SSERC, 12 February 2014

Immunology Discussion

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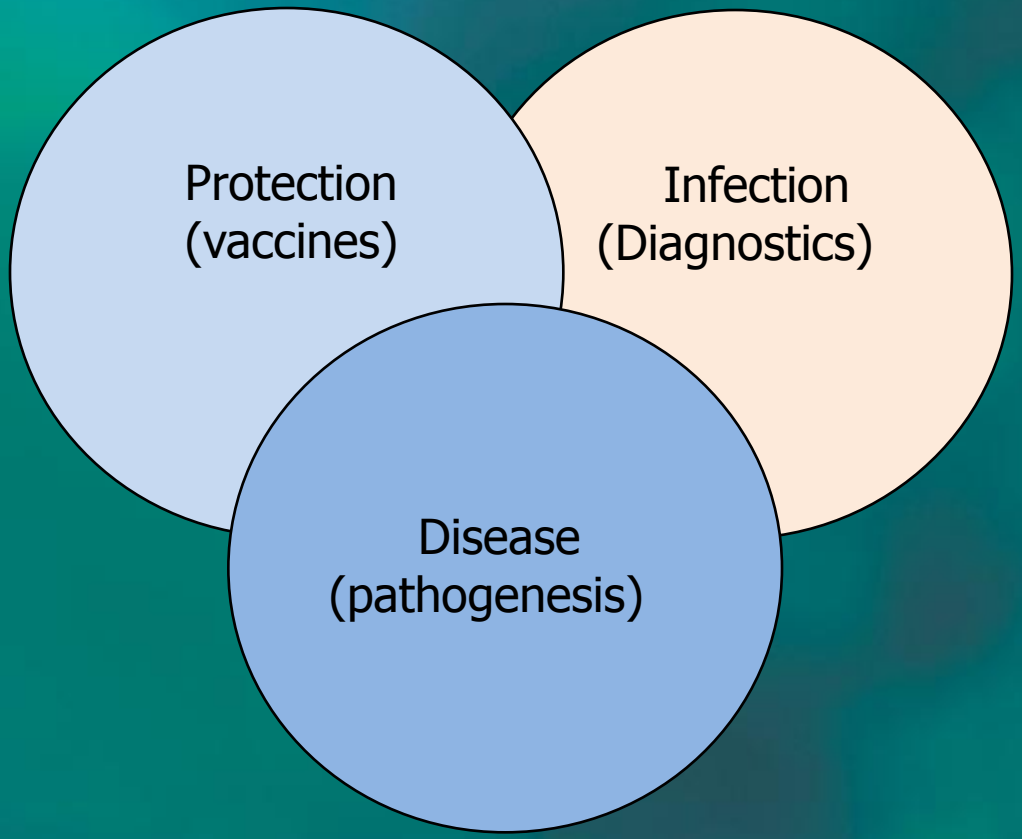
Uses of immunology



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Curriculum elements relating to the immune system

Non-specific defences
(i) Physical and chemical defences
(ii) Inflammatory response
(iii) Non-specific cellular responses

Specific cellular defences
(i) Immune surveillance
(ii) Clonal selection theory
(iii) T and B lymphocytes (Recognition of self and non self)
(iv) Memory cells (immunological memory)

Infectious diseases and immunity

The transmission of infectious diseases
Epidemiology of infectious diseases
Active immunisation and vaccination
Vaccine clinical trials
Herd immunity
Public health medicine
Evasion of specific immune responses by pathogens:
- Antigenic variation
- Direct attack on the immune system

Higher Biology: Cell Biology Unit

Cellular response in defence in animals and plants

(i) The nature of viruses and their invasion of cells. Alteration of cell instructions to produce more viruses.
(ii) Cellular defense mechanisms in animals. Phagocytosis. Antibody production.
(iii) Cellular defence mechanisms in plants.

The importance of lysosomes in phagocytosis should be noted. Reference need not be made to different types of phagocytes. The production of antibodies by lymphocytes, and antibody action in response to the presence of foreign antigens, should be given simple treatment without reference to specific types of lymphocytes or to antigen-antibody reactions. The problem of tissue rejection and the use of suppressors in tissue transplantation.

Advanced Higher Biology (revised): Immune response to parasites

Non-specific defences of mammals: physical barriers, chemical secretions, inflammatory response, phagocytes and natural killer cells destroying abnormal cells.

Specific cellular defence in mammals involves immune surveillance by white blood cells, clonal selection of T lymphocytes, T lymphocytes targeting immune response and destroying infected cells by inducing apoptosis, phagocytes presenting antigens to B lymphocytes, the clonal selection of B lymphocytes, production of specific antibody by B lymphocyte clones, long term survival of some members of T and B lymphocyte clones to act as immunological memory cells.

Epidemiology is the study of the outbreak and spread of infectious disease. The herd immunity threshold is the density of resistant hosts in the population required to prevent an epidemic.

Endoparasites mimic host antigens to evade detection by the immune system, and modify host-immune response to reduce their chances of destruction. Antigenic variation in some parasites allows them to evolve fast enough for them to be one step ahead of host immune cell clonal selection.

Advanced Higher Biology (revised): Antibody techniques

Antibodies are widely used in the detection and identification of specific proteins. Immunoassay techniques use antibodies linked to reporter enzymes to cause a colour change in the presence of a specific antigen. Fluorescent labelling of antibodies in blotting and (immunohistochemical) staining of tissue. Use of monoclonal antibodies in the diagnosis and detection of disease. Use the ELISA technique to identify the presence of specific antigens. To produce stocks of a particular antibody, hybridomas are formed by fusion of a B lymphocyte with a myeloma cell using polyethelene glycol (PEG).

Use of monoclonal antibodies in the diagnosis and detection of disease. Use the ELISA technique to identify the presence of specific antigens.

Advanced Higher Biology: Biotechnology Unit

Protein nature of antibodies produced in response to specific antigens by B lymphocytes. Site and production of B lymphocytes. Preparation of polyclonal sera and its disadvantages. Monoclonals produced from a single B line secreting one specific antibody. Nature of myeloma cells and their hybridisation with lymphocytes using polyethylene glycol (PEG) to produce hybridomas. Use of selective media and screening. Hybridomas only produce one particular monoclonal. Batch culture of secreting hybridomas in fermenters and extraction of pure antibody.

Uses of monoclonal antibodies

Use of monoclonal antibodies in diagnosis and detection of disease. Use of immunoassay (ELISA) techniques involving monoclonal antibodies joined to enzyme; coloured product used to quantify presence of antigen specific to pathogen eg AIDS, meningitis, *Botrytis.* Treatment of disease: tumour-specific antibodies joined to toxins, combine with tumour cells and kill them.

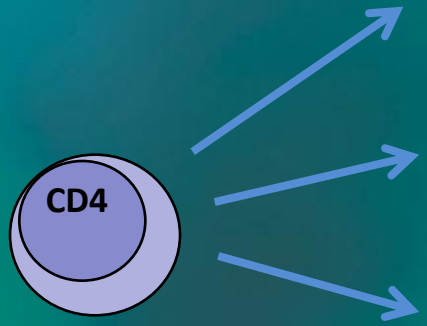
Immune responses

- What triggers immune responses?
  - Signals that ‘alert’ the innate immune system
- How can we make immune responses to effectively combat many different infections (viruses, bacteria, parasites?)
  - Selective activation of the adaptive immune system
- What controls immune responses once they have started?
  - Without regulation the immune system can cause disease (autoimmunity)

Host immunity

- B lymphocytes make antibodies
- Antibodies work against infections when they are outside cells (neutralising virus infectivity)
- T lymphocytes are important for cell-mediated immunity (CMI)
- CMI works against infections inside cells (can be viruses, bacteria, protozoa). T lymphocytes can recognise and kill infected cells or programme the cells to kill microbes inside them themselves (via cytokines)
- Lymphocyte activation is required – how?

