



Immunology & ELISAs



2013 BIOMAN Workshop, MC³

Lori Dodson, Ph.D.

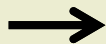
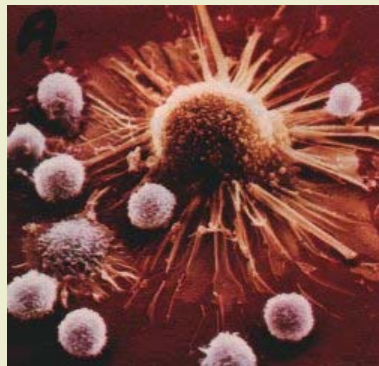
Show N Tell
& freebies
at end of
workshop ☺



Disclaimers



➤ I am NOT an immunologist



Killer T cells attacking a cancer cell



➤ Nobody is paying me!

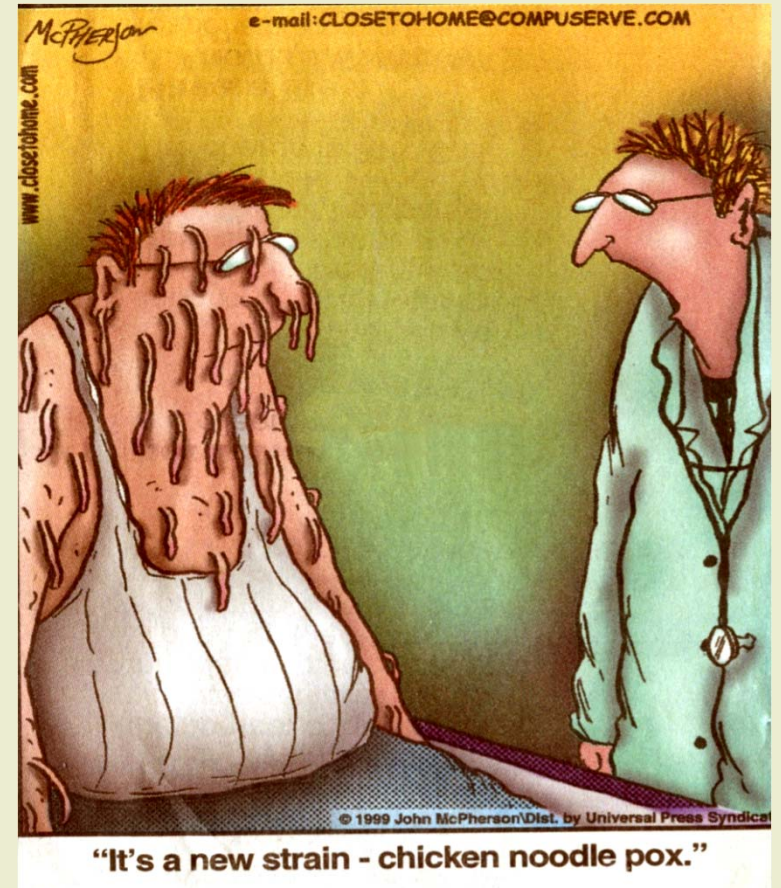


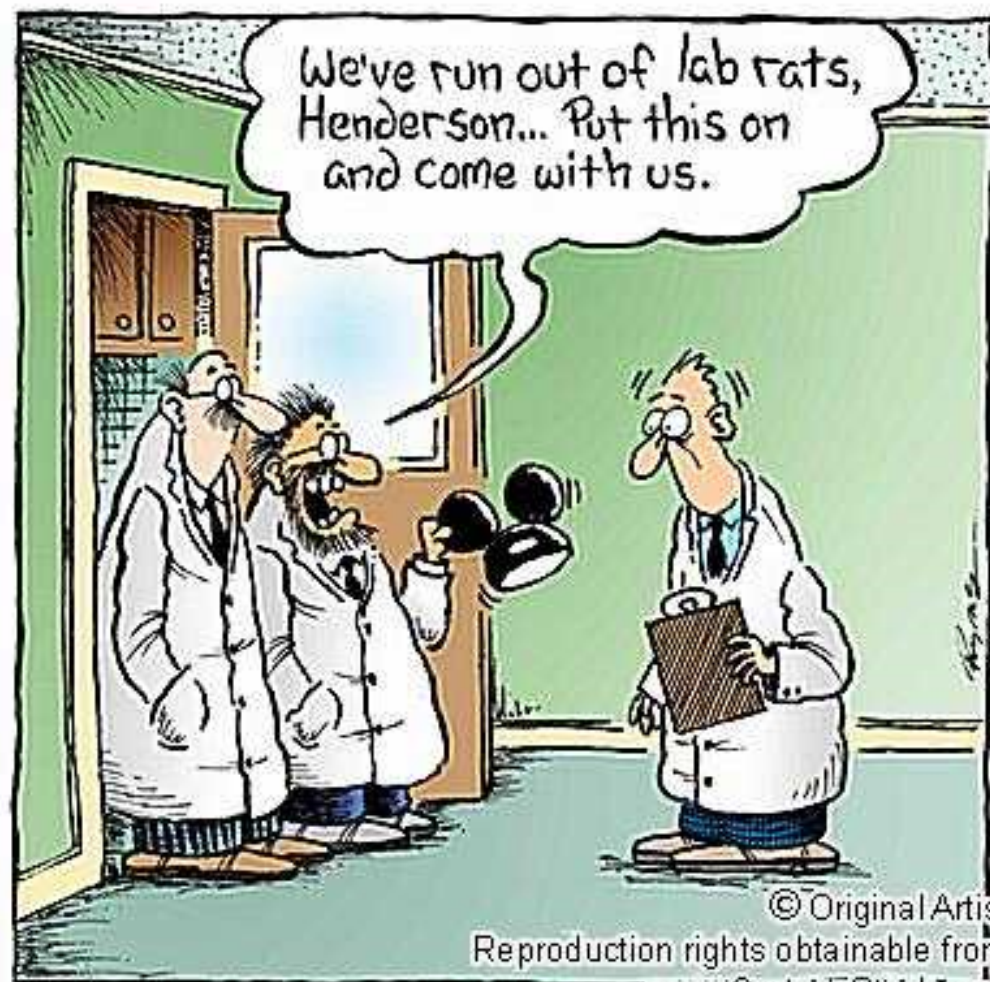


Workshop Content

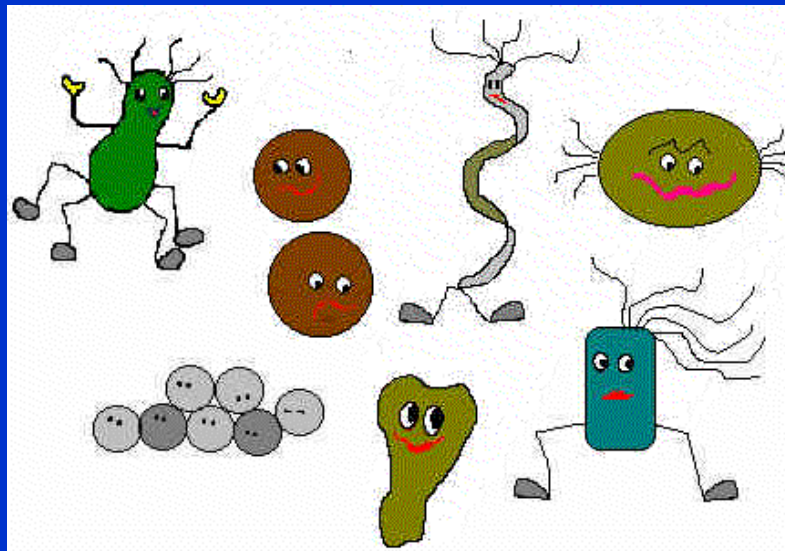
- Immunology & ELISAs
 - General Overview
 - Resources
 - Commercial Kits
 - Bio-Rad, Edvotek, Carolina
 - On-line sites
 - Simulations, Animations
 - Hands-on Component
 - Kits: Bio-Rad & Edvotek
 - Indirect ELISA
 - Multi-plate Reader
 - Quantitative Assay

