



40 minutes total

## Lesson Plan 08 Dilutions

### Overview of Activities

1. Introduction
2. Dilution Theory
3. Dilution Factors
4. Review

### Learning Outcomes

At the end of this session participants will be able to:

1. Discuss the importance of diluting water samples
2. Calculate a Dilution Factor

#### Materials & Preparation

- Activity: Introduction Demonstration
- Powdered or liquid drink concentrate
- Six clear drinking glasses (plastic) or containers
- Water for filling water glasses (one litre)
- Spoon for mixing
- Prepare: Flip chart paper with the title: When to Dilute
- Activity: Dilution Factors
- Handout: Dilution Exercise, Option 1 and Option 2, see end of lesson (one page; one copy per group), cut the paper to separate the two options.
- PowerPoint: Dilutions
- Activity: Review
- Handout: Dilution Chart, see end below (one page, one copy per participant)

#### Recommended Reading

- Technical Brief: Quality Control in Drinking Water Quality Testing

5 minutes



## Introduction

Use PowerPoint: Dilutions for this presentation (optional).

1. Pour water into six plastic water glasses (or containers) to two-thirds full.
2. Demonstrate adding powdered drink concentrate into the first glass of water to fill the glass (or fill an empty glass with full strength liquid drink concentrate). Make sure it is **much** too concentrated. Stir.
3. Pour from the concentrated glass to the next glass in the series to dilute the drink concentrate. Continue the process until the final glass in the series is significantly diluted.
4. Ask: “What do you call the process just demonstrated?”
  - Dilution
5. Ask: “Why is this done?”
  - To reach a concentration that is appropriate (in this case, good to drink)
6. Use PowerPoint to explain dilutions (optional).
  - Adding water to a mixture to decrease the concentration in a sample
7. Present the learning outcomes or an overview of the lesson.

10 minutes



## Dilution Theory

Use PowerPoint: Dilutions for this presentation (optional).

1. Ask: “When are dilutions needed in drinking water quality testing?”
2. Divide participants into groups of three or four people. Allow groups two minutes to discuss.
3. Share ideas with the large group.
4. Record responses on the flip chart under the heading *When to Dilute*. Responses should include:
  - Microbiological testing – too many bacteria make plates difficult to count
  - Physical Testing – turbidity too high (out of range on the meter)
  - Chemical Testing – chemical concentration too high (out of range)

### Key Points

- Microbiological testing: Ideal number of colonies on a plate = 20-80 , dilute if >200 cfu/100 mL.
- Turbidity testing: Reading should be less than 40 NTU. If higher, dilute the sample.
- Chemical testing with a colour comparator: Water samples must be diluted if the result is at the top of the range on the colour disc.

5. Ask: "What type of water can be used for diluting samples for testing?"
  - Chemical testing: distilled, deionized or boiled water
  - Turbidity testing: turbidity-free water (or filtered water)
  - Microbiological testing: sterile water, sterile stock phosphate buffer solution or boiled water. Do NOT use distilled or chlorinated water (may harm or kill organisms in the sample)

20 minutes

## Dilution Factors



Use PowerPoint: Dilutions for this presentation (optional).

1. Explain: "After diluting a sample, you must adjust the test result by multiplying by a dilution factor."
2. Explain how to calculate a dilution factor using the trainer tips below if PowerPoint is not available.

### Trainer Tips:

Step 1: Calculate the dilution factor for a sample.

$$\text{Total Volume/Sample Volume} = \text{Dilution Factor}$$

Step 2: Apply the dilution factor to the test result.

$$\text{Dilution Factor} \times \text{Test Result} = \text{Final Result}$$

Example calculation for a microbiological sample:

You have used a sample volume of 10 mL and have a colony count of 15.

What are the total Colony Forming Units (CFU) / 100 mL in your sample?

