Molecular Detection of Disease: ELISA

@CAPSVetMed

Spring 2018

To Accompany Carolina ELISA kit

Photo Credit:
https://www.medicalnewstoday.com/articles/316230.php

BACKGROUND INFO

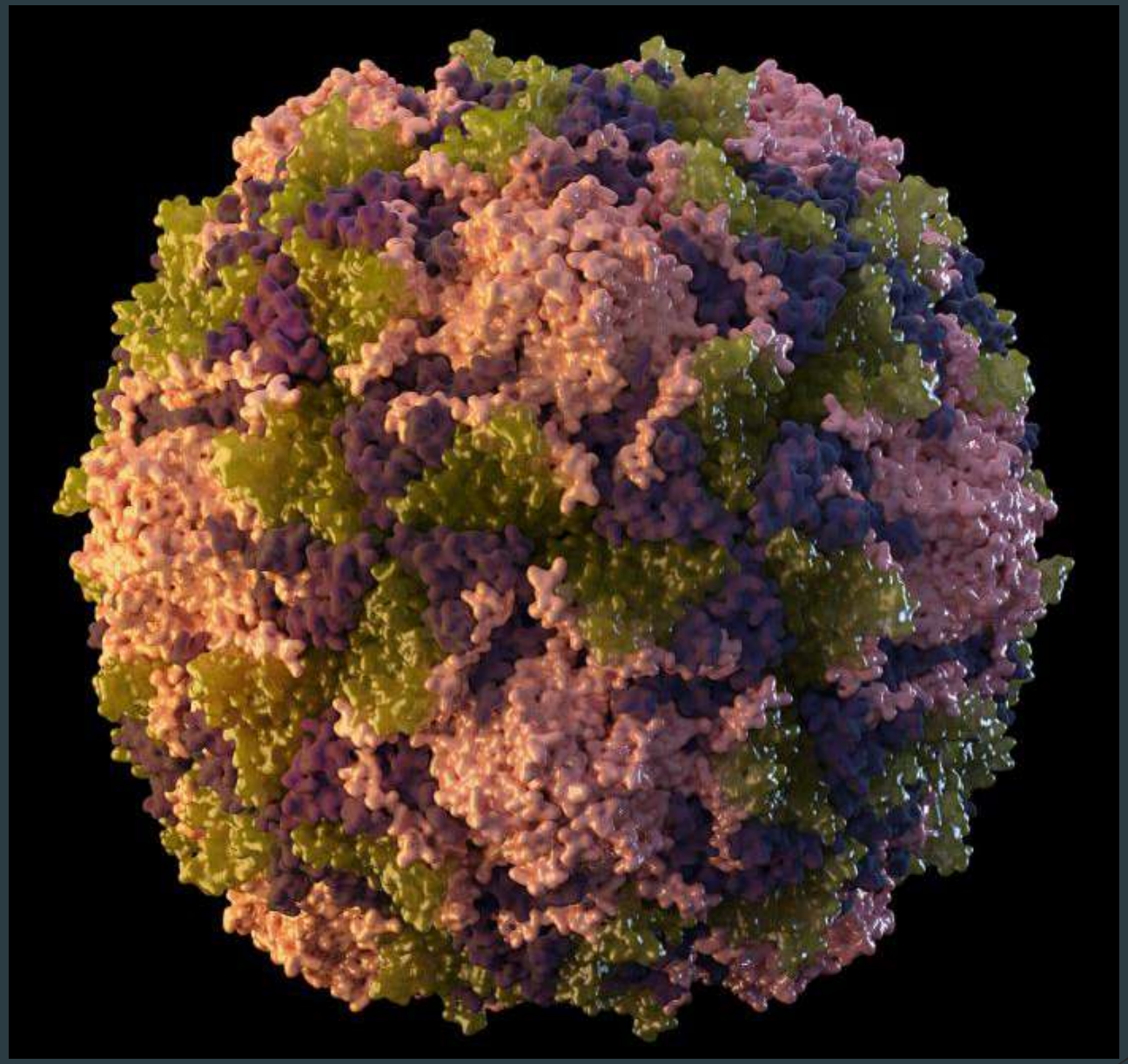


Photo Credit:
https://phil.cdc.gov/Details.aspx?pid=21325

Photo credit:
https://si.wsj.net/public/resources/images/PJ-BY980_FIXBUR_G_20141215113400.jpg

