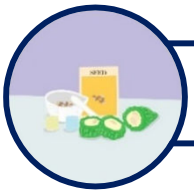
Lipase Inhibitor Investigation



Lipase Inhibitor Investigation

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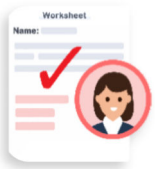
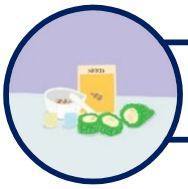


Notes for teachers

- Scan the QR code to get the electronic files.
- Teachers are strongly encouraged to adapt and modify these resources as necessary.

Innovations in Biology Investigations



Lipase Inhibitor Investigation

Overview

- The *Lipase Inhibitor Investigation* is about the search for an anti-obesity agent. Students investigate the inhibitory effects of different types of bitter melon seed extracts on lipase activity using the milk–pH indicator system (Royal Society of Biology Nuffield Foundation, 2019).
- Students are given the opportunity to design and carry out experiments in which they set up controls, consider the need for replicates, and identify limitations of using visual inspection to determine the end point of a reaction and an *in vitro* system to study the effects of the seed extracts on enzyme activity *in vivo*.

Teaching Plan & Key Features

Prerequisite knowledge (scientific ideas)
- Food substances and energy requirement in humans
- Digestion and absorption of fats in humans

Prerequisite manipulative skills
- Using an autopipette to transfer a small volume of solution

Lesson	Lesson sequence	Duration (mins)	Resources
Stage ❶ Preparing for the investigation • It is situated in an authentic context related to the search for anti-obesity drugs (**Contextualisation**). • Students read information about the background of the investigation (*Reading Materials*).			
Before Lesson 1	• The teacher distributes *Worksheet 1* for students to complete at home so that they can be familiar with the background of the investigation.		*Worksheet 1*
1	• The teacher discusses the investigation context with students. • The teacher provides feedback on students' responses in *Worksheet 1*. • The teacher distributes *Worksheet 2* for students to complete at home.	40	*Worksheet 2*
Before Lesson 2	• The teacher distributes *Worksheet 2* for students to complete at home.		*Worksheet 2*
Stage ❷ Designing the investigation • Students interact with a virtual laboratory to familiarise themselves with the materials and apparatuses they would use in the investigation (*Virtual Laboratory*). • Students use a template to design their own experimental set-ups (*Investigation Planning Template*). • Students have the chance to evaluate their own and their peers' experimental set-ups (*Self & Peer Evaluation*).			
2	• Teacher provides feedback on students' experimental designs in *Worksheet 2*.	40	Student Samples 1
3	• The teacher discusses with the students some questions related to the experimental design. • The teacher provides students with laboratory manual for preparation at home.	40	Teacher Notes 1

Stage ❸ Carrying out the investigation • Students use microscale instrumentation that reduces the time of the experiments (*Microscale Instrumentation*). • Students collect more complex data sets by setting up replicates (**Complex Data Set**). • Students use camera to collect data (*Digital Tool*).			
4	• Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills. • Students carry out the investigation.	40	Laboratory Manual
Stage ❹ Explaining and evaluating data • Students use *Google Sheet* for data recording and manipulation (*Digital Tool*). • Students use data to identify seed extracts with the highest inhibitory effect on lipase activity. • Students considering the limitations of using an *in vitro* system to study the effects of the seed extracts on enzyme activity *in vivo*.			
Before Lesson 5	• Students complete data reporting and analysis at home. • Teacher collects and marks student responses.		Teacher Notes 2
5	• Teacher provides feedback on students' performance related to data reporting and analysis**.**	40	Teacher Notes 2

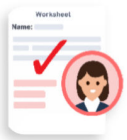
Important Notes

- Students are *not* required to learn the detailed mechanism of enzyme inhibition. Rather, they are expected to use data to support their claims about the inhibitory effect.
- Students should avoid skin contact with the solutions and quickly rinse any splashes of lipase solution or sodium carbonate from their skin.

Instructional Materials

Stage 1 Preparing for the investigation

Student Worksheet 1



Notes for teachers

- The teachers can distribute *Worksheet 1* and ask students to read the background information related to the investigation at home.
- Students' responses can be collected using a *Google Form*.

Task 1

- Read the following information and source materials in the *Data File*.
- Answer the questions that follow.

Obesity is a major risk factor of cardiovascular diseases, musculoskeletal disorders, and some cancers. According to the World Health Organization, approximately 650 million adults were obese, and more than 1.9 billion were overweight in 2016.

Orlistat is a drug approved by the United States Food and Drug Administration for the long-term treatment of obesity; it inhibits lipase activity in the alimentary canal. Orlistat reduces the absorption of dietary fat in the human body. However, it may cause side effects such as gastrointestinal discomfort.

Scientists are now searching for natural alternatives as anti-obesity drugs. Read the information in the *Data File* to familiarise yourself with the investigation background.

Scan the QR code to get a copy of the *Google Form*.

Teachers can diagnose students' difficulties in understanding the relevant content and methods for measuring lipase activity, and then provide feedback before students design the experiments.

Data File

Your biology teacher asks you to read the following source materials to prepare you to design a scientific investigation related to lipase activity:

Source 1:

Most dietary fats are made up of triglycerides. Dietary fat cannot be directly consumed by the human body and must be digested for absorption. Fats consumed by the human body are digested by lipases in the alimentary canal and broken up into smaller molecules, fatty acids, and glycerol for absorption into the body. *Figure 1* shows the action of lipases in fat absorption in the human body.

