SNAB Topic 6
Infection, immunity and forensics

This teaching scheme is divided into three parts.
- Introduction.
- Road map: a suggested route through Topic 6.
- Guidance notes for teachers and lecturers. These include a commentary that runs parallel with the student book with hints and tips on teaching and references to the associated activities.

Note: There are more detailed notes about individual activities in the teacher/lecturer sheets that accompany most of the activities.

Introduction

The Road map starting on page 2 is a suggested route through Topic 6.

The learning outcomes are numbered as in the specification.

If two teachers/lecturers are sharing a group, the first could start at the beginning (Session 1) with the second starting at Session 7 and working through to 13, presenting the material on the immune system and TB. The first teacher/lecturer, after completing Session 6, would leapfrog to cover Sessions at 14 to 18 inclusive, with the second teacher/lecturer picking up the final Sessions 19 to 21 inclusive on antibiotics.

It is assumed that each session is approximately an hour in length. There are more activities than can be done in the time available in most centres, so select a balanced collection according to your and your students' interests, and the time and resources available. Some activities are labelled 'Core'. Core activities contain experimental techniques included in the specification, and may appear in questions on the unit exam for this topic. These learning outcomes are in **bold** in the specification, and in the Road map grid below. They are underlined in the Guidance notes below. In the Road map grid, activities are in *italics* if there is an additional activity covering the same material more directly. Choose which activities students complete, and substitute your own activities as appropriate.

There are various activities – particularly the interactive tutorials associated with some of the activities – which could be completed by students outside of class time. These activities are shown in the lower half of each 'Possible activities' box.

There is an A2 summary chart at the end of the guidance notes. This shows where concepts are introduced and revisited in later topics. .

SNAB Road map:
a route through Topic 6 Infection, immunity and forensics

Session	Learning outcomes to be covered	Possible activities
1	Introduction	The introduction of the context could be relatively short and combined with session 2 below if the interactive introduction is completed outside of class time.
		Interactive introduction
2	DNA profiling 5 Describe how DNA profiling is used for identification and determining genetic relationships between organisms (plants and animals). **7 Describe how gel electrophoresis can be used to separate DNA fragments of different length.**	Activity 6.1 Restriction enzymes and gel electrophoresis (A6.01L) (Interactive tutorial) You might decide to just do part one of this activity and then complete Activity 6.4 on the polymerase chain reaction before considering gel electrophoresis. See guidance notes below. Checkpoint question 6.1
		Activity 6.3 DNA profiling summary (A6.03L)
3	DNA profiling Describe how DNA profiling is used for identification and determining genetic relationships between organisms (plants and animals). **7 Describe how gel electrophoresis can be used to separate DNA fragments of different length.**	**Activity 6.2 Practical DNA gel electrophoresis (Core) (A6.02L) (Practical)**
4	Polymerase chain reaction **6 Describe how DNA can be amplified using the polymerase chain reaction (PCR).**	**Activity 6.4 DNA photocopying: the polymerase chain reaction (Core) (A6.04L) (Interactive tutorial)**
5/6	Determining time of death, forensic entomology and succession 20 Describe how to determine the time of death of a mammal by examining the extent of decomposition, stage of succession, forensic entomology, body temperature and degree of muscle contraction.	Activity 6.5 Crime investigation (A6.05L) (Interactive tutorial) Note that this activity needs to be introduced in advance to give students preparation time. Checkpoint question 6.2

