

## Serial Dilutions Method

**Scope and application:** For non-potable water and wastewater.



### Test preparation

## MPN Dilution Guidelines

It is necessary to dilute non-potable water samples so that an MPN test has one positive or one negative tube (at minimum) out of the 15 tubes.

If all of the tubes in an MPN test are positive, dilute the samples and do the test again until the dilution series gives both positive and negative tubes. Refer to the dilution tables in [Serial sample dilutions](#) on page 1.

If all of the tubes in an MPN test are positive or negative, then the sample was not correctly diluted. Do the test again and use less serial dilutions.

## Sterile buffered dilution water

Use dilution water that is buffered to a neutral pH and sterilized for microbiological testing. Hach dilution water is recommended for dilution of most non-potable and wastewater samples. Each bottle contains 99 mL of sterile buffered dilution water.

When 11 mL of sample is added to a 99-mL bottle of dilution water, the sample is diluted by a factor of 10 (10-fold dilution). Before and after the sample is added, make sure to fully mix the bottles. The dilution factor of an undiluted sample = 1.

## Serial sample dilutions

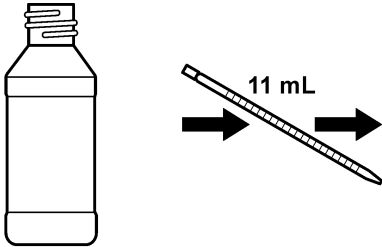
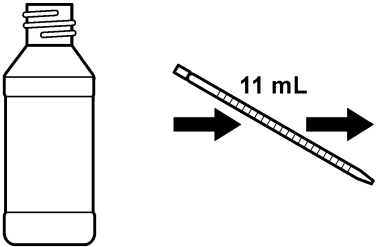
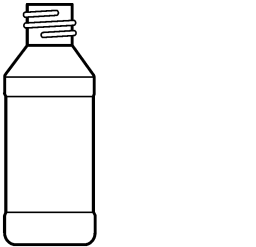
Do the steps that follow to make serial dilutions of the sample. Refer to the tables that follow to find the number of dilutions for different sample types.

**Example:** For Class A sludge, add 10 mL of the 100x sample dilution into five tubes, 10 mL of the 1000x sample dilution into another five tubes and 10 mL of the 10,000x sample dilution into the last five tubes. If the coliform density is not known, add five separate dilutions to five sets of five MPN tubes.

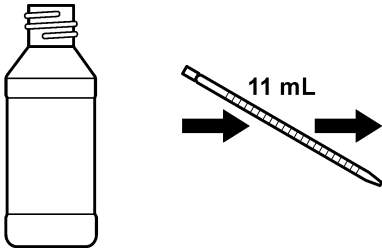
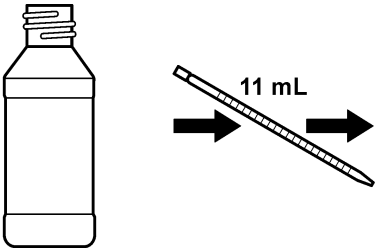
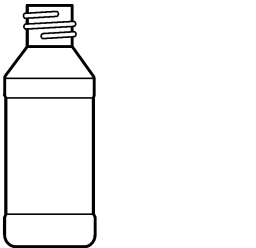
1. Wash hands thoroughly with soap and water. Gloves are optional.
2. Vigorously mix the sample for 30 seconds.
3. Open a bottle of sterile buffered dilution water.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert for 30 seconds (25 times). This is a 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. Continue to make dilutions until there are three bottles that contain the dilutions shown in the tables that follow.

**Note:** Do not shake the sample too vigorously because this will injure or stress the organisms.

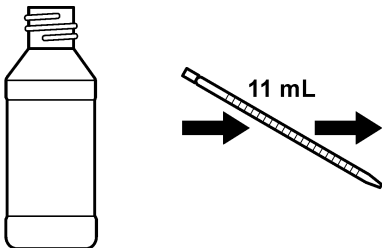
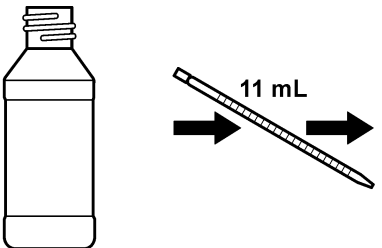
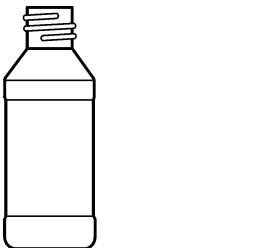
**Table 1 Swimming pool water, chlorinated—Lowest Dilution Factor (LDF) = 1**

		
<b>Bottle A</b> Undiluted sample Inoculate 5 tubes	<b>Bottle B</b> 10x dilution 99 mL dilution water Inoculate 5 tubes	<b>Bottle C</b> 100x dilution 99 mL dilution water Inoculate 5 tubes

**Table 2 Bathing beach water; lake water, unpolluted river water—LDF = 10**

		
<b>Bottle B (from Table 1)</b> 10x dilution 99 mL dilution water Inoculate 5 tubes	<b>Bottle C</b> 100x dilution 99 mL dilution water Inoculate 5 tubes	<b>Bottle D</b> 1000x dilution 99 mL dilution water Inoculate 5 tubes

**Table 3 Final wastewater effluent, chlorinated—LDF= 100**

		
<b>Bottle C (from Table 2)</b> 100x dilution 99 mL dilution water Inoculate 5 tubes	<b>Bottle D</b> 1000x dilution 99 mL dilution water Inoculate 5 tubes	<b>Bottle E</b> 10,000x dilution 99 mL dilution water Inoculate 5 tubes

**Table 4 River water, polluted—LDF = 1000**

<p><b>Bottle D</b> (from <a href="#">Table 3</a>)            1000x dilution            99 mL dilution water            Inoculate 5 tubes</p>	<p><b>Bottle E</b>            10,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>	<p><b>Bottle F</b>            100,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>

**Table 5 Storm water or unchlorinated final wastewater effluent—LDF= 10,000**

<p><b>Bottle E</b> (from <a href="#">Table 4</a>)            10,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>	<p><b>Bottle F</b>            100,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>	<p><b>Bottle G</b>            1,000,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>

**Table 6 Raw sewage—LDF= 100,000**

<p><b>Bottle F</b> (from <a href="#">Table 5</a>)            100,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>	<p><b>Bottle G</b>            1,000,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>	<p><b>Bottle H</b>            10,000,000x dilution            99 mL dilution water            Inoculate 5 tubes</p>



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