



Immunology & ELISAs



2013 BIOMAN Workshop, MC³

Lori Dodson, Ph.D.

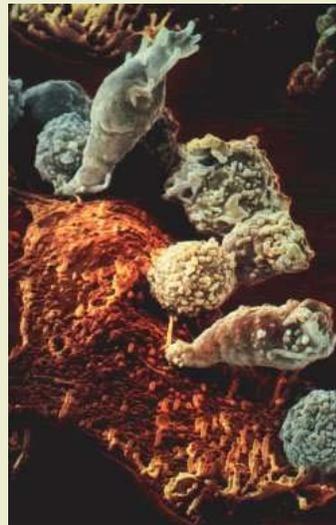
Show N Tell
& freebies
at end of
workshop 😊



Disclaimers



➤ I am NOT an immunologist



Killer T cells attacking a cancer cell



➤ Nobody is paying me!

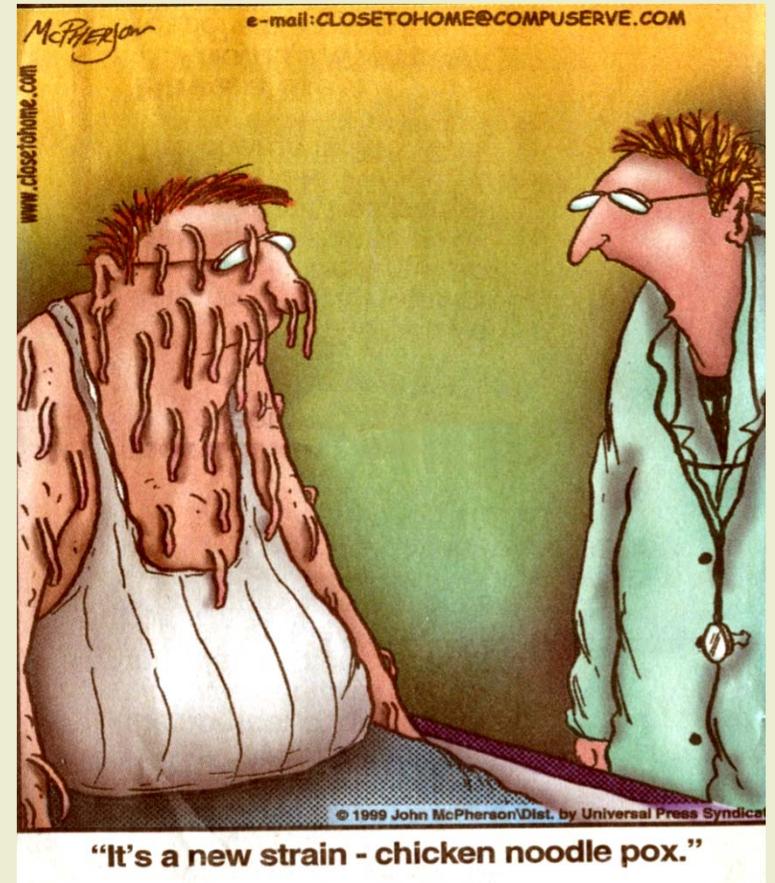




Workshop Content

- Immunology & ELISAs
 - General Overview
 - Resources
 - Commercial Kits
 - Bio-Rad, Edvotek, Carolina
 - On-line sites
 - Simulations, Animations
 - Hands-on Component
 - Kits: Bio-Rad & Edvotek
 - Indirect ELISA
 - Multi-plate Reader
 - Quantitative Assay