- Miscellaneous
 - E-Gels
 - Freebies



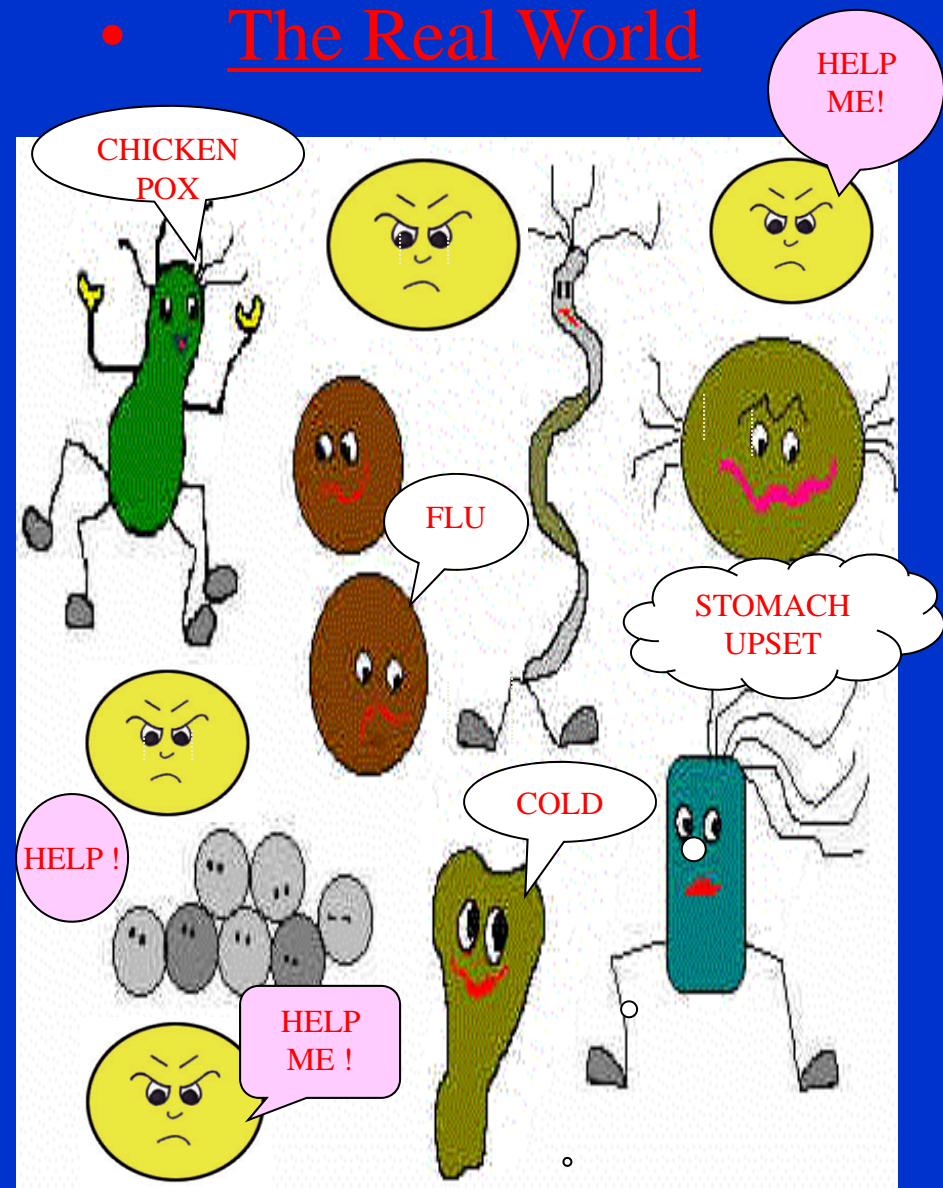


- The Perfect World



NATIONAL
CANCER
INSTITUTE

- The Real World



NCI's *Understanding The Immune System*

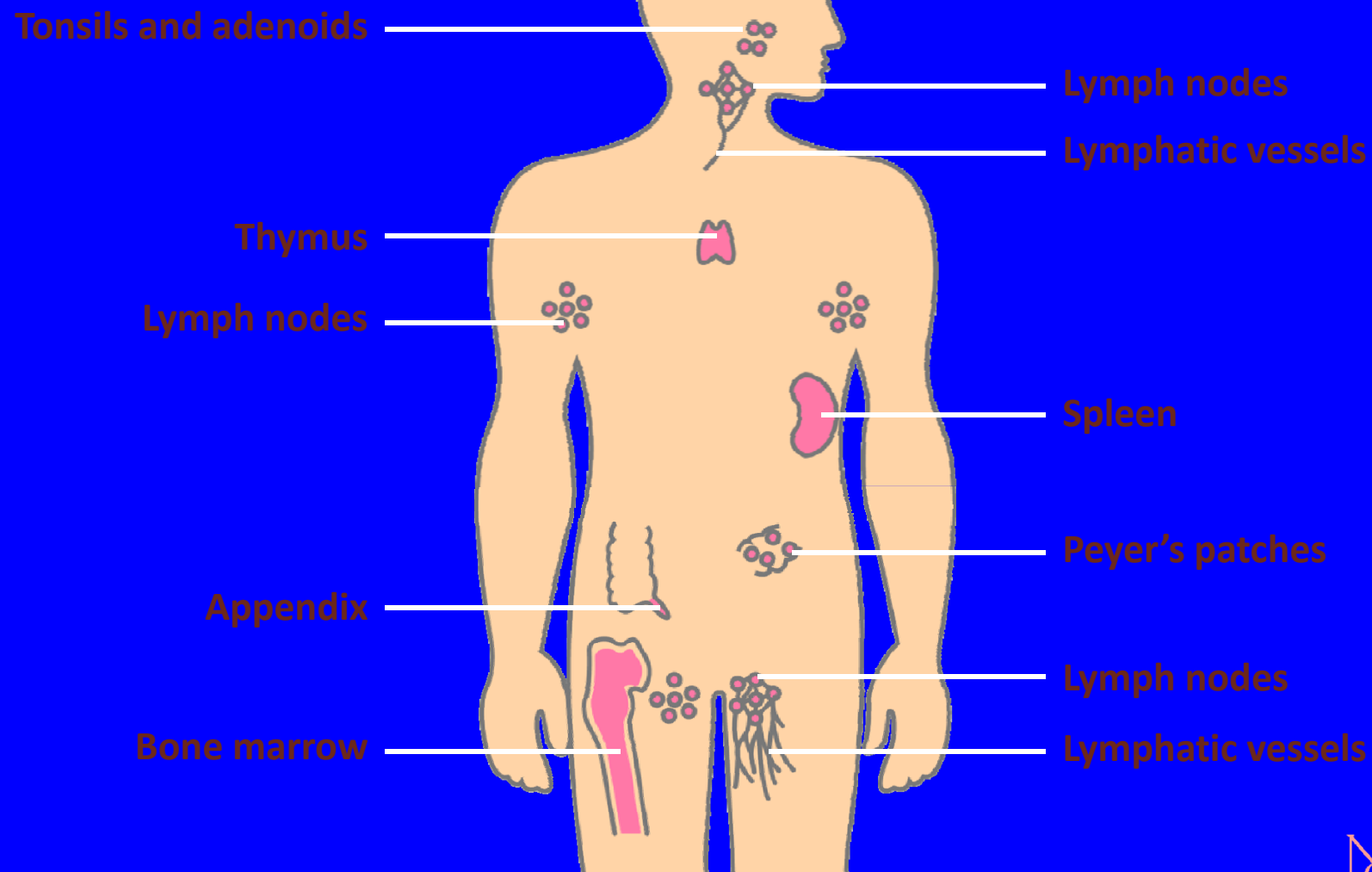


