



***eBioLabs***  
Integrated tools for  
laboratory teaching

# Bristol BSc Honours Programmes in BioMedSci.

- BSc = 120 credits / year / 3 years.
- Students typically take three 40 credit point Units per year.
- BioMedSci First year is a selection of Units from:

Microbiology, Biochemistry, Chemistry,  
Physiology, Pathology, Anatomy etc etc

# Departmental Teaching: Biochemistry Dept

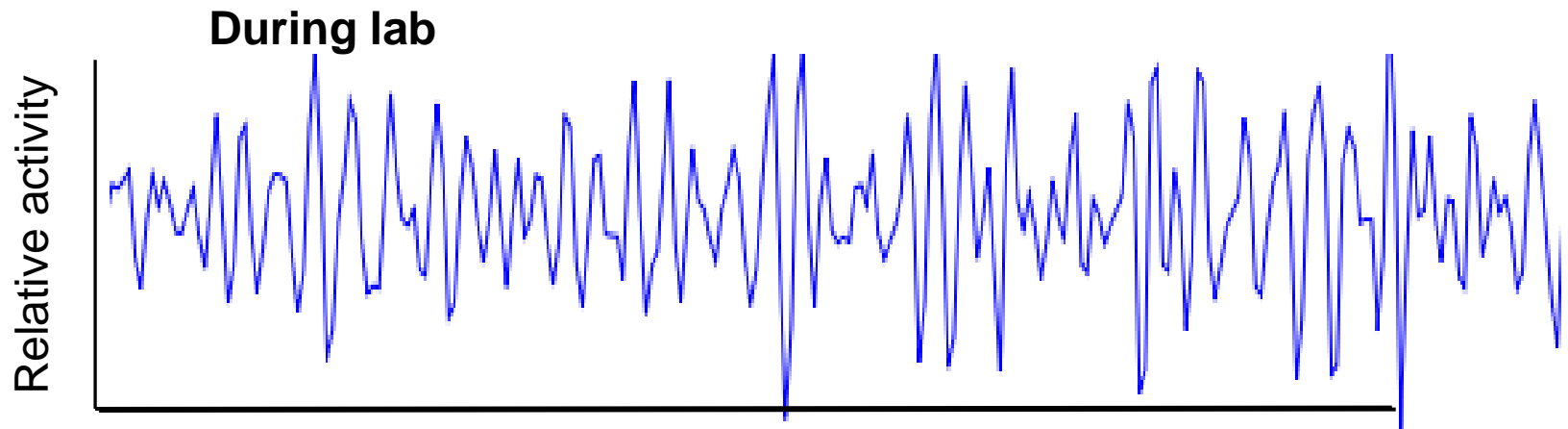
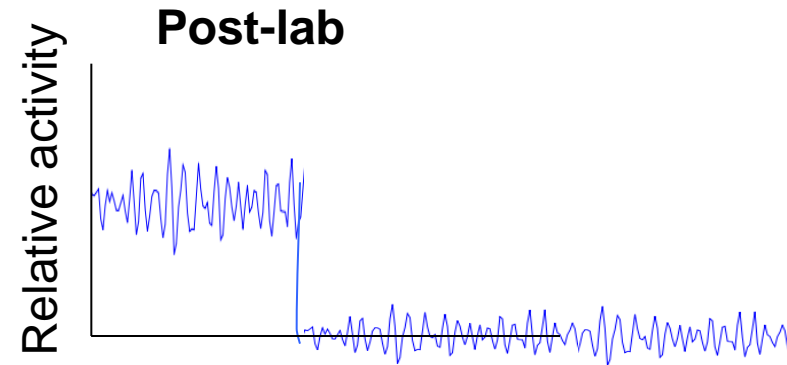
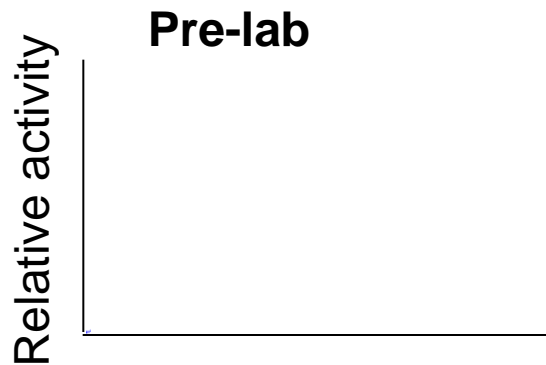
- BSc programme:
  - Single first year 40 credit-point unit (“1G Biochemistry”).
  - 245 students.
  - 45 “honours biochemists”
- Professional programmes:
  - Medics (250).
  - Vets (105).
  - Dentists (80).

