OCR
RECOGNISING ACHIEVEMENT

ADVANCED SUBSIDIARY GCE **2858/01**

HUMAN BIOLOGY

Case Studies

INSERT 1

MONDAY 4 JUNE 2007

Morning

Time: 45 minutes

CUP/T24088

INSTRUCTIONS TO CANDIDATES

- Questions 1 and 2 are based on the articles which follow on pages 2 to 5 of this insert.

This insert consists of **6** printed pages and **2** blank pages.

Case Study 1

CYTOLOGICAL STAINING

The diagnosis of many conditions often depends on the analysis of tissue samples removed during a biopsy or other surgical procedures. It is essential that the tissues do not degrade before examination and so all the samples are placed immediately in labelled plastic pots containing a fixative. This fixative is normally a solution of formalin in a buffer. This solution causes the proteins in the tissue cells to denature. The solution also contains various salts and it is isotonic. Each pot is immediately labelled with the patient's details, details of the procedure used and the tissue sampled. These details are in code and every slide taken from this sample will carry this code.

In most cases, the tissues will be prepared for examination by cutting thin sections and then staining the sections. To make the cutting of thin sections easier, the tissues must be embedded in wax. Since cytoplasm is water-based and wax is non-polar, the water must be gradually removed from the tissues by a series of alcohol 'soaks' followed by a period in xylene and finally in paraffin wax. This process is largely automated but the cutting and selection of sections is done by hand. This is a skilled job carried out by Biomedical Scientists in Histopathology and Cytology laboratories. The wax is then removed from the sections by reversing the steps carried out earlier. After staining the sections, they are examined using a light microscope.

The two commonest stains used are haematoxylin and eosin. Most other dyes used in histology and cytology are synthetic but there is no synthetic equivalent of haematoxylin. Haematoxylin is extracted from the heartwood of a tree, *Haematoxylum campechianum*, which was discovered by Spanish explorers in 1525 at Campeche Bay in the Gulf of Mexico. The genus name *Haematoxylum* is derived from two greek words: '*aimatos*' which means *blood*, and '*xylon*' which means *wood*. The haematoxylin which is used is extracted from the heartwood of this *bloodwood* tree. The tree can be found widely distributed in Central America in areas of low forest. This is not rainforest but it is flooded during the rainy season. Like many other types of forest, the low forest of Central America is influenced by human actions but is intact in areas designated as National Parks, for example, Sierra del Lacandon National Park in Guatemala.

In slides stained with a combination of haematoxylin and eosin, (H & E), nuclei stain purple and cytoplasm stains pink. Collagen fibres also stain pink. The H & E stain is often used to stain sections of blood vessels. If the elastic fibres in the walls of blood vessels need to be distinguished from collagen fibres, then a combination of haematoxylin and orange G-erythrosin is used. This second combination stains collagen orange and the elastic tissues bright pink.

Other stains can be used to highlight elastic fibres and one such stain is orcein. The standard method for the demonstration of elastic fibres is to apply orcein in an acidic, alcoholic solution. This same solution has recently been shown to be effective for staining the rough endoplasmic reticulum of liver cells infected with the Hepatitis-B virus, and it is now commonly used for that purpose. The appearance of the slides can be very distinctive with the cytoplasm having the appearance of ground glass. Orcein was originally obtained from lichens, although synthetic orcein is now used.

Different tissues may require different staining procedures. H & E stains the cytoplasmic organelles in all types of white blood cells similar colours. For this reason, stains based on methylene blue and eosin, such as Wright's stain and Leishman's stain, are preferred specifically for staining blood. These stains allow Biomedical Scientists to identify the different kinds of white blood cells present.

Case Study 2

CYTOLOGICAL SCREENING

Rob, an AS level student, is interested in applying for University courses in Biomedical Science. As part of a work placement in his local hospital he spends a day in the Cytology laboratory. He interviews Anna, a young scientist working in Pathology, on the work carried out by the laboratory.

Rob: *So is everyone working in the lab a Biomedical Scientist?*

Anna: Not at all. We have MLAs – medical lab assistants – who book in and log the samples and assist with the labelling and mounting, but most of the people you see working at the moment are Cytoscreeners.

Rob: *Cytoscreeners? Is that a separate job then?*

Anna: Well some Cytoscreeners can be eligible for state registration as Biomedical Scientists, but the intention is to make Cytology Screening a state registered profession in its own right – hopefully by 2007!

