FAO/ASTF Project: GCP/RAF/510/MUL:

Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture: Intensive Training Course on TiLV

4-13 December 2018. Kisumu, Kenya

in cooperation with Kenya Marine Fisheries Research Institute (KMFRI) and Kenya Fisheries Service (KeFS)

Session:

Diagnostic PCR method for Tilapia Lake Virus (TiLV)

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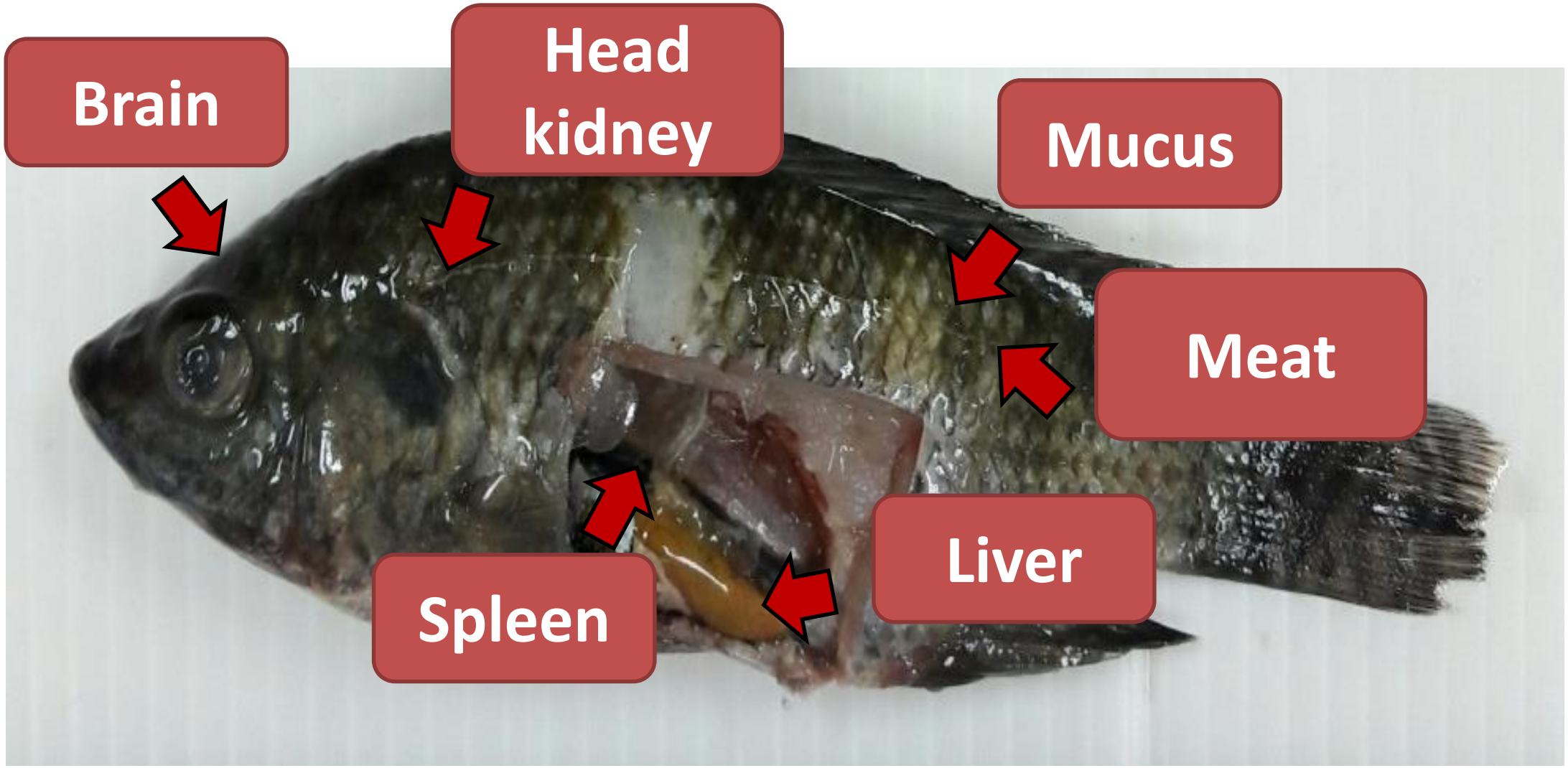
Food and Agriculture Organization of the United Nations

Acknowledgements

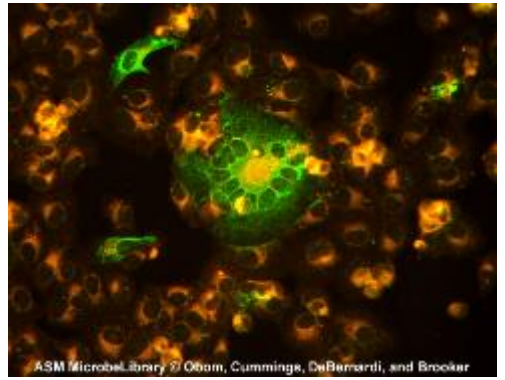
Sample collection for diagnostic procedures

Organs be used for TiLV diagnosis



Direct Examination

1. Electron microscopy
2. Light microscopy
3. Immunofluorescence
4. Molecular techniques



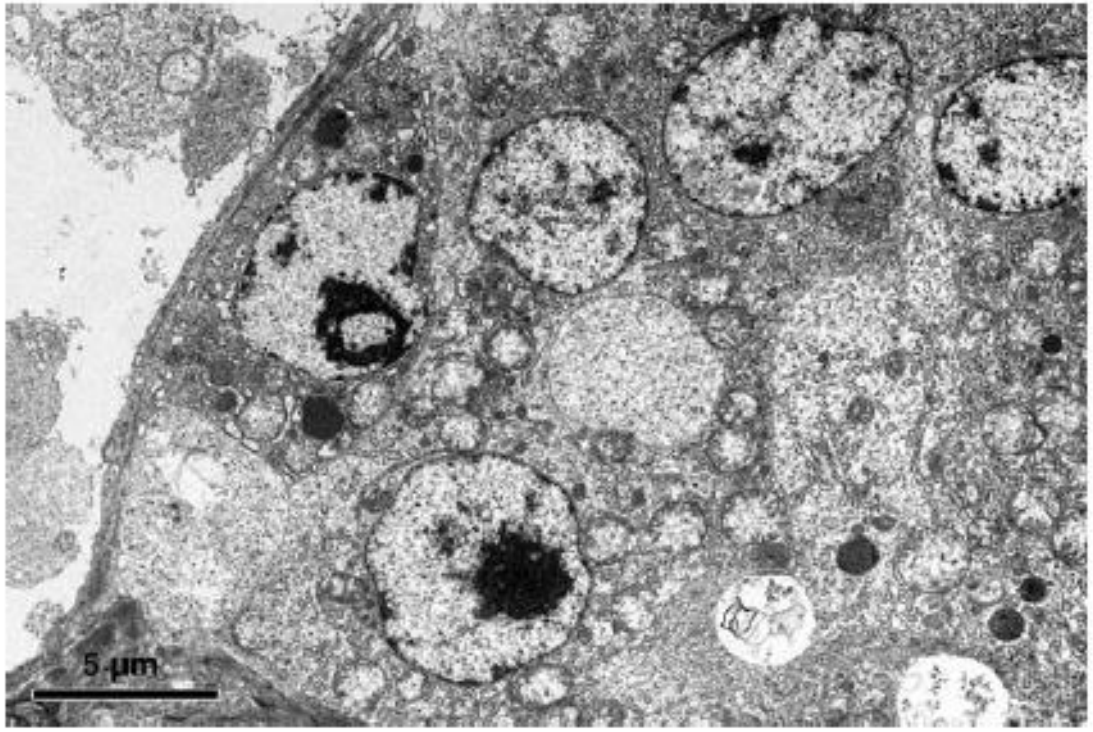
1. Transmission electron microscopy

- Examination of virus preparations in which their **morphological identity** is unknown

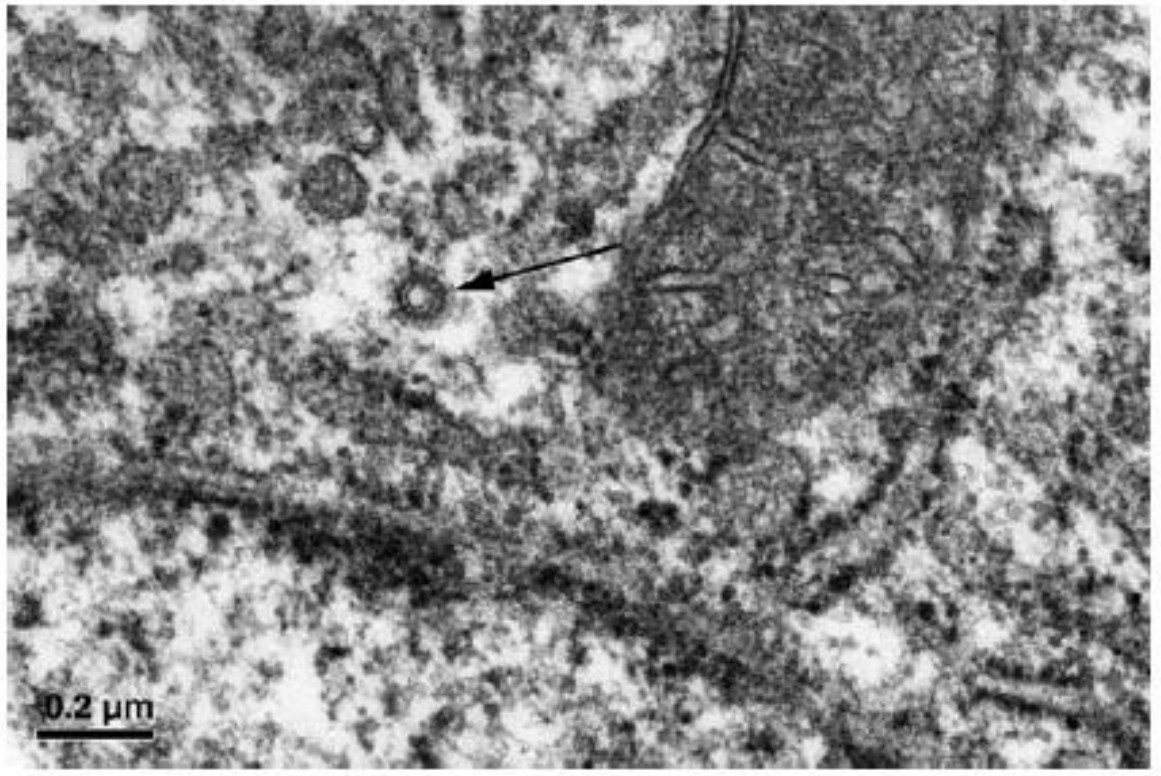
- A beam of electrons is transmitted through an ultra thin specimen → An image is formed, magnified, and focused in an imaging device

SHV morphology

Transmission electron microscopy



Liver from moribund tilapia showing multinucleated hepatocyte

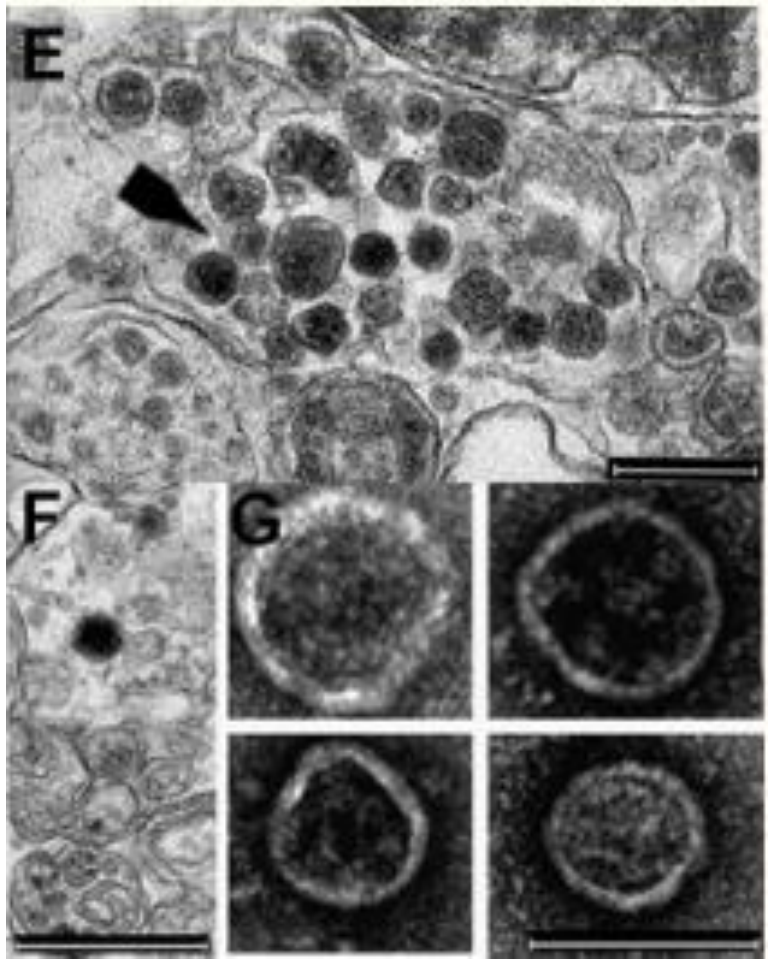


EM of hepatocyte from moribund tilapia showing typical appearance of virus-like particle within cytoplasm (arrow)

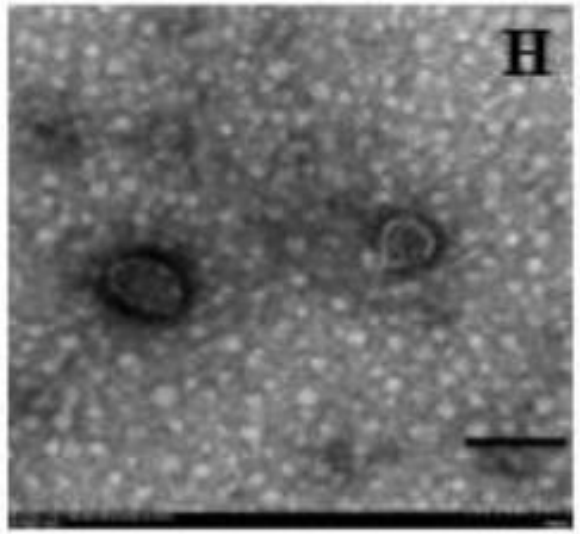
Ferguson et al., 2014 J Fish Dis.