# A typical Unit is composed of

- 66 lectures (66 hrs)
- Twelve tutorials (12 hrs)
- Ten practicals (30 hrs)
- Assessment:
  - Progress exam (10 - 15 %)
  - Two three-hour papers (80-90 %)
  - Lab work and tutorials (0-10 %)

# First-year students and attitudes to lab work

# Early Work - Student Encephalography Traces



# Biochemistry Teaching Labs

- Max 120 students (typically 80)
- Each experiment runs up to three times
- Two technicians
- One academic
- One full-time lead demonstrator
- Two to four postgraduate demonstrators



# First-year students and attitudes to lab work



# First-year students and attitudes to lab work





# The lab book before eBiolabs.

## 2. Loading the support plate with centrifuge tubes.

The minimum number of centrifuge tubes you can use is 2 as this allows the load to be evenly balanced. You should make sure that all the tubes contain approximately the same amount of liquid and that they are firmly closed. If you only wish to centrifuge one sample tube then fill the other tube with an equal amount of water. If you are using more than two tubes, spread them evenly around the support plate. This will make sure that the centrifuge stays balanced. The maximum number of tubes you can centrifuge at one time is between 12 and 18 depending on the size of the tubes and the support plate you are using.

## 3. Closing the lid.

Once you have placed the tubes in the support plate, close the lid and push it firmly down. You will hear a 'click' that means that the lid has locked down.

## 4. Choosing the centrifuge speed.

You can choose any speed of centrifuge up to 13,000 RPM.

## 5. Choosing the centrifuge time.

You can either use the pulse switch for times of less than 1 minute or the automatic timer for longer periods of up to 30 minutes.

## 6. How and when to use the pulse switch.

If you want to centrifuge for short times of less than 1 minute you should use the pulse switch instead of the timer. Once you have loaded the centrifuge, close the lid and set the speed, press the 'pulse' switch to start the centrifuge. The 'LID' light will go out and the centrifuge will spin for as long as you keep your finger on the switch. When you release the switch the centrifuge will slow down and stop. The 'LID' light will come on again to show you that you can now open the lid. The pulse feature can also be used for runs of more than 1 minute if you wish.

## 7. Using the automatic timer.

If you want to centrifuge for between 1 minute and 30 minutes you can set the time on the automatic timer. Once set and running the timer will display remaining time until coming to rest then it will revert back to its pre-set value ready for the next run.

## 8. Starting and stopping the centrifuge.

Once the timer is set you should press the green start button to begin centrifuging. The 'LID' light will go out. The centrifuge will spin until the set time has elapsed, when it will slow down and stop, displaying 'END'. When it has stopped the 'LID' light will come on again to show that you can open the lid.

If you want to stop the centrifuge during its spin, you can press the red STOP button.

NOTE: if at the end of a run 'END' is not displayed, then the unit has not completed the set run time.

### **N.B.** Lid 'sleep' mode.

Should the Micro be left on for more than 1 minute without use, the unit will shut down. No attempt should be made to open the lid whilst the Micro is in the 'sleep' mode. To 'wake up' the Micro, simply press any button on the control panel.

## Practical 1

### MEASUREMENTS WITH A SPECTROPHOTOMETER

Dr. P. M. Wood  
(p.wood@bris.ac.uk)

This practical covers two important substances - haemoglobin and ethanol. One is coloured while the other is colourless. The differences in their absorption spectra will be studied. Please make sure you have graph paper before you start.

#### Aims

To determine the absorbance (A) of a given wavelength in proportion to concentration (c). A graph of absorbance is very useful for determining a concentration. For a substance that does not absorb light, the concentration can often be determined by a reaction involving an enzyme.

#### Learning Objectives

After completing this practical you should be able to: determine an absorbance measurement and determine a concentration of a substance from a graph. You should know the assay equation by use of the enzyme dehydrogenase.

#### Minor objectives (for revision)

- Wavelengths for different colours. Dilution factors. Accuracy in biochemistry.

#### Skills:

- Familiarization with the class spectrophotometers; numeracy

#### Introduction

##### 1. The Beer-Lambert Law: $A = \epsilon c l$

When a beam of light passes through a coloured substance, a proportion of it is absorbed and the remainder passes through unchanged. If  $I_0$  is the intensity of the incident light and  $I$  the intensity of light emerging from the sample, then  $(I_0 - I)/I_0$  is the fraction of light absorbed.

Lambert's law states that the fraction of light absorbed by a thin layer of a substance is independent of the light intensity,  $I$ .

Beer's law states that the fraction of light absorbed by a substance is proportional to its concentration,  $c$ .

When a finite thickness of the absorbing substance is considered, the relationship between  $I$  and  $c$  becomes logarithmic, because of the progressive absorption of the light. An integration (see physical chemistry texts) leads to the Beer-Lambert law:

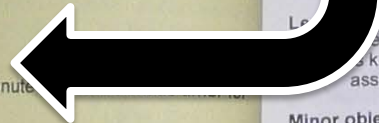
$$\log_{10} (I_0/I) = \epsilon c l$$

Here  $\epsilon$  (epsilon) is a constant, the **absorption coefficient** (also known as extinction coefficient) and  $l$  is the pathlength (= thickness of the absorbing substance).

