Clinical Biochemistry

- Measurement of components of plasma to identify or monitor disease.
- Monitor organ function
- Measuring proteins
  - Albumin
  - Immunoglobulins
  - Enzymes
  - Hormones

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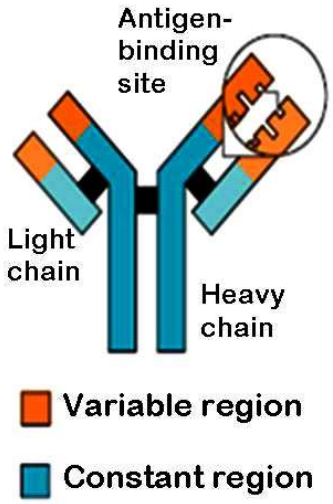
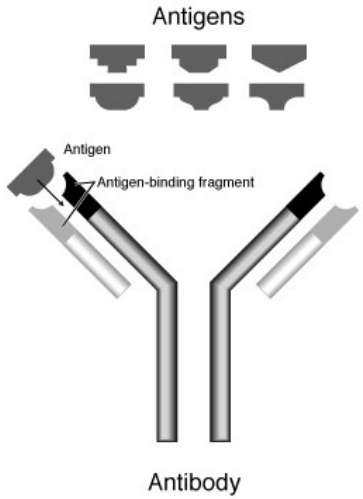
Immunoglobulins

- Synthesised by B lymphocytes
- 5 different types – IgA, IgD, IgE, IgG & IgM





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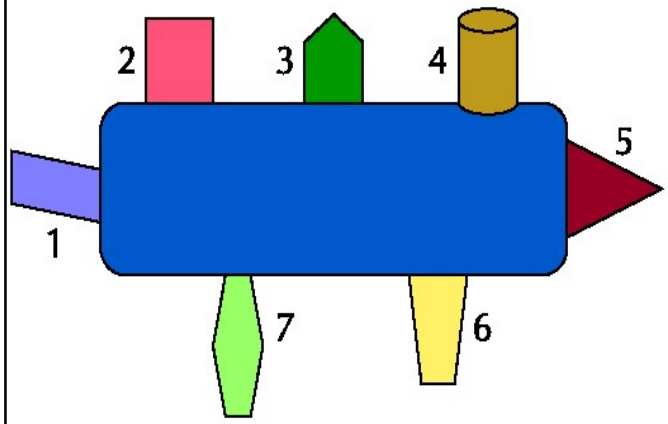
Antigens and Antibodies



- Most antigens have many epitopes
- Injecting antigens into mammals produces serum of polyclonal antibodies
  - Bind to many different epitopes and with different affinities
  - A uniform, high affinity, highly specific antibody preparation would be preferable
- Monoclonal antibodies

