



## Monoclonal Antibodies: An update

As a topic, monoclonal antibodies only feature explicitly on AQA BUT, exam questions frequently appear on OCR, Edexcel, WJEC and the IB too. On these specs, the examiners set them as **application** questions (e.g. pregnancy testing) for which you don't need to know anything specific about monoclonal antibodies because they are testing your ability to apply basic biological principles to this topic. However, there are two good reasons for learning this stuff:

1. Tackling any type of question is easier if you actually already know the theory
2. It's fascinating Biology

This Factsheet:

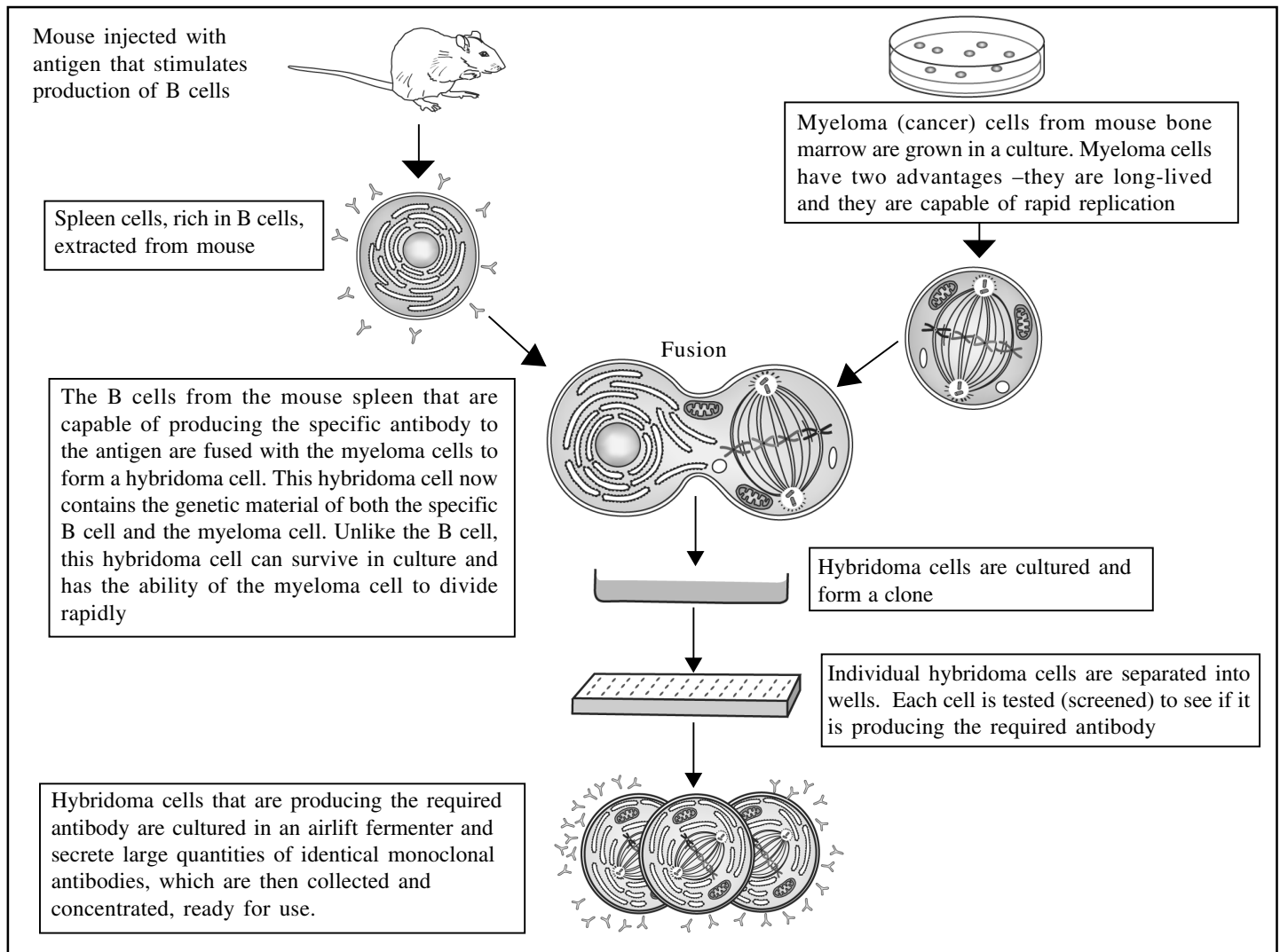
- Explains what monoclonal antibodies are
- Describes how they are produced
- Explains how they can be used in pregnancy testing and in the detection of prostate cancer

When **antigens** (foreign proteins, glycoproteins or polysaccharides) enter the body, specialist white blood cells (**B lymphocytes or B cells**) respond by producing specialized proteins (**antibodies**) that bind specifically to the antigens. For any antigen that enters, the B cells can produce antibodies with the **exact complementary shape** and size to fit them.

