Country: _____	Student Code: _____

19th INTERNATIONAL BIOLOGY OLYMPIAD

13th – 20th July, 2008

Mumbai, INDIA

PRACTICAL TEST 3

實作 3

BIOCHEMISTRY AND CELL BIOLOGY

生物化學與細胞學

Total Points: 43

總分：43

Duration: 60 minutes

時間：60 分鐘

Dear Participants, 親愛的參賽者

- In this test, you have been given the following task:
- 在本次的測驗中，你必須回答下列題目：

Task 1: A: Study of β-lactamase activity and its inhibition (35 points)

試題一：**A**：**β - lactam** 酶的活性與抑制研究 **(35** 分**)**

B: Correlating β-lactamase expression to resistance (4 points)

B：有關 **β - lactam** 酶的表現與抗性 **(4** 分**)**

C: Correlating K_i values of pesticides to bacterial growth (4 points)

C：殺蟲劑的 **Ki** 值對細菌生長的影響 **(4** 分**)**

- **You have to write down your results and answers in the ANSWER SHEET. Answers written in the Question Paper will not be evaluated.**
- 你必須將答案回答於答案紙卷上，如果在試題上作答，將不予計分
- Please make sure that you have received all the materials and equipment listed for the task. In case any of these items is missing, please raise the yellow card.
- 請務必檢查所有的材料與儀器，他們都必須與試題上所記載完全相同，如有不符或不足，請舉起黃牌。
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.
- 作答完畢後，將試卷與答案卷放入信封中，監試人員會收回該信封。

Good Luck!! 祝好運 !!

Country 國家 : _____

Country Code 國家編號 : _____

First Name 名 : _____

Middle Name: _____

Family Name 姓 : _____

Student Code 學號 : _____

Task 1 試題一

PART A (35 points) 第一部分 (35 分)

Study of β-lactamase activity and its inhibition

β - lactam 酶的活性與抑制研究

第一部分：

Materials and equipment 材料與方法	**Quantity** 數量
1. Colorimeter, with a set of seven cuvettes 比色計，含一組 (七隻) 比色管	1 組
2. Test tubes 試管	8 支
3. Test tube stand 試管架	1 個
4. Micropipette (10 – 100 µl capacity) 微量吸管 (容量 10 – 100 µl)	1 支
5. Micropipette (100 – 1000 µl capacity) 微量吸管 (容量 100 – 1000 µl)	1 支
6. Micropipette tips (10 – 100 µl capacity) 微量吸管吸頭 (容量 10 – 100 µl)	20 根
7. Micropipette tips (100 – 1000 µl capacity) 微量吸管吸頭 (容量 100 – 1000 µl)	20 根
8. Photographs of Petri plates 培養皿相片	6 張
9. Permanent marker 油性筆	1 支
10. Tissue paper roll 衛生紙	1 卷

11. Wash bottle containing distilled water 1 個
    含蒸餾水的洗瓶
12. Container for wash and discard 1 個
    廢棄物與洗液容器
13. Graph paper 1 張
    繪圖紙

Reagents (please see the next page)
溶劑 (請參見下表)

Label **(標示)**	**Reagent** **(溶劑)**	**Container** **(容器)**
A	β – Lactamase enzyme (1.85 mg/ml) β – lactam 酶	Vial 微量滴管
B	Inhibitor (100 mM) 抑制劑	Vial 微量滴管
C	Penicillin G (0.54 mM) 盤尼西林 G	Blue-stoppered tube 藍色蓋子的大離心管
D	Sodium phosphate buffer, pH 7.0 (10 mM) 磷酸鈉緩衝液	Blue-stoppered tube 藍色蓋子大離心管
E	$CuSO_4$-Neocuproine reagent 硫酸銅 – 新氧化銅溶劑	Blue-stoppered tube 藍色蓋子大離心管
F	HCl (2 M) 鹽酸	White-stoppered tube 白色蓋子小離心管

Handling of micropipette:

