

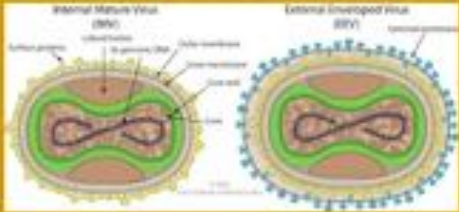



## Our Scenario: Smallpox

- Virus – DNA
- Person to person; aerosol
- Viable for 24 hours
- 12-24 day incubation
- Fever, headache, backache, abdominal pain, rash
- Postexposure vaccination in 2-4 days provides protection



You and your partner are a family unit (2 adults and two children). You are taking your family to the zoo aboard the metro. While on the metro, we were exposed to smallpox, deliberately released in aerosol form. It is important to determine as soon as possible which people have been exposed. Vaccination within 2-3 days of exposure can prevent smallpox, but the vaccine is in short supply. Also, the vaccination can have nasty side effects, so no one should be vaccinated unnecessarily. Vaccination of exposed individuals is essential to prevent further spread of the disease. We will look for exposure by evaluating how much antigen is present in the plasma of students and teachers before the person has had the ability to make much antibody yet.

Smallpox is caused by infection with variola virus, which belongs to the genus Orthopoxvirus, the family Poxviridae and subfamily Chordopoxvirinae. Variola is a large brick-shaped virus measuring approximately 302 to 350 nanometers by 244 to 270 nm,[26] with a single linear double stranded DNA genome 186 kilobase pairs (kbp) in size and containing a hairpin loop at each end.[27][28] The two classic varieties of smallpox are variola major and variola minor.

Four orthopoxviruses cause infection in humans: variola, vaccinia, cowpox, and monkeypox. Variola virus infects only humans in nature, although primates and other animals have been infected in a laboratory setting. Vaccinia, cowpox, and monkeypox viruses can infect both humans and other animals in nature.[20]

The lifecycle of poxviruses is complicated by having multiple infectious forms, with differing mechanisms of cell entry. Poxviruses are unique among DNA viruses in that they replicate in the cytoplasm of the cell rather than in the nucleus. In order to replicate, poxviruses produce a variety of specialized proteins not produced by other DNA viruses, the most important of which is a viral-associated DNA-dependent RNA polymerase.

Both enveloped and unenveloped virions are infectious. The viral envelope is made of modified Golgi membranes containing viral-specific polypeptides, including hemagglutinin.[27] Infection with either variola major or variola minor confers immunity against the other.

## Family Name:

ID #	Person's Name	Age	Favorite Personality Characteristic	Diagnosis: Exposed? Antigen Levels	Need Vaccine? Yes or No

"Typical Family" – Two Adults plus two children



Technique:  
**ELISA**  
*Enzyme  
Linked  
Immunosorbent  
Assay*

What does that mean?

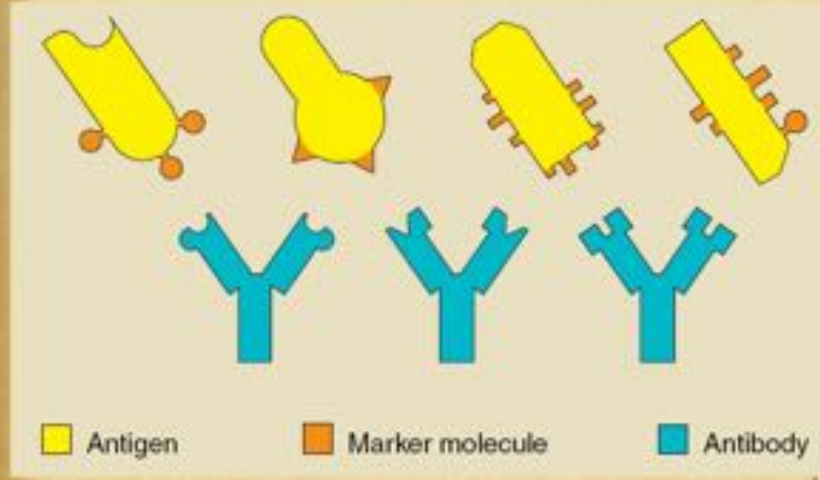
Good Animation:

<http://www.sumanasinc.com/webcontent/animations/content/ELISA.html>

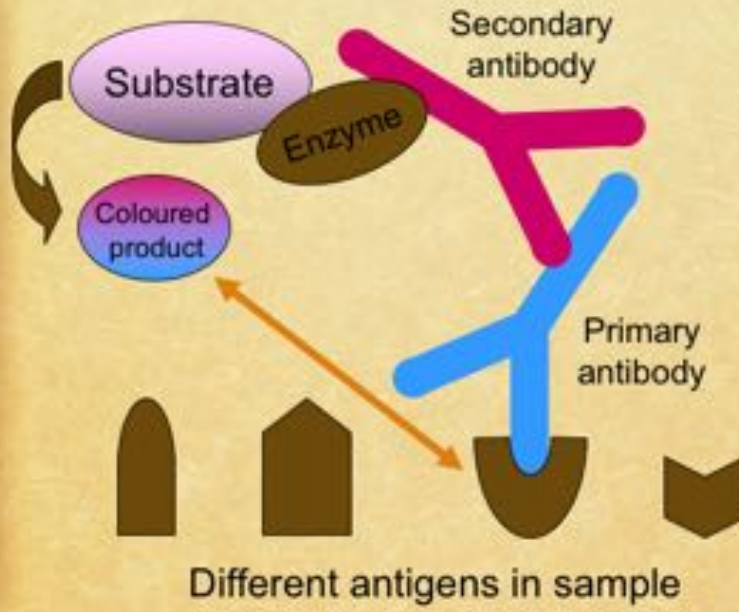


SARS – Severe Acute Respiratory Sndrome

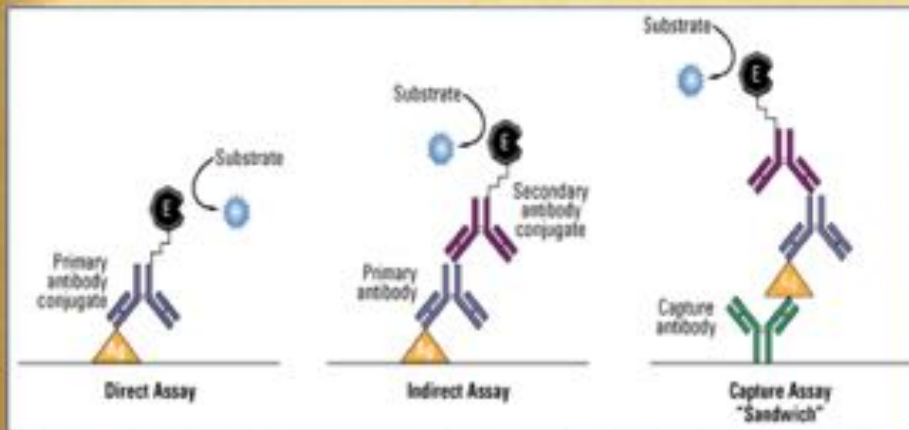
# Putting Antibodies & Antigens to Work



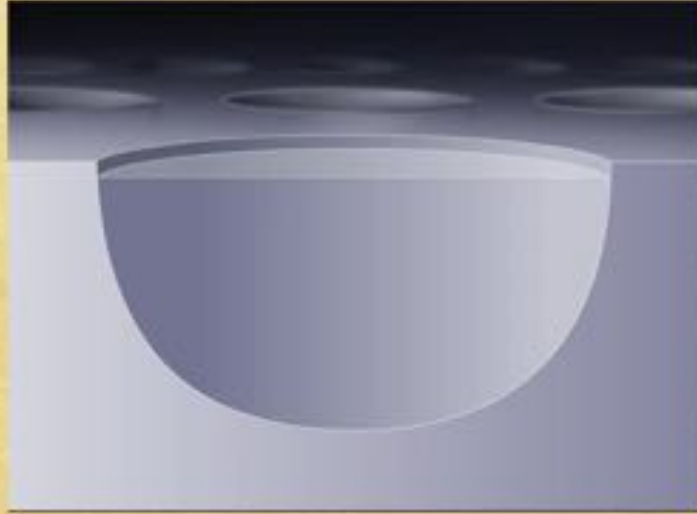
# Basic Scheme



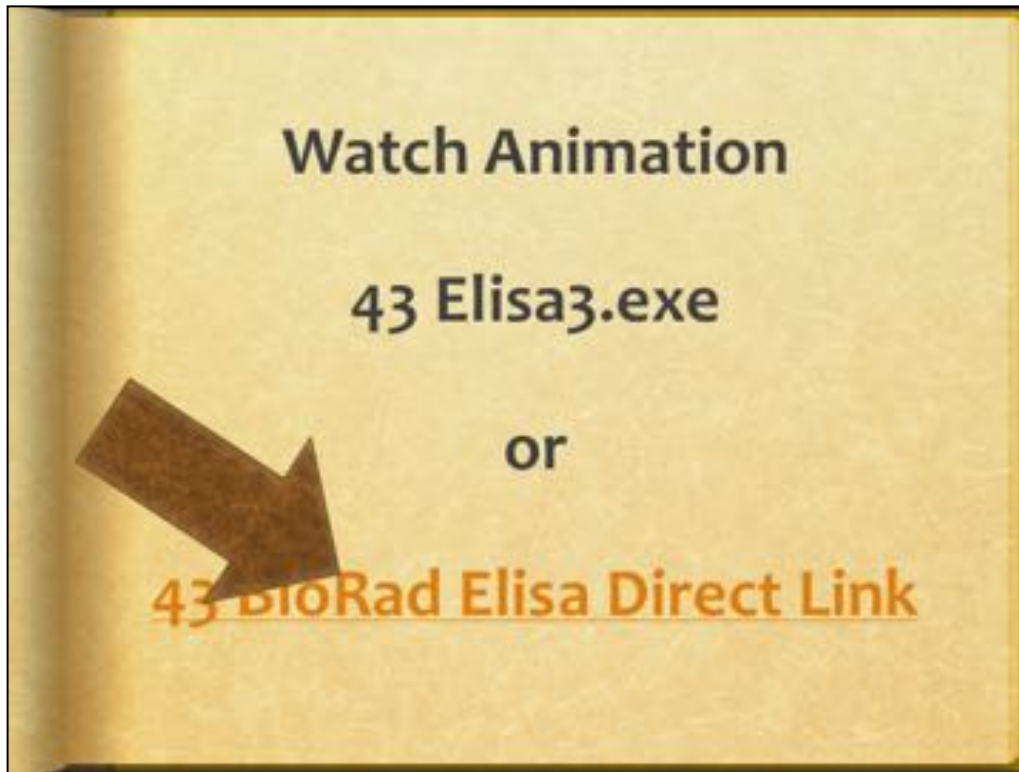
# Basic Scheme



## ELISA Animation: Direct & Indirect







[http://www.bio-rad.com/LifeScience/jobs/2004/04-0522/04-0522\\_ELISA.html](http://www.bio-rad.com/LifeScience/jobs/2004/04-0522/04-0522_ELISA.html)

**Indirect ELISA**

These reporter antibodies bind to the bound autoantibodies from the sample, forming a complex consisting of immobilised antigen, autoantibody and labelled reporter antibody. The wells are again rinsed to remove any unbound antibodies.

**Direct ELISA**

The secondary antibody attaches to the bound protein from the sample, forming a complex consisting of immobilised antibody, protein and labelled secondary antibody.

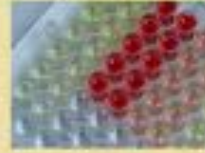
Good Animation at:

[http://www.chemgapedia.de/vsengine/vlu/vsc/en/ch/25/orgentec/autoimmunodiagnostik.vlu/Page/vsc/en/ch/25/orgentec/diagnostik\\_elisa\\_varianden.vscml.html](http://www.chemgapedia.de/vsengine/vlu/vsc/en/ch/25/orgentec/autoimmunodiagnostik.vlu/Page/vsc/en/ch/25/orgentec/diagnostik_elisa_varianden.vscml.html)