Organs of the Immune System

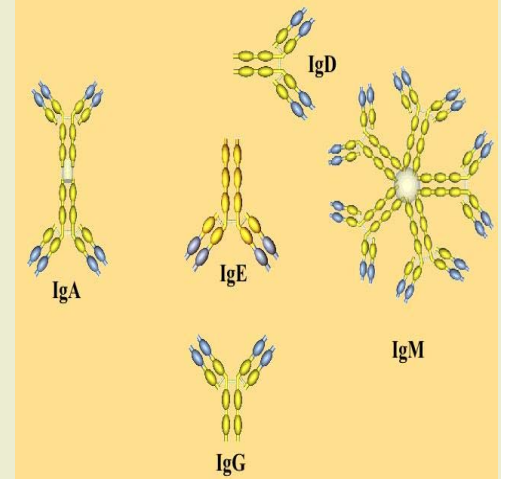
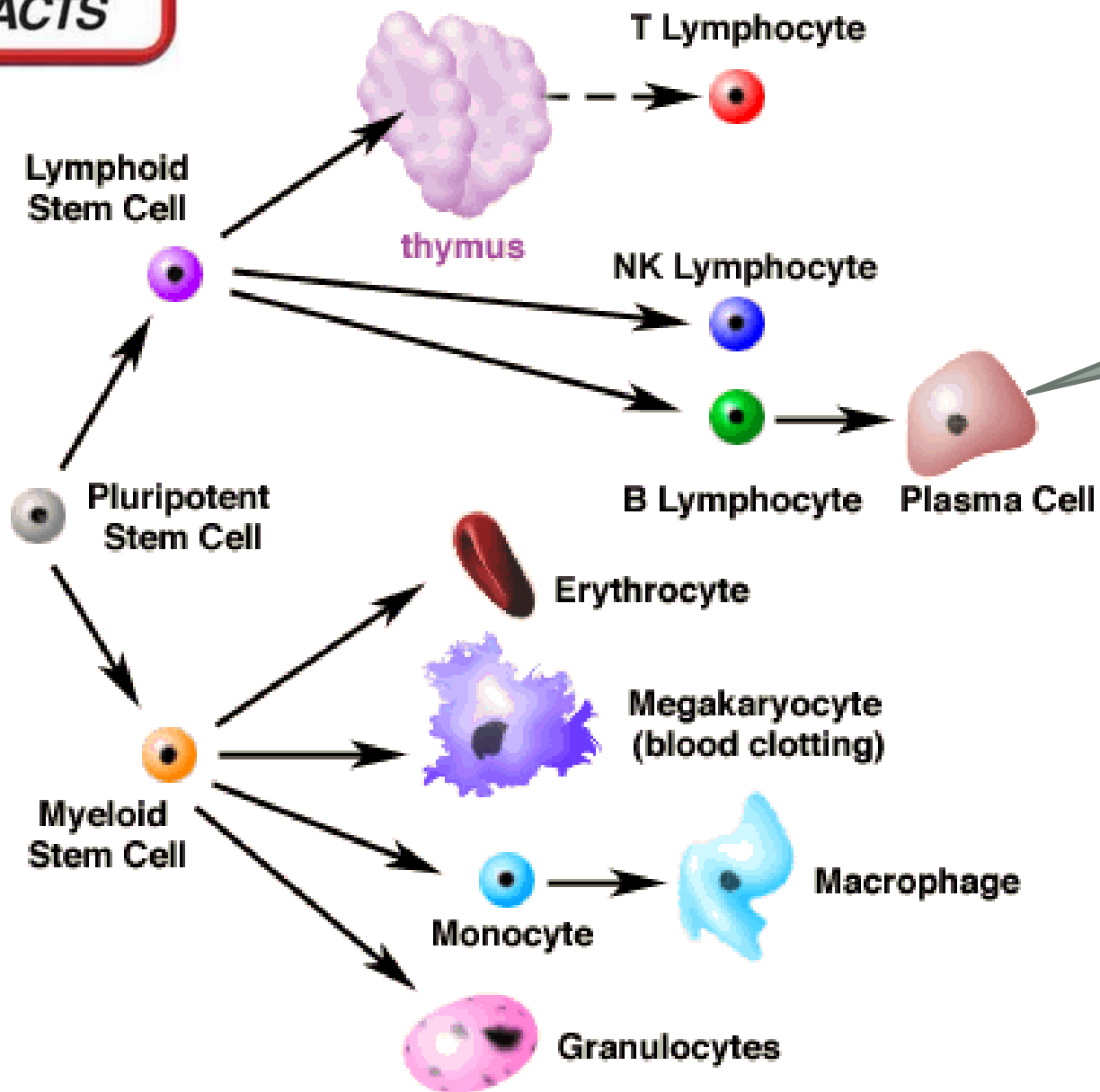
Understanding the Immune System

NIH, NIAID, NCI

booklet /tutorial:

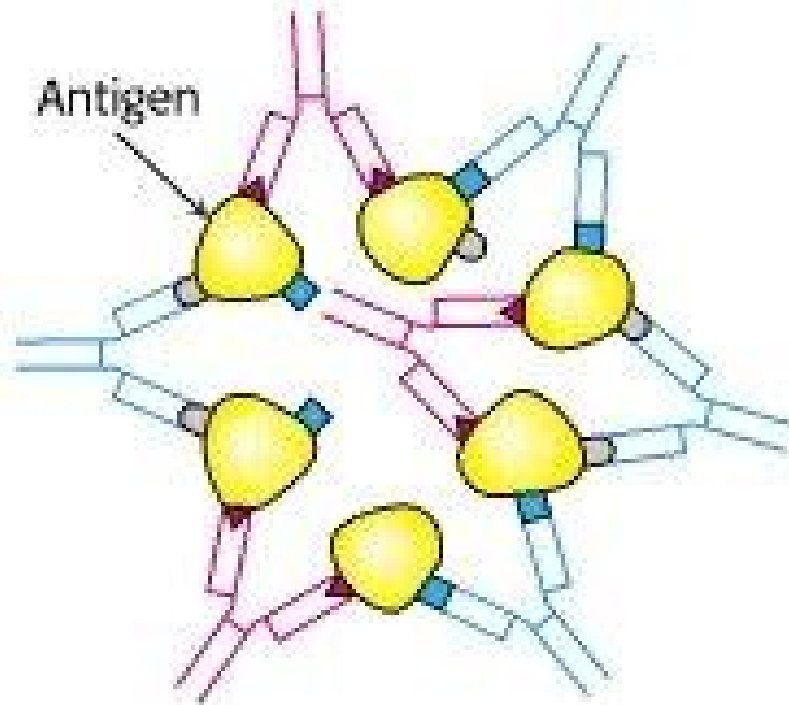


Artwork by Jeanne Kelly. ©2004.