The quantity  $\log_{10} (I_0/I)$  is defined as the **absorbance**, denoted  $A$ . Hence,

$$A = \epsilon c l$$

# Centrifuge Instructions



# The “lab book” after eBioLabs.

It is important to make sure the centrifuge is balanced before running it; an unbalanced rotor is dangerous.

You have **1** more tube that needs spinning – click the rotor where you want to add it to ensure the centrifuge is balanced.



## Centrifuge Instructions are now online and interactive

Make sure you understand the steps involved in using a centrifuge safely.

Use of the microfuge

Balance microfuge tubes

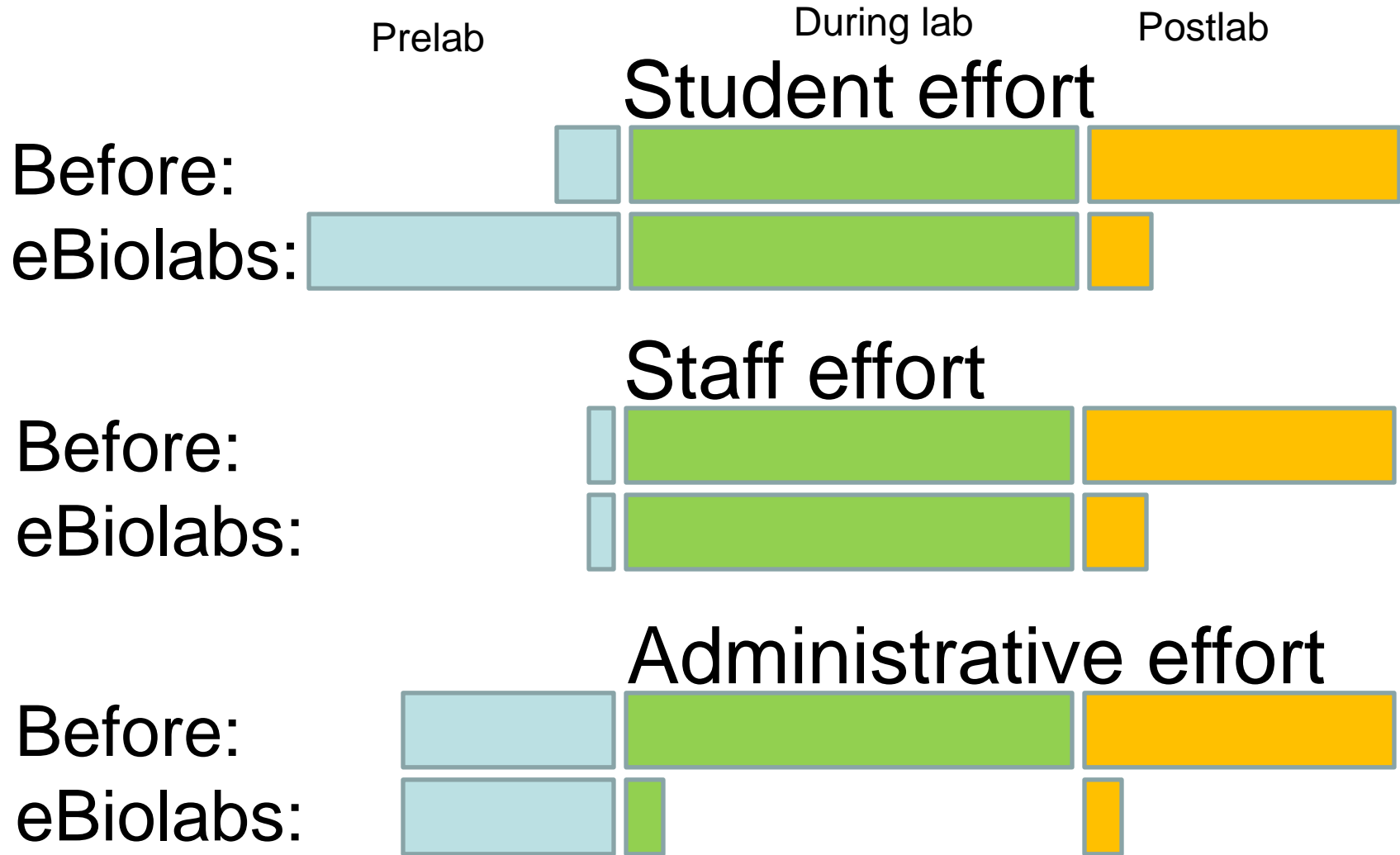
Set controls on microfuge

Start microfuge

Balance microfuge tubes



# eBioLabs aims:

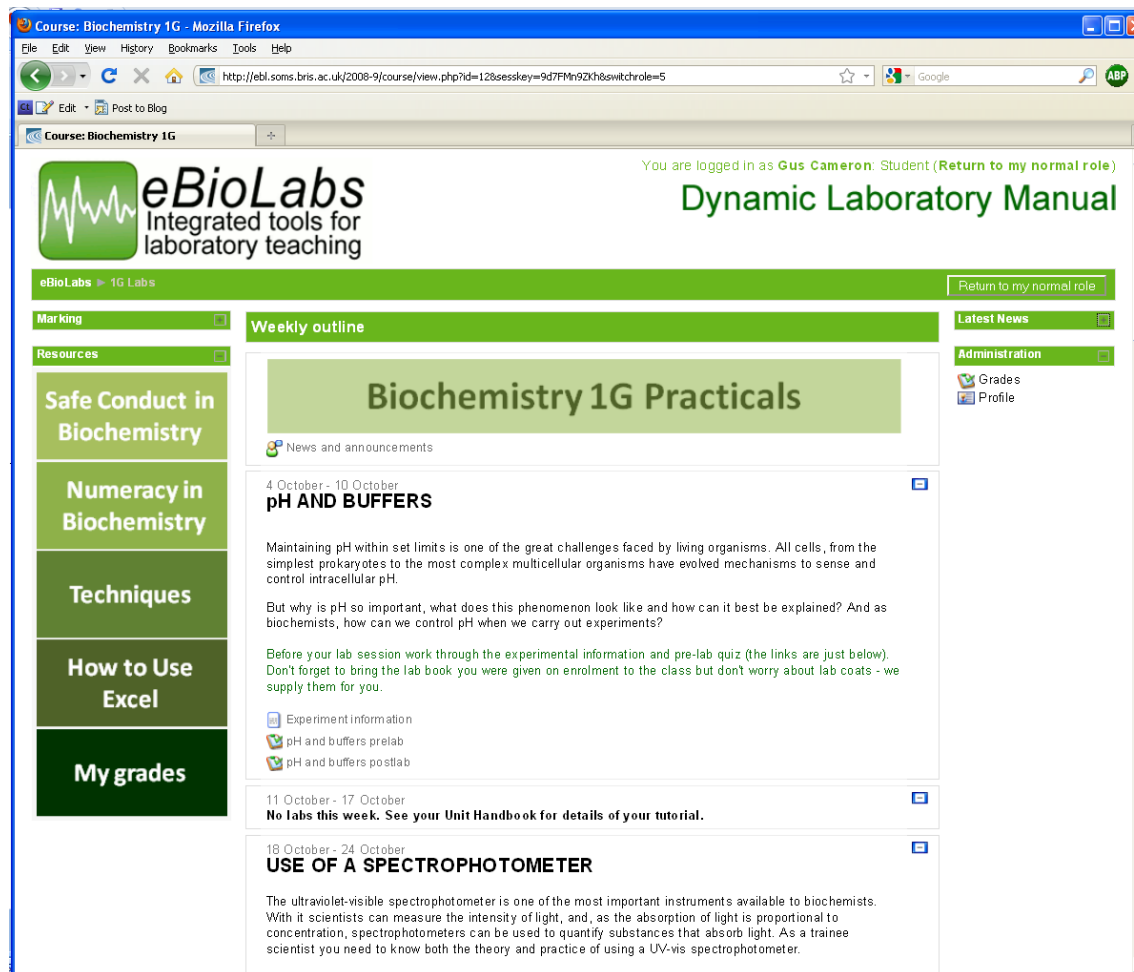


# eBiolabs combines

- Lab instructions.
- Pre-lab tasks.
- On-line submission of work.
- On-line marking and feedback.
- Electronic registers.
- Electronic grade-books.
- Communication tools.



# eBioLabs website



The screenshot shows a web browser window titled "Course: Biochemistry 1G - Mozilla Firefox". The address bar contains the URL: <http://ebl.soms.lbris.ac.uk/2008-9/course/view.php?id=12&sesskey=9d7Fm92Kh&switchrole=5>. The page content includes:

- Header:** "eBioLabs Integrated tools for laboratory teaching" logo on the left, and "You are logged in as Gus Cameron: Student (Return to my normal role) Dynamic Laboratory Manual" on the right.
- Navigation:** A green bar with "eBioLabs > 1G Labs" and a "Return to my normal role" button.
- Left Sidebar (Resources):** A vertical list of green buttons: "Safe Conduct in Biochemistry", "Numeracy in Biochemistry", "Techniques", "How to Use Excel", and "My grades".
- Main Content Area:**
  - Weekly outline** header.
  - Biochemistry 1G Practicals** section header.
  - News and announcements** section:
    - 4 October - 10 October: pH AND BUFFERS**

Maintaining pH within set limits is one of the great challenges faced by living organisms. All cells, from the simplest prokaryotes to the most complex multicellular organisms have evolved mechanisms to sense and control intracellular pH.