Good animation: <http://www.wiley.com/college/fob/quiz/quiz05/5-3.html>

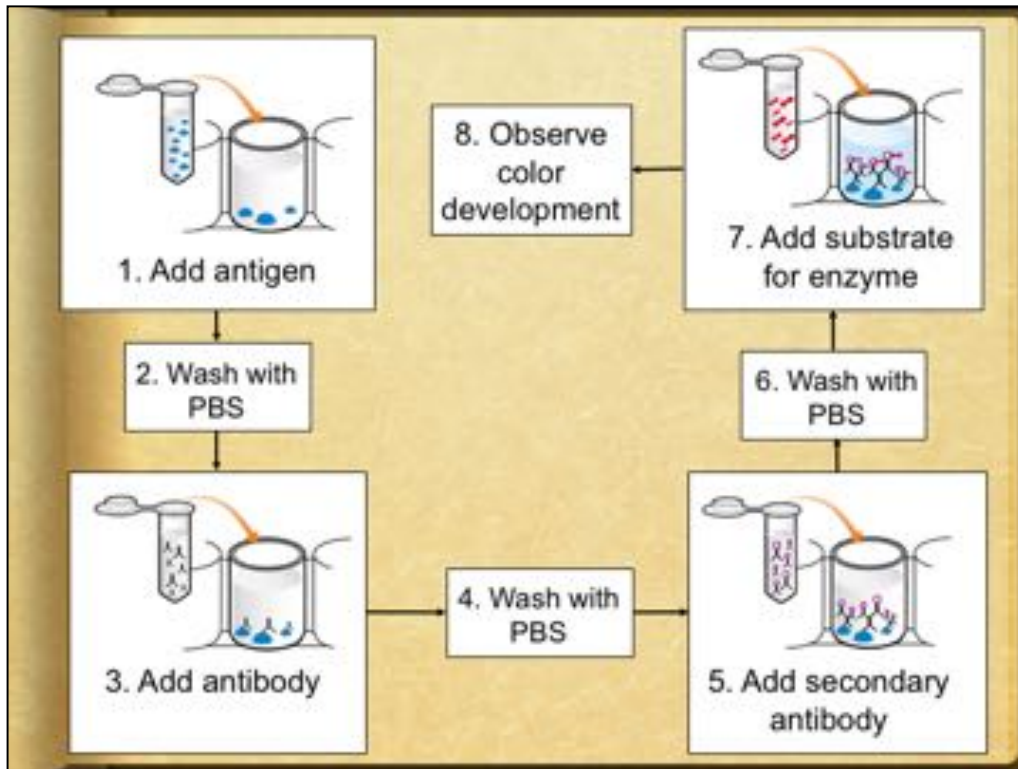
# Basic steps of ELISA

Enzyme Linked Immunosorbent Assay

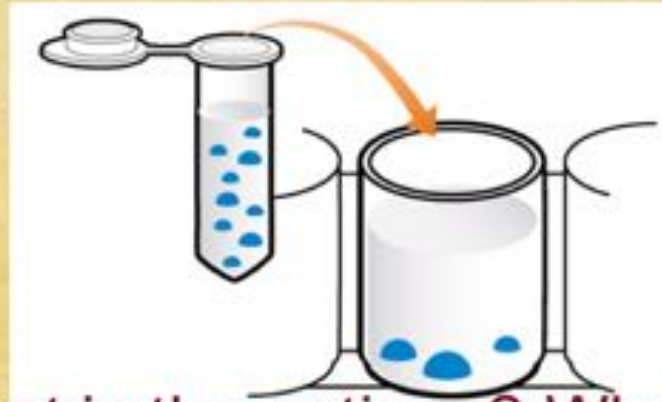


1. Antigen of interest is absorbed on to plastic surface ('*sorbent*').
2. Antibody is added specific to the antigen ('*immuno*').
3. This antibody (acting as an antigen) is recognised by second antibody ('*immuno*') which has enzyme attached ('*enzyme-linked*').
4. Substrate reacts with enzyme to produce product, usually colored.

**Degree of Colored product =  
measure (*assay*) of antigen present**



Coating the wells with antigen & incubate



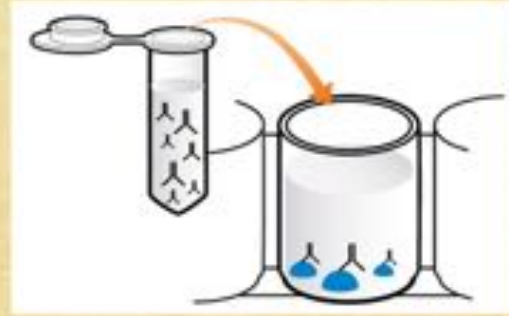
What is the antigen? Where is this antigen coming from?

## Remove liquid & Wash wells

- Remove the liquid from each well with plastic pipette.
- Using pipette, fill wells with WASH buffer then empty out.
- **CAUTION: Do NOT Overfill**
- Tap wells upside down on paper towel. Change towels often
- Label the pipette WASH and keep for later PBS pipetting.

## Add Primary Antibody: +, - controls, patients

- Using a clean pipette.



What antigen are we looking for?  
Give a name to the antibody.

Remove liquid &  
Wash wells of excess antibody



## Add secondary antibody: Anti-(Anti-smallpox virus)

- Conjugated to the enzyme peroxidase.
- Using a clean pipette, secondary antibody to each well.
- Incubate.



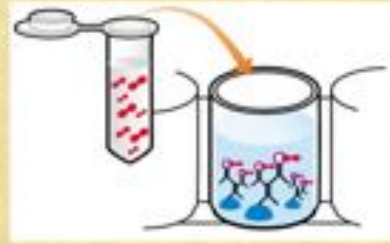
Remove liquid &  
Wash wells of excess antigen

## Add substrate for enzyme

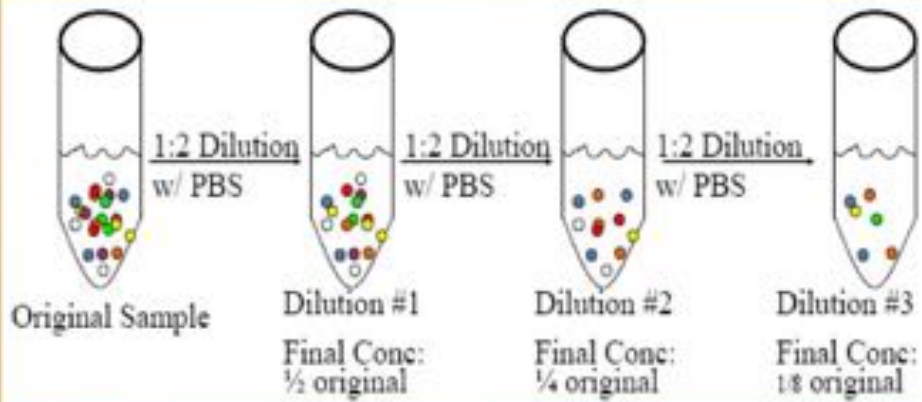
- Add substrate to each well

- Incubate for 5 min.

- Observe color development.

