Research
Based
Curricula

The Role of Human Papillomavirus (HPV) in Cancer

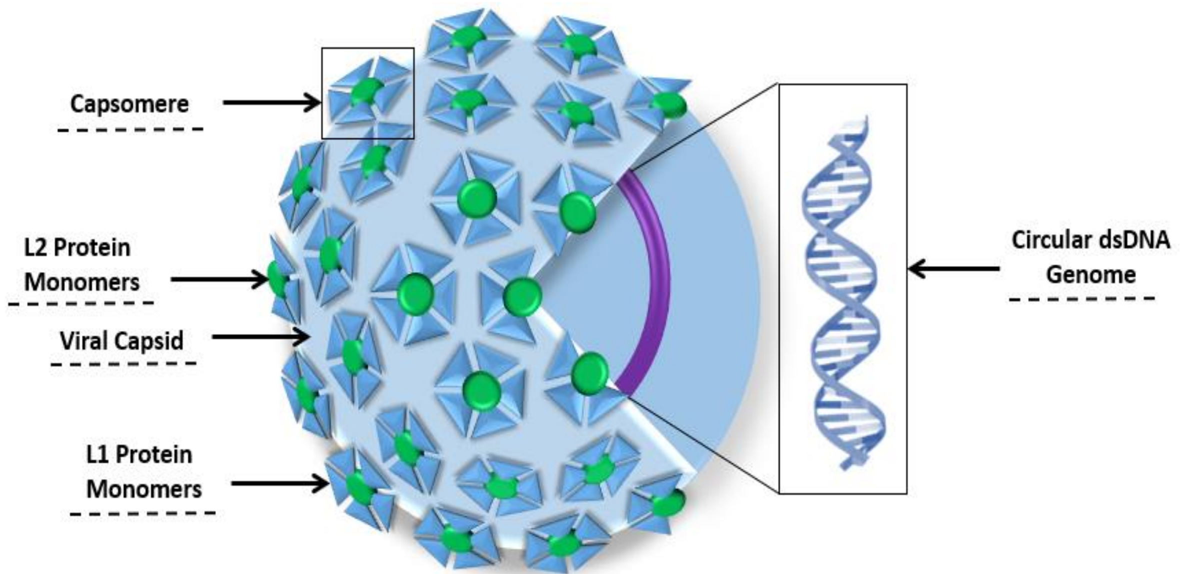
Key Stage 5 Biology

Model Answers

2019

Resource One
Model Answers

Answers 1. Labels –



2. Table -

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
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Answers

3. Please note: key points and terms in the answers are bolded.

a) There are over **200** different HPV genotypes.

b) Low-risk HPV genotypes can cause **benign tumours** like warts whilst high-risk HPV genotypes can cause **cancerous tumours** due to oncogenic properties.

c) HPV has been found to cause **genital** (especially cervical), **anal**, **lung** and **head and neck cancers**.

d) HPV is transmitted from person to person by **sexual contact, touch and perinatally**. It is also possible that HPV may be transmitted through blood.

e) Enveloped viruses **obtain components of the lipid bilayer from a host cell**, therefore, must remain **within a host to survive for long periods of time** whilst **non-enveloped viruses** like HPV can **exist outside of host cells**.

4. Examples of possible questions (can also be multiple choice):

- What is your age?
- What is your gender?
- What is your sexual orientation?
- Are you sexually active and have you had sex within the last year? (Remember HPV infections can be cleared within 18 months of exposure)
- What is the total number of sexual partners you have had?
- How many people have you had sex or sexual contact with in the last year?
- Are you currently in a monogamous relationship and how long have you been in this relationship?
- What types of sexual activity have you engaged in?
- Do you use barrier contraception and how often?