But why is pH so important, what does this phenomenon look like and how can it best be explained? And as biochemists, how can we control pH when we carry out experiments?

Before your lab session work through the experimental information and pre-lab quiz (the links are just below). Don't forget to bring the lab book you were given on enrolment to the class but don't worry about lab coats - we supply them for you.

      - Experiment information
      - pH and buffers prelab
      - pH and buffers postlab
    - 11 October - 17 October: No labs this week. See your Unit Handbook for details of your tutorial.**
    - 18 October - 24 October: USE OF A SPECTROPHOTOMETER**

The ultraviolet-visible spectrophotometer is one of the most important instruments available to biochemists. With it scientists can measure the intensity of light, and, as the absorption of light is proportional to concentration, spectrophotometers can be used to quantify substances that absorb light. As a trainee scientist you need to know both the theory and practice of using a UV-vis spectrophotometer.

# Student workflow

1. Log in via Single Sign On.
2. Study experimental information.
3. Check understanding with pre-lab test.
4. Attend practical and record results.
5. Log in and submit post lab assignment.
6. Await feedback and marks.

# Experimental Information

The respiratory chain - Mozilla Firefox

http://ebl.soms.bris.ac.uk/2008-9/file.php/12/mitochondria/Practical 9 Ox Phos Revised GC v2/page\_05.htm

## Mitochondrial respiration studied using the oxygen electrode

**Table of Contents**

- A. Overview
- B. Introduction
  - 1. The Citric Acid Cycle
  - 2. Oxidation is coupled to phosphorylation.
  - 3. The respiratory chain**
  - 4. Calculation of P/O ratios 1.
- C. Experimental setup
  - 1. Electrode theory
  - 2. The Clark oxygen electrode
  - 3. Calculation of P/O ratios 2.
  - 4. Key points
- D. Experimental protocol
  - 1. Getting started
  - 2. Investigation of oxidative phosphorylation
- E. After you leave the lab

### The respiratory chain

A diagram of the respiratory chain and how this is linked to the citric acid cycle is shown [here](#).

The effect of various respiratory chain inhibitors is shown [here](#).

**Explore the following interactive animation making sure you follow the instructions and fully understand the processes that are represented before you continue.**

Legend:

- Red line: Movement of H<sup>+</sup>
- Blue line: Movement of electrons

NADH	succinate	NAD <sup>+</sup>
FADH <sub>2</sub>	FAD	malate

Complex I: 4H<sup>+</sup> (intermembrane space)

Complex IV: 2H<sup>+</sup> (intermembrane space)

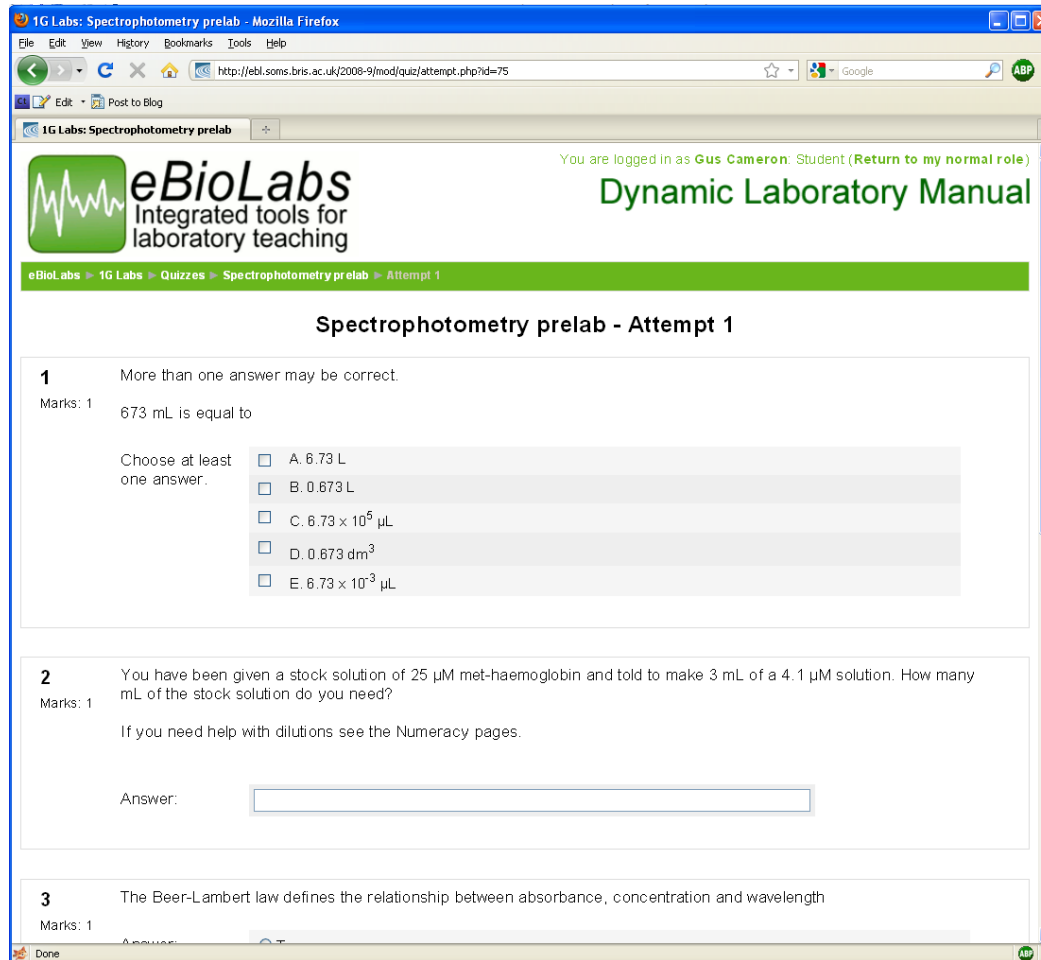
ATP synthase: 3H<sup>+</sup> (intermembrane space) → ATP