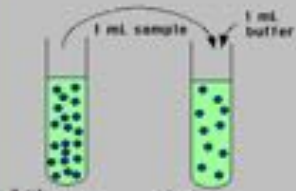


## Creating a Comparison: Serial Dilution



### Dilutions

$$\frac{1}{2} = \frac{1 \text{ sample}}{1 \text{ sample} + 1 \text{ buffer}}$$



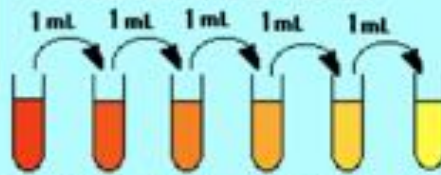
2 ml  
20 Particles  
10 Particles/mL

2 ml  
10 Particles  
5 Particles/mL  
1 ml buffer added  
1/2 dilution

• = Particles, molecules, grams, or moles.

## Making Our Serial Dilution

### Serial 1/2 Dilution



	1	1/2	1/2	1/2	1/2	1/2
Total Dilution	1	1/2	1/4	1/8	1/16	1/32
Buffer added (mL)	1	1	1	1	1	1

# **Perform your ELISA Test on your 4 Family Members**

**Make Serial Dilution**

**Report your Results**

**False Positives?**

**False Negatives?**

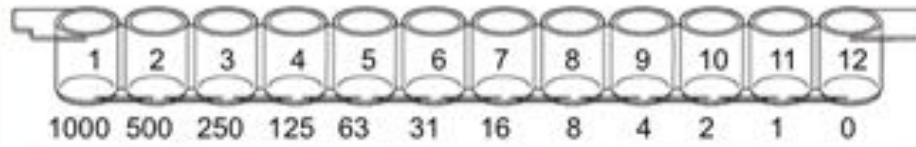
**What are your controls?**

## Family Name:

ID #	Person's Name	Age	Favorite Personality Characteristic	Diagnosis: Exposed? Antigen Levels	Need Vaccine? Yes or No