Resource One
Model Answers

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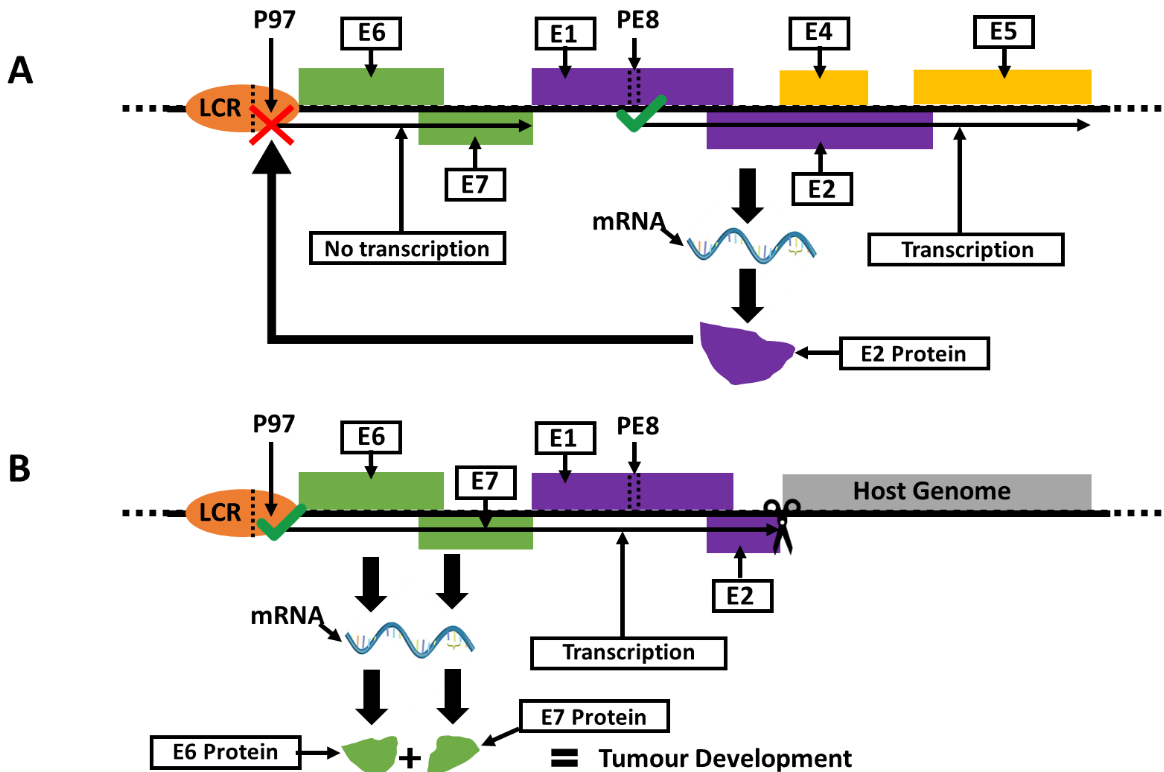
- How often do you have "one-night stands"?
- How many of your total number of sexual partners were "one-night stands"?
- Have you ever had a sexually transmitted infection, to your knowledge (including HPV)?
- Have you ever had sex with someone who, to your knowledge, has had a sexually transmitted infection?
- Do you currently have an HPV infection, to your knowledge?
- Have you ever been diagnosed with an HPV-positive related cancer or precursor?
- Have you had the HPV vaccination?

Resource Two
Model Answers

Answers

1. Labels –



2. Answers –

a) Part A shows **initial HPV gene expression** after the genome has entered the host's nucleus where **E2 is expressed which means E6 and E7's transcription is disrupted**. Part B then shows after the **HPV genome integrates** with the host's genome, the **E2 sequence is partially lost** so no longer can be transcribed. Instead, **E6 and E7 transcription** is no longer regulated so expression of the oncogenes starts.

b) It is not completely understood why this is the case, but one theory is to allow the virus to **firstly replicate its genome** to create many copies for future HPV virions that will be produced later, in order to spread the infection and **continue its life cycle**. If the virus integrated straight away, it would not be possible to continue its life cycle.