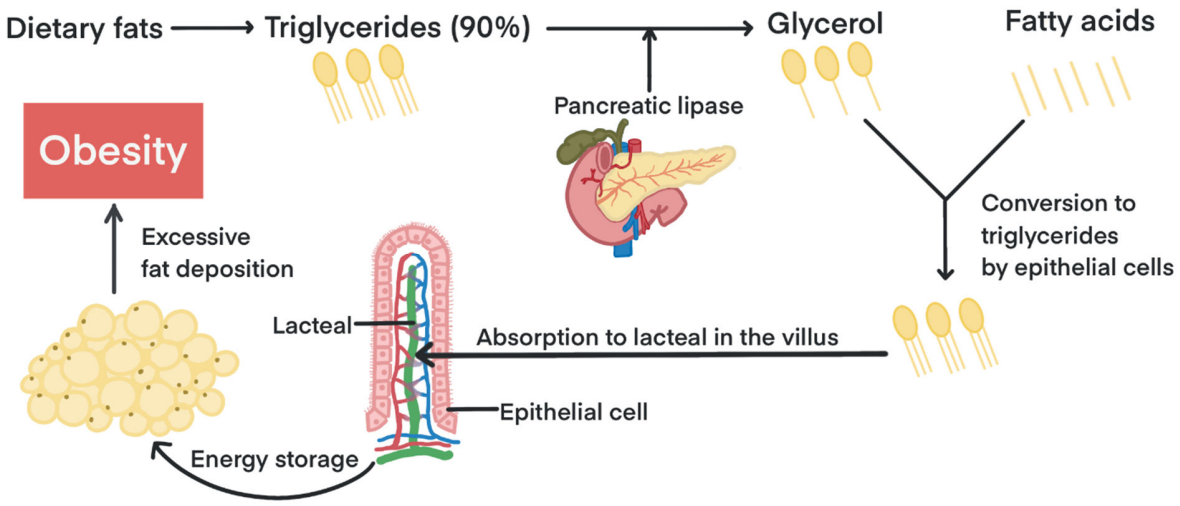


Figure 1. Metabolic pathways related to fats in the human body

Lipase inhibitors are substances that can inhibit lipase activity by reducing the breakdown of fats and their absorption into the human body.

Source 2:

Student scientists in Hong Kong discover anti-obesity agents present in bitter melon seed extract

Some plant tissues contain lipase inhibitors such as polyphenols and saponins.

A team of secondary school student scientists screened more than 60 plant samples to identify natural inhibitors that effectively reduce pancreatic lipase activity *in vitro*. They performed a simple experiment using whole milk and an alkaline solution containing a pH indicator (which is blue at alkaline pH values and yellow at acidic pH values). They first prepared different seed extracts by grinding the seeds with a pestle in a mortar using water and a spoonful of sand.

Scan the QR code to access the material used by the student scientists.

The plant extract was then incubated with alkaline pancreatic lipase containing the pH indicator for 5 minutes. After adding whole milk to initiate fat digestion, the time taken for the reaction mixture to change colour from blue to yellow was recorded. The recorded data were then used to determine the activity of the pancreatic lipase preincubated with different plant extracts.

Scan the QR code to watch their investigation.

The student scientists' findings revealed that bitter melon (*Momordica charantia*) seed extracts contain pancreatic lipase inhibitors. Their findings show potential for addressing the global obesity problem.

Answer the questions below *after* reading the source materials:

(a) Explain why inhibiting the lipase activity in the alimentary canal can help reduce body weight.

(b) In which part of the alimentary canal can you find pancreatic lipase? Explain the conditions that favour the pancreatic lipase activity in this part.

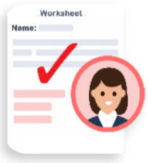
(c) Whole milk contains triglycerides. Write a word equation to show the actions of pancreatic lipase on triglycerides in whole milk.

(d) Explain why whole milk containing an alkaline solution and the pH indicator described in *Source 2* would turn from blue to yellow after the addition of pancreatic lipase.

(e) How is the time taken for the alkaline solution to turn from blue to yellow related to the rate of lipase activity?

(f) After reading the source material, propose *one* investigation question related to the material you have read.

Student Worksheet 2



Notes for teachers

- After discussing with students their responses in *Worksheet 1*, teachers can distribute *Worksheet 2* and ask students to design the investigation at home.
- An *Investigation Planning Template* can be provided to students.
- Student work samples are shown below to illustrate possible student thinking.
- Scan the QR code to get a copy of the *Google Form*.

Task 2

- Answer the questions that follow.

Scenario

Bitter melon (*Momordica charantia*) belongs to the Cucurbitaceae family. Different varieties of bitter melons have different shapes and bitterness. The Cucurbitaceae family is composed of different types of melons.

Your biology teacher asks you to design an investigation to compare the inhibitory effect of the seed extracts of three different types of melons within the Cucurbitaceae family on pancreatic lipase. The goal is to identify the seed extract sample with the highest inhibitory effect on pancreatic lipase activity.

You received the following materials:

Alkaline solution containing a pH indicator (blue under alkaline pHs and yellow under acidic pHs)	Glass vials	Test sample 1 [Bitter melon 1 (*Momordica charantia*) seed extract]
Pancreatic lipase	Timer	Test sample 2 [Bitter melon 2 (*Momordica charantia*) seed extract]
Orlistat (a drug that inhibits pancreatic lipase)	Distilled water	Test sample 3 [Angled Luffa (*Luffa acutangular*) seed extract]
Whole milk	Tablet (to be used as a camera)	Tablet stand
Autopipette	Autopipette tips	

Scan the QR code to view the materials.

The virtual laboratory provides students with opportunities to get familiar with materials used in the investigation.

(a) Briefly describe how you would use the materials to design an investigation to achieve the aim. You can also draw your experimental design.
(For this purpose, the *Investigation Planning Template* may be helpful.)

The *Investigation Planning Template* provides a structured framework and scaffolding to help students express their design decisions.

Brief explanation of my design:

Scan the QR code to get a copy of the *Investigation Planning Template.*

Is a fair test required in this investigation? Why?

A fair test is/is not needed because

Question

How **X:**_____ **affects Y:**_____ ?

Independent variable

How to change and measure X?

Control variables

Anything else that likely affects Y?

How to ensure they are the same between different trials?

Anything that cannot be controlled?

Dependent variables

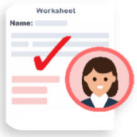
How to change and measure Y?

Is it the best way to measure Y?

Are there any other ways?

Are repeat measurements needed?