Antibody-mediated Immune Response

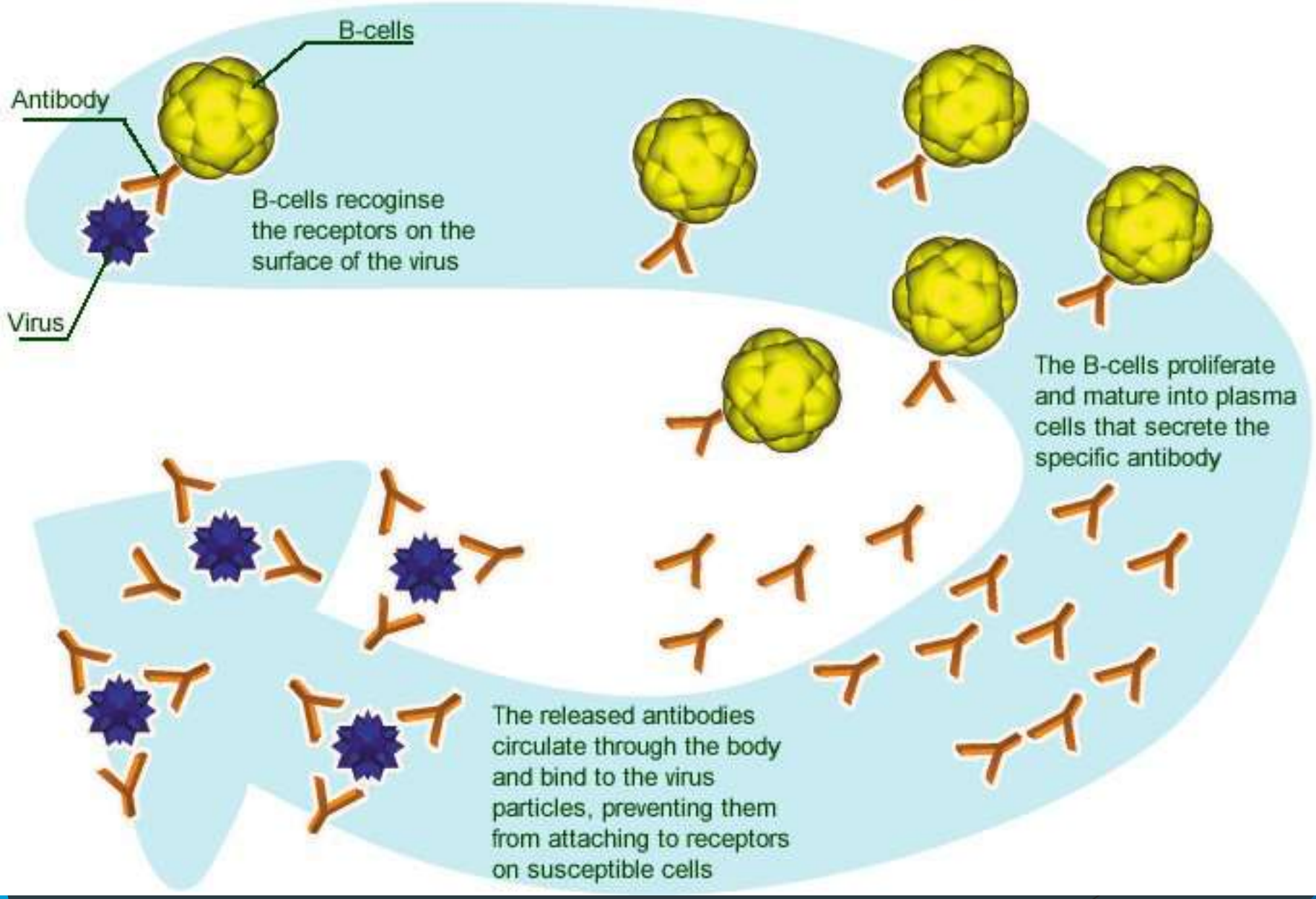
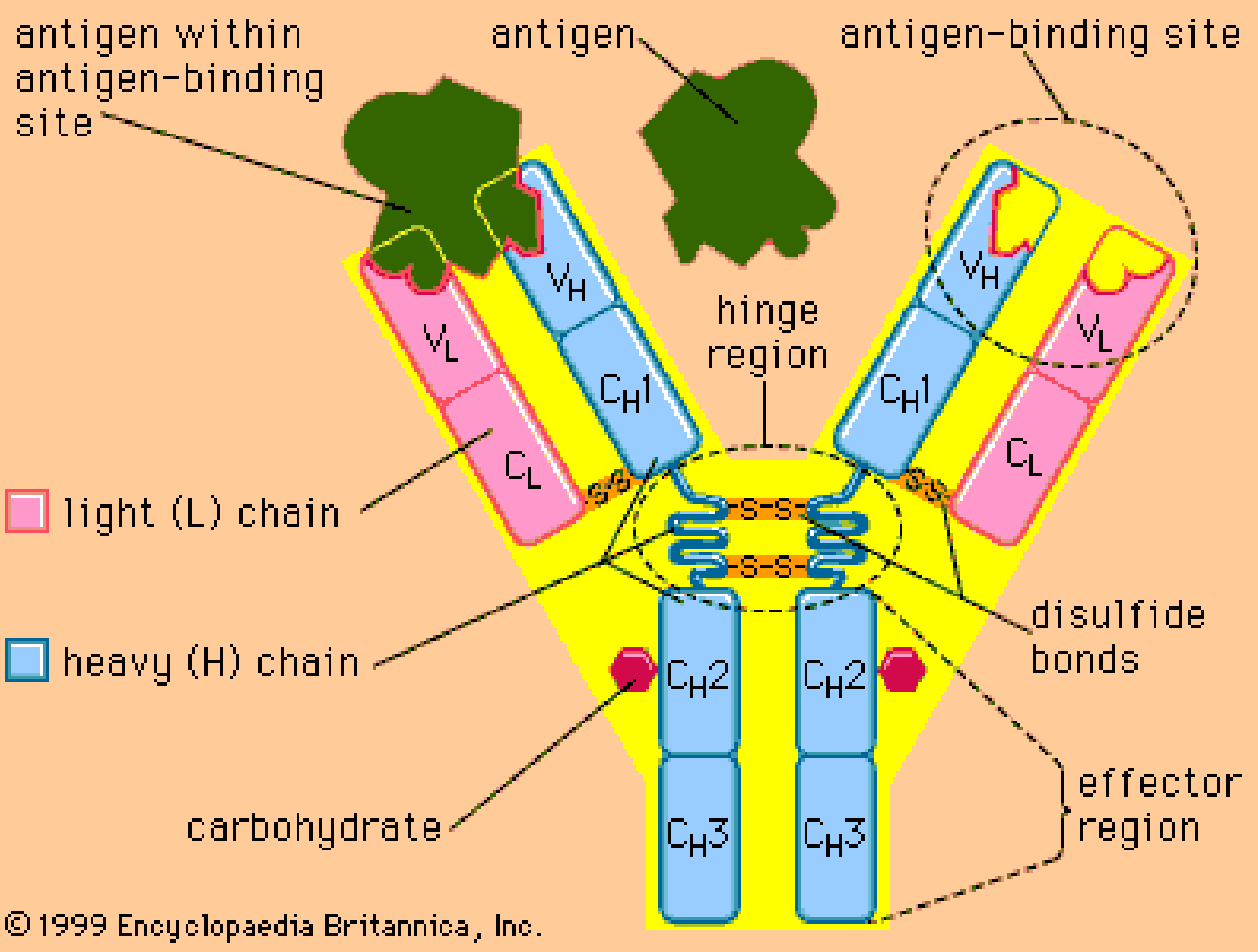


