HARDNESS (0 to 4.00 mg/L Ca and Mg as CaCO₃) For water, wastewater and seawater

Calcium and Magnesium; Calmagite Colorimetric Method

1. Pour 100 mL of water sample in a 100-mL graduated mixing cylinder.

Note: For the most accurate magnesium test results the sample temperature should be between 21–29 °C (70–84 °F).

2. Add 1.0 mL of Calcium and Magnesium Indicator Solution using a 1.0 mL measuring dropper. Stopper. Invert several times to mix.

3. Add 1.0 mL of Alkali Solution for Calcium and Magnesium Test using a 1.0 mL measuring dropper. Stopper. Invert several times to mix.

4. Pour 25 mL of the solution into each of three sample cells.

Note: The test will detect any calcium or magnesium contamination in the mixing cylinder, measuring droppers of sample cells. To test cleanliness, repeat the test multiple times until you obtain consistent results.

5. Add one drop of 1 M EDTA Solution to one cell (the blank). Swirl to mix.

6. Add one drop of EGTA Solution to another cell (the prepared sample). Swirl to mix.

7. Enter a stored program number for magnesium.

Press: 2 2 5 READ/ENTER for units of mg/L Mg as CaCO₃.

Press: 2 2 6 READ/ENTER for units of mg/L Mg.

The display will show:

DIAL nm TO 522

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 9. Proceed with Step 10.
9. Press: **READ/ENTER**

The display will show:

- mg/l CaCO₃ Mg
- mg/l Mg

**Note:** The Pour-Thru Cell can be used if rinsed well with demineralized water between the blank and prepared sample.

10. Place the blank into the cell holder. Close the light shield.

11. Press: **ZERO**

The display will show:

- WAIT
- 0.00 mg/l CaCO₃ Mg
- 0.00 mg/l Mg

12. Place the prepared sample into the cell holder. Close the light shield.

13. Press: **READ/ENTER**

The display will show:

- WAIT
- then the result in mg/L Mg as CaCO₃ or mg/L Mg will be displayed.

**Note:** mg/L magnesium equals mg/L Mg as CaCO₃ multiplied by 0.243.


The display will show:

- two times.

**Note:** Do not remove the sample cell.

15. Enter a stored program number for calcium.

Press: **2 2 0 READ/ENTER**

for units of mg/L Ca as CaCO₃

Press: **2 2 1 READ/ENTER**

for units of mg/L Ca.

The display will show:

- DIAL nm TO 522

**Note:** DR/2000s with software versions 3.0 and greater will display “P” and the program number.

**Note:** Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 16. Proceed with Step 17.

**Note:** Do not remove sample cell.
17. Press: ZERO

The display will show:

WAIT

then:

0.00 mg/l CaCO₃ Ca
OR
0.00 mg/l Ca

18. Place the third sample cell into the cell holder.

19. Press: READ/ENTER

The display will show:

WAIT

then the result in mg/L Ca as CaCO₃ or mg/L Ca will be displayed.

Note: mg/L hardness equals
mg/L Ca as CaCO₃ plus mg/L Mg as CaCO₃.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear.
When the display stabilizes, read the result.

<table>
<thead>
<tr>
<th>Table 1 Conversion Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>To convert from</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>mg/L Ca as CaCO₃</td>
</tr>
<tr>
<td>mg/L Mg as CaCO₃</td>
</tr>
<tr>
<td>mg/L MgCO₃</td>
</tr>
</tbody>
</table>

SAMPLING AND STORAGE
Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Cool samples to 4 °C. Preserved samples can be stored up to six months. Adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution just before analysis. Correct the test results for volume additions (see Correction for Volume Additions in Section 1).

PRECISION
In a single laboratory, using a standard solution of 1.5 mg/L Mg as CaCO₃ and 3 mg/L Ca as CaCO₃ with the DR/2000, a single operator obtained a standard deviation of ± 0.006 mg/L Mg as CaCO₃ and ± 0.02 mg/L Ca as CaCO₃.

INTERFERENCES
For the most accurate calcium test result, the test should be rerun on a diluted sample if the calcium is over 1.0 and the magnesium is over 0.25 mg/L as CaCO₃. No retesting is needed if either is below those respective concentrations.

The following cause a detectable error in test results.

Cr³⁺ 0.25 mg/L
Cu²⁺ 0.75 mg/L
EDTA, chelated 0.2 mg/L as CaCO₃
Fe²⁺ 1.4 mg/L
Fe³⁺ 2.0 mg/L
Mn²⁺ 0.20 mg/L
Zn²⁺ 0.050 mg/L

Traces of EDTA or EGTA remaining in sample cells from previous tests will give erroneous results. Rinse cells thoroughly before using.
SUMMARY OF METHOD
The colorimetric method for measuring hardness supplements the more conventional titrimetric method through its ability to measure very low levels of calcium and magnesium. Also some interfering metals (those listed above) in the titrimetric method will be rendered inconsequential when diluting the sample to bring it within the range of this test. The indicator dye used is calmagite which forms a purplish-blue color in a strongly alkaline solution and changes to red when contacting free calcium or magnesium. Calcium and magnesium determinations are made by chelating calcium with EGTA to destroy any red color due to calcium and then chelating the calcium and magnesium with EDTA to destroy the red color due to both calcium and magnesium. By measuring the red color in the different states, calcium and magnesium concentrations are determined.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness Reagent Set (100 Tests)</td>
<td>........................</td>
<td>23199–00</td>
<td></td>
</tr>
<tr>
<td>Includes: (1) 22417–32, (1) 22418–32, (1) 22419–26, (1) 22297–26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali Solution for Calcium and Magnesium Test</td>
<td>1 mL</td>
<td>100 mL</td>
<td>22417–32</td>
</tr>
<tr>
<td>Calcium and Magnesium Indicator Solution</td>
<td>1 mL</td>
<td>100 mL</td>
<td>22418–32</td>
</tr>
<tr>
<td>EDTA Solution, 1 M</td>
<td>1 drop</td>
<td>59 mL</td>
<td>22419–26</td>
</tr>
<tr>
<td>EGTA Solution</td>
<td>1 drop</td>
<td>59 mL</td>
<td>22297–26</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder, 100–mL mixing</td>
<td>1</td>
<td>each</td>
<td>1896–42</td>
</tr>
<tr>
<td>Dropper, measuring, 0.5 and 1.0 mL</td>
<td>2</td>
<td>10/pkg</td>
<td>21247–10</td>
</tr>
</tbody>
</table>

OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
<td></td>
</tr>
<tr>
<td>Thermometer, −20 to 105 °C</td>
<td>each</td>
<td>1877–01</td>
<td></td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
HARDNESS, TOTAL, ULTRA LOW RANGE (0 to 1,000 µg/L Ca and Mg as CaCO₃)

Calcium and Magnesium; Chlorophosphonazo Colorimetric Method

1. Enter the stored program number for ultra low range hardness.

Press: 2 2 7 READ/ENTER

The display will show:
DIAL nm TO: 669

Note: Or, use the up and down arrows to scroll the display to:
227 µg/L as CaCO₃ and press:
READ/ENTER

Note: For DR/2000s without this stored program, see Instrument Setup following these steps.

2. Rotate the wavelength dial until the small display shows:

669 nm

3. Press: READ/ENTER

The display will show:
µg/L as CaCO₃

4. Rinse a plastic sample cell and the cap three times with the water to be tested. Do not allow the underside of the cap to come in contact with surfaces that may contaminate it.

5. Fill the plastic sample cell to the 25–ml mark with sample.

6. Add the contents of one Chlorophosphonazo Solution Pillow to the sample cell.

Note: A small amount of solution may remain in the pillow. This will not affect results.

Note: One mL of Chlorophosphonazo Solution (25895–40) may be used instead of the solution pillow.

7. Cap the cell and swirl to mix.

8. Place the sample cell into the cell holder. Close the light shield.
9. Press: ZERO

The display will show: WAIT
then:
0 μg/l as CaCO₃

10. Remove the cell from the instrument. Add one drop of CDTA Reagent for Ultra Low Range Hardness.

Note: Complete Steps 11–13 within 1–2 minutes.

11. Cap the cell and swirl to mix

12. Place the sample cell into the cell holder. Close the light shield.

13. Press: READ/ENTER

The display will show: WAIT
then the result in μg/L as CaCO₃ will be displayed.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
HARDNESS, TOTAL, ULTRA LOW RANGE, continued

INSTRUMENT SETUP
For DR/2000s with software versions less than 3.0
The calibration program for this method cannot be installed in DR/2000s with software versions before 3.0. The following modifications to the procedure are necessary for these earlier software versions.

a) In place of Step 1, press 0 at the METHOD # prompt, then press READ/ENTER to place the instrument in the absorbance mode.

The display will show:

Abs

b) Follow the instructions of Step 2.

c) Follow the instructions of Step 3. The display will instead show:

ZERO SAMPLE

d) Follow the instructions of Steps 4 and 5.

Before proceeding to Step 6, cap the cell containing 25 mL of sample, place it into the cell holder and close the light shield.

Press: ZERO

The display will show:

WAIT
then:

0.000 Abs

e) Follow the instructions for Steps 6, 7 and 8.

f) In place of Step 9, press: READ/ENTER.

The display will show:

WAIT
then the absorbance value will be displayed.

If the amount of hardness in this sample is within the range of the method (0 to 1,000 μg/L as CaCO₃), the absorbance value displayed will be between 0.500 Abs and 2.500 Abs.

For example, the display may show an absorbance value of:

1.273

Record the displayed value. This value will be referred to as Abs1.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, record the result.

g) Follow the instructions of the remaining steps (10 through 13).

However, after completing Step 13 the display will not show μg/L as CaCO₃. It will show an absorbance value between 0.500 Abs and 1.000 Abs.

For example, the display may show an absorbance value of:

0.786

Record the displayed value. This value will be referred to as Abs2.

h) To calculate total hardness as CaCO₃, subtract Abs2 from Abs1 and multiply the result by the slope factor 0.759 (μg/L/Abs).

For example, if
Abs1 = 1.273
Abs2 = 0.786
then μg/L as CaCO₃ = (1.273 Abs - 0.786 Abs) x 0.759 (μg/L/Abs) = 370 μg/L as CaCO₃

Note: Analysis may determine their own slope factor by performing the Standard Solution Accuracy Check given below. The slope will be the value of the standard (500 μg/L as CaCO₃) divided by the difference of the two recorded absorbance values.

For DR/2000s with software versions 3.0 or 3.1:
1. Turn the instrument on and press

![SHIFT] [CONFIG] [METH]

to enter configuration mode. The display will show:
MOMENTARY or CONSTANT ON

2. Press the UP arrow twice to select HACH UPDATE. Press READ/ENTER. The display will show:
ENTER #:

3. Press:

![EDIT] 2 [EDIT] 2 [TIMER] 7 [READ] ENTER

The display will show:
P227 ENTER nm

4. Press:

![CONC] 6 [CONC] 6 9 [READ] ENTER

The display will show:
P227 DECIMAL? 00.00

199
Note: If you make an error, press SHIFT CLEAR and re-enter the number. When the number is correct, press READ/ENTER.

5. For this method, press the DOWN ARROW key twice to correctly position the decimal point. The display will show:
   **P227 Decimal? 0000.**

6. Press READ/ENTER when the decimal is correct. The display will show:
   **P227 UNITS?**

7. For this method, press the DOWN ARROW three times to select the unit of measure. The display will show:
   **P227 μg/l**

8. Press READ/ENTER. The display will show:
   **P227 μg/l**

9. Construct the correct symbol display:
   **as CaCO₃**
   a) Select the correct character symbol by scrolling with the arrow keys.

b) To make a letter upper case, press the SHIFT key.

c) To make a number subscript or regular, press SHIFT until the symbol is correct.

d) Make a space by pressing the DOWN ARROW key once.

e) Make sure you enter the display line exactly as shown, including spaces. Each dot indicates a character. Do not enter trailing spaces.

f) Accept each symbol by pressing READ/ENTER.

g) To end symbol entry, press READ/ENTER a second time after accepting the last character.

10. After accepting the symbol, the display will show:
    **P227 TIMER?**

11. This method has no timing periods, so press READ/ENTER. The display will show:
    **#0 STANDARD**

12. Press READ/ENTER to display the zero data pair. The display will show:
    **0.000 Abs 0000. μg/l**

13. Press READ/ENTER. The display will show:
    **#1 STANDARD**

14. Press READ/ENTER. The display will prompt for entry of the first concentration point:
    #1 0000. μg/l

15. Enter concentration point #1 from the table below (Step 20) by pressing **1000** so the display shows:
    #1 1000. μg/l

16. Press READ/ENTER. The display will prompt for entry of the first absorbance point:
    **#1 0.000 Abs**

17. Press SHIFT – to set the negative sign for the bleaching chemistry. Then enter the absorbance point #1 from the table below by pressing **1318** so the display shows:
    **#1 –1.318 Abs**

18. Press READ/ENTER. The display should show the first data pair:
    **–1.318 Abs 1000. μg/l**

19. Press READ/ENTER to accept the first data pair. The display will show:
    **#2 STANDARD**

20. The data pair values from the table below are now entered.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Data Pair Table</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>#0</td>
<td>0 μg/L</td>
<td>0.000 Abs</td>
</tr>
<tr>
<td>#1</td>
<td>1000 μg/L</td>
<td>–1.318 Abs</td>
</tr>
</tbody>
</table>

21. Press SHIFT READ/ENTER to complete data point entry. The display will show:
    **#:**

22. Enter the validation number **3791** so the display shows
    **#: 3791**

23. Press READ/ENTER. The display should show:
    **COMPLETED**
    then
    **P227 μg/l as CaCO₃**

If the display shows
    **INCORRECT #**

then prompts again for the validation number, you may have made a mistake during data entry. Make sure the validation number is correct. If it is, the error occurred during some other part of the method entry. Press METH and respond to the ABORT? message by pressing READ/ENTER. Then re-enter the method.
HARDNESS, TOTAL, ULTRA LOW RANGE, continued

After correct entry, the instrument is ready for use with method 227.

**SAMPLING AND STORAGE**

Do not use glass containers. Collect samples in clean plastic containers, preferably with screw-type closures. Rinse containers several times with water to be analyzed before capturing final sample. Seal to avoid contamination during transport. Analyze as soon as possible.

**ACCURACY CHECK**

**Standard Additions Method**

a) Use a TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL, of the 20 mg/L (as CaCO₃) Calcium Chloride standard to 25–mL aliquots of sample, respectively.

b) Perform the hardness test on each sample as described above.

c) Each 0.1 mL addition of standard should cause an increase of 80 µg/L hardness as CaCO₃.

d) If these increases do not occur, see **Standard Additions** in Section I for more information.

**Standard Solution Method**

Use the 0.50 mg/L (as CaCO₃) Calcium Chloride Standard Solution listed under **Optional Reagents**. Analyze this solution according to the above procedure.

The strength of this standard solution is 0.50 mg/L as CaCO₃ (or 500 µg/L); the analytical result should be between 460 µg/L as CaCO₃ and 540 µg/L as CaCO₃.

**PRECISION**

In a single laboratory using a standard solution of 600 µg/L as CaCO₃ and two representative lots of reagents, a single operator obtained a standard deviation of 3 µg/L.

**INTERFERENCES**

Interference studies were conducted at various hardness levels between 0 and 500 µg/L as CaCO₃. Various cations and anions were evaluated at levels in the range appropriate to ultra pure water applications.

An ion is said to interfere when the resulting concentration is changed by ±10%.

**Negative Interference:**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Level above which it interferes (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>150</td>
</tr>
<tr>
<td>Sodium</td>
<td>79,000</td>
</tr>
</tbody>
</table>

**Positive Interference:**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Level above which it interferes (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>250</td>
</tr>
<tr>
<td>Silicon</td>
<td>1,000</td>
</tr>
</tbody>
</table>

**No Interference:**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Highest Concentration Tested (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>1,000</td>
</tr>
<tr>
<td>Ammonium</td>
<td>1,000</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>47,000</td>
</tr>
</tbody>
</table>

**DISPOSAL INFORMATION**

To dispose of sample cell contents, turn the cold tap water on, and pour the material carefully down the drain. Let the water run one minute to flush the system.

**SUMMARY OF METHOD**

Calcium and magnesium combine equivalently with the chlorophosphonazo III indicator to form a complex which absorbs light very strongly at 669 nm. One drop of the CDTA reagent breaks up this complex, and the resultant decrease in absorbance is linearly related to the amount of calcium and magnesium in the sample (as CaCO₃).

---

**REQUIRED REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Unit</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophosphonazo Indicator Solution Pillows</td>
<td>1 pillow</td>
<td>100(pkg)</td>
<td>25895–99</td>
</tr>
<tr>
<td>for Ultra Low Range Hardness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDTA Reagent for Ultra Low Range Hardness</td>
<td>1 drop</td>
<td>10 mL</td>
<td>25896–36</td>
</tr>
</tbody>
</table>

201
HARDNESS, TOTAL, ULTRA LOW RANGE, continued

REQUIRED APPARATUS
Clippers (Shears) for opening Solution Pillows ............ 1 ................. each ............ 23694–00
Sample Cell, 1-inch, polystyrene w/ cap .................. 1 .................. 12/pkg ............ 24102–12

OPTIONAL REAGENTS
Calcium Standard Solution, 20 mg/L as CaCO₃ .................. 946 mL ............ 21246–16
Calcium Standard Solution, 0.50 mg/L as CaCO₃ .............. 946 mL ............ 20580–16
Chlorophosphonazo Indicator Solution ..................... 1 mL .................. 500 mL ............ 25895–49

OPTIONAL APPARATUS
Dispenser, 1.0 mL fixed volume, Repipet Jr ..................... each ............ 21113–01
Pipet, TenSette, 0.1 to 1.0 mL .................................. each ............ 19700–01
Pipet Tips, for 19700–01 TenSette Pipet ..................... 50/pkg ............ 21856–96

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
p-Dimethylaminobenzaldehyde Method*

1. Enter the stored program number for hydrazine (N₂H₄).

Press: 2 3 1 READ/ENTER

The display will show:
DIAL nm TO 455

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: For DR/2000s without this stored program, see Instrument Setup following this procedure.

Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

2. Rotate the wavelength dial until the small display shows:

455 nm

3. Press: READ/ENTER

The display will show:
μg/l N₂H₄

4. Pour 25.0 mL of demineralized water into a sample cell (the blank) using a graduated cylinder.

*Adapted from ASTM Manual of Industrial Water, D1385–78, 376 (1979)
5. Pour 25.0 mL of sample into a second sample cell (the prepared sample) using a graduated cylinder.

Note: For proof of accuracy, use a 100 μg/L hydrazine standard solution (preparation given in the Accuracy Check) in place of the sample.

Note: Sample temperature should be 21 ±4 °C (70 ±7 °F).

6. Add 1 mL of HydraVer 2 Hydrazine Reagent to each sample cell. Swirl to mix.

Note: A yellow color will develop if hydrazine is present.

Note: HydraVer 2 Hydrazine Reagent will cause a faint yellow color to appear in the blank.

7. Press: SHIFT TIMER

A 12-minute reaction period will begin.

8. When the timer beeps, the display will show:

μg/L N₂H₄

Immediately after the timer beeps, insert the blank into the cell holder. Close the light shield.

Note: The Pour-Thru Cell can be used with this procedure.

9. Press: ZERO

The display will show: WAIT

then:

0, μg/L N₂H₄

10. Place the prepared sample into the cell holder. Close the light shield.

11. Press: READ/ENTER

The display will show: WAIT

then the result in μg/L hydrazine will be displayed.

Note: For a DR2000 in the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
**HYDRAZINE, continued**

**INSTRUMENT SETUP**
For a DR/2000 with software versions 1.261 and 1.27, enter the following calibration as an operator–programmed calibration. Follow the steps in the *Operation* section of the *DR/2000 Instrument Manual*. Store the method as follows:

\[
\begin{align*}
nm &= 455 \\
\text{Decimal} &= 0000. \\
\text{Units} &= \mu g/l \\
\text{Symbol} &= N_2H_4 \\
\text{Timer 1} &= 12:00
\end{align*}
\]

The calibration is first entered with 0.000 absorbance values for the #0 through #9 standards. To do this, do not place anything in the sample cell compartment. Begin by storing standards #0 through #9 as the concentrations shown in the table below, with nothing in the sample cell compartment.

Accept 0.000 Abs. as the value for all standards. Store the calibration by pressing **SHIFT READ/ENTER**. Next, the values for the #1 through #9 standard must be changed to the values given below.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>#0</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>#1</td>
<td>41</td>
<td>0.125</td>
</tr>
<tr>
<td>#2</td>
<td>82</td>
<td>0.250</td>
</tr>
<tr>
<td>#3</td>
<td>122</td>
<td>0.375</td>
</tr>
<tr>
<td>#4</td>
<td>164</td>
<td>0.500</td>
</tr>
<tr>
<td>#5</td>
<td>206</td>
<td>0.625</td>
</tr>
<tr>
<td>#6</td>
<td>248</td>
<td>0.750</td>
</tr>
<tr>
<td>#7</td>
<td>333</td>
<td>1.000</td>
</tr>
<tr>
<td>#8</td>
<td>420</td>
<td>1.250</td>
</tr>
<tr>
<td>#9</td>
<td>509</td>
<td>1.500</td>
</tr>
</tbody>
</table>

The method is now stored as an operator–programmed method with a method number between 950 and 999. Record the method number for future reference.

For a DR/2000 with software versions 2.0 and 2.2, enter the calibration as an update to Hach–stored programs. (Stored program number 230 has been replaced with number 231.)

1. Press: **1 0**

2. Press: **SHIFT CONFIG METH**

3. Press: **EDIT PROG BATT READ ENTER**

4. Within 3 seconds, press:

   ![SHIFT PROG CONFIG METH]

   The display will show:

   **ENTER** *nm*

   If the display returns to the METHOD prompt, repeat the sequence.

5. Press:

   ![4 5 5]

   If you make an error, press **SHIFT CLEAR** and re-enter the number. When the number is correct, press **READ/ENTER**. The display will show:

   **DECIMAL? 00.00**

6. Use the arrow keys to correctly position the decimal point. For this method, press the **DOWN ARROW** key twice. The display will show:

   **DECIMAL? 0000.**

7. When the decimal point is correctly positioned, press: **READ/ENTER**. The display will show:

   **UNITS?**

8. Use the arrow keys to select the appropriate unit of measure. For this method, press the **DOWN ARROW** key 3 times. The display will show:

   **\( \mu g/l \)**

9. With the proper unit of measure displayed, press **READ/ENTER**. The display will show:

   **SYMBOL?**

10. Use the arrow keys to construct the correct symbol display. For this method, press the **DOWN ARROW** key repeatedly until you see:

    **\( \mu g/l \ ^n \)**

11. Press **SHIFT** to make the “\( n \)” uppercase. The display will show:

    **\( \mu g/l \ N \)**

12. Press the **READ/ENTER** key to accept the capital “\( N \).”

13. Press 2 on the numeric keypad. The display will show:

    **\( \mu g/l \ N_2 \)**

14. Press the **READ/ENTER** key to accept the subscript 2.
15. Continue to construct the display:
\[ \mu g/l \text{ N}_2\text{H}_4 \]
The space is the character displayed after one press of the down arrow.

16. When the last character of the symbol is accepted with the READ/ENTER key, the display will show:
Timer?

17. This method uses one timer, so press SHIFT TIMER. The display will show:
MM:SS TIME 1 ?

18. To enter the timer value of 12 minutes, press:

1 2 0 0

19. Press READ/ENTER to accept the timer value.
The display will show:
MM:SS TIME 2 ?

20. Press READ/ENTER to complete the timer entry.
The display will show:
# 1 Data

21. Enter the following 12 numbers as shown.
Complete each number entry by pressing READ/ENTER.

<table>
<thead>
<tr>
<th># 1 Data</th>
<th># 2 Data</th>
<th># 3 Data</th>
<th># 4 Data</th>
<th># 5 Data</th>
<th># 6 Data</th>
<th># 7 Data</th>
<th># 8 Data</th>
<th># 9 Data</th>
<th># 10 Data</th>
<th># 11 Data</th>
<th>Checksum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10537</td>
<td>10282</td>
<td>10794</td>
<td>10795</td>
<td>11052</td>
<td>11564</td>
<td>65535</td>
<td>65535</td>
<td>5461</td>
<td>512</td>
<td>60077</td>
</tr>
</tbody>
</table>

The final number is a check value which is used to determine if the data sequence was correctly entered. If an error is made during number entry, the display will return to the prompt for date number 1 and the entire sequence must be re-entered. If all numbers are correctly entered, the display will return to the method prompt and is ready for use.

22. Once the new method 231 has been successfully entered, block access to the now obsolete method 230.
Press:

SHIFT METH

Press:

2 3 0

Within 3 seconds press:

SHIFT PROG METH

The display will show:

800 CONFIGURE
Press: READ/ENTER three times to return to:
METHOD #?

Access to method 230 is blocked.

SAMPLING AND STORAGE
Samples collected in glass or plastic bottles should be filled completely and capped tightly. Avoid excessive agitation or exposure to air. Samples must be analyzed immediately after collection and cannot be preserved for late analysis.

ACCURACY CHECK
Standard Solution Method
To assure the accuracy of the test, prepare the following solutions:
a) Prepare a 25 mg/L stock solution by dissolving 0.1016 g of hydrazine sulfate in demineralized water then diluting to 1000 mL. Prepare stock solution daily.

b) Prepare a 0.1 mg/L (1000 μg/L) hydrazine working solution by diluting 0.4 mL of the 25 mg/L stock solution to 100 mL with demineralized water. Prepare just before analysis.

c) Use the working solution in place of the sample in Step 5. The result should be 100 μg/L hydrazine.

PRECISION
In a single laboratory, using standard solutions of 204 μg/L hydrazine (N\textsubscript{2}H\textsubscript{4}) and 2 representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±4.2 μg/L hydrazine. For better precision, ±0.7 μg/L hydrazine, use a TenSette Pipet to deliver the 1.0 mL HydraVer 2 Hydrazine reagent instead of the calibrated eyedropper.

INTERFERENCES
For highly colored or turbid samples, prepare a blank by oxidizing the hydrazine in a portion of the sample. This can be accomplished with a 1:1 mixture of demineralized water and a household bleach such as Clorox. Add one drop of the mixture to 25 mL of sample in a graduated mixing cylinder and invert to mix. Use this solution in Step 4, in place of demineralized water, to prepare the blank. There are no other common interferences.
SUMMARY OF METHOD
Hydrazine in the sample reacts with the
p-dimethylaminobenzaldehyde from the HydraVer 2
Reagent to form a yellow color which is proportional
to the hydrazine concentration.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HydraVer 2 Hydrazine Reagent</td>
<td>2 mL</td>
<td>100 mL* MDB</td>
<td>1790–32</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>25 mL</td>
<td>4 L</td>
<td>272–56</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder, graduated, 25 mL</td>
<td>1 each</td>
<td></td>
<td>508–40</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrazine Sulfate, ACS</td>
<td>100 g</td>
<td></td>
<td>742–26</td>
</tr>
</tbody>
</table>

OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder, graduated, mixing, 25 mL</td>
<td>each</td>
<td></td>
<td>1896–40</td>
</tr>
<tr>
<td>Flask, volumetric, 100 mL</td>
<td>each</td>
<td></td>
<td>547–42</td>
</tr>
<tr>
<td>Flask, volumetric, 1000 mL</td>
<td>each</td>
<td></td>
<td>547–53</td>
</tr>
<tr>
<td>Pipet, serological, 1 mL</td>
<td>each</td>
<td></td>
<td>532–35</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td></td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td></td>
<td>21856–96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 1.00 mL</td>
<td>each</td>
<td></td>
<td>14515–35</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td></td>
<td>14651–00</td>
</tr>
<tr>
<td>Pour-Thru Cell Assembly Kit</td>
<td>each</td>
<td></td>
<td>45215–00</td>
</tr>
<tr>
<td>Thermometer, –20 to 105 °C</td>
<td>each</td>
<td></td>
<td>1877–01</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes.
IODINE (0 to 7.00 mg/L)  For water, wastewater and seawater

DPD Method* (Powder Pillows or AccuVac Ampuls)

USING POWDER PILLOWS

1. Enter the stored program number for iodine (I₂)–powder pillows.
   
   Press 2 4 0 READ/ENTER
   
   The display will show:
   DIAL nm TO 530

   Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

   Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

   Note: Samples must be analyzed immediately and cannot be preserved for later analysis.

2. Rotate the wavelength dial until the small display shows:
   530 nm

3. Press: READ/ENTER
   
   The display will show:
   mg/l I₂

4. Fill a cell with 25 mL of sample.

   Note: The Pour–Thru Cell can be used with this procedure if it is rinsed shortly after each analysis with demineralized water.

*Adapted from Palin, A.T., *Inst Water Eng.*, 21 (6), 537–547 (1967)
5. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

*Note:* A pink color will develop if iodine is present.

*Note:* Accuracy is unaffected by undissolved powder.

6. Press: **SHIFT TIMER**
A 3-minute reaction period will begin.

7. When the timer beeps the display will show: 
   **mg/L I₂**
   Fill a second sample cell with 25 mL of sample (the blank). Place it into the cell holder.

8. Press: **ZERO**
The display will show: 
   **WAIT**
   then: 
   **0.00 mg/L I₂**

9. Within three minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield.

10. Press: **READ/ENTER**
The display will show: 
    **WAIT**
    then the result in mg/L I₂ will be displayed.

*Note:* If the sample temporarily turns yellow after reagent addition, or reads 
OVER-RANGE, dilute a fresh sample. Repeat the test. A slight loss of iodine may occur because of the dilution. Multiply by the appropriate dilution factor (see Sample Dilution Techniques in Section I).

*Note:* In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
USING ACCUVAC AMPULS

1. Enter the stored program number for
iodine (I₂)–AccuVac
ampul.

2. Rotate the wavelength
dial until the small display
shows:
530 nm

3. Press: READ/ENTER
The display will show:
mg/l I₂ AV

4. Fill a zeroing vial (the
blank) with at least 10 mL
of sample. Collect at least
40 mL of sample in a
50–mL beaker.

Note: DR/2000s with software
versions 3.0 and greater will
display “P” and the program
number.

Note: Instruments with software
versions 3.0 and greater will not
display “DIAL nm TO message
if the wavelength is already set
correctly. The display will show
the message in Step 3. Proceed
with Step 4.

5. Fill a DPD Total
Chlorine Reagent
AccuVac Ampul with
sample.

6. Quickly invert the
ampul several times to
mix. Wipe off any liquid
or fingerprints.

7. Press: SHIFT TIMER
A 3–minute reaction
period will begin.

8. Place the AccuVac
Vial Adapter into the cell
holder of the instrument.

Note: Place the grip tab at the
rear of the cell holder.

Note: Keep the tip immersed
while the ampul fills completely.

Note: A pink color will form if
iodine is present.

Note: Accuracy is unaffected by
undissolved powder.
9. When the timer beeps the display will show: mg/L $\text{I}_2$ AV
   Place the blank into the cell holder. Close the light shield.

