



**UTF/077/ZAM: Technical Assistance to the Zambia Aquaculture Enterprise Development Project (ZAEDP)**

# **Session 3: TiLV diagnostics (level I, II, III)**

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- **Tilapia lake virus (TiLV) was discovered in sick tilapia in Israel in 2009.**



- **Diagnosis:**
  - 1) **gross signs (Level I)**
  - 2) **histology (Level II)**
  - 3) **PCR-based and other molecular methods (Level III)**
  - 4) **cell-culture (level III) and laboratory infection**

# Gross signs (Level I)



- Shrinkage of the eye and loss of ocular functioning
- Dermal erosions and ulcers

Eyngor et al. 2014

# Gross signs (level I)

## Gross signs (farm outbreaks)

## Gross signs (lab-infected fish)



Experimental infection of Tilapia Lake Virus (TiLV) in Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis spp.*)

Puntanat Tattiyapong<sup>a,b</sup>, Worawan Dachavichitlead<sup>a,b</sup>, Win Surachetpong<sup>a,b,\*</sup>

# Gross signs (level I)



## Naturally diseased fish

- ✓ discoloration
- ✓ loss of scales
- ✓ skin erosion
- ✓ skin hemorrhage

## Experimentally diseased fish

- ✓ exophthalmia
- ✓ abdominal swelling
- ✓ scale protrusion

Behera et al. Aquaculture 484 (2018): 168-174

Jansen et al. Review in Aquaculture 2018

# TiLV diagnosis by histopathology (level II)

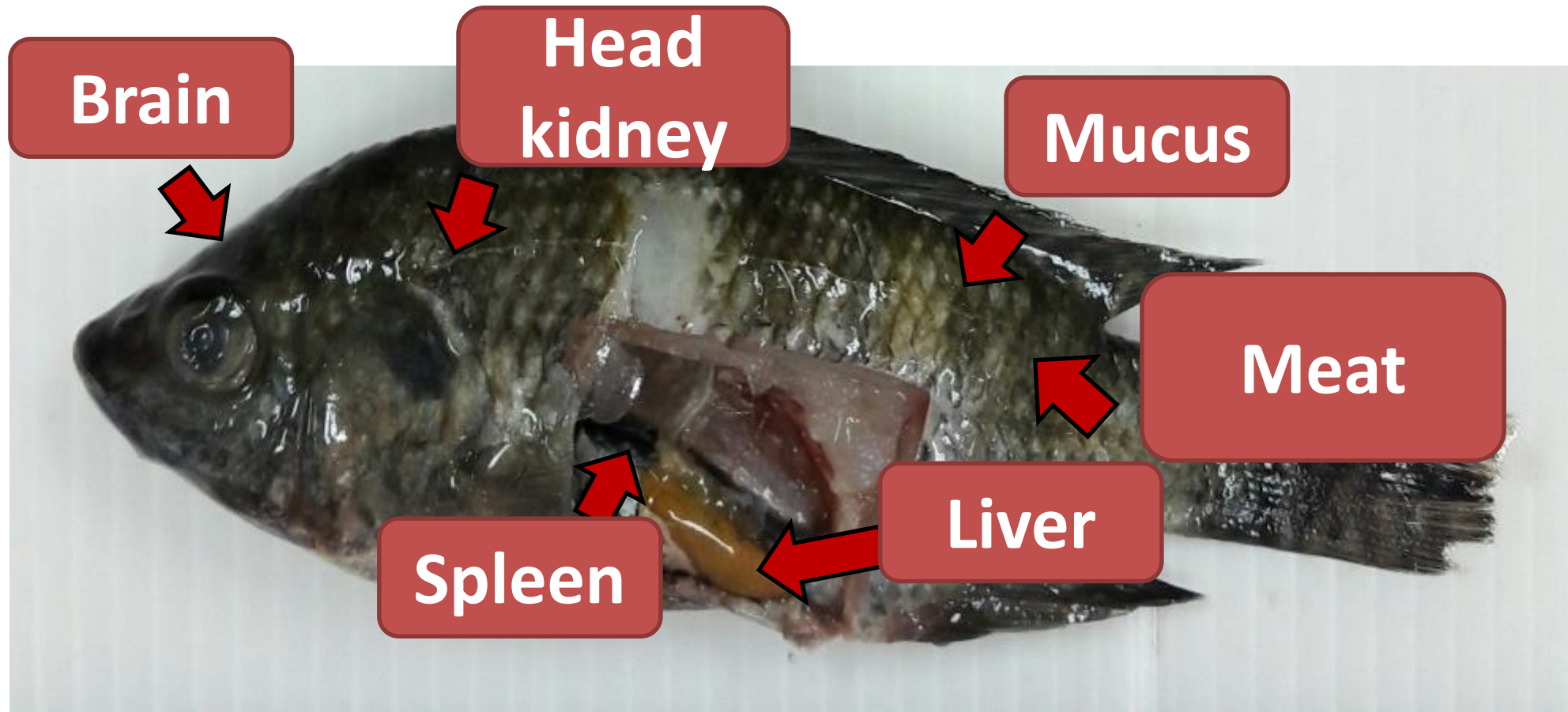
## What are target tissues of TiLV?

- **Liver**
- **Kidney**
- **Brain**
- **Spleen**
- **Gills**
  
- **Mucus**



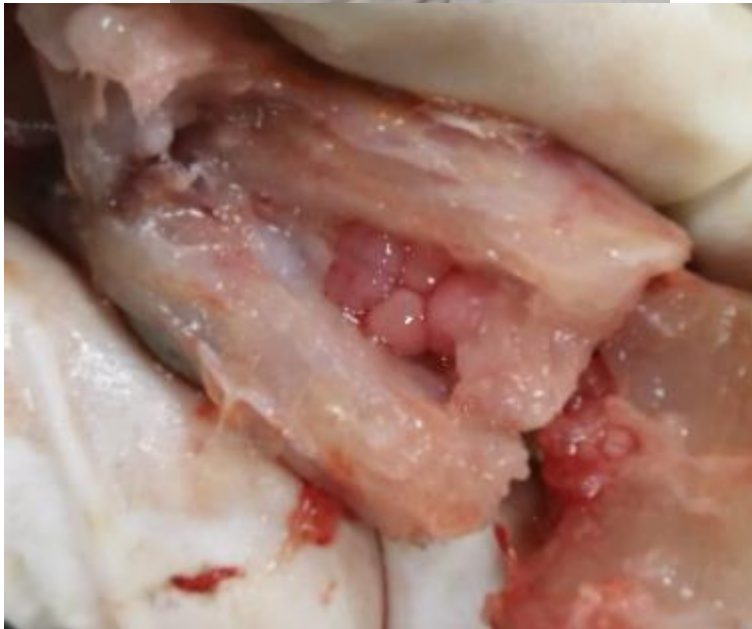
# Sample collection for diagnostic procedures

## Organs for TiLV diagnosis





**Brain → show redness**

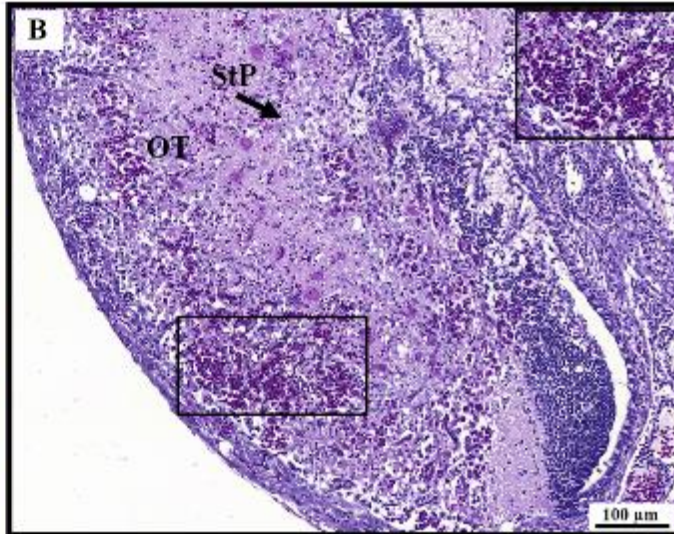
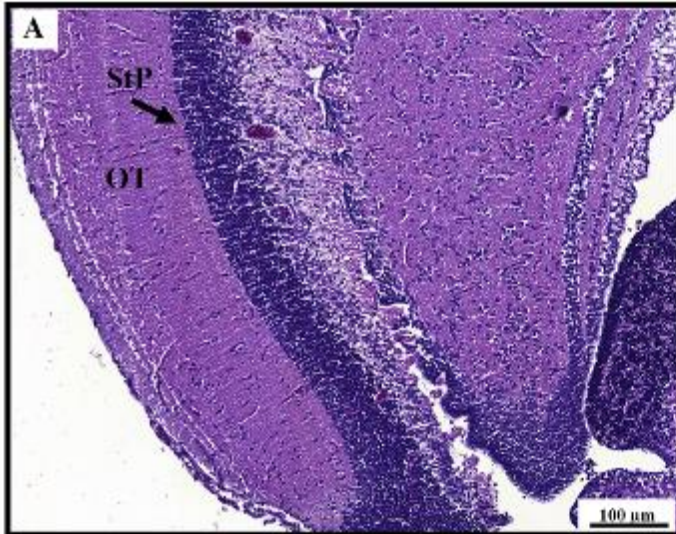


