STANDARD METHODS
FOR THE
EXAMINATION
OF
WATER AND SEWAGE

FOURTH EDITION
Revised by committees of the American Public Health Association, American Chemical Society, and referees of the Association of Official Agricultural Chemists

AMERICAN PUBLIC HEALTH ASSOCIATION
169 MASSACHUSETTS AVENUE
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PREFACE TO FOURTH EDITION.

The Committee on Standard Methods of Bacteriological Water Analysis was reorganized in 1918 with the following membership: F. P. Gorham, chairman, L. A. Rogers, W. G. Bissell, H. E. Hasseltine, H. W. Redfield, with M. Levine as adjunct member. This committee made a report in 1918 which was not acted on by the Laboratory Section, and in 1919 made a revised report, recommending certain changes in Standard Methods, which were adopted by the section and which are now incorporated in this present fourth edition.

Following are the more important changes:
New brands of peptone authorized.
Phenol Red Method of Hydrogen-ion Concentration.
Five-tenths per cent of sugar specified for broths instead of 1 per cent.
Sterilization of sugar is media specified in greater detail.
Preparation of Endo Medium.
Synthetic Medium for the Methyl Red Test.
There are no changes in the chemical methods in this edition.
AMERICAN PUBLIC HEALTH ASSOCIATION.

LABORATORY SECTION.

STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE.

Compiled and revised by committees of the American Public Health Association and the American Chemical Society and referees of the Association of Official Agricultural Chemists.

COLLECTION OF SAMPLES.

QUANTITY REQUIRED FOR ANALYSIS.

The minimum quantity necessary for making the ordinary physical, chemical, and microscopical analyses of water or sewage is 2 liters; for the bacteriological examination, 100 cc. In special analyses larger quantities may be required.

BOTTLES.

The bottles for the collection of samples shall have glass stoppers, except when physical, mineral, or microscopical examinations only are to be made. Jugs or metal containers shall not be used.

Sample bottles shall be carefully cleansed each time before using. This may be done by treating with sulfuric acid and potassium bichromate, or with alkaline permanganate, followed by a mixture of oxalic and sulfuric acids, and by thoroughly rinsing with water and draining. The stoppers and necks of the bottles shall be protected from dirt by tying cloth, thick paper or tin foil over them.

For shipment bottles shall be packed in cases with a separate compartment for each bottle. Wooden boxes may be lined with corrugated fibre paper, felt, or similar substance, or provided with spring corner strips, to prevent breakage. Lined wicker baskets also may be used.
Collection of Samples

Bottles for bacteriological samples shall be sterilized as directed on page 98.

Interval Before Analysis.

In general, the shorter the time elapsing between the collection and the analysis of a sample the more reliable will be the analytical results. Under many conditions analyses made in the field are to be commended, as data so obtained are frequently preferable to data obtained in a distant laboratory after the composition of the water has changed.

The time that may be allowed to elapse between the collection of a sample and the beginning of its analysis cannot be stated definitely. It depends on the character of the sample, the examinations to be made, and other conditions. The following are suggested as fairly reasonable maximum limits.

Physical and chemical analysis.

<table>
<thead>
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<th>Time</th>
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<td>Ground waters</td>
<td>72</td>
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<tr>
<td>Fairly pure surface waters</td>
<td>48</td>
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<td>Polluted surface waters</td>
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<td>Sewage effluents</td>
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<td>Raw sewages</td>
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Microscopical examination.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground waters</td>
<td>72</td>
</tr>
<tr>
<td>Fairly pure surface waters</td>
<td>24</td>
</tr>
<tr>
<td>Waters containing fragile organisms</td>
<td>Immediate examination</td>
</tr>
</tbody>
</table>

Bacteriological examination.

Samples kept at less than 10°C. 24 hours

If a longer period elapses between collection and examination the time should be noted. If sterilized by the addition of chloroform, formaldehyde, mercuric chloride, or some other germicide samples for sanitary chemical examination may be allowed to stand for longer periods than those indicated, but as this is a matter which will vary according to circumstances, no definite procedure is recommended. If unsterilized samples of sewage, sewage effluents, and highly polluted surface waters are analyzed after greater intervals than those suggested caution must be used.
in interpreting analyses of the organic content, which frequently changes materially upon standing.

Determinations of dissolved gases, especially oxygen, hydrogen sulfide, and carbon dioxide, should be made at the time of collection in order to be reasonably accurate, in accordance with the directions given hereafter in connection with each determination.

REPRESENTATIVE SAMPLES.

Care should be taken to obtain a sample that is truly representative of the liquid to be analyzed. With sewages this is especially important because marked variations in composition occur from hour to hour. Satisfactory samples of some liquids can be obtained only by mixing together several portions collected at different times or at different places—the details as to collection and mixing depending upon local conditions.
PHYSICAL EXAMINATION.

TEMPERATURE.

The temperature of the sample, if taken, shall be taken at the time of collection, and shall be expressed preferably in degrees Centigrade, to the nearest degree, or closer if more precise data are required. The thermophone is recommended for obtaining the temperature of water at various depths below the surface.

TURBIDITY.

The turbidity of water is due to suspended matter, such as clay, silt, finely divided organic matter, microscopic organisms, and similar material.

TURBIDITY STANDARD.

The standard of turbidity shall be that adopted by the United States Geological Survey, namely, a water which contains 100 parts per million of silica in such a state of fineness that a bright platinum wire 1 millimeter in diameter can just be seen when the center of the wire is 100 millimeters below the surface of the water and the eye of the observer is 1.2 meters above the wire, the observation being made in the middle of the day, in the open air, but not in sunlight, and in a vessel so large that the sides do not shut out the light so as to influence the results. The turbidity of such water is arbitrarily fixed at 100 parts per million.

For preparation of the silica standard dry Pearl's "precipitated fuller's earth" and sift it through a 200-mesh sieve. One gram of this preparation in 1 liter of distilled water makes a stock suspension which contains 1,000 parts per million of silica and which should have a turbidity of 1,000. Test this suspension, after diluting a portion of it with nine times its volume of distilled water, by the platinum-wire method to ascertain if the silica has the necessary degree of fineness and if the suspension has the necessary degree of turbidity. If not, correct by adding more silica or more water as the case demands.*

Standards for comparison shall be prepared from this stock suspension by dilution with distilled water. For turbidity readings

*This method of correction very slightly alters the coefficient of fineness of the standard, but does not noticeably affect its use.
below 20, standards of 0, 5, 10, 15, and 20 shall be kept in clear glass bottles of the same size as that containing the sample; for readings above 20, standards of 20, 30, 40, 50, 60, 70, 80, 90, and 100 shall be kept in 100 cc. Nessler tubes approximately 20 millimeters in diameter.

Comparison with the standards shall be made by viewing both standard and sample sidewise toward the light by looking at some object and noting the distinctness with which the margins of the object can be seen.

The standards shall be kept stoppered, and both sample and standards shall be thoroughly shaken before making the comparison.

In order to prevent any bacterial or algal growths from developing in the standards a small amount of mercury dichloride may be added to them.

**PLATINUM WIRE METHOD.**

This method requires a rod with a platinum wire 1 mm. in diameter inserted in it about 1 inch from one end of the rod and projecting from it at a right angle at least 25 mm. Near the other end of the rod, at a distance of 1.2 meters from the platinum wire, a small ring shall be placed directly above the wire through which, with his eye directly above the ring, the observer shall look when making the examination.

The rod shall be graduated as follows: The graduation mark of 100 shall be placed on the rod at a distance of 100 mm. from the center of the wire. Other graduations shall be made according to Table 1, which is based on the best obtainable data. The distances recorded in Table 1 are intended to be such that when the water is diluted the turbidity readings will decrease in the same proportion as the percentage of the original water in the mixture. These graduations are those on what is known as the U. S. Geological Survey Turbidity Rod of 1902.\(^{106}\)
## TURBIDITY

### Table 1.—GRADUATION OF TURBIDITY ROD.

<table>
<thead>
<tr>
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<th>Vanishing depth of wire (mm.)</th>
<th>Turbidity (parts per million)</th>
<th>Vanishing depth of wire (mm.)</th>
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</table>

**Procedure.**—Lower the rod vertically into the water as far as the wire can be seen and read the level of the surface of the water on the graduated scale. This will indicate the turbidity.

The following precautions shall be taken to insure correct results:

Observations shall be made in the open air, preferably in the middle of the day and not in direct sunlight. The wire shall be kept bright and clean. If for any reason observations cannot be made directly under natural conditions a pail or tank may be filled with water and the observation taken in that, but if this is done care shall be taken that the water is thoroughly stirred before the observation is made, and no vessel shall be used for this purpose unless its diameter is at least twice as great as the depth to which the wire is immersed. Waters which have a turbidity greater than 500
shall be diluted with clear water before the observations are made, but if this is done the degree of dilution shall be reported.

TURBIDIMETRIC METHOD.

Several forms of turbidimeter or diaphanometer\textsuperscript{73} have been suggested for use. The simplest and most satisfactory form is the candle turbidimeter.\textsuperscript{18} This consists of a graduated glass tube with a flat polished bottom, enclosed in a metal case. This is supported over an English standard candle and so arranged that one may look vertically down through the tube at the flame of the candle. The observation is made by pouring the sample of water into the tube until the image of the flame of the candle just disappears from view. Care shall be taken not to allow soot or moisture to accumulate on the lower side of the glass bottom of the tube so as to interfere with the accuracy of the observations. The graduations on the tube correspond to turbidities produced in distilled water by certain numbers of parts per million of silica standard. In order to insure uniform results it is necessary to have the distance between the top rim of the candle and the bottom of the tube constant, and this distance shall be 7.6 cm. or 3 inches. The observations shall be made in a darkened room or with a black cloth over the head.

It is allowable to substitute for the candle an electric light. Calibrate the apparatus to correspond with the United States Geological Survey scale. The figures in Table 2 on page 8 are believed to be approximately correct for the candle turbidimeter but should be checked by the experimenter. It is allowable to calibrate the tube of the instrument with waters of known turbidity prepared by making a series of dilutions of the silica standard with distilled water. From the figures obtained in calibrating plot a curve from which the turbidity of a sample may be read when the depth of water in the tube has been obtained.


<table>
<thead>
<tr>
<th>Depth of liquid (cm)</th>
<th>Turbidity (parts per million of silica)</th>
<th>Depth of liquid (cm)</th>
<th>Turbidity (parts per million of silica)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>1000</td>
<td>7.7</td>
<td>291</td>
</tr>
<tr>
<td>1.6</td>
<td>900</td>
<td>7.4</td>
<td>290</td>
</tr>
<tr>
<td>1.8</td>
<td>800</td>
<td>6.6</td>
<td>277</td>
</tr>
<tr>
<td>2.0</td>
<td>700</td>
<td>6.2</td>
<td>262</td>
</tr>
<tr>
<td>2.2</td>
<td>650</td>
<td>6.0</td>
<td>254</td>
</tr>
<tr>
<td>2.4</td>
<td>600</td>
<td>5.7</td>
<td>240</td>
</tr>
<tr>
<td>2.6</td>
<td>550</td>
<td>5.4</td>
<td>227</td>
</tr>
<tr>
<td>2.8</td>
<td>500</td>
<td>5.0</td>
<td>214</td>
</tr>
<tr>
<td>3.0</td>
<td>450</td>
<td>4.7</td>
<td>201</td>
</tr>
<tr>
<td>3.2</td>
<td>400</td>
<td>4.4</td>
<td>188</td>
</tr>
<tr>
<td>3.4</td>
<td>350</td>
<td>4.0</td>
<td>175</td>
</tr>
<tr>
<td>3.6</td>
<td>300</td>
<td>3.7</td>
<td>162</td>
</tr>
<tr>
<td>3.8</td>
<td>250</td>
<td>3.4</td>
<td>149</td>
</tr>
<tr>
<td>4.0</td>
<td>200</td>
<td>3.0</td>
<td>136</td>
</tr>
<tr>
<td>4.2</td>
<td>150</td>
<td>2.7</td>
<td>123</td>
</tr>
<tr>
<td>4.4</td>
<td>100</td>
<td>2.4</td>
<td>110</td>
</tr>
<tr>
<td>4.6</td>
<td>100</td>
<td>1.1</td>
<td>100</td>
</tr>
</tbody>
</table>

The results of turbidity observations shall be expressed in whole numbers which correspond to parts per million of silica and recorded as follows:

<table>
<thead>
<tr>
<th>Turbidity between</th>
<th>1 and 50 recorded in nearest unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10-90</td>
</tr>
<tr>
<td>0</td>
<td>100-499</td>
</tr>
<tr>
<td>0</td>
<td>500-999</td>
</tr>
<tr>
<td>0</td>
<td>1000-1999</td>
</tr>
<tr>
<td>0</td>
<td>greater</td>
</tr>
</tbody>
</table>

COEFFICIENT OF FINENESS

The quotient obtained by dividing the weight of suspended matter in the sample by the turbidity, both expressed in the same unit, shall be called the coefficient of fineness. If the quotient is greater than unity the matter in suspension is coarser and if it is less than unity it is finer than the standard.
COLOR.

The "color," or the "true color," of water shall be considered the color that is due only to substances in solution; that is, it is the color of the water after the suspended matter has been removed. In stating results the word "color" shall mean the "true color" unless otherwise designated.

The "apparent color" shall be considered as including not only the true color but also any color produced by substances in suspension. It is the color of the original unfiltered sample.

The platinum-cobalt method of measuring color shall be considered as the standard, and the unit of color shall be that produced by 1 part per million of platinum.

COMPARISON WITH PLATINUM-COBLANT STANDARDS.

Reagents.—Dissolve 1.246 grams of potassium platinic chloride (PtCl₄2KCl), containing 0.5 gram platinum, and 1.00 gram crystallized cobalt chloride (CoCl₄.6H₂O), containing 0.25 gram of cobalt, in water with 100 cc. concentrated hydrochloric acid, and dilute to 1 liter with distilled water. This solution has a color of 500. Dilute this solution with distilled water in 50 cc. Nessler tubes to prepare standards having colors of 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 70. Keep these standards in Nessler tubes of such diameter that the graduation mark is between 20 and 25 cm. above the bottom and of such uniformity that they match within such limit that the distance from the bottom to the graduation mark of the longest tube shall not exceed that of the shortest tube by more than 6 mm. Protect the tubes from dust and light when not in use.

Procedure.—The color of a sample shall be observed by filling a standard Nessler tube to the height equal to that in the standard tubes with the sample and by comparing it with the standards. The observation shall be made by looking vertically downward through the tubes upon a white or mirrored surface placed at such angle that light is reflected upward through the column of liquid.

Water that has a color greater than 70 shall be diluted before making the comparison, in order that no difficulties may be encountered in matching the hues.

Water containing matter in suspension shall be filtered, before the color observation is made, until no visible turbidity remains.
COLOR

If the suspended matter is coarse, filter paper may be used for this purpose; if the suspended matter is fine, the use of a Berkefeld filter is recommended. The Pasteur filter shall not be used as it exerts a marked decolorizing action.

The apparent color, if determined, shall be determined on the original sample without filtration. The true and the apparent color of clear waters or waters with low turbidities are substantially the same.

The results of color determinations shall be expressed in whole numbers and recorded as follows:

<table>
<thead>
<tr>
<th>Color between 1 and 50 recorded to nearest unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; &quot; 51 &quot; 100 &quot; &quot; 5</td>
</tr>
<tr>
<td>&quot; &quot; 101 &quot; 250 &quot; &quot; 10</td>
</tr>
<tr>
<td>&quot; &quot; 251 &quot; 500 &quot; &quot; 20</td>
</tr>
</tbody>
</table>

COMPARISON WITH GLASS DISKS, 108

As the platinum-cobalt standard method is not well adapted for field work, the color of the water to be tested may be compared with that of glass disks held at the end of metallic tubes through which they are viewed by looking toward a white surface. The glass disks are individually calibrated to correspond with colors on the platinum scale. Experience has shown that the glass disks used by the U. S. Geological Survey give results in substantial agreement with those obtained by the platinum determinations, and their use is recognized as a standard procedure.

COMPARISON WITH NESSLER STANDARDS.

Inasmuch as the Nessler scale, 22 49 which agrees with it except for colors less than 20, have been largely used in the past, the old results may be converted 117 into terms of the platinum standard by means of the ratios in Table 3, but they must not be considered as universally applicable as the variable sensitiveness of the Nessler solution introduces an uncertain factor.
Table 3.—Values for converting colors by the natural water scale into colors by the platinum standard in parts per million.*

<table>
<thead>
<tr>
<th>Modified Nessler or natural-water standard</th>
<th>0.00</th>
<th>0.01</th>
<th>0.02</th>
<th>0.03</th>
<th>0.04</th>
<th>0.05</th>
<th>0.06</th>
<th>0.07</th>
<th>0.08</th>
<th>0.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum-cobalt standard color.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>31</td>
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<td>32</td>
</tr>
<tr>
<td>.30</td>
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<td>34</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>37</td>
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<td>38</td>
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<td>51</td>
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<td>.60</td>
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<td>79</td>
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</tr>
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</tr>
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<td>106</td>
<td>107</td>
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</tr>
<tr>
<td>1.40</td>
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</tr>
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<td>120</td>
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</tr>
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<td>1.60</td>
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<td>125</td>
<td>125</td>
<td>126</td>
<td>127</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>1.70</td>
<td>129</td>
<td>130</td>
<td>130</td>
<td>131</td>
<td>132</td>
<td>132</td>
<td>133</td>
<td>134</td>
<td>135</td>
<td>136</td>
</tr>
<tr>
<td>1.80</td>
<td>136</td>
<td>137</td>
<td>137</td>
<td>138</td>
<td>139</td>
<td>139</td>
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<td>142</td>
</tr>
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<td>1.90</td>
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<td>2.00</td>
<td>150</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Zero on the true Nessler scale is about 15 on the platinum scale.

LOVIBOND TINTOMETER

The value of the readings of tint and shade by the Lovibond tintometer has not been commensurate with the labor involved, but it is necessary to make a record of the reflected tint and shade of some waters. The standard color disks used in teaching optics may be used for the purpose.

Procedure.—The white disk supports three movable standard color sectors, red, yellow, and blue, and one movable black sector. All are mounted on a device which can be revolved rapidly, blending the colors into a uniform tint or shade. A scale around the circumference of the disk is used to indicate the percentage of each color or white or black in the blend.

Place the sample in a battery jar on a white ground; adjust the sectors so that when blended the tint or shade will match the reflected tint or shade of the sample. Report the percentages of red, yellow, blue, white, and black in the blended tint or shade.
The observation of the odor, cold and hot, of samples of surface water is important as the odors are usually indicative of organic growths or sewage contamination or both. The odor of some ground waters is caused by the earthy constituents of the water-bearing strata. The odor of a contaminated well water is often contributory evidence of its pollution. A study of the organisms as directed under Microscopical Examination (p. 90) is a valuable adjunct to physical and chemical examination of water. Certain odors distinguish or identify certain organisms, as, for example, the "fishy" odor of Uroglena, the "aromatic" or "rose geranium" odor of Asterionella and the "pig pen" odor of Anabaena. Observe and record the odor, both at room temperature and at just below the boiling-point, as follows:

COLD ODOR.

Shake the sample violently in one of the collecting bottles, when it is half to two-thirds full and when the sample is at room temperature (about 20° C.). Remove the stopper and smell the odor at the mouth of the bottle.

HOT ODOR.

Pour about 150 cc. of the sample into a 500 cc. Erlenmeyer flask. Cover the flask with a well-fitting watch glass. Heat the water almost to boiling on a hot plate. Remove the flask from the plate and allow it to cool not more than five minutes. Then agitate it with a rotary movement, slip the watch glass to one side, and smell the odor.

EXPRESSION OF RESULTS

Express the quality of the odor by a descriptive epithet like the following, which may be abbreviated in the record:

a—aromatic
C—free chlorine
D—disagreeable
e—earthy
f—fishy
g—grassy
m—moldy
M—musty
p—peaty
s—sweetish
S—hydrogen sulfide
v—vegetable.

Express the intensity of the odor by a numeral prefixed to the term expressing quality, which may be defined as follows:
<table>
<thead>
<tr>
<th>Numerical Value</th>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None.</td>
<td>No odor perceptible.</td>
</tr>
<tr>
<td>1</td>
<td>Very faint.</td>
<td>An odor that would not be detected ordinarily by the average consumer, but that could be detected in the laboratory by an experienced observer.</td>
</tr>
<tr>
<td>2</td>
<td>Faint.</td>
<td>An odor that the consumer might detect if his attention were called to it, but that would not attract attention otherwise.</td>
</tr>
<tr>
<td>3</td>
<td>Distinct.</td>
<td>An odor that would be detected readily and that might cause the water to be regarded with disfavor.</td>
</tr>
<tr>
<td>4</td>
<td>Decided.</td>
<td>An odor that would force itself upon the attention and that might make the water unpalatable.</td>
</tr>
<tr>
<td>5</td>
<td>Very strong.</td>
<td>An odor of such intensity that the water would be absolutely unfit to drink. (A term to be used only in extreme cases.)</td>
</tr>
</tbody>
</table>
CHEMICAL EXAMINATION.

EXPRESSION OF RESULTS.

The results of chemical analyses shall be expressed in parts per million, which in most analyses is practically equivalent to milligrams per liter. In some laboratories other forms of expression have been used. Results expressed in parts per 100,000 or in grains per gallon may be transformed to parts per million, or conversely, by the use of the following table:

Table 4.—Factors for transforming results of analyses.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Equivalent.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grains per U. S. gallon.</td>
</tr>
<tr>
<td>1 grain per U. S. gallon</td>
<td>1.000</td>
</tr>
<tr>
<td>1 grain per Imperial gallon</td>
<td>.835</td>
</tr>
<tr>
<td>1 part per 100,000</td>
<td>.585</td>
</tr>
<tr>
<td>1 part per million</td>
<td>.058</td>
</tr>
</tbody>
</table>

The following general rules shall govern the use of significant figures in the expression of results:

1. If the results show quantities greater than 10 parts per million use no decimals; record only whole numbers. If the quantities reach hundreds and thousands of parts record only two significant figures.

2. If the results are between 1 and 10 parts do not retain more than one decimal place.

3. If the results are between 0.1 and 1 part do not retain more than two decimal places.

4. Estimates of ammonia, albuminoid, and nitrite nitrogen alone justify the use of three decimals.

5. If the results of analyses are tabulated ciphers should not be added at the right of the decimal point to make the column uniform.
Ammonia Nitrogen

Forms of Nitrogen.

Nitrogenous organic matter passes through several intermediate compounds during its natural decomposition, and that which does not gasify ultimately forms nitrate. Nitrogen in organic matter is determined by the Kjeldahl process. An indication of the amount present is obtained by the albuminoid nitrogen determination. It has not been found possible to differentiate the nitrogen in the organic matter that readily decomposes from that in stable or non-putrefiable compounds. Decomposition of organic matter produces nitrogen combined in ammonia, which is the first step between nitrogenous organic matter and the completely mineralized nitrate. Ammonia nitrogen may be determined by distillation and Nesslerization or by direct Nesslerization of the clarified sample. The next step is oxidation to nitrite, and the final step, oxidation to nitrate. It is recommended that all forms of nitrogen be reported as the element nitrogen (N).

