

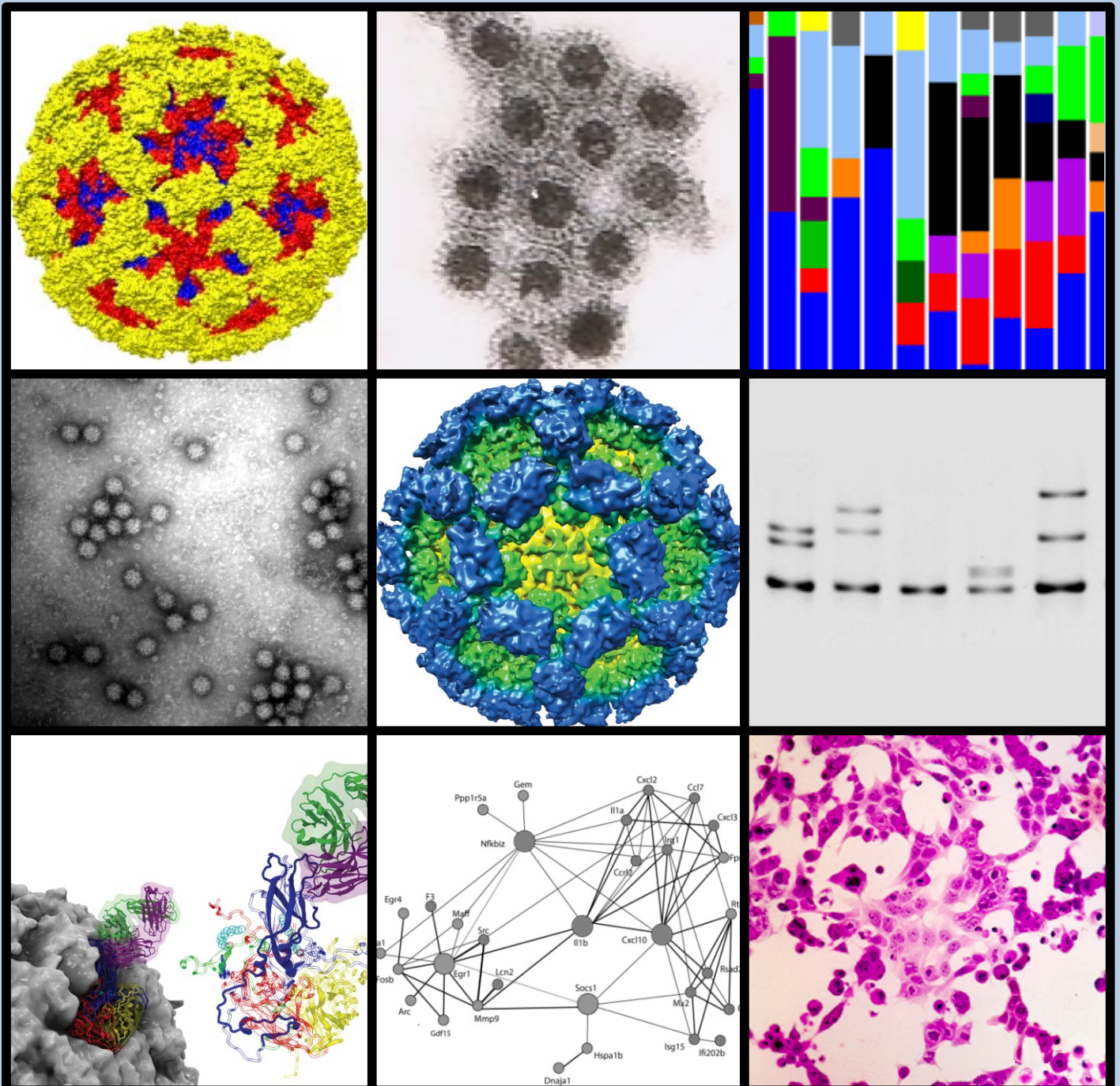
VIRUSES AND DISEASE

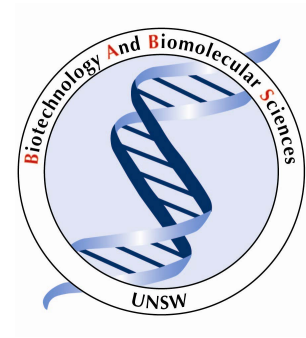
MICR3061

School of Biotechnology and Biomolecular Sciences
UNSW

Session 3, 2020

Coordinator: Prof Peter White





UNSW

MICR 3061

VIRUSES AND DISEASE

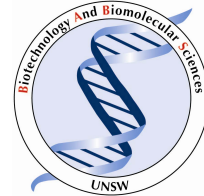
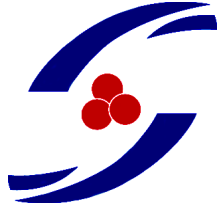
2020

Coordinator

Professor Peter White

**SCHOOL OF BIOTECHNOLOGY AND
BIOMOLECULAR SCIENCES**

School of Biotechnology and Biomolecular Sciences



In association with

The Prince of Wales Hospital

Viruses and Disease, MICR3061

Trimester 3, 2020

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Prof Peter White (UNSW)

Dr Rowena Bull (UNSW)

Prof William Rawlinson (POWH)

Dr Caroline Ford (UNSW)

Dr Sacha Stelzer-Braid (POWH)

A/Prof Noel Whitaker (UNSW)

Alice Russo (UNSW)

Course Tutors:

Prof Peter White

Alice Russo

Grace Yan

Emma Harding

Technical Staff:

Dr Tammy Tang

SCHOOL OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES**VIRUSES AND DISEASE, MICR 3061****2020 LECTURE TIMETABE**Course convener: Prof Peter White (Tel: 9385 3780; e-mail: p.white@unsw.edu.au)

Lectures: Mon 3-4pm, Wed 1-2pm and Fri 9-10am

Week	Date	Time	Lecture Topic	Lecturer
1	14-9	3-4pm	Introduction I – The course, viral properties and classification	PW
	16-9	1-2pm	Introduction II – Viruses and viral structure	PW
	18-9	9-10pm	Viral genomes and replication	PW
2	21-9	3-4pm	Vaccines	SSB
	23-9	1-2pm	Serology and viral cell culture	PW
	25-9	9-10pm	Viral pathogenesis	PW
3	28-9	3-4pm	The Coronavirus Pandemic	PW
	30-9	1-2pm	Hepatitis Viruses 1 – HCV	PW
	2-10	9-10pm	Influenza A and B	SSB
4	5-10	3-4pm	<i>No Lecture - public holiday, Labour Day</i>	
	7-10	1-2pm	Hepatitis Viruses 2 - HAV, HBV, delta, HEV	PW
	9-10	9-10pm	Gastrointestinal infections	PW
5	12-10	3-4pm	Human papillomavirus	NW
	14-10	1-2pm	Herpesviruses – part 1	WR
	16-10	9-10pm	Herpesviruses – part 2	WR
6	19-10	3-4pm	No lecture	
	21-10	1-2pm	No lecture	
	23-10	9-10pm	No lecture	
7	26-10	3-4pm	Antiviral agents	PW
	28-10	1-2pm	Prions, virophages and subviral particles	PW
	30-10	9-10pm	Viral discovery and its applications	AR
8	2-11	3-4pm	Respiratory viruses	WR
	4-11	1-2pm	Immunology in virology	PW
	6-11	9-10pm	Childhood Infections – including measles, mumps, rubella	PW
9	9-11	3-4pm	Emerging/re-emerging viruses	RB
	11-11	1-2pm	Arboviruses	RB
	13-11	9-10pm	Human tumour viruses	CF
10	16-11	3-4pm	Human immunodeficiency virus and HTLV	PW
	18-11	1-2pm	Course Overview - Revision Lecture	PW
	20-11	9-10pm	No lecture	

Lecturers:

PW: Prof Peter White (BABS, UNSW)
NW: A/Prof Noel Whitaker (BABS, UNSW)
CF: Dr Caroline Ford (SWCH, UNSW)
RB: Dr Rowena Bull (KIRBY, UNSW)
SSB: Dr Sacha Stelzer-Braid (POWH, UNSW)
WR: Prof William Rawlinson (POWH/BABS, UNSW)
AR: Alice Russo (BABS, UNSW)

SCHOOL OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
MICR 3061, VIRUSES AND DISEASE
2020 PRACTICAL CLASS AND TUTORIAL TIMETABLE

Tutorials: Friday: 1-2pm (online through Teams)
 Laboratory: Tuesday: 10-1pm (Videos and online through Teams)

Week	Date	*Tutorials (Fri 1-2pm)	Practical/Consultancy (Tues 10am-1pm)	Demonstrators
1	17-9	Tutorial 1	No Practical	
2	24-9	Tutorial 2 (optional)	Consultancy briefs. Group assignment for write and present a consultancy report. Overview of task and marking.	PW, AR, GY, EH
3	1-10	Tutorial 3	Practical 1 (Dry Prac) - Creating a Virus 10-1pm Online	PW, AR, GY, EH
4	8-10	Tutorial 4 (optional)	Practical 2 - HCV genotyping 10-1pm Online video of practical	PW, AR, GY, EH
5	15-10	Mid-session exam	Computer Prac - Viral Recombination, 10-12:30pm Online Computer practical POW Hospital talk, 12:30-1pm	AR, GY, PW POWH staff
6	22-10	No tutorial	No Practical	
7	29-10	Tutorial 5	Practical 3 – Antiviral agents 10-1pm Online video of practical	PW, AR, GY, EH
8	5-11	Tutorial 6	Lab Practical 4 - Virus cultivation and bacteriophages, part 1 10am -1pm Online video of practical	PW, AR, GY, EH
9	12-11	Tutorial 7	Lab Practical 4 - part 2 Virus cultivation and bacteriophages, part 2 10am -1pm Online video of practical	PW, AR, GY, EH
10	19-11	Tutorial 8	Consultancy briefs presentations 10am-1pm, All groups Online presentations in Microsoft Teams	PW, AR, GY, EH

*Practicals should be viewed from 10-11am or beforehand. From 11-1pm synchronous sessions will be run with your tutor through Microsoft Teams. Friday tutorials are compulsory on weeks 1, 3, 5, 7, 9 and otherwise optional.

Tutors:

PW: Peter White AR: Alice Russo
 GY: Grace Yan EH: Emma Harding

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ASSESSMENT

Please read this information carefully.

	<i>Contribution to final mark</i>
1. Written Examination	40%
2. Consultancy Work	30%
3. Mid-session exam (MCQ)	30%
	100%

In this course 60% of the total mark is in the form of continuous assessment and 40% is based on the final written examination held at the end of the course. Students must obtain an aggregate mark of 50% in the continuous assessment and the final examination to pass the subject.

1) Final written examination – total possible mark – 40%

Basis for examination: All sections of the course.

The exam format will be 20 short answer questions over 2 hours. The exam will be held in the exam period at the end of the semester.

The Supplementary exam will be held between the 11th to the 15th January 2021

2) Continuous assessment – total mark possible – 60%

1) Consultancy briefs (30%)

This is a major part of the assessment. It contains an extensive collaborative review of a virological topic of importance to one of several professional/commercial interests in Australia. Students, in groups of four or five are required to research the literature, consult with available experts, participate in UNSW Moodle and face to face discussions, and then to:

- a) Write an integrated report on the topic of the consultancy brief**
- b) Give a verbal exposition to “clients”**

<i>All written consultancy reports must be submitted by 4 pm, Thursday 5th November 2020.</i>

Assessment of consultancy briefs

The details of the consultancy brief and assessments will be discussed in the tutorial classes. Each student will receive a mark on the basis of:

Written report - draft	5% of total course mark
Individual written report	12.5%
Individual verbal presentation	10.0%
Assessment by peers within the group	2.5%

Total marks possible	30%
-----------------------------	------------

2) *Mid-session exam Tuesday 22nd Oct (wk 6) (30%)*

This will be based on material from weeks 1-5.

Basis for test: Lectures and associated parts of textbook and other reading material. Tutorials and Practicals.

3) *Laboratory reporting*

Laboratory manuals: Students will be expected to keep accurate recordings of their laboratory experimentation in their laboratory manual provided online. Laboratory manuals will be checked periodically by tutors throughout the trimester. All questions in the laboratory manual must be answered and will form part of the assessment. A hardcover lab book is recommended for taking notes.

Tutorials

Tutorials will enable you to learn about diagnostics in virology, virology news and the world of research. You will also gain communication and writing skills aimed at helping you with your consultancy work. Quizzes and feedback sessions also form an integral part of tutorials. **Attendance and participation in tutorials is compulsory and will be recorded; 80% participation is required to pass the course.** Students are expected to participate in tutorial discussions and complete short tutorial assignments.

Hospital Excursion

In this course, part of the practical class will be an excursion to the Virology Division, Department of Microbiology, SEALS at the Prince of Wales Hospital. The excursion will be a virtual excursion with a Q&A session this year, rather than visiting the hospital. Virtual afternoon tea will be provided, if you want to make it, over an Honours discussion.

These laboratories will also be toured:

Area Virology Laboratory (AVL)

Group 2 - Area Serology Laboratory (ASL)

Group 3 - Virology Research Lab (VRL)

POWH Staff:

Professor Bill Rawlinson

A/Prof Sacha Stelzer-Braid

Susanne Booth

and others



SPECIAL CONSIDERATION AND FURTHER ASSESSMENT

TRIMESTER 3, 2020

Special Consideration

Students who believe that their performance, either during the session or in the end of session exams, may have been affected by illness or other circumstances may apply for special consideration. Applications can be made for compulsory class absences such as (laboratories and tutorials), in-session assessments tasks, and final examinations.

You must submit the application prior to the start of the relevant exam, or before a piece of assessment is due, except where illness or misadventure prevent you from doing so. If you become unwell on the day of the exam or fall sick during an exam, you must provide evidence dated within 24 hours of the exam, with your application.

UNSW has a fit to sit/submit rule which means that if you sit an exam or submit a piece of assessment, you are declaring yourself fit to do so.

You must obtain and attach Third Party documentation before submitting the application. Failure to do so may result in the application being rejected.

Further information on special consideration can also be found at <https://student.unsw.edu.au/specialconsideration>.