Resource Two
Model Answers

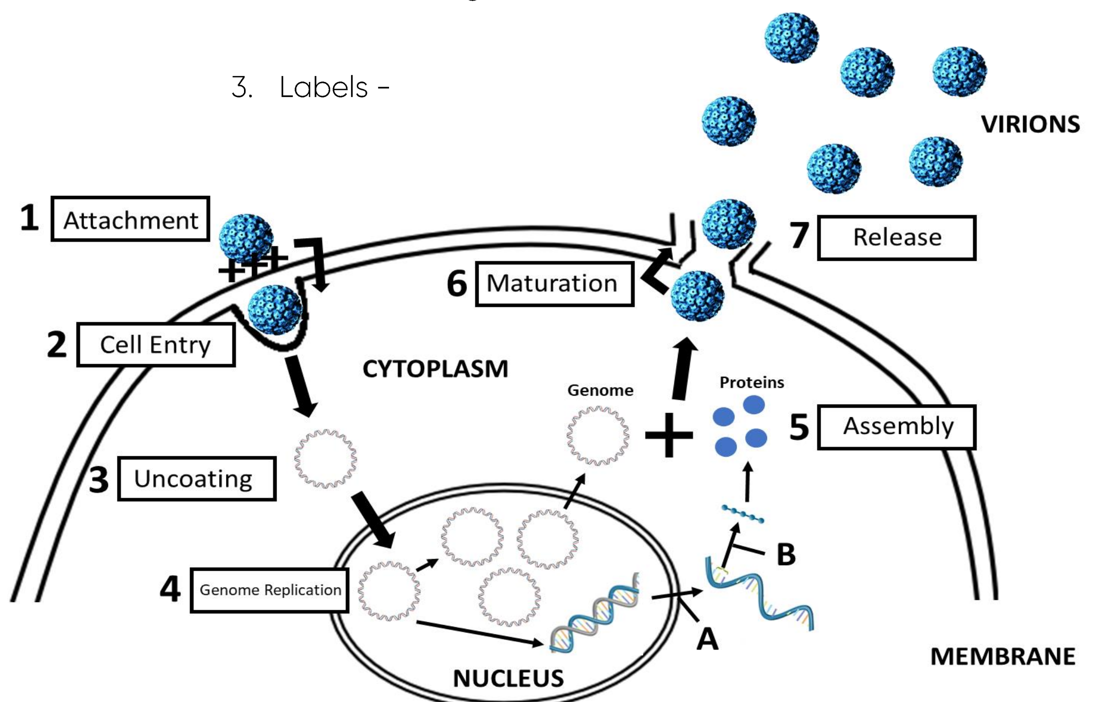
Answers

c) **Both proteins will be expressed when the sequence is translated.** Also, please note, that multiple proteins can be expressed from one sequence of DNA dependent on how it is read.

-Explanation- If you continue to study Genetics in the future, you will come across a term known as a "reading frame". One dsDNA sequence has six reading frames; three on either strand (5' and 3'). To translate genetic code into an amino acid, we read codons which are triplet sets of bases (e.g. ATC). A sequence can start being "read" from any one of those bases in the first codon of the sequence. So, in this case, A is the first frame, T is the second and C is the third. Because sequences' bases are always read in triplet, reading from the second (T) or third (C) will produce a completely new code of amino acids. For example, say our sequence was... ATC ATT CAT... If we read from T instead of A (it hasn't disappeared, it is just ignored!), the new triplet sequence would be... TCA TTC AT... and the same with reading from C instead of AT, the new triplet sequence would be... CAT TCA T... This way, we can produce multiple proteins from the same sequence of DNA just by reading it differently.

d) They would need to test for **E6 and E7** <u>**proteins**</u> and the loss of the **E2** <u>**gene**</u>.

3. Labels -



Resource Two Model Answers

Answers

4. The first arrow labelled (A) represents transcription of viral dsDNA into mRNA whilst the second arrow labelled (B) represents translation of mRNA into viral proteins. These two processes are important for viral replication because they allow capsid proteins to be expressed which when assembled, create new viral particles to continue spreading the virus and infecting new cells.

5. Firstly, at **stage 4** of the life cycle (**genome replication**), the infection is **latent within basal epithelial cells**. At this stage, **E1 and E2 are being expressed** in the host's nucleus for **low level viral genome replication** which does **not disrupt the host cell**.

As the cell differentiates into **spinous and/or granular epithelial cells**, **stage 4** of the life cycle continues but the **rest of the early genes are now also expressed**. **E4 and E5** are expressed in preparation of the production of new virions (used in **assembly, release and immune system evasion** meaning **stage 5 has also been entered**), whilst **E6 and E7 bind** to and disrupt host's proteins (**p53 and pRB**) but only in the case of **high-risk genotypes and if viral genome has integrated into the host's genome**.