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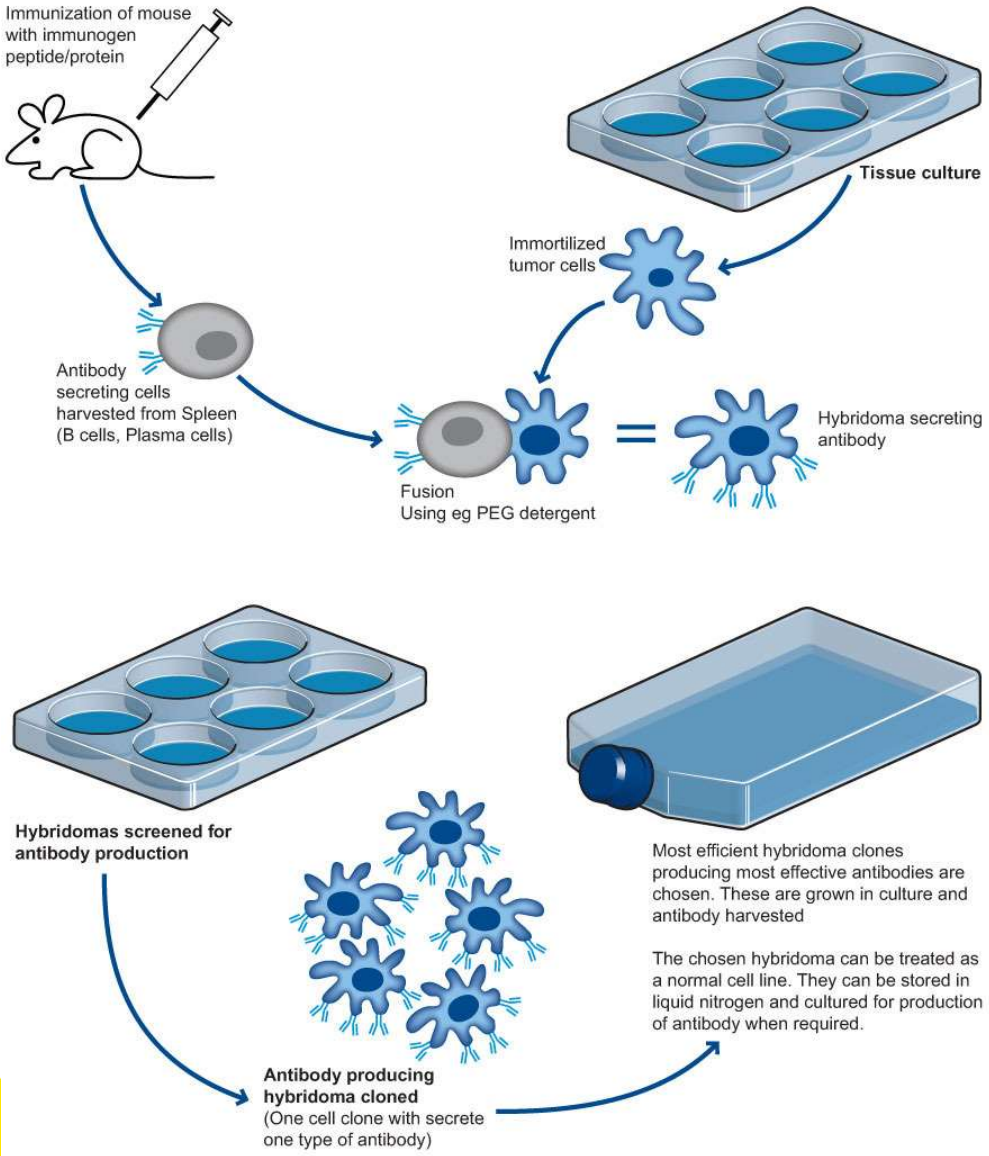
Monoclonal Antibodies

- MAb production



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Targets for Monoclonal Antibody-based Diagnostic Tests

- **Hormones**
  - Chorionic gonadotropin

  Growth hormone
  - Luteinizing hormone
  - Follicle-stimulating hormone
  - Thyroid-stimulating hormone
- **Tumour markers**
  - Interleukin-2 receptor
  - Epidermal growth factor receptor
- **Drug Monitoring**
  - Theophylline
  - Gentamicin
  - Cyclosporin
- **Infectious diseases**
  - Herpes
  - Rubella
  - Hepatitis B
  - Legionella
  - HIV

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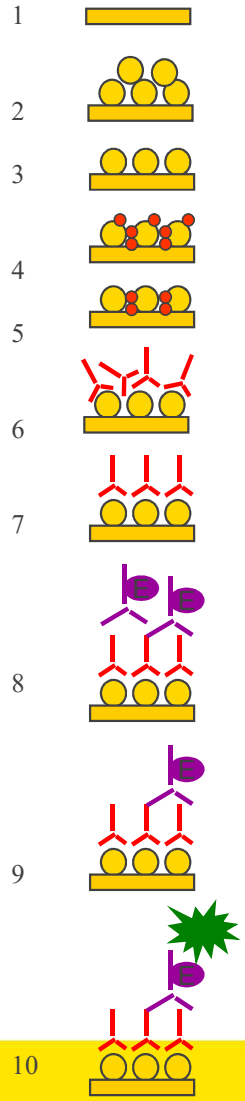
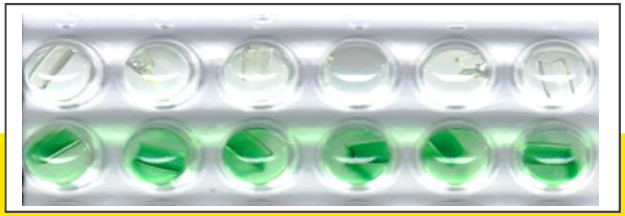
Diagnostics in solution