Antibodies are clearly invaluable weapons against disease-causing organisms (pathogens). For decades, scientists investigated ways of manufacturing antibodies by culturing white blood cells outside the body. But the white blood cells wouldn't divide outside the body. The breakthrough came in 1975 when scientists devised a technique to produce clones of a single type of antibody, known as monoclonal antibodies.

**Monoclonal antibodies:** identical antibodies that are all descendants of one specific B cell. One type of antibody is produced for each type of antigen

### Manufacture of monoclonal antibodies



**Early use of monoclonal antibodies**

So, for any antigen that we wanted to kill, we could now produce a specific set of monoclonal antibodies. The antigen could be a protein on a bacterium or a cancer cell, for example. All we needed to do was get a sample of the antigen, inject it into the mouse and then manufacture a clone of antibodies, whose only function is to kill that antigen.

However, there was one problem. The monoclonal antibodies manufactured from mice spleen cells were recognized as foreign by the patient's immune system and provoked an immune response in the patient. The antibodies would then either be destroyed by the immune system or they would have to be removed from the patient's system before they caused, for example kidney damage.

It took the scientists at the Medical Research Institute in Cambridge 11 years to solve this problem. Eventually, they "humanized" the monoclonal antibodies by replacing the parts of the antibody that was provoking the immune response with human antibody.

These antibodies are 90% human and don't provoke an immune response but are still capable of binding to the antigen.

**Advantages of the latest monoclonal antibodies**

- Can target any molecule, including human ones
- Unlike some anti-cancer drugs, they don't kill adjacent cells
- Can be produced in huge quantities, quickly

The uses of monoclonal antibodies include:

- Attacking cancer cells e.g. myeloid leukaemia
- Helping prevent transplant rejection e.g. of kidney, liver and bone marrow
- Attacking cells that are causing harm in auto-immune diseases e.g. rheumatoid arthritis
- Attacking viruses

**Case Study 1. Home pregnancy testing kits**

During pregnancy a hormone called human chorionic gonadotrophin (HCG) is released from the placenta. It builds up in the blood stream and is released in the urine. Detection of this HCG in the urine is the basis of some home pregnancy kits in which HCG binds to specific monoclonal antibodies. The monoclonal antibodies are incorporated into the surface of a plastic strip.

Why it's useful:

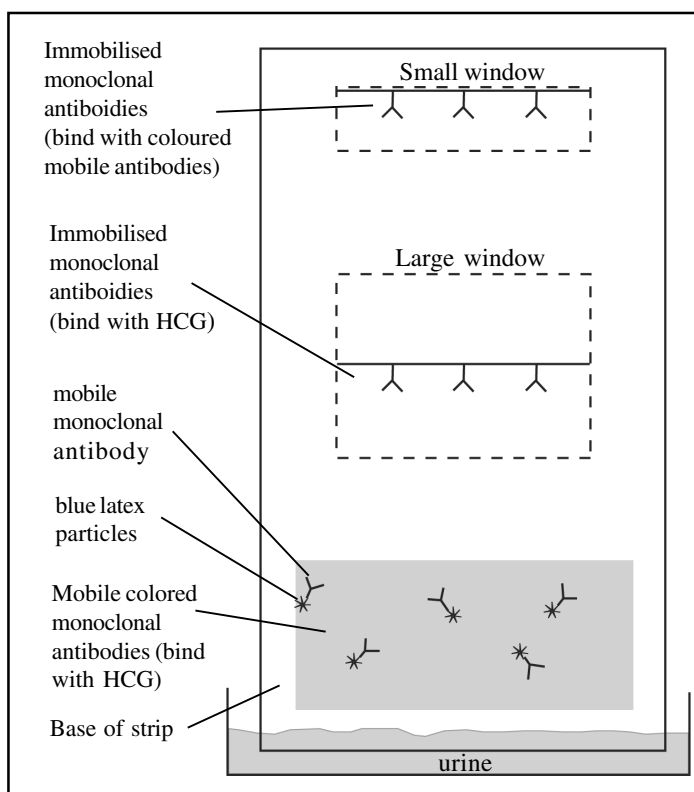
- HCG only found in urine if woman is pregnant
- Non-invasive technique
- Can be done at home