The final cell differentiation into **cornified cells** sees life cycle **stages 5, 6 and 7** (assembly, maturation and release) where **L1 and L2 are now expressed** to create the viral capsid. As the cells degrade, newly produced **HPV virions are released** and go on to spread the infection further

Resource Three Model Answers

Answers

Answers to Activity 1:

Answers to part 1:

From the largest genome to smallest genome…

EBV, KSHV, HCV, HTLV-1, HPV, MCPyV, BKPyV, JCPyV, HBV

Answers to part 2 questions:

a) Ordered by **year of discovery**

b) Polyomaviruses -*Explanation- Polyomaviridae is the family name*

c) It means that the single-stranded RNA genome is **transcribed in the 5'-3' direction** -*Explanation- like it was a dsDNA genome that has been unzipped, also known as the "sense strand"*

d) Hepatitis B Virus (HBV), family name = *Hepadnaviridae*

e) Merkel Cell Polyomavirus (MCPyV)

f) Similarities: **(2)**

Name & both are associated with hepatocellular carcinomas (liver cancer)

Differences: **(5)**

Family names (Taxonomy), HBV is a DNA virus with two DNA genome structure whilst HCV is a RNA virus with one genome structure, HCV has a larger genome than HBV, HCV is also linked to Non-Hodgkin Lymphoma unlike HBV & HCV was discovered 24 years after HBV

g) EBV, JCPyV, HTLV-1, HCV and KSHV

h) BKPyV, and the two forms of bone cancer listed are Osteosarcomas and Ewing's Sarcoma

Answers to Activity 2:

Examples of good discussion points and research questions:

- Does having two or more oncogenic viruses in the same cell speed up oncogenesis, and worsen the prognosis of that particular type of cancer?

Resource Three Model Answers

Answers

- Do different oncogenic virus pairs induce oncogenesis more efficiently than others/not at all?
- Do they combine transformation mechanisms (i.e. functions of the oncoproteins) or promote oncogenesis separately but at the same time in the same cell?
- Does co-infection and ability to transform cells depend on the cell/tissue type that the viruses have infected i.e. does unstable conditions such as frequent cell differentiation provide a more suitable environment for oncogenesis?
- Do the viral oncoproteins from different viruses actually compete for the tumour suppressor proteins (like inhibitors of enzymes) and hinder oncogenesis instead?
- If the ability to transcribe oncogenes has been lost by integrating into the host genome, does another oncogenic virus have the ability to reverse this and effectively "help" the other?
- What oncoproteins work together i.e. do ones targeting different cell cycle proteins work better or does oncogenesis speed up as a result of both/all targeting the same host protein?
- What effect does two or more oncogenic viruses have on establishing latency or maintaining a persistent infection?
- Does abundance/the amount of oncoprotein expression effect the ability to work together to transform cells?
- Do other environmental factors such as location of the cells, cell type, prior cellular DNA damage, host immunity, host gender and host genetics play a role in helping or hindering oncogenesis via two or more viruses?

Answers to Activity 3:

a) A reference gene/protein acts as a control for the blot to **ensure that the method worked in every cell line**, that there is **no damage to the cell lines/extracted proteins**, and as a comparison of "normal expression levels".

b) The control cell line

c) Actin = "normal expression", pRB = significant reduction in the normal amount present, and p53 = "normal expression"

Resource Three Model Answers

Answers

d) The 16 E6E7 cell line because it must be expressing E6 and E7 which causes **downregulation of both tumour suppressor proteins** which is shown in the blot.

e) 1) The HPV infection could be low-risk so the oncoproteins are not expressed or 2) The viral genome may have not integrated into the host genome yet (if high-risk) so the E2 proteins are still preventing transcription of the oncogenes.

f) There are two possible answers as the topic is controversial: 1) No change if LT-ag expression is lost after integration or 2) p53 would be downregulated in response to the LT-ag.

Resource Four
Model Answers

Answers

Answers to Activity 1:

Possible answers to part 1:

- **Metastasis** = Cancerous tumours can form secondary malignant tumours at new sites within the host by transporting cancerous cells through the blood and/or lymphatic vessels, usually through the invasion of tumour masses into the walls of blood and/or lymph vessels.
- **Angiogenesis** = Cancerous tumours can stimulate the growth of new blood vessels from pre-existing ones and draw them to them for a nutrient supply. This process is caused by the release of chemicals by the tumour and by host cells near the tumour.
- **Invasive** = Cancerous tumours grow rapidly until they extend and penetrate neighbouring normal healthy tissues, vessels or organs.
- **Immortal** = In terms of cancerous tumours, this is defined as recurrent growth through the introduction of new gene mutations or faults, rapid cell growth, metastasis, and becoming resistant to treatment. Cancerous cells can ignore the signals for apoptosis (programmed cell death) so just continue growing.
- **Proliferation** = An increase in the number of cells by cell growth and cell division. Cancerous tumours undergo unchecked cell growth as mutations in genes (or interactions by oncoproteins) accelerate cell division rates and/or inhibit normal controls on the system, such as cell cycle arrest or apoptosis.
- **Genetic/Genome Instability** = There is a high frequency of gene mutations or DNA damage in tumours which can be hereditary, caused by pathogen like a virus or due to recurrent unchecked cell divisions.
- **Undifferentiated Cells** = Cancerous tumours can control cells or tissues to stop them differentiating into specialized ("mature") structures. These immature cells (anaplastic cells) are more malignant as they can often grow and spread quickly.