Making the ‘right’ response

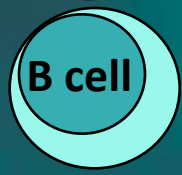


Intracellular bacteria

Parasites

Bacteria, fungi

Virus-infected cells



Extracellular pathogens

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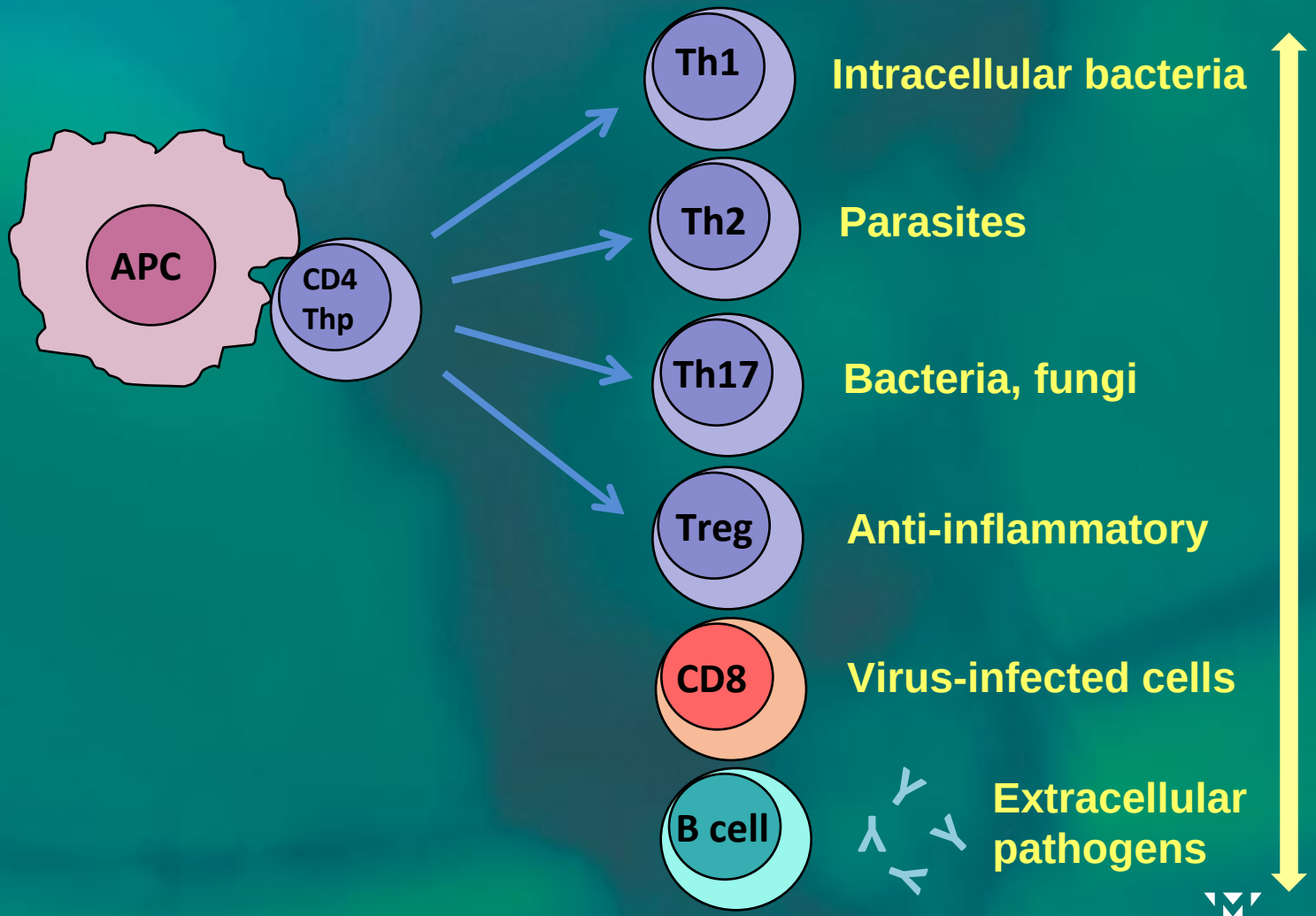
Making the ‘right’ response



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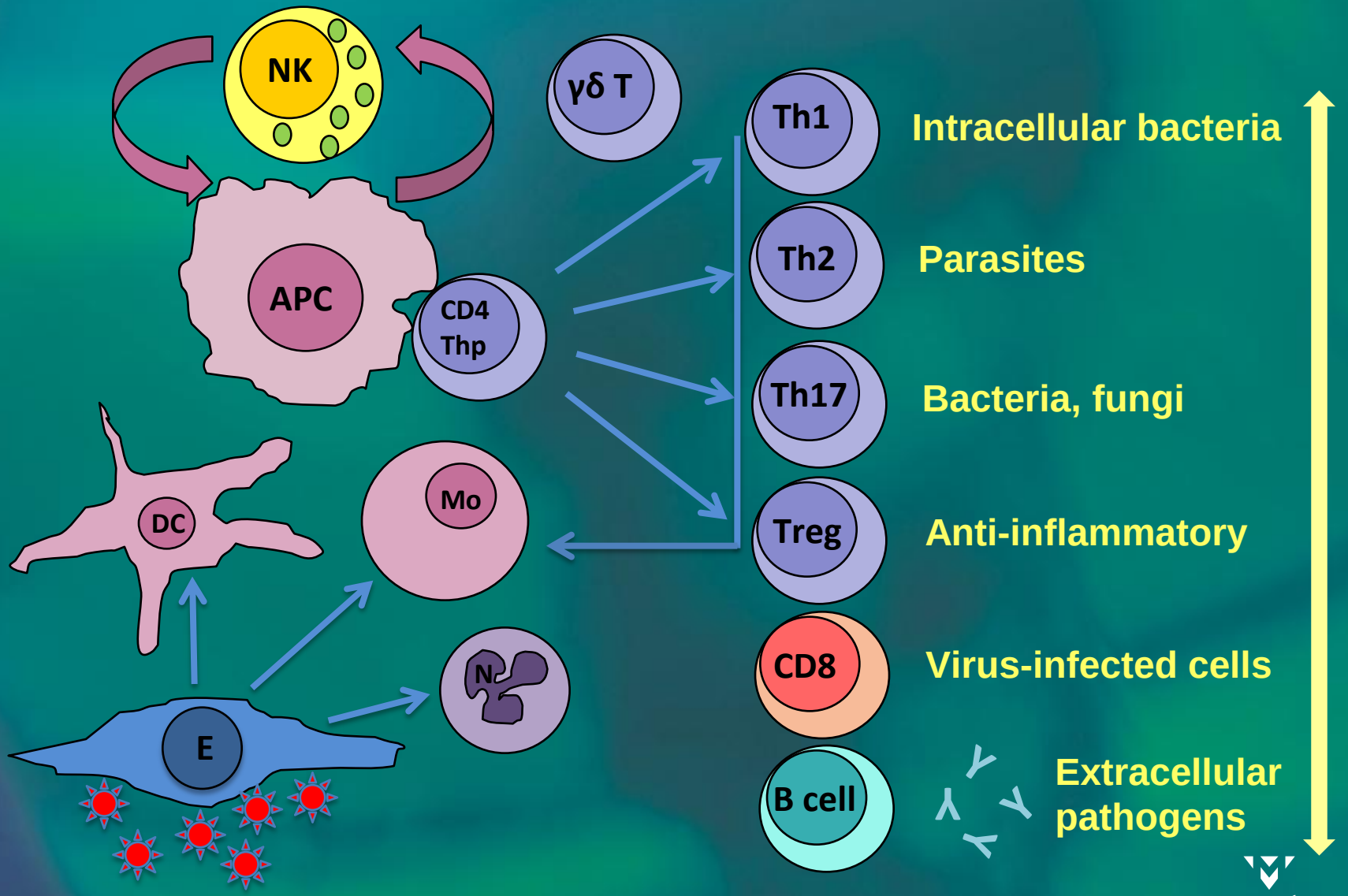
Making the ‘right’ response