Student Samples 1 (Worksheet 2)



Notes for teachers

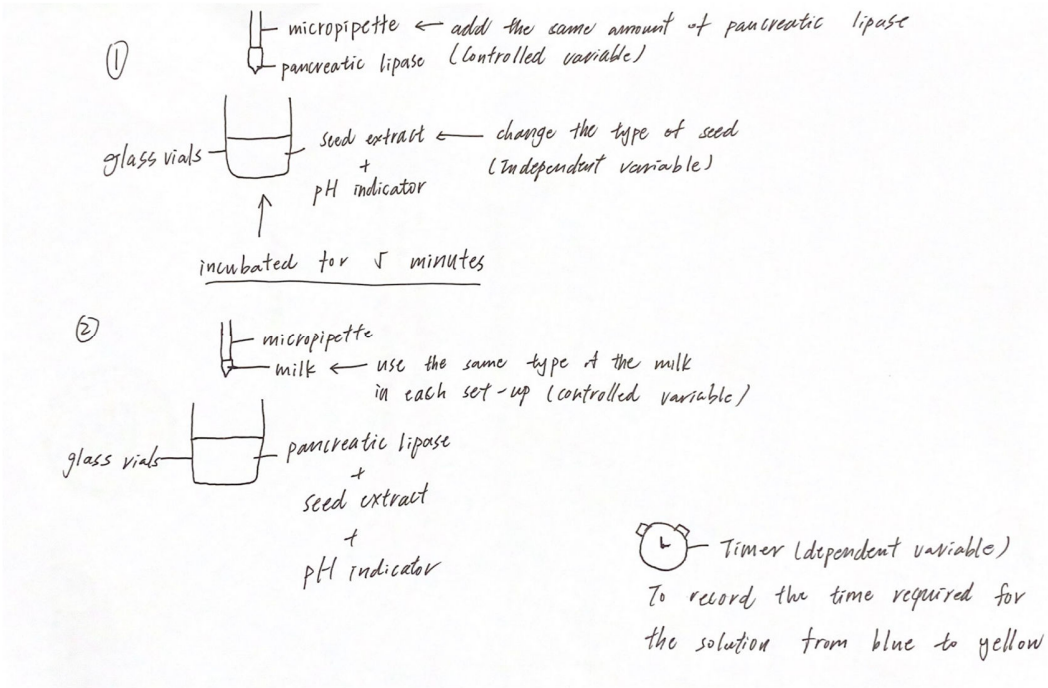
- After collecting students' designs, teachers can select student drawings (anonymised) for discussion.
- The following shows three samples with varying sophistication in responses. Some guiding questions can be included to facilitate students' evaluation of experimental designs.

Examples of students' experimental designs

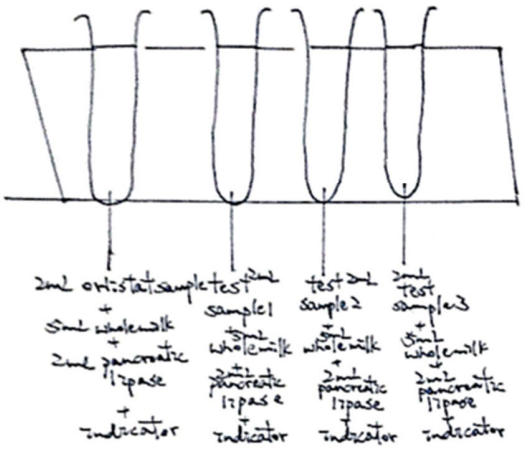
Possible guiding questions

- Which design(s) accurately represent the independent variable and provide methods for manipulating it? Why does your group think so?
- Which design(s) accurately represent the dependent variable and specify the parameters for measuring it? Why does your group think so?
- Which design(s) demonstrates the correct sequence for adding the chemicals? Why does your group think so?
- Which design(s) incorporate the appropriate control set-up(s), if necessary? Why does your group think so?
- What are the ways to enhance the designs to ensure that the data collected are accurate and reliable?

Design ❶:



Design ❷:



① Pipette 5mL whole milk to 4 vials respectively

② Pipette 2mL orlistat, test sample 1, test sample 2, test sample 3 to 4 vials respectively.

③ Put each sample into vials, record the time of indicator colour change afterwards
Orlistat → sample 1 → sample 2 → sample

④ Repeat the tests twice

⑤ Repeat steps above for 3 different temperatures

Independent variable: Test sample 1, 2, 3 and temperature

↳ temperature: put glass vials for required temperature of water bath for 5 mins before tests, incubate them throughout the test.

Dependent variable: Time for indicator colour change
's^{-1}' as unit, the smaller the value, the larger the inhibit effect of the sample
Use timer to record required time.

Control variable: ① amount of pancreatic lipase used
↳ more enzymes will increase the catalyzing rate to the breakdown reaction which greatly lowers the required time by lowering the activation energy. The time recorded may be underestimated.

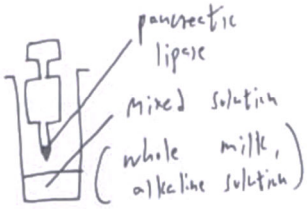
② Total surface area of test sample used
↳ the larger the surface area, the higher rate of inhibitory effect.
Time recorded may be underestimated.

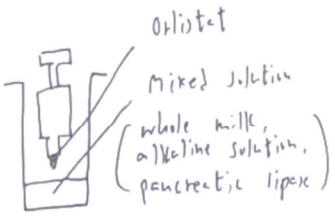
Design Consideration → ↳ crush in same period / length of time (5 mins) to ensure it's more or less the same.

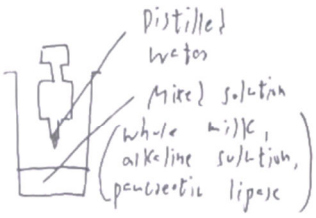
Assumption: Other component in the seed extract does not contain inhibitory effect on pancreatic lipase.

Design ❸ :

To investigate the effect of three different types on melons within the Cucurbitaceae family on the pancreatic lipase activities.

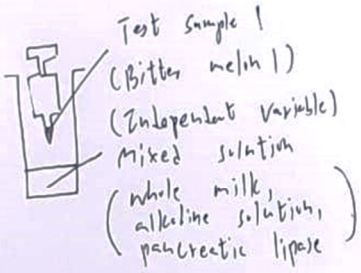




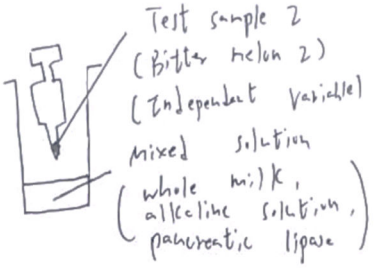


Note: Set a timer for each setup for one minute once the solution in the micropipette tip is added to the mixed solution inside the glass vial.