Resource Four
Model Answers

Answers

Answer to part 2:

A = Normal Cervix

B = Cervical Intraepithelial Neoplasia (CIN)

C = Cancer (Invasive)

D = Grade 2

E = Virions/Viral Particles

F = Superficial Zone/Layer

G = Midzone/Intermediate Layer

H = Basal Layer

I = Viral Genome/Episome Present

J = Genome Integration

K = Nuclei with episomal viral DNA/genome present

Answers to Activity 2:

a) It's around 450bp as it is **between 400bp and 500bp** on the DNA ladder.

b) The **larger the DNA sequence** of the product/fragment from the PCR/qPCR, the **slower they separate** from each other and move through the gel, so the largest sequences of the ladder will be closer together.

c) It represents **copy number/amount of the DNA** product/fragment present. It tells scientists that 1) the target gene is **present** in the samples and 2) gives them idea **how much** is present.

d) Controls are used to **check the method** has worked and **samples are compared** against them.

e) The products are **less than 100bp in length** and are **another product that has been amplified** in the PCR/qPCR reaction.

-Explanation- These products are actually called "primer-dimers" and are around 20-50bp in length. Primers are short DNA sequences of 20bp, on average, that are designed to attach to a section of a target gene sequence during a PCR/qPCR reaction for amplification to

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occur. There are always at least two types of primers as a minimum; a forward primer and a reverse primer for both strands of dsDNA. Sometimes the primers are not specific enough to the target and can either 1) bind to another sequence of DNA in the sample which is called non-specific binding, or 2) bind to each other causing primer-dimers, especially in the absence of the target DNA sequence (otherwise known as "template") and/or with adding a high concentration of primers to the reaction.

f) The gel thickness should be **increased because smaller DNA products/fragments will travel faster** through the agarose gel.

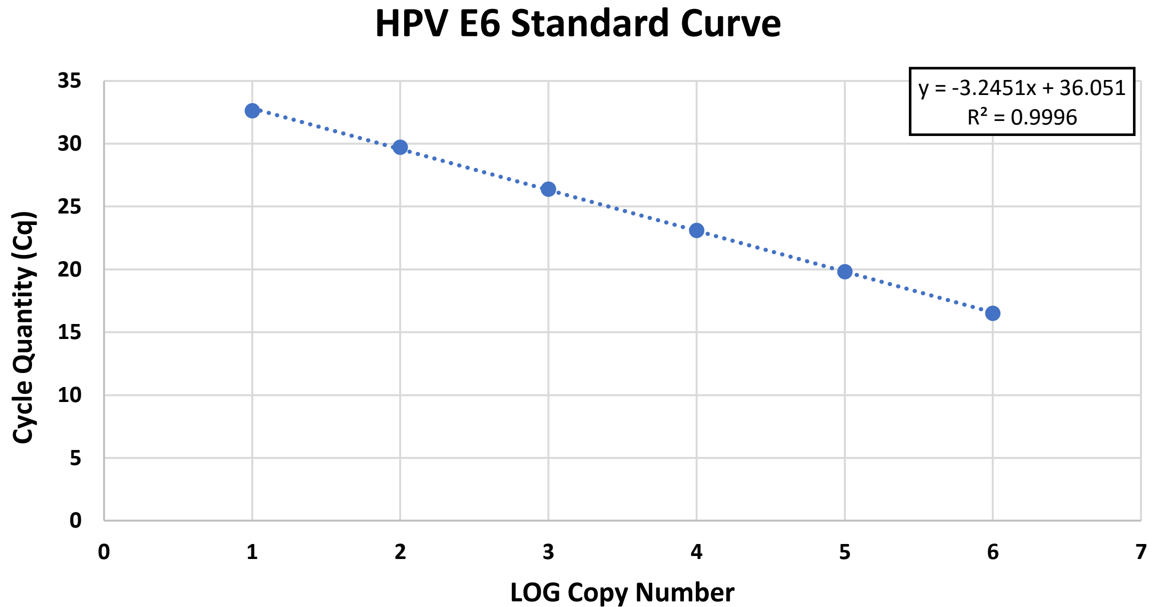
-Explanation- If the gel is too thin, the smaller products could quickly end up running over the edge of the gel. They cannot be recovered if this happens. Scientists also adjust the voltage to determine how quickly DNA will travel through the gel but if this is turned up too high, the bands usually curve into a sort of "upside down smile shape" affecting separation.