Matrix: oxaloacetate, fumarate

Central box: Study the following diagram of the electron transport chain and drag the missing components into the correct place. Continue

eBioLabs - Integrated Tools for Laboratory Teaching

# Testing understanding



1G Labs: Spectrophotometry prelab - Mozilla Firefox

http://ebl.soms.bris.ac.uk/2008-9/mod/quiz/attempt.php?id=75

You are logged in as Gus Cameron: Student (Return to my normal role)

**eBioLabs**  
Integrated tools for laboratory teaching

Dynamic Laboratory Manual

eBioLabs > 1G Labs > Quizzes > Spectrophotometry prelab > Attempt 1

### Spectrophotometry prelab - Attempt 1

**1** More than one answer may be correct.  
Marks: 1  
673 mL is equal to

Choose at least one answer:

- A. 6.73 L
- B. 0.673 L
- C.  $6.73 \times 10^6 \mu\text{L}$
- D.  $0.673 \text{ dm}^3$
- E.  $6.73 \times 10^{-3} \mu\text{L}$

**2** You have been given a stock solution of  $25 \mu\text{M}$  met-haemoglobin and told to make 3 mL of a  $4.1 \mu\text{M}$  solution. How many mL of the stock solution do you need?  
Marks: 1  
If you need help with dilutions see the Numeracy pages.

Answer:

**3** The Beer-Lambert law defines the relationship between absorbance, concentration and wavelength  
Marks: 1  
Answer:

# Gradebook

1G Labs: Grades: View - Mozilla Firefox  
 http://ebl.soms.bris.ac.uk/2008-9/grade/report/grader/index.php?id=12&perpage=10&page=0&sortitemid=78










**eBioLabs** Integrated tools for laboratory teaching  
 Dynamic Laboratory Manual

eBioLabs > 1G Labs > Grades > View > Grader report

Grader report

Visible groups: All participants

Page: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 ...26 (Next)

First name / Surname	Biochemistry 1G					
	Aggregation	Course total	pH and buffers Full view	Spectrophotometry		
			pH and buffers prelab	pH and buffers postlab	Experiment 1	Spectrophotometry prelab
 Olivia Lambert		741	20	69	93	16
 Jenny Sharpe		854	20	74	97	17
 Emma Still		760	20	70	87	17
 Eleanor Bird		529	20	70	87	14
 Luke Dickerson		496	20	60	79	17
 Vesislava Petrova		768	20	71	99	19
 Paul Lissowski		685	20	71	81	18
 Nicholas Hough		779	20	75	90	13
 Sarah Cumming		740	20	64	91	18

# Lab book

5

You may need to adjust the **Cal** control during the practical to maintain full scale deflection (with fresh buffer and NO mitochondria – see 1), but **do not** touch the zero control.

**B. Effect of various inhibitors** - note these are toxic, but the amounts that you are using pose no health risk. If you do get any on your skin, wash with methanol and then water.

Perform the following experiments:

- (i) Set up the electrode with medium + phosphate in the cell. Add glutamate + malate + mitochondria before lowering the electrode. Then add two additions of ADP and while respiration is proceeding rapidly, add **oligomycin**. Add more ADP and observe the effect. Finally add **dinitrophenol** and note what happens.
- (ii) Set up the electrode with medium + malate + glutamate + phosphate in the cell. Add mitochondria then add DNP. After the rapid rate has been obtained, add **rotenone**, then succinate, then **antimycin**, then ascorbate, allowing time for any effect to be observed in each case

