Color Comparator Box	1732-00
Color Disc, ammonia salicylate	23938-00
Color Viewing Tubes, plastic (4)	46600-04
Stoppers, color viewing tube (6)*	1731-06
Water, deionized, 100 mL*	272-42

* Not included in kit.

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. Gently pour sample into a clean 250-mL Erlenmeyer flask. See *Note B.*
4. 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. Divide the digital counter reading by 5 to determine mg/L carbon dioxide (CO_2). See *Note C.*

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.*

CARBON DIOXIDE, continued

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	16900-02
Digital Titrator Cartridge, 0.3636 N Sodium Hydroxide	14378-01
Flask, Erlenmeyer, 250-mL	505-46
Phenolphthalein Powder Pillows (100)	942-99

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 marine 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. Divide the digital counter reading 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
Clippers	968-00
Cylinder, graduated, 100-mL, poly	1081-42
Delivery Tubes, 180° hook (5)	17205-00
Digital Titrator	16900-02
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	349-32

* Not included in kit.

DISSOLVED OXYGEN, continued

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. 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. Filling a clean 100-mL graduated cylinder to the 10-mL mark with sample. Then add deionized water to the 100-mL mark. Pour 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).
7. Multiply the digital counter reading by 10 to get total hardness as mg/L $CaCO_3$.

* 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	16900-02
Digital Titrator Cartridge, 0.800 M EDTA	14399-01
Flask, Erlenmeyer, 250-mL	505-46
Hardness 1 Buffer Solution, 100-mL	424-32
ManVer® 2 Powder Pillows (100)	851-99
Water, deionized, 100 mL*	272-42

* Not included in kit.

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. This test is very sensitive to low nitrite concentrations.

Procedure

1. Rinse a viewing tube several times with the sample, then fill to the 5-mL mark.
2. Add the contents of one NitriVer®* 3 Powder Pillow for 5-mL Sample.
3. Stopper the tube and shake vigorously for exactly one minute. A red color will develop if nitrite is present.
4. Allow the prepared sample to sit undisturbed for 10 minutes, then place the tube into the right-hand opening of the comparator.
5. Fill another viewing tube to the 5-mL mark with untreated sample. Stopper and place it into the left-hand opening.
6. Place the Nitrite Nitrogen Color Disc into the Color Comparator if it is not already in place and close the Comparator door.
7. Hold the Comparator up to light and view through the openings in front. Rotate the disc until a color match is obtained.
8. 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 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
Stoppers, color viewing tube (6)**	1731-06

* NitriVer is a registered trademark of Hach Company.

** Not included in kit.

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 marine 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 marks with sample. *It is imperative that the tubes be rinsed completely free of any solutions that may have been used previously.*
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. Place the Wide Range pH Color Disc into the Color Comparator if it is not already in place and close the door.
6. Hold the Color Comparator up to light and view through the two openings in the front. Rotate the color disc until a color match is obtained.
7. 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
Stoppers, color viewing tube (6)*	1731-06
Wide Range 4 pH Indicator Solution, 100-mL	23293-32

* Not included in kit.

SALINITY

Salinity is an important variable in saline water aquaculture. It is widely recognized that salinity can be estimated from the chloride concentration.

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

0-100 ppt salinity (ppt = parts per thousand)

1. Attach a clean delivery tube to a 2.570 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. Using the 3-mL syringe, collect a 2.0-mL sample. Add to the vial provided.
4. Fill the vial to the 10-mL mark with demineralized water. See *Note A.*
5. Add the contents of one Diphenylcarbazone Reagent Powder Pillow and swirl to mix. See *Note B.*
6. Titrate the sample with mercuric nitrate until the color changes from yellow to light pink.
7. Divide the digital counter reading by 10 to get salinity in parts per thousand (ppt). See *Note C*.

Note A *Demineralized water is produced by adding tap water to the demineralizer bottle and shaking for 30 seconds. This water can then be dispensed from the flip-top dispenser.*

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 chloride (Cl^-) by multiplying the ppt salinity by 569. Results may be expressed as mg/L sodium chloride (NaCl) by multiplying the ppt salinity by 940.*

REAGENTS AND APPARATUS

Description	Cat. No.
Delivery Tubes, 180° hook (5)	17205-00
Demineralizer Bottle, 473 mL	21846-00
Digital Titrator	16900-02
Digital Titrator Cartridge, 2.570 N Mercuric Nitrate	23937-01
Diphenylcarbazone Powder Pillows (100)	967-99
Syringe, 3-cc luer lock tip	43213-00
Vial, 2, 5, 10, 15, 20, 25 mL	2193-00
Water, deionized, 100 mL*	272-42

* Not included in kit.

TEMPERATURE

Temperature has a great effect on the chemical and biological systems in water. Lower temperatures will lower growth rates of fish food organisms and fish. At higher temperatures, processes such as dissolved oxygen uptake by marine 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.

APPARATUS

Description	Cat. No.
Thermometer, pocket, non-mercury, –10 to 110 °C (0 to 220 °F)	26764-00

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.

REPLACEMENT REAGENTS AND APPARATUS

Description	Cat. No.
Ammonia Salicylate Reagent Powder Pillows (50)	23952-66
Ammonia Cyanurate Reagent Powder Pillows (50)	23954-66
BOD Bottle, 60-mL, glass	1909-00
Bromcresol Green-Methyl Red Indicator Pillows (100)	943-99
Bromphenol Blue Powder Pillows (100)	14550-99
Clippers	968-00
Color Comparator Box	1732-00
Color Disc, ammonia salicylate	23938-00
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
Demineralizer Bottle, 473 mL	21846-00
Digital Titrator	16900-02
Digital Titrator Cartridge, 0.800 M EDTA	14399-01
Digital Titrator Cartridge, 2.570 N Mercuric Nitrate	23937-01
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 Powder Pillows (100)	967-99
Dissolved Oxygen 1 Powder Pillows (100)	981-99
Dissolved Oxygen 2 Powder Pillows (100)	982-99
Dissolved Oxygen 3 Powder Pillows (25)	987-68
Flask, Erlenmeyer, 250-mL	505-46
Saltwater Aquaculture Manual, Test Kit Model FF–3	2430-90
Hardness 1 Buffer Solution, 100-mL	424-32
ManVer® 2 Powder Pillows (100)	851-99
NitriVer® 3 Powder Pillows (100)	14078-99
Phenolphthalein Powder Pillows (100)	942-99
Starch Indicator Solution, 100-mL	349-32
Syringe, 3-cc luer lock tip	43213-00
Thermometer, pocket, non-mercury, –10 to 110 °C (0 to 220 °F)	26764-00
Vial, 2, 5, 10, 15, 20, 25 mL	2193-00
Wide Range 4 pH Indicator Solution, 100-mL	23293-32

Optional Reagents and Apparatus

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
Dissolved Oxygen Sampler	1962-00
sension™***6*** Dissolved Oxygen Meter with Probe	51850-10

REPLACEMENT REAGENTS AND APPARATUS, continued

Optional Reagents and Apparatus, continued

Stoppers, color viewing tube (6) 1731-06
TitraStir® Stir Plate, 115 Vac 19400-00
TitraStir® Stir Plate, 230 Vac 19400-10
Water, deionized, 100 mL 272-42

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