HOW TO APPLY FOR SPECIAL CONSIDERATION

The application must be made through Online Services in myUNSW (My Student Profile tab > My Student Services > Online Services > Special Consideration).

Students will be contacted via their official university email as to the outcome of their application.

SUPPLEMENTARY EXAMINATIONS:

The University does not give deferred examinations. However, further assessment exams may be given to those students who were absent from the final exams through illness or misadventure and received Special Consideration approval. Mid-term supplementary exam will be held during the term at the convenient period determined by the course convenor. Final supplementary exam will be run by The Exam Office and in supplementary exam period.

*For Term 3 2020, Supplementary Exams will be scheduled between
11th Jan –15th Jan, 2021*

It is the responsibility of all students to regularly consult their official student email accounts and myUNSW in order to ascertain whether or not they have been granted further assessment. Failure to sit for the appropriate exam may result in an overall failure for the course. **Further assessment will NOT be offered on any alternative dates.**

COURSE AIMS, LEARNING OUTCOMES AND OBJECTIVES

COURSE AIMS

This course aims to present students with the background to viruses the technologies currently used to study them. Overall, the course aims to advance student understanding of virology, and human and animal diseases. Students will also gain an insight into the scientific development pathways of vaccines and antivirals used to combat viruses. The course presents students with opportunities for further investigation through group-based Consultancy Brief work. The ability to understand virological concepts and scientific thought processes are provided through practical work, assessment tasks and a hospital excursion.

COURSE LEARNING OUTCOMES

1. Describe the major themes in virology. Understand the virus replication cycle from infection to transmission.
 2. Accurately communicate virology ideas using both written reports and oral presentations.
 3. Critically evaluate virology information and arguments in both the scientific media and the mainstream media.
 4. Perform and design experiments, record accurately the results and critically evaluate the data. Be confident with the use of laboratory equipment used in virological experiments.
 5. Have knowledge in a range of virology sub-disciplines including; morphology, viral replication, epidemiology, genomes, pathogenesis, host response, treatment, vaccines and diagnosis
-

COMPONENTS

- Lectures
- Tutorials
- Practicals
- Consultancy briefs and presentations
- Moodle
- Prince of Wales Hospital excursion (virtual)

Viruses and Disease is a single unit course designed to complement the other School courses in microbiology. The goals of the Virology course are in keeping with the overall goals for science students in the School of Biotechnology and Biomolecular Sciences. Specific goals and objectives for the course are given below.

OBJECTIVES

Classification and Morphology

- Describe viral classification, taxonomy and morphology.
- Effectively recognise and distinguish between virion morphologies presented as electron micrographs.

History

- Knowledge of the important historical aspects of viruses described in this course.
- Describe the important historical events leading to the discovery of a virus, major breakthroughs and vaccinations

Epidemiology

- Understand the epidemiology of important human viruses, including their incidence, prevalence, population and geographic distribution and their mode of transmission.

Genome and proteins

- Describe the viral genomes of human and animal viruses and bacteriophages.
- Have an understanding of the proteins encoded by the viral genome and their functions.

Lifecycle and entry

- Be able to describe the keys steps of the viral life cycle starting with attachment and entry.
- Have knowledge pertaining to differences between different virus lifecycles.
- Understand how bacteriophages introduce their nucleic acid into host cells.

Replication

- Effectively describe details of the replication strategy for important human viruses.
- The ability to discuss the differences in replication of eukaryotic DNA and RNA viruses with particular reference to control of transcription and translation.

Pathogenesis

- Understand the pathogenesis of important human viruses and some animal viruses, including the types of diseases associated with viral infection and the areas of the body affected. Co-infection with two different viruses will be discussed in the context of Hepatitis viruses and HIV.

Host Response

- Describe the general features of the human host response to viral infections.

Diagnosis –EM, Culture, serology and nucleic acid tests

- Give an account of the major diagnostic approaches used in virology and serology laboratories.
- Recognise the difference in morphology of uninfected cell cultures and those infected by specified viruses.
- Distinguish clearly between the use of serology to identify and classify viruses and its use in diagnosis and epidemiology.
- Describe the mechanisms of at least two serological tests, the nature of the antigens detected and situations where the test is especially useful.
- Explain the principles involved in nucleic acid testing, including PCR and RT-PCR detection of viruses
- List the problems and limitations in the application of molecular diagnostic tests in virology.
- Describe the principles of heteroduplex mobility analysis (HMA) and describe an application for the assay.

Treatment

- List the chemicals currently showing promise as chemotherapeutic agents and describe their mode of action, including acyclovir, gancyclovir, foscarnet, AZT, zidovudine, interferon, ribavirin, protease inhibitors and the new HCV antivirals.
- List the chemicals and disinfectants that have a virocidal effect and state under what circumstances they are used in the laboratory.

Vaccines

- Outline the types of vaccines available for human viruses and their advantages and disadvantages.
- List the human viral diseases for which vaccines are available, with particular reference to influenza, hepatitis A and B, mumps, measles and rubella.

Oncogenic viruses

- Assess the current status of viruses as causes of cancer in animals and man.
- List the most important oncogenic viruses and outline their mechanism of oncogenesis.
- Describe the range of possible effects on a host cell of having a viral genome integrated into the chromosome.

Prions satellite viruses, virophages and bacteriophages

- Describe the classification, general features and biology of prions and bacteriophages. In addition, describe how bacteriophages can be used to treat bacterial infections.

Presentation/written skills

- Have the ability to prepare a scientific presentation using slides.
- Be able to present and communicate verbally to a scientific audience.
- Have the skills to write scientifically.

RECOMMENDED TEXT AND MOODLE

Optional: S.J. Flint, L.W. Enquist, V.R. Racaniello and A.M. Skalka. Principles of Virology (two volumes). (4th edition). ASM Press 2015.

USING UNSW MOODLE IN MICR 3061

Take some time to tour through the MICR3061 UNSW Moodle site. All students should have a UNSW Moodle user account and password. Participation in UNSW Moodle discussions and group sharing of resources is encouraged to facilitate consultancy work. Additional resources that are relevant to the material covered in lecture and practical classes will be placed on the UNSW Moodle site throughout the session to complement your studies in MICR3061. Students are encouraged to contribute other relevant information (articles, viruses mentioned in the media, etc).

The goals of the UNSW Moodle site are:

- To provide supplementary resources to facilitate your understanding of lectures and practical work.
- To enable you to conduct group discussions and share resources for your consultancy work.
- To provide you with the opportunity to initiate and/or participate in class discussions on topics in MICR3061.
- To review of topics in MICR3061 and clarify areas of uncertainty.

CONTINUAL COURSE IMPROVEMENT

Periodically student evaluation feedback on the course is gathered, using among other means, UNSW's MyExperience. Student feedback is taken seriously, and continual improvements are made to the course based in part on such feedback. Significant changes to the course will be communicated to subsequent cohorts of students taking the course.

Students are asked 6 questions about the quality of the course. The scoring system works as follows: 6 is strongly agree, 5 is agree, 4 is moderately agree, 3 is moderately disagree, 2 is disagree and 1 is strongly disagree.

The questions were:

- 1) I felt part of a learning community
- 2) The feedback helped me learn
- 3) The digital resources helped me learn
- 4) The assessment tasks were appropriate
- 5) The amount of assessment was appropriate
- 6) Overall I was satisfied with the quality of the teaching

2017. "Overall I was satisfied with the quality of the course" 5.17/6.0

2018. "Overall I was satisfied with the quality of the course" 4.73/6.0

Improvements based on last year's feedback are as follows:

- Less assessment tasks for 2020
- Reducing the number of viruses needed to be learnt with more focus on concepts
- Further improvements to student feedback during the course
- More assessment weighting for the consultancy brief presentations

ACADEMIC HONESTY AND PLAGIARISM

Plagiarism is the presentation of the thoughts or work of another as one's own.*

Examples include:

- direct duplication of the thoughts or work of another, including by copying work, or knowingly permitting it to be copied. This includes copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, web site, Internet, other electronic resource, or another person's assignment without appropriate acknowledgement;
- paraphrasing another person's work with very minor changes keeping the meaning, form and/or progression of ideas of the original;
- piecing together sections of the work of others into a new whole;
- presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor; and,
- claiming credit for a proportion a work contributed to a group assessment item that is greater than that actually contributed.†

Submitting an assessment item that has already been submitted for academic credit elsewhere may also be considered plagiarism.

The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does *not* amount to plagiarism.

Students are reminded of their Rights and Responsibilities in respect of plagiarism, as set out in the University Undergraduate and Postgraduate Handbooks, and are encouraged to seek advice from academic staff whenever necessary to ensure they avoid plagiarism in all its forms.

The Learning Centre website is the central University online resource for staff and student information on plagiarism and academic honesty. It can be located at: www.lc.unsw.edu.au/plagiarism

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- correct referencing practices;
- paraphrasing, summarising, essay writing, and time management;
- appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre. Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

* Based on that proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle.

† Adapted with kind permission from the University of Melbourne.

IMPORTANT VIRUSES IN AUSTRALIA AND OTHER

ENTITIES:

Viral family	Virus species
<i>Picornaviridae</i>	Enteroviruses, EV71, hepatitis A, rhinoviruses and cane toad Rhimavirus
<i>Caliciviridae</i>	Norovirus, feline calicivirus and Sapovirus
<i>Hepeviridae</i>	Hepatitis E virus
<i>Orthomyxoviridae</i>	Influenza type A, B and C
<i>Paramyxoviridae</i>	Parainfluenza viruses, respiratory syncytial virus, mumps, measles
<i>Coronaviridae</i>	Human coronavirus, SARS, MERS
<i>Togaviridae</i>	Rubella, Ross River virus, Chikungunya virus
<i>Flaviviridae</i>	Hepatitis C virus, Yellow Fever virus, Dengue virus, Zika virus Bovine Viral Diarrhoea Virus (BVDV)
<i>Reoviridae</i>	Rotaviruses, orbiviruses, reoviruses
<i>Retroviridae</i>	Human immunodeficiency virus (HIV-1 and HIV- 2) Human T Lymphotropic virus type 1 and type 2
<i>Rhabdoviridae</i>	Rabies
<i>Arenaviridae</i>	Lassa fever, lymphocytic choriomeningitis (LCM) viruses
<i>Herpesviridae</i>	Herpes simplex 1 and 2, varicella zoster, Epstein Barr, cytomegaloviruses and human herpes virus (HHV)-6, HHV7 and HHV8.
<i>Adenoviridae</i>	Adenoviruses A32, F40 and F41
<i>Parvoviridae</i>	Parvovirus B-19, Adeno-associated viruses
<i>Papillomaviridae</i>	Papillomavirus
<i>Poxviridae</i>	Smallpox, molluscum contagiosum, vaccinia, orf virus
<i>Hepadnaviridae</i>	Hepatitis B virus
Prion causing subacute spongiform encephalopathies	Kuru, Creutzfeldt-Jacob Disease, Scrapie, “Mad Cow” disease or BSE and new variant CJD
Other viruses	Delta virus

LABORATORY RULES AND SAFETY

In the laboratory individuals are exposed to hazards not normally found in a regular classroom. It is essential that students follow all laboratory rules and regulations. Failure to follow established rules may result in dismissal of the individual from the class.

Laboratories have certain standard safety equipment. These typically include:

- general-purpose fire extinguisher
- fire blanket
- eyewash ^{[[[]]]}_[SEP]
- safety shower ^{[[[]]]}_[SEP]
- cut-off switches for electrical and gas outlets. ^{[[[]]]}_[SEP]

It is the responsibility of the student to locate and know how to use the general safety equipment in the laboratory. Additionally, students should be aware of exits from the room in case of emergency, how to summon Campus Security (9385 6666), and how to obtain emergency medical assistance. The virology lab has some additional safety considerations. Since individuals work with potentially pathogenic organisms care must be taken to prevent possible infection or transmission of the organisms from the laboratory. Students must wear protective clothing (including lab coats and closed in shoes) while working in the laboratory. Lab coats may not be worn outside the laboratory. Intact skin is an adequate barrier against microorganisms so gloves are not necessary in the lab. Gloves will be provided and students must wear gloves when handling chemicals. Aseptic technique must be followed while working with microorganisms and viruses, and appropriate instruction in aseptic technique will be provided. Bench-tops must be disinfected after lab classes using the disinfectant provided. You **MUST** wash your hands before leaving the lab.

MICROBIOLOGY LABORATORY SAFETY RULES:

All materials and clothes other than those needed for the laboratory are to be kept away from the work area. ^{[[[]]]}_[SEP]

A lab coat or other protective clothing must be worn during the lab. Lab clothing is not to be worn outside of the laboratory. Items heavily contaminated with bacteria must be disposed of properly. Disposable items are to be placed in the BIOHAZARD container. Reusable items

are to be placed in the designated area for autoclaving prior to cleaning. Sharps are to be disposed of in the appropriate container.

- Aseptic technique must be observed at all times. No eating, drinking, application of cosmetics or smoking is allowed. Mouth pipetting is not allowed.
- Cuts and scratches must be covered with appropriate dressing. Disposable gloves will be provided on request.
- Long hair should be tied back while in the lab.
- All accidents, cuts, and any damaged glassware or equipment should be reported to the lab demonstrator immediately.
- Sterilization techniques will involve the use of Bunsen burners, which constitute a fire and burn hazard. Keep all combustibles away from the Bunsen burners.
- It is the responsibility of the student to know the location and use of all safety equipment in the lab (eyewash, fire extinguisher, etc.)
- Cultures may not be removed from the lab. Visitors are not allowed in the lab.
- Health and Safety Precautions for Electronic devices including laptop computers and mobile phones.

Mobile phones: For your own safety when using your mobile phone in class please ensure it is placed in a plastic zip lock bag. Every student will be provided with a zip lock bag in their first practical class, which they should continue to use during the session (keep it with your lab book). If you misplace or loose this bag you will be expected to provide your own zip lock bag.

Computers and tablets: this applies to either your own device or those supplied by the school. Please cut a section of benchcoat (this will be provided in the lab class) and place your device on this on the lab bench to separate it from your other laboratory work. DO NOT wear gloves when using these devices. If the lab is being used as a dedicated DRY LAB (please check signage on the door) you will not be required to do this as the benches will be cleaned and decontaminated before your class.

