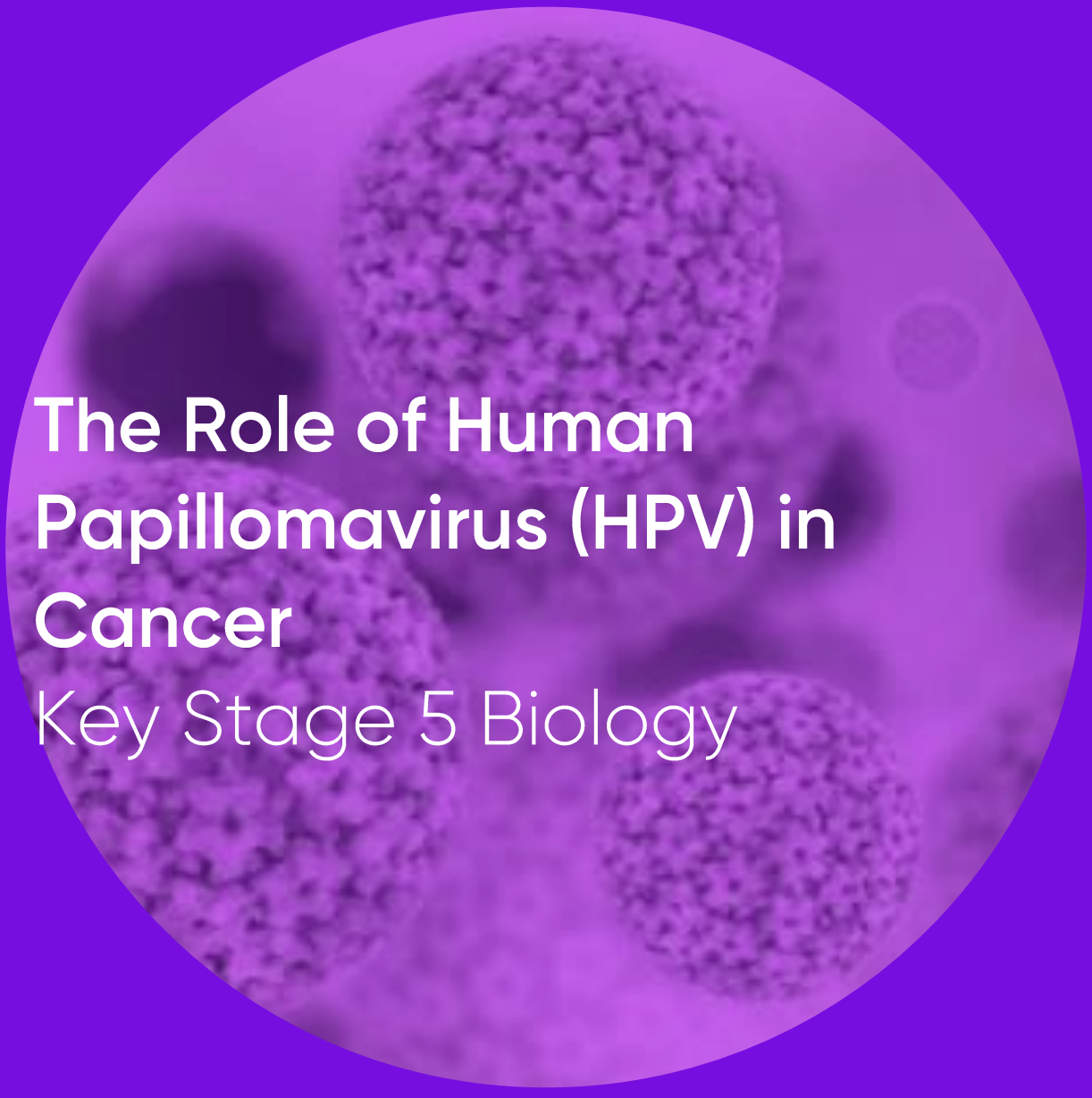
Research
Based
Curricula



The Role of Human Papillomavirus (HPV) in Cancer

Key Stage 5 Biology

2019

BROUGHT TO YOU BY RBC ACCESS ED

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For Pupils
Welcome

To get into the best universities, you must demonstrate that you are intellectually curious, and will make the most of the wonderful academic opportunities available to you.

One of the best ways of demonstrating this, is by going above and beyond what is taught in school and studying something that is not on the curriculum.

This resource will give you exactly such an opportunity. You will have something interesting to write about in your application to university, something interesting to talk about in a university interview, and open whole new areas of study you might be interested in!

You will develop valuable academic skills as you go, that we have marked out with gold badges (see the next page on university skills). As you work through the resource you can look out for these badges so that you can explain which skills you have developed and what you did to demonstrate them. Developing these skills will help you get university ready!

If you have any questions while you are using the resources in this pack, you can contact your teacher or email us directly at schools@access-ed.ngo.

Good luck with your journey to higher education!

For Pupils
University Skills

To complete this resource, you will have to demonstrate impressive academic skills. When universities are looking for new students, they will want young people who can study independently and go above and beyond the curriculum. All of these skills that you will see here will demonstrate your abilities as a university student – while you're still at school!

Every time you have to look something up, or write up a reference you are showing that you can work independently. Every time that you complete a challenging problem or write an answer to a difficult question, you might demonstrate your ability to think logically or build an argument. Every time that you evaluate the sources or data that you are presented with, you are showing that you can "dive deep" into an unfamiliar topic and learn from it.

Here are the skills that you will develop in this course:

independent research	your ability to work on your own and find answers online or in other books
creativity	your ability to create something original and express your ideas
problem solving	your ability to apply what you know to new problems
building an argument	your ability to logically express yourself
providing evidence	your ability to refer to sources that back up your opinions/ ideas
academic referencing	your ability to refer to what others have said in your answer, and credit them for their ideas
deep dive	your ability to go above and beyond the school curriculum to new areas of knowledge
source analysis	your ability to evaluate sources (e.g. for bias, origin, purpose)
data interpretation	your ability to discuss the implications of what the numbers show
active reading	your ability to engage with what you are reading by highlighting and annotating

For Teachers
RBC Guide

Programme Aims

The Research-Based Curriculum aims to support student attainment and university progression by providing classroom resources about cutting-edge research at local universities. The resources are designed to:

- ✓ promote intellectual curiosity through exposure to academic research
- ✓ stretch and challenge students to think deeply about content that may be beyond the confines of the curriculum
- ✓ develop core academic skills, including critical thinking, metacognition, and written and verbal communication
- ✓ inform students about how subjects are studied at university, and provide information, advice and guidance on pursuing subjects at undergraduate level

Content

The programme represents a unique collaboration between universities and schools. Trained by AccessEd, PhD Researchers use their subject expertise to create rich resources that help bring new discoveries and debates to students.

The Research-Based Curriculum offers ten modules suitable for either KS4 or KS5 study. The modules span a range of disciplines, including EBacc and A-level subjects, as well as degree subjects like biochemistry. Each module includes six hours of teaching content, supported by student packs, teacher notes and slides. All modules are available online and free of charge for teachers at select schools.

Delivery

Resources are designed to be used flexibly by teachers. The resources can be completed by students individually or in groups, in or out of the classroom.

For Teachers
RBC Guide

Here are five examples of delivery options:

Extra-Curricular Subject Enrichment Clubs

The resources can be completed in small groups (4-8 pupils) across a series of weekly lunch clubs or after-school clubs. Groups can reflect on their learning by presenting a talk or poster on the subject matter at the end of the course.

University Access Workshops

The resources can be used by students to explore subjects that they are interested in studying at university. This can inform their decision making with regards to university degree courses, and allow students to write more effective personal statements by including reflections on the Research-Based Curriculum.

Research Challenge

The resources can be used to ignite curiosity in new topics and encourage independent research. Schools could hold a research challenge across a class or year group to submit a piece of work based on the resources. Pupils could submit individually or in small groups, with a final celebration event.

Summer Project

Resource packs can function as 'transition' projects over the summer, serving as an introduction to the next level of study between KS3 and KS4, or KS4 and KS5. Students could present their reflections on the experience in a journal.

Evidence

The Research-Based Curricula programme builds on the University Learning in Schools programme (ULiS), which was successfully delivered and evaluated through the London Schools Excellence Fund in 2015. The project was designed in a collaboration between Achievement for All and The Brilliant Club, the latter of which is the sister organisation of AccessEd. ULiS resulted in the design and dissemination of 15 schemes of work based on PhD research for teachers and pupils at Key Stage 3. The project was evaluated by LKMCo. Overall, pupils made higher than expected progress and felt more engaged with the subject content. The full evaluation can be found here: ULiS Evaluation.

Questions?

For more information contact hello@access-ed.ngo

Introduction to Topic Human Papillomavirus (HPV) & Cancer

Molecular biology is an important scientific area which overlaps many sub-disciplines of biology and chemistry. Researchers who work in this field concern themselves with understanding the interactions between the various systems of a cell, including the interactions between DNA, RNA and proteins as well as learning how these interactions are regulated. Since the early 1960s, molecular biologists have learnt how to characterise, isolate, and manipulate the molecular components of cells and organisms. An example of this can be seen within research on oncogenic (cancer-causing) viruses such as Human papillomavirus (HPV), where scientists have identified which viral proteins interact with our own to disrupt cellular pathways, which in turn, can cause cancer. So not only are molecular techniques used to identify causes of disease, allowing us to examine what cannot be seen, but also help to discover treatments and develop methods of protection such as vaccines against infectious diseases like HPV.

The topics within this pack will include:

- What is the Human Papillomavirus (HPV)?
- Viral Genes & Replication
- Oncogenesis: Cellular Transformation
- HPV Related Cancers
- Viral Screening Techniques
- HPV Vaccination

In order to understand the intricate cellular interactions of molecules such as DNA and proteins, you require a broad base knowledge of topics covered in biology at GCSE and A-Level. This knowledge will then be utilised and developed through completing these six resources. Within this pack, you will learn about the structure and life cycle of papilloma viruses, how viruses like HPV replicate, the molecular abilities of some viruses to transform cells into cancerous cells, what types of cancer HPV causes, current viral screening techniques, and the importance of vaccination. Developing an insight into all of these areas of molecular biology and virology will hopefully then introduce you to the way in which scientists theorise and answer important questions through research.

Introduction to Subject Molecular Biology at University

Molecular biology is the study of biology on a molecular level including the structure, function, and makeup of fundamental biologically important molecules such as DNA, RNA, and proteins. The field of molecular biology is what is known as a sub-discipline of biology that often overlaps many other sub-disciplines such as virology, biochemistry and genetics. As molecular biology tends to link many other areas within biology (and chemistry), it can also be called an interdisciplinary subject. Because of this overlap with other subjects, you tend to find molecular biologists working in many different environments such as universities, in the pharmaceutical sector, in the healthcare industry, or even in the modern high-tech food industry.

The study of molecular biology and other subjects related to it such as oncology, immunology and virology (to name a few) has allowed scientists to discover much about the world around us that would be otherwise invisible to the naked eye. For instance, molecular biology research is responsible for discoveries such as finding out how to transport medicine into specific organs in the body, finding out how plants convert atmospheric nitrogen into fertiliser, or my personal favourite, discovering new viruses and finding out how manipulate these into treating diseases instead of causing them.

Whilst much of the research conducted within molecular biology is quantitative and traditionally based within a laboratory, recently there has been more focus on molecular biology and computer science creating subjects such as bioinformatics and proteomics, allowing complex biological data to be collected, classified and analysed which could not have been previously. These recent advances in computational biology has also contributed to development of revolutionary techniques used in laboratory such as

Introduction to Subject Molecular Biology at University

CRISPR/Cas9 for targeted genome editing and real-time quantitative PCR which has numerous applications such as determining the level and severity of a viral infections.

Overall, the field of molecular biology is constantly progressing and has many real-world applications, and I hope that after studying these six resources, that you will start to appreciate how versatile and fascinating this type of science can be. These topics may seem a little tricky at first, but I hope that as you progress through the pack you will discover that you can understand more than you thought you could and hopefully enjoy the insight into current molecular research.

Good luck!

Aimee

Meet the PhD Researcher Aimee Whitton

I've always had an uncontrollable curiosity and analytically-wired brain which is why I think I enjoyed science so much at school and subsequently chose to study Biology and Chemistry at A-Level. Following this, I actually applied to study Zoology but changed my mind last minute and moved all the way from Bristol to a city I had never visited, Derby, to study BSc Biology instead. I certainly do not regret the decision as my degree transcended into an MRes in Biological Science, and then into a PhD.

