

### Immunofluorescence Assays

#### Fluorescent Antibody Test (FA)

**Mechanism** Fluorescently labeled mAb to bind and illuminate a target Ag/Ab

**Designed to Detect** A sample's Antigen OR Antibody

**Reagent** mAb-FITC conjugate ~ *Ab tagged with a Fluorescein*

**Compatible Sample Types** Serum or tissue section

#### Types

**1. Direct (DFA)** Detection of sample's **antigen**

**Sample** Unknown **Antigen** in blood

**2. Indirect (IFA)** Detection of sample's **antibody**

**Sample** Unknown **Antibody** in blood (Ag = known)

**Reagent** Secondary mAb-FITC conjugate

#### Direct Fluorescent Antibody

##### Uses

**Bovine Viral Diarrhea Virus (BVDV)** Detection of **live** BVDV in bovine blood

#### Direct Fluorescent Antibody (cont)

**Rabies in Brain Necropsy** Detection of the Rabies **virus** in the brain tissue

**Sample** Ag from culture/slide

**Known** mAb-FITC conjugate against antigen of interest

**Detects (unknown reactant)** Antigen from sample

**Reagent** mAb-FITC conjugate

#### Results

**Positive Test** Fluorescence = Ag present

**Negative Test** No fluorescence = No Ag

\*Only ONE 'known' Ab is used in this test ~ so the known test component IS the Reagent

#### DFA Mechanism of Action

1 Antigen is fixed to a surface.

2 Patient serum is added: if antibodies are present, they bind to the antigen.

3 Secondary antibody (with fluorescent label) is added: if patient antibodies are present, the secondary antibody binds to the patient antibodies.

(a)

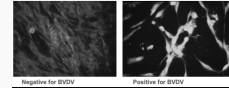
(b)

ASML MicroLibrary © Odom, Cummings, DeBernardi, and Brooker

#### DFA Example ~ BVDV

1. Incubate patient serum (containing the virus) with a cultured cell line ~ *Cell-line must be permissive to BVDV infection*
2. Probe with mAb-FITC conj. that targets the viral Ag of BVDV

#### BVDV DFA Results

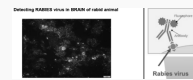


#### DFA ~ Rabies Brain Necropsy Dx

**DFA is required for an official Rabies Dx**

- An impression or tissue section of the euthanized animal's **Cerebellum, Hippocampus, and/or Brainstem** is collected
- mAb-FITC targeting the Rabies virus' antigen

#### Positive DFA of Rabies in the Brain



#### Indirect Fluorescent Antibody Test (IFA)

##### Uses

**Porcine Reproductive and Respiratory Virus (PRRSV)** Detection of **PRRSV Antibody** in Porcine serum

**Titers** Highest serial dilution of serum with Ab - that fluoresces

### Indirect Fluorescent Antibody Test (IFA) (cont)

*Dengue Fever/ChikV/Zika Virus*

<b>Sample</b>	Serum
<b>Known</b>	Antigen
<b>Detects (unknown reactant)</b>	Antibody from serum (1°Ab)
<b>Reagent</b>	Anti-spp. Ab-FITC conjugate (2°Ab)

#### Results

<b>Positive Test</b>	Fluorescence = Ab present
<b>Negative Test</b>	No fluorescence = No Ab

\*This test uses TWO antibodies ~ a 1° and 2° antibody

### IFA Mechanism

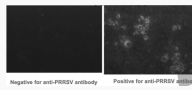


### IFA Example ~ PRRSV

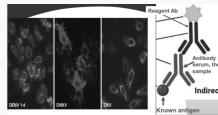
#### Detection of Antibody against PRRSV in Swine serum

- > **Known:** PRRSV infected cell line
- > **Sample:** Porcine serum incubated with cell line
- > **2°Ab Probe:** Anti-pig IgG conjugated with FITC
- > **Unknown:** Antibody against PRRSV

### Results of PRRSV IFA Test



### IFAs Results for DENV 1-4/CHIKV/ZIKV



### Enzyme-Linked Immunosorbent Assay (ELISA)

#### ELISA

High *sensitivity* / Low *specificity*

#### Test Types that Detect Antigen

- > Direct ELISA
- or Sandwich ELISA
- or Antigen Capture ELISA
- or Antigen ELISA