Lab Session 10. Mitochondrial respiration

6

## Results

### 1. Calculation of P/O ratios:

i) nmols of ADP in 20 $\mu$ l addition =

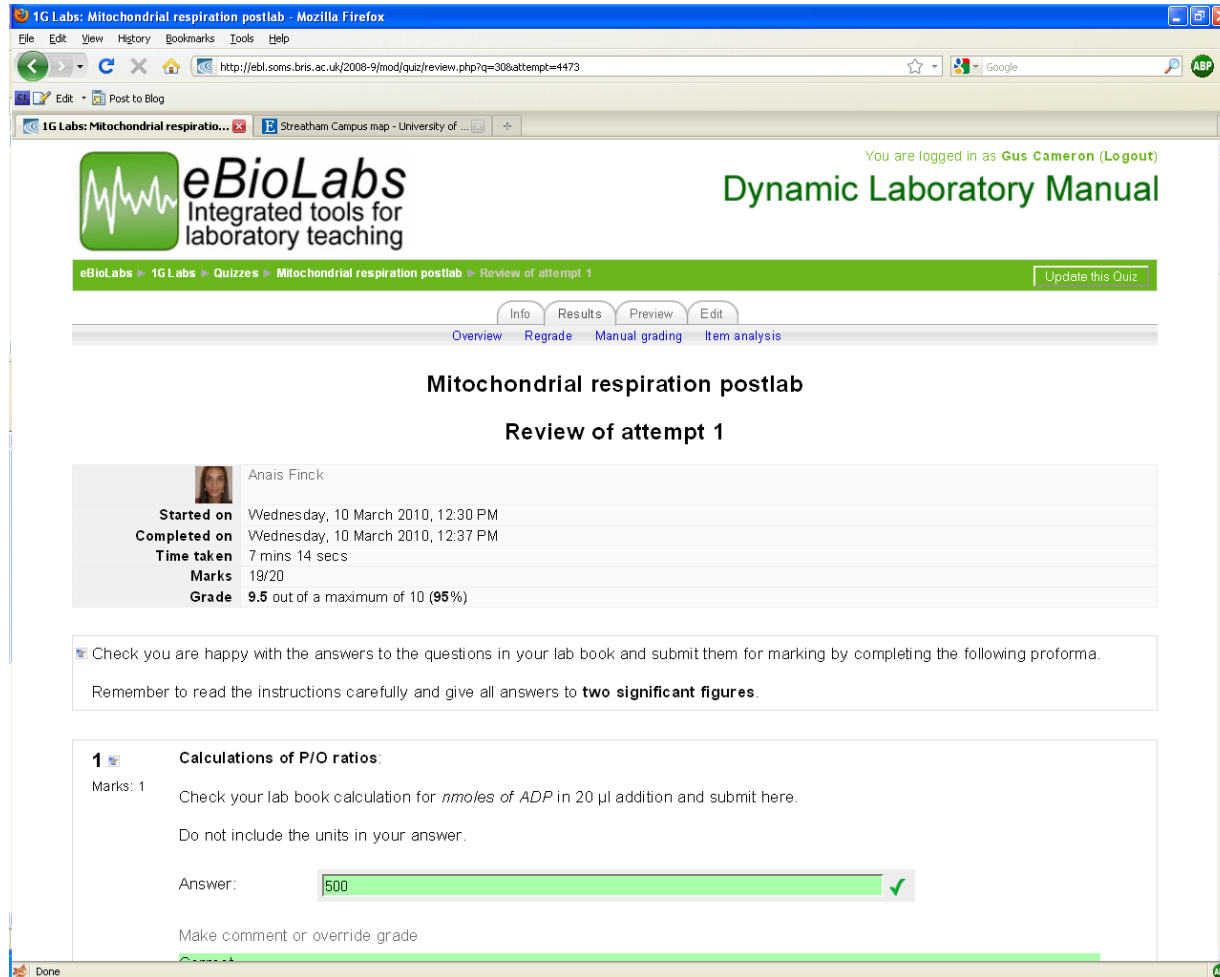
ii) nmols O atoms in 3.0 ml air saturated medium =

iii) nmols O atoms represented by each small division on the chart recorder =

		Substrate		
		Glutamate + malate	Succinate	Ascorbate + TMPD
Number of chart recorder divisions	ADP addition 1			
	ADP addition 2			
	ADP addition 3			
Mean value				
Mean P/O				
Expected P/O				

Lab Session 10. Mitochondrial respiration

# Post-lab tasks. *Integrated.*



The screenshot shows a Mozilla Firefox browser window displaying the eBioLabs website. The page title is "1G Labs: Mitochondrial respiration postlab". The user is logged in as "Gus Cameron". The page content includes a navigation menu with "Info", "Results", "Preview", and "Edit" tabs. Below the navigation, there are buttons for "Overview", "Regrade", "Manual grading", and "Item analysis". The main heading is "Mitochondrial respiration postlab" followed by "Review of attempt 1". A table shows the user's performance: Anais Finck, Started on Wednesday, 10 March 2010, 12:30 PM, Completed on Wednesday, 10 March 2010, 12:37 PM, Time taken 7 mins 14 secs, Marks 19/20, and Grade 9.5 out of a maximum of 10 (95%). Below the table, there is a checkbox for "Check you are happy with the answers to the questions in your lab book and submit them for marking by completing the following proforma." and a text box for "Remember to read the instructions carefully and give all answers to two significant figures." The first question is "Calculations of P/O ratios:" with a mark of 1. The question text is "Check your lab book calculation for *nmoles* of ADP in 20  $\mu$ l addition and submit here. Do not include the units in your answer." The answer field contains "500" and is marked as correct with a green checkmark. There is a "Make comment or override grade" link below the answer field.