微量吸管操作

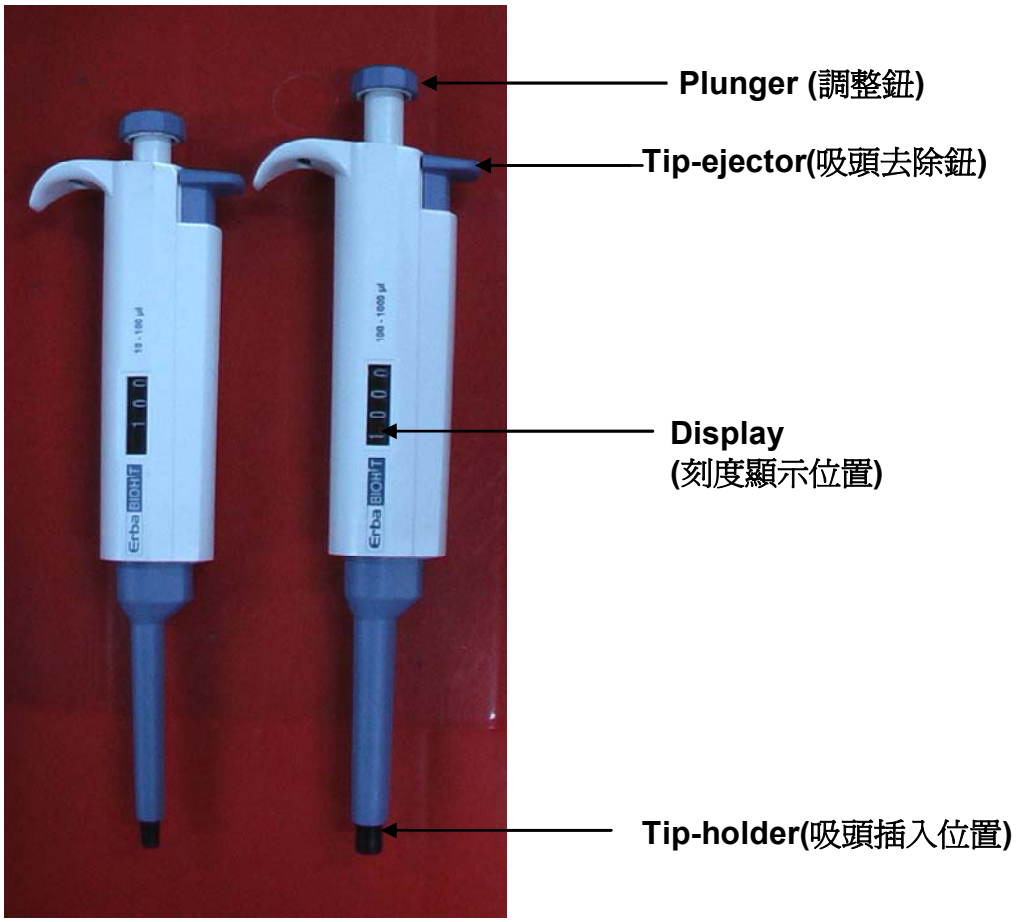


Figure 1 (圖一)

Adjustment method

調整方法

Turn the plunger (Figure 1) to set the value to the desired volume, which can be seen in the display.

如圖一所示，轉動調整紐 (plunger) 調整到所需要的體積數。可以由刻度顯示位置得知 (Display)。

Remember that each micropipette has a fixed range of volumes as indicated on the pipette. DO NOT CROSS THE LIMITS OF THIS RANGE.