Session	Learning outcomes to be covered	Possible activities
	9 Describe the role of micro-organisms in the decomposition of organic matter and the recycling of carbon.	
7	Cause of death Structure of bacteria and viruses 8 Distinguish between the structure of bacteria and viruses.	Q6.22 Activity 6.10 Gram staining bacteria (Optional) (A6.10L) (Practical) could be done at this stage or later when considering TB in detail. Checkpoint question 6.3
		Activity 6.6 Bacteria and viruses (A6.06L)
8	Non-specific responses of the body to infection 12 Describe the non-specific responses of the body to infection, including inflammation, lysozyme action, interferon, and phagocytosis.	Checkpoint question 6.4
		Activity 6.7 Phagocytosis (A6.07L) (Interactive tutorial) Note that this contains detail covered in the specific immune response.
9/10/11	The specific immune response 13 Explain the roles of antigens and antibodies in the body's immune response including the involvement of plasma cells, macrophages and antigen-presenting cells. 14 Distinguish between the roles of B cells (including B memory and B effector cells) and T cells (T helper, T killer and T memory cells) in the body's immune response.	Activity 6.8 The specific immune response (A6.08L) (Interactive tutorial) Checkpoint question 6.5
12/13	Tuberculosis – symptoms 11 Explain how bacterial and viral infectious diseases have a sequence of symptoms that may result in death, including the diseases caused by *Mycobacterium tuberculosis* (TB) and Human Immunodeficiency Virus (HIV).	Activity 6.10 Gram staining bacteria (Optional) (A6.10L) (Practical) could be done at this stage or earlier when considering the basic structure of bacteria.
		Activity 6.9 Tuberculosis (A6.09L)
14/15	HIV/AIDS – symptoms 11 Explain how bacterial and viral infectious diseases have a sequence of symptoms that may result in death, including the diseases caused	Activity 6.11 HIV worksheet (A6.11L) Checkpoint question 6.5

Session	Learning outcomes to be covered	Possible activities
	by *Mycobacterium tuberculosis* (TB) and Human Immunodeficiency Virus (HIV).	
16/17	Protein synthesis 2 Explain the nature of the genetic code (triplet code, non-overlapping and degenerate). 3 Explain the process of protein synthesis (transcription, translation messenger RNA, transfer RNA, ribosomes and the role of start and stop codons) and explain the roles of the template (antisense) DNA strand in transcription, codons on messenger RNA, anticodons on transfer RNA. 4 Explain how one gene can give rise to more than one protein through post-transcriptional changes to messenger RNA.	Activity 6.13 DIY protein synthesis kit (A6.13L) Activity 6.12 Protein synthesis (A6.12L)
18	Preventing pathogen entry to the body 10 Describe the major routes pathogens may take when entering the body and explain the role of barriers in protecting the body from infection, including the roles of skin, stomach acid, gut and skin flora.	Activity 6.14 Preventing infection (A6.14L)
19	Immunity 15 Explain how individuals may develop immunity (natural, artificial, active, passive)	Checkpoint question 6.7 and Q6.38
20	The effect of antibiotics on bacterial growth **18 Describe how to investigate the effect of different antibiotics on bacteria.**	**Activity 6.15 Which antibiotic is most effective? (Core) (A6.15) (Practical)**

Session	Learning outcomes to be covered	Possible activities
21/22	How antibiotics work and evolution of antibiotic resistance by bacteria 17 Distinguish between bacteriostatic and bactericidal antibiotics. 16 Discuss how the theory of an 'evolutionary race' between pathogens and their hosts is supported by the evasion mechanisms as shown by Human Immunodeficiency Virus (HIV) and *Mycobacterium tuberculosis* (TB). 19 Describe how an understanding of the contributory causes of hospital acquired infections have led to codes of practice relating to antibiotic prescription and hospital practice relating to infection prevention and control. (Activity 6.14)	Activity 6.16 Classifying antibiotics (A6.16L) Activity 6.17 TB and macrophages (A6.17L)
23		End-of-topic test

Guidance notes for teachers and lecturers

There are no GCSE style reviews and tests at the start of the A2 topics.

Introduction

This topic has a clear storyline (we hope!) that runs throughout. It concerns the discovery of two dead bodies. A series of questions is posed, such as: How are these people identified? When did they die? What caused their deaths? Could their deaths have been prevented? The topic then presents the biology that is needed to answer these questions. The introductory pages in the textbook briefly outline these ideas although the bodies are not introduced until the beginning of Section 6.1.

The interactive introductory presentation gives a general overview of the topic, it does not introduce the two bodies that appear in the textbook. The topic revisits and builds on areas covered at AS and in Topic 5, for example cell structure and function, succession, evolution and DNA technology. The topic title is 'Infection, immunity and forensics'. However, these three areas of biology are covered in the reverse order in the topic: forensics, infection and immunity.

6.1 Forensic biology

This first section starts with an online-style newspaper report of the discovery of two dead bodies and poses the questions that any police investigation would have to answer in the course of their enquiries: Who are the dead people? When did they die? How did they die?

Each of these three questions is then taken in turn and the biological techniques that would be used to help answer them are considered.