# Post-lab tasks. *Excel.*

Macromedia Fireworks 8 - [Untitled-3.png @ 73% (Bitmap)\*]  
 Kate\_Binley\_marked\_RBC\_kb9213\_1\_xlsx [Read-Only] - Microsoft Excel

Home Insert Page Layout Formulas Data Review View Developer Add-Ins Acrobat

Normal Page Layout Page Break Preview Custom Views Full Screen Workbook Views

Message Bar Show/Hide

Ruler Formula Bar Gridlines Headings

Zoom 100% Zoom to Selection Zoom

New Window Arrange All Freeze Panes Unhide Hide Synchronise Reset Wind

X3

**eBiolabs Session 6: Membrane Permeability**  
 This spreadsheet is automatically marked. Do not add additional rows, columns or formatting of any kind.  
 You MUST save this spreadsheet in Excel format on your computer before to uploading it to eBiolabs.

Enter your name here: Kate Binley Your lab partners name: Cherie Leung  
 Enter your Bristol Username here: kb9213 Did you do experiment A or B? A

**A1. Osmotic fragility curve with NaCl (5 marks)**

Tube	Final [NaCl] (M)	A <sub>420</sub>	% Haemolysis	% Haemolysis Best Fit	Square of Error
1	0.147	0.016	1.39	0.029	1.854
2	0.14	0.027	2.34	0.085	5.087
3	0.13	0.028	2.43	0.398	4.130
4	0.12	0.036	3.13	1.849	1.642
5	0.11	0.067	5.82	8.161	5.478
6	0.0999	0.326	28.3	29.861	2.435
7	0.09	0.817	70.9	66.414	20.123
8	0.08	0.972	84.4	90.318	35.023
9	0.06	1.112	96.5	99.521	9.124
10	0	1.152	100	100.000	0.000
Sum Squares					2.914
EC50_A1					0.094
Slope_A1					-67.37

**A2. Osmotic fragility curve with Sucrose (5 marks)**

Tube	Final [Sucrose] (M)	A <sub>420</sub>	% Haemolysis	% Haemolysis Best Fit	Square of Error
1	0.294	0.064	6.15	0.013	37.664
2	0.24	0.068	6.54	0.452	37.060
3	0.22	0.045	4.33	1.670	7.074
4	0.2	0.065	6.25	5.971	0.078
5	0.18	0.078	7.5	19.186	136.557
6	0.16	0.614	59	47.021	143.498
7	0.14	0.743	71.4	76.841	29.609
8	0.12	0.917	88.2	92.540	18.834
9	0.0789	1.05	101	99.466	2.352
10	0	1.04	100	99.997	0.000
Sum Squares					6.424
EC50_A2					0.158
Slope_A2					-28.63

**Osmotic fragility curves**

The osmolarity required to obtain 50% lysis (the EC50) is the same for all three compounds. Thus when concentration is measured in molarity the EC50 of sucrose (one species) will be three times the EC50 of Na<sub>2</sub>HPO<sub>4</sub> (three species) and 1.5 times that of NaCl (two species). The EC50 of NaCl should be twice that of Na<sub>2</sub>HPO<sub>4</sub>. We have calculated the EC50 from the data you presented and used that to assign marks.

EC50 for the compounds	EC50_A1	0.094	M NaCl
	EC50_A2	0.158	M Sucrose
Ratio of the EC50s	EC50_A2 / EC50_A1	1.68	M Na <sub>2</sub> HPO <sub>4</sub>
	EC50_A1 / EC50_B1	2.80	
Expected ratio	EC50_A2 / EC50_A1	1.5	
	EC50_A1 / EC50_B1	3	
% Error	% Error	11.7	
	% Error	6.5	
Marks / 5	Marks / 5	Marks / 5	

# eBioLabs year one. Results.

- ~7000 student contact hours supported.
- ~5000 student assignments managed.
- Significant differences between eBioLabs students and non-eBioLabs students.
- Reflects way students want to learn.
- Staff report higher level conversations.
- ~ 40 staff days saved.

# eBioLabs. Next.

- Physiology first year.
- Pharmacology first year.
- Biochemistry second year.
- Cellular and Molecular Medicine /  
Microbiology pending.