I have read and understand the above rules and agree to follow them.

Signed _____ Date _____

Name _____

Risk management:

For every task we perform in the laboratory that involves new equipment or substances that are potentially hazardous we are legally required to prepare a risk management plan (Workplace Health and Safety Act and Regulation 2011).

What is Risk Management?

It is about identifying hazards, assessing them and controlling the risk of the hazards. When identifying hazards consider where, how and who is carrying out work. The level of risk is assessed and used to prioritise implementation of risk control measures.

The risk control measures are:

- **Eliminate the hazard(s)**
 - **Substitute for something less hazardous**
 - **Isolate the hazard from people**
 - **Engineering controls e.g. plant guarding, work in fume cupboard or biosafety cabinet**
 - **Administrative controls e.g. training, safe work procedures, signage**
 - **Personal protective equipment (PPE) e.g. lab coat, safety glasses, gloves**
- To prepare a risk management plan/assessment you need to do the following:**
- **Identify Hazards: Source of potential harm to people, property or environment**
 - **Assess Risks: Chance of something happening - consequence vs likelihood**
 - **Eliminate or Control Risks**

For the practical based studies, you will be undertaking in this course you will be required to read and sign the risk management plan associated with each individual laboratory class to provide evidence you have understood the associated risks and the control measures that have been put in place to minimise these risks. The risk management plans have been prepared by experienced staff however to help you understand this process we will ask you to identify the hazards and risks you may encounter in a microbiology lab and control measures we can use to minimise these risks and then discuss this with your fellow students and demonstrator.

CONSULTANCY BRIEFS -

Group Assignment - Consultancy Brief Instructions

Premise of the consultancy brief

You are a team of contracted virologists working for consultancy firm Unisearch. You have been approached by a company/agency with pressing concerns about a non-human virus. Unisearch has accepted the case on your behalf. Your team must firstly write a brief then submit a final written consultancy report (due week 8, 5th Nov, 2020), as well as a verbal presentation to the clients on 17th Nov 2020 (**see attached A for details**).

Please ensure that you include the briefing letter in your actual consultancy brief. You will also be required to meet fortnightly milestones to ensure client satisfaction (**see attached B for details**). Your final consultancy brief and verbal presentation will be assessed with the following components in mind (**see attached C for details**).

A



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Written report:

- Length:** A maximum of 40 typed pages (including figures and tables). You should aim for around 8 pages per person. (References may be additional to this length).
- Format:** Formatted in the style of the Journal of Virology. Bound board covers, spiral backing.
- Deadline:** Thursday 5th November 2020, 4pm. (Turn-it-in submission)

Verbal Presentation to clients:

- Duration:** 40 minutes, including questions and discussion time (Unisearch usually recommends 30 minutes presentation and 10 minutes discussion)
- Summary:** (of verbal presentation) one double-sided A4 page per consultancy group.
- Venue:** Microsoft Teams
- Time:** 17th November 2020

Facilities are usually available for computer and slide projection and white board activity, but it likely these presentations will be done in Microsoft Teams this year.

Level of report and verbal presentation:

Briefs should be presented in clear language, understandable by highly intelligent successful business people, most of whom are not science graduates. However, you may assume that they understand biology at the HSC level, and further, are conversant with the general aspects of immunity and viral replication. Assume that most of the clients will have taken the effort to read a primer in virology. Rather than aim too low, target a level approximating a 2nd year biological science student (end of year), but be prepared to clarify concepts they do not understand at discussion time in the verbal presentation.

The final report should include detailed information on the virus could include, but is not limited to, the following categories:

- **What is the problem and location**
- **Economic impact**
- **Virus classification**
- **History**
- **Epidemiology/Transmission**
- **Morphology/structure**
- **Genome**
- **Lifecycle/Replication**
- **Pathogenesis**
- **Diagnosis**
- **Protection methods**
- **Other**

A comprehensive and objective report addressing the above issues and others should be provided. In addition, should there be further relevant information that would be of use to interested sectors their inclusion is welcomed.

Please do not rely on non-peer reviewed internet searches, that will make you unsure of the reliability of the data presented. Your report to be based on solid scientific peer reviewed manuscripts, other peer reviewed literature, books and experts in the area.

B

Milestones and Key Dates for consultancy brief.

The brief is worth 30% of the final mark for the course.

- Week 2: “Contract” start date, decide on virus
- Week 3: Division of workload amongst team member with a detailed plan of sections, as well as subheadings within sections
- Week 5: Consultancy brief at ~50% completion (will be checked by demonstrators) **(5% of final course grade)**
- Week 8: Final written consultancy brief due **(12.5% of final course grade)**
- Week 9: Preparation of consultancy brief presentation
- Week 10: Final verbal presentation to client **(10% of final grade)** and peer review **(2.5% of final grade)**

C

Criteria for marking of brief.

Marks will be assigned based on the quality of the sections completed by each student, a mark will also be assigned for the cohesiveness of the overall brief.

Individual

Content/answering brief: Sections appropriately answer the brief, necessary and relevant information is covered. There is no unnecessary repetition.

Referencing: Minimum of 10 references. References are mostly from relevant peer-reviewed journal articles from reputable sources. Journal of Virology referencing style. All figures and tables are appropriately referenced in text. If a figure was taken/adapted from another source, the source must be referenced.

Formatting: Formatting is consistent and professional (font, size, colour, margins etc.). Subheadings are used in each section appropriately. Brief is pleasant to the read.

Writing: Writing is clear and concise. Spelling, grammar and punctuation should be correct.

Group

Cohesiveness/overall brief: Previous four sections are assessed for the entire brief. Make sure formatting, referencing and writing is consistent. Some sections may have some overlap, make sure there isn't unnecessary repetition.

Verbal presentation

Good presentation skills (eye contact, clarity, projection, minimal "umms" and "ahhs", posture).

Good slides (slides are clear and easy to understand).

Ability to answer questions from the audience.

Peer review

You will also be asked to give each of your group members a peer review mark.

CREATING A VIRUS

AIMS OF CLASS:

This is a dry practical focused on viral structures and genomes. The aim of the class is to create and design a virus. This will aid you to relate viral structure with the viral life cycle, transmission, epidemiology and pathogenesis.

CONTENTS:

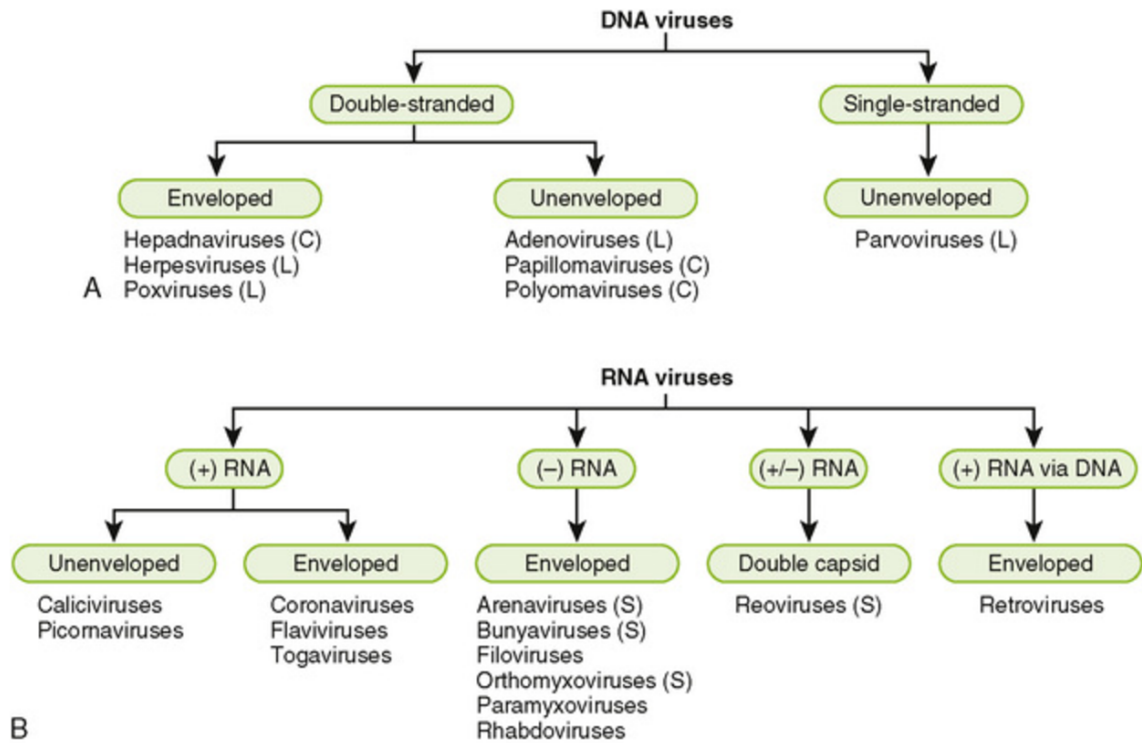
- PART A: QUIZ ON VIRAL STRUCTURE, TRANSMISSION AND PATHOLOGY**
PART B: CREATE A VIRUS
PART C: CDC INVESTIGATION OF VIRAL INFECTION
PART D: ROUND UP

ASSOCIATED LEARNING RESOURCES

1. Lectures (Viruses and viral structure, viral replication strategies and viral proteins)
 2. White & Fenner: pp 4 –15.
 3. UNSW Moodle: Web sites on viral structure.
 4. Flint, L.W. et al. Principles of Virology. ASM Press 2004. pp 82-124.
-

FAMILY GROUPS - THE BALTIMORE METHOD

- Group I: dsDNA Viruses
 - Group II: The ssDNA Viruses
 - Group III: dsRNA Viruses
 - Group IV: (+)sense RNA Viruses
 - Group V: (-) sense RNA Viruses
 - Group VI: RNA Reverse Transcribing Viruses
 - Group VII: DNA Reverse Transcribing Viruses
 - Subviral Agents: Satellites, Viroids, and Agents of Spongiform Encephalopathies
-



PART A: QUIZ ON VIRAL STRUCTURE, TRANSMISSION AND PATHOLOGY

Complete the following quiz

- 1) Describe the possible types and arrangement of nucleic acids found within a virion

- 2) Describe the main types of structural architecture used by viruses and name examples of viruses that use the architecture

- 3) Sometimes non-structural or other proteins are carried within the virion. Give some examples of these proteins. In terms of replication why are these “on board” proteins needed?
- 4) What is a viral envelope, what is its function and where does it come from?
- 5) Describe the properties and function of viral envelope proteins. How does the presence or absence of an envelope affect the transmission properties of a virus?
- 6) In terms of viral genomes what is meant by ambisense?

Discuss the answers to these questions. One person in each group will be an expert on one of the six questions and will lead that part of the discussion.

PART B: CREATE A VIRUS

MATERIALS

-Per bench

You will be provided with six trays. Select items from each tray to build your virus:

Tray #:

1. Nucleic acid
2. Capsid (size)
3. Accessory proteins
4. Envelope
5. Envelope proteins
6. Other structural features

Procedure

YOU WILL NOW NEED TO BUILD A VIRUS.

1. Decide on the type and size of nucleic acid.
 - Is the virus single stranded, double stranded or does it have some other configuration?
2. Decide on the structure and make it. Don't forget to put your nucleic acid into virion.
 - What type of structural architecture does the virus have?
 - How big is the virus?
 - Are any matrix proteins part of the structure?
3. Decide on what type of accessory proteins (if any) the virion carries. Add them to your virus
 - What is the function of these proteins?
4. Does your virus have an envelope? If so, use the material provided to create an envelope for your virus

5. Does your virus have any envelope proteins? If so add them to your virus.

6. Finally, based on the structure of your virus, and with knowledge of viruses with similar properties, write on the card provided something about each of the following:
 - Organism infected
 - Transmission route
 - Mode of replication
 - Epidemiology
 - Pathogenesis

Name the virus on the card and below

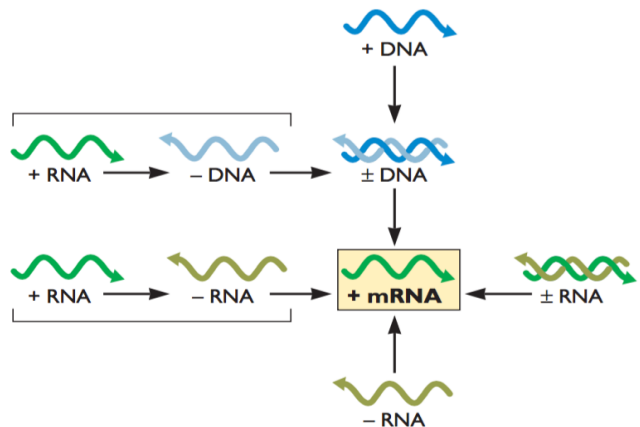
.....

Give this card to your tutor.

7. What viral family best fits with the virus you have created? (see the last page of this manual)

8. Is your virus viable and does it fit within the principles of the Balimore classification system?

Figure 1.11 The Baltimore classification. All viruses must produce mRNA that can be translated by cellular ribosomes. In this classification system, the unique pathways from various viral genomes to mRNA define specific virus classes on the basis of the nature and polarity of their genomes.



From Flint, Principles of Virology, 2015.

PART C: INVESTIGATION**CENTRES FOR DISEASE CONTROL (CDC) INVESTIGATION OF VIRAL INFECTION**

You are part of a CDC investigation team and called in to investigate a disease of possible viral aetiology. Swap your virus with a virus from another group. This is the virus under investigation.

Procedure

1. Investigate the virus and identify all features including:

- Nucleic acid
- Capsid (size)
- Accessory proteins
- Envelope
- Envelope proteins
- Other structural features

Describe the features of the virus here:

2. Using the structure of the virus match it with the appropriate card and give your reasoning.

3. Can you say anything about the following after investigating the structure of the virus?

- Organism infected
- Transmission route
- Mode of replication
- Epidemiology
- Pathogenesis

PART D: ROUND UP AND PRESENTATION

General discussion

Place your virus at the front of the class for viewing and have a look at the other viruses

Each group will be asked to name their unknown virus, describe the features of the unknown virus and comment on their investigation. Say something about why the viral structure link to information on the card.