Identifying the body
Conventional methods of identification such as skin fingerprinting and dental required are covered in a Did you know box and in two extensions, they are not in the specification. The specification requires students to know how DNA profiling could be used for identification purposes.

Extension 6.1 Fingerprint analysis (X6.01L)
This activity gets students to look at their own fingerprints and those of others in the class, with an opportunity to use χ^2 again. The weblink box in the textbook refers students to some Radio 4 programmes that can be listened to online. The weblink can be found in the book-related links for Topic 6.

Extension 6.2 Identifying the Romanovs (X6.02L)
Students have to match the descriptions of skeletal remains with the correct members of the Russian Royal family, the Romanovs.

DNA profiling
The textbook describes DNA fingerprinting techniques that produce a traditional banded pattern 'fingerprint' and a graph-style profile. The latter is used routinely by the forensic science service, using short tandem repeats and PCR techniques. The textbook first explains what short tandem repeats are, and how they differ among individuals allowing virtually unique DNA profiles to be produced. The text book goes on to describe how the DNA profile is made how the DNA is obtained, how fragments are created using restriction enzymes or PCR, how the fragments are separated and visualised.

There are four activities associated with this section. Activity 6.1 looks at the use of restriction enzymes and gel electrophoresis. If one follows the order that ideas are presented in the text in the book, looking first at creating the fragments it would be best to complete the first part of Activity 6.1 about restriction enzymes and then complete Activity 6.4 on the polymerase chain reaction. Then go on to look at separating the fragments using gel electrophoresis using the interactive tutorial in Activity 6.1 and the practical in Activity 6.2. Activity 6.3 provides a summary of DNA profiling.

These ideas are quite challenging and teacher/lecturer support is likely to be needed.

Activity 6.1 Restriction enzymes and gel electrophoresis (A6.01L) Core practical
The worksheet accompanying this interactive simulation explains how restriction enzymes are used to cut DNA at precise sequences of bases. This is followed by detailed instructions for the restriction enzyme simulation, using a Word document containing the Lambda DNA base sequence. Simulations of gel electrophoresis experiments using single and double digests follow this. The worksheet asks a series of questions to ensure that the student focuses on the key features of these techniques.

This simulation allows students to predict the gels they should get before they do it as a practical.

Activity 6.2 DNA gel electrophoresis (A6.02L)
The teacher/lecturer notes give details about equipment suppliers for the practical on restriction enzymes and gel electrophoresis. The main companies produce their own worksheets, so a detailed student sheet is not included within the SNAB resources.

Activity 6.3 DNA profiling summary (A6.03L)
This activity summarises formation of a DNA profile, it can be used as an outline of the process when introducing the process or as a revision exercise at the end of the topic.

Activity 6.4 DNA photocopying: the polymerase chain reaction (A6.04L) Core practical
This interactive tutorial explains what is happening in the polymerase chain reaction to produce the short repeated sequences. Unlike many A level textbooks which skate over the detail, the figure in the textbook and the interactive tutorial provide a detailed description of what is really happening. This may prove too difficult for some students and a more 'black box' approach may be needed which concentrates on the idea that primers and DNA polymerase attach adjacent to the repeated sequence and produce a copy of it. Please note that the figure on the Teacher/lecturer sheet includes the term minisatellites it should read STRs to match the term used in the book.

Checkpoint 6.1 requires students to summarise the ideas covered on DNA profiling in a flow chart.

Determining time of death
This section of the topic looks at the techniques used by forensic pathologists in determining the time of death. A brainstorm of possible techniques will probably identify body temperature, rigor mortis and decomposition; use of forensic entomology may be less well known. The student book describes each of the techniques in turn with associated questions that require students to use the information in the text. There are not separate activities associated with each technique but in Activity 6.5 all the techniques are drawn upon in a class crime investigation activity.

Activity 6.5 Crime investigation (A6.05L)
It is assumed that the class will be divided into groups and given time to prepare for the discussion aspect of this activity. Each group is given one role within the crime investigation team to prepare a presentation on. Together these presentations will draw together the different forensic techniques used in identifying the victim and ascertaining the time and cause of death. To ensure that every student has a solid grasp of the principles, each student writes an article explaining the techniques used or they could complete Checkpoint question 6.2.

There is a good article 'Maggots and murder' in *Biological Sciences Review* April 2003.