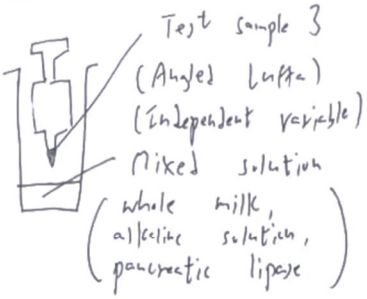
Test sample 1



Test sample 2



Test sample 3



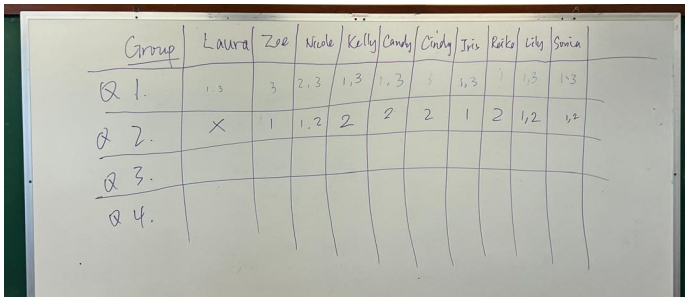
Note: Set a timer for each setup for one minute once the test sample in the micropipette tip is added to the mixed solution inside the glass vial.

Control variables: Initial pancreatic lipase and concentration of test sample. This is to ensure the accuracy of the experiment.

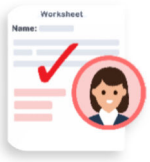
Bacteria may enter the solution through air during experiment.

Notes for teachers

- Teachers can capture and represent student thinking using public displays (e.g. whiteboards) and then work with students to explore their divergent thinking.



Group	Laura	Zoe	Nicole	Kelly	Candy	Cindy	Iris	Peter	Lily	Sonia
Q 1.	1,3	3	2,3	1,3	1,3	3	1,3	1	1,3	1,3
Q 2.	X	1	1,2	2	2	2	1	2	1,2	1,2
Q 3.										
Q 4.										



Notes for teachers

- After receiving feedback on their experimental designs, the following shows questions that teachers may use to guide students in thinking about and assessing the scientific inquiry skills related to their experimental designs.
- Student work samples are shown below to illustrate possible student thinking to some questions.

Task 3

Possible questions

1. The following shows two methods to measure the dependent variable:

Method A:	Record the colour of the alkaline solution containing the pH indicator in glass vials, both *with* and *without* the test samples, after 10 min.
Method B:	Measure the time it takes for the colour of the alkaline solution containing the pH indicator to change (i.e. reach the end point) in the glass vials, both *with* and *without* the test samples.

Your teacher suggests that you should use *Method B*.

(a) What is the limitation of using *Method A* to compare the inhibitory effects of different types of seed extract on pancreatic lipase activity?

(b) Explain how the inhibitory effects of different types of seed extract on pancreatic lipase can be compared using *Method B*.

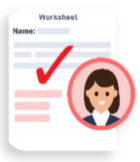
2. Jeffrey proposes two methods for setting up a control to compare the degree of inhibition on alkaline lipase activity:

Set-up A: Replacing the test samples with orlistat.
Set-up B: Replacing the alkaline lipase with boiled alkaline lipase in the glass vials containing the test samples.

Which set-up, *A* or *B*, enables a more accurate determination of the degree of inhibition? Explain your answer.
(Put a '✓' in the appropriate box.)

I will choose ☐ *Set-up A* ☐ *Set-up B*

The reasons:

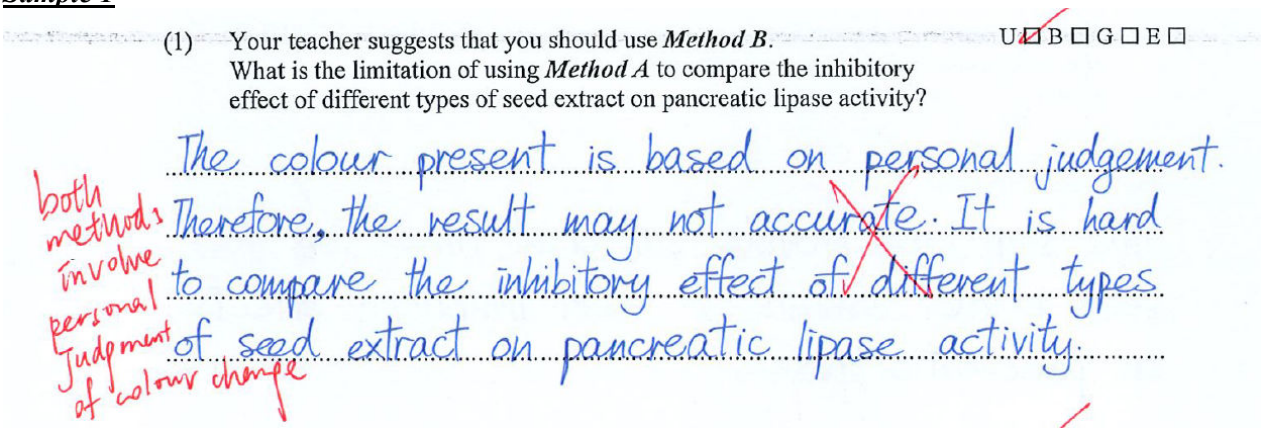


Notes for teachers

- Q.1 assesses students' ability to connect the methods of measurement to the dependent variable and the limitations related to the measurement method.
- Q.2 assesses students' ability to set up the control and explain its function.

