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## Activity

# Your microbiome

***Martin Rowland***

The questions in this worksheet relate to Elle Lindsay's article 'Your biome: what it is and why it matters'.

Elle's article touches on several topics in the A-level biology specifications.

This worksheet helps you to explore the following topics:

- microbiology
- immunity
- ecology
- gene sequencing

Students studying the following A-level biology specifications will not be able to attempt question 7: AQA; Edexcel A; OCR A. The Gram-stain is not subject content in these specifications.

## Questions

1 Four ecological terms are listed below.

Tick the **one** that corresponds with the term microbiota. (1 mark)

- Community
- Ecosystem
- Population
- Species

2 Three terms are listed.

- A Coral reef
- B Desert
- C Your large intestine

Tick the **one** box below that contains a true statement. (1 mark)

- Only A and B are natural biomes
- Only A and C are natural biomes
- Only B and C are natural biomes
- A, B and C are natural biomes
- None of A, B or C is a natural biome

**3** Elle opens her article with 'You might not think of your body as a habitat' (lines 1–2).

Define the term habitat. [2 marks]

**4** Later, when Elle tells us that, 'The most diverse sites include the gut, nostrils and vagina' (lines 10–11), she is referring to biodiversity. This can be measured as species richness and as species diversity.

Compare and contrast species richness and species diversity. (2 marks)

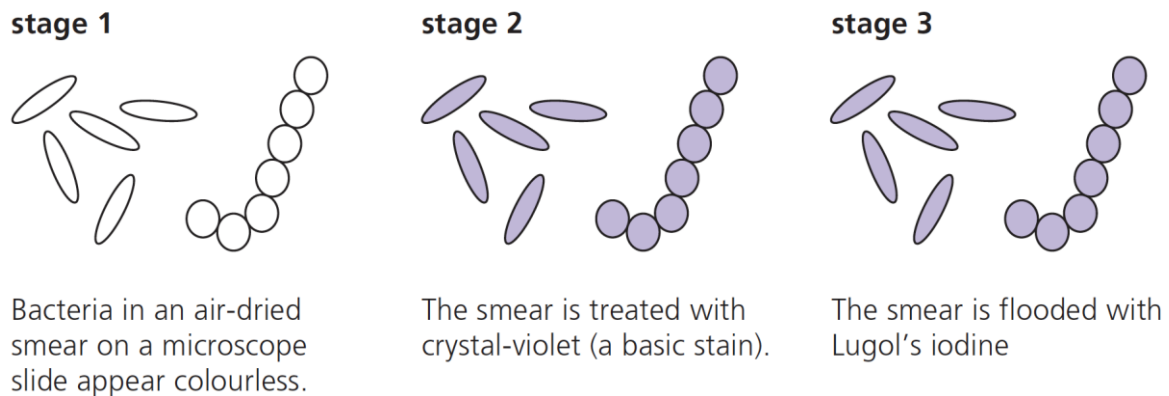
**5** 'The density of microbes also differs depending on location' (line 12).

Devise and outline a method by which you could determine the density of bacteria on your skin.

Do **not** give experimental details in your answer. (5 marks)

**6** Suggest why the microbiota of babies born naturally is dominated by species of *Lactobacillus* but those of babies born by C-section is dominated by species of *Staphylococcus*. (2 marks)

**7** Obese people have a higher proportion of Gram-negative to Gram-positive microbes in their guts.



**Figure 1**

(a) Figure 1 shows three steps in the Gram-staining process.

Describe the final two steps of the Gram-staining process. (2 marks)

(b) Describe how the results of the Gram-staining process would enable you to distinguish between Gram-positive and Gram-negative bacteria on the slide. (1 mark)

(c) Explain why the results of the Gram-staining process are different for Gram-positive and Gram-negative bacteria. (4 marks)

**8** People with Crohn's disease suffer prolonged swelling of the lining of the large intestine. This swelling is thought to be linked to T lymphocytes reacting to pathogenic bacteria in the gut.

