



## Chemistry 251

### Immunology

Fall Semester, 2009

## COURSE OUTLINE

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This course describes the basic concepts of immunology and the application of immunochemistry to molecular, medical and veterinary biotechnology. Topics include antigens and antibodies, immune responses, vaccines, antibody diagnostics, immunosuppression, hypersensitivity, transplants, cancer, auto-immune diseases, immunodeficiencies (including AIDS), and current immunological techniques. (T)

The Approved Course Description is available on the web @  
<http://www.camosun.bc.ca/calendar/chem.php#251>

*Please note: This outline will not be kept indefinitely. It is recommended students keep it for their records.*

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### 1. Instructor Information

(a) Instructor    Jamie Doran, Ph.D.

(b) Office hours:    Monday, 2:30 to 4:20 pm  
                              Thursday, 2:30 to 4:20 pm  
                              Friday, 10:30 am to 12:20 pm & 2:30 to 3:20 pm

*Students are welcome whenever my office door is open.*

*Appointments may be made to meet at other than posted times.*

*Office hours will be extended prior to exam times.*

(c) Location        Room 342C, Fisher Building, Lansdowne Campus

(d) Phone            250.370.3441 (voicemail available)

(e) E-mail            [jdoran@camosun.bc.ca](mailto:jdoran@camosun.bc.ca)

## 2. Intended Learning Outcomes

- Students successful in this course will be able to evaluate fundamental aspects of the human immune system, and relate these to a wide variety of immunologically-based clinical conditions including allergies, transplant rejections, autoimmune diseases, and immunodeficiencies including AIDS.
- Students will be able to compare and contrast various types of antibody-based diagnostic tests, and various vaccine formulations.
- Students will have the hands-on experimental skills required to conduct the most commonly used immunological techniques including enzyme-linked immunosorbent assays (ELISA), latex bead agglutination assays, and Western-blotting detection of antigens.
- Students will have the ability to evaluate experimental design, design control experiments, and interpret data arising from basic immunological technologies.
- Students will be capable of working in a level-1 biosafety laboratory.
- Students will be experienced in the preparation, handling and storage of many types of solutions, buffers, reagents, and with equipment used immunological experimentation.

## 3. Required Materials

### (a) Course Text Book

*The Immune System*. Third Edition (2009). Au. Peter Parham. Garland Science. London.

This book is available in the Lansdowne Campus Bookstore. Also, a copy of the textbook is available for loan through the reserve library.

Supplementary information from articles recently published in relevant journals, including Nature Immunology Reviews, will be provided as required, by request, or for general interest.

*(b) Laboratory Manual and Selected Course Notes & Lecture Slides*

A required booklet of experimental procedures, selected course notes and selected lecture slides from the textbook is available through the Lansdowne Campus College Bookstore.

*(c) General Materials and Supplies*

Safety glasses Safety glasses are required when handling hazardous chemicals or biochemicals. Each student is required to provide her or his pair of safety glasses. Students lacking safety glasses when they are required will not be permitted to work in the laboratory.

Lab coats Lab coats are required for all experimental work in the laboratory. Each student is required to provide her or his own lab coat. Students lacking lab coats will not be permitted to work in the laboratory.

Latex gloves Latex or similar gloves will be available in the lab and are to be used when appropriate to protect hands from potentially hazardous chemicals or to protect valuable immunochemicals from becoming degraded by enzymes from the skin. Hypoallergenic gloves are available for people with allergies to some types of latex gloves.

Calculator Scientific calculators may be required occasionally in the lab, in class and during exams. Each student must provide her or his own calculator.

#### **4. Course Content and Schedule**

Credits	4 credits
In-class workload	6 hours per week <ul style="list-style-type: none"><li>• There are three 50-minute lectures per week.</li><li>• Laboratory periods are 2 hours and 50 minutes.</li></ul>
Out-of-class workload	6 hours per week
Number of weeks	14 weeks
Pre-requisite	Chem 120 - College Chemistry 1

## Course times and locations

Lecture times	Tuesday 9:30 to 10:20 AM Fisher Building, Room F360
	Wednesday 9:30 to 10:20 AM Fisher Building, Room F360
	Thursday 9:30 to 10:20 AM Fisher Building, Room F360
Laboratory Periods	Tuesday 2:30 to 5:20 PM Fisher Building, Rooms F360 and F358