The following are some examples of students' responses to Q.1(a):

Sample 1

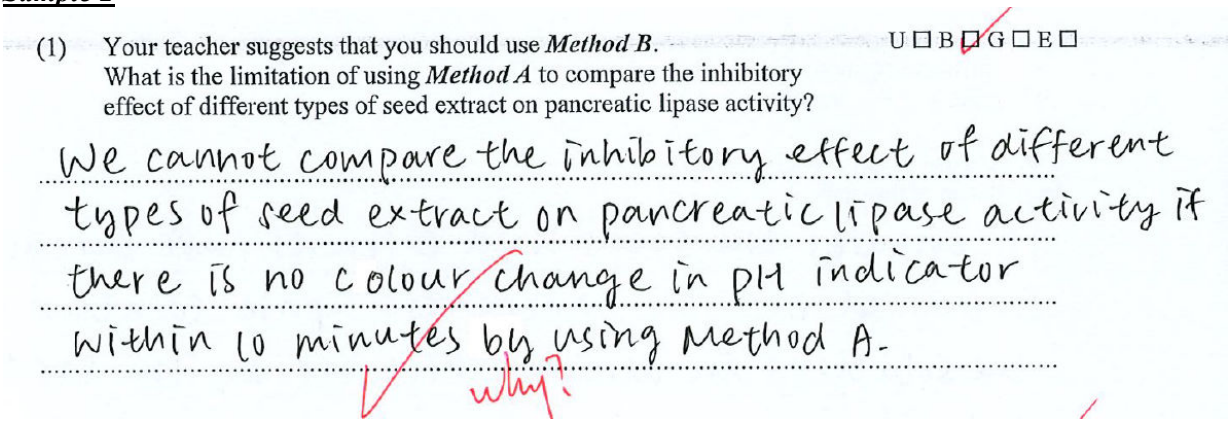

(1) Your teacher suggests that you should use *Method B*. What is the limitation of using *Method A* to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity?

U☑B☐G☐E☐

The colour present is based on personal judgement. Therefore, the result may not accurate. It is hard to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity.

both methods involve personal judgment of colour change

Sample 2

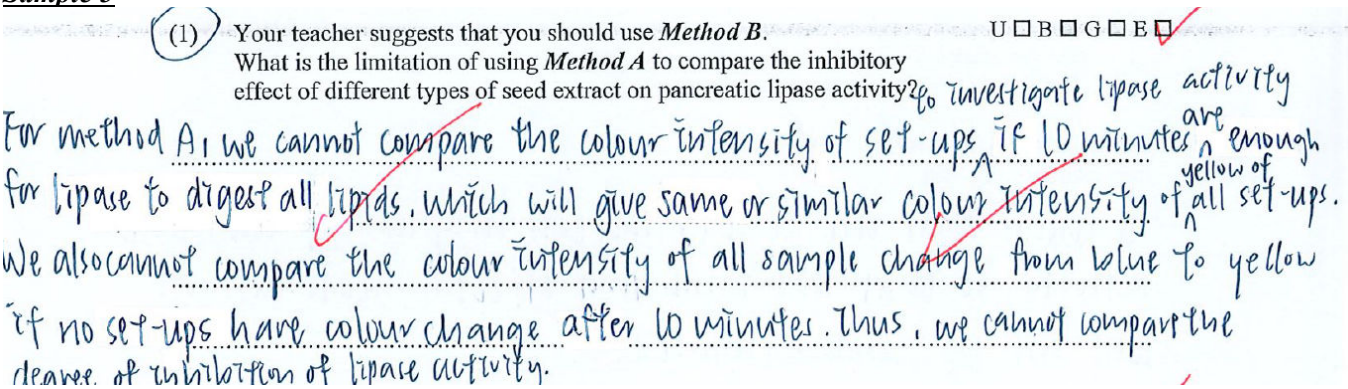

(1) Your teacher suggests that you should use *Method B*. What is the limitation of using *Method A* to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity?

U☐B☑G☐E☐

We cannot compare the inhibitory effect of different types of seed extract on pancreatic lipase activity if there is no colour change in pH indicator within 10 minutes by using Method A.

why?

Sample 3


(1) Your teacher suggests that you should use *Method B*. What is the limitation of using *Method A* to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity?

U☐B☐G☐E☐

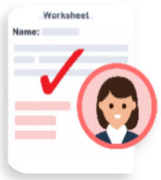
To investigate lipase activity

For method A, we cannot compare the colour intensity of set-ups if 10 minutes are enough for lipase to digest all lipids, which will give same or similar colour intensity of yellow all set-ups. We also cannot compare the colour intensity of all sample change from blue to yellow if no set-ups have colour change after 10 minutes. Thus, we cannot compare the degree of inhibition of lipase activity.

About the samples

- Sample 1 incorrectly cited the limitation arising from the subjectivity of colour judgment, a limitation inherent in both methods.
- Sample 2 correctly identified the limitation but lacked details whereas Sample 3 provided a detailed explanation, such as the absence of colour difference because all lipids were digested within the specified time frame.

Laboratory Manual



Notes for teachers

- It is suggested that seed extracts are prepared for students. See the *Supplementary Resource* section for the protocol.
- Students may also be asked to prepare the seed samples. If so, one more lesson will be required.
- Teachers can distribute the manual for students to read and prepare before the investigation.
- Teachers can ask questions to check if students fully understand the procedures.
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

Task 4

- Read the following procedures to carry out the investigation.

Procedure

Determining the inhibitory effects of seed extracts

1. Use your mobile phone/tablet to start recording a video.
2. Label 15 vials (A1–3 to E1–3).
3. Add the seed extracts/orlistat/distilled water, alkaline solution containing the pH indicator, and pancreatic lipase into the vials, according to the following table:

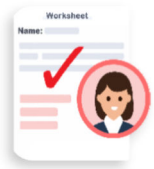
Vial	Sample	(mL)	Alkaline solution containing the pH indicator (mL)	Pancreatic lipase (mL)
A	Seed extract 1	1	2	2
B	Seed extract 2	1	2	2
C	Seed extract 3	1	2	2
D	Orlistat	1	2	2
E	Water	1	2	2

4. Incubate the vials at room temperature for 5 minutes.
5. Add 3 mL of whole milk to the vials, and shake the vials well.
6. Start the timer.
7. Shake the vials occasionally.
8. Repeat *Steps 4–8* two more times.
9. Stop the video recording when the colour of the solution in all of the vials turns yellow from blue.
10. Fill in the data in the *Google Sheet*.

Scan the QR code to get a copy of the *Google Sheet*.

The *Google Sheet* helps students process and visualise the data they collected.