#### USES

*Heartworm Test (HWTM)* Using the Anti-HTWM-Ab-HRP

#### Test Types that Detect Antibody

- > Indirect ELISA
- or Antibody ELISA

#### USES

*Titration (titers)* Quantifies the amount of Ab present

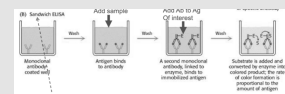
### DIRECT (Ag Capture) ELISA

<b>Sample</b>	Serum from patient
<b>Known</b>	1° Capture Ab (coats wells in tray)
<b>Detection of (unknown)</b>	Antigen
<b>Reagent + Substrate*</b>	2° Detection Ab ~ <i>specific to disease conjugated to enzyme</i>
<b>Positive Test</b>	Color change = Ag present
<b>Negative Test</b>	No color change = No Ag

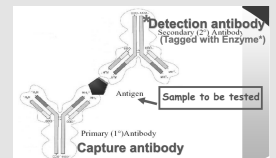
\* substrate = activates enzyme

\*\* The capture Ab and the detection Ab may be the same Ab > BUT ONLY the detection Ab will be tagged with the enzyme

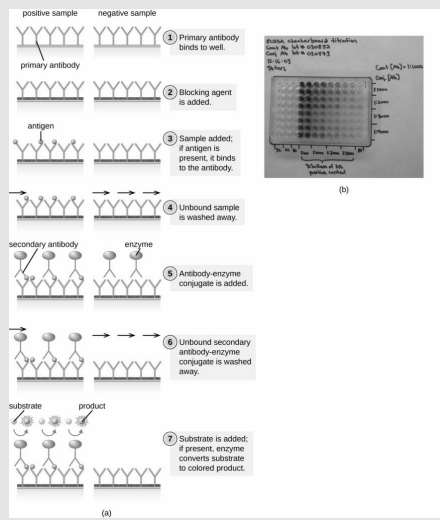
### Ag-Capture ELISA ~ MOA



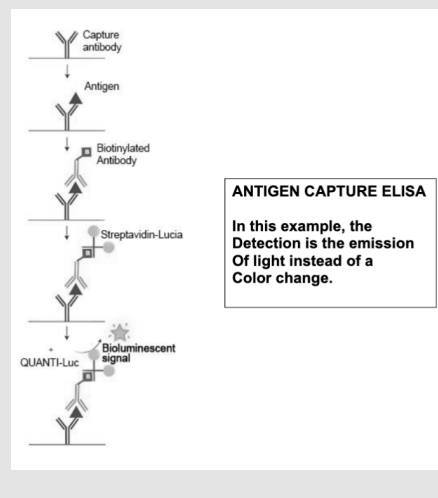
### Ag-Capture ELISA ~ MOA



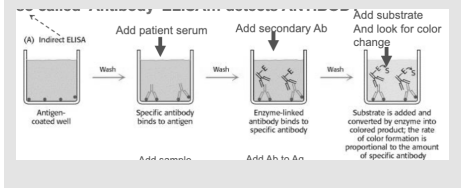
### Ag-Capture ELISA ~ MOA



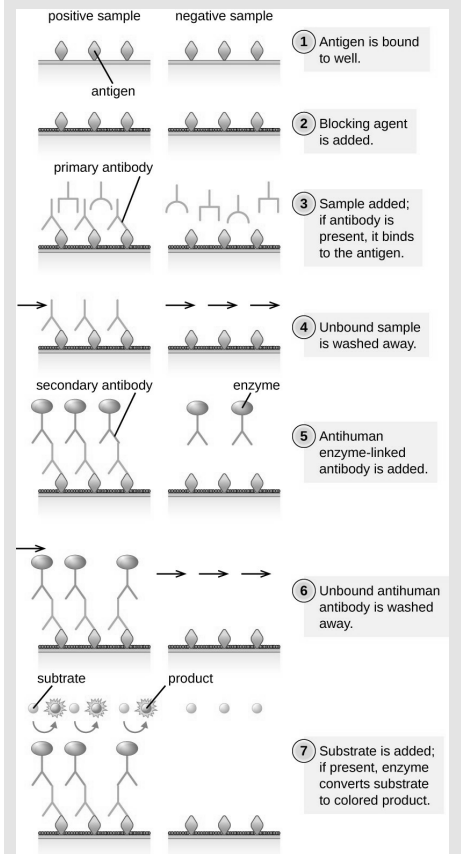
### Ag-Capture ELISA ~ MOA



### Ab ELISA ~ MOA



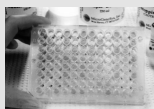
### Ab ELISA ~ MOA



### Ag Capture ELISA ~ HTWM Ag Detection

- > **1° Capture Ab:** Ab that targets Ab
- > **Sample with unknown:** Serum with HTWM Ag
- > Wash slide
- > **2° Detection Ab:** Anti-HTWM-Ab-HRP
- > Add substrate to activate enzyme to show color change if bound