# Histopathology (level II)

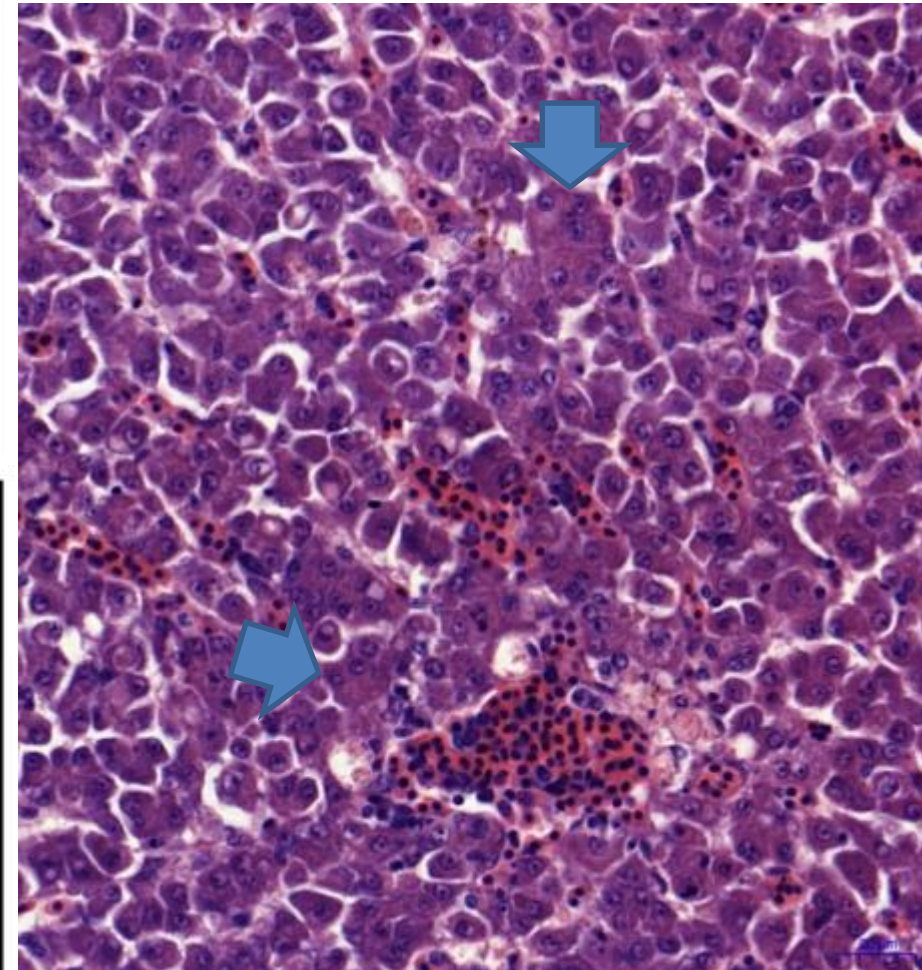
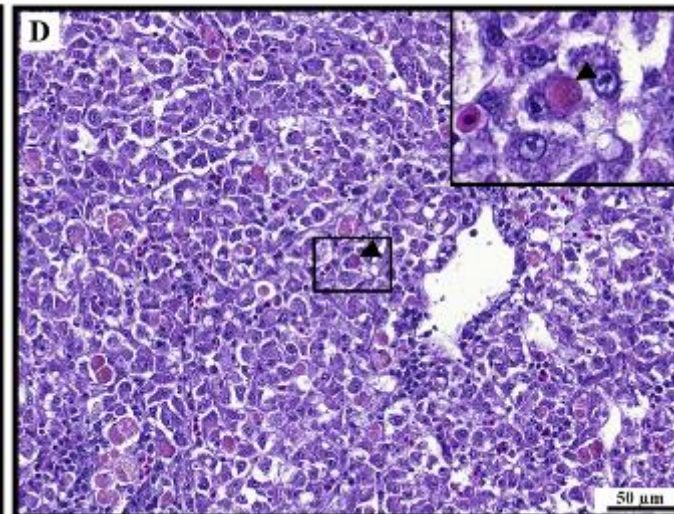
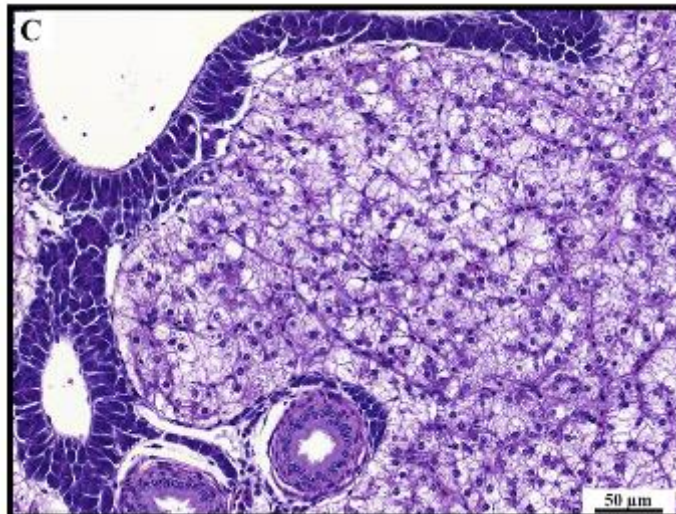
Normal

Infected

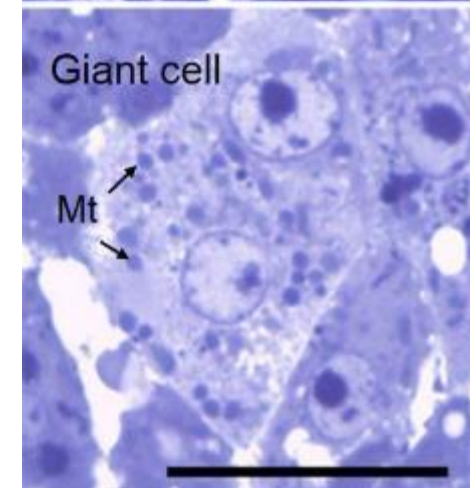
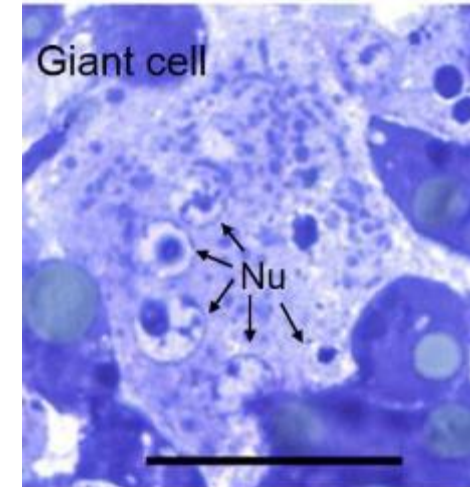
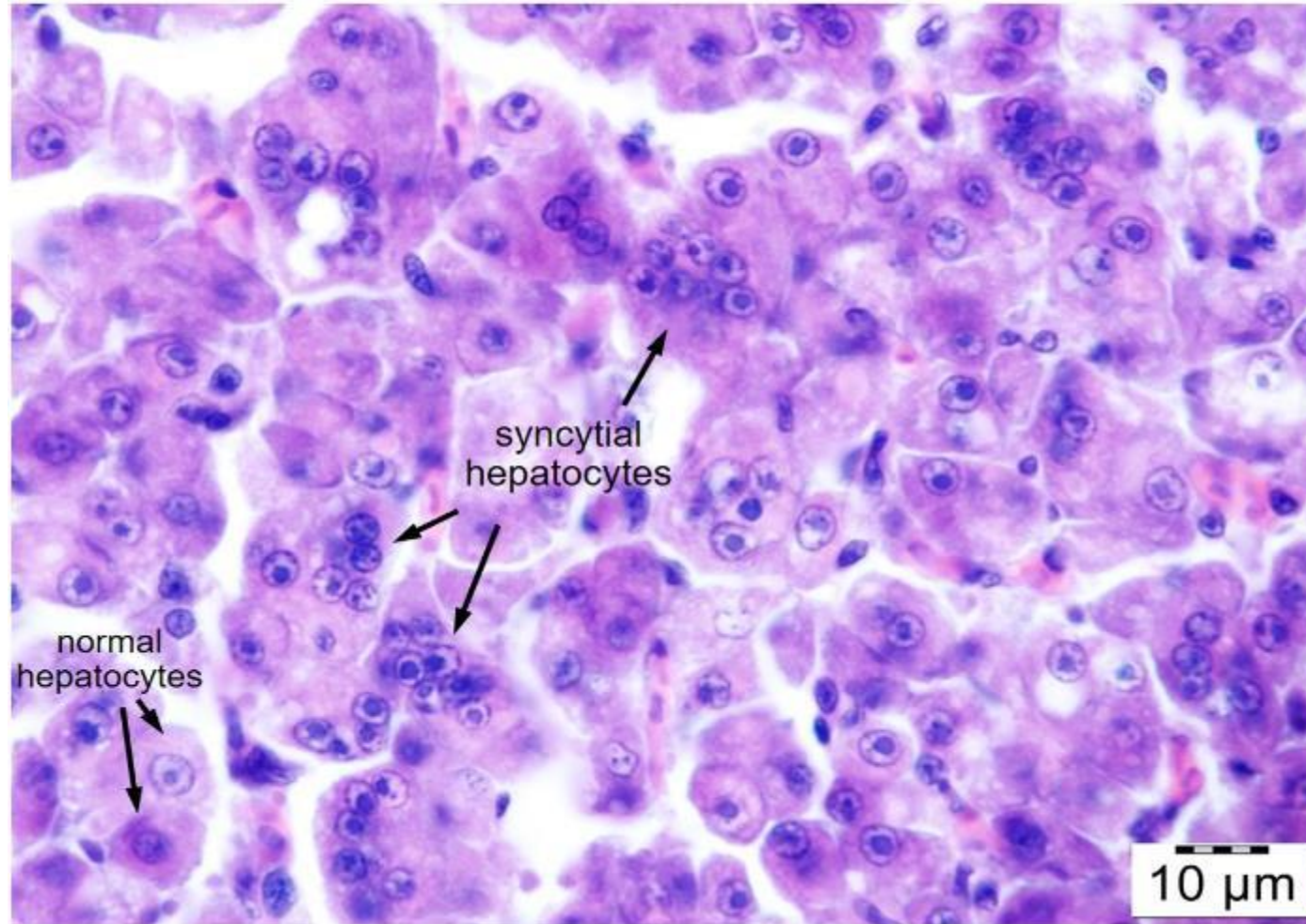
Brain