- The chemistry can also be performed in solution and detected using an ELISA
- The presence of anti-hepatitis A antibodies in plasma
- Usually reported as a dilution of your plasma, titrating the reactivity of the IgG



Enzyme Linked Immuno-Sorbent Assay

1.Blank plate
2. Incubate with protein/antigen
3. Rinse with buffer
4. Block free binding sites on plate with a small protein such as casein or albumin
5. Rinse with buffer
6. Add primary antibody
7. Rinse with buffer
8. Add secondary antibody (enzyme linked)
9. Rinse with buffer
10. Chromogen or substrate which changes color when cleaved by the enzyme attached to the second antibody.



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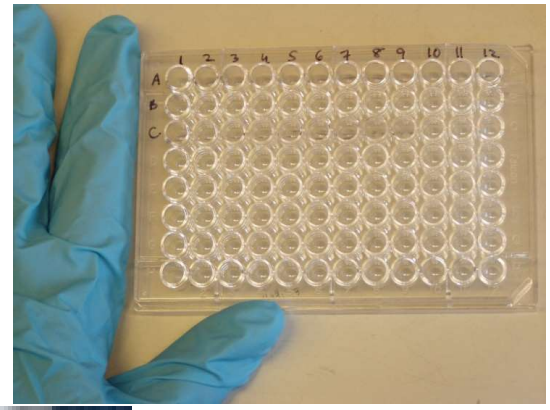
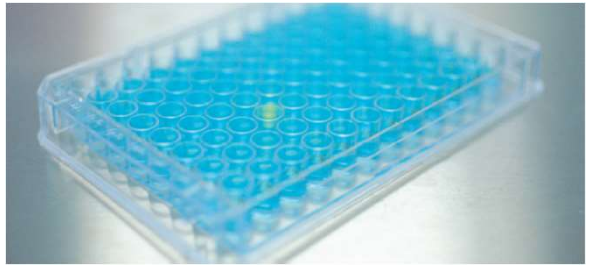
- Beer – Lambert Law
- A = Kcl





Antibodies as diagnostics

- Hormones (Proteins) usually measured by an antibody detection system
- β – Human Chorionic Gonadotrophin (βHCG) detection in pregnancy – made of 2 protein subunits; α and β
- Luteinising hormone (LH) has an α subunit with similarities in 3D structure
- Both control oocyte maturation and release
  - LH causes ovulation and is raised post menopause
  - β – HCG results in the maintenance of the placenta