Ammonia Nitrogen.

There are two methods for estimating ammonia nitrogen—distillation and direct Nesslerization. Distillation is recommended for most waters and direct Nesslerization is recommended for sewages, sewage effluents, and highly polluted surface waters.

Determination by Distillation.

Procedure.—Use a metal or a glass flask connected with a condenser so that the distillate may drop from the condenser tube directly into a Nessler tube or a flask. Free the apparatus from ammonia by boiling distilled water in it until the distillate shows no trace of ammonia. After this has been done empty the distilling flask and measure into it 500 cc. of the sample, or a smaller portion diluted to 500 cc. with ammonia-free water. If the sample is acid or if the presence of urea is suspected add about 0.5 gram of sodium carbonate before distillation. Omit this if possible as it tends to increase “bumping.” Apply heat so that the distillation may proceed at the rate of not more than 10 cc. nor less than 6 cc. per minute. Collect the distillate in four Nessler tubes, 50 cc. to each tube, or if the nitrogen is high in a 200 cc. graduated flask. These receptacles contain the ammonia nitrogen to be measured as hereafter described.
AMMONIA NITROGEN

Use Nessler tubes of such diameter that the graduation mark is between 20 and 25 cm. above the bottom and of such uniformity of diameter that the distance from the bottom to the graduation mark of the longest tube shall not exceed that of the shortest tube by more than 6 mm. The tubes must be of clear white glass with polished bottoms.

MEASUREMENT OF AMMONIA NITROGEN.

The amount of ammonia in the distillates may be measured either by (1) comparison of the Nesslerized distillates with Nesslerized solutions containing known quantities of nitrogen as ammonium chloride, or by (2) comparison of the Nesslerized distillates with permanent standard solutions in which the colors of Nesslerized standard ammonia solutions are duplicated by solutions of platinum and cobalt chlorides.

COMPARISON WITH AMMONIA STANDARDS.

Reagents.—1. Ammonia-free water.

2. Standard ammonium chloride solution. Dissolve 3.82 grams of ammonium chloride in ammonia-free water and dilute to 1 liter; dilute 10 cc. of this to 1 liter with ammonia-free water. One cc equals 0.00001 gram of nitrogen.

3. Nessler reagent. Dissolve 50 grams of potassium iodide in a minimum quantity of cold water. Add a saturated solution of mercuric chloride until a slight precipitate persists permanently. Add 400 cc. of 50 per cent solution of potassium hydroxide, made by dissolving the potassium hydroxide and allowing it to clarify by sedimentation before using. Dilute to 1 liter, allow to settle, and decant. This solution should give the required color with ammonia within five minutes after addition and should not produce a precipitate with small amounts of ammonia within two hours.

Procedure.—Prepare a series of 16 Nessler tubes containing the following amounts of the standard ammonium chloride solution, diluted to 50 cc. with ammonia-free water, namely: 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 6.0 cc. These solutions will contain 0.00001 gram of nitrogen for each cubic centimeter of the standard solution.

Nesslerize the standards and the distillates by adding approximately 1 cc. of Nessler reagent to each tube. Do not stir the contents of the tubes. The temperature of the tubes should be
practically the same as that of the standards; otherwise the colors will not be directly comparable. Allow the tubes to stand at least 10 minutes after Nesslerizing. Compare the color produced in the tubes with that in the standards by looking vertically downward through them at a white or mirrored surface placed at an angle in front of a window so as to reflect the light upward.

If the color obtained by Nesslerizing the distillates is greater than that of the darkest tube of the standards, mix the contents of the tube thoroughly, pour out half of the liquid, and dilute the remainder to the original volume with ammonia-free water; then make the color comparison and multiply the result by two. If the color is still too dark after pouring out half the liquid, repeat this process of division until a reading can be made. The process of dilution may be shortened by mixing together the distillates from one sample before making the comparison and comparing an aliquot portion with the standards.

After the readings have been recorded add the results obtained by Nesslerizing each portion of the entire distillate. If 500 cc. of the sample is distilled this sum, expressed in cubic centimeters and multiplied by 0.02, will give the number of parts per million of ammonia nitrogen in the sample. If \( x \) cc. of sample is used multiply the sum of the readings by \( \frac{10}{x} \).

If the ammonia is known to be high the distillate may be collected in 200 cc. flasks and an aliquot part Nesslerized.

**Comparison with Permanent Standards.**

**Reagents.**—Platinum solution. Dissolve 2.00 grams of potassium platinic chloride (\( \text{PtCl}_4.2\text{KCl} \)) in a small amount of distilled water, add 100 cc. of strong hydrochloric acid, and dilute to 1 liter.

Cobalt solution. Dissolve 12 grams of cobaltous chloride (\( \text{CoCl}_2.6\text{H}_2\text{O} \)) in distilled water, add 100 cc. of strong hydrochloric acid, and dilute to 1 liter.

Prepare standards by putting various amounts of these two solutions into Nessler tubes and diluting to the 50 cc. mark with distilled water as indicated in Table 5. These standards may be kept for several months if protected from dust.
<table>
<thead>
<tr>
<th>Value in standard ammonium chloride.</th>
<th>Solution of platinum.</th>
<th>Solution of cobalt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
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<tr>
<td>0.0</td>
<td>1.2</td>
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<tr>
<td>.1</td>
<td>1.8</td>
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<td>.2</td>
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<td>.4</td>
<td>4.7</td>
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<td>1.4</td>
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<td>1.7</td>
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<td>20.0</td>
<td>10.4</td>
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<td>20.0</td>
<td>15.0</td>
</tr>
<tr>
<td>7.0</td>
<td>20.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

The amounts in Table 5 are approximate, and the actual amount necessary will differ with the character of the Nessler solution, the color sensitiveness of the analyst's eye, and other conditions. The final test of the standard is best obtained by comparing it with Nesslerized standards and modifying the tint accordingly. Such comparison should be made for each new batch of Nessler solution and should be checked by each analyst.

Procedure.—In comparison with permanent standards, Nesslerize the distillates in the manner above described and compare the resulting colors at the end of about 10 minutes with the permanent standards. The method of calculating results is precisely the same as with the ammonia standards.

MODIFICATION FOR SEWAGE.

Ammonia nitrogen and albuminoid nitrogen in sewages, soils, and other materials of high nitrogen content may be satisfactorily determined by diluting the sample with ammonia-free distilled water and proceeding as described in the preceding sections, but it is permissible to distill with steam.40
Procedure.—Use a 200 cc. long-necked Kjeldahl flask connected with a condenser so that the distillate may drop from the condenser tube directly into a Nessler tube or a flask. Connect the Kjeldahl flask with a steam generator by a tube reaching almost to the bottom of the flask.

After the apparatus is freed from ammonia put the sample to be tested into the flask. Use 10 to 100 cc. of the sample according to its ammonia content. Pass ammonia-free steam through the liquid in the Kjeldahl flask and collect the distillate in the usual way. It is usually convenient to collect the distillate in a 200 cc. flask and to take an aliquot part of it for Nesslerization. Compare with standards and calculate the nitrogen content in the usual manner.

This method has the advantage, when the sample is treated with an alkaline solution of potassium permanganate, of avoiding bumping, permitting the assay of solid matter, and yielding the ammonia more rapidly than by the ordinary process of distillation.

DETERMINATION BY DIRECT NesslerIZATION.21 75

Reagents.—1. Ten per cent solution of copper sulfate \((\text{CuSO}_4 \cdot 5\text{H}_2\text{O})\).

2. Ten per cent solution of lead acetate \((\text{Pb(C}_2\text{H}_5\text{O}_2)_2 \cdot 3\text{H}_2\text{O})\).

3. Fifty per cent solution of sodium hydroxide \((\text{NaOH})\) or potassium hydroxide \((\text{KOH})\).

Procedure.—To 50 cc. of the sample to be tested, diluted if necessary with an equal volume of ammonia-free water, in a short tube, add a few drops of the copper sulfate solution. After thoroughly mixing, add 1 cc. of the alkali hydroxide solution and again thoroughly mix. Allow the tube to stand for a few minutes, when a heavy precipitate should fall to the bottom, leaving a colorless supernatant liquid. Nesslerize an aliquot part. Compare with standards and compute the ammonia nitrogen in the same manner as in the distillation procedure.

Samples containing hydrogen sulfide may require the use of lead acetate in addition to the copper sulfate. Some samples may require a few trials before the right combination of the three solutions to bring about the best results can be found.

Instead of adding copper sulfate to sewages of high magnesium content satisfactory clarification of the sample can be obtained by mixing it with the alkali hydroxide alone.54
ALKALIMINO NITROGEN

The addition of an alkaline permanganate solution to a mixture containing nitrogenuous organic matter causes the formation of ammonia, which can be distilled and determined by Nesslerization of the distillate. The nitrogen of the ammonia thus formed is called albuminoid nitrogen. As the ratio of nitrogenuous organic matter to the ammonia obtained by distillation is generally variable, 0.7 to 1.0, sewage and other substances containing much nitrogenuous organic matter albuminoid nitrogen results in such substances are less accurate than organic nitrogen. Therefore in sewage work, including analysis of influents and effluents of purification plants and the water of luxury polluted streams, it is recommended that determinations of organic nitrogen be substituted for determinations of albuminoid nitrogen. For ground waters and surface waters containing but little pollution, the albuminoid nitrogen is approximated one-half the organic nitrogen; accordingly the continuance of albuminoid nitrogen determinations for this class of work is approved.

Reagents.—Alkaline potassium permanganate. Pour 1.200 cc. of distilled water into a porcelain dish holding 2,500 cc. and boil 10 minutes, and turn off the gas. Add 16 grams of C. P. potassium permanganate and stir until solution is complete. Then add 500 cc. of 50 per cent clarified solution of potassium hydroxide or an equivalent amount of sodium hydroxide and enough distilled water to fill the dish. Boil down to 2,000 cc. Test this solution for ammonia by making a blank determination. Correct determinations by the amount of this blank.

Procedure.—After the collection of the distillate for ammonia nitrogen described on page 15 add 50 cc. (or more if necessary to insure the complete oxidation of the organic matter) of alkaline potassium permanganate and continue the distillation until at least four portions, and preferably five portions, of 50 cc. each, of distillate have been collected in separate tubes. Determine the albuminoid nitrogen in the distillate by Nesslerization. If the albuminoid nitrogen is known to be high it is convenient to collect the distillate in a 200 cc. flask and to Nesslerize an aliquot part of it.

Dissolved albuminoid nitrogen may be determined in a sample from which suspended matter has been removed by filtration either through filter paper or through a Berkefeld filter. Suspended
albuminoid nitrogen is the difference between the total and the dissolved albuminoid nitrogen.

ORGANIC NITROGEN

Procedure for water.—Boil 500 cc. of the sample in a round-bottomed flask to remove ammonia nitrogen. This usually causes the loss of 200 cc. of the sample, which may be collected for the determination of ammonia nitrogen. Add 5 cc. of nitrogen-free concentrated sulfuric acid and a small piece of ignited pumice. Mix by shaking and place over a flame under a hood. Digest until copious fumes of sulfuric acid are given off and the liquid finally becomes colorless or pale straw color. Remove from the flame, and add potassium permanganate crystals in small portions until a heavy green precipitate persists in the liquid. Cool. Dilute to about 300 cc. with ammonia-free water. Make alkaline with 10 per cent ammonia-free sodium hydroxide. Distill the ammonia, collect the distillate in Nessler tubes, Nesslerize, and compare with standards as described (pp. 16–18).

First procedure for sewage.—Distill the ammonia nitrogen directly from 100 cc. or less of the sample, diluted to 500 cc. with nitrogen-free water. Collect the distillate and determine the ammonia nitrogen in it. Add 5 cc. of nitrogen-free sulfuric acid and 1 cc. of 10 per cent nitrogen-free copper sulfate, and digest the liquid for half an hour after it has become colorless or pale straw color. Add 0.5 gram of potassium permanganate crystals to the hot acid solution, and dilute to 500 cc. with ammonia-free water. Dilute 10 cc. or more of this liquid, in a Kjeldahl distilling flask, to about 300 cc. with ammonia-free water. Make alkaline with 10 per cent sodium hydroxide, distill, and Nesslerize. With some samples direct Nesslerization may be used. (See p. 19.)

In this determination care must be taken to digest thoroughly, to add potassium permanganate to the point of precipitation, to sample carefully after dilution, and to add enough sodium hydroxide to insure the separation of the ammonia from the precipitated manganese hydroxide. Potassium permanganate should not be added during digestion because it causes loss of nitrogen.

Second procedure for sewage.—Omit the separation of ammonia nitrogen and determine the ammonia nitrogen and organic nitrogen together. Determine the ammonia nitrogen in a separate sample
by direct Nesslerization as described on page 19. The organic nitrogen is equal to the difference.

NITRITE NITROGEN

Reagents. 1. Sulfanilic acid solution. Dissolve 8.00 grams of the purest sulfanilic acid in 1,000 cc. of 5 N acetic acid (sp. gr. 1.041) or in 1,000 cc. of water containing 50 cc. of concentrated hydrochloric acid. This is practically a saturated solution.

2. a-naphthylamine acetate or chloride solution. Dissolve 5.00 grams solid a-naphthylamine in 1,000 cc. of 5 N acetic acid or in 1,000 cc. of water containing 8 cc. of concentrated hydrochloric acid. Filter the solution through washed absorbent cotton or an alundum filter.

3. Sodium nitrite stock solution. Dissolve 1.1 gram silver nitrite in nitrite-free water; precipitate the silver with sodium chloride solution and dilute the whole to 1 liter.

4. Standard sodium nitrite solution. Dilute 100 cc. of solution 3 to 1 liter, then dilute 50 cc. of this solution to 1 liter with sterilized nitrite-free water, add 1 cc. of chloroform, and preserve in a sterilized bottle. One cc. = 0.0005 mg. nitrogen.

5. Fuchsin solution. 0.1 gram per liter.

Procedure. Place in a standard Nessler tube 50 cc. of the sample, decolorize if necessary with nitrite-free aluminum hydroxide (see p. 42) or a smaller amount diluted to 50 cc. At the same time prepare in Nessler tubes a set of standards, by diluting to 50 cc. with nitrite-free water, various amounts of the standard nitrite solution. The following amounts of standard solution are suggested: 0.0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0, and 2.5 cc. Add 1 cc. of the sulfanilic acid solution and 1 cc. of the a-naphthylamine acetate or hydrochloride solution to the sample and to each standard. Mix thoroughly and allow to stand 10 minutes; then compare the sample with the standards. Do not allow the sample to stand more than one-half hour before making the comparison. If the color of the sample is deeper than that of the highest standard repeat the test on a diluted sample. If 50 cc. of the sample is used, multiply the number of cc. of the standard solution given per cc. of sample times the number of cc. of the standard solution given times per milligram of nitrite nitrogen. Satisfactory results can be obtained by using either hydrochloric or acetic acid in preparing the test solution, but the speed of the reaction is more rapid if acetic acid is used.
NITRATE NITROGEN

Permanent standards may be prepared by matching the nitrite standards with dilutions of the fuchsine solution. Fuchsine standards have been found to be sufficiently accurate for waters high in nitrite and for sewage. The standards should be checked once a month and kept out of bright sunlight.

NITRATE NITROGEN

Two methods are recommended for the determination of nitrate nitrogen in water, sewage, and sewage effluents.

PHENOLDISULFONIC ACID METHOD.

Reagents.—1. Phenoldisulfonic acid. Dissolve 25 grams of pure white phenol in 150 cc. of pure concentrated sulfuric acid. Add 75 cc. of fuming sulfuric acid (15 per cent SO₃), stir well, and heat for 2 hours at about 100°C.

2. Potassium hydroxide solution. Prepare an approximately 12 N solution, 10 cc. of which will neutralize about 4 cc. of the phenoldisulfonic acid.

3. Standard nitrate solution. Dissolve 0.72 gram of pure recrystallized potassium nitrate in 1 liter of distilled water. Evaporate cautiously to dryness 10 cc. of the solution on the water bath. Moisten residue quickly and thoroughly with 2 cc. of phenoldisulfonic acid and dilute to 1 liter. This is the standard solution, 1 cc. of which equals 0.001 mg. of nitrate nitrogen.

4. Standard silver sulfate solution. Dissolve 4.4 grams of silver sulfate free from nitrate in 1 liter of water. One cc. of this solution is equal to 1 mg. of chloride.

Procedure.—The alkalinity, chloride, and nitrite content, and color of the sample must first be determined. If the sample is highly colored decolorize it with freshly precipitated aluminium hydroxide. Measure into an evaporating dish 100 cc. of the sample, or if nitrate is very high such volume as will contain about 0.01 mg. of nitrate nitrogen. Add sufficient N/50 sulfuric acid nearly to neutralize the alkalinity. Then add sufficient standard silver sulfate to precipitate all but about 0.1 mg. of chloride. The removal of chloride may be omitted if the sample contains less than 30 parts per million of chloride. Heat the mixture to boiling, add a little aluminium hydroxide, stir, filter, and wash with small amounts of hot water. Evaporate the filtrate to dryness, and add 2 cc. of the phenoldisulfonic acid, rubbing with a glass rod to insure intimate
contact. If the residue becomes packed or appears vitreous because of the presence of much iron, heat the dish on the water bath for a few minutes. Dilute the mixture with distilled water, and add slowly a strong solution of potassium hydroxide or ammonium hydroxide until the maximum color is developed. Transfer the solution to a Nessler tube, filtering if necessary. If nitrate is present a yellow color will be formed. Compare the color with that of standards made by adding 2 cc. of strong potassium hydroxide or ammonium hydroxide to various amounts of standard nitrate solution and diluting them to 50 cc. in Nessler tubes. The following amounts of standard nitrate solution are suggested: 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, and 40.0 cc. These standards may be kept several weeks without deterioration. If 100 cc. of water is used the number of cubic centimeters of the standard multiplied by 0.01 is equal to parts per million of nitrate nitrogen.

Standards that will remain permanent for several years if stored in the dark may be prepared from tripotassium nitrophenoldisulfo-
ate. If nitrite nitrogen is present in excess of 1 part per million it should be oxidized by heating the samples a few minutes with a few drops of hydrogen peroxide free from nitrate repeatedly added or by adding dilute potassium permanganate in the cold until a faint pink coloration appears; the nitrogen equivalent of the nitrite thus oxidized to nitrate is then subtracted from the final nitrate nitrogen reading.

REDUCTION METHOD.

Reagents.—1. Sodium or potassium hydroxide solution. Dissolve 250 grams of the hydroxide in 1.25 liters of distilled water. Add several strips of aluminium foil and allow the evolution of hydrogen to continue over night. Concentrate the solution to 1 liter by boiling.

2. Aluminium foil. Use strips of pure aluminium about 10 cm. long, 6 mm. wide, and 0.33 mm. thick and weighing about 0.5 gram.

Procedure.—To 100 cc. of the sample in a 300 cc. casserole add 2 cc. of the hydroxide solution and concentrate by boiling to about 20 cc. Pour the contents of the casserole into a test tube about 16 cm. long and 3 cm. in diameter, or of approximately 100 cc. capacity. Rinse the casserole several times with nitrogen-free water and add the rinse water to the liquid already in the tube,
thus making the contents of the tube approximately 75 cc. Add a
strip of aluminium foil. Close the tube by means of a rubber
stopper through which passes a bent glass tube about 5 mm. in
diameter. Put the shorter arm of the tube flush with the lower
side of the rubber stopper and let the longer arm extend below
the surface of distilled water in another test tube. This apparatus
serves as a trap through which the evolved hydrogen escapes freely.
The small amount of ammonia escaping into the trap may be
neglected. Allow the action to proceed for a minimum period of
four hours or over night. Pour the contents of the tube into a
distilling flask, dilute with 250 cc. of ammonia-free water, distill,
collect the distillate in Nessler tubes, and Nesslerize. If the nitrate
content is high collect the distillate in a 200 cc. flask and Nesslerize
an aliquot part. If the supernatant liquid in the reduction tube is
clear and colorless the solution may be diluted to a definite volume
and an aliquot part Nesslerized without distillation.

TOTAL NITROGEN.\textsuperscript{46}

In sewage work it is frequently of assistance to know the total
nitrogen content. This is ordinarily computed by adding together
the organic, ammonia, nitrite, and nitrate nitrogen, each of which
is determined as already described.

OXYGEN CONSUMED.\textsuperscript{47 \textsuperscript{54} 56 \textsuperscript{58} 75 \textsuperscript{94} 101 \textsuperscript{105}}

Oxygen consumed means the oxygen that the oxidizable com-
pounds of sewage and water consume when treated in an acid
solution with potassium permanganate. The expression is synony-
mous with oxygen required, oxygen absorbed, and oxygen-consuming
capacity. It should not be confused with biochemical oxygen
demand.

As the carbon, not the nitrogen, in organic matter is oxidized
by potassium permanganate, oxygen consumed is considered by
some an indication of the amount of carbonaceous organic matter
present. The determination indicates, however, only part of
the carbon, the proportion varying in different samples because
the carbon in nitrogenous matter is not so readily oxidized as that
in carbonaceous organic matter. Furthermore, it does not directly
differentiate the carbon present in unstable organic matter from
that in fairly stable organic matter, such as is sometimes referred
Oxygen Consumed

to as residual humus matter. As nitrite nitrogen, ferrous iron, sulfide, and other oxidizable mineral substances reduce potassium permanganate, corrections for them should be made in the determination.

RECOMMENDED METHOD.

Reagents.—1. Dilute sulfuric acid. Dilute 1 part of concentrated sulfuric acid with 3 parts of distilled water and free the solution from oxidizable matter by adding potassium permanganate until a faint pink color persists after the solution has stood several hours.

2. Standard ammonium oxalate. Dissolve 0.888 gram of the pure salt in 1 liter of distilled water. One cc. is equivalent to 0.1 mg. of oxygen. An equivalent quantity of oxalic acid or sodium oxalate may be used.

3. Standard potassium permanganate. Dissolve 0.4 gram of the crystallized salt in 1 liter of distilled water. Add 10 cc. of the dilute sulfuric acid and 10 cc. of this solution of potassium permanganate to 100 cc. of distilled water, and digest 30 minutes. Add 10 cc. of the ammonium oxalate solution, and then add potassium permanganate till a pink coloration appears. This destroys the oxygen-consuming capacity of the water used. Now add another 10 cc of ammonium oxalate solution and titrate with potassium permanganate. Adjust the potassium permanganate solution so that 1 cc is equivalent to 1 cc of ammonium oxalate solution or 0.1 mg. of available oxygen.

