COURSE TITLE: BIOC 2169 – Practical Skills in Biochemistry II
SEMESTER: II
LEVEL: II
NO OF CREDITS: 1.5

PRE-REQUISITES: BIOL1362 Biochemistry I or BIOL1061 Cell Biology and Genetics and, either CHEM1066 Introduction to Chemistry I and CHEM1067 Introduction to Chemistry II or CHEM1060 Introductory Chemistry.

Course Description

Practical Skills in Biochemistry is a yearlong course run through semester I and II. This course is geared to equip students with the necessary laboratory skills to adequately perform routine practical manipulations in biochemistry. It introduces students to common laboratory instruments and familiarizes them with common protocols and techniques used in biochemistry laboratories. Many of the laboratory exercises complement the theory components covered in other Level II courses, which should reinforce biochemistry concepts taught in other courses. New practical concepts are also introduced which will be supported by theory taught in lectures with tutorial support within this course. A Course manual will be available online, alongside lecture handouts and further reading. The course will be fully supported by myelearning. The course will be assessed fully by coursework with no final examination. Coursework assessment will include a combination of two incourse theory examinations, laboratory quizzes, laboratory etiquette and laboratory reports.

Rationale

Biochemistry is a laboratory based science and as such an understanding of basic biochemistry techniques is essential in the training of a biochemist. The techniques and calculations covered in this course are taught within a series of individual laboratory exercises but are

Instructor’s Information

Instructor: Details to be provided
E-mail: 
Phone:
Letter to the Student:
Many of you will be continuing in Biochemistry at the graduate level, while many others will find employment in Biochemistry Laboratories. This course provides the practical skills which are absolutely essential to be able to carry out the research required to discover new knowledge in Biochemistry and to work in a Biochemistry laboratory. It would also enhance your appreciation of the information you are exposed to in the classroom. It actually brings the subject to life as you verify for yourself some of the ‘proven’ knowledge, while at the same time it gives you the opportunity to discover new information and to ask new questions. You will be required to work in small groups which allow you to share ideas and to problem-solve together, but it is your responsibility to ensure that you experience all the necessary ‘hands-on’ activities. Obey ALL safety guidelines and rules and enjoy the experience.

Course Content

PRACTICALS:

SEMESTER I

1. Instrumentation and Safety in the Biochemistry Laboratory (3 hrs)
   Objectives: Students should be able to:
   - describe standard safety precautions and safe work practices for biochemical laboratories
   - calibrate pH meters and spectrophotometers
   - identify and use various instruments in the lab
   - construct a calibration curve and perform the necessary calculations to quantify various biomolecules.

2. pH & Buffers (4 hrs)
   Objectives: Students should be able to:
   - construct a titration curve and extract physical information from them.
   - apply the theory behind the Henderson-Hasselbach equation to experimentally determine pKa values and prepare a buffer accordingly.

3. Proteins & Amino Acids (5 hrs)
   Objectives: Students should be able to:
   - accurately quantify proteins using the Lowry/Bradford test.
   - detect the presence of amino acids and infer structure based on the Ninhydrin test.
   - show that protein(s) can be isolated or separated from solutions by the use of various precipitants.
   - separate mixtures of amino acids and proteins using various chromatographic and electrophoretic techniques.

4. The Hill Reaction in Isolated Chloroplasts (5 hrs)
Objectives: Students should be able to:
- isolate chloroplasts and quantify chlorophyll content from leaf tissues.
- show the effect of light intensity on the rate of the light reactions using the Hill reaction.
- illustrate that photosynthesis can be inhibited by using inhibitors which uncouple ATP synthesis and hinder electron transport.

5. Measurement of Arginase Activity (4 hours)
Objectives: Students should be able to:
- prepare a crude extract of an enzyme from living tissue.
- estimate the activity of an enzyme using a fixed time incubation method and spectrophotometry.

6. Assay of Tissue Glycogen (5 hours)
Objectives: Students should be able to:
- isolate glycogen from liver, muscle, kidney and heart tissues.
- quantify glycogen indirectly using the glucose oxidase-peroxidase reaction.
- use the acquired data to explain how glucose is metabolized in the fed and fasting states.