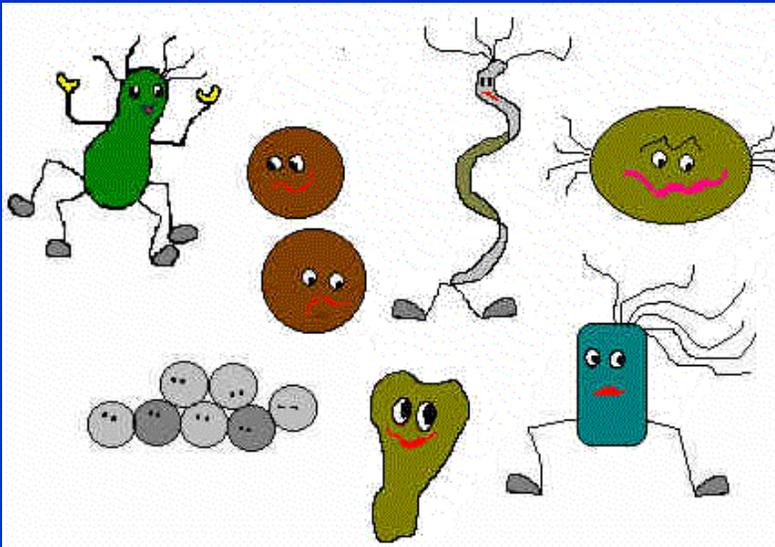
- Miscellaneous
 - E-Gels
 - Freebies



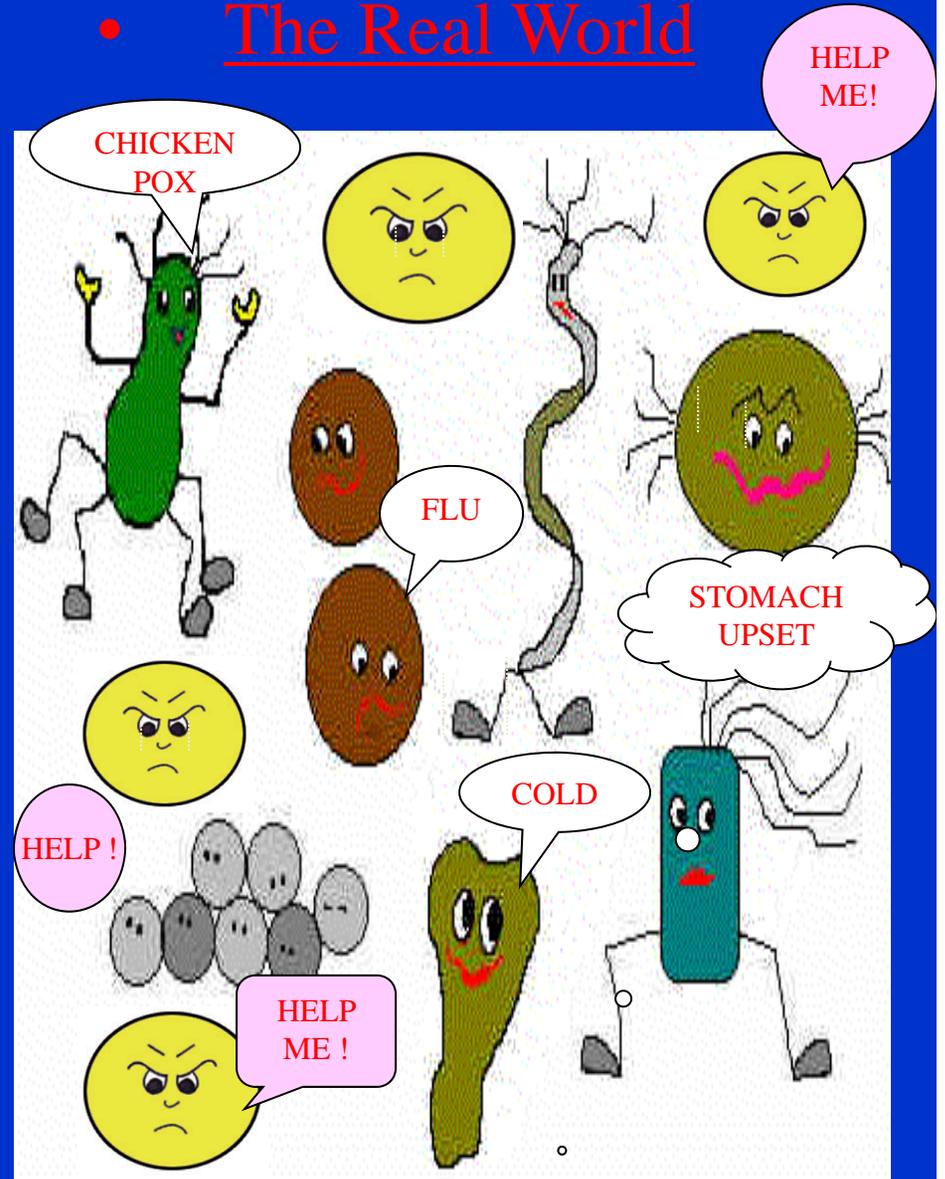


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- The Perfect World



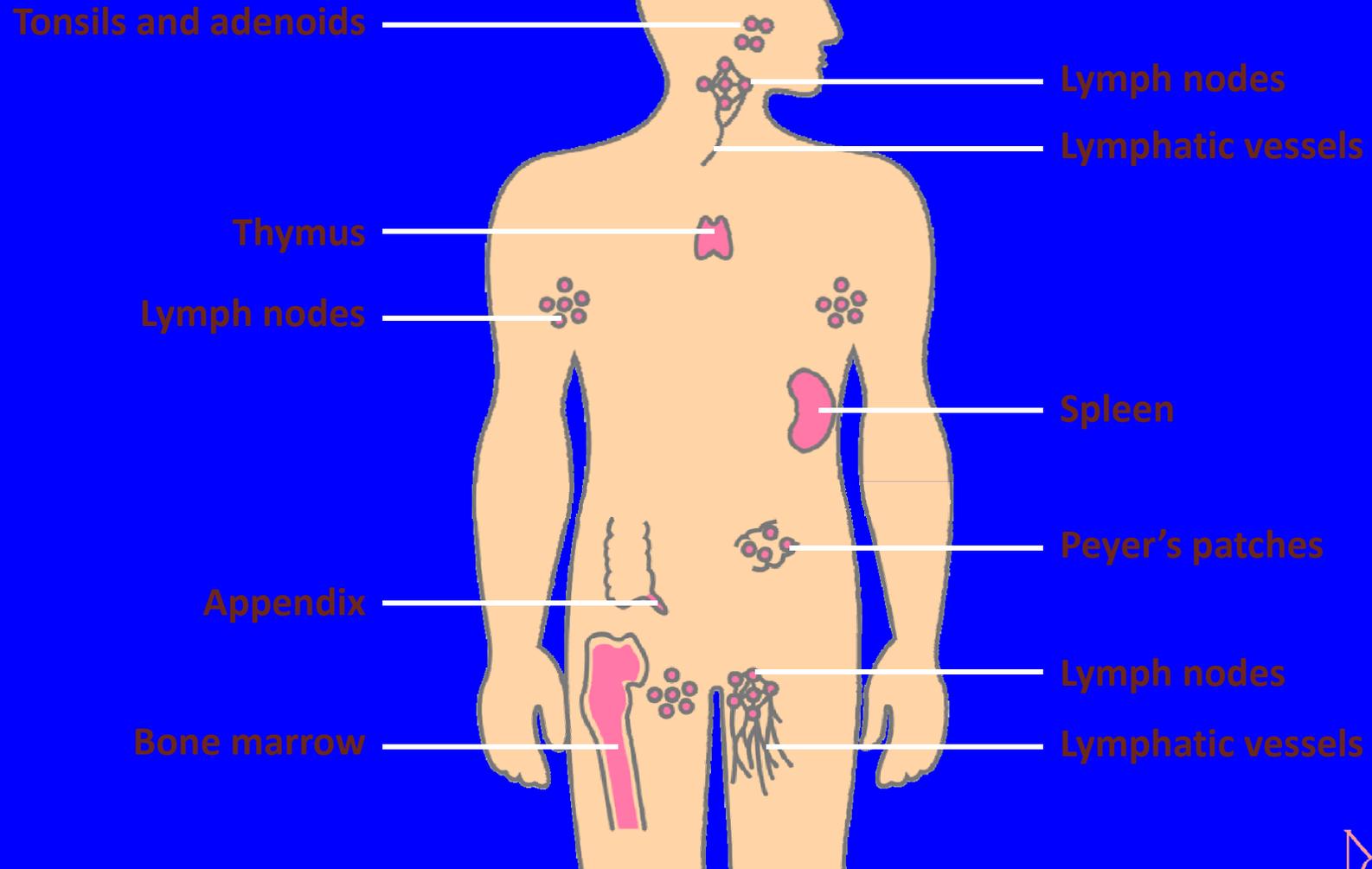
- The Real World



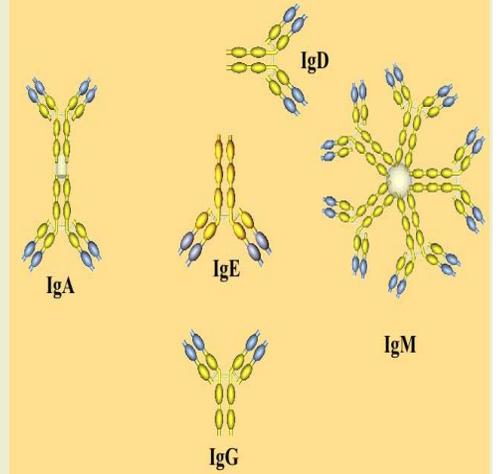
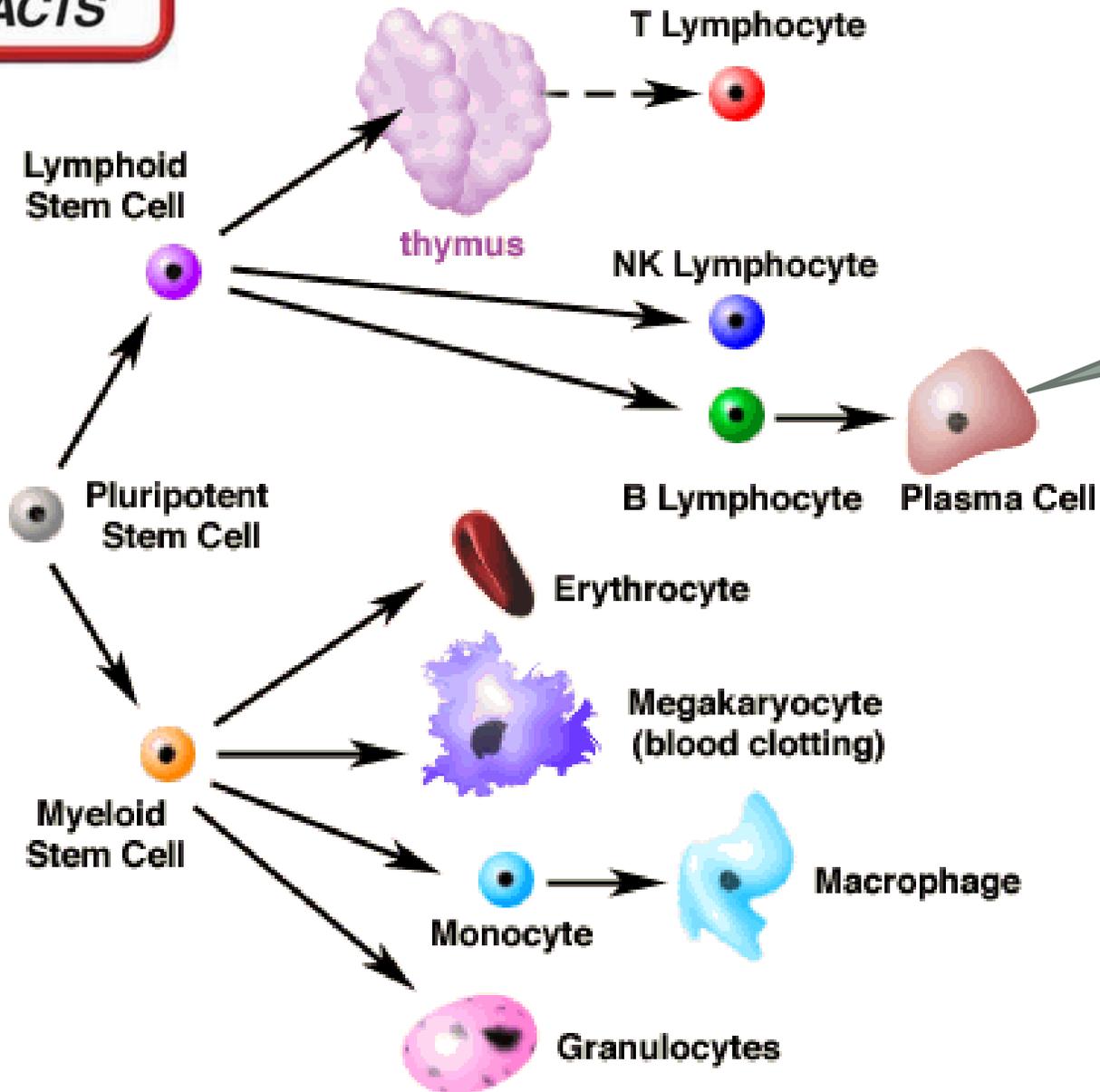