### HTWM Ag-Capture ELISA Results



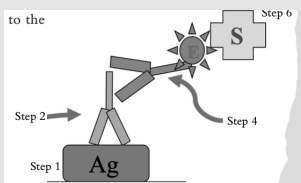
### INDIRECT (Antibody) ELISA

<b>Sample</b>	Serum from patient
<b>Known</b>	Antigen (coating wells)
<b>Detection of (unknown)</b>	1° Ab in serum (spec. for Ag)
<b>Reagent + Substrate*</b>	Anti-Ab 2° Detection Antibody- HRP conj. ~ *Targets hosts own Ab (1° Ab from serum)
<b>Positive Test</b>	Color change = Ab present
<b>Negative Test</b>	No color change = No Ab

### Ab ELISA Titration

- > Coat ELISA wells with Ag (can get commercial Ag of interest)
- > Add serial dilutions of patient's serum into wells
- > wash off unbound Ab
- > Add 2° Ab like Rabbit-Anti-Horse-Ig that's conjugated w enzyme, to the wells
- > wash off unbound Ab
- > add substrate

### Ab ELISA ~ MOA



- Can run serum from multiple patients at once
- Can determine titer by running serial dilutions of the serum

### Indirect ELISA Ab Titer



### Western Blot (WB)

### WB

Higher *specificity* than ELISA

#### Designed to ID/Detect PROTEINS:

1. **Detection of Antibody** if patient's serum contains Ab against a *specific* protein in a complex protein mixture
2. **ID specific protein antigen in mix** Use of a known reagent Ab to the protein of interest

**MOA** This is a three-stage primary binding test

**Stage I** Electrophoresis of a protein mixture on gels so that each component is resolved into a single band

**Stage II** Blotting of these protein bands to an immobilizing nitrocellulose membrane

### WB (cont)

**Stage III** Visualization of transferred Ag by either directly or indirectly probing the membrane with Ab's

#### WB Probing Methods

**Direct** Detection of the **Protein Antigen**

**Indirect** Detection of the **Antibody**

### WB MOA

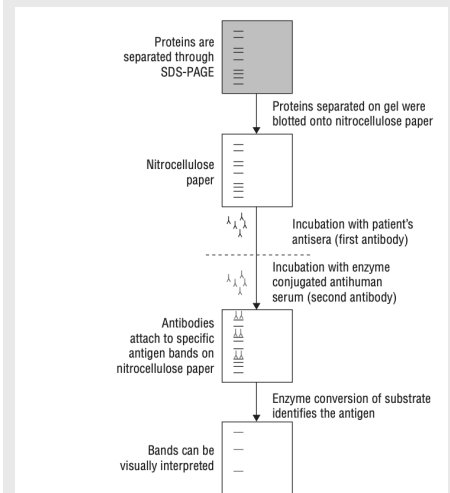


FIG. 14-16. Western blot test.

By Carsonmccall

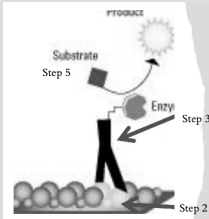


[cheatography.com/carsonmccall/](https://cheatography.com/carsonmccall/)

Not published yet.  
Last updated 28th November, 2024.  
Page 4 of 6.

Sponsored by **ApolloPad.com**  
Everyone has a novel in them. Finish Yours!  
<https://apollopad.com>

### DIRECT WB



1. Separate out proteins by size and charge in the unknown antigen using gel electrophoresis
2. Transfer molecules to secondary matrix
3. Probe with enzyme-labeled known antibody to the antigen of interest
4. Wash
5. Add substrate and observe change (color or light)

### DIRECT WB ~ Bovine Spongiform Encephalopathy (BSE)

<b>Sample</b>	Serum w Ag from pt. (separated by electroph.)
_____ -	Brain tissue
_____ -	
_____ -	
<b>Known</b>	BSE specific Ab-tagged with an enzyme (reagent)
<b>Detects</b>	BSE Prion protein <b>Antigen</b> ( <i>Unknown</i> )

#### RESULTS

<b>Positive Test</b>	Banding that match the positive band pattern = Antigen present
----------------------	--

### DIRECT WB ~ Bovine Spongiform Encephalopathy (BSE) (cont)

**Negative Test** Banding that match the negative band pattern = No Antigen

#### Direct WB Required for Dx of BSE! ~

##### Because:

- The BSE Ag that causes a disease is a normal brain protein in Bovine that is malfunctioning because it is folded incorrectly
- Since this is a normal protein in the Cow brain ~ There is NO IMMUNE RESPONSE that will generate

>>> THUS: We **have** to test for the Ag since the Ab will never be produced

#### Direct WB BSE Results

- 3 brain preps from 3 suspect cow with brain proteins separated
- Abnormal BSE-specific prion protein molecules can be detected using antibodies linked to an enzyme that results in a chemical reaction
- For this test a monoclonal antibody was made that recognizes BSE-specific abnormal prion protein >>> This antibody is a reagent antibody (tagged with an enzyme).