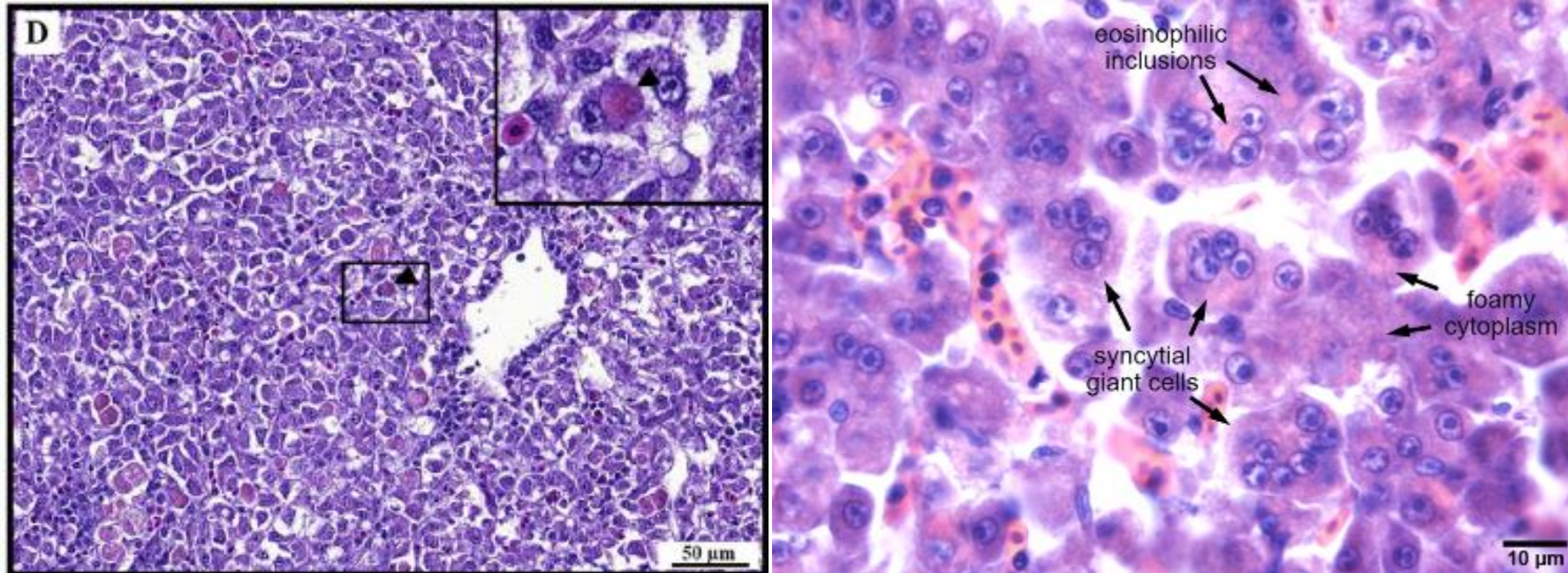
Liver



# TiLV histopathology (level II)



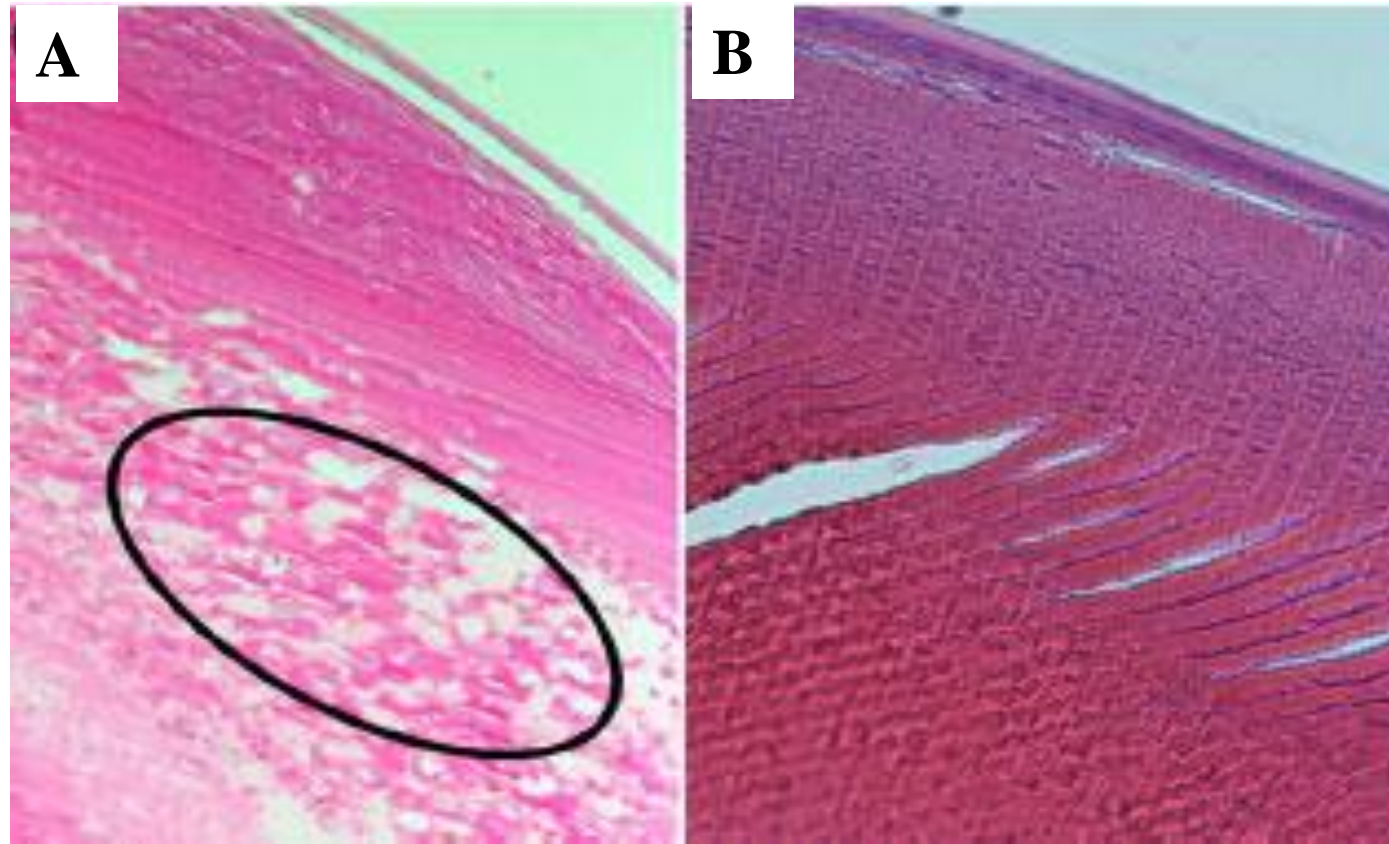
# histopathological changes in liver



Intracytoplasmic inclusion bodies  
Tattiyapong et al. 2017

**Syncytial giant cells,  
intracytoplasmic inclusion bodies,  
foamy cytoplasm (HT Dong)**

# Histopathological changes in eye



H&E histology of eye lens in TiLV-infected tilapia. (A) Cataractous changes characterized by formation of eosinophilic spherical structures accompanied by degeneration of crystalline fibers (encircled); (B) control lens from healthy fish.

# TiLV Diagnostics: molecular tests (level III)

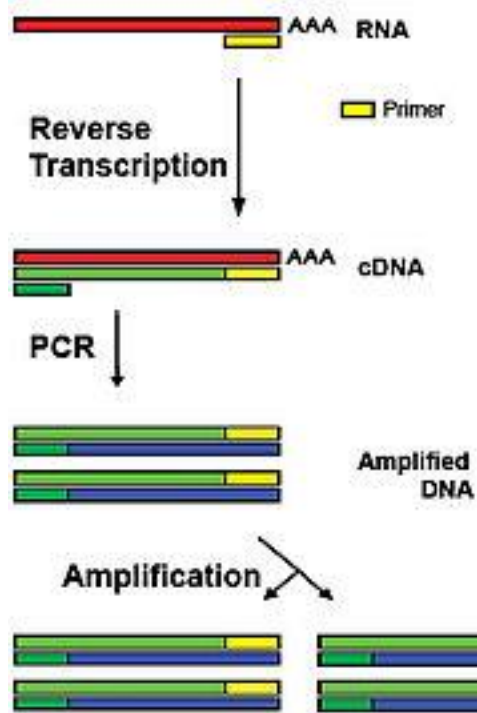
- **PCR-based**
  - Conventional nested RT-PCR**
  - Real-time RT-PCR**
  - RT-LAMP**
  - iiPCR**
- **LAMP (loop-mediated isothermal amplification) -based**
- **In situ hybridization (ISH)**
- **Antibody-based:**
  - Immunohistochemistry (IHC)**
  - Immunofluorescence**
  - ELISA (enzyme-linked immunosorbent assay)**