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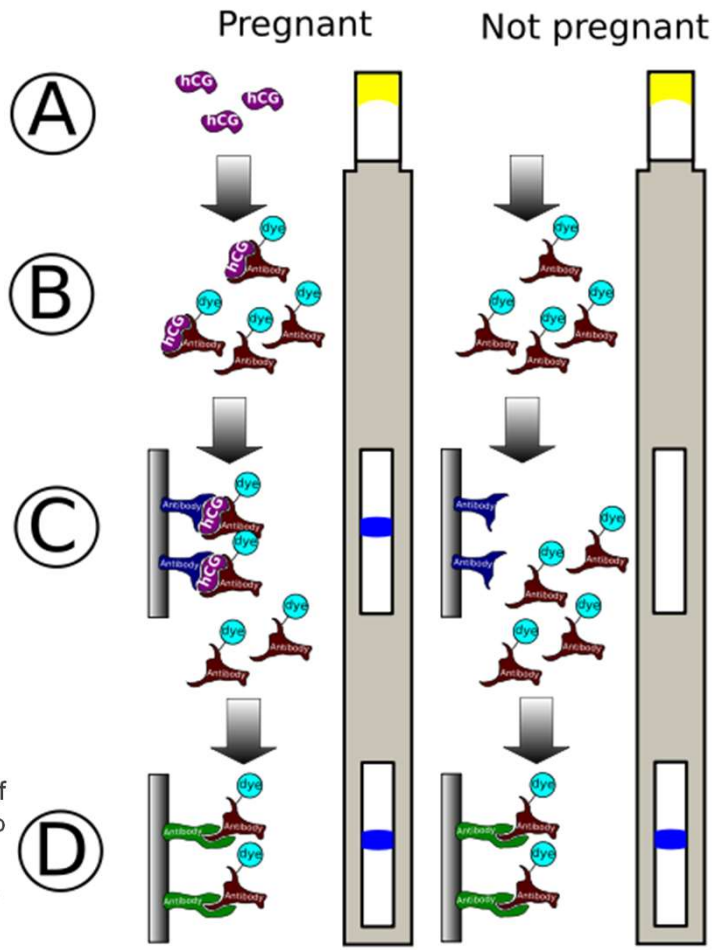
Pregnancy test strip

- A
  - Pregnancy results in an increase in human chorionic gonadotrophin hormone in urine
  - No hCG – not pregnant
- B
  - If human hCG present – binds to soluble anti-hCG Ab conjugated to insoluble blue dye
  - If not, dye conjugated anti-hCG AB flows thru strip with urine
- C
  - If human hCG bound to anti-hCG Ab (dye) then binds to another anti-hCG Ab coupled to the membrane in a strip
  - The dye conjugated anti-hCG Ab continues to flow past this strip if hCG absent
- D
  - The dye conjugated hCG is added in excess in the early part cf the strip leaving free antibody to flow past step C and to bind to an anti antibody coupled to the membrane in a strip.
  - In both cases there is excess Ab so we expect a strip of colour to appear – CONTROL strip



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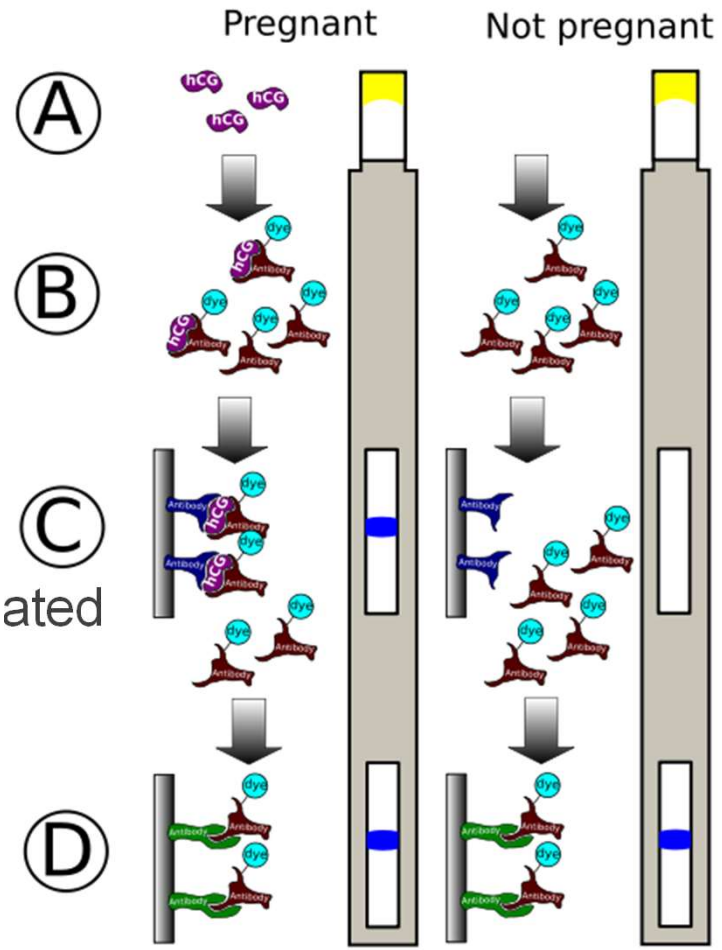
Pregnancy test strip – design strategy