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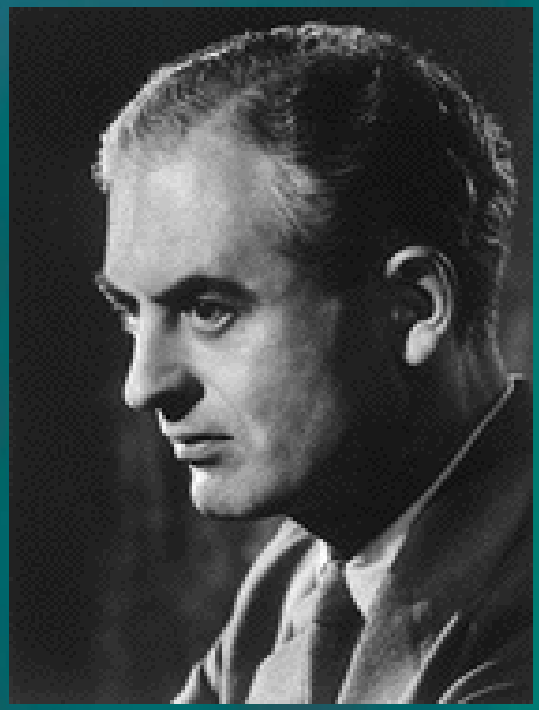
The clonal selection model

The clonal selection model

Macfarlane Burnet

Peter Medawar



Nobel Prize in Physiology or Medicine 1960

“for the discovery of immunological tolerance”

Clonal selection model

Lymphocytes express receptors of a single antigenic specificity

This specificity is genetically determined and precedes antigen encounter

Antigen only stimulates cells with specific receptors and induces clonal expansion of that lymphocyte population

Diversity is achieved by random gene rearrangement (*RAG* genes) and somatic mutation

Clonal selection model

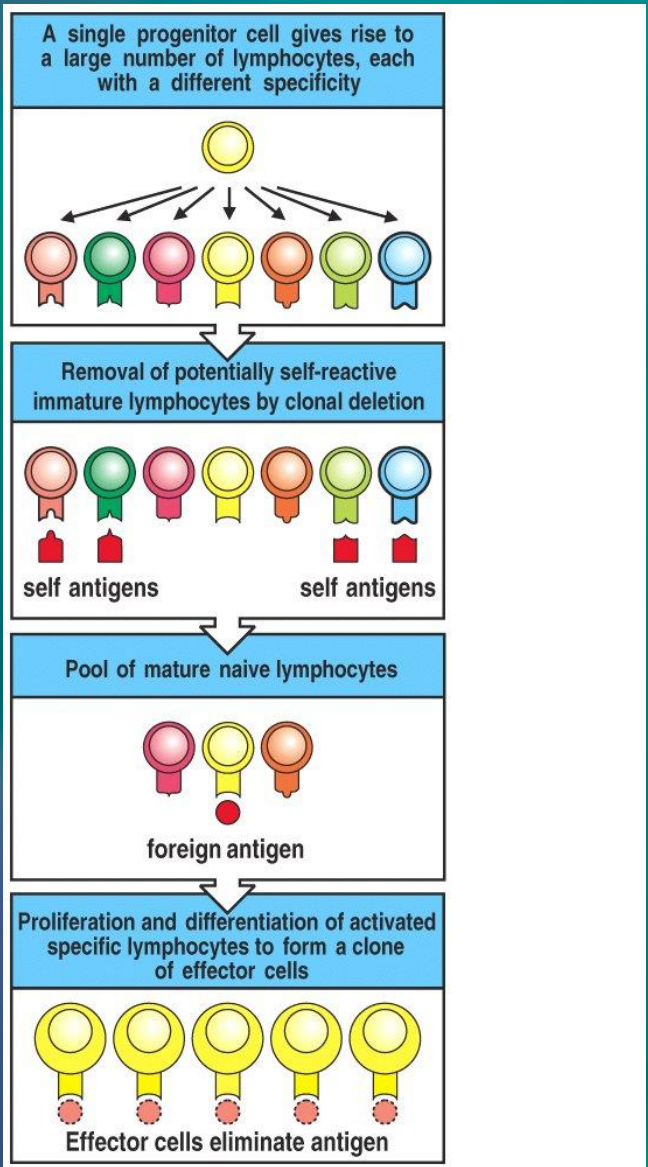


Figure 1-14 Immunobiology, 6/e. (© Garland Science 2005)

This theory has underpinned the formulation of paradigms relating to immunological self tolerance and immunological memory as well as practical applications of vaccine design

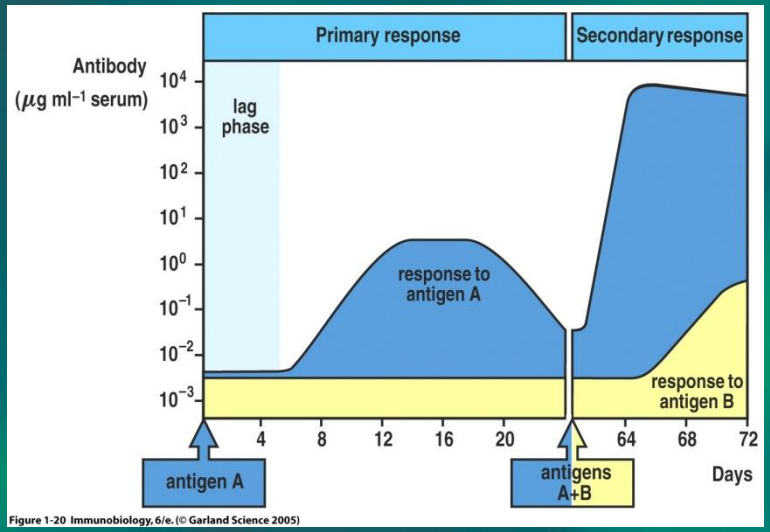


Figure 1-20 Immunobiology, 6/e. (© Garland Science 2005)

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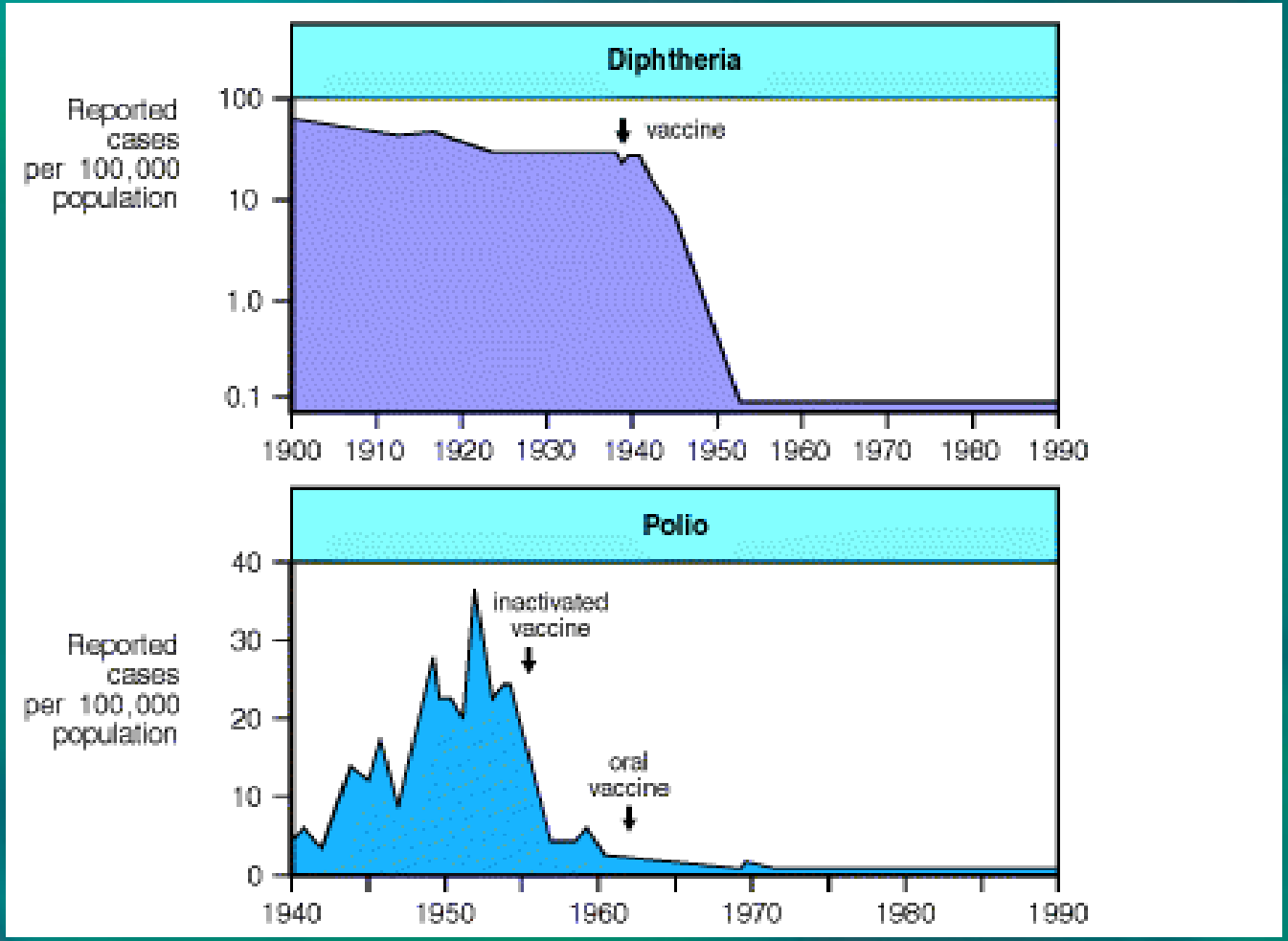
Vaccination is a very effective means of preventing infectious diseases