Discussion Notes:

PRACTICAL 2 - DIAGNOSTIC MOLECULAR VIROLOGY

HETERODUPLEX MOBILITY ANALYSIS GENOTYPING OF HEPATITIS C VIRUS

AIMS OF CLASS:

Molecular virology, including techniques such as the polymerase chain reaction (PCR), is routinely used in virology diagnostic settings. PCR is a rapid and sensitive technique that can replace viral culture in a number of circumstances. Molecular virological diagnostic techniques include agarose gel electrophoresis (AGE), polyacrylamide gel electrophoresis (PAGE), Southern blotting, northern blotting, PCR and heteroduplex mobility analysis (HMA).

In this practical we will PCR-amplify a region of the genome from an unknown sample of hepatitis C virus (HCV). The PCR products will be run on an agarose gel to verify success of the PCR reaction and to determine the length of the amplified product. In the second part of the practical, heteroduplex mobility analysis (HMA) will be utilised to genotype the unknown HCV sample.

CONTENTS:

- 1. BACKGROUND**
- 2. THE SCENARIO**
- 3. MATERIALS**
- 4. PCR AMPLIFICATION OF HCV RNA**
- 5. AGAROSE GEL ELECTROPHORESIS (AGE)**
- 6. POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)**
- 7. HETERODUPLEX MOBILITY ANALYSIS (HMA) GENOTYPING**

ASSOCIATED LEARNING RESOURCES:

1. White, P. A., X. Zhai, I. Carter, Y. Zhao and W. D. Rawlinson. 2000. Simplified hepatitis C virus genotyping using heteroduplex mobility analysis. *J. Clin. Microbiol.* **38**: 477-482.
2. White, P. A., Z. Li., X. Zhai, G. Marinos and W. D. Rawlinson. 2000. Mixed viral infection identified using heteroduplex mobility analysis (HMA). *Virology* **271**: 382-389.
3. White, P. A., Z. Li. and W. D. Rawlinson. 1999. Sequence diversity in the 5'-UTR region of GB virus C/hepatitis G virus as assessed by sequencing, heteroduplex mobility assays and Single-strand conformation polymorphism. *J. Virol. Methods.* **83**: 91-101.
4. Delwart, E. L., E. G. Shpaer, J. Louwagie, F. E. McCutchan, M. Grez, H. Rubsamen-Waigmann, and J. I. Mullins. 1993. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. *Science.* **262**:1257-61.

1. BACKGROUND

Hepatitis C virus is the major cause of non-A, non-B hepatitis and was first definitively identified by molecular cloning of the virus genome in 1989. HCV is a member of the Flaviviridae. The 9.5 kb, ssRNA positive sense genome contains a single long open reading frame which encodes a polyprotein of about 3000 amino acids. There is an untranslated region (UTR) of 324-341 nucleotides at the 5' end and a 3' UTR of variable length. The nucleotide sequence of HCV is highly variable, with the most divergent isolates sharing only 60% nucleotide sequence homology over the whole genome. Based on nucleotide sequence data there are six main HCV genotypes each with several subtypes.

HCV infection occurs in approximately 250,000 of the Australian population and chronic HCV infection affects an estimated 135 million individuals worldwide. Several diagnostic tests have been developed: serological- using recombinant antigens, and PCR detection. The parenteral route of infection seems to be most prevalent, with high rates of infection observed in intravenous drug abusers, haemophiliacs and recipients of unscreened blood transfusions. In Australia IDU accounts for 82% of HCV transmission.

In the minority of cases HCV infection gives rise to an acute illness; ~60% of such cases develop into chronic hepatitis. Almost all patients develop a vigorous antibody and cell-mediated immune response that fails to clear the infection but may contribute towards liver damage. Patients with chronic disease are at risk of developing hepatocellular carcinoma (HCC). The duration from the onset of acute hepatitis until the time of diagnosis of cirrhosis of the liver and of HCC is about 20 and 30 years, respectively.

Before the recent direct acting antivirals (10 have been approved in the USA), the only therapy with any demonstrated efficacy against HCV-induced liver disease involves the use of pegylated alpha-IFN in combination with ribavirin, but this approach was not that successful. Even in the best trials reported, around 50%-80% of chronic sufferers demonstrate a sustained response. Treatment is much less effective against some HCV genotypes than others, i.e genotypes 1 and 4. The virus could not be cultured *in vitro* until 2005 and this has hampered investigations. No vaccine has yet been developed.

Genotype, viral load and liver histology are important parameters used when selecting patients for antiviral therapy with the greatest chance of success. Genotype 1 isolates, in particular 1b, are known to respond poorly to interferon treatment, whereas other HCV genotypes such as genotype 3 respond more favourably. The highly conserved 5'-untranslated region (5'-UTR) is almost exclusively used for routine reverse transcription-polymerase chain reaction (RT-PCR) detection of HCV and is currently the most sensitive and reliable method of establishing ongoing infection. Many genotyping assays utilise the 5'-UTR PCR product. However, the initial RT-PCR detection step, or downstream processing of the PCR product remains too complicated, costly or time consuming for routine genotyping.

Heteroduplex mobility analysis (HMA) relies on the formation of mismatches when two divergent DNA molecules are mixed, denatured and allowed to reanneal. This results in the formation of homoduplexes and heteroduplexes that migrate at different speeds during polyacrylamide gel electrophoresis (PAGE). The mismatches reduce the mobility of the heteroduplexes, which are retarded roughly in proportion to the divergence between the two sequences. Genotyping by HMA involves mixing a PCR product of unknown genotype separately with a panel of reference products of each genotype and the resultant heteroduplexes are then separated by PAGE. Genotype determination for HCV relies on the identification of specific patterns or “**finger prints**” of heteroduplexes in the reference lanes.

2. THE SCENARIO

Background

You are a molecular biologist in Miami. Due to the incompetence of the CSI team and their inability to perform routine molecular biology testing, you have been called into an investigation.

The case

Horatio was found fatally injured at the bottom of a flight of stairs. He survived just long enough to implicate a colleague from another CSI division, who he claimed pushed him down the stairs unprovoked. The colleague, Grissom claims to have been held at needlepoint with a syringe full of HCV infected blood (of Horatio's own). He said he was threatened by Horatio and said that Horatio had stalked Grissom, and was jealous because Grissom was a better detective. An argument and subsequent scuffle ensued. Grissom claims to have been punctured in the neck with the syringe by Horatio and in the panic that followed, Horatio fell down a flight of stairs whereupon Grissom promptly fled the scene. The syringe (if it existed) could not be retrieved.

Your task

CSI Miami are struggling to gather evidence, possibly due to the unfortunate demise of their leader, hence any information that you can gather about the HCV status of Grissom will be of assistance in their investigation.

- It is unknown if Grissom is infected with HCV
- Grissom claims to never have injected drugs or been under circumstances where HCV infection was possible
- Serum from Horatio showed infection with HCV genotype 3a
- Horatio was known to be an injecting drug user

Therefore, you postulate that if Horatio infected Grissom they should have the same viral genotype. You are aware that if they are both infected with the same genotype, this does not necessarily confirm the guilt of Horatio, nor mean Grissom cannot have been infected by a different means, but in the pursuit of thoroughness, you decide to:

- A) Test for HCV by PCR to determine if indeed Grissom has been infected with HCV and in the case that he is,
- B) Perform HMA testing to determine the viral genotype

You do not have access to the Horatio's serum sample but you have a number of reference HCV genotypes (1a, 2b, 3a, 4a, 6a) for comparison.

Is Grissom lying? Was Horatio to blame? Can you come up with a scenario that fits the evidence?

3. MATERIALS

-Per bench

You will be provided with:

- A sample of RNA extracted from a patient (Grissom) with HCV infection of unknown genotype. **You will determine the genotype of this patient sample.**
- Five tubes are provided with plasmid DNA containing cloned PCR products from genotypes 1a, 2b, 3a, 4a and 6a (reference strains).

PCR reagents

1× beaker (150 ml size) per bench containing 0.1 ml PCR tubes {sterile}

1× beaker (150 ml size) per bench containing 0.5 ml Eppendorf tubes {sterile}

3× Eppendorf tube racks

Pipettes and tips

Per CLASS

3× 1L sterile D.H₂O

Plastic bags for the gel

2× thermocyclers

2× acrylamide gel tanks

2× power packs

glassware

S, M & L gloves

running buffer

1× masking tape

2× Eppendorf centrifuges

4× agarose gel tanks

1× microwave

6-8 eskies with ice

PCR markers and loading buffer

Notes:

4. PCR AMPLIFICATION OF HEPATITIS C VIRUS (HCV) RNA**Procedure (Steps 1 to 3 are already done for you)**

1. Label seven PCR tubes (0.2ml) with your initials, and: unknown, 1b, 2b, 3a, 4a, 6a and negative control.

Using a **new sterile tip** for each addition:

2. Add 2 μ l of unknown sample to the PCR tube labelled “unknown”.
3. Add 2 μ l of plasmid DNA samples to the corresponding PCR tubes (labelled accordingly) and 2 μ l of H₂O to the PCR tube labelled “negative control”. Briefly spin down the contents of the tubes.
4. Then make the following reaction mix (indicated in **bold** in Table 1) in a separate 1.5 ml Eppendorf tube provided which contains 2 μ l of Taq. To this tube you should add the reagents listed in column 3 (Quantity for 8 reactions).
5. Aseptically add 38 μ l of this reaction mix into each of the seven tubes (there should be some left).
6. Place sample tubes in a thermocycler (demonstration to be given by tutor).

Table 1

Reagent	Quantity/40 μ l reaction	Quantity for 8 reactions	Final Concentration
PCR buffer 5 x	8 μ l	64 μl	1 x
dNTP (10mM)	0.8 μ l	6.4 μl	200 μ M
Primer hep21b (10 pmol/ μ l)	2 μ l	16 μl	0.5 pmol/ μ l
Primer hep22 (10 pmol/ μ l)	2 μ l	16 μl	0.5 pmol/ μ l
H ₂ O	24.95	199.6 μl	-
template	2 μ l	-	-
<i>Taq</i> (5 Units/ μ l)		2 μl already in Eppendorf	1 unit/reaction
	Total =40 μ l	Total =304 μl	

PCR Cycling Conditions:

The thermocycler will be programmed to follow the conditions below:

94°C for 2 min then:

25 PCR amplification cycles at:

- denaturation at 94°C for 30 s
- annealing at 60°C for 30 s
- extension at 72°C for 30 s

Final extension at 72°C for 5 min

5. AGAROSE GEL ELECTROPHORESIS (AGE)

On completion of the PCR reaction (while the HMA reaction is progressing) separate your products by agarose gel electrophoresis.

Procedure

1. Pouring and running of the agarose gels will be supervised by a tutor. The gel contains 1.5% agarose in TBE buffer.
2. Load products on to a 1.5 % agarose gel.
3. Also load 10 µl of marker – plasmid pGEM-3 cut with *Hinf* I, *Rsa* I and *Sin* I.
4. Note down your group name, the order you loaded the products and the gel number.
5. Run the gel for ~14 min at 220 volts.
6. Stain the gel with Gel Red
7. View the gel on a UV transilluminator: the size of the HCV PCR product is **175 bp** (caution: wear protective eye goggles and gown to protect from harmful UV rays, handle gel with gloves).
8. Photograph gel for lab notes.

6. DEMONSTRATION: PREPARATION OF A POLYACRYLAMIDE GEL

Your tutors will demonstrate (with volunteer help) how to assemble the polyacrylamide gel equipment.

Stick your final PAGE and AGE gel pictures in the space provided below:

7. HETEROduplex MOBILITY ANALYSIS (HMA) GENOTYPING

Procedure

1. Add 3 μl of each of the five reference products to a fresh PCR tube (labelled accordingly; put your initials on the tubes [*NOT ON THE LIDS*]).
2. Add 3 μl of unknown PCR product to each of the above tubes and add 3 μl of unknown sample PCR product to a separate PCR tube (HMA control).
3. Place tubes in a thermocycler to carry out the denaturation/reannealing reaction (demonstration to be given by tutor).

HMA Conditions:

Denaturation at 94°C for 5 min

Reannealing at 60°C for 20 min

4. After the reaction has finished, add 4 μl of loading dye to each tube and mix well. Samples are now ready for separation of homoduplexes and heteroduplexes by PAGE.
5. Load 6 μl of the HMA loading dye mix onto an 8% polyacrylamide gel.
6. Run the gel at 100V for 70 min.

The following will be completed by tutors and gel pictures provided for HCV genotype determination next week.

7. Stain with SYBR Green I (5μl/100 ml 1x TBE) (**caution, wear gown and gloves**).

Using reference 1 and the HCV HMA genotyping picture provided below, determine the genotype of your unknown virus.