Photo credit: https://www.eduhk.hk/biotech/eng/classrm/explain/health1.jpg

Key Definitions for *Acquired Immune Defense*

1. Antigens: Foreign invader into the body. Examples include viruses, bacteria, foreign proteins, and drugs.
2. Antibodies (Immunoglobulins – Ig): Proteins produced by B-Lymphocytes that recognize and bind with specific antigens.
3. Epitope: The specific portion of the antigen that is chemically recognized by the antibody.


antigen within antigen-binding site
antigen
antigen-binding site
V_H
V_L
C_H1
C_L
hinge region
S-S
S-S
S-S
light (L) chain
heavy (H) chain
carbohydrate
C_H2
C_H3
disulfide bonds
effector region
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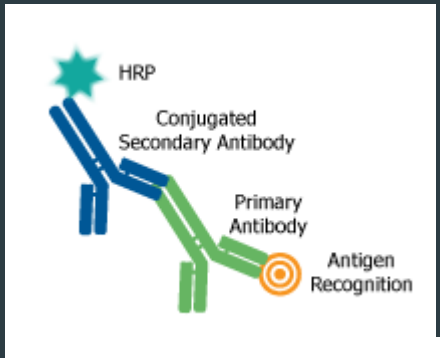
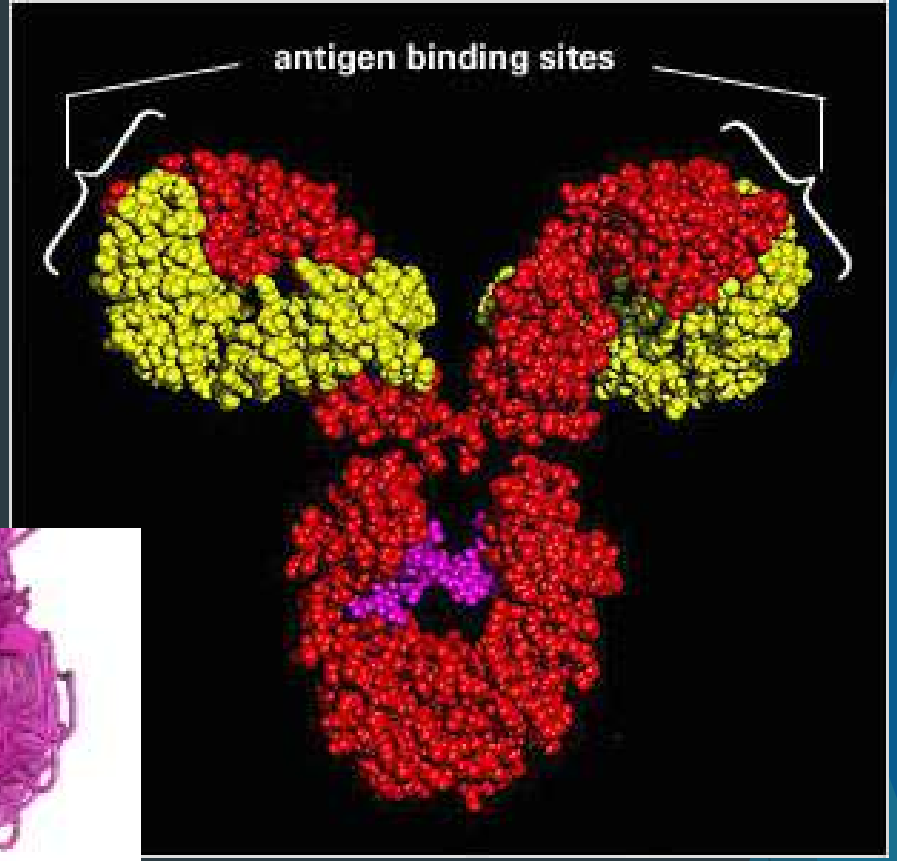
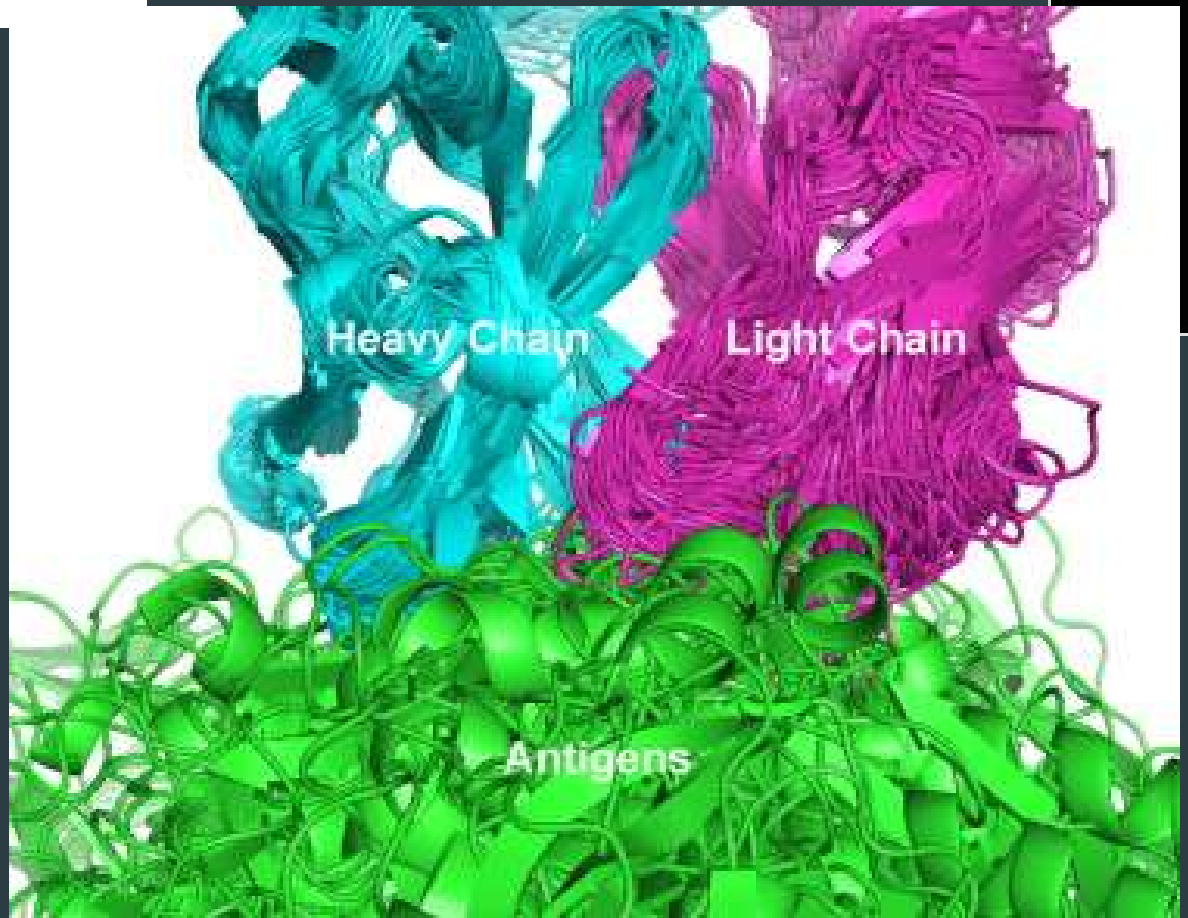