(a) Explain why a reaction by T lymphocytes in the gut could lead to swelling of the intestinal lining of someone suffering Crohn's disease. (2 marks)

(b) Describe how pathogenic bacteria would cause the reaction by T lymphocytes. (4 marks)

Questions 9, 10 and 11 relate to *Clostridium difficile* infection.

*Clostridium difficile* is a bacterium that infects the human gut. *Clostridium difficile* infection (CDI) can cause cramps, diarrhoea, colitis and, in severe cases, death.

**9** The use of antibiotics is a major risk factor for CDI.

Suggest why. (3 marks)

**10** Standard treatment using vancomycin fails to resolve CDI in 20–40% of patients. Many of these patients suffer further CDI with increased severity of symptoms and increased death rates.

A group of scientists investigated the effectiveness of a new antibiotic, metronidazole, in the treatment of CDI. They collected clinical data from CDI treatments over a number of years.

The scientists used their raw data to compare the success rate of vancomycin with the success rate of metronidazole. They calculated:

- the relative risk of patients suffering further CDI
- the relative risk of patients dying within 30 days of treatment

Their results are shown in Table 1.

**Table 1** A comparison of two antibiotics used to treat *Clostridium difficile* infection

Severity of symptoms	Number of patients	Relative risk as $\frac{\text{vancomycin}}{\text{metronidazole}}$ (with 95% confidence intervals)	
		Recurrence of CDI	Death within 30 days
Mild to moderate	5452	1.07 (0.93 to 1.15)	0.91 (0.72 to 1.14)
Severe	3130	0.96 (0.76 to 1.23)	0.79 (0.65 to 0.92)

What can you conclude from the data in Table 1? (4 marks)

**11** A different group of scientists investigated the use of faecal microbiota transplants (FMTs) as treatment for persistent CDI.

They chose a group of patients who had been treated unsuccessfully with vancomycin or metronidazole. Each was given an FMT using a sample of faeces from a donor. The scientists analysed the species richness of the faeces of each patient, pre- and post-FMT and the faeces of the donors.

(a) The scientists screened the donors before using their faeces for FMTs. Suggest **two** criteria they would use in this screening. (2 marks)

(b) The scientists measured species richness by sequencing the genes of 16S ribosomes in each sample of faeces.

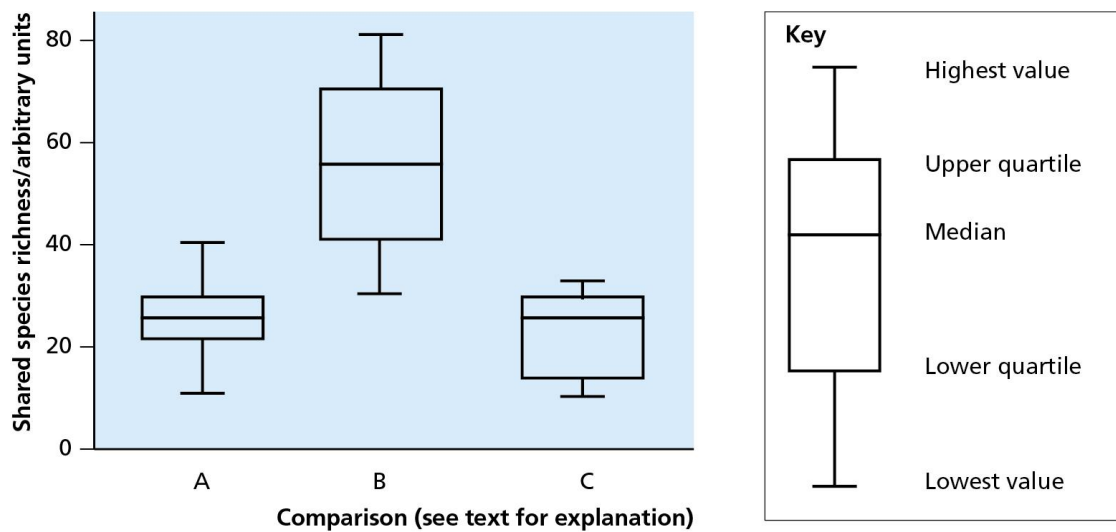
(i) Suggest why they chose to use gene sequencing to determine species richness. (2 marks)

(ii) Suggest why the scientists chose to sequence the genes of 16S ribosomes. (2 marks)

(c) The scientists compared the species richness of faeces:

- between recipient pre-FMT and post-FMT (Comparison **A**)
- between recipient pre-FMT and donor (Comparison **B**)
- between recipient post-FMT and donor (Comparison **C**)

They presented their data as box-and-whisker plots, shown in Figure 2.