Questions:

1) The genotype of the unknown HCV isolate is:

2) Based on my results, the reason for this is as follows:

Sequences of 175 bp products derived from different HCV genotypes:

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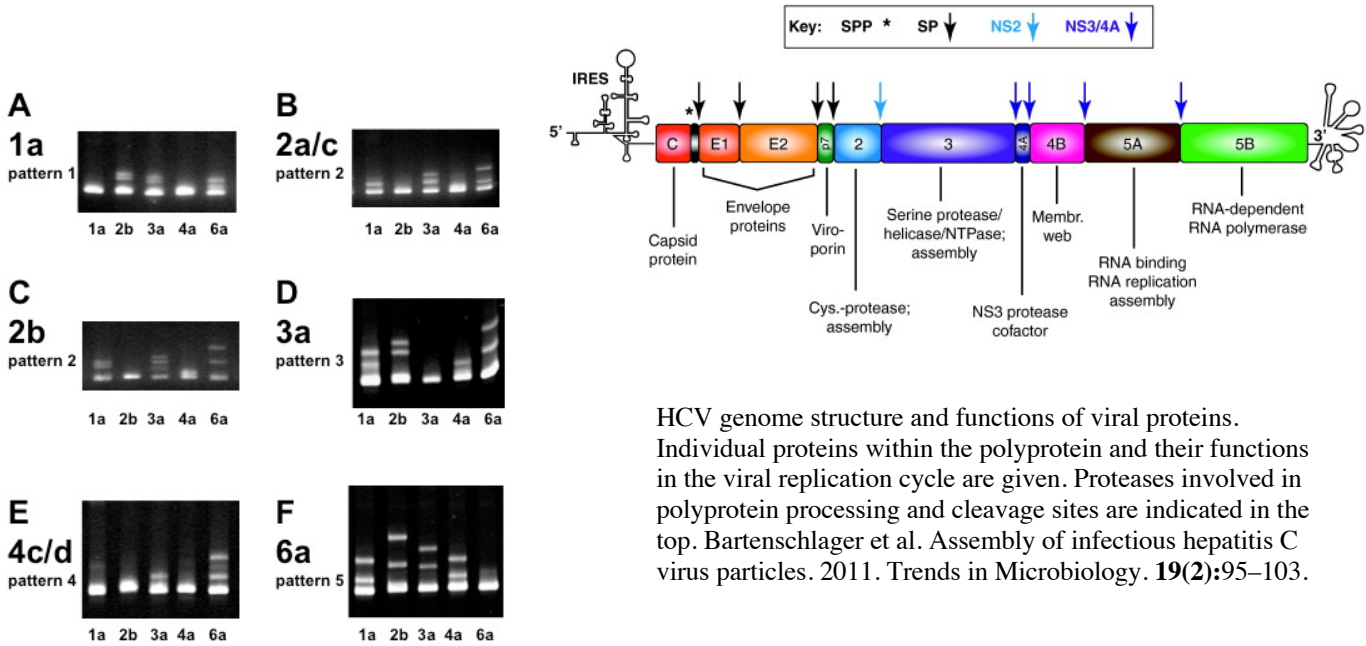
GAGTGT GT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC
1a : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
1b : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
2a : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
2b : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
3a : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
3b : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
4a : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
4cd : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
5 : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
6a : GAGTGTGT CAGCCTCCAGG CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACC : 60
    
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GGTGAGTACACCGGAAT C G GAC GGGTCCTTTC TTGGA AA CCC CTC
1a : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
1b : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
2a : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
2b : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
3a : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
3b : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
4a : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
4cd : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
5 : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 117
6a : GGTGAGTACACCGGAATTG CAG GAC GAC GGGTCCTTTC TTGGA TCAA CCC CTTCA : 120
    
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AT C G ATTTGGGCGTGCCCCCGC AGA CTAGCCGAGTAG GTTGGGT GC
1a : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
1b : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
2a : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
2b : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
3a : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
3b : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
4a : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
4cd : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
5 : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 175
6a : ATGCC TGGAG ATTTGGGCGTGCCCCCGC AGACTG CTAGCCGAGTAG GTTGGGT GGC : 178
    
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HCV genome structure and functions of viral proteins. Individual proteins within the polyprotein and their functions in the viral replication cycle are given. Proteases involved in polyprotein processing and cleavage sites are indicated in the top. Bartenschlager et al. Assembly of infectious hepatitis C virus particles. 2011. Trends in Microbiology. 19(2):95–103.

HCV HMA genotyping picture

HMA Ethidium bromide stained 8% polyacrylamide gels showing genotyping by HMA with typical heteroduplex patterns formed for test samples of each group, 1, 2, 3a, 4, and 6. The reference panel of subtypes used is shown along the bottom and the test sample and pattern number is indicated on the left-hand side. For examples of HMA of genotype 2 isolates, note the larger shifts in mobility seen with subtype 2b. An example of subtype 3a shows heteroduplexes in all lanes except the reference 3a lane. Similar results are seen for genotype 6a. HMA of a 4c/d HCV subtype shows heteroduplexes present in only 3a and 6a reference lanes.

Recipe for 8% polyacrylamide gel for heteroduplex mobility analysis (HMA). In this practical we use commercially available gels.

	2 small gels (15ml)
Water	9.4 ml
30% acrylamide bis solution	4.0 ml
10 × TBE	1.5 ml
PEG 6000 (500g/l)	150 μ l
To polymerise add 10% APS	200 μ l
TMED	20 μ l

PCR questions

1. Describe briefly, with the aid of small diagrams, the sequential steps from HCV genomic RNA → PCR-amplified sample.

2. Why are the following reagents added to PCR:
 - i. dNTP:
 - ii. MgCl₂:

3. What is RT-PCR and how does it differ from standard PCR?

4. What is in TBE buffer?

- 5a. What is nested PCR?

- 5b. Why is it useful in diagnostic virology?

- 5c. What are the advantages/disadvantages of this method?

6. Why is the RT-PCR reaction heated to 94°C between the RT step and the PCR step?

7. Describe three methods for detecting PCR products.

8. Describe three methods to confirm that the PCR product is not a non-specific product.

9. What is a negative control in PCR and why do we use one?

10. Briefly explain viral genotyping and describe its utility in virology

VIRAL RECOMBINATION - COMPUTER PRACTICAL

Mechanisms of Viral Evolution

Background

RNA viruses exist as genetically diverse populations with an ability to rapidly introduce new sequence variation. The high level of genetic diversity in RNA viruses is recognized as the basis for their ubiquity and adaptability. The molecular mechanisms underlying RNA virus diversity are mutation, recombination and, genome reassortment in viruses with segmented genomes.

Influenza virus utilizes all three strategies to rapidly evolve, change its antigenic profile and create novel strains. Often, these new strains appear when either an existing animal flu virus spreads to humans, or undergoes recombination/reassortment with genes from an existing human flu strain. The arrival of novel antigenic variants coincides with seasonal epidemics, resulting in the deaths of hundreds of thousands annually — millions in pandemic years.

Influenza is a member of the family Orthomyxoviridae, that affects birds and mammals. The most common symptoms of the disease are chills, fever, sore throat, muscle pains, severe headache, coughing, weakness and general discomfort. In more serious cases, influenza causes pneumonia, which can be fatal, particularly for the young and the elderly.

Influenza genome contains 11 genes on eight pieces of RNA, encoding for 11 proteins: haemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M1, M2, NS1, NS2 (NEP), PA, PB1, PB1-F2 and PB2. HA and NA are the two large glycoproteins on the outside of the viral particles to which antibodies are raised. HA mediates binding and entry of the virus into target cells, while NA is involved in the release of progeny virus from infected cells.

Influenza A viruses are classified into subtypes based on antibody responses to HA and NA. These different types of HA and NA form the basis of the *H* and *N* distinctions in, for example, *H5N1*. There are 16 H and 9 N subtypes known, but only H 1, 2 and 3, and N 1 and 2 are commonly found in humans. However, due to the segmented nature of Influenza's genome it is able to swap segments of its genome, in a process called antigenic shift, with other co-infecting influenza strains.

Antigenic shift is of particular concern because human influenza strains that undergo reassortment/recombination with zoonotic species, especially avian strains, are likely to have increased virulence. An avian strain named H5N1 raised the concern of a new influenza pandemic, after it emerged in Asia in the 1990s, but it has not evolved to a form that spreads easily between people. In April 2009 a novel flu strain evolved that combined genes from human, pig, and bird flu, initially dubbed "swine flu", emerged in Mexico, the United States, and several other nations. WHO officially declared the outbreak to be a "pandemic" on June 11, 2009.

Antigenic drift is also a problem for vaccine design as the existing vaccine may not induce immunity against the new antigenic variant. The trivalent influenza vaccine (TIV) contains purified and inactivated material from three viral strains. Typically, this vaccine includes material from two influenza A virus subtypes and one influenza B virus strain. The TIV carries no risk of transmitting the disease, and it has very low reactivity. A vaccine

formulated for one year may be ineffective in the following year, since the influenza virus evolves rapidly, and new strains quickly replace the older ones.



**WHO Collaborating Centre for
Reference & Research on Influenza**
Melbourne, Australia



The Australian WHO Collaborating Centre for Reference and Research on Influenza is part of the World Health Organization influenza surveillance network. The network was established in 1947 to monitor the frequent changes in influenza viruses with the aim of reducing the impact of influenza through the use of vaccines containing currently circulating strains.

The two major original objectives of the WHO program were:

To study the origins of epidemic and pandemic influenza strains;

To provide new virus strains quickly for the production of vaccines in the face of outbreaks.

Since then the WHO influenza surveillance network has grown to involve approximately 110 National Influenza Centres in 80 countries and four WHO Collaborating Centres for Influenza Reference and Research located in London, Atlanta, Melbourne and Tokyo.

The Melbourne Centre was originally established as a Regional Influenza Centre in 1951 and was subsequently upgraded to Collaborating Centre status in 1992.

The Practical

You are a molecular biologist at the Influenza surveillance network in Melbourne, Australia. Diagnostic labs from across Australia have reported to your department that they have seen an uncharacteristic rise in the number of influenza cases being detected. These strains appear to be of H1N1 origin. Your lab has already sequenced representative strains from interstate and found the same variant to be circulating. There is however a concern that the strain may be a novel antigenic variant of previously circulating H1N1 strains and this may only be the start of the influenza outbreak and potential pandemic.

You have been provided with the full length sequence of the influenza strain associated with the recent epidemics. The sequence file that you have been given also contains, the recent human influenza strains and some zoonotic influenza strains, in particular swine and avian strains. These sequences contain all eight segments in the following order; HA, NA, MP, NP, NS, PA, PB1 and PB2.

In order to determine if it is a new antigenic variant this strain will need to be analysed for evidence of antigenic drift and shift. Antigenic drift is a result of small point mutations in amino acid coding sequence and antigenic shift is a result of large sections of the viral genome being swapped by either reassortment or recombination.

To determine the origin of the individual segments you will need to analyse the sequence of each segment to other human and animal influenza strains. To do this you will plot the sequence identity of previously circulating strains against the new variant. This will be done in the program Simplot.

1. Open the program Simplot
2. Go to “file” and click “open”.
3. Load the file name “influenza.fas” from the desktop.
4. Click “Use first character to identify groups” and select “use first N characters to identify groups” and change 1 to 10.
5. Click the “Simplot” tab.
6. Go to “commands” and then “query” and select the strain called California/04/09 (H1N1) (this is the new strain currently in circulation).
7. Click “window size” at the bottom left side of the screen and change to 500.
8. Click “step size” and change to 50.
9. Click “start scan”.
10. Multiple lines will appear across the graph. The x-axis represents the genome position and y-axis represents the identity of the line (influenza strain) to the new strain. The higher the line is to 100 the closer in identity it is to our new influenza variant.
11. The bar on the right indicates the line of each strain.
12. If you click on the coloured lines it will tell you the genomic position for that strain.
13. If you double click on the white background a red line will appear – to remove it just double click. Add lines at the junction for the different gene segments as listed in Table 1.
14. For each gene from the new H1N1 variant, determine its closest relative from the Simplot graph and fill in the table and answers the questions below.

The strains included in the analysis are:

- Swine/England/WVL7/92 (H1N1) (swine virus of avian origin)
- Swine/Illinois_100085A/01 (H1N2) (swine virus of swine origin)
- Human/Hong_Kong/24/85 (H3N2) (human virus of human origin)

Q1. Is there any evidence of antigenic shift? Is it a result of recombination or reassortment?

Q2. Is there any evidence of antigenic drift?

Q3. In gene PB1 both Swine/Illinois_100085A/01 and Human/Hong_Kong/24/85 share high sequence identity to the new H1N1 variant Human/California/04/09. Provide a possible explanation for this, use figure 1 at the end of this prac to help you answer this question.

Q4. The influenza vaccine components for the year 2009 season contain the following viruses:

- * A (H1N1): an A/Brisbane/59/2007 (H1N1) - like strain, 15 µg HA per dose
- * A (H3N2): an A/Brisbane/10/2007 (H3N2) - like strain, 15 µg HA per dose
- * B: a B/Florida/4/2006 - like strain, 15 µg HA per dose

Is this likely to be effective against the new influenza variant?

Q5. Influenza virus induces life long immunity and provides partial protection (ie. Reduces the severity of illness) to closely related strains. Use the graph (Figure 1) below to find out what age group is most at risk, explain your answer?

Table 1. Simplot analysis of new Influenza variant

Influenza gene	Location of gene in the full length RNA segment	Closest Relative strain		Any evidence of		
		Name	Species of Origin	Anti-genic drift	Recomb -ination	Reass-ortment
HA	1 - 1792					
NA	1793 - 3285					
M	3286 – 4333					
NP	4334 – 5878					
NS	5879 - 6764					
PA	6765 - 8993					
PB1	8994 - 11336					
PB2	11337 - 13650					