HRP
Conjugated
Secondary Antibody
Primary
Antibody
Antigen
Recognition


antigen binding sites


Heavy Chain
Light Chain
Antigens

ELISA:

Enzyme-Linked Immunoabsorbant Assay

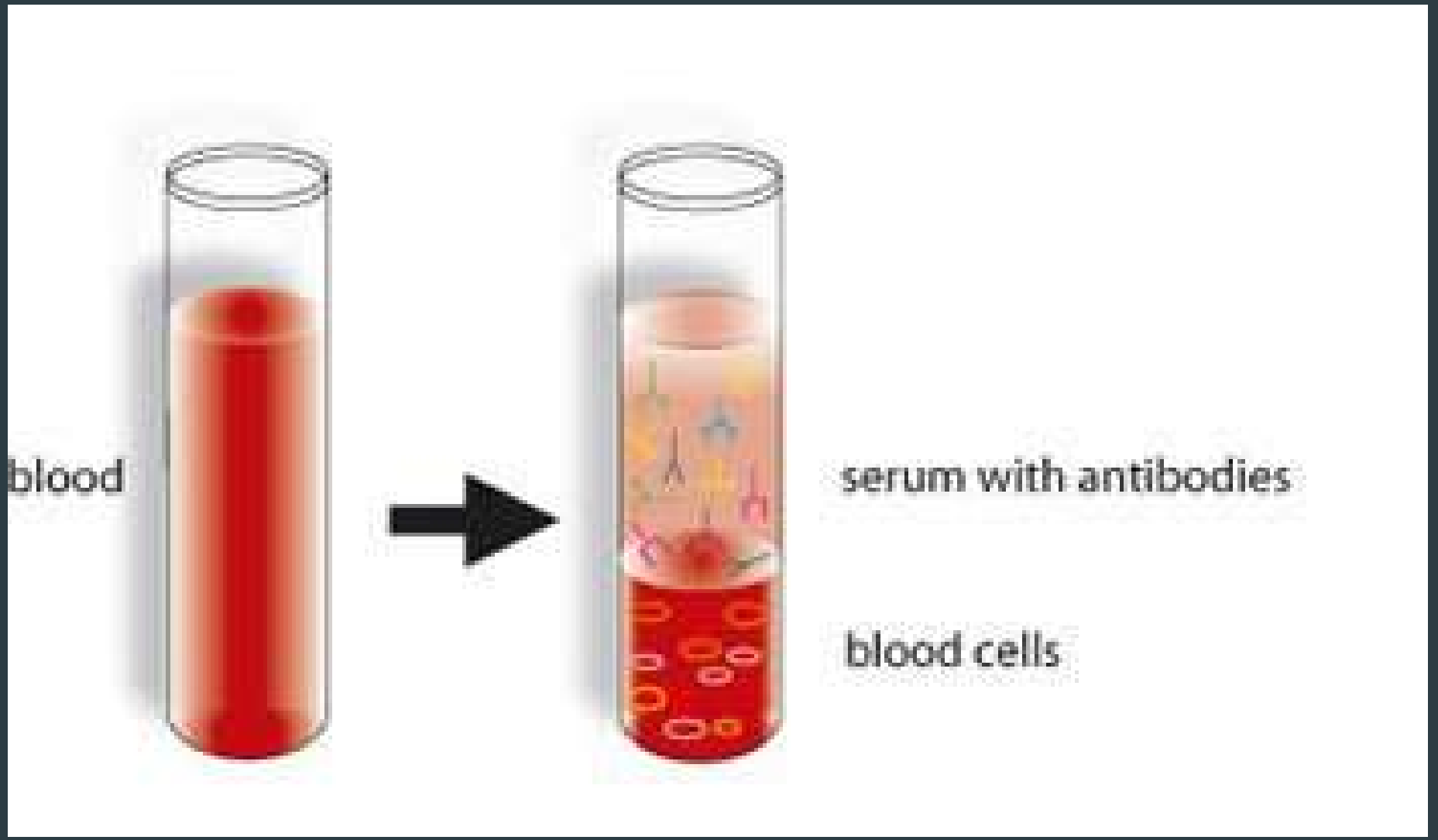


Photo credit: http://www.differencebetween.info/difference-between-blood-and-serum

WATCH THIS:
https://www.youtube.com/watch?v=UF6rAoWcvZM

What is a common medical application of ELISA?