# Reverse transcription polymerase chain reaction (RT-PCR)

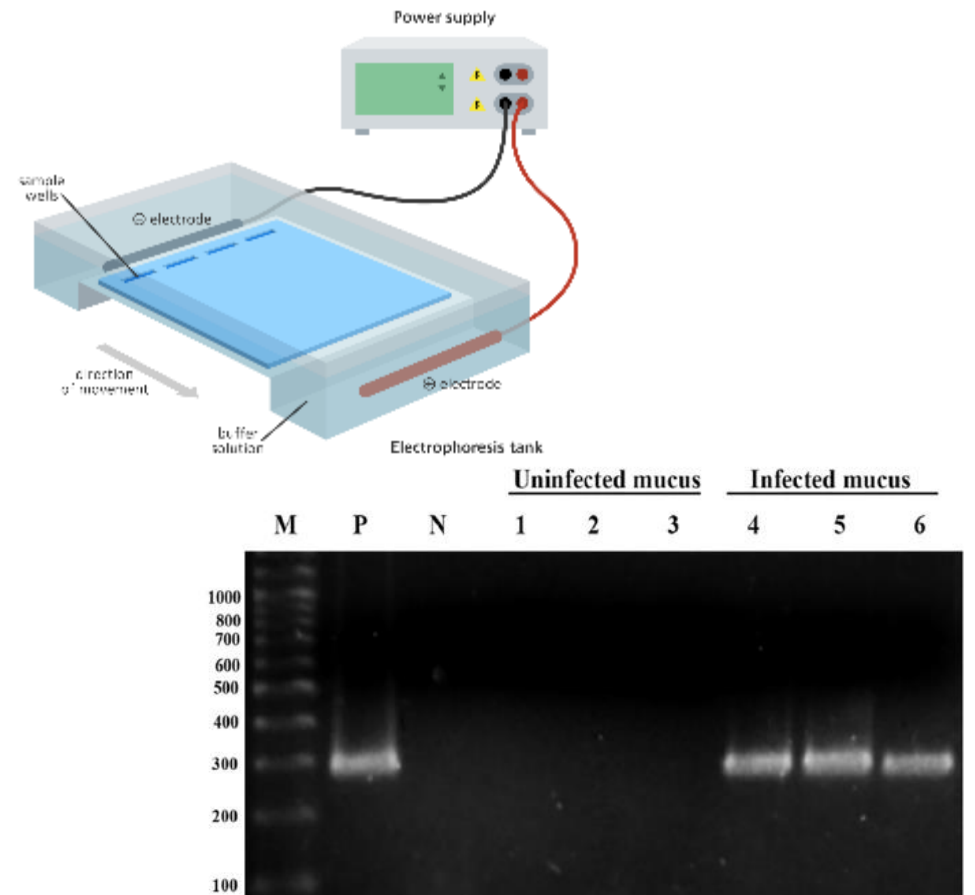
TiLV = negative sense RNA genome



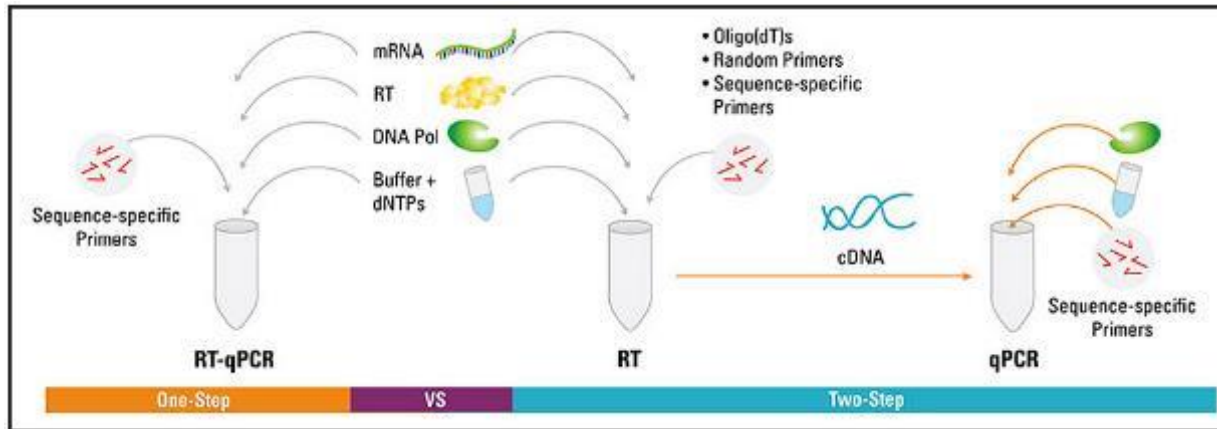
Thermo cycler



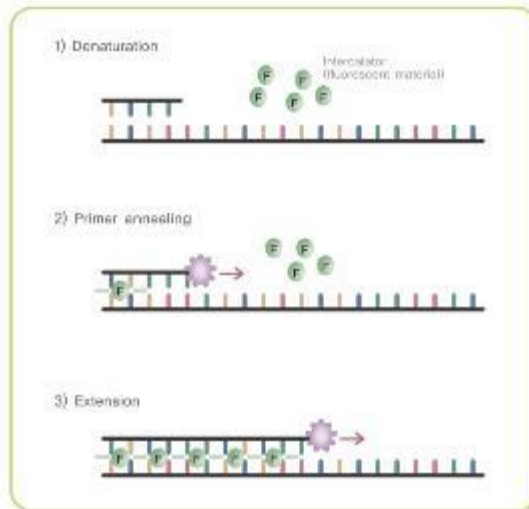
## Gel electrophoresis



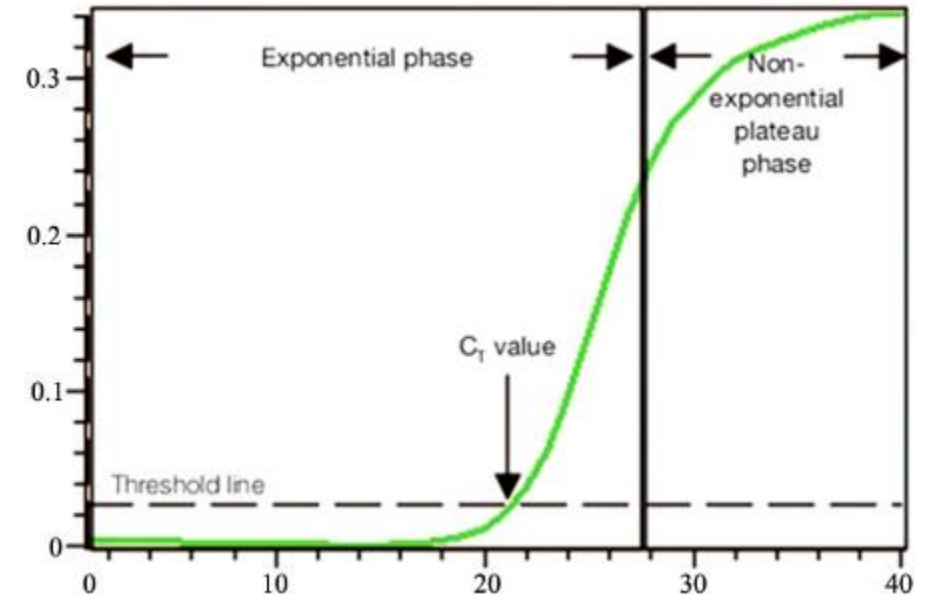
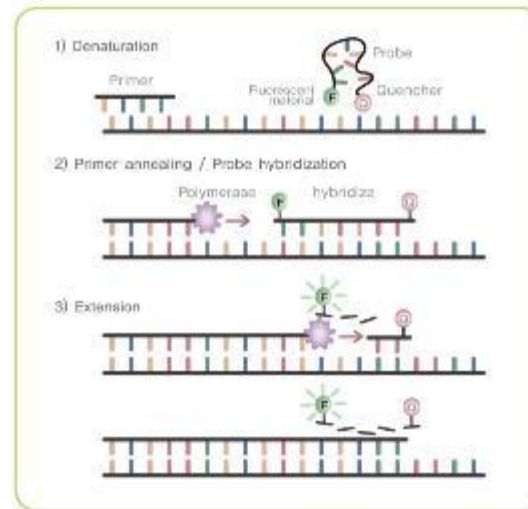
# Quantitative reverse transcription polymerase chain reaction (RT-qPCR)



SYBR Green Detection



Taqman-Probe Detection



# Detection of TiLV in clinical samples using RT-qPCR method

Fish samples	Number of samples	TiLV positive (%)	Mean Ct values (range)	Estimated viral loads (Copy numbers) <sup>c</sup>
Clinical samples <sup>a</sup>	30	30/30 (100)	22.86 (12.83 - 32.89)	$1.65 \times 10^4$ ( $1.37 \times 10^7$ - $1.97 \times 10^1$ )
TiLV-challenged fish	10	10/10 (100)	23.65 (20.08 - 27.28)	$9.72 \times 10^3$ ( $1.00 \times 10^5$ - $8.50 \times 10^2$ )
Non-challenged fish	10	0/10 (0)	ND <sup>b</sup>	ND <sup>b</sup>

<sup>a</sup>Clinical samples were collected from 30 field outbreaks with history of massive mortality.

<sup>b</sup>ND = No detection.

<sup>c</sup>Copy numbers of TiLV template per  $\mu\text{g}$  of total RNA.

## **On farm diagnostic PCR for TiLV detection**

- **A commercial pond-site TiLV RT-PCR detection assay based on insulated isothermal PCR (iiPCR) is available**
- **POCKIT™ Micro (GeneBeach Biotechnology Corp.)**
- **Assay can be completed in 45 min**
- **Rapid, inexpensive, sensitive, easy to maintain**

# POCKIT™ Micro (GeneBeach Biotechnology Corp.) Insulated isothermal PCR (iiPCR)

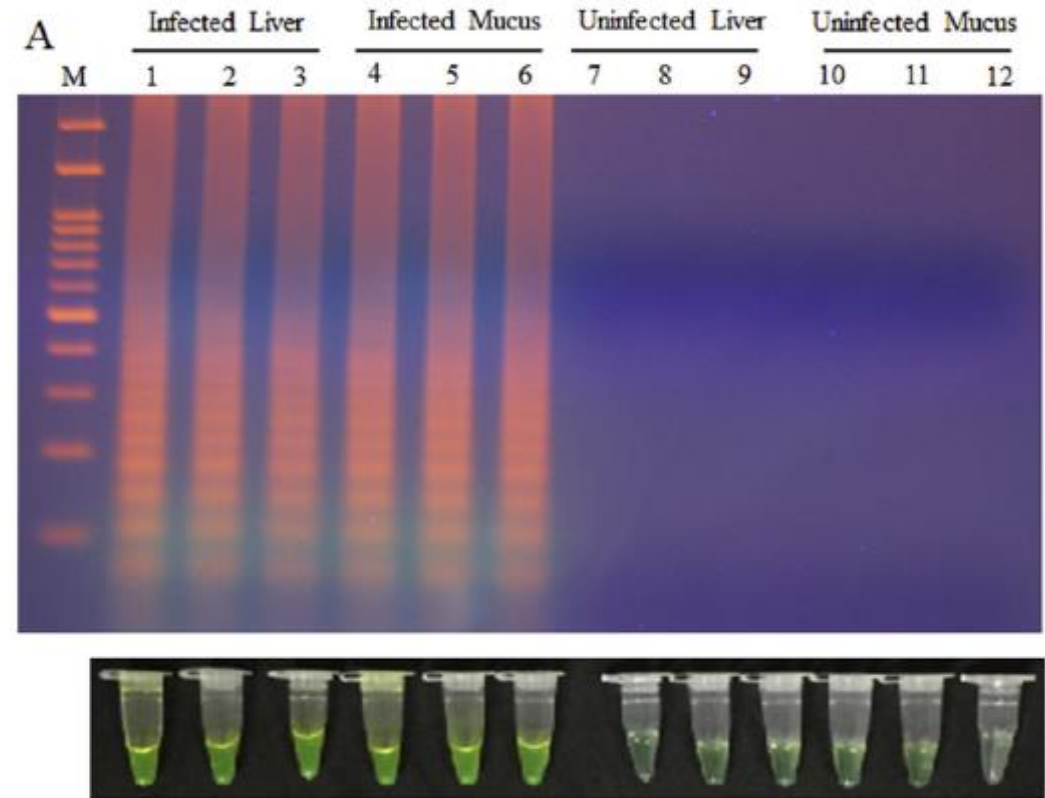
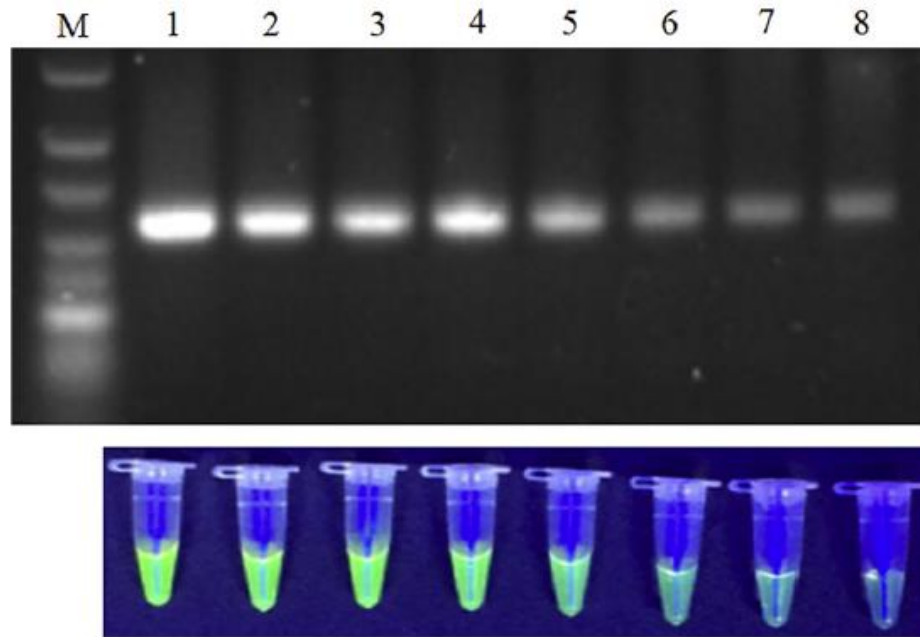