"Typical Family" – Two Adults plus two children

# Our Experiment & Serial Dilution

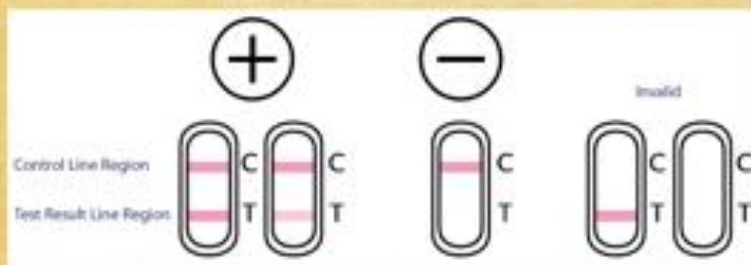




## ELISA, Reproductive Hormones & Pregnancy



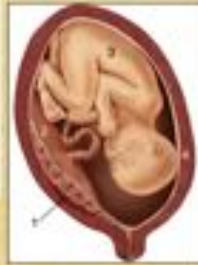
# ELISA & The Pregnancy Test



# The Important Hormones

FSH

LH



hCG

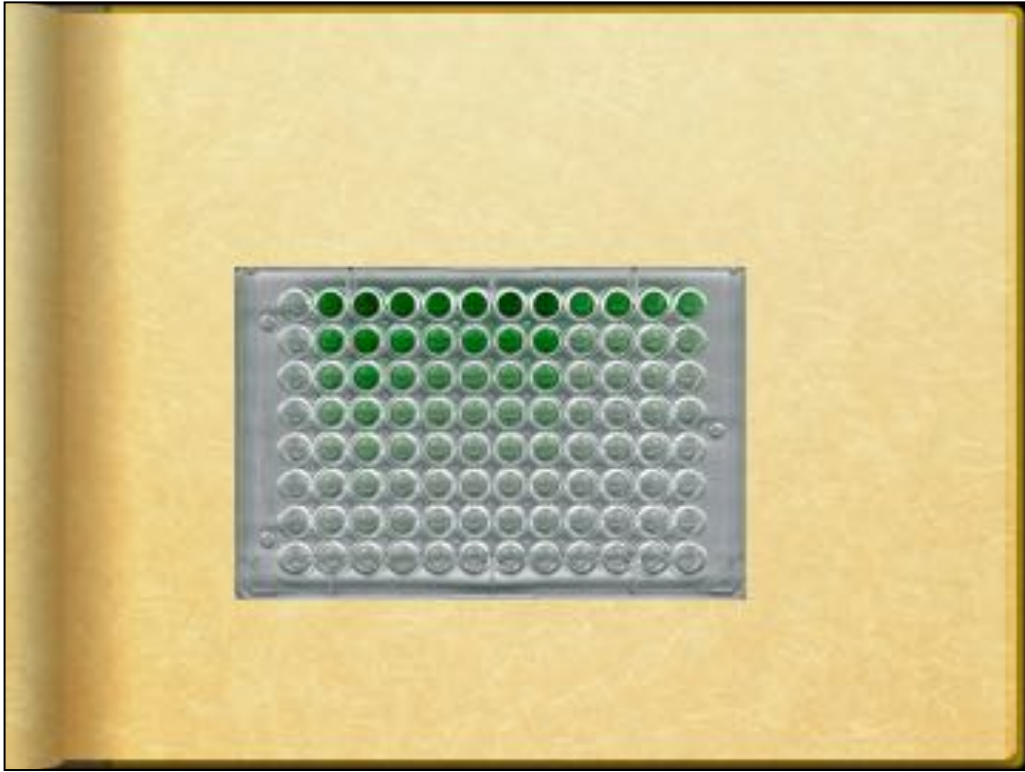


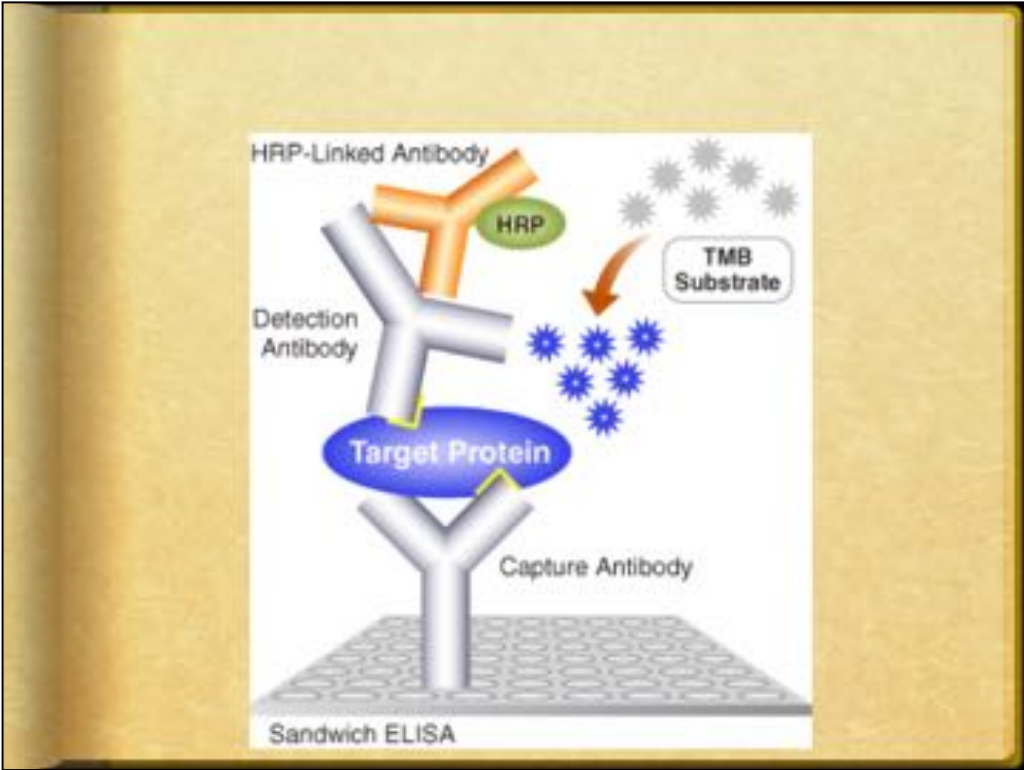
Estrogen

Progesterone

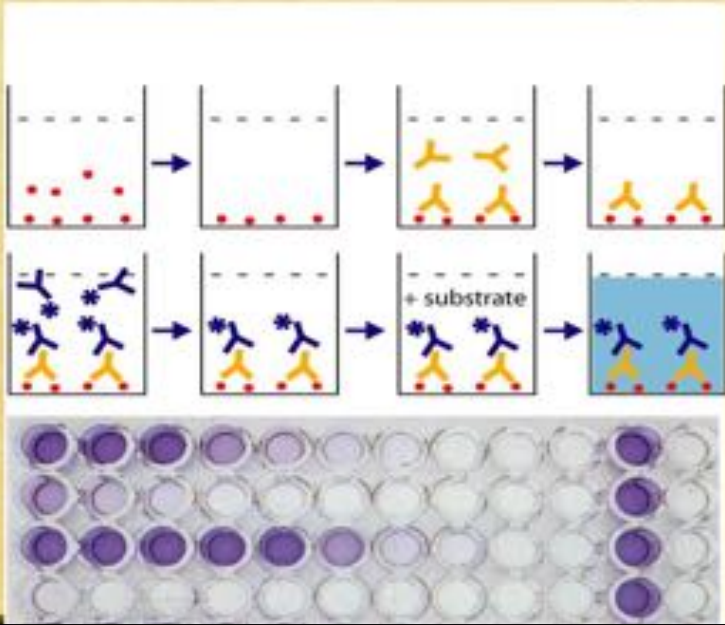
## Watch Flash Animation







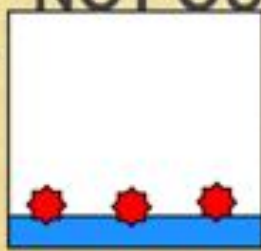
# NOT Bio A TECHNIQUE



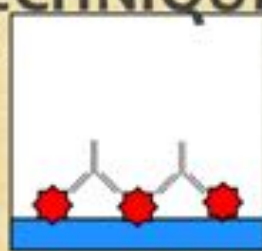


# NOT OUR TECHNIQUE

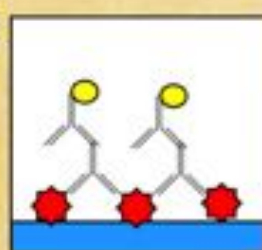
1



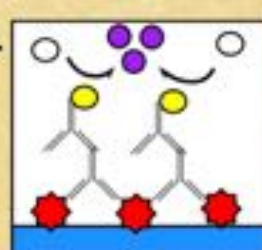
2



3

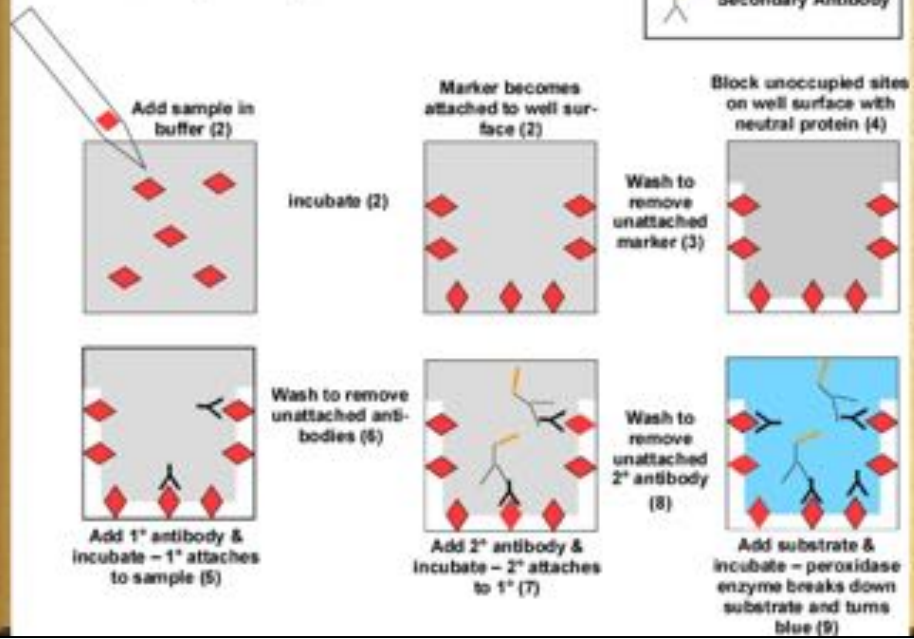
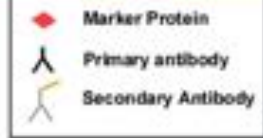