Organs of the Immune System

Understanding the Immune System
NIH, NIAID, NCI
booklet /tutorial:

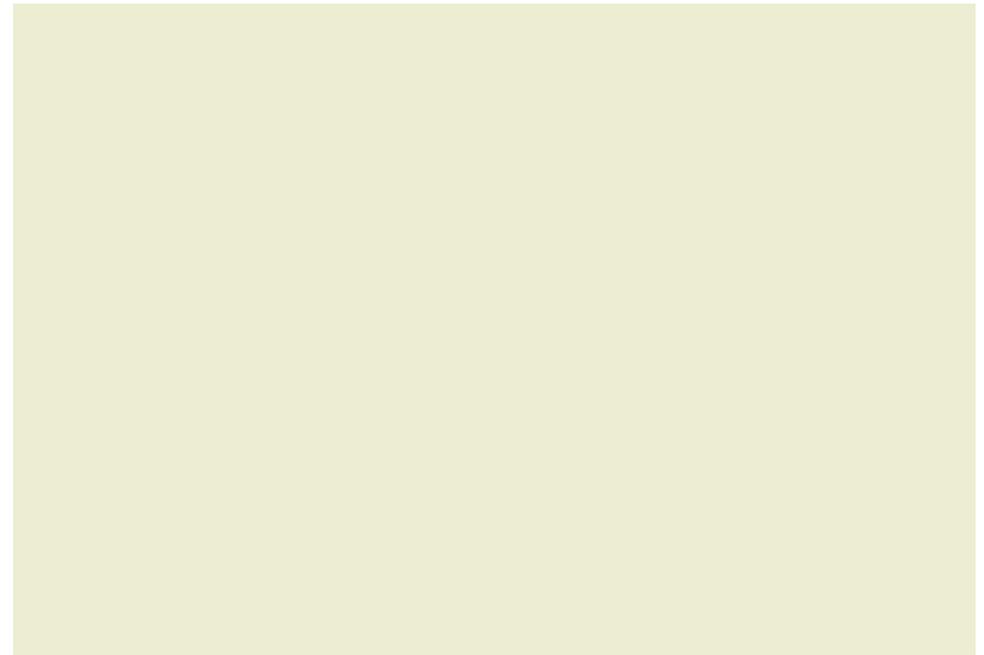
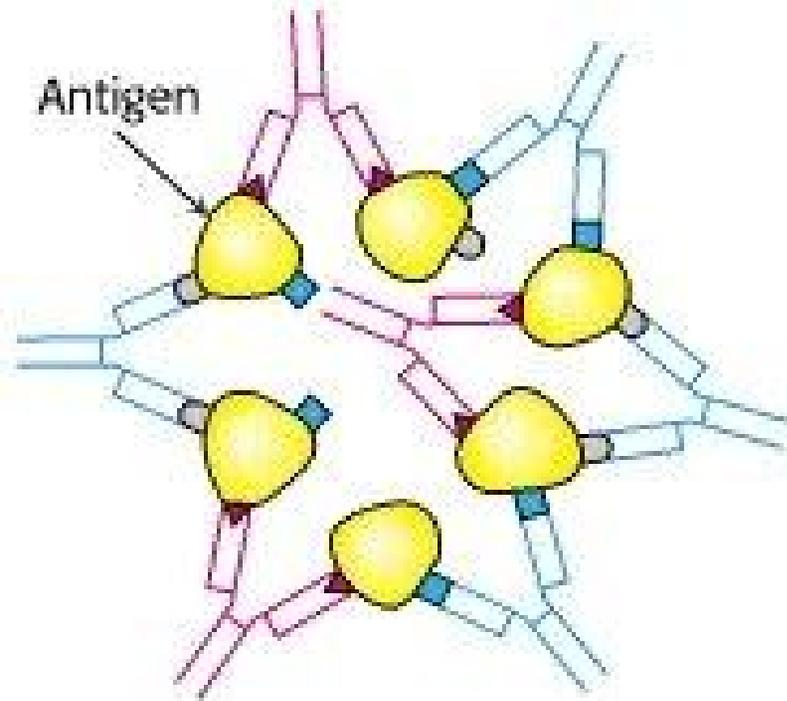


Artwork by Jeanne Kelly. ©2004.