For my MRes, I studied two viruses that are known to cause different types of cancer; Human Papillomavirus (HPV) and the lesser-known, Merkel Cell Polyomavirus (MCPyV). I now use the screening methodologies developed in this project in my current PhD research which aims to determine prevalence and abundance of oral HPV within healthy UK adult population, to identify those who are at risk of developing head and neck cancers (HNSCCs).

A-Level Subjects	Biology, Chemistry, Retail, English Language & EPQ
Undergraduate	BSc (Hons) Biology
Postgraduate	MRes in Biological Sciences & PhD in Molecular Biology

Glossary

Term	Definition
HPV	Human papillomavirus; a virus known to infect the human body
Genome	The complete set of genes or genetic material present within an organism
Gene	The basic physical unit of heredity; a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA, which is translated into protein
Genotype	The genetic constitution (genome) of a cell, an individual, or an organism. For viruses, genetic variation through mutations in the viral DNA can result in different "geno" types of a virus
Transcription	The process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA)
Translation	The process by which a protein is synthesized from the information contained in a molecule of mRNA
Endocytosis	A type of active transport that moves large particles, such as viruses, into a cell through the plasma membrane via a vesicle
Latent Infection	The dormant and inactive stage of a viral life cycle where the virus does not produce particles and only expresses a minimal number of viral genes
Oncogenesis	The transformation of normal healthy cells into cancerous cells, otherwise known as Tumorigenesis
Oncogenic	The ability to transform cells into cancerous cells
CIN	Cervical Intraepithelial Neoplasia; involving three grading stages of how abnormal the cervix cells look under the microscope and how severe the cell changes are from normal mucosal lining

Glossary

Term	Definition
HNSCCs	Head & Neck Squamous Cell Carcinomas; cancerous tumours of the mouth, nose and throat
PCR	Polymerase Chain Reaction; a molecular biology technique used to copy (amplify) and detect DNA and/or RNA sequences
Real-time qPCR/RT-qPCR	Real-time Quantitative Polymerase Chain Reaction; same as PCR but the amplification can be visualised in "real-time" and DNA and/or RNA quantified to find out how many copies of a gene you have
Vaccination	An injection of antigenic material given to a person to induce long-term immunity to a particular infectious disease

Resource One Overview

Topic

What is the Human Papillomavirus (HPV)?

A-Level Modules

Structure of viruses, HIV, DNA & Genes

Objectives

By the end of this resource, you will be able to:

- ✓ Give an overview of what HPV is, the differences between low and high-risk HPV genotypes, what cancers can be caused by HPV, and transmission methods.
- ✓ Define key words and terminology used within the data source and the glossary.
- ✓ Determine the structure of a non-enveloped virus by labelling a diagram representation of HPV & matching important components to their explanations.
- ✓ Interpret the information on HPV given in the data source, identifying the key areas of research and trends in the field, to be able to design a questionnaire for scientists to give to study participants as a method of data analysis.
- ✓ Explore and utilise the further reading section on general information about HPV.

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context

Viruses are acellular, non-living particles. There are millions of different families of viruses, identified by their molecular structure, what type of organisms they infect, and what diseases they cause. Human papillomavirus (HPV) is an example of a virus from the Papillomaviridae family that infects humans and causes papillomas or epithelial growths such as tumours (usually benign warts).

It is important for scientists to gain such knowledge about a virus as it allows them to be studied on a molecular level allowing determination of cellular interactions, how they cause diseases and how to produce vaccines for protection against them.

Resource One
Data Source

Section A

Introduction to Human Papillomavirus (HPV)

Human papillomavirus (HPV) is an infectious human virus. There are over 200 different types of the virus that have been identified, known as **viral genotypes**. Of those types, almost a quarter are transmitted sexually. The virus, as most papillomaviruses do, can cause skin lesions or warts. When transmitted sexually, HPV can be the cause of genital warts, usually by **"low-risk"** HPV types 6 and 11. On the other hand, some HPV infections causes no symptoms at all, making it nearly impossible for infected individuals to know they have it. Approximately 80% of people will have encountered HPV by their mid-twenties but will be completely oblivious to it. The body, alike most viral infections, can clear HPV within 18 months from becoming infected.

Unfortunately, there are some more dangerous forms of HPV, otherwise known as **"high-risk"** types, that the body does not always successfully clear and instead of warts, can cause cancer. In fact, HPV is known to be the causative agent of cervical cancer, usually by HPV types 16 and 18. Men can also experience HPV genital cancers, but these are less common than in women. Other cancers that can be caused by high-risk HPV infections include anal, lung, and **head and neck cancers (HNSCCs)**. HNSCCs, in particular, are on the rise but scientists have yet to discover exactly why and what types cause these. Because of the ability to cause cancer, HPV is what is known as an oncogenic virus.

Section B

How is HPV Spread?

- Skin-to-skin contact between an infected and non-infected individual, this includes;

- Sexual contact (intercourse & oral)
- Touching (of warts, lesions or infected mucosal linings - sometimes even handshakes can transfer HPV!)

Resource One
Data Source

- Other possible transmission methods;

- Perinatal transmission (from mother to baby - can cause respiratory papillomatosis which is defined as warts of the lungs).
- Blood as a vector (a recent discovery so the extent of this is still under investigation)

Section C

Structure of HPV

Deep Dive

HPV is a non-enveloped DNA virus as shown in Figure 1. Instead of a protective envelope, HPV just has a viral capsid surrounding its genetic material. HPV particles, or **virions**, are quite small with a diameter of only about 30 nanometres (nm). In comparison, the human immunodeficiency virus (HIV), which is enveloped by a lipid bilayer (components of which are derived from the host cell), is approximately four times larger than HPV.

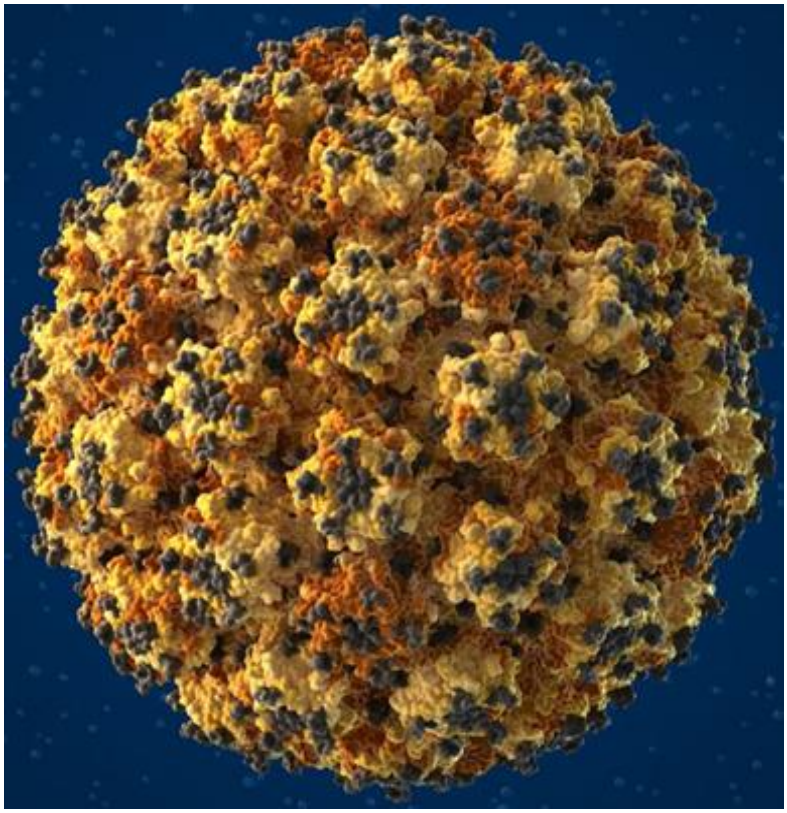
The HPV **genome** consists of circular double-stranded DNA (dsDNA) approximately 8000 base pairs (bp) in length (dependent on genotype) which encodes for two types of protein; early (E) proteins and late (L) proteins. The early proteins maintain regulatory functions (and are responsible for **oncogenesis** in the case of high-risk types), whilst the late proteins form the capsid of the HPV virion. There are six early proteins (E1, E2, E4, E5, E6 and E7) and two late proteins (L1 and L2). In terms of structure, the L1 proteins make up most of the viral capsid by assembling into pentagonal **capsomeres**, each with one L2 protein in the centre.

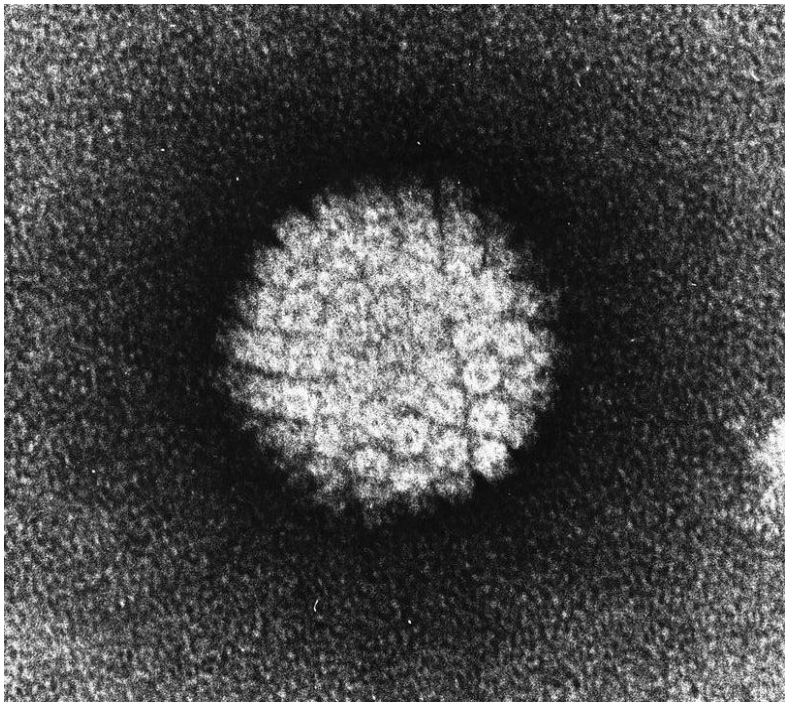
Using this newly acquired knowledge, complete the activities on the next page(s) and for more information on what the role of each HPV protein is and how viruses like HPV replicate, move onto Resource 2 of this pack.

Resource One
Data Source

Figure 1

From top to bottom; a 3D model and an electron micrograph of a negatively stained HPV virion. Image credit: Public Domain.

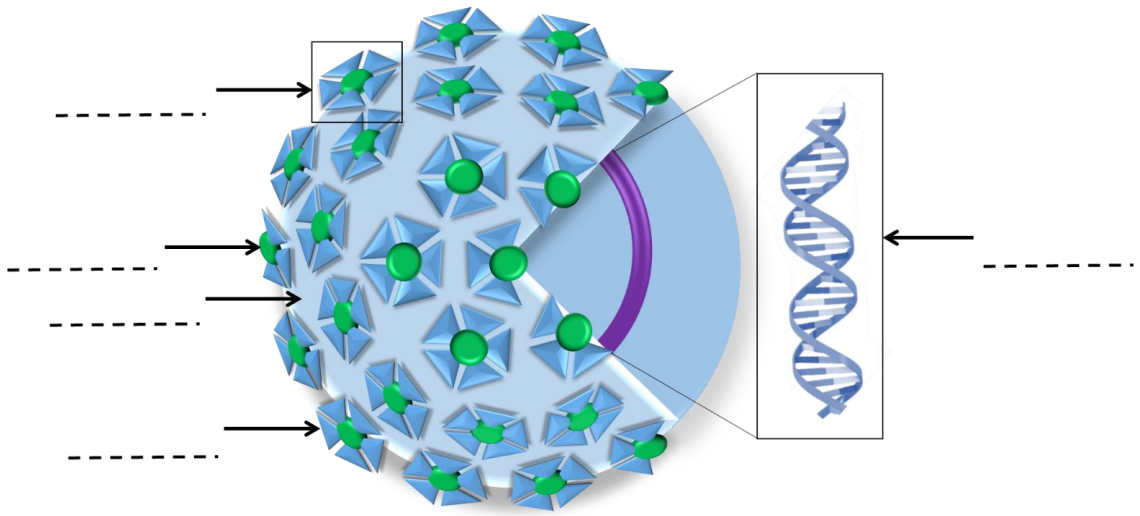




Resource One Activities

Activities

1. Label the components of an HPV virus on the diagram below using the data source and table of key words (provided underneath) to help you.