Students need to be able to describe the role of micro-organisms in the decomposition of organic matter and the recycling of carbon. This is covered in the textbook and builds on the ideas about the carbon cycle covered in Topic 5. There is no activity linked to this specification point but it could be added as an addition to Checkpoint question 6.2.

6.2 Cause of death
The two bodies found at the start of the topic have now been identified. In each case a pathologist performing a post mortem examination would determine the cause of death. The virtual autopsy referred to in the textbook is produced by HBO (the US TV company); most of this interactive autopsy uses a model body rather than pictures of real cadavers. There are a few pictures that some students might not like but, as indicated in the weblink box, there is nothing that is too gruesome. There is a series of case studies on this website. An interesting one, concerns the testing of maggots found on a body but the pictures, although small, might be off-putting for some people. There is no learning outcome related to autopsies.

The death of George Watson, the Canal Lane body, from an aortic aneurysm provides a link back to the material covered in Topic 1. There are no revision notes in the textbook but

Q6.21 requires the student to think about atherosclerosis and the risk factors discussed in Topic 1.

Nicki Overton, the second body, was discovered to have been infected with *Mycobacterium tuberculosis* and HIV. The remainder of the topic is concerned with infection and immunity addressing the questions: Could these infections have been the cause of death? and Could anything have been done to prevent her early death? Before looking in detail at how the body responds to these infections, students must first know the difference between bacteria and viruses.

Students have met prokaryotes before but not viruses; a Key biological principles box considers the difference between a virus and a bacterium. Students can also look back at the information in Topic 3 to refresh their memory of prokaryotic cell structure; the interactive cell will provide most of the detail necessary to complete Q6.22. A visit to the Science Photo Library website or Google images with a general virus search will demonstrate the wide variety of possible virus structures. You can search the photo library without registering. The website can be found in the general weblinks for Topic 6.

Activity 6.6 Bacteria and viruses (A6.06L)
This activity asks students to prepare an animation brief that could be used to create a new section of the interactive cell animation. Alternatively, students could be asked to draw up a simple table of comparisons as suggested in Checkpoint question 6.3; the key features are outlined on the teacher/lecturer notes for this activity. Activity 6.10, bacterial staining, could be used at this stage as a practical introduction to bacteria.

Students do not have to learn examples of infections caused by bacteria and viruses. Q6.23 is meant just to show that a number of common illnesses result from microbes.

How might Nicki have become infected?
The specification requires students to know about barriers to infections; this is dealt with later in the topic. At this stage the methods of transmission of *M. tuberculosis* and HIV are described. This is to provide an introduction to how the body responds to infection. It is hoped that a short question-and-answer session would throw up the methods of transmission for both types of microbes. Although not mentioned separately in the specification there is health education value in discussing transmission, particularly of HIV.

6.3 How does the body respond to infection?
A summary of the immune response is followed by a detailed account of the non-specific responses and the specific responses to infection.

Non-specific responses to infection
The text covers the role of lysozyme, inflammation, phagocytosis and interferon. Checkpoint question 6.4 asks students to summarise the sequence of events in the non-specific immune response using a flowchart. Alternatively, a cartoon drawing exercise for the artistic could be used to summarise the sequence of events when a microbe enters the body, for example through a cut. This can include blood clotting to provide a link back to Topic 1.

There is an interactive tutorial on phagocytosis; this is referred to within the specific immune response section but it could be used at this point. To avoid confusion it is probably worth pointing out to students that it covers more detail than presented in the non-specific immune response text.

The textbook describes action to prevent the spread of infection; this provides a link back to tissue fluid and the lymph system encountered in Topic 1.

Specific immunity
Specific immunity is a challenging subject and we have included a lot of detail here. However, there are some aspects we have omitted in an attempt to prevent information overload. In particular, complement proteins and detail of different immunoglobins are not included. Antigen presentation by cells in the immune system is included but the term major histocompatibility protein is no longer required.

The whole process has been broken down into small units to help students. First, B cells are introduced along with the structure and role of antibodies. This is followed by a very brief introduction to T helper and T killer cells before embarking on the detail of the primary immune response. This includes the role of antigen presentation, activation of T helper cells, clonal selection of B cells and the role of T killer cells. All these aspects are covered in the interactive tutorials in Activities 6.7 and 6.8.