Acid digestion.—Place in a flask 100 cc. of the water, or, if the water is of high organic content, a smaller portion diluted to 100 cc. Add 10 cc. of sulfuric acid solution and 10 cc. of standard potassium permanganate and digest the liquid exactly 30 minutes in a bath of boiling water the level of which is kept above the level of the contents of the flask. If the quantity of permanganate is insufficient for complete oxidation repeat the digestion with a larger quantity; at least 5 cc. excess of the standard permanganate should be present when the ammonium oxalate solution is added. Remove the flask, add 10 cc. of the ammonium oxalate solution, and titrate with the standard permanganate until a faint but distinct color is obtained. If 100 cc. of water is used the number of cubic centimeters of potassium permanganate solution in excess of the
OXYGEN CONSUMED

number of cubic centimeters of ammonium oxalate solution is equal to parts per million of oxygen consumed. If oxidizable mineral substances, such as ferrous iron, sulfide, or nitrite, are present in the sample corrections should be applied as accurately as possible by suitable procedures. Direct titration of the acidified sample in the cold, using a three-minute period of digestion, serves this purpose quite well for polluted surface waters and fairly well for purified sewage effluents. Few raw sewages containing no trade wastes need such a correction, but raw sewages containing "pickling" liquors do need it. If the sample contains both oxidizable mineral compounds and gaseous organic substances the latter should be driven off by heat and the sample allowed to cool before applying this test for the correction factor. If such corrections are made the fact should be stated with the amount of correction.

Period and temperature of digestion.—As the practice in regard to the period and temperature of digestion has varied widely it is difficult to compare the results obtained at one laboratory with those obtained at another. None of the methods gives absolute results. They are all relative46 29 57 at best. Digesting 30 minutes at the boiling temperature is herein designated the recommended method. If samples are analyzed by any other method the method should be noted, and, representative results by the standard method should be placed on record for purposes of comparison.

OTHER METHODS.

Additional reagents.—1. Potassium iodide solution. Ten per cent solution, free from iodate.
2. Standard sodium thiosulfate. Dissolve 1.0 gram of the pure crystallized salt in 1 liter of distilled water. Standardize this solution against the standard potassium permanganate. As the thiosulfate solution does not keep well determine its actual strength at frequent intervals.
3. Starch indicator. Prepare as directed in the section on dissolved oxygen (pp. 65–66).
4. Sodium hydroxide solution. Dissolve 1 part of pure sodium hydroxide in 2 parts of distilled water.

Certain widely practiced deviations from the standard procedure just described are noted in the following paragraphs.
1. Heat the acidified sample to boiling, add the permanganate
solution, and digest for two minutes at boiling temperature. This procedure is facilitated by agitating the liquid constantly with a small current of air to guard against bumping.

2. Same method as No. 1 except that the period of digestion is five minutes.12

3. Same method as No. 2 except that the permanganate solution is added to the acidified sample when cold, and digestion is continued five minutes after the sample reaches the boiling point. The advantage of this method is that there is included the oxygen-consuming power of the volatile matter present in some sewages and sewage effluents, which is driven off by heat and thus escapes when the test is made in accordance with procedures 1 and 2.

4. Same method as No. 3 except that the period of digestion is 10 minutes.62 63c

5. Digestion of the sample after the acid and permanganate solutions are added is carried out abroad, especially in England, at approximately the room temperature, apparently to guard against decomposition of permanganate in the presence of high chloride, for periods of three minutes, fifteen minutes, and four hours; many observers record the oxygen consumed after all three periods, while some record the result only for the four-hour period. At the end of the period of digestion, add 0.5 cc. of potassium iodide solution to discharge the pink color; mix; titrate the liberated iodine with thiosulfate until the yellow color is nearly destroyed, then add a few drops of starch solution and continue titration until the blue color is just discharged. The number of cubic centimeters of potassium permanganate solution in excess of the number of cubic centimeters of sodium thiosulfate solution is equal to parts per million of oxygen consumed.

6. Digestion in alkaline solution is preferable to digestion in acid solution for brines or waters high in chlorine. Place in a flask 100 cc. of the sample, or if it is of high organic content a smaller portion diluted to 100 cc. Add 0.5 cc. of sodium hydroxide solution and 10 cc. of standard potassium permanganate and digest exactly 30 minutes. Remove the flask, add 5 cc. of sulfuric acid and 10 cc. of the standard ammonium oxalate, and titrate with the standard potassium permanganate as in the acid digestion.
RESIDUE ON EVAPORATION.

TOTAL RESIDUE.\textsuperscript{14}

Ignite and weigh a clean platinum dish, and measure into it 100 cc. of the thoroughly shaken sample. Evaporate to dryness on a water bath. Then heat the dish in an oven at 103° C. or 180° C: for one hour. Cool in a desiccator and weigh. The temperature of drying should be mentioned in the report. The increase in weight gives the total solids or residue on evaporation. If 100 cc. of the sample was taken this weight expressed in milligrams and multiplied by 10 is equal to parts per million of residue on evaporation. The residue from waters low in organic matter but relatively high in iron may be used, as a matter of convenience, for the determination of iron.

FIXED RESIDUE AND LOSS ON IGNITION.\textsuperscript{15} \textsuperscript{16}

The residue from sewages and waters high in organic matter may be ignited to burn off the organic matter, which, with some volatile inorganic matter, constitutes the loss on ignition.

\textit{Procedure}.—Ignite the residue in the platinum dish at a low red heat. If great accuracy is desired this should be done in an electric muffle furnace or in a radiator, which consists of a platinum or a nickel dish large enough to allow an air space of about half an inch between it and the dish within it, the inner dish being supported by a triangle of platinum wire laid on the bottom of the outer dish. A disc of platinum or nickel foil large enough to cover the outer dish is suspended over the inner dish to radiate the heat into it. The larger dish is heated to bright redness until the residue is white or nearly so. Allow the dish to cool, and moisten the residue with a few drops of distilled water. Dry the residue in the oven, cool in a desiccator, and weigh. The fixed residue on evaporation is the difference between this weight and the weight of the dish.

The loss on ignition is the difference between the total residue on evaporation and the fixed residue on evaporation.

If the odor and color on ignition of some residues give helpful clues to the character of the organic matter record them.
solution to stand several days before standardizing. Pure potassium oleate made from lead plaster and potassium carbonate may be used in place of Castile soap.

First method of standardization.—Dilute 20 cc. of the calcium chloride solution in a 250 cc. glass-stoppered bottle to 50 cc. with distilled water which has been recently boiled and cooled. Add soap solution from a burette, 0.2 or 0.3 cc. at a time, shaking the bottle vigorously after each addition until a lather remains unbroken for five minutes over the entire surface of the water while the bottle lies on its side. Then adjust the strength of the stock solution with 70 per cent alcohol so that the resulting diluted soap solution will give a permanent lather when 6.40 cc. of it is properly added to 20 cc. of standard calcium chloride solution diluted to 50 cc. Usually 75 to 100 cc. of the stock soap solution is required to make 1 liter of the standard soap solution. The quantity of calcium carbonate equivalent to each cubic centimeter of the standard soap solution consumed in the titration is indicated in Table 6.

Table 6.—Total hardness in parts per million of CaCO$_3$ for each tenth of a cubic centimeter of soap solution when 50 cc. of the sample is titrated.

<table>
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<th>0.2</th>
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<th>0.5</th>
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<td>28.6</td>
<td>29.9</td>
<td>31.2</td>
</tr>
<tr>
<td>3.0</td>
<td>32.5</td>
<td>33.8</td>
<td>35.1</td>
<td>36.4</td>
<td>37.7</td>
<td>38.0</td>
<td>40.3</td>
<td>41.6</td>
<td>42.9</td>
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<tr>
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<td>47.1</td>
<td>48.6</td>
<td>50.0</td>
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<td>52.9</td>
<td>54.3</td>
<td>55.7</td>
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<td>58.6</td>
</tr>
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<td>62.9</td>
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<td>67.1</td>
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<td>94.3</td>
<td>95.7</td>
<td>97.1</td>
<td>98.6</td>
<td>100.0</td>
<td>101.5</td>
</tr>
</tbody>
</table>

This table does not provide for the use of so large volume of soap solution for a single determination as former ones because the end-point becomes somewhat obscured in the presence of magnesium if more than 7 cc. is used.

Second method of standardization.—Dilute 100 cc. of the stock soap solution to 1 liter with 70 per cent alcohol. This dilute solution should be of such strength that approximately 6.4 cc. of it will give a permanent lather when 20 cc. of standard calcium chloride
solution diluted to 50 cc. with distilled water is titrated with it. Determine the amount of soap solution required to give a permanent lather with 50 cc. of distilled water and with 5, 10, 15, and 20 cc. of standard calcium chloride solution diluted to 50 cc. with distilled water. Finally plot on cross-section paper a curve showing the relation of various quantities of soap solution to corresponding quantities of standard calcium carbonate solution and therefore to parts per million of hardness.

Procedure.—Measure 50 cc. of the water into a 250 cc. bottle and add to it soap solution in small quantities in precisely the same manner as described under the standardization of the soap solution. From the number of cubic centimeters of soap solution used obtain from Table 6 or from the plotted curve the total hardness of the water in parts per million of calcium carbonate.

To avoid mistaking the false or magnesium end-point for the true one when adding the soap solution to waters containing magnesium salts, read the burette after the titration is apparently finished, and add about 0.5 cc. more of soap solution. If the end-point was due to magnesium the lather will disappear. Soap solution must then be added until the true end-point is reached. Usually the false lather persists for less than five minutes.

If more than 7 cc. of soap solution is required for 50 cc. of the water take less of the sample and dilute it to 50 cc. with distilled water which has been recently boiled and cooled. This step reduces somewhat the disturbing influence of magnesium, which consumes more soap than an equivalent weight of calcium.

At best the soap method is not a precise test on account of the different relative amounts of calcium and magnesium in different waters. For hard waters, especially in connection with processes for purification and softening, it is advised that this method be not exclusively used. If the same water is frequently analyzed it may be of assistance to standardize the soap solution against a mixture of calcium and magnesium salts, the relative proportions of which approximate those found in the water.

The strength of the soap solution should be determined from time to time, to make sure that it has not materially changed. Record all results in parts per million of calcium carbonate.

One English degree of hardness, Clark's scale, is equivalent to 1 grain per Imperial gallon of calcium carbonate. One French degree of hardness is equivalent to 1 part per 100,000 of calcium carbonate.
TEMPORARY HARDNESS

One German degree of hardness is equivalent to 1 part per 100,000 of calcium oxide, and multiplied by 17.9 gives parts per million of calcium carbonate. The relations of these various scales are indicated in Table 7.

Table 7.—Conversion table for hardness.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Equivalent.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parts per million.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>One part per million</td>
<td>1.00</td>
</tr>
<tr>
<td>One Clark degree</td>
<td>14.3</td>
</tr>
<tr>
<td>One French degree</td>
<td>10.0</td>
</tr>
<tr>
<td>One German degree</td>
<td>17.9</td>
</tr>
</tbody>
</table>

TOTAL HARDNESS BY SODA REAGENT METHOD. Add standard sulfuric acid to 200 cc. of the sample until the alkalinity is neutralized. (See Procedure with methyl orange, p. 37.) Then apply the non-carbonate hardness method (pp. 34–35). This method gives fairly satisfactory estimates of total hardness of hard waters.

TEMPORARY HARDNESS BY TITRATION WITH ACID.

Determine the alkalinity in presence of methyl orange (see p. 37) in the original sample and also in the sample after boiling, cooling, restoring to the original volume with boiled distilled water, and filtering. The difference between the two, if any, is the temporary hardness. This is the most accurate method of determining the temporary hardness of ordinary waters. Iron bicarbonate is included as a part of the temporary hardness.

NON-CARBONATE HARDNESS BY SODA REAGENT METHOD. The use of soda reagent does not avoid entirely the error due to solubility of the salts of calcium and magnesium; consequently, if much depends on the results, as in water softening, gravimetric determinations of the calcium and magnesium that remain in solution should be made and a correction should be applied for those amounts.

Reagent.—Prepare soda reagent from equal parts of sodium
Temporary Hardness—Alkalinity

hydroxide and sodium carbonate. It should be approximately tenth normal.

Procedure.—Measure 200 cc. of the sample and 200 cc. of distilled water into 500 cc. Jena or similar glass Erlenmeyer flasks. Treat the contents of each flask in the following manner. Boil 15 minutes to expel free carbon dioxide. Add 25 cc. of soda reagent. Boil 10 minutes, cool, rinse into 200 cc. graduated flasks, and dilute to 200 cc. with boiled distilled water. Filter, rejecting the first 50 cc., and titrate 50 cc. of each filtrate with N/50 sulfuric acid in the presence of methyl orange or erythrosine indicator. The non-carbonate hardness in parts per million of calcium carbonate is equal to 20 times the difference between the number of cubic centimeters of sulfuric acid required for the soda reagent in distilled water and the number of cubic centimeters of N/50 sulfuric acid required for the soda reagent in the sample.

Water naturally containing bicarbonate and carbonate in excess of calcium and magnesium requires a larger amount of acid to neutralize the sample after it has been treated than is required to neutralize the volume of soda reagent originally added. (See p. 39.)

Non-carbonate Hardness by Soap Method.

Non-carbonate hardness may be calculated for waters which are soft or moderately hard in a fairly satisfactory manner by deducting the total alkalinity from the total hardness by the soap method (pp. 31–34). For waters that are very hard, and particularly those that contain much magnesium, this method is not advised.

Alkalinity.

The alkalinity of a natural water represents its content of carbonate, bicarbonate, borate, silicate, phosphate, and hydroxide. Alkalinity is determined by neutralization with standard sulfuric acid or potassium bisulfate in the presence of phenolphthalein and either methyl orange, erythrosine, or lacmoid as indicators. Methyl orange may be used except in waters containing aluminium sulfate or iron sulfate. The relations between estimates in presence of these indicators and the carbonate, bicarbonate, and hydroxide radicles are indicated in Table 8. The alkalinity of carbonates in the presence of phenolphthalein is different from that in the presence of methyl orange, partly because of loss of carbon dioxide.
and partly because of defects in phenolphthalein as an indicator in such conditions.

Table 8.—Relations Between Alkalinity to Phenolphthalein and That to Methyl Orange, Erythrosine, or Lacmoid, in Presence of Bicarbonate, Carbonate, and Hydroxide.

<table>
<thead>
<tr>
<th>Result of titration.*</th>
<th>Value of radicle expressed in terms of calcium carbonate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P = 0</td>
<td>T</td>
</tr>
<tr>
<td>P &lt; (\frac{T}{2})</td>
<td>T - 2P</td>
</tr>
<tr>
<td>P = (\frac{T}{2})</td>
<td>0</td>
</tr>
<tr>
<td>P &gt; (\frac{T}{2})</td>
<td>0</td>
</tr>
<tr>
<td>P = T</td>
<td>0</td>
</tr>
</tbody>
</table>

*\(T\) = Total alkalinity in presence of methyl orange, erythrosine, or lacmoid.
\(P\) = Alkalinity in presence of phenolphthalein.

Reagents.—1. Sulfuric acid or potassium bisulfate. A N/50 solution.

2. Phenolphthalein indicator. Dissolve 5 grams of a good quality of phenolphthalein in 1 liter of 50 per cent alcohol. Neutralize with N/10 potassium hydroxide. The alcohol should be diluted with boiled distilled water.

3. Methyl orange indicator. Dissolve 0.5 gram of a good grade of methyl orange in 1 liter of distilled water. Keep the solution in the dark.

4. Lacmoid indicator. Dissolve 2.0 grams of lacmoid in 1 liter of 50 per cent alcohol. Dilute the alcohol with freshly boiled distilled water.

5. Erythrosine indicator. Dissolve 0.5 gram of erythrosine (the sodium salt) in 1 liter of freshly boiled distilled water.

PROCEDURE WITH PHENOLPHTHALEIN.

Add 4 drops of phenolphthalein indicator to 50 or 100 cc. of the sample in a white porcelain casserole or an Erlenmeyer flask over a white surface. If the solution becomes colored, hydroxide or normal carbonate is present. Add N/50 sulfuric acid from a burette until the coloration disappears.

The phenolphthalein alkalinity in parts per million of calcium carbonate is equal to the number of cubic centimeters of N/50
sulfuric acid used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

PROCEDURE WITH METHYL ORANGE.

Add 2 drops of methyl orange indicator to 50 or 100 cc. of the sample, or to the solution to which phenolphthalein has been added, in a white porcelain casserole or an Erlenmeyer flask over a white surface. If the solution becomes yellow, hydroxide, normal carbonate, or bicarbonate is present. Add N/50 sulfuric acid from a burette until the faintest pink coloration appears. The methyl orange alkalinity in parts per million of calcium carbonate is equal to the total number of cubic centimeters of N/50 sulfuric acid used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

PROCEDURE WITH LACMOID.

Add 4 drops of lacmoid indicator to 50 or 100 cc. of the sample in a porcelain casserole or an Erlenmeyer flask. Add N/50 sulfuric acid from a burette until within 1 or 2 cc. of the amount necessary for neutralization has been added. Heat the solution until bubbles of steam begin to break at the surface. Remove the dish from the source of heat and continue the titration until a drop of the acid striking the surface of the liquid and sinking to the bottom of the vessel produces no change in the uniform reddish or purple color of the solution. The calculation is the same as for phenolphthalein alkalinity.

PROCEDURE WITH ERYTHROSINE.

Add 5 cc. of neutral chloroform and 1 cc. of erythrosine indicator to 50 or 100 cc. of the sample in a 250 cc. clear glass-stoppered bottle: If the chloroform becomes rose colored on shaking, hydroxide, bicarbonate, or normal carbonate is present. Add N/50 sulfuric acid from a burette until the chloroform becomes colorless. A white surface behind the bottle facilitates detection of a trace of color as the end-point is approached. The calculation is the same as with phenolphthalein alkalinity.

BICARBONATE.

Bicarbonate is present if the alkalinity to phenolphthalein is less than one-half the alkalinity to methyl orange, erythrosine, or lac-
2. N/22 sodium carbonate. Dissolve 2.41 grams of anhydrous sodium carbonate in 1 liter of boiled distilled water that has been cooled in an atmosphere free from carbon dioxide. Preserve this solution in bottles of resistant glass protected from the air by tubes filled with soda-lime. One cc. is equivalent to 1 mg. of CO₂.

3. Phenolphthalein indicator (see p. 36).

4. Methyl orange indicator (see p. 36).

**TOTAL ACIDITY.**

*Procedure.*—Add 4 drops of phenolphthalein indicator to 50 or 100 cc. of the sample in a white porcelain casserole or an Erlenmeyer flask over a white surface. Add N/50 sodium carbonate until the solution turns pink. The total acidity in parts per million of calcium carbonate is equal to the number of cubic centimeters of N/50 sodium carbonate used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

**FREE CARBON DIOXIDE.**

Carbon dioxide may exist in water in three forms—free carbon dioxide, bicarbonate (pp. 37–38), and carbonate (p. 38). One-half the carbon dioxide as bicarbonate is known as the half-bound carbon dioxide. The carbon dioxide as carbonate plus one-half that as bicarbonate is known as the bound carbon dioxide.

*Procedure.*—Pour 100 cc. of the sample into a tall narrow vessel, preferably a 100 cc. Nessler tube. Add 10 drops of phenolphthalein indicator, and titrate rapidly with N/22 sodium carbonate, stirring gently, until a faint but permanent pink color is produced. The free carbon dioxide (CO₂) in parts per million is equal to 10 times the number of cubic centimeters of N/22 sodium carbonate used.

Because of the ease with which free carbon dioxide escapes from water, particularly when the gas is present in large amount, a special sample should be collected for this determination, which should preferably be made at the time of collection. If the analysis cannot be made at the time of collection approximate results with water not too high in free carbon dioxide may be obtained on samples collected in bottles completely filled so as to leave no air space under the stopper. Bottled samples should be kept, until tested, at a temperature lower than that of the water when collected. If mineral acids or certain salts are present correction must be made.
At best, the results of the titration are uncertain because the proper end-point for correct results differs in color with different types of water.

**FREE MINERAL ACIDS.**

*Procedure.*—Add 2 drops of methyl orange indicator to 50 or 100 cc. of the sample in a white porcelain casserole or an Erlenmeyer flask over a white surface. Add N/50 sodium carbonate from a burette until the pink coloration of the solution disappears. The acidity due to free mineral acids, expressed in terms of calcium carbonate, is equal to the number of cubic centimeters of N/50 sodium carbonate used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

**MINERAL ACIDS AND SULFATES OF IRON AND ALUMINIUM.**

*Procedure.*—Modify the method for free mineral acids by titrating the water at boiling temperature in the presence of phenolphthalein indicator. The acidity due to free mineral acids and sulfates of iron and aluminium, expressed in terms of calcium carbonate, is equal to the number of cubic centimeters of N/50 sodium carbonate used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

The acidity due to sulfates of iron and aluminium is equal to the acidity due to mineral acids and sulfates minus the acidity due to mineral acids. The acidity due to ferrous and ferric sulfate can be calculated from the determined amount of these salts (pp. 43–48). The acidity due to aluminium sulfate is equal to the acidity due to total acid sulfates minus that due to iron sulfates.

Acidity shall be reported in parts per million of calcium carbonate (CaCO₃). Sulfate (SO₄) equals parts per million of calcium carbonate multiplied by 0.96.

Carbon dioxide (CO₂) equals parts per million of calcium carbonate multiplied by 0.44.

**CHLORIDE.**

Chloride in water and sewage has its origin in common salt, from mineral deposits in the earth, from ocean vapors carried inland by the wind, or from polluting materials like sewage and trade wastes, which contain the salt used in the household and in manufacturing.
Comparison of the chloride content of a water with that of other waters in the vicinity known to be unpolluted frequently affords useful information as to its sanitary quality. If, however, the chloride normally exceeds 20 parts per million because of chloride-bearing mineral deposits the chloride content of a water has little sanitary significance.

_Reagents._—1. Standard sodium chloride solution. Dissolve 16.48 grams of pure fused sodium chloride in 1 liter of distilled water. Dilute 100 cc. of this stock solution to 1 liter in order to obtain a standard solution each cubic centimeter of which contains 0.001 gram of chloride.

2. Standard silver nitrate solution. Dissolve about 2.40 grams of silver nitrate crystals in 1 liter of distilled water. Standardize this with the standard salt solution, and adjust, correcting for volume (see p. 43), so that 1 cc. will be exactly equivalent to 0.0005 gram of chloride.

3. Potassium chromate indicator. Dissolve 50 grams of neutral potassium chromate in a little distilled water. Add enough silver nitrate to produce a slight red precipitate. Filter and dilute the filtrate to 1 liter with distilled water.

4. Aluminium hydroxide. Electrolyze ammonia-free water, using aluminium electrodes. Wash the precipitate until it is free from chloride, ammonia, and nitrite. Or dissolve 125 grams of potassium or ammonium alum in 1 liter of distilled water. Precipitate the aluminium by adding cautiously ammonium hydroxide. Wash the precipitate in a large jar by successive additions and decantations of distilled water until free from chloride, nitrite, and ammonia.

_Procedure._—Add 1 cc. of potassium chromate indicator to 50 cc. of the sample in a 6-inch white porcelain evaporating dish or a 150 cc. Erlenmeyer flask over a white surface. Titrating with the silver nitrate solution under similar conditions of volume, light, and temperature as were used in standardizing the silver nitrate until a faint reddish coloration is perceptible. The detection of the end-point is facilitated by comparison of the contents of the porcelain dish with those of another dish containing the same quantity of potassium chromate indicator in 50 cc. of distilled water. Some analysts prefer to make the titration in a dark-room provided with a yellow light. The end-point is very sharp by electric light and also by daylight with photographic yellow glass. The titration may
be made in Nessler tubes if the solutions are standardized under similar conditions.