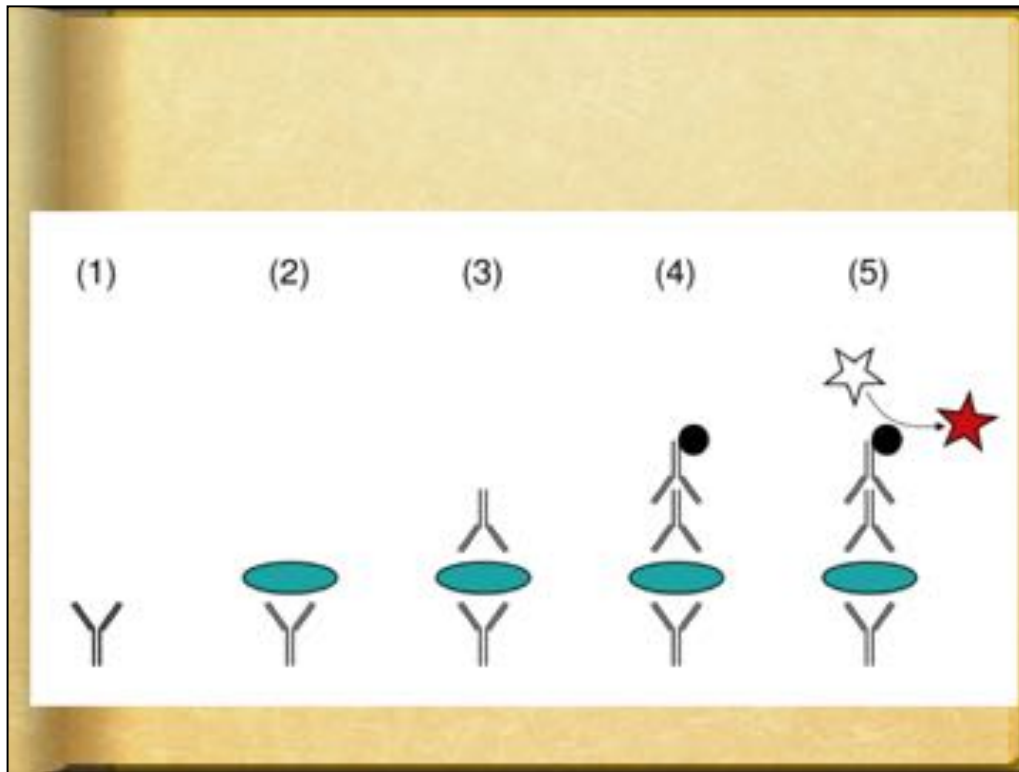
4



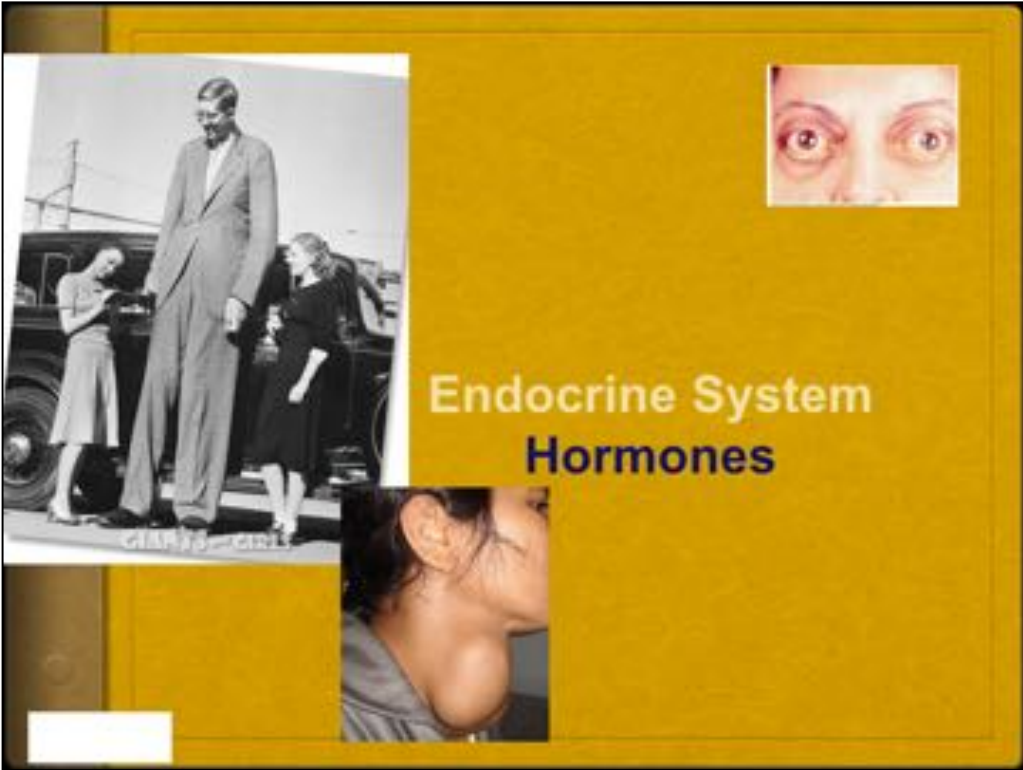
# Indirect ELISA

Numbers correspond to steps in text





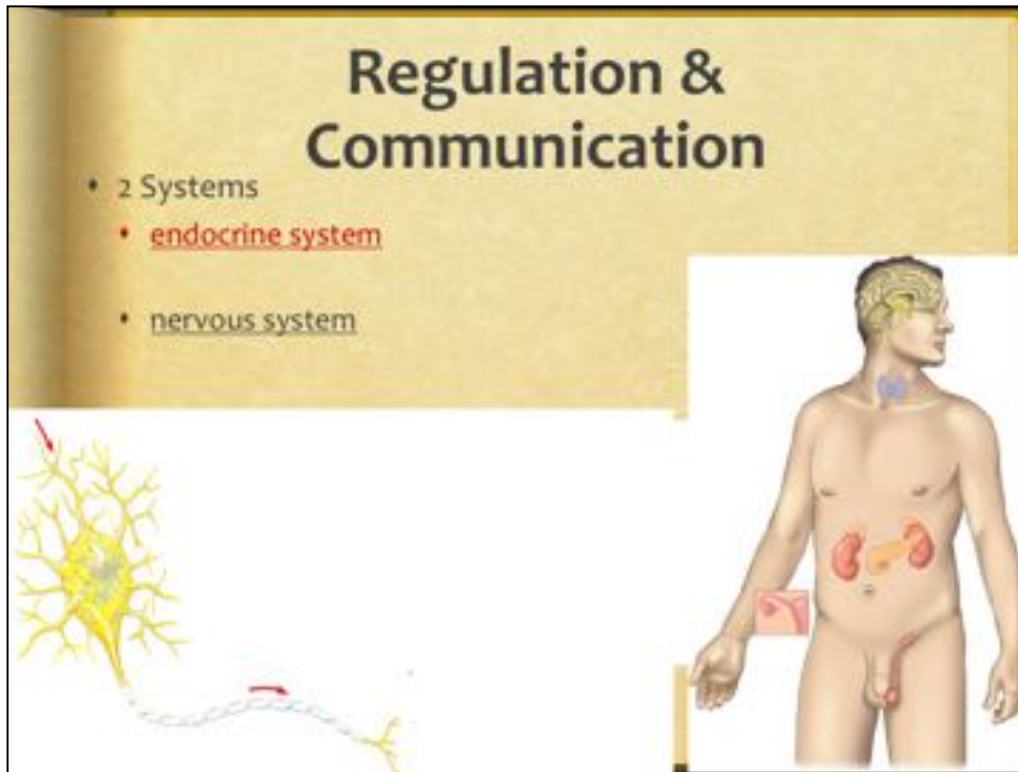
Not Ours - We are adding antigen first.