How it works

- The base of a pregnancy testing strip is coated with monoclonal antibodies capable of binding to HCG. Each monoclonal antibody has a blue latex particle attached to it and is capable of moving up the strip
- The strip with mobile, coloured monoclonal antibodies attached is dipped into a sample of urine, which begins to diffuse up the strip
- If HCG is present in the urine, it will become bound to the mobile, coloured antibodies
- The urine and antibodies diffuse up the strip to the large window
- The large window has a row of immobilised monoclonal antibodies which bind with the HCG on the mobile antibodies
- Thus the appearance of a fixed blue line in the large window is an indication of a positive test – HCG must be present in her urine, indicating she is pregnant
- The small window has a row of immobilised monoclonal antibodies which bind with coloured, mobile antibodies. The woman has to wait for the appearance of the blue colour in the small window to confirm that the test is complete and that the monoclonal antibodies have reached the top i.e. they were viable

**Results of positive and negative pregnancy tests**

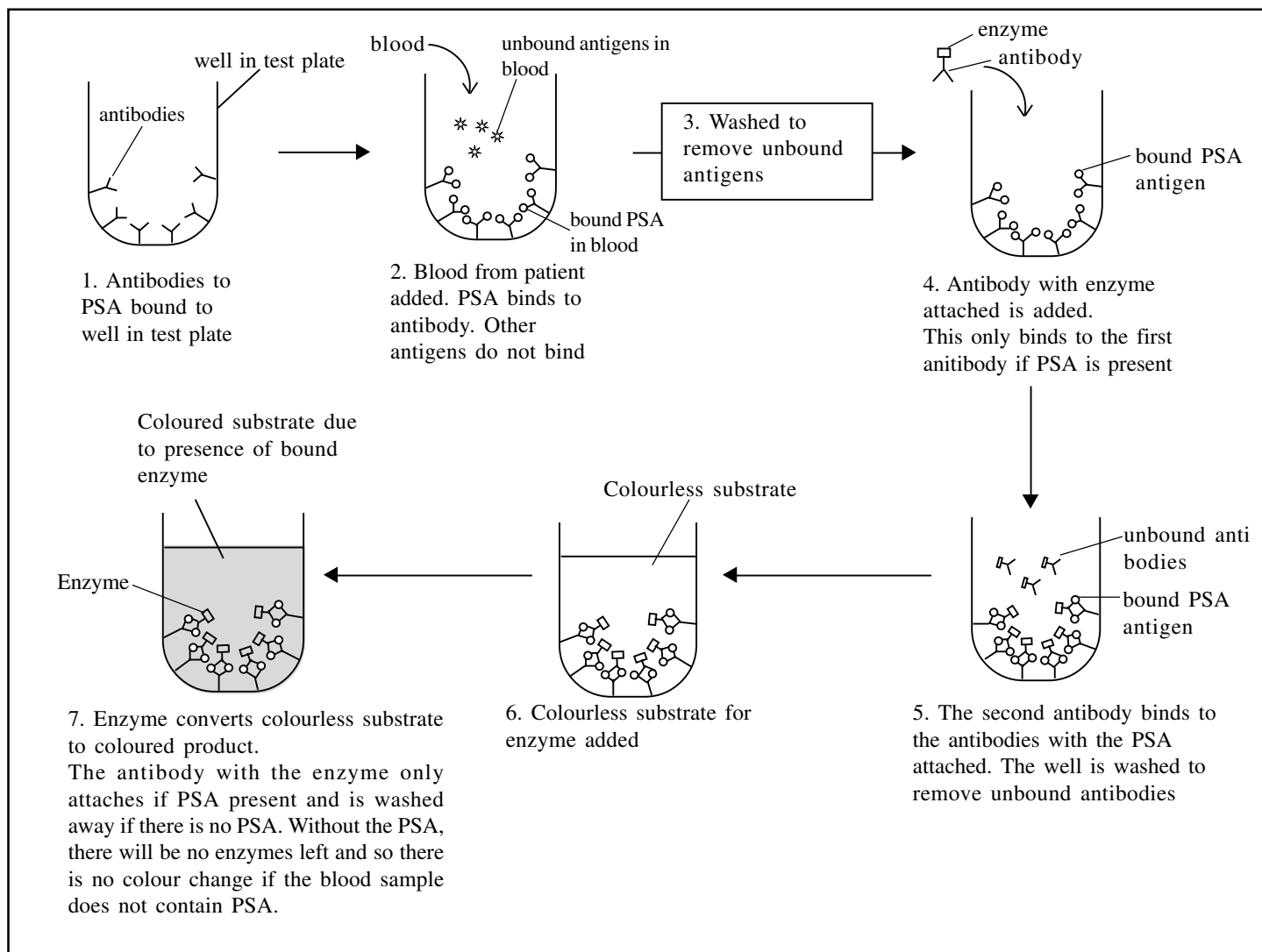
Result of test	Appearance of large window	Appearance of small window
<b>Positive (pregnant)</b>	Blue line	Blue line
<b>Negative (not pregnant)</b>	No blue line	Blue line