2. Opposite each key word in the table below, there is an explanation of a function, but they are all currently mixed up. Draw arrows to match each of these explanations to their key words.

Term		Definition
Circular dsDNA Genome		The major capsid protein of HPV, five of which make up the majority of the capsomere
Viral Capsid		The minor capsid protein of HPV, involved in stabilisation of the capsid
L1 Protein Monomers		The complete sequence of nucleic acids, or genetic material, of the HPV virus
L2 Protein Monomers		A subunit of the capsid made up of two HPV proteins, L1 and L2, arranged in a pentagonal shape
Capsomere		An outer shell of protein that protects the genetic material of a virus, composed of structural units called capsomeres

Resource One Activities

Activities

3. You were provided with some paragraphs of general information about HPV in the data source. Using this information, answer the following questions:

    a) How many different genotypes of HPV are there?

    b) What is the difference between high and low risk genotypes?

    c) What types of cancer can HPV cause?

    d) Can you describe how HPV can be transmitted from person to person?

    e) Researchers have found that HPV is more virulent and can thrive in harsher environments in comparison to some enveloped viruses such as HIV. Why do you think this is?

4. Imagine you have to design a questionnaire on sexual activity for scientists to give to individuals that are being screened for HPV. The purpose of the questionnaire is to enable the scientists to make correlations between sexual behaviour and risk of infection.

    a) What questions would you include in the questionnaire?

    b) Read the data source, think about what a scientist need to know and how they would draw conclusions from the data collected. Please give a **minimum** of 5 question examples.

Resource One
Further Reading

Explore

- A broad overview of information about the HPV virus:

www.nhs.uk/common-health-questions/sexual-health/what-is-hpv/

- A video overview of viruses, their structure and replication (nicely links this resource to the next one!):

www.youtube.com/watch?v=7KXHwhTghWI

- A book chapter about the structure of viruses:

Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 6.3, Viruses: Structure, Function, and Uses. Available from: www.ncbi.nlm.nih.gov/books/NBK21523/

Resource Two Overview

Topic	Viral Genes & Replication
A-Level Modules	Transcription, Translation & Gene Regulation

Objectives

By the end of this resource, you will be able to:

- ✓ Define key words and terminology used within the data source and the glossary.
- ✓ Understand and explain how the complex cellular processes of transcription and translation, and their involvement in gene expression, is crucial to viral replication.
- ✓ Understand and explain what gene regulation is and how it affects downstream gene transcription, particularly within dsDNA viruses such as HPV.
- ✓ Utilise the knowledge gained about gene regulation, transcription and translation to explain what happens during integration of the HPV genome in the host genome; via written and visual communication using a diagram representation.
- ✓ Describe the main stages of a viral life cycle using HPV as an example.

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context

In order for viruses to reproduce and replicate themselves, they must infect the cells of an organism. Viruses are not alive so to copy themselves, a virus must hijack host cell's cellular machinery to replicate their genetic material (the most important stage of the viral replication life cycle!). There are many complex cellular processes involved in replication including gene transcription and translation, all of which usually effect the host cell and can give rise to certain diseases like cancer.

Understanding these cellular processes and interactions allows scientists to develop detection methods for the viruses and determine possible disease treatments.

Resource Two
Data Source

Section A

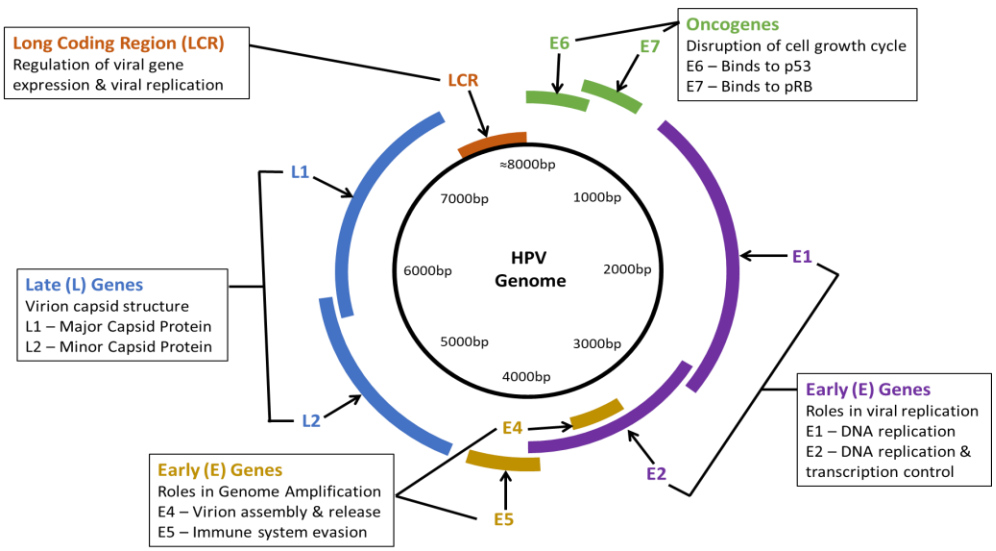
The HPV Genome

Genes are sections of DNA that contain the code for making amino acids; chains of these make up polypeptides, whilst polypeptide chains make up proteins. There are many types of proteins that have different functions dependent on the combination of amino acids and protein structure. The **transcription** and **translation** of DNA sequences into proteins from genes is called **gene expression** (or protein expression). For a gene to be expressed, a control region is required, and this is case for all living and non-living organisms. These regions contain transcription regulatory components such as **promoters**, **enhancers**, **silencers** and a transcription start site.

Below, Figure 2 reveals a diagram example of the HPV circular dsDNA genome, showing where each viral gene is located within the full 8000bp sequence, and a brief description of the function of each gene's protein following expression. As previously stated in Resource 1, the genome is organised into two main types of genes; the late (L) genes and the early (E) genes (as you can now see, they are named according to where they are located in the genome!). HPV's genome also contains a control region called the **Long Coding Region (LCR)** which regulates the transcription and replication of the HPV viral DNA.

Figure 2

HPV genome organisation and explanations of the functions of each viral gene



Resource Two
Data Source

It is important note that these genes are not all expressed at the same time, and the expression of some can actually lead to the disruption of the transcription of others. For instance, the expression of the HPV E2 protein supresses the transcription of the oncogenes E6 and E7. In some cases, such as with low-risk HPV genotypes, this is all that happens after infecting a cell but in others like high-risk genotypes, E2 expression can be disrupted by the viral genome integrating into the host's genome. Only after this happens, can E6 and E7 be expressed to then disrupt the host's normal cell growth cycle which then can cause tumour development. You will find more about HPV's ability to transform cells in cancerous cells within Resource 3.

Section B

HPV: The Viral Life Cycle

There are many stages of a viral life cycle; all rely on infecting a host organism. **Viral replication** is an important part of this cycle but it a complex process in comparison to eukaryotic cells as viruses cannot reproduce by division. To survive, instead they must replicate all viral components and then assemble these to produce new virions. The key stages of a viral life cycle include;

1. Attachment (to cell membrane)
2. Entry into a host cell
3. Uncoating (of the viral capsid)
4. Genome replication
5. Assembly
6. Maturation (note: for some viruses this occurs after release)
7. Release

After infecting a new cell by either injecting its genetic material or by **endocytosis**, a virus must then replicate its genome to continue with its viral life cycle. To do this it must transport its genetic material into the host's cell nucleus and where transcription and translation of viral genes can occur.

Resource Two
Data Source

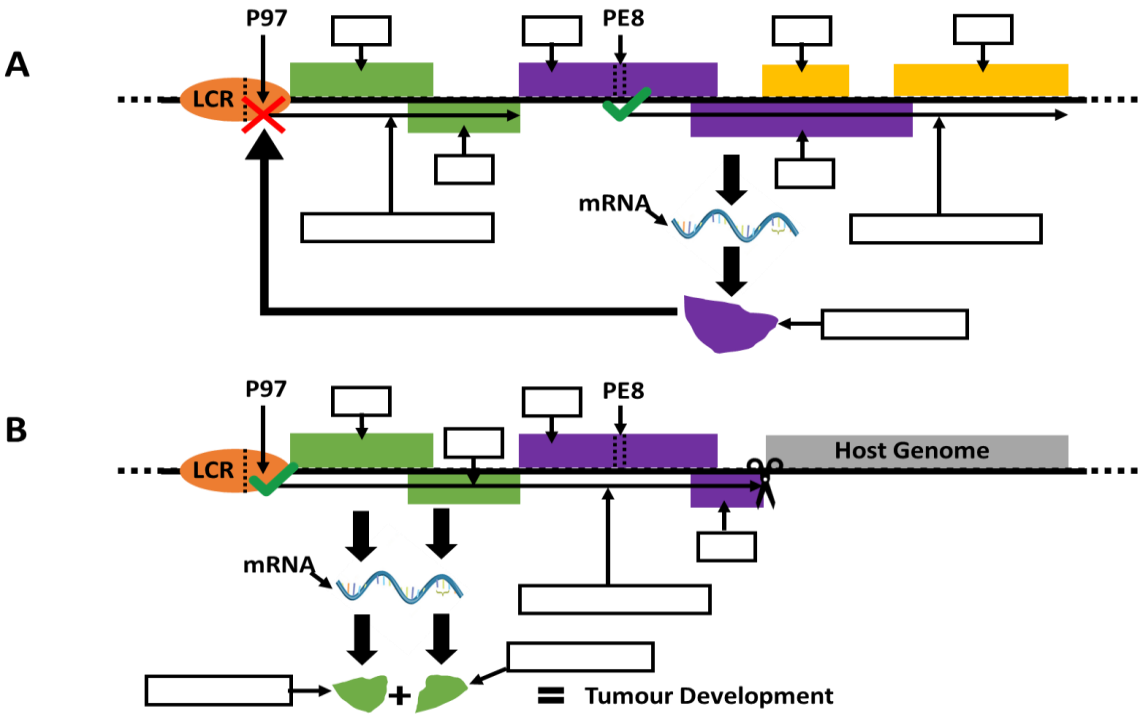
In the case of HPV in cutaneous epithelium (skin), the initial infection occurs within the basal layer of epithelial cells in skin (deepest layer). Here, it establishes a latent infection with low level replication of the viral genome and minimal viral gene expression of E1 and E2. The host basal cells then differentiate into other types of epithelial cells (spinous and granular), moving closer to the surface of the skin. At this stage, early HPV genes are expressed (E1, E2, E4, E5, E6 and E7) and the viral genome is amplified with high level genome replication. Finally, the last cell differentiation (creating the cornified layer) allows late viral gene expression (L1 and L2) to produce new capsid proteins and virus assembly occurs. Thereafter, the capsid matures (stabilises by forming disulphide bonds between the L1 proteins) and the virions are released as the outermost layer of skin cells naturally degrade and shed, spreading the virus further.

Using this newly acquired knowledge, complete the activities on the next page(s) and for more information on how the HPV E6 and E7 proteins cause cancer, move onto Resource 3 of this pack.

Resource Two Activities

Activities

1. Below is a diagram of the HPV genome in linear form showing early gene expression and regulation. You have been given the labels for the Long Coding Region, mRNA and two promoters, P97 and PE8 (these initiate downstream gene expression). Fill in the rest of the boxes for parts A and B using the data source to help you.



Source Analysis

2. Using the now completed diagram, answer the following questions:

   a) Part A and B of the diagram are showing two different scenarios in terms HPV gene expression and regulation, what are these scenarios, and can you describe what is happening?

   b) Why do you think that HPV regulates its own transcription?