Teacher Notes 2



Notes for teachers

- The following are possible questions that teachers can use to guide students in thinking about or assessing their scientific inquiry skills related to data analysis and interpretation.
- Student work samples are shown below to illustrate possible student thinking to some questions.

Task 5

Possible questions

1. Plot a graph to show the effect of the seed extracts on pancreatic lipase activity.
   Consider the following questions when plotting the graph:
   - Which type of graph (bar graph, line graph, pie chart, etc.) would you choose? Why?
   - Which axis (x-axis/y-axis) should contain the independent variable?
   - Which axis (x-axis/y-axis) should contain the dependent variable?
   - What would be a suitable title for your graph?

Some reminders are added to guide students in constructing graphical representations appropriately.

2. Boris found that an outlier was present in a replicate of one of the test samples. Suggest *one* possible reason for why this occurred.

3. Your classmate claims that the seed extract that showed the highest inhibitory effect on pancreatic lipase in this investigation should be used as an anti-obesity drug.

   Discuss whether you agree with this claim.
   (Put a '✓' in the appropriate box.)

   ☐ Agree
   ☐ Disagree

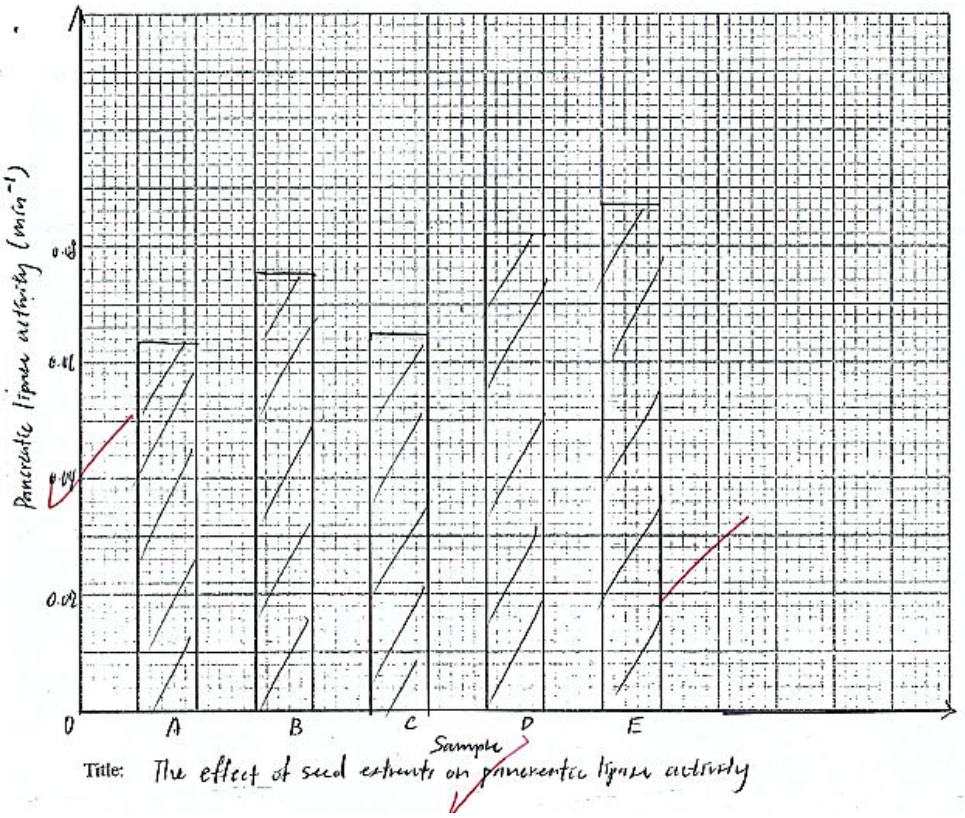
4. Suggest one *new* investigation that needs to be conducted before the seed extract(s) can be used as an anti-obesity drug.

Notes for teachers

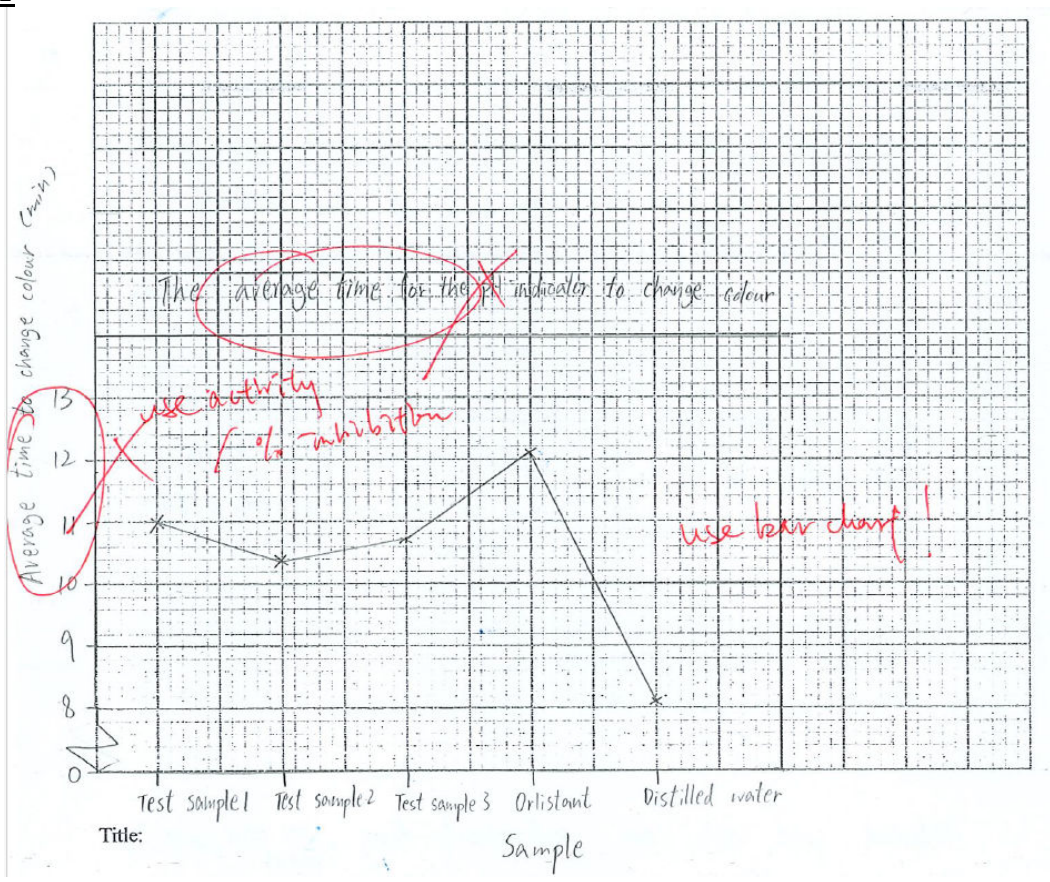
- Q.1 assesses students' ability to construct appropriate graphic representations.
- Q.2 assesses students' ability to propose reasons to explain the occurrence of an outliner.
- Q.3 assesses students' ability to identify the limitations of the generalisability of the results from an *in vitro* to an *in vivo* system.
- Q.4 assesses students' ability to generate a new investigation question that extends the present investigation.