*Alternatively, this 3 h time period is used to host two term exams and a final exam review. Occasionally, some lab time will be used for lecture or lab-lecture early in the semester, and lecture time will be used to complete one or more experiments later in the semester. Please see the laboratory and term test schedule below.*

## Lecture Outline

### HISTORICAL PERSPECTIVE

*This topic is briefly introduced in the introduction (pages 1-2) to Chapter One of The Immune System, 3<sup>rd</sup> ed. by P. Parham. More extensive reading relevant to the initial lectures is provided in the selected course notes in the course package See "Historical Perspective on the Field of Immunology" on pages 186 to 196.*

- Early historical evidence of immunity in humans
  - Meaning of the term 'immunity'
  - Recognition of the four basic tenants of adaptive immunity
- Variolation & the early evidence of vaccination
- Development of Jenner's small pox vaccine
- Development of the field of immunology
  - Louis Pasteur (1860's- 1890's) creates the field of immunology with seminal experiments demonstrating vaccination and acquired immunity in animals and humans.
  - Pasteur and Koch compete to create widely-accepted vaccines.
  - Metchnikoff establishes the field of cellular immunology (1880's)
  - Nuttall (1888) & Von Behring (1888-1890's): humoral immunity
  - Wright (1903): synergy of cellular and humoral immunity.
  - Paul Ehrlich (early 1900's-1915): furtherance of understanding.
  - Border: immune responses to non-pathogenic cells
  - Lansteiner: blood group ABO antigens.

- Ramon (1928): toxoids (attenuated chemicals) as vaccines
- Kabat (1930's): isolated immunoglobulins (antibodies) from blood
- Chase (1940's): demonstrates transfer of cellular immunology
- (Note the list of Nobel Prize winning immunologists on page 226.)
- A history of vaccine use proves the efficacy of stimulating immunity to prevent major human infectious diseases.

## GENERAL ROLE FOR THE IMMUNE SYSTEM IN MAINTAINING BODY INTEGRITY

Refer to Chapter 1, sections 1-1, 1-2, 1-5, 1-6 & 1-8 to 1-13

- Challenges to health: infectious organisms, cancer, toxins
- Innate immunity 'versus' adaptive immunity
- The lymphatic system
  - Primary and secondary lymphatic tissues
  - Structure and function of the lymphatic system as it relates to immunity
- Primary immune responses vs. secondary immune responses
- Introduction to Protective Immunity & Vaccination

## THE INNATE IMMUNE RESPONSE

Refer to:

Chapter 1, sections 1-3, 1-4 & 1-7;

Chapter 2, sections 2-1, 2-5, 2-10, 2-11, 2-13 to 2-16, 2-2, 2-3, 2-6 to 2-9, 2-18, 2-17, 2-20 to 22;

Chapter 9, sections 9-17 & 9-19;

"Innate Immunity" on pages 193 to 200 in the selected course notes in the course package.

- General characteristics of nonspecific physical and chemical defenses
  - Physical barriers
    - Skin and mucous membranes
  - Defensive chemicals
    - pH, lysozyme, iron-binding compounds, O<sub>2</sub>
  - Natural bacterial flora and microbial antagonism
- White blood cells (leukocytes) involved in innate immunity
  - Phagocytic cell types: monocytes & macrophage, neutrophils (PMN's), dendritic cells, Langerhans cells
  - Nonphagocytic leukocytes: eosinophils, natural killer cells
  - Inflammatory leukocytes: mast cells, basophils
  - Lymphocytes: B-cells and T-cells
  - Origins of myeloid and lymphoid cell lines
- The innate, acute, inflammatory response
  - Constriction and local dilation of vessels
  - Roles for cells and soluble factors from the blood
  - Margination, extravasation (diapedesis), chemotaxis
  - Mast cell activity, soluble mediators
- The process of phagocytosis by macrophage
  - Antigen presentation links innate immunity with adaptive immunity
  - Oxygen-dependent and oxygen-independent killing mechanisms
  - Microbial strategies for the prevention of phagocytic killing