Resource Five
Model Answers

Answers

Answers to Activity 1:

Ideal figure produced for parts 1 & 2:



Answers to part 2 questions:

a) Negative correlation

b) As cycle quantity (Cq) increases, copy number decreases.

-Explanation- The higher the number of copies of the target gene in a sample, the quicker the amplification fluorescence is detected by the thermocycler resulting in low Cq values for high copy numbers and high Cq values for low copy numbers.

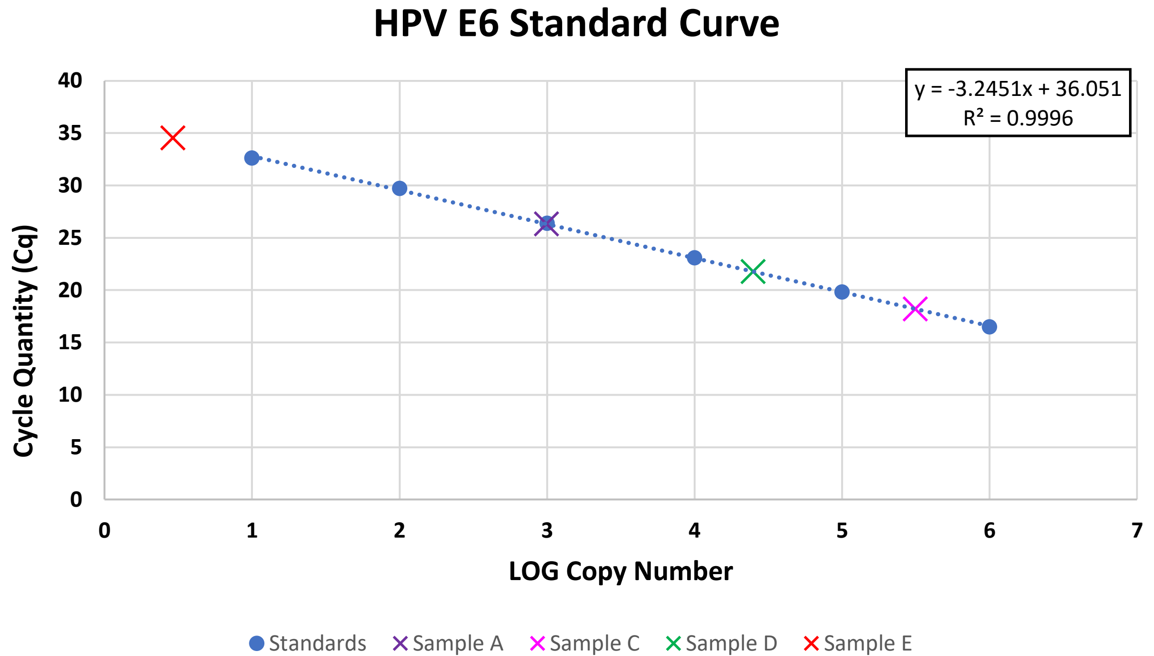
c) Both accuracy indicators of the standard curve are within the ideal recommended parameters. The R2 value is 0.9996 which is only 0.0004 away from the ideal value of 1, whilst the slope (m) value is -3.2451 which is only 0.0749 from -3.32, the ideal number of cycles between each of the standards. Due to this, the standard curve can be deemed accurate enough to use for sample quantification.

Resource Five
Model Answers

Answers

Answers to Activity 2:

Ideal table & figure produced for parts 1 & 2:



Answers to part 2 questions:

a) For samples B and F, no amplification fluorescence passed through the thermocycler's threshold meaning no Cq value production. This could indicate HPV negative samples.

b) Sample C has the most abundant HPV infection as the highest number of copies of the E6 gene were calculated from the Cq value detected. Sample C's actual copy number is 314649.82 copies/reaction.

c) Sample E cannot be accurately quantified as it has a copy number so low that it does not sit on the line of best fit, so it does not fall between the highest and lowest known copy number standard.

d) Increase the number of replicates used per sample, calculate an average Cq value and standard deviation.