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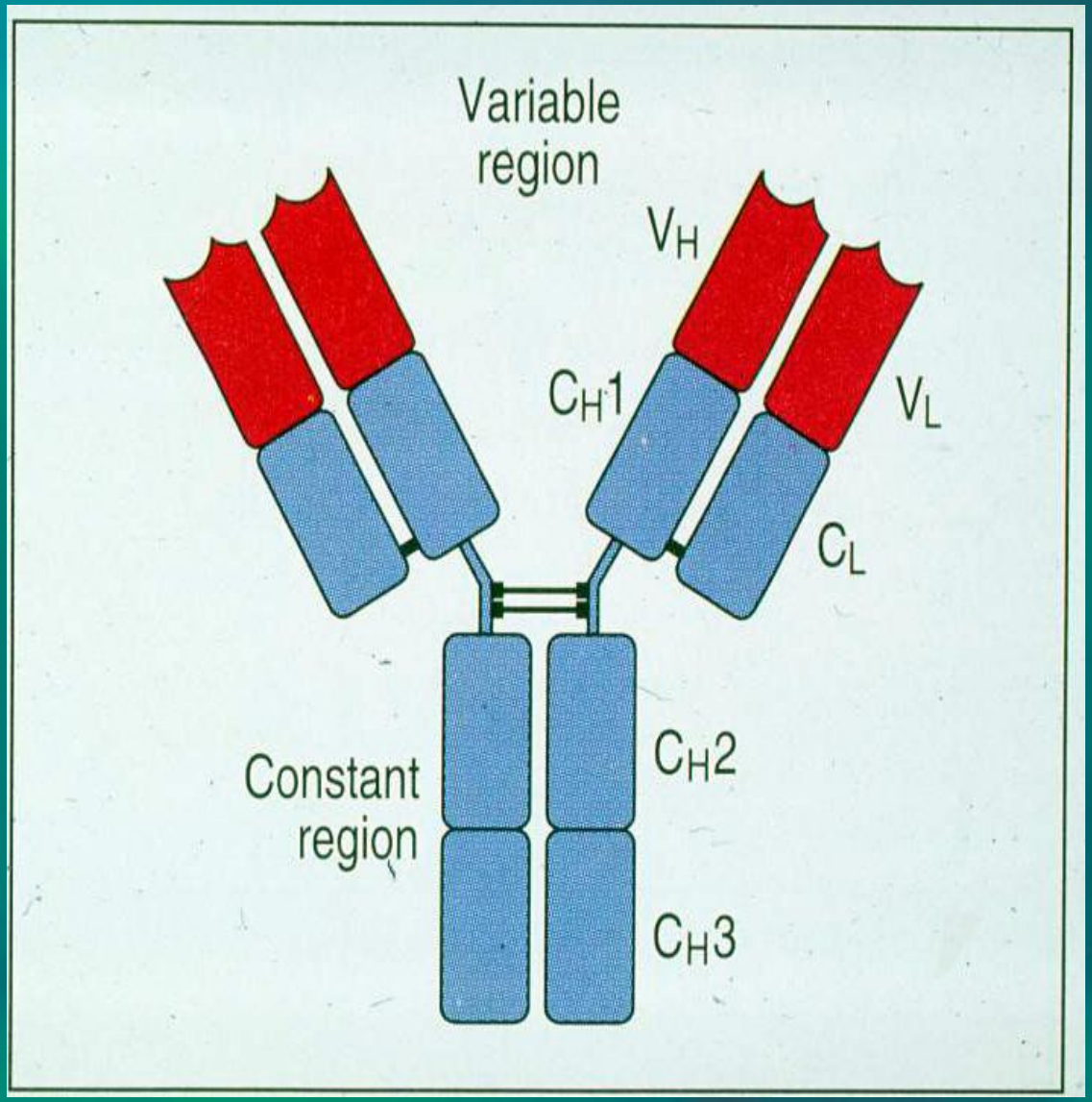
Production of monoclonal antibodies

Antibody structure



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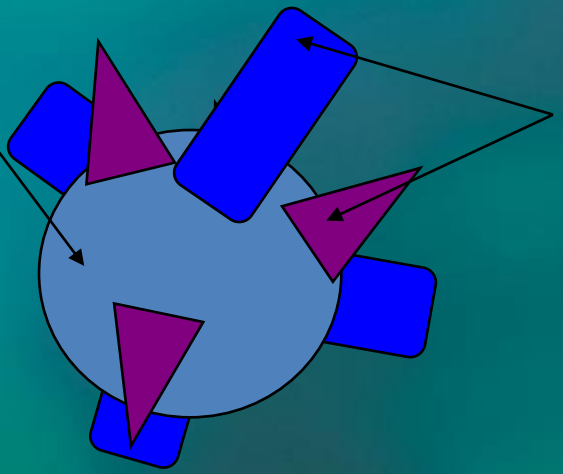
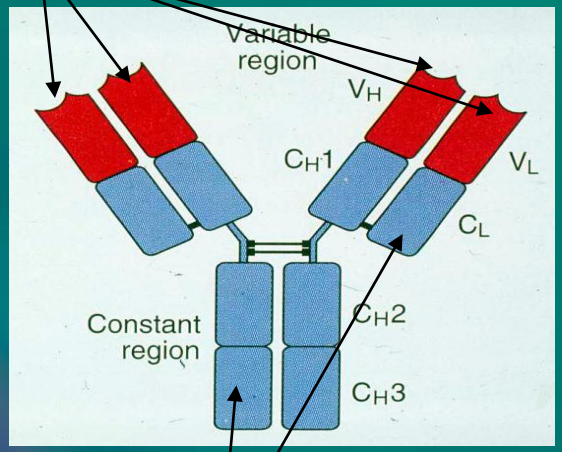
Some technical terms:

Antigen- Any molecule which may be a protein that stimulates antibody production.



Epitope- Unique marker on antigen surface responsible for stimulating antibody production.

Antigen binding sites



Constant region

Antibody or Immunoglobulin (Ig)- Protein that binds specifically to a particular antigen.