**Extract from Chief Examiner's Markscheme**

Few candidates were able to explain how monoclonal antibodies are used in a pregnancy test. Some suggested that the antibodies were introduced into the uterus or even into the fetus

**Case Study 2. Prostate cancer**

Prostate cancer is the most common form of cancer found in men and there are about 35,000 new cases found each year –about one every 15 minutes. Men who are in the early stages of prostate cancer possess an antigen called PSA in their blood. The blood test for PSA uses monoclonal antibodies (Fig 3)

**Fig 3 Using monoclonal antibodies to test for PSAs****Other uses of monoclonal antibodies****1. Separating one type of molecule from a mixture**

- The monoclonal antibodies are immobilised on resin beads
- The mixture is poured over the beads
- Only the target molecules become attached to the antibodies on the beads
- The beads can be washed with a substance that releases the molecule

**2. Immunoassays**

An immunoassay is a biochemical test to identify and quantify a molecule.

- A fluorescent dye or radioactive substance is attached to the monoclonal antibodies
- The antibodies are added to the test sample and attach only to their target antigen
- Unattached antibodies are washed away
- The quantity of the target antigen is measured by the degree of fluorescence or level of radioactivity

**3. Antibody Direct Enzyme Prodrug Therapy (ADEPT)**

This uses an enzyme to change a harmless version of a drug into one capable of killing cells e.g. cancer cells. The aim is only to kill the cancer cells, not any adjacent healthy ones!

- An enzyme is attached to the monoclonal antibodies. The enzyme converts an inactive cytotoxic drug (prodrug) into its active form
- The monoclonal antibodies attach to the target cancer cells
- A large dose of the inactive prodrug is then injected into the patient
- Around healthy cells, the prodrug remains in its inactive form
- When the prodrug reaches cancer cells which have the antibodies and enzyme attached, the enzyme converts the prodrug into its cytotoxic form and it kills the cancer cells

**Practice Questions**

- Gonorrhoea bacteria are sexually transmitted pathogens that respond to the antibiotic penicillin. Chlamydia is another sexually transmitted disease that produces similar symptoms to gonorrhoea. Diagnosis before treatment is important because chlamydia does not respond to the antibiotic penicillin. A quick diagnosis is possible by the addition of monoclonal antibodies to a sample taken from the infected region.
  - What is a monoclonal antibody?
  - With reference to gonorrhoea and chlamydia, suggest why monoclonal antibodies can be used to help to make the diagnosis.
- (a) Myeloid leukaemia is a type of cancer. Monoclonal antibodies are used in treating it. A monoclonal antibody will bind to an antigen on a myeloid leukaemia cell. It will not bind to other types of cell. Explain why this antibody binds only to an antigen on a myeloid leukaemia cell.
  - Calichaemicin is a substance which is very toxic and kills cells. Scientists have made a drug by joining calichaemicin to the monoclonal antibody that attaches to myeloid leukaemia cells. Explain why this drug is effective in treating myeloid leukaemia.
- Complete the correct sequence of the stages shown below, by writing the letter of each stage in the boxes provided below. The first two have been completed for you.

Sequence	1	2	3	4	5	6	7	8
Letter of stage			A		B			

**A**  
HCG has bound to antibody on stick. All other antigens washed away

**B**  
Second antibody binds to first.

**C**  
Stick held in flowing urine.

**D**  
Stick appears blue - a positive pregnancy test

**E**  
Chemical turns blue with specific enzyme

**F**  
HCG in urine of pregnant female binds to antibody on surface of stick

**G**  
Stick dipped into second antibody with enzyme attached

**F**  
Stick dipped into chemical

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Sequence	1	2	3	4	5	6	7	8
Letter of stage	C	F	A	G	B	H	E	D

- Correct sequence is
  - Calichaemicin delivered specifically to cancer cells/less likely to/will not harm normal/healthy cells;
  - Lower dose of calichaemicin needed to be effective;
- Therefore detects presence of gonorrhoea bacteria because different antigen on chlamydia/ora;

- An antibody that is of just one/all same type;
  - Antigens on cell surface membrane;

Monoclonal antibody reacts with specific antigen only;

Therefore detects presence of gonorrhoea bacteria because different antigen on chlamydia/ora;
- (a) Molecule/part of molecule/protein/glycoprotein;
 

[Allow: polysaccharide]

Stimulates immune response;

These antigens/antibodies have complementary/particular shape;

[Reject: Active site]

Allow fitting/binding with (relevant) antibody/antigen;