The following are some examples of students' responses to Q.1:

Sample 1



Sample 2



About the samples:

- Sample 1 mistakenly used a line graph for data presentation while Sample 2 correctly used a bar chart to present the data. A bar chart is appropriate as the independent variable is the type of seed extract, which is a categorical variable.
- Sample 1 also has a proper title and labelling of the *x* and *y* axes.

The following are some examples of students' responses to Q.2:

Sample 1

(2) Boris found that an outlier was present in one of the replicates of one of the test samples. Suggest ***one*** possible reason for why this occurred. E2: U ☐ B ☑ G ☐ E ☐

The colour change is difficult to observe. We can't exactly tell when the colour chang the time

Will it result in a great difference in one of the data?

Sample 2

(2) Boris found that an outlier was present in one of the replicates of one of the test samples. Suggest ***one*** possible reason for why this occurred. E2: U ☐ B ☐ G ☑ E ☐

The sample used does not have equal amount of inhibitary effect within the same sample used. The part of sample taken has much more or much less inhibitor than other part of the same sample.

used the set up

due to...

Sample 3

(2) Boris found that an outlier was present in one of the replicates of one of the test samples. Suggest ***one*** possible reason for why this occurred. E2: U ☐ B ☐ G ☐ E ☑

There might be human error occurs. The person might have made mistake in adding a different amount of milk into the sample, which requires a longer or shorter period of time for the breakdown of lipid and the colour change.

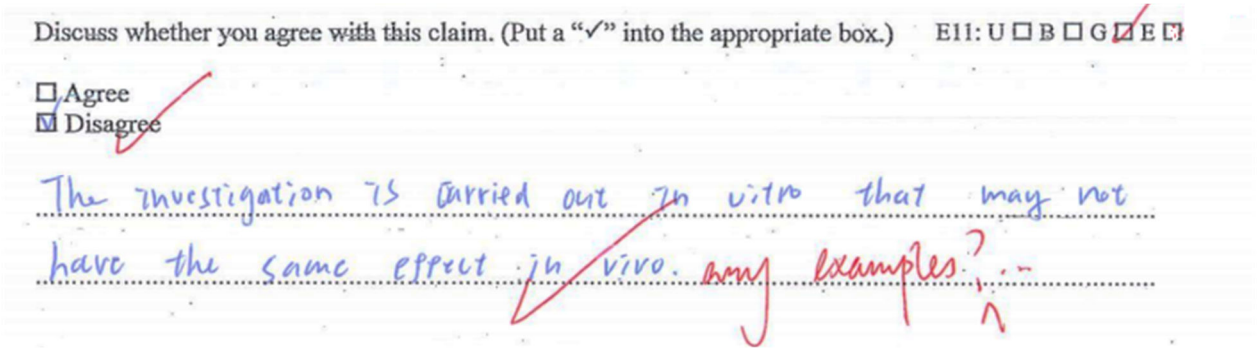
means a different amount of lipid is added, thu

About the samples

- Sample 1 proposed a reason that was not sufficiently convincing in explaining the occurrence of the outlier.
- Sample 2 proposed a plausible reason for the occurrence of the outliers but lacked a detailed explanation.
- Sample 3 not only provided a plausible reason but also offered a more thorough explanation.

The following are some examples of students' responses to Q.3:

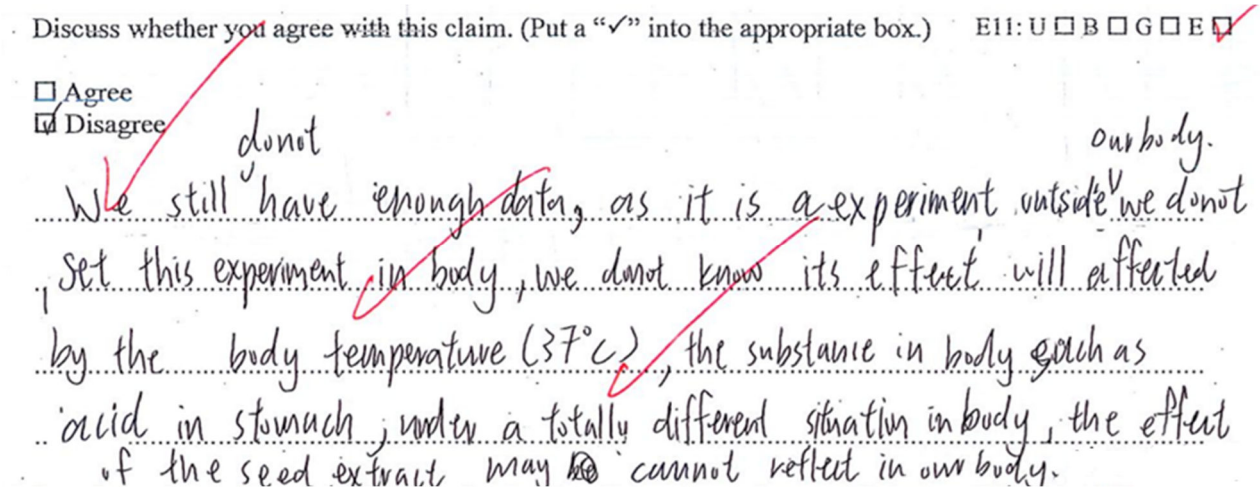
Sample 1


Discuss whether you agree with this claim. (Put a "✓" into the appropriate box.) E11: U ☐ B ☐ G ☑ E ☐

☐ Agree
☑ Disagree

The investigation is carried out in vitro that may not have the same effect in vivo. any examples?