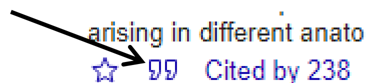
Resource Six
Model Answers

Answers

Activity 1 (Parts 1-2):

-Advisory section for the students and the teacher of the resource rather than answers due to independent research being required-

- Make sure the articles found are scientifically reliable and accurate from pages that are peer-reviewed and cannot be edited like Wikipedia. If the original journal article that the information has been taken from can be found, this is preferable as this will be expected from students at university.
- Try to use Google Scholar to find the most recent scientific journal articles from 2017-2018. The dates can be selected to the left-hand side of the search engine.
- Start trying to get to grips with citing and referencing in different styles by clicking on the speech marks sign under the articles, and recording them in a referencing style of the student's choice. Make sure the same is used for all articles though for consistency i.e. Harvard (most commonly used in UK universities).



- Would stick to about 10 mins maximum for the debate between different groups' findings and record the information on a board or some paper for them to then use to collate the data from everyone into a table in part 3.
- The teacher of the resource should act as the chair of the debate, keeping the students on topic and allowing all students with valid points and reliable research to contribute to the discussion.
- The students should discuss their findings, and the differences and similarities between other groups' findings. The discussion should focus on why the results published in the articles differ (or are similar) looking at the study cohort and demographics, the methodology used to determine the results, and the conclusions. Students should start to critically evaluate the sources of the information in terms of reliability and accuracy of the data within.

Resource Six Model Answers

Answers

For example:

Source A revealed a reduction rate in detecting HPV of 80% but used a younger study cohort and a more-sensitive DNA detection method compared to source B.

Activity 1 (Parts 3-4):

-Advisory section for the students and the teacher of the resource rather than answers due to independent research being required-

- The teacher should either construct the table on a board with the whole group completing it together or encourage the students to produce a table in their in their smaller groups/pairs. Either way, the table should be constructed per continent or by grouping the main countries looked at together rather than every individual country as it may take too long if all are included.
- It is recommended that the similarities are highlighted in one colour and differences in another, or arrows drawn linking two or more countries/continents, or have the table laid out with a column per one and the countries/continents as the rows. Students can even draw a spider diagram instead, if they would prefer.
- The teacher should then start to encourage the students to start thinking about the wider impacts and affecting factors of vaccinations in part 4; such as convincing governments to issue laws and licenses, and then funding to produce the vaccines. There are many variables that must be considered to make an impact with scientific research.
- If there is time, perhaps the suggestions for improvement in part 4 could also be discussed as a whole group as well to compare and contrast ideas, to help to determine the practicalities of the suggestions and to examine ideas that other groups may have not thought about.

Final Reflection Model Answers

Guidance

Masters Level Research Proposal Example

Title:

Determining the level of infectivity and localisation of Merkel Cell Polyomavirus (MCPyV) in multiple cancerous and healthy tissues.

Aim & Objectives:

The overall aim of the study is to determine the level of infectivity, abundance and incidence of MCPyV in multiple cancerous and healthy tissues. In order to do this, several objectives need to be outlined and met first.

Objectives:

- To collect a range of different types of cancerous and healthy human tissues using available tissue banks sources. Potential tissues will vary in type from cutaneous to mucosal dependent on availability. Lung, vulva, ovarian and skin samples would be ideal for MCPyV detection. Skin cells samples may also be collected from healthy volunteers to determine the rate of MCPyV infection within the population.
- To design suitable MCPyV viral DNA primers to enable the detection of MCPyV by PCR in collected tissues to determine rates of infection.
- To isolate MCPyV proteins from healthy and cancerous tissues to investigate viral protein expression levels within samples in order to determine if expression levels of the oncogenic protein LT-ag varies in tissue type and/or disease status.
- To investigate cellular localisation of MCPyV viral proteins in donated tissues using fluorescence in-situ hybridisation (FISH) to establish if viral localisation changes between tissue type and/or disease status.