If the amount of chloride is very high use 25 cc., or even a smaller quantity, diluting the volume taken to 50 cc. with distilled water. If the amount of chloride is very low concentrate 250 cc. of the sample to 50 cc. by evaporation. Rotate the liquid to make sure that no residue remains undissolved on the walls of the dish, and, if necessary, use a rubber-tipped glass rod to assist in this operation.

Chloride is determined by some observers by extracting with hot distilled water the residue in the platinum dish in the determination of the residue on evaporation and proceeding as just described. This is permissible if a little sodium carbonate is added before evaporation to prevent loss of chloride through decomposition of magnesium chloride in the residue.

If the sample has a color greater than 30 it should be decolorized by shaking it thoroughly with washed aluminium hydroxide (3 cc. to 500 cc. of the sample) and allowing the precipitate to settle. Make the determination on a portion of the clarified sample, filtered if necessary. If the sample is acid neutralize it with sodium carbonate; if hydroxide is present add dilute sulfuric acid until the cold liquid will just discharge the color of phenolphthalein. If the presence of sulfide and sulfocyanate renders it necessary make proper corrections or modifications in treatment.

Make correction for the error due to variations in the volume of the liquid and precipitate by means of the formula in cubic centimeters of silver nitrate solution and V = cubic centimeters of liquid at the end of the titration. If 50 cc. of the sample is titrated chloride (Cl) in parts per million is equal to the number of cubic centimeters of silver nitrate solution multiplied by 10. The correction to be applied is 0.2 cc. unless unusual accuracy is required.

IRON

Iron occurs in natural waters in both ferrous and ferric condition, depending on the source of the sample. In ground waters the iron is usually in an unoxidized and soluble condition, sometimes combined with carbonic or sulfuric acid, and also in combination with organic matter. Many waters, especially those that have been
exposed to the air, contain the iron in the form of a colloidal hydroxide. Silt-bearing waters often contain much iron in suspension, usually in an oxidized form. Sewages and sewage effluents, particularly those receiving manufacturing wastes, contain various forms of iron of different degrees of solubility, oxidation, and coagulation.

TOTAL IRON.\textsuperscript{40,41b}

COLORIMETRIC METHOD.

Reagents.—1. Standard iron solution. Dissolve 0.7 gram of crystallized ferrous ammonium sulfate in 50 cc. of distilled water to which 20 cc. of dilute sulfuric acid has been added. Warm the solution slightly and add potassium permanganate until the iron is completely oxidized. Dilute the solution to 1 liter. One cc. of the standard solution equals 0.1 mg. Fe.

2. Potassium sulfocyanide solution. Dissolve 20 grams of the salt in 1 liter of distilled water.

3. Dilute hydrochloric acid. One volume of acid (Sp. gr. 1.2) and one volume of distilled water. This shall be free from nitric acid.

4. N/5 potassium permanganate. Dissolve 6.30 grams of the salt in distilled water and dilute to 1 liter.

5. Hydrochloric acid. Concentrated, free from iron.


7. Nitric acid. 5N, free from iron.

First procedure.—Evaporate 100 cc. of the water to dryness, or use the residue left after the determination of residue on evaporation (p. 29). Ignite the residue at a low red heat taking care not to heat it hot enough to make the iron difficultly soluble. Cool the dish and add 5 cc. of concentrated hydrochloric acid. Moisten the inner surface of the dish. Warm the solution for two or three minutes, and again moisten the inner surface of the dish by permitting the hot acid to flow over it. Wash the hot solution from the dish into a 50 cc. Nessler tube, filtering if necessary through paper that has been washed with hot water. Dilute to 50 cc., and add 3 drops of potassium permanganate solution. Add 5 cc. of potassium sulfocyanide solution, mix, and compare with standards.

If it is not convenient to use the residue on evaporation and if the sample is relatively free from organic matter boil 50 cc. of the sample with 5 cc. of 5N nitric acid for five minutes. Add a few drops of permanganate and 5 cc. of potassium sulfocyanide and
compare with standards, using nitric acid in place of hydrochloric acid in the standards. This method is excellent for ground waters. The permanganate and acid liberate chlorine in water high in chloride, and produce a permanent yellow color which interferes with the determination, unless the sample is first diluted to 50 cc. An excess of permanganate, reacting with hydrochloric acid, causes similar trouble. The amounts of hydrochloric acid, 5 cc., and of sulfocyanide, 5 cc., should be approximately measured because more acid lightens the color whereas more sulfocyanide deepens it. This is especially important if permanent standards are used.

Second procedure.—For surface waters containing small amounts of organic matter, the method of Klut¹⁰ is recommended. Samples containing small amounts of iron should be concentrated, if possible, until at least 0.5 mg. of iron is present in the volume tested. Boil the sample in a beaker with 2 to 3 cc. of concentrated nitric acid free from iron, adding permanganate if necessary to destroy the organic matter. To the hot liquid add ammonia in slight excess and warm until the smell of ammonia is hardly discernible. Filter and wash with water at 70° to 80° C. containing a little ammonia. Dissolve the iron in the beaker and on the filter paper in 5 cc. of concentrated hydrochloric acid, and wash with hot water until the iron is all dissolved, collecting the filtrate in a 50 cc. Nessler tube. Dilute to 50 cc. Add potassium sulfocyanide and determine the iron by comparison with standards.

Comparison with iron standards.

First procedure.—Prepare standards containing amounts of standard iron solution ranging from 0.05 to 4 cc. according to the quantity of iron in the sample. Dilute these amounts with water to about 40 cc. Add 5 cc. of dilute hydrochloric acid and 3 drops of potassium permanganate to each tube and dilute to 50 cc. Add 5 cc. of the potassium sulfocyanide to each of the standard solutions at the same time that it is added to the samples of water under examination, and compare immediately after mixing. If 100 cc. of the sample is used the iron in parts per million is equal to the number of cubic centimeters of the standard iron solution in the standard that the sample matches.

Second procedure.—For a small number of determinations it is more convenient to run the standard iron solution into a Nessler
tube containing the acid, distilled water, and potassium sulfocyanide until the color matches that of the sample tested. When determining iron in three or four samples the colors may be matched in the order of their intensity and the volumes of standard iron solution required for each tube may be read from the burette.

**Comparison with permanent standards.**

*Reagents.*—1. Platinum solution. Dissolve 2 grams of potassium platinic chloride (PtCl₄·2KCl) in distilled water, add 100 cc. of concentrated hydrochloric acid, and dilute to 1 liter with distilled water.

2. Cobalt solution. Dissolve 24 grams of dry cobaltous chloride crystals (CoCl₂·6H₂O) in a small amount of distilled water, add 100 cc. of strong hydrochloric acid, and dilute to 1 liter with distilled water.

*Procedure.*—Prepare a series of permanent standards by diluting to 50 cc. with distilled water the amounts of platinum and cobalt solutions, in 50 cc. Nessler tubes, indicated in Table 9. Compare the sample with these standards, and calculate the parts per million of iron.

Table 9.—Preparation of permanent standards for the determination of iron.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>.1</td>
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</tr>
<tr>
<td>3.5</td>
<td>40</td>
<td>55.0</td>
</tr>
</tbody>
</table>

**Volumetric method.**

Some samples of sewage and water mixed with trade wastes and mine drainage contain so much iron that it is preferable to use the volumetric method described on page 57 for the determination of
both total and dissolved iron, rather than to work with quantities small enough to permit application of the colorimetric methods just described. If iron is present in large quantities in suspension, as in some sewages and septic tank effluents, it may be filtered off and the residue washed, ignited, and fused with potassium and sodium carbonate. The fusion is then extracted with hydrochloric acid and the iron determined as on page 57.

Samples containing much organic matter should be evaporated to dryness with 0.5 cc. of concentrated sulfuric acid and the residue then ignited before estimation of iron.

**DISSOLVED IRON.**

Determine, by the method described for total iron, the iron in the sample after filtration. Iron may precipitate from some samples during filtration.

**SUSPENDED IRON.**

The suspended iron is the difference between total iron in the unfiltered sample and dissolved iron in the filtered sample.

**FERROUS IRON.**

Determine the total ferrous iron in an unfiltered sample and the dissolved ferrous iron in a filtered sample.

*Reagents.*—1. Standard iron solution. Dissolve 0.7 gram of crystallized ferrous ammonium sulfate in a large volume of freshly boiled distilled water to which 10 cc. of dilute sulfuric acid has been added and dilute to 1 liter. This solution should be freshly prepared when needed. One cc. of this standard solution contains 0.1 mg. of Fe.

2. Potassium ferricyanide solution. Dissolve 5 grams of the salt in 1 liter of distilled water. Use a freshly prepared solution.

3. Dilute sulfuric acid. Dilute 1 part of sulfuric acid, specific gravity 1.84, with 5 parts of distilled water.

*Procedure.*—Add 10 cc. of dilute sulfuric acid to 50 cc. of the sample, remove the suspended matter by filtration if necessary, and add 15 cc. of potassium ferricyanide solution. Dilute the solution to 100 cc. with distilled water. Compare the color developed in the sample with that in standards made at the same time from the ferrous iron solution. Place in 100 cc. Nessler tubes, in the following order, 75 cc. of distilled water, 10 cc. of dilute sulfuric
acid, and 15 cc. of potassium ferricyanide solution, and mix well the contents of each tube. Prepare as many tubes in this way as are needed. Add various quantities of standard ferrous iron solution to several tubes, mix well, and compare the resulting colors with the samples immediately.

FERRIC IRON.

The amount of ferric iron in solution and suspension is equal to the difference between the total iron and the ferrous iron obtained by the methods described.

MANGANESE.

If the sample contains less than 10 parts per million of manganese, use a colorimetric method in which the manganous salt is oxidized to permanganate and the color produced thereby is compared with that of a standard solution similarly treated. The persulfate method and the bismuthate method are suitable. If the sample contains more than 10 parts per million of manganese it is sometimes preferable to use a volumetric or gravimetric method.

PERSULFATE METHOD.

Reagents.—1. Nitric acid. Dilute concentrated nitric acid with an equal volume of distilled water. Free the diluted acid from brown oxides of nitrogen by aeration.

2. Silver nitrate. Dissolve 20 grams of silver nitrate in 1 liter of distilled water.

3. Standard manganous sulfate. Dissolve 0.288 gram of purest potassium permanganate in about 100 cc. of distilled water. Acidify the solution with sulfuric acid and heat to boiling. Add slowly a sufficient quantity of dilute solution of oxalic acid to discharge the color. Cool and dilute to 1 liter. One cc. of this solution contains 0.1 mg. of manganese.


Procedure.—Use an amount of the sample that contains not more than 0.2 mg. of manganese. Add 2 cc. of nitric acid and boil down to about 50 cc. Precipitate the chloride with silver nitrate solution, adding at least 1 cc. in excess. Shake and heat to coagulate the precipitate, and filter. A sample that contains much chloride should be evaporated with a few drops of sulfuric acid until
white fumes appear and then diluted before the nitric acid and silver nitrate are added as directed above. If the sample is highly colored by organic matter it should be evaporated with sulfuric acid, and the residue ignited and dissolved in dilute nitric acid. Add about 0.5 gram of ammonium persulfate crystals and warm the solution until the maximum permanganate color is developed. This usually takes about ten minutes. At the same time prepare standards by diluting portions of 0.2, 0.4, 0.6 cc., etc. of the standard manganous sulfate solution to about 50 cc. and treating them exactly as the sample was treated. Transfer the sample and the standards to 50 cc. Nessler tubes, and compare the colors immediately. Manganese in parts per million is equal to the number of cubic centimeters of standard manganous sulfate solution in the tube that the sample matches multiplied by 100, divided by the number of cubic centimeters of the sample used.

**BISMUTHATE METHOD.**

**Reagents.**—1. Nitric acid. Dilute 1 part of concentrated nitric acid with 4 parts of distilled water. Free the dilute acid from brown oxides of nitrogen by aeration.

2. Sulfuric acid. Dilute 1 part of concentrated sulfuric acid with 3 parts of distilled water.

3. Dilute sulfuric acid. Dilute 25 cc. of concentrated acid to 1 liter with distilled water. Add enough permanganate solution to color faintly the dilute acid.

4. Standard manganous sulfate. The standard solution of manganous sulfate prepared as described under persulfate method (p. 48) should be used and the standards should be prepared by following the same procedure as is used for the sample. This solution is more permanent than a solution of potassium permanganate, which may, however, be used. To prepare it dissolve 0.288 gram of potassium permanganate in distilled water and dilute the solution to 1 liter.

5. Sodium bismuthate. Purest dry salt.

**Procedure.**—Use an amount of the sample that contains not more than 0.2 mg. of manganese. Add 0.5 cc. of sulfuric acid and evaporate to dryness. Heat until the sulfuric acid is volatilated and ignite the residue. Dissolve in 40 cc. of nitric acid, add about 0.5 gram of sodium bismuthate, and heat until the permanganate color disappears. Add a few drops of a solution of ammonium or
sodium bisulfate to clear the solution and again boil to expel oxides of nitrogen. Remove from the source of heat, cool to 20° C., again add 0.5 gram of sodium bismuthate, and stir. When the maximum permanganate color has developed, filter through an alundum or Gooch crucible containing an asbestos mat ignited and washed with potassium permanganate. Wash the precipitate with dilute sulfuric acid until the washings are colorless. Transfer the filtrate to a 50 cc. Nessler tube and compare the color of it with that of standards prepared from the potassium permanganate solution. To prepare the standards, dilute portions of 0.2, 0.4, 0.6 cc., etc. of the permanganate solution to 50 cc. with dilute sulfuric acid. The content of manganese is calculated as described under persulfate method (p. 49).

LEAD, ZINC, COPPER, AND TIN

Determinations of lead, zinc, copper, and tin are important in certain mining regions and in places where the water has a solvent action on pipes and other containers. The use of certain "germicides" also makes it necessary to test for some of these metals.

Lead, zinc, and copper may be determined colorimetrically or electrolytically. The colorimetric methods are not so accurate as a combination of both, and are chiefly of value as qualitative tests.

It is possible to make a rough estimation of the amount of lead in clear waters by acidifying with acetic acid, saturating with hydrogen sulfide, and comparing the color produced with that produced by standard lead solutions in Nessler tubes, treated in similar manner. This method, however, is not applicable if the water is colored or contains iron.

Reagents.—1. Standard lead solution. Dissolve 1.60 grams of lead nitrate (Pb(NO₃)₂) in 1 liter of distilled water. One cc. of this solution contains 1 mg. of lead (Pb). As a check it is desirable to determine lead as sulfate in a measured portion of this solution.

2. Standard copper solution. Dissolve about 0.8 gram of copper sulfate crystals (CuSO₄.5H₂O) in water and, after the addition of 1 cc. of concentrated sulfuric acid, dilute the solution to 1 liter. Determine the copper in 100 cc. of this solution in the usual way by electrolytic deposition. Dilute the solution so that 1 cc. contains 0.2 milligram copper (Cu). This solution is permanent.

3. Ammonium chloride. Twenty-five per cent solution.

4. Ammonium acetate. Fifty per cent solution.
5. Ammonium hydroxide. (Sp. gr. 0.96.)
7. Potassium sulfide. An alkaline solution of potassium sulfide
   made by mixing equal volumes of 10 per cent potassium hydroxide
   and a saturated aqueous solution of hydrogen sulfide.
10. Alcohol. Ninety-five per cent.
11. Alcohol. Fifty per cent.
14. Nitric acid. Dilute 1 part of the concentrated acid to 10
   parts with distilled water.
15. Hydrochloric acid. (Sp. gr. 1.20.)
16. Sulfuric acid. Concentrated acid (Sp. gr. 1.84).
17. Sulfuric acid. Dilute the concentrated acid with an equal
   volume of distilled water.

LEAD.

Concentrate (1)* rapidly by boiling in a 7-inch porcelain dish
over a free flame 3 or 4 liters of the sample to be tested, or more if
very small amounts of the metals are present, to a volume of about
30 cc. Add 10 or 15 cc. of ammonium chloride solution to assist in
the separation of the sulfides, then add a few drops of concentrated
ammonium hydroxide, and saturate with hydrogen sulfide. Allow
to stand some time, preferably over night, add a little more am-
onium hydroxide and hydrogen sulfide, boil the contents of the
dish a few minutes, and filter. The precipitate (2) may consist
of lead, zinc, copper, and iron sulfides and the suspended organic
matter. The soluble coloring matter is in the filtrate (3). Wash
the precipitate a few times with hot water, place the precipitate
and the filter paper in the original dish and boil with dilute nitric
acid, rubbing down the sides of the dish, if necessary, to detach
any adhering sulfide precipitate. After again filtering and washing
several times with hot water, evaporate the filtrate and washings
in the original dish to a bulk of 10 to 15 cc., cool, add 5 cc. of con-
centrated sulfuric acid, and heat until copious fumes of sulfuric
acid are evolved.

*The numbers in parentheses refer to tables 10-12, pages 55-56.
If lead is present dilute the contents of the dish slightly with water, and treat them with 150 cc. of 50 per cent alcohol, in which the lead sulfate is insoluble. Allow to stand some time, preferably over night, filter off the lead sulfate, and wash it with 50 per cent alcohol. Save the filtrate for the determination of zinc.

Dissolve the precipitate of lead sulfate by boiling the filter containing it in ammonium acetate solution in a porcelain dish. (4). Filter into a 50 cc. Nessler tube and wash the filter with boiling water containing a little ammonium acetate. Divide this filtrate in halves and treat one-half with saturated hydrogen sulfide water in order to get an approximation of the amount of lead present. To the other half, or an aliquot portion, if a large amount of lead is present, add a few drops of acetic acid, then an excess of saturated hydrogen sulfide solution, and compare the color with that of standards made by treating known amounts of the standard lead solution with a little acetic acid, ammonium acetate, and hydrogen sulfide.

ZINC.

If zinc is present and copper is absent concentrate the filtrate from the lead sulfate to expel the alcohol, and remove the iron by adding an excess of ammonium hydroxide. Filter, wash, and acidify the filtrate with sulfuric acid. Concentrate the filtrate to about 150 cc. and transfer to a weighed platinum dish. Add 2 grams of potassium oxalate and 1.5 grams of potassium sulfate. Deposit the zinc electrolytically by means of a current of about 0.3 amperc for three hours. After deposition is complete and while the current is on, siphon off the solution and at the same time run into the dish a stream of distilled water in order to expel the free sulfuric acid, which might dissolve some of the zinc if the circuit were broken. After the acid has been removed break the circuit, wash the dish with water, then with 95 per cent alcohol, dry at 70° C., cool, and weigh it. The difference between this weight (10) and the weight of the platinum dish equals the amount of metallic zinc. Some difficulty has been experienced in this determination in obtaining pure reagents. It is therefore advisable to make blank determinations with each new lot of reagents and to correct the results if necessary.

If copper also is present (5) concentrate the filtrate from the lead sulfate until the alcohol is expelled, and add an excess of
ammonium hydroxide. (6) Remove any iron precipitate by filtration. Neutralize the filtrate (7) with sulfuric acid, and add 2 cc. of concentrated sulfuric acid and 1 gram of urea. Electrolyze the solution and determine copper colorimetrically as described in the procedure for copper (p. 54). After the copper has been deposited add ammonium hydroxide to the solution containing the zinc until nearly all the sulfuric acid has been neutralized, concentrate to slightly less than the capacity of the platinum dish, add 1.5 grams of potassium sulfate and 2 grams of potassium oxalate, and electrolyze for zinc. As this solution is usually saturated with ammonium salts due to neutralizing the large quantity of sulfuric acid, it is frequently impossible to get the zinc deposited firmly on the dish before the salts interfere by crystallization. To avoid this difficulty, dilute half the solution and electrolyze it for zinc; or, if the amount of zinc is very small, precipitate the zinc as sulfide in acetic acid solution, wash, ignite to oxide, and weigh the precipitate. This difficulty will not be encountered if copper is absent as there will then be no excess of ammonium salts.

If lead and copper are known to be absent and zinc alone is to be determined (13), after treating with sulfuric acid for separation of lead, slightly dilute the contents of the dish. Add an excess of ammonium hydroxide to precipitate iron and filter. Make the filtrate slightly acid with sulfuric acid, concentrate to about 150 cc., transfer to a weighed platinum dish, add potassium oxalate and sulfate, and electrolyze the solution as described for deposition of zinc.

COPPER.**

Use 1 liter of a sample containing 0.1 to 1.0 part per million of copper, and proportionate amounts for other concentrations. Evaporate to about 75 cc., and wash into a 100 cc. platinum dish. Add 2 cc. of dilute sulfuric acid for clear and soft waters; add more acid to very alkaline waters to offset the alkalinity; add 5 cc. of acid to waters carrying much organic matter or clay to insure the formation of a soluble copper salt. Then place the dish as the anode in a direct current circuit, suspend a spiral wire cathode in the solution so that it is parallel to and about half an inch from the bottom of the dish, and close the circuit.

Electrolyze for about four hours with occasional stirring, or over night, if convenient. The current may be supplied by two gravity
cells in series, yielding a current through the solution of about 0.02 ampere. Lift out the cathode without previously opening the circuit, and immerse the spiral in a small amount of dilute nitric acid previously heated to boiling. Wash off the wire and evaporate the nitric acid solution to dryness on the water-bath. If the presence of silver is suspected add a few drops of hydrochloric acid before evaporation. Dissolve the residue in water and wash it into a 50 cc. Nessler tube. Dilute to 50 cc. and add 10 cc. of the potassium sulfide solution. The color of the copper sulfide develops at once and is fairly permanent, lasting at least several hours. Add 10 cc. of the potassium sulfide solution to a similar tube containing 50 cc. of distilled water, and then add to it standard copper solution in 0.2 cc. portions until the colors of the two tubes match. If 1 liter of the sample is used copper in parts per million is equal to the number of cubic centimeters of standard copper solution required to match the color of the sample multiplied by 0.2.

**TIN.**

Small quantities of tin are occasionally found in waters that have passed through tin or tin-lined pipes. This metal, if present, is precipitated with the iron by ammonia in the lead, zinc, and copper separations. In the method for copper alone, it is removed in the same way and may be further avoided by dissolving the sulfides in concentrated nitric acid. Any tin present will then separate as an insoluble compound, which may be ignited and weighed as the oxide (SnO₂).

The following schematic tables illustrate the procedures given.
**LEAD, ZINC, COPPER**

Table 10.—**Scheme for the Separation of Lead, Zinc, and Copper.**

1. Concentrate sample. Add 10 cc. NH₄Cl, a few drops NH₄OH and saturate with H₂S. Allow to stand, add more NH₄OH and H₂S. Boil, filter, and wash.

2. Dissolve the precipitate in dilute HNO₃. Filter and wash. Evaporate to 10 or 15 cc. Cool. Add 5 cc. concentrated H₂SO₄ and heat until white fumes are given off. Dilute slightly and treat with 150 cc. of 50 per cent alcohol. Allow to stand; filter, and wash with 50 per cent alcohol.