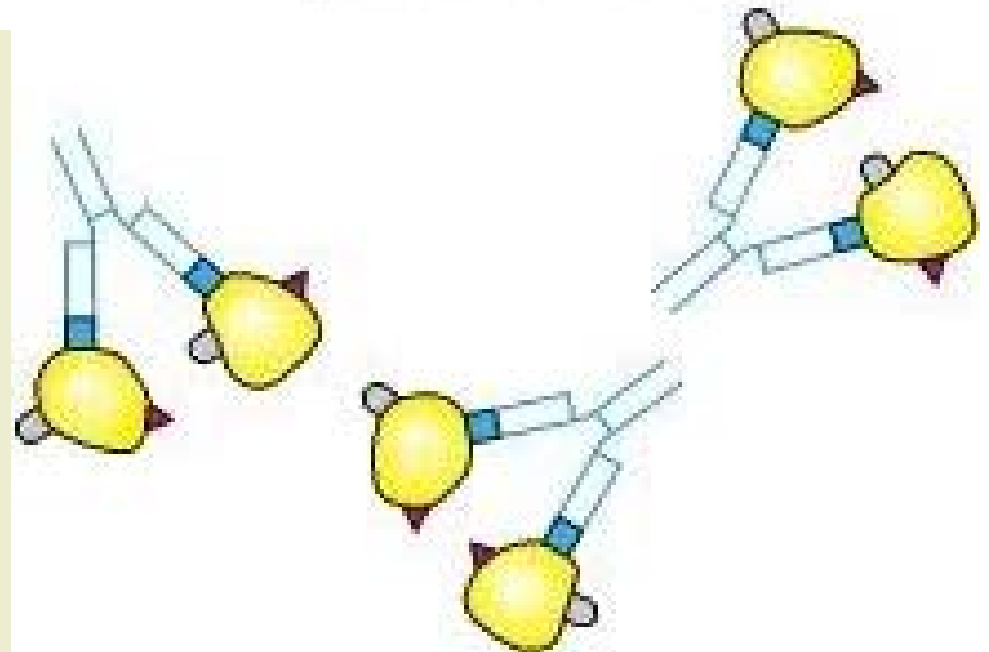


Ab-mediated
Acquired Immunity
2^o Response

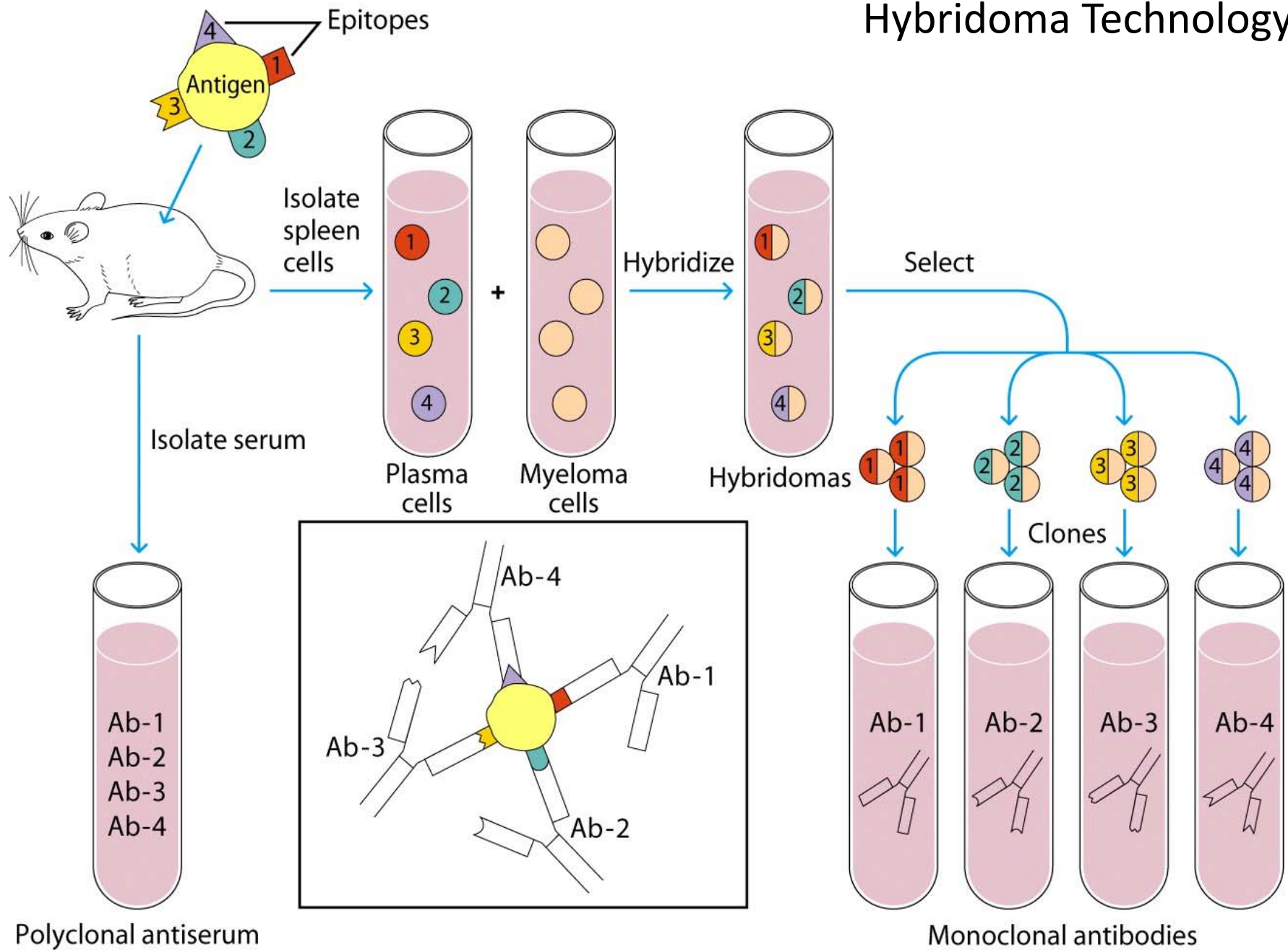
Polyclonal Antibodies

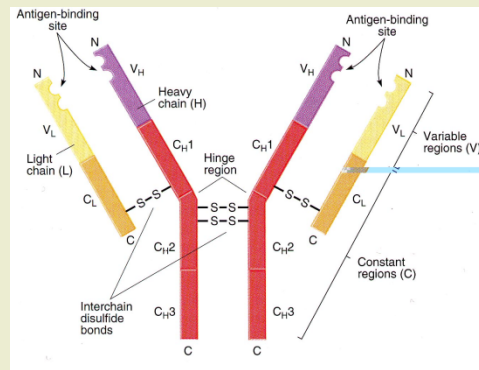
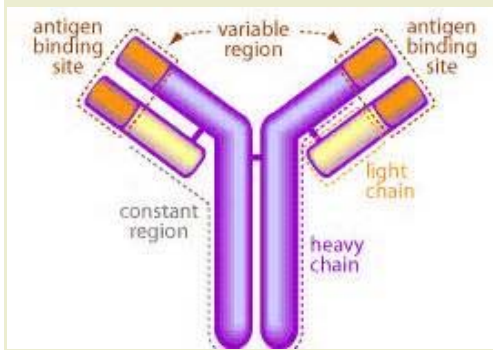