Monoclonal – single epitope specificity

Polyclonal- multiple epitope specificities

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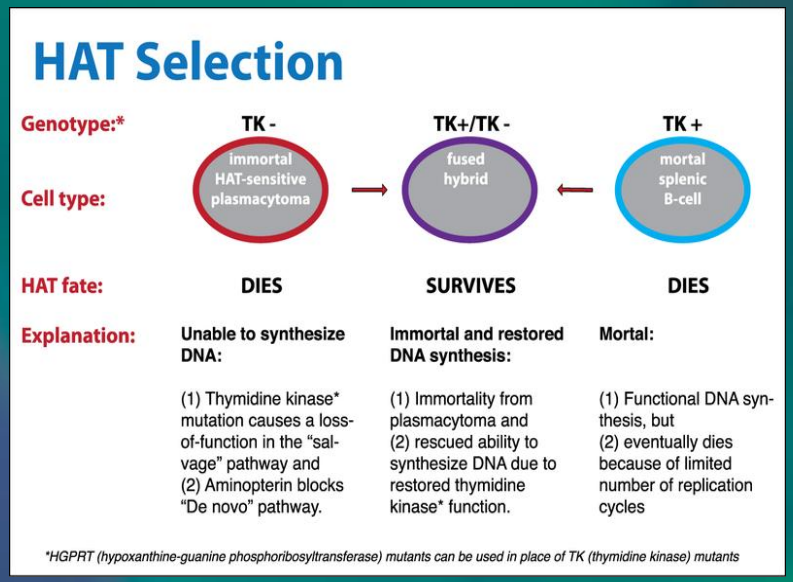
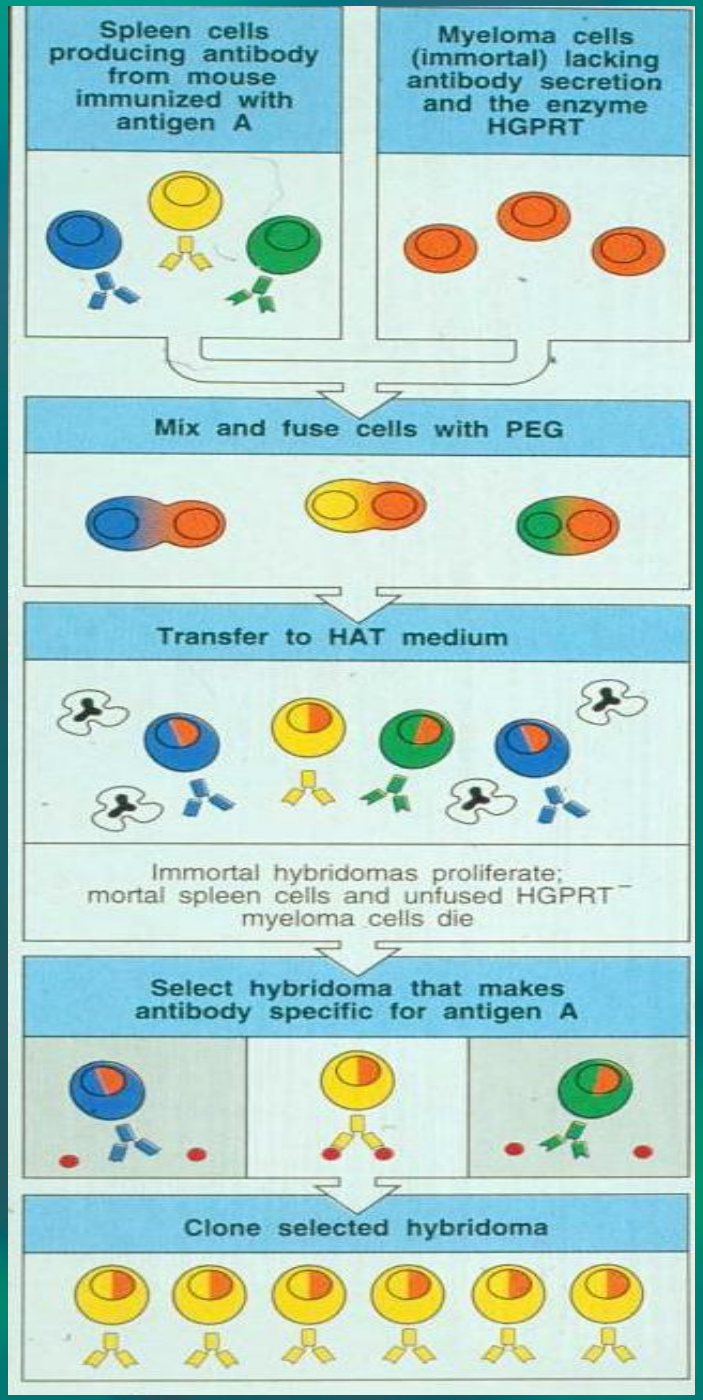
Production of monoclonal antibodies





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Use of monoclonal antibodies for therapeutics

Therapeutic monoclonal antibodies

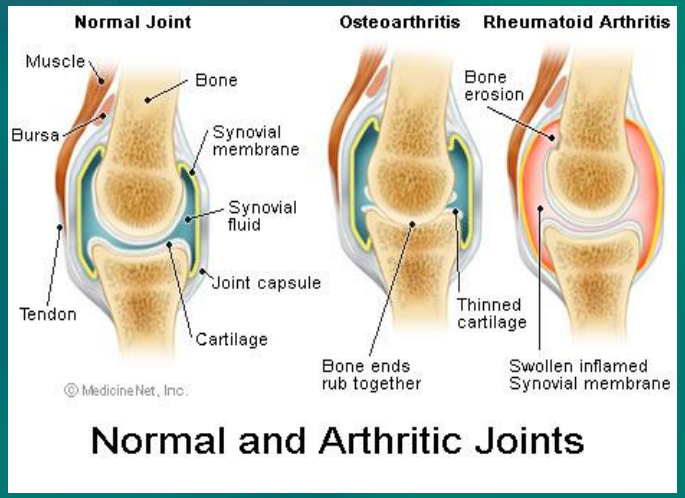
Clinically-approved therapies for cancer, autoimmunity/inflammation, infection, transplant rejection

Exert their effects by:

- Blocking biological function by neutralising soluble factors or inhibiting cell-cell interactions.
- Killing target cells through receptor blockage, activating cytotoxic immune cells or via selective targeting of a cytotoxic compound

Immunotherapy for rheumatoid and psoriatic arthritis

Rheumatoid and psoriatic arthritis are chronic progressive conditions characterised by inflammation in the joints. TNF-α is commonly observed in both conditions.



Normal and Arthritic Joints

Conventional therapy relies on non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate. DMARDs are most effective when used early. In patients where DMARDs fail, alternative therapeutics include Infliximab (Remicade®), a chimaeric antibody that neutralises TNF-α. It is an antagonist.

Cancer immunotherapy

Approximately 20-30% of breast cancers over-express the human epidermal growth factor receptor-2 (HER-2) on their cell surface

Trastuzumab is a humanised monoclonal antibody that binds HER-2 and stops cells dividing. It also suppresses angiogenesis (blood vessel development)