3. Reject the filtrate which contains the coloring matter.

4. The precipitate contains the Pb. Dissolve in NH₄C₂H₅O₂ solution. Filter into a 50 cc. Nessler tube and wash with water containing NH₄C₂H₅O₂. Divide filtrate in halves. Saturate one half with H₂S. Determine the Pb in the other half by adding H₂C₂H₂O₂ and H₂S and comparing with standards containing known amounts of Pb.

5. The filtrate contains the Zn and Cu. Concentrate to expel alcohol. Add excess of NH₄OH, filter and wash precipitate.

6. Reject the precipitate which contains the Fe.

7. The filtrate contains the Zn and Cu. Neutralize with H₂SO₄. Add 10 cc. concentrated H₂SO₄ and 1 g. urea. Electrolyze for two hours with a current of 0.5 ampere. Break circuit, empty dish and wash.

8. The deposit is Cu. Immerse the cathode in a small amount of hot, dilute HNO₃; wash off and evaporate to dryness. Take up in water and wash into a Nessler tube. Make up to mark, and add 10 cc. of potassium sulfide solution. Compare with standard. If large amount is present, dry and weigh as Cu.

9. The solution contains the Zn. Nearly neutralize with NH₄OH. Concentrate to less than the capacity of the dish. Add 2 g. K₂C₂O₄ and 1.5 g. K₂SO₄. Electrolyze for 3 hours with a current of 0.3 ampere. Siphon off solution, break circuit, wash with water, then alcohol, dry at 70° C., cool and weigh.

10. The weighed residue is metallic Zn.

Table 11.—**Scheme for Determination of Copper Only.**

11. Concentrate sample to 75 cc. Add 2 cc. conc. H₂SO₄ for clear, soft waters and 5 cc. for alkaline or turbid waters. Electrolyze following procedure in 7 and 8.
Table 12.—Scheme for determination of zinc only.

13. Follow scheme for all three metals as given in Table 10 through section 5. Nearly neutralize the filtrate with H₂SO₄, concentrate to less than the capacity of the dish and electrolyze as directed in section 9.

MINERAL ANALYSIS.

RESIDUE ON EVAPORATION.

See description of method (p. 29). The residue should be dried one hour at 180° C. Turbid waters should be filtered, and the composition of the suspended matter should be determined separately or the amount of it reported as suspended matter.

ALKALINITY AND ACIDITY.

See description of method (pp. 35–41).

CHLORIDE.

See description of method (pp. 41–43).

NITRATE NITROGEN.

See description of method (pp. 23–25).

SEPARATION OF SILICA, IRON, ALUMINIUM, CALCIUM, AND MAGNESIUM.¹⁰⁴⁸

SILICA.

Evaporate in platinum 100 to 1,000 cc. of the sample or sufficient if possible to form a residue weighing 0.4 to 0.6 gram, and preferably containing 0.1 to 0.2 gram of calcium. When the residue is nearly dry add 1 cc. of hydrochloric acid (1 to 1) and, after moistening the sides of the dish, evaporate to dryness. Dry at 180° C. and if much organic matter is present char it in a radiator. Moistlen the residue with dilute hydrochloric acid and expel the excess of acid by heating on the water bath. Add a few drops of hydrochloric acid, dissolve in hot water, and filter. Wash the residue with hot water. Evaporate the filtrate to dryness, repeat the filtration, and combine the two residues. If great accuracy is not required the second evaporation with hydrochloric acid may be omitted. Ignite and weigh the insoluble residue. Add 2 drops of concentrated sulfuric acid and a little hydrofluoric acid, volatilize the acids, ignite, and weigh again. Report the loss in weight
as silica (SiO₂). A weight of non-volatile matter exceeding 0.5 mg. should be analyzed.

IRON AND ALUMINIUM.

Heat to boiling the filtrate from the insoluble residue, oxidize with concentrated nitric acid or bromine, and concentrate to about 25 cc. Add ammonium hydroxide in slight excess, boil one minute, and filter. Dissolve the precipitate on the filter in a small amount of hot dilute hydrochloric acid. Reprecipitate with ammonium hydroxide, filter, and wash. Unless very accurate results are necessary this solution and reprecipitation may be omitted. Unite the two filtrates for determination of calcium. Ignite and weigh the precipitate. It will comprise oxides of iron and aluminium and phosphate. If much phosphate is present it should be determined in a separate sample and a correction for the amount applied; otherwise it may be neglected. Determine the iron in the ignited precipitate by fusion with sodium or potassium pyrosulfate, reduction with zinc, and titration with potassium permanganate. Aluminium (Al) is calculated as follows:

\[
Al = 0.53[(Fe₂O₃ + Al₂O₃) - 1.43 \text{ Fe}] 
\]

CALCIUM.

Concentrate the filtrate from the separation of iron and aluminium to about 100 cc., and add an excess of concentrated solution of ammonium oxalate, little by little, to the hot ammoniacal solution. Keep the solution warm and stir at intervals till the precipitate settles readily and leaves a clear supernatant liquid. Filter, dissolve the precipitate in a little hot dilute hydrochloric acid, and reprecipitate with ammonium hydroxide and ammonium oxalate. If great accuracy is not required this solution and reprecipitation may be omitted, and the first precipitate may be washed clean with hot water⁴⁴⁸. Save the filtrate for determination of magnesium. Ignite the precipitate and weigh it as calcium oxide, 71.5 per cent of which is the equivalent of calcium (Ca); or dissolve the precipitate in hot 2 per cent sulfuric acid and titrate with a standard solution of potassium permanganate.

MAGNESIUM.

Acidify with hydrochloric acid the filtrate from the separation of calcium and concentrate it to about 100 cc. Add 20 cc. of a saturated solution of microcosmic salt, cool, and make slightly but
distinctly alkaline by adding ammonium hydroxide, drop by drop. Allow the solution to stand four hours, then filter and wash with 3 per cent ammonium hydroxide. Dissolve the precipitate, especially in the presence of large amounts of sodium or potassium, in a slight excess of dilute hydrochloric acid and reprecipitate the magnesium with ammonium hydroxide and a few drops of microcosmic salt solution. If great accuracy is not required this solution and reprecipitation may be omitted. Ignite the precipitate and weigh it as magnesium pyrophosphate (Mg₃P₂O₇), 21.9 per cent of which is the equivalent of magnesium (Mg). If manganese is present it is precipitated with the magnesium and a correction for it should be applied after having determined manganese in a separate sample. The weight of manganese pyrophosphate (Mn₃P₂O₇) is 2.58 times the weight of manganese.

SEPARATION OF SULFATE, SODIUM, AND POTASSIUM.

SULFATE.

Evaporate to dryness 100 to 1,000 cc. of the sample, or sufficient to obtain a residue weighing 0.4 to 0.6 gram and containing preferably 0.05 to 0.2 gram of sodium. Acidify the residue with hydrochloric acid and remove the silica, iron, and aluminium (pp. 56-57). Make acid and add a hot solution of barium chloride in slight excess to the hot filtrate, and warm it, stirring at intervals for one-half hour, until the precipitate settles readily and leaves a clear supernatant liquid. Dry, ignite, and weigh the precipitate of barium sulfate, 41.1 per cent of which is equal to the content of sulfate (SO₄).

SODIUM, POTASSIUM, AND LITHIUM.

Evaporate to dryness the filtrate from the precipitation of barium sulfate. Add a few cubic centimeters of hot water and then a saturated solution of barium hydroxide until a slight film collects on the top of the solution. Filter and wash the precipitate with hot water. Add to the filtrate an excess of ammonium hydroxide and ammonium carbonate solution. Filter, evaporate the filtrate to dryness, dry, and ignite at low red heat to expel ammonium salts. Repeat the operations including the addition of barium hydroxide until no precipitate is obtained by barium hydroxide or by ammonium hydroxide and ammonium carbonate. Evaporate the final filtrate to dryness in a weighed platinum dish, dry, cool,
and weigh the residue. Dissolve the residue in a few cubic centimeters of water, filter, wash the filter paper twice with hot water, then ignite the filter paper in the platinum dish. Cool and weigh the residue. Subtract this weight from the first weight including the residue. The difference is the weight of the chlorides of sodium and potassium and lithium. If it is not desired to separate sodium and potassium the weight of sodium and potassium as sodium may be calculated from this difference by multiplying it by 0.394.

**POTASSIUM.**

*First procedure.*—Add to the solution of the chlorides of sodium and potassium a few drops of dilute hydrochloric acid (1 to 3) and 1 cc. of 10 per cent platinic chloride (PtCl₆) for each 30 mg. of the combined chlorides. Evaporate to a thick syrup on the water bath, then remove dish and allow it to come to dryness at laboratory temperature. Treat the residue cold with 80 per cent alcohol and filter. Wash the precipitate with 80 per cent alcohol until the filtrate is no longer colored. Dry the precipitate and dissolve it in hot water. Evaporate the solution to dryness in a platinum dish and weigh it as potassium platinic chloride (K₂PtCl₆). The weight of potassium (K) is 16.1 per cent of this weight and the equivalent of potassium chloride (KCl) is 30.7 per cent of this weight. Subtract the equivalent weight of potassium chloride from the weight of the combined chlorides. The weight of the sodium is 39.4 per cent of the difference.

*Second procedure.*—Add to the hot solution of the combined chlorides 20 per cent perchloric acid (HClO₄) slightly in excess of the amount required to combine with the bases. One cubic centimeter of 20 per cent perchloric acid is equivalent to 90 mg. of potassium. Evaporate the solution to dryness, dissolve the residue in 10 cc. of hot water and a small amount of perchloric acid, and again evaporate to dryness. Repeat the addition of water, perchloric acid, and evaporation until white fumes appear on evaporating to dryness. Add to the residue 25 cc. of 96 per cent alcohol containing 0.2 per cent of perchloric acid (1 cc. of 20 per cent perchloric acid in 100 cc. of 98 per cent alcohol). Break up the residue with a stirring rod. Decant the supernatant liquid through a weighed Gooch crucible that has been washed with 0.2 per cent perchloric acid in alcohol. If the precipitate is unusually large dissolve it in hot water and repeat the evaporation with perchloric acid. Wash
Lithium

the precipitate once by decantation with the 0.2 per cent perchloric acid in alcohol, transfer the precipitate to the crucible, and wash it several times with a 0.2 per cent perchloric acid in alcohol. Dry the crucible at 120–130° C. for one hour, cool, and weigh it. The increase in weight is potassium perchlorate (KClO₄). The equivalent weight of potassium is 28.2 per cent and the equivalent weight of potassium chloride is 53.8 per cent of the potassium perchlorate. Calculate the content of sodium by difference.

Lithium.*

Use a large quantity of the sample. Obtain the combined chlorides of sodium, potassium, and lithium (see pp. 58–59). Transfer the combined chlorides to a small Erlenmeyer flask (50 or 100 cc. capacity) and evaporate the solution nearly, but not quite, to dryness. Add about 30 cc. of redistilled amyl alcohol. Connect the flask, the stopper of which carries a thermometer, with a condenser* and boil until the temperature rises approximately to the boiling point of amyl alcohol (130° C.), showing that all the water has been driven off. Cool slightly and add a drop of hydrochloric acid to convert small amounts of lithium hydroxide to lithium chloride. Connect with the condenser and continue the boiling to drive off again all water and until the temperature reaches the boiling point of amyl alcohol. The content of the flask at this time is usually 15 to 20 cc. Filter through a small paper or a Gooch crucible into a graduated cylinder and note exact quantity of filtrate, which determines the subsequent correction. Wash the precipitate with small quantities of dehydrated amyl alcohol. Evaporate the filtrate and washings in a platinum dish to dryness on the steam bath, dissolve the residue in water, and add a few drops of sulfuric acid. Evaporate on a steam bath and expel the excess of sulfuric acid by gentle heat over a flame. Repeat until carbonaceous matter is completely burned off. Cool and weigh the dish and contents. Dissolve in a small quantity of hot water, filter through a small filter, wash, and return filter to dish, ignite, and weigh. The difference between the original weight of dish and contents and the weight of the dish and small amount of residue equals the weight of impure lithium sulfate. The purity of the lithium sulfate should be tested by adding small amounts of

*The amyl alcohol may be boiled off without the use of a condenser, but the vapors are very disagreeable.
ammonium phosphate and ammonium hydroxide, which will precipitate any magnesium present with the lithium sulfate. Any precipitate appearing after standing over night should be collected on a small filter and weighed as magnesium pyrophosphate, calculated to sulfate, and subtracted from the weight of impure lithium sulfate. From this weight subtract 0.00113 gram for every 10 cc. of amyl alcohol filtrate exclusive of the amyl alcohol used in washing residue because of the slight solubility of solid mixed chlorides in amyl alcohol. Calculate lithium from the corrected weight of lithium sulfate. Dissolve the mixed chlorides from flask and filter with hot water, evaporate to dryness, ignite gently to remove amyl alcohol, filter and thoroughly wash; concentrate the filtrates and washings to 25 to 50 cc.

To the weight of potassium chloride add 0.00051 gram for every 10 cc. of amyl alcohol used in the extraction of the lithium chloride, which corrects for the solubility of the potassium chloride in amyl alcohol. Calculate to potassium.

The weight of sodium chloride is found by subtracting the combined weights of lithium chloride and potassium chloride (corrected) from the total weight of the three chlorides. Calculate sodium chloride to sodium.

BROMINE, IODINE, ARSENIC, AND BORIC ACID.

Evaporate to dryness a large quantity of the sample to which a small amount of sodium carbonate has been added. Boil the residue with distilled water, transfer it to a filter, and thoroughly wash it with hot water. Dilute the alkaline filtrate to a definite volume, and determine bromine and iodine, arsenic, and boric acid in aliquot portions of it.

BROMINE AND IODINE.¹⁸

Reagents.—1. Sulfuric acid. 1 to 5.
2. Potassium nitrite or sodium nitrite. Two per cent solution.
4. Iodine standards. Acidify with dilute sulfuric acid measured quantities of a standard solution of potassium iodide in small tubes. Add 3 or 4 drops of the potassium nitrite solution and extract with carbon bisulfide as in the actual determination. Transfer to small flasks the standards from which the iodine has been removed.
I. Fermentation standards, will measured quantities of a standard portion of 100 cc. in accordance with the small flasks from which the sample has been removed, and to each 5 cc. of purified carbon bisulfide, and proceed exactly as with the sample.

1. Dissolve the portion of the sample in 100 cc. of water, and add enough sodium hydroxide to make the percentage of alcohol about 40. Heat and filter, and repeat the extraction of the residue with an equal volume of water, and 1 or 2 drops of sodium hydroxide at the temperature to acetic acid, and compare to a hydrometer. Dissolve the residue in 2 or 3 cc. of water, and repeat the extraction with water and the addition of a drop of sodium hydroxide at the temperature to the sample and compare it to a hydrometer. Dissolve the residue in a little water, add 1 cc. of sodium hydroxide, and transfer it to a small flask. Add 4 drops of the solution of potassium nitrate in sodium nitrite, and about 5 cc. of carbon bisulfide. Shake the mixture until all the iodine is extracted. Separate the undissolved from the carbon bisulfide by filtration. Wash the flask, filter, and contents with cold filtered water and transfer the carbon bisulfide containing the iodine in solution to Nessler tubes by means of about 5 cc. of pure carbon bisulfide. In washing the filter, dilute the contents of the tube to a definite volume, usually 12 or 15 cc., and compare the color with that of known amounts of iodine dissolved in carbon bisulfide for other times.

Transfer to a small flask the sample from which the iodine has been removed. Add saturated sodium water, 1 cc. at a time, shaking after each addition until all the bromine is freed. Care must be taken not to add much more chlorine than is necessary to free the bromine since an excess of reagent may form a bromochloride that spoil the color reaction. Separate the water solution from the carbon bisulfide by filtration through a moistened filter, wash the contents of the filter two or three times with water, and then transfer them to a Nessler tube by means of about 1 cc. of carbon bisulfide. Repeat this extraction of the filtrate twice, using 3 cc. of carbon bisulfide each time. The combined carbon bisulfide extracts usually amount to 11.5 to 12 cc. Add enough carbon bisulfide to the tubes to bring them to a definite volume, usually 12 to 15 cc., and compare the sample with the standards. If much bromine is present it is not always completely extracted.
by the amounts of carbon bisulfide recommended. If the extraction is incomplete, therefore, make one or two extra extractions with carbon bisulfide, transfer the extracts to another tube, and compare the color with that of the standards.

ARSENIC.

Evaporate to dryness an aliquot portion of the alkaline filtrate (p. 61). Acidify the residue with arsenic-free sulfuric acid, and subject it to the action of arsenic-free zinc and sulfuric acid in a Marsh-Berzelius apparatus. Compare the mirror obtained with a mirror obtained from an arsenious oxide solution of known strength.

BORIC ACID.

Evaporate to dryness an aliquot portion of the alkaline filtrate (p. 61), treat the residue with 1 or 2 cc. of water, and slightly acidify the solution with hydrochloric acid. Add about 25 cc. of absolute alcohol, boil, filter, and repeat the extraction of the residue. Make the filtrate slightly alkaline with sodium hydroxide, and evaporate it to dryness. Add a little water, slightly acidify with hydrochloric acid, and place a strip of turmeric paper in the liquid. Evaporate to dryness on the steam bath, and continue the heating until the turmeric paper is dry. If boric acid is present the turmeric paper becomes cherry red. It is not usually necessary to determine quantitatively boric acid; the quantitative method devised by Gooch is recommended.

HYDROGEN SULFIDE.

Hydrogen sulfide should be determined preferably in the field; the procedure as far as the final titration with sodium thiosulfate must be carried out in the field.

Reagents.—1. N/100 sodium thiosulfate.
2. Standard iodine. A N/100 solution containing potassium iodide standardized against the N/100 sodium thiosulfate. To standardize, add 10 cc. of the iodine solution to 500 cc. of boiled distilled water. Add about 1 gram of potassium iodide, and titrate with N/100 sodium thiosulfate in the presence of starch indicator. One cc. of N/100 iodine is equivalent to 0.17 mg. H₂S.
4. Starch. A freshly prepared solution for use as indicator.
Procedure.—Add 500 cc. of the sample to 10 cc. of the standard iodine solution and 1 gram of potassium iodide in a large glass-stoppered bottle or flask. If the sample is to be collected from a tap lead the water into the bottle through a rubber tube extending to the bottom of the bottle so as to eliminate errors due to aeration. Shake the bottle, allow it to stand for a few minutes, and then titrate the excess of iodine with sodium thiosulfate in the presence of starch indicator. Hydrogen sulfide (H₂S) in parts per million is equal to 0.34 times the difference in cubic centimeters between the amount of iodine solution added and the amount of N/100 thiosulfate used in the titration.

CHLORINE.

In waters that have been treated with calcium hypochlorite or liquid chlorine it is frequently advisable to ascertain the presence or absence of chlorine. As the reagents which have been proposed for its detection are not specific for chlorine but give similar or identical reactions with oxidizing agents or reducible substances care must be exercised in interpreting the results of such tests: nitrites and ferric salts are of common occurrence, and chlorates also may lead to misinterpretation in waters treated with calcium hypochlorite.

Reagents.—1. Tolidin solution. One gram of o-tolidin, purified by being recrystallized from alcohol, is dissolved in 1 liter of 10 per cent hydrochloric acid.

2. Copper sulfate solution. Dissolve 1.5 grams of copper sulfate and 1 cc. of concentrated sulfuric acid in distilled water and dilute the solution to 100 cc.

3. Potassium bichromate solution. Dissolve 0.025 gram of potassium bichromate and 0.1 cc. of concentrated sulfuric acid in distilled water and dilute the solution to 100 cc.

Procedure.—Mix 1 cc. of the toolidin reagent with 100 cc. of the sample in a Nessler tube and allow the solution to stand at least 5 minutes. Small amounts of free chlorine give a yellow and larger amounts an orange color.

For quantitative determination compare the color with that of standards in similar tubes prepared from the solutions of copper sulfate and potassium bichromate. The amounts of solution for various standards are indicated in Table 13.
### Table 13.—Preparation of Permanent Standards for Content of Chlorine.

<table>
<thead>
<tr>
<th>Chlorine.</th>
<th>Solution of copper sulfate.</th>
<th>Solution of potassium bichromate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parts per million.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.10 0.20 0.30 0.40 0.50</td>
<td>0.0 0.0 0.0 0.0 0.4 0.8 1.2 1.5 1.7 1.8 1.9 1.9 2.0 2.0</td>
<td>0.8 2.1 3.2 4.3 5.5 6.6 7.5 8.7 9.0 10.0 20.0 30.0 38.0 45.0</td>
</tr>
</tbody>
</table>

### DISSOLVED OXYGEN

Reagents.—1. Sulfuric acid, concentrated. (Sp. gr. 1.83–1.84.)
2. Potassium permanganate. Dissolve 6.32 grams of the salt in water and dilute the solution to 1 liter.
3. Potassium oxalate. A 2 per cent solution.
4. Manganous sulfate. Dissolve 480 grams of the salt in water and dilute the solution to 1 liter.
5. Alkaline potassium iodide. Dissolve 700 grams of potassium hydroxide and 150 grams of potassium iodide in water and dilute the solution to 1 liter.
6. Hydrochloric acid. Concentrated (Sp. gr. 1.18–1.19).
7. Sodium thiosulfate. A N/40 solution. Dissolve 6.2 grams of chemically pure recrystallized sodium thiosulfate in water and dilute the solution to 1 liter with freshly boiled distilled water. Each cc. is equivalent to 0.2 mg. of oxygen or to 0.1395 cc. of oxygen at 0° C. and 760 mm. pressure. Inasmuch as this solution is not permanent it should be standardized occasionally against a N/40 solution of potassium bichromate. The keeping qualities of the thiosulfate solution are improved by adding to each liter 5 cc. of chloroform and 1.5 grams of ammonium carbonate before diluting to the prescribed volume.
8. Starch solution. Mix a small amount of clean starch with cold water until it becomes a thin paste and stir this mass into 150 to 200 times its weight of boiling water. Boil for a few minutes,
The procedure to this point must be carried out in the field, but
after the acid has been added and the stopper replaced there is no further change, and the rest of the test may be performed within a few hours, as convenient. Transfer 200 cc. of the contents of the bottle to a flask and titrate with N/40 sodium thiosulfate, using a few cubic centimeters of the starch solution as indicator toward the end of the titration. Do not add the starch solution until the color has become faint yellow, and titrate until the blue color disappears.

The use of potassium permanganate is made necessary by high nitrite or organic matter. The procedure outlined must be followed in all work on sewage and partly purified effluents or seriously polluted streams or samples whose nitrite nitrogen exceeds 0.1 part per million. In testing other samples the procedure may be shortened by beginning with the addition of the manganous sulfate solution and proceeding from that point as outlined, except that only 1 cc. of alkaline potassium iodide need be added.