Final Reflection Model Answers

Guidance

Literature Review & Rationale:

The current literature indicates that MCPyV (Merkel Cell Polyomavirus) may play an important role in oncogenesis, especially within MCCs (Merkel cell carcinomas) (Errichetti *et al.*, 2013). However, the virus has also been detected in several non-MCCs such as cervical carcinomas (Imajoh *et al.*, 2012) and extrapulmonary small

cell carcinomas (Hourdequin *et al.*, 2013) revealing some degree of target cell non-specificity and suggesting some involvement in non-MCC tumorigenesis also. With limited studies into what cell type can be affected by MCPyV, there is a need to research into all possibilities; cancerous and healthy tissues alike. By determining what tissues are targeted by MCPyV; an insight into infectivity rates within the population can be established, MCPyV's mode of transmission could be confirmed, and evidence could be discovered to indicate a primary target cell to function as its viral reservoir.

Furthermore, MCPyV's association with cervical carcinomas and cutaneous squamous cell carcinomas could provide possible links to vulvar cancer and/or the precursor VIN (vulval intraepithelial neoplasia). This is due to locality and cell type infectivity; one type of vulvar cancer is an adenocarcinoma which MCPyV DNA has been detected in but within non-small cell lung cancer (Joh *et al.*, 2010). Vulvar cancer is thought to be caused by two main factors; a persistent HPV infection (Insinga *et al.*, 2008) and VIN (also highly linked to HPV infection) (Kokka *et al.*, 2011). Unfortunately, the main treatment is surgery for tumour excision and a vulvectomy (Höckel *et al.*, 2010), however, potential new treatments could be developed if viruses, perhaps collectively, were found to play a major role in the transformation of the vulval squamous cells. HPV and MCPyV have only been detected together in two types of carcinomas; non-small cell lung cancer and one cutaneous

Guidance

squamous cell carcinoma sample (Joh *et al.*, 2010); further research into multiple types of cancerous and healthy tissues could lead to discoveries to consolidate previous findings and provide evidence of MCPyV acting as an oncogenic co-factor.

Fortunately, viral screening molecular techniques that can detect viral DNA have already been established at the institution, which can be applied to this project as laboratory protocol would be very similar. The techniques were developed whilst screening human tissue for HPV using PCR so the same strategy could be applied to detect MCPyV DNA by PCR within a range of cancerous and healthy tissues. The project also would aim to establish methods of viral protein expression detection by aiming to detect the LT-ag oncoprotein within both types of tissues. By screening multiple types of cancerous and healthy human tissues, there is potential to discover MCPyV viral DNA and/or protein expression present within tissues in which may have not been presented before.

Study Design & Methods:

- Collect a range of cancerous & healthy tissue samples from tissue bank sources.
- Extraction of DNA and protein from the cancerous and healthy samples. DNA extraction will occur via the Qiagen DNeasy kit, whilst protein extraction methods will be devised depending on the previous fixation method (ethanol or Formaldehyde) of the tissue.
- Epithelial skin cell samples may also be collected by swabbing the foreheads of human participants, DNA and protein will also be isolated from these samples by similar methods as briefly stated above.

Guidance

- PCR will be performed in order to detect any MCPyV infection within the tissues and skin samples whilst using a cell line for MCPyV as +ve control.
- Extracted viral proteins will be examined by western blotting to look at MCPyV large T protein expression levels within the tissues.
- FISH (fluorescence in situ hybridization) using antibodies designed to detect a range of viral proteins such as the major capsid (VP1) or large T antigens (LT-ag). FISH combined with fluorescence microscopy will allow detection and localisation of the specific viral proteins within the cells of healthy and cancerous tissues.
- Data collection and results interpretation.
- Project write-up and completion.

Ethical Considerations:

Overall, the project will have two types of ethical considerations dependent on tissue sample availability and participants. The first consideration will be obtaining consent from human participants to allow the collection of their skin samples for use in the project; screening for MCPyV DNA/protein expression. The consent will be collected via forms signed by the human participants for permission to use skin samples. All consent forms will be kept confidential and only linked to the participant via a unique identification number to ensure anonymity. These forms will then also be destroyed at the end of the study for data protection purposes. Also, in terms of the human participants themselves, no physical harm will occur through sample collection.

The second type of ethical considerations relevant to this study would be NHS ethics in terms of handling human tissue samples and patient details derived from the XXXXXX Hospital* and/or other NHS tissue bank sources.

**Please note, some details have been removed for confidentially of the study.*

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