Resource Two Activities

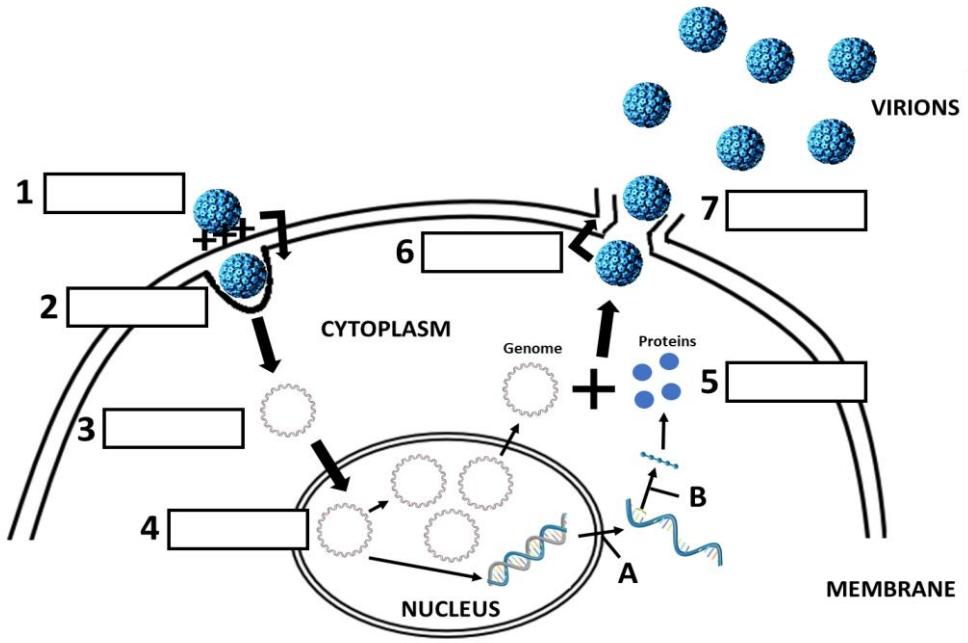
Activities

Problem solving

c) Some of the genes within Figure 2 of the data source and this diagram overlap. What do you think that means in terms of gene expression?

d) If a scientist wanted to examine cells that they suspected to be infected with a high-risk HPV genotype and that the viral genome was integrated into the host's genome, what gene(s) or protein(s) would they need to test for?

3. Below is a diagram that depicts a general viral life cycle. Fill in the numbered boxes labelling what stages are shown using the data source to help you.



Building an argument

4. Within the diagram, there are also two letters (A and B) pointing to arrows representing two important processes required during viral replication. Write a small paragraph explaining what these two processes are and why they are important for replication to occur.

Resource Two Activities

Activities

5. Imagine the diagram specifically represents the viral life cycle of HPV and that this cell differentiates over time. With each cutaneous cell differentiation (basal, spinous/granular, and cornified), describe what stage the viral life cycle is at (from stages 4–7 on the diagram), what HPV genes/proteins are being expressed, and explain what effect these have on the host cell and virus itself. Write a couple of paragraphs and use the knowledge you have gained about the HPV genome, different HPV genotypes, and HPV viral life cycle to help you answer.

Resource Two
Further Reading

Explore

- General information about gene expression and regulation:

www2.le.ac.uk/projects/vgec/highereducation/topics/gene expression-regulation

- A video on the life cycle of viruses and viral replication:

www.youtube.com/watch?v=ulut0oVWCEg

- An article explaining the life cycle of HPV after infecting epithelial cells with a helpful visual aid:

www.nature.com/articles/nrc.2018.13/figures/1

Resource Three Overview

Topic	Oncogenesis: Cellular Transformation
A-Level Modules	Tumour Suppressor Genes & Gene Expression

Objectives

By the end of this resource, you will be able to:

- ✓ Define key words and terminology used within the data source and the glossary.
- ✓ Identify and describe viruses associated with cancer using information provided.
- ✓ Understand and compare the differences between oncogenic (cancer-causing) viruses and cancer-associated viruses, giving specific examples of both.
- ✓ Understand and explain of how oncogenic viral gene expression can cause cancer, describing specific cellular interactions, using HPV as an example.
- ✓ Describe how oncogenic gene expression and signs of an active/integrated HPV infection can be detected using western blotting in the laboratory.
- ✓ Explore and utilise the further reading section on HPV and viral oncogenesis.

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context

A number of viruses are suspected of causing cancer in organisms, including humans (such as certain types of HPV!), so are frequently referred to as oncogenic viruses or cancer-associated viruses.

To be able to determine if a virus is oncogenic, scientists need to have an understanding of complex cellular pathways involved in the normal cell cycle and be able to detect if the presence of a virus is disrupting these pathways via viral gene expression. Using this knowledge, scientists are able to determine populations at risk of developing a cancer caused by a virus before tumours even begin to arise!

Resource Three
Data Source

Section A

Oncogenic Viruses

An **oncogenic** virus is a virus that has the potential to transform normal healthy cells of its host into cancerous cells. They do this by expressing oncogenic proteins that interact and interfere with important cell cycle pathways. This causes genetic instability of the host cell, compromises the ability of the immune system to recognise cancerous cells, and forces host cells to grow and rapidly divide before they are ready to (otherwise known as **cell proliferation**). For each oncovirus, the mechanisms for **oncogenesis** differ and are thought to have been adapted over time due to their ability to establish long-term infections within their host's cells. The more that scientists uncover about these viruses, the more we come to appreciate just how complex our cells' everyday processes are and how the introduction of one factor can cause a devastating molecular chain reaction.

On the next page, Table 1 describes nine viruses associated with different types of cancer in humans but not all of these have been shown to directly cause cancer via disrupting cell cycle pathways. As a result, only some of these viruses can truly be defined as oncogenic. The others have been detected in tumours or linked to cancer in individuals that are already immunocompromised such as the **Hepatitis B & C Viruses (HBV & HCV)**, and the **JC & BK Polyomaviruses (JCPyV & BKPyV)**. High-risk types of HPV, on the other hand, have been shown repeatedly to be the causative agent of some cancers via expression of oncogenic proteins, E6 and E7. Similarly, **Merkel Cell Polyomavirus (MCPyV)** produces a large and a small T antigen (LT-ag and sT-ag), whilst the **Human T-Lymphotropic Virus-1 (HTLV-1)** produces Tax, all uniquely designed to interfere with our normal cellular pathways. The viruses do not always get it right though. When some integrate their viral genome into the host genome, similarly to E2 of HPV, parts of viral genes sequences can be lost so

Resource Three
Data Source

cannot be transcribed. For instance, in some studies of MCPyV in cancer, the virus has been detected in tumours but has been found to be unable to replicate it's viral DNA after genome integration. This has contributed to the theory of multiple viruses infecting the same cells (**co-infection**), and potentially working together to cause cancer.

Table 1

Information about nine cancer-associated or cancer-causing viruses

Virus	Taxonomy	Genome	Length (bp)	Associated Cancers	Year of Discovery
Epstein-Barr Virus (EBV or HHV4)	Herpesviridae	dsDNA herpesvirus	171,823	Hodgkin lymphoma, Burkitts lymphoma and nasopharyngeal carcinomas	1964
Hepatitis B Virus (HBV)	Hepadnaviridae	ssDNA & dsDNA hepadenovirus	3,182	Hepatocellular carcinomas	1965
JC Polyomavirus (JCPyV) or John Cunningham Virus (JCV)	Polyomaviridae	dsDNA polyomavirus	5,130	Non-Hodgkin lymphoma, brain tumours, colorectal, prostatic and bladder cancer	1965
BK Polyomavirus (BKPyV or BKV)	Polyomaviridae	dsDNA polyomavirus	5,153	Brain tumours, osteosarcomas, Ewing's sarcomas, neuroblastomas, prostatic and bladder cancer	1971
Human T-Lymphotropic Virus-1 (HTLV-1)	Retroviridae	Positive-Stranded ssRNA retrovirus	8,507	Adult T-cell leukaemia/ lymphoma	1980
Human Papillomavirus (HPV)	Papillomaviridae	dsDNA papillomavirus	≈8000	Anogenital, cervical, skin, lung & oropharyngeal (HNSCCs) carcinomas	1956 (HPV), 1984 (HPV16 & HPV18)
Hepatitis C Virus (HCV)	Flaviviridae	Positive-Stranded ssRNA flavivirus	9,646	Non-Hodgkin lymphoma & hepatocellular carcinomas	1989
Kaposi's Sarcoma Herpesvirus (KSHV or HHV8)	Herpesviridae	dsDNA herpesvirus	137,969	Kaposi's sarcomas, primary effusion lymphoma, Multicentric Castleman Disease (MCD)	1994
Merkel Cell Polyomavirus (MCPyV or MCV)	Polyomaviridae	dsDNA polyomavirus	5,387	Merkel cell carcinomas (skin cancer)	2008

Resource Three
Data Source

Section B

High-risk HPV Oncogenesis

We already know that high-risk HPV genotypes can infect cells and cause oncogenesis but how exactly do they do this? After the viral genome integrates into the host genome, this leads to a significant increase in oncogenic protein expression, E6 and E7. The oncoproteins then bind to **p53** and **pRB** (host cell **tumour suppressor proteins**) in the cytoplasm to inactivate them; E6 to p53 and E7 to pRB. The combined functions of p53 and pRB are to stop the progress of the normal cell cycle of a host cell if there is DNA damage. In the absence of p53, DNA damage can accumulate without repair and without pRB, cells with DNA damage are able to undergo cell division. As a result, the host cell will undergo unregulated cell division, cell growth, and cell survival; all of which are characteristics of cancerous cells.

With this knowledge, scientists can not only test for oncogenic protein expression to see if an oncovirus is present and active, but they can also determine the severity of the infection by testing for **down-regulation** (reduction in quantities) of tumour suppressor proteins before tumours even develop, allowing early diagnosis of an oncogenic viral infection.

Using this newly acquired knowledge, complete the activities on the next page(s) and for more information on different types of cancer caused by HPV, move onto Resource 4 of this pack.

Resource Three Activities

Activities

1. Using the information given in Table 1 in the data source, write a list ordering the nine viruses by genome size starting with the largest and ending with the smallest.
2. Answer the following questions about the data in Table 1:

a) How has the viruses been ordered?

b) Which **type** of viruses has the second smallest genomes (use your answer the Q1 to help)?

c) What does it mean by "positive-stranded" when describing the genomes of two viruses in the table?

d) Which virus has two different genome structures and what is the **family** of this virus?

e) Which virus was the most recently identified?

f) Can you state all of the similarities and differences, given in Table 1, between Hepatitis B Virus and Hepatitis C Virus?

g) Can you list all of the viruses, by name, that have been linked to a type of lymphoma?

h) Which is the only virus in this list to be linked to bone cancer and identify the name(s) of the cancer?

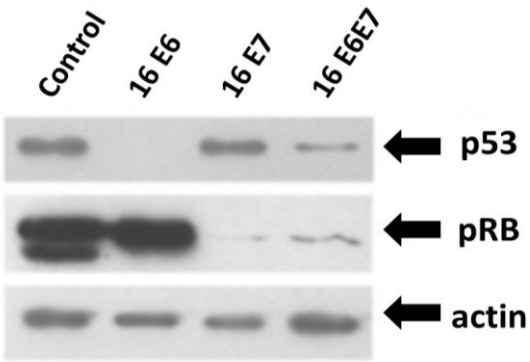
3. Discuss in pairs or small groups the theory of co-infection and come up with **three** research questions that scientists may want to address in area of oncogenic viruses working together to cause cancer. Think about how viral DNA is expressed, the areas of the body that the viruses infect, and the long-term effects of an infection within a cell. This will give you an idea how scientists use what we already know within a subject to find out what knowledge is missing, and then address this through scientific research.

Resource Three Activities

Activities

Deep Dive

4. Below is a nitrocellulose membrane (or blot) used in a technique called western blotting. Western blotting is used to detect specific protein molecules within a mixture (i.e. from a sample). It will also reveal the size of the protein of interest (against a ladder of known sizes) and measure the amount of protein present in the sample (how dark the bands are)..The blot shown here reveals the expression levels/amount of the two tumour suppressor proteins (p53 and pRB) and a reference protein (actin) in three different HPV positive cell lines in comparison to an HPV negative control cell line. Looking at this blot, can you answer the following questions:



a) What do you think the purpose of showing actin expression levels in every cell line is?
b) Which cell line shows "normal" expression of all three cellular proteins?
c) Can you describe the results for all three proteins within the "16 E7" cell line?
d) Which cell line shows reduced levels of both p53 and pRB, and can you explain why this is?
e) Say the control cell line was infected with a type of HPV but the blot still revealed the same results, could you suggest two reasons why this may happen?
f) What would the blot results be for all three proteins if the control cell was infected with MCPyV and the viral genome had integrated?