10. Press: **ZERO**
    The display will show: **WAIT**
    Then: 0.00 mg/L $\text{I}_2$ AV

11. Within three minutes after the timer beeps, place the AccuVac ampul into cell holder. Close the light shield.

12. Press: **READ/ENTER**
    The display will show: **WAIT**
    Then the result in mg/L $\text{I}_2$ will be displayed.

   **Note:** If the sample temporarily turns yellow after sample addition, or shows OVER–RANGE, dilute a fresh sample. Repeat the test. A slight loss of iodine may occur because of the dilution. Apply the appropriate dilution factor (see Sample Dilution Techniques in Section I).

   **Note:** In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

---

**ACCURACY CHECK**

*Standard Additions Method*

a) Snap the top off the Chlorine Voluette Ampule Standard Solution, 50 to 75 mg/L $\text{Cl}_2$.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 25–mL water samples. Swirl gently to mix. (For AccuVac ampuls, use 50–mL beakers.)

c) Analyze each sample as described above. Each 0.1 mL of standard should cause an incremental increase in iodine, the exact value of which depends on the chlorine concentration in the Voluette. Check the certificate enclosed with the Voluette for the incremental chlorine value. Multiply by 3.6 to obtain the value for iodine.

d) If these increases do not occur, see Standard Additions in Section I for more information.

---

**PRECISION**

In a single laboratory, using a standard solution of 1.00 mg/L chlorine and two representative lots of reagent with the DR2000, a single operator obtained a standard deviation of ±0.012 chlorine. This is equivalent to ±0.04 mg/L iodine.

In a single laboratory, using a standard solution of 1.10 mg/L chlorine and two representative lots of AccuVac ampuls with the DR2000, a single operator obtained a standard deviation of ±0.009 mg/L chlorine. This is equivalent to ±0.03 mg/L iodine.

**INTERFERENCES**

Samples containing more than 300 mg/L alkalinity or 150 mg/L acidity as CaCO$_3$ may not develop the full amount of color, or it may instantly fade. Neutralize these samples to a pH of 6 to 7 with 1 N sulfuric acid or 1 N sodium hydroxide. Determine the amount required on a separate 25–mL sample. Add the same amount to the sample to be tested. Correct for volume additions.
iodine, continued

Bromine, chlorine, ozone and oxidized forms of manganese and chromium also may react and read as iodine. To compensate for the effects of manganese (Mn^{4+}) or chromium (Cr^{6+}), adjust pH to 6 to 7 as described above. Add three drops of potassium iodide, 30 g/L, to 25 mL of sample, mix and wait one minute. Add three drops of sodium arsenite, 5 g/L, and mix. Analyze this sample as described above. (If chromium is present, allow the same reaction period with the DPD for both analyses.) Subtract the result of this test from the original analysis to obtain the accurate iodine result. DPD Reagent Powder Pillows and AccuVac ampuls are formulated with a buffer which will withstand high levels (>1000 mg/L) of hardness without interference.

SUMMARY OF METHOD
Iodine reacts with DPD (N, N-diethyl-p-phenylenediamine) to form a red color which is proportional to the total iodine concentration.

**REQUIRED REAGENTS** (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPD Total Chlorine Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>100/pkg</td>
<td>14064–99</td>
</tr>
</tbody>
</table>

**REQUIRED REAGENTS** (Using AccuVac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPD Total Chlorine Reagent AccuVac Ampuls</td>
<td>25/pkg</td>
<td>25030–25</td>
</tr>
</tbody>
</table>

**REQUIRED APPARATUS** (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>each</td>
</tr>
</tbody>
</table>

**REQUIRED APPARATUS** (Using AccuVac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac vial</td>
<td>each</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>each</td>
</tr>
<tr>
<td>Sample Cell, 10 mL, with cap</td>
<td>each</td>
</tr>
</tbody>
</table>

**OPTIONAL REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine Standard Solution Voluette Ampule, 50 to 75 mg/L Cl₂, 10 mL</td>
<td>16/pkg</td>
<td>14268–10</td>
</tr>
<tr>
<td>Potassium Iodide Solution, 30 g/L</td>
<td>100 mL*MDB</td>
<td>343–32</td>
</tr>
<tr>
<td>Sodium Arsenite Solution, 5 g/L</td>
<td>100 mL*MDB</td>
<td>1047–32</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 1 N</td>
<td>100 mL*MDB</td>
<td>1045–32</td>
</tr>
<tr>
<td>Sulfuric Acid Standard Solution, 1 N</td>
<td>100 mL*MDB</td>
<td>1270–32</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
</tr>
</tbody>
</table>

**OPTIONAL APPARATUS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuVac Snaper Kit</td>
<td>each</td>
<td>24052–00</td>
</tr>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
</tr>
<tr>
<td>Cylinder, graduated, 25 mL, poly</td>
<td>each</td>
<td>1081–40</td>
</tr>
<tr>
<td>Graph Paper, linear</td>
<td>100/pkg</td>
<td>22313–00</td>
</tr>
<tr>
<td>pH Meter, EC10, portable</td>
<td>each</td>
<td>50050–00</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes.*
IRON, FERROUS (0 to 3.00 mg/L)  
For water, wastewater and seawater

1.10 Phenanthroline Method* (Powder Pillows or AccuVac Ampuls)
USING POWDER PILLOWS

1. Enter the stored program number for ferrous iron, (Fe²⁺)– powder pillows.

Press: 2 5 5 READ/ENTER

The display will show:
DIAL nm TO 510

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined.

2. Rotate the wavelength dial until the small display shows:
510 nm

3. Press: READ/ENTER
The display will show:
mg/l Fe²⁺

4. Fill a sample cell with 25 mL of sample.

Note: For proof of accuracy, use a 1.0 mg/L ferrous iron standard solution (preparation given in the Accuracy Check) in place of the sample.

*Adapted from Standard Methods for the Examination of Water and Wastewater
5. Add the contents of one Ferrous Iron Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: An orange color will form if ferrous iron is present.

Note: Undissolved powder does not affect accuracy.

6. Press: **SHIFT TIMER**
A 3-minute reaction period will begin.

7. When the timer beeps, the display will show:

\[ \text{mg/L Fe}^{2+} \]

Fill a second sample cell (the blank) with 25 mL of sample.

8. Place the blank into the cell holder. Close the light shield.

*Note: The Pour-Thru Cell can be used with this procedure.*

9. Press: **ZERO**
The display will show:

\[ \text{WAIT} \]
then:

\[ 0.00 \text{ mg/L Fe}^{2+} \]

10. Place the prepared sample into the cell holder. Close the light shield.

11. Press: **READ/ENTER**
The display will show:

\[ \text{WAIT} \]
then the result in mg/L Fe\(^{2+}\) will be displayed.

*Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*
IRON, FERROUS, continued

USING ACCUVAC AMPULS

1. Enter the stored program number for ferrous iron (Fe²⁺) Accuvac ampuls.

Press: 2 5 7 READ/ENTER

The display will show:
DIAL nm TO 510

Note: DR2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: Analyze samples as soon as possible to prevent air oxidation of ferrous iron to ferric iron, which is not determined.

2. Rotate the wavelength dial until the small display shows:

510 nm

3. Press: READ/ENTER

The display will show:
mg/l Fe²⁺ AV

4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50–mL beaker.

Note: For proof of accuracy, a 1.0 mg/L ferrous iron standard solution (preparation given in the Accuracy Check) can be used in place of the sample.
5. Fill a Ferrous Iron AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.

6. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: An orange color will form if ferrous iron is present.

Note: Undissolved powder does not affect accuracy.

7. Press: SHIFT TIMER

A 3-minute reaction period will begin.

8. Place the AccuVac Vial Adapter into the cell holder.

Note: Place the grip tab at the rear of the cell holder.

9. When the timer beeps, the display will show:

   mg/l Fe²⁺ AV

Place the blank into the cell holder. Close the light shield.

10. Press: ZERO

The display will show: WAIT

then:

   0.00 mg/l Fe²⁺ AV

11. Place the AccuVac ampul into the cell holder.

Close the light shield.

12. Press: READ/ENTER

The display will show: WAIT

then the result in mg/L Fe²⁺ will be displayed.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
ACCUACY CHECK
Standard Solution Method
Prepare a ferrous iron stock solution (100 mg/L Fe) by dissolving 0.7022 grams of ferrous ammonium sulfate, hexahydrate, in demineralized water. Dilute to 1 liter. Prepare immediately before use. Dilute 1.00 mL of this solution to 100 mL with demineralized water to make a 1.0 mg/L standard solution. Prepare this immediately before use.

PRECISION
In a single laboratory, using an iron standard solution of 1.000 mg/L Fe\(^{2+}\) and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.006 mg/L Fe\(^{2+}\). In a single laboratory using a standard solution of 1.000 mg/L Fe\(^{2+}\) and two representative lots of AccuVac ampuls with the DR/2000, a single operator obtained a standard deviation of ±0.009 mg/L Fe\(^{2+}\).

SUMMARY OF METHOD
The 1,10 phenanthroline indicator in Ferrous Iron Reagent reacts with ferrous iron in the sample to form an orange color in proportion to the iron concentration. Ferric iron does not react. The ferric iron (Fe\(^{3+}\)) concentration can be determined by subtracting the ferrous iron concentration from the results of a total iron test.

REQUIRED REAGENTS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous Iron Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>100/pkg</td>
<td>1037–69</td>
</tr>
</tbody>
</table>

REQUIRED REAGENTS (Using AccuVac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous Iron Reagent AccuVac Ampuls</td>
<td>1 ampul</td>
<td>25/pkg</td>
<td>25140–25</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968–00</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using AccuVac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac Vial</td>
<td>1</td>
<td>each</td>
<td>43784–00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>1</td>
<td>each</td>
<td>500–41</td>
</tr>
<tr>
<td>Sample Cell, 10 mL, with cap.</td>
<td>1</td>
<td>each</td>
<td>21228–00</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous Ammonium Sulfate, hexahydrate, ACS</td>
<td>113 g</td>
<td>11256–14</td>
<td></td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
<td></td>
</tr>
</tbody>
</table>

OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuVac Snapper Kit</td>
<td></td>
<td>24052–00</td>
<td></td>
</tr>
<tr>
<td>Clippers, shears, 7–1/4”</td>
<td></td>
<td>23694–00</td>
<td></td>
</tr>
<tr>
<td>Flask, volumetric, 100 mL, Class B</td>
<td></td>
<td>547–42</td>
<td></td>
</tr>
<tr>
<td>Flask, volumetric, 1000 mL, Class B</td>
<td></td>
<td>547–53</td>
<td></td>
</tr>
<tr>
<td>Pipet, volumetric, 1 mL</td>
<td></td>
<td>515–35</td>
<td></td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td></td>
<td>14651–00</td>
<td></td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td></td>
<td>45215–00</td>
<td></td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
IRON, TOTAL (0 to 3.00 mg/L)  

For water, wastewater and seawater

FerroVer Method* (Powder Pillows or AccuVac Ampuls); USEPA approved for reporting wastewater analysis (digestion is required; see Section 1)**

USING POWDER PILLOWS

1. Enter the stored program number for iron (Fe), FerroVer, powder pillows.

Press: 265 READ/ENTER

The display will show:  
DIAL nm TO 510

Note: Determination of total iron needs a prior digestion; use the mild, vigorous or Digesdahl digestion (Section 1).

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:  
510 nm

The display will show:  
mg/l Fe FV

Note: For proof of accuracy, use a 1.0 mg/L iron standard solution (preparation given in the Accuracy Check) in place of the sample.

3. Press: READ/ENTER

4. Fill a cell with 25 mL of sample.

*Adapted from Standard Methods for the Examination of Water and Wastewater

**Federal Register: 45 (126) 43459 (June 27, 1980)
5. Add the contents of one FerroVer Iron Reagent Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

*Note:* An orange color will form if iron is present.

*Note:* Accuracy is not affected by undissolved powder.

6. Press: **SHIFT TIMER**

A 3-minute reaction period will begin.

*Note:* Samples containing visible rust should be allowed to react at least five minutes.

7. When the timer beeps, the display will show: **mg/L Fe FV**

Fill another sample cell (the blank) with 25 mL of sample.

8. Place the blank into the cell holder. Close the light shield.

*Note:* For turbid samples, treat the blank with one 0.2-gram scoop of RoVer Rust Remover. Swirl to mix.

*Note:* The Pour-Thru Cell can be used with this procedure.

9. Press: **ZERO**

The display will show: **WAIT**

then: **0.00 mg/L Fe FV**

10. Within thirty minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield.

*Note:* If more than five minutes elapse after the timer beeps, **ZERO SAMPLE** may appear. If so, remove the prepared sample. Insert the blank. Press: **ZERO**. Insert the prepared sample.

11. Press: **READ/ENTER**

The display will show: **WAIT**

then the result in mg/L iron will be displayed.

*Note:* In the constant-on mode, pressing **READ/ENTER** is not required. **WAIT** will not appear. When the display stabilizes, read the result.
USING ACCUVAC AMPULS

1. Enter the stored program number for iron (Fe), AccuVac ampuls.
   Press: 2 6 7 READ/ENTER
   The display will show: DIAL nm TO 510

   Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

   Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

   Note: If samples cannot be analyzed immediately, see Sampling and Storing, below. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows: 510 nm

   Note: Determination of total iron needs a prior digestion; use the mild, vigorous or Digesdahl digestion (Section I).

3. Press: READ/ENTER
   The display will show: mg/l Fe FV AV

   Note: For proof of accuracy, use a 1.0 mg/L iron standard solution (preparation given in the Accuracy Check) in place of the sample.

4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50–mL beaker.
5. Fill a FerroVer AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills completely.

6. Quickly invert the ampul several times to mix. Wipe off any liquid or fingerprints.

Note: An orange color will form if iron is present.

Note: Accuracy is not affected by undissolved powder.

7. Press: SHIFT TIMER

A 3-minute reaction period will begin.

Note: Samples containing visible rust should be allowed to react at least five minutes.

Note: Place the grip tab at the rear of the cell holder.

8. When the timer beeps, the display will show:

mg/l Fe FV AV

Place the AccuVac Vial Adapter into the cell holder of the instrument.

9. Place the blank into the cell holder. Close the light shield.

10. Press: ZERO

The display will show: WAIT

then:

0.00 mg/l Fe FV AV

11. Within thirty minutes after the timer beeps, place the AccuVac ampul into the cell holder. Close the light shield.

Note: If more than five minutes elapse after the timer beeps, ZERO SAMPLE may appear. If so, remove the prepared sample. Insert the blank. Press: ZERO. Insert the prepared sample.

12. Press: READ/ENTER

The display will show:

WAIT

then the result in mg/L iron will be displayed.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
IRON, TOTAL, continued

SAMPLING AND STORAGE
Collect samples in acid-cleansed glass or plastic containers. No acid addition is necessary if analyzing the sample immediately. To preserve samples, adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples may be stored up to six months at room temperature. Before analysis, adjust the pH to between 3 and 5 with 50.0 N Sodium Hydroxide Standard Solution. Correct the test result for volume additions (see Correction for Volume Additions in Section I).

If only dissolved iron is to be determined, filter the sample before acid addition using the labware listed under Optional Apparatus.

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off an Iron Voluette Ampule Standard Solution, 50 mg/L.

b) Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 25-mL water samples and mix thoroughly. (For AccuVac ampuls, use 50-mL beakers.)

c) Analyze each sample as described above. The iron concentration should increase 0.2 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method
Prepare a 1.0 mg/L iron standard by diluting 1.00 mL of Iron Standard Solution, 100 mg/L Fe, to 100 mL with demineralized water. Or, use the TenSette Pipet to dilute 1.0 mL of an Iron Voluette Ampule Standard Solution (50 mg/L) to 50 mL in a volumetric flask. Prepare this solution daily.

PRECISION
In a single laboratory, using a standard solution of 1.000 mg/L Fe and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.006 mg/L.

In a single laboratory, using a standard solution of 1.000 mg/L Fe and two representative lots of AccuVac ampuls with the DR/2000, a single operator obtained a standard deviation of ±0.009 mg/L Fe.

INTERFERENCES
The following will not interfere below the levels shown:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>185,000 mg/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>10,000 mg/L as CaCO₃</td>
</tr>
<tr>
<td>Magnesium</td>
<td>100,000 mg/ as CaCO₃</td>
</tr>
<tr>
<td>Molybdate Molybdenum</td>
<td>50 mg/L as Mo</td>
</tr>
</tbody>
</table>

A large excess of iron will inhibit color development. A diluted sample should be tested if there is any doubt about the validity of a result.

FerroVer Iron Reagent Powder Pillows and AccuVac ampuls contain a masking agent which eliminates potential interferences from copper.

Samples containing some forms of iron oxide require the mild, vigorous or Digesdahl digestion (Section I). After digestion, adjust the pH to between 2.5 and 5 with ammonium hydroxide.

Samples containing large amounts of sulfide should be treated as follows in a fume hood, or well ventilated area:

a) Add 5 mL of hydrochloric acid to 100 mL of sample and boil for 20 minutes.

b) Adjust the pH to between 2.5 and 5 with 5 N sodium hydroxide and readjust the volume to 100 mL with demineralized water. Analyze as described above.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment (see pH Interference in Section I).

REAGENT STORAGE
FerroVer Reagent Powder Pillows are stable indefinitely if stored properly. A cool, dry atmosphere is recommended. The reagent can be checked by adding the contents of a pillow to about 25 mL of water containing visible rust (such as a few drops of Rust Suspension). If the orange color does not form, the reagent should be replaced.

SUMMARY OF METHOD
FerroVer Iron Reagent reacts with all soluble iron and most insoluble forms of iron in the sample, to produce soluble ferrous iron. This reacts with the 1,10 phenanthroline indicator in the reagent to form an orange color in proportion to the iron concentration.
REQUIRED REAGENTS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FerroVer Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>854-66</td>
</tr>
</tbody>
</table>

REQUIRED REAGENTS (Using AccuVac Ampulls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FerroVer Iron Reagent AccuVac Ampuls</td>
<td>25/pkg</td>
<td>25070-25</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>each</td>
<td>968-00</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using AccuVac Ampulls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac vial</td>
<td>each</td>
<td>43784-00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>each</td>
<td>500-41</td>
</tr>
<tr>
<td>Sample Cell, 10-mL with screw cap</td>
<td>each</td>
<td>21228-00</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Hydroxide, ACS</td>
<td>500 mL</td>
<td>106-49</td>
</tr>
<tr>
<td>Hydrochloric Acid Standard Solution, 6 N</td>
<td>500 mL</td>
<td>884-49</td>
</tr>
<tr>
<td>Hydrochloric Acid, ACS</td>
<td>500 mL</td>
<td>134-49</td>
</tr>
<tr>
<td>Iron Standard Solution, 100 mg/L</td>
<td>100 mL</td>
<td>14175-42</td>
</tr>
<tr>
<td>Iron Volutte Ampule Standard, 50 mg/L</td>
<td>16/pkg</td>
<td>14254-10</td>
</tr>
<tr>
<td>Nitric Acid, ACS</td>
<td>500 mL</td>
<td>152-49</td>
</tr>
<tr>
<td>Nitric Acid Solution, 1:1</td>
<td>500 mL</td>
<td>2540-49</td>
</tr>
<tr>
<td>RoVer Rust Remover</td>
<td>454 g</td>
<td>300-11</td>
</tr>
<tr>
<td>Rust Suspension</td>
<td>15 mL DB</td>
<td>1279-36</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>100 mL MDB</td>
<td>2450-32</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272-56</td>
</tr>
</tbody>
</table>

OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuVac Snapper Kit</td>
<td>each</td>
<td>24052-00</td>
</tr>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968-00</td>
</tr>
<tr>
<td>Clippers, Shears 7-1/4&quot;</td>
<td>each</td>
<td>23694-00</td>
</tr>
<tr>
<td>Cylinder, graduated, poly, 25 mL</td>
<td>each</td>
<td>1081-40</td>
</tr>
<tr>
<td>Cylinder, graduated, poly, 100 mL</td>
<td>each</td>
<td>1081-42</td>
</tr>
<tr>
<td>Filter Discs, glass, 47 mm</td>
<td>each</td>
<td>2530-00</td>
</tr>
<tr>
<td>Filter Holder, membrane</td>
<td>100/pkg</td>
<td>2340-00</td>
</tr>
<tr>
<td>Filter Pump</td>
<td>each</td>
<td>2131-00</td>
</tr>
<tr>
<td>Flask, erlenmeyer, 250 mL</td>
<td>each</td>
<td>505-46</td>
</tr>
<tr>
<td>Flask, filtering, 500 mL</td>
<td>each</td>
<td>546-49</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 50 mL</td>
<td>each</td>
<td>14574-41</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 100 mL</td>
<td>each</td>
<td>14574-42</td>
</tr>
<tr>
<td>Hot Plate, 3 1/2&quot; diameter, 120 Vac</td>
<td>each</td>
<td>12067-01</td>
</tr>
<tr>
<td>Hot Plate, 3 1/2&quot; diameter, 240 Vac</td>
<td>each</td>
<td>12067-02</td>
</tr>
<tr>
<td>pH Meter, EC10, portable</td>
<td>each</td>
<td>50050-00</td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>each</td>
<td>391-33</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651-00</td>
</tr>
<tr>
<td>Pipet, serological, 2 mL</td>
<td>each</td>
<td>532-36</td>
</tr>
<tr>
<td>Pipet, serological, 5 mL</td>
<td>each</td>
<td>532-37</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700-01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700-01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856-96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 1.00 mL</td>
<td>each</td>
<td>14515-35</td>
</tr>
<tr>
<td>Pour-Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215-00</td>
</tr>
<tr>
<td>Spoon, measuring, 0.1 g</td>
<td>each</td>
<td>511-00</td>
</tr>
<tr>
<td>Spoon, measuring, 0.2 g</td>
<td>each</td>
<td>638-00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800-227-4224 to place an order.
**IRON** (0 to 1.300 mg/L)  For water and seawater

**FerroZine Method***

1. Enter the stored program number for total iron (Fe), FerroZine method.

   **Press: 2 6 0 READ/ENTER**

   The display will show: **DIAL nm to 562**

   **Note:** If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

   **Note:** Total iron determination needs a prior digestion; use any of the three procedures given in Digestion (Section I).

2. Rotate the wavelength dial until the small display shows: **562 nm**

   **Note:** Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3 Proceed with Step 4.

3. Press: **READ/ENTER**

   The display will show: **mg/l Fe FZ**

4. Fill a sample cell to the 25-mL mark with sample.

   **Note:** Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with demineralized water. These two steps will remove iron deposits which can cause slightly high results.

   **Note:** For proof of accuracy, use a 0.4 mg/L iron standard solution (preparation given in the Accuracy Check) in place of the sample.

---

5. Add the contents of one FerroZine Iron Reagent Solution Pillow to the cell (the prepared sample). Swirl to mix.

*Note:* Do not allow the clippers to come into contact with the contents of the pillow.

*Note:* 0.5 mL of FerroZine Iron Reagent Solution can be used in place of the solution pillow if preferred.

*Note:* If the sample contains rust, see Interferences below.

6. Press **SHIFT TIMER**

A 5-minute reaction period will begin.

*Note:* A violet color will develop if iron is present.

7. Fill another sample cell (the blank) with 25 mL of sample.

8. When the timer beeps, the display will show:

\[ \text{mg/l Fe FZ} \]

Insert the blank into the cell holder. Close the light shield.

*Note:* The Pour-Thru Cell can be used with this procedure.

9. Press **ZERO**

The display will show:

\[ \text{WAIT} \]

then:

\[ 0.000 \text{ mg/l Fe FZ} \]

10. Place the prepared sample into the cell holder. Close the light shield.

11. Press **READ/ENTER**

The display will show:

\[ \text{WAIT} \]

then the result in mg/L iron will be displayed.

*Note:* In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
IRON, continued

SAMPLING AND STORAGE
Collect samples in acid–washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with nitric acid (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature. If only dissolved iron is to be reported, filter sample immediately after collection and before addition of nitric acid.

Before testing, adjust the sample pH to 3 to 5 with ammonium hydroxide, ACS. Do not exceed pH 5 as iron may precipitate. Correct test results for volume additions (see Correction for Volume Additions, in Section I).

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off an Iron Voluette Ampule Standard, 25 mg/L Fe.

b) Use the TenSette Pipet to add 0.1 mL of standard to the prepared sample measured in Step 11.

c) Swirl to mix and allow another five–minute reaction period, then measure the iron concentration as in Step 11.

d) Add two additional 0.1 mL standard increments, taking a concentration reading after allowing the five–minute reaction period for each.

e) Each additional 0.1 mL increment of standard added should cause a 0.1 mg/L increase in the concentration reading.

f) If these increases do not occur, see Standard Additions (Section I) for more information.

Standard Solution Method
Prepare a 0.4 mg/L iron working solution as follows:

a) Pipet 1.00 mL of iron standard solution, 100 mg/L Fe, into a 250–mL volumetric flask.

b) Dilute to volume with demineralized water. This solution should be prepared daily. Analyze the working solution according to the above procedure.

PRECISION
In a single laboratory, using a standard solution of 0.80 mg/L iron and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.0027 mg/L iron.

INTERFERENCES
Copper and cobalt may interfere to give slightly high results.

Strong chelants, such as EDTA, will interfere in the FerroZine method for determining iron. The FerroVer or TPTZ methods should be used for these samples. The TPTZ method is suggested for low concentrations. Any of the three digestions give in Digestion (Section I) used in place of the treatments given below will eliminate the following interferences.

If rust or hydroxides are present, the sample, with the FerroZine Iron Reagent from Step 5, should be boiled for one minute in a boiling water bath then cooled to 24 °C (75 °F) before proceeding with Step 6. The reduced sample volume should be returned to 25 mL with demineralized water.

If the sample contains magnetite (black iron oxide) or ferrites, perform the following procedure.

a) Fill a 25–mL graduated cylinder with 25 mL of sample.

b) Transfer the sample water into a 125–mL erlenmeyer flask.

c) Add the contents of one FerroZine Iron Reagent Solution Pillow and swirl to mix.

d) Place the flask on a hot plate or over a flame and bring to a boil.

e) Continue boiling gently for 20 to 30 minutes.

Note: Do not allow to boil dry.
Note: A purple color will develop if iron is present.

f) Return the boiled sample to the graduated cylinder. Rinse the erlenmeyer flask with small amounts of demineralized water and empty into the graduated cylinder.

g) Return the sample volume to the 25–mL mark with demineralized water.

h) Pour the solution into a sample cell and swirl to mix.

i) Proceed with Steps 6 through 11.

SUMMARY OF METHOD
The FerroZine Iron Reagent forms a purple–colored complex with trace amounts of iron in samples that are buffered to a pH of 3.5. This method is applicable for determining trace levels of iron in chemical reagents and glycols and can be used to analyze samples containing magnetite (black iron oxide) or ferrites.
## REQUIRED REAGENTS AND APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FerroZine Iron Reagent Solution Pillows</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>2301–66</td>
</tr>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968–00</td>
</tr>
</tbody>
</table>

### OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Hydroxide, ACS</td>
<td>500 mL</td>
<td>106–49</td>
<td></td>
</tr>
<tr>
<td>Hydrochloric Acid Solution, 1:1 (6 N)</td>
<td>500 mL</td>
<td>884–49</td>
<td></td>
</tr>
<tr>
<td>FerroZine Iron Reagent Solution</td>
<td>1000 mL</td>
<td>2301–53</td>
<td></td>
</tr>
<tr>
<td>Iron Standard Solution, 100 mg/L Fe</td>
<td>100 mL</td>
<td>14175–42</td>
<td></td>
</tr>
<tr>
<td>Iron Standard Solution, Voluette ampule, 25 mg/L Fe, 10 mL</td>
<td>16/pkg</td>
<td>14253–10</td>
<td></td>
</tr>
<tr>
<td>Nitric Acid, ACS</td>
<td>500 mL</td>
<td>152–49</td>
<td></td>
</tr>
<tr>
<td>Nitric Acid Solution, 1:1</td>
<td>500 mL</td>
<td>2540–49</td>
<td></td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
<td></td>
</tr>
</tbody>
</table>

### OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
<td></td>
</tr>
<tr>
<td>Clippers, shears, 7–1/4”</td>
<td>each</td>
<td>23694–00</td>
<td></td>
</tr>
<tr>
<td>Cylinder, graduated, 25 mL</td>
<td>each</td>
<td>508–40</td>
<td></td>
</tr>
<tr>
<td>Dropper, calibrated, 0.5–mL &amp; 1.0–mL mark</td>
<td>6/pkg</td>
<td>23185–06</td>
<td></td>
</tr>
<tr>
<td>Flask, erlenmeyer, 125 mL</td>
<td>each</td>
<td>505–43</td>
<td></td>
</tr>
<tr>
<td>Flask, erlenmeyer, 50 mL</td>
<td>each</td>
<td>505–41</td>
<td></td>
</tr>
<tr>
<td>Flask, volumetric, 250 mL, Class B</td>
<td>each</td>
<td>547–46</td>
<td></td>
</tr>
<tr>
<td>Hot plate, 3 1/2” diameter, 120 Vac</td>
<td>each</td>
<td>12067–01</td>
<td></td>
</tr>
<tr>
<td>Hot plate, 3 1/2” diameter, 240 Vac</td>
<td>each</td>
<td>12067–02</td>
<td></td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>5 rolls/pkg</td>
<td>391–33</td>
<td></td>
</tr>
<tr>
<td>pH Meter, EC10, portable</td>
<td>each</td>
<td>50050–00</td>
<td></td>
</tr>
<tr>
<td>Pipet, serological, 2 mL</td>
<td>each</td>
<td>532–36</td>
<td></td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
<td></td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21866–96</td>
<td></td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 0.5 mL</td>
<td>each</td>
<td>14515–34</td>
<td></td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 1.00 mL</td>
<td>each</td>
<td>14515–35</td>
<td></td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651–00</td>
<td></td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
<td></td>
</tr>
<tr>
<td>Thermometer, –20 to 105 °C</td>
<td>each</td>
<td>1877–01</td>
<td></td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
IRON, TOTAL  
(0 to 1.80 mg/L)  
For water, wastewater and seawater

TPTZ Method* (Powder Pillows or AccuVac Ampuls)  
USING POWDER PILLOWS

1. Enter the stored program number for iron (Fe)–TPTZ powder pillow method.
Press: 2 7 0 READ/ENTER
The display will show:  
DIAL nm to 590

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:  
590 nm

Note: Total iron determination needs a prior digestion; use any of the three procedures given in Digestion (Section I).

3. Press: READ/ENTER
The display will show:  
mg/l Fe TPTZ

Note: Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with demineralized water. These two steps will remove iron deposits which can cause slightly high results.

4. Fill a 25–mL graduated mixing cylinder with 25 mL of sample.

Note: Sample pH is important in this test (see pH Interference in Section I).

Note: For proof of accuracy, use a 0.4 mg/L iron standard solution (preparation given in the Accuracy Check) in place of the sample.