Calculation of Results.—Oxygen shall be reported in parts per million by weight. It is sometimes convenient to know the number of cubic centimeters per liter of the gas at 0° C. temperature and 760 mm. pressure and also to know the percentage which the amount of gas present is of the maximum amount capable of being dissolved by distilled water at the same temperature and pressure. If 200 cc. of the sample is taken the number of cubic centimeters of N/40 thiosulfate used is equal to parts per million of oxygen. Corrections for volume of reagents added amount to less than 3 per cent and are not justified except in work of unusual precision. To obtain the result in cubic centimeters per liter multiply the number of cubic centimeters of thiosulfate used by 0.698. To obtain the result in percentage of saturation divide the number of cubic centimeters of thiosulfate by the figure in Table 14 opposite the temperature of the water and under the proper chlorine figure. The last column of Table 14 permits interpolation for intermediate chlorine values. At elevations differing considerably from mean sea level and for accurate work attention must be given to barometric pressure, the normal pressure in the region being preferable to the specific pressure at the time of sampling. The term “saturation” refers to a condition of equilibrium between the solution and an oxygen pressure in the atmosphere corresponding to 158.8 millimeters, or approximately one-fifth atmosphere. The true saturation or equilibrium between the solution and pure oxygen is nearly five times this value, and
consequently values in excess of 100 per cent saturation frequently occur in the presence of oxygen-forming plants.

Table 14.—Solubility of Oxygen in Fresh Water and in Sea Water of Stated Degrees of Salinity at Various Temperatures When Exposed to an Atmosphere Containing 20.9 Per Cent of Oxygen under a Pressure of 760 mm.*

(Calculated by G. C. Whipple and M. C. Whipple from measurements of C. J. Fox.)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Chloride in sea water (milligrams per liter)</th>
<th>Difference per 100 parts of chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.000</td>
<td>5000.</td>
</tr>
<tr>
<td>0</td>
<td>14.62</td>
<td>13.79</td>
</tr>
<tr>
<td>1</td>
<td>14.23</td>
<td>13.41</td>
</tr>
<tr>
<td>2</td>
<td>13.84</td>
<td>13.05</td>
</tr>
<tr>
<td>3</td>
<td>13.48</td>
<td>12.72</td>
</tr>
<tr>
<td>4</td>
<td>13.13</td>
<td>12.41</td>
</tr>
<tr>
<td>5</td>
<td>12.80</td>
<td>12.09</td>
</tr>
<tr>
<td>6</td>
<td>12.48</td>
<td>11.79</td>
</tr>
<tr>
<td>7</td>
<td>12.17</td>
<td>11.51</td>
</tr>
<tr>
<td>8</td>
<td>11.87</td>
<td>11.24</td>
</tr>
<tr>
<td>9</td>
<td>11.59</td>
<td>10.97</td>
</tr>
<tr>
<td>10</td>
<td>11.33</td>
<td>10.73</td>
</tr>
<tr>
<td>11</td>
<td>11.08</td>
<td>10.49</td>
</tr>
<tr>
<td>12</td>
<td>10.83</td>
<td>10.28</td>
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<tr>
<td>13</td>
<td>10.60</td>
<td>10.05</td>
</tr>
<tr>
<td>14</td>
<td>10.37</td>
<td>9.85</td>
</tr>
<tr>
<td>15</td>
<td>10.15</td>
<td>9.65</td>
</tr>
<tr>
<td>16</td>
<td>9.95</td>
<td>9.46</td>
</tr>
<tr>
<td>17</td>
<td>9.74</td>
<td>9.26</td>
</tr>
<tr>
<td>18</td>
<td>9.54</td>
<td>9.07</td>
</tr>
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<td>19</td>
<td>9.35</td>
<td>8.89</td>
</tr>
<tr>
<td>20</td>
<td>9.17</td>
<td>8.73</td>
</tr>
<tr>
<td>21</td>
<td>8.99</td>
<td>8.57</td>
</tr>
<tr>
<td>22</td>
<td>8.83</td>
<td>8.42</td>
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<tr>
<td>23</td>
<td>8.68</td>
<td>8.27</td>
</tr>
<tr>
<td>24</td>
<td>8.53</td>
<td>8.12</td>
</tr>
<tr>
<td>25</td>
<td>8.38</td>
<td>7.96</td>
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<tr>
<td>26</td>
<td>8.22</td>
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<td>28</td>
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<td>7.53</td>
</tr>
<tr>
<td>29</td>
<td>7.77</td>
<td>7.39</td>
</tr>
<tr>
<td>30</td>
<td>7.63</td>
<td>7.25</td>
</tr>
</tbody>
</table>

* Under any other barometric pressure, B, the solubility can be obtained from the corresponding value in the table by the formula:

\[ S' = S - \frac{S - S'}{760 - 29.22} \]

in which

- B = Solubility at B or B',
- S = Solubility at 760 mm. or 29.22 inches,
- B = Barometric pressure in mm.,
- and B' = Barometric pressure in inches.
ETHER-SOLUBLE MATTER

ETHER-SOLUBLE MATTER.44

Evaporate 500 cc. of the sample in a porcelain evaporating dish to a volume of about 50 cc. By means of a rubber-tipped glass rod remove to the bottom of the dish the solid matter attached to the sides, and add normal sulfuric acid to neutralize the alkalinity. Do not use an excess of acid. Then evaporate the contents of the dish to dryness. Treat the dry residue with boiling ether, rubbing the bottom and sides of the dish to insure complete solution of fat. Three extractions with ether are required. Filter the ether solution through a 5 cm. filter into a weighed flask having a wide mouth. Evaporate the ether slowly, and dry the flask at 100° C. for 30 minutes. The increase in weight of the flask gives the amount of fats, or, in more precise language, the ether-soluble matter.

An excess of acid gives too high results because of the formation of fatty-acid residues.

RELATIVE STABILITY OF EFFLUENTS.73

Reagent.—Methylene blue solution. A 0.05 per cent aqueous solution of methylene blue, preferably the double zinc salt or commercial variety.60b

Collection of sample.—Collect the sample in a glass-stoppered bottle holding approximately 150 cc. If the dissolved oxygen is low observe precautions similar to those used in collecting samples for dissolved oxygen (p. 66).

Procedure.—Add 0.4 cc. of the methylene blue solution to the sample in the 150 cc. bottle. As methylene blue has a slightly antiseptic property be careful to add exactly 0.4 cc. Add the methylene blue solution preferably below the surface of the liquid after filling the bottle with the sample. If the methylene blue is added first do not allow the liquid to overflow as coloring matter will thus be lost. Incubate the sample at 20°C. for ten days. Four days' incubation may be considered sufficient for all practical purposes in routine plant-control work. If quick results are desired incubate the sample at 37° C. for five days using suitable stoppers18 2a to prevent the loss and reabsorption of dissolved oxygen. The bacterial flora at 37° C. is different from that at 20° C. The lower temperature is more nearly the average temperature of surface waters and therefore the higher temperature should be used only when quick approximate results are essential.
Observe the sample at least twice a day during incubation. Give a sample in which the methylene blue becomes decolorized a relative stability corresponding to the time required for reduction (see Table 15). For routine filter control ordinary room or cellar temperature gives fairly satisfactory results. For accurate studies, room temperature incubation is very undesirable, as the fluctuations in temperature which are ordinarily not noticed are responsible for appreciable deviations from the true values of relative stability. If the samples are incubated less than 10 days at 20° C. and are not decolorized place a plus sign after the stability value in order to indicate that the stability might have been higher if more time had been allowed. In applying this test to river waters it often happens that the blue coloring matter is removed either partly or completely through absorption by the clay which many rivers carry in suspension. True relative stabilities cannot be obtained for such waters except by determining the initial available oxygen at the start and the biochemical oxygen demand on incubation at 20° C. for 10 days (pp. 71–73). Germicides, such as hypochlorite of lime, if present in sufficient quantity, vitiate the results. If a sample contains free chlorine, therefore, store it about 2 hours, or until the chlorine is gone, and then add methylene blue.

Table 15 gives the relation between the time in days to decolorize methylene blue at 20° C. ($t_{50}$) and the relative stability number or ratio of available oxygen to oxygen required for equilibrium, expressed in percentage (S).

<table>
<thead>
<tr>
<th>Days</th>
<th>Percentage</th>
<th>Days</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>11</td>
<td>8.0</td>
<td>84</td>
</tr>
<tr>
<td>1.0</td>
<td>21</td>
<td>9.0</td>
<td>87</td>
</tr>
<tr>
<td>1.5</td>
<td>30</td>
<td>10.0</td>
<td>90</td>
</tr>
<tr>
<td>2.0</td>
<td>37</td>
<td>11.0</td>
<td>92</td>
</tr>
<tr>
<td>2.5</td>
<td>44</td>
<td>12.0</td>
<td>94</td>
</tr>
<tr>
<td>3.0</td>
<td>50</td>
<td>13.0</td>
<td>95</td>
</tr>
<tr>
<td>4.0</td>
<td>60</td>
<td>14.0</td>
<td>96</td>
</tr>
<tr>
<td>5.0</td>
<td>68</td>
<td>16.0</td>
<td>97</td>
</tr>
<tr>
<td>6.0</td>
<td>75</td>
<td>18.0</td>
<td>98</td>
</tr>
<tr>
<td>7.0</td>
<td>80</td>
<td>20.0</td>
<td>99</td>
</tr>
</tbody>
</table>
The theoretical relation is, \( S = 100 \left( 1 - 0.794e^{-0.06} \right) \)

The relation between the time of reduction at 20° C. and that at 37° C. is approximately two to one, but if an observer incubates at 37° C. he should work out his own comparative 37° C. table or factor.

A relative stability of 75 signifies that the sample examined contains a supply of available oxygen equal to 75 per cent of the amount of oxygen which it requires in order to become perfectly stable. The available oxygen is approximately equivalent to the dissolved oxygen plus the available oxygen of nitrate and nitrite. Nitrite in sewage is usually so low as to be negligible.

**BIOCHEMICAL OXYGEN DEMAND OF SEWAGE AND EFFLUENTS**

**RELATIVE STABILITY METHOD.**

The relative stability method may be employed to obtain a measure of the putrescible material in sewages and effluents in terms of oxygen demand.

**Procedure for effluents.**—Divide the total available oxygen, including the oxygen of nitrite and nitrate, by the relative stability expressed as a decimal.

**Procedure for sewages.**—Make one or two dilutions with fully aerated distilled water of known dissolved oxygen content. Tap water may be employed if it is free from nitrates. Vary the relative proportions of sewage and water to be employed to give a relative stability of 50 to 75. Unless sealed \( ^1b \) \( ^2b \) \( ^3a \) are used bring the water as well as the sewage to the temperature at which the mixtures are to be incubated before preparing the dilutions. During the manipulation avoid aeration. Having made the proper dilutions, determine the relative stability of each.

Calculate the oxygen demand in parts per million by the formula:

\[
\text{Oxygen demand} = \frac{O (1 - p)}{R_p}
\]

In this formula, \( O \) is the initial dissolved oxygen of the diluting water, \( p \) is the proportion of sewage; and \( R_p \) is the relative stability of the mixture. Ordinarily the available oxygen in crude sewages, septic tank effluents, settling tank effluents, and trade wastes can be neglected.
72  BIOCHEMICAL OXYGEN DEMAND

SODIUM NITRATE METHOD.

For the determination of the biochemical oxygen demand the sodium nitrate method may be used. The method is based on the biochemical consumption of oxygen from sodium nitrate by a sewage or polluted water during an incubation period of ten days at 20°C. A reasonable excess of sodium nitrate does not give a higher oxygen demand, as do higher dilutions with aerated water. The oxygen absorbed from the air in applying the method to sewages is negligible.

Reagent.—Sodium nitrate solution. Dissolve 26.56 grams of pure sodium nitrate in 1 liter of distilled water. One cc. of this solution in 250 cc. of sewage represents 50 parts per million of available oxygen. The strength of the sodium nitrate solution may be varied to suit conditions.

Procedure for sewages.—Ordinarily disregard the initial available oxygen as it is very small compared with the total biochemical oxygen demand. Add measured amounts of the sodium nitrate solution to the sewage in bottles holding approximately 250 cc. which have been completely filled and stoppered. Incubate for 10 days at 20°C. A seal is not required during incubation. The appearance of a black sediment and the development of a putrid odor during incubation indicates that too little sodium nitrate has been added. Methylene blue solution in proper proportion may be added at the start to serve as an indicator during the incubation. Domestic sewage usually varies in its oxygen demand from 100 to 300 parts per million, approximately 30 per cent of which is used up at 20°C. in the first 24 hours. At the end of the incubation period determine the residual nitrite and nitrate. Determine the nitrate by the aluminium reduction method and direct Nesslerization. To convert the nitrogen into oxygen equivalents, multiply the nitrite nitrogen by 1.7 and the nitrate nitrogen by 2.9. The difference between the available oxygen added as sodium nitrate and that found as nitrite and nitrate at the end of the incubation period is the biochemical oxygen demand.

Procedure for industrial wastes.—Employ the same procedure using larger quantities of the sodium nitrate solution. Make the reaction alkaline to methyl orange and acid to phenolphthalein. Adjust an acid reaction with sodium bicarbonate and a caustic alkaline reaction with weak hydrochloric acid. If the liquid is
ANALYSIS OF SEWAGE SLUDGE

ANALYSIS OF SEWAGE SLUDGE AND MUD DEPOSITS.

COLLECTION OF SAMPLE.

Collect a representative sample of the material. In general more than one sample should be taken from a spot and a large number of samples should be collected rather than a few large samples. If the surface layer is darker and a lower layer consists of pure clay sample only the surface layer. Samples may be analyzed either separately or as composites of careful mixtures. After the sample has settled a few minutes roughly drain or siphon the excess water. Allow sewage sludge to stand for one hour before draining it free from excess water unless it is essential to determine the moisture content of the sample originally collected. If sludge cannot be analyzed within twenty-four hours it is better not to use air-tight bottles and to add small quantities of chloroform and keep in the ice box to retard decomposition. At the time of collection carefully examine mud from the bottom of surface water for evidence of sewage pollution and macroscopic and microscopic animal and plant organisms. Record the predominant species. Note the physical appearance of the material, particularly its color, odor, and consistency. Express all analytical results in percentage on a dry basis.

REACTION.

Determine the reaction by diluting a definite quantity of the wet sludge and titrating by the methods given under alkalinity and acidity (pp. 35–39 and 39–41).
SPECIFIC GRAVITY.

Weigh to the nearest tenth of a gram a wide-mouthed flask of 100 to 300 cc. capacity, according to the quantity of material available. Then completely fill the flask with distilled water to the brim and weigh it again. Empty the flask and fill it completely with fresh sewage sludge or mud. If the material is of such consistency that it flows readily fill the flask to the brim and weigh. The specific gravity is equal to the weight of the sludge or mud divided by the weight of an equal volume of distilled water.

If the material does not flow readily fill the weighed flask as completely as possible without exerting pressure during the procedure. Weigh and then fill the flask to the brim with distilled water. Let it stand for a few minutes, until trapped air has escaped, then add more water if necessary and weigh. Subtract the weight of the added water from the weight of the water that completely fills the flask; the specific gravity is equal to the weight of the material divided by this difference. Record the specific gravity only to the second decimal place.

MOISTURE.

Heat approximately 25 grams of sludge or mud in a weighed nickel dish on the water bath until it is fairly dry. Dry the residue in an oven at 100° C., cool, and weigh. Repeat to approximate constant weight. The loss in weight is moisture.

VOLATILE AND FIXED MATTER.

Ignite, at dull red heat in a hood, the residue from the determination of moisture until all the carbon has disappeared. Cool the residue in a desiccator and weigh it. The residue is the fixed matter. The volatile matter is the difference in weight between the original dried sludge and the ignited sludge.

TOTAL ORGANIC NITROGEN.

Preparation of sample.—For the determination of organic nitrogen and fats dry approximately 50 to 75 grams of the sludge or mud in a porcelain dish first on the water bath and finally in the hot-water oven until all the moisture has disappeared. Grind the dry material to a fine powder and keep it in a glass-stoppered bottle.
Reagents.—1. Sulfuric acid. Concentrated, nitrogen free.  
2. Copper sulfate solution. Ten per cent.  

First procedure.—Weigh accurately 0.5 gram of dried sludge or 5.0 grams of dried mud and put it into a 500 cc. Kjeldahl flask. Digest it with 20 cc. of sulfuric acid, or more if necessary, and 1 cc. of copper sulfate solution to assist the oxidation. Boil for several hours until the liquid becomes colorless or slightly yellow. Oxidize the residue with 0.5 gram of potassium permanganate, and follow the “Procedure for Sewage” (pp. 21–22).  

Second procedure.—The following method is convenient for routine work at sewage disposal plants. After digestion as described in the first procedure, cool, transfer to a glass-stoppered 100 cc. flask, dilute with distilled water to 100 cc., and mix well. Transfer 50 cc. with a pipette into another 100 cc. volumetric flask, and make this portion alkaline with 50 per cent sodium hydroxide, testing a drop of the liquid on a porcelain plate with phenolphthalein to insure neutralization. The formation of a floc usually indicates that neutralization is complete. Dilute the solution to 100 cc., pour it into a small glass-stoppered bottle and permit it to stand until the next day. Nesslerize an aliquot portion of the clear supernatant liquid, and calculate the percentage of nitrogen in the material.

ETHER-SOLUBLE MATTER.  

Fats are usually determined only on sewage sludge, but some mud deposits contain small quantities due to the presence of trade wastes.  

Procedure.—Weigh 0.5 to 25 grams of dry material according to the quality of the sludge or mud. Add water to the weighed portion in a porcelain dish and acidify the mixture with N/50 sulfuric acid in the presence of litmus tincture or azolitmin solution as indicator. Avoid adding too much acid as an excess gives too high results on account of fatty acid residues. Evaporate the acidified mixture to dryness on the water bath, and heat it in the hot air oven at 100° C. two to three hours. Extract the dry residue with boiling ether, rubbing the sides and bottom of the dish to insure complete solution of the fat. Three extractions with ether are usually sufficient. Filter the ether solution through a 5 cm. filter paper into a small flask. Evaporate the ether slowly, dry the fatty
extract for half an hour at 100° C., cool in a desiccator, and weigh. If it is desirable, particularly with certain industrial wastes, to determine the quantity of saponified fat determine the fats with and without the addition of acid. The difference between the quantities found by the two determinations is the content of saponified fat.

FERROUS SULFIDE.

The liberation of hydrogen sulfide on adding dilute hydrochloric acid to a sludge indicates the presence of ferrous sulfide. As ferrous sulfide quickly oxidizes on exposure to air a quantitative determination of this constituent must be made immediately after collection of the sample.

Procedure.—Heat a definite portion of the sludge with hydrochloric acid in a flask. Pass the liberated gas through bromine water or hydrogen peroxide. Determine gravimetrically the sulfate in the oxidizing solution, and calculate the equivalent of ferrous sulfide by multiplying the weight of barium sulfate by 0.376.

BIOCHEMICAL OXYGEN DEMAND.

The quantity of river mud most suitable for the determination of the biochemical oxygen demand ranges within certain limits, largely according to the amount of oxidizable matter present. For examinations of river mud prepare a 1 per cent stock suspension in distilled water or tap water saturated with oxygen and free from nitrate; use in the test a dilution of this stock suspension equivalent to a concentration of 1 to 10 grams per liter of mud. For examinations of fresh sewage sludge prepare a 1 per cent stock suspension in a similar manner, but use in the test a dilution equivalent to only 0.1 to 1.0 gram per liter of wet material. For examinations of dried sludges, which have undergone more or less oxidation higher concentrations may be required.

Procedure.—Place a measured portion of the sample, or the proper amount of the 1 per cent stock suspension of the sample, in a 300 cc. narrow-mouth glass-stoppered bottle, and dilute it to the desired concentration with water saturated with oxygen. Determine the oxygen content at 20° C. of the waters that are used for dilution. This determination must be made before the mud or sludge is added because iron sulfide in the mud or sludge rapidly consumes part of the dissolved oxygen. Incubate at 20° C. for five days.
Shortly before the determination of the oxygen remaining in solution at the end of five days rotate the bottle once or twice to mix its contents and allow sedimentation for about 30 minutes. Siphon the greater part of the liquid through a narrow-bore siphon into a 150 cc. bottle, which has been filled with carbon dioxide. Reject the first 25 cc. of the siphoned liquid and allow a little to overflow at the end of siphoning. Determine the oxygen content of the solution in the bottle in the usual way (pp. 65-68). Report the oxygen demand in percentage of the dried mud or sludge.

ANALYSIS OF CHEMICALS.

The following sections describe the accepted methods for the analysis of the chemicals commonly used in the treatment of water.

REAGENTS.

1. Distilled water. In practically all the tests of chemicals it is necessary to use exclusively distilled water that has been freshly boiled to free it from carbon dioxide and oxygen.
3. Hydrochloric acid, N/2.
4. Hydrochloric acid, N/10.
5. Ammonium hydroxide. Redistilled; sp. gr. 0.90.
6. Dilute sulfuric acid. Dilute 1 part of concentrated sulfuric acid with 3 parts of freshly boiled distilled water.
7. Methyl orange indicator. See page 36.
10. Stannous chloride, N/20. This should be frequently standardized by titration against a standard iron solution. One cc. of N/20 stannous chloride is equal to 0.0028 gram of iron (Fe) estimated in the ferrous state.
11. Sodium hydroxide, N/1. Free from carbonate. This should be frequently standardized by titration against a standard acid solution in presence of phenolphthalein indicator. One cc. of N/1 sodium hydroxide is equal to 0.049 gram of sulfuric acid (H2SO4), or to 0.03645 gram of hydrochloric acid (HCl).
13. Standard potassium permanganate. A N/10 solution. One
SULFATE OF ALUMINIUM.

cc. of N/10 potassium permanganate is equal to 0.0056 gram of iron (Fe) estimated in the ferrous state.

15. Sugar. Solid granulated cane sugar.

SULFATE OF ALUMINIUM.

Determine and report insoluble matter, aluminium oxide (Al₂O₃), ferric oxide (Fe₂O₃), ferrous oxide (FeO), basicity ratio, and, if present, free acid as H₂SO₄. If the material is what is known as "granular" sulfate mix it well before sampling. If it is in lump form crush it to 1/8 to 1/4 inch size, mix, and sample it. It is unnecessary to grind the sample to a fine powder, but it is preferable to have the particles fairly uniform in size.

INSOLUBLE MATTER.

Treat 10 grams of the sample with 100 cc. of distilled water and digest one hour at boiling temperature. Filter through a weighed Gooch crucible and wash the insoluble matter with hot water freshly boiled to free it from carbon dioxide. Dry the crucible to constant weight at 100° C., cool, and weigh. Report the percentage of insoluble matter.

OXIDES OF IRON AND ALUMINIUM.

