Required Practical Review

Separate science only - Biology Practical - Antiseptics

Free science lessons: https://www.youtube.com/watch?v=BkbLI2mAMP8
GCSEpod: https://members.gcsepod.com/shared/podcasts/title/11567

Know it

Investigating the effect of antiseptics on the growth of bacteria

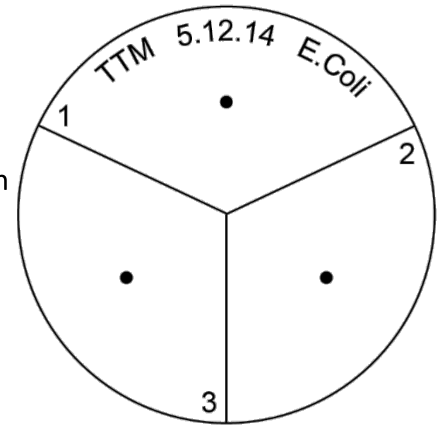
Method

You are provided with the following:

- a nutrient agar plate
- a Bunsen burner
- a heatproof mat
- a disposable plastic pipette
- a culture of bacteria (*E. coli*)
- a glass spreader
- filter paper discs
- three antiseptics (such as mouthwash, TCP, and antiseptic cream)
- disinfectant bench spray
- a 'discard beaker' of disinfectant
- 1% VirKon disinfectant
- forceps
- clear tape
- hand wash
- a wax pencil
- access to an incubator (set to 30°C).

Read these instructions carefully before you start work.

1. Spraying the bench where you are working with disinfectant spray. Then wipe with paper towels.



2. Put the Bunsen burner on the heatproof mat in the middle of where you are working. Light the Bunsen on a yellow flame.

3. Mark the underneath of a nutrient agar plate (not the lid) with the wax pencil as follows (make sure that the lid stays in place to avoid contamination):
   - divide the plate into three equal sections and number them 1, 2 and 3 around the edge
   - place a dot into the middle of each section
   - around the edge write your initials, the date and the name of the bacteria (*E. coli*)
   -

4. Wash your hands with the antibacterial hand wash.

5. Turn the Bunsen flame to blue.

6. Remove the lid of the bottle containing the culture of bacteria (keep the lid in your hand). Then flame the neck of the bottle through the Bunsen flame. Do this by quickly twisting the bottle from side to side.
   Use the disposable pipette to collect approximately 1 ml of the bacterial culture.

7. Quickly flame the neck of the bottle again and replace the lid.

8. Carefully lift the lid of the agar plate at an angle. Do **not** open it fully.
   The lid should only be fully open on the Bunsen burner side.

9. Pipette the bacteria onto the agar plate and replace the lid.

10. Place the pipette into the 'discard beaker'. Turn the Bunsen burner flame back to yellow.

11. Dip the glass spreader into the VirKon disinfectant.
    Remove the glass spreader and tap off the excess. Then pass the glass spreader through the flame. Hold the glass spreader horizontally to ensure nothing drips down onto your hand.

12. Allow the spreader to cool for a count of 20 seconds.

13. Lift the lid of the agar plate. Again, the lid should be at an angle so only the side next to the Bunsen burner is fully open.
    Spread the bacteria around the plate using the glass spreader.

14. Remove the glass spreader and put into the discard beaker. Lower the lid of the agar plate.

15. Put different antiseptics onto the three filter paper discs. This can be done by either soaking them in the liquid **or** spreading the cream or paste onto them.