Activity 6.7 Phagocytosis (A6.07L)
This interactive tutorial includes phagocytosis and antigen presentation by macrophages.

Activity 6.8 The specific immune response (A6.08L)
The worksheet that accompanies this activity is designed for use with the interactive tutorial on the specific immune response, but it could be completed using just the textbook. An alternative strategy for getting students to grasp these ideas would be to get them to make cardboard cut-out pieces that represent each of the cells and then use these to explain to another student in the group what is happening.

Completing Checkpoint question 6.5 should help ensure that students understand how the different aspects of the immune response fit together. The artwork in the Checkpoint answer does not quite match that found in the textbook – the layout of the diagram is correct but the answer contains the proof versions of the diagrams and not the final artwork, and there are some minor differences, for example the shape of the bacteria.

Up to this point the principles of immunity have been considered with no reference to particular infections. The textbook now covers how the body responds to TB and HIV.

6.4 The body's response to TB

What is tuberculosis?
The symptoms and course of TB are described in the textbook. The websites that can be accessed through the Activity 6.9 weblinks give up-to-date statistics and information about the disease.

Activity 6.9 Tuberculosis (A6.09L)
The extract on the student sheet comes from a very good article on TB from *Biological Sciences Review*. Please note that not all the answers to the questions are found in the article – students have to use the textbook.

Activity 6.10 Gram staining bacteria (A6.10L) Optional
The section 'How is TB diagnosed?' and the associated activity on bacterial staining techniques are not featured as learning outcomes within the specification. However, they highlight how antibodies in the blood are used in diagnosis. If time permits, students could perform the practical on staining bacteria but they should be aware that this technique is not required knowledge. It could be used earlier in the section on bacteria and viruses, with students producing write-ups that include the key features of prokaryotes.

Checkpoint question 6.6 sits within Section 6.5 but requires summaries of the course of both TB and AIDS.

6.5 The body's response to HIV and AIDS

What are HIV and AIDS?
The information on the number of cases of HIV around the world does not have to be learnt by students but it does provide an insight for students into the scale of the global epidemic. The structure of HIV, how it enters cells and reproduces, and the course of infection are all described in the textbook; use this in conjunction with Activity 6.11

Activity 6.11 HIV worksheet (A6.11L)
This fill-the-gaps exercise provides a summary of the basic features of HIV and of how it infects cells. There follows a series of questions on HIV. Question 6 is challenging and could be considered as extension.

Protein synthesis, introduced in Topic 2, is revisited and extended in the context of the synthesis of viral proteins. A Biological Principles box starts with a diagram used in Topic 2 and goes on to give more detail of what is happening on the ribosome including post-transcriptional changes.

Activity 6.12 Protein synthesis (A6.12L)
The worksheet requires students to recall complementary base pairing and the genetic code; the coverage of Topic 2 is extended by considering the non-overlapping and degenerative nature of the code. The interactive tutorial takes the student through the process of protein synthesis. The worksheet requires students to summarise the process.

Activity 6.12 could be completed outside of class time, with students completing Activity 6.13 in class to check their learning.

Activity 6.13 DIY protein synthesis kit (A6.13L)
The successful construction of this jigsaw-style diagram is only possible if students understand post-transcriptional changes and the process of translation.

Please note that the use of the sense and antisense in the textbook is correct (in the sense that we have used the terms as they are commonly used in the academic and industrial literature). Many A-level textbooks are wrong in their use of these terms.

Testing for HIV has not been included in the textbook. It is worth pointing out to students that when someone is said to be HIV positive it means that HIV antibodies have been detected in their blood, indicating that they have the virus. The use of ELISA (enzyme-linked immunosorbent assay) tests for HIV testing could be included.

6.6 Could the infections have been prevented?
Returning to the two bodies discovered at the start of the topic, the question is posed as to whether the infections could have been prevented. The prevention of entry of pathogens is described in the textbook.

Activity 6.14 Preventing infection (A6.14L)
This activity provides a summary diagram to which students have to add annotations. Students should be alerted to the fact that a couple of the label lines go to parts of the body that are not discussed in the section on preventing entry of pathogens in the textbook. They could be told that there are two such boxes (the ears and vagina) and they could make educated guesses about what reduces the likelihood of pathogen entry (antibacterial ear wax and acidic vaginal secretions).