TiLV morphology

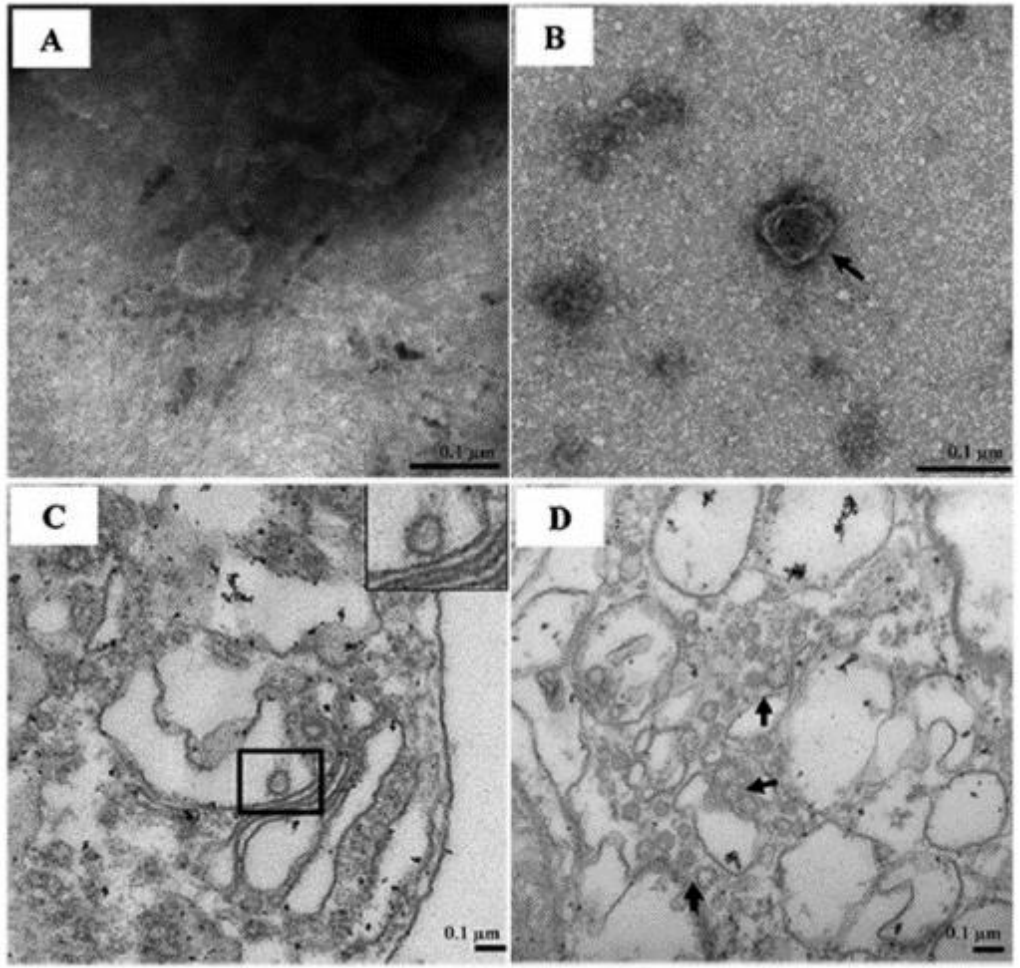
Transmission electron microscopy



Eyngor et al J Clin Micro 2014



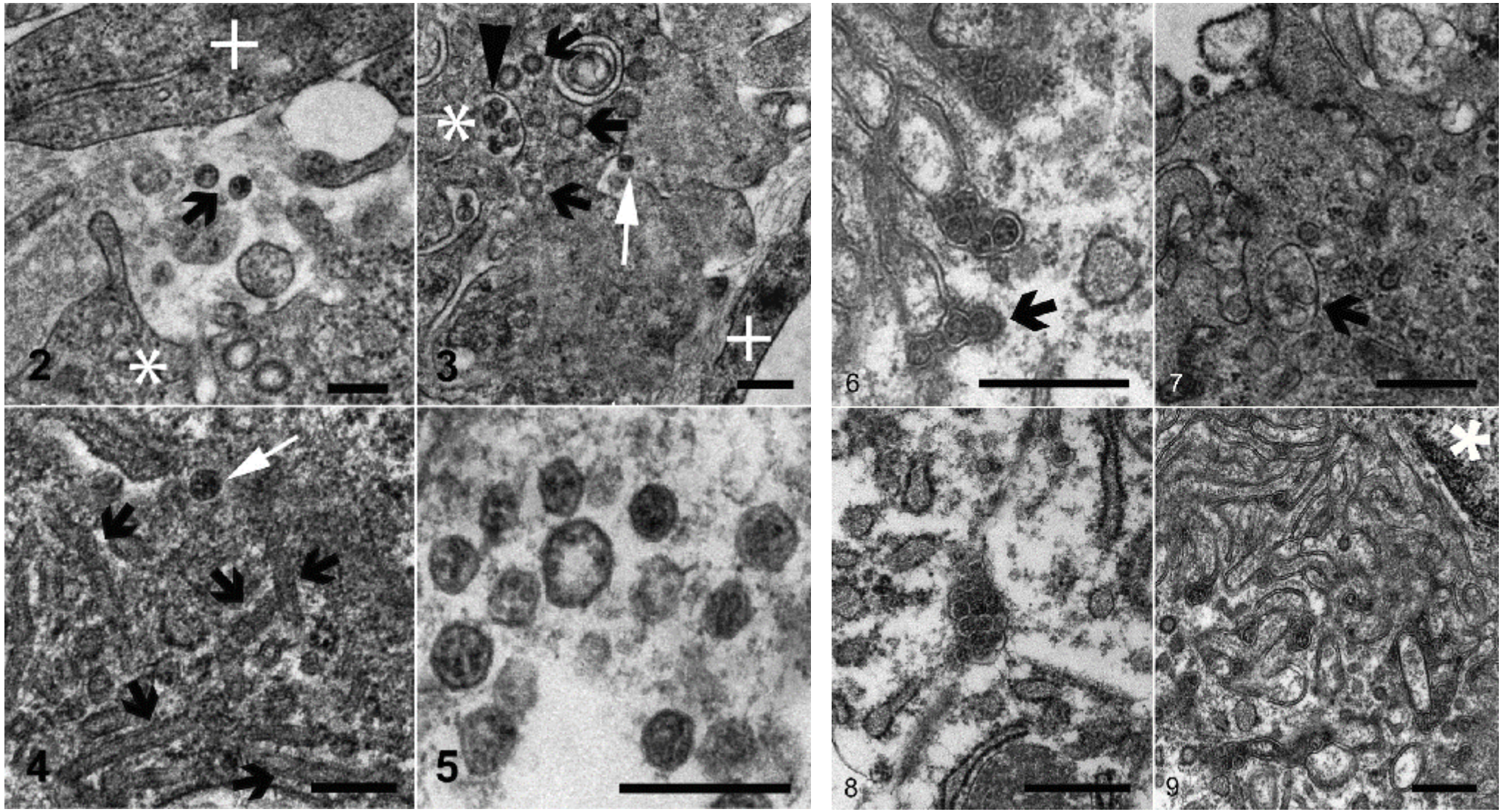
Surachetpong et al Emerging Infectious Dis 2017



Tattiyapong et al Vet Micro 2017

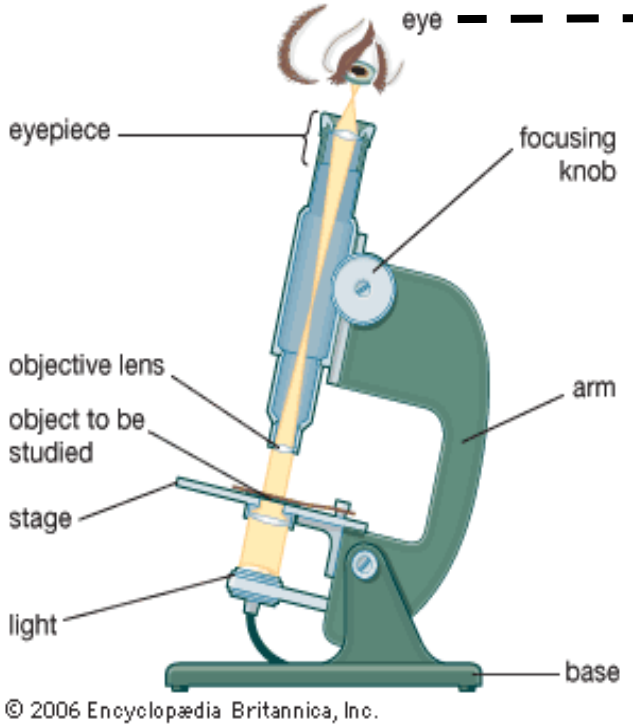
TiLV morphology

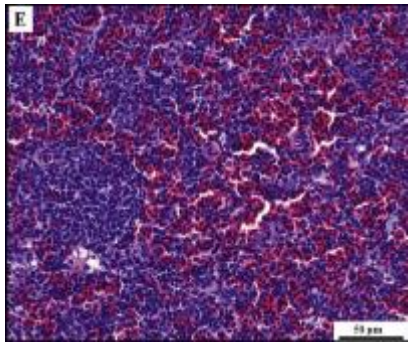
Transmission electron microscopy



Del-Pozo et al., 2016 Vet. Pathol.