*Trainer Tip: CFU = colony forming units = # colonies on the test plate*

Step 1: Calculate the dilution factor.

$$100 \text{ mL (total volume)}/10 \text{ mL (sample volume)} = 10 \text{ (dilution factor)}$$

Step 2: Apply the dilution factor.

$$10 \text{ (dilution factor)} \times 15 \text{ (# of colonies)} = 150 \text{ CFU}/100 \text{ mL}$$

*Trainer Tip: The total volume for a microbiological sample is always reported as per 100 mL.*

3. Divide participants into small groups. Try to make sure each group includes one person who understands the material well.
4. Hand out one Dilution Exercise to each group based on the participants' ability. Option 1 is easier and Option 2 is harder.
5. Alternatively, start with the easier exercise, then hand out the harder exercise after the first one is completed. If participants are having trouble with this exercise, direct them to the Dilution Chart in the workbook to help them.
6. Allow 10-15 minutes for groups to work on the activity.
7. Review the answers with the group. Answer key below:

Table 1: Dilution Exercise Option 1 Answer Key

Option 1 (Easier)			
Sample Volume	Colony Count	Dilution Factor	Total Colony Count in CFU/100 mL
100 mL	6	1	6
5 mL	150	20	3,000
50 mL	35	2	70
1 mL	3	100	300
10 mL	12	10	120

Note: Total Volume = 100 mL

Table 2: Dilution Exercise Option 2 Answer Key

Option 2 (Harder)		
Sample Volume	Volume of Dilution Water	Dilution Factor
100 mL	0 mL	X 1
50 mL	50 mL	X 2
10 mL	90 mL	X 10
5 mL	95 mL	X 20
1 mL	99 mL	X 100

Note: Total Volume = 100 mL

5 minutes

## Review



1. If you have not already done so, give one Dilution Chart handout to each participant.
2. Explain: "This dilution chart can be used to help you determine if dilution is required and how much to dilute when testing for microbiological contamination."
3. Ask: "You are sampling a protected shallow well. What is the likely contamination level and what sample volume would you use?" *Trainer Tip: Direct participants to refer to the Dilution Chart to answer this question.*
  - Contamination level = moderate
  - Sample volume = 50 mL (x 2 dilution factor)

**Key Points**

- Standard for reporting microbiological test results is CFU/100 mL
- Typical contamination points listed in the dilution chart are a guide only. You may have to test samples at different dilutions to determine the optimal dilution level.

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# Dilution Exercise

Handout

## Option 1

Fill in the blanks. (Optional: use dilution chart)

Option 1			
Sample Volume	Colony Count	Dilution Factor	Total Colony Count in CFU/100 mL
100 mL	6		
5 mL	150		
50 mL	35		
1 mL	3		
10 mL	12		

Note: Total Volume = 100 mL

Cut ✂

## Option 2

Fill in the blanks.

Option 2		
Sample Volume	Volume of Dilution Water	Dilution Factor
100 mL		x 1
	50 mL	x 2
10 mL	90 mL	
5 mL		x 20
	99 mL	

Note: Total Volume = 100 mL

# Dilution Chart

Handout

## Diluting a sample for microbiological testing

### Dilution Chart for Microbiological Testing

Dilution Chart			
Contamination Level	Water Sample Volume	Dilution Water Volume	Dilution Factor
Low	100 mL	0 mL	x 1 (no dilution)
Moderate	50 mL	50 mL	x 2
Moderate – High	10 mL	90 mL	x 10
High	5 mL	95 mL	x 20
Extreme	1 mL	99 mL	x 100

Total Volume = 100 mL

### Typical Contamination Levels

Contamination Level	Description
Low	Borehole; treated water; e.g., directly from a biosand filter or other HWT technology
Moderate	Protected hand dug well or shallow well
Moderate - High	Unprotected hand dug well or shallow well
High	Unprotected hand dug well or shallow well with evidence of infiltration by runoff
Extreme	Open water source; e.g., river, pond or stream