5. Add the contents of one TPTZ Iron Reagent Powder Pillow (the prepared sample). Stopper and shake the cylinder for 30 seconds. Remove stopper.

Note: A blue color will develop if iron is present.

6. Press: **SHIFT TIMER**
A 3-minute reaction period will begin.

Note: Continue with Steps 7 to 9 while the timer is running.

7. Fill a second 25–mL graduated mixing cylinder with 25 mL of demineralized water.

8. Add the contents of one TPTZ Iron Reagent Powder Pillow to the demineralized water (the blank). Stopper and shake for 30 seconds. Remove stopper.

9. Transfer the prepared sample and blank into two matched 25–mL sample cells.

Note: The Pour–Thru Cell can be used with this procedure.

10. When the timer beeps, the display will show:

    \[ \text{mg/L Fe TPTZ} \]

    Insert the blank into the cell holder. Close the light shield.

11. Press: **ZERO**
The display will show: **WAIT**
then:

    \[ 0.00 \text{ mg/L Fe TPTZ} \]

12. Place the prepared sample into the cell holder. Close the light shield.
Press: **READ/ENTER**

The display will show: **WAIT**
then the result in mg/L iron will be displayed.

Note: In the constant–on mode, pressing **READ/ENTER** is not required. **WAIT** will not appear. When the display stabilizes, read the result.
1. Enter the stored program number for iron (Fe)-TPTZ AccuVac method.

Press: 272 READ/ENTER

The display will show: DIAL nm TO 590

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows: 590 nm

Note: Total iron determination needs a prior digestio; use any of the three procedures given in Digestion (Section I).

3. Press: READ/ENTER

The display will show: mg/L Fe TPTZ AV

Note: Rinse glassware with a 1:1 Hydrochloric Acid Solution. Rinse again with demineralized water. These two steps will remove iron deposits which can cause slightly high results.

4. Collect at least 40 mL of sample in a 50-mL beaker. Fill a zeroing vial with at least 10 mL of sample.

Note: Sample pH is important in this test (see pH Interference in Section I).

Note: For proof of accuracy, use a 0.4 mg/L iron standard solution (preparation given in the Accuracy Check) in place of the sample.
5. Insert the AccuVac Vial Adapter into the cell holder.
   
   Note: Insert the vial adapter so the grip tab is at the rear of the instrument.

6. Fill a TPTZ Iron AccuVac Ampul with sample.
   
   Note: Keep the tip immersed while the ampul fills completely.

7. Invert the ampul (the prepared sample) repeatedly to mix. Wipe off any liquid or fingerprints.
   
   Note: A blue color will develop if iron is present.

8. Press: SHIFT TIMER
   A 3-minute reaction period will begin.

---

9. When the timer beeps the display will show:
   **mg/l Fe TPTZ AV**
   Place the zeroing vial into the adapter. Close the light shield.

10. Press ZERO
    The display will show: WAIT
    then:
    **0.00 mg/l Fe TPTZ AV**

11. Place the prepared sample into the adapter. Close the light shield.

12. Press: READ/ENTER
    The display will show: WAIT
    then the result in mg/L iron will be displayed.

   Note: For the most accurate results, run the procedure using iron-free demineralized water instead of sample in Step 4. Subtract the value obtained in Step 12 from all later tests. Repeat for each new lot of AccuVac reagent.

   Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
IRON, TOTAL, continued

SAMPLING AND STORAGE
Collect samples in acid—washed glass or plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 2 mL per liter). Store samples preserved in this manner up to six months at room temperature. If reporting only dissolved iron, filter sample immediately after collection and before addition of nitric acid.

Before testing, adjust the pH of the stored sample to between 3 to 4 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 5 as iron may precipitate. Correct the test result for volume addition (see Correction for Volume Additions in Section I).

ACCURACY CHECK
Using Powder Pills
a) Snap the neck off an Iron Voluette Ampule Standard, 25 mg/L Fe.

b) Use the TenSette Pipet to add 0.1 mL of standard to the prepared sample measured in Step 12. Swirl to mix.

c) Measure the iron concentration as in Step 12.

Note: The measurement may be taken immediately, without the three–minute reaction period of Step 6.

d) Add two additional 0.1–mL standard increments, taking a concentration reading after each addition. The iron concentration reading should increase by 0.1 mg/L for each 0.1 mL addition of standard.

e) If these increases do not occur see Standard Additions in Section I for more information.

Using AccuVac Ampuls
a) Measure 25.0 mL of sample using a graduated cylinder into each of three 50–mL beakers.

b) Snap the neck off a Voluette Ampule Standard for Iron, 25 mg/L Fe.

c) Add 0.1, 0.2 and 0.3 mL of standard, respectively, to the three 50–mL beakers using the TenSette Pipet. Swirl to mix.

d) Fill a TPTZ Iron AccuVac Ampul completely from each beaker.

e) Measure the concentration of each ampul according to the above procedure. The iron concentration reading should increase by 0.1 mg/L for each 0.1 mL addition of standard.

f) If these increases do not occur, see Standard Additions, (Section I) for more information.

Prepare a 0.4 mg/L iron working solution as follows:

a) Pipet 1.00 mL of Iron Standard Solution, 100 mg/L Fe, into a 250–mL volumetric flask.

b) Dilute to volume with demineralized water. Prepare this solution fresh daily. Analyze the working solution according to the above procedure.

PRECISION
In a single laboratory, using a standard solution of 1.00 mg/L Fe and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.009 mg/L Fe.

In a single laboratory, using a standard solution of 1.00 mg/L Fe and one representative lot of AccuVac ampuls with a DR/2000, a single operator obtained a standard deviation of ±0.007 mg/L Fe.

INTERFERENCES
In the powder pillow procedure, if the sample, without a TPTZ Iron Reagent Powder Pillow, has a color or turbidity greater than the blank of Step 8 (demineralized water plus TPTZ Iron Reagent), use the sample as the blank.

A sample pH of less than 3 or greater than 4 after the addition of reagent may inhibit color formation, cause the developed color to fade quickly or result in turbidity. Adjust the sample pH in the sample cell before the addition of reagent to between 3 to 8 by using a pH meter or pH paper and adding, dropwise, an appropriate amount of iron–free acid or base such as 1.0 N Sulfuric Acid Standard Solution or 1.0 N Sodium Hydroxide Standard Solution. Make a volume correction if significant volumes of acid or base are used (see Correction for Volume Additions in Section I).

Interference tests were performed using an iron concentration of 0.5 mg/L. When interferences occurred, the color formation was inhibited or a precipitate formed. The following do not interfere with the test when present up to the levels listed:

<table>
<thead>
<tr>
<th>Element</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>4.0 mg/L</td>
</tr>
<tr>
<td>Chromium (3+)</td>
<td>0.25 mg/L</td>
</tr>
<tr>
<td>Chromium (6+)</td>
<td>1.2 mg/L</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.05 mg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>0.6 mg/L</td>
</tr>
<tr>
<td>Cyanide</td>
<td>2.8 mg/L</td>
</tr>
<tr>
<td>Manganese</td>
<td>50.0 mg/L</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.4 mg/L</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>4.0 mg/L</td>
</tr>
<tr>
<td>Nickel</td>
<td>1.0 mg/L</td>
</tr>
<tr>
<td>Nitrate Ion</td>
<td>0.8 mg/L</td>
</tr>
</tbody>
</table>
IRON, TOTAL, continued

SUMMARY OF METHOD
The TPTZ Iron Reagent forms a deep blue-purple color with ferrous iron. The indicator is combined with a reducing agent which converts precipitated or suspended iron, such as rust, to the ferrous state. The amount of ferric iron present can be determined as the difference between the results of a ferrous iron test and the concentration of total iron.

REQUIRED REAGENTS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPTZ Iron Reagent Powder Pillows</td>
<td>2 pillows</td>
<td>25/pkg</td>
<td>22756–68</td>
</tr>
</tbody>
</table>

REQUIRED REAGENTS (Using AccuVac Ampulls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPTZ Low Range Iron Reagent AccuVac Ampulls</td>
<td>1 ampul</td>
<td>25/pkg</td>
<td>25100–25</td>
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</tbody>
</table>

REQUIRED APPARATUS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968–00</td>
</tr>
<tr>
<td>Stopper, hollow, No. 1</td>
<td>2</td>
<td>6/pkg</td>
<td>14480–01</td>
</tr>
<tr>
<td>Cylinder, graduated, mixing, 25 mL</td>
<td>2</td>
<td>each</td>
<td>1896–40</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using AccuVac Ampulls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac Vial</td>
<td>1</td>
<td>each</td>
<td>43784–00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>1</td>
<td>each</td>
<td>500–41</td>
</tr>
<tr>
<td>Sample Cell, 10–mL with cap</td>
<td>1</td>
<td>each</td>
<td>21228–00</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric Acid Solution, 1:1 6.0 N</td>
<td>500 mL</td>
<td>884–49</td>
<td></td>
</tr>
<tr>
<td>Iron Standard Solution, 100 mg/L Fe</td>
<td>105 mL</td>
<td>14175–42</td>
<td></td>
</tr>
<tr>
<td>Iron Standard Solution, Voluette ampule, 25 mg/L Fe, 10 mL</td>
<td>16/pkg</td>
<td>14253–10</td>
<td></td>
</tr>
<tr>
<td>Nitric Acid, ACS</td>
<td>500 mL</td>
<td>152–49</td>
<td></td>
</tr>
<tr>
<td>Nitric Acid Solution, 1:1</td>
<td>500 mL</td>
<td>2540–49</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 1.0 N</td>
<td>100 mL MDB</td>
<td>1045–32</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>100 mL MDB</td>
<td>2450–32</td>
<td></td>
</tr>
<tr>
<td>Sulfuric Acid Standard Solution</td>
<td>100 mL MDB</td>
<td>1270–32</td>
<td></td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
<td></td>
</tr>
</tbody>
</table>

OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuVac Snapper Kit</td>
<td></td>
<td>24052–00</td>
<td></td>
</tr>
<tr>
<td>Ampule Breaker Kit</td>
<td></td>
<td>21968–00</td>
<td></td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td></td>
<td>500–41</td>
<td></td>
</tr>
<tr>
<td>Clippers, shears, 7-1/4&quot;</td>
<td></td>
<td>23694–00</td>
<td></td>
</tr>
<tr>
<td>Cylinder, graduated, 25 mL</td>
<td></td>
<td>1081–40</td>
<td></td>
</tr>
<tr>
<td>Dropper, graduated, 0.5 and 1.0 mL marks</td>
<td></td>
<td>21247–10</td>
<td></td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 250 mL</td>
<td></td>
<td>14574–46</td>
<td></td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>5 rolls/pkg</td>
<td>391–33</td>
<td></td>
</tr>
<tr>
<td>pH Meter, EC10, portable</td>
<td></td>
<td>50050–00</td>
<td></td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td></td>
<td>14651–00</td>
<td></td>
</tr>
<tr>
<td>Pipet, serological, 2 mL</td>
<td></td>
<td>532–36</td>
<td></td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td></td>
<td>19700–01</td>
<td></td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
<td></td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 1.00 mL</td>
<td></td>
<td>14515–35</td>
<td></td>
</tr>
<tr>
<td>Pour-Thru Cell Assembly Kit</td>
<td></td>
<td>45215–00</td>
<td></td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
IRON, TOTAL (0 to 1.80 mg/L) For cooling water containing molybdate–based treatment

FerroMo Method*

1. Enter the stored program number for iron (Fe)–FerroMo method.
   Press: 2 7 5 READ/ENTER
   The display will show: DIAL nm to 590

   Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows: 590 nm

3. Press: READ/ENTER
   The display will show: mg/l Fe FM

   Note: Total Iron determination needs a prior digestion. Use any of the three procedures given in the Chemical Analysis section.

4. Fill a 50–mL graduated mixing cylinder with 50 mL of water to be tested.
   Note: Sample pH is important in this test (see pH Interference in Section I).

   Note: For proof of accuracy, use a 0.4 mg/L iron standard solution (preparation given in Accuracy Check) in place of the sample.

5. Add the contents of one FerroMo Iron Reagent Powder Pillow to the graduated cylinder. Stopper and invert several times to dissolve the reagents. This is the prepared sample.

6. Transfer 25 mL of the prepared sample into a 25–mL matched sample cell.

7. Add the contents of one FerroMo Iron Reagent 2 Powder Pillow to the sample cell. Swirl to dissolve the reagents. This is the developed sample.

   Note: A blue color will develop if iron is present.

8. Press: SHIFT TIMER
   A 3–minute reaction period will begin.

9. When the timer beeps, the display will show:

\[
\text{mg/l Fe FM}
\]

Fill a second sample cell with 25 mL of the prepared sample (the blank).

10. Insert the blank into the cell holder. Close the light shield.

*Note: The Pour-Thru Cell can be used with this procedure.*

11. Press: **ZERO**

The display will show:

**WAIT**

then:

\[
0.00 \text{ mg/l Fe FM}
\]

12. Place the developed sample into the cell holder. Close the light shield.

*Note: For samples containing high levels of molybdate (\( \geq 100 \text{ mg/L} \)), read the sample immediately after zeroing the blank.*

13. Press: **READ/ENTER**

The display will show:

**WAIT**

then the result in mg/L iron will be displayed.

*Note: For the most accurate results, run the procedure using iron-free demineralized water instead of sample in Step 4. Subtract the value obtained in Step 13 from all later tests. Repeat for each new lot of FerroMo reagents.*

*Note: In the constant-on mode, pressing **READ/ENTER** is not required. **WAIT** will not appear. When the display stabilizes, read the result.*
IRON, TOTAL, continued

SAMPLING AND STORAGE
Collect samples in acid–washed glass or plastic bottles. To preserve samples, adjust the sample pH to 2 or less with hydrochloric acid (about 2 mL per liter). Samples preserved in this manner can be stored up to six months at room temperature. If only dissolved iron is to be reported, filter sample immediately after collection and before addition of hydrochloric acid.

Before testing, adjust the sample pH to 3 to 4 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 5 as iron may precipitate. Correct test results for volume addition (see Correction for Volume Additions in Section I).

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off an Iron Voluette Ampule Standard, 25 mg/L Fe.

b) Use the TenSette Pipet to add 0.1 mL of standard to the prepared sample measured in Step 13.

c) Swirl to mix, then measure the iron concentration as in Step 13.

d) Add two additional 0.1–mL increments of standard, taking a reading after each addition. The iron concentration should increase by 0.1 mg/L after each addition of 0.1 mL addition of standard.

e) If these increases do not occur, see Standard Additions in Section I for more information.

Prepare a 0.4 mg/L iron working solution as follows:

a) Pipet 1.00 mL of Iron Standard Solution, 100 mg/L Fe, into a 250–mL volumetric flask.

b) Dilute to volume with demineralized water. Prepare this solution fresh daily. Analyze the working solution according to the above procedure.

PRECISION
In a single laboratory, using a standard solution of 1.00 mg/L Fe and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.007 mg/L Fe.

INTERFERENCES
A sample pH of less than 3 or greater than 4 after the addition of reagents may inhibit color formation, cause the developed color to fade quickly or result in turbidity. Adjust the sample pH in the graduated cylinder before the addition of reagent to between 3 to 8 by using a pH meter or pH paper and adding, dropwise, an appropriate amount of iron–free acid or base such as 1.0 N Sulfuric Acid Standard Solution or 1.0 N Sodium Hydroxide Standard Solution. Make a volume correction if significant volumes of acid or base are used (see Correction for Volume Additions in Section I).

SUMMARY OF METHOD
FerroMo Iron Reagent 1 contains a reducing agent combined with a masking agent. The masking agent eliminates interference from high levels of molybdate. The reducing agent converts precipitated or suspended iron, such as rust, to the ferrous state. FerroMo Reagent 2 contains the indicator combined with a buffering agent. The indicator reacts with ferrous iron in the sample, buffered between pH 3 and 4, resulting in a deep blue–purple color.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>FerroMo Reagent Set (100 tests)</td>
<td>25448–00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>25437–68, 25438–68</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td></td>
<td>1</td>
<td>each</td>
</tr>
<tr>
<td>Cylinder, graduated, mixing, 50 mL</td>
<td></td>
<td>1</td>
<td>each</td>
</tr>
</tbody>
</table>
OPTIONAL REAGENTS
Hydrochloric Acid Solution, 1:1 6.0 N .................................................. 500 mL ............ 884–49
Hydrochloric Acid, ACS .......................................................... 500 mL ............ 134–49
Iron Standard Solution, 100 mg/L Fe ........................................... 105 mL ............ 14175–42
Iron Standard Solution, Voluette ampule, 25 mg/L Fe, 10 mL ............. 16/pkg ............ 14253–10
Sodium Hydroxide Standard Solution, 1.0 N ................................ 105 mL MDB ............ 1045–32
Sulfuric Acid Standard Solution, 1.0 N ........................................... 105 mL MDB ............ 1270–32
Water, demineralized ............................................................... 4 L ............ 272–56

OPTIONAL APPARATUS
Ampule Breaker Kit ................................................................. each ............ 21968–00
Flask, volumetric, 250 mL ......................................................... each ............ 14574–46
pH Indicator Paper, 1 to 11 pH ................................................... 5 rolls/pkg ............ 391–33
pH Meter, EC10, portable .......................................................... each ............ 50050–00
Pipet Filler, safety bulb ............................................................... each ............ 14651–00
Pipet, serological, 2 mL ............................................................ each ............ 532–36
Pipet, TenSette, 0.1 to 1.0 mL ..................................................... each ............ 19700–01
Pipet Tips, for 19700–01 TenSette Pipet .................................... 50/pkg ............ 21856–96
Pipet, volumetric, Class A, 1.00 mL ............................................ each ............ 14515–35
Four-Thru Cell Assembly Kit ................................................... each ............ 45215–00
Sample Cell, disposable, polystyrene ....................................... 12/pkg ............ 24102–12

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
LEAD (0 to 160 μg/L)  
Method 8033  
For water and wastewater

Dithizone Method*, USEPA accepted for reporting wastewater analysis (digestion is required; see Section I).**

1. Enter the stored program number for lead (Pb).

Press: 280 READ/ENTER

The display will show: DIAL nm to 515

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

2. Rotate the wavelength dial until the small display shows: 515 nm

Note: If sample cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust the pH of stored samples before analysis.

3. Press: READ/ENTER

The display will show: μg/l Pb

4. Fill a 250-mL graduated cylinder to the 250-mL mark with sample.

Note: Clean all glassware with a 1:1 Nitric Acid Solution. Rinse with demineralized water.

Note: Cloudy and turbid samples may require filtering before running test. Results should be reported as μg/L soluble lead. Use a glass membrane filter to avoid loss of lead by adsorption on filter paper.

Note: For proof of accuracy, use a 100 μg/L lead standard solution (preparation given in the Accuracy Check) in place of the sample.

*Adapted from Snyder, L.J., Analytical Chemistry, 19 684 (1947)

**Procedure is equivalent to Standard Method 3500–Pb D for wastewater.
5. Transfer the sample into 500–mL separatory funnel.

6. Add the contents of one Buffer Powder Pillow, citrate type for heavy metals. Stopper the funnel. Shake to dissolve. **Note:** Spilled reagent will affect test accuracy and is hazardous to skin and other materials.

7. Add 50 mL of chloroform to a 50–mL graduated mixing cylinder. Add the contents of one DithiVer Metals Reagent Powder Pillow. Stopper. Invert repeatedly to mix. This is the dithizone solution. **Note:** Use adequate ventilation. The DithiVer Powder will not all dissolve in the chloroform. For further notes see DithiVer Solution Preparation, Storage and Reagent Blank.

8. Add 30 mL of the dithizone solution. Stopper. Invert. Open stopcock to vent. Add 5 mL of 5.0 N Sodium Hydroxide Standard Solution. Stopper. Invert. Open stopcock to vent. Shake the funnel once or twice and vent again. **Note:** Add a few drops of 5.25 N Sulfuric Acid Standard Solution if the solution turns orange on shaking. The blue-green color will reappear. To avoid higher blanks, repeat procedure on new sample and use less sodium hydroxide in this step. **Note:** Large amounts of zinc cause the color transition at the end point to be indistinct.
9. Continue adding 5.0 N Sodium Hydroxide Standard Solution dropwise until the color of the solution being shaken changes from blue–green to orange. Then add 5 more drops of 5.0 N Sodium Hydroxide Standard Solution.

Note: A pink color in the bottom (chloroform) layer at this point does not necessarily indicate lead is present. Only after shaking with cyanide in next step will a pink color in the chloroform layer confirm the presence of lead. For more accurate results, adjust the sample to pH 11.0 to 11.5 using a pH meter, omitting the five additional drops of Sodium Hydroxide Standard Solution.

10. Add two heaping 1.0-g scoops of potassium cyanide to the funnel. Stopper. Shake vigorously until the potassium cyanide is all dissolved (about 15 seconds).

Note: Wait one minute for the layers to separate. The bottom (chloroform) layer will be pink if lead is present.

11. Insert a cotton plug the size of a pea into the delivery tube of the funnel and slowly drain the bottom (chloroform) layer into a dry 25–mL sample cell (the prepared sample). Stopper.

Note: The lead–dithizone complex is stable for hours if the sample cell is kept tightly capped and out of direct sunlight.

12. Fill another sample cell (the blank) with chloroform. Stopper.
13. Place the blank into the cell holder. Close the light shield.

Note: The Pour-Thru Cell cannot be used with this procedure.

14. Press: **ZERO**

The display will show: **WAIT**

then:

0. µg/L Pb

Note: The Pour-Thru Cell cannot be used with this procedure.

15. Place the prepared sample into the cell holder. Close the light shield.

Note: See DithiVer Solution Preparation, Storage and Reagent Blank for information on preparing a reagent blank.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

16. Press: **READ/ENTER**

The display will show: **WAIT**

then the result in µg/L lead will be displayed.

DITHIVER SOLUTION PREPARATION, STORAGE AND REAGENT BLANK

Store DithiVer Powder Pillows away from light and heat. A convenient way to prepare this solution is to add the contents of 10 DithiVer Metals Reagent Powder Pillows to a pint bottle of chloroform and invert several times until well mixed (carrier powder may not dissolve). Store dithizone solution in an amber glass bottle. This solution is stable for 24 hours.

A reagent blank using demineralized water should be carried out through the entire method to obtain the most accurate results. The amount of reagent blank determined on each lot of DithiVer Metals Reagent Powder Pillow is then subtracted from the reading obtained in Step 16.

SAMPLING AND STORAGE

Collect samples in acid-cleaned glass or plastic containers. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 2.5 to 4.5 with 5.0 N Sodium Hydroxide. Correct the test result for volume additions (see Correction for Volume Additions in Section I).

ACCURACY CHECK

**Standard Additions Method**

a) Snap the neck off a Lead Voluette Ampule Standard Solution, 50 mg/L as Pb.

b) Use the TenSette Pipet to add 0.1 mL, 0.2 mL, and 0.3 mL of standard to each of three 250-mL samples and mix each thoroughly.

c) Analyze each sample as described above. The lead concentration should increase 20 µg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

**Standard Solution Method**

Prepare a 100.0-µg/L lead standard solution by pipetting 5.00 mL of Lead Standard Solution, 100 mg/L as Pb, into a 100-mL volumetric flask. Dilute to mark with demineralized water to make a 5-mg/L lead standard. Pipet 5.00 mL of the 5.0-mg/L Lead Standard Solution into 245 mL of demineralized water in a 500-mL separatory funnel. This solution should result in a reading of 100 µg/L lead when analyzed according to the above procedure. Prepare these solutions daily.
LEAD, continued

PRECISION
In a single laboratory, using a standard solution of 40 μg/L Pb and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±1.3 μg/L Pb.

INTERFERENCES
The following do not interfere.

<table>
<thead>
<tr>
<th>Aluminum</th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimony</td>
<td>Chromium</td>
<td>Manganese</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Cobalt</td>
<td>Nickel</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Iron</td>
<td>Zinc</td>
</tr>
</tbody>
</table>

The following interfere.

<table>
<thead>
<tr>
<th>Bismuth</th>
<th>Mercury</th>
<th>Tin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>Silver</td>
<td></td>
</tr>
</tbody>
</table>

Eliminate interference from these metals by the following treatment, beginning after procedure Step 7:

a) Measure about 5 mL of the prepared dithizone solution into the separatory funnel. Stopper the funnel, invert and open the stopcock to vent. Close the stopcock and shake the solution vigorously for 15 seconds. Allow the funnel to stand undisturbed until the layers separate (about 30 seconds). A yellow, red, or bronze color in the bottom (chloroform) layer confirms the presence of interfering metals. Draw off and discard the bottom (chloroform) layer.

b) Repeat extraction with fresh 5–mL portions of prepared dithizone solution (discarding the bottom layer each time) until the bottom layer shows a pure dark green color for three successive extracts. Extractions can be repeated a number of times without appreciably affecting the amount of lead in the sample.

c) Extract the solution with several 2 or 3 mL portions of pure chloroform to remove any remaining dithizone, again discarding the bottom layer each time.

d) Continue the procedure, substituting 28.5 mL of prepared dithizone solution for the 30 mL in Step 8.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment (see pH Interference in Section I).

WASTE MANAGEMENT
Collect all cyanide–containing waste for proper disposal. To prevent release of hydrogen cyanide gas, store cyanide wastes in a strong solution of sodium hydroxide. In the event of a spill or release, clean up the area by the following steps:

a) Use a fume hood or supplied–air or self–contained breathing apparatus.

b) While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).

c) Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.

d) Neutralize and flush the solution down the drain with a large excess of water.

SUMMARY OF METHOD
The DithiVer Metals Reagent is a stable powder form of dithizone. Lead ions in basic solution react with dithizone to form a pink to red lead–dithizonte complex, which is extracted with chloroform.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Required Reagent</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead Reagent Set (100 Tests)</td>
<td></td>
<td></td>
<td>22431–00</td>
</tr>
<tr>
<td>Includes: (1) 14202–99, (2) 14458–17, (4) 1216–68, (1) 767–14, (1) 2450–53, (2) 2450–26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer Powder Pillows, Citrate for heavy metals</td>
<td>1 pillow</td>
<td>100/pkg</td>
<td>14202–99</td>
</tr>
<tr>
<td>Chloroform, ACS</td>
<td>50 mL</td>
<td>500 mL</td>
<td>14458–49</td>
</tr>
<tr>
<td>DithiVer Metals Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>25/pkg</td>
<td>12616–68</td>
</tr>
<tr>
<td>Potassium Cyanide, ACS</td>
<td>2 g</td>
<td>113 g</td>
<td>767–14</td>
</tr>
<tr>
<td>Sodium Hydroxide Solution, 5 N</td>
<td>5 mL</td>
<td>1000 mL</td>
<td>2450–53</td>
</tr>
<tr>
<td>Sodium Hydroxide, 5 N</td>
<td>drops</td>
<td>59 mL DB</td>
<td>2450–26</td>
</tr>
</tbody>
</table>
### REQUIRED APPARATUS
- Clippers, for opening powder pillows: 1 each: 968–00
- Cotton balls, absorbent: 100/pkg: 2572–01
- Cylinder, mixing graduated, 50 mL: 1 each: 1896–41
- Cylinder, graduated, 5 mL: 1 each: 508–37
- Cylinder, graduated, 250 mL: 1 each: 508–46
- Funnel, separatory, 500 mL: 1 each: 520–49
- pH Meter, Hach One: 1 each: 43800–00
- Ring, support, 4": 1 each: 580–01
- Spoon, measuring, 1.0 g: 1 each: 510–00
- Stand, support, 5 X 8": 1 each: 563–00
- Stopper, hollow, No. 1: 2 pkg: 6 each: 14480–01

### OPTIONAL REAGENTS
- Chloroform, ACS: 4 L: 14458–17
- Lead Standard Solution, 100 mg/L Pb: 100 mL: 12617–42
- Lead Standard Solution, Voluette ampuls, 50 mg/L Pb: 16 pkg: 14262–10
- Nitric Acid Solution, 1:1: 500 mL: 2540–49
- Nitric Acid, ACS: 500 mL: 152–49
- Sodium Hydroxide Standard Solution, 5.0 N: 59 mL: 2450–26
- Sodium Hydroxide Standard Solution, 5.0 N: 100 mL: 2450–32
- Water, demineralized: 4 L: 272–56

### OPTIONAL APPARATUS
- Ampule Breaker Kit: each: 21968–00
- Filter Discs, glass membrane, 47 mm: 100/pkg: 2530–00
- Filter Holder, graduated, 47 mm: each: 2340–00
- Flask, erlenmeyer, 500 mL: each: 505–49
- Flask, filtering, 500 mL: each: 546–49
- Flask, volumetric, Class B, 100 mL: each: 547–42
- pH Indicator Paper, 1 to 11 pH: 5 rolls/pkg: 391–33
- pH Meter, EC10, portable: each: 50050–00
- Pipet Filler, safety bulb: each: 14651–00
- Pipet Tips, for 19700–01 TenSette Pipet: 50/pkg: 21856–96
- Pipet, TenSette, 0.1 to 1.0 mL: each: 19700–01
- Pipet, serological, 2 mL: each: 532–36
- Pipet, volumetric, Class A, 5.00 mL: each: 14515–37
- Sample Cell, with 25–mL mark, matched pair: each pair: 20950–00

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
LEAD (0 to 150 µg/L)  
For drinking water

LeadTrak®* Fast Column Extraction Method

1. Enter the stored program number for lead (Pb), column extraction method.

Press: 2 8 3 READ/ENTER

The display will read:

DIAL nm TO 477

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: For DR/2000s without this stored program, see Instrument Setup following these steps.

2. Rotate the wavelength dial until the small display reads: 477 nm

3. Press: READ/ENTER

The display will read: µg/l Pb FC

4. Fill a 100–mL plastic graduated cylinder with 100 mL of the water to be tested. Pour the measured sample into a 250–mL plastic beaker.

Note: The sampling requirements for “first-draw” analysis are detailed in the Sampling and Storage section.

Note: For proof of accuracy, use a 50-µg/L Lead Standard Solution (preparation given in Accuracy Check) in place of the sample.

* U.S. Patent number 5,019,516

247
5. Using a plastic 1–mL dropper, add 1.0 mL of pPb–1 Acid Preservative Solution to the sample and swirl to mix.

Note: If the sample has been preserved previously with pPb–1 Acid Preservative at a ratio of 1.0 mL per 100 mL sample, omit Steps 5 and 6.

Note: Samples preserved with Nitric Acid also require Steps 5 and 6.

6. Press **SHIFT TIMER**
A 2–minute reaction period will begin.

7. When the timer beeps, use a second 1–mL plastic dropper to add 2.0 mL of pPb–2 Fixer Solution to the sample. Swirl to mix.

Note: Field samples that have been preserved with nitric acid or samples that have been digested, may exceed the buffer capacity of the Fixer Solution. After Step 7 check the pH of these samples and adjust with 5 N sodium hydroxide to a pH of 6.7–7.1 before proceeding with Step 8.

Note: A Fast Column Extractor is included in the LeadTrak Reagent Set.

Note: A new extractor is required for each test.