請注意每支微量吸管各有其使用範圍，請勿超出其使用範圍使用。

Usage method

使用方法

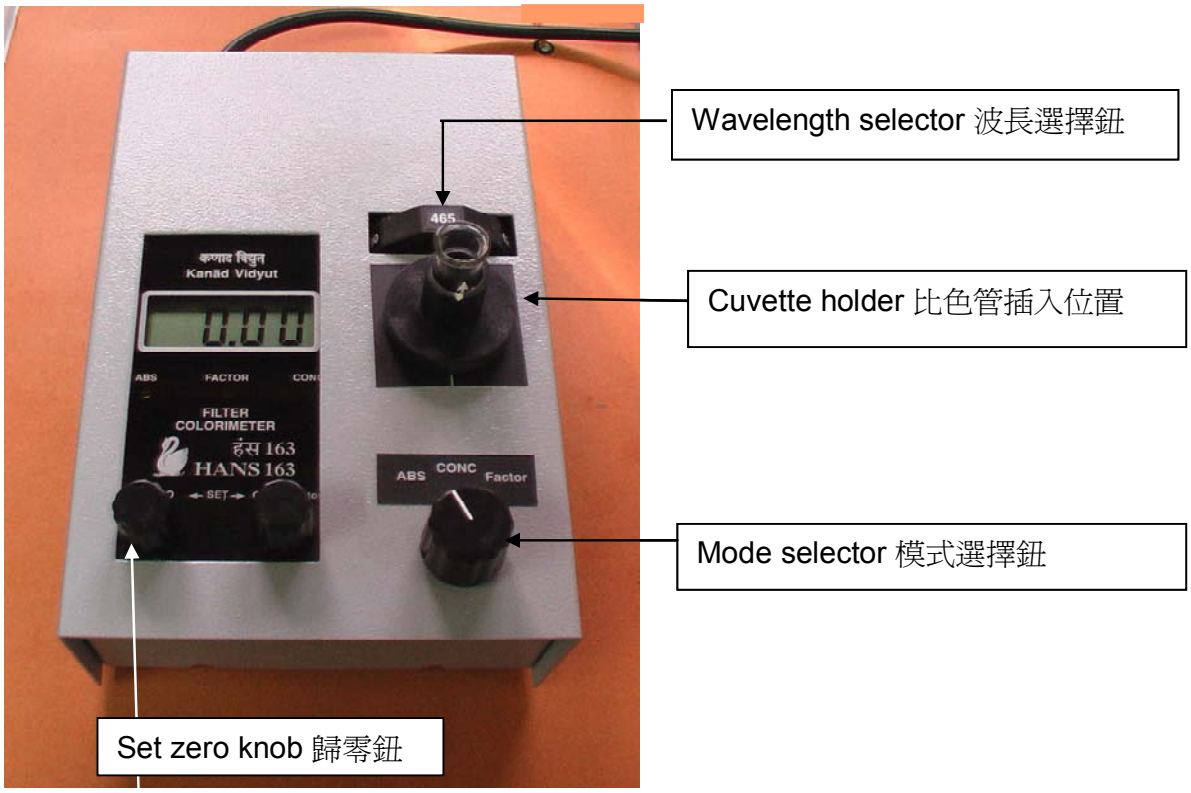
Secure the pipette tip to the tip holder (Figure 1). Gently push down the plunger to the first stop, hold, and dip the tip into the solution vertically to a depth of 2 - 4 mm. Release the plunger slowly and make it return to the original position. Remove the pipette from the liquid and transfer the contents to the desired tube. Make sure that the tip is close to the inner wall of the tube. Push the plunger to the first stop and then push further to discharge the solution completely from the tip. Remove the pipette from the tube. Eject the used tip into the discard container by pressing the tip-ejector.

將吸頭準確而完全插入吸頭插入位置 (tip holder)。按下調整鈕 (plunger) 到第一段，此時不要放開調整鈕，將吸頭前端插入液面下約 2 – 4 mm 位置處。慢慢鬆開調整鈕，讓液體流入吸頭內，直到原先位置。將吸頭內的液體移入待裝入的試管中。讓吸頭接觸試管內壁，壓下調整鈕 (plunger)到剛剛第一段的位置，再用力壓下直到液體完全被吹出吸頭。將微量吸管移開，壓下吸頭去除鈕 (tip-ejector)，讓用過的吸頭射入廢棄物與洗液容器中。