Becoming immune
The textbook does not provide a traditional description of the different types of immunity. Instead, a series of case studies is used to illustrate them. Checkpoint question 6.7 asks students to write a definition for each type. Students could then answer Q6.38 to confirm that they have understood the four different types.

Being vaccinated
Vaccination is described in further detail; reference is made to the question of whether or not vaccination is dangerous but this has not been given extensive coverage.

6.7 Are there treatments for AIDS and TB?

A short section on AIDS is followed by an extensive section that concentrates on antibiotics; a short history of their discovery and development is followed by a summary of how they work.

Activity 6.15 Which antibiotic is most effective? (A6.15L) Core practical
This practical technique is included in the specification, so students do need to be able to describe the technique to allow them to establish which of a number of antibiotics is most effective. The technique is similar to the one used in Topic 5 when investigating antibacterial properties of plants.

Activity 6.16 Classifying antibiotics (A6.16L)
This activity provides a set of questions about the action of antibiotics.

Why do we still have diseases like TB?
This final section of the topic deals with the development of antibiotic resistance and the ongoing evolutionary race between pathogens and their hosts. This section concentrates on evolution of bacteria and the evasion mechanisms in TB. These are referred to in Activity 6.17. Evasion mechanisms in HIV are not discussed in this section but are included in the earlier text on HIV. These include intracellular infection, particularly of cells of the immune system. HIV also has a virus envelope created from the cell surface membrane, which helps the virus attach to and enter the host cell. Students are likely to need this link highlighted. Students could be given the learning outcomes as titles for extended pieces of prose writing.

Points that students might include:

Discuss how the theory of an 'evolutionary race' between pathogens and their hosts is supported by the evasion mechanisms as shown by Human Immunodeficiency Virus (HIV) and Mycobacterium tuberculosis (TB).
Host evolves mechanism to combat pathogen;
e.g. feature of immune system;
Pathogen reproduces very quickly producing some bacteria/viruses with mutations that give pathogen advantage;
e.g. use different food resources; reproduce more quickly; infect other cells more successfully; or produce symptoms in the host, such as coughing and sneezing, which aid the spread of the disease;
Some (mutations) may allow the pathogen to overcome the host's immune system;
e.g. slight changes in the pathogen's antigens mean that any reservoir of antibodies, and memory B and T cells from a previous infection, will be useless in combating a second infection;
e.g. intracellular infection to evade the action of antibodies and macrophages;
e.g. HIV intracellular infection of immune cells avoids the action of the immune system, and also disables the immune system;

e.g. HIV virus envelope created from the cell surface membrane; which helps the virus attach to and enter the host cell;

Describe how an understanding of the contributory causes of hospital acquired infections have led to codes of practice relating to antibiotic prescription and hospital practice relating to infection prevention and control.
Increasing use of antibiotics;
Increased exposure of bacteria to antibiotics;
Patients frequently fail to complete their course of antibiotics;
These drugs provide a selection pressure;
Rapid multiplication of bacteria (or other pathogens) produces numerous bacteria with mutations;
Any bacteria with mutations that confer resistance survive; those without resistance do not survive*;*
Removal of competitors allows rapid increase in number of resistant bacteria;
Antibiotics ineffective against resistant strains;
Spread of resistance by conjugation;
Possibility of antibiotic-resistance genes used in genetic modification conferring resistance on pathogenic bacteria;
Formation of multiple-resistant strains;

In the weblinks accompanying Activity 6.15 there is a link to 'Bioethics bytes - how clean is your hospital' based on a 2008 Panorama special about the rise of *Clostridium difficile* in UK hospitals.

Activity 6.17 TB and macrophages (A6.17L)
The aim of this activity is to encourage students to apply the ideas learnt earlier about the immune system to the specific example of TB. The activity also refers to the evasion mechanism used by TB.

Activity 6.18 Check your notes
Students can use the checklist of learning outcomes in this activity in their revision.

End-of-topic tests

There is an online interactive end-of-topic test. This test is not accessible to students initially unless set by their teacher/lecturer. There is also a paper-based test for Topic 6 with examination-style questions on the teachers' and technicians' sites. A mark scheme is also available on these sites. The questions are similar in layout and style to those that are found on exam papers. However, the restriction of questions to only one topic in each test has meant that it has not been possible to include some types of questions that draw on material from different topics.