8. Mount a new Fast Column Extractor in a ring stand with a clamp. Place a 150–mL plastic beaker under the Extractor.

9. Pour the prepared sample slowly into the Column Extractor. Wait for the sample to flow through.

10. After the flow has stopped, fully compress the absorbent pad in the Extractor with the plunger. Discard the contents of the beaker. Withdraw the plunger slowly from the Extractor.

Note: The absorbent pad should remain at the bottom of the Extractor when the plunger is removed. Recompress with the plunger if the pad has retracted with the plunger.

11. Place a 25–mL sample cell under the Extractor. Using a 25–mL plastic graduated cylinder, add 25 mL of pPb–3 Eluant Solution to the Extractor.

12. After the Eluant Solution has started to drip from the Extractor, insert the plunger and slowly force the remaining Eluant Solution through the Extractor. Fully compress the absorbent pad. The volume in the sample cell should be 25 mL.
13. Using a 1-mL plastic dropper, add 1.0 mL of pPb–4 Neutralizer Solution to the sample cell. Swirl thoroughly to mix and proceed immediately to Step 14.

14. Add the contents of one pPb–5 Indicator Powder Pillow to the sample and swirl thoroughly to mix.

*Note:* The solution color will turn brown.

15. Split the sample by filling two 10-mL vials to the 10-mL lines with the prepared sample.

16. Press: **SHIFT TIMER**

A second 2-minute reaction period will begin.

17. When the timer beeps, add three drops of pPb–6 Decolorizer Solution to one of the vials. Cap and invert to mix. Mark this vial as the blank.

*Note:* There will be little visual difference between the reagent blank and the sample. Both vials will be colored.

18. Place the AccuVac Vial Adapter into the cell holder.

*Note:* Place the grip tab at the rear of the cell holder.

19. Place the vial containing the blank into the cell holder. Close the light shield.

*Note:* The Pour–Thru Cell cannot be used with this procedure.

20. Press: **ZERO**

The display will show: **WAIT** then: **0 μg/l Pb FC**
21. Place the other vial with the prepared sample into the cell holder. Close the light shield.

22. Press READ/ENTER

The display will read:

WAIT

then the results in μg/L lead (Pb) will be displayed.

Note: For a DR/2000 in the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

Note: Running a reagent blank with lead-free, reagent-grade water is required for USEPA reporting purposes. Each new lot of reagents should have a reagent blank determined. The reagent blank is then subtracted from each test result.

INSTRUMENT SETUP
For a DR/2000 with software versions 1.261 and 1.27, the Lead, Fast Column Extraction Method, Stored Method #283, has not been included. Enter the following calibration as an operator–programmed calibration. Follow the steps given in the Operation section of the DR/2000 Instrument Manual. Store the method as follows:

\[ \text{nm} = 477 \]
\[ \text{Decimal} = 0000. \]
\[ \text{Units} = \mu g/l \]
\[ \text{symbol} = \text{Pb FC} \]
\[ \text{Timer 1} = 02:00 \]
\[ \text{Timer 2} = 02:00 \]

The calibration is entered with 0.000 absorbance values for zero, #1 standard and #2 standard. To do this, do not place anything in the sample cell compartment. Begin by storing zero, #1 standard and #2 standard as concentrations of 0, 3, and 164, respectively, with nothing in the cell compartment. Accept 0.000 Abs as the value for zero and the standards. Next, the absorbance values for the #1 standard and #2 value must be changed to the values given below.

<table>
<thead>
<tr>
<th>Std.</th>
<th>Abs.</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.063</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>1.000</td>
<td>164</td>
</tr>
</tbody>
</table>

The method is now stored as an operator–programmed method with a method number between 950 and 999. Record the method number for future reference when using this method.

APPARATUS/SAMPLE PREPARATION
Because lead is very common to our environment, care must be taken to prevent sample contamination. Follow these steps for greatest test accuracy:
a) Lead–free water is necessary to minimize sample contamination when rinsing apparatus or diluting sample. The water may be either distilled or deionized. If the water is obtained from a grocery store, verify the lead concentration is zero from the label. If the lead concentration is uncertain, determine the lead concentration with the LeadTrak test.

c) Rinse glassware used in this test with a small amount of dilute lead–free nitric acid or Pb–1 Acid Preservative followed by rinsing with lead–free water.

b) Plastic or glass sample containers and lids may be checked for contamination by rinsing with 1 mL of Pb–1 Acid Preservative. Add 100 mL of lead–free water. After 24 hours, analyze this solution using the LeadTrak test to confirm the absence of lead.

d) Pb–5 Indicator may be rinsed from the glass sample cells with a few drops of Pb–1 Acid Preservative or a small amount of dilute lead–free nitric acid.

**SAMPLING**

Samples may be collected either from household pipes (point–of–use) or from water sources. Samples may be stored up to six months.

**Sampling for lead contamination in household pipes for point–of–use drinking water**

a) The sample should be collected after sitting in pipes with no flow for 8 to 18 hours.

b) Add 10 mL of Pb–1 Acid Preservative to a one–liter bottle.

c) Turn on tap and collect exactly the first liter of water in the bottle containing lead preservative.

d) Cap and invert several times to mix.

e) After two minutes the sample is ready for analysis. Steps 5 and 6 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in Step 7.

**Sampling for lead contamination from drinking water sources such as well water or water from main supply lines**

a) Add 10 mL of Pb–1 Acid Preservative to a one–liter bottle.

b) Turn on the tap for 3–5 minutes or until the water temperature has been stable for 3 minutes.

c) Collect exactly one liter of water into the bottle containing the acid preservative.

d) Cap and invert several times to mix.

e) After two minutes the sample is ready for analysis. Steps 5 and 6 are skipped in the analysis procedure. Use 100 mL of this preserved sample directly in Step 7.

**Notes**

a) At least one liter should be collected to obtain a representative sample. If less than one liter is collected, use 1 mL of Pb–1 Acid Preservative per 100 mL of sample.

b) If nitric acid is to be substituted for Pb–1 as a preservative or the sample is digested, the buffering capacity of the Pb–2 Fixer Solution may be exceeded. Adjust the sample pH to 6.7 to 7.1 pH with 5 N sodium hydroxide after Step 7.

c) Each sample type typically requires different sampling procedures. Consult with the appropriate regulatory agency in your area for more information about your specific sampling requirements.

**ACCURACY CHECK**

**Standard Additions Method**

The standard additions method for checking the validity of the test results can be performed as follows:

a) Use a TenSette Pipet to add 0.1 mL of a 10 mg/L Lead Standard Solution (included in the reagent set) to a second 100–mL portion of the sample.

b) Swirl the sample to mix. Then test the sample as described in the procedure. Each 0.1–mL of standard added should increase the lead concentration determined in Step 22 by 10 µg/L.

**Standard Solution Method**

A 50–µg/L lead standard solution can be prepared by first pipetting 1.00 mL of Lead Standard Solution, 1000 mg/L as Pb, into a 100–mL plastic volumetric flask and diluting to the mark with lead–free water to make a 10 mg/L lead working solution (included in the reagent set). Pipet 5.00 mL of this working solution into a 1–liter plastic volumetric flask. Dilute to the mark with lead–free water. This 50–µg/L standard solution should be prepared immediately before use.

Alternatively, a 50–µg/L lead standard solution can be prepared by using a TenSette Pipet and pipetting 0.1 mL from a Lead Volutette Ampule Standard Solution, 50 mg/L as Pb, into a 100–mL plastic volumetric flask and diluting to volume with demineralized water. This solution should be prepared immediately before use.
INTERFERENCE
Interference studies were conducted by preparing a known lead solution of approximately 25 µg/L as well as the potential interfering ion. The ion was said to interfere when the resulting concentration changed by ±10%.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Interference Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum, Al$^{3+}$</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Barium, Ba$^{2+}$</td>
<td>6 mg/L</td>
</tr>
<tr>
<td>Calcium, Ca$^{2+}$</td>
<td>500 mg/L</td>
</tr>
<tr>
<td>Chloride, Cl$^{-}$</td>
<td>1000 mg/L</td>
</tr>
<tr>
<td>Copper, Cu$^{2+}$</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>Fluoride, F$^{-}$</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Iron, Fe$^{2+}$</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>Magnesium, Mg$^{2+}$</td>
<td>500 mg/L</td>
</tr>
<tr>
<td>Manganese, Mn$^{2+}$</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Nitrogen, Ammonium, NH$_4^+$</td>
<td>500 mg/L</td>
</tr>
<tr>
<td>Nitrogen, Nitrate, NO$_3^-$</td>
<td>1000 mg/L</td>
</tr>
<tr>
<td>Sulfate, SO$_4^{2-}$</td>
<td>1000 mg/L</td>
</tr>
<tr>
<td>Zinc, Zn$^{2+}$</td>
<td>1 mg/L</td>
</tr>
</tbody>
</table>

Sample containing levels exceeding these concentration values may be diluted 1:1 and re-analyzed. Multiply the value obtained by the factor of 2 to determine the lead present in the original sample.

Every effort has been made to prevent contamination in packaging the reagents. Use of black rubber stoppers, black dropper bulbs and droppers with inked graduations may contaminate the sample and should be avoided. Use the plastic droppers provided in the reagent set.

Glassware and plastic ware should be rinsed with a dilute nitric acid solution such as 0.1 N Nitric Acid Standard Solution or a few drops of pPb–1 Acid Preservative Reagent to prevent sample contamination, especially if the previous sample had a high lead level. The sample cell walls will become colored from the pPb–5 Indicator and should be rinsed. The Extractor plunger is intended to be used for more than one test and should be rinsed as well.

SUMMARY OF METHOD
Acid soluble lead, as Pb$^{2+}$, in a potable water sample is first concentrated on a Fast Column Extractor. The lead is then eluted from the Extractor and determined colorimetrically with an indicator.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LeadTrak, reagent set</td>
<td>1</td>
<td>20 tests/pkg</td>
<td>23750-00</td>
</tr>
<tr>
<td>Lead Standard Solution, 10 mg/L</td>
<td></td>
<td>25 mL</td>
<td>23784-20</td>
</tr>
<tr>
<td>pPb–1, Acid Preservative</td>
<td></td>
<td>236 mL</td>
<td>23685-31</td>
</tr>
<tr>
<td>pPb–2, Fixer Solution</td>
<td></td>
<td>43 mL</td>
<td>23686-55</td>
</tr>
<tr>
<td>pPb–3, Eluant</td>
<td></td>
<td>500 mL</td>
<td>23687-49</td>
</tr>
<tr>
<td>pPb–4, Neutralizer</td>
<td></td>
<td>22 mL</td>
<td>23688-55</td>
</tr>
<tr>
<td>pPb–5, Indicator Reagent Powder Pillows</td>
<td></td>
<td>20/pkg</td>
<td>23689-64</td>
</tr>
<tr>
<td>pPb–6, Decolorizer</td>
<td></td>
<td>10 mL SCDB</td>
<td>23748-20</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Unit(s)</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac</td>
<td>1</td>
<td>each</td>
<td>43784-00</td>
</tr>
<tr>
<td>Beaker, polypropylene, 250 mL</td>
<td>1</td>
<td>each</td>
<td>1080-46</td>
</tr>
<tr>
<td>Beaker, polypropylene, 150 mL</td>
<td>1</td>
<td>each</td>
<td>1080-44</td>
</tr>
<tr>
<td>Clamp, two–prong extension</td>
<td>1</td>
<td>each</td>
<td>21145-00</td>
</tr>
<tr>
<td>Clamp, holder</td>
<td>1</td>
<td>each</td>
<td>326-00</td>
</tr>
<tr>
<td>Clippers, small</td>
<td>1</td>
<td>each</td>
<td>936-00</td>
</tr>
<tr>
<td>Cylinder, graduated, polypropylene, 100 mL</td>
<td>1</td>
<td>each</td>
<td>1081-42</td>
</tr>
<tr>
<td>Cylinder, graduated, polypropylene, 25 mL</td>
<td>1</td>
<td>each</td>
<td>1081-40</td>
</tr>
<tr>
<td>Dropper, DPDE, 0.5 and 1 mL marks</td>
<td>3</td>
<td>10/pkg</td>
<td>21247-10</td>
</tr>
<tr>
<td>Pipet tip, for 100 µL Pipettor</td>
<td>varies</td>
<td>10/pkg</td>
<td>22754-10</td>
</tr>
<tr>
<td>pPB Fast Column Extractor</td>
<td>1</td>
<td>each</td>
<td>23749-00</td>
</tr>
<tr>
<td>Sample Cell, 10–mL, with cap</td>
<td>2</td>
<td>each</td>
<td>21228-00</td>
</tr>
<tr>
<td>Support, ring stand</td>
<td>1</td>
<td>each</td>
<td>563-00</td>
</tr>
<tr>
<td>Syringe plunger</td>
<td>1</td>
<td>each</td>
<td>23764-00</td>
</tr>
</tbody>
</table>

252
LEAD, continued

OPTIONAL REAGENTS
Lead Standard Solution, 1000 mg/L as Pb .......................................................... 100 mL .... 12796–42
Lead Standard Solution, Volutette ampule, 50 mg/L as Pb²⁺, 10 mL .................. 16/pkg .... 14262–10
Nitric Acid, ACS .................................................................................................. 500 mL .... 152–49
Nitric Acid Standard Solution, 0.1 N .................................................................... 100 mL .... 23328–42
pPb–1 Acid Preservative Reagent ......................................................................... 237 mL .... 23685–31
Sodium Hydroxide Standard Solution, 5.0 N ......................................................... 1 L .... 2450–53
Water, demineralized .......................................................................................... 4 L .... 272–56

OPTIONAL APPARATUS
Ampule Breaker Kit ............................................................................................... each .... 21968–00
Bottle, sampling, 125 mL ..................................................................................... each .... 23240–43
Bottle, sampling, 125 mL ..................................................................................... 48/pkg .... 23240–73
Bottle, sampling, 1000 mL .................................................................................. each .... 23242–53
Bottle, sampling, 1000 mL .................................................................................. 24/pkg .... 23242–83
Dropper, 0.5 & 1.0 mL ......................................................................................... 10/pkg .... 21247–10
Flask, volumetric, plastic, 100 mL ..................................................................... each .... 20995–42
Flask, volumetric, plastic, 1000 mL ................................................................... each .... 20995–53
pH Meter, EC10, portable .................................................................................... each .... 50050–00
Pipet, serological, 5 mL ....................................................................................... each .... 532–37
Pipet, TenSette, 0.1 to 1.0 mL ............................................................................. each .... 19700–01
Pipet Tips, for 19700–01 TenSette Pipet ............................................................... 50/pkg .... 21856–96
Pipet, volumetric, Class A, 1.00 mL ................................................................. each .... 14515–35
Pipet, volumetric, Class A, 5.00 mL ................................................................. each .... 14515–37
Pipet Filler, 3–valve ............................................................................................. each .... 12189–00
Pipetter, 100 µL .................................................................................................. each .... 22753–00
Stopper, hollow .................................................................................................... 6/pkg .... 14480–00

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
MANGANESE, HR (0 to 20.0 mg/L)

Periodate Oxidation Method*; USEPA approved for reporting wastewater analysis (digestion is required; see Section I.)**

1. Enter the stored program number for manganese (Mn) periodate oxidation.

Press: 2 9 5 READ/ENTER

The display will show:

DIAL nm TO 525

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:

525 nm

3. Press: READ/ENTER

The display will show:

mg/l Mn H

4. Fill a cell with 25 mL of sample.

*Adapted from Standard Methods for the Examination of Water and Wastewater

**Federal Register, 44 (116) 34193 (June 14, 1979)
5. Add the contents of one Buffer Powder Pillow, citrate type. Swirl to mix.

Note: For proof of accuracy, use a 5.0 mg/L manganese standard solution (preparation given in Accuracy Check) in place of the sample.

6. Add the contents of one Sodium Periodate Powder Pillow to the sample cell (the prepared sample). Swirl to mix.

Note: A violet color will form if manganese is present.

Note: Accuracy is not affected by undissolved powder.

7. Press: **SHIFT TIMER**
A 2-minute reaction period will begin.

8. When the timer beeps, the display will show:
   **mg/l Mn H**
Fill another sample cell (the blank) with 25 mL of sample.

9. Place the blank into the cell holder. Close the light shield.

Note: The Pour-Thru Cell can be used with this procedure.

10. Press: **ZERO**
This display will show: **WAIT**
then:
   **0.0 mg/l Mn H**

11. Within eight minutes after the timer beeps, place the prepared sample into the cell holder. Close the light shield.

Note: If more than five minutes elapses after the prepared sample, insert the blank and press: **ZERO**. Insert the prepared sample and press: **READ/ENTER**.

12. Press: **READ/ENTER**
The display will show:
   **WAIT**
then the result in mg/L Mn will be displayed.

Note: Results may be expressed as mg/L permanganate (MnO₄⁻) or as mg/L potassium permanganate (KMnO₄) by multiplying the mg/L Mn by 2.16 or 2.88, respectively.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
MANGANESE, HR, continued

SALENDING AND STORAGE
Collect samples in acid—washed plastic bottles. Manganese may be lost by adsorption to glass
container walls. Adjust the pH to 4 to 5 with 5.0 N
sodium hydroxide before analysis. Do not exceed pH
5, as manganese may be lost as a precipitate. Correct
the test result for volume additions (see Correction for
Volume Additions in Section I). If only dissolved
manganese is to be determined, filter the sample
before acid addition.

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off a Manganese Voluette Ampule
Standard Solution, High Range, 250 mg/L Mn.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL
of standard to three 25–mL water samples. Mix
thoroughly.

c) Analyze each sample as described above. The
manganese concentration should increase 1.0 mg/L for
each 0.1 mL of standard added.

d) If these increases do not occur, see Standard
Additions in Section I for more information.

Standard Solution Method
Prepare a 5.0–mg/L manganese standard solution by
pipetting 5.00 mL of Manganese Standard Solution,
1000 mg/L Mn, into a 1000–mL volumetric flask.

Dilute to the mark with demineralized water. Or,
prepare this standard by diluting 1.00 mL of the
contents of a Voluette Ampule For High Range
Manganese to 50 mL, using the TenSette Pipet.
Prepare these solutions daily.

INTERFERENCES
The following may interfere when present in
concentrations exceeding these listed below:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>700 mg/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>70,000 mg/L</td>
</tr>
<tr>
<td>Iron</td>
<td>5 mg/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>100,000 mg/L</td>
</tr>
</tbody>
</table>

Highly buffered samples or extreme sample pH may
exceed the buffering capacity of the reagents and
require sample pretreatment (see pH Interference in
Section I).

PRECISION
In a single laboratory, using a standard solution of
10.00 mg/L manganese and two representative lots of
reagent with the DR/2000, a single operator obtained a
standard deviation of ±0.06 mg/L manganese.

SUMMARY OF METHOD
Manganese in the sample is oxidized to the purple
permanate state by sodium periodate, after
buffering the sample with citrate. The purple color is
directly proportional to the manganese concentration.

If only dissolved manganese is to be determined, filter
the sample before acid addition.

REQUIRED REAGENTS
High Range Manganese Reagent Set (100 Tests*) ........................................ 22432–00
Includes: (2) 983-66, (1) 984-99

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer Powder Pillows, citrate type for manganese</td>
<td>1 pillow</td>
<td>100/pkg</td>
<td>983-99</td>
</tr>
<tr>
<td>Sodium Periodate Powder Pillows for manganese</td>
<td>1 pillow</td>
<td>100/pkg</td>
<td>984-99</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS
Clippers, for opening powder pillows .................... 1 each 968–00

OPTIONAL REAGENTS
Hydrochloric Acid, 6N ........................................ 500 mL 884–49
Manganese Standard Solution, 1000 mg/L Mn ............ 100 mL** 12791–42
Manganese Standard Solution, Voluette ampule, High Range, 250 mg/L Mn, 10 mL 16/pkg 14258–10
Nitric Acid, ACS ........................................ 500 mL 152–49
Nitric Acid Solution 1:1 .................................. 500 mL 2540–49
Sodium Hydroxide Solution, 5.0 N ....................... 100 mL** MDB 2450–52
Water, demineralized ...................................... 4 L 272–56

257
**OPTIONAL APPARATUS**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968-00</td>
</tr>
<tr>
<td>Dropper, plastic, 0.5 and 1.0 mL marks</td>
<td>10/pkg</td>
<td>21247-10</td>
</tr>
<tr>
<td>Flask, erlenmeyer, 250 mL</td>
<td>each</td>
<td>505-46</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 50 mL</td>
<td>each</td>
<td>14574-41</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 100 mL</td>
<td>each</td>
<td>14574-42</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 1000 mL</td>
<td>each</td>
<td>14574-53</td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>5 rolls/pkg</td>
<td>391-33</td>
</tr>
<tr>
<td>pH Meter, Hach One</td>
<td>each</td>
<td>50050-00</td>
</tr>
<tr>
<td>Pipet, serological, 1 mL</td>
<td>each</td>
<td>532-35</td>
</tr>
<tr>
<td>Pipet, serological, 5 mL</td>
<td>each</td>
<td>532-37</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700-01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856-96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 5.0 mL</td>
<td>each</td>
<td>14515-37</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651-00</td>
</tr>
<tr>
<td>Pour–Thru Cell Kit</td>
<td>each</td>
<td>45215-00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

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*100 tests equal 100 samples and 100 blanks.
**Contact Hach for larger sizes.
MANGANESE, LR (0 to 0.700 mg/L)

For water and wastewater

PAN Method*

1. Enter the stored program number for manganese (Mn).

Press: 290 READ/ENTER

The display will show:

DIAL nm TO 560

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust the pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:

560 nm

Note: Total manganese determination requires a prior digestion; use any of the three digestion procedures given in Digestion (Section I).

3. Press: READ/ENTER

The display will show:

mg/l Mn L

Note: Rinse all glassware with 1:1 Nitric Acid Solution. Rinse again with demineralized water.

4. Pour 25.0 mL of demineralized water into a sample cell (the blank).

*Adapted from Goto, K., et al., Talanta, 24, 752–3 (1977)
5. Pour 25.0 mL of sample into another sample cell (the prepared sample).

Note: For proof of accuracy, use a 0.5 mg/L manganese standard solution (preparation given in the Accuracy Check) in place of the sample.

6. Add the contents of one Ascorbic Acid Powder Pillow to each cell. Swirl to mix.

Note: For samples containing hardness greater than 300 mg/L CaCO₃, add ten drops of Rochelle Salt Solution to the sample after addition of the Ascorbic Acid Powder Pillow.

7. Add 1.0 mL of Alkaline–Cyanide Reagent Solution to each cell. Swirl to mix.

Note: A cloudy or turbid solution may form in some samples after addition of the Alkaline–Cyanide Reagent Solution. The turbidity should dissipate after Step 8.

8. Add 1.0 mL of 0.1% PAN Indicator Solution to each sample cell. Swirl to mix.

Note: An orange color will develop in the sample if manganese is present.

Note: Use the plastic dropper supplied because droppers with rubber bulbs may contaminate solution.


A 2-minute reaction period will begin.

Note: If the sample contains high amounts of iron (greater than 5 mg/L), allow ten minutes for complete color development. To set the timer for ten minutes, press SHIFT PROG TIMER 1 0 0 0. To begin the new reaction period press: SHIFT TIMER.

Note: The Pour–Thru Cell can be used if rinsed well with demineralized water between the blank and prepared sample.

10. When the timer beeps, the display will show:

mg/L Mn L

Place the blank into the cell holder. Close the light shield.

11. Press: ZERO

The display will show: WAIT
then: 0.000 mg/L Mn L

12. Place the prepared sample into the cell holder. Close the light shield.

Press: READ/ENTER

The display will show: WAIT
then the result in mg/L manganese will be displayed.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

Note: See Waste Disposal below for proper disposal of cyanide-containing wastes.
MANGANESE, LR, continued

SAMPLING AND STORAGE
Collect samples in a clean glass or plastic container. Adjust the pH to 2 or less with nitric acid (about 2 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the pH to 4.0 to 5.0 with 5.0 N sodium hydroxide. Correct the test result for volume additions (see Correction for Volume Additions in Section I).

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off a Manganese Voluette Ampule Standard, 25 mg/L Mn^{2+}.

b) Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 25–mL samples. Mix each thoroughly.

c) Analyze each sample as described above. The manganese concentration should increase 0.1 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method
Prepare a 0.5 mg/L manganese standard solution as follows:

a) Pipet 5.00 mL of Manganese Standard Solution, 1000 mg/L Mn, into a 1000–mL volumetric flask.

b) Dilute to the mark with demineralized water. This solution should be prepared daily.

c) Pipet 10 mL of the above dilution into a 100–mL volumetric flask.

d) Dilute to the mark with demineralized water. This second dilution is equivalent to 0.5 mg/L Mn.

e) Perform the manganese procedure as described above. The reading in Step 12 should be 0.5 mg/L Mn.

PRECISION
In a single laboratory, using a standard solution of 0.5 mg/L Mn and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.0049 mg/L Mn.

INTERFERENCES
The following do not interfere up to the indicated concentrations:

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>20 mg/L</td>
</tr>
<tr>
<td>Cadmium</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>1000 mg/L as CaCO₃</td>
</tr>
<tr>
<td>Cobalt</td>
<td>20 mg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>50 mg/L</td>
</tr>
<tr>
<td>Iron</td>
<td>25 mg/L</td>
</tr>
<tr>
<td>Lead</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>300 mg/L as CaCO₃</td>
</tr>
<tr>
<td>Nickel</td>
<td>40 mg/L</td>
</tr>
<tr>
<td>Zinc</td>
<td>15 mg/L</td>
</tr>
</tbody>
</table>

WASTE MANAGEMENT
Collect all cyanide–containing waste for proper disposal. To prevent release of hydrogen cyanide gas, store cyanide wastes in a strong solution of sodium hydroxide. In the event of a spill or release, clean up the area by following the steps:

a) Use a fume hood or supplied–air or self–contained breathing apparatus.

b) While stirring, add the waste to a beaker containing a strong solution of sodium hydroxide and calcium hypochlorite or sodium hypochlorite (household bleach).

c) Maintain a strong excess of hydroxide and hypochlorite. Let the solution stand for 24 hours.

d) Neutralize and flush the solution down the drain with a large excess of water.

SUMMARY OF METHOD
The PAN method is a highly sensitive and rapid procedure for detecting low levels of manganese. An ascorbic acid reagent is used initially to reduce all oxidized forms of manganese to Mn^{2+}. An alkaline–cyanide reagent is added to mask any potential interferences. PAN Indicator is then added to combine with the Mn^{2+} to form an orange–colored complex.
REQUwRED REAGENTS

Manganese Reagent Set (100 Tests) ......................................................... 22433-00
   Includes: (2) 21223-32, (2) 14577-99, (2) 21224-39

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline–Cyanide Reagent</td>
<td>2 mL</td>
<td>100 mL</td>
<td>21223-32</td>
</tr>
<tr>
<td>Ascorbic Acid Powder Pillows</td>
<td>2 pillows</td>
<td>100/pkg.</td>
<td>14577-99</td>
</tr>
<tr>
<td>PAN Indicator Solution, 0.1%</td>
<td>2 mL</td>
<td>118 mL</td>
<td>21224-39</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>25 mL</td>
<td>4 L</td>
<td>272-56</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

Clippers, for opening powder pillows ............................................. 1 each 968-00
Cylinder, graduated, 25 mL ......................................................... 1 each 508-40

OPTIONAL REAGENTS

Hydrochloric Acid Solution, 1:1 (6 N) ........................................... 500 mL 884-49
Manganese Standard Solution, 1000 mg/L Mn ................................... 100 mL 12791-42
Manganese Standard Solution, Voluette ampule, 25 mg/L Mn, 10 mL ....... 16/pkg 21128-10
Nitric Acid Solution, 1:1 ......................................................... 500 mL 2540-49
Rochelle Salt Solution .............................................................. 29 mL 1725-33
Sodium Hydroxide Solution, 50% .................................................. 500 mL 2180-49
Nitric Acid, ACS ................................................................. 500 mL 152-49

OPTIONAL APPARATUS

Ampule Breaker Kit ........................................................................... each 21968-00
Beaker, glass, 1000 mL ................................................................. each 500-53
Dropper, plastic calibrated, 1.0 mL ............................................... 10/pkg 21247-10
Flask, volumetric, Class A, 1000 mL ........................................... each 14574-53
Flask, volumetric, Class A, 100 mL ............................................ each 14574-42
Pipet, TenSette, 0.1 to 1.0 mL ..................................................... each 19700-01
Pipet Tips, for 19700-01 TenSette Pipet ....................................... 50/pkg 21856-96
Pipet, volumetric, 10.0 mL, Class A ........................................... each 14515-38
Pipet, volumetric, 5.0 mL, Class A ............................................. each 14515-37
Pipet Filler, safety bulb ......................................................... each 14651-00
Pour–Thru Cell Kit ........................................................................ each 45215-00

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
MERCAPTOACETIC ACID METHOD* USING ACCUVACS®

1. Enter the stored program number for high range molybdenum (Mo⁶⁺) using AccuVac Ampuls.
   Press: 9 ?? READ/ENTER
   OR
   Press: 3 2 2 READ/ENTER
   The display will show:
   DIAL nm TO 420

Note: This method requires a user-entered program number. See Instrument Setup following these steps.

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: Collect samples in glass or plastic bottles.

2. Rotate the wavelength dial until the small display shows:
   420 nm

3. Press: READ/ENTER
   The display will show:
   mg/l Mo⁶⁺ H AV

4. Collect 40 mL of sample in a 50–mL beaker.

Note: For proof of accuracy, use a 10.0 mg/L Molybdenum Standard Solution (listed under Optional Reagents) in place of the sample.

Note: Filter turbid samples using the labware listed under Optional Apparatus.

*Adapted from Analytical Chemistry, 25 (9) 1963 (1953)
5. Add 4 drops of 0.4 M CDTA Solution to the beaker. Swirl to mix.

6. Fill a MolyVer® 6 Molybdenum AccuVac with sample.
   
   **Note:** Keep the tip immersed while the ampul fills.

7. Invert the ampul repeatedly to mix.
   
   **Note:** If molybdenum is present a yellow color will develop.

8. Press: **SHIFT TIMER**
   
   A five–minute reaction period will begin.

9. When the timer beeps, the display will show:
   
   mg/l Mo$^{6+}$ H AV
   
   Fill a Zeroing Vial with at least 10 mL of sample (the blank).

10. Place the AccuVac Vial Adapter into the cell holder of the instrument.

11. Insert the blank into the cell holder. Close the light shield.

12. Press: **ZERO**
   
   The display will show:
   
   WAIT then:
   
   0.0 mg/l Mo$^{6+}$ H AV
13. Place the prepared sample into the cell holder. Close the light shield.

Press: **READ/ENTER**

The display will read:

**WAIT**

then the results in mg/L molybdate molybdenum will be displayed.