Monoclonal Antibodies



Hybridoma Technology





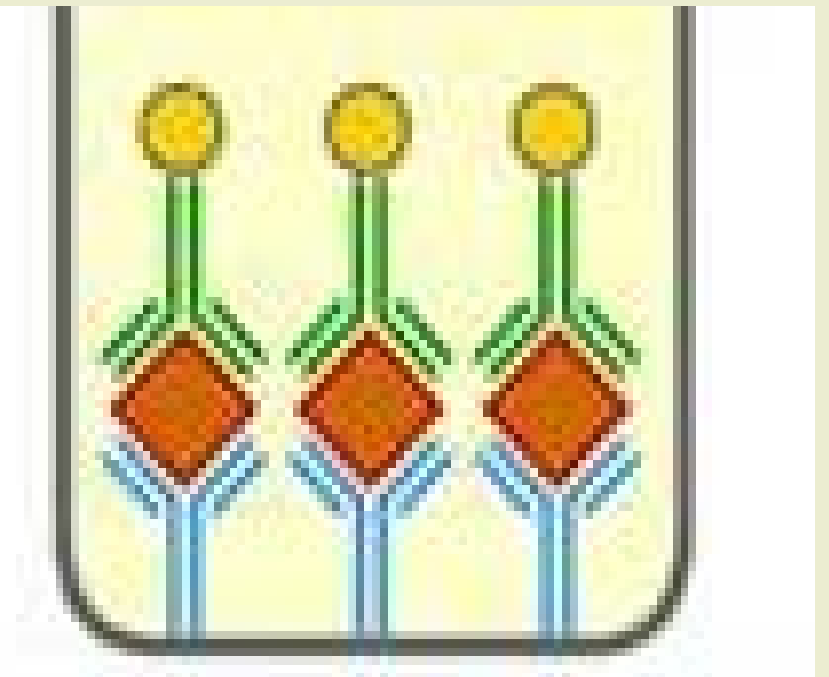
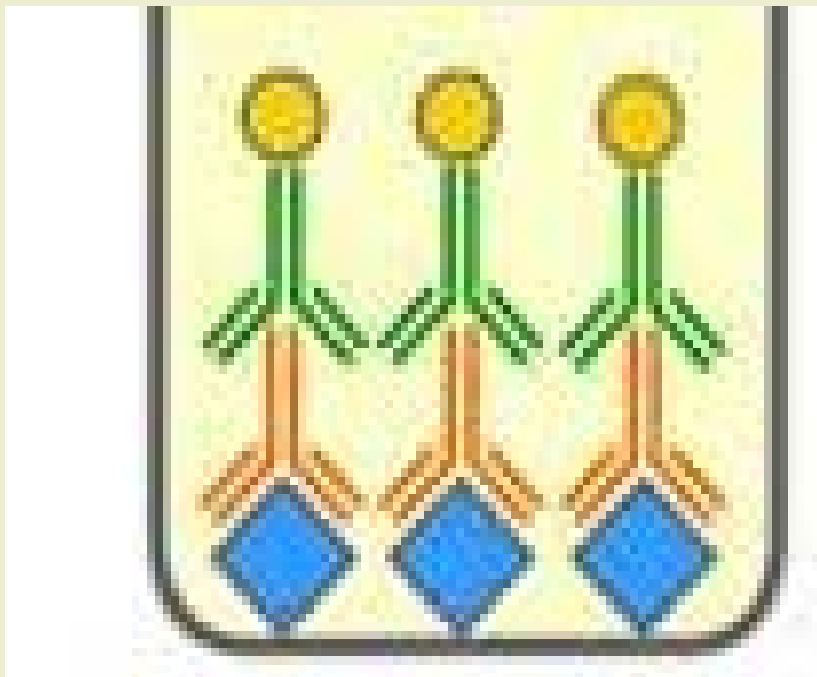
Ab-Ag
Specificity



ELISA

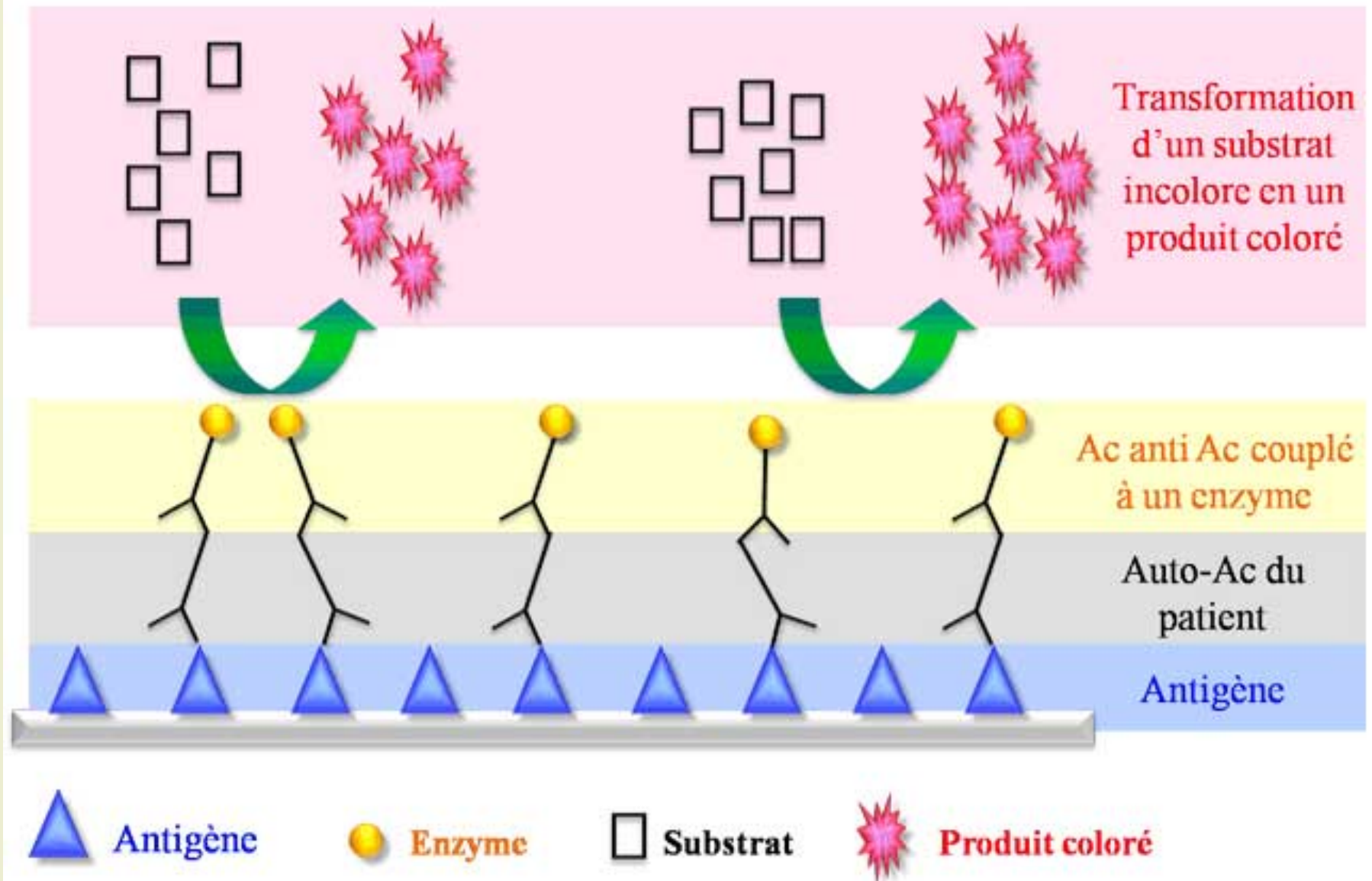
Indirect

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-immunosorgent-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>

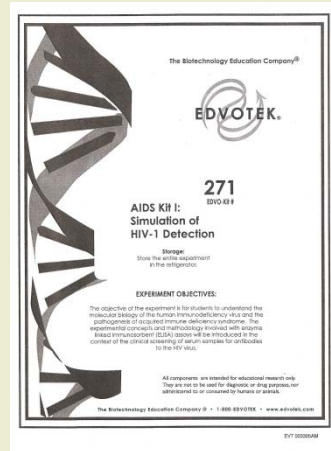
<http://www.pbs.org/wgbh/nova/aids/textindex.html>