- Complement
  - Classical complement pathway
  - Alternative complement pathway
  - Lectin-mediated complement activation pathway
  - Roles of products of complement activation and other acute phase proteins in the inflammatory response and other aspects of immunity.
- Other humoral factors involved in innate immunity
  - Complement activation products
  - Coagulation
  - Kinins
  - Defensins
  - Toll-like, and other surface receptors
  - Cytokines
  - Chemokines
  - Adhesion molecules
  - Interferons:  $\alpha$  &  $\beta$
  - Other acute phase proteins
- Cytokines
  - General nature and characteristics
  - Autocrine and paracrine functions
  - Classic characteristics: pleotrophy, redundancy, synergy, antagonism
- Natural killer (NK) cells
  - NK-cells: roles in innate immunity: killing mechanism

#### ADAPTIVE IMMUNITY & THE PRINCIPLE OF CLONAL SELECTION

*Browse Chapter 3 for a general overview of material detailed in following chapters.*

- Basic nature of antibodies & T-cell receptors
- Antigens, immunogens, and haptens
  - Epitopes (antigenic determinants)
  - Characteristics and properties of immunogens
  - Experimental conditions that affect the immunogenicity of immunogens
  - Vaccination conditions that affect the immunogenicity of immunogens

#### ANTIBODIES - STRUCTURE & DIVERSITY

*Refer to Chapter 4, sections 4-1 to 4-16.*

- Antibodies
  - Structure and function of a prototypic, divalent Ab molecule
    - ◇ Fab and Fc fragments
    - ◇ Globular constant domains
    - ◇ Variable and hypervariable (CDR) regions
  - Isotypes (classes) of antibodies
    - ◇ Classes of heavy and light chains
    - ◇ Immunological characteristics and functions
- Genetics of antibody diversity Antibody production by B-cells
  - Multi-gene organization of immunoglobulin genes
  - Variable region gene rearrangements
  - Generation of antibody diversity
  - Class switching

## B-CELL ACTIVATION & ANTIBODY EFFECTOR FUNCTIONS

Refer to:

Chapter 6, Introduction, sections 6-1 & summary (browse chapter for interest);

Chapter 9, sections 9-1 to 9-17 & 9-20 to 9-25;

Chapter 10, sections 10-5 to 10-9, 10-11 to 10-19.

- Development & processing of B-cells
- Antibody production by B-cells
  - Clonal selection and antibody synthesis
  - B-cell receptors and antigen binding
  - B-cell activation and maturation
    - Plasma cells
    - Memory B-cells
  - Affinity maturation
  - Relationship of affinity maturation to class switching
  - Relationship of affinity maturation to memory B-cells
- Antibody effector functions
  - Roles as adaptor molecule
  - Roles specific to classes (isotypes) of antibodies
- Antibody interactions with Fc receptors on macrophage, mast cells, basophils, eosinophils and natural killer (NK) cells.
  - ADCC (antibody-dependent cell-mediated cytotoxicity)
- B-cell Receptors & cell adhesion molecules
- Role of CD4 Helper T<sub>H</sub>2-cells in antibody production
- Role of CD4 Helper T<sub>H</sub>2 -cells in CD4 B-cell activation
- T-independent B-cell antigens
- Role of the lymphatic system
- The role of T-helper cell - B-cell interactions
  - Affinity maturation and isotype switching
  - Prevention of harmful effects of affinity maturation

## T-CELL ANTIGEN RECOGNITION AND ACTIVATION, AND T-CELL MEDIATED IMMUNITY

Refer to:

Chapter 7, Introduction and sections 7-1, 7-8 & 7-13;

Chapter 5, Introduction and sections 5.1, 5-4, 5-5 to 5.17 (browse the rest of Chapter 5);

Chapter 8, Introduction and sections 8-1 to 8-6;

Chapter 10, sections 10-5 to 10-6, 10-19 to 10-23, & 10-28 to 10-29.