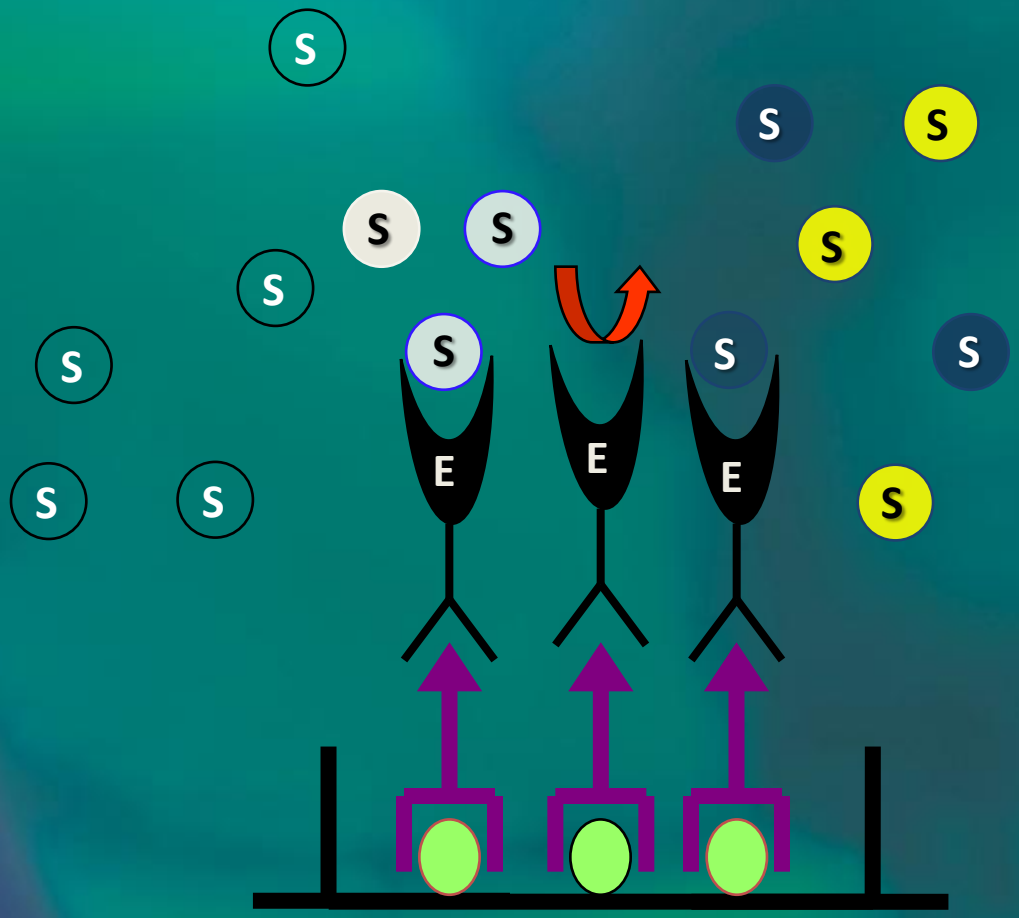
Trastuzumab is marketed under the trade name Herceptin®

Use of monoclonal antibodies for diagnostics

ELISA: the definition

Enzyme-**L**inked **I**mmuno**S**orbent **A**ssay…

…..is a test that can measure antibodies from biological fluids such as blood and milk.



Well of serological plate

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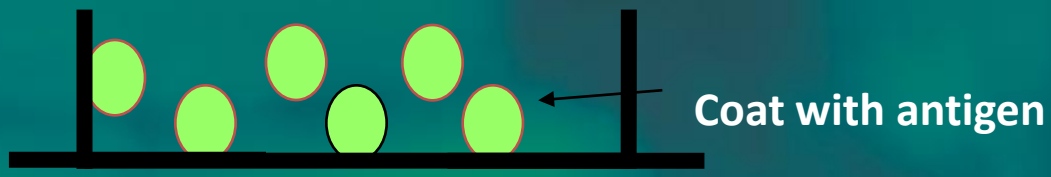
Why do we use an ELISA test?

- We can use ELISAs to measure antibodies or antigens present in the blood of humans and animals

- ELISA process involves multiple steps

ELISA: The works- step 1 of 11

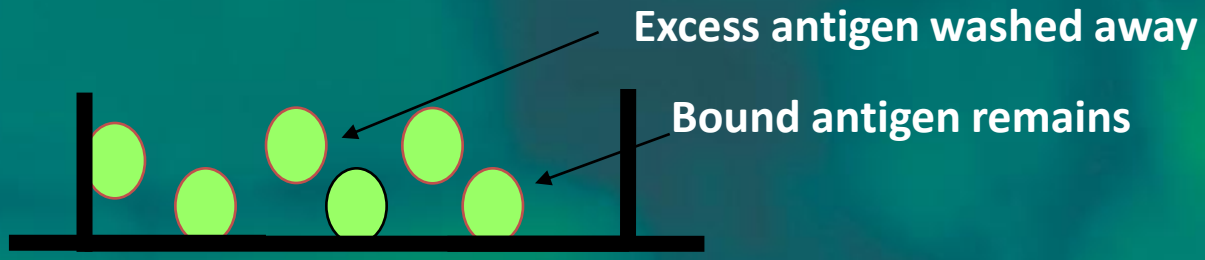
1. Specific antigen is bound to wells of an ELISA plate



Representative well of ELISA plate

ELISA: step 2 of 11

2. Any excess antigen washed away



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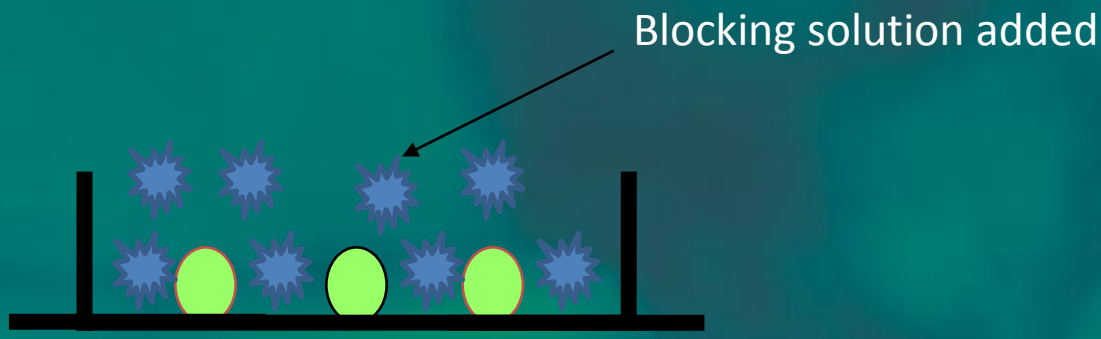
ELISA: step 3 of 11

3. Blocking solution added to ensure the test sample binds correctly



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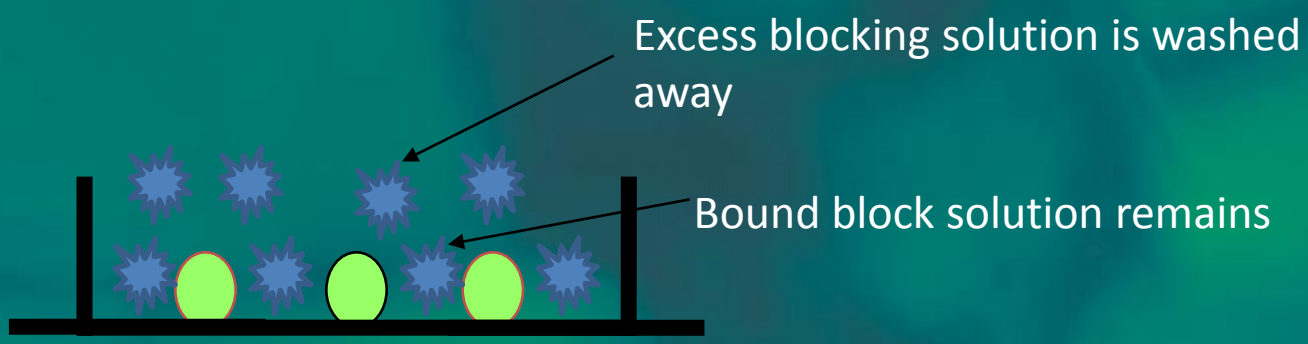
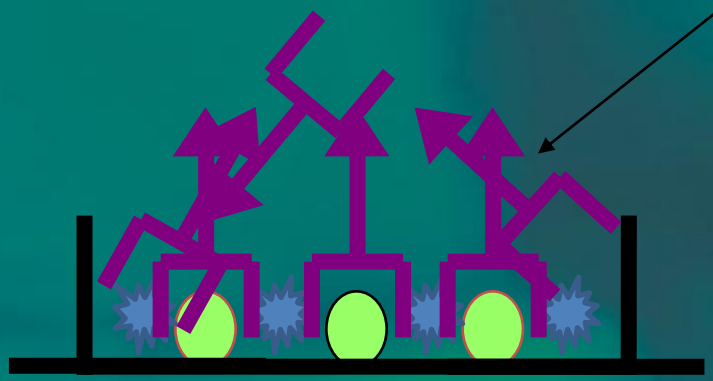
4. Excess blocking solution is washed away