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

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

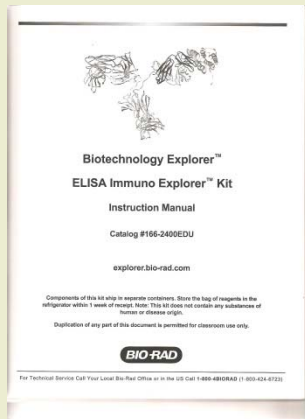
Commercial Kits

Edvotek's AIDS Kit

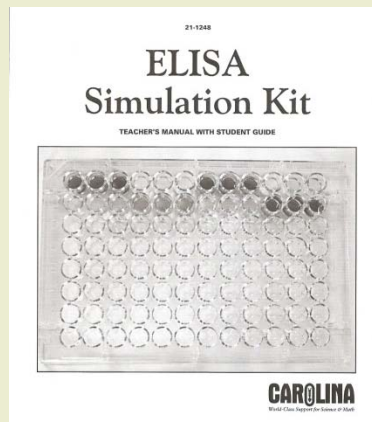


Used 16 months past expiration; great results (even with ½ volumes so x2 the # rxns)

Bio-Rad's ELISA Immuno Explorer Kit



Carolina's ELISA Simulation Kit



\$104.95 (Reagent refill \$46.95)

Storage: --20°C

(or 4°C for 1 month)

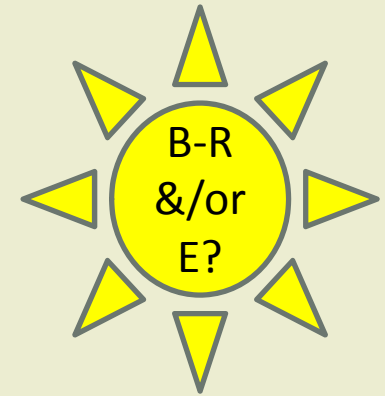
Purple/Green {no Ab/Ag}

No pre-lab reagent prep

16 pairs of students

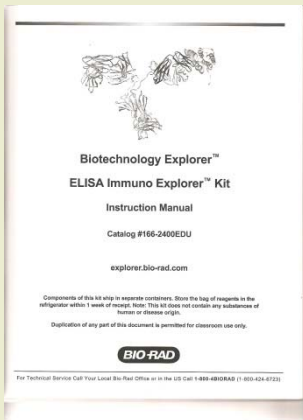
No washing steps

Commercial Kits

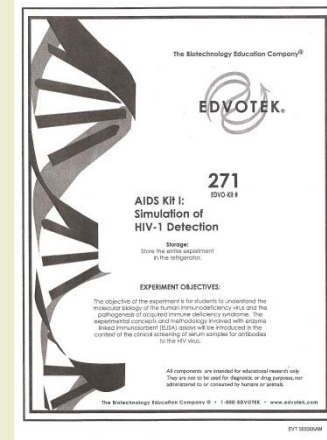
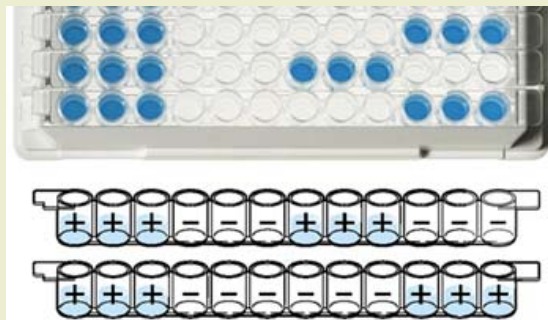


Bio-Rad's ELISA Immuno Explorer Kit

Edvotek's AIDS 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}



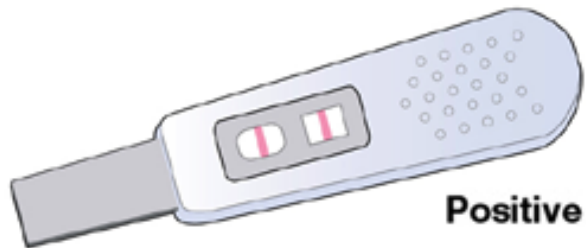
\$99
Storage: 4°C
Peroxidase/brown
Pre-lab reagent prep
10 student groups
Transfer pipet removal



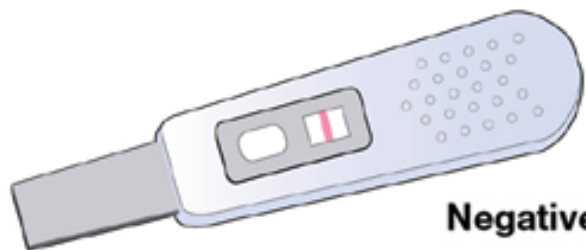
Bio-Rad: provides detailed information with Kit