#### RESULTS

Cow #3 has BSE-specific prion proteins in its brain.

### INDIRECT WB

#### USES

**ELISA Dx** Feline Immunodeficiency  
**Confirmation** Virus (FIV)

Human Immunodeficiency  
Virus (HIV)

**Sample** Patient serum w Ab  
(separated by

**Known** HIV Ag (from known HIV-infected cells)

**Detects (unknown reactant)** Ab spec. to HIV Ag

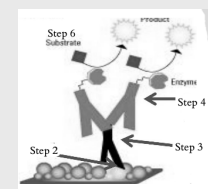
**Reagent** Anti-spp Ab conj. to enzyme

#### INTERPRETATION

**Positive Test** Banding Pattern matches that of known positive = Ab present

**Negative Test** Banding Pattern matches that of known negative = No Ab

### INDIRECT WB MOA



1. Separate out proteins by size and charge in the known antigen using gel electrophoresis
2. Transfer molecules to secondary matrix
3. Probe with patient's serum antibody
4. Add enzyme-labeled antibody to patient's antibody
5. Wash
6. Add substrate and observe change (color or light)



By Carsonmccall

[cheatography.com/carsonmccall/](https://cheatography.com/carsonmccall/)

Not published yet.

Last updated 28th November, 2024.

Page 5 of 6.

Sponsored by [ApolloPad.com](https://apollopad.com)

Everyone has a novel in them. Finish Yours!

<https://apollopad.com>

### INDIRECT WB ~ FIV Confirmation

<b>Source for Ag</b>	Proteins from FIV-infected cells (separated out by electrophoresis)
<b>Sample with unknown Ab</b>	Cat's serum w/ Ab
<b>Detection Reagent</b>	Anti-cat Ab conj. w enzyme

### Immunohistochemistry (IHC)

#### IHC Test

- Always detects antigen

- Horseradish peroxidase (brown color)

- (-) Controls = irrelevant Ab OR normal tissue section

<b>Sample</b>	Thin tissue section
<b>Known ~ 1° reagent</b>	1° Reagent Antibody ~ probes Ag
<b>Detects (unknown)</b>	Antigen in the Tissue sect.
<b>2° Detection reagent</b>	2° Detection Ab conj. ~ spec. for 1° Ab

#### CONTROLS

Positive Control

<b>1° Reagent</b>	1° Reagent Ab ~ spec. for tissue Ag
-------------------	-------------------------------------

### IHC Test (cont)

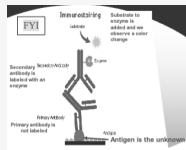
**Positive Result** Brown in color = Ag is present

#### Negative Control

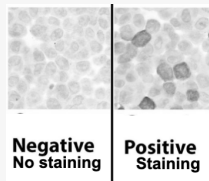
**1° Reagent** 1° Reagent Ab ~ spec. for tissue Ag NOT IN SAMPLE

**Negative Result** No color change = No Ag

### IHC MOA



### IHC Microscopy Result



### IHC Negative Control

#### USES

Detection of *mammary tumor Ag\** Use of an irrelevant rabbit mAb spec. to tumor Ag

Detection of *Brucella Melitensis*

### IHC ~ Brucella Melitensis

**Sample** Histo section of a Goat's tissue from the Prepuce of the Penis and the Seminal Vesicular Gland

**1° Reagent Ab** mAb spec. to *B. melitensis* Ag

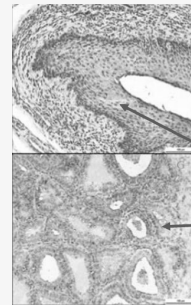
**2° Detection Ab** 2° mAb spec. to 1° Ab

#### RESULTS

**Positive Test** Observed brown color when compared to controls = Ag present

**Negative Test** No color change from controls = No Ag

### B. Melitensis IHC Results



Top: Mucosal epithelium of the Prepuce of the Penis

Bottom: Seminal Vesicular Gland epithelia



By Carsonmccall

[cheatography.com/carsonmccall/](https://cheatography.com/carsonmccall/)

Not published yet.

Last updated 28th November, 2024.

Page 6 of 6.

Sponsored by [ApolloPad.com](https://apollopad.com)

Everyone has a novel in them. Finish Yours!

<https://apollopad.com>