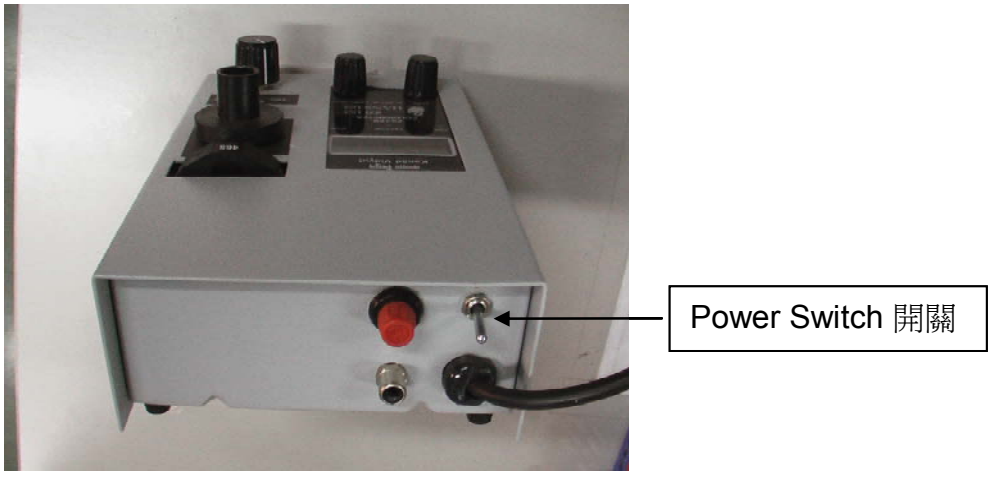
Operating Instructions for the colorimeter:

比色計操作指南

Figure 2 (圖二)



比色計鳥瞰圖



比色計背面

1) Turn the power switch (Figure 2) of the colorimeter ON.
   打開比色計開關。 (如圖二，比色計背面)
2) Set the instrument to Absorbance mode (“ABS”) using the mode selector.
   將模式選擇鈕轉到 ABS 處 (Absorbance mode)
3) Set the wavelength to 465 nm using the wavelength selector.
   利用波長選擇鈕將波長設定在 465 nm 處
4) Put the blank solution in a cuvette. Clean the outside surface of the cuvette with tissue paper and insert it into the cuvette holder. Gently push the cuvette all the way down.
   取一支空白的比色管，利用衛生紙將比色管外圍擦拭乾淨。之後，將該比色管插入比色管插入位置處，輕輕的將比色管整支完全插入。
5) Rotate the ‘set zero’ knob to set the reading to zero. The instrument is now ready for measuring the absorbance of the test solutions.
   利用歸零鈕將讀數調整到 “ 0 “。此時你可以利用它去測定吸光值。

Introduction

簡介

Penicillins are antibiotics with a characteristic β-lactam ring in their structure. This antibiotic kills bacteria by inhibiting the cell wall synthesis. However, these molecules are rendered inactive by some bacteria, which synthesize an enzyme called β-lactamase. These bacteria, which produce β-lactamases, are resistant to penicillins. Due to this, penicillin treatment is ineffective in patients infected with such resistant bacteria. One approach to overcome this problem is to develop effective β-lactamase inhibitors.

盤尼西林是一種具有 β - lactam 環結構的抗生素。該抗生素殺死細胞的機制爲抑制細菌細胞壁的生成。有些細菌會合成 β-lactam 酶，進而破壞盤尼西林的活性。這些細菌因爲會分泌 β-lactam 酶而對盤尼西林產生抗藥性。當病人受到該細菌感染後，盤尼西林的治療對這類感染者將是無效的。要解決此種問題，研發 β-lactam 酶抑制劑便是一項重要的工作。

The effectiveness of a β-lactamase inhibitor can be evaluated by determining its IC_{50} and K_i values. The IC_{50} of an inhibitor is defined as the concentration of the inhibitor required to inhibit the enzyme activity by 50 percent. The K_i of an inhibitor is a measure of its binding affinity for the enzyme.

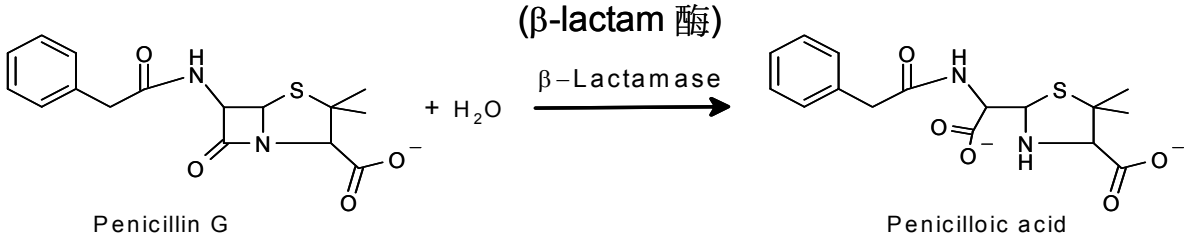
有效的研發 β-lactam 酶抑制劑必須依靠兩種指標，IC_{50} 與 K_i 值。IC_{50} 指抑制 50% 酵素活性的濃度。K_i 指抑制劑對酶的親和力。

