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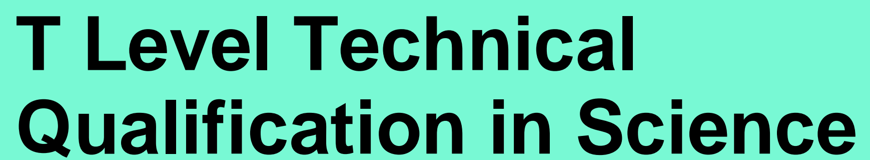

T Level Technical Qualification in Science

Occupational specialism assessment (OSA)

Laboratory Sciences

Assignment 2 – Part B

Assignment brief

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Assignment brief

Assignment 2

Part B

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Experimental practical

Scenario

A microbiology laboratory has decided to investigate whether the higher levels of radiation within the Fukushima Daiichi area have prevented the biological contamination of food samples by microorganisms. Food is often irradiated in order to increase its longevity, and a research scientist has suggested that the disaster could have had a similar effect.

In order to test this hypothesis, spinach samples that originated in the Fukushima Daiichi area were washed with deionised water and the run-off collected. A senior technician has asked you to carry out the appropriate microbiological techniques on the collected water samples.

As part of your role within the laboratory, you will need to complete the following tasks:

- use aseptic techniques to culture any bacteria present in the samples
- analyse data to determine the number of colony forming unit (CFU) per ml

Task 1 – performing aseptic techniques

Task 1 (a)

You need to determine whether there is any bacterial contamination of samples from the Fukushima prefecture.

- use aseptic techniques to make a streak plate using the samples of water provided

During the practical you will be observed by an assessor who will make judgements on your ability to carry out the standard operating procedure (SOP) with due care.

(23 marks) (3 hours total for task 1 (a) and task 1 (b))

Task 1 (b)

For quality assurance purposes, use results provided by the senior technician to:

- calculate the mean CFU/ml
- use a LIMS system to perform relevant statistical techniques

(12 marks) (3 hours total for task 1 (a) and task 1 (b))

Standard operating procedure (SOP)

Process title: aseptic culturing of bacteria

Introduction

Risk assessment

Substance, equipment or procedure	Hazard	Risk	Controls
70% ethanol	Flammable	Proximity to open flame	Ensure ethanol has completely dried before lighting the Bunsen burner. Once used, ensure the ethanol is covered and kept away from the Bunsen burner. Wear suitable PPE.
Bunsen burner	Hot flame	Risk of burns	Once flame is lit, care is taken to **not** work above the flame. Do not overheat equipment during flaming. Equipment requiring flaming is given sufficient time to cool.

Ethanol 70%	

Apparatus

- 70% ethanol
- 2x prepared and sterile agar plates
- mechanical pipette
- sterile pipette tips
- 2x inoculating loops
- control sample (labelled C) stoppered with cotton wool plug
- test sample (labelled T) stoppered with cotton wool plug
- boiling tube rack
- Bunsen burner and heatproof mat
- tape

- marker pen
- waste beaker containing disinfectant solution

Preparing the work area

1) Wipe down the work surface and sterilise pieces of equipment with 70% ethanol
2) Once the ethanol has dried, light the Bunsen burner
3) Ensure all equipment is located close to the Bunsen burner

Making a spread plate

1) Turn the Bunsen burner to a roaring flame
2) Hold the boiling tube containing the control sample and remove the cotton wool plug
3) Flame the neck of the boiling tube
4) Use pipette to withdraw 1ml of the control sample
5) Reflame the neck of the boiling tube
6) Replace the cotton wool plug on the boiling tube and return to the rack
7) Lift the lid of the agar plate away from your body and pipette the 1ml of control sample at the very top of the agar plate and close the lid
8) Discard the pipette tip in the disinfectant solution
9) Pick up the inoculating loop
10) Flame the inoculating loop and allow it to cool
11) Lift the lid of the agar plate away from your body
12) Streak the sample backwards and forwards across the very top of the plate
13) Remove the loop and close the lid of the agar plate
14) Reflame the loop and allow it to cool
15) Turn the agar plate 90˚ anticlockwise
16) Lift the lid of the agar plate away from body and streak the plate 3 or 4 times in parallel lines at the top, making sure some of the original sample is carried over
17) Remove the loop and close the agar plate lid
18) Reflame the loop, allow it to cool and turn the agar plate 90˚ anticlockwise
19) Lift the lid of the agar plate away from body and streak the plate in 3 or 4 parallel lines, starting from the second smear and moving away
20) Close the lid on the agar plate and reflame the inoculating loop, which can now be set down
21) Tape the agar plate shut and invert
22) Label appropriately ready for incubation
23) Repeat the process for the sample of water taken from spinach

Determining the colony forming unit (CFU) concentration of samples

For quality assurance purposes, a senior colleague performed a similar technique in order to determine the number of CFUs in the sample. Results from the control sample of pure water and 4 repeats using the run-off samples were subsequently recorded in a lab book:

Control sample

Volume spread on plate: 0.8ml

Dilution factor: 10^3

No of CFUs identified: 15

Test sample 1

Volume spread on plate: 0.8ml

Dilution factor: 10^3

No of CFUs identified: 167

Test sample 2

Volume spread on plate: 0.8ml

Dilution factor: 10^3

No of CFUs identified: 180

Test sample 3:

Volume spread on plate: 0.8ml

Dilution factor: 10^3

Number of CFUs identified: 174

Test sample 4:

Volume spread on plate: 0.8ml

Dilution factor: 10^3

Number of CFUs identified: 158

For each of the 4 test samples, determine the number of CFUs per ml (CFU/ml) in the original sample using the below equation, showing your working out:

$$\text{CFU/ml} = \frac{\text{number of colony forming units} \times \text{dilution factor}}{\text{volume spread on the plate}}$$

Transfer the calculated CFU results to a suitable table within a LIMS system and calculate:

- the mean CFU/ml for the 4 test samples
- the standard deviation

Evidence to be submitted

At the end of the task, ensure the following records are submitted to the invigilator:

- written results labelled with (Provider_number)_(Student registration number)_(Surname)_(First name)_Assignment 2 Part B
- LIMS system data, saved using the following file name format: (Provider_number)_(Student registration number)_(Surname)_(First name)_Assignment 2 Part B

References

SOP amended from: https://practicalbiology.org/standard-techniques/making-a-streak-plate

Document information

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