Commercial pond-site  
TiLV RT-PCR detection



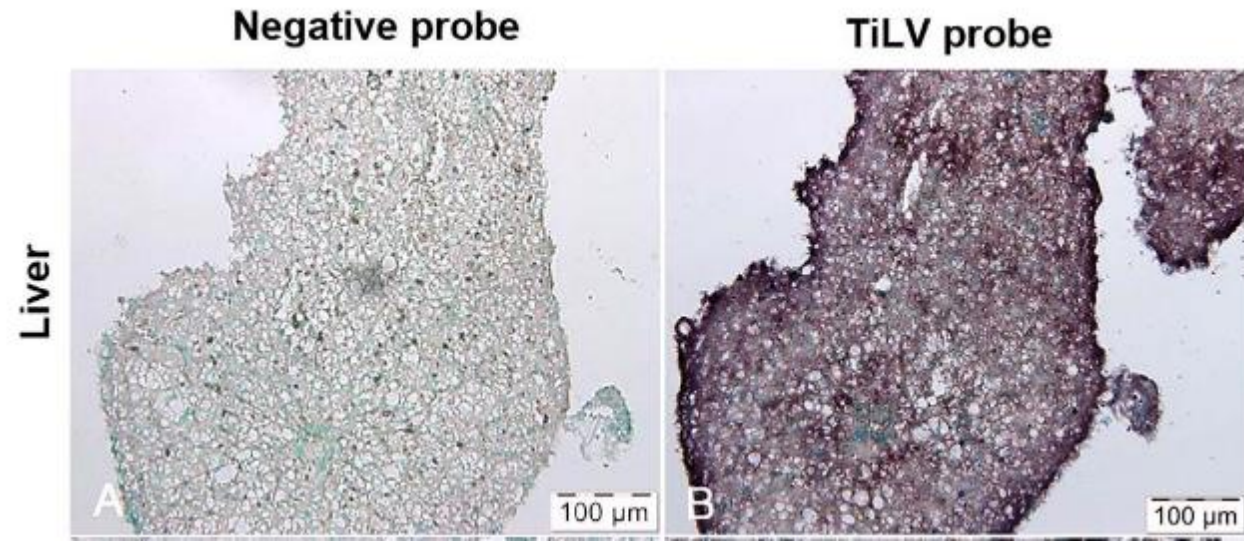
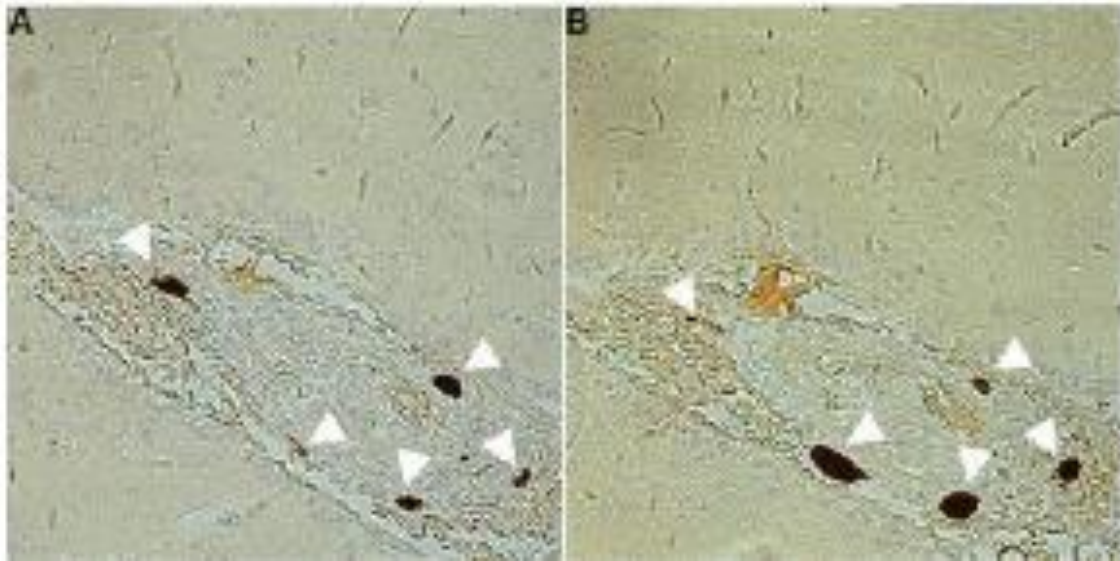
The TiLV RT-PCR has a limit of detection  
LoD95% of **12 genome**

# RT loop-mediated isothermal amplification (RT-LAMP)

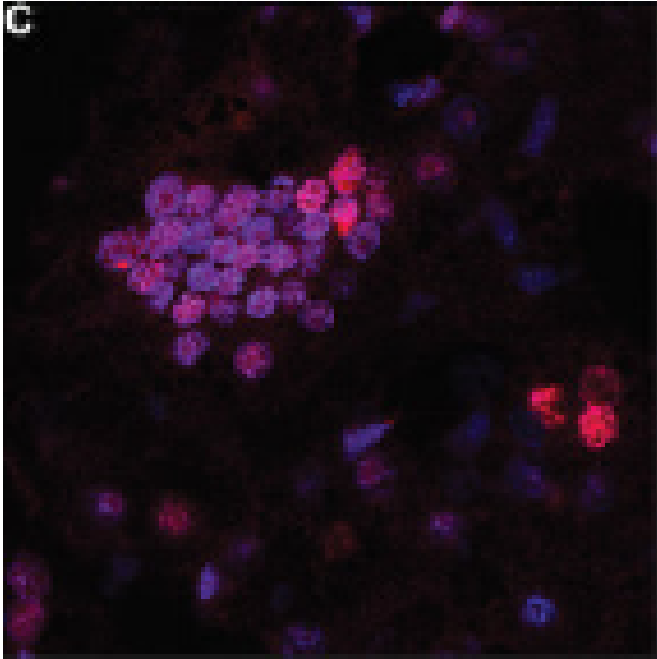


- Color change
- Easy to observe and interpret
- Field application

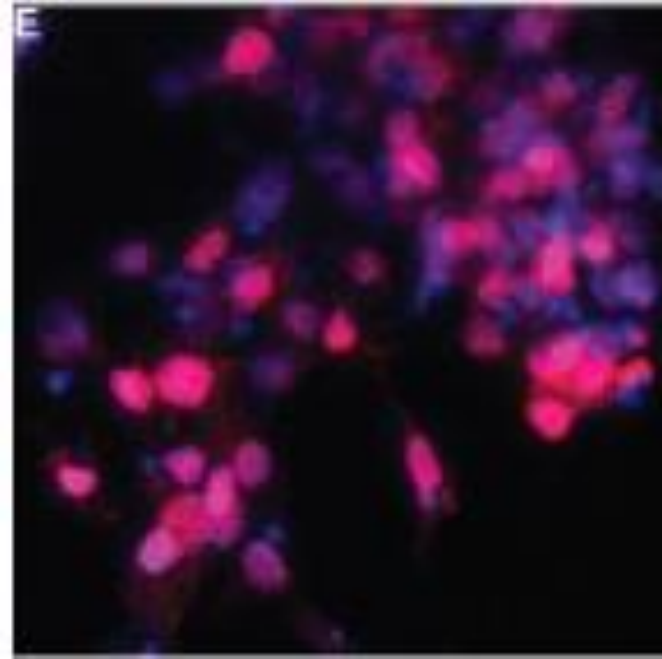
# In situ hybridization (level III)



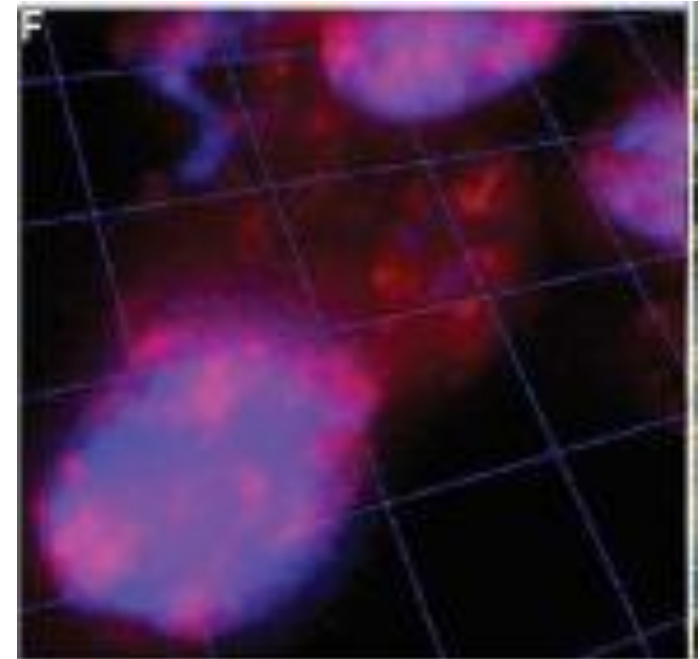
# In situ hybridization (ISH) with fluorescence probes



Liver sections  
Cy3-conjugated (red) Stellaris probes to segment 3 to detect mRNA. Nuclei are stained with DAPI (blue).

