

T Level Technical Qualification in Science

Occupational specialism assessment (OSA)

Laboratory Sciences

Assignment 2 - Part B

Assignment brief

v1.1: Additional sample material
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Experimental practical

Scenario

Following on from the identification of the likely pathogenic bacteria the clinician is concerned it may be resistant to the antibiotic penicillin. As such you are to perform an assay to detect the level of beta-lactamase (β -lactamase) activity present in the samples provided. β -lactamases are enzymes capable of hydrolysing the β -lactam rings present in some antibiotics, including penicillin. Hydrolysis of the β -lactam ring deactivates the antibiotic, causing it to lose its potency. This loss of potency results in the antibiotic no longer being able to kill the bacteria as effectively, resulting in the bacteria becoming resistant to the effects of the antibiotic.

SAMPLE

Task 1

You have 3 hours to complete these tasks

Task 1(a)

Your assay is based upon a colorimetric assay, using nitrocefin, an antibiotic which when hydrolysed undergoes a shift in its UV absorbance. This shift causes a change in colour which can be measured.

The colour is detectable at OD=490 nm and is directly proportional to the amount of β -lactamase activity.

You should measure the coloured product in a colorimeter that is already calibrated and ready for use.

(23 marks)

Task 1(b)

Record your results in a suitable table and carry out any necessary calculations.

(12 marks)

Standard operating procedure (SOP)

Process title: setting up a standard curve to determine β -lactamase quantity in a sample

Introduction

Reagents and equipment

- 0.5 mg/ml nitrocefin standard in DMSO (diluted from 10 mg/ml in 1 x PBS)
- beta-lactamase enzyme standard (10 units/ml)
- 1 x PBS
- bacterial sample – spun down and resuspended in 1 ml 1 x PBS, sonicated, spun down and supernatant retained as sample
- colorimeter
- 8 cuvettes
- air-displacement pipettes and tips.
- Eppendorfs/test tubes
- timer/stopwatch
- marker pen

Protocol

- prepare a serial dilution in 1 x PBS of the β -lactamase standard at, 10 units/ml, 5 units/ml, 2.5 units/ml, 1.25 units/ml, and 0 units/ml. Show your calculations for preparing the serial dilutions
- prepare a 1 ml serial dilution of your sample in 1 x PBS at 1:10, 1:100 and 1:1000 dilution
- for each standard or sample add 0.1 ml of 0.5mg/ml Nitrocefin to a cuvette then add 0.9 ml of the relevant standard or sample to the cuvette and read the absorbance at 490 nm immediately on the colorimeter
- record the absorbance for each cuvette every 15 minutes for one hour

Analysis

- plot the absorbances over time for each sample and standard
- determine the β -lactamase concentration of the bacterial sample by comparing it to the curves generated by the known quantity of β -lactamase

Risk assessment

Substance, equipment, or procedure	Hazard	Risk	Control(s)
Nitrocefin	Causes skin irritation and serious eye damage.	Very low	Limit handling. Wear correct PPE (gloves and eye protection).

SAMPLE

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Change History Record

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v1.0	Additional sample material		01 September 2023
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