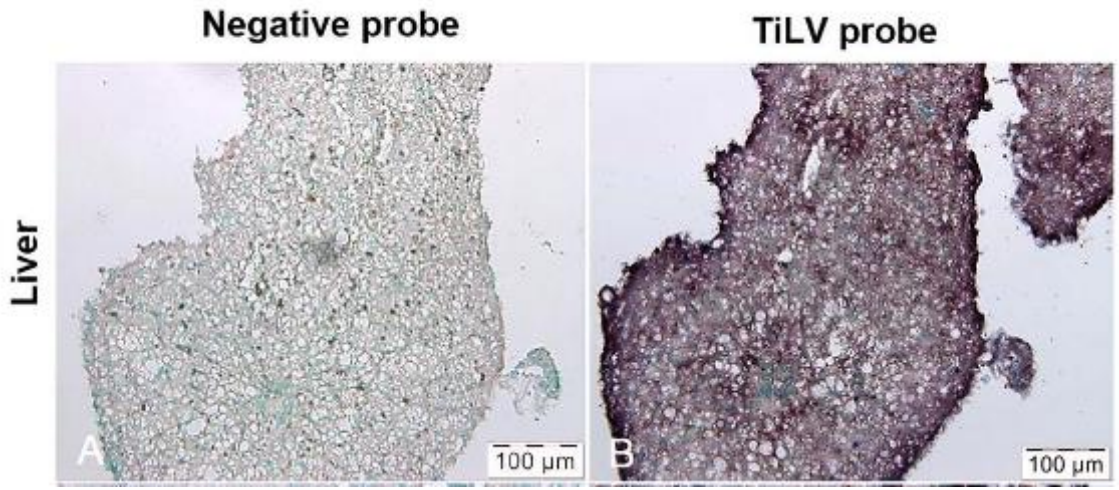
2. Light microscopy





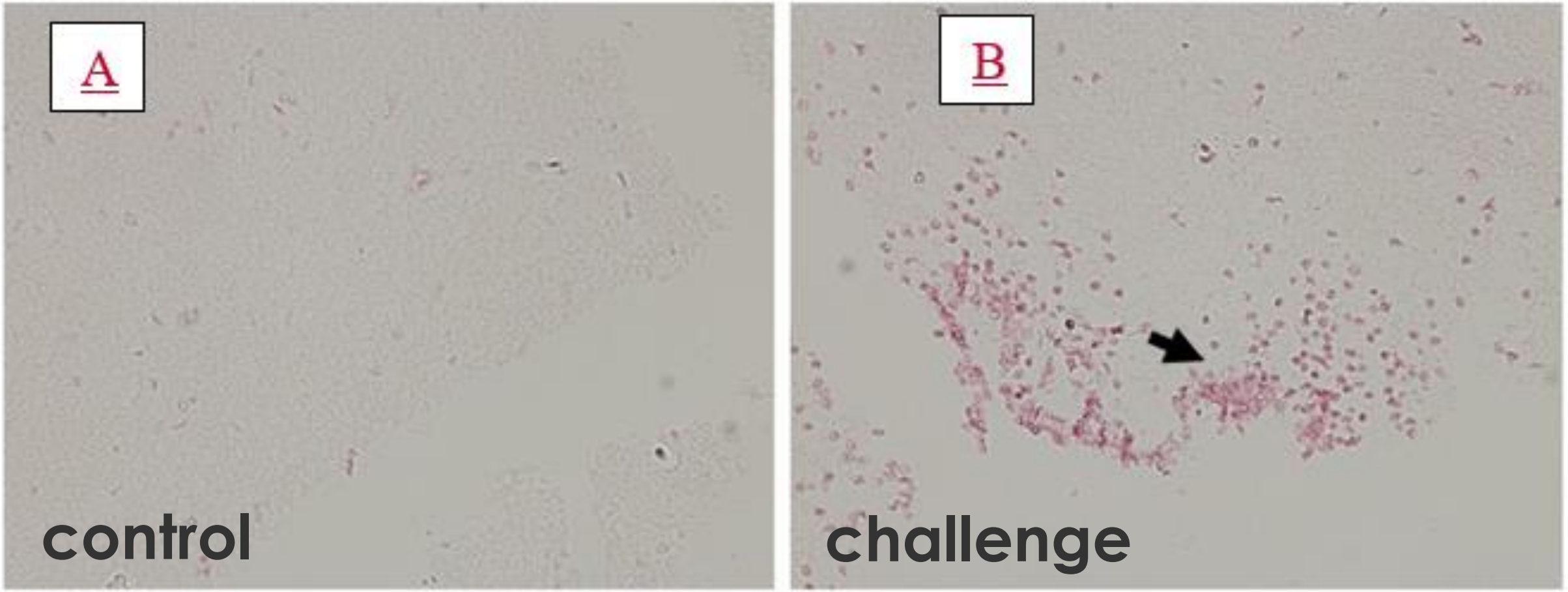
In situ hybridization

Bacharach et al 2016



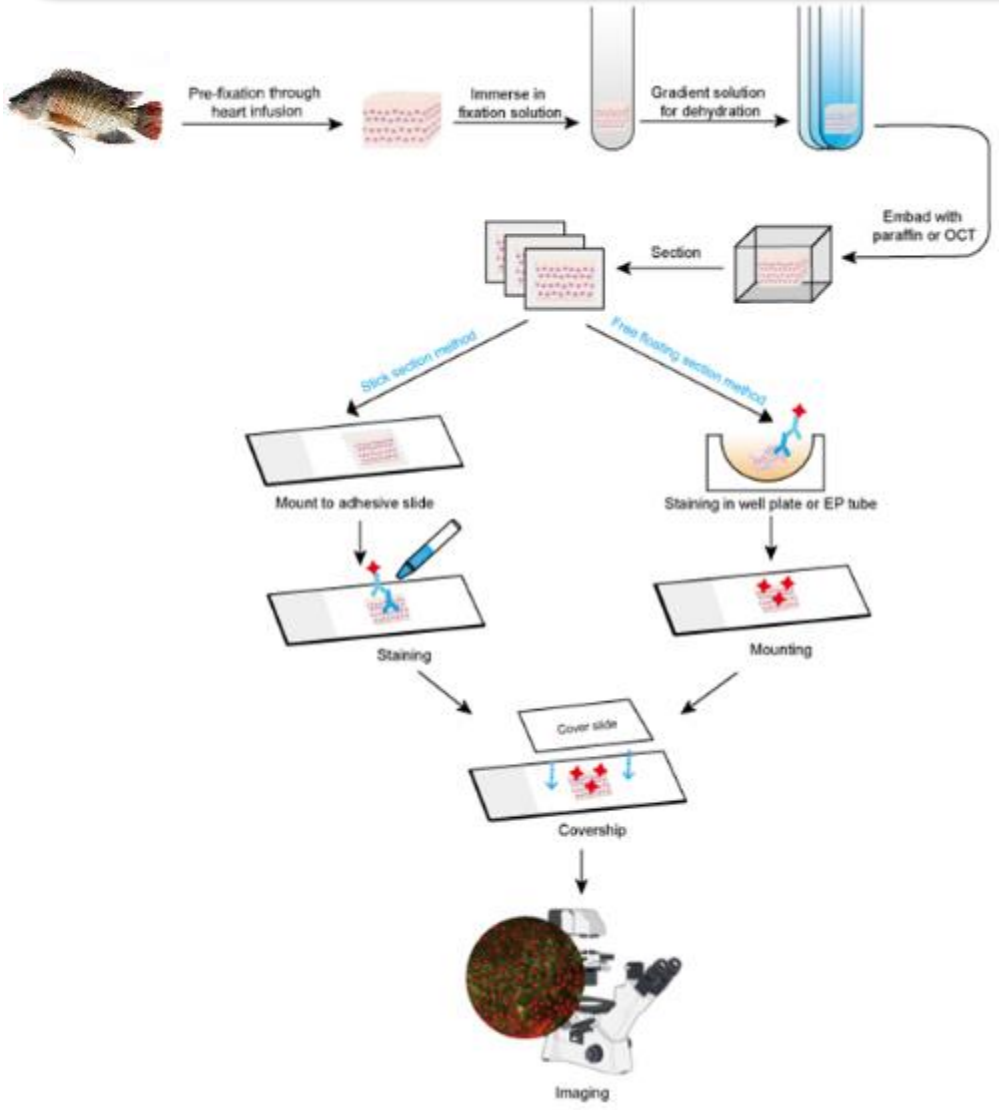
- **Viral detection**
- **Microscopic pathological examination**

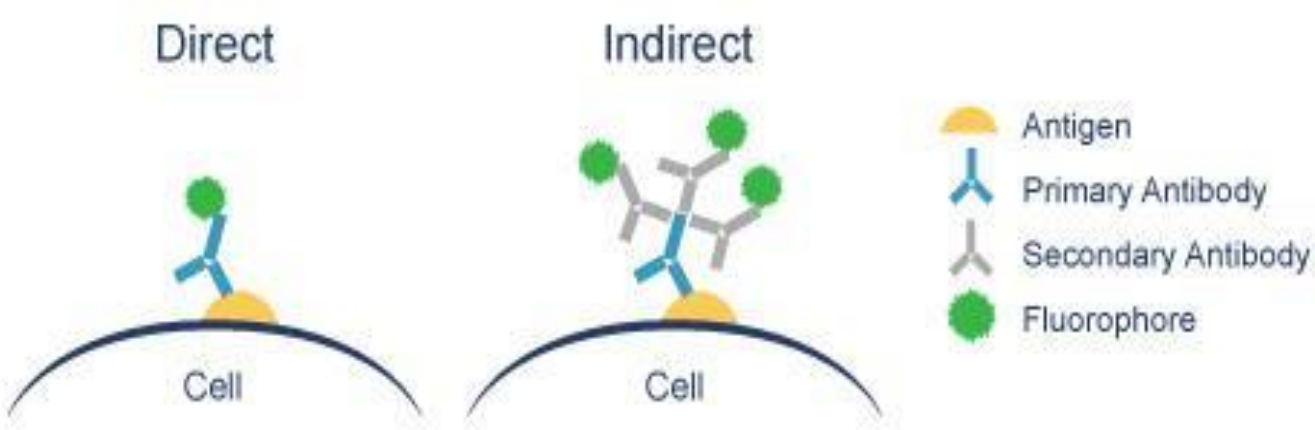
In situ hybridization of TiLV in the brain of infected fish



Credit: Dr.Attapon Kamlungdee Vet Patho KU

3. Immunofluorescence Immunohistochemistry staining

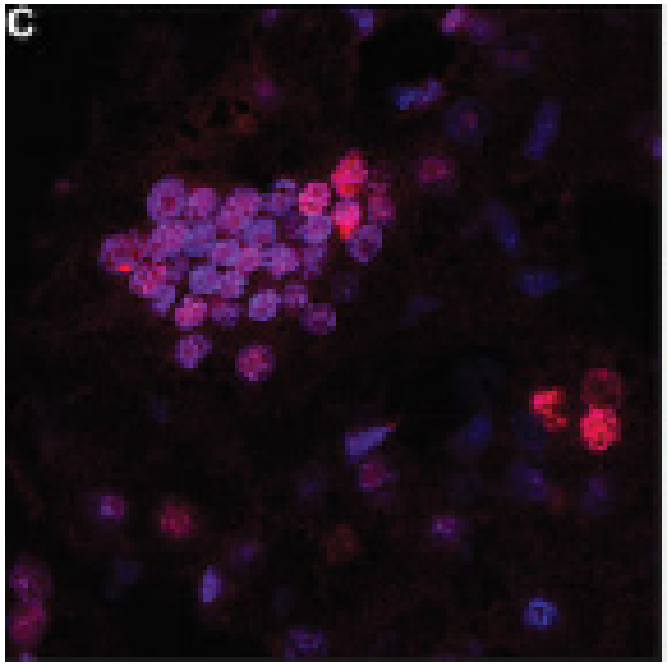




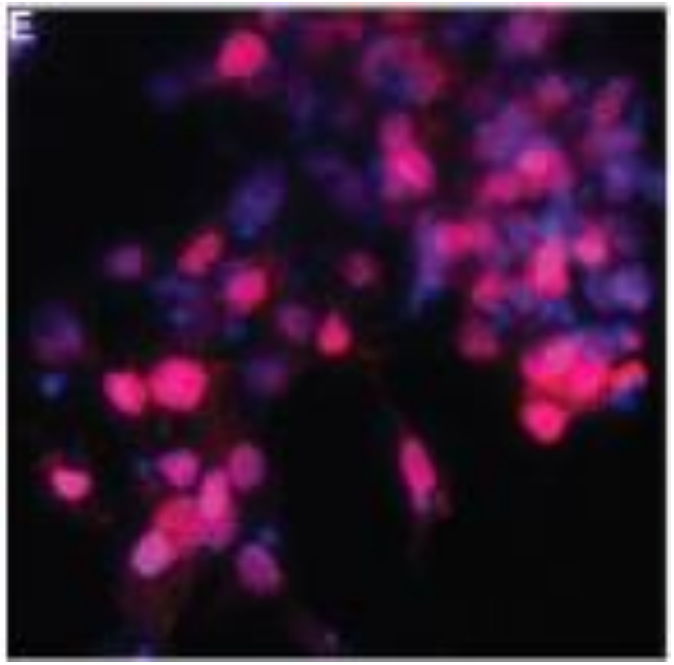
Concept: detection of antigen

Material: frozen tissue, cells (smear) or cultured cells

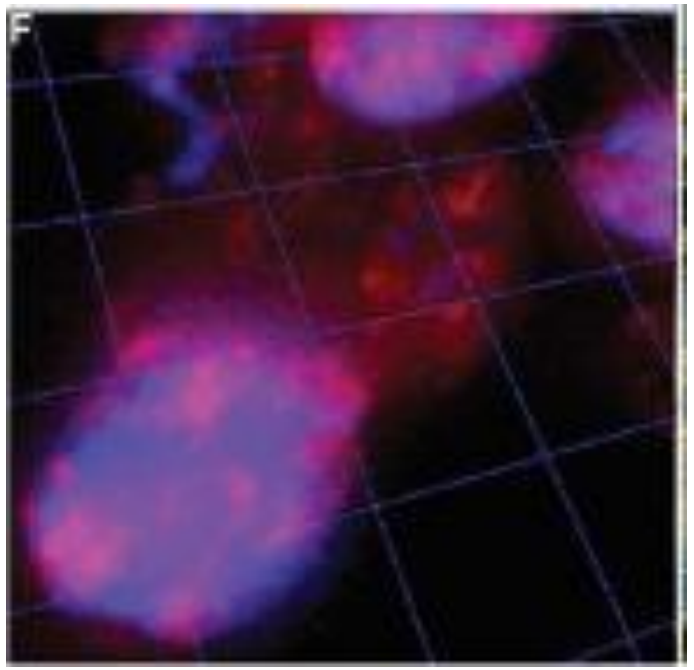
In situ hybridization with fluorescence probes



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).