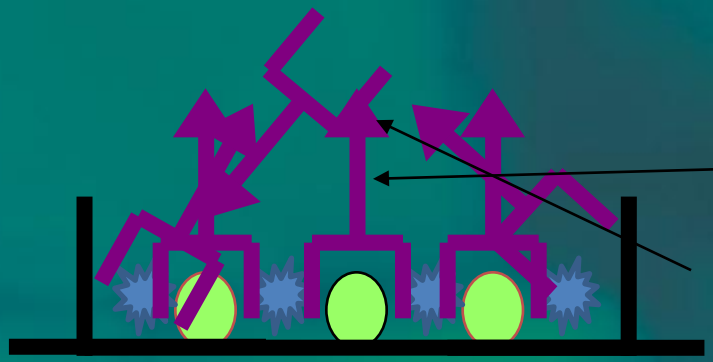
ELISA: step 5 of 11

5. Test sample added to well. Each well is used to test a separate sample



ELISA: step 6 of 11

6. Specific antibodies bind to antigen, anything else is washed away



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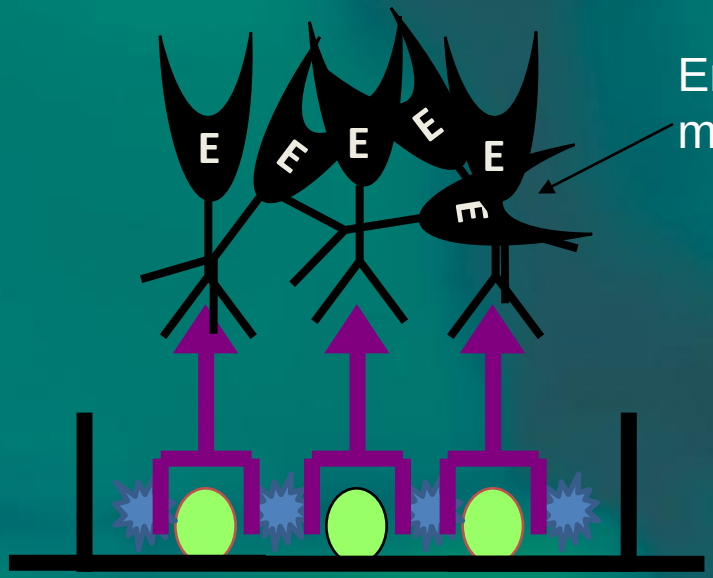
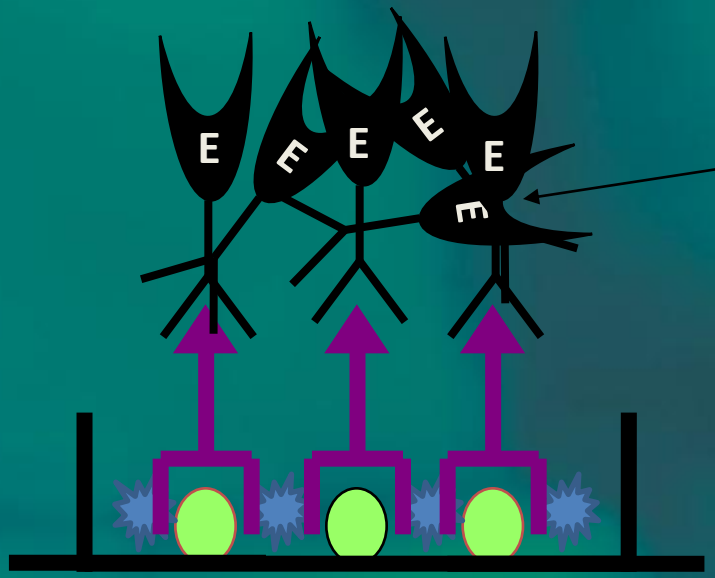
7. Enzyme-coupled detection molecules are added



ELISA: step 8 of 11

8. Wash away unbound enzyme-coupled detection molecules



Wash away unbound enzyme-coupled detection molecules

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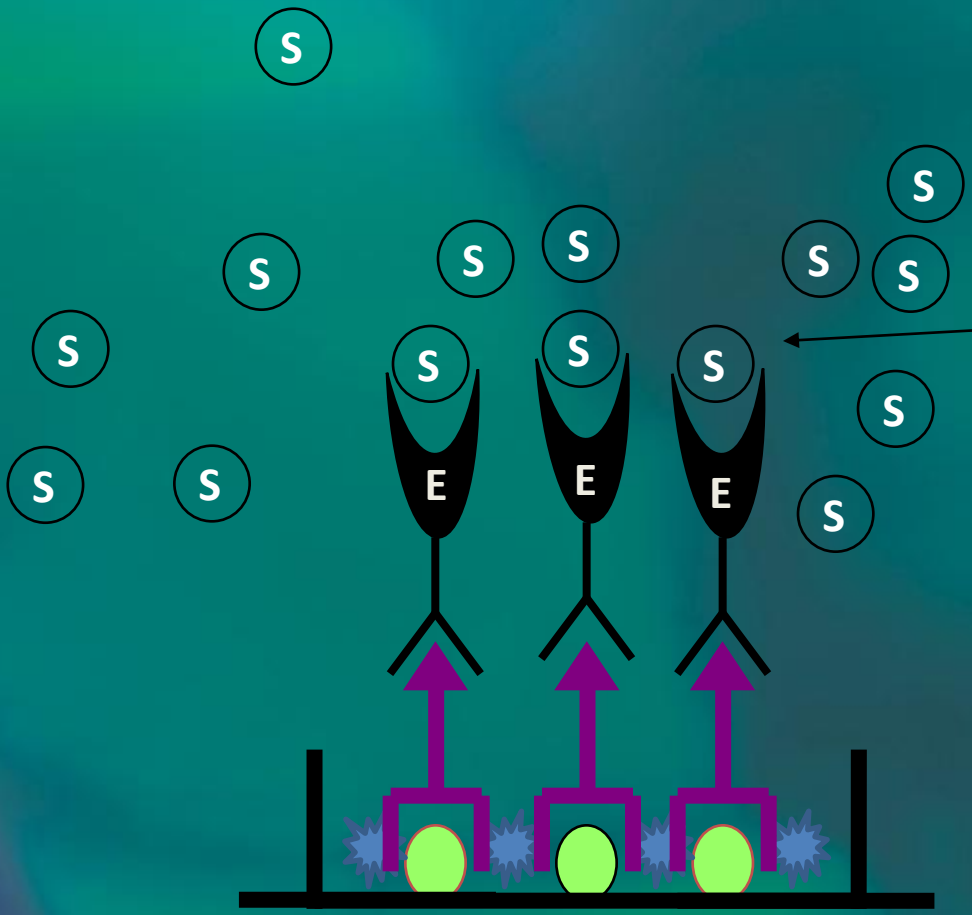
ELISA: step 9 of 11