Figure 1. Pandemics of influenza, 1900-2000

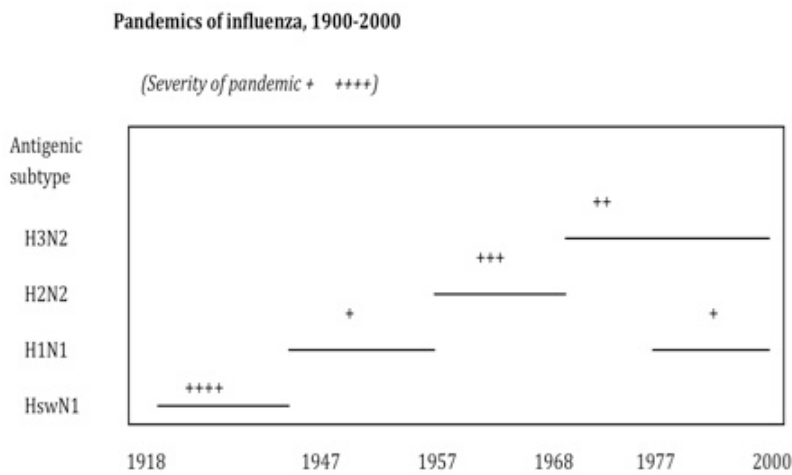
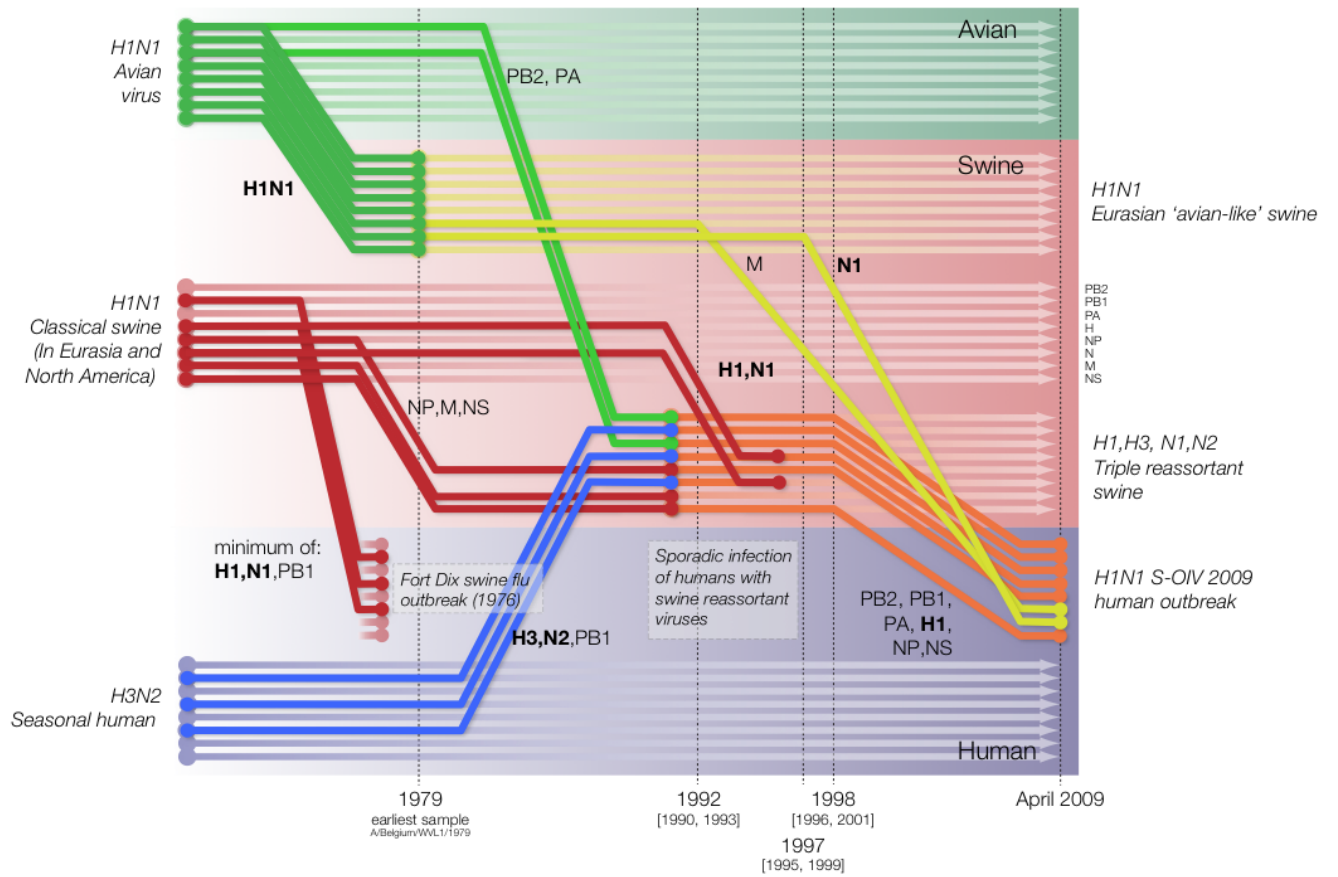


Figure 2



SCREENING FOR SMALL COMPOUND VIRAL POLYMERASE INHIBITORS

Background

The hepatitis C virus (HCV) life cycle offers a number of potential targets for antiviral therapy, and several direct acting antiviral (DAA) therapies for HCV are in preclinical and clinical stages. Results from clinical trials carried out in the past few years on DAA agents in combination with standard therapy with pegylated interferon and ribavirin provide promise of higher rates of viral clearance and, potentially, shorter duration of therapy than standard therapy alone (4, 5). However, in the short-term pegylated interferon and ribavirin are likely to remain as the cornerstone of therapeutic regimens (2). One target for hepatitis C virus (HCV) antivirals is the HCV RNA dependent RNA polymerase (RdRp) because of its key role in replication (3). Currently, the nucleoside analogues R7128 and the non-nucleoside inhibitor Dasabuvir (ABT-333) are the most advanced towards clinical use. Others that have completed phase II trials have been abandoned because of adverse-effects, including Valopicitabine (NM283) (gastrointestinal adverse events) and R1626 (Roche-phase II) (toxicity). Thus there is still an extremely active hunt for new polymerase inhibitors (4, 5).

With recent advances, replicons and cell culture systems exist for genotypes 1 and 2, but do not exist for any other genotype. Antiviral screening programs rely very heavily on *in vitro* biochemical assays using *Escherichia coli* expressed RdRps to look for inhibitors of other HCV genotypes.

Strategy. *The aim is to conduct a screening campaign against the Hepatitis C Virus (HCV) RNA polymerase.*

The Chemical Compound Library. The chemical compound library to be used in this application is co-owned by the Children's Cancer Institute Australia (CCIA) and the Walter and Eliza Hall Medical Research Institute (WEHI). It is a unique collection of 12 (normally this would be 100s of thousands) small molecule compounds selected for "lead-like" chemical structures and is based around the concept that smaller and polar compounds act as precursors for the development of successful drugs. These compounds have physio-chemical properties and occupy pharmacophore space suitable for hit identification and lead compound development.

Screen Design. Recombinant HCV RdRps expressed in bacterial systems possess biochemical properties indistinguishable from HCV RdRps expressed in either mammalian or insect cell systems. This suggests that a bacterially expressed enzyme assay is the simplest and most effective way of studying the properties of the enzyme and developing high throughput screening assays. Methods have been established in the laboratory that successfully isolate highly purified and active recombinant HCV RdRp from bacterial cell cultures (1). Using an *in vitro* RdRp activity assay we will screen 12 small compounds for their ability to inhibit RdRp mediated incorporation of GTP into a polyC template, as described in (1), but with a fluorescent readout instead of a radioactive one.

References

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2. Eltahla, A. A., K. Lackovic, C. Marquis, J. S. Eden, and P. A. White. 2013. A fluorescence-based high-throughput screen to identify small compound inhibitors of the genotype 3a hepatitis C virus RNA polymerase. *Journal of Biomolecular Screening*. 18:1027-1034.
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4. Pereira, A. A., and I. M. Jacobson. 2009. New and experimental therapies for HCV. *Nat Rev Gastroenterol Hepatol* 6:403-11.
5. Legrand-Abrevanel, F., N. Florence and J. Izopet. 2010. New NS5B polymerase inhibitors for hepatitis C. *Expert Opinion on Investigational Drugs* 19:963-975.

Annals of Translational Medicine, Vol 4, No 2 January 2016

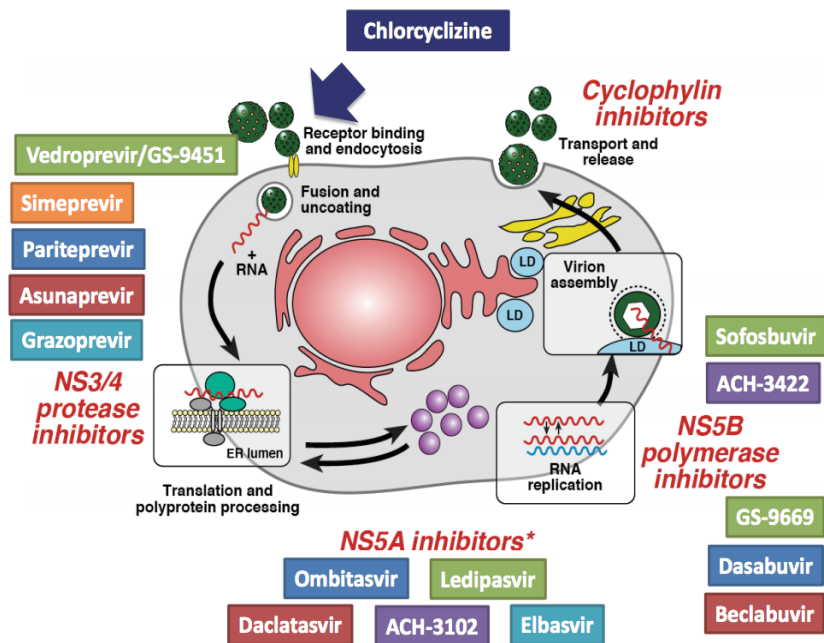


Figure 1 The major sites of action of existing and newer antiviral agents being developed for the management of hepatitis C. Chlorcyclizine allows us a novel mode of action and could complement existing DAA therapy.

How many classes of direct acting antivirals are available for HCV and how does each class work?

Materials:

POLARstar OPTIMA microplate reader (BMG Labtech)

Quant-iT™ PicoGreen dsDNA Assay Kit (Invitrogen)

Fluorescent plate: 96-well (P8741, Sigma)

RdRp Reaction Conditions:

There are three components for the RdRp reaction: component A (polymerase) and component B (reaction mix) and the compounds.

1. Select four compounds from the library of 12 small compounds at the front of the class.
2. Label 12 PCR tubes (0.2ml) with your initials, and **duplicates for:** unknown 1, unknown 2, unknown 3, unknown 4, positive and negative control, all done in duplicates
3. Prepare the polymerase component (component A) in a 1.5 ml tube according to the following table:

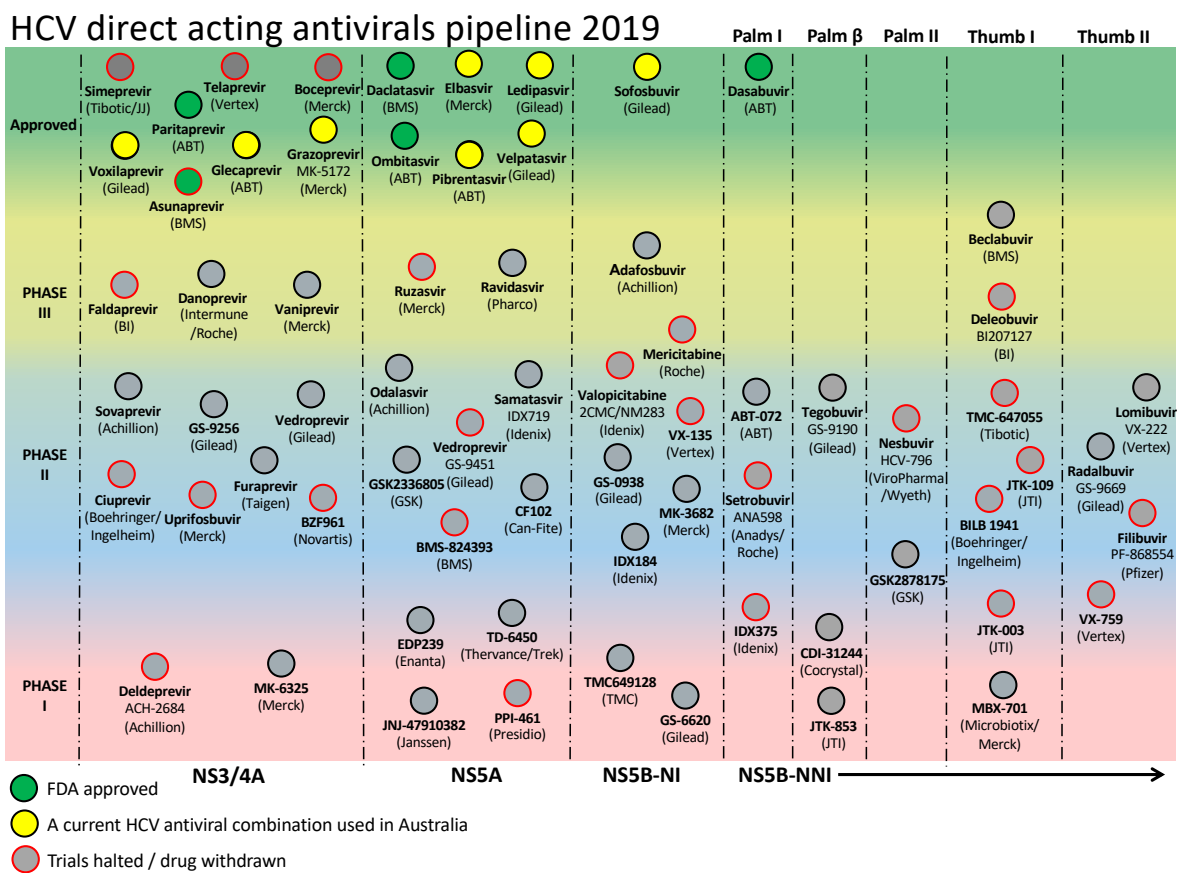
Component	Stock Conc.	Volume (μ l)	Volume for 14 reactions (μ l)	Final Conc.
RdRp	200 ng/ μ l	2	28	400 ng
Tween20	0.1% (v/v)	0.25	3.5	0.005% (v/v)
BSA	0.2% (w/v)	0.25	3.5	0.01% (w/v)
H₂O		2.5	35	~
Total		5	70	

4. Prepare the reaction reagents, component B in a 1.5 ml tube according to the following table:

Component	Stock Conc.	Volume (μ l)	Volume for 14 reactions (μ l)	Final Conc.
RNA*	250 ng/ μ l	1	14	250ng
Tris-HCl (pH 7.5)	100 mM	5	70	20 mM
DTT	100 mM	1.25	17.5	5 mM
MnCl₂	50 mM	1.25	17.5	2.5 mM
rGTP	4.6 mM	1.25	17.5	0.23 mM
Tween 20	0.1% (v/v)	0.75	10.5	0.005% (v/v)
BSA	0.2% (w/v)	0.75	10.5	0.01% (w/v)
H₂O	~	3.75	52.5	~
Total		15	210	

*Sigma's Polycytidylic acid (~200 to 400 bases)

5. Add 5 µl compound or control to the bottom of each PCR tube.
6. Add 5 µl of RdRp (component A) to the bottom of the PCR tube and incubate for 10 mins at room temperature
7. Add 15 µl reaction mix (component B) to each of tubes to start the reaction
8. Incubate at 30°C in a PCR machine for 30 minutes
9. Stop reaction by adding 10 µl 17.5 mM EDTA (Final Concentration 5 mM)



Using a computer search, can you name the most effect drugs shown in this schematic of the HCV drug-development pipeline that are used in Australia for the treatment of HCV patients?

How many people will be cured in Australia in 2020?