- Development and processing of T-cells.
- T-cell receptors
  - T-cell receptor diversity
  - Role of  $\alpha\beta$  receptors
  - role of  $\gamma\delta$  receptors
- MHC Presentation and T-cell Surface Proteins CD4 and CD8
  - Endogenous antigen processing
  - Exogenous antigen processing
  - Role of CD4 in recognition of MHC II
  - Role of CD8 in recognition of MHC I

- 'T-cell restriction'
- MHC polymorphism
- CD4 & CD8 T-cell subclasses
  - Cytotoxic T-cells, helper T-cells, regulatory T-cells
  - Clonal selection applies to cytotoxic T-cells
  - MHC I presentation & Tc-cell Activation
  - MHC I presentation & Tc-cell Activation
- Roles of antigen-presenting cells (APC's)
  - Macrophage
  - Dendritic cells
  - Langerhans cells
  - B-cells
- Adhesion molecules: CD molecules, selectins, integrins, toll-like receptors
- Role of CD4 Helper T<sub>H</sub>1-cells in CD8 cytotoxic T-cell activation
- Activity of cytotoxic CD8 T-cells
- Role of CD4 Helper T-cells in CD8 cytotoxic macrophage activation
- Role of CD4 Helper T<sub>H</sub>2 -cells in CD4 B-cell activation
- T<sub>H</sub>1 vs. T<sub>H</sub>2 Responses
  - Humoral vs. cellular immune responses
  - Cytokine profiles
  - Polarization (humoral vs. cellular) of immune responses
  - Functions of cytokines in mediating polarization
- Activity of cytotoxic CD8 T-cells

## EVASION OF THE IMMUNE SYSTEM BY PATHOGENS

*Refer to:*

*Chapter 11, sections 11-1 to 11-25;*

*Chapter 10, sections 10-1 to 10-4.*

*Handout materials will present very recent developments.*

- Influenzae virus
- Trypanosomes
- Herpes virus
- Other pathogens

## IMMUNODEFICIENCY

*Refer to Chapter 11, sections 11-8, 11-11 to 11-25.*

*Handout materials will present very recent developments.*

- Primary immunodeficiencies
- Secondary immunodeficiencies
  - HIV & AIDS



## HYPERSENSITIVITY (Allergy)

Refer to Chapter 12, sections 12-1 to 12-24.

Handout materials will present very recent developments.

- The nature of hypersensitivity and allergens
- Types of hypersensitivity
  - Immediate-type hypersensitivity
    - Type 1 - Anaphylactic hypersensitivity
      - ◇ Systemic anaphylaxis
      - ◇ Localized anaphylaxis
    - Type 2 - Antibody-dependent cytotoxicity hypersensitivity
    - Type 3 - Complex-mediated hypersensitivity
      - ◇ Systemic
      - ◇ Localized
  - Delayed type hypersensitivity
    - Type 4 - Cell-mediated hypersensitivity

## AUTOIMMUNITY

Refer to Chapter 13, sections 13-1 to 13-12, 13-13 to 13-17, 13-20 to 13-26.

Also refer to the Selected Course Notes.

Handout materials will present very recent developments.

- Major sources of autoimmunity
- Autoimmune diseases
  - Tissue-specific diseases
    - Aspermatogenesis
    - Sympathetic ophthalmia
    - Hashimoto's thyroiditis
    - Insulin-dependent diabetes
    - Autoimmune anemias
      - Pernicious anemia
      - Hemolytic anemias
    - Goodpasture's syndrome
    - Graves disease
  - Systemic autoimmune diseases
    - SLE (Lupus)
    - MS
    - Rheumatoid arthritis

## VACCINES

Refer to Chapter 14, sections 14-1 to 14-10.

Handout materials will present very recent developments.

- Needs, benefits, and potential risks
- Type of vaccines
  - Killed or otherwise inactivated vaccines
  - Live attenuated vaccines
  - Subunit vaccines
    - Purified biomolecules
    - Recombinant vaccines

- Heterologous vaccines
- Peptide vaccines
- DNA vaccines

## TRANSPLANTATION IMMUNOLOGY

*Refer to Chapter 15, sections 15-1 to 15-7, 15-9, 15-11, 15-18, 15-24 & 15-25.*

*(Browse the remainder of Chapter 15).*

- Autograft, isograft, allograft, xenograft
- Privileged sites & privileged tissues
- Graft rejection
  - Hyperactive rejection
  - Acute rejection
    - First-set rejection
    - Second-set rejection
  - Chronic rejection
- Prevention of rejection
  - Tissue typing
  - Immunosuppressive agents
- Clinical transplantation
  - Current status
  - Graft vs. host reaction
- Immunosuppression
  - Immunological silence
    - Central tolerance
      - ◇ Thymic processing
      - ◇ Neonatal tolerance
    - Peripheral tolerance
  - Acquired immunotolerance
    - Low-zone tolerance
    - High-zone tolerance
    - Immunotolerance created by certain immunization regimes
    - Natural acquisition of 'immunotolerance' in people
- Blood Group Antigens
  - Rh antigens and fetal hemolytic disease
  - ABO antigens and compatible blood donors