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

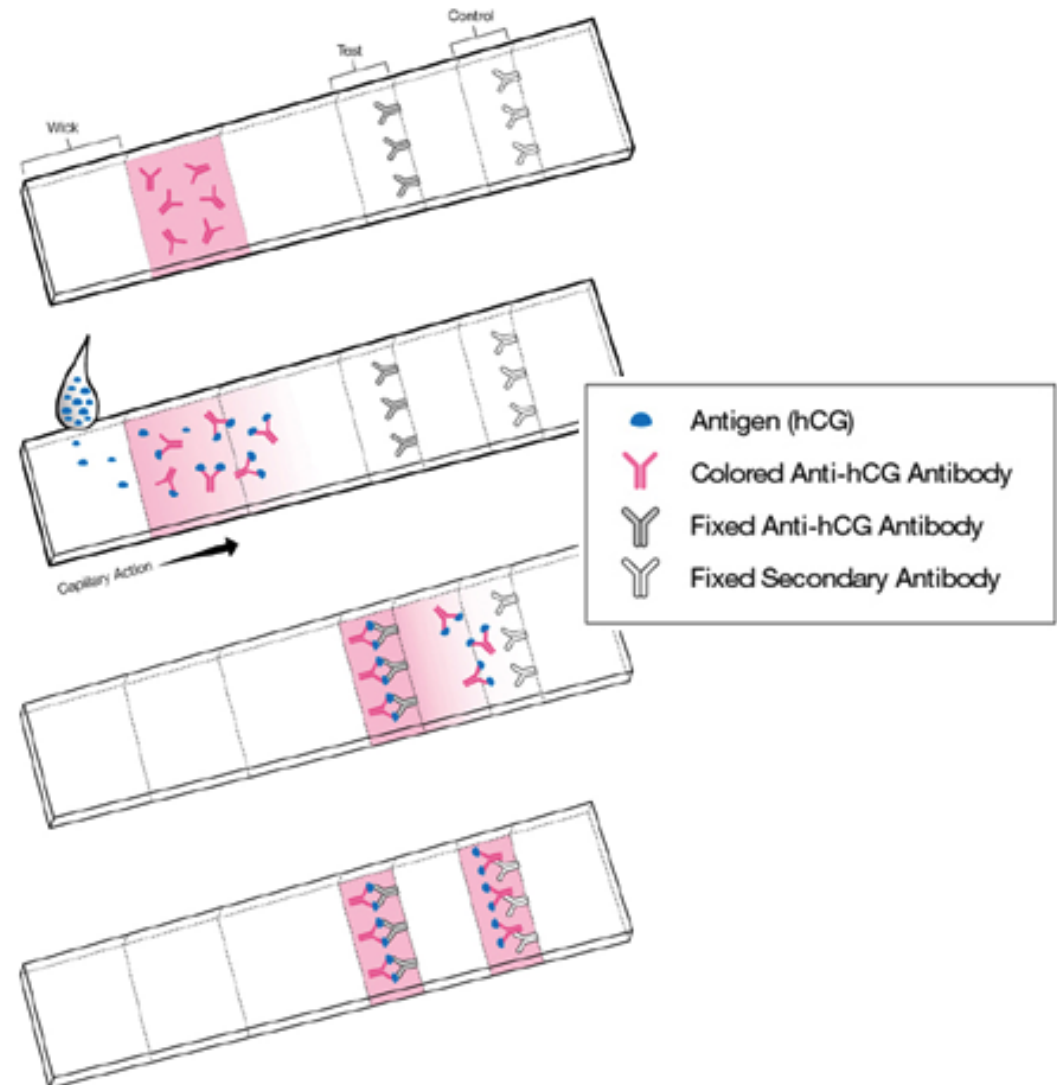
Example: Pregnancy Test



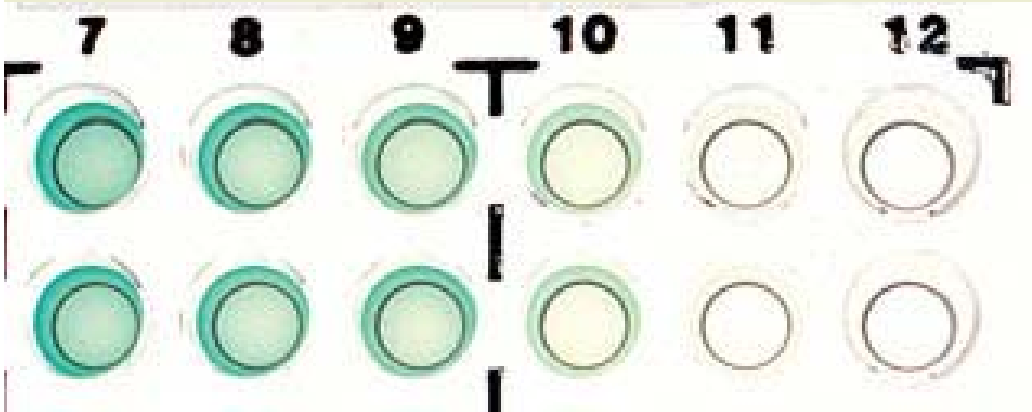
Positive



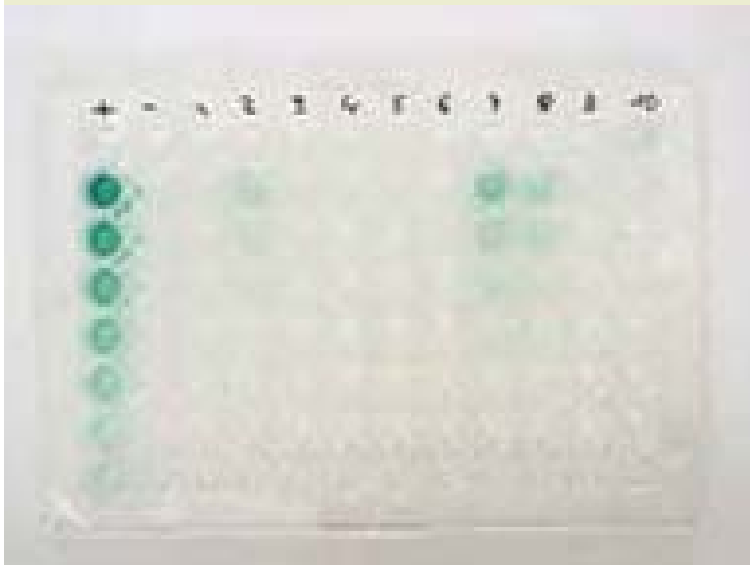
Negative



Quantitative ELISA

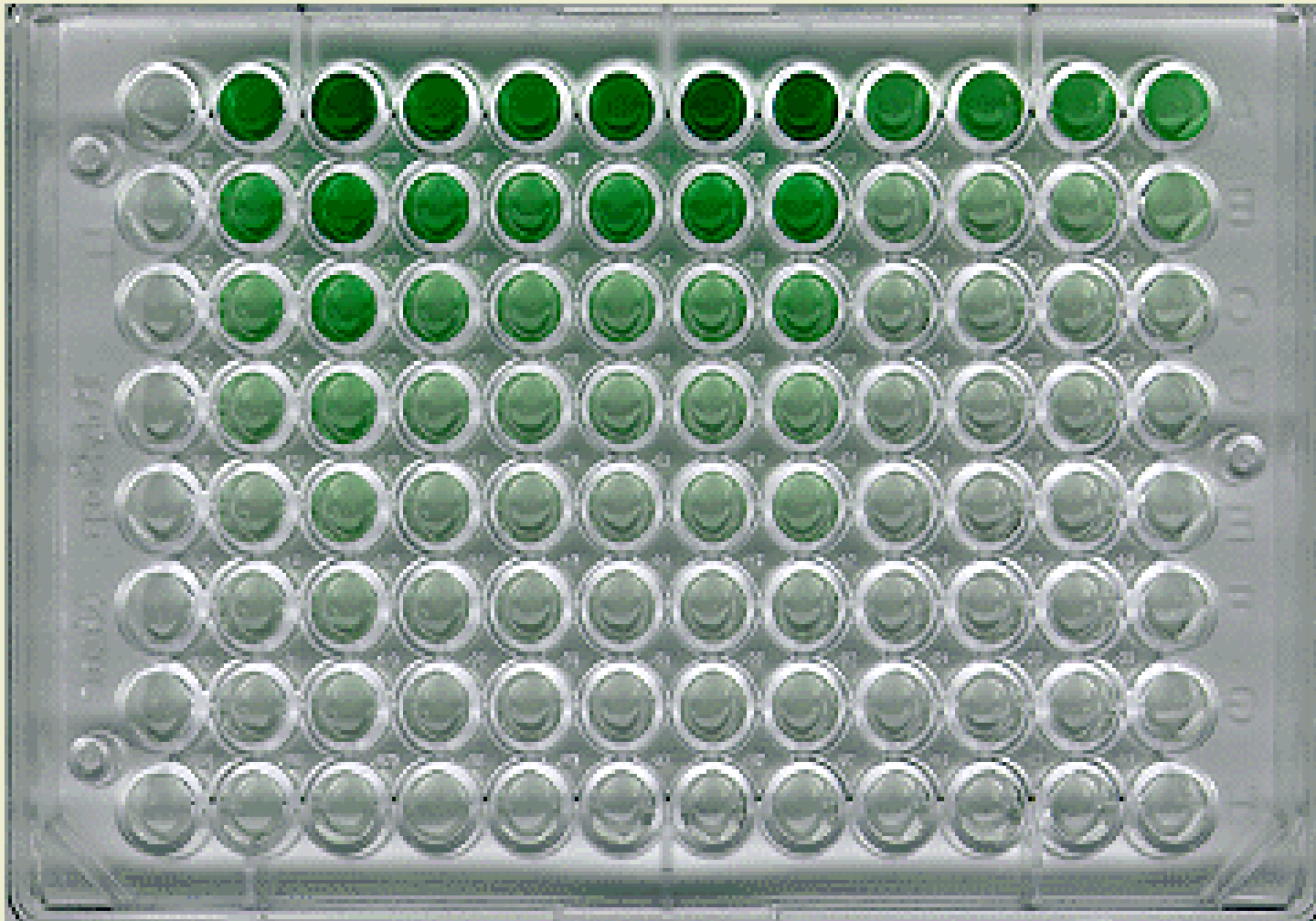


Enzyme-Linked
Immunosorbent Assay
(ELISA)-Multi-Lingual



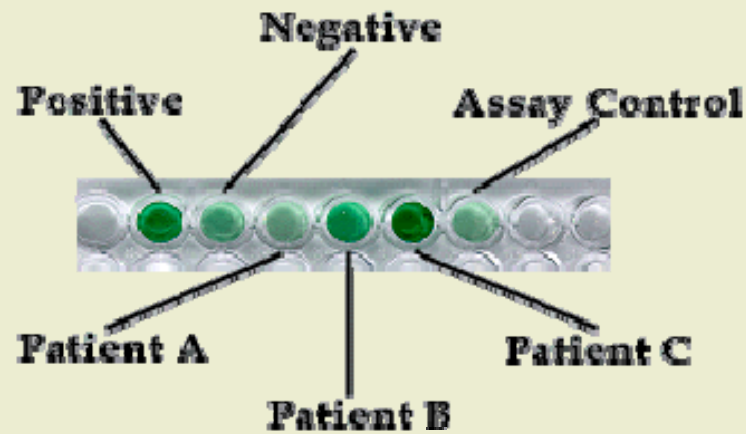
www.youtube.com/watch?v=RRbuz3VQ100

?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



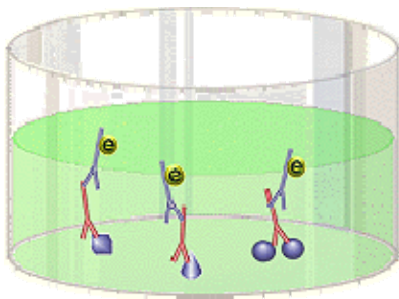
Anti-human immunoglobulin coupled to an enzyme.

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

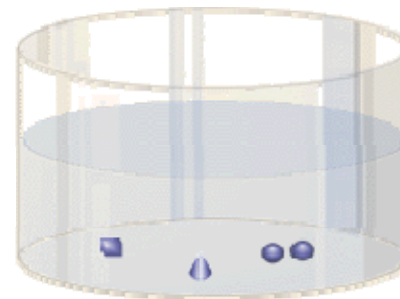