Resource Three
Further Reading

Explore

- An educational website page with an overview of many of the oncogenic viruses:

www.cubocube.com/dashboard.php?a=340&b=418&c=1

- A scientific journal article about co-infection of three of the viruses described in this resource and the effects of this on oropharyngeal cancer (this paper contains some tricky science, try reading the introduction and discussion first):

Drop, B., Strycharz-Dudziak, M., Kliszczewska, E. and Polz-Dacewicz, M. (2017). Coinfection with Epstein–Barr Virus (EBV), Human Papilloma Virus (HPV) and Polyoma BK Virus (BKPyV) in Laryngeal, Oropharyngeal and Oral Cavity Cancer. *International journal of molecular sciences*, **18**(12), p.2752.

- A factsheet including instructions on western blotting:

www.onlinebiologynotes.com/western-blotting-technique-principle-procedure-application/

Resource Four Overview

Topic	HPV Related Cancers
A-Level Modules	Cancer, Risk Factors & Gel Electrophoresis

Objectives

By the end of this resource, you will be able to:

- ✓ Identify and describe the characteristics of cancer and explain the differences between malignant and benign tumours.
- ✓ Determine what types of cancer can be caused by HPV and their population incidence rates by locating key information in the data source provided.
- ✓ Explain what the different stages of CIN are by severity of dysplasia and link each stage to the HPV life cycle, determining which genes are being expressed & when.
- ✓ Describe what gel electrophoresis is and how it is used in current HPV research.
- ✓ Explore and analyse emerging oral HPV research to identify what key information is missing from the field to understand how scientists develop research questions

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context

Oncology is the study of cancer including prevention, diagnosis, and treatments. Oncology is closely linked to virology in relation to oncogenic viruses. Discovering as much as scientists can about a virus that causes cancer, allows for prevention strategies, new diagnosis methods and treatments to be developed; all specifically tailored to the virus responsible.

High-risk types of HPV have been repeatedly shown to cause a range of cancers, some of which have had methods of prevention and treatment already established. Others, like head and neck cancers, have yet to be fully explored. Read on for more!

Resource Four
Data Source

Section A

HPV & Cervical Cancer

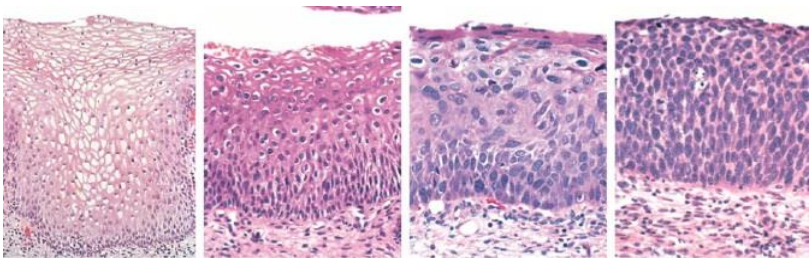
Cancer occurs when abnormal cells divide in an uncontrolled way producing growths in the form of tumours. Cancerous tumours are **malignant** meaning they have properties specific for **invasion** and **metastasis**. Invasion refers to cancer cells extending and penetrating neighbouring tissues, whilst metastasis refers to the ability to form secondary malignant tumours at new sites within the host by transporting cancerous cells through the blood and/or lymphatic vessels. Further characteristics of cancer include **angiogenesis**; the process of drawing new blood vessels towards a tumour for a nutrient supply, uncontrolled cell proliferation, genetic instability, and eventually **immortality**.

High-risk genotypes of HPV have been well-known for causing almost all cervical cancer cases. Other associated cancers are of the vulva, vagina, penis, anus, and **head and neck region (HNSCCs)** but research is still being conducted to understand how and to what extent HPV causes these cancers. Overall, HPV is attributed to more than 90% of anal and cervical cancers, 70% of vaginal and vulvar cancers, and 60% of penile cancers.

Fortunately, not only is there a vaccination to protect against the high-risk genotypes of HPV (see Resource 6), there is also a cervical screening programme in the UK to detect abnormal cells on the cervix. If abnormal cells are found via **cytobrushing**, usually cervical biopsies are then taken and looked at under a microscope to determine the severity of the situation.

Figure 3

From left to right; a biopsy of a healthy cervix and those with CIN (1, 2 & 3)



Resource Four
Data Source

Figure 3 shows four cervical biopsy samples revealing the progression from a normal healthy cervix to **Cervical Intraepithelial Neoplasia (CIN)** Grade 3. CIN refers to lesions on the surface of the cervix where there is no invasion of the tissue like cancer. Because of this, CIN is known to be a **precursor** of cervical cancer, not cancer itself.

CIN is divided into three grades of **dysplasia**; defined as an abnormal disordered growth, development and maturation of cells. These grades determine the severity of the dysplasia and how much of the cervix is affected. This is also usually indicative of how severe the high-risk HPV infection is causing the transformation of the cells. The following guidelines are used to classify the grade of CIN;

Deep Dive

- **CIN1** – mild dysplasia; affecting only 1/3 of the thickness of the surface layer of the cervix.
- **CIN2** – moderate dysplasia; affecting 2/3 of the thickness of the surface layer of the cervix.
- **CIN3** – severe dysplasia with undifferentiated cells; affecting >2/3 to the full thickness of the surface layer of the cervix.

CIN1 has high regression rates of 70-90% within 1-2 years without treatment, particularly if the host clears the HPV infection responsible. CIN2 can also regress but has a much lower rate of about 50%, whilst CIN3 is highly unlikely to regress at all. The treatment for CIN2 and CIN3, in the absence of regression, is the removal of the abnormal cells. If left untreated, CIN3 can become cervical cancer.

Section B

Oral HPV, Cancer & Current Research

As previously stated, HPV has also been linked to HNSCCs. HPV positive HNSCCs are predominately found in the tonsils, base of the tongue and throat (all make up the **oropharynx**). New research is showing that HPV positive HNSCCs are

Resource Four
Data Source

presenting more frequently in men than women. HNSCCs have also been linked to heavy smoking and high levels of alcohol consumption due to carcinogens and chemicals damaging the cells, therefore, DNA within. The combination of this and an active high-risk HPV infection is thought to lead to oncogenesis.

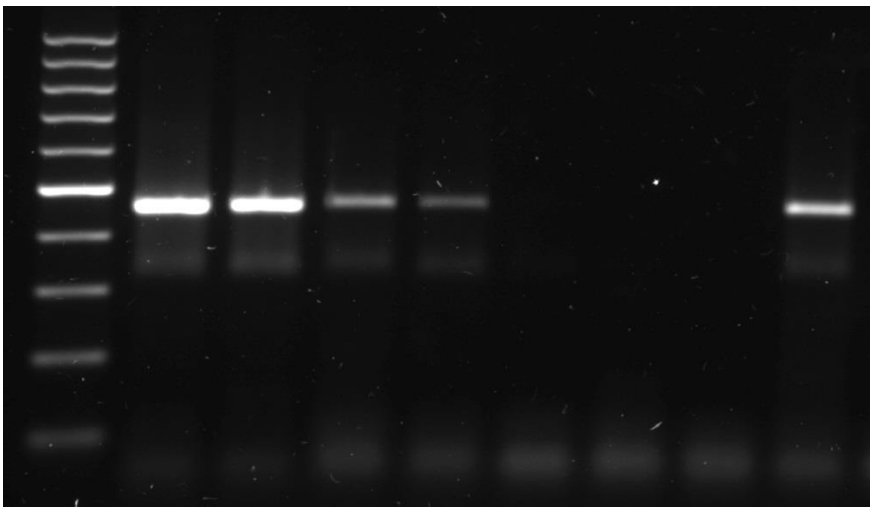
As this is cutting edge research, there is however, still a lot that is unknown about oral HPV and the link to HNSCCs. We do not know the HPV genotypes causing oral infections and if they are the same as the genital genotypes, if the vaccination against genital HPV is effective against oral HPV, what it is about the structure of the oropharynx that makes it more susceptible to oncogenesis, what it is about the morphology of men's mouths that encourages oncogenesis with HPV present, and finally, the incidence and prevalence rates of oral HPV in healthy individuals (pre-cancer). Answering these questions will allow scientists to identify those at risk of developing an HPV positive oral cancer.

Figure 4 (next page) is a 1% agarose gel, used in a method called **gel electrophoresis** which separates sequences of DNA from a mixture (i.e. from a sample like western blotting but for DNA) based on sequence length, determining size and number of copies. Gel electrophoresis is used after samples have been screened for a specific target gene in **PCR/qPCR**. It works by running an electric current through the gel with an **anode** (positively-charged rod) and a **cathode** (negatively-charged rod) either end, and any molecules that are negatively-charged like DNA sequences, will be pull towards the anode. Larger sequences will move slower through the gel than smaller sequences so can be distinguished from one another and compared against a known copy number ladder to confirm the length of the expected PCR product. UV is then used to visualise the product bands on the gel.

Resource Four
Data Source

Figure 4

Demonstration of screening for HPV using gel electrophoresis



Scientists use methods such as gel electrophoresis in combination with PCR/qPCR to screen samples for viral infections such as HPV. This method is used in combination to determine viral presence within the cervical biopsies and within oral screening. This gel contains real data showing the detection of the L1 gene of HPV in oral cell samples collected from the inside of the cheeks (mucosal lining) of participants in a research study. This is an ongoing study contributing to finding out who is at risk of HPV positive HNSCCs.

Using this newly acquired knowledge, complete the activities on the next page(s) and for more information on viral screening techniques used within the laboratory such as PCR/qPCR, move onto Resource 5 of this pack.

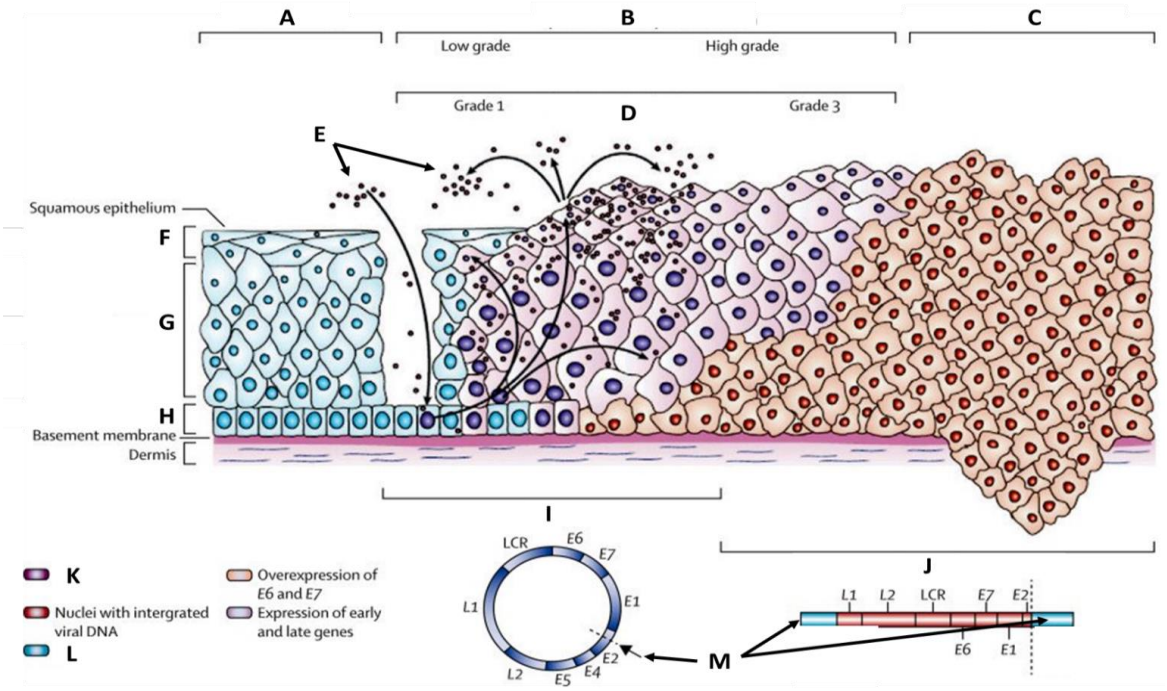
Resource Four Activities

Activities

1. Identify three main characteristics of malignant tumours and give a complete description for each.

Independent research

2. Below is a diagram representation of a cervix at different stages of transformation after being infected with HPV. Using the data source and the knowledge gained in Resource 2 of this pack, label what **A-M** stand for. Please note that this is a **mucosal epithelium** (inside lining) rather than cutaneous epithelium (skin; outside lining) described in Resource 2 so some of the cell layers are different and **may require internet sources** to help you.