Dilute the filtrate from the determination of insoluble matter to 500 cc. with water free from carbon dioxide and thoroughly mix the solution. Transfer 50 cc. of the solution to a 250 cc. beaker, add about 150 cc. of water and 5 cc. of concentrated hydrochloric acid, and heat to boiling. Add ammonium hydroxide in slight excess; when the solution has been almost neutralized it is convenient to add a drop of methyl orange indicator and then to add about 0.5 cc. of ammonium hydroxide after the solution is neutral to the indicator. Digest at about 100° C. for a few minutes and filter. Some analysts prefer to wash this gelatinous precipitate with hot water by decantation, and some to wash it evenly distributed over the surface of a filter paper; either method may be used. It is difficult to filter it completely from impurities and it is not necessary to do so unless unusual quantities of calcium, magnesium, sodium, or potassium are present. While the precipitate is being washed do not allow it to become dry, as it then packs and can not
be washed clean. After most of the water has drained drying the
filter may be hastened by placing it on a sheet of blotting paper.
If much iron is present completely dry the precipitate, remove it
from the paper, and ignite the paper separately. Finally, blast
the precipitate, with free access of air to the crucible, for five or
ten minutes, cool, and weigh as oxides of iron and aluminium
(Fe₂O₃ + Al₂O₃).

Subtract the content of total iron, expressed as ferric oxide
(Fe₂O₃), from the weight of the combined oxides and report the
difference as aluminium oxide (Al₂O₃), in percentage.

TOTAL IRON.

As filter alum usually contains 0.2 to 0.3 per cent of iron use a
10 gram sample for the determination of total iron. Treat the
sample with 50 cc. of freshly boiled distilled water and add 5 cc. of
concentrated hydrochloric acid and 1 cc. of bromine water. Evapo-
rate the solution to dryness, dissolve the residue in water, and wash it
into a flask with sufficient water to make the volume about 50 cc.
Add 50 cc. of concentrated hydrochloric acid, boil to expel oxygen,
and titrate, as hot as possible, with N/20 stannous chloride.

If a 10 gram sample is used the percentage of iron (Fe) is equal
to the number of cubic centimeters of stannous chloride used
multiplied by 0.028, and the percentage of iron expressed as ferric
oxide is equal to the number of cubic centimeters of stannous
chloride used multiplied by 0.040.

FERRIC IRON.

As filter alum usually contains 0.02 to 0.04 per cent of ferric iron
use a 20 gram sample. Boil 50 cc. of distilled water to expel
oxygen, add 50 cc. of concentrated hydrochloric acid, and add the
sample while the solution is boiling. Keep it boiling till the sample
is dissolved. The flask should be kept filled with carbon dioxide
during this process by dropping in occasionally small amounts of
sodium carbonate. When solution of the sample is complete
titrate it hot immediately with N/20 stannous chloride.

If a 20 gram sample is used the percentage of ferric oxide (Fe₂O₃)
is equal to the number of cubic centimeters of stannous chloride
used multiplied by 0.020.
FERROUS IRON.

The content of ferrous iron is the difference between total and ferric iron. The percentage of ferrous oxide (FeO) is, therefore, equal to 0.90 times the difference between the percentage of total iron expressed as ferric oxide and the percentage of ferric iron expressed as ferric oxide. Report the percentage of ferrous oxide (FeO).

BASICITY RATIO.

Transfer 50 cc. of the filtrate from the determination of insoluble matter to a 200 cc. casserole and dilute it to 100 cc. Boil the solution and titrate it at boiling temperature with N/1 sodium hydroxide in presence of phenolphthalein indicator. The percentage of equivalence of sulfuric acid (H₂SO₄) is equal to the number of cubic centimeters of sodium hydroxide used multiplied by 4.9. In this titration iron and aluminium are precipitated as hydroxides and any free acid is neutralized.

Calculate the percentage of sulfuric acid equivalent to the determined percentages of aluminium oxide, ferric oxide, and ferrous oxide by the following formula:

\[ 2.88 \times Al₂O₃ - 1.53 \times Fe₂O₃ + 1.36 \times FeO. \]

If this percentage of acid equivalent is less than that found by titration report the difference as percentage of free acid. If the percentage of acid equivalent is greater than that found by titration the difference found by 2.88 is the percentage equivalent to the excess of aluminium oxide present. Divide this excess by the percentage of total aluminium oxide and report the quotient as the basicity ratio.

LIME.

Mix well the sample, which should contain no lumps. If foreign matter is present grind the sample to pass a 100-mesh sieve.

Place 20 grams of granulated cane sugar and 1 gram of the sample in a 250 cc. glass-stoppered bottle, tightly stopped, and mix the mass by rolling. Do not shake hard as much of the lime could thus be lost as dust. Then add 187.4 cc. of distilled water freshly boiled to expel carbon dioxide. This makes 200 cc. of sugar solution. The lime is mixed dry with the sugar and the water added later to keep the lime from lumping. After shaking the sugar solution one hour titrate 50 cc. of it with N/2 hydrochloric
acid in presence of methyl orange indicator. The acid used is equivalent to the carbonate and hydroxide in 0.25 gram of the sample.

Filter the remainder of the sugar solution, discarding the first 25 cc. of filtrate. Titrate 50 cc. of the filtrate with N/2 hydrochloric acid in presence of methyl orange indicator. The acid used is equivalent to the hydroxide in 0.25 gram of the sample.

If a 1 gram sample is used the percentage of calcium oxide (CaO) is equal to 5.6 times the number of cubic centimeters of hydrochloric acid used in the second titration; and the percentage of calcium carbonate (CaCO₃) equivalent to the carbonate present is equal to 10 times the difference in cubic centimeters between the results of the two titrations.

SULFATE OF IRON.

INSOLUBLE MATTER.

Treat 10 grams of the sample with 100 cc. of freshly boiled distilled water cooled to 30° C. or less. When solution is complete filter through a weighed Gooch crucible, wash, dry, cool, and weigh. Report the weight of the residue, in percentage, as insoluble matter.

IRON AS FERROUS SULFATE.

Dissolve 1 gram of the sample and dilute to 200 cc. with freshly boiled distilled water cooled to 30° C. or less. Add 5 cc. of dilute sulfuric acid (1 to 3) to a 50 cc. portion of the solution and titrate with N/10 potassium permanganate. The percentage of ferrous sulfate (FeSO₄·7H₂O) is equal to 11.12 times the number of cubic centimeters of potassium permanganate used.

ACIDITY.

Shake 12.25 grams of the sample in a 150 cc. bottle with 75 cc. of 95 per cent alcohol for ten minutes. Run a blank. Filter rapidly both sample and blank and wash rapidly with alcohol sufficient to make 100 cc. of filtrate. Titrate with N/20 sodium hydroxide in presence of phenolphthalein and subtract the result of titrating the blank from that of titrating the solution of the sample. The percentage of acidity, expressed as sulfuric acid (H₂SO₄), is equal to 0.02 times the number of cubic centimeters of sodium hydroxide used.
SODA ASH, CHEMICAL BIBLIOGRAPHY

SODA ASH.

INSOLUBLE MATTER.

Treat 5.305 grams of the sample with 200 cc. of freshly boiled and cooled distilled water. When solution is complete filter through an asbestos mat in a weighed Gooch crucible, dry, cool, and weigh. Report the weight of the residue, in percentage, as insoluble matter.

AVAILABLE ALKALI.

Dilute the filtrate from the determination of insoluble matter to 1,000 cc. and thoroughly mix. Titrate 25 cc. of this dilution with N. 10 hydrochloric acid in presence of methyl orange indicator. The percentage of available alkali, expressed as sodium carbonate (Na₂CO₃), is equal to 4 times the number of cubic centimeters of hydrochloric acid used.

CHEMICAL BIBLIOGRAPHY.

The subjoined bibliography comprises the publications cited in the text of this report. The references are arranged alphabetically by authors' names and under each author in order of dates of publication. When different pages of a single work are cited letters are used in connection with the number that refers to the work.


13. DROWN, T. M. The chemical examination of waters and the interpretation of analyses: Examinations by the State Board of Health of water supplies of Mass. 1887–90, pt. 1, Examination of water supplies, pp. 519–78, 1890.


15. ———, and HAZEN, ALLEN. A report of the chemical work done at the Lawrence Experiment Station: Examinations by the State Board of Health of water supplies of Mass. 1887–90, pt. 2, Purification of sewage and water, pp. 707–34, 1890.


22. ———. Sewage works analyses, pp. 21–37, John Wiley & Sons, New York, 1902; (a) pp. 31–4; (b) pp. 58–60; (c) pp. 86–9; (d) pp. 89–95; (e) pp. 96–7; (f) pp. 98–100.

23. FOWLER, G. J. Univ. of Manchester Lecture, March, 1904, Pamphlet, p. 7.


71. Report of the University of Illinois on the report of surveys examination, sanitary dist. Chicago, p. 60, Chicago, 1903; (by p. 56; (by p. 54). 1903.


92. Stearns, F. P., and Drown, T. M. Discussion of special topics relating to the quality of public water supplies: *Examinations by the State Board of Health of water supplies of Mass., 1887–90*, pt. 1, Examination of water supplies, pp. 740–9, 1890.


100. ————. The examination of water and water supplies, p. 200, Philadelphia, 1904; (a) p. 219; (b) p. 195; (c) p. 282.


MICROSCOPICAL EXAMINATION.

The microscopical examination of water consists of the enumeration of the kinds of microscopic organisms (Plankton), and an estimation of their quantity.

It may serve any one or more of the following purposes:

1. To explain the presence of objectionable odors and tastes.
2. To indicate the progress of the self purification of streams.
3. To indicate the presence of sewage contamination.
4. To explain the chemical analysis.
5. To identify the source of a water.
6. To aid in the study of the food of fish, shellfish, and other aquatic organisms.

The term “Microscopic Organisms” shall include all organisms microscopic or barely visible to the naked eye, with the exception of the bacteria. It includes the diatomaceae, chlorophyceae, cyanophyceae, fungi, protozoa, rotifera, crustacea, bryophyta, and spongidae found in water.

Fragments of organic matter, silt, mineral matter, zoöglea, etc., shall be considered as amorphous matter. The recording of amorphous matter usually serves no useful purpose and shall not be considered a part of the standard method.

Apparatus.—1. A cylindrical funnel about two inches in diameter at the top, with a straight side for nine inches, narrowed over a distance of three inches to a bore of one-half inch in diameter, and terminating in a straight portion of this diameter two and one-half inches in length. The capacity of this funnel is 500 cc. It shall be provided at the bottom with a tightly fitting rubber stopper with a single perforation and a disk of silk bolting cloth over the hole about three eighths of an inch in diameter.

2. A counting cell consisting of a brass rim closely cemented to a plate of optical glass. The shape and size of this cell are not essential but its depth shall be one millimeter. A convenient capacity is about one cubic centimeter.

3. An ocular micrometer ruled as follows: The ocular micrometer is commonly of such a size that with a 16 mm. objective and a situ-
consisting of a metal funnel slung to a pivoted handle, with a disk of wire gauze in the detachable lower end to support the sand. Filtration is hastened by imparting a whirling motion to the whole and utilizing the centrifugal force thus generated.

THE OCULAR MICROMETER.

MICROSCOPICAL BIBLIOGRAPHY.


c. ———. A report of the biological work of the Lawrence Experiment Station: *Examinations by the State Board of Health of water supplies of Mass.*, 1887–90, pt. 2, Purification of sewage and water, pp. 793–862, 1890.

d. Parker, G. H. Report upon the organisms, excepting the bacteria found in the waters of the State: *Examinations by the State Board of Health of water supplies of Mass.*, 1887–90, pt. 1, Examination of water supplies, pp. 579–620, 1890.

BACTERIOLOGICAL EXAMINATION.

I. APPARATUS.

1. Sample Bottles.—Any size, shape or quality of bottle may be used for a bacterial sample, provided it holds a sufficient amount to carry out all the tests required and is such that it may be properly washed and sterilized and will keep the sample uncontaminated until the analysis is made. Four- or eight-ounce, ground-glass-stoppered bottles are recommended. These should be protected by being wrapped in paper, or their necks covered with tin-foil, and should be placed in proper boxes for transportation.

2. Pipettes.—Pipettes may be of any convenient size or shape provided it is found by actual test that they deliver accurately the required amount in the manner in which they are used. The error of calibration shall in no case exceed 2 per cent. Protecting the pipettes with a cotton stopper is recommended.

3. Dilution Bottles.—Bottles for use in making dilutions should preferably be of tall form, of such capacity as to hold at least twice the volume of water actually used. Close-fitting ground-glass stoppers are preferable, but tight-fitting cotton stoppers may be used, provided due care is taken to prevent contamination and to avoid loss of volume through wetting of the stopper before mixing has been accomplished.

4. Petri Dishes.—Petri dishes ten centimeters in diameter shall be used with glass or porous tops\(^{11}\) as preferred. The bottoms of the dishes shall be as flat as possible so that the medium shall be of uniform thickness throughout the plate.

5. Fermentation Tubes.—Any type of fermentation tube\(^3\) may be used provided it holds at least three times as much medium as the amount of water to be tested.
CULTURE MEDIA

II. MATERIALS.

1. Water.—Distilled water shall be used in the preparation of all culture media and reagents.

2. Meat Extract.—Liebig's meat extract shall be used in place of meat infusion. Other brands may be substituted for Liebig's when comparative tests have shown that they give equivalent results.

3. Peptone.—Armour's, Digestive Ferments Company's, Fairchild's, or any other peptone which gives equivalent results may be used.

4. Sugars.—All sugars used shall be of the highest purity obtainable.

5. Agar.—The agar used shall be of the best quality and shall be dried for one-half hour at 105° C. before weighing. Much of the agar on the market contains considerable amounts of sea salts. These may be removed by soaking in water and draining before use.

6. Gelatin.—The gelatin used shall be of light color, shall contain not more than a trace of arsenic, copper, sulfids, and shall be free from preservatives, and of such a melting point that a 10 per cent. standard nutrient gelatin shall have a melting point of 25° C. or over. Gelatin shall be dried for one-half hour at 105° C. before weighing.

7. Litmus.—Reagent litmus of highest purity (not litmus cubes) or azolitmus (Kahlbaum) shall be used for all media requiring a litmus indicator.

8. General Chemicals.—Special effort shall be made to have all the other ingredients used for culture media chemically pure.

III. METHODS.

1. PREPARATION OF CULTURE MEDIA.

   a. Adjustment of Reaction.

      aa. Phenol Red Method for adjustment to a hydrogen-ion concentration of \( P_{H^+} = 6.8-8.4 \). Withdraw 5 cc. of the medium, dilute with 5 cc. of distilled water, and add 5 drops of a solution of phenol red (phenol sulphone phthalein). This solution is made by dissolving 0.04 grams of phenol red in 30 cc. of alcohol and diluting to 100 cc. with distilled water.

      Titrate with a 1:10 dilution of a standard solution of NaOH (which need not be of known normality) until the phenol red shows a slight but distinct pink color. Calculate the amount of the standard NaOH solution which must be added to the medium to reach
this reaction. After the addition check the reaction by adding 5 drops of phenol red to 5 cc. of the medium and 5 cc. of water.

\textit{bb. Titration with phenolphthalein.} (For the convenience of those who wish to retain the use of this method for the present it is given here, but it is recommended that as soon as possible the more accurate method of determining the hydrogen-ion concentration be substituted.)

In a white porcelain dish put 5 cc. of the medium to be tested, add 45 cc. of distilled water. Boil briskly for one minute. Add 1 cc. of phenolphthalein solution (5 grams of commercial salt to one liter of 50 per cent. alcohol). Titrate immediately with a n/20 solution of sodium hydrate. A faint but distinct pink color marks the true end point. This color may be precisely described as a combination of 25 per cent. of red (wave length approximately 658) with 75 per cent. of white as shown by the disks of the standard color top made by the Milton Bradley Educational Co., Springfield, Mass.

All reactions shall be expressed with reference to the phenolphthalein neutral point and shall be stated in percentages of normal acid or alkali solutions required to neutralize them. Alkaline media shall be recorded with a minus (−) sign before the percentage of normal acid needed for their neutralization and acid media with a plus (+) sign before the percentage of normal alkali solution needed for their neutralization.

The standard reaction for culture media for water analysis shall be +1.0 per cent., as determined by tests of the sterilized medium. As ordinarily prepared, broth and agar will be found to have a reaction between +0.5 and +1.0. For such media no adjustment shall be made. The reaction of media containing sugar shall be neutral to phenolphthalein. Whenever reactions other than the standard are used, it shall be so stated.

\textbf{b. Sterilization.}

All media and dilution water shall be sterilized in the autoclave at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs. All air must be forced out of the autoclave before the pressure is allowed to rise. As soon as possible after sterilization the media shall be removed from the autoclave and cooled rapidly. Rapid and immediate cooling of gelatin is imperative.

Media shall be sterilized in small containers, and these must not be closely packed together. No part of the medium shall be more
than 2.5 cm. from the outside surface of the glass. All glassware shall be sterilized in the dry oven at 170° C. for at least 1½ hours.

c. Nutrient Broth. To Make One Liter.

1. Add 3 grams of beef extract and 5 grams of peptone to 1,000 cc. of distilled water.
2. Heat slowly on a steam bath to at least 65° C.
3. Make up lost weight and adjust the reaction to a faint pink with phenol red, or if the phenolphthalein titration is used, and the reaction is not already between +0.5 and +1, adjust to +1.
5. Distribute in test-tubes, 10 cc. to each tube.
6. Sterilize in the autoclav at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

d. Sugar Broths.

Sugar broths shall be prepared in the same general manner as nutrient broth with the addition of 0.5 per cent. of the required carbohydrate just before sterilization. The removal of muscle sugar is unnecessary as the beef extract and peptone are free from any fermentable carbohydrates. The reaction of sugar broths shall be a faint pink with phenol red or, if on titration with phenolphthalein the reaction is not already between neutral and +1, adjust to neutral. Sterilization shall be in the autoclav at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs., provided the total time of exposure to heat is not more than one-half hour; otherwise a 10 per cent. solution of the required carbohydrate shall be made in distilled water and sterilized at 100° C. for 1½ hours, and this solution shall be added to sterile nutrient broth in amounts sufficient to make a 0.5 per cent. solution of the carbohydrate and the mixture shall then be tubed and sterilized at 100° C. for 30 minutes, or it is permissible to add by means of a sterile pipette directly to a tube of sterile neutral broth enough of the carbohydrate to make the required 0.5 per cent. The tubes so made shall be incubated at 37° C. for 24 hours as a test for sterility.

e. Nutrient Gelatin. To Make One Liter.

1. Add 3 grams of beef extract and 5 grams of peptone to 1,000 cc. of distilled water and add 100 grams of gelatin dried for one-half hour at 105° C. before weighing.
2. Heat slowly on a steam bath to 65° C. until all gelatin is dissolved.*

3. Make up lost weight and adjust the reaction to a faint pink with phenol red, or if the phenolphthalein titration is used, and the reaction is not already between +0.5 and +1, adjust to +1.

4. Filter through cloth and cotton until clear.

5. Distribute in test-tubes, 10 cc. to each tube, or in larger containers as desired.

6. Sterilize in the autoclav at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

1. Nutrient Agar. To Make One Liter.

1. Add 3 grams of beef extract, 5 grams of peptone and 12 grams of agar, dried for one-half hour at 105° C. before weighing, to 1,000 cc. of distilled water. Boil over a water bath until all agar is dissolved, and then make up the loss by evaporation.

2. Cool to 45° C. in a cold water bath, then warm to 65° C. in the same bath, without stirring.

3. Make up lost weight and adjust the reaction to a faint pink with phenol red, or if the phenolphthalein titration is used, and the reaction is not already between +0.5 and +1, adjust to +1.

4. Filter through cloth and cotton until clear.

5. Distribute in test-tubes, 10 cc. to each tube, or in larger containers, as desired.

6. Sterilize in the autoclav at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

g. Litmus or Azolitmin Solution.

The standard litmus solution shall be a 2 per cent. aqueous solution of reagent litmus. Powder the litmus, add to the water and boil for five minutes. The solution usually needs no correction in reaction and may be at once distributed in flasks or test-tubes and sterilized as is culture media. It should give a distinctly blue plate when 1 cc. is added to 10 cc. of neutral culture medium in a Petri dish.

The standard azolitmin solution shall be a 1 per cent. solution of Kahlbaum’s azolitmin. Add the azolitmin powder to the water and boil for five minutes. The solution may need to be corrected in reaction by the addition of sodium hydrate solution so that it will be approximately neutral and will give a distinctly blue plate

*The solution of the gelatin will be facilitated by allowing it to soak in the cold one-half hour before heating.
when 1 cc. is added to 10 cc. of neutral culture medium in a Petri dish. It may be distributed in flasks or test-tubes and sterilized as is culture media.

h. Litmus-lactose-agar.

Litmus-lactose-agar shall be prepared in the same manner as nutrient agar with the addition of 1 per cent. of lactose just before sterilization. The reaction shall be a faint pink with phenol red, or, if on titration with phenolphthalein the reaction is not already between neutral and +1, adjust to neutral. One cc. of sterilized litmus or azolitmin solution shall be added to each 10 cc. of the medium just before it is poured into the Petri dish, or the mixture may be made in the dish itself.

i. Endo's Medium. To Make One Liter.

1. Add 5 grams of beef extract, 10 grams of peptone and 30 grams of agar dried for one-half hour at 105° C. before weighing, to 1,000 cc. of distilled water. Boil on a water bath until all the agar is dissolved and then make up the loss by evaporation.

2. Cool the mixture to 45° C. in a cold water bath, then warm to 65° C. in the same bath without stirring.

3. Make up lost weight, titrate, and if the reaction is not already between neutral and +1 adjust to neutral.

4. Filter through cloth and cotton until clear.

5. Distribute 100 cc. or larger known quantities in flasks large enough to hold the other ingredients which are to be added later.

6. Sterilize in the autoclave at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

7. Prepare a 10 per cent. solution of basic fuchsin in 95 per cent. alcohol, allow to stand 20 hours, decant and filter the supernatant fluid. This is a stock solution.

8. When ready to make plates melt 100 cc. of agar in streaming steam or on a waterbath. Dissolve 1 gram of lactose in 15 cc. of distilled water, using heat if necessary. Dissolve 0.25 gram anhydrous sodium sulphite in 10 cc. water. To the sulphite solution add 0.5 cc. of the fuchsin stock solution. Add the fuchsin-sulphite solution to the lactose solution and then add the resulting solution to the melted agar. The lactose used must be chemically pure and the sulphite solution must be made up fresh.

9. Pour plates and allow to harden thoroughly in the incubator before use.
2. COLLECTION OF SAMPLE.

Samples for bacterial analysis shall be collected in bottles which have been cleansed with great care, rinsed in clean water, and sterilized with dry heat for at least one hour and a half at 170° C., or in the autoclave at 15 lbs. (120° C.) for 15 minutes or longer after the pressure reaches 15 lbs.

Great care must be exercised to have the samples representative of the water to be tested and to see that no contamination occurs at the time of filling the sample bottles.

3. STORAGE AND TRANSPORTATION OF SAMPLES.

Because of the rapid and often extensive changes which may take place in the bacterial flora of bottled samples when stored even at temperatures as low as 10° C., it is urged, as of importance, that all samples be examined as promptly as possible after collection.

The time allowed for storage or transportation of a bacterial sample between the filling of the sample bottle and the beginning of the analysis should be not more than six hours for impure waters and not more than twelve hours for relatively pure waters. During the period of storage, the temperature shall be kept as near 10° C. as possible. Any deviation from the above limits shall be so stated in making reports.

