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**Through a microbiologist's eyes: optimal time to add honey to tea**

# Procedure sheet for students

## Activity 1

### Materials

For each group:

- Worksheet 1
- Three types of honey, approximately 1 g of each
- Artificial honey analogue: a sugar solution containing the same amount of sugar as the tested honey (grams of sugar labelled on the package) to mimic the main sugar composition of natural honey
- One fresh liquid culture of a non-pathogenic teaching strain, approximately 0.5 ml
- One sterile 90 mm LB agar plate
- 3 small, labelled glass beakers
- Micropipette and sterile tips
- Sterile spreader or disposable sterile swab
- Sterile pipette tips or a sterile cork borer for making agar wells
- Water bath or dry-block heater set to approximately 50°C (alternatively, use a hot plate with a beaker of water and a thermometer to maintain the temperature)
- Permanent marker
- Ruler or calliper
- Parafilm or adhesive tape
- Personal protective equipment
- Surface disinfectant
- Biological-waste container
- Optional:
    - Incubator set to 37°C
    - Additional plates for three independent replicates

### Procedure

1. Allow the fresh bacterial liquid culture and LB agar plates, which have been prepared in advance by the teacher, to reach room temperature.
2. Label the bottom of each plate with the date and group name. Mark and label one well for each honey sample, one as 'W' for the sterile water negative control and one as 'AH' for the artificial honey analogue. Use no more than five wells per Petri dish. If more wells are required, use additional plates.
3. Place approximately 1 g of each honey sample into separate labelled small glass containers.

4. Warm the honey samples to approximately 50°C for 5 minutes in a water bath or dry-block heater to make them easier to transfer, then allow them to cool slightly.
5. Working aseptically, transfer the same volume of bacterial culture onto each agar plate. If a micropipette is available, use approximately 100 µl. Otherwise, use a sterile graduated or Pasteur pipette and try to keep the volume as consistent as possible between plates.
6. Spread the bacterial suspension evenly across the agar surface using a sterile spreader or sterile swab. Close the plates and allow the liquid to absorb for a few minutes.
7. Using a sterile pipette tip or cork borer, make wells of equal size at the marked positions (figure 1).
8. Add the same volume of the corresponding honey sample to each labelled well. Add the same volume of sterile water to the well labelled 'W' and the same volume of artificial honey analogue to the well labelled 'AH' (figure 2).
9. Close and seal the plates with parafilm or adhesive tape. **Do not invert the plates.**
10. Incubate the plates at 37°C for approximately 24 hours or for longer at room temperature.
11. Observe the sealed plates without opening them. Measure the inhibition zones and record the results in Worksheet 1.
12. Keep the plates sealed and sterilise all cultures and contaminated materials before disposal.

## Activity 2

### Materials

For each group:

- Worksheet 2
- Approximately 1 g of each honey sample per temperature tested
- Approximately 0.5 ml of a fresh liquid culture of a non-pathogenic teaching strain
- Sterile 90 mm LB agar plates: one plate can be used to test honey heated to up to four different temperatures. Use additional plates if more than four temperatures are tested.
- One small, labelled glass beaker or heat-resistant container per temperature tested
- Micropipette with sterile tips or sterile Pasteur pipette
- Sterile spreader or disposable sterile swab
- Sterile pipette tips or a sterile cork borer
- Water bath or dry-block heater (alternatively, use a hot plate with beakers of water and thermometers)
- Permanent marker
- Ruler or callipers

- Parafilm or adhesive tape
- Personal protective equipment
- Surface disinfectant
- Biological-waste container
- Optional:
    - Incubator set to 37°C
    - Additional plates for three independent replicates

### Procedure

1. Fresh bacterial liquid culture and sterile LB agar plates will be provided by the teacher. Let them cool down to room temperature and spread the bacterial suspension evenly across the agar surface as described in steps 1–7 of Activity 1.
2. Label the bottom of each plate with the date, group name, honey type, and the temperatures to be tested. Use no more than four wells per plate.
3. Place approximately 1 g of the selected honey into separate labelled glass containers, one for each temperature.
4. Heat the samples for 5 minutes at the selected temperatures (for example, 70°C, 80°C, 90°C and 100°C). If more temperatures are tested, use additional plates (figure 3).
5. Allow the heated honey samples to cool slightly so that residual heat does not affect bacterial growth.
6. Using a sterile pipette tip or cork borer, make wells of equal size at the marked positions.
7. Add the same volume of each temperature-treated honey sample to the corresponding well.
8. Close and seal the plates with parafilm or adhesive tape. **Do not invert them**.
9. Incubate at 37°C for approximately 24 hours, or longer at room temperature.
10. Observe the sealed plates, measure the inhibition zones, and record the results in Worksheet 2.
11. Keep the plates sealed and sterilise all cultures and contaminated materials before disposal.

## Activity 3

### Materials

For each group

- 250 ml ceramic cup
- 200 ml freshly boiled water
- Laboratory or kitchen thermometer that can measure temperatures of at least 100°C
- Measuring cylinder

- Stopwatch or timer
- Laboratory stand and clamp to hold the thermometer
- Heat-resistant gloves
- Safety goggles
- Paper towel or cloth for spills
- Worksheet 3

### Procedure (with a thermometer)

1. Measure 200 ml of freshly boiled water and pour it carefully into a 250 ml ceramic cup.
2. Place the thermometer in the water without touching the bottom or sides of the cup.
3. Start the timer immediately after pouring.
4. Record the temperature at regular intervals in Worksheet 3 (for example, every 30 seconds).
5. Continue measuring until the water reaches the target temperature identified in Activity 2.
6. Record the time taken to reach this temperature.
7. Repeat the measurement at least three times and calculate the average cooling time.
8. Use the result to recommend how long to wait before adding the honey to the tea.