- Detector strip
  - **Mouse** anti-human Chorionic Gonadotrophin
- Control strip
  - **Sheep** anti-mouse IgG
- What antibody is the dye conjugated soluble antibody (in excess)?
- What dye do you use?
  - Colour, solubility
- What does the control strip do?



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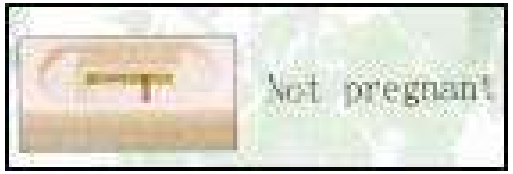
Diagnostics



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Limitations

- Sensitivity = the probability of detecting the clinical event in those who have the protein marker
- Specificity = the probability of not detecting the clinical event in those who don't have the protein marker
- False negatives
  - dilute urine specimens, low levels of hCG (less than 50 mIU/mL) - first morning urine
  - Early in pregnancy
- False positives – some cancers cause elevated levels of hCG.
- Confirmation with other test - physical examination

Specificity

- The antibody is specific for the β subunit, cross reactivity with the α subunit will cause false positives.
- Test for LH?

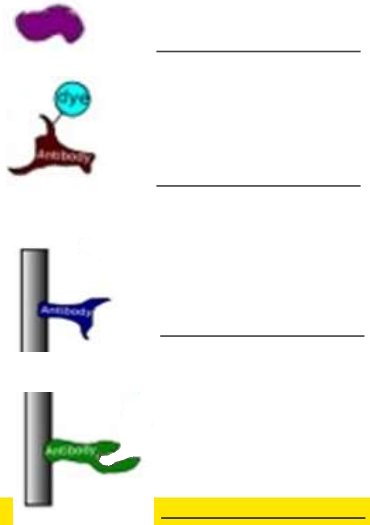
Exercise: Design an ovulation test strip

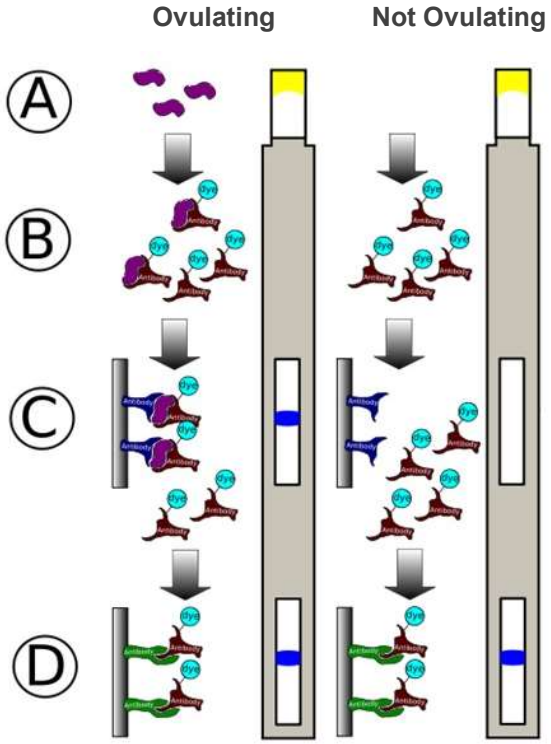
Using pregnancy test strip as an example, how can we design a test strip to test for ovulation?

Things to consider:

- Which antigen shall we target?
- What antibody shall we use to avoid cross reactivity with HCG hormone?

Label each component in the test strip.





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