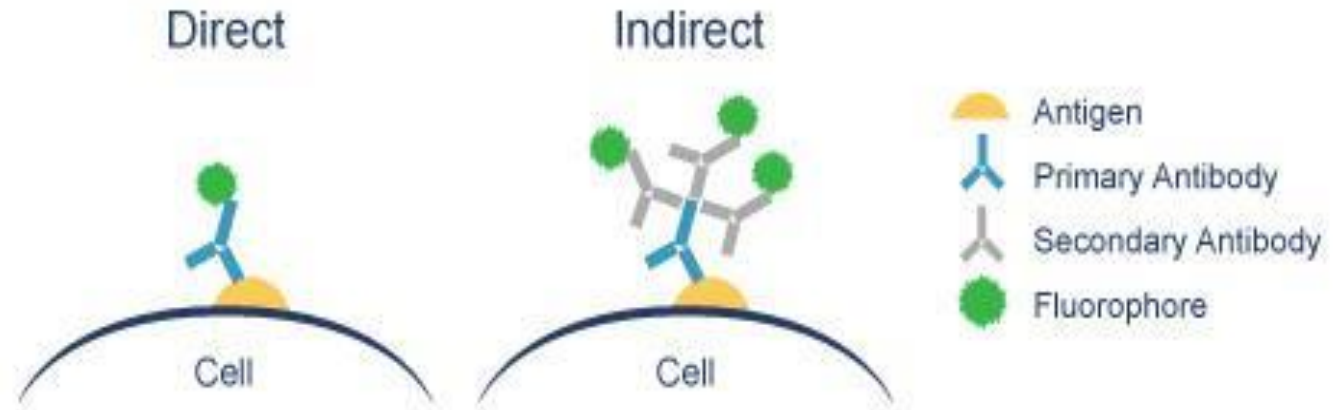
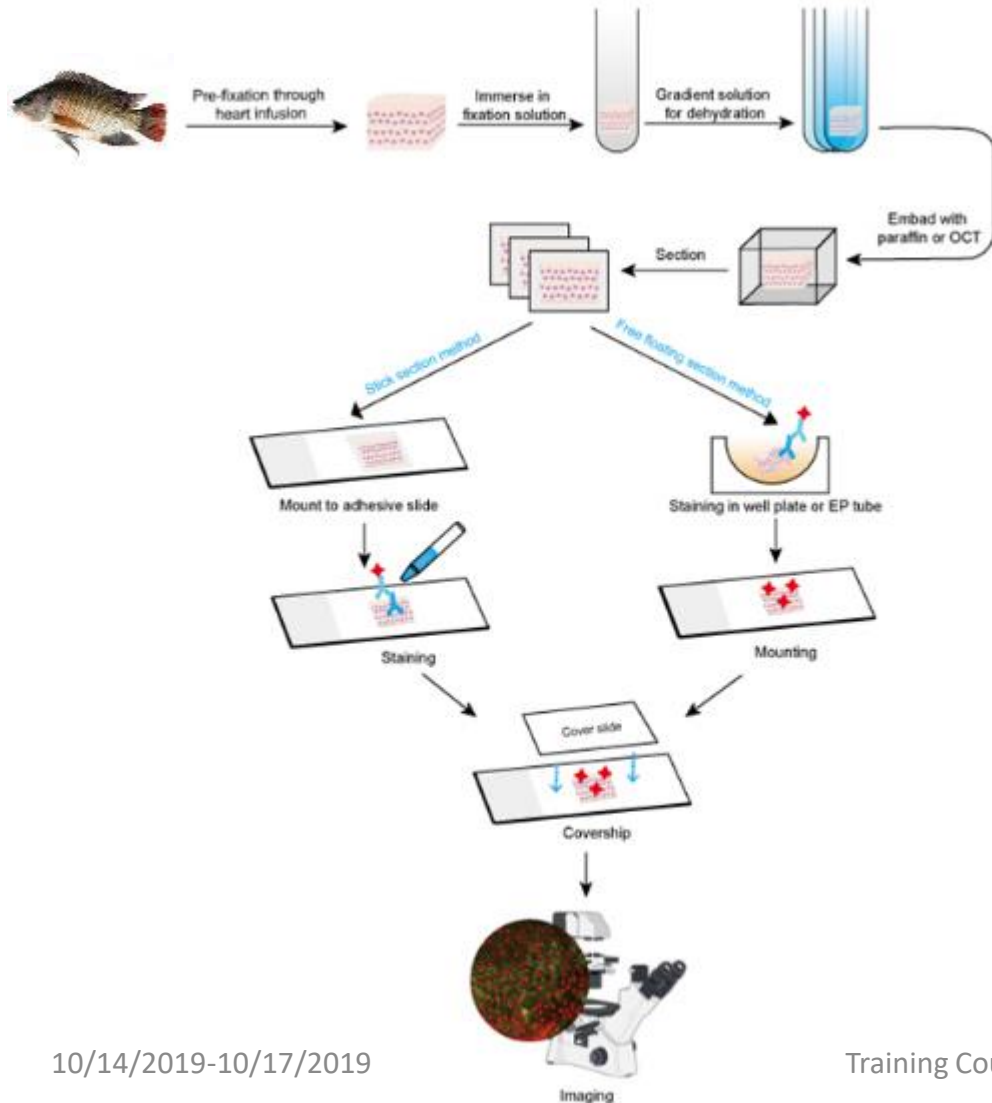


TiLV-infected E-11 cells  
Quasar 670-conjugated (red) Stellaris probe to segment 3 to detect TiLV mRNA. Nuclei are stained with DAPI (blue).



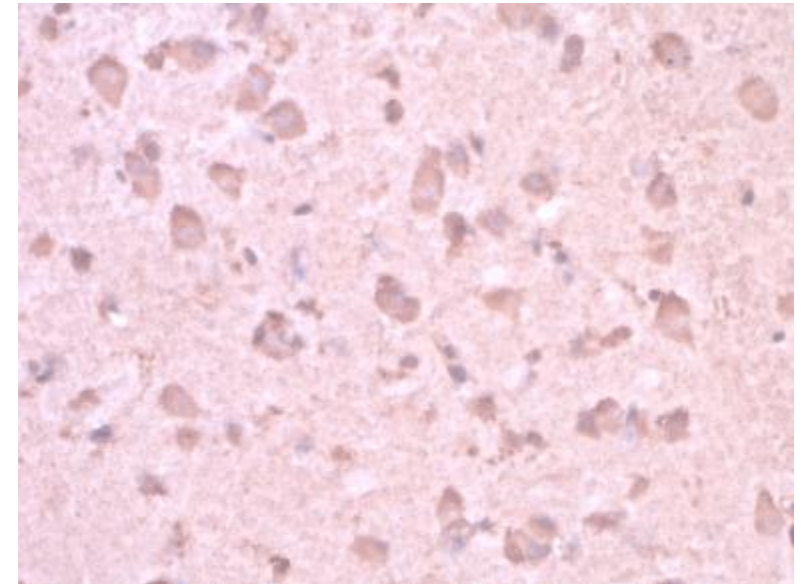
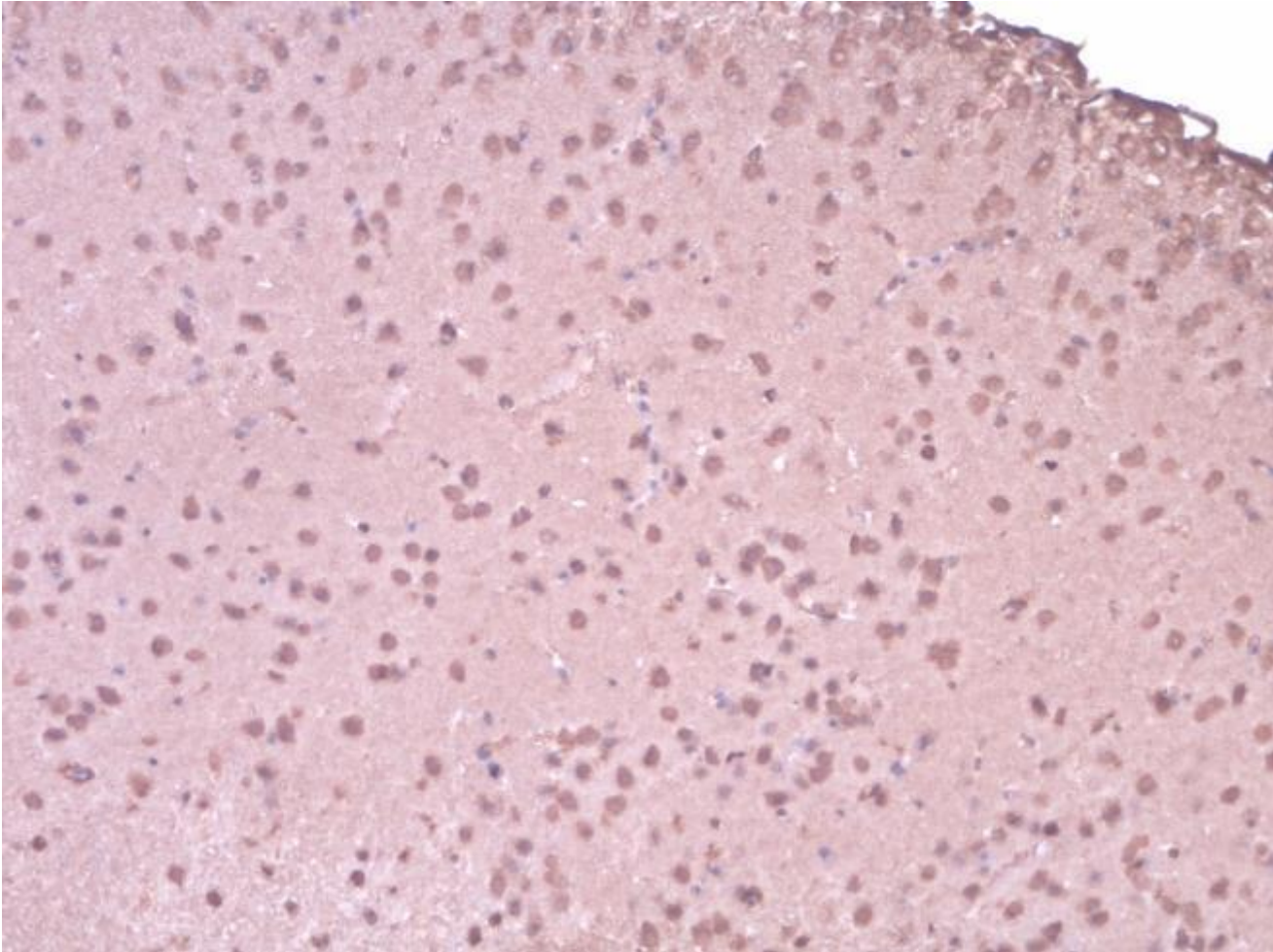
Images of confocal sections of cells in panel E were reconstituted into a 3D image.