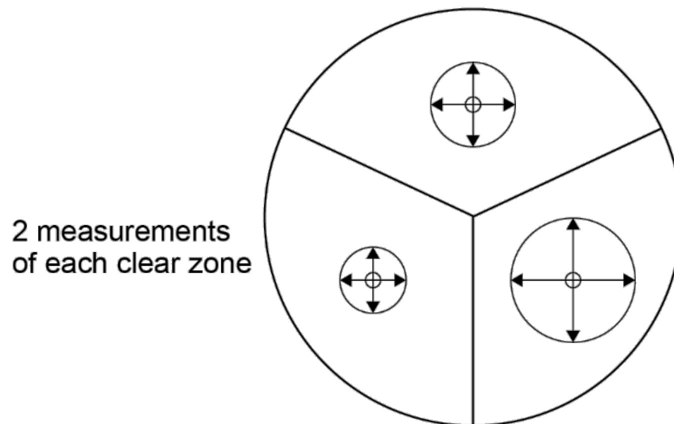
16. Lift the lid of the agar plate as in step **8**. Use forceps to carefully put each disc onto one of the dots drawn on with the wax pencil.

17. Make a note of which antiseptic is in each of the three numbered sections of the plate.

18. Secure the lid of the agar plate in place using two small pieces of clear tape.
    Do **not** seal the lid all the way around as this creates anaerobic conditions. Anaerobic conditions will prevent the *E. coli* bacteria from growing and can encourage some other very nasty bacteria to grow.

1. Incubate the plate at 30 °C for 48 hours.

20. Measure the diameter of the clear zone around each disc by placing the ruler across the centre of the disc. Measure again at 90° to the first measurement so that the mean diameter can be calculated.



21. These results were obtained.

Type of antiseptic	Diameter of clear zone in mm		
	1	2	Mean
Mouthwash (1)	10	11	
TCP (2)	25	23	
Antiseptic cream	12	2	

Notes on additional technical information

Techniques requiring practice	Additional information
Flaming the neck of the culture bottle	This must be done whilst still holding the pipette and the lid of the culture bottle in your other hand (neither should be placed down on the bench at any point). The bottle must not be held still in the flame as the glass will crack – it should be rotated as it is very briefly passed through the flame.
Lifting the lid of the agar plate at an angle	The lid should only be opened at the side facing the Bunsen burner to avoid contamination
Practice placing drops of culture from the pipette onto the agar.	This needs to be done while carefully holding the lid over the plate.
Spreading the bacteria thoroughly around the agar plate right to the edges	This is best done by holding the glass spreader still up to the edge of the plate and rotating the plate. The lid of the plate must be held over it at the same time to avoid contamination.
Placing the filter paper discs onto the agar plate in the right positions	Students should hold the first disc with the forceps. They should lift the lid of the agar plate at an angle (as before) and place the disc flat onto the central dot in the first third of the plate. The lid of the agar plate should be replaced whilst the next disc is collected. This is repeated so that all three discs are in position.

Clear zones are not always perfectly circular so students should measure the diameter twice (at 90° to each other) and calculate a mean diameter for each clear zone.

Review it

Complete the tasks below into your book.

Up to grade 4

1. Calculate the mean diameter of clear zone.
2. Describe the pattern in the results
3. Identify a potentially anomalous result.

Grade 5-7

1. Describe how anomalous results can be identified and what should be done with them
2. Describe how the experiment is carried out to minimize risk to health

Grade 7+

1. Explain why the petri dishes are incubated at 21°C and not closer to 35-40°C.
2. Describe the technique used to measure the clear zone if it is not perfectly circular.

Test it

Answer the exam questions below into your book.

Q1.

Some students investigated the effect of pH on the growth of one species of bacterium.

They transferred samples of bacteria from a culture of this species to each of eight flasks. Each flask contained a solution of nutrients but at a different pH.

After 24 hours, the students measured the amount of bacterial growth.

(a) It was important that the flasks in which the bacteria grew were not contaminated with other microorganisms.

Describe **two** precautions the students should have taken to prevent this contamination.

1. ____

____

2. ____

____

(2)

(b) To see the effect of pH on the growth of the bacteria, other conditions should be kept constant.