*Note:* In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

---

**INSTRUMENT SETUP**

For DR/2000s with software versions 1.261 and 1.27

Enter the following calibration as an operator–programmed calibration. Follow these steps in the Operation section of the *DR/2000 Instrument Manual* under *User Methods*. Store the method as follows:

\[
\begin{align*}
\text{nm} & = 420 \\
\text{Decimal} & = 000.0 \\
\text{Units} & = \text{mg/L} \\
\text{Symbol} & = \text{Mo}^{6+} \text{ H A V} \\
\text{Timer 1} & = 05:00
\end{align*}
\]

Enter the calibration with 0.000 absorbance values for the #0 and #1 standards. To do this, leave the sample cell compartment empty. Begin by storing #0 and #1 standard as the concentrations shown in the table below (with nothing in the sample cell compartment).

Accept 0.000 Abs as the value for all standards. Store the calibration by pressing **SHIFT READ/ENTER**.

Next, edit the absorbance values for the standards to the values given in the table below. Follow the steps in the Operations section of the *DR/2000 Instrument Manual*.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>#0</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>#1</td>
<td>500</td>
<td>0.334</td>
</tr>
</tbody>
</table>

The method is now stored as an operator–programmed method with a method number between 950 and 999. Record the method number for future reference.

**DR/2000 with Software Versions 2.0 and 2.2.**

Enter the calibration as an update to Hach stored programs.

1. Press: **1 0**

2. Press: **SHIFT** **CONFIG** **METH**

3. Press: **PROG** **EDIT** **EDIT** **READ ENTER**
4. Within 3 seconds, press:

```
SHIFT  PROG 3  CONFIG
```

The display will show:

```
ENTER nm
```

5. Press:

```
%T  EDIT  0  READ ENTER
```

Note: If you make an error, press SHIFT CLEAR and re-enter the number. When the number is correct, press READ/ENTER.

The display will show:

```
DECIMAL? 00.00
```

6. Use the arrow keys to correctly position the decimal point. Press the DOWN ARROW key once. The display will show:

```
DECIMAL? 000.0
```

Press: READ/ENTER

7. The display will show:

```
UNITS?
```

8. Use the arrow keys to select the appropriate unit of measure. Press the DOWN ARROW key once. The display will show:

```
mg/l
```

9. Press READ/ENTER when the correct unit of measure is displayed. The display will show:

```
SYMBOL?
```

10. Construct the correct symbol display:

```
MO+ H AV
```

a. Select letters and regular numbers by scrolling to the correct symbol with the arrow keys.

b. To make a letter or number uppercase, press the SHIFT key.

c. The space is the character displayed after one press of the DOWN ARROW key.

d. Accept each symbol by pressing READ/ENTER.

e. To end symbol entry, press READ/ENTER a second time after accepting the last character.

11. When the instrument is out of symbol entry mode, the display will show:

```
TIMER?
```

12. This method has one timed step, so press SHIFT TIMER. The display will show:

```
MM:SS TIME 1?
```

13. Enter a timer value of 5 minutes. Press:

```
0 5 ABS 0 0
```

14. Press READ/ENTER to accept the timer value. The display will show:

```
MM:SS TIME 2?
```

15. Press READ/ENTER to complete the timer entry. The display will show:

```
#1 Data
```

16. Enter the following 12 numbers as shown. Complete each number entry by pressing the READ/ENTER key.

```
#1 Data  0
#2 Data  7710
#3 Data  7710
#4 Data  7710
#5 Data  7710
#6 Data  7710
#7 Data  7710
#8 Data  65535
#9 Data  65535
#10 Data 9362
#11 Data  512
Checksum  9404
```

The final number is a check value which determines if the data sequence was correctly entered. If an error was made during number entry, the display will return to the prompt for data # 1 and the entire sequence must be re-entered. If all numbers are correctly entered, the display will return to the method prompt and is ready for use.

**DR/2000 with Software Version 3.0 and 3.1**

1. Turn the instrument on. Press SHIFT METHOD to enter configuration mode. The display will show:

```
MOMENTARY or CONSTANT ON
```

2. Press the UP ARROW key twice to select HACH UPDATE. Press READ/ENTER. The display will show:

```
ENTER #:
```
MOLYBDENUM, MOLYBDDATE, HR, continued

3. Press:  
   \[
   \begin{array}{ccc}
   \text{PROG} & \text{EDIT} & \text{EDIT} \\
   3 & 2 & 2 \\
   \text{READ ENTER}
   \end{array}
   \]

   The display will show:  
   \[
   \text{P322 ENTER nm}
   \]

4. Press:  
   \[
   \begin{array}{ccc}
   \%T & \text{EDIT} & 0 \\
   4 & 2 & 0 \\
   \text{READ ENTER}
   \end{array}
   \]

   Note: If you make an error, press SHIFT CLEAR and re-enter the number. When the number is correct, press READ/ENTER.

   The display will show:  
   \[
   \text{P322 DECIMAL? 00.00}
   \]

5. Use the arrow keys to correctly position the decimal point. Press the DOWN ARROW key once. The display will show:  
   \[
   \text{DECIMAL? 000.0}
   \]

   Press READ/ENTER. The display will show:  
   \[
   \text{P322 UNITS?}
   \]

6. Use the arrow keys to select the appropriate unit of measure. Press the DOWN ARROW key twice. The display will show:  
   \[
   \text{P322 mg/l}
   \]

7. Press READ/ENTER when the correct unit of measure is displayed. The display will show:  
   \[
   \text{P322 mg/l}
   \]

8. Construct the display to read the correct symbol. The symbol must be entered EXACTLY as shown including dashes and spaces between characters.  
   \[
   \text{Mo}^{6+} \text{ H AV}
   \]

   a) Select letters and numbers by scrolling to the correct character with the arrow keys.

   b) To make a letter or number uppercase, press the SHIFT key.

   c) The space is the character displayed after one press of the DOWN ARROW key.

   d) Make sure to enter the display line EXACTLY as shown, including all spaces. Do not enter trailing spaces.

   e) Accept each symbol by pressing READ/ENTER.

   f) To end symbol entry, press READ/ENTER a second time after accepting the last character.

9. When the instrument is out of symbol entry mode, the display will show:  
   \[
   \text{P322 TIMER?}
   \]

10. This method has one timed step, so press SHIFT TIMER. The display will show:  
    \[
    \text{MM:SS TIME 1?}
    \]

11. Enter a timer value of 5 minutes. Press:  
    \[
    0 \text{ ABS} 5 \text{ 0} 0 \text{ 0}
    \]

12. Press READ/ENTER to accept the timer value. The display will show:  
    \[
    \text{MM:SS TIME 2?}
    \]

13. Press READ/ENTER to complete the timer entry. The display will show:  
    \[
    \#0 \text{ STANDARD}
    \]

14. Press READ/ENTER to display the zero data pair. The display will show:  
    \[
    0.000 \text{ Abs} 000.0 \text{ mg/l}
    \]

15. Press READ/ENTER. The display will show:  
    \[
    \#1 \text{ STANDARD}
    \]

16. Enter concentration point #1 from the table below by pressing 0350 so that the display shows:  
    \[
    \#1 035.0 \text{ mg/l}
    \]

17. Press READ/ENTER. The display will prompt for entry of the first absorbance point:  
    \[
    \#1 0.000 \text{ Abs}
    \]

18. Enter absorbance point #1 from the table below by pressing 1460 so that the display shows:  
    \[
    \#1 1.460 \text{ Abs}
    \]

19. Press READ/ENTER. The display will show the first data pair:  
    \[
    1.460 \text{ Abs} 035.0 \text{ mg/l}
    \]

20. Press READ/ENTER to accept the first data pair. The display will show:  
    \[
    \#2 \text{ STANDARD}
    \]
21. The data pair values from the table below are now entered.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td># 0</td>
<td>0.0 mg/l</td>
<td>[0.000] Abs</td>
</tr>
<tr>
<td># 1</td>
<td>35.0 mg/l</td>
<td>[1.460] Abs</td>
</tr>
</tbody>
</table>

22. When the last point pair is entered the display will show:

# 2 STANDARD

23. Press SHIFT READ/ENTER to complete data point entry. The display will show:

#:

24. Enter the validation number: **3372** so that the display shows:

#: 3372

25. Press READ/ENTER. The display will show:

COMPLETED

P322 mg/l MO6+ H AV

Note: If the display shows:

INCORRECT #

then prompts again for the validation number you may have made an error during data entry. Make sure the validation number is correct. If so, then the error occurred during some other portion of the method entry. You must press METH and respond to the ABORT? message by pressing READ/ENTER then re-enter the method.

The instrument is now ready for use with method 322.

**SAMPLING AND STORAGE**
Collect samples in glass or plastic bottles.

**ACCURACY CHECK**

**Standard Additions Method**

a) Snap the neck off a Molybdenum Voluette Ampule Standard Solution, 500 mg/L Mo6+.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 25–mL samples contained in 50–mL beakers. Mix thoroughly.

c) Analyze the spiked sample according to the above procedure. The molybdenum concentration reading should increase by 2.0 mg/L for each 0.1 mL addition of standard.

d) If these increases do not occur, see Standard Additions (Section I of the DR/2000 Procedures Manual) for more information.

**Standard Solution Method**
To ensure test accuracy, use a Molybdenum Standard Solution, 10.0 mg/L Mo6+, listed under Optional Reagents.

**PRECISION**
In a single laboratory using standard solutions of 10.0 mg/L Mo6+ and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ± 0.10 mg/L Mo6+.

**INTERFERENCES**
Samples containing 10 mg/L copper or more will exhibit an increasing positive interference upon standing. Read these samples as soon as possible after the five-minute reaction period.

Aluminum, iron and nickel do not interfere in concentrations up to 50 mg/L.

Chromium does not interfere in concentrations up to 1000 mg/L.

Interference from nitrite up to 2000 mg/L as NO2− can be eliminated by adding one Sulfamic Acid Powder Pillow in Step 4.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment; see Interferences, pH (Section I of the DR/2000 Procedures Manual).

**SUMMARY OF METHOD**
The CDTA Solution masks metal interferences. The MolyVer 6 reagent provides the mercaptoacetic acid which reacts with molybdate molybdenum to form a yellow color proportional to the molybdenum concentration.
REQUIRED REAGENTS

Molybdenum Reagent Set (25 Tests) ............................................. 25220–98
Includes 25 MolyVer 6 AccuVac Ampuls and 0.4 M CDTA Solution, 15 mL

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Unit</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac Vial</td>
<td>1</td>
<td>each</td>
<td>43784–00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>1</td>
<td>each</td>
<td>500–41</td>
</tr>
<tr>
<td>Zeroing Vial</td>
<td>1</td>
<td>each</td>
<td>21228–00</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

CDTA Solution, 0.4 M .......................................................... 26154–36
Molybdenum Standard Solution, 10 mg/L Mo\(^{6+}\) ............................................ 14187–42
Molybdenum Standard Solution, Voluette ampule, 500 mg/L Mo\(^{6+}\), 10 mL ........... 14265–10
MolyVer\(^{®}\) 6 AccuVac Ampuls ................................................... 25220–25
Sulfamic Acid Powder Pillows ......... 1055–99

OPTIONAL APPARATUS

AccuVac Snapper Kit ................................................................. 24052–00
AccuVac Drainer (for disposal) ......................... Each .................. 41036–00
Filter Paper, folded, 12.5 cm ......................... Each .................. 1894–57
Flask, erlenmeyer, 250 mL ......................... Each .................. 505–46
Funnel, poly, 65 mm ......................................................... Each .................. 1083–67
Pipet, TenSette, 0.1 to 1.0 mL ......................... Each .................. 19700–01
Pipet Tips, for 19700–01 Tensette Pipet ............ Each .................. 21856–96

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
Mercaptoacetic Acid Method*

1. Enter the stored program number for molybdate molybdenum (Mo\(^{6+}\)).

   **Press:** 3 2 0 READ/ENTER

   The display will read:
   
   **DIAL nm TO 420**

   **Note:** DR2000s with software versions 3.0 and greater will display “P” and the program number.

   **Note:** Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

   **Note:** Collect samples in glass or plastic bottles.

2. Rotate the wavelength dial until the small display reads:

   **420 nm**

3. Press: READ/ENTER

   The display will read:
   
   **mg/l Mo\(^{6+}\) H**

   **Note:** For proof of accuracy, use a 10.0 mg/L Molybdenum Standard Solution (listed under Optional Reagents) in place of the sample.

   **Note:** Filter turbid samples using the labware listed under Optional Apparatus.

4. Fill a sample cell with 25 mL of sample.

5. Add the contents of one MolyVer 1 Reagent Powder Pillow. Swirl to mix.

6. Add the contents of one MolyVer 2 Reagent Powder Pillow. Swirl to mix.

7. Add the contents of one MolyVer 3 Reagent Powder Pillow. Swirl to mix. This is the prepared sample.

   **Note:** Molybdenum will cause a yellow color to form.

8. **Press:** SHIFT TIMER

   A 5-minute reaction period will begin.

*Adapted from *Analytical Chemistry*, 25 (9) 1363 (1953)
9. When the timer beeps, the display will show: mg/l Mo⁶⁺ H
   Fill a second sample cell with 25 mL of sample (the blank).

10. Insert the blank into the cell holder. Close the light shield.
    *Note: The Pour-Thru Cell can be used with this procedure.*

11. Press: ZERO
    The display will show: WAIT then:
    0.0 mg/l Mo⁶⁺ H

12. Place the prepared sample into the cell holder. Close the light shield.
    Press: READ/ENTER
    The display will read: WAIT
    then the results in mg/L molybdate molybdenum will be displayed.
    *Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*

---

**ACCURACY CHECK**

**Standard Additions Method**

a) Snap the neck off a Molybdenum Voluette Ampule Standard Solution, 500 mg/L Mo⁶⁺.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 25–mL samples. Mix thoroughly.

c) Analyze the spiked sample according to the above procedure. The molybdenum concentration reading should increase by 2.0 mg/L for each 0.1 mL addition of standard.

d) If these increases do not occur, see **Standard Additions** in Section I for more information.

**Standard Solution Method**

To assure the accuracy of the test, use a Molybdenum Standard Solution, 10.0 mg/L Mo⁶⁺, listed under Optional Reagents.

**PRECISION**

In a single laboratory, using standard solutions of 10.0 mg/L Mo⁶⁺ and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.07 mg/L Mo⁶⁺.

**INTERFERENCES**

Samples containing 10 mg/L copper or more will exhibit an increasing positive interference upon standing. Read these samples as soon as possible after the five-minute reaction period of Step 8.

Aluminum, iron and nickel do not interfere in concentrations up to 50 mg/L. Chromium does not interfere in concentrations up to 1000 mg/L.

Interference from nitrite up to 2000 mg/L as NO₂⁻ can be eliminated by adding one Sulfamic Acid Powder Pillow in Step 4.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment (see pH Interference in Section I).

**SUMMARY OF METHOD**

MolyVer 1 and 2 Reagents are added to buffer and condition the sample. MolyVer 3 provides the mercaptoacetic acid which reacts with molybdate molybdenum to form a yellow color proportional to the molybdenum concentration.
**REQUIRED REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molybdenum Reagent Set (100 Tests)</td>
<td>1 pillow</td>
<td>100/pkg</td>
<td>22434–00</td>
</tr>
<tr>
<td>Includes (1) 14146–69, (1) 14148–69, (1) 14178–69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**REQUARED APPARATUS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1 each</td>
<td>968–00</td>
</tr>
</tbody>
</table>

**OPTIONAL REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molybdenum Standard Solution, 10 mg/L Mo&lt;sup&gt;6+&lt;/sup&gt;</td>
<td>100 mL</td>
<td>14187–42</td>
<td></td>
</tr>
<tr>
<td>Molybdenum Standard Solution, Voluette ampule, 500 mg/L Mo&lt;sup&gt;6+&lt;/sup&gt;, 10 mL</td>
<td>16/pkg</td>
<td>14265–10</td>
<td></td>
</tr>
<tr>
<td>Sulfamic Acid Powder Pillows</td>
<td>100/pkg</td>
<td>1055–99</td>
<td></td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
<td></td>
</tr>
</tbody>
</table>

**OPTIONAL APPARATUS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
</tr>
<tr>
<td>Filter Paper, folded, 12.5 cm</td>
<td>100/pkg</td>
<td>1894–57</td>
</tr>
<tr>
<td>Flask, erlenmeyer, 250 mL</td>
<td>each</td>
<td>505–46</td>
</tr>
<tr>
<td>Funnel, poly, 65 mm</td>
<td>each</td>
<td>1083–67</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
MOLYBDENUM, MOLYBDATE, LR (0 to 3.00 mg/L) For boiler and cooling tower waters

Ternary Complex Method

1. Enter the stored program number for molybdate molybdenum (Mo\(^{6+}\)), low range.

   Press: 3 1 5 READ/ENTER

   The display will read:
   DIAL nm TO 610

   Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

   Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed to Step 4.

   Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps.

2. Rotate the wavelength dial until the small display reads:
   610 nm

3. Press: READ/ENTER
   The display will read:
   mg/\(\ell\) Mo\(^{6+}\) L

4. Fill a 50–mL graduated mixing cylinder with 50 mL of the water to be tested.

   Note: For proof of accuracy, use a 2.0 mg/L Molybdenum Standard Solution (preparation given in Accuracy Check) in place of the sample.

   Note: Filter turbid samples using the labware listed under Optional Apparatus.

5. Add the contents of one Molybdenum 1 Reagent Powder Pillow to the graduated cylinder. Stopper, then shake the graduated cylinder to dissolve the reagents. This is the prepared sample.

6. Pour 25 mL of the prepared sample into one sample cell of a matched pair.

7. Add 1.0 mL of Molybdenum 2 Reagent to the sample cell. Swirl to mix. This is the developed sample.

   Note: Molybdenum will cause a green color to form.

8. Press: SHIFT TIMER
   A 2–minute reaction period will begin.
9. When the timer beeps, the display will show:
   mg/l Mo⁶⁺  L
   Fill a second sample cell with 25 mL of prepared sample (the blank).

10. Insert the blank into the cell holder. Close the light shield.
    Note: The Pour-Thru Cell can be used with this procedure.

11. Press: ZERO
    The display will show: WAIT
    then:
    0.00 mg/l Mo⁶⁺  L

12. Place the developed sample into the cell holder. Close the light shield.
    Press: READ/ENTER
    The display will read: WAIT
    then the results in mg/L molybdate molybdenum (Mo⁵⁺) will be displayed.
    Note: The results can be expressed as mg/L molybdate (MoO₄²⁻) or mg/L sodium molybdate (Na₂MoO₄) by multiplying the mg/L molybdenum (Mo⁵⁺) by 1.67 or 2.15, respectively.
    Note: For a DR/2000 in the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

---

**SAMPLING AND STORAGE**
Collect samples in glass or plastic bottles.

**ACCURACY CHECK**

a) Snap the neck off a Molybdenum Voluette Ampule Standard Solution, 500 mg/L Mo⁶⁺.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 250–mL samples. Mix thoroughly.

c) Analyze 50 mL of each sample according to the above procedure. The molybdenum concentration reading should increase by 0.2 mg/L for each 0.1 mL addition of standard.

**Standard Solution Method**
To assure the accuracy of the test, use a 2–mg/L Molybdenum Standard Solution. Prepare a 2–mg/L Molybdenum Standard Solution by first pipetting 10 mL of a 10–mg/L Molybdenum Standard Solution into a 50–mL graduated mixing cylinder. Next, dilute to a final volume of 50 mL using demineralized water. Mix thoroughly. Analyze the standard according to the above procedure.

**PRECISION**
In a single laboratory, using standard solutions of 2.0 mg/L Mo⁶⁺ and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.015 mg/L Mo⁶⁺.

**INTERFERENCES**
Interference studies were conducted by preparing a molybdenum standard solution (2 mg/L Mo⁶⁺) as well as a solution of the potential interfering ion. When the standard solution concentration changed by ±5% with a given ion concentration, the ion was considered an interference.
### MOLYBDENUM, MOLYBDATE, LR, continued

<table>
<thead>
<tr>
<th>Negative Interference:</th>
<th>Level above which it interferes (mg/L)</th>
<th>No Interference: (continued)</th>
<th>Highest Concentration Tested (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion</td>
<td></td>
<td>Ion</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>200</td>
<td>Chlorine</td>
<td>7.5</td>
</tr>
<tr>
<td>Copper</td>
<td>98</td>
<td>PBTC (phosphonate)</td>
<td>500</td>
</tr>
<tr>
<td>Chromium (Cr6+)</td>
<td>4.5*</td>
<td>Sulfate</td>
<td>12,800</td>
</tr>
<tr>
<td>Chloride</td>
<td>1,400</td>
<td>Bisulfite</td>
<td>9,600</td>
</tr>
<tr>
<td>AMP (Phosphonate)</td>
<td>15</td>
<td>Nickel</td>
<td>250</td>
</tr>
<tr>
<td>Phosphonohydroxyacetic Acid</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisulfate</td>
<td>3,300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>350*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminum</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylates</td>
<td>790</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alum</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin Sulfonate</td>
<td>105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>4,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>5,650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>1,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borate</td>
<td>5,250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>2% (by volume)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfite</td>
<td>6,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethanolthiocarbamate</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Read the molybdenum concentration immediately after the beep of the two-minute reaction period.

<table>
<thead>
<tr>
<th>Positive Interference:</th>
<th>Level above which it interferes (mg/L)</th>
<th>Ion</th>
<th>Highest Concentration Tested (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion</td>
<td></td>
<td>chlorine</td>
<td></td>
</tr>
<tr>
<td>Carbonate</td>
<td>1,325</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silica</td>
<td>600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzotriazole</td>
<td>210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morpholine</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| No Interference:       | Highest Concentration Tested (mg/L)    |
| Ion                    |                                        |
| Zinc                   | 400                                     |
| Calcium                | 720                                     |
| Magnesium              | 8,000                                   |
| Manganese              | 1,600                                   |

The presence of the phosphonate HEDP at concentrations up to 30 mg/L will increase the apparent molybdenum concentration reading by approximately 10% (positive interference). For these samples, multiply the value obtained in step 12 by 0.9 to obtain the actual molybdenum concentration. As the concentration of HEDP increases above 30 mg/L, a decrease in the molybdenum concentration reading occurs (negative interference).

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagent and require sample pretreatment. Adjust the sample pH to between 3 and 5 by using a pH meter or pH paper and adding, dropwise, an appropriate amount of acid or base such as 1.0 N Sulfuric Acid Standard Solution, or 1.0 N Sodium Hydroxide Standard Solution. If significant volumes of acid or base are used, a volume correction should be made by dividing the total volume (sample + acid + base) by the original volume and multiplying the test result by this factor.

After a number of samples have been analyzed, the sample cells may exhibit a build-up of a slight blue color. A rinse using 1:1 Hydrochloric Acid Solution will eliminate the build-up if it occurs.

### SUMMARY OF METHOD
The ternary complex method for molybdenum determination is a method in which molybdate molybdenum reacts with an indicator and sensitizing agent to give a stable blue complex.

### REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molybdenum 1 Reagent for Low Range Molybdate</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>23527–66</td>
</tr>
<tr>
<td>Powder Pillows, for 50 mL sample size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molybdenum 2 Reagent for Low Range Molybdate Solution</td>
<td>1.0 mL</td>
<td>100 mL MDB</td>
<td>23525–32</td>
</tr>
</tbody>
</table>

### REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder, mixing, graduated, 50 mL</td>
<td>1</td>
<td>each</td>
<td>1896–41</td>
</tr>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968–00</td>
</tr>
</tbody>
</table>

277
### OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molybdenum Standard Solution, 10 mg/L Mo&lt;sub&gt;6&lt;/sub&gt;⁺</td>
<td>100 mL</td>
<td>14187–42</td>
</tr>
<tr>
<td>Molybdenum Standard Solution, Voluette ampule, 500 mg/L Mo&lt;sub&gt;6&lt;/sub&gt;⁺, 10 mL</td>
<td>16/pkg</td>
<td>14265–10</td>
</tr>
<tr>
<td>Hydrochloric Acid Solution, 1:1, 6.0 N</td>
<td>500 mL</td>
<td>884–49</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
</tr>
</tbody>
</table>

### OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
</tr>
<tr>
<td>Filter Paper, folded, 12.5 cm</td>
<td>100/pkg</td>
<td>1894–57</td>
</tr>
<tr>
<td>Flask, volumetric, 250 mL, Class B</td>
<td>each</td>
<td>547–46</td>
</tr>
<tr>
<td>Funnel, poly, 65 mm</td>
<td>each</td>
<td>1083–67</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pipet, volumetric, 10 mL, Class A</td>
<td>each</td>
<td>14515–38</td>
</tr>
<tr>
<td>Pipet Filler</td>
<td>each</td>
<td>12189–00</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.

In the U.S.A. call 800–227–4224 to place an order.
Heptoxime Method*: USEPA accepted for reporting wastewater analysis
(digestion required – see Section I)**

1. Enter the stored program number for nickel (Ni)–heptoxime method.
Press: 3 3 5 READ/ENTER

The display will show:
DIAL nm TO 430

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust the pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:
430 nm

3. Press: READ/ENTER
The display will show:
mg/l Ni Hept

4. Measure 300 mL of sample in a 500–mL graduated cylinder. Pour into a 500–mL separatory funnel.

Note: For proof of accuracy, use a 1.0 mg/L nickel standard solution (preparation given in Accuracy Check) in place of the sample.

5. Add the contents of one Nickel 1 Reagent Powder Pillow to the funnel. Stopper. Shake to mix.

6. Press: SHIFT TIMER
A 5–minute reaction period will begin.

7. When the timer beeps, add the contents of one Nickel 2 Reagent Powder Pillow to the funnel. Stopper. Shake to mix.

8. Press: SHIFT TIMER
A second 5–minute reaction period will begin.

*Adapted from Chemie Analytique, 36 43 (1954)
**Procedure is equivalent to Standard Method 3500-Ni D for wastewater.
9. When the timer beeps, add 10 mL of chloroform. Stopper. Shake gently. Invert. Open the stopcock to vent.


11. Press: SHIFT TIMER
A third 5-minute reaction period will begin. Shake the funnel several times over the five-minute period.

12. When the timer beeps, the display will show:
   mg/l Ni Hept
   Wait for the layers to separate. Insert a pea-sized cotton plug into the delivery tube of the funnel. Drain the chloroform layer into a sample cell (the prepared sample). Stopper.

13. Repeat Steps 9 to 12 two additional times with 10–mL portions of chloroform.
   Note: The five-minute reaction period is not necessary. Shake with chloroform to separate; then continue. Wait for layers to separate, then continue.
   Note: The Pour-Thru Cell cannot be used with this procedure.

14. Fill a second cell (the blank) with 25 mL of chloroform. Stopper. Place the blank into the cell holder. Close the light shield.
   Note: The final volume of extract will be about 25 mL due to the slight solubility of chloroform in water.
   Note: Swirl sample cell to mix extracts.

15. Press: ZERO
   The display will show: WAIT
   then: 0.00 mg/l Ni Hept

16. Place the prepared sample into the cell holder. Close the light shield.
   Press: READ/ENTER
   The display will show: WAIT
   then the result in mg/L nickel will be displayed.
   Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
SAFETY AND STORAGE
Collect samples in acid-washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the sample pH to between 3 and 8 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 8 as this may cause some loss of nickel as a precipitate. Correct the test results for volume additions (see Correction for Volume Additions in Section I).

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off a Nickel Volumette Ampule Standard Solution, 300 mg/L Ni.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 300–mL samples.

c) Analyze each sample as described above. The nickel concentration should increase 0.10 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method
Prepare a 1.0 mg/L nickel standard solution by diluting 50.0 mL of a 10–mg/L working standard solution to 500 mL in a volumetric flask. The working stock solution should be prepared daily by diluting 10.00 mL of Nickel Standard Solution, 1000 mg/L as Ni, to 1000 mL with demineralized water.

Or, use the TenSette Pipet to add 1.0 mL of a Nickel Volumette Ampule Standard Solution, 300 mg/L Ni, into a 500–mL volumetric flask and dilute to volume with demineralized water. This solution is 0.6 mg/L nickel.

PRECISION
In a single laboratory, using standard solutions of 0.83 mg/L nickel and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation ±0.022 mg/L nickel.

INTERFERENCEs
Cobalt, copper and iron interferences can be overcome by adding one or more additional Nickel 1 Reagent Powder Pillows in Step 5. The tolerance limits of these interferences are shown in the following table:

<table>
<thead>
<tr>
<th>Tolerance Limits vs. Number of Nickel 1 Reagent Powder Pillows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pills of Nickel 1 Reagent</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

A preliminary acid digestion is required to determine any suspended or precipitated nickel and to eliminate interference by organic matter. To eliminate this interference or to determine total recoverable nickel, perform the EPA approved digestion in Digestion (Section I).

SUMMARY OF METHOD
Nickel ion reacts with heptoxime to form a yellow-colored complex which is then extracted into chloroform to concentrate the color and enable a more sensitive determination. Chelating agents are added to the sample to overcome the interferences caused by cobalt, copper and iron.

REQUIRED REAGENTS
Nickel Reagent Set (50 Tests) ................................................................. 22435–00

Includes: (3) 14458–49, (2) 2123–68, (2) 2124–68

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform, ACS</td>
<td>55 mL</td>
<td>500 mL</td>
<td>14458–49</td>
<td></td>
</tr>
<tr>
<td>Nickel 1 Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>25/pkg</td>
<td>2123–68</td>
<td></td>
</tr>
<tr>
<td>Nickel 2 Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>25/pkg</td>
<td>2124–68</td>
<td></td>
</tr>
</tbody>
</table>
### REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968-00</td>
</tr>
<tr>
<td>Cotton balls, absorbent</td>
<td>1</td>
<td>100/pkg</td>
<td>2575-01</td>
</tr>
<tr>
<td>Cylinder, graduated, 10 mL</td>
<td>1</td>
<td>each</td>
<td>508-49</td>
</tr>
<tr>
<td>Cylinder, graduated, 500 mL</td>
<td>1</td>
<td>each</td>
<td>508-49</td>
</tr>
<tr>
<td>Funnel, separatory, 500 mL</td>
<td>1</td>
<td>each</td>
<td>520-49</td>
</tr>
<tr>
<td>Ring, support, 4&quot;</td>
<td>1</td>
<td>each</td>
<td>580-01</td>
</tr>
<tr>
<td>Stand, support, 127 x 203 mm</td>
<td>1</td>
<td>each</td>
<td>563-00</td>
</tr>
<tr>
<td>Stopper, hollow, poly, Size 0</td>
<td>2</td>
<td>6/pkg</td>
<td>14480-00</td>
</tr>
</tbody>
</table>

### OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel Standard Solution, 1000 mg/L Ni</td>
<td>100 mL</td>
<td></td>
<td>14176-42</td>
</tr>
<tr>
<td>Nickel Standard Solution, Voluette ampule, 300 mg/L Ni, 10 mL</td>
<td>16/pkg</td>
<td></td>
<td>14266-10</td>
</tr>
<tr>
<td>Nitric Acid, ACS</td>
<td>500 mL</td>
<td></td>
<td>152-49</td>
</tr>
<tr>
<td>Nitric Acid Solution, 1:1</td>
<td>500 mL</td>
<td></td>
<td>2540-49</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>1 L</td>
<td></td>
<td>2450-53</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td></td>
<td>272-56</td>
</tr>
</tbody>
</table>

### OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Unit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flask, erlenmeyer, 500 mL</td>
<td></td>
<td>each</td>
<td>505-49</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 500 mL</td>
<td></td>
<td>each</td>
<td>14574-49</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 1000 mL</td>
<td></td>
<td>each</td>
<td>14574-53</td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td></td>
<td>5 rolls/pkg</td>
<td>391-33</td>
</tr>
<tr>
<td>Pipet, serological, 1 mL</td>
<td></td>
<td>each</td>
<td>532-35</td>
</tr>
<tr>
<td>Pipet, serological, 5 mL</td>
<td></td>
<td>each</td>
<td>532-37</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td></td>
<td>each</td>
<td>19700-01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700-01 TenSette Pipet</td>
<td></td>
<td>50/pkg</td>
<td>21856-96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 10.00 mL</td>
<td></td>
<td>each</td>
<td>14515-38</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td></td>
<td>each</td>
<td>14651-00</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 50.00 mL</td>
<td></td>
<td>each</td>
<td>14515-41</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800-227-4224 to place an order.
1—(2 Pyridylazo)—2—Naphthol (PAN) Method*

1. Enter the stored program number for nickel (Ni), PAN method.
   Press: **3 4 0 READ/ENTER**

The display will show:
   **DIAL nm TO 560**

*Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.*

*Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.*

*Note: If sample cannot be analyzed immediately, see Sampling and Storage following this procedure. Adjust the pH of stored samples before analysis.*

2. Rotate the wavelength until the small display shows: **560 nm**

3. Press: **READ/ENTER**
   The display will show: **mg/l Ni PAN**

4. Measure 25 mL of sample in a mixing graduated cylinder. This will be the prepared sample.

*Note: If sample is less than 10 °C (50 °F), warm to room temperature before analysis.*

*Note: For proof of accuracy, use a 0.5 mg/L nickel standard solution (preparation given in Accuracy Check) in place of the sample.*

---

5. Measure 25 mL of demineralized water in a second cylinder (the blank).

6. Add the contents of one Phthalate–Phosphate Reagent Powder Pillow to each cylinder. Stopper. Immediately shake to dissolve.