PicoGreen Staining:

10. Once reaction is finished, transfer contents to a black 96 well microplate at the front of the class and add PicoGreen (1:680 v:v) to the reaction
11. Incubate for 5-8 mins at room temp (~25°C) protected from light
12. On the POLARSTAR, perform a quick “gain adjustment” run for the whole plate
13. Measure fluorescence at standard wavelengths (excitation 480 nm, emission 520 nm)
14. Record the relative fluorescence for all five samples
15. Calculate percentage inhibition as follows:

$$\text{percentage inhibition} = 100 - (100 \times (\text{sample well} - \text{Mean}_{\text{NSA}}) \div (\text{Mean}_{\text{TA}} - \text{Mean}_{\text{NSA}}))$$
 where NSA = Non Specific Activity, TA = Total Activity
16. Record your results in the table below:

Compound	Relative fluorescence of 4 compounds	Percentage inhibition of RdRp activity	Class results – Average % inhibition (for compounds not tested)
MICR-1			
MICR-2			
MICR-3			
MICR-4			
MICR-5			
MICR-6			
MICR-7			
MICR-8			
MICR-9			
MICR-10			
MICR-11			
MICR-12			
Negative control		0%	0%
Positive control		100%	100%

17. Describe the compounds with potential for development as antiviral agents and give your reasoning:

VIRUS AND BACTERIOPHAGE CULTIVATION –I

PRACTICAL 5

PART A: EGG INOCULATION TECHNIQUES

PART B: BACTERIOPHAGES

AIMS OF CLASS:

INOCULATION TECHNIQUES

The range of hosts affected is characteristic of a given virus and may be used in tentative identification of either viruses or the host they infect. This class provides experience in inoculation techniques for the propagation of a virus in embryonated eggs, plants and cell cultures and the propagation of a bacteriophage. In next week's class the effects of these viruses will be examined.

NOTE: LIVING VIRUSES AND CELLS ARE USED. STRICT ASEPTIC TECHNIQUES ARE REQUIRED.

CONTENTS:

A:

1. Inoculation of embryonated eggs with fowlpox virus.
 - 1.1 Examination of the structure of an 10 day egg.
 - 1.2 Practice inoculation using dye
 - 1.3 Actual inoculation onto chorioallantoic membrane (CAM) of 10 day egg.

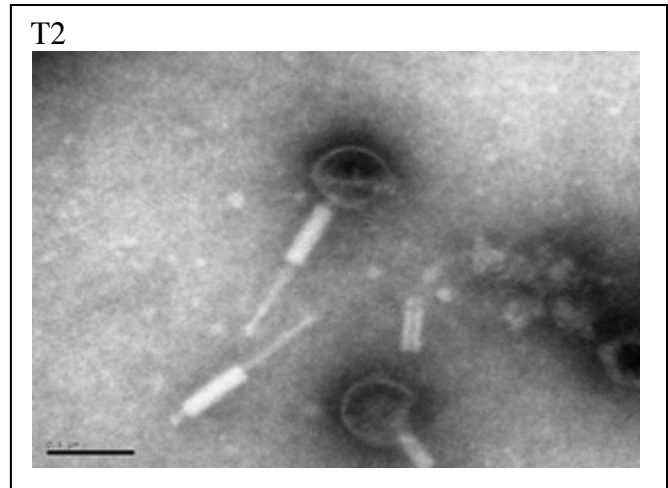
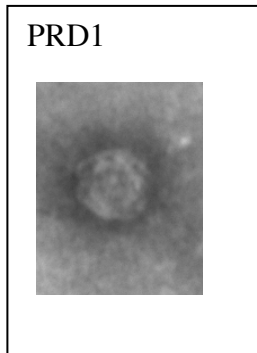
B: Identification of unknown bacteria using bacteriophages and determination of phage titre.

ASSOCIATED LEARNING RESOURCES

1. Lectures (cultivation of viruses and bacteriophages)
 2. White & Fenner: pp 205 – 208; 210 – 216; 394 – 398.
 3. Voyles: pp 347 – 349.
 4. UNSW Moodle: Web sites on laboratory techniques.
 5. Prescott, Harley and Klein. Microbiology: pp 364; 381-390
 6. Flint, L.W. et al. Principles of Virology. ASM Press 2004. pp 13-15; 27-30; 454.
-

Background

Enterobacteria phage T2 is a virulent bacteriophage of the T4-like viruses genus, in the family *Myoviridae*. It infects *Escherichia coli* and is the best known of the T-even phages. Its virion contains linear double-stranded DNA this is terminally redundant. This phage can quickly turn an *E. coli* cell into a T2-producing factory that releases phages when the cell ruptures.

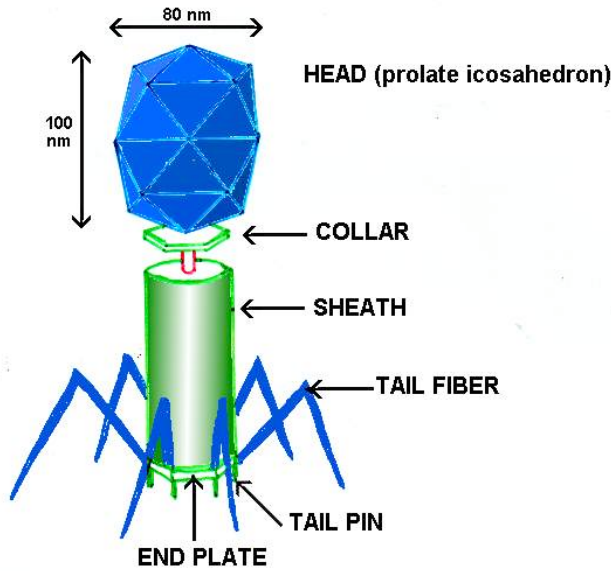


PRD1 belongs to a family of lipid-containing bacteriophages with double capsids which infect both Gram-negative and Gram-positive bacteria. It has one genus, Tectivirus. Tectiviruses have morphological similarities to tailed phages (capsid size, tail) and corticoviruses (capsid size, thick inner component). They differ from tailed phages by their double capsid and the transitory nature of their “tail”, and from corticoviruses by their ability to produce a “tail” or nucleic acid ejection device.

Enterobacteria phages T2 virus and PRD1 virus classification

Group:	Group I (<u>dsDNA</u>)	Group I (<u>dsDNA</u>)
Order:	<i>Caudovirales</i>	<i>Caudovirales</i>
Family:	<i>Myoviridae</i>	<i>Tectiviridae</i>
Genus:	<i>T4-like viruses</i>	<i>Tectivirus</i>
Species:	<i>Enterobacteria phage T2</i>	<i>Enterobacteria phage PRD1</i>

STRUCTURE OF T-even PHAGE



Chapter 2, *The Bacteriophages* 2nd edition, R. Calendar (ed), Oxford University Press

brought to you by www.thebacteriophages.org & www.phage.org

Table 2-1. Classification and Basic Properties of Bacteriophages^a

Symmetry	Nucleic Acid	Order and Families	Genera	Members	Particulars
Binary (tailed)	DNA, ds, L	<i>Caudovirales</i>	15	4950	
		<i>Myoviridae</i>	6	1243	Tail contractile
		<i>Siphoviridae</i>	6	3011	Tail long, noncontractile
		<i>Podoviridae</i>	3	696	Tail short
Cubic	DNA, ss, C	<i>Microviridae</i>	4	40	
		<i>Corticoviridae</i>	1	3?	Complex capsid, lipids
		<i>Tectiviridae</i>	1	18	Internal lipoprotein vesicle
Helical	RNA, ss, L	<i>Leviviridae</i>	2	39	
	ds, L, S	<i>Cystoviridae</i>	1	1	Envelope, lipids
	DNA, ss, C	<i>Inoviridae</i>	2	57	Filaments or rods
	ds, L	<i>Lipothrixviridae</i>	1	6?	Envelope, lipids
	ds, L	<i>Rudiviridae</i>	1	2	Resembles TMV
Pleomorphic	DNA, ds, C, T	<i>Plasmaviridae</i>	1	6	Envelope, lipids, no capsid
		<i>Fuselloviridae</i>	1	8?	Spindle-shaped, no capsid

C, circular; L, linear; S, segmented; T, superhelical; 1, single-stranded; 2, double-stranded.

^aModified from Ackermann, H.-W. 2001. Le matin des bactériophages. *Virologie* 5:35-43. With permission of John Libbey-Eurotext . Phage numbers are from reference 3.

PART A: INOCULATION TECHNIQUES

Work in groups of two; three groups per bench.

1. INOCULATION OF EMBRYONATED EGGS

Eggs have been largely supplanted for diagnostic purposes by cell cultures but are still used for specialised purposes; e.g. production of viral haemagglutinin for influenza serology; distinction between types 1 and 2 of herpes simplex virus. They are also used for the production of influenza vaccine. It is common to refer to fertile eggs that have been incubated as being 3, 5, 10, etc. days old. This does not refer to the time that has elapsed since the egg was laid but to the time that it has been incubated.

1.1. Examination of the structure of an 10 day egg

Materials – (per bench)

- 3 eggs incubated at 37°C degrees for 10 days
- Petri dish and forceps
- per **CLASS** (front bench) –
- Dark hood and egg candler

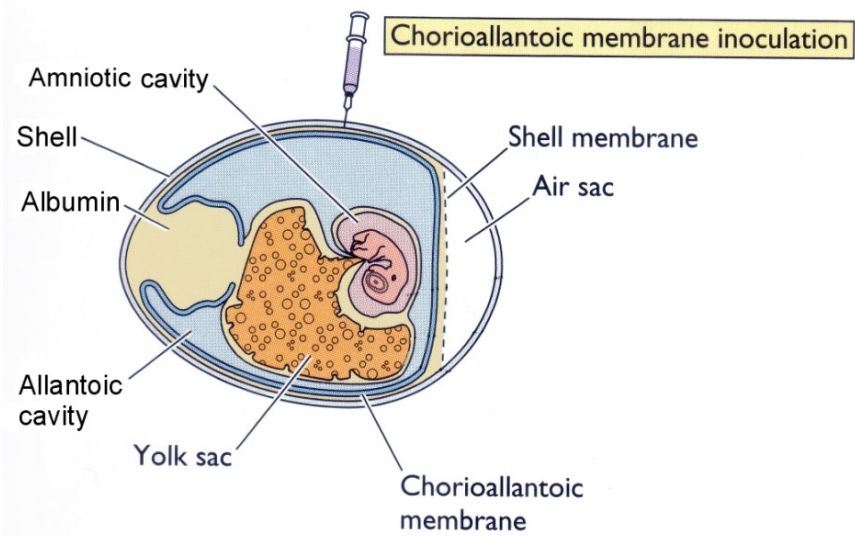


Procedure

1. Take the first of three eggs. Using the forceps and scissors chip away the shell and shell membrane from the entire air sac (blunt end) to expose the chorioallantoic membrane (CAM).
2. Tip the embryo into the Petri dish.
3. Identify as many of the structures shown on the diagram below as possible:

Tick the structures off as you identify them

Structure of fertile hen egg. Adapted from Flint et al., Principles of Virology (ASM Press, Washington DC, 2000).



4. At some time in the afternoon at the front bench each student should use the egg candler to examine the egg to determine fertility and identify the embryonic eye, air sac, blood vessels in embryo (see earlier diagram). One egg is already provided for this next to the candler.
 5. Hold a candling lamp securely against the blunt end of the egg. The light should illuminate the air cell and the egg contents. All eggs should remain in a vertical (blunt end up) position.
 6. Note: the egg has been marked (with pencil) around the air-sac border, and a “cross” is over an area of the CAM where there are no blood vessels. Identify the embryonic eye (ask tutor).
-

1.2. Practice inoculation onto chorioallantoic membrane (CAM) of 10 day egg

2nd egg –you will need to drop the CAM

Certain viruses (e.g. poxviruses, herpes simplex) produce pocks (localised areas of cellular proliferation) on the CAM, enabling detection and quantitation (one virion can produce one pock). This method is recommended for viruses that produce localised lesions in animal hosts. These are likely to produce localised lesions (pocks) on CAMs.

1.2. Practice at inoculation and harvesting

Materials – (per Bench)

- 3 x 10 day eggs with (CAM needs to be “dropped” with only this egg) and with “X” over inoculation site (in incubator)
- 1 Petri dish
- 1 x 1 ml syringe and needle
- 1 large plastic container
- 1 pair scissors, 70% alcohol, sticky labels

Procedure

1. Swab inoculation site carefully with 70% alcohol (See diagram on previous page).
2. Now you will need to drop the CAM. Make a small hole with the tool provided in the wider end of the egg, where the air sack is
3. Make another small hole at site of inoculation
4. Use a sucker to suck to draw back the CAM into the air sack, thus “dropping the CAM” away from the side of the egg shell (the site is now ready for inoculation)
5. Ask your tutor if you need help with dropping the CAM
6. Using a syringe needle inoculate 0.2 ml of any dye (from the stains rack) through the hole above CAM (this is the virus). **IF YOU GO TOO FAR YOU WILL PIERCE THE CAM**
Note: In a “real” inoculation the CAM needs to be re-establish into position by sucking with rubber teat over the hole in the air sac.
7. Seal holes with adhesive tape.
8. Spread inoculum on CAM by rocking gently.
9. Label egg using removable sticky label with your name, date and tutor’s name.
10. At this stage you would incubate at 37°C for 4 days. As this is a practice go to 12.
11. Open eggs. Harvest CAMs.
12. For harvesting, place egg with the inoculated side up on “cradle” of cotton wool in lid of Petri dish.

13. Insert sharp-pointed scissors into the hole in the blunt end, and cut the shell along the horizontal axis completely around the egg. Discard the bottom half. Using the forceps harvest the CAM from the upper half where the top hole is positioned. Place the CAM in a Petri dish containing sterile saline and gently agitate with forceps to wash free of mess.
14. Examine the CAM against a dark background, recording the appearance for future comparisons (next page). Has the dye penetrated the membrane, if so your needle has gone too far and you have not inoculated the CAM.