3. Use the data source and Figure 4 to answer the following questions:

a) If you knew that the top band on the DNA ladder was 1000bp and the bottom band was 100bp, can you estimate what length the L1 sequence fragment is?

Resource Four Activities

Activities

b) Towards the top of the DNA ladder, the bands are closer together, why is this?

c) What do you think the brightness of the bands represents and what does that tell scientists about those particular samples?

d) Scientists always run controls as well as samples in agarose gels, why is this?

e) Along the bottom of the gel, there are a series of faint bands for most of the samples and controls, what size do you think these products are, and thinking about the PCR/qPCR reaction, could you suggest a possible reason for these?

f) Scientists also can change the thickness of the gel (e.g. 0.8%, 1% & 2%) dependent on the size of the fragments they are looking to detect. If scientists were looking to detect a product smaller than the L1 fragments, do you think the gel thickness would need to be increased or decreased and why?

Resource Four
Further Reading

Explore

- A webpage containing links to topics about cervical cancer including CIN, treatments, symptoms, and much more:

www.cancerresearchuk.org/about-cancer/cervical-cancer

- An educational video explaining how HPV transforms cervical cells into cancerous cells through the stages of CIN1-3:

www.youtube.com/watch?v=WSL8rBMWW1Y

- A scientific journal article reviewing oral HPV and HNSCCs:

D'Souza, G. and Dempsey, A. (2011). The role of HPV in head and neck cancer and review of the HPV vaccine. *Preventive medicine*, **53**, pp.S5-S11

Resource Five Overview

Topic

Viral Screening Techniques

A-Level Modules

PCR, Plasmids, Probes & Genetic Screening

Objectives

By the end of this resource, you will be able to:

- ✓ Identify and describe what PCR is, how it works and what key components are required for successful gene amplification to occur.
- ✓ Define key words and terminology used within the data source and the glossary.
- ✓ Understand & explain the differences between end-point PCR & real-time qPCR.
- ✓ Explain how scientists use qPCR in viral screening & what information it provides.
- ✓ Critically analyse and evaluate real qPCR data for accuracy by generating a standard curve to determine reliability for copy number quantification.
- ✓ Interpret qPCR data to determine if samples are positive for HPV and use an independently generated standard curve to calculate gene copy number.

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context

A key laboratory technique widely used in molecular biology, for any area of research, is PCR. Over the last three decades, many new versions have been developed including, the superior and highly specific, real-time qPCR.

This technique is frequently used in viral screening research as it provides a quick, reliable and quantifiable way of determining the presence of a virus within a sample, identifying the strain of virus, the severity of the infection via viral load, and in some cases, if viral genome integration has occurred which is an indicator of an active infection. Determining these epidemiological aspects is essential for disease control.

Resource Five
Data Source

Section A

End-point PCR

Polymerase Chain Reaction (PCR) technology is used in molecular biology and virology to analyse sequences of DNA or RNA in samples containing very low concentrations. PCR amplifies targeted sections of the DNA or RNA sequence (of a gene) in question to high concentrations so that they can be detectable at the end of the thermocycling processes via gel electrophoresis (known as "**end-point PCR**"), described in Resource 4.

For PCR amplification to occur:

- Two **primers** are required; short single-stranded DNA sequences that correspond to the beginning and ending of the DNA sequence to be copied.
- An enzyme called **DNA polymerase**; it moves along the DNA template (containing the target sequence), reading the code and assembling copies.
- DNA **nucleotides**; individual DNA base pairs used to build the new copies of the target sequence.

There are three stages to PCR, **denaturation**, **annealing**, and **elongation/extension**, which all occur at different temperatures and for different lengths of time. One amplification cycle is when all three stages have completed, then the cycle starts again over and over for approximately 40 cycles. Unfortunately, using end-point PCR you cannot visualise any of these thermocycling processes and can only interpret qualitative data by eye at the end which is subjective and prone to human error.

Section B

Real-time qPCR

Over the last 10-15 years, end-point PCR has been developed into a more quantitative, highly specific and reliable method producing **Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)** technology. In RT-qPCR, fluorescent dyes (probes) that attach to DNA

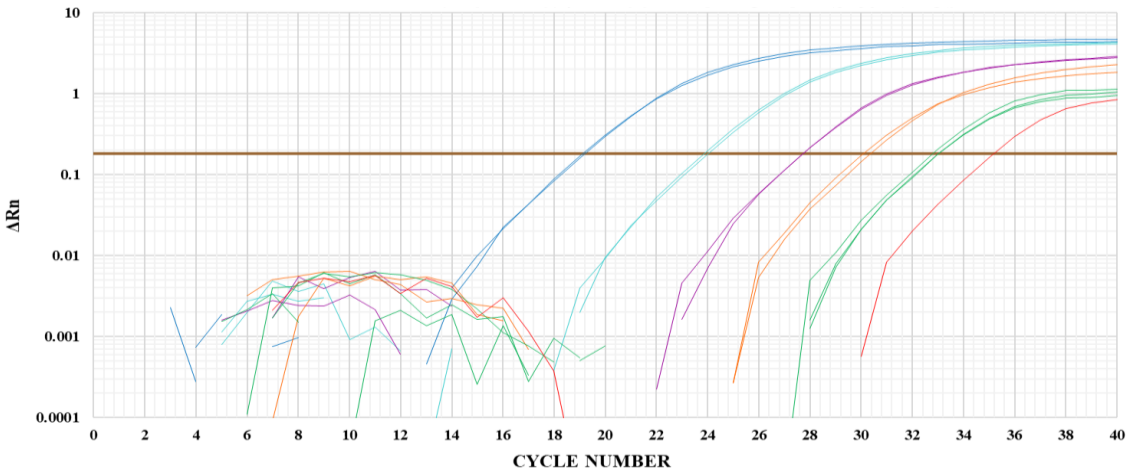
Resource Five
Data Source

sequences enable the collection of data as the PCR thermocycling progresses, hence the name "real-time". When the thermocycler detects emitted fluorescence, the target DNA sequences are amplifying, allowing scientists to determine the presence of that target in a sample. Sequences can also be characterised by using different dyes for different targets and quantified to find out exactly how many copies of the target sequence is in the sample.

For scientists to be able to accurately interpret the results of RT-qPCR, a series of controls must be run at the same time as the samples in question for comparisons to be made. There are three types of controls, 1) **positive** for the target sequence, 2) **negative** for the target sequence, and 3) a **non-template control (NTC)** which contains no DNA. To quantify samples, scientist must know the exact copy number of the target gene of their positive controls. The easiest way is to use **plasmid DNA**; circular dsDNA vectors containing an insert of the gene in question. A series of known copy number controls are called **standards** and range from a very high copy number to the lowest possible copy number (e.g. 1 million copies to 1 copy) that a sample could contain.

Figure 5

An amplification plot of fluorescence signals of 6 positive copy number controls

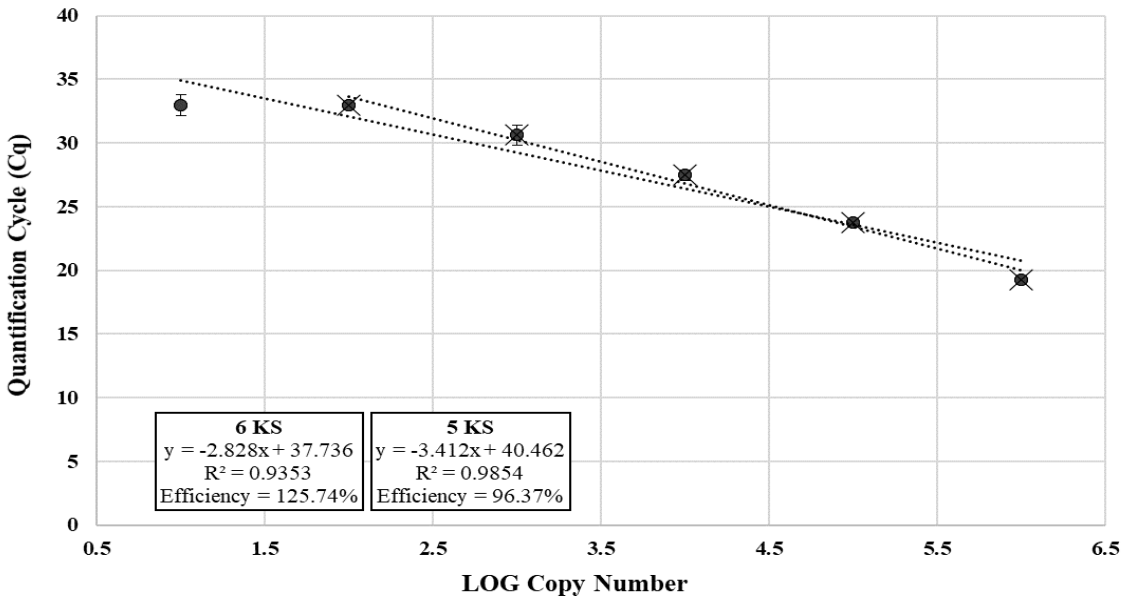


Resource Five
Data Source

Figure 5 represents how scientists can visualise the amplification of samples in real-time. As each cycle progress, more and more of the **fluorescence signal** (coloured lines) is revealed. The samples and controls with the highest copy numbers of the target sequence will be detected earlier in the cycling then the ones with the lowest copy numbers. When the fluorescence signal for amplification is given off, it hits the **threshold** (brown horizontal line), and produces a number known as a **Cq (cycle quantity)** value. These Cq values allow scientists to then quantify samples based on what number cycle the target was detected by the thermocycler machine. If there is no amplification fluorescence signal so no Cq value produced, the target gene is not present.

Figure 6

A copy number standard curve produced to calculate target quantity in samples



To quantify samples, the standard control Cq values produced in the amplification plot must be plotted against their known copy numbers in **logarithmic** form. This produces a copy number **standard curve** (despite the name, there is no curve!) as shown in Figure 6. Adding a trendline to the plotted

Resource Five
Data Source

data allows for the generation of the **equation of the line** and **R^2 value**; both mathematical indicators of accuracy. The closer to 1 the R^2 value is, the less variance there is in the data, whilst the closer the slope value (m in y=mx+c) to -3.32 (perfect value in this case), the more likely the standard curve can be used to quantify samples. Providing these indicators have been met, rearranging the equation of a line allows scientists to work out the exact copy number of the target sequence in the samples being compared, as long as the sample Cq values sit on that line of best fit (within the highest and lowest standard).

This method is frequently used to screen individuals for viruses in research and diagnostic settings, predominately due to the sensitivity and specificity of the method to pick up an infection as small as 10 copies of a viral gene. It may seem complicated at first but hopefully after working through the activities provided, it will start to make more sense.

Using this data source, complete the activities on the next page(s) and for more information on HPV and vaccinations, move onto Resource 6 of this pack.

Resource Five Activities

Activities

*Please note, that in order to complete this activity, you require a computer with Microsoft Excel

1. Imagine you have just run a real-time qPCR experiment to detect the E6 oncogene of HPV in human mucosal samples (you are trying to find out if they are HPV positive) and known copy number plasmid controls (these are HPV positive and known as "standards"). The thermocycler has detected a fluorescence signal from the amplification of your target gene in some of your samples and all your known standard controls. When the fluorescence signal passes a threshold in the linear phase of the amplification, the thermocycler produces Cq values (cycle quantities) for every HPV positive sample and known standard control from this experiment. The Cq values for the known copy number standard controls are written in the table of numbers below against their known copy numbers in logarithmic format.

In Excel, create an HPV E6 copy number standard curve using both columns of numbers provided in the table below. Use the instructions on the right to help you.

Standards	
Cq Values	LOG Copy Number
16.5	6
19.82	5
23.11	4
26.39	3
29.72	2
32.62	1

Instructions for creating a standard curve in Excel:

a) Go to insert, charts, X-Y scatter charts and click on the first scatter chart option available. A blank chart square should appear in the spreadsheet.
b) In the same spreadsheet, create an identical table of the one provided to the left.
c) Click on the chart and go to design, select data and a box should appear allowing you to input your data series (look at the data source for help on which axis should be which and don't forget to title them!)