**Endocrine System**  
**Hormones**

GIANT GIRL

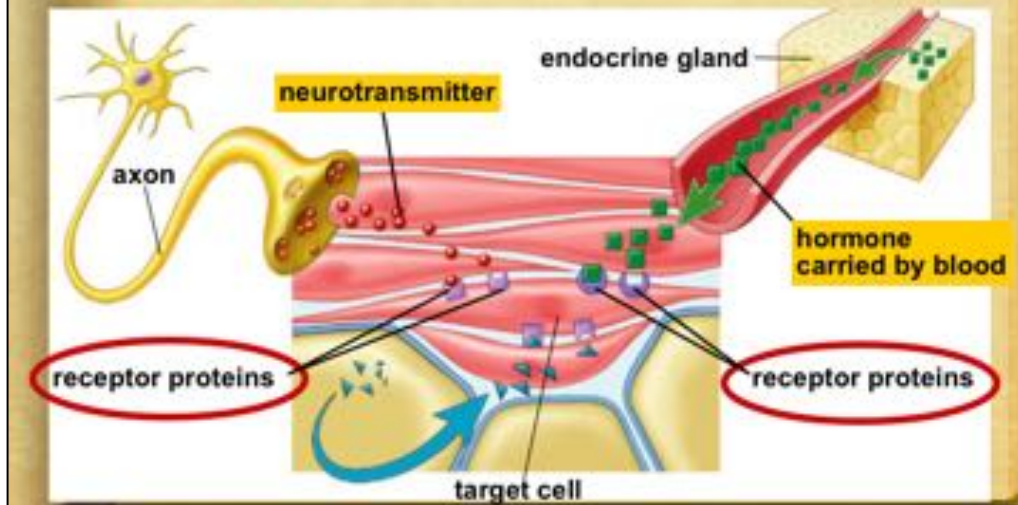
[Redacted]



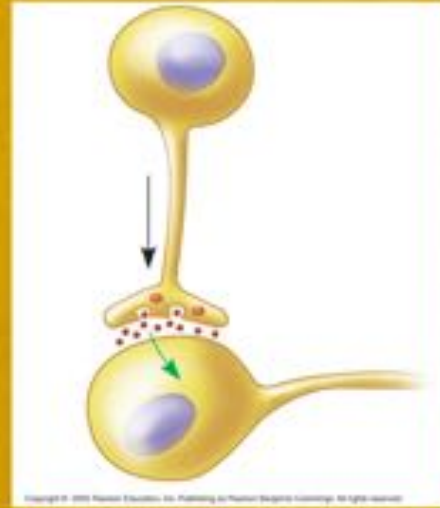
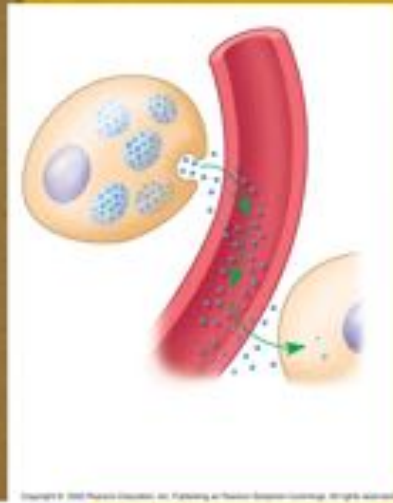
Hormones coordinate slower but longer-acting responses to stimuli such as stress, dehydration, and low blood glucose levels. Hormones also regulate long-term developmental processes by informing different parts of the body how fast to grow or when to develop the characteristics that distinguish male from female or juvenile from adult. Hormone-secreting organs, called endocrine glands, are referred to as ductless glands because they secrete their chemical messengers directly into extracellular fluid. From there, the chemicals diffuse into the circulation.