*Note:* If sample contains iron (Fe²⁺), it is important that all powder be dissolved completely before continuing with Step 7.

7. Add 1.0 mL of 0.3% PAN Indicator Solution to each cylinder. Stopper. Invert several times to mix.

*Note:* Use the plastic dropper provided.

8. Press: **SHIFT TIMER**

A 15-minute reaction period will begin.

*Note:* During color development, the sample solution color may vary from yellowish-orange to dark red depending on the chemical make-up of the sample. The demineralized water blank should be yellow.

9. When the timer beeps, the display will show: **mg/l Ni PAN**

Add the contents of one EDTA Reagent Powder Pillow to each cylinder. Stopper. Shake to dissolve.

10. Fill a sample cell with 25 mL of the blank. Place it into the cell holder. Close the light shield.

*Note:* The Pour-Through Cell can be used if rinsed well with demineralized water between the blank and prepared sample.

11. Press: **ZERO**

The display will show: **WAIT**

then: **0.000 mg/l Ni PAN**

12. Fill a second sample cell with the prepared sample. Place it into the cell holder. Close the light shield.
13. Press: READ/ENTER

The display will show: WAIT
then the result in mg/L nickel will be displayed.

Note: If the sample contains cobalt, continue with Steps 14 to 19.

14. To correct for the presence of cobalt, rotate the wavelength dial to change the wavelength display to show: 620 nm and continue.

15. Place the blank into the cell holder. Close the light shield.

16. Press: ZERO

The display will show: WAIT
then 0.000 mg/l Ni PAN

17. Place the prepared sample into the cell holder. Close the light shield.

18. Press: READ/ENTER

The display will show: WAIT
then the apparent nickel concentration in mg/L nickel due to the cobalt present.

19. Subtract the mg/L apparent nickel (Ni) found in Step 18 from that obtained in Step 13 to obtain the actual nickel concentration in the sample.

Note: A determination of the cobalt concentration may be made with the same prepared samples by using the Cobalt Stored Program No. 110.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
NICKEL, continued

SAMPLING AND STORAGE
Collect samples in acid–washed plastic bottles. Adjust the sample pH to 2 or less with nitric acid (about 5 mL per liter). Preserved samples can be stored up to six months at room temperature. Before analysis, adjust the sample pH to between 3 to 8 with 5.0 N Sodium Hydroxide Standard Solution. Do not exceed pH 8 as this may cause some loss of nickel as a precipitate. Correct test results for volume additions (see Correction for Volume Additions in Section I).

ACCURACY CHECK
Standard Solution Method
Prepare a 0.5 mg/L nickel standard solution by diluting 10.0 mL of a 5–mg/L working stock solution to 100 mL in a 100–mL volumetric flask. The working stock solution should be prepared daily by diluting 5.00 mL of Nickel Standard Solution, 1000 mg/L as Ni, to 1000 mL with demineralized water.

Or, using the TenSette Pipet, add 0.2 mL of a Voluette Ampule Standard Solution for Nickel, 300 mg/L Ni, into a 100–mL volumetric flask. Dilute to volume with demineralized water. This is a 0.6 mg/L standard solution.

PRECISION
In a single laboratory, using standard solutions of 0.500 mg/L nickel and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.0037 mg/L nickel.

INTERFERENCES
The following may interfere when present in concentrations exceeding those listed at right:

- Al\(^{3+}\): 32 mg/L
- Ca\(^{2+}\): 1000 mg/L as (CaCO\(_3\))
- Cd\(^{2+}\): 20 mg/L
- Cl\(^{-}\): 8000 mg/L
- Cr\(^{3+}\): 20 mg/L
- Cr\(^{6+}\): 40 mg/L
- Cu\(^{2+}\): 15 mg/L
- F\(^{-}\): 20 mg/L
- Fe\(^{3+}\): 10 mg/L
- Fe\(^{2+}\) interferes directly and must not be present.
- K\(^{+}\): 500 mg/L
- Mg\(^{2+}\): 400 mg/L
- Mn\(^{2+}\): 25 mg/L
- Mo\(^{6+}\): 60 mg/L
- Na\(^{+}\): 5000 mg/L
- Pb\(^{2+}\): 20 mg/L
- Zn\(^{2+}\): 30 mg/L

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and required sample pretreatment (see pH Interference in Section I).

Chelating agents, such as EDTA, interfere. Use either the Digesdahl or vigorous digestion (Section I) to eliminate this interference.

SUMMARY OF METHOD
After buffering the sample and masking any Fe\(^{3+}\) with pyrophosphate, the nickel is reacted with 1–(2–Pyridylazo)–2–Naphthol indicator. The indicator forms complexes with most metals present. After color development, EDTA is added to destroy all metal–PAN complexes except nickel and cobalt. This method is unique because both nickel and cobalt can be determined on the same sample.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel Reagent Set (100 Tests*)</td>
<td></td>
<td></td>
<td>22426–00</td>
</tr>
<tr>
<td>Includes: (4) 7005–66, (4) 21501–66, (2) 21502–32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA Reagent Powder Pillows</td>
<td>2 pillows</td>
<td>50/pkg</td>
<td>7005–66</td>
</tr>
<tr>
<td>Phthalate–Phosphate Reagent Powder Pillows</td>
<td>2 pillows</td>
<td>50/pkg</td>
<td>21501–66</td>
</tr>
<tr>
<td>P.A.N. Indicator Solution, 0.3%</td>
<td>2 mL</td>
<td>100 mL</td>
<td>21502–32</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>25 mL</td>
<td>4 L</td>
<td>272–56</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

- Clippers, for opening powder pillows 1 each 968–00
- Cylinder, graduated, 25 mL 2 each 20886–40
<table>
<thead>
<tr>
<th>OPTIONAL REAGENTS</th>
<th>Quantity</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel Standard Solution, 1000 mg/L Ni</td>
<td>100 mL</td>
<td>14176–42</td>
</tr>
<tr>
<td>Nickel Standard Solution, Voluette ampule, 300 mg/L Ni, 10 mL</td>
<td>16/pkg</td>
<td>14266–10</td>
</tr>
<tr>
<td>Nitric Acid, ACS</td>
<td>500 mL</td>
<td>152–49</td>
</tr>
<tr>
<td>Nitric Acid Solution, 1:1</td>
<td>500 mL</td>
<td>2540–49</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>100 mL**</td>
<td>2450–32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OPTIONAL APPARATUS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 100 mL</td>
<td>each</td>
<td>14574–42</td>
</tr>
<tr>
<td>Flask, volumetric, Class A, 1000 mL</td>
<td>each</td>
<td>14574–53</td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>5 rolls/pkg</td>
<td>391–33</td>
</tr>
<tr>
<td>pH Meter, EC10, portable</td>
<td>each</td>
<td>50050–00</td>
</tr>
<tr>
<td>Pipet, serological, 1 mL</td>
<td>each</td>
<td>532–35</td>
</tr>
<tr>
<td>Pipet, serological, 5 mL</td>
<td>each</td>
<td>532–37</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 5.0 mL</td>
<td>each</td>
<td>14515–37</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 10.0 mL</td>
<td>each</td>
<td>14515–38</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651–00</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section. In the U.S.A. call 800–227–4224 to place an order.

*100 Tests equals 50 sample and 50 blanks.

**Contact Hach for larger sizes.
Cadmium Reduction Method (Powder Pillows or AccuVac Ampuls)

USING POWDER PILLOWS

1. Enter the stored program number for high range nitrate nitrogen (NO₃⁻–N)–powder pillows.

   Press: 3 5 5 READ/ENTER

   The display will show:

   DIAL nm TO 500

   *Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

   *Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

   *Note: If sample cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust the pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:

   500 nm

   3. Press: READ/ENTER

   The display will show:

   mg/l N NO₃⁻– H

4. Fill a sample cell with 25 mL of sample.

   *Note: For proof of accuracy, use a 10 mg/L Nitrate Nitrogen Standard Solution (listed under Optional Reagents) in place of the sample.

   *Note: A reagent blank must be determined on each new lot of NitraVer 5. Perform Steps 4 to 12 using demineralized water as the sample. Subtract this value from each result obtained with this lot of reagent.

*For seawater, a manual calibration is required; see Interferences.
5. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow to the cell (the prepared sample). Stopper.

6. Press: **SHIFT TIMER**

Shake the cell vigorously until the timer beeps in one minute.

**Note:** A deposit of unoxidized metal will remain after the NitraVer 5 Nitrate Reagent Powder dissolves. This deposit will have no effect on test results.

**Note:** Shaking time and technique influence color development. For most accurate results, make successive tests on a 10 mg/L Nitrate Nitrogen Standard Solution listed under Optional Reagents. Adjust the shaking time to obtain the correct result.

7. When the timer beeps, press: **SHIFT TIMER**

A 5-minute reaction period will begin.

**Note:** An amber color will develop if nitrate nitrogen is present.

8. Fill another sample cell with 25 mL of sample (the blank).
9. When the timer beeps, the display will show:
   \[ \text{mg/L N} \text{ NO}_3^- \text{ H} \]
   Place the blank into the cell holder. Close the light shield.

Note: The Pour-Thru Cell can be used if rinsed well with
demineralized water after use.
Avoid pouring any cadmium particles into the cell.

10. Press: ZERO
    The display will show:
    \[ \text{WAIT} \]
    then:
    \[ 0.0 \text{mg/L N} \text{ NO}_3^- \text{ H} \]

11. Remove the stopper.
    Place the prepared sample into the cell holder. Close
    the light shield.

12. Press: READ/ENTER
    The display will show:
    \[ \text{WAIT} \]
    then the result in mg/L nitrate nitrogen (NO$_3^-$-N)
    will be displayed.

Note: In the constant-on mode, pressing READ/ENTER is not
required. WAIT will not appear. When the display stabilizes, read
the result.

Note: The results can be expressed as mg/L nitrate
(NO$_3^-$) by multiplying the mg/L nitrate nitrogen (NO$_3^-$-N) by 4.4.

Note: Rinse the sample cell immediately after use to remove
all cadmium particles.
USING ACCUVAC AMPULS

1. Enter the stored program number for high range nitrate nitrogen (NO₃⁻–N)–AccuVac ampuls.

Press: 3 6 1 READ/ENTER

The display will show:
DIAL nm TO 500

*Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

*Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

*Note: If your instrument does not have program number 361, see Instrument Setup following these steps.

2. Rotate the wavelength dial until the small display shows:
500 nm

3. Press: READ/ENTER

The display will show:
mg/l N NO₃⁻ H AV

4. Collect at least 40 mL of sample in a 50–mL beaker. Fill a NitraVer 5 Nitrate AccuVac Ampul with sample.

*Note: Keep the tip immersed while the ampul fills completely.

*Note: For proof of accuracy, use a 10 mg/L Nitrate Nitrogen Standard Solution (listed under Optional Reagents) in place of the sample.

*Note: A reagent blank must be determined on each new lot of NitraVer 5. Repeat Steps 4 to 12 using demineralized water as the sample. Subtract this value from each result obtained with this lot of reagent.
5. Press: **SHIFT TIMER**

A one-minute mixing period will begin. Invert the ampul repeatedly until the timer beeps. Wipe off any liquid or fingerprints.

**Note:** Shaking time and technique influence color development. For most accurate results, make successive tests on a 10 mg/L Nitrate Nitrogen Standard Solution listed under Optional Reagents. Adjust the shaking time to obtain the correct result.

6. When the timer beeps, press: **SHIFT TIMER**

A 5-minute reaction period will begin.

**Note:** A deposit of oxidized metal will remain after the NitroVer 5 Nitrate Reagent Powder dissolves. This deposit will have no effect on test results.

**Note:** An amber color will develop if nitrate nitrogen is present.

7. Fill a zeroing vial with at least 10 mL of sample (the blank).

**Note:** Place the grip tab at the rear of the cell holder.

8. Place the AccuVac Vial Adapter into the cell holder.

9. When the timer beeps, the display will show:

$\text{mg/L } \text{NO}_3^- \text{ H AV}$

Place the blank into the cell holder. Close the light shield.

10. Press: **ZERO**

The display will show: **WAIT**

Then

$0.0 \text{ mg/L } \text{NO}_3^- \text{ H AV}$

11. Place the AccuVac ampul into the cell holder. Close the light shield.

12. Press: **READ/ENTER**

The display will show: **WAIT**

Then the nitrate result in mg/L nitrate nitrogen ($\text{NO}_3^- \text{-N}$) will be displayed.

**Note:** The results can be expressed as mg/L nitrate ($\text{NO}_3^-$) by multiplying the mg/L nitrate nitrogen ($\text{NO}_3^- \text{-N}$) by 4.4.

**Note:** In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
INSTRUMENT SETUP
For a DR/2000 with software versions 1.27 or 1.265, enter the following calibration as an operator-programmed calibration for high range nitrate nitrogen AccuVac ampuls (method 361). Follow steps in the Operation section of the DR/2000 Instrument Manual. Store the method as follows:

\[
\begin{align*}
\text{nm} & = 500 \\
\text{Decimal} & = 000.0 \\
\text{Units} & = \text{mg/l} \\
\text{Symbol} & = \text{NO}_3^-\text{N} \\
\text{Timer 1} & = 01:00 \\
\text{Timer 2} & = 05:00
\end{align*}
\]

At first, enter the calibration with 0.000 absorbance values for zero and standards #1–4. To do this, do not place anything in the sample compartment. Begin by storing zero, #1 standard, #2 standard, #3 standard and #4 standard as concentrations of 0, 6.0, 12.5, 20.0 and 35.0, respectively, with nothing in the sample compartment. Accept 0.000 Abs. as the value for all standards. Next, the values for the standards must be changed to the values given below.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>1</td>
<td>6.0</td>
<td>0.125</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>0.250</td>
</tr>
<tr>
<td>3</td>
<td>20.0</td>
<td>0.375</td>
</tr>
<tr>
<td>4</td>
<td>35.0</td>
<td>0.563</td>
</tr>
</tbody>
</table>

The method is now stored as an operator-programmed method with a method number between 950 and 999. Record the method number for future reference.

For a DR/2000 with software version 2.0 and 2.2, enter the calibration as an update to Hach-stored programs. (Stored program number 360 has been replaced with number 361.)

1. Press:  

2. Press:  

3. Press:  

4. Within 3 seconds, press:  

The display will show:  

\[\text{ENTER } \text{nm}\]

If the display returns to the METHOD prompt, repeat the sequence.

5. Press:  

If you make an error, press \text{SHIFT CLEAR} and re-enter the number. When the number is correct, press \text{READ/ENTER}. The display will show:  

\[\text{DECIMAL? 000.0}\]

6. Use the arrow keys to correctly position the decimal point. For this method, press the \text{RIGHT/DOWN ARROW} key once. The display will show:  

\[\text{DECIMAL? 000.0}\]

7. When the decimal point is correctly positioned, press: \text{READ/ENTER}. The display will show:  

\[\text{UNITS?}\]

8. Use the arrow keys to select the appropriate unit of measure. For this method, press the \text{RIGHT/DOWN ARROW} key twice. The display will show:  

\[\text{mg/l}\]

9. With the proper unit of measure displayed, press \text{READ/ENTER}. The display will show:  

\[\text{SYMBOL?}\]

10. Use the arrow keys to construct the correct symbol display. For this method, press the \text{RIGHT/DOWN ARROW} key repeatedly until you see:  

\[\text{mg/l n}\]

11. Press \text{SHIFT} to make the “n” uppercase. The display will show:  

\[\text{mg/l N}\]

12. Press the \text{READ/ENTER} key to accept the capital “N.”

13. Using the arrow keys, continue to construct the display:  

\[\text{mg/l N NO}_3^-\text{H AV}\]

The space is the “character” displayed after one press of the \text{RIGHT/DOWN ARROW} key. To enter subscript 3, press the number 3 key. It will enter as a subscript.
14. When the last character of the symbol is accepted with the READ/ENTER key, the display will show: TIMER?

15. There are two timers for this method, so press SHIFT TIMER. The display will show: MM:SS TIME 1?

16. To enter the first timer value of 1:00 minute, press:

```
0 1 0 0
```

The display will then read: 01:00 TIME 1?

17. Press READ/ENTER to accept the timer value. The display will show: MM:SS TIME 2?

18. To enter the second timer value of 5:00 minutes, press:

```
0 5 0 0
```

The display will then read: 05:00 TIME 2?

19. Press READ/ENTER to accept the timer value.

20. The display will then read: MM:SS TIME 3?

21. Press READ/ENTER to complete the timer entry. The display will show: # 1 Data 0

22. Enter the following 12 numbers as shown. Complete each number entry by pressing READ/ENTER.

<table>
<thead>
<tr>
<th># 1 Data</th>
<th># 2 Data</th>
<th># 3 Data</th>
<th># 4 Data</th>
<th># 5 Data</th>
<th># 6 Data</th>
<th># 7 Data</th>
<th># 8 Data</th>
<th># 9 Data</th>
<th># 10 Data</th>
<th># 11 Data</th>
<th>Checksum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15425</td>
<td>20324</td>
<td>25855</td>
<td>65535</td>
<td>65535</td>
<td>65535</td>
<td>65535</td>
<td>65535</td>
<td>65535</td>
<td>512</td>
<td>60769</td>
</tr>
</tbody>
</table>

The final number is a check value which is used to determine if the data sequence was correctly entered. If an error is made during number entry, the display will return to the prompt for data number 1 and the entire sequence must be re-entered. If all numbers are correctly entered, the display will return to the method prompt and is ready for use: METHOD #?

23. Once the new method 361 has been successfully entered, block access to the now obsolete method 360. Press:

```
SHIFT  CONFIG  METH
```

Press:

```
PROG  CONC  0  READ  ENTER
```

Within 3 seconds press:

```
SHIFT  PROG  3  CONFIG  METH
```

The display will show: 800 CONFIGURE

Press READ/ENTER three times to return to: METHOD #?

Access to method 360 is blocked. Method 361 is now a stored method.

**SAMPLING AND STORAGE**

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods, up to 14 days, adjust sample pH to 2 or less with sulfuric acid, ACS, (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature. Neutralize the sample with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct test results for volume additions (see Correction for Volume Additions in Section I).
ACCURACY CHECK
Standard Additions Method
a) Snap the neck off a fresh High Range Nitrate Nitrogen Voltette Ampule Standard, 500 mg/L NO₃⁻-N.

b) Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 25-mL samples. Mix each thoroughly. (For Accu-Vac ampuls, use 50-mL beakers.)

c) Analyze each sample as described above. The nitrogen concentration should increase 2.0 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method
Use a 10.0 mg/L Nitrate Nitrogen Standard Solution listed under Optional Reagents to check test accuracy. Or, this can be prepared by diluting 1.0 mL of solution from a High Range Nitrate Nitrogen Voltette Ampule Standard Solution, 500 mg/L NO₃⁻-N, to 50.0 mL with demineralized water.

PRECISION
In a single laboratory, using standard solutions of 20.0 mg/L nitrate nitrogen (NO₃⁻-N) and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.8 mg/L nitrate nitrogen.

Using standard solutions of 30.0 mg/L (NO₃⁻-N) and one representative lot of Accu-Vac ampuls with the DR/2000, a single operator obtained a standard deviation of ±2.3 mg/L nitrate nitrogen.

INTERFERENCES
Compensate for nitrite interference as follows:

a) Add Bromine Water, 30 g/L, drop-wise to the sample in Step 4 until a yellow color remains.

b) Add one drop of Phenol Solution, 30 g/L, to destroy the color.

c) Proceed with Step 4. Report results as total nitrate and nitrite.

Strong oxidizing and reducing substances will interfere. Ferric iron causes high results and must be absent. Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride levels (i.e., seawater), but a calibration must be performed using standards spiked to the same chloride concentration. See User Stored Programs in the DR/2000 Instrument Manual for more information.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment (see pH Interference in Section I).

SUMMARY OF METHOD
Cadmium metal reduces nitrates present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. This salt couples to gentisic acid to form an amber-colored product. Nitrate can be determined directly using the Nitrate Ion Selective Electrode (Cat. No. 44560-71).

REQUIRED REAGENTS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitraVer 5 Nitrate Reagent Powder Pills</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>14034–66</td>
</tr>
</tbody>
</table>

REQUIRED REAGENTS (Using Accu-Vac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitraVer 5 Nitrate Reagent Accu-Vac Ampul</td>
<td>1 ampul</td>
<td>25/pkg</td>
<td>25110–25</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968–00</td>
</tr>
<tr>
<td>Stopper, rubber, size 2</td>
<td>1</td>
<td>12/pkg</td>
<td>2118–02</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS (Using Accu-Vac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, Accu-Vac Vial</td>
<td>1</td>
<td>each</td>
<td>43784–00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>1</td>
<td>each</td>
<td>500–41</td>
</tr>
<tr>
<td>Zeroing Vial</td>
<td>1</td>
<td>each</td>
<td>21228–00</td>
</tr>
</tbody>
</table>
### OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Reagent Description</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromine Water, 30 g/L</td>
<td>29 mL*</td>
<td>2211-20</td>
</tr>
<tr>
<td>Nitrate Nitrogen standard Solution, 10 mg/L NO₃-N</td>
<td>500 mL</td>
<td>307-49</td>
</tr>
<tr>
<td>Nitrate Nitrogen Standard Solution, Voluette Ampule, 500 mg/L (NO₃-N), 10 mL</td>
<td>16/pkg</td>
<td>14260-10</td>
</tr>
<tr>
<td>Phenol Solution, 30 g/L</td>
<td>29 mL</td>
<td>2112-20</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>59 mL*</td>
<td>2450-26</td>
</tr>
<tr>
<td>Sulfuric Acid, ACS</td>
<td>500 mL*</td>
<td>979-49</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272-56</td>
</tr>
</tbody>
</table>

### OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Apparatus Description</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuVac Snapper Kit</td>
<td>each</td>
<td>24052-00</td>
</tr>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968-00</td>
</tr>
<tr>
<td>Cylinder, graduated, 25 mL</td>
<td>each</td>
<td>1081-40</td>
</tr>
<tr>
<td>Dropper, for 1–oz bottle</td>
<td>each</td>
<td>2258-00</td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>5 rolls/pkg</td>
<td>391-33</td>
</tr>
<tr>
<td>Pipet, serological, 2 mL</td>
<td>each</td>
<td>532-36</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700-01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856-96</td>
</tr>
<tr>
<td>Pipet, volumetric, 1.0 mL, Class A</td>
<td>each</td>
<td>14515-35</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651-00</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215-00</td>
</tr>
<tr>
<td>Sample Cells, 1–inch, polystyrene, disposable</td>
<td>12/pkg</td>
<td>24102-12</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes*
1. Enter the stored program number for low range nitrate nitrogen (NO₃⁻–N).

Press: 3 5 1 READ/ENTER

The display will show:

DIAL nm TO 507

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage following this procedure. Adjust the pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows: 507 nm

3. Press: READ/ENTER

The display will show:

mg/l N NO₃⁻ L

4. Fill a 50–mL graduated mixing cylinder to the 30–mL mark with sample.

Note: For proof of accuracy, use a 0.20 mg/L nitrate nitrogen standard solution (preparation given in the Accuracy Check) in place of the sample.

Note: Determine a reagent blank for each new lot of powder pillows. Repeat Steps 4 to 14 of the procedure, using demineralized water as the sample. Subtract this value from each result obtained with this lot of reagent.

*Seawater requires a manual calibration; see Interferences.
5. Add the contents of one NitraVer 6 Nitrate Reagent Powder Pillow to the cylinder. Stopper.

6. Press: **SHIFT TIMER**
   A 3-minute reaction period will begin. Shake the cylinder continuously during the three-minute period.

   *Note:* A deposit of unoxidized metal will remain after the NitraVer 6 Nitrate Reagent powder dissolves. This is normal and does not affect test results.

7. When the timer beeps, press: **SHIFT TIMER**
   A 2-minute period allows the cadmium to settle.

8. When the timer beeps, pour 25 mL of sample into a sample cell.

   *Note:* Take care not to transfer any cadmium particles.

   *Note:* Shaking time and technique influence color development. For most accurate results, make successive tests on a solution containing a known amount of nitrate and adjust the shaking time to obtain the correct result. See the Accuracy Check for more information.

9. Add the contents of one NitraVer 3 Nitrite Reagent Powder Pillow to the sample cell (the prepared sample). Stopper. Shake to dissolve.

10. Press: **SHIFT TIMER**
    A 10-minute reaction period will begin.

11. When the timer beeps, the display will show:
    \[ \text{mg/L} \text{ NO}_3^- \]
    Fill another sample cell (the blank) with 25 mL of sample.

12. Place the blank into the cell holder. Close the light shield.

   *Note:* The Pour-Thru Cell can be used with this procedure.
13. Press: **ZERO**

The display will show: **WAIT**

then: **0.00 mg/l N NO₃⁻ L**

14. Within ten minutes after the timer beeps, remove the stopper from the prepared sample. Place the prepared sample into the cell holder. Close the light shield.

**Note:** If more than five minutes elapse after the timer beeps, ZERO SAMPLE may appear. If so, remove the prepared sample. Insert the blank. Press: **ZERO.** Insert the prepared sample.

15. Press: **READ/ENTER**

The display will show: **WAIT**

then the result in mg/L nitrate expressed as nitrate nitrogen (NO₃⁻-N) will be displayed.

**Note:** In the constant--on mode, pressing READ/ENTER is not required. **WAIT** will not appear. When the display stabilizes, read the result.

**Note:** The results can be expressed as mg/L nitrate (NO₃⁻) by multiplying the mg/L nitrate nitrogen (NO₃⁻-N) by 4.4.

**Note:** Rinse the sample cell immediately after use to remove all cadmium particles.

---

**SAMPLING AND STORAGE**

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods, adjust sample pH to 2 or less with sulfuric acid, ACS (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct the test result for volume additions (see Correction for Volume Additions in Section I).

---

**ACCURACY CHECK**

**Standard additions Method**

a) Measure 30 mL of sample into three cylinders.

b) Using the TenSette Pipet, add 0.1, 0.2, and 0.3 mL of Nitrate Nitrogen, Voluette Ampule Standard Solution, 12 mg/L as NO₃⁻-N, to the three samples. Mix well.

c) Analyze each sample as described above. The nitrate nitrogen concentration should increase 0.04 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

**Standard Solution Method**

Prepare a 0.20 mg/L nitrate nitrogen standard by diluting 2.00 mL of the 10-mg/L Nitrate Nitrogen Standard Solution to 100 mL with demineralized water.
Or, using the TenSette Pipet, make a 0.12 mg/L nitrate nitrogen standard by diluting 1.0 mL of a Nitrate Nitrogen Volute Standard Solution, 12 mg/L, to 100 mL with demineralized water.

**PRECISION**
In a single laboratory, using standard solutions of 0.25 mg/L nitrate nitrogen (NO₃⁻N) and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.010 mg/L nitrate nitrogen.

**INTERFERENCES**
This method registers both the nitrate and nitrite nitrogen present in the sample. If nitrite is present, the nitrite nitrogen test using program number 371 should be done on the sample. The amount of nitrite nitrogen found should be subtracted from the results of the nitrate nitrogen test when the following pretreatment is used:

a) Add Bromine Water drop-wise to 30–mL of sample until a yellow color persists. Mix after adding each drop.

b) Add one drop of Phenol Solution. Swirl to destroy the yellow color.

c) Continue with Step 4 of the nitrate procedure.

Calcium interferes in amounts over 100 mg/L as CaCO₃.

Chlorides in amounts over 100 mg/L cause low results. To determine nitrate in high chloride samples or seawater, a manual calibration must be performed. Prepare nitrate standard solutions with the approximate chloride concentration of the samples to be tested. See User Stored Programs in Section I of the Instrument Manual.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment (see pH Interference Section I).

**SUMMARY OF METHOD**
Cadmium metal reduces nitrites present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt which couples to chromotropic acid to form a pink-colored product.