# Immunohistochemistry (IHC) (level III)



**Concept: detection of antigen**  
**Material: frozen tissue, cells (smear) or cultured cells**

# Immunohistochemistry (IHC) of TiLV-infected brain



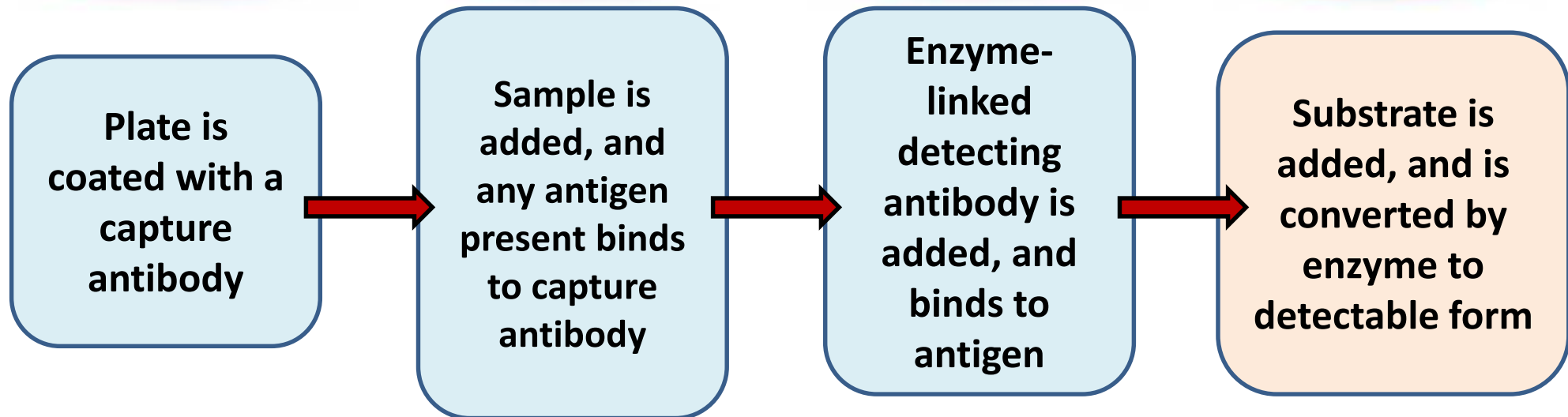
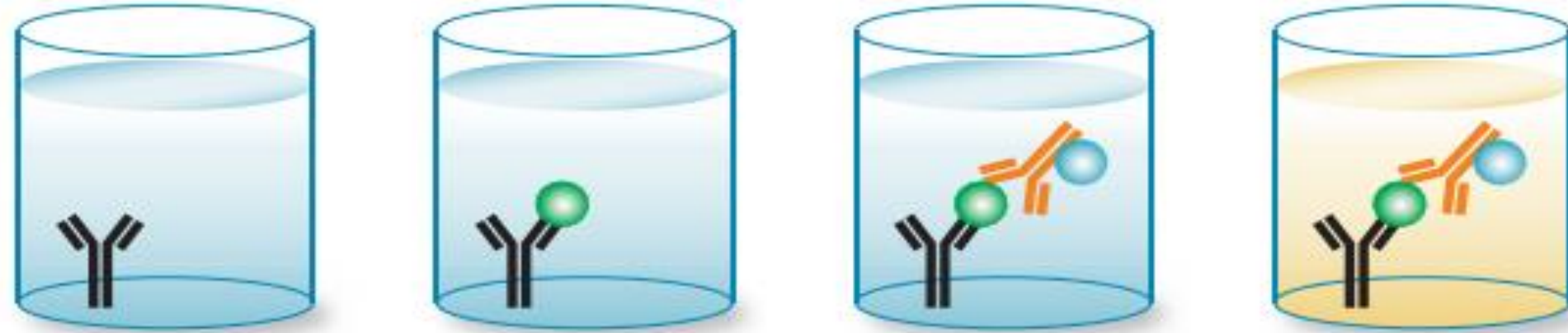
Credit: Dr.Promptorn Raksaseri Faculty of Veterinary Science, Chulalongkorn University

# Enzyme-linked immunosorbent assay (ELISA)

- Uses antibodies and color change to identify a substance (antigen)
- Adsorb certain components onto an immobilized solid phase
- Color development by the product of an enzymatic reaction

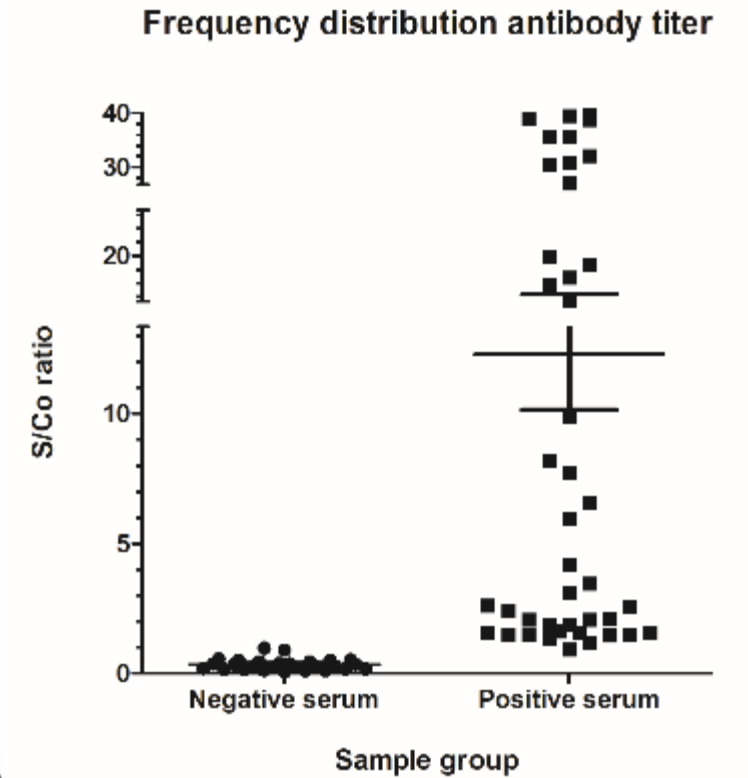
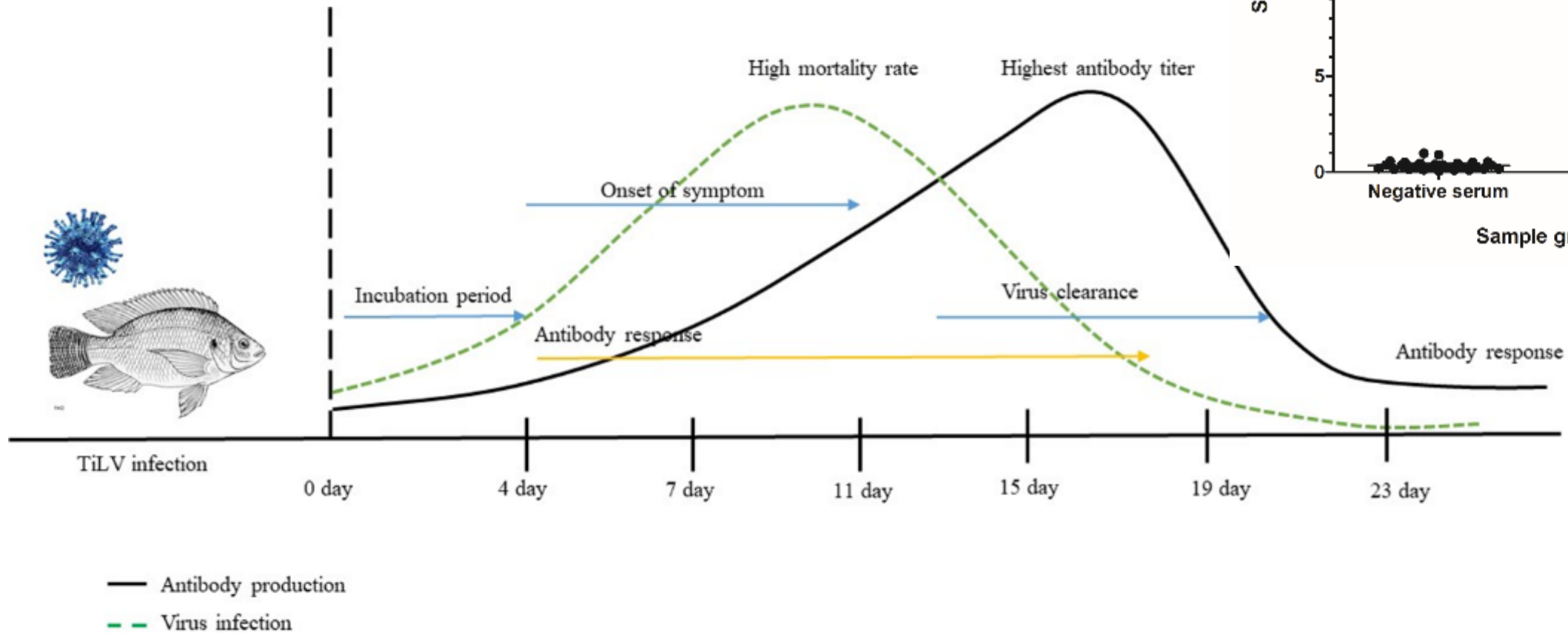
# Sandwich ELISA