Principle of β-lactamase assay

β - lactam 酶分析原理

β-Lactamase inactivates penicillin by catalyzing the following reaction:

β-lactam 酶藉由催化下列反應進行盤尼西林的去活化作用：



Penicillin G (盤尼西林 G)

Penicilloic acid (盤尼西林酸)

The penicilloic acid generated is complexed with $CuSO_4$ in the presence of neocuproine. The yellow-colored product formed can be monitored by measuring its absorbance at 465 nm using a colorimeter.

在新氧化銅 (neocuproine) 存在下，盤尼西林酸會與 $CuSO_4$ 形成化合物。這種黃色的物質可以在 465 nm 波長下藉由比色計所測得。

In this task, you will:

- determine the IC_{50} value of a given inhibitor by generating a dose-response curve, and
- calculate the K_i value for the inhibitor.

本試題中你將進行下列計算：

- 藉由不同濃度抑制劑所產生的反應曲線，計算抑制劑的 IC_{50} 值與 K_i 值。

A dose-response curve for the inhibitor is generated by measuring the activity of β-lactamase in the presence of varying concentrations of the inhibitor at a fixed concentration of the substrate.

抑制劑的濃度反應曲線是利用不同濃度的抑制劑作用下進行 β-lactam 酶活性測定後得知。

Q. 1.A.1. (18 points) (18 分)

Follow the protocol given below and enter the absorbance values **in Table 1.A.1. in the Answer Sheet**.

依照下列步驟進行實驗，並將吸光值填寫於答案卷 表 **1.A.1.** 中

I. Prepare the following reaction mixtures:
準備下列反應混合液

Test tube (試管)	Sodium phosphate buffer, pH 7.0 (磷酸鈉緩衝液)	Inhibitor (100 mM) (抑制劑)	β-lactamase enzyme (β – lactam 酶)	Distilled water (蒸餾水)
1	1.48 ml	-	20 µl	-
2	1.46 ml	20 µl	20 µl	-
3	1.44 ml	40 µl	20 µl	-
4	1.42 ml	60 µl	20 µl	-
5	1.40 ml	80 µl	20 µl	-
6	1.38 ml	100 µl	20 µl	-
Blank 空白管	1.43 ml	50 µl	-	20 µl

II. Mix gently and incubate at room temperature for 5 minutes. You may use the wall clock or your wrist watch to keep track of the incubation time.
均勻混合上述混合液，並於室溫中靜置 5 分鐘。你可以使用牆上的掛鐘或手錶計時。

III. Add 1 ml of penicillin G (0.54 mM) to each tube and mix gently. Incubate at room temperature for 10 minutes.
在每支試管中加入 1 ml 盤尼西林 G (0.54 mM)，均勻混合，並於室溫中靜置 10 分鐘。

IV. Add 1.5 ml of the $CuSO_4$-neocuproine reagent to each tube and mix gently. Incubate at room temperature for 5 minutes.

在每支試管中加入 1.5 ml 硫酸銅 – 新氧化銅溶劑，均勻混合，並於室溫中靜置 5 分鐘。

V. Stop the color development by adding 100 μl of HCl to each tube and mix gently.

在每支試管中加入 100 μl 鹽酸停止反應進行，鹽酸加入後需均勻混合。

VI. Set the colorimeter to 465 nm.

將比色計波長設於 465 nm。

VII. Use the Blank to set the absorbance to zero.

利用空白比色管將吸光值歸零。

VIII. Measure the absorbance values of the solutions in Test tubes 1 to 6, and enter these values in the table. **You should get any one absorbance reading countersigned by the supervisor. To call the supervisor, raise the yellow card.**

分別測量 1 至 6 號試管的吸光值。<u>每一個吸光值都必須獲得監試人員確認。舉起你的黃牌，請監試人員確認。</u>

Table 1.A.1.