SEMESTER II
7. DNA and RNA Isolation in Animal Tissues
Objectives: Students should be able to:
- extract DNA from various animal tissues
- quantify DNA using UV and visible spectrometry
- to determine purity of various DNA samples

Lectures pertaining to this lab to be given during downtime of labs:-
- Principles of agarose gel electrophoresis
- Principles of DNA extraction from different sources i.e. plant, animal, microbes
- Other methods of estimating DNA integrity

8. Partial Purification and Characterization of Yeast Invertase
Exercise Summary
This is a project that will be completed in five (5) laboratory sessions. From this exercise, students should be able to isolate and purify an enzyme from a living organism using the knowledge and technical skills acquired in Semester I. Fractions of varying levels of purity are prepared and total protein in each fraction is quantified and the enzyme of interest is assayed for activity. Students will perform gel electrophoresis to visualize the effect of purification and determine the size of the invertase enzyme. They will also perform experiments using the purest fraction to determine the kinetics of the isolated enzyme.

Sessions I – Extraction, heat treatment and alcohol fractionation
Objectives: Students should be able to:
- prepare fractions of invertase of various levels of purity using a heat treatment to coagulate/precipitate contaminating proteins and alcohol fractionation to separate the
protein of interest.

Session II – DEAE – Cellulose Column Chromatography
Objectives: Students should be able to:
- purify an enzyme using DEAE-Cellulose column chromatography
- indirectly quantify an enzyme using spectrophotometry.
- to estimate enzyme activity in various fractions using diagnostic Clinistix.

Session III – Protein Determination, Nelson’s Assay and Activity Determinants
Objectives: Students should be able to:
- determine protein concentrations in the various fractions using the Lowry method.
- use their data to calculate and explain the relevance of – Units of enzyme, Specific Activity, Enzyme Yield and Fold Purification.

Session IV & V- Enzyme Kinetics Using Fraction 4 and Acrylamide Electrophoresis.
Objectives: Students should be able to:
- determine and discuss the effect of invertase concentration on initial velocity.
- determine and discuss the effect of increasing incubation time on product formation.
- determine and explain the kinetics of invertase by increasing substrate concentration.
- determine and explain the inhibitory kinetics of invertase using urea.
- to use polyacrylamide gel electrophoresis (PAGE) to separate, identify and determine the molecular size of yeast invertase as well as to visualize the effect of purification in enzyme isolation.

**Goals/Aims:**
To equip students with the necessary laboratory and practical skills to function in a biochemistry laboratory.

**Course Learning Outcomes**

At the end of the course students should be able to:
- structure and communicate ideas effectively in a standardized report format.
- manage time and work to deadlines.
- work as a member of a group or team.
- work independently and be self-reliant.
- solve numerical problems.
- find and filter information.
- calibrate and use common instruments found in a biochemistry laboratory.
- use various techniques for the identification, quantification, isolation and purification of biomolecules.
- assay and elucidate the kinetics of enzymes.
- work efficiently and safely with various animal and plant tissues knowing the safety procedures employed in handling both groups of organisms.
- perform the accompanying calculations with each lab and present, interpret and discuss
the data scientifically.

- plan, design and carry out experiments to solve a real world biochemistry problem.
- generate, collect and analyze scientific data.
- identify sources of experimental error.
- interpret results and discuss in relation to objectives of investigation.
- demonstrate correct lab skills.
- apply rules of safety in practice of lab investigation.
- demonstrate proper protocols, SOP’s of common scientific equipment.
- conduct experiments with accuracy and practical competence.
- test and perform various experiments to isolate and quantify various biomolecules.
- to study the kinetics of various enzymes and the various factors that affects their activity.
- use appropriate computer software to write reports, graph and display data.
- Write reports in a scientific style

**Intellectual Skills**

Students should be able to:

- reason critically.
- generate, analyze and interpret scientific data.
- draw conclusions from available data and be able to determine whether such conclusions are justified.
- apply theoretical knowledge to research/lab based exercises.
- devise solutions to problems using a scientific approach.

**Assignments**

Course work - 100%

- Laboratory Reports (including in lab performance) 70%
- laboratory Quizzes 10%
- In-course theory examinations 20%

**Teaching Strategies**

Practical exercises assessed primarily via the submission of laboratory reports and pop quizzes. There will be twelve hours of lectures/tutorials and two in-course exams per semester.