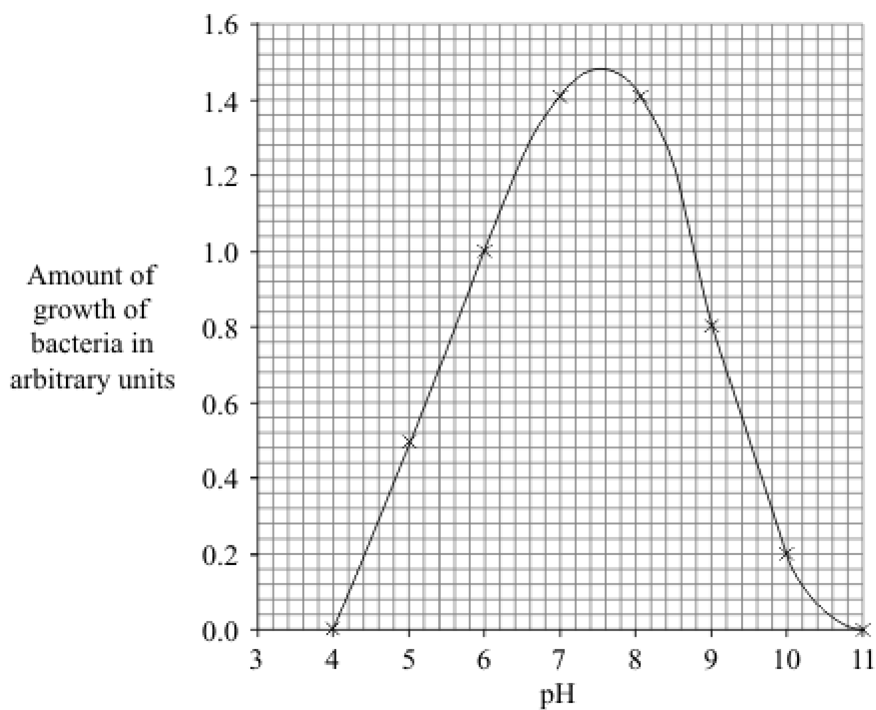
Suggest **two** conditions which should have been kept constant for all eight flasks.

1. ____

2. ____

(2)

(c) The graph shows the results of the investigation.



The students wanted to find the best pH for the growth of this species of bacterium.

(i) Use the graph to estimate the pH at which the bacteria would grow best.

pH ________________

(1)

(ii) What could the students do to find a more accurate value for the best pH for growth of the bacteria?

____

____

(1)

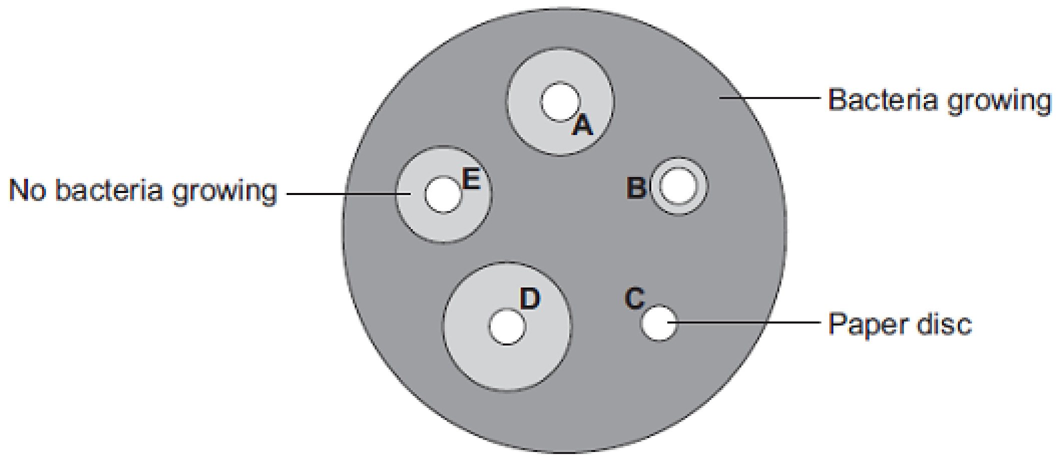
(Total 6 marks)

Q2.
Students in a school investigated the effect of five different antibiotics, **A**, **B**, **C**, **D** and **E**, on one type of bacterium.