\*A detecting antibody and an enzyme-linked secondary antibody may also be used



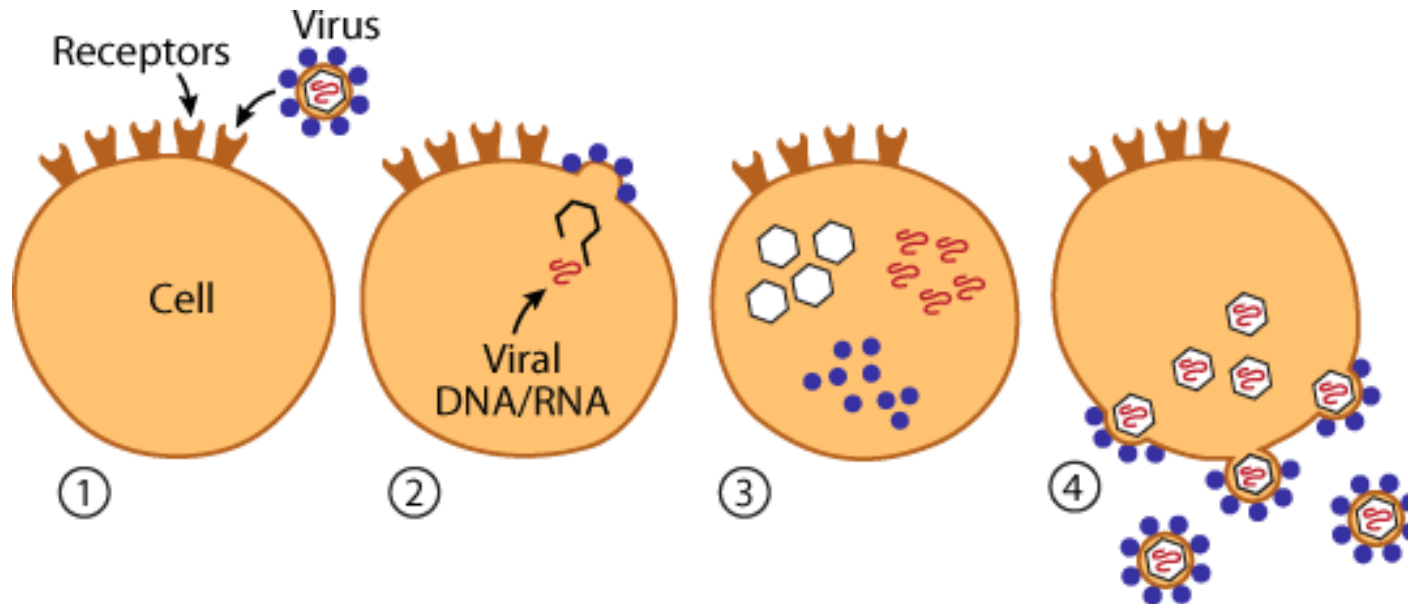
# ELISA

- Detect developing antibody in serum of TiLV-infected fish



# Indirect Examination

1. Cell Culture methods
2. Laboratory infection



# TiLV viral isolation and cell culture

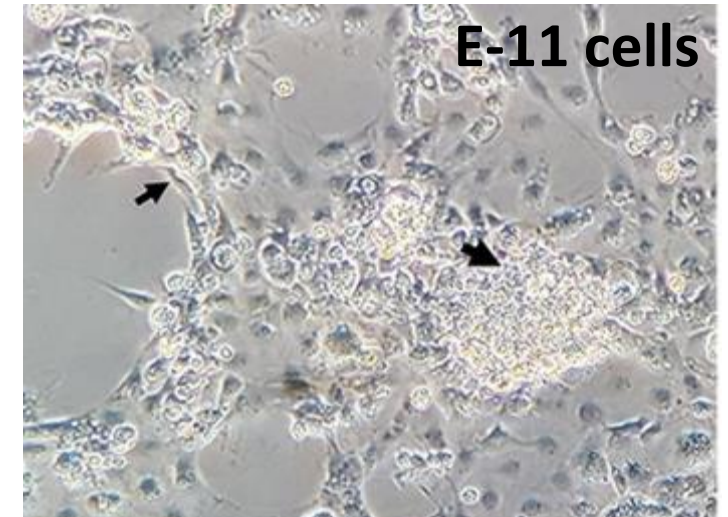


## Clinical specimens

- Organ and tissue
- Mucus



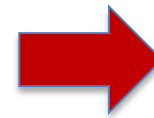
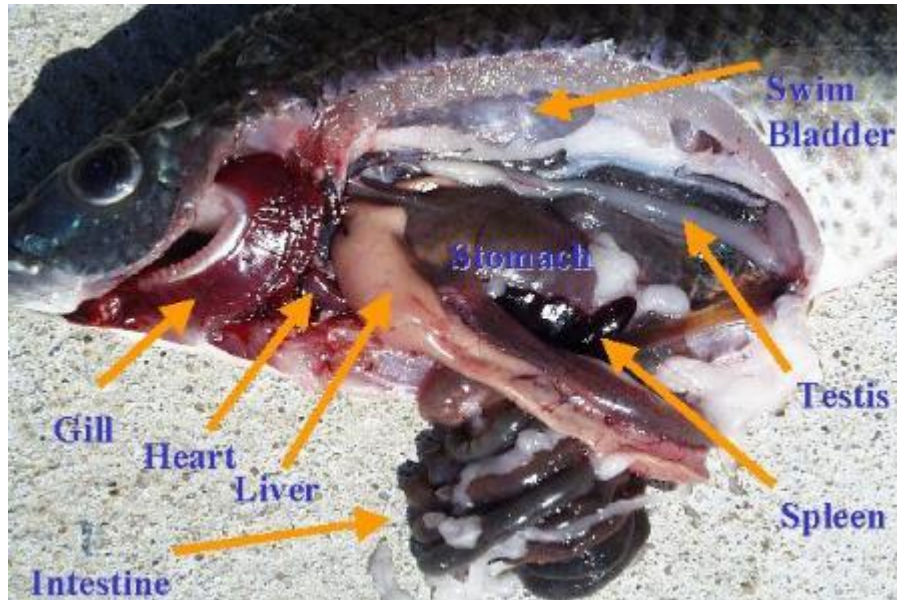
## Virus cultivation in cell culture



## Observe CPE in viral replicated cells

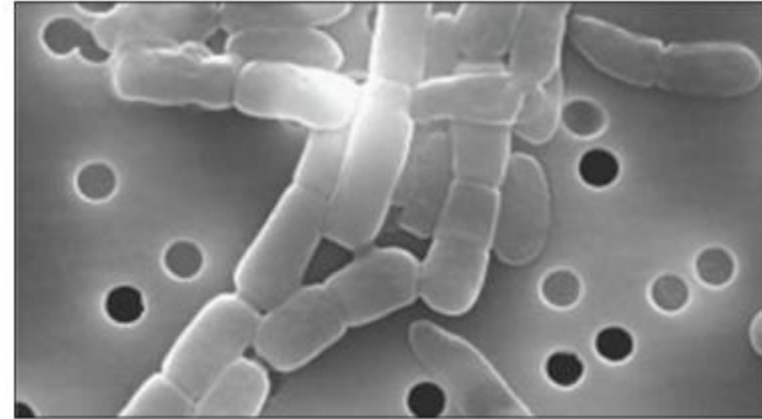
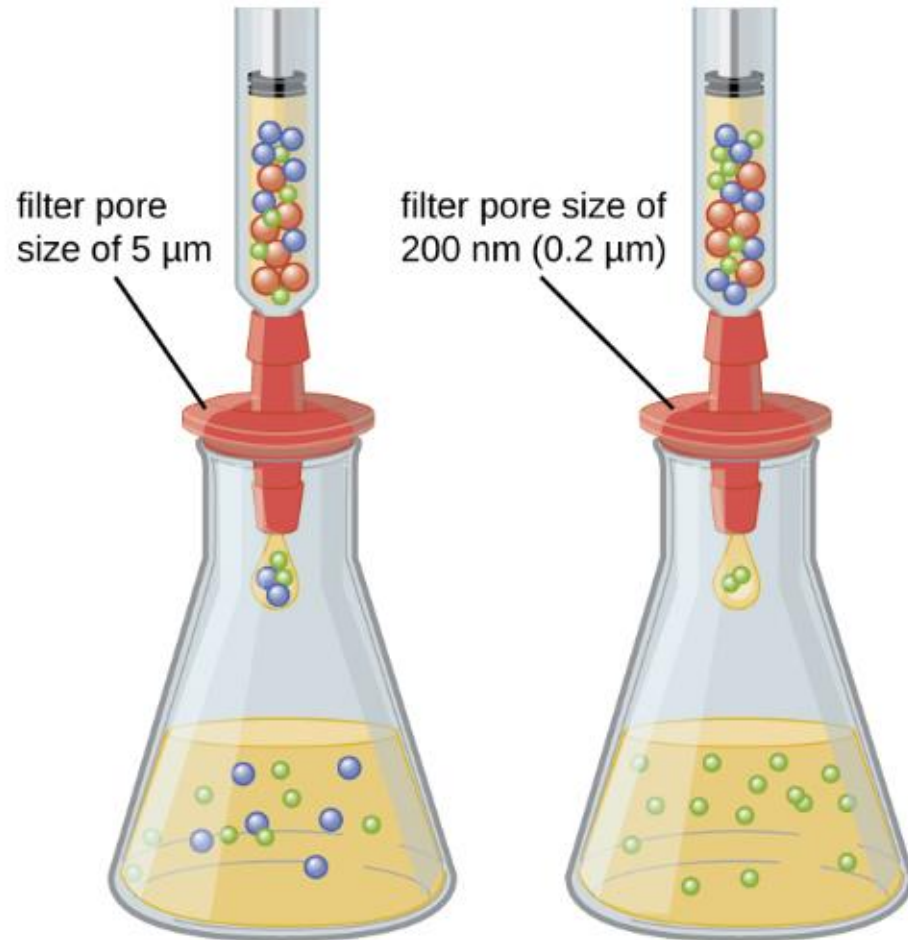
# Virus isolation

## Tissue collection



## Tissue grinding

# Filtration of virus through membrane



# Virus isolation: general concept

Viruses are obligate intracellular parasites and can replicate only within living host cells. These include cell cultures, embryonated eggs, and animals.

**Cell cultures are the host system most frequently used for virus cultivation.**

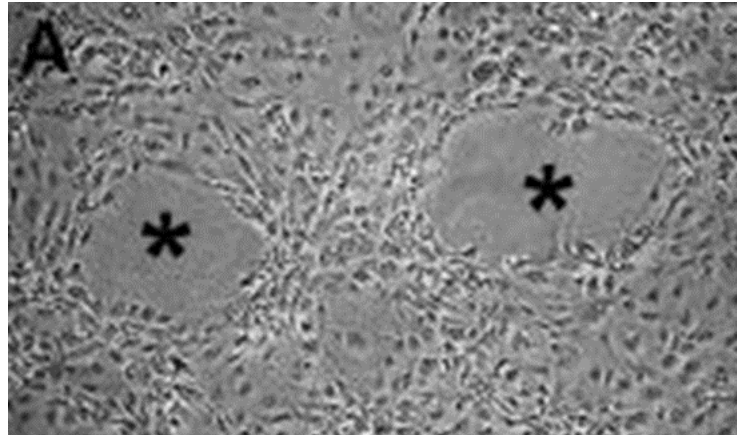


Leland D.S., French M.L.V. 1988. Virus Isolation and Identification. In: Laboratory Diagnosis of Infectious Diseases Principles and Practice. Springer, NY

# Cell lines for TiLV isolation

## e.g. E-11 cell line

E-11 from the striped snakehead (*Ophicephalus striatus*). The commercial fibroblast cell from whole fry tissue. Because the adherent type, the clear CPE show the cytoplasmic vacuole formation followed by intensive disintegration.



**Plaque formation and vacuolated cells at the rims of the plaques. The centers of two plaques are marked with asterisks.**

## 2. Laboratory infection

- **Play an essential role in the studies of viral pathogenesis**
- **Routes of viral inoculation**
  - **Intracerebral**
  - **Subcutaneous**
  - **Intraperitoneal**
  - **Intranasal**
- **After inoculation, the animal is:**
  - **Observed for signs of disease or visible lesions**
  - **Euthanized so that infected tissues can be examined**



# General overview of diagnostic methods for TiLV

## Direct methods

- Electron microscopy
- Light microscopy
- Immunofluorescence
- Molecular techniques

## Indirect methods

- Cell culture
- Laboratory infection

## Serology

Advanced techniques

- Immunoassay (e.g. ELISA)

# References

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**Thank you for your attention**