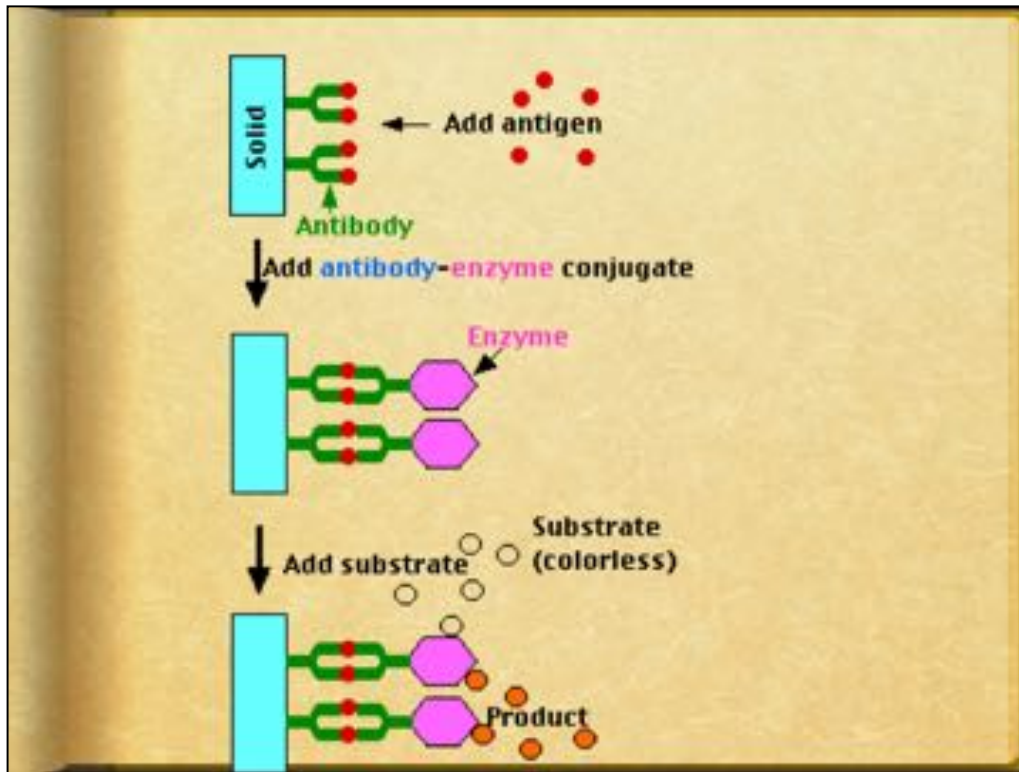
# Regulation by chemical messengers

- Neurotransmitters
- Hormones



## Endocrine vs. Nervous







# The Importance of Shape

Antibodies latch onto antigens on the surface

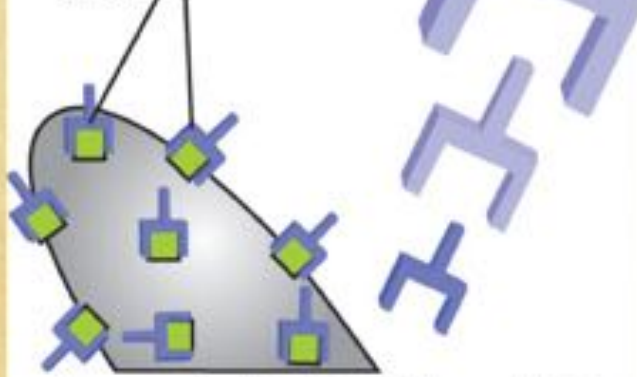
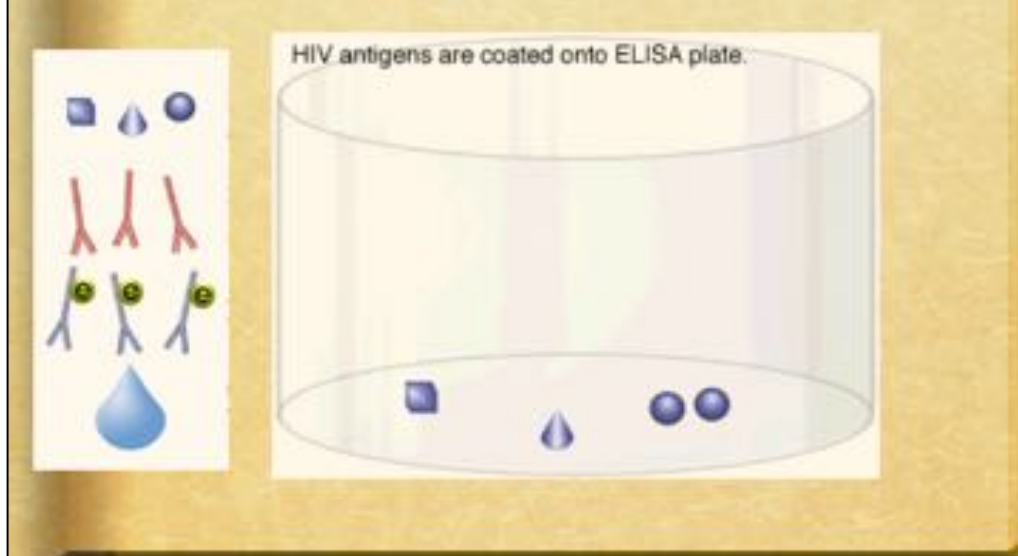


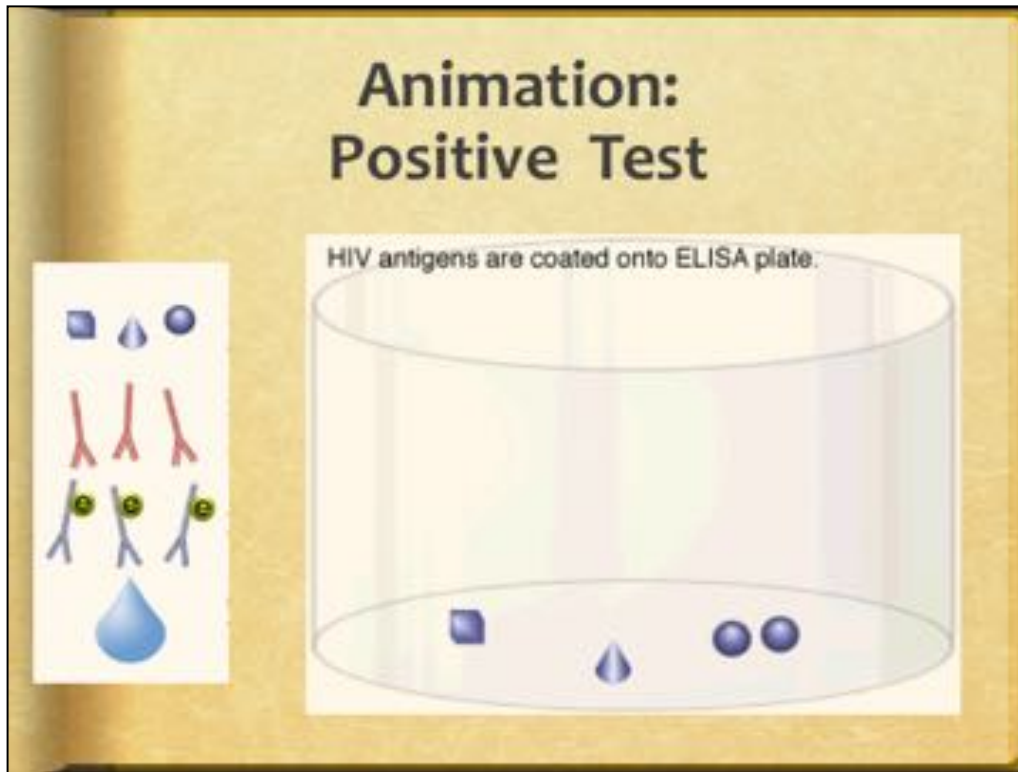
Image source: Association of the British Pharmaceutical Industry.  
Reproduced with kind permission, [www.abpischools.org.uk](http://www.abpischools.org.uk)

## Animation: Negative Test



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

## Animation: Positive Test



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

# Researchers

Patient Letter (number)

Researchers		A (1)	B (2)	C (3)	D(4)
Chase	Caroline	Caroline	Faraz	Lindsay	Colin
Jharnae	Spencer	Spencer	Eliza	Jharnae	Max
		Jacqueline	Sina	Emma	Emily
Max	Nick	Sarah	Nick	Chase	M. Bahe
Eliza	Emily	Caroline	Faraz	Lindsay	Colin
Emma	Sina	Spencer	Eliza	Jharnae	Max
Lindsay	Faraz	Jacqueline	Sina	Emma	Emily
Jacqueline	Colin	Sarah	Nick	Chase	M. Bahe