## CANCER IMMUNOLOGY

*Refer to Chapter 16, sections 16-1 to 16-14.*

*Handout materials will present very recent developments.*

- Tumour-Specific transplantation antigens
  - Viral antigens
  - Chemically-induced tumour antigens
- Tumour-associated transplantation antigens
  - Carcinofoetal antigens
  - Embryonic antigens
  - Alpha-feto protein antigen
- Immune response to tumours

- Cancer immunotherapy
  - Cytokine therapy
  - Interferon therapy
  - Tumour necrosis factor therapy
  - Monoclonal antibody-based therapies
  - Anti-cancer vaccines

### **Semester-ending, laboratory-lecture**

Other Immuno-Diagnostic Formats:

Radioimmunoassay (RIA)

Immunofiltration assays

Immunochromatographic assays

Affinity chromatography

Immuno-electron microscopy

Immuno-fluorescence microscopy

Fluorescence-activated cell sorter.

## Laboratory and Term Exam Schedules

- Week 1            Tuesday, September 8<sup>th</sup>.  
Organization of the Laboratory Portion of the Course; Perspective on Inter-Relatedness of Experiments; Overall Lab Orientation; Explanation of Proper Use of Various Micropipettors.  
*Lecture material will also be presented in this initial period.*
- Week 2            Tuesday, September 15<sup>th</sup>.  
***Gel Immunodiffusion and the Identification of Antigens by Precipitin Reactions***  
Pre-Lab Talk: Nature of Precipitin Reactions  
***Experiment 1. The Ouchterlony Reaction***  
***Experiment 2. The Radial Immunodiffusion Assay***  
*Lecture or lab-lecture material will also be presented in this lab period.*
- Week 3            Tuesday, September 22<sup>nd</sup>.  
***Experiment 1. (continued). Interpretation of the Ouchterlony Reaction***  
***Experiment 2. (continued). Interpretation of the Radial Immunodiffusion Assay***  
Post-Lab Discussion - Interpretation of Precipitin Reactions  
Pre-Lab Talk - Nature of Agglutination Reactions  
***Experiment 3. Use of a Latex Bead Agglutination Assay to Identify Aeromonas salmonicida, a Bacterial Pathogen of Salmon and Trout.***  
*Lecture or lab-lecture material will also be presented in this lab period.*
- Week 4            Tuesday, September 29<sup>th</sup>.  
***Experiment 4. Detection of A. salmonicida Antigens and Determination of Anti-A. salmonicida Polyclonal Antibody Titre Using an Indirect Enzyme-Linked Immunosorbent Assay (ELISA).***  
Pre-Lab Talk: Principles of ELISA.  
***Part I. Coating of microtiter plate wells with antigens.***  
*Lecture or lab-lecture material will also be presented in this lab period.*
- Week 5            Tuesday, October 6<sup>th</sup>.  
***Experiment 4. Detection of A. salmonicida Antigens and Determination of Anti-A. salmonicida Polyclonal Antibody Titre Using an Indirect Enzyme-Linked Immunosorbent Assay (ELISA).***  
***Part II. Conducting ELISA.***
- Week 6            Tuesday, October 13<sup>th</sup>.  
***Experiment 4. Detection of A. salmonicida Antigens and Determination of Anti-A. salmonicida Polyclonal Antibody Titre Using an Indirect Enzyme-Linked Immunosorbent Assay (ELISA).***  
Post-Lab Discussion. Interpretation and Discussion of ELISA results.  
Review for Term Test #1.
- Week 7            **Tuesday, October 20<sup>th</sup>            Term Test #1**



## 5. Basis of Student Assessment (Weighting)

### (a) Laboratory Experiments

**Attendance in the lab periods is mandatory.** No laboratory experiment can be missed without an acceptable reason submitted in writing, such as a suitable note from Medical Doctor.

NB. There are no laboratory reports to be handed but *students are responsible for understanding the principles, technical bases, and results of each experiment.* These aspects of the laboratory work will be subject to examination on the term exams and the final exam.