Sample 2


Discuss whether you agree with this claim. (Put a "✓" into the appropriate box.) E11: U ☐ B ☐ G ☐ E ☑

☐ Agree
☑ Disagree

We still donot have enough data, as it is a experiment outside our body. Set this experiment in body, we donot know its effect will effected by the body temperature (37°C), the substance in body such as acid in stomach, under a totally different situation in body, the effect of the seed extract may cannot reflect in our body.

About the samples

- Both samples correctly disagreed with the claim. Both samples identified the limitations of generalising results produced in an *in vitro* system to the *in vivo* conditions.
- Sample 2 further provided an explanation for the difference between the *in vivo* and *in vitro* conditions.

The following are some examples of students' responses to Q.4:

Sample 1

(i) Suggest ***one*** new investigation that needs to be conducted before the seed extract(s) in (g) can be used as an anti-obesity drug. G12: U ☐ B ☒ G ☐

Investigate whether have side effect on human's body. how...

END

Sample 2

(i) Suggest ***one*** new investigation that needs to be conducted before the seed extract(s) in (g) can be used as an anti-obesity drug. G12: U ☐ B ☐ G ☒

Carry out the whole investigation in vivo (e.g. white rat) or at a set temperature of 37°C.

Sample 3

(i) Suggest ***one*** new investigation that needs to be conducted before the seed extract(s) in (g) can be used as an anti-obesity drug. G12: U ☐ B ☐ G ☒

To investigate whether the extracts will inhibit other enzymes like amylase present in pancreatic juice.

END

About the samples

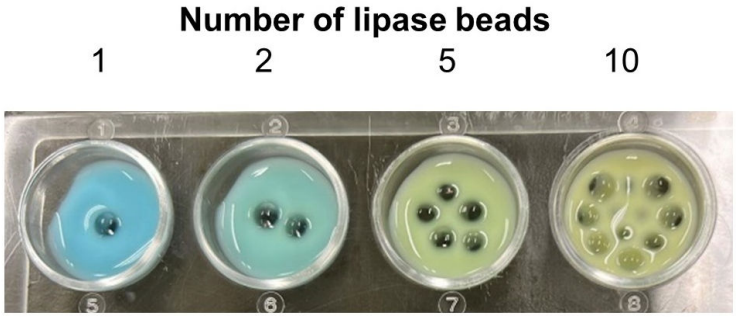
- All the samples were able to generate a new investigation question that expanded upon the current investigation. However, the question posed by Sample 1 was somewhat vague.

Supplementary Resources

Possible Modifications

1. Using immobilised lipase beads to investigate lipase activity

- Lipase can be immobilised using 3% sodium alginate solution. Immobilised lipase beads can be used to study lipase activity using a milk–pH indicator system.
- The following shows the sample results of the investigation that examined the effect of increasing the number of lipase beads on the digestion of milk.
- See Chan et al. (2024) for procedures of how to make immobilised enzyme beads.



Technician Notes

1. Materials for Task 4

Preparation of seed extract

1. Weigh 2 g of seeds using an electronic balance.
2. Place the seeds in a mortar and pestle.
3. Add a spoonful of sand.
4. Add 10 mL of distilled water.
5. Grind the seeds into powder.
6. Filter the extract/Centrifuge the extract at top speed (13, 500 rpm) to obtain the supernatant.

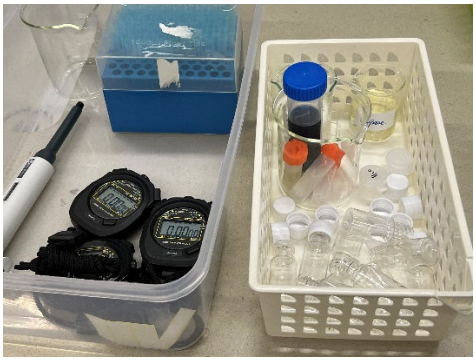
(A grinder can be used to grind the seeds.)

Chemicals to be prepared

- Alkaline solution with a pH indicator (a master mix comprising 100 mL of 2% sodium carbonate [2 g of sodium carbonate in 100 mL distilled water] and 200 mL of 0.04% bromothymol blue [0.1 g of bromothymol blue in 16 mL of 0.01 M sodium hydroxide, with the volume made up to 250 mL with distilled water])
- 5% porcine pancreatic lipase (0.5 g in 10 mL distilled water)
- 10 mg/mL orlistat (120 mg tablet dissolved in 1 mL of absolute ethanol, with the volume made up to 12 mL with distilled water)

Materials for each group

• Whole milk (>35 mL)	• Vials X 15	• Tablet stand
• Distilled water (>3 mL)	• Timer	• Tablet/mobile phone
• Seed extract 1 to 3 (>3 mL)	• Orlistat (>3 mL)	• Rubbish bin
• Autopipette (P-1000)	• Autopipette tips (P-1000)	• Labels
• Pen	• Alkaline solution with a pH indicator (>30 mL)	• Pancreatic lipase solution (>30 mL)



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Chan, K. K. H., Ho, D. T. S., & Lau, D. S. P. (2024). Using amylase beads to investigate factors affecting enzyme activity. *The American Biology Teacher*, *86*(3), 153–160.

Chan, P. C., & Chan, K. K. H. (2023). Inquiry on a potential anti-obesity agent: Investigating pancreatic lipase inhibitors in seed extracts *The American Biology Teacher*, *85*(5):265–269

Royal Society of Biology Nuffield Foundation. (2019). Investigating effect of temperature on the activity of lipase. https://practicalbiology.org/bio-molecules/factors-affecting-enzyme-activity/investigating-effect-of-temperature-on-the-activity-of-lipase

This project is supported by Quality Education Fund (Project No. 2019/0283)

此計劃由優質教育基金贊助 (計劃編號 2019/0283)

nnovations in Biology Investigatior

Faculty of Education
The University of Hong Kong
香港大學教育學院

優質教育基金
Quality Education Fund