---

**REQUIRED REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitrVer 3 Nitrite Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>14065–66</td>
</tr>
<tr>
<td>NitrVer 6 Nitrate Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>14119–46</td>
</tr>
</tbody>
</table>

**REQUIRED APPARATUS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td>each</td>
<td>968–00</td>
</tr>
<tr>
<td>Cylinder, graduated, mixing, 50 mL</td>
<td>1</td>
<td>each</td>
<td>1896–41</td>
</tr>
<tr>
<td>Stopper, hollow, No.1</td>
<td>1</td>
<td>6/pkg</td>
<td>14480–01</td>
</tr>
</tbody>
</table>

**OPTIONAL REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromine Water</td>
<td>29 mL*</td>
<td>2211–20</td>
<td></td>
</tr>
<tr>
<td>Nitrate Nitrogen Standard Solution, 1 mg/L as NO₃⁻N</td>
<td>500 mL</td>
<td>2046–49</td>
<td></td>
</tr>
<tr>
<td>Nitrate Nitrogen Standard Solution, 10 mg/L as NO₃⁻N</td>
<td>500 mL</td>
<td>307–49</td>
<td></td>
</tr>
<tr>
<td>Nitrate Nitrogen Standard Solution, Voluette ampule, 12 mg/L as NO₃⁻N, 10 mL</td>
<td>16/pkg</td>
<td>14333–10</td>
<td></td>
</tr>
<tr>
<td>Phenol Solution, 30 g/L</td>
<td>30 mL</td>
<td>2112–20</td>
<td></td>
</tr>
<tr>
<td>Pretreatment Kit, contains: (1) 2112–20, (1) 2211–20</td>
<td>each</td>
<td>2268–00</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>59 mL*</td>
<td>2450–26</td>
<td></td>
</tr>
<tr>
<td>Sulfuric Acid, ACS</td>
<td>500 mL*</td>
<td>979–49</td>
<td></td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
<td></td>
</tr>
</tbody>
</table>
NITRATE, LR, continued

OPTIONAL APPARATUS
Nitrate at these levels can be determined directly using the Nitrate Ion Selective Electrode (Cat. No. 44560–71)

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
</tr>
<tr>
<td>Dropper, for 1–oz bottle</td>
<td>each</td>
<td>2258–00</td>
</tr>
<tr>
<td>Flask, volumetric, 100 mL</td>
<td>each</td>
<td>547–42</td>
</tr>
<tr>
<td>pH Indicator Paper, 1 to 11 pH</td>
<td>5–roll/pkg</td>
<td>391–33</td>
</tr>
<tr>
<td>Pipet, serological, 2 mL</td>
<td>each</td>
<td>532–36</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 2.00 mL</td>
<td>each</td>
<td>14515–36</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651–00</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes.
NITRATE, MR (0 to 4.5 mg/L NO₃⁻—N) For water, wastewater and seawater*

Cadmium Reduction Method (Using Powder Pillows or AccuVac Ampuls)

USING POWDER PILLOWS

1. Enter the stored program number for medium range nitrate nitrogen (NO₃⁻—N) powder pillows.

Press: 3 5 3 READ/ENTER

The display will show:
DIAL nm TO 400

2. Rotate the wavelength dial until the small display shows:

400 nm

Note: If samples cannot be analyzed immediately, see Sampling and Storage. Adjust the pH of stored samples before analysis.

3. Press: READ/ENTER

The display will show:
mg/l N NO₃⁻ M

Note: For proof of accuracy, use a 1.0 mg/L Nitrate Nitrogen Standard Solution listed under Optional Reagents in place of the sample.

4. Fill a sample cell with 25 mL of sample (the prepared sample).

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

*Seawater requires a manual calibration; see Interferences.
5. Fill another cell with 25 mL of demineralized water (the blank).

6. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow to each cell. Stopper

7. Press: **SHIFT TIMER**
A 1-minute reaction period will begin. Shake until the timer beeps.

*Note: Shaking time and technique influence color development. For most accurate results, make successive tests on a solution containing a known amount of nitrate and adjust the shaking time to obtain the correct result. See the Accuracy Check section for more information.*

8. When the timer beeps, press: **SHIFT TIMER**
A 5-minute reaction period will begin.

*Note: A deposit of unoxidized metal will remain after the NitraVer 5 Nitrate Reagent Powder dissolves and will have no effect on test results.*

*Note: An amber color will develop if nitrate nitrogen is present.*

9. When the timer beeps, the display will show:
\[
\text{mg/L N NO}_3^- \text{ M}
\]
Remove the stopper.
Place the blank into the cell holder. Close the light shield.

*Note: The Pour-Thru Cell can be used if rinsed well with demineralized water after use.*

10. Press: **ZERO**
The display will show: **WAIT**
then:
\[
0.0 \text{ mg/L N NO}_3^- \text{ M}
\]

11. Place the prepared sample into the cell holder. Close the light shield.

12. Press: **READ/ENTER**
The display will show: **WAIT**
then the result in mg/L nitrate expressed as nitrogen (NO$_3^-$–N) will be displayed.

*Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*

*Note: The results can be expressed as mg/L nitrate (NO$_3^-$) by multiplying the mg/L nitrate nitrogen (NO$_3^-$–N) by 4.4.*

*Note: Rinse the sample cell immediately after use to remove all cadmium particles.*
USING ACCUVAC AMPULS

1. Enter the stored program number for medium range nitrate nitrogen (NO₃⁻-N) AccuVac Ampuls.

   Press: 3 5 9 READ/ENTER

   The display will show:
   DIAL nm TO 400

   Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

   Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

   Note: If sample cannot be analyzed immediately, see Sampling and Storage. Adjust the pH of stored samples before analysis.

   Note: If your instrument does not have program number 359, see Instrument Setup following these steps.

2. Rotate the wavelength dial until the small display shows:

   400 nm

3. Press: READ/ENTER

   The display will show:
   mg/l N NO₃⁻ M AV

4. Collect at least 40 mL of sample in a 50 mL beaker. Fill a NitraVer 5 Nitrate AccuVac Ampul with sample.

   Note: Keep the tip immersed while the ampul fills.

   Note: For proof of accuracy, use a 1.0 mg/L Nitrate Nitrogen Standard Solution (listed under Optional Reagents) in place of the sample.

   Note: A reagent blank must be determined for each lot of NitraVer 5 AccuVacs. Perform Steps 4 to 12 using demineralized water as the sample. Subtract this value from each result obtained with this reagent lot.
5. Press: **SHIFT TIMER**
A 1-minute shaking period will begin.

6. Invert the ampul repeatedly back and forth until the timer beeps. Wipe off any liquid or fingerprints.

   **Note:** Shaking time and technique influence color development. For most accurate results, make successive tests on a solution containing a known amount of nitrate and adjust the shaking time to obtain the correct result. See the Accuracy Check section for more information.

7. When the timer beeps, press: **SHIFT TIMER**
A 5-minute reaction period will begin.

   **Note:** A deposit of unoxidized metal will remain after the NitraVer S Nitrate Reagent Powder dissolves and will have no effect on test results.

   **Note:** An amber color will develop if nitrate nitrogen is present.

8. When the timer beeps, the display will show:
   \[ \text{mg/L N NO}_3^- \text{ M AV} \]
   Fill a zeroing vial with at least 10 mL of sample (the blank).

9. Place the AccuVac Vial Adapter into the cell holder of the instrument.

   **Note:** Place the grip tab at the rear of the cell holder.

10. Place the blank into the cell holder. Close the light shield.

    Press: **ZERO**

    The display will show:
    \[ \text{WAIT} \]

    then:
    \[ 0.0 \text{ mg/L N NO}_3^- \text{ M AV} \]

11. Place the AccuVac ampul into the cell holder. Close the light shield.

12. Press: **READ/ENTER**

    The display will show:
    \[ \text{WAIT} \]
    then the result in mg/L nitrate expressed as nitrogen (NO$_3^-$-N) will be displayed.

   **Note:** In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

   **Note:** The results can be expressed as mg/L nitrate (NO$_3^-$) by multiplying the mg/L nitrate nitrogen (NO$_3^-$-N) by 4.4.
INSTRUMENT SETUP
For a DR/2000 with software versions 1.27 or 1.265, enter the following calibration as an operator-programmed calibration for medium range nitrate nitrogen AccuVac ampouls (method 359). Follow steps in the operation section of the instrument manual. Store the method as follows:

\[
\begin{align*}
\text{nm} &= 400 \\
\text{Decimal} &= 000.0 \\
\text{Units} &= \text{mg/l} \\
\text{Symbol} &= \text{N NO}_3^- \text{ M} \\
\text{Timer} 1 &= 01:00 \\
\text{Timer} 2 &= 05:00
\end{align*}
\]

At first, enter the calibration with 0.000 absorbance values for zero and standards #1–4. To do this, do not place anything in the sample cell compartment. Begin storing zero, #1 standard, #2 standard and #3 standard as concentrations of 0, 0.9, 3.0 and 4.8, respectively, with nothing in the sample compartment. Accept 0.000 Abs. as the value for all standards. Next, the values for the standards must be changed to the values given below.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>0.250</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>0.750</td>
</tr>
<tr>
<td>3</td>
<td>4.8</td>
<td>1.125</td>
</tr>
</tbody>
</table>

The method is now stored as an operator-programmed method with a method number between 950 and 999. Record the method number for future reference.

For a DR/2000 with software version 2.0 and 2.2, enter the calibration as an update to Hach-stored programs. (Stored program number 358 has been replaced with number 359.)

1. Press: \[ \text{I} \]
2. Press: \[ \text{SHIFT} \quad \text{CONFIG} \quad \text{METH} \]
3. Press: \[ \text{PROG} \quad \text{ABS} \quad + \quad \text{READ} \quad \text{ENTER} \]
4. Within 3 seconds, press:

\[ \text{SHIFT} \quad \text{PROG} \quad \text{CONFIG} \quad \text{METH} \]

The display will show:

\[ \text{ENTER nm} \]

If the display returns to the METHOD prompt, repeat the sequence.

5. Press:

\[ \text{4} \quad \text{0} \quad \text{0} \quad \text{0} \]

If you make an error, press \text{SHIFT CLEAR} and re-enter the number. When the number is correct, press \text{READ/ENTER}. The display will show:

\[ \text{DECIMAL? 00.00} \]

6. Use the arrow keys to correctly position the decimal point. For this method, press the \text{RIGHT/DOWN ARROW} key once. The display will show:

\[ \text{DECIMAL? 000.0} \]

7. When the decimal point is correctly positioned, press: \text{READ/ENTER}. The display will show:

\[ \text{UNIT?} \]

8. Use the arrow keys to select the appropriate unit of measure. For this method, press the \text{RIGHT/DOWN ARROW} key twice. The display will show:

\[ \text{mg/l} \]

9. With the proper unit of measure displayed, press \text{READ/ENTER}. The display will show:

\[ \text{SYMBOL?} \]

10. Use the arrow keys to construct the correct symbol display. For this method, press the \text{RIGHT/DOWN ARROW} key repeatedly until you see:

\[ \text{mg/l n} \]

11. Press \text{SHIFT} to make the “n” uppercase. The display will show:

\[ \text{mg/l N} \]

12. Press the \text{READ/ENTER} key to accept the capital “N.”

13. Using the arrow keys, continue to construct the display:

\[ \text{mg/l N NO}_3^- \text{ M AV} \]

The space is the "character" displayed after one press of the right/down arrow key. To enter subscript 3, press the number 3 key. It will enter as a subscript.
14. When the last character of the symbol is accepted with the **READ/ENTER** key, the display will show: **TIMER**?

15. There are two timers for this method, so press **SHIFT TIMER**. The display will show: **MM:SS TIME 1**?

16. To enter the first timer value of 1:00 minute, press:

```
0 1 0 0
```

The display will then read:

```
01:00 TIME 1
```

17. Press **READ/ENTER** to accept the timer value. The display will show:

```
MM:SS TIME 2 ?
```

18. To enter the second timer value of 5:00 minutes, press:

```
0 5 0 0
```

The display will then read:

```
05:00 TIME 2
```

19. Press **READ/ENTER** to accept the timer value. The display will then read:

```
MM:SS TIME 3 ?
```

20. Press **READ/ENTER** to complete the timer entry. The display will show:

```
# 1 Data 0
```

21. Enter the following 12 numbers as shown. Complete each number entry by pressing **READ/ENTER**.

```
# 1 Data 0
# 2 Data 1029
# 3 Data 1286
# 4 Data 1285
# 5 Data 1542
# 6 Data 1542
# 7 Data 65535
# 8 Data 65535
# 9 Data 65535
#10 Data 32760
#11 Data 512
Checksum 25583
```

The final number is a check value which is used to determine if the data sequence was correctly entered. If an error is made during number entry, the display will return to the prompt for data number 1 and the entire sequence must be re-entered. If all numbers are correctly entered, the display will return to the method prompt and is ready for use:

```
METHOD #?
```

22. Once the new method 359 has been successfully entered, block access to the now obsolete method 358.

Press:

```
SHIFT CONFIG METH
```

Press:

```
PROG 3 ABS 5 8 READ ENTER
```

Within 3 seconds press:

```
SHIFT PROG 3 CONFIG METH
```

The display will show:

```
800 CONFIGURE
```

Press **READ/ENTER** three times to return to:

```
METHOD #?
```

Access to method 358 is blocked. Method 359 is now a stored method.

**SAMPLING AND STORAGE**
Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods, adjust sample pH to 2 or less with sulfuric acid, ACS (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct the test result for volume additions (*see Correction for Volume Additions in Section I*).
ACCURACY CHECK
Standard Additions Method
a) Measure 25 mL of sample into three sample cells or 50-mL beakers.

b) Use the TenSette Pipet to add 0.2, 0.4, and 0.6 mL of Nitrate Nitrogen Standard Solution, 100 mg/L as NO₃⁻−N, to the three samples. Mix well.

c) Analyze each sample as described above. The nitrate nitrogen (NO₃⁻−N) concentration should increase 0.8 mg/L for each 0.2 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section 1 for more information.

Standard Solution Method
A 1.0 mg/L Nitrate Nitrogen Standard Solution is available from Hach. Or, dilute 1.00 mL of Nitrate Nitrogen Standard Solution, 100 mg/L as NO₃⁻−N, to 100.0 mL with demineralized water.

PRECISION
In a single laboratory, using standard solutions of 2.0 mg/L nitrate nitrogen (NO₃⁻−N) and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.10 mg/L nitrate nitrogen.

In a single laboratory, using a standard solution of 1.5 mg/L (NO₃⁻−N) and two representative lots of AccuVac ampuls with the DR/2000, a single operator obtained a standard deviation of ±0.03 mg/L nitrate nitrogen.

INTERFERENCES
Compensate for nitrite interference as follows:

a) Add Bromine Water dropwise to the sample in Step 4 until a yellow color remains.

b) Add one drop of Phenol Solution to destroy the color.

c) Proceed with Step 4. Report results as total nitrate and nitrite.

Strong oxidizing and reducing substances will interfere. Ferric iron causes high results and must be absent. Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride levels and in seawater, but a calibration must be performed using standards spiked to the same chloride concentration.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment (see pH Interference in Section 1).

SUMMARY OF METHOD
Cadmium metal reduces nitrates present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt which couples to gentisic acid to form an amber-colored product.

REQUIRED REAGENTS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitraVer 5 Nitrate Reagent Powder Pillows</td>
<td>1 pillow</td>
<td></td>
<td>50/pkg</td>
<td>14034–99</td>
</tr>
</tbody>
</table>

REQUIRED REAGENTS (Using AccuVac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitraVer 5 Nitrate Reagent AccuVac Ampuls</td>
<td>1 ampul</td>
<td></td>
<td>25/pkg</td>
<td>25110–25</td>
</tr>
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</table>

REQUIRED APPARATUS (Using Powder Pillows)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td></td>
<td>each</td>
<td>968–00</td>
</tr>
<tr>
<td>Stopper, rubber, size 2</td>
<td>2</td>
<td></td>
<td>12/pkg</td>
<td>2118–02</td>
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REQUIRED APPARATUS (Using AccuVac Ampuls)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac</td>
<td>1</td>
<td></td>
<td>each</td>
<td>43784–00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>1</td>
<td></td>
<td>each</td>
<td>500–41</td>
</tr>
<tr>
<td>Vial, zeroing</td>
<td>1</td>
<td></td>
<td>each</td>
<td>21228–00</td>
</tr>
</tbody>
</table>
NITRATE, MR, continued

OPTIONAL REAGENTS
Bromine Water 30 g/L .......................... 29 mL* .... 2211–20
Nitrate Nitrogen Standard Solution, 1 mg/L as (NO₃⁻–N) ........................................ 500 mL .... 2046–49
Nitrate Nitrogen Standard Solution, 100 mg/L as (NO₃⁻–N) ........................................ 500 mL .... 1947–49
Phenol Solution .................................. 29 mL .... 2112–20
Sodium Hydroxide Standard Solution, 5.0 N ........................................ 59 mL* .... 2450–26
Sulfuric Acid, ACS ............................ 500 mL* .... 979–49
Water, demineralized ....................... 4 L .... 272–56

OPTIONAL APPARATUS
AccuVac Snapper Kit .......................... each .... 24052–00
Cylinder, graduated, 25 mL ................. each .... 1081–40
Dropper, for 1–oz bottle .................... each .... 2258–00
pH Indicator Paper, 1 to 11 pH .......... each .... 391–33
Pipet Filler, safety bulb .................. 5 rolls/pkg .... 14651–00
Pipet, serological, 2 mL ................ each .... 532–36
Pipet, TenSette, 0.1 to 1.0 mL ... each .... 19700–01
Pipet Tips, for 19700–01 TenSette Pipet .......... 50/pkg .... 21856–96
Pipet, volumetric, Class A, 1.0 mL ........ each .... 14515–35
Pour–Thru Cell Assembly Kit .......... each .... 45215–00
Sample Cells, 1–inch, polystyrene, disposable .......... 12/pkg .... 24102–12

Nitrate at these levels can be determined directly with the Nitrate Ion Selective Electrode (Cat. No. 44430-01).

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes.
Ferrous Sulfate Method*

1. Enter the stored program number for high range nitrite (NO$_2^-$).

Press: 373 READ/ENTER

The display will show:
DIAL nm TO 585

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage following this procedure.

2. Rotate the wavelength dial until the small display shows:

585 nm

3. Press: READ/ENTER

The display will show:
mg/l NO$_2^-$ H

4. Fill a sample cell with 25 mL of sample.

Note: For proof of accuracy, use a 100–mg/l nitrite standard solution (preparation given in the Accuracy Check) in place of the sample.

*Adapted from McAlpine, R. and Soule, B., Quisitative Chemical Analysis, New York, 476, 575 (1933)
5. Add the contents of the NitriVer 2 Nitrite Reagent Powder Pillow, topper and shake to dissolve (the prepared sample).

*Note:* A greenish-brown color will develop if nitrite is present.

6. Press: **SHIFT TIMER**

A 10-minute reaction period will begin.

7. Fill another sample cell with 25 mL of sample (the blank). Place it into the cell holder.

*Note:* The Pour-Thru Cell cannot be used with this procedure.

8. When the timer beeps the display will show:

   mg/L NO₂⁻ H

   Press: **ZERO**

   The display will show:

   **WAIT**

   then:

   0. mg/L NO₂⁻ H

9. Invert the prepared sample twice. Remove the stopper. Place the prepared sample into the cell holder. Close the light shield.

10. Press: **READ/ENTER**

    The display will show:

    **WAIT**

    then the result in mg/L NO₂⁻ will be displayed.

*Note:* The results can be expressed as mg/L nitrite nitrogen (N) or as mg/L sodium nitrite (NaNO₂) by multiplying the mg/L nitrite by 0.3 or by 1.5, respectively.

*Note:* In the constant-on mode, pressing **READ/ENTER** is not required. **WAIT** will not appear. When the display stabilizes, read the result.
SAMPLING AND STORAGE
Collect samples in clean plastic or glass bottles. If prompt analysis is not possible, store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. Samples stored for longer than 48 hours may not be used for reporting purposes.

For longer storage periods, add 4.0 mL of Mercuric Chloride Solution for each liter of sample taken and mix. Sample refrigeration is still required. Do not use acid preservatives.

ACCURACY CHECK
Standard Solution Method
Dissolve 0.150 grams of fresh sodium nitrite and dilute to 1000 mL with demineralized water to prepare a 100 mg/L nitrite standard solution. Prepare this solution daily.

PRECISION
In a single laboratory, using standard solutions of 100 mg/L nitrite and two representative lots of reagents with the DR/2000, a single operator obtained a standard deviation of ±2.2 mg/L nitrite.

INTERFERENCES
This test does not measure nitrates nor is it applicable to glycol based samples. Dilute glycol based samples and follow the Nitrite, Low Range Procedure (Stored Program No. 371).

SUMMARY OF METHOD
The method uses ferrous sulfate in an acidic medium to reduce nitrite to nitrous oxide. Ferrous ions combine with the nitrous oxide to form a greenish–brown complex in direct proportion to the nitrite present.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitriVer 2 Nitrite Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>2219–66</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1</td>
<td></td>
<td>968–00</td>
</tr>
<tr>
<td>Stopper, hollow, polyethylene, No. 1</td>
<td>1</td>
<td>6/pkg</td>
<td>14480–01</td>
</tr>
</tbody>
</table>

OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercuric Chloride Solution</td>
<td>100 mL</td>
<td>14994–42</td>
</tr>
<tr>
<td>Sodium Nitrite, ACS</td>
<td>454 g</td>
<td>2452–01</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td>272–56</td>
</tr>
</tbody>
</table>

OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balance, analytical</td>
<td></td>
<td>22310–00</td>
</tr>
<tr>
<td>Flask, volumetric, 1000 mL</td>
<td></td>
<td>547–53</td>
</tr>
<tr>
<td>Pipet, serological, 10 mL</td>
<td></td>
<td>532–38</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td></td>
<td>14651–00</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
Diazotization Method (Powder Pillows or AccuVac Ampuls),
USEPA approved for reporting wastewater analysis*
USING POWDER PILLOWS

1. Enter the stored program number for low range nitrite nitrogen (NO$_2^-$-N)—powder pillows.

Press: 3 7 1 READ/ENTER

The display will show: DIAL nm TO 507

Note: DR/2000s with software versions 3.0 and greater will display “P” and the program number.

Note: DR/2000s with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage following this procedure.

2. Rotate the wavelength dial until the small display shows: 507 nm

3. Press: READ/ENTER

The display will show: mg/L N NO$_2^-$

4. Fill a sample cell with 25 mL of sample.

Note: For proof of accuracy, use a 0.10-mg/L nitrite nitrogen standard solution (preparation given in the Accuracy Check) in place of the sample.

*Federal Register, 44(85) 25505 (May 1, 1979)

**Note:** A pink color will develop if nitrite nitrogen is present.

6. Press: **SHIFT TIMER**
A 15-minute reaction period will begin.

7. When the timer beeps, the display will show:
**mg/l N NO₂⁻**
Fill a second sample cell with 25 mL of sample (the blank).

8. Place the blank into the cell holder. Close the light shield.

**Note:** The Pour-Thru Cell can be used with this procedure.

9. Press: **ZERO**
The display will show: **WAIT**
then:
**0.000 mg/l N NO₂⁻**

10. Remove the stopper. Place the prepared sample into the cell holder. Close the light shield.

11. Press: **READ/ENTER**
The display will show: **WAIT**
then the result in mg/L nitrite expressed as nitrogen (NO₂⁻–N) will be displayed.

**Note:** In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

**Note:** The result can be expressed as mg/L nitrite (NO₂⁻) by multiplying the mg/L nitrite nitrogen (NO₂⁻–N) by 3.3.
USING ACCUVAC AMPULS

1. Enter the stored program number for low range nitrite – AccuVac Ampuls.

Press: 3 7 5 READ/ENTER

The display will show:
DIAL nm TO 507

Note: DR 2000s with software versions 3.0 and greater will display “P” and the program number.

Note: Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage following this procedure.

2. Rotate the wavelength dial until the small display shows: 507 nm

3. Press: READ/ENTER

The display will show:
mg/l N NO₂⁻ L AV

4. Collect at least 40 mL of sample in a 50–mL beaker. Fill a NitriVer 3 Nitrite AccuVac Ampul with sample.

Note: Keep the tip immersed while the ampul fills.

Note: For proof of accuracy, use a 0.10 mg/L nitrite nitrogen standard solution (preparation given in the Accuracy Check) in place of the sample.
5. Invert the ampul several times to mix. Wipe off any liquid or fingerprints.
   Note: A pink color will develop if nitrite nitrogen is present.

6. Press: **SHIFT TIMER**
   A 15-minute reaction period will begin.

7. When the timer beeps, the display will show:
   \[ \text{mg/L N NO}_2^- \text{ L AV} \]
   Fill a zeroing vial (the blank) with at least 10 mL of sample.
   Note: Place the grip tab at the rear of the cell holder.

9. Place the blank into the cell holder. Close the light shield.
   Press: **ZERO**
   The display will show: **WAIT**
   then:
   \[ 0.000 \text{ mg/L N NO}_2^- \text{ L AV} \]

10. Place the AccuVac Ampul into the cell holder. Close the light shield.

11. Press: **READ/ENTER**
    The display will show: **WAIT**
    then the result in mg/L nitrite expressed as nitrogen (NO$_2^-$-N) will be displayed.
    Note: In the constant-on mode, pressing **READ/ENTER** is not required. **WAIT** will not appear. When the display stabilizes, read the result.
    Note: The results can be expresses as mg/L nitrite (NO$_2^-$) by multiplying the mg/L nitrite nitrogen (NO$_2^-$-N) by 3.3.
**NITRITE, LR, continued**

**SAMPLING AND STORAGE**
Collect samples in clean plastic or glass bottles.

Store at 4 °C (30 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods, add 4.0 mL of Mercuric Chloride Solution* for each liter of sample taken and mix. Sample refrigeration is still required. **This storage method may not be used when reporting results to regulatory agencies.** Do not use acid preservatives.

*Use of mercuric chloride is not recommended due to environmental and health concerns.

**ACCURACY CHECK**

**Standard Solution Method**
Prepare a nitrite nitrogen standard solution by dissolving 0.493 grams of sodium nitrite, ACS, in 1000 mL of nitrite-free demineralized water to give a 100 mg/L nitrite nitrogen (NO₂⁻-N) standard solution. This solution is not stable and should be prepared daily. Use a TenSette Pipet to dilute 1.00 mL of the stock solution to 1000 mL with nitrite-free demineralized water to give a 0.10-mg/L (NO₂⁻-N) nitrite nitrogen standard solution. Prepare this solution immediately before use.

**PRECISION**
In a single laboratory, using a standard solution of 0.100 mg/L nitrite nitrogen and two representative lots of powder pillow reagent with the DR/2000, a single operator obtained a standard deviation of ±0.0011 mg/L nitrite nitrogen.

In a single laboratory, using a standard solution of 0.100 mg/L nitrite nitrogen and two representative lots of AccuVac Ampuls with the DR/2000, a single operator obtained a standard deviation of ±0.0007 mg/L nitrite nitrogen.

**INTERFERENCES**
Strong oxidizing and reducing substances interfere. Cupric and ferrous ions cause low results. Ferric, mercurous, silver, bismuth, antimonious, lead, auric, chloroplatinate and metavanadate ions interfere by causing precipitation.

Very high levels of nitrate (100 mg/L nitrate as N or more) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.

**SUMMARY OF METHOD**
Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

---

**REQUIRED REAGENTS (Using Powder Pillows)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitriVer 3 Nitrite Reagent Powder Pillows</td>
<td>1 pillow</td>
<td>50/pkg</td>
<td>14065–66</td>
</tr>
</tbody>
</table>

**REQUIRED REAGENTS (Using AccuVac Ampuls)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NitriVer 3 Nitrite Reagent AccuVac Ampul</td>
<td>1 ampul</td>
<td>25/pkg</td>
<td>25120–25</td>
</tr>
</tbody>
</table>

**REQUIRED APPARATUS (Using Powder Pillows)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clippers, for opening powder pillows</td>
<td>1 each</td>
<td></td>
<td>968–00</td>
</tr>
<tr>
<td>Stopper, hollow, polyethylene, No.1</td>
<td>1 each</td>
<td>6/pkg</td>
<td>14480–01</td>
</tr>
</tbody>
</table>

**REQUIRED APPARATUS (Using AccuVac Ampulls)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapter, AccuVac Vial</td>
<td>1 each</td>
<td></td>
<td>43784–00</td>
</tr>
<tr>
<td>Beaker, 50 mL</td>
<td>1 each</td>
<td></td>
<td>500–41</td>
</tr>
<tr>
<td>Vial, zeroing</td>
<td>1 each</td>
<td></td>
<td>21228–00</td>
</tr>
</tbody>
</table>

**OPTIONAL REAGENTS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercuric Chloride Solution</td>
<td>100 mL</td>
<td></td>
<td>14994–49</td>
</tr>
<tr>
<td>Sodium Nitrite, ACS</td>
<td>454 g</td>
<td></td>
<td>2452–01</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>4 L</td>
<td></td>
<td>272–56</td>
</tr>
</tbody>
</table>
**OPTIONAL APPARATUS**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuVac Snapper Kit</td>
<td>each</td>
<td>24052–00</td>
</tr>
<tr>
<td>Balance, analytical</td>
<td>each</td>
<td>22310–00</td>
</tr>
<tr>
<td>Flask, volumetric, 1000 mL</td>
<td>each</td>
<td>547–53</td>
</tr>
<tr>
<td>Pipet, serological, 10 mL</td>
<td>each</td>
<td>532–38</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 1.0 mL</td>
<td>each</td>
<td>14515–35</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651–00</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
<tr>
<td>Sample Cells, 1–inch, polystyrene, disposable</td>
<td>12/pkg</td>
<td>24012–12</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.
1. Enter the stored program number for ammonia nitrogen (NH₃–N).

Press: 3 8 0 READ/ENTER

The display will show: DIAL nm TO 425

**Note:** DR/2000s with software versions 3.0 and greater will display “P” and the program number.

**Note:** Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

**Note:** If samples cannot be analyzed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows:

425 nm

**Note:** This test is sensitive to the wavelength setting. To assure accuracy, run the test using a 1.0 mg/L standard solution and demineralized water blank. Repeat Steps 9 to 12 at slightly different wavelengths, setting the dial from higher to lower values, until the correct result is obtained. The wavelength should be 425 ±2 nm. Always set this wavelength by approaching from high to low values.

3. Press: READ/ENTER

The display will show: mg/l N NH₃ Ness

4. Fill a 25–mL graduated mixing cylinder to the 25–mL mark with sample (the prepared sample).

**Note:** For proof of accuracy, use a 1.0-mg/L Nitrogen Ammonia Standard Solution (listed under Optional Reagents) in place of the sample.

---

*Requires distillation

** Adapted from Standard Methods for the Examination of Water and Wastewater.

***Procedure is equivalent to USEPA method 350.2 and Standard Method 4500–NH₃ B and C for wastewater.
5. Fill another 25–mL mixing graduated cylinder with demineralized water (the blank).

6. Add three drops of Mineral Stabilizer to each cylinder. Invert several times to mix. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder (hold the dropping bottle exactly vertical). Invert several times to mix.

7. Pipet 1.0 mL of Nessler Reagent into each cylinder. Stopper. Invert several times to mix.

Note: Nessler Reagent is toxic and corrosive. Pipet carefully and use a pipet filler.

Note: A yellow color will develop if ammonia is present. (The reagent will cause a faint yellow color in the blank.)

8. Press: SHIFT TIMER

A 1–minute reaction period will begin.

Note: Continue with Step 9 while timer is running.

9. Pour each solution into respective blank and prepared sample cells.

Note: The Pour–Thru Cell can be used with this procedure. If the Pour–Thru Cell Assembly Kit is used, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush it through the funnel and cell with enough demineralized water to dissolve. Rinse out the crystals.

10. When the timer beeps, the display will show:

\[ \text{mg/L } \text{NH}_3 \text{ Ness} \]

Place the blank into the cell holder. Close the light shield.