Resource Five Activities

Activities

2. Add a line of best fit (trendline) on to your standard curve by going to "add chart element" in design, then right-click your trendline to select "format trendline" to add the equation of the line and R2 value to your standard curve.

Once you have added all the required indicators of accuracy to your standard curve, answer the following questions:

a) What type of correlation is shown?
b) What does this type of correlation mean in terms the relationship between Cq values and copy number?
c) Write a small paragraph to explain why the standard curve you have produced can be used to quantify unknown samples?

(Tip: use the equation of a line and the R2 value to help you explain your answer!)

3. Plot a new data series for each HPV positive sample onto your standard curve (remember, the only values you need to plot are the Cq values against log copy number!) and then, answer the following questions:

a) Why do you think sample B and sample F have "Undetermined" Cq values?

b) Which sample out of the six screened has the most abundant HPV infection and how have you determined this?

c) One **positive** sample cannot be accurately quantified, can you identify which sample this is and explain why?

d) If you were to screen these exact samples again using the same standard curve, how do you think you could improve the reliability of these results?

Resource Five
Further Reading

Explore

- An educational article explaining the basics of PCR:

www.sciencelearn.org.nz/resources/2347-what-is-pcr

- A short video explanation of qPCR:

www.youtube.com/watch?v=1kvy17ugl4w

- A scientific journal article describing PCR and real-world applications of it in various scientific fields:

Valones, MA., Guimarães, RL., Brandão, LAC., Souza, PRED., Carvalho, ADAT., and Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fiel), ds: a review. *Brazilian Journal of Microbiology*, **40**(1pp.1-11.

Resource Six Overview

Topic	HPV Vaccination
A-Level Modules	Vaccination, Immune System & Viral Diseases

Objectives

By the end of this resource, you will be able to:

- ✓ Describe what a vaccine is, what they contain, and how they create immunity within the human body whilst highlighting key terminology.
- ✓ Define the difference between each HPV vaccine and discuss the success rates in the UK and across other countries with specific statistical examples.
- ✓ Independently conduct scientific research on HPV vaccination, exploring scientific journal articles and communicate research ideas via group discussion.
- ✓ Identify the differences and similarities in studies conducted on HPV vaccination.
- ✓ Analyse sources and suggest strategies for improving global vaccination success rates by exploring wider economical and governmental concepts.

Instructions

1. Read the data source
2. Complete the activities
3. Explore the further reading

Context

Vaccines have been developed as a result of the research conducted by molecular biologists studying viruses that cause human diseases. The first vaccine developed was the actually the smallpox vaccine by Edward Jenner in 1796, although this was accidental. Due to world-wide vaccination, smallpox has now been eradicated proving how successful this method of prevention can be. Since the first vaccine, many more have been developed leading to dramatic reductions in incidence rates of diseases caused by viruses (even with some anti-vaccine controversy in specific areas of the world!). To demonstrate this further, the HPV vaccination, that was only introduced ten years ago, is now being linked to the elimination of cervical cancer!

Resource Six
Data Source

Section A

What's Vaccination?

Anyone can be immunised against a pathogen through something we call **vaccination**. There tend to be one **vaccine** per pathogen, but these can be combined into one like the DTP vaccine which protects us from diphtheria, tetanus, and pertussis. Vaccines are made by putting a small amount of an inactive or weakened form of the specific pathogen into the body. They can contain:

- **"Dead" pathogens**; viruses are not alive will be classification as inactive
- **Live pathogens** but treated to make them harmless to the recipient
- **Fragments of the pathogen**; harmless to the recipient
- **Toxins produced by pathogens**; usually excretory products of a bacterium

All these function as **antigens**. When antigens are injected into the body, they produce a primary immune response, stimulating white blood cells (WBC) to divide and produce **antibodies** against the specific pathogen our body believes we have encountered. There are a few types of WBC involved, two of which are **plasma cells** and **memory B cells**. The plasma cells produce the antibodies which are only temporary until the body believes it has cleared the infection, whilst the memory B cells multiple and remain dormant for years, ensuring that if we encounter the actual pathogen our bodies will be able to recognise it straight away and destroy it immediately.

Section B

UK HPV Vaccination

In attempt to reduce the risk of developing cervical cancer caused by an HPV infection, a **bivalent** vaccination was introduced in the UK in 2008 for 12-13-year-old females known as **Cervarix**. The antigenic material in this was simply synthesised viral capsids. This vaccination only protected

Resource Six
Data Source

individuals from the two most common high-risk genotypes though; HPV16 and HPV18. This did not protect individuals from acquiring genital warts though as the low-risk genotypes were not targeted also. Because of this, a **quadrivalent** vaccination known as **Gardasil-4** was produced and replaced Cervarix in 2012, providing additional protection for the two most common low-risk genotypes, HPV6 and HPV11, thus protecting individuals against benign genital warts as well. Since then, a **nine-valent** vaccination was developed and licenced in 2014 known as **Gardasil-9**, which protects individuals from an additional five high-risk genotypes; HPV31, HPV33, HPV45, HPV52 and HPV58.

Currently, Gardasil-9 is only available on request from GPs and from some private independent companies such as Superdrug and Boots UK. Due to this, males aged between 12 to 44 years old have been allowed the vaccination, for the first time in the UK, which could also help to protect them against the development of HPV-associated anal, penile and head and neck cancers. It was also announced in the Summer 2018, that 12-13-year-old boys would also be given the vaccination in UK schools along with girls but there has been no update (as of December 2018) or indication as to when this will begin. The decision was made as other countries were already providing **gender-neutral vaccination**, **herd immunity** does not apply to men who have sex with men (MSM), and emerging research was showing that HPV was causing HNSCCs, especially in men.

Section C

Vaccination Success?

Following the introduction of HPV vaccination programme in the UK, there has been a significant fall in HPV infections in young women. HPV type 16 and 18 infections, which cause cervical cancer, decreased by 86% in women aged between

Resource Six
Data Source

16 to 21 who were eligible for the vaccination at school between 2010 and 2016. In addition, there has also been a reduction in the prevalence of HPV types 31, 33 and 45, which can also cause cancer (high-risk genotypes).

In Australia, there has been a 77% reduction in HPV genotypes detected responsible for almost 75% of cervical cancer, almost 50% reduction in the incidence of high-grade cervical abnormalities in girls under 18 years of age, and a 90% reduction in genital warts in heterosexual men and women under 21 years of age.

Despite all the success with genital HPV cancers and warts, we still do not know about the effects of the vaccination on oral HPV, if it is effective and to what extent. There is a vast amount research currently being conducted on oral HPV, HNSCCs and the effective of the HPV vaccination (including my own research!) in order to answer some of the questions we molecular biology scientists still have.

Resource Six Activities

Activities

*Please note, that in order to complete this activity, you require a computer with internet access

1. In pairs or small groups, research into the success of the HPV vaccination in terms of reduction rates of cervical cancer cases, reduction rates of CIN cases, and reduction rates in the detection of high-risk genotypes. Try and find some scientific journal articles as well as general news articles (approximately 4-5 per pair/group), preferably from 2017 onwards. Write down the references.

2. Discuss and debate with the whole class/group your findings for part 1. Please note, you will find some conflicting data from either the same country or different countries. Try to critically analyse the articles and discuss why you have found conflicting data (or even similar data). **Tip:** look at the study cohort demographics used and methods for detecting HPV, do they differ?
3. Draw up a table of a list of statistics that have been found organised by country or continent, identifying similarities and differences between areas of the world.

4. In pairs or small groups again, discuss where improvement is needed in terms of HPV vaccination success and come up with some of your own strategies on how to address this, and how these can be implemented (e.g. high oral HPV incidence in men = give boys the HPV vaccination in the UK).

Resource Six
Further Reading

Explore

- A video explanation of the HPV vaccination and how it works:

www.youtube.com/watch?v=FaHrhKvPZ2w

- A news article on the HPV vaccination, information and controversies surrounding the programme:

www2.philly.com/philly/health/the-cancer-preventing-hpv-vaccine-a-dozen-years-on-progress-fear-and-loathing-20181107.html

- A video explanation of a study conducted showing how successful the HPV vaccination is in preventing cervical cancer:

www.youtube.com/watch?v=qF7pBzU4D20

- A scientific journal article discussing the successes and challenges of vaccination programmes:

Mallory, ML., Lindesmith, LC. and Baric, RS. (2018). Vaccination-Induced Herd Immunity: Successes and Challenges. *Journal of Allergy, Asthma & Immunology*, **142**(1), pp64-66.

Final Reflection

Deep Dive

BROUGHT TO YOU BY RBC ACCESS ED

Topic

Writing your Own Scientific Research Proposal...

Objectives

Using everything you have learnt over the last six resources about oncoviruses like HPV, screening methods and how molecular biology/virology research is conducted, write a research proposal for a scientific study on an oncovirus of your choosing. It is completely up to you what virus you choose to study and why, but you will need to write, what scientists call, a "rationale" for the project which is an introduction into the current research around the topic, highlighting what knowledge is missing, and how you proposed to find it out.

You could do a comparison study between two screening methods for a virus, or presence/absence study of the virus in different tissue types, or even a comparison of the prevalence rates of two different viruses. You could do a longitudinal study looking at the effect of a vaccination (an existing one though!) over a time period or investigate how long it takes for a persistent infection to develop in a certain cell type, or even a study on oncogenesis rates of oncoproteins using an animal model. The possibilities are endless!

Whatever you chose, you need to make sure that the results are 1) not already know, 2) there is enough evidence already indicating that this area needs researching into, and 3) you are genuinely interested and passionate about it – scientific research (especially laboratory-based) can be frustrating at times so a passion for the subject is what keeps most scientists going!

Instructions

Structuring the research proposal:

1. **Title**

Sometimes it is easier to write this last. To help, think about what the overall aim of your study will be and the methods you are wanting to use. Molecular biologists tend to be quite literal and concise with titles despite them being quite lengthy. I would suggest having a look at some examples online to help you construct this.

Final Reflection

Deep Dive

BROUGHT TO YOU BY RBC ACCESS ED

2. Aim & Objectives

This is easier to write after the literature review, rationale and methods has been started. The objectives are smaller checkpoints of success to achieve the overall aim (closely linked to the title). Think about what information is missing (i.e. incidence rates, prevalence rates, persistence, abundance, or all aspects of epidemiology) and the methods you want to employ to determine that information.

3. Literature Review & Rationale

The largest section of a research proposal where you gather and collate information on other people's studies in the area, you cite them (not required unless you what the practice for university!), compare them against each other, and analyse how you could improve on them. This usually includes your research questions written into the paragraphs with all the other information.

4. Study Design & Methods

Where you lay down all the finer details like time-frame, number of participants, sample type, costs and the proposed methodology (would help if you take note of the current methodologies being used in the area).

5. Ethical Considerations

As a research scientist, you will need to adhere to certain rules and regulations to ensure you inflict no harm upon anyone in anyway. There are several branches to ethics, but you are simply required to give a brief overview of what ethical issues you think may encounter and how you are planning on alleviating them e.g. recruiting participants under 18 = get their parents to sign the consent for them to participate or cutting tumours out of the mouths of participants = under anaesthetic, with written consent and after obtaining NHS ethical approval.

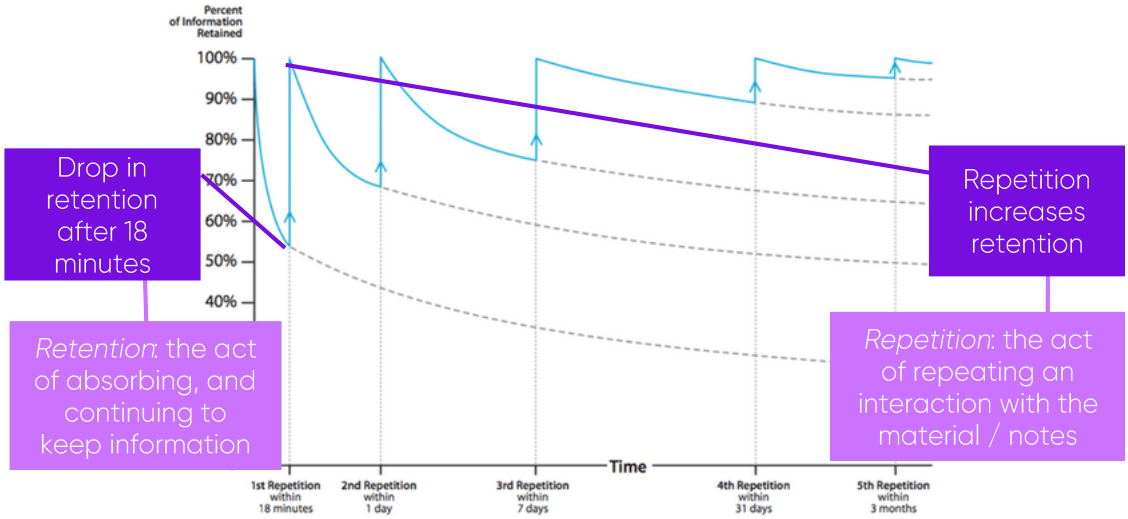
An example of a Master's level research proposal has been provided as a guide. You do not need to produce one as in depth as this one but it is a good length and will help you visualise how a scientific research proposal should be structured and what it should contain.