9. Add colourless substrate. The substrate will react with the enzymes



Add colourless substrate and wait for enzyme reactions to take place

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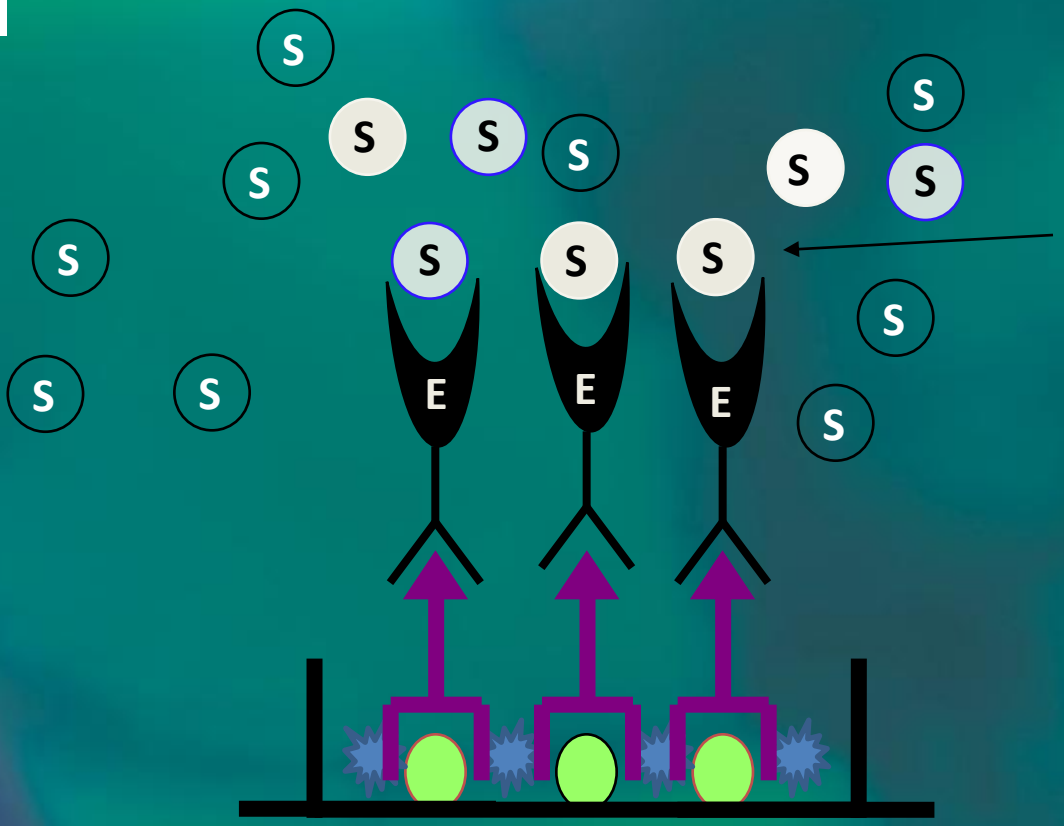
ELISA: step 10 of 11

10. Colourless substrate will undergo a reaction and change colour. The stronger the colour, the more antibody is detected in the well



Substrate will be converted to a blue liquid if it binds to the enzyme

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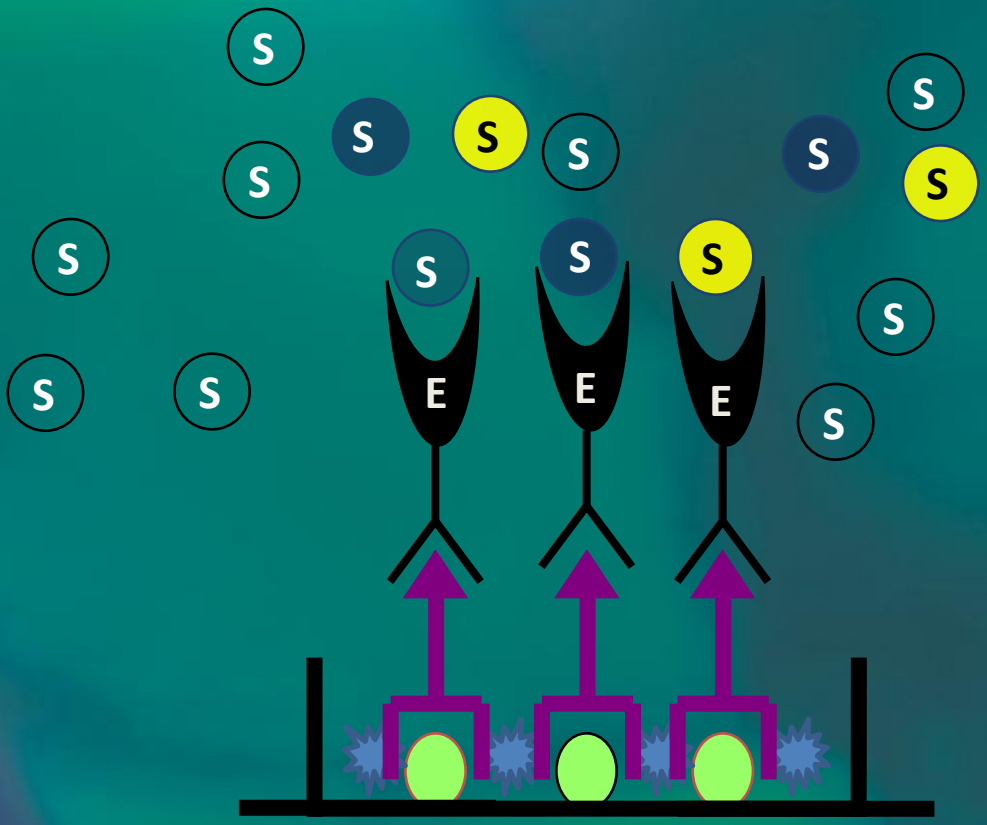
ELISA: step 11 of 11

11. The reaction is stopped by adding acid, converting blue substrate solution to yellow



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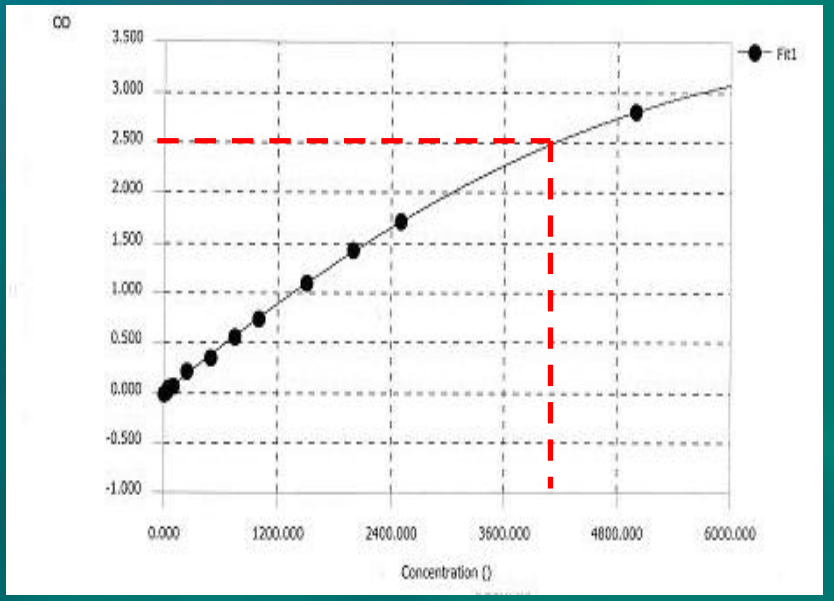
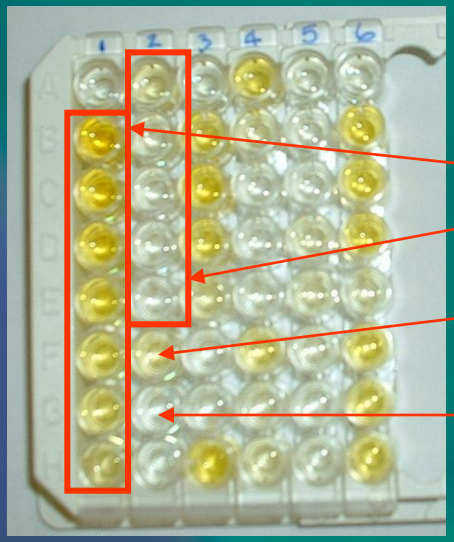
Understanding the test results:

- A spectrophotometer is used to measure intensity of the colour-change. The readings from the machine can be read off the curve to reveal the antibody concentration.





Range of standards to produce curve

Kit positive

Kit negative

- For example, colour value 2.5 gives 4200 units of antibody

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Advantages of ELISAs as diagnostic tests:

- Only a small sample is needed
- Quick results: usually 2-4hrs
- Simple technique that can be accurately reproduced
- Many samples can be tested at the same time
- Relatively inexpensive