Press: ZERO

The display will show:

\[ \text{WAIT} \]

then:

\[ 0.00 \text{ mg/L } \text{NH}_3 \text{ Ness} \]

11. Place the prepared sample into the cell holder. Close the light shield.

12. Press: READ/ENTER

The display will show:

\[ \text{WAIT} \]

then the result in mg/L ammonia expressed as nitrogen (NH$_3$–N) will be displayed.

Note: Do not wait more than five minutes after reagent addition (Step 7) before performing Step 12.

Note: The results may be expressed as mg/L ammonia (NH$_3$) or mg/L ammonium (NH$_4^+$) by multiplying the result by 1.22 or 1.29 respectively.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.
NITROGEN, AMMONIA, continued

SAMPLING AND STORAGE
Collect samples in clean glass or plastic bottles. If chlorine is present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl₂ in a 1-liter sample. Preserve the sample by reducing the pH to 2 or less with sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5 N sodium hydroxide. Correct the test result for volume additions (see Correction for Volume Additions in Section I).

ACCURACY CHECK
Standard Additions Method
a) Snap the neck off a Nitrogen Ammonia Volumette Ampule Standard Solution, 50 mg/L NH₃-N.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 25–mL samples. Mix each thoroughly.

c) Analyze each sample as described above. The nitrogen concentration should increase 0.20 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method
To check accuracy, use a 1.0-mg/L NH₃-N Nitrogen Ammonia Standard Solution listed under Optional Reagents. Or, this can be prepared by diluting 1.00 mL of solution from a Volumette Ampule Standard For Nitrogen Ammonia to 50.0 mL with demineralized water.

PRECISION
In a single laboratory, using standard solutions of 1.00 mg/L ammonia nitrogen (NH₃-N) and two representative lots of reagent with the DR2000, a single operator obtained a standard deviation of ±0.015 mg/L.

INTERFERENCES
A solution containing a mixture of 500 mg/L CaCO₃ and 500 mg/L Mg as CaCO₃ does not interfere. If the hardness concentration exceeds these concentrations, extra Mineral Stabilizer should be added.

Iron and sulfide interfere by causing a turbidity with Nessler Reagent.

Residual chlorine must be removed by addition of sodium arsenite solution. Use two drops to remove each mg/L Cl from a 250–mL sample. Sodium thiosulfate can be used in place of sodium arsenite. See Sampling and Storage section.

Less common interferences, such as glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols may cause greenish or other off colors or turbidity. It may be necessary to distill the sample if these compounds are present.

Seawater samples may be analyzed by addition of 1.0 mL (27 drops) of Mineral Stabilizer to the sample before analysis. This will complex the high magnesium concentrations found in sea water, but the sensitivity of the test will be reduced by 30 percent due to the high chloride concentration. For best results, perform a calibration, using standards spiked to the equivalent chloride concentration, or distill the sample as described below.

DISTILLATION
a) Measure 250 mL of sample into a 250–mL graduated cylinder and pour into a 400–mL beaker. Destroy chlorine, if necessary, by adding 2 drops of Sodium Arsenite Solution per mg/L Cl₂.

b) Add 25 mL of Borate Buffer Solution and mix. Adjust the pH to about 9.5 with 1.0 N Sodium Hydroxide Standard Solution. Use a pH meter.

c) Set up the general purpose distillation apparatus as shown in the Hach Distillation Apparatus Manual. Pour the solution into the distillation flask. Add a stir bar.

d) Use a graduated cylinder to measure 25 mL of demineralized water into a 250–mL erlenmeyer flask. Add the contents of one Boric Acid Powder Pillow. Mix thoroughly. Place the flask under the still drip tube. Elevate so the end of the tube is immersed in the solution.

e) Turn on the heater power switch. Set the stir control to 5 and the heat control to 10. Turn on the water and adjust to maintain a constant flow through the condenser.

f) Turn off the heater after collecting 150 mL of distillate. Immediately remove the collection flask to avoid sucking solution into the still. Measure the distillate to assure 150 mL was collected (total volume 175 mL).

g) Adjust the pH of the distillate to about 7 with 1.0 N Sodium Hydroxide Standard Solution. Use a pH meter.

h) Pour the distillate into a 250–mL volumetric flask. Rinse the erlenmeyer with several small volumes of demineralized water and add the rinsings to the volumetric flask.
NITROGEN, AMMONIA, continued

i) Dilute to the mark with ammonia-free demineralized water. Stopper. Mix thoroughly. Analyze as described above.

SUMMARY OF METHOD
The Mineral Stabilizer complexes hardness in the sample. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nessler Reagent</td>
<td>2 mL</td>
<td>500 mL</td>
<td>21194-49</td>
</tr>
<tr>
<td>Mineral Stabilizer</td>
<td>6 drops</td>
<td>59 mL* SCDB</td>
<td>23766-26</td>
</tr>
<tr>
<td>Polyvinyl Alcohol Dispersing Agent</td>
<td>6 drops</td>
<td>59 mL* SCDB</td>
<td>23765-26</td>
</tr>
<tr>
<td>Water, demineralized</td>
<td>25 mL</td>
<td>4 L</td>
<td>272-56</td>
</tr>
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REQUIRED APPARATUS

<table>
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<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
<td>Cylinder, graduated, mixing, tall form, 25 mL</td>
<td>2</td>
<td>each</td>
</tr>
<tr>
<td>Pipet, serological, 1 mL</td>
<td>2</td>
<td>each</td>
</tr>
<tr>
<td>Pipet Filler, safety bulb</td>
<td>1</td>
<td>each</td>
</tr>
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OPTIONAL REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borate Buffer Solution</td>
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<td>14709-53</td>
<td></td>
</tr>
<tr>
<td>Boric Acid Powder Pilows</td>
<td>50/pkg</td>
<td>14817-66</td>
<td></td>
</tr>
<tr>
<td>Nitrogen, Ammonia Standard Solution, 1 mg/L NH₃-N</td>
<td>500 mL</td>
<td>1891-49</td>
<td></td>
</tr>
<tr>
<td>Nitrogen, Ammonia Standard Solution, Volutte Ampule, 50 mg/L NH₃-N</td>
<td>16/pkg</td>
<td>14791-10</td>
<td></td>
</tr>
<tr>
<td>Sodium Arsenite Solution, 5 g/L</td>
<td>100 mL MDB</td>
<td>1047-32</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>100 mL* MDB</td>
<td>2450-32</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 1.0 N</td>
<td>100 mL* MDB</td>
<td>1045-32</td>
<td></td>
</tr>
<tr>
<td>Sodium Thiosulfate Solution, 0.1 N</td>
<td>100 mL* MDB</td>
<td>323-32</td>
<td></td>
</tr>
<tr>
<td>Sulfuric Acid, ACS</td>
<td>500 mL*</td>
<td>979-49</td>
<td></td>
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OPTIONAL APPARATUS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
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<td>21968-00</td>
</tr>
<tr>
<td>Beaker, 400 mL</td>
<td>each</td>
<td>500-48</td>
</tr>
<tr>
<td>Cylinder, graduated, 25 mL</td>
<td>each</td>
<td>508-40</td>
</tr>
<tr>
<td>Cylinder, graduated, 250 mL</td>
<td>each</td>
<td>508-46</td>
</tr>
<tr>
<td>Distillation apparatus general purpose accessories</td>
<td>each</td>
<td>22653-00</td>
</tr>
<tr>
<td>Distillation heater and support apparatus set, 115 V</td>
<td>each</td>
<td>22744-00</td>
</tr>
<tr>
<td>Distillation heater and support apparatus set, 230 V</td>
<td>each</td>
<td>22744-02</td>
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<tr>
<td>Dropper, plastic, 0.5 and 1.0–mL marks</td>
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<td>21247-10</td>
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<tr>
<td>Flask, erlenmeyer, 250–mL</td>
<td>each</td>
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<tr>
<td>Flask, volumetric, 50 mL</td>
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<tr>
<td>Flask, volumetric, 250 mL</td>
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<td>547-46</td>
</tr>
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<td>pH Meter, EC10, portable</td>
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<td>50050-00</td>
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<td>Pipet, serological, 2 mL</td>
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<td>532-36</td>
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<tr>
<td>Pipet, TenSette, 0.1 to 1.0 mL</td>
<td>each</td>
<td>19700-01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856-96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 1 mL</td>
<td>each</td>
<td>14515-35</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215-00</td>
</tr>
<tr>
<td>Thermometer, –20 to 105 °C</td>
<td>each</td>
<td>1877-01</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes.
1. Enter the stored program number for ammonia nitrogen (NH₃–N), salicylate method.

Press: **3 8 5 READ/ENTER**

The display will show:

DIAL nm TO 655

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If samples cannot be analysed immediately, see Sampling and Storage following these steps. Adjust pH of stored samples before analysis.

2. Rotate the wavelength dial until the small display shows: **655 nm**

3. Press: **READ/ENTER**

The display will show:

mg/l N NH₃ Salic

4. Pour 25 mL of sample into a 25–mL graduated mixing cylinder (the prepared sample).

Note: For proof of accuracy, use a 0.20 mg/L NH₃–N solution (preparation given in the Accuracy Check) in place of the sample.

5. Add 25 mL of deionized water into a second cylinder (the blank).

6. Add the contents of one Ammonia Salicylate Reagent Powder Pillow to each cylinder. Stopper. Shake to dissolve.

7. Press **SHIFT TIMER**
A 3-minute reaction period will begin.

8. When the timer beeps, add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each cylinder. Stopper. Shake to dissolve.

Note: A green color will develop if ammonia nitrogen is present.

9. Press **SHIFT TIMER**
A 15-minute reaction period will begin.

10. When the timer beeps, pour the blank into a sample cell. Place the cell into the cell holder. Close the light shield.

Note: The Pour-Thru Cell can be used with this procedure.

11. Press **ZERO**
The display will show: WAIT then: 0.00 mg/L N NH₃ Salic

12. Fill a second cell with the prepared sample. Place the cell into the cell holder. Close the light shield.
13. Press: READ/ENTER

The display will show:

WAIT

then the result in mg/L ammonia as nitrogen (NH₃–N) will be displayed.

Note: Results may be expressed as mg/L ammonia (NH₃) or mg/L ammonium (NH₄⁺) by multiplying the above result by 1.22 or by 1.29, respectively.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

SAMPLING AND STORAGE

Collect samples in clean plastic or glass bottles. Most reliable results are obtained when samples are analyzed as soon as possible after collection.

If chlorine is known to be present, the sample must be treated immediately with sodium thiosulfate. Add one drop of 0.1 N Sodium Thiosulfate Standard Solution for each 0.3 mg of chlorine present in a one liter sample.

To preserve samples, adjust the pH to 2 or less with concentrated sulfuric acid (about 2 mL per liter). Store samples at 4 °C or less. Samples preserved in this manner can be stored up to 28 days. Just before testing the stored sample, warm to room temperature and neutralize with 5.0 N Sodium Hydroxide Standard Solution. Correct the test result for volume additions (see Correction for Volume Additions in Section I).

ACCURACY CHECK

Standard Additions Method

a) Measure 25 mL of sample into three 25–mL mixing cylinders.

b) Use the TenSette Pipet to add 0.2, 0.4 and 0.6 mL of Ammonium Nitrogen Standard, 10 mg/L as NH₃–N to the three samples. Mix well.

c) Analyze each sample as described above. The ammonia nitrogen concentration should increase 0.08 mg/L for each 0.2 mL of standard added.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method

Prepare a 0.20 mg/L ammonia nitrogen standard by diluting 2.00 mL of the Nitrogen Ammonia Standard Solution, 10 mg/L, to 100 mL with demineralized water. Or, using the TenSette Pipet, prepare a 0.20 mg/L ammonia nitrogen standard by diluting 0.4 mL of a Nitrogen Ammonia Voluette Standard Solution, 50 mg/L as NH₃–N, to 100 mL with demineralized water.
PRECISION
In a single laboratory, using a standard solution of 0.20 mg/L ammonia nitrogen (NH₃–N) and two representative lots of reagent with the DR2000, a single operator obtained a standard deviation of ±0.015 mg/L ammonia nitrogen.

INTERFERENCES
The following ions may interfere when present in concentrations exceeding those listed below:

- Calcium 1000 mg/L as CaCO₃
- Magnesium 6000 mg/L as CaCO₃
- Nitrite 12 mg/L as NO₂⁻–N
- Nitrate 100 mg/L as NO₃⁻–N
- Orthophosphate 100 mg/L as PO₄³⁻–P
- Sulfate 300 mg/L as SO₄²⁻

Sulfide will intensify the color. Eliminate sulfide interference as follows:

a) Measure about 350 mL of sample in a 500–mL erlenmeyer flask.

b) Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix.

c) Filter the sample through a folded filter paper.

d) Use the filtered solution in Step 4.

Iron interferes with the test. Eliminate iron interference as follows:

a) Determine the amount of iron present in the sample following one of the Total Iron procedures.

b) Add the same iron concentration to the demineralized water blank in Step 5.

The interference from iron in the sample will then be successfully blanked out in Step 11.

Adjust extremely acidic or alkaline samples to approximately pH 7. Use 1.0 N Sodium Hydroxide Standard Solution for acidic samples or 1.0 N Sulfuric Acid Standard Solution for basic samples.

Less common interferences such as hydrazine and glycine will cause intensified colors in the prepared sample. Turbidity and sample color will give erroneous high values. Samples with severe interferences require distillation. Albuminoid nitrogen samples also require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set (see Optional Apparatus listing). The distillation procedure is detailed in the Nitrogen, Ammonia—Nessler Method.

SUMMARY OF METHOD
Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Nitrogen Ammonia Reagent Set (100 Tests)</th>
<th>22437-00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity Required</td>
<td></td>
</tr>
<tr>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>Ammonia Cyanurate Reagent Powder Pills</td>
<td></td>
</tr>
<tr>
<td>Ammonia Salicylate Reagent Powder Pills</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REQUIRED APPARATUS</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Clippers, large</td>
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<tr>
<td>Cylinder, graduated, mixing, 25 mL</td>
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### Optional Reagents

<table>
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<tr>
<th>Item</th>
<th>Quantity</th>
<th>Price</th>
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</thead>
<tbody>
<tr>
<td>Nitrogen Ammonia Standard Solution, 10 mg/L as (NH₃–N)</td>
<td>500 mL</td>
<td>153–49</td>
</tr>
<tr>
<td>Nitrogen Ammonia Voluette Ampule, 50 mg/L as (NH₃–N), 10 mL</td>
<td>16/pkg</td>
<td>14791–10</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 1.0 N</td>
<td>100 mL MDB</td>
<td>1045–32</td>
</tr>
<tr>
<td>Sodium Hydroxide Standard Solution, 5.0 N</td>
<td>59 mL</td>
<td>2450–26</td>
</tr>
<tr>
<td>Sodium Thiosulfate Standard Solution, 0.1 N</td>
<td>100 mL MDB</td>
<td>323–32</td>
</tr>
<tr>
<td>Sulfide Inhibitor Reagent Powder Pills</td>
<td>100/pkg</td>
<td>2418–99</td>
</tr>
<tr>
<td>Sulfuric Acid, concentrated, ACS</td>
<td>500 mL</td>
<td>979–49</td>
</tr>
<tr>
<td>Sulfuric Acid Standard Solution, 1.0 N</td>
<td>100 mL MDB</td>
<td>1270–32</td>
</tr>
<tr>
<td>Water, demineralized</td>
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### Optional Apparatus

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>Ampule Breaker Kit</td>
<td>each</td>
<td>21968–00</td>
</tr>
<tr>
<td>Cylinder, graduated, polypropylene, 500 mL</td>
<td>each</td>
<td>1081–49</td>
</tr>
<tr>
<td>Distillation Heater and Support Apparatus, 115 V</td>
<td>each</td>
<td>22744–00</td>
</tr>
<tr>
<td>Distillation Heater and Support Apparatus, 230 V</td>
<td>each</td>
<td>22744–02</td>
</tr>
<tr>
<td>Distillation Set, General Purpose</td>
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</tr>
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<tr>
<td>Funnel, poly, 65 mm</td>
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<td>1083–67</td>
</tr>
<tr>
<td>pH Meter, EC10, portable</td>
<td>each</td>
<td>50050–00</td>
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<tr>
<td>Pipet Filler, safety bulb</td>
<td>each</td>
<td>14651–00</td>
</tr>
<tr>
<td>Pipet, TenSette, 0.1 to 1.0 ml</td>
<td>each</td>
<td>19700–01</td>
</tr>
<tr>
<td>Pipet Tips, for 19700–01 TenSette Pipet</td>
<td>50/pkg</td>
<td>21856–96</td>
</tr>
<tr>
<td>Pipet, volumetric, Class A, 2.0 mL</td>
<td>each</td>
<td>14515–36</td>
</tr>
<tr>
<td>Pour–Thru Cell Assembly Kit</td>
<td>each</td>
<td>45215–00</td>
</tr>
<tr>
<td>Thermometer, –20 to 105 °C</td>
<td>each</td>
<td>1877–01</td>
</tr>
</tbody>
</table>

For additional ordering information, see final section. In the U.S.A. call 800–227–4224 to place an order.
**NITROGEN, TOTAL KJELDAHL** (0 to 150 mg/L) For water, wastewater and sludge

**Nessler Method**, Digestion Required

1. Enter the stored program number for nitrogen total Kjeldahl.

   **Press: 3 9 9 READ/ENTER**

   for the factory stored program

   OR

   **9 ??** for your user stored program previously determined during the calibration below.

   The display will show:

   **DIAL nm TO 460**

**Note:** DR/2000s with software versions 3.0 and greater will display “P” and the program number.

   **Note:** Instruments with software versions 3.0 and greater will not display “DIAL nm TO” message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

2. Rotate the wavelength dial until the small display shows:

   **460 nm**

   **Note:** For process control, use precalibrated program number.

   For greater accuracy, a user-stored program should be entered because the test is sensitive to wavelength setting. Always set wavelength by approaching from high to low values. For even greater accuracy, run the accuracy check and a demineralized water blank. If the correct result is not obtained, repeat the accuracy check at slightly different wavelengths, again setting the dial from higher to lower values. The wavelength should be 460 ±2 nm.

3. Press: **READ/ENTER**

   The display will show:

   **mg/l TKN**

4. Select the appropriate sample amount from Table I following these steps. Digest the sample amount as described under Digestion Using Digesdahl following these steps. Digest an equal amount of demineralized water as the blank.

---

5. Select the appropriate analysis volume of the digested sample given in Table 1. Pipet the analysis volume from the sample and the blank into separate 25–mL graduated mixing cylinders.

6. Add 12.0 N KOH at one-tenth of the aliquot volume added to the cylinders in Steps 4 and 5. See the Digestion Table for proper volume of 12.0 N KOH.

Example: If a 10 mL sample aliquot was taken in Step 5, then add 1.0 mL of 12.0 N KOH to each cylinder.

Note: If aliquot is less than 1 mL, KOH does not need to be added. Continue with Step 7.

7. Fill both cylinders to the 20–mL mark with demineralized water. Add three drops of Mineral Stabilizer to each cylinder. Invert several times to mix. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder. Invert several times to mix.

Note: Hold the dropping bottles upright while dispensing.

8. Fill both cylinders to the 25–mL mark with demineralized water.

9. Pipet 1 mL of Nessler's Reagent Stopper to each cylinder. Stopper, invert repeatedly. The solution should be clear.


A 2-minute reaction period will begin.

11. When the timer beeps, the display will show:

mg/l TKN

Pour the contents of each cylinder into respective 25–mL sample cells.

Note: The Pour-Thru Cell can be used with this procedure.

Note: If the Pour-Thru Cell Assembly Kit is used, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush it through the funnel and cell with enough demineralized water to dissolve. Rinse out the crystals.

12. Place the blank into a cell holder. Close the light shield.
NITROGEN, TOTAL KJELDAHL, continued

13. Press: ZERO

The display will show:
WAIT
then:
0. mg/L TKN

14. Place the prepared sample into the cell holder.
Close the light shield.

15. Press: READ/ENTER

The display will show:
WAIT
then the result in mg/L total kjeldahl nitrogen as N will be displayed.

Note: The readout is the actual concentration of total kjeldahl nitrogen when the sample amount is 25 mL and the analysis volume is 3 mL. If other volumes are used, the true concentration must be calculated using the formula in Step 16.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

16. Calculation formula:

\[ \text{mg/L TKN} = \frac{75 \times A}{B \times C} \]

Where:
- \( A \) = mg/L read from the display
- \( B \) = mL (or grams) samples taken for digest
- \( C \) = mL digest taken for analysis

Note: For maximum accuracy, the reagent blank value may be determined by repeating the digestion and colorimetric procedures using reagents only; omit addition of sample. Subtract the value obtained for the reagent blank from the reading on the display.

CALIBRATION
A new calibration may be performed for each lot of Nessler Reagent as follows:

a) Prepare standards of 0, 20, 40, 60, 80, 100, 120 and 140 mg/L N by diluting 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mL of a Nitrogen Ammonia Voluette Ampule Standard Solution, 150 mg/L NH₃-N, to 20.0 mL with demineralized water as indicated to Step 7. Add 3 drops of Mineral Stabilizer and 3 drops of Polyvinyl Alcohol Dispersing Agent and dilute to the 25–mL mark with demineralized water. Use a TenSette Pipet to measure the standard solution. Mix well.

b) Store the calibration in the instrument memory using the procedure in the Operation section of the DR/2000 Instrument Manual. Follow the procedure described, choosing a wavelength of 460 nm, the decimal position as 0000, units as mg/L TKN, and a Timer 1 interval of 02:00. Note the program number assigned to the procedure.

c) Add one mL of Nessler Reagent to the 0 standard, reagent blank, and to the prepared standards. Use the 0 standard to perform the zero calibration. Enter the TKN concentration of the first standard (20 mg/L) and measure the remaining standards.

d) Use this stored program number in the procedure above. Prepare a new calibration for each new lot of reagent, using the same stored program number.

DIGESTION USING DIGESDAHL
For safe Digesdaahl operation, do not deviate from these instructions in any way. Sample size, acid volumes, heating periods and step sequence must be followed. Additional safety precautions are given in General Digesdaahl Digestion (Section 1).

a) Transfer a preweighed or a premeasured amount of sample (see Digestion Table for sample amount) into a 100–mL volumetric flask. The sample cannot contain more than 0.5 g of solids. Oils and organic liquids
should be considered as solids when determining samples sizes. Add several boiling chips to prevent bumping.

**Note:** The maximum sample volume is 50 mL (0.5 g of solids). Several 50–mL sample aliquots may be digested in succession to concentrate a dilute sample.

b) Turn on the water aspirator. Check to be sure there is suction in the fractionating head.

**Warning:** Always operate the Digesdahl with a safety shield in place or inside a closed fume hood. Always wear safety glasses.

c) Add 3 mL of concentrated sulfuric acid to the sample in the volumetric flask. Add boiling chips for liquid samples. Immediately place the flask weight and the head on the volumetric flask.

d) Place the volumetric flask on the heater. Turn the temperature dial to 440 °C (825 °F). Once the acid starts to reflux and/or white acid vapors are present, allow the sample to char for three to five minutes. **Do not boil to dryness.** Do not proceed with Step e if sulfuric acid is not present.

**Note:** Liquid samples will require the total evaporation of water before refluxing mark is visible.

**Note:** Discard sample if it evaporates to dryness. Begin the test again using a larger volume of concentrated sulfuric acid or a smaller sample size.

e) Add 10 mL of 50% Hydrogen Peroxide to the sample via the capillary funnel on the fractionating head.

**Note:** Visually confirm the presence of sulfuric acid in the flask before adding hydrogen peroxide.

f) Boil off excess hydrogen peroxide by heating for two more minutes after addition of hydrogen peroxide is complete.

**Note:** If the sample goes to dryness, turn off the Digesdahl and cool completely. Add water to the flask before handling. Repeat digestion from the beginning.

**Note:** Digestion is complete when the digestate is colorless or the color of the digestate does not change upon addition of hydrogen peroxide.

g) Remove the manifold from the digestion flask. Take the flask off the heater. Allow the flask to cool.

h) If the digestate is turbid, it should be filtered at this time. Quantitatively transfer the filtrate to a 100–mL volumetric flask. Dilute to the mark with demineralized water. The sample is now ready for analysis.

**SAMPLING AND STORAGE**
Collect samples in a cleaned glass or plastic container. Adjust the pH to 2 or less with sulfuric acid (about 2 mL per liter) and cool to 4 °C. Preserved samples can be stored up to 28 days.

**ACCURACY CHECK**

**Standard Additions Method**
a) Snap off the neck of a Voluette Ampule Standard For Nitrogen Ammonia, 150 mg/L as NH₃–N.

b) Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25–mL samples. Mix each thoroughly.

c) Analyze each sample as described above. The nitrogen concentration should increase 20 mg/L for each 0.1 mL of standard added.

**Note:** Do not use this value in the formula given in Step 16.

d) If these increases do not occur, see Standard Additions in Section I for more information.

**Standard Solution Method**
Fill a 25–mL graduated mixing cylinder to the 25–mL mark with a 1.0 mg/L NH₃–N solution. Perform the nitrogen procedure as described in Steps 5 to 15. The display should show 33 mg/l TKN in Step 15.

**PRECISION**
In a single laboratory, using a standard solution of 64 mg/L TKN and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.8 mg/L TKN.
TABLE 1. DIGESTION TABLE

LIQUID SAMPLES (Solutions or suspensions in water – less than 1% solids)

<table>
<thead>
<tr>
<th>Expected Nitrogen</th>
<th>Sample Amount</th>
<th>Analysis Volume</th>
<th>Analysis Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3–20 ppm</td>
<td>50 mL</td>
<td>10 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>1–60 ppm</td>
<td>30 mL</td>
<td>6 mL</td>
<td>.6 mL</td>
</tr>
<tr>
<td>2–150 ppm</td>
<td>20 mL</td>
<td>3 mL</td>
<td>.3 mL</td>
</tr>
<tr>
<td>10–500 ppm</td>
<td>10 mL</td>
<td>2 mL</td>
<td>.2 mL</td>
</tr>
<tr>
<td>50–2000 ppm</td>
<td>5 mL</td>
<td>1 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td>500–20000 ppm</td>
<td>1 mL</td>
<td>0.5 mL</td>
<td>0 mL</td>
</tr>
</tbody>
</table>

DRY SAMPLES (Including organic liquids such as oils, etc.)

<table>
<thead>
<tr>
<th>Expected Nitrogen</th>
<th>Sample Amount</th>
<th>Analysis Volume</th>
<th>Analysis Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–2000 ppm</td>
<td>0.5 g</td>
<td>10 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>100–4500 ppm</td>
<td>0.4 g</td>
<td>6 mL</td>
<td>.6 mL</td>
</tr>
<tr>
<td>150–9000 ppm</td>
<td>0.3 g</td>
<td>4 mL</td>
<td>.4 mL</td>
</tr>
<tr>
<td>400–25000 ppm</td>
<td>0.2 g</td>
<td>2 mL</td>
<td>.2 mL</td>
</tr>
<tr>
<td>1500–110000 ppm</td>
<td>0.1 g</td>
<td>1 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td>6500–450000 ppm</td>
<td>0.05 g</td>
<td>0.5 mL</td>
<td>0 mL</td>
</tr>
</tbody>
</table>

*1 ppm = 1 mg/L

SUMMARY OF METHOD
The term "Total Kjeldahl Nitrogen" refers to the combination of ammonia and organic nitrogen. However, only the organic nitrogen compounds appearing as organically bound nitrogen in the trinegative state are determined in this test. Nitrogen in this form is converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide. The ammonia is then analyzed by a modified nessler method test.

REQUIRED REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity Required Per Test</th>
<th>Units</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Peroxide, 50%</td>
<td>20 mL</td>
<td>500 mL</td>
<td>21196–49</td>
</tr>
<tr>
<td>Mineral Stabilizer</td>
<td>6 drops</td>
<td>59 mL SCDB</td>
<td>23766–26</td>
</tr>
<tr>
<td>Nesslers Reagent</td>
<td>2 ml</td>
<td>500 mL</td>
<td>21194–49</td>
</tr>
<tr>
<td>Polyvinyl Alcohol Dispersing Agent</td>
<td>6 drops</td>
<td>59 mL SCDB</td>
<td>23765–26</td>
</tr>
<tr>
<td>Potassium Hydroxide Standard Solution, 12.0 N</td>
<td>varies</td>
<td>100 mL MDB</td>
<td>230–32</td>
</tr>
<tr>
<td>Sulfuric Acid, ACS</td>
<td>6 mL</td>
<td>500 mL</td>
<td>979–49</td>
</tr>
</tbody>
</table>

REQUIRED APPARATUS

Boiling Chips, silicon carbide            | 2–3                         | 500 g       | 20557–34 |
Cots, finger                             | 2                           | 2/pkg       | 14647–02 |
Cylinder, graduated, mixing, tall-form, 25 mL | 2                         | each        | 21190–40 |
Pipe, TenSette, 0.1 to 1.0 mL            | 1                           | each        | 19700–01 |
Pipe Tips, for 19700–01 TenSette Pipe    | 2                           | 50/pkg      | 21856–96 |
Safety Shield, for Digesdahl              | 1                           | each        | 20974–00 |

Select one based on available voltage:

Digesdahl digestion apparatus, 115 Vac   | 1                           | each        | 23130–20 |
Digesdahl digestion apparatus, 230 Vac    | 1                           | each        | 23130–21 |
OPTIONAL REAGENTS
Nitrogen Ammonia Standard Solution, 1 mg/L NH₃–N .......................... 500 mL ...... 1891–49
Nitrogen Ammonia Standard Solution, Voluette Ampule, 150 mg/L NH₃–N, 10 mL .. 16/pkg ...... 21284–10
Potassium Hydroxide, 12.0 N .................................................. 500 mL ...... 230–49
Sodium Thiosulfate, pentahydrate, ACS ........................................ 454 g ...... 460–01

OPTIONAL APPARATUS
Ampule Breaker Kit ........................................................................... each ...... 21968–00
Bottle, glass dispenser, 118 mL .................................................. each ...... 591–00
Bottle, plastic wash, 32–oz (1000 mL) ........................................ each ...... 620–16
Cylinder, graduated, 50 mL .................................................. each ...... 508–41
Mini Grinder, 120 Vac ................................................................. each ...... 20991–00
Pipet, volumetric, Class A, 0.50 mL ........................................ each ...... 14515–34
Pipet, volumetric, Class A, 1.00 mL ........................................ each ...... 14515–35
Pipet, volumetric, Class A, 2.00 mL ........................................ each ...... 14515–36
Pipet, volumetric, Class A, 3.00 mL ........................................ each ...... 14515–03
Pipet, volumetric, Class A, 4.00 mL ........................................ each ...... 14515–04
Pipet, volumetric, Class A, 5.00 mL ........................................ each ...... 14515–37
Pipet, volumetric, Class A, 10.00 mL ........................................ each ...... 14515–38
Pour–Thru Cell Assembly Kit ................................................ each ...... 45215–00
Safety Glasses ............................................................................... each ...... 18421–00

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.