Watch (stop around 3:39)

https://www.youtube.com/watch?v=aOfWTscU8YM

Veterinary Application: "Snap Test"

https://www.idexx.com/small-animal-health/products-and-services/snap-4dx-plus-test.html

PART 4: PERFORM THE LAB

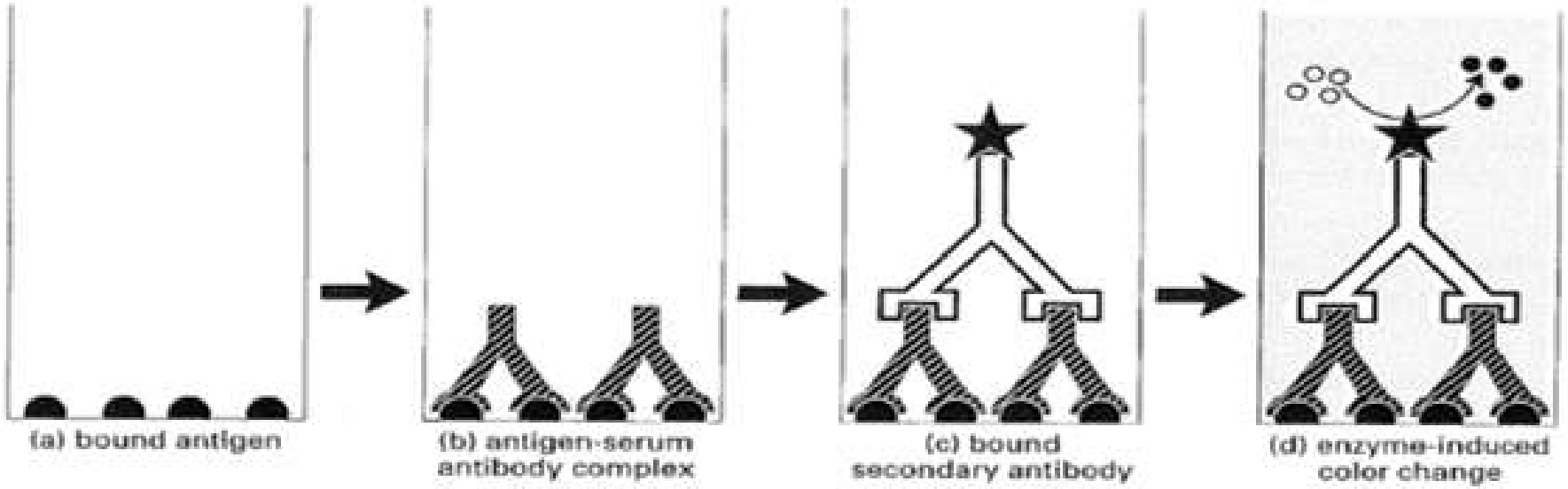


Figure 2. ELISA schematic of a positive result

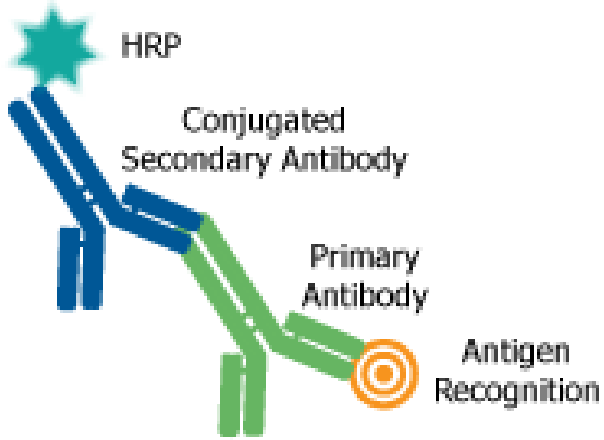


Photo credits:

- Carolina student guide, scanned
- https://rockland-inc.com/fit-for-purpose-antibodies.aspx

“Foot and Mouth Disease” (FMD)
AKA “Hoof and Mouth Disease”

Photo credit: Wikipedia

Etiology/Infectious agent

- Virus, picornavirus family
- Highly contagious
- Infection when virus particle is taken into host cell
- Cell forced to manufacture thousands copies of virus > eventually bursts > releasing new particles in blood

Affected species

- Cloven-hooved animals…

Signs

- Fever
- Blisters on tongue, lips, in mouth, on teats, between hooves (heal ~7 days)
- Ruptured blisters can lead to lameness or reluctance to move

Photo credit: Merck Veterinary Manual

“Foot and Mouth Disease” (FMD)
AKA “Hoof and Mouth Disease”

Photo credit: North Dakota State Government

Transmission

- Virus found in all excretions and secretions of infected animal
- Virus may be in milk and semen up to 4 days before signs
- Recovered animals may serve as carriers of virus

- Indirect transmission via
  - Contaminated pens, buildings, vehicles used to house/move susceptible animals
  - Infected animals breathe out aerosolized virus > infect other animals via respiratory or oral routes
  - Contaminated materials such as hay, feed, water, milk or biologics
  - People wearing contaminated clothes or footwear, or using contaminated equipment
  - Meat or animal products, raw or improperly cooked food infected with the virus and fed to susceptible animals, and; – aerosol spread of virus from an infected property via air currents.

“Foot and Mouth Disease” (FMD)
AKA “Hoof and Mouth Disease”

Prevalence

- In susceptible population, morbidity approaches 100%
- Intensively reared animals are more susceptible to the disease than traditional breeds
- Rarely fatal in adult animals
- High mortality in young animals due to myocarditis or lack of milk when the dam is infected by the disease
- *Endemic:* several parts of Asia, most of Africa and the Middle East
- *In zones:* Latin America
- *Currently free:* Australia, New Zealand and Indonesia, Central and North America and continental Western Europe (can occur sporadically in typically free areas)

Human Impact

- Disease causes severe production losses
- Majority of affected animals recover, the disease often leaves them weakened and debilitated
- FMD not readily transmissible to humans.

"Foot and Mouth Disease" (FMD)
AKA "Hoof and Mouth Disease"

Prevention

7strains (A, O, C, SAT1, SAT2, SAT3, Asia1)

- each one requiring specific vaccine strain to provide immunity to vaccinated animal

Global strategy for dealing with FMD

Early detection and warning systems and prevention measures in place according to OIE Guidelines for the Surveillance of Foot and Mouth Disease (OIE Terrestrial Animal Health Code)

Monitoring the occurrence, prevalence and characterisation of FMD viruses

Protection of FMD free countries, areas or zones is enhanced with *stringent import and cross-border animal movement controls and surveillance*

Essential for livestock owners and producers to *maintain sound biosecurity practices* to prevent introduction/spread of the virus. Measures that are recommended at the farm level include: - control over access to livestock by people and equipment; - control the introduction of new animals to existing stock; - maintain sanitation of livestock pens, buildings, vehicles and equipment ; - monitor and report illness; - appropriate disposal of manure and dead carcasses.