### (b) Term Exams

#### Term Exam #1

This exam covers relevant material from approximately the first third of the course. The delineation of material that students may be responsible for on this exam will be provided in class about one week before the date of the exam.

This is a 110 minute test written on Tuesday, October 16<sup>th</sup> in the adjoining rooms F360 & F358 during the 2:30 to 4:20 PM time period.

The value this exam contributes to the final grade is **30%**.

#### Term Exam #2

This exam covers relevant material from approximately the second third of the course. The delineation of material that you may be responsible for on this exam will be provided in class about one week before the date of the exam.

This is a 110 min. test written on Tuesday, November 20<sup>th</sup> in the adjoining rooms F360 & F358 during the 2:30 to 4:20 PM time period.

The value this exam contributes to the final grade is **30%**.

If either of the midterm exams is missed due to illness or for any other justifiable reason (accompanied by appropriate documentation), a student may either take a substitute test to be written at a mutually agreeable time, or choose to add the percentage value of that midterm exam (30%) to the percentage value of the final exam.

### (d) Final Exam

The final exam is a comprehensive exam.

The value this exam contributes to the final grade is **40%**.

The time and location of the final exam will be published by the College during the Fall Semester.

**Attendance at the final exam is mandatory.** Appropriate documentation must accompany any explanation for absence.

## 6. Grading System

### Standard Grading System (GPA)

Percentage	Grade	Description	Grade Point Equivalency
90-100	A+		9
85-89	A		8
80-84	A-		7
77-79	B+		6
73-76	B		5
70-72	B-		4
65-69	C+		3
60-64	C		2
50-59	D		1
0-49	F	Minimum level has not been achieved.	0

### Temporary Grades

Temporary grades are assigned for specific circumstances and will convert to a final grade according to the grading scheme being used in the course. See Grading Policy at [camosun.ca](http://camosun.ca) or information on conversion to final grades, and for additional information on student record and transcript notations.

Temporary Grade	Description
I	<i>Incomplete:</i> A temporary grade assigned when the requirements of a course have not yet been completed due to hardship or extenuating circumstances, such as illness or death in the family.
IP	<i>In progress:</i> A temporary grade assigned for courses that are designed to have an anticipated enrollment that extends beyond one term. No more than two IP grades will be assigned for the same course.
CW	<i>Compulsory Withdrawal:</i> A temporary grade assigned by a Dean when an instructor, after documenting the prescriptive strategies applied and consulting with peers, deems that a student is unsafe to self or others and must be removed from the lab, practicum, worksite, or field placement.

Temporary grades are assigned for specific circumstances and will convert to a final grade according to the grading scheme being used in the course. See Grading Policy E-1.5 at [camosun.ca](http://camosun.ca) for information on conversion to final grades, and for additional information on student record and transcript notations.

## 7. Recommended Materials or Services to Assist Students to Succeed Throughout the Course

A reading guide to the course text is provided for each topic (above). Supplementary course notes and copies of lecture slides that primarily present figures or tables from the text are provided in the course manual which includes the laboratory experiment protocols. These notes support lectures and laboratory experiments by the provision of material on subjects that are not addressed in the sufficient detail in the text, or are addressed in less detail or from a different perspective. The copies of many selected lecture slides that present figures, tables or other complex or somewhat information-intensive materials will facilitate efficient note taking, and promote in-class learning and discussion.

### LEARNING SUPPORT AND SERVICES FOR STUDENTS

There are a variety of services available for students to assist them throughout their learning. This information is available in the College Calendar, Registrar's Office or the College web site at <http://www.camosun.bc.ca>

#### *Please Note:*

*Students may not use recording devices in the classroom without the prior permission of the instructor. However, the instructor's permission is not required when the use of a recording device is sanctioned by the College's Resource Centre for Students with Disabilities in order to accommodate a student's disability and when the instructor has been provided with an instructor notification letter which specifies the use of a recording device. Recordings made in the classroom are for the student's personal use only, and distribution of recorded material is prohibited.*

### ACADEMIC CONDUCT POLICY

There is an Academic Conduct Policy. It is the student's responsibility to become familiar with the content of this policy. The policy is available in each School Administration Office, Registration, and on the College web site in the Policy Section.

[www.camosun.bc.ca/divisions/pres/policy/2-education/2-5.html](http://www.camosun.bc.ca/divisions/pres/policy/2-education/2-5.html)