Rob: *So is cytoscreening an important job?*

Anna: I should say so. Cytoscreeners are responsible for examining the 23 000 or so smears we get in each year, not to mention the 3 000 non-smear slides.

Rob: *I have heard of smears but I'm afraid I don't know much about them. Can you explain why they take up so much of the work in here?*

Anna: The smear test – or Pap test as they call it in other parts of the world including the USA – after George Papanicolaou who invented it – is used to screen for changes to cells in the cervix and it is basically a dye which detects different cells present in the cervix. You could call it differential staining.

Rob: *So you are looking for cervical cancer then?*

Anna: Indeed not! Mostly these will be healthy women. This is a **screening** programme. We are looking for changes in cells in the cervix that suggest a risk of cancer developing. Let me try to explain what is found normally. Where the inner lining of the cervix meets the lining of the outer part there's a transformation zone. Here the epithelial tissues change from ciliated columnar to stratified squamous. On the boundary the cells are metaplastic – they are very active and can differentiate into either type. If we find some of these ciliated or 'honeycomb cells' we know we have sampled the right area.

Rob: *So are all the cells in the smear like this?*

Anna: Not usually. Most are stratified squamous cells. We can even tell from the colour of these if they are from the outer layers, or the basal layer that divides to replace cells lost at the surface.

[Turn over

Rob: *That's fascinating. Can you see any other cells on these slides?*

Anna: Oh yes – all sorts! We find neutrophils – you will have heard of these? *(Rob nods)* We find lactobacilli – bacterial cells? *(Rob nods again)* and we sometimes find stripped nuclei.

Rob: *How come?*

Anna: Well, some of the cells contain glycogen. Bacteria may invade these cells to get at the glycogen, causing the cells to burst leaving free nuclei. However, what the Cytoscreeners are really looking for is signs of diskaryosis. This means cells that appear abnormal – abnormally large or misshapen nuclei or clumped chromatin. They also look for signs of HPV infection.

Rob: *HPV?*

Anna: Human Papilloma Virus – causes genital warts. There are over 100 strains of this virus but not every infected person gets warts. About 13 strains carry oncogenes *(Rob nods)* so HPV infection is one risk factor for diskaryosis.

Rob: *So these cells with diskaryosis can go on to become cancer cells?*

Anna: It's not that straightforward. These cells can still return to normal but they can progress and become cancerous although it might take 10 years for the cancer to develop. If re-testing after six months still shows moderate diskaryosis then a colposcopy will probably be in order. That's why it's so important to check regularly. Women should have a smear test every three years between the ages of 25 and 50.

Rob: *You've lost me this time – what's a colposcopy?*

Anna: Sorry Rob – colposcopy uses a binocular microscope called a colposcope which can view the tissues around the cervix. After gently wiping with acetic acid, any abnormal cells are stained white. Samples can then be taken for biopsy. At a re-test, sometimes the abnormal cells are no longer present. If the abnormal cells go deeper into the lining then they may be removed at the colposcopy clinic even though they are not necessarily cancerous. However, if abnormal cells break through the basement membrane to the tissues beneath, cervical cancer is diagnosed and will then be treated in the same ways as many other cancers.

Rob: *So does screening like this work? I mean does it cut down the numbers of women who get cervical cancer?*

Anna: Absolutely! Cervical screening has probably prevented an epidemic that would have killed about 1 in 65 of all British women born since 1950! The biggest risk factor for cervical cancer these days is failing to go for regular smear tests.

Rob: *I never thought tissues and cell identification could be so important! But it seems to me that we are talking about a cancer which is caused mostly by a virus – is this true?*

Anna: Certainly HPV is one risk factor. The DNA of HPV can be detected in virtually all cervical cancer cells. This raises a number of issues though and remember that not all HPV is oncogenic. However, there is a very high prevalence of HPV in young women – I think I have seen figures that report 33% of 20-29 year olds have the virus. You could say there is an HPV epidemic. There is research being carried out on developing a vaccine and I did read that one would probably be on the market within the next few years which raises still more issues!

Rob: *So do you think Cytoscreeners will be out of a job then?*

Anna: (laughs) Probably not – and they will probably be called "Cytotechnologists" by then and you might even be one!

Rob: *Thanks for the encouragement and for the opportunity to see a career opportunity I never knew existed!*

BLANK PAGE

BLANK PAGE