**Readings**

Laboratory Manual available on myeLearning

Lehninger Principles of Biochemistry – Nelson and Cox
Experiments in Biochemistry-a hands-on approach - Shawn O. Farrell and Lynn E. Taylor
Basic Biochemical Methods – Renee R. Alexander and Joan M. Griffiths
Modern Experimental Biochemistry – Rodney Boyer
EVALUATION: BIOL2XXX will be evaluated in two ways – (a) through the offices of the Class Representative and the Life Sciences Student-Staff Liaison Committee, and (b) an end of semester course evaluation survey. The class will elect two class representatives, whose role is to act as a mediator between the Life Sciences academic staff and the students in the class. The representatives will attend Liaison Committee meetings (held at least twice per semester), where they will present feedback on the course to the Department for action. The UWI performs a course evaluation survey at the end of every semester, and this information will also be used for overall assessment of the course and guide possible actions for improvement in subsequent semesters.

Course Calendar

Semester I

In Semester I, the lab schedule leaves seven (7) free hours for Lectures/Post Lab Discussions at a designated venue. All sessions would be 50 minutes long.

<table>
<thead>
<tr>
<th>Week</th>
<th>Exercise</th>
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<tbody>
<tr>
<td>1</td>
<td>Course Introduction - Instrumentation and Safety in the Biochemistry Laboratory</td>
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<tr>
<td>2</td>
<td>Lecture: Protein Purification</td>
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</tbody>
</table>
| 3    | pH & Buffers  
      | Lecture: Protein Purification |
| 4    | Lecture: Protein Structure determination |
| 5    | Proteins & Amino Acids  
      | Post-Lab Discussion 1: Proteins & Amino Acids |
| 6    | Incourse Exam I |
| 7    | The Hill Reaction in Isolated Chloroplasts |
| 8    | Lecture: The Scientific Paper |
| 9    | Measurement of Arginase Activity |
| 10   | Incourse Exam II |
| 11   | Assay of Tissue Glycogen |
| 12   |  |
| 13   | Course review- tutorials |

Laboratory Reports to be handed in one week following the practical exercise

Semester II
In Semester II, the lab schedule leaves six (6) free hours for Lectures/Post Lab Discussions at a designated venue. All sessions would be 50 minutes long.

<table>
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<th>Week</th>
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<tbody>
<tr>
<td>1</td>
<td>DNA and RNA Isolation in Animal Tissues</td>
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<tr>
<td>2</td>
<td>Lecture: Writing a Scientific Paper</td>
</tr>
<tr>
<td>3</td>
<td>Session I – Extraction, heat treatment and alcohol fractionation</td>
</tr>
<tr>
<td>4</td>
<td>Post Lab Discussion: Yeast Invertase Calculations</td>
</tr>
<tr>
<td>5</td>
<td>Session II – DEAE – Cellulose Column Chromatography</td>
</tr>
</tbody>
</table>
| 6    | **Incourse Exam I**
|      | Lecture: Nucleic Acid Isolation & Visualization |
| 7    | Session III – Protein Determination, Nelson’s Assay and Activity Determinants |
| 8    | Lecture: Preparation of Laboratory Reagents : Storage & Safety |
| 9    | Session IV– Enzyme Kinetics Using Fraction 4 and Acrylamide Electrophoresis |
| 10   | **Incourse Exam II** |
| 11   | Session V– Enzyme Kinetics Using Fraction 4 and Acrylamide Electrophoresis |
| 12   | | |
| 13   | Course review- tutorials |

**Laboratory Reports to be handed in one week following the practical exercise**

**How to Study for this Course:**

- Attend and participate in all laboratory sessions and submit lab reports in a timely fashion.
- Take notes and tips while listening to lab talks.
- Review the materials covered. Clarify any unclear concepts through interaction with instructors/lecturers/peers and reading the text(s).
- Pre-read the topic to be covered in the next class/lab.
- Attend ALL lectures - make brief notes.
- Read over the materials covered, preferably on the same day, making additions to notes taken during class and prepare questions for clarification/discussion.
- Prepare for the tutorial sessions- work through the tutorial sheet.
- Read over notes just after pre-reading the topic for the next class.
- Take a self-quiz on the topic covered- ensure you understand all the solutions/’right’ answers.