**Figure 2**

Do the data in Figure 2 show that FMT was successful? Explain your answer. (4 marks)

## Answers

1  Community

2  A, B and C are natural biomes

3 A full answer would contain two parts.

- A habitat is the area or natural environment in which an organism/a species/a population lives. (1)
- It is characterised by abiotic and biotic factors. (1)

4 To gain marks, each statement must make a clear comparison or a clear contrast between the two terms.

- Species richness and species diversity both include a measure of the number of different species. (1)
- Species diversity also takes into account the number of individuals in each species, but species richness does not. (1)

5 Any five from the following.

- Swab/take a sample from a known area of skin. (1)
- Transfer the sample to a known volume of sterile water. (1)
- Carry out a serial dilution/dilution series of this water. (1)
- Plate out the same volume of each dilution onto separate agar plates. (1)
- After incubation, choose which plate to count and use the result to calculate the density in the original sample. (1)
- Method of choosing which plate to count, e.g. individual colonies must be seen and there must be enough of them to make the count a representative sample. (1)

6 Corresponds with natural microbiota encountered during birth. (1)

Urogenital tract dominated by *Lactobacillus*, but skin/hand-held instruments dominated by *Staphylococcus*.

7 (a) Treat the smear with a decolourising agent/with acetone and alcohol. (1)

Add the red dye safranin. (1)

(b) Gram-positive bacteria appear purple and Gram-negative bacteria appear red. (1)

(c) Crystal violet stains peptidoglycan in bacterial cell walls. (1)

Gram-positive bacteria have very thick walls that retain the stain. (1)

Gram-negative bacteria have very thin cell walls, so stain is washed away by decolouriser. (1)

Red stain visible in Gram-negative but masked by purple in Gram-positive bacteria. (1)

8 (a) T lymphocytes release cytokines. (1)

Cytokines stimulate the (non-specific) inflammatory immune response. (1)

(b) Bacteria ingested by phagocyte/macrophage. (1)

Bacterial proteins displayed on surface membrane of macrophage/antigen-presenting cell. (1)

T-cell with complementary receptor binds to specific bacterial antigen on APC. (1)

Binding activates T lymphocyte. (1)

**9** Healthy gut microbiota reduces risk of colonisation by *Clostridium difficile*/provides colonisation resistance. (1)

By competitive exclusion/by outcompeting *C. difficile*. (1)

Antibiotic disrupts (healthy) microbiota/kills 'good' bacteria. (1)

**10** Large number of patients, so statistical analysis will be valid. (1)

Metronidazole reduces the risk of recurrence in severe group, but vancomycin reduces the risk of recurrence in the mild group. (1)

Vancomycin reduces the risk of death within 30 days in both groups. (1)

Differences in recurrence and death rates not/probably not significant. (1)

**11** (a) Donor must not have previously suffered a CDI. (1)

Donor must be free of any other pathogen. (1)

(b) (i) Any two of:

Bacteria difficult to identify. (1)

Gene sequencing will show genetic variation. (1)

Gene sequencing faster. (1)

(ii) 16S ribosomes found only in bacteria/in prokaryotic cells. (1)

Avoids contamination by human gene sequences/by host DNA. (1)

(c) Yes (no mark)

Shared species richness low for recipients pre-and post-FMT. (1)

Shared species richness low for recipients pre-FMT and donor. (1)

Shared species richness highest for recipients post-FMT and donor. (1)

Shows that microbiota of donor has colonised gut of recipient. (1)

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