University Study Skills
Cornell Notes

Why is good note taking important?

If it feels like you forget new information almost as quickly as you hear it, even if you write it down, that's because we tend to lose almost 40% of new information within the first 24 hours of first reading or hearing it.

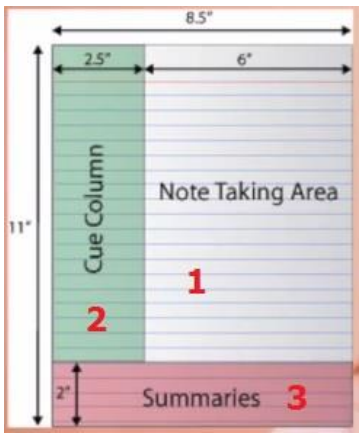
If we take notes effectively, however, we can retain and retrieve almost 100% of the information we receive. Consider this graph on the rate of forgetting with study/repetition:



Learning a new system

The Cornell Note System was developed in the 1950s at the University of Cornell in the USA. The system includes interacting with your notes and is suitable for all subjects. There are three steps to the Cornell Note System.

Step 1: Note-Taking



1. <u>Create Format</u>: Notes are set up in the Cornell Way. This means creating 3 boxes like the ones on the left. You should put your name, date, and topic at the top of the page.

2. <u>Write and Organise</u>: You then take your notes in area on the right side of the page. You should organise these notes by keeping a line or a space between 'chunks' /main ideas of information. You can also use bullet points for lists of information to help organise your notes.

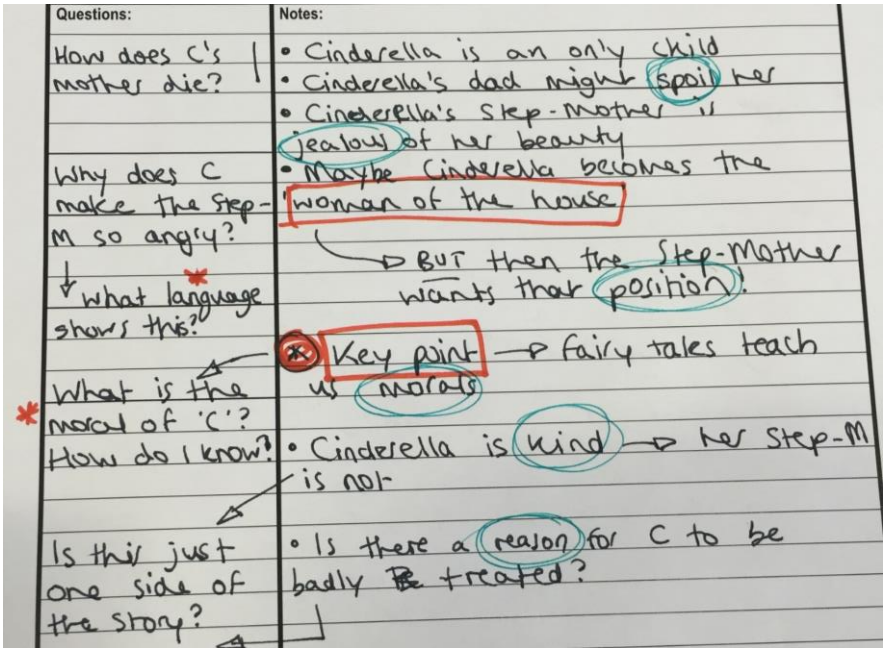
Step 2 Note-Making

1. Revise and Edit Notes: Go back to box 1, the note taking area and spend some time revising and editing. You can do this by: highlighting 'chunks' of information with a number or a colour; circling all key words in a different colour; highlighting main ideas; adding new information in another colour

2. Note Key Idea: Go to box 2 on the left hand side of the page and develop some questions about the main ideas in your notes. The questions should be 'high level'. This means they should encourage you to think deeper about the ideas. Example 'high level' questions would be:

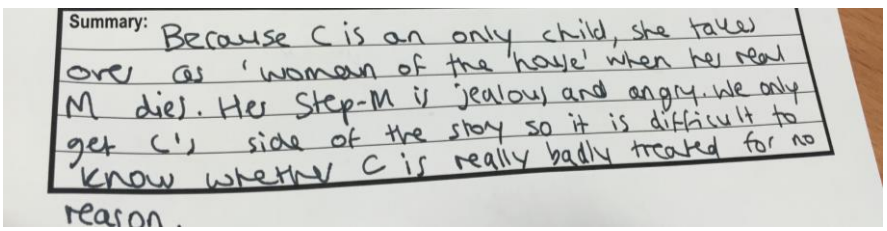
- Which is most important / significant reason for...
- To what extent...
- How does the (data / text / ideas) support the viewpoint?
- How do we know that...

Here is an example of step 1 and step 2 for notes on the story of Cinderella:



Step 3 Note-Interacting

1. Summary: Go to box 3 at the bottom of the page and summarise the main ideas in box 1 and answer the essential questions in box 2.



Give the Cornell Note Taking System a try and see if it works for you!

University Study Skills
Key Instruction Words

These words will often be used when university tutors set you essay questions - it is a good idea to carefully read instruction words before attempting to answer the question.

Analyse – When you analyse something you consider it carefully and in detail in order to understand and explain it. To analyse, identify the main parts or ideas of a subject and examine or interpret the connections between them.

Comment on – When you comment on a subject or the ideas in a subject, you say something that gives your opinion about it or an explanation for it.

Compare – To compare things means to point out the differences or similarities between them. A comparison essay would involve examining qualities/characteristics of a subject and emphasising the similarities and differences.

Contrast – When you contrast two subjects you show how they differ when compared with each other. A contrast essay should emphasise striking differences between two elements.

Compare and contrast – To write a compare and contrast essay you would examine the similarities and differences of two subjects.

Criticise – When you criticise you make judgments about a subject after thinking about it carefully and deeply. Express your judgement with respect to the correctness or merit of the factors under consideration. Give the results of your own analysis and discuss the limitations and contributions of the factors in question. Support your judgement with evidence.

Define – When you define something you show, describe, or state clearly what it is and what it is like, you can also say what its limits are. Do not include details but do include what distinguishes it from the other related things, sometimes by giving examples.

Describe – To describe in an essay requires you to give a detailed account of characteristics, properties or qualities of a subject.

Discuss – To discuss in an essay consider your subject from different points of view. Examine, analyse and present considerations for and against the problem or statement.

University Study Skills
Key Instruction Words

Evaluate – When you evaluate in an essay, decide on your subject's significance, value, or quality after carefully studying its good and bad features. Use authoritative (e.g. from established authors or theorists in the field) and, to some extent, personal appraisal of both contributions and limitations of the subject. Similar to **assess**.

Illustrate – If asked to illustrate in an essay, explain the points that you are making clearly by using examples, diagrams, statistics etc.

Interpret – In an essay that requires you to interpret, you should translate, solve, give examples, or comment upon the subject and evaluate it in terms of your judgement or reaction. Basically, give an explanation of what your subject means. Similar to **explain**.

Justify – When asked to justify a statement in an essay you should provide the reasons and grounds for the conclusions you draw from the statement. Present your evidence in a form that will convince your reader.

Outline – Outlining requires that you explain ideas, plans, or theories in a general way, without giving all the details. Organise and systematically describe the main points or general principles. Use essential supplementary material, but omit minor details.

Prove – When proving a statement, experiment or theory in an essay, you must confirm or verify it. You are expected to evaluate the material and present experimental evidence and/or logical argument.

Relate – To relate two things, you should state or claim the connection or link between them. Show the relationship by emphasising these connections and associations.

Review – When you review, critically examine, analyse and comment on the major points of a subject in an organised manner

University Guidance

Exploring Careers and Study Options

- ✓ Find job descriptions, salaries and hours, routes into different careers, and more at https://www.startprofile.com/
- ✓ Research career and study choices, and see videos of those who have pursued various routes at http://www.careerpilot.org.uk/
- ✓ See videos about what it's like to work in different jobs and for different organisations at https://www.careersbox.co.uk/
- ✓ Find out what different degrees could lead to, how to choose the right course for you, and how to apply for courses and student finance at https://www.prospects.ac.uk/
- ✓ Explore job descriptions and career options, and contact careers advisers at https://nationalcareersservice.direct.gov.uk/
- ✓ Discover which subjects and qualifications (not just A levels) lead to different degrees, and what careers these degrees can lead to, at http://www.russellgroup.ac.uk/media/5457/informed-choices-2016.pdf

Comparing Universities

- ✓ https://www.whatuni.com/
- ✓ http://unistats.direct.gov.uk/
- ✓ https://www.thecompleteuniversityguide.co.uk/
- ✓ Which? Explorer tool – find out your degree options based on your A level and BTEC subjects: https://university.which.co.uk/

UCAS

- ✓ Key dates and deadlines: https://university.which.co.uk/advice/ucas-application/ucas-deadlines-key-application-dates
- ✓ Untangle UCAS terminology at https://www.ucas.com/corporate/about-us/who-we-are/ucas-terms-explained
- ✓ Get advice on writing a UCAS personal statement at https://www.ucas.com/ucas/undergraduate/getting-started/when-apply/how-write-ucas-undergraduate-personal-statement
- ✓ You can also find a template to help you structure a UCAS statement, at https://www.ucas.com/sites/default/files/ucas-personal-statement-worksheet.pdf
- ✓ How to survive Clearing: https://university.which.co.uk/advice/clearing-results-day/the-survivors-guide-to-clearing

Subject Guidance

Biomedical Science at University

- ✓ Biomedical scientists utilise molecular biology to carry out a range of scientific tests to help diagnose and treat diseases.
- ✓ Biomedical scientists work within a laboratory, using a variety of technical equipment, so need good analytical skills and technical abilities.
- ✓ You can find out more about different courses and entry requirements by exploring the UCAS Biology Guide online: https://www.ucas.com/ucas/subject-guide-list/biological-sciences
- ✓ You can find out more about the different careers by exploring the UCAS Biomedical Scientists Careers online; https://www.ucas.com/ucas/after-gcses/find-career-ideas/explore-jobs/job-profile/biomedical-scientist

A Deeper Look Into HPV

- ✓ **Watch:** Published in July 2018, a news broadcast stating how the UK government has announced that the vaccination will become gender-neutral: www.youtube.com/watch?v=WJU95mFDRtw
- ✓ **Listen:** A podcast from 2015, interviewing Dr. Marcus Monroe about HPV and the cancers it can cause, specifically HNSCCs, and about the HPV vaccination: https://healthcare.utah.edu/the-scope/shows.php?shows=0_1p5siftj
- ✓ **Read:** In October 2018, HPV researchers co-wrote a HPV newsletter discussing HPV screening strategies to help eliminate HPV positive cervical cancer. It is a comprehension read but a pivotal moment in the history of HPV research: http://cogi-congress.org/wp-content/uploads/2018/11/HPW_2018_final.pdf
- ✓ **Think About This Exciting Prospect:** Disease Elimination
  - How close we are to eliminating HPV positive cervical cancer
  - How successful the HPV vaccination programmes have been
  - How males all over the world are starting to receive the vaccination too
  - How much research is being conducted on all the other types of HPV cancer

One day scientists may be able to **eliminate ALL HPV positive cancers in every continent**, and if this can be done for HPV, what is stopping it being done for other viruses that cause diseases?

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