**Examination of uninfected chorioallantoic membrane (CAM)
(Draw labelled diagram here)**

1.3. Inoculation of Fowlpox virus

3rd egg –CAM IS DROPPED FOR YOU

Material – (per Bench)

- 3 x 10 day eggs with CAM “dropped” and “X” over inoculation site (in incubator)
- 1 dilution of a 10-fold dilution series of fowlpox virus
- 1 x 1 ml syringe and needle

Procedure

1. Inoculate 0.2 ml of virus onto CAM as described above. (**NOTE the dilution of fowlpox virus that your group has**).
2. Label (name, bench, virus, dilution) your egg using a sticky label and place in designated area of 37°C incubator.
3. CAMS will be harvested next week with the help of technical staff and demonstrators as described earlier.

Questions:

1. This practical describes the inoculation of two different sites. Why are different sites used?

 2. What is the function of the CAM

 3. At what sites would you inoculate:
 - mumps virus

 - an influenza A virus
-

PART B: BACTERIOPHAGES - IDENTIFICATION OF BACTERIA

Work in groups of three (two groups per bench).

You will be provided with one of two unknown bacteria. Your task is to identify which bacterial strain you have using a strain specific bacteriophage. You will also be asked to determine the titre of the phage sample used in the identification.

Material - (per bench)

Each group is provided with an overnight culture of one of two bacteria:

- A) *Escherichia coli*
- B) *Salmonella typhimurium* LT2

Each group is provided with two bacteriophages:

- 1) T2 - infects only *E. coli*.
- 2) PRD1 – infects only *S. typhimurium* LT2.

Other materials:

- 2 × overnight (O/N) cultures (5 ml) of unknown bacteria.
- 2 × 10 µl of each phage; T2 or PRD1.
- Plastic-ware, saline, pipettes and tips.
- 2 × set of six LB agar plates
- Top layer agar (Front bench-molten)

(per Class)

Microwave, water bath at 50°C, 30°C incubator.

Procedure

1. For each phage preparation, serially dilute phage in saline:
 - Add 100µl to 900 µl of saline and gently mix, this is the 10⁻¹ dilution.
 - Take 100 µl of this and add to 900µl of saline, this is the 10⁻² dilution.
 - Take 100 µl of this and add to 900µl of saline, this is the 10⁻³ dilution.
 - Continue this until you get to the 10⁻⁸ dilution.

You will now have dilutions of 10⁻¹ to 10⁻⁸ for each phage.

2. Label two sets of three plates (six plates) with phage name and dilution – use **ONLY** 10⁻⁶, 10⁻⁸ and 10⁻¹⁰.
3. Mix 100µl of 10⁻⁶, 10⁻⁸ and 10⁻¹⁰ dilutions with 200µl of overnight culture
4. Add this mixture to 2.5ml of top layer agar.
5. Pour on to a bottom layer agar LB plate.
6. Incubate overnight at 30°C for 24 hours. Plates will be removed and stored in the fridge for next week by the technical support staff.

VIRUS AND BACTERIOPHAGE CULTIVATION - II

PRACTICAL 4 - CONTINUED

- PART A: VIRAL ACTIVITY IN EGGS**
PART B: BACTERIOPHAGES
PART C: VIRAL CELL CULTURE

AIMS OF CLASS:

Recognition of viral activity

To characterise and identify viral activity in eggs, cell cultures and bacteria inoculated last week.

CONTENTS:

- A:** Examination of infected eggs - pock formation by fowlpox virus.
B: Bacteriophage typing of unknown bacteria and determination of phage titres
C: Observation of viral-infected cell cultures.

ASSOCIATED LEARNING RESOURCES

1. Lectures (cultivation of viruses and bacteriophages)
 2. White & Fenner: pp 205 – 208; 210 – 216; 394 – 398.
 3. Voyles: pp 347 – 349.
 4. UNSW Moodle: Web sites on laboratory techniques.
 5. Prescott, Harley and Klein. Microbiology: pp 364; 381-390.
 6. Flint, L.W. et al. Principles of Virology. ASM Press 2004. pp 13-15; 27-30; 454.
-

PART A: EXAMINATION OF INFECTED EGGS

Pock formation by fowlpox virus on CAM

Materials – (per Bench)

- 3 x 10 day eggs inoculated last week with fowlpox and incubated for 4 days.
- 1 Petri dish
- 1 x 1 ml syringe and needle
- 1 large plastic container
- 1 pair scissors

(per Class)

Demonstration of pock formation in a harvested CAM.

Procedure

1. For harvesting, place egg with the inoculated side up on “cradle” of cotton wool in lid of Petri dish.
2. Insert sharp-pointed scissors into the hole in the blunt end, and cut the shell along the horizontal axis completely around the egg. The lower half of the shell is removed with forceps and the rest is placed in the bottom of Petri dish containing sterile saline. Gently agitate with forceps to wash free of mess.
3. Examine your CAM, draw diagram (below) and count number of pocks. Record your results on the blackboard.
4. Estimate, by collaboration with other students, the approximate infectivity of the fowlpox suspension from which the dilutions were made.
5. Record results in the table provided on board and in manual.

Indicate number of pocks

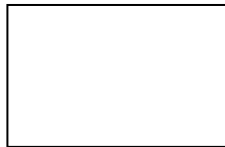


Diagram of pock formation on CAM

Infectivity of fowlpox suspension (class results)

Fowlpox dilution	Number of pox	Average
10^{-1}		
10^{-2}		
10^{-3}		
10^{-4}		

What is the infectivity of the fowlpox?

Infectivity is measured in Pock forming units (pfu) of fowlpox virus per ml (pfu/ml)

$\text{pfu}/0.2 \text{ ml} = \text{number of pox} \times 1/ \text{dilution of fowlpox}$

$\times 5 = \text{_____ pfu/ml}$

PART B: BACTERIOPHAGES**Materials – (per Bench)**

Two sets of six plates inoculated from last week.

(per Class)

Demonstration showing plaque formation on a bacterial lawn and a control plate that has not been inoculated.

Procedure

1. Examine all of your plates. Describe your finding below and record number of plaques in the table provided.

Bacteriophage	Number of plaques		
	10 ⁻⁶ dilution	10 ⁻⁸ dilution	10 ⁻¹⁰ dilution
T2			
PRD1			

Questions

- 1) If PRD1 infects *S. typhimurium* LT2 and T2 infects only *E. coli*.

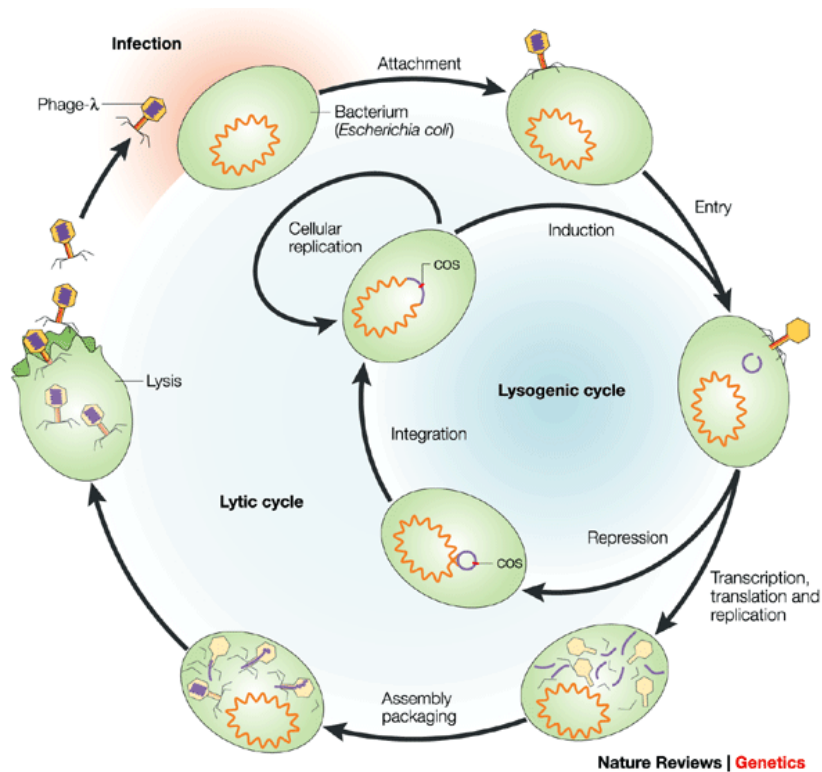
What is the identification of your bacterium?

- 2) The plaque forming units (pfu.) per ml of phage can be determined from the number counted (around 10⁸- 10¹⁰ is on the higher end of the scale). What is the titre of any phage that produced bacterial plaques?

3) Describe the major phage families and how they are classified.

4) In some detail describe below an application, commercial or otherwise, for a given bacteriophage.

Life cycle of a lysogenic bacteriophage



CONSULTANCY BRIEF PRESENTATIONS

INTRODUCTION

WORKING TOGETHER IN GROUPS

It is essential that groups are able to work well together during the course of preparing the consultancy briefs. Group collaboration in UNSW Moodle discussions is also encouraged. Peer assessment within groups will form part of the final consultancy brief mark and students who have not participated in the group activities will be penalised. Students are expected to keep a log of hours worked on their consultancy briefs.

INFORMATION GATHERING

Consultancy work uses literature, the internet and people as resources. Other sources of information may include the media, museums, etc. We will discuss the process of gathering and culling information.

PLANNING FOR ACTION

In the first week students in each group will be expected to set a time for a preliminary meeting with members of a group. Briefs will be presented during week 10. A significant amount of planning and work must be invested in the briefs, including rehearsals for final oral presentations. Allocation of tasks within each group so that thorough coverage with no overlaps occurring requires careful consideration.

VERBAL PRESENTATION OF CONSULTANCY GROUPS –week 10

Consultancy Presentation will be held in tutorial rooms unless otherwise stated.

Each member of a consultancy group will speak for less than 10 minutes, and each group's total presentation cannot exceed 40 minutes. Following the presentation, the group will be required to answer questions from the Client Audience.

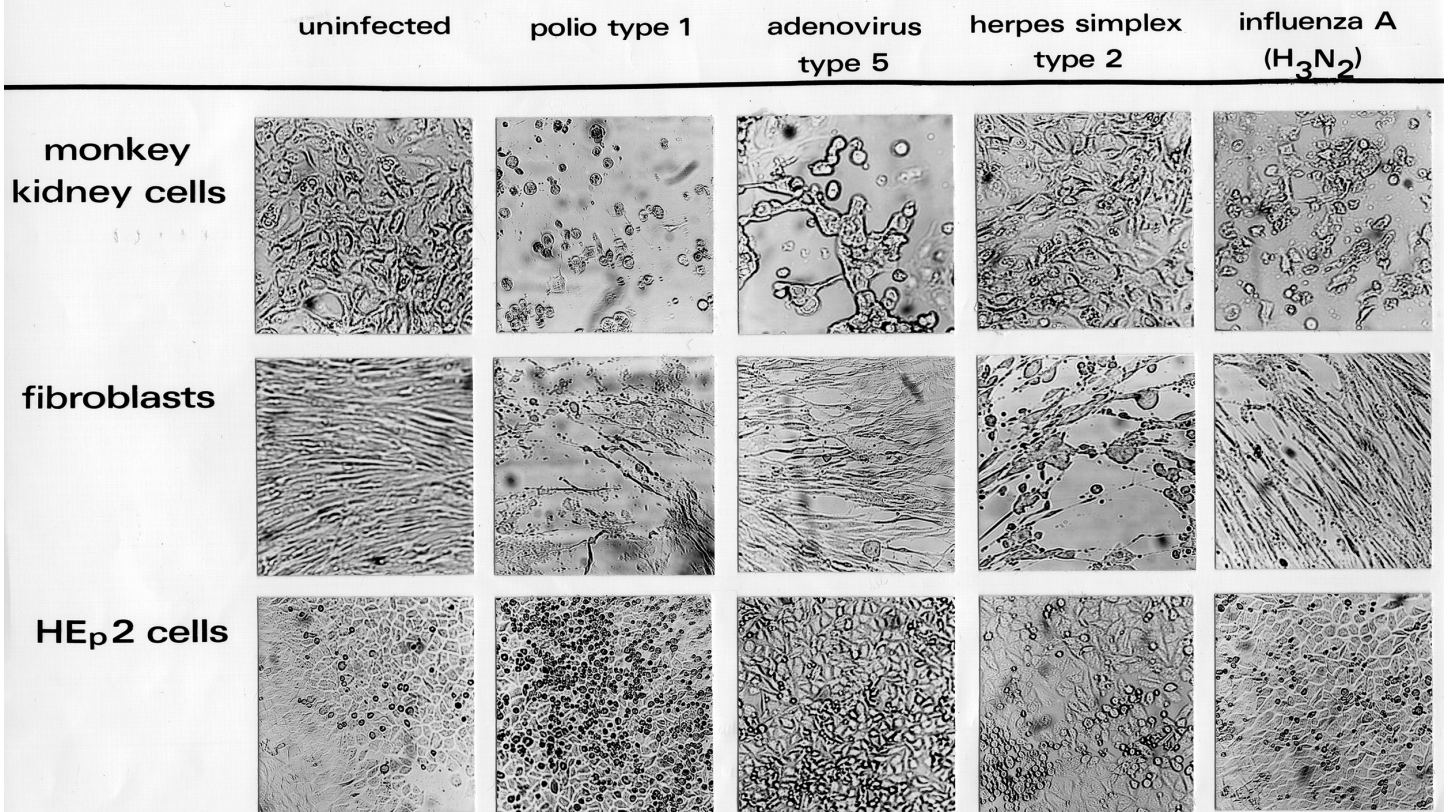
Prior to each group's presentation, each of the speakers will distribute a handout consisting of an outline of her/his presentation (headings, subheadings, summary of material) together with any tabulations or figures that may be helpful to the audience. **The handout may NOT exceed 1 page, but can be double sided.**

It is essential that the entire presentation has been rehearsed prior to this performance, and that the Powerpoint files, slides, or transparencies are in the hands of the chairperson before the commencement of the afternoon's session. The whole performance should be "polished".

Each student will be assessed on her/his performance, in accordance with the criteria discussed at the beginning of session and during meetings with tutors and Prof White.

NB: All written consultancy reports must be submitted by 4pm, Thurs 5-11-20

VIRUS INFECTED CELL CULTURES



Notes:

Viral Classification