The students:

- grew the bacteria on agar jelly in a Petri dish
- soaked separate paper discs in each of the antibiotics
- put the paper discs onto the bacteria in the Petri dish
- put the Petri dish into an incubator.

The diagram shows what the Petri dish looked like after 3 days.



(a) (i) What is the maximum temperature the incubator should be set at in the school?

Draw a ring around your answer.

10°C25°C50°C

(1)

(ii) Draw a ring around the correct answer to complete the sentence.

The incubator should **not** be set at a higher temperature because the higher

temperature might help the growth of	pathogens. toxins. viruses.

(1)

(b) Which antibiotic, **A**, **B**, **C**, **D** or **E**, would be best to treat a disease caused by this type of bacterium?

Write your answer in the box. ☐

Give the reason for your answer.

____

____

(2)

(c) Antibiotics **cannot** be used to treat diseases caused by viruses.

Why?

Tick (✓) **one** box.

Viruses are not pathogens	☐
There are too many different types of virus	☐
Viruses live inside cells	☐

(1)

(Total 5 marks)

Mark it

Q1.

(a) any **two** from:

- sterilise / kill microorganisms
  ignore 'cleaning' / 'disinfect'
  ignore 'germs'
- method of sterilisation eg apparatus / media sterilised in oven / autoclave
  allow pressure cooker / boiling water
- pass flask mouth / pipette tip / loop / test tube mouth through flame
- work near a flame
- minimise opening of flask / test tube **or** hold non-vertical
  *allow idea of sealing / covering **or** prevent entry of air*

2

(b) any **two** from:

- temperature
  ignore references to time / type of bacterium
- concentration / amount of nutrients / ions
- type of nutrient
- volume / amount of solution
- amount of bacteria added
- agitation **or** amount of oxygen

2

(c) (i) 7.5
accept in range 7.4 – 7.6

1

(ii) use more pH values around / close to pH 7.5 / between 7 and 8

1

[6]

Q2.

(a) (i) 25°C

1

(ii) pathogens

1

(b) **D**

1

more / most bacteria killed

accept biggest area / ring where no bacteria are growing

1

(c) viruses live inside cells

1

[5]

Examiner reports

Q1.

In part (a) sealing the apparatus was the most common precaution that examiners rewarded as being equivalent to preventing the entry of other microorganisms, although various methods of sterilising apparatus and media were also frequently described. Nearly all of the candidates were able to make at least one sensible suggestion.

A surprising number of candidates in part (b) suggested that pH, the independent variable, should be held constant, but most were to suggest at least one appropriate control variable eg temperature, concentrations of various named substances, volumes of media and the amount of bacteria added.

Reading of the optimum pH from the graph, in part (c)(i), was sometimes imprecise. Examiners accepted values in the range 7.4 to 7.6.

In part (c)(ii), only a quarter of candidates could suggest that the way to improve the accuracy of the answer to part (c)(i) was to use several more pH values at smaller intervals around pH 7.5. Some lost the mark through imprecise communication, for example: do the experiment at pH 3, pH 3.5 and pH 4 does not imply it should also be done at pH 7.5. Many thought that mere repetition of the original experiment would improve its accuracy reliability, perhaps, but not accuracy.

Q2.

(a) (i) The maximum temperature for a school incubator, ‘25°C’,was widely known. ‘10°C’ was the most commonly selected distracter.

(ii) The growth of pathogens was generally known to be the reason for the temperature setting. Although ‘toxins’ was the most commonly chosen distracter, ‘viruses’ was selected by a considerable number of students.

(b) A significant number of students chose E, incorrectly, possibly because of the ’no bacteria growing’ label, without realising that D had a larger area with no bacteria growing or bacteria having been killed. Stating that D had ‘fewer bacteria’ did not gain the second mark since the grey area around D had *no* bacteria in it.

(c) Most students knew that antibiotics are ineffective against viruses because the viruses live inside cells, although many appeared to believe that ‘viruses are not pathogens’.