4. DILUTIONS.

Dilution bottles shall be filled with the proper amount of tap water so that after sterilization they shall contain exactly 9 cc. or 99 cc. as desired. The exact amount of water can only be determined by experiment with the particular autoclave in use. If desired, the 9 cc. dilution may be measured out from a flask of sterile water with a sterile pipette.

Dilution bottles shall be sterilized in the autoclave at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

The sample bottle shall be shaken vigorously 25 times and 1 cc. withdrawn and added to the proper dilution bottles as required. Each dilution bottle after the addition of the 1 cc. of the sample, shall be shaken vigorously 25 times before a second dilution is made from it or before a sample is removed for plating.
5. PLATING.

All sample and dilution bottles shall be shaken vigorously 25 times before samples are removed for plating. Plating shall be done immediately after the dilutions are made. One cc. of the sample or dilution shall be used for plating and shall be placed in the Petri dish, first. Ten cc. of liquefied medium at a temperature of 40° C. shall be added to the 1 cc. of water in the Petri dish. The cover of the Petri dish shall be lifted just enough for the introduction of the pipette or culture medium, and the lips of all test-tubes or flasks used for pouring the medium shall be flamed. In making litmus-lactose-agar plates, 1 cc. of sterile litmus or azolitmin solution shall be added to each 10 cc. of culture medium either in the Petri dish or before pouring into the Petri dish. The medium and sample in the Petri dish shall be thoroughly mixed and uniformly spread over the bottom of the Petri dish by tilting or rotating the dish. All plates shall be solidified as rapidly as possible after pouring and gelatin plates shall be placed immediately in the 20° C. incubator and the agar plates in the 37° C. incubator. Endo plates shall be made by placing one loopful of the material to be tested on the surface of the plate and distributing the material with a sterile loop or glass rod.

6. INCUBATION.

All gelatin plates shall be incubated for 48 hours at 20 C. in a dark, well-ventilated incubator in an atmosphere practically saturated with moisture.  

All agar plates shall be incubated for 24 hours at 37° C. in a dark, well-ventilated incubator in an atmosphere practically saturated with moisture. Glass covered plates shall be inverted in the incubator. Any deviation from the above described method shall be stated in making reports.

7. COUNTING.

In preparing plates, such amounts of the water under examination shall be planted as will give from 25 to 250 colonies on a plate, and the aim should be always to have at least two plates giving colonies between these limits. Where it is possible to obtain plates showing colonies within these limits, only such plates should be considered in recording results, except where the same amount of water has been planted in two or more plates, of which one gives colonies
within these limits, while the others give less than 25 or more than 250. In such case, the result recorded should be the average of all the plates planted with this amount of water. Ordinarily it is not desirable to plant more than 1 cc. of water in a plate; therefore, when the total number of colonies developing from 1 cc. is less than 25, it is obviously necessary to record the results as observed, disregarding the general rule given above.

Counting shall in all cases be done with a lens of 2½ diameter's magnification, 3½X. The Engraver's Lens No. 146 made by the Bausch & Lomb Optical Company fills the requirements, and is a convenient lens for the purpose.

8. THE TEST FOR THE PRESENCE OF MEMBERS OF THE B. COLI GROUP.

It is recommended that the B. coli group be considered as including all non-spore-forming bacilli which ferment lactose with gas formation and grow aerobicically on standard solid media.

The formation of 10 per cent. or more of gas in a standard lactose broth fermentation tube within 24 hours at 37° C. is presumptive evidence of the presence of members of the B. coli group, since the majority of the bacteria which give such a reaction belong to this group.

The appearance of aerobic lactose-splitting colonies on lactose-litmus-agar or Endo's medium plates made from a lactose-broth fermentation tube in which gas has formed confirms to a considerable extent the presumption that gas-formation in the fermentation tube was due to the presence of members of the B. coli group.

To complete the demonstration of the presence of B. coli as above defined, it is necessary to show that one or more of these aerobic plate colonies consists of non-spore-forming bacilli which, when inoculated into a lactose-broth fermentation tube, form gas.

It is recommended that the standard tests for the B. coli group be either (A) the Presumptive, (B) the Partially Confirmed, or (C) the Completed test as hereafter defined, each test being applicable under the circumstances specified.

A. PRESumptIVE TEST.

1. Inoculate a series of fermentation tubes with appropriate graduated quantities of the water to be tested. In every fermentation tube there must always be at least three times as much medium as the amount of water to be tested. When necessary to examine larger amounts than 10 cc. as many tubes as necessary shall be inoculated with 10 cc. each.

2. Incubate these tubes at 37° C. for 48 hours. Examine each
tube at 24 and 48 hours, and record gas-formation. The records should be such as to distinguish between:
(a) Absence of gas-formation.
(b) Formation of gas occupying less than ten per cent. (10%) of the closed arm.
(c) Formation of gas occupying more than ten per cent. (10%) of the closed arm.
More detailed records of the amount of gas formed, though desirable for purposes of study, are not necessary for carrying out the standard tests prescribed.
3. The formation within 24 hours of gas occupying more than ten per cent. (10%) of the closed arm of fermentation tube constitutes a positive presumptive test.
4. If no gas is formed in 24 hours, or if the gas formed is less than ten per cent. (10%), the incubation shall be continued to 48 hours. The presence of gas in any amount in such a tube at 48 hours constitutes a doubtful test, which in all cases requires confirmation.
5. The absence of gas formation after 48 hours' incubation constitutes a negative test. (An arbitrary limit of 48 hours' observation doubtless excludes from consideration occasional members of the B. coli group which form gas very slowly, but for the purposes of a standard test the exclusion of these occasional slow gas forming organisms is considered immaterial.)

B. PARTIALLY CONFIRMED TEST.
1. Make one or more Endo's medium or lactose-litmus-agar plates from the tube which, after 48 hours' incubation, shows gas formation from the smallest amount of water tested. (For example, if the water has been tested in amounts of 10 cc., 1 cc., and 0.1 cc., and gas is formed in 10 cc., and 1 cc., not in 0.1 cc., the test need be confirmed only in the 1 cc. amount.)
2. Incubate the plates at 37° C., 18 to 24 hours.
3. If typical colon-like red colonies have developed upon the plate within this period, the confirmed test may be considered positive.
4. If, however, no typical colonies have developed within 24 hours, the test cannot yet be considered definitely negative, since it not infrequently happens that members of the B. coli group fail to form typical colonies on Endo's medium or lactose-litmus-agar plates, or that the colonies develop slowly. In such case, it is always necessary to complete the test as directed under "C" 2 and 3.
C. COMPLETED TEST.

1. From the Endo's medium or lactose-litmus-agar plate made as prescribed under "B," fish at least two typical colonies, transferring each to an agar slant and a lactose broth fermentation tube.

2. If no typical colonies appear upon the plate within 24 hours, the plate should be reincubated another 24 hours, after which at least two of the colonies considered to be most likely B. coli, whether typical or not, shall be transferred to agar slants and lactose broth fermentation tubes.

3. The lactose broth fermentation tubes thus inoculated shall be incubated until gas formation is noted; the incubation not to exceed 48 hours. The agar slants shall be incubated at 37° C. for 48 hours, when a microscopic examination shall be made of at least one culture, selecting one which corresponds to one of the lactose broth fermentation tubes which has shown gas-formation.

The formation of gas in lactose broth and the demonstration of non-spore-forming bacilli in the agar culture shall be considered a satisfactory completed test, demonstrating the presence of a member of the B. coli group.

The absence of gas-formation in lactose broth or failure to demonstrate non-spore-forming bacilli in a gas-forming culture constitutes a negative test.

APPLICATION OF PRESumptIVE, PARTIALLY CONFIRMED, AND COMPLETED TESTS.

A. The Presumptive Test.

1. When definitely positive, that is showing more than 10 per cent. (10%) of gas in 24 hours, is sufficient:

   (a) As applied to all except the smallest gas-forming portion of each sample in all examinations.

   (b) As applied to the smallest gas-forming portion in the examination of sewage or of water showing relatively high pollution, such that its fitness for use as drinking water does not come into consideration. This applies to the routine examinations of raw water in-connection with control of the operation of purification plants.

2. When definitely negative, that is showing no gas in 48 hours, is final and therefore sufficient in all cases.
3. When doubtful, that is showing gas less than 10 per cent. (10%) (or none) in 24 hours, with gas either more or less than 10 per cent. in 48 hours, must always be confirmed.

B. The Partially Confirmed Test.

1. When definitely positive, that is, showing typical plate colonies within 24 hours, is sufficient:
   (a) When applied to confirm a doubtful presumptive test in cases where the latter, if definitely positive, would have been sufficient.
   (b) In the routine examination of water-supplies where a sufficient number of prior examinations have established a satisfactory index of the accuracy and significance of this test in terms of the completed test.

2. When doubtful, that is, showing colonies of doubtful or negative appearance in 24 hours, must always be completed.

C. The Completed Test.

The completed test is required as applied to the smallest gas-forming portion of each sample in all cases other than those noted as exceptions under the "presumptive" and the "partially confirmed" tests.

The completed test is required in all cases where the result of the confirmed test has been doubtful.

9. EXPRESSION OF RESULTS.

In order to avoid fictitious accuracy and yet to express the numerical results by a method consistent with the precision of the work, the numbers of colonies of bacteria per cubic centimeter shall be recorded as follows:

<table>
<thead>
<tr>
<th>Number of bacteria per cc.</th>
<th>Range</th>
<th>Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 1 to 50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>51</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>101</td>
<td>5</td>
<td>500</td>
</tr>
<tr>
<td>251</td>
<td>25</td>
<td>1,000</td>
</tr>
<tr>
<td>501</td>
<td>50</td>
<td>10,000</td>
</tr>
<tr>
<td>1,001</td>
<td>100</td>
<td>50,000</td>
</tr>
<tr>
<td>10,001</td>
<td>500</td>
<td>100,000</td>
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<tr>
<td>50,001</td>
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<td>100,001</td>
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<tr>
<td>500,001</td>
<td>50,000</td>
<td>10,000,000</td>
</tr>
<tr>
<td>1,000,001</td>
<td>100,000</td>
<td></td>
</tr>
</tbody>
</table>

This applies to the gelatin count at 20° C. and to the agar count at 37° C.
### Summary of Steps Involved in Making Presumptive, Partially Confirmed and Completed Tests for B. coli

<table>
<thead>
<tr>
<th>Steps in procedure</th>
<th>Further procedure required</th>
</tr>
</thead>
</table>
| I. Inoculate lactose broth fermentation tubes; incubate 24 hours at 37°C; observe gas-formation in each tube.  
1. Gas-formation, 10 per cent. or more; constitutes positive presumptive test.  
   (a) For other than smallest portion of any sample showing gas at this time, and for all portions, including smallest, of sewage and raw water this test is sufficient.  
   (b) For smallest gas-forming portion, except in examinations of sewage and raw water. | None |
| 2. Gas-formation less than 10 per cent. in 24 hours; inconclusive | III |
| II. Incubate an additional 24 hours, making a total of 48 hours' incubation; observe gas-formation.  
1. Gas-formation, any amount; constitutes doubtful test, which must always be carried further | III |
| 2. No gas-formation in 48 hours; constitutes final negative test | None |
| III. Make plate from smallest gas-forming portion of sample under examination; incubate 18 to 24 hours; observe colonies.  
1. One or more colonies typical in appearance.  
   (a) If only "partially confirmed" test is required  
   (b) If completed test is required, select two typical colonies for identification. | None |
| 2. No typical colonies | V |
| IV. Replace plate in incubator for an additional 18 to 24 hours; then, whether colonies appear typical or not, select at least two of those which most nearly resemble B. coli. | IV |
| V. Transfer each colony fished to:  
1. Lactose broth fermentation tube; incubate not more than 48 hours at 37°C. Observe gas-formation. | None |
| 2. Agar slant; incubate 48 hours at 37°C.  
   (a) If gas formed in lactose broth tube inoculated with corresponding culture.  
   (b) If no gas formed in corresponding lactose broth tube, test is completed and negative. | VI |
| VI. Make stained cover-slip or slide preparation, and examine microscopically.  
1. If preparation shows non-spore-forming bacilli in apparently pure culture, demonstration of B. coli is completed. | None |
| 2. If preparation fails to show non-spore-forming bacilli or shows them mixed with spore-bearing forms or bacteria of other morphology | VII |
| VII. Replate, to obtain assuredly pure culture, select several colonies of bacilli and repeat steps V and VI. |    |
In order that tests for B. coli may have quantitative significance, the following general principles and rules should be observed:

Ordinarily not less than three portions of each sample should be tested, the portions being even decimal multiples or fractions of a cubic centimeter; for example, 10 cc., 1 cc., 0.1 cc., .01 cc., etc. It is essential that the dilutions should be such that the largest amount gives a positive test (unless the water is such as to give negative tests in 10 cc.), and the smallest dilution, a negative result. To insure this result, it is often necessary to plant four or five dilutions, especially in the examination of a sample of entirely unknown quality. The quantitative value of a series of tests is lost, unless all or at least a large proportion of the smallest dilutions tested have given negative results.

In reporting a single test, it is preferable merely to record results as observed, indicating the amounts tested and the result in each, rather than to attempt expression of the result in numbers of B. coli per cc. In summarizing the results of a series of tests, however, it is desirable, for the sake of simplicity, to express the results in terms of the numbers of B. coli per cc., or per 100 cc. To convert results of fermentation tests to this form, the result of each test is recorded as indicating a number of B. coli per cc. equal to the reciprocal of the smallest decimal or multiple fraction of a cubic centimeter giving a positive result. For example, the result: 10 cc. +; 1 cc. +; 0.1 cc. –; would be recorded as indicating one B. coli per cc. An exception should be made in the case where a negative result is obtained in an amount larger than the smallest portion giving a positive result; for example, in a result such as: 10 cc. +; 1 cc. –; 0.1 cc. +. In such case, the result should be recorded as indicating a number of B. coli per cc. equal to the reciprocal of the dilution next larger than the smallest one giving a positive test, this being a more probable result.

Where tests are made in amounts larger than 1 cc., giving average results less than one B. coli per cc., it is more convenient to express results in terms of the numbers of B. coli per 100 cc.

The following table illustrates the method of recording and averaging results of B. coli tests:
The above method of expressing results is not mathematically altogether correct. The average number of B. coli per cc., as thus estimated, is not precisely the most probable number calculated by application of the theory of probability. To apply this theory to a correct mathematical solution of any considerable series of results involves, however, mathematical calculations so complex as to be impracticable of application in general practice. The simpler method given is therefore considered preferable, since it is easily applied and the results so expressed are readily comprehensible.

In order that results as reported may be checked and carefully valued, it is necessary that the report should show not only the average number of B. coli per cc., but also the number of samples examined; and, for each dilution, the total number of tests made, and the number (or per cent.) positive.

10. INTERPRETATION OF RESULTS.

While it is not within the province of this report to suggest the proper interpretation of results obtained by the use of the methods herein specified as standard, the committee feels that a word of caution should be given regarding the significance of the presence in a water of members of the B. coli group as defined in this report. Recent work seems to indicate that the B. coli group as herein defined consists of organisms of both fecal and non-fecal origin. Therefore care must be exercised in judging the sanitary quality of a water solely from the determination of the presence of members of the group.

11. DIFFERENTIATION OF FECAL FROM NON-FECAL MEMBERS OF THE B. COLI GROUP.

(1) At least 10 cultures should be used. If possible these should be subcultured from plates made direct from the water since all of the cultures obtained by plating from fermentation tubes may be
descendants of a single cell in the water. If cultures from water plates are not available those obtained from plates made as prescribed under B (p. 101) may be used.

(2) Inoculate each culture into dextrose potassium phosphate broth,* adonite broth, and gelatin. For additional confirmatory evidence inoculation may be made into tryptophane broth,† and saccharose broth. The dextrose broth must be incubated at 30°. Other sugar broths may be incubated at 30° or 37° as convenient. Gelatin should be incubated at 20°.

(3) After 48 hours record gas formation in adonite and saccharose broths. Determine indol formation in tryptophane broth by adding drop by drop, to avoid mixing with the medium, about 1 cc. of a 2 per cent. alcoholic solution of p-dimethyl amido-benzaldehyd, then a few drops of concentrated hydrochloric acid. The presence of indol is indicated by a red color which is soluble in chloroform. There may be some unconverted tryptophane still present which will give a distinctly blue color which is insoluble in chloroform. A mixture of the two will be either blue or violet. If from such a mixture of colors the red of indol be extracted with chloroform proof of the presence of indol will be complete.

(4) After 5 days apply methyl red test and Voges-Proskauer test to dextrose broth.

Methyl Red Test.*

Indicator solution.—Dissolve 0.1 gram methyl red in 300 cc. alcohol and dilute to 500 cc. with distilled water.

Procedure in test.—1. To 5 cc. of each culture add 5 drops of methyl red solution.

* *(a) Peptone Medium for the Methyl Red Test. To Make One Liter.
1. To 800 cc. of distilled water add 5 grams of Proteose-Peptone, Difco., or Witte's Peptone (other peptones should not be substituted), 5 grams c. p. dextrose, and 5 grams dipotassium hydrogen phosphate (K₂HPO₄). A dilute solution of the K₂HPO₄ should give a distinct pink with phenolphthalein.
2. Heat with occasional stirring over steam for twenty minutes.
3. Filter through folded filter paper, cool to 20° C. and dilute to 1,000 cc. with distilled water.
4. Distribute 10 cc. portions in sterilised test tubes.
5. Sterilize by the intermittent method for 20 minutes on three successive days.

(b) Synthetic Medium for the Methyl Red Test. To Make One Liter. Dissolve 7 grams Na₂HPO₄ (anhydrous) or 8.8 grams Na₂HPO₄.2H₂O, 2 grams KH₂PO₄, 1 gram aspartic acid, and 4 grams dextrose in about 800 cc. of warm distilled water. When solution is complete, cool and make up to 1 liter at room temperature. Heat in an autoclave for 15 minutes after the pressure has reached 15 pounds, provided the total time of exposure to heat is not more than one-half hour. The hydrox- ion concentration of the medium is fixed by the composition. It should be very close to pH 7.0, slightly red with phenol red. All materials should be recrystallised or if used from stock furnished by manufacturers, should be carefully examined. The di-sodium hydrogen phosphate may be used either as the anhydrous salt obtained by desiccation in vacuo at 100° C. or else as the salt containing two molecules of water of crystallisation. This is obtained by exposing the recrystallised Na₂HPO₄.2H₂O for two weeks. Use 0.38 per cent. of Na₂HPO₄.2H₂O.

† Tryptophane Broth for Indol Test.
To 1,000 cc. of distilled water add 0.3 gram tryptophane, 5 grams dipotassium hydrogen phosphate (K₂HPO₄), and 1 gram peptone. Heat until ingredients are thoroughly dissolved, tube (6 to 8 cc.), and place in autoclave for 15 minutes after the pressure reaches 15 pounds. Some American peptones are standardised to contain a uniform amount of tryptophane. If such peptone is used the tryptophane in the above formula may be omitted and the peptone increased to 5 grams.
2. Record distinct red color as methyl red +, distinct yellow color as methyl red −, and intermediate colors as ?.

*Voges-Proskauer Test.*

To the remaining 5 cc. of medium add 5 cc. of a 10 per cent. solution of potassium hydroxide. Allow to stand over night. A positive test is indicated by an eosin pink color.

(5) Gelatin tubes should not be pronounced negative until they have been incubated at least 15 days.

The following group reactions indicate the source of the culture with a high degree of probability:

<table>
<thead>
<tr>
<th>Methyl red +</th>
<th>Voges-Proskauer −</th>
<th>Gelatin −</th>
<th>Adonite −</th>
<th>Indol, usually +</th>
<th>Saccharose, usually −</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. coli of fecal origin.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methyl red −</th>
<th>Voges-Proskauer +</th>
<th>Gelatin −</th>
<th>Adonite +</th>
<th>Indol, usually −</th>
<th>Saccharose +</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. aërogenes of fecal origin.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methyl red −</th>
<th>Voges-Proskauer +</th>
<th>Gelatin −</th>
<th>Adonite −</th>
<th>Indol, usually −</th>
<th>Saccharose +</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. aërogenes, probably not of fecal origin.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methyl red −</th>
<th>Voges-Proskauer +</th>
<th>Gelatin −</th>
<th>Adonite +</th>
<th>Indol, usually −</th>
<th>Saccharose +</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cloacae, may or may not be of fecal origin.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. ROUTINE PROCEDURE FOR EXAMINATION OF SAMPLES OF WATER.

*First Day:*

1. Prepare dilutions as required.
2. Make two (2) gelatin plates from each dilution, and incubate at 20° C.
3. Make two (2) agar plates from each dilution, and incubate at 37° C.
4. Inoculate lactose broth fermentation tubes with appropriate amounts for B. coli tests, inoculating two (2) tubes with each amount.

Note:—Where repeated tests are made of water from the same source, as is customary in the control of public supplies, it is not necessary to make duplicate plates or fermentation tubes in each dilution. It is sufficient, in such circumstances, to make duplicate plates only from the dilution which will most probably give from 25 to 250 colonies per plate.

Second Day:
1. Count the agar plates made on the first day.
2. Record the number of lactose broth fermentation tubes which show 10 per cent. (10%) or more of gas.

Note:—In case only the presumptive test for B. coli is required, fermentation tubes showing more than 10 per cent. (10%) of gas at this time may be discarded.

Third Day:
1. Count gelatin plates made on first day.
2. Record the number of additional fermentation tubes which show 10 per cent. (10%) or more of gas.
3. Make a lactose-litmus-agar or Endo’s medium plate from the smallest portion of each sample showing gas. Incubate plate at 37° C.

Note:—In case the smallest portion in which gas has been formed shows less than 10 per cent. (10%) of gas, it is well to make a plate also from the next larger portion, so that, in case the smallest portion gives a negative end result it may still be possible to demonstrate B. coli in the next larger dilution.

Fourth Day:
1. Examine Endo’s medium or lactose-litmus-agar plates. If typical colonies have developed, select two and transfer each to a lactose broth fermentation tube and an agar slant, both of which are to be incubated at 37° C.
2. If no typical B. coli colonies are found, incubate the plates another 24 hours.

Fifth Day:
1. Select at least two colonies, whether typical or not, from the Endo’s medium or lactose-litmus-agar plates which have been incubated an additional 24 hours; transfer each to a lactose
broth fermentation tube and an agar slant, and complete the test as for typical colonies.

2. Examine lactose broth fermentation tubes inoculated from plates on the previous day. Tubes in which gas has been formed may be discarded after the result has been recorded. Those in which no gas has formed should be incubated an additional 24 hours.

**Sixth Day:**

1. Examine lactose broth fermentation tubes reincubated the previous day.

2. Examine microscopically agar slants corresponding to lactose fermentation tubes inoculated from plate colonies and showing gas-formation.

**BACTERIOLOGICAL BIBLIOGRAPHY.**


33. Jones, H. M. A rapid hydrogen-ion electrode method for the determination of hydrogen-ion concentrations in bacterial cultures or other turbid or colored solutions. Jour. of Inf. Diseases, 25, 1919, 262.
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