Reference: http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/FMD-EN.pdf

INSTRUCTIONS

Materials

- Plastic Pipettes – **don't touch the sides of the wells!**
- Microtiter Plate/Strip

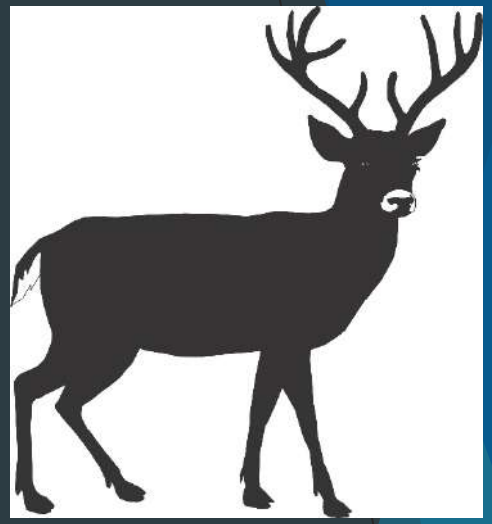
  - Antigen Sample
  - Secondary Antibody Sample
  - Chromogen Sample
- Positive Control – "+" (Pink)
- Negative Control – "-" (Blue)

- Our Veterinary Patient Serum Samples – FIND OUT: with/without antibody?
  - "A"
  - "B"
  - "C"
  - "D"
  - "E"
  - "F"