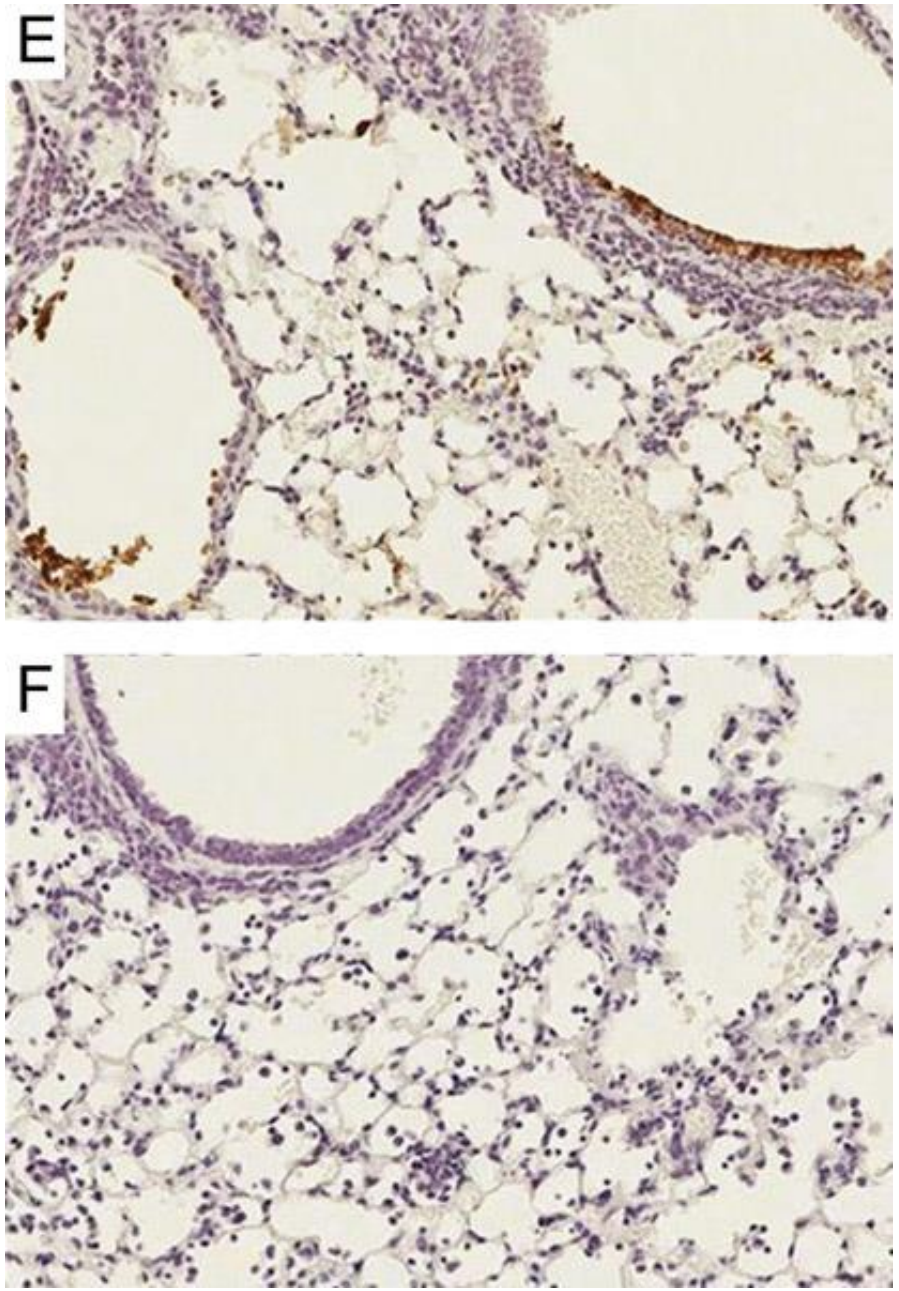


Images of confocal sections of cells in panel E were reconstituted into a 3D image.

Bacharach et al., 2016 MBIO

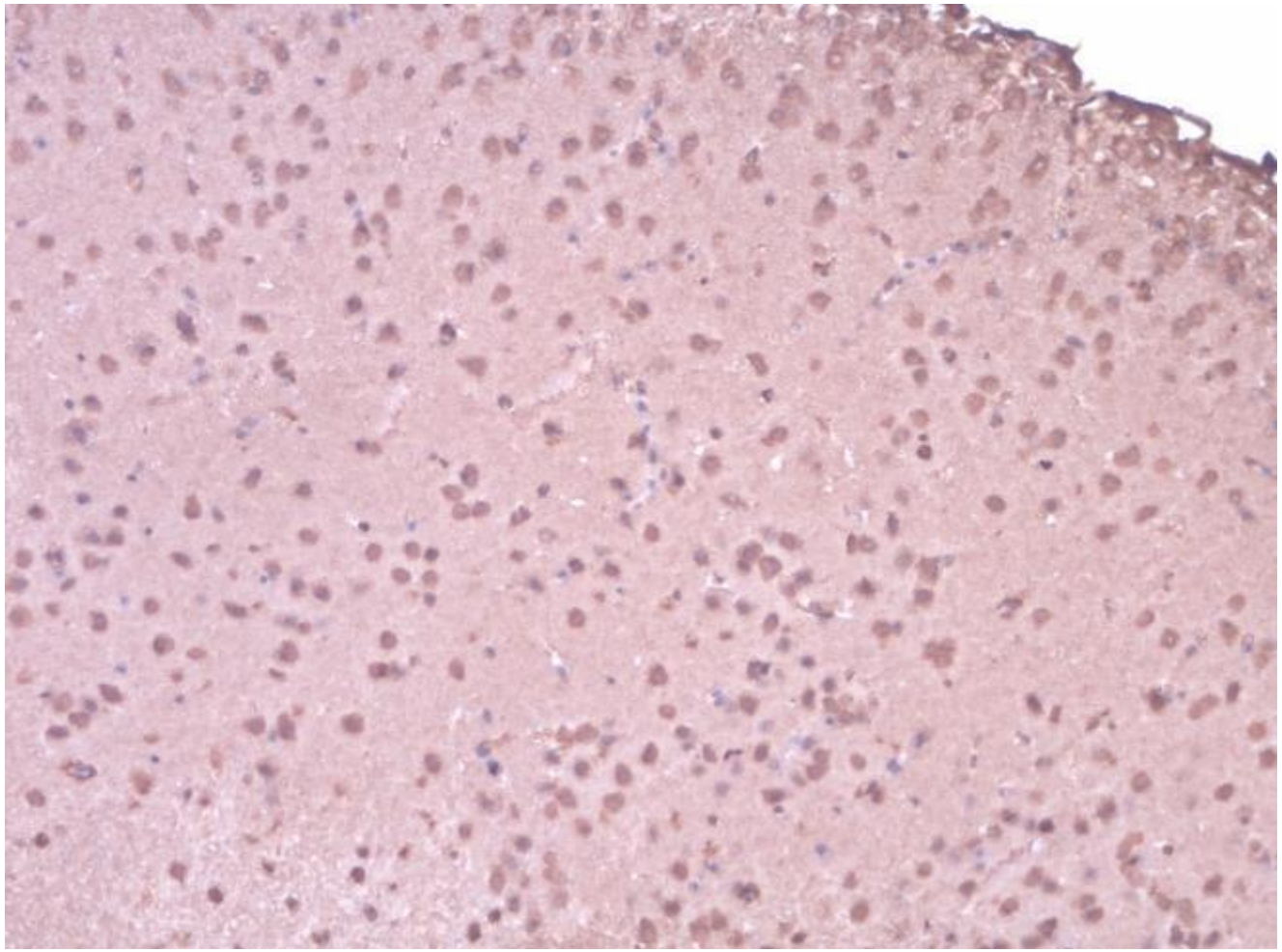
IHC staining of influenza A virus in mouse lung tissue

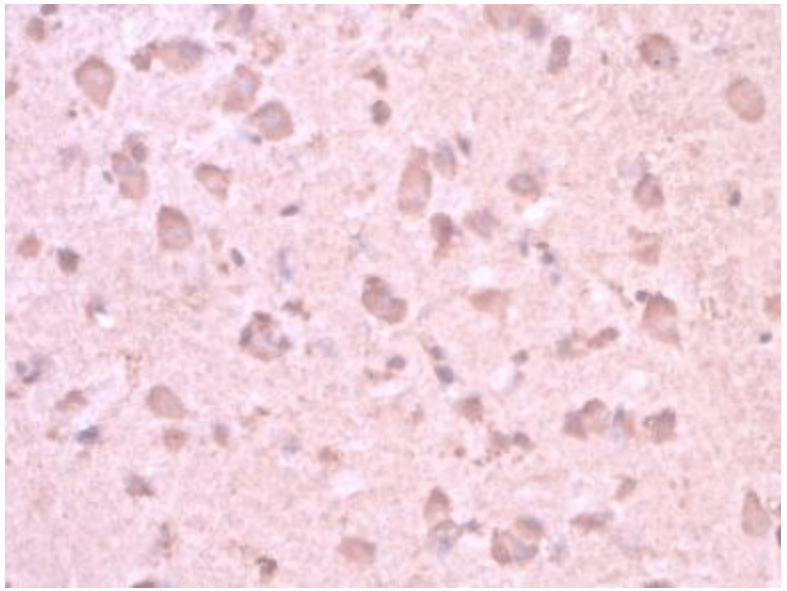
Brown staining indicates positive staining in cell's cytoplasm and/or nucleus.



Kash et al., 2013
Free Radic Biol Med.

Immunohistochemistry of TiLV-infected brain





Credit: Dr.Promporn Raksaseri Faculty of Veterinary Science

Chulalongkorn University

4. Molecular techniques

- Reverse transcription polymerase chain reaction (RT-PCR)
- Quantitative reverse transcription polymerase chain reaction (RT-qPCR)
- Insulated isothermal PCR (iiPCR)

Reverse transcription polymerase chain reaction (RT-PCR)

- Several PCR methods have been described for the detection of TiLV including

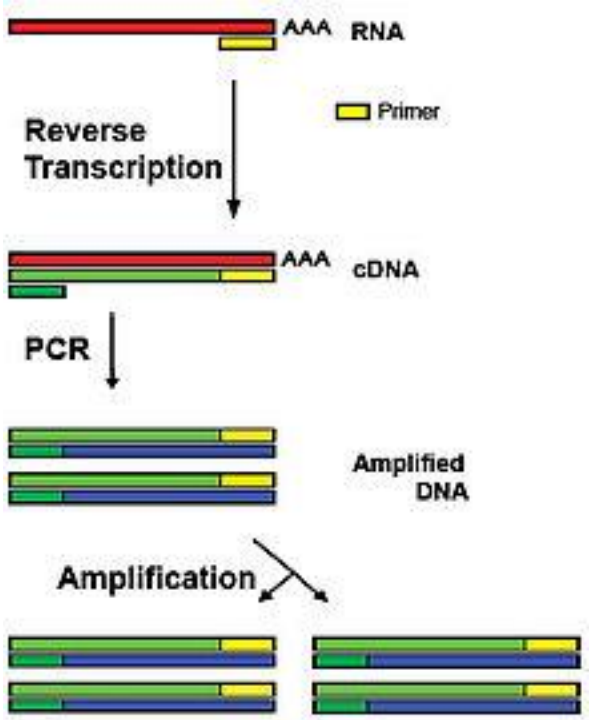
  conventional RT-PCR, semi-nested RT-PCR

- Recently, a SYBR green-based RT-qPCR method targeting the same segment was developed with a reported sensitivity of 2 copies

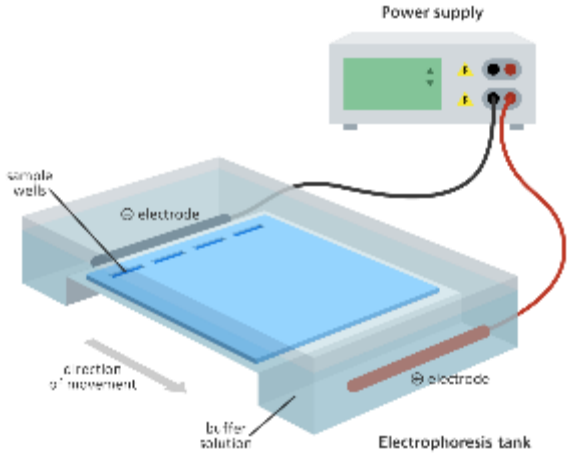
Reverse transcription polymerase chain reaction (RT-PCR)

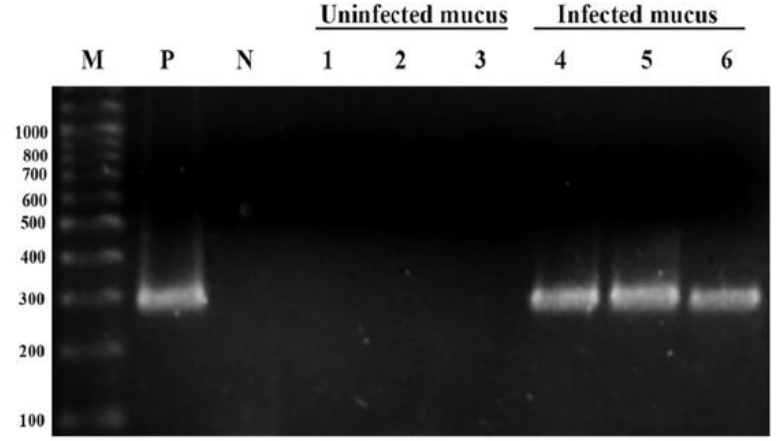
TiLV = negative sense RNA genome

Thermo cycler



Gel electrophoresis





JCM
Journals.ASM.org

Journal of Clinical Microbiology p. 4137–4146

Identification of a Novel RNA Virus Lethal to Tilapia

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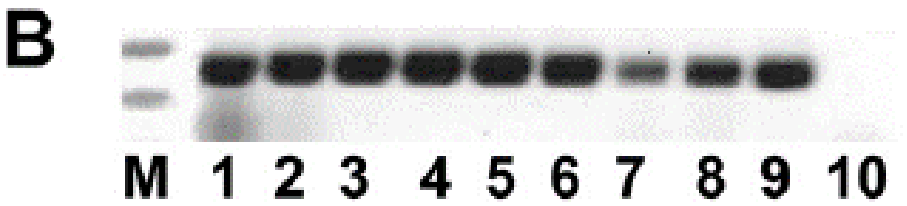
Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan, Israel[a]; Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel[b]; The Hebrew University-Hadassah Medical School, Jerusalem, Israel[c]; Department of Animal Facility, Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel[d]; Ein Gev Fisheries, Kibbutz Ein Gev, Israel[e]; Dan Fish Farms, Kibbutz Dan, Upper Galilee, Israel[f]; Department of Food Science, Section of Veterinary Pathology, University of Udine, Udine, Italy[g]

1326 bases clone of 7450 segment 3

GAAATGGACTCGCGGTTGCACAGCTAACTGGGGTTTTCTGTGACGATTTCACTTATAGCGAA
GGGAGCCGAAGGTTCCTAAGTTCTTACAGTACAGTAGAGAGACGTCCAGGAGTCCCCGTAGAG
GGTGACTGTTATGACTGTTTGAAGAATAAGTGGATTGCCTTTGAGCTGGAAGGCCAGCCGCGG
AAATTTCCAAAGGCAACAGTTCGTTGCATTTTGAACAATGATGCTACATACGT*TTGCTCTGAG*
*CAAGAGTACC*AGCAGATTTGTAAGGTACAATTCAAGGATTATTTGGAGATCGACGGGGTTGTT
AAA*GTTGGGCACAAGGCATCCTAC*GATGCTGAGCTAAGGGAACGGCTATTGGAACTACCACAT
CCAAAGAGTGGCCCGAAGCCTCGTATTGAGTGGGTGGCACCACCCAGACTTGCGGACATATCC
AAGGAAACAGCTGAGCTAAAGAGGCAATATGGATTCTTCGAGTGCTCAAAGTTCCTCGCCTGC
GGTGAGGAGTGTGGTCTTGACCAAGAGGCAAGAGAACTTA*TACTGAACGAGTACGCACGTGAT*
AGAGAATTTGAGTTCCGCAATGGAGGGTGGATACAAAGGTATACAGTTGCTTCTCACAAGCCT
GCTACACAGAAGATATTACCTCTACCGGCTAGTGCTCCACTTGCTCGTGAGCTTTTGATGTTG
ATTGCTAGA*AGCACAACTCAGGCAGGGAAAGTACTGCATA*GCGATAATACCAGCATACTAGCT
GTACCGGTCATGCGCGACTCTGGAAAGCACAGTAAAAGGAGACCAACCGCCTCCACTCACCAC
TTAGTTGTAGGTCTAAGTAAACCTGGCTGTGAACACGATTTTGAGTTTGACGGGTACAGGGCA
GCTGTGCATGTGATGCACCTAGATCCCAAGCAATCGGCTAATATAGGGGAGCAAGACTTTGTG
AGTACCCGAGAAATTTACAAGCTGGATATGTTGGAACTACCTCCCATAAGTAGGAAGGGTGAT
CTGGACAGAGCTAGTGGTCTTGAGACAAGATGGGACGTCATCTTACTTCTGGAATGCCTCGAC
TCTACAAGGGTTAGCCAAGCAGTGGCTCAACATTTTAATAGGCACCGGCTAGCACTTAGCGTC
TGTAAGGACGAGTTCAGGAAAGGCTACCAGCTGGCTTCTGAGATAAGGGGTACAATACCCTTA
AGCTCACTTTATTATTCACTTTGTGCAGTAAGATTGCGGATGACAGTACACCCATTGCGAGA
TGATCGCTTTCGACGCCTTCGCTAAAGGTTACGACGTTCTAATAGAGGATTATGGGAAAAATT
TGC

EMDSRFAQLTGVFCDDFTYSEGSRRFLSSYSTVERRPGVPVEGDCYDCLKNKWIAFELEGQPR
KFPKATVRCILNNDATYVCSEQEYQQICKVQFKDYLEIDGVVKVGHKASYDAELRERLLELPH
PKSGPKPRIEWVAPPRLADISKETAELKRQYGFFECSKFLACGEECGLDQEARELILNEYARD
REFEFRNGGWIQRYTVASHKPATQKILPLPASAPLARELLMLIARSTTQAGKVLHSDNTSILA
VPVMRDSGKHSKRRPTASTHHLVVGLSKPGCEHDFEFDGYRAAVHVMHLDPKQSANIGEQDFV
STREIYKLDMLELPPISRKGDLDRASGLETRWDVILLLECLDSTRVSQAVAQHFNRHRLALSV
CKDEFRKGYQLASEIRGTIPLSSLYYSLCAVRLRMTVHPPAR

RT-PCR method based on the design of segment 3 of TiLV



Lane 1-7 infected fish

Lane 8-9 infected E-11 cells

Lane 10 healthy fish

Detection of Tilapia Lake Virus in Clinical Samples by Culturing and Nested Reverse Transcription-PCR

Japhette Esther Kembou Tsofack,[a] Rachel Zamostiano,[a] Salsabeel Watted,[b] Asaf Berkowitz,[b] Ezra Rosenbluth,[b] Nischay Mishra,[c] Thomas Briese,[c] W. Ian Lipkin,[c] Richard M. Kabuusu,[d] Hugh Ferguson,[d] Jorge del Pozo,[e] Avi Eldar,[b] Eran Bacharach[a]

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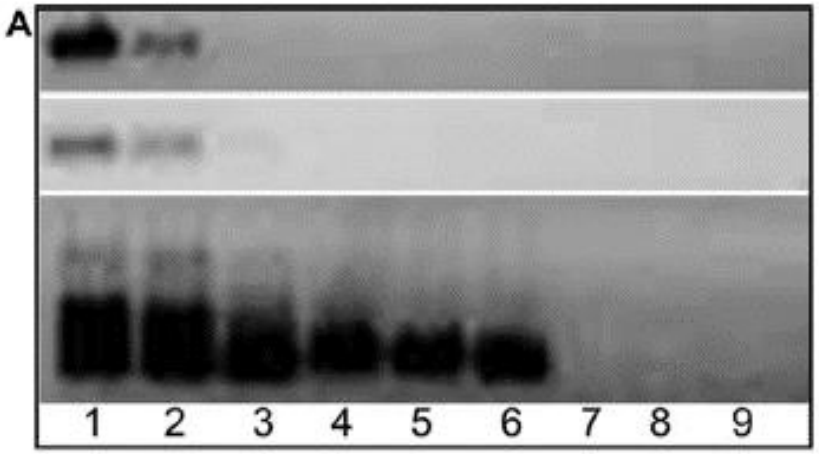
Sensitivity of PCR, and nested PCR



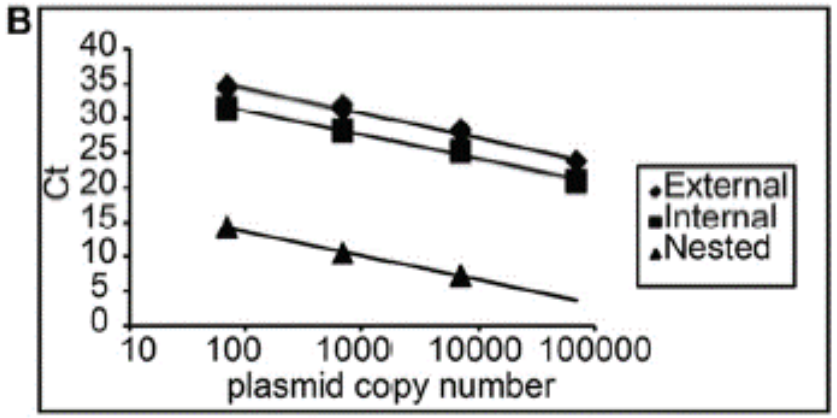
491 bp

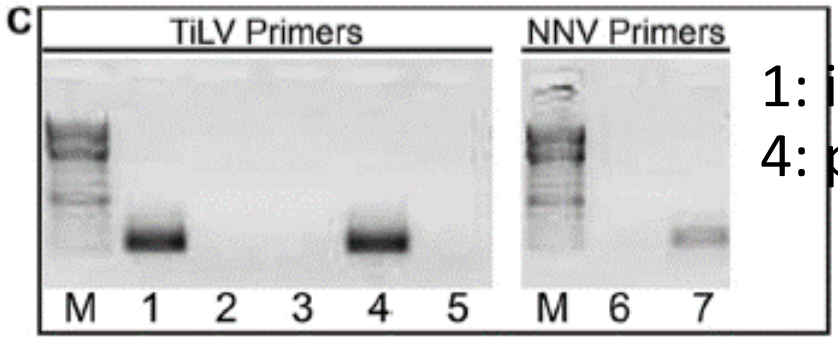
250 bp

Nested

RT-PCR method based on the design of segment 3 of TiLV

qPCR reaction using primers in (A)

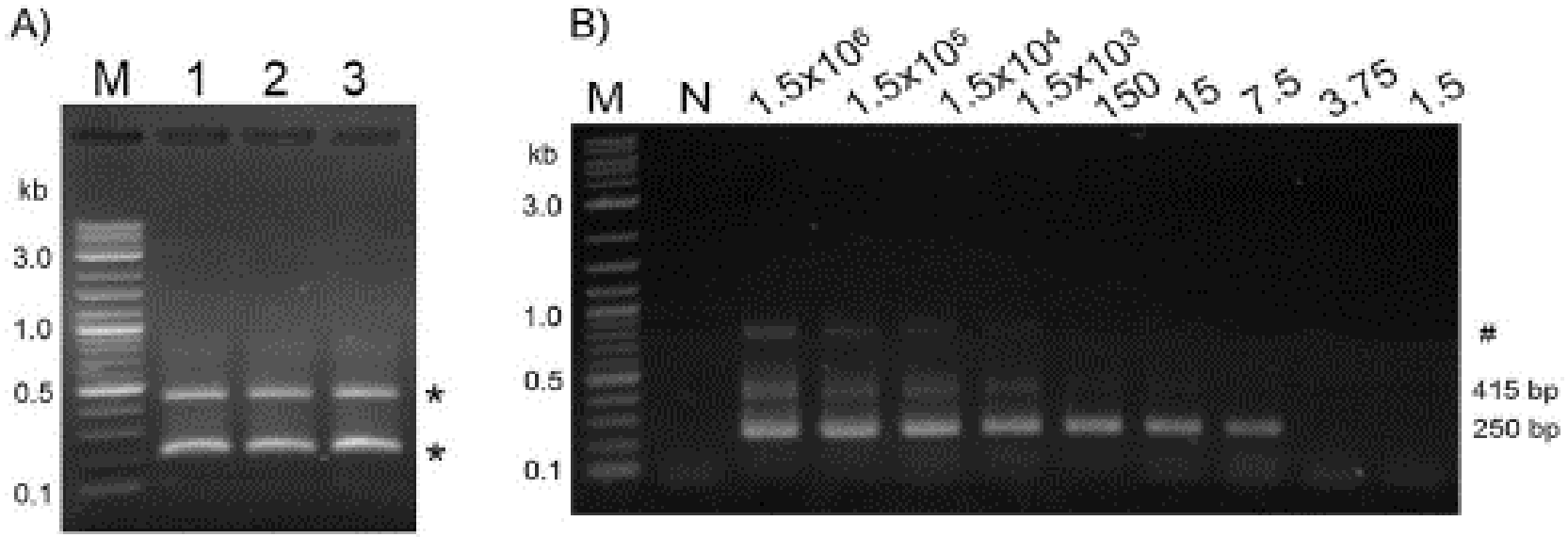




1: infect E-11

4: plasmid

Semi-nested RT-PCR for TiLV detection

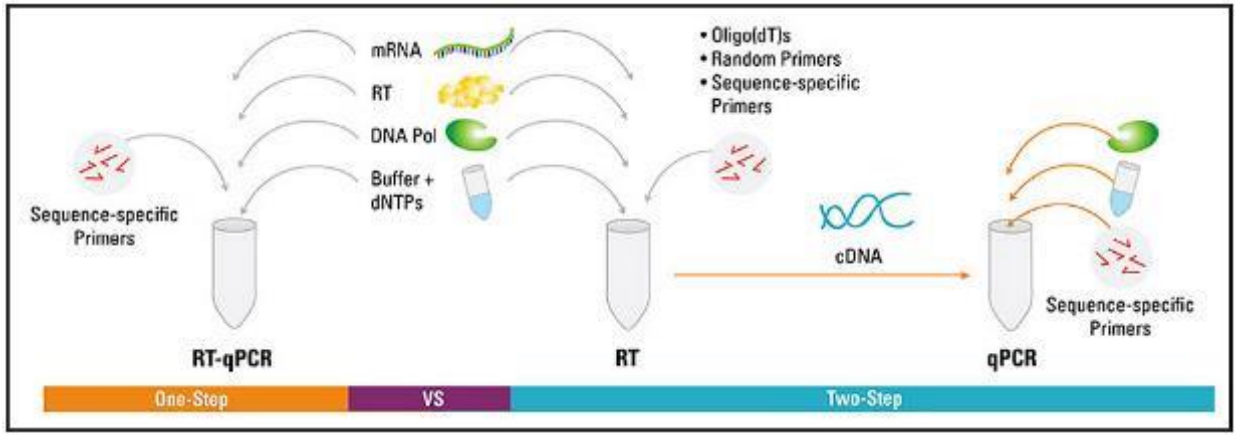


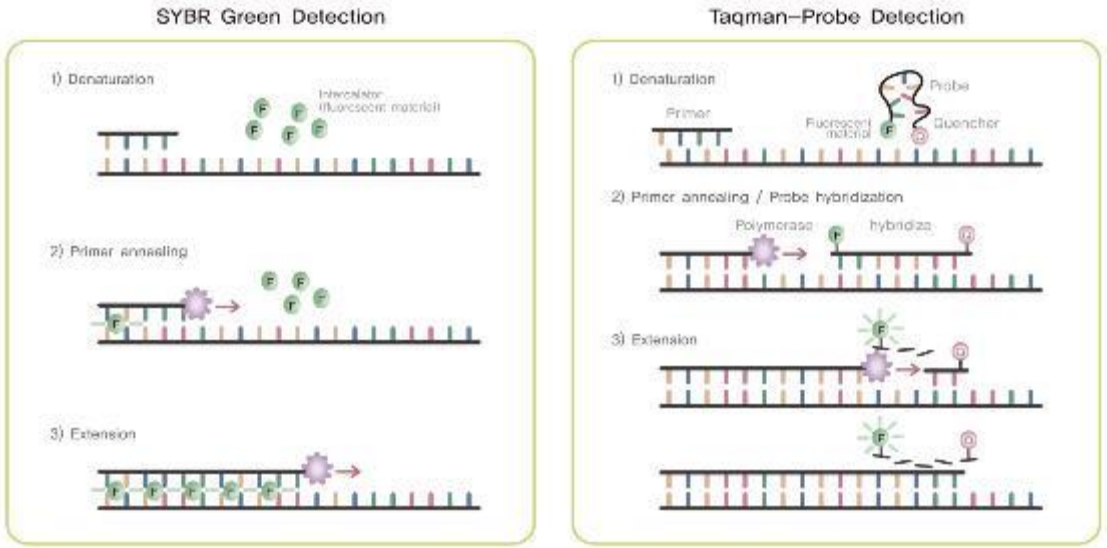
Three nested RT-PCR reactions run at an annealing temperature 56 °C non-specifically amplified fish mRNA

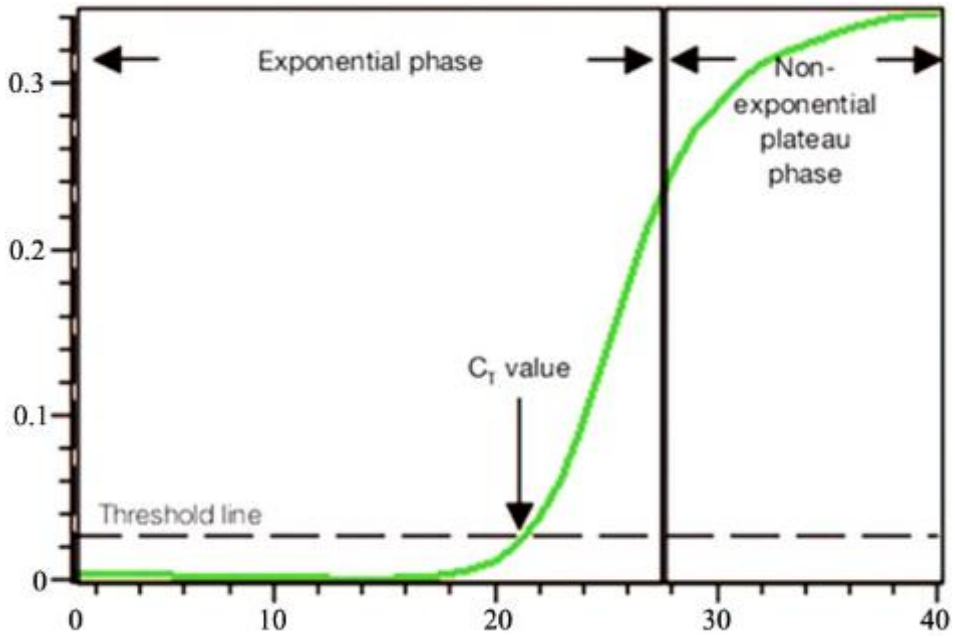
- #Marks band probably derived from cross hybridizations of the amplified products. M, DNA marker
- Serial dilutions of positive control plasmid (pGEM-415_bp) are indicated. Expected band sizes of 415 bp and 250 bp represent amplicons from the first and seminested.
- Expected band sizes of 415 bp and 250 bp represent amplicons from the first and seminested PCR

Dong et al 2017

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)







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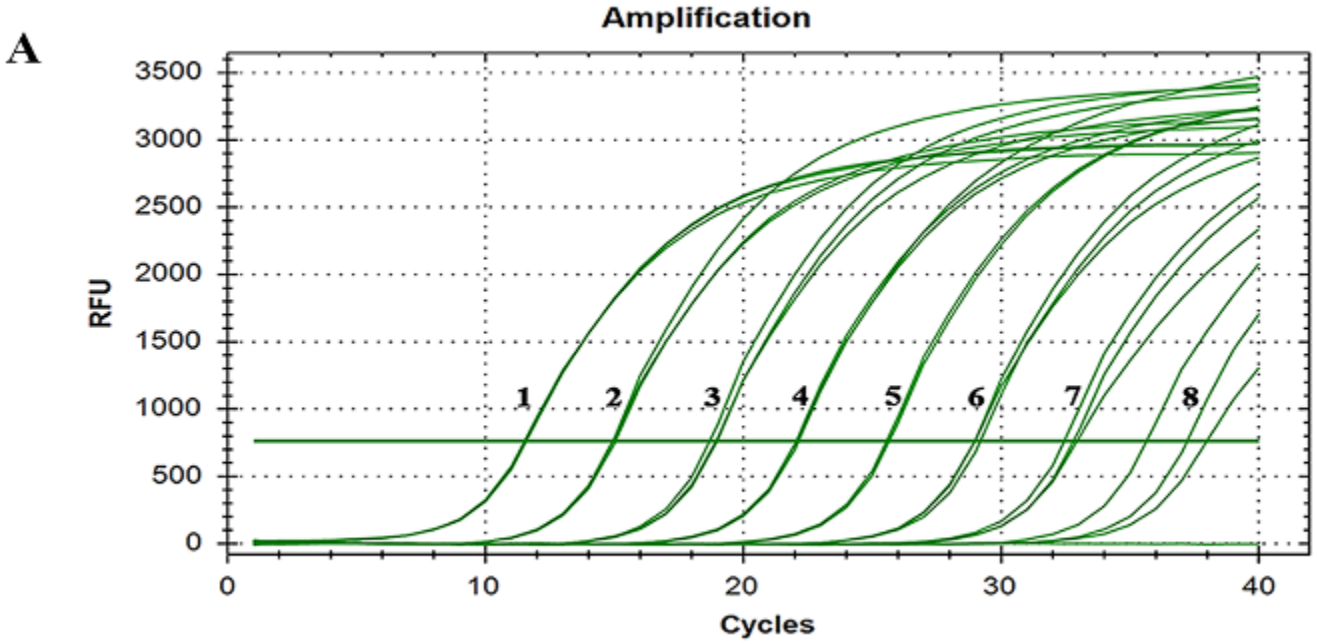
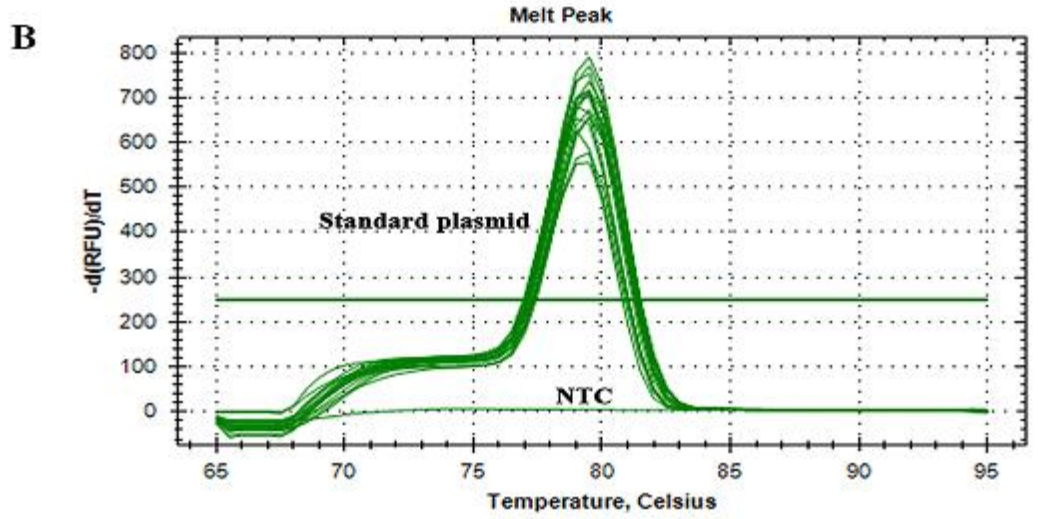
Journal of Fish Diseases

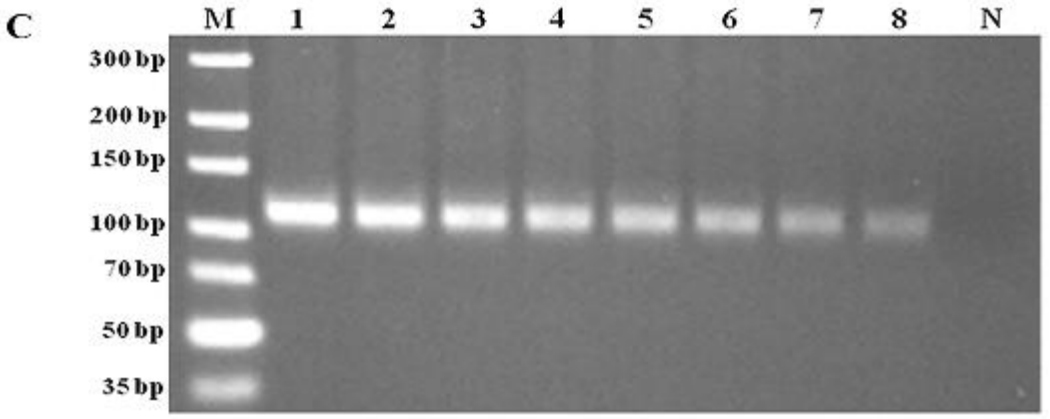
Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish

P Tattiyapong[1,2] | K Sirikanchana[3,4] | W Surachetpong[1,2]



Amplification curve of serially ten-fold diltuion





Melt curve and gel electrophoresis

Standard curve of SYBR green-based RT-qPCR

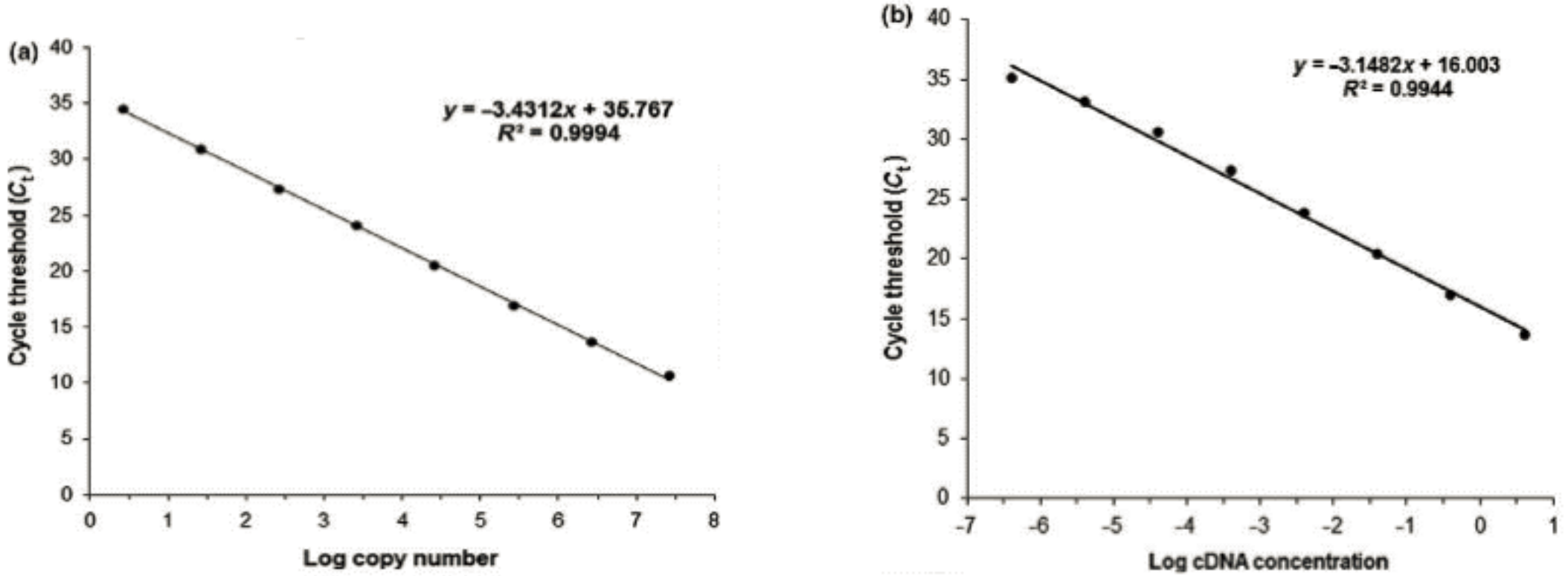
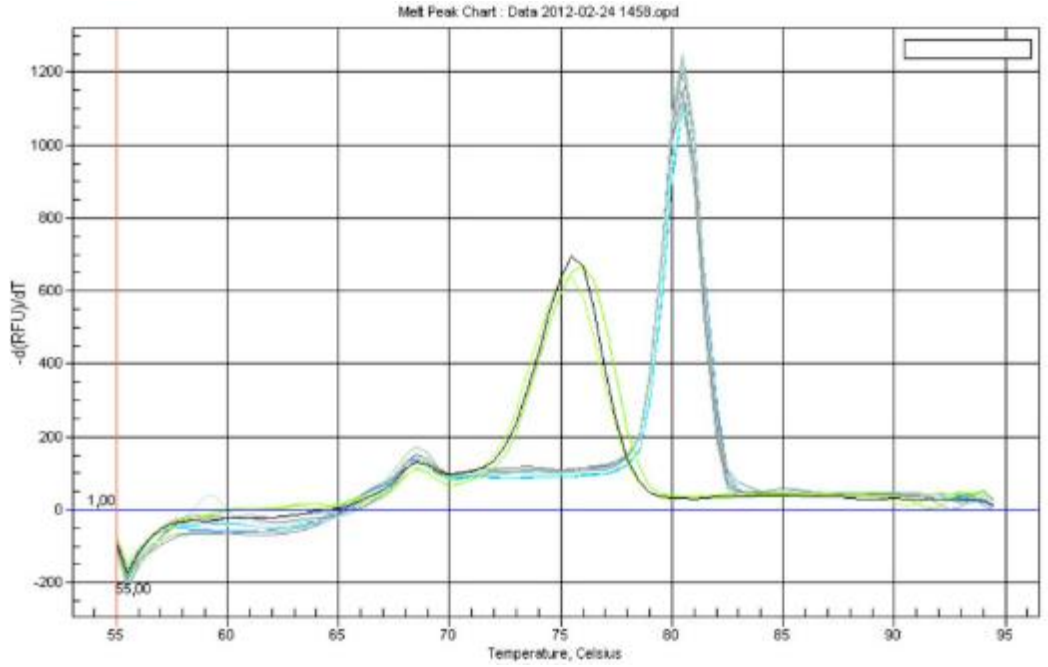


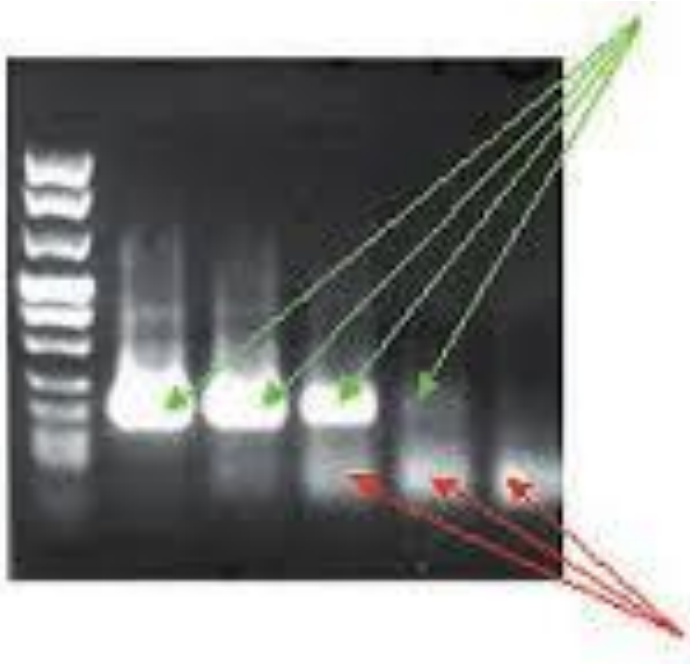
FIGURE 1 Standard curve of SYBR green-based RT-qPCR amplification of plasmid pTiLV containing segment three of TiLV and infected tissue. (a) Standard curve was plotted between mean Ct values obtained from each dilution of standard plasmid pTiLV against calculated log copy number (slope = -3.4312, R^2 = 0.9994). (b) Standard curve of cDNA prepared from TiLV-infected fish tissue showed slopes = -3.1482, R^2 = 0.994

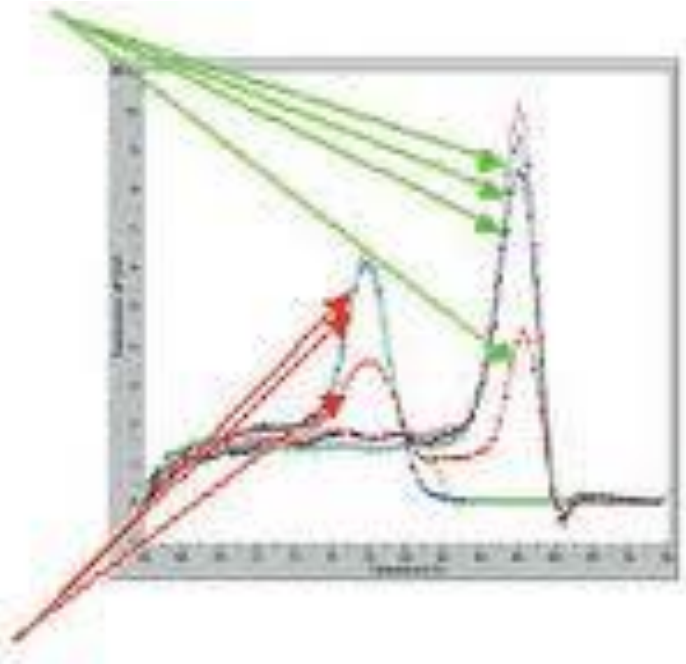
Tattiyapong et al., 2018 J. Fish Dis.

Primer-dimer formation (SYBR)

- **Must run melting curve analysis**







Validation of qPCR assay with field collected samples

Journal of Fish Diseases

Supplementary Table 1 List of field samples and estimated TiLV copies in liver

Sample No.	Collection date	Locations	Mean Ct values	Viral loads[a]
1	30/10/2015	Ang Thong	23.52	1.06×10^{4}
2	11/11/2015	Ang Thong	22.75	1.77×10^{4}
3	05/01/2016	Pathum Thani	24.70	4.75×10^{3}
4	19/01/2016	Ratchaburi	22.37	2.28×10^{4}
5	02/08/2016	Pathum Thani	15.21	2.80×10^{6}
6	05/08/2016	Pathum thani	26.66	1.28×10^{3}
7	16/08/2016	Ratchaburi	13.31	9.95×10^{6}
8	22/08/2016	Ang Thong	18.09	4.00×10^{5}
9	24/08/2016	Nakhon Pathom	13.52	8.67×10^{6}
10	27/08/2016	Nakhon Pathom	28.82	3.00×10^{2}
11	02/09/2016	Suphanburi	25.13	3.60×10^{3}
12	16/09/2016	Pathum Thani	22.30	2.39×10^{4}
13	23/09/2016	Nong Khai	13.66	7.90×10^{6}
14	02/10/2016	Pathum Thani	12.83	1.37×10^{7}
15	05/10/2016	Pathum Thani	19.46	1.61×10^{5}

Supplementary Table 1 (Cont.) List of field samples and estimated TiLV copies in liver

Sample No.	Collection date	Locations	Mean Ct values	Viral loads[a]
16	16/10/2016	Pathum Thani	19.20	1.94×10^{5}
17	25/11/2016	Ang Thong	14.56	4.35×10^{6}
18	26/11/2016	Pathum Thani	13.46	9.08×10^{6}
19	21/12/2016	Petchaburi	17.16	7.55×10^{5}
20	14/11/2016	Khon Kaen	19.45	1.62×10^{5}
21	01/01/2017	Pathum Thani	21.11	5.35×10^{4}
22	14/02/2017	Nakhon Sawan	16.72	1.01×10^{6}
23	20/02/2017	Uthai Thani	13.26	1.04×10^{7}
24	24/02/2017	Phitsanulok	27.84	5.85×10^{2}
25	24/02/2017	Uttaradit	32.89	1.97×10^{1}
26	25/02/1017	Ratchaburi	25.39	3.01×10^{3}
27	25/02/2017	Nakhon Si Thammarat	19.87	1.22×10^{5}
28	25/02/2017	Prachinburi	16.21	1.43×10^{6}
29	25/02/2017	Nakhon Nayok	14.36	4.98×10^{6}
30	25/02/2017	Ang Thong	18.76	2.55×10^{5}

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)[c]
Clinical samples[a]	30	30/30 (100)	22.86 (12.83 - 32.89)	1.65×10^4 (1.37×10^7-1.97×10^1)
TiLV-challenged fish	10	10/10 (100)	23.65 (20.08 - 27.28)	9.72×10^3 (1.00×10^5-8.50×10^2)
Non-challenged fish	10	0/10 (0)	ND[b]	ND[b]

[a]Clinical samples were collected from 30 field outbreaks with history of massive mortality.
[b]ND = No detection.
[c]Copy numbers of TiLV template per μg of total RNA.

Tattiyapong et al., 2018 J. Fish Dis.

Received: 1 June 2017 | Revised: 20 July 2017 | Accepted: 23 July 2017
DOI: 10.1111/jfd.12708

Journal of Fish Diseases

Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish

P Tattiyapong[1,2] | K Sirikanchana[3,4] | W Surachetpong[1,2]

Table 4 Comparison of RT-qPCR, conventional RT-PCR and virus isolation in cell culture

Detection method	Template dilution							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
RT-qPCR	+	+	+	+	+	+	+	-
Conventional RT-PCR	+	+	+	+	+	-	-	-
Virus isolation in cell culture	+	+	+	-	-	-	-	-

Received: 1 June 2017 | Revised: 20 July 2017 | Accepted: 23 July 2017
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Journal of Fish Diseases

Development and validation of a reverse transcription quantitative polymerase chain reaction for tilapia lake virus detection in clinical samples and experimentally challenged fish

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Table 3 Analysis of viral loads in different tissues

Sample No.	Viral loads (copies μg^{-1} of total RNA)					
	Gills	***Liver***	***Brain***	***Heart***	***Anterior kidney***	***Spleen***
1	2.2×10^5	1.7×10^5	3.4×10^5	6.3×10^5	1.6×10^5	2.3×10^4
2	3.1×10^5	6.3×10^3	1.3×10^6	3.9×10^5	3.1×10^5	2.8×10^4

Aquaculture 497 (2018) 184–188

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

A TaqMan RT-qPCR assay for tilapia lake virus (TiLV) detection in tilapia

Pitchaporn Waiyamitra[a,b], Puntanat Tattiyapong[a,b], Kwanrawee Sirikanchana[c,d], Skorn Mongkolsuk[c,d], Pamela Nicholson[e], Win Surachetpong[a,b,*]

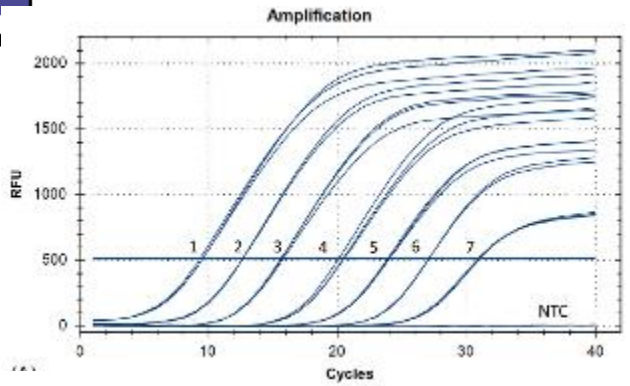


Table 2

Detection of TiLV-infected fish collected from different geographic locations using TaqMan RT-qPCR.

Fish samples	Number of samples	TiLV positive (%)
Positive sample		
Nile tilapia	7	100
Red tilapia	10	100
Negative sample		
Nile tilapia	5	0
Red tilapia	6	0

ND: no fluorescence detection.

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Non-lethal sampling for Tilapia Lake Virus detection by RT-qPCR and cell culture

Pavarit Liamnimitr[a], Worrayanee Thammatorn[a], Sonicha U-thoomporn[a], Puntanat Tattiyapong[b], Win Surachetpong[a,b,*]

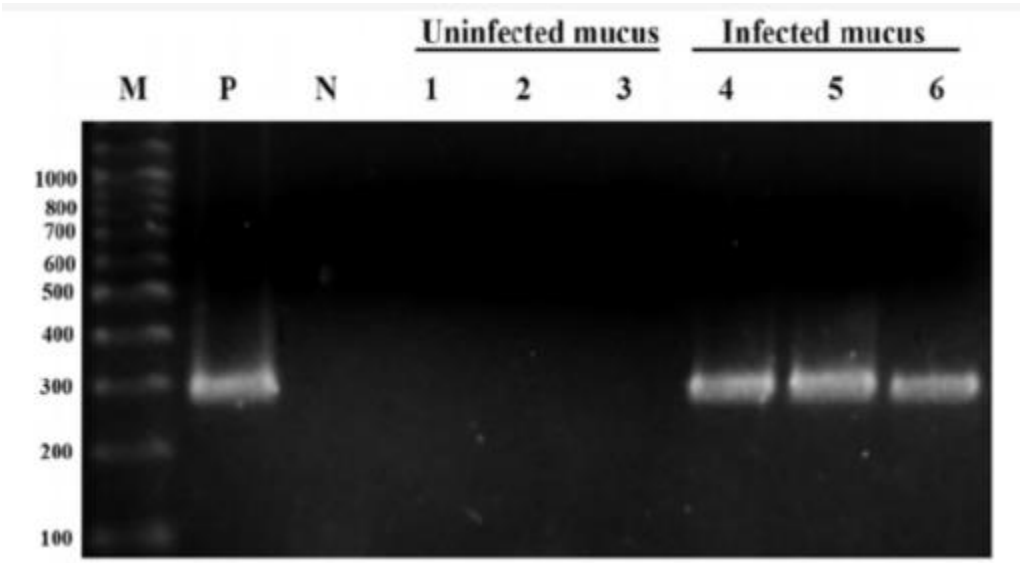
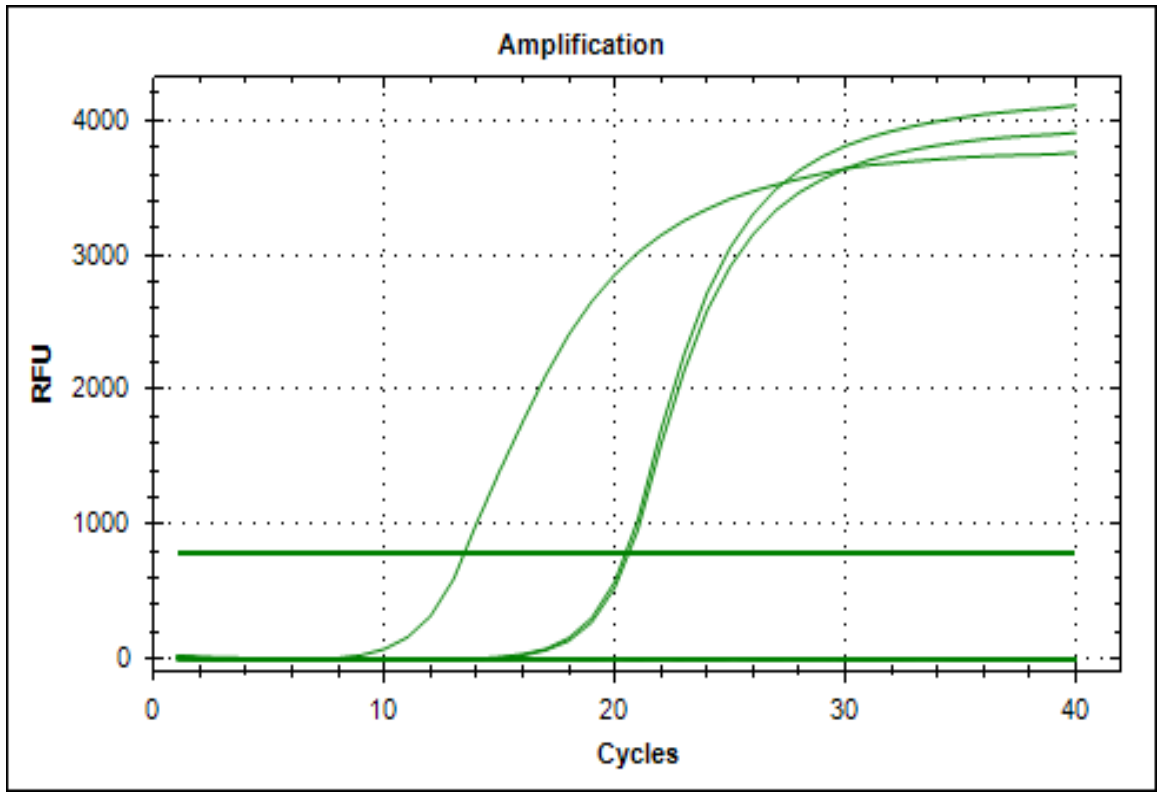


Fig. 2. Amplification of PCR products from E-11 cells inoculated with uninfected or TiLV-infected mucus. (M) 100 bp marker; P = Positive control (pTiLV), N = No template control, Lanes 1–3; E-11 inoculated with mucus from normal fish (uninfected mucus), Lanes 4–6; E-11 inoculated with mucus from TiLV-positive fish (infected mucus). The uninfected and infected mucus analysis was based on three clinical samples representing different geographic locations.



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)

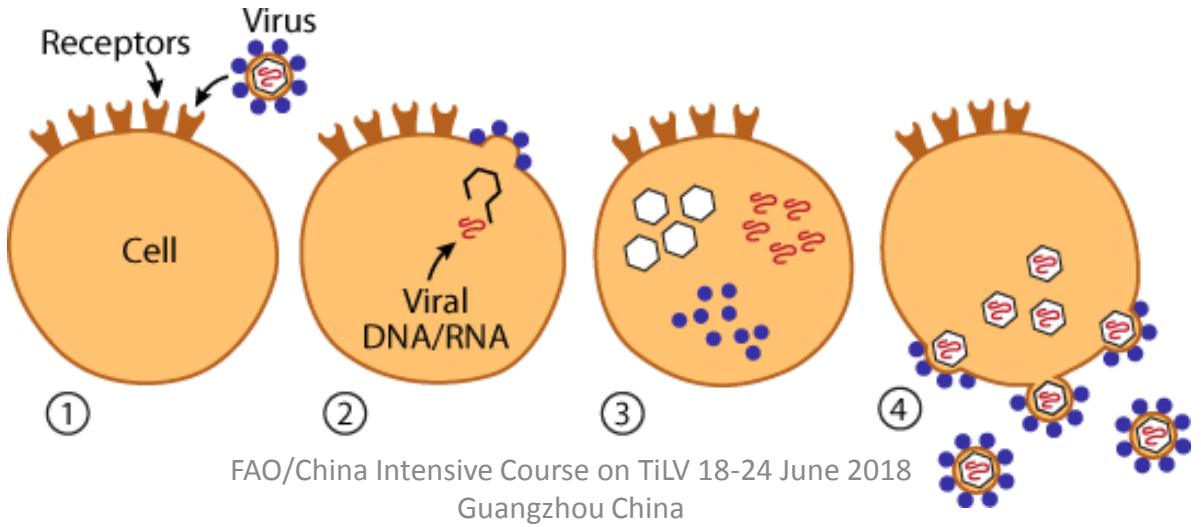
www.genereach.com

Commercial pond-site
TiLV RT-PCR detection

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

Indirect Examination

1. Cell Culture methods
2. Laboratory animals



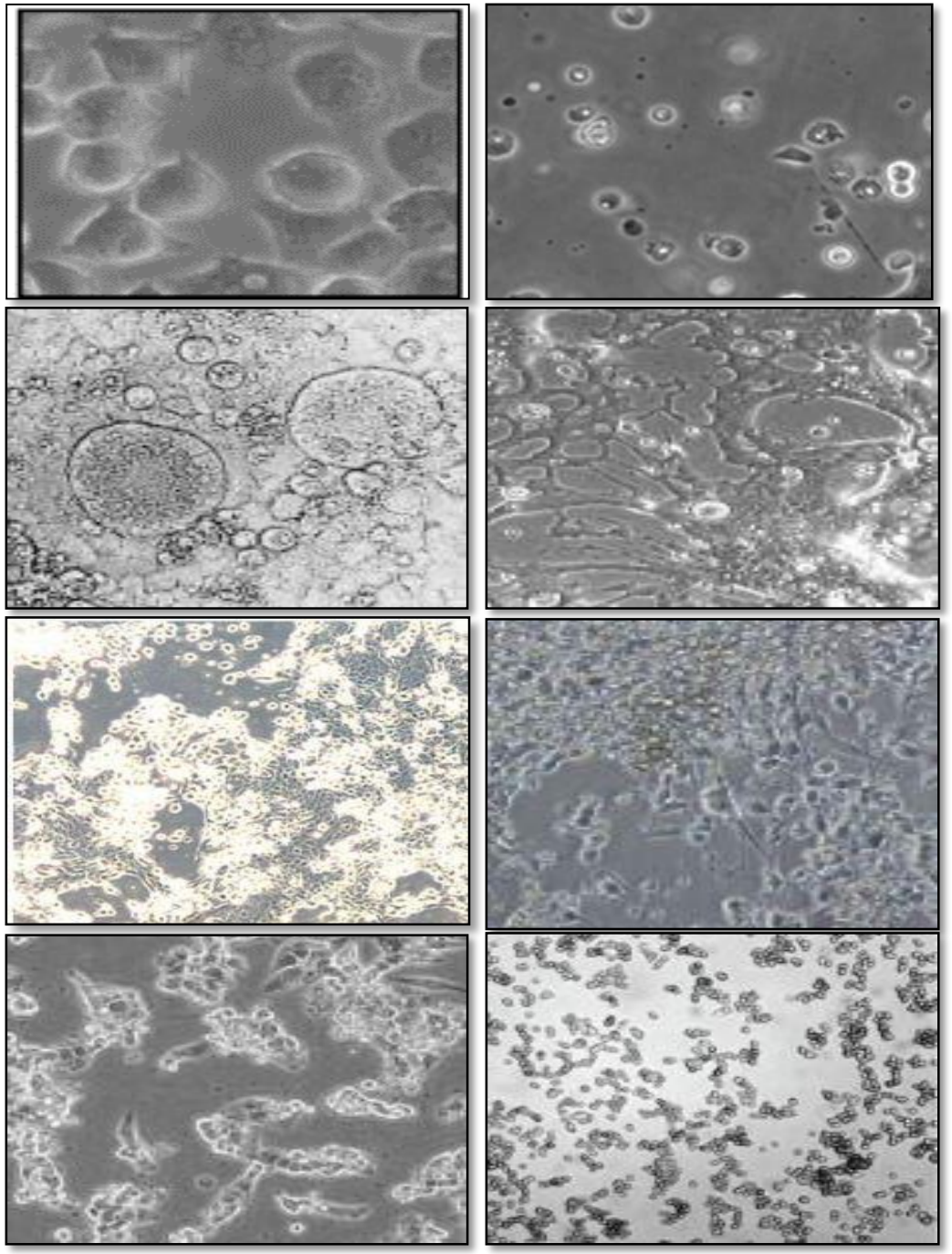
1. Cell culture methods

- Since the discovery by Enders (1949) that polioviruses could be cultured in cells, cell culture has become a very useful and convenient method for isolating viruses *in vitro*
- **Gold standard for virus isolation and identification**

