

6.2 IA2 practical methods and measurement critique

Name: _____ Class: _____ Date: _____

Total: 25 marks

KEY WORDS redox indicator

• dilution plating

• abiotic

Objective

Justify IA2 practical methods: the redox-indicator respiration investigation (CP15), respirometers and RQ (CP16), dilution plating for bacterial numbers (CP13), and habitat sampling with random quadrats and abiotic controls (CP11). Original practice for the Unit 6 implementation criteria.

1 Key knowledge and worked examples

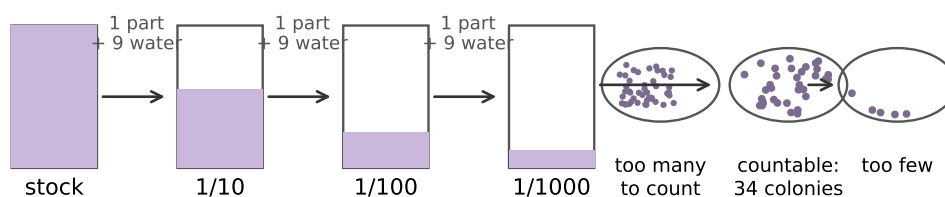
■ A redox indicator measures respiration rate

Yeast respiring a sugar substrate reduces the artificial hydrogen carrier – **TTC**, **methylene blue** or **DCPIP** – whose colour change depends on the indicator used. For temperature: five temperatures across a stated range (e.g. 0–60 °C), tubes equilibrated in water baths before mixing, same yeast strain and volume, same indicator volume, glucose added, and a stopwatch timing each colour change. The **dependent variable is the time to the colour change**; 1/time compares rates.

■ The respirometer and RQ

A sealed chamber with soda lime absorbs CO₂, so oxygen uptake pulls the marker; readings per unit time per mass give the rate. Without the absorber, the gas-volume decrease reflects O₂ used minus CO₂ produced: $RQ = \text{CO}_2 \text{ produced} \div \text{O}_2 \text{ consumed}$. Control temperature (water bath) – a temperature change alone moves the marker.

■ Dilution plating, justified step by step

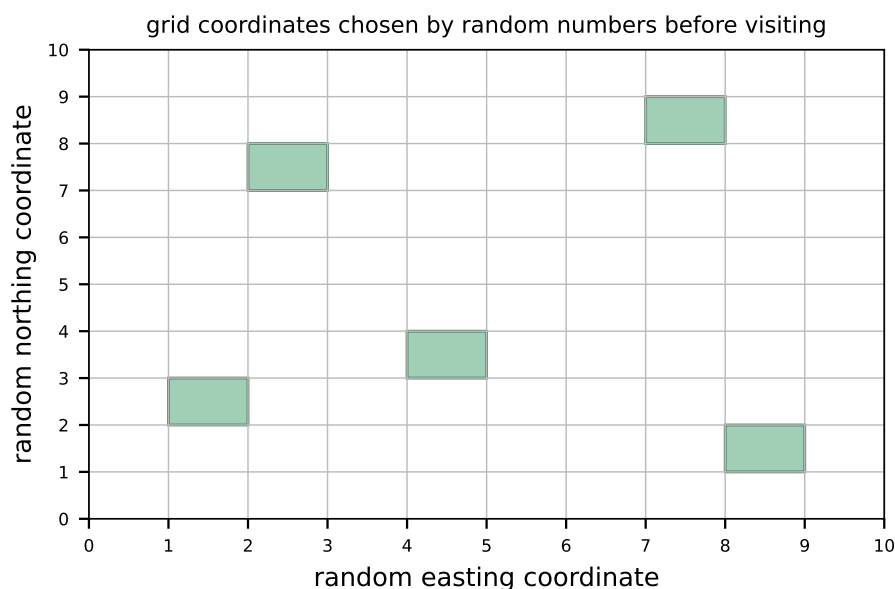


spread a known volume of each dilution on agar, incubate at a fixed temperature for a fixed time,

count the plate with 30-300 separate colonies, then multiply back by the dilution factor

Serially dilute (1 part + 9 parts water, repeated) – raw samples have too many bacteria to count; plate a known volume of a suitable dilution – so separate colonies can be counted; incubate at a fixed temperature for a fixed time – so colonies become visible; aseptic technique throughout – avoiding contamination and counting errors. Use the method's accepted colony-count range. Colony count $\times$ dilution factor $\div$ plated volume estimates colony-forming units per unit volume.

■ Sampling and magnification



Random sites by grid coordinates from random numbers, before visiting. Abiotic variables – soil pH, moisture, minerals, light, season – affect the organisms; control by comparing matched sites. Magnification = image $\div$ actual, and can be below 1 for a photograph smaller than life.

2 Practice

2.1 Name one artificial hydrogen carrier, and state the dependent variable in a yeast-respiration investigation using it at different temperatures. [2]

2.2 Give three method points the scheme credits in the temperature investigation (not the dependent variable). [3]

2.3 Explain why a respirometer must be kept in a water bath even when temperature is not the independent variable. [2]

2.4 Describe how to select five random sampling sites in a plantation, and name two abiotic variables (not temperature) to record. [4]

3 Exam-style questions

3.1 Justify each step of a dilution-plating method used to follow the number of oil-degrading bacteria in soil over 180 days. Six justifications are credited. [6]

3.2 An earthworm is 13.5 cm long and appears 5.4 cm in a photograph. Calculate the magnification, and explain why the answer can be smaller than 1. [3]

3.3 Describe how a simple respirometer determines the rate of respiration and the RQ of germinating seeds, including one control and one safety point. [5]

Solutions

Original-task marking: M1 method, A1 accuracy, B1 reasoning.

2.1 TTC / methylene blue / DCPIP. Dependent variable: the time for the indicator to change colour.

2.2 Any three: five temperatures across a stated range; equilibrate tubes to each temperature before mixing; same strain/age/mass of yeast and volume of culture; add glucose/sugar substrate; same volume of indicator; time each colour change with a stopwatch.

2.3 A temperature change changes gas volume/pressure inside the chamber, moving the marker independently of respiration; a water bath holds temperature constant so marker movement reflects oxygen uptake only.

2.4 Lay a grid over the area and generate five random coordinate pairs with random numbers (or numbers from a bag), then visit only those points. Two of: soil pH, soil moisture, soil minerals, light intensity, season.

3.1 Serially dilute the soil suspension – the raw sample holds too many bacteria to count as separate colonies; plate a known volume of a suitable dilution – so separated colony-forming units produce countable colonies; incubate approved teaching cultures at a fixed school-approved temperature and time – so colonies grow visibly under identical conditions each sampling round; use aseptic technique – preventing contamination and overgrowth that would corrupt counts. Use the method's accepted count range and calculate colony count $\times$ dilution factor $\div$ plated volume to compare colony-forming units per unit volume across the 180 days.

3.2 Magnification = image / actual = $5.4/13.5 = 0.40$. The photograph shows the worm smaller than life, so the image is shorter than the object and the ratio is below 1.

3.3 Seeds in a sealed chamber connected to a graduated tube with the marker; soda lime (or KOH) absorbs the CO_2 the seeds release; oxygen uptake lowers the gas volume and moves the marker – record distance per time to get the rate of oxygen uptake; to find RQ, run the same setup without the absorber: volume change = O_2 used – CO_2 produced, and CO_2 produced = O_2 used (with absorber) – volume change (without), so $\text{RQ} = \text{CO}_2 \div \text{O}_2$; control: water bath for temperature, and a chamber with inert material for comparison; safety: keep absorbent from skin (caustic) and away from the seeds.