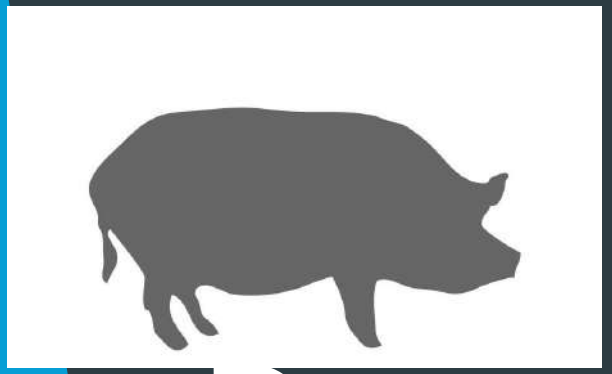
Our FMD Scenario…

A

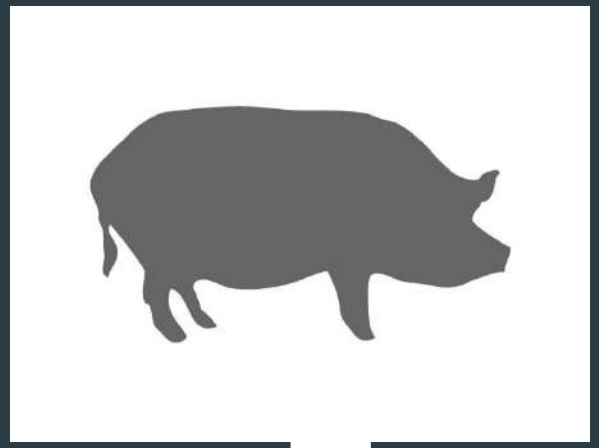
B



C



D



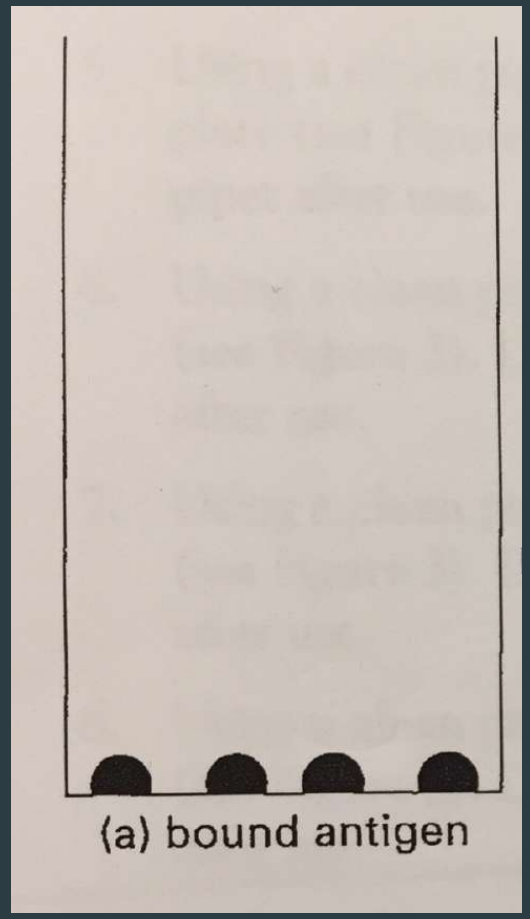
E

F

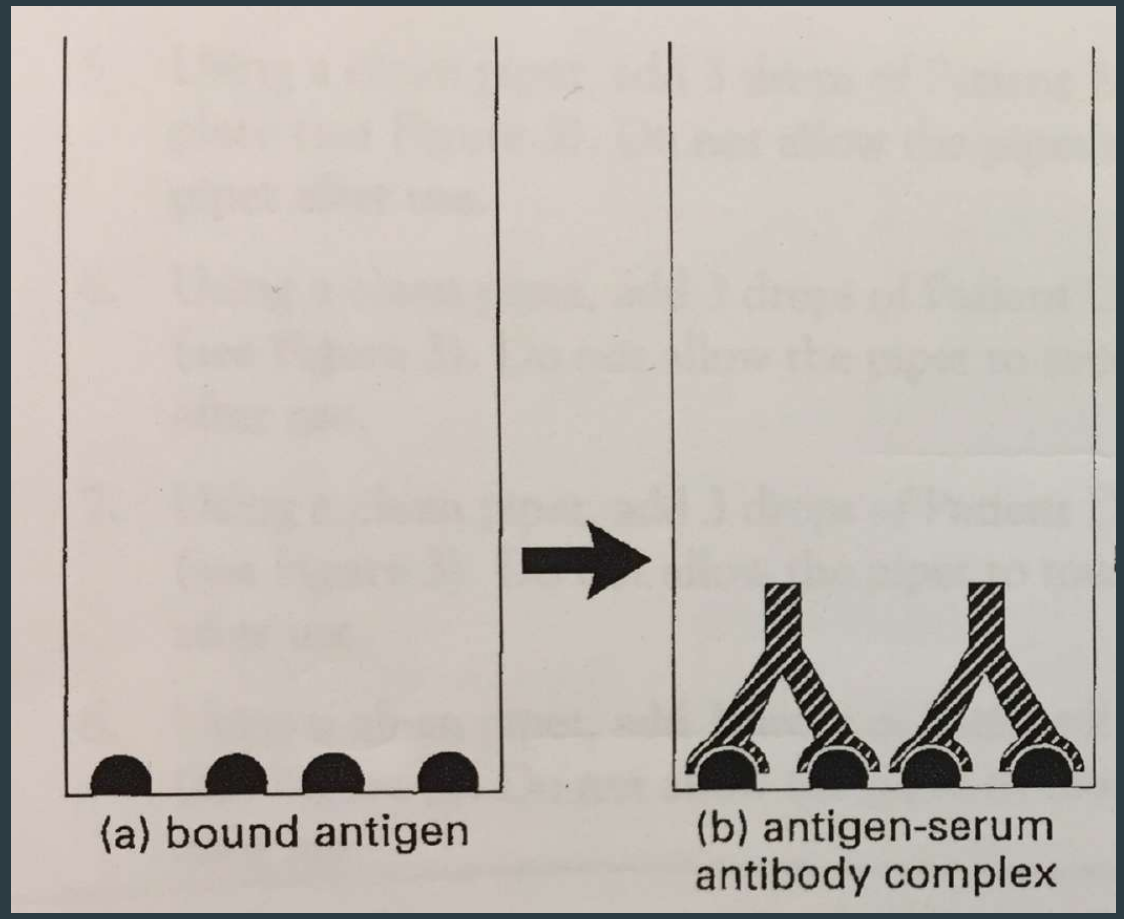
This is DIFFERENT than human disease…

- Hand, Foot & Mouth
- https://www.cdc.gov/hand-foot-mouth/about/transmission.html

INSTRUCTIONS



INSTRUCTIONS



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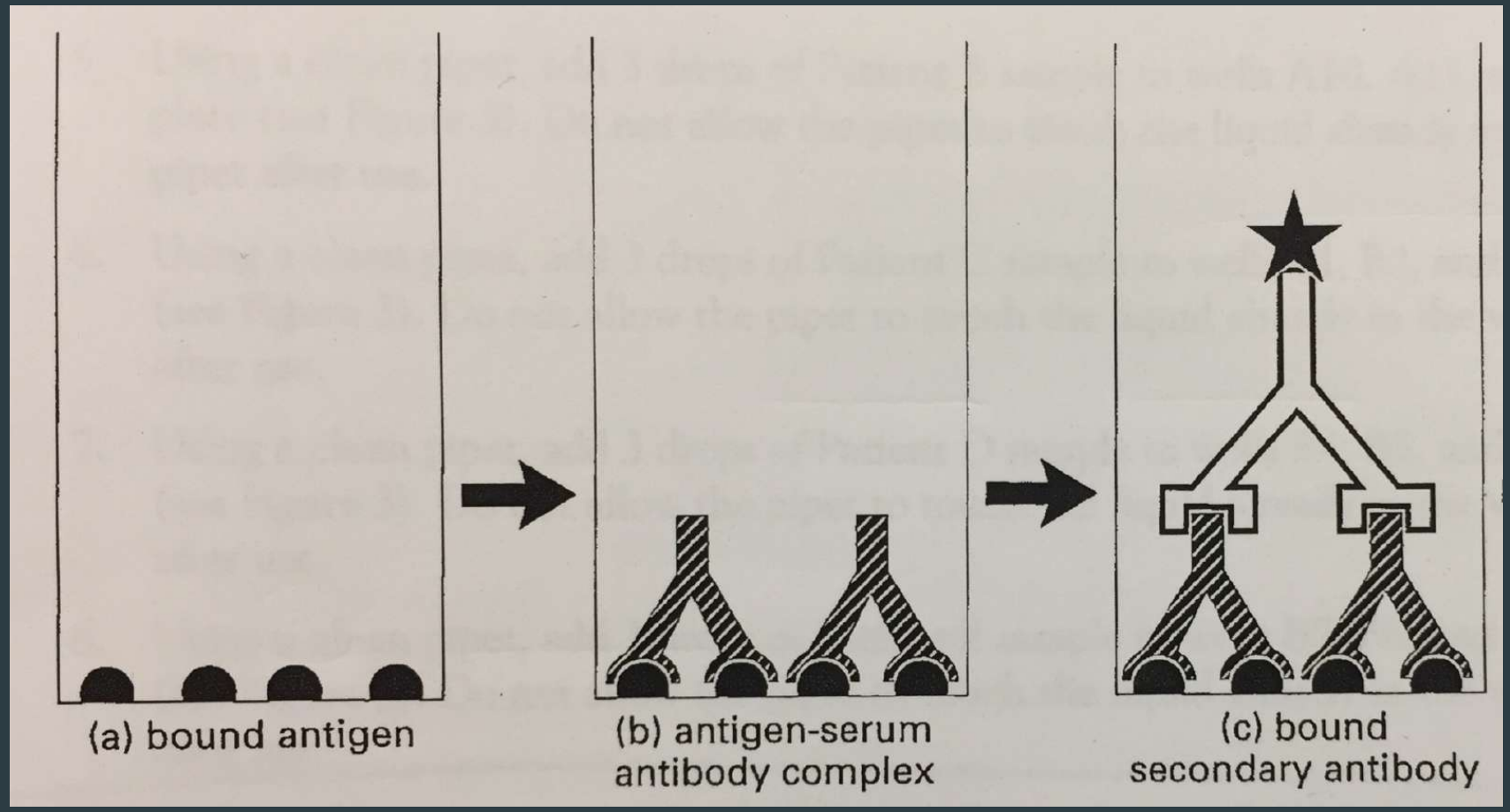