Test tube (試管)	Absorbance (吸光值)
1	
2	
3	
4	
5	
6	

Data analysis and interpretation

資料分析與計算

Q. 1.A.2. (6 points)(6 分)

I. Calculate the concentrations (in mM) of the inhibitor [I] in 2.5 ml of the enzyme reaction in Test tubes 1 to 6 and enter these values **in Table 1.A.2. in the Answer Sheet**.

計算 2.5 ml 酵素反應體積下，試管 1 至 6 號中抑制劑[I]的濃度 (單位：mM)。**將答案填在答案卷表 1.A.2. 中**

II. Consider the absorbance values to be the rates of hydrolysis of penicillin G. Now calculate v_i/v_0, where:

v_0 is the rate of hydrolysis of penicillin G by β-lactamase in the absence of the inhibitor, and v_i is the rate of penicillin G hydrolysis in the presence of the inhibitor. Note that for Test tube 1, $v_i = v_0$.

由於吸光值是水解盤尼西林 G 後的值，在此要進行 v_i/v_0,的計算。

v_0 是無抑制劑下盤尼西林 G 被 β-lactam 酶水解的值。

v_i 是有抑制劑下盤尼西林 G 被 β-lactam 酶水解的值。

注意：試管 1 的條件下 $v_i = v_0$.

Enter these values (up to two decimals) **in Table 1.A.2. in the Answer Sheet**.

Table 1.A.2.

將上述數值，單位：小數點後兩位，填在答案卷 表 **1.A.2.** 中

Test tube (試管)	[I] (mM)	v_i/v_0
1		
2		
3		
4		
5		
6		

Q. 1.A.3. (5 points)(5 分) Plot a graph of v_i/v_0 versus [I] in the **Graph Paper attached to the Answer Sheet**.

將 v_i/v_0 對 [I] 的值進行繪圖。**繪圖紙會附在答案卷上。**

Determination of the IC_{50} and K_i value of the inhibitor

抑制劑 IC_{50} 值與 K_i 值的測定

Q. 1.A.4. (3 points)(3 分) Determine the IC_{50} value by interpolation of the data points in the graph. Write the value (up to two decimals) in the box **in the Answer Sheet**.

利用內插法將 IC_{50} 值自圖形中求出。**將正確答案，數值要達小數點後兩位，填入答案卷 Q. 1.A.4. 的方格中。**

IC_{50} = _____mM

Q. 1.A.5. (3 points)(3 分)

Calculate the dissociation constant K_i of the inhibitor using the equation:
利用下列公式計算 K_i 值

$$IC_{50} = K_i\left(1+\frac{[S]}{K_m}\right)$$

where K_m is the Michaelis-Menten constant of β-lactamase for penicillin G and [S] is the initial concentration of substrate (penicillin G) present in the enzyme reaction mixture.
Assume the K_m of β-lactamase for penicillin G to be **0.05 mM**. Write down your answer (up to two decimals) in the box **in the Answer Sheet**.

Km 是 β-lactam 酶的馬歇-馬坦常數 (Michaelis-Menten constant)，[S] 爲酵素混合液中受質 (盤尼西林 G) 的起始濃度。本實驗中，β-lactam 酶對盤尼西林 G 的 Km 值爲 **0.05 mM**。**將正確答案，數值要達小數點後兩位，填入答案卷 Q. 1.A.5. 的方格中。**

K_i = _____mM

PART B (4 points)

Correlating β-lactamase expression to resistance

第二部分：有關 β-lactam 酶的表現與抗性 (4 分)

When penicillin-resistant bacteria are grown in liquid culture media, β-lactamase is secreted into the medium. The supernatant of such a medium can be assayed for β-lactamase activity. Culture supernatants from four different organisms (P, Q, R and S), which are suspected to be penicillin-resistant, were obtained and 20 μl of each was assayed for β-lactamase activity. The corresponding absorbance values were measured at 465 nm and are given in the table below.