Chromogen or substrate which changes color when cleaved by the enzyme

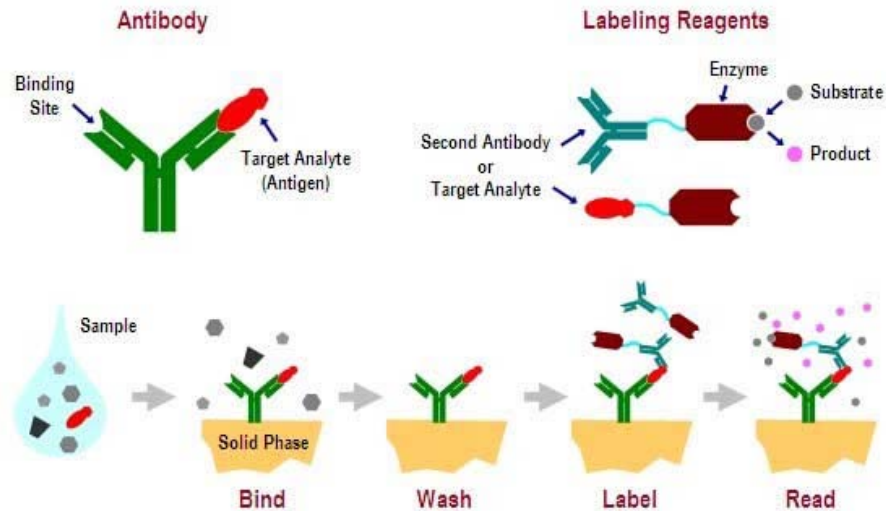
Positive



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.

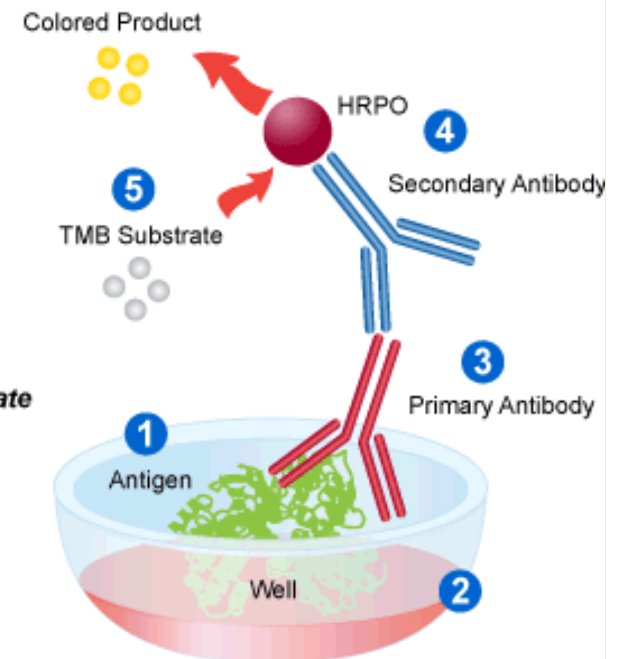
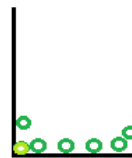


Diagram 1: Illustration of Indirect ELISA method.

Indirect ELISA

1. protein coated container



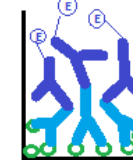
2. Antibodies bind to protein



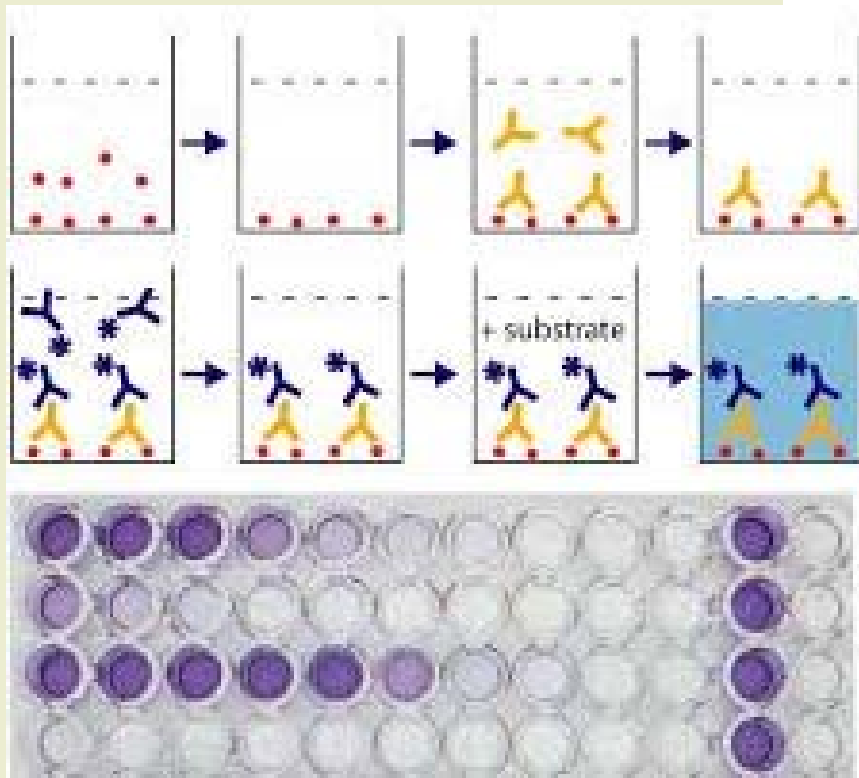
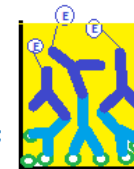
3. Excess washed away



4. second antibody binds to first



5. visual change confirms correct protein is present



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