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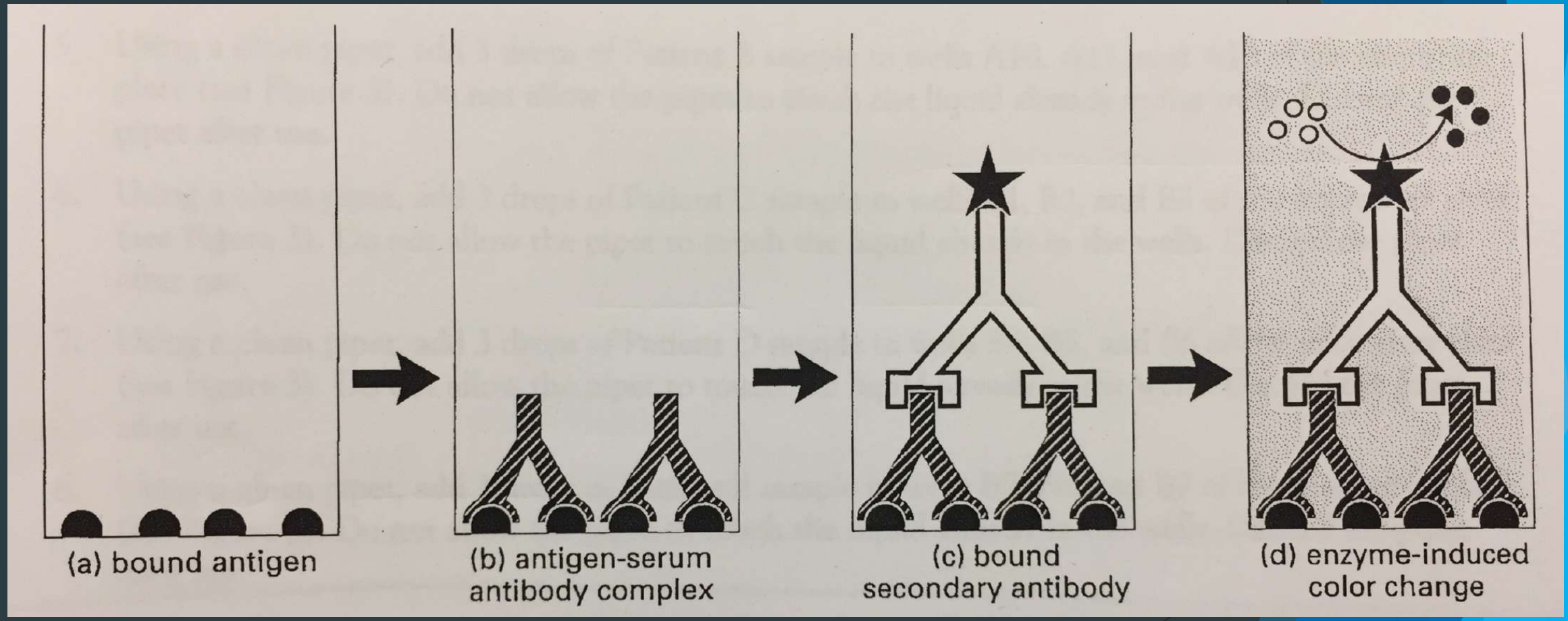


Results

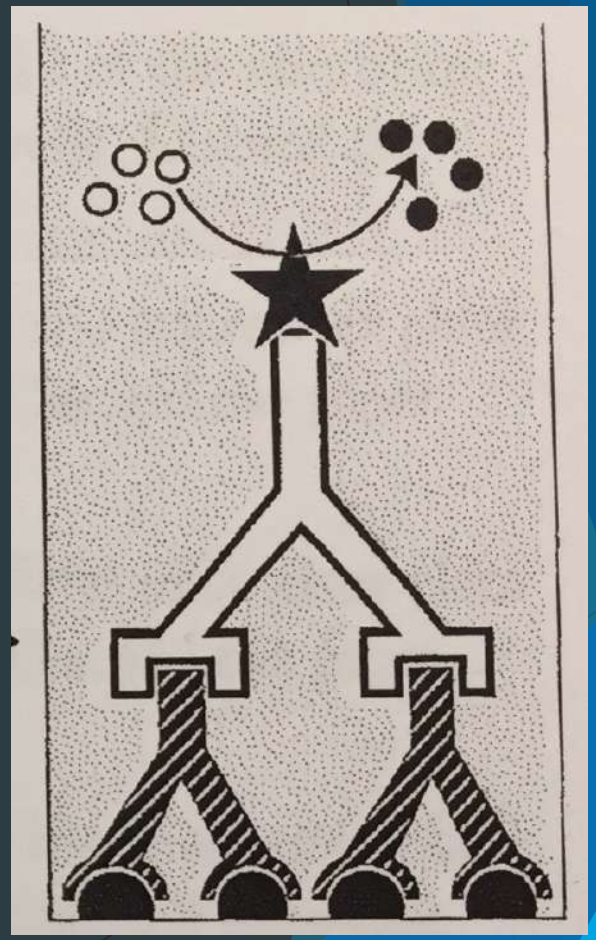
Sample	Color	Test Result
Positive Control	Dark Purple	Positive
Negative Control	Light Green	Negative
Patient A	Dark purple	Positive
Patient B	Light green or clear	negative
Patient C	Clear or light green	Negative
Sample D	Light purple	positive
Sample E	Clear	Negative
Sample F	Dark purple	positive

ELISA

- ELISA - Enzyme-Linked Immunoabsorbant Assay
- Indirect ELISA – Tests for the presence/absence of patient antibodies against a pathogen.
- Direct ELISA – Tests for the presence/absence the pathogen's antigens.

https://www.youtube.com/watch?v=Z1uAGLJkcu4

- **Which type of ELISA did we just do?**
- **…discuss**



Extension Questions

1. What are vaccines and how do they work?

2. Explain how a home pregnancy test or drug test uses similar principles as the simulated ELISA to determine the presence/absence of a pregnancy/drug.

Photo credit: Google image search

Photo credit: https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcQhSTdyJ_BnaHNTuilCJsM6taXlycDZ9W2qkbZmkMZZLZ-VP7yhBQ