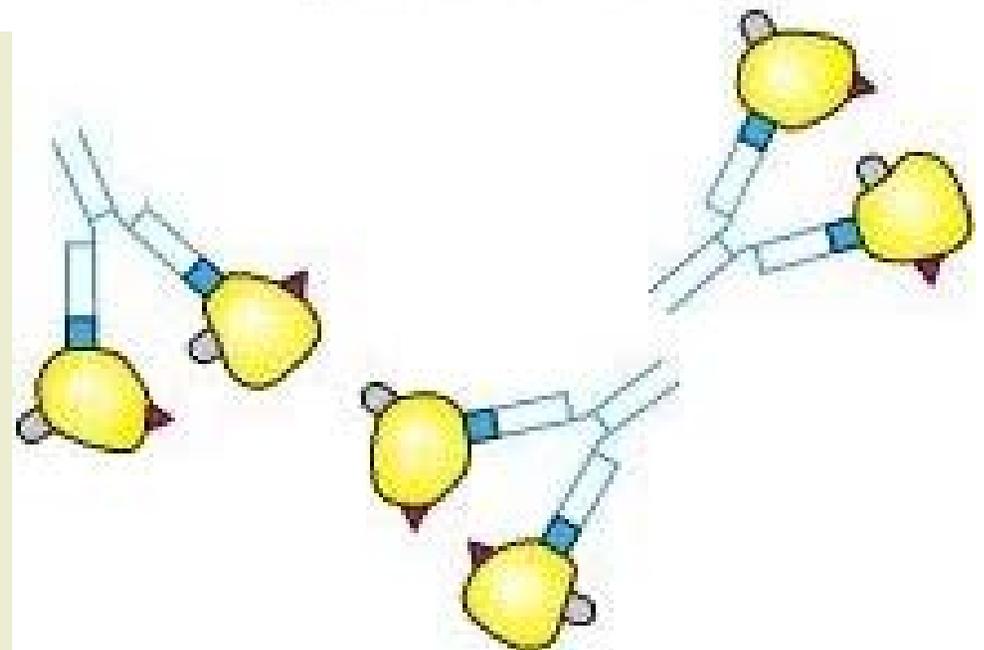


Ab-mediated
Acquired Immunity
2^o Response

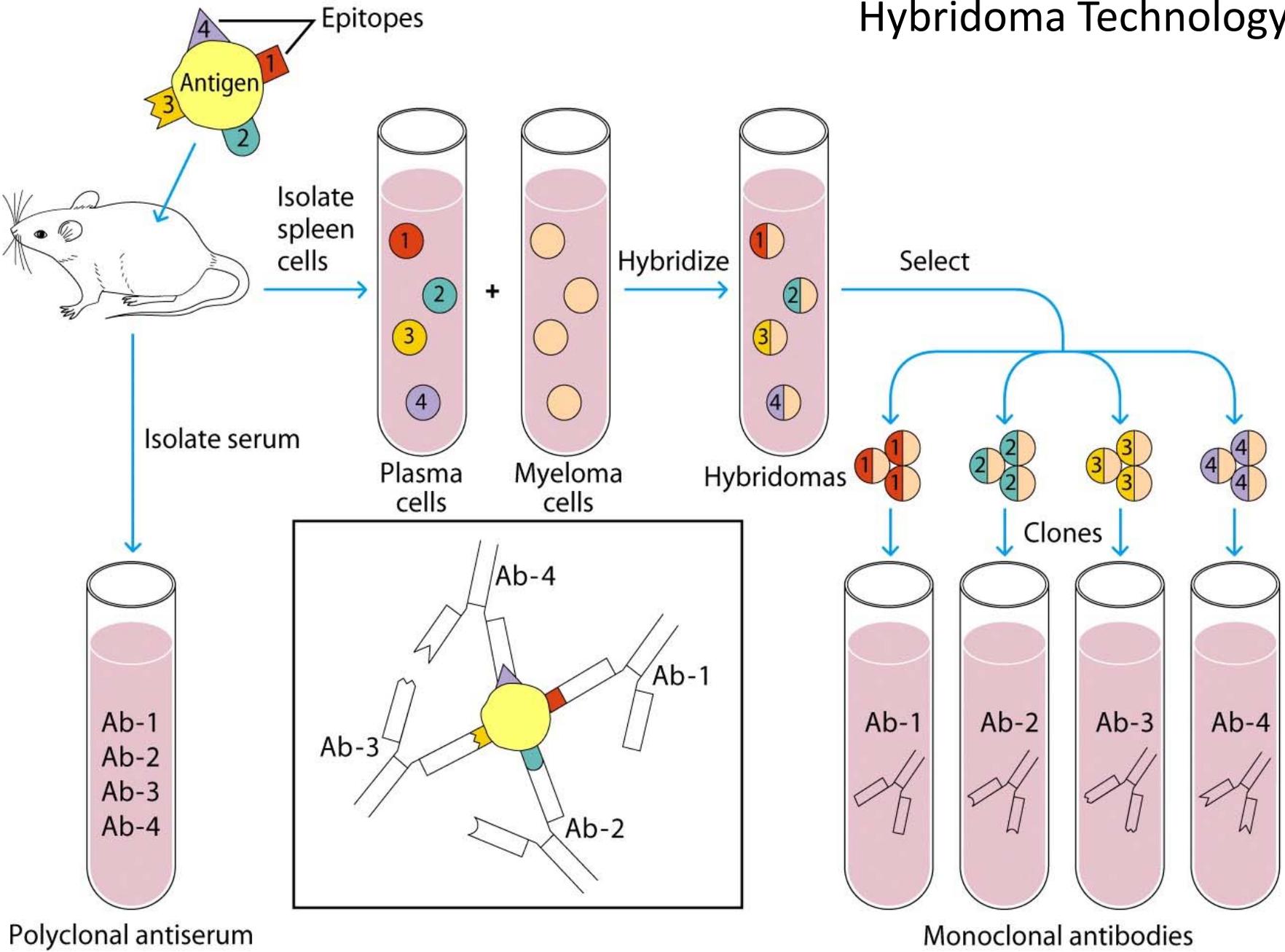
Polyclonal Antibodies

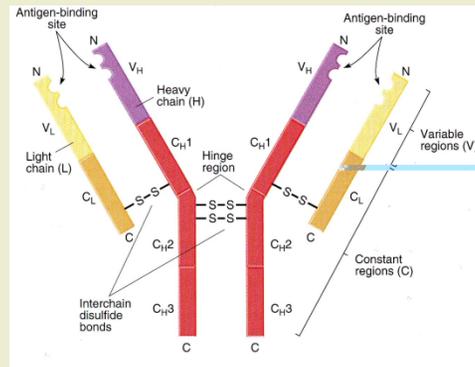
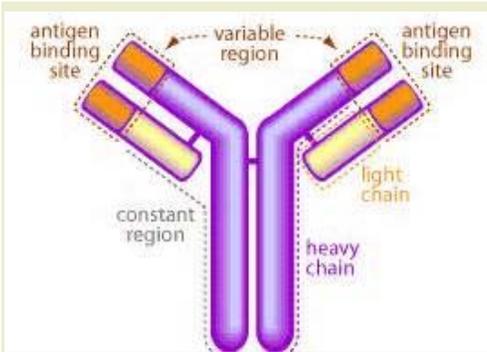


Monoclonal Antibodies

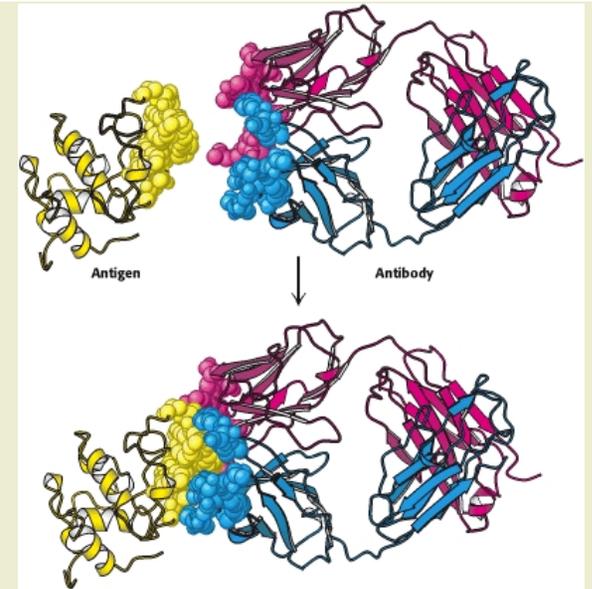


Hybridoma Technology





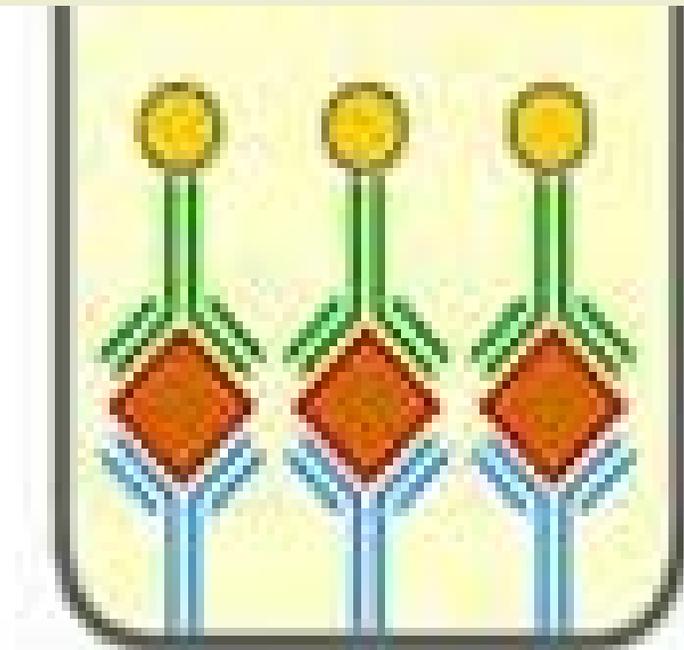
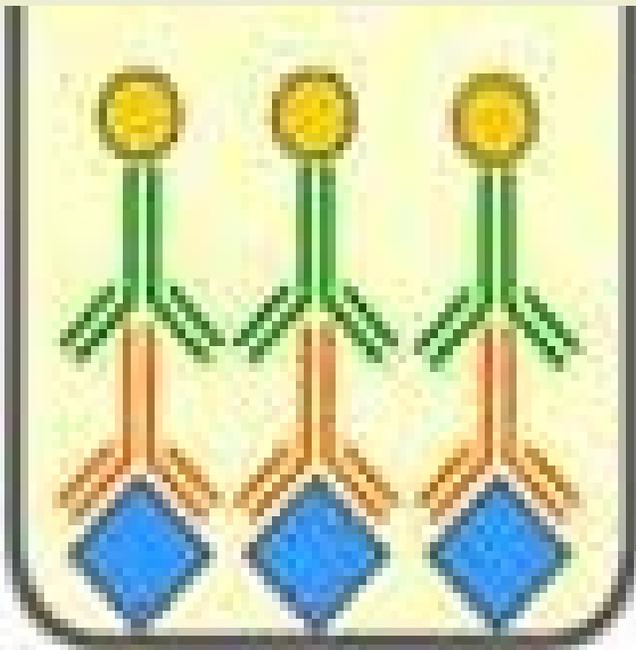
Ab-Ag
Specificity



ELISA

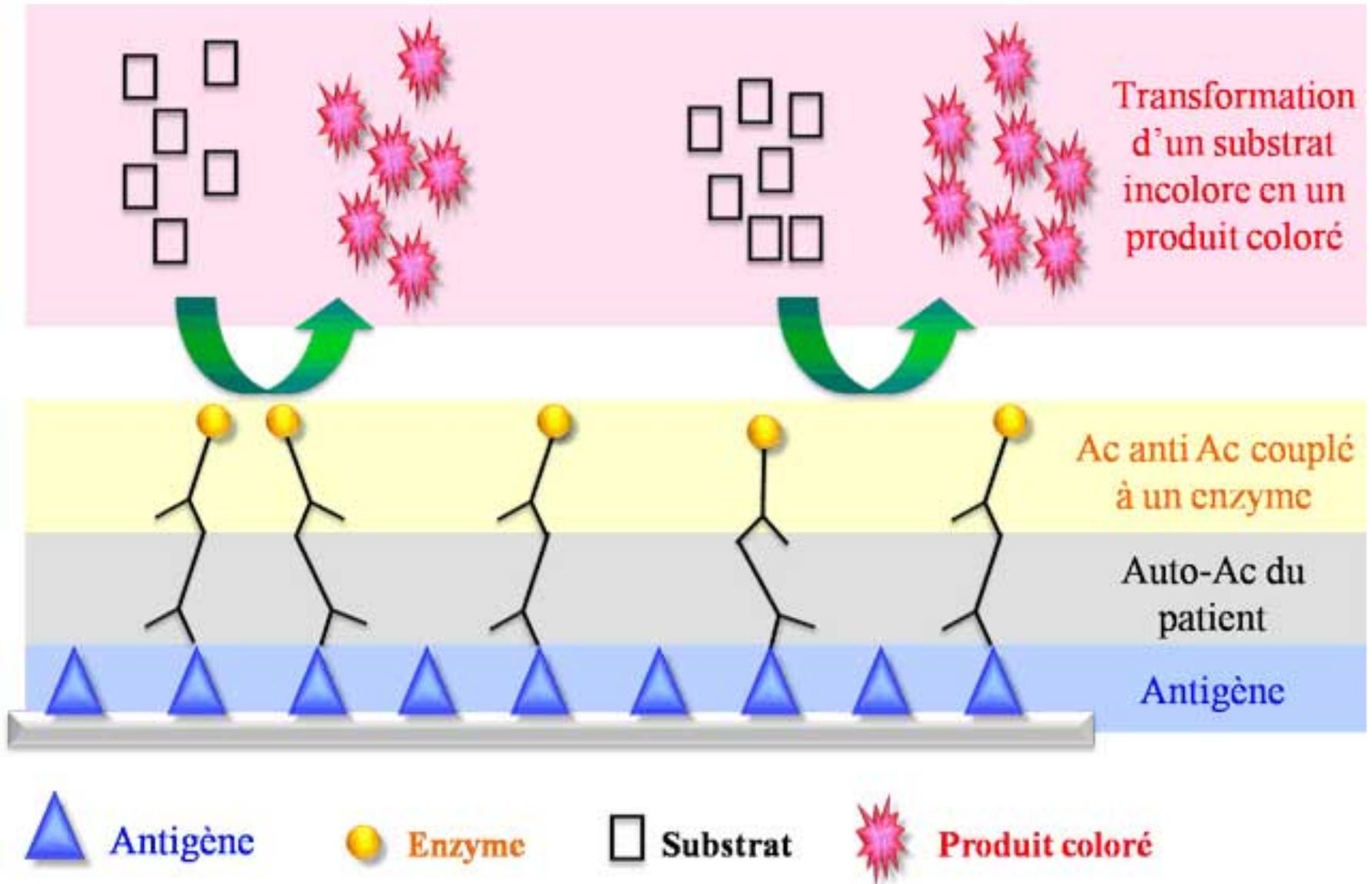
Indirect

Direct



A Picture is worth a 1000 words!

ELISA : Enzyme Linked Immuno Sorbent Assay



Home-made Simulations

- Connecticut's BioBus

- BPB pH indicator 
- Coat with Tween or dish detergent

- Univ of Calif Davis

Biotechnology in the Classroom:

“Disease Epidemic—ELISA Technique”

- TMB substrate/Streptavidin peroxidase

Internet Animations

- www.biology.arizona.edu/immunology/activities/elisa/main.html
- www.sumanasinc.com/webcontent/animations/content/ELISA.html
- www.edumedia-sciences.com/en/a543-direct-enzyme-linked-immunosorbent-assay-elisa

➤ \$8 for download

Immunology/ELISA/HIV

<http://www.biology.arizona.edu/immunology/activities/elisa/main.html>

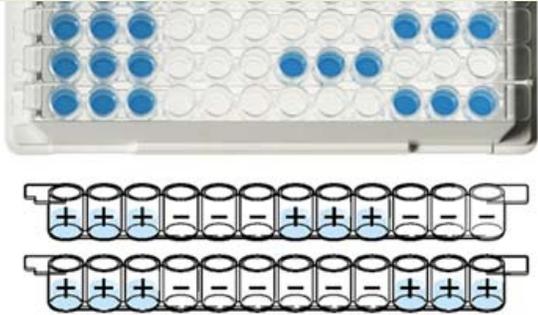
<http://www.biology.arizona.edu/immunology/activities/AIDS2003/main.html>

<http://www.pbs.org/wgbh/nova/aids/textindex.html>

<http://www.teachersdomain.org/9-12/sci/life/gen/doubleimmunity/index.html>

<http://www.learner.org/channel/courses/biology/units/hiv/images.html>

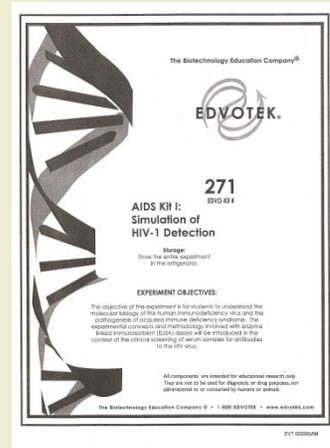
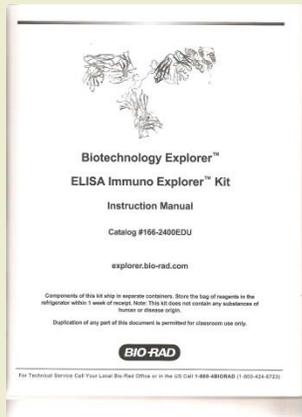
Commercial Kits



Edvotek's AIDS Kit

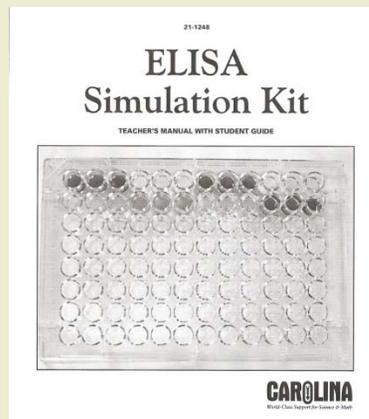


Bio-Rad's ELISA Immuno Explorer Kit



Used 16 months past expiration; great results (even with 1/2 volumes so x2 the # rxns)

Carolina's ELISA Simulation Kit



\$104.95 (Reagent refill \$46.95)

Storage: --20°C

(or 4°C for 1 month)

Purple/Green {no Ab/Ag}

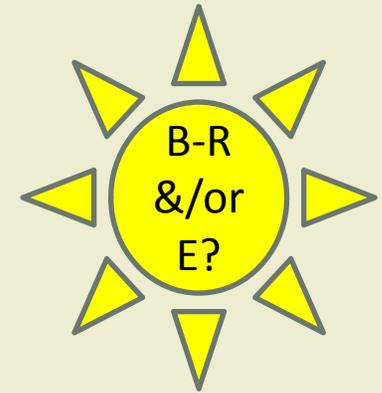
No pre-lab reagent prep

16 pairs of students

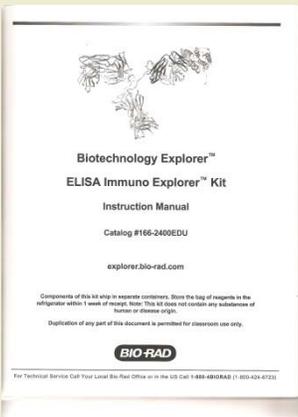
No washing steps



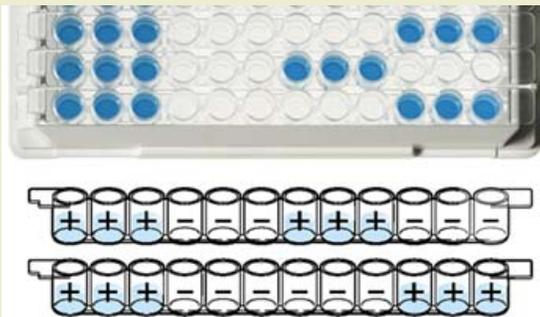
Commercial Kits



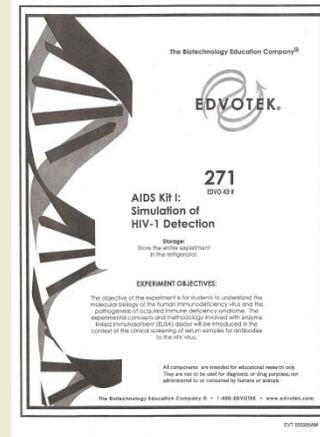
Bio-Rad's ELISA Immuno Explorer Kit



\$162.50 (Reagent refill \$120)
 Storage: 4°C
 HRP/TMB blue
 Pre-lab reagent prep
 12 student groups
 Invert strips
 Color-coding of reagent tubes
 Protocols I, II, III
 Quant option {655nm}



Edvotek's AIDS Kit



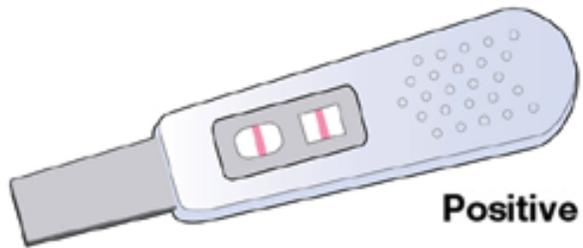
\$99
 Storage: 4°C
 Peroxidase/brown
 Pre-lab reagent prep
 10 student groups
 Transfer pipet removal



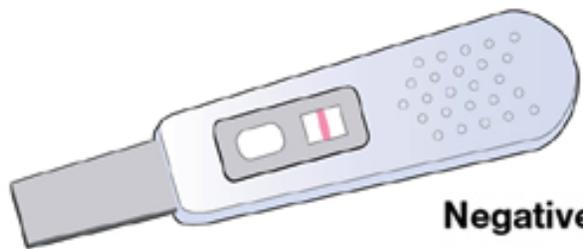
Bio-Rad: provides detailed information with Kit

Protocol	Type of ELISA	Real-World Application
I	Tracking outbreaks of disease by detecting antigens	HIV, SARS, Smallpox, anthrax
II	Detecting antigens	Pregnancy, drugs, GMO, BSE
III	Detecting antibodies in serum	Lyme disease, HIV, smallpox, West Nile virus

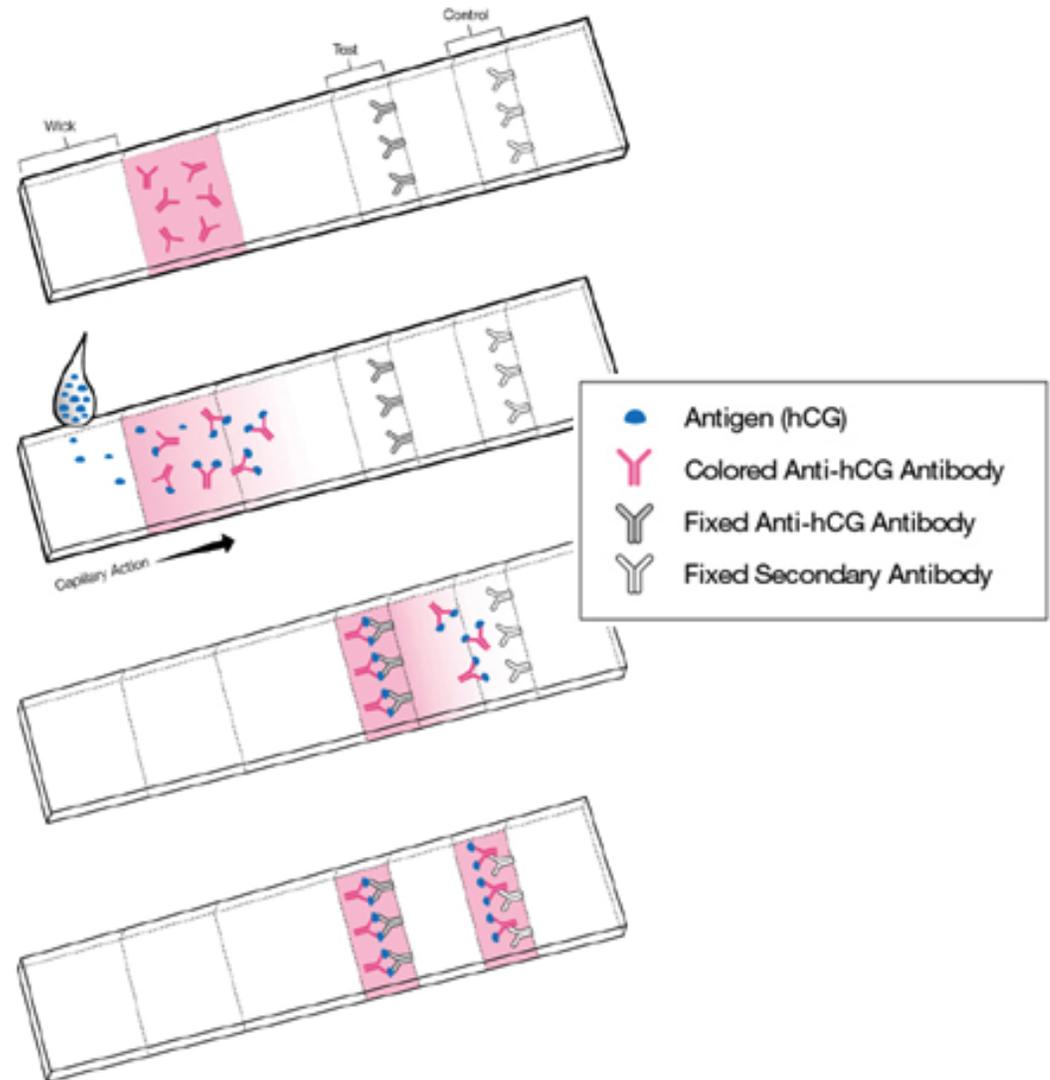
Example: Pregnancy Test



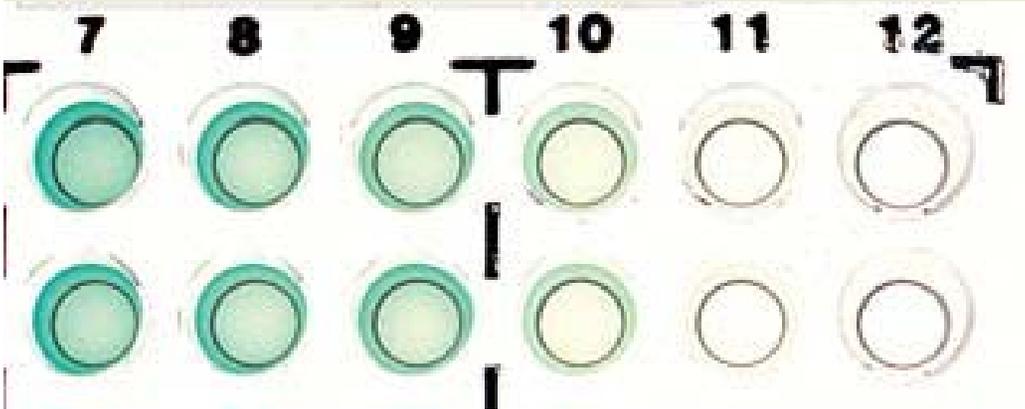
Positive



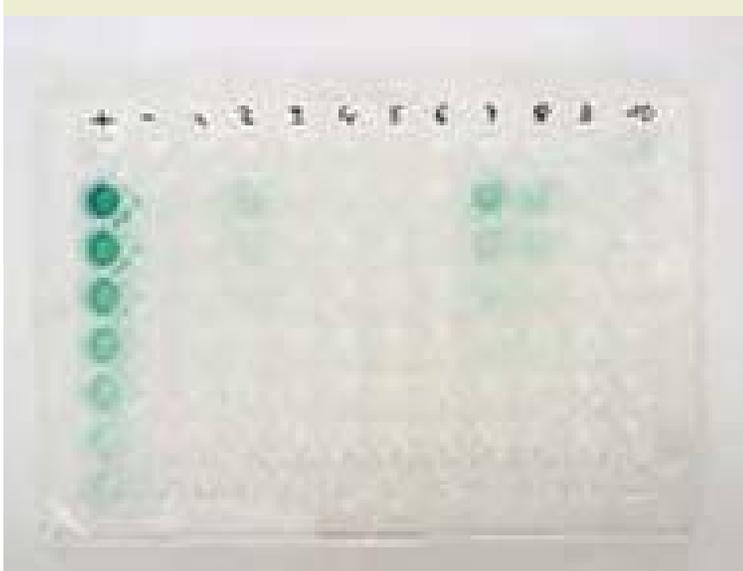
Negative



Quantitative ELISA

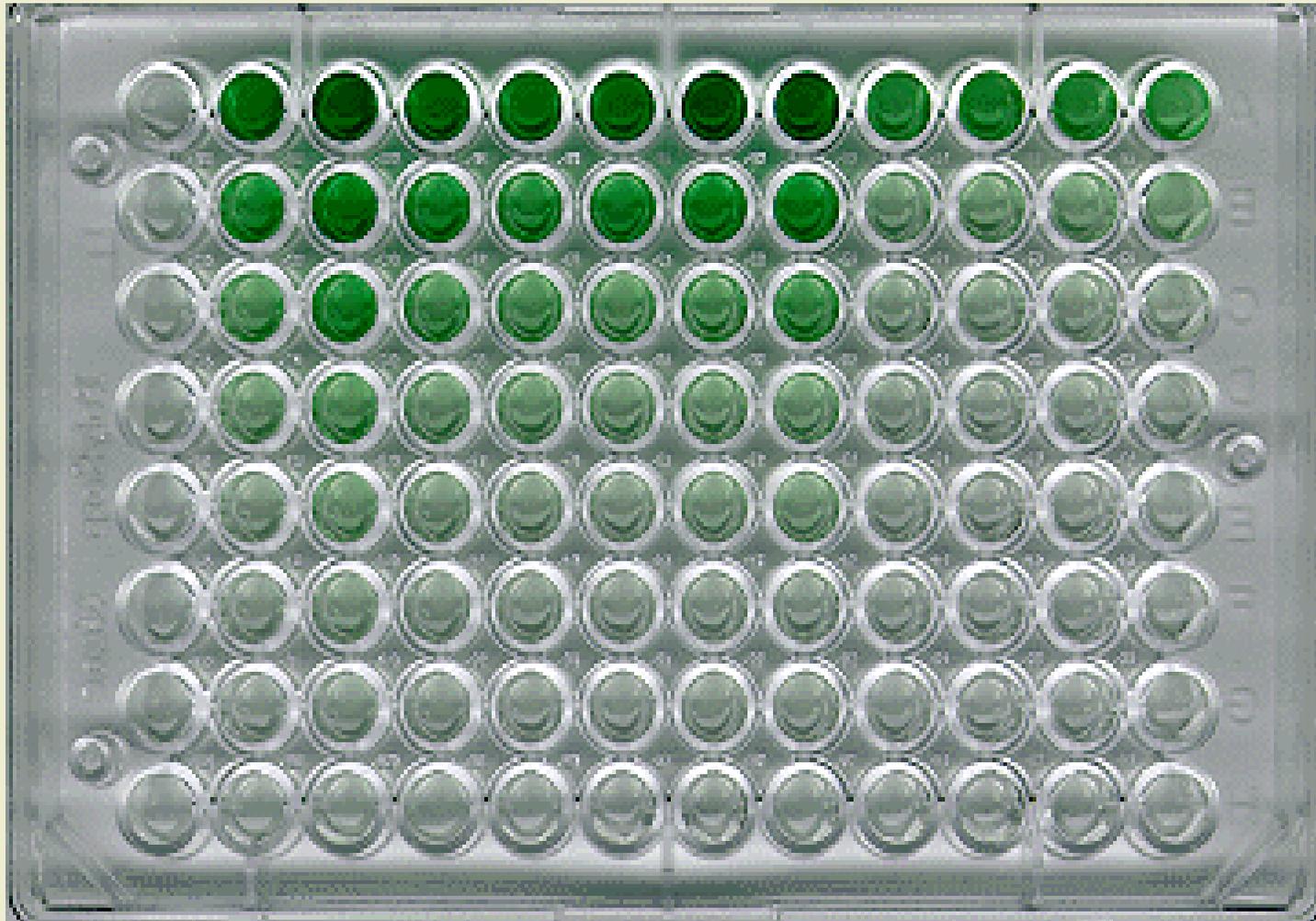


Enzyme-Linked
Immunosorbent Assay
(ELISA)-Multi-Lingual



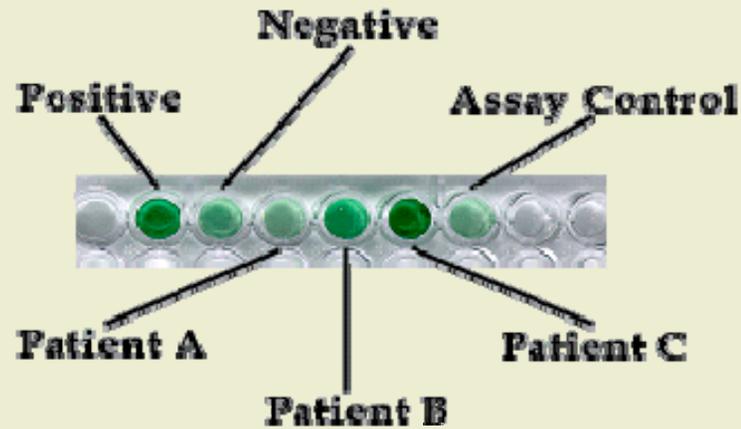
www.youtube.com/watch?v=RRbuz3VQ100

?Results determined?



A 96-well ELISA plate (8 cm x 12 cm)

OD_{450 nm}



Positive Control	Negative Control	Patient A	Patient B	Patient C	Assay Control
1.689	0.153	0.055	0.412	1.999	0.123

- The cutoff value indicating a positive result is 0.500
- Optical densities of 0.300 → 0.499 are indeterminate and need to be retested

Indirect ELISA: To detect HIV antibodies in serum



Inactivated HIV antigens pre-coated onto an ELISA plate



Patient serum



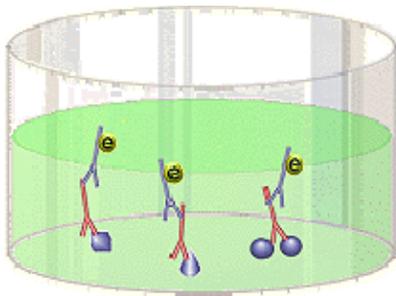
Anti-human immunoglobulin coupled to an enzyme.

This is the second antibody, and it binds to human antibodies

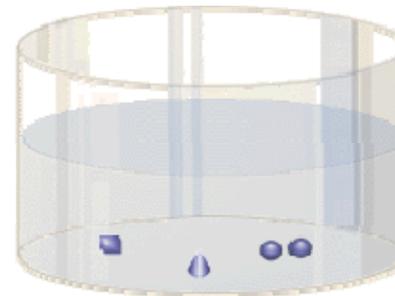


Chromogen or substrate which changes color when cleaved by the enzyme

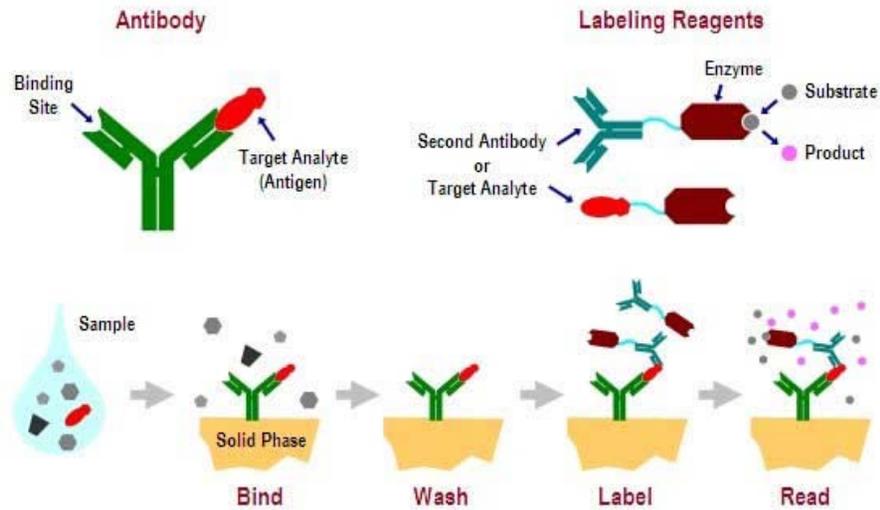
Positive



Negative



ELISA



Indirect ELISA

- 1 Antigen/sample is added to plate.
- 2 Blocking buffer is added to block remaining protein-binding sites.
- 3 Next a suitable **primary antibody** is added.
- 4 A suitable **secondary antibody – HRPO conjugate** is then added which recognizes and binds to the primary antibody.
- 5 TMB substrate (*Leinco Prod. No. T118*) is added and is converted by HRPO to detectable form.

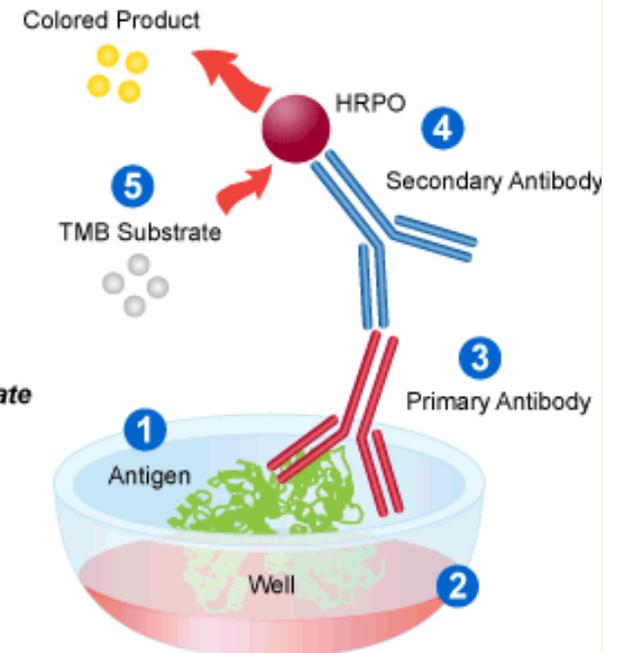
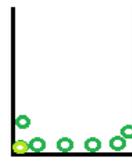


Diagram 1: Illustration of Indirect ELISA method.

Indirect ELISA

1. protein coated container



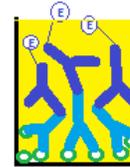
2. Antibodies bind to protein



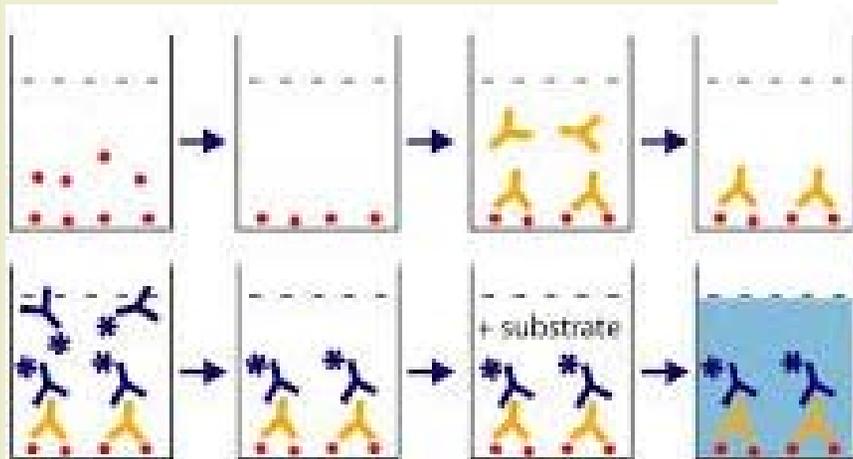
3. Excess washed away



5. visual change confirms correct protein is present



4. second antibody binds to first



CD available from HHMI
Virtual Lab:
Immunology/ELISA
Order online