利用液態培養液培養抗盤尼西林細菌時，β-lactam 酶會被釋放到培養基中。培養基的上清液將會出現 β-lactam 酶的活性。利用液態培養液方法培養疑似抗盤尼西林生物 P, Q, R 與 S (organisms P, Q, R and S) 時，分別取 20 μl 的培養液進行 β-lactam 酶活性測定，他們在 465 nm 下的吸光值分別記錄於下表中。

Organism (生物)	Absorbance (吸光值)
P	0.090
Q	0.450
R	0.075
S	0.220

These four organisms were tested for their resistance to penicillin G by the disc diffusion plate assay as follows:

上述四種生物的盤尼西林 G 抗生素盤 (disc) 擴散分析，敘述如下：

1. Each organism was separately inoculated into warm growth medium and poured into a sterile Petri plate. On cooling, the medium solidified.
   每種生物分別接種在溫的生長培養基中，倒入無菌的培養皿裡，待溫度降低後，培養基會凝固。

2. Filter paper discs impregnated with varying concentrations of penicillin G were then placed on the surface of the medium.
   盤型濾紙片先浸泡在不同濃度的盤尼西林 G 中，之後分別置於上述步驟 1 中培養基表面。
3. The plates were incubated allowing penicillin to diffuse and organisms to grow.
   培養皿將持續進行培養，並讓盤尼西林擴散，此時生物仍持續生長。
4. Organisms sensitive to penicillin will not be able to grow in the vicinity of the antibiotic disc and hence, a clear zone will be obtained around the disc.
   培養的生物如果對盤尼西林是敏感的，生物將無法生長，此時在抗生素盤周圍會出現透明環。

You have been given labeled photographs of six plates I - VI.
你將會獲得分別標有 I – VI 的六張照片。

Plate I is a control plate showing uniform mat growth of organisms in the absence of penicillin G.
第一張 (I) 爲對照組，你看到的將是在無盤尼西林 G 添加情況下，生物將長成一片。

Plate II is also a control plate that contains media without the growth of any organism.
第二張 (II) 也是對照組，培養基中沒有添加生物。

Plates III to VI show the growth of the four organisms in the presence of penicillin G. 2.5, 5, 7.5, 10 and 12.5 are the micrograms of penicillin G present in the respective discs.
第三張到第六張，顯示四種生物分別在含有不同濃度的盤尼西林 G 盤，2.5, 5, 7.5, 10 與 12.5 下生物生長的狀況。

Q. 1.B.1. (4 points) (4 分) Observe these plates and infer which organism is growing in each plate. Write your answers **in Table 1.B.1. in the Answer Sheet**.
觀察上述照片後，分別將正確的生物名稱填入照片 III 到 VI 的表中。將正確答案填入答案卷的表 1.B.1.。

Table 1.B.1.

Plate (照片)	Organism (生物)
III	
IV	
V	
VI	

PART C (4 points)

Correlating K_i values of pesticides to bacterial growth

第三部分：殺蟲劑的 **Ki** 值對細菌生長的影響

Four pesticides P1 to P4 are reversible inhibitors of an enzyme E that is essential for the growth of a bacterium B. Their K_i values are given in the table below. Each of these four pesticides is used in four geographically different regions R1 to R4. The residual concentrations of these four pesticides in the respective regions are also shown in the table below:

四種殺蟲劑 P1 到 P4 爲酵素 E 的可逆抑制劑，對於細菌 B 的生長是必須的。他們的 K_i 分別表示在下表。四種殺蟲劑分別施用在四個不同的地理區域 R1 到 R4。四種殺蟲劑的殘留量表示也分別表示在下表中。

Region (地理區域)	R1	R2	R3	R4
Pesticide (殺蟲劑)	P1	P2	P3	P4
K_i for the enzyme E (酵素 E 的 K_i)	1 nM	5 nM	0.45 μM	0.55 μM
Residual concentration (殘留量)	60 nM	100 pM	30 nM	5.5 μM

Q. 1.C.1. (4 points)(4 分)

Indicate whether the bacterium B would grow or not in each of the four regions by putting tick marks (√) in the appropriate boxes **in the Table 1.C.1. in the Answer Sheet**.

判斷細菌 **B** 能或不能在上述四種地區生長，並在答案卷中表 **1.C.1.** 的正確位置打鉤 **(√)**。

Table 1.C.1.

Region (地理區域)	R1	R2	R3	R4
Bacterium B grows (細菌 B 能在該地區生長)				
Bacterium B does not grow (細菌 B 不能在該地區生長)				

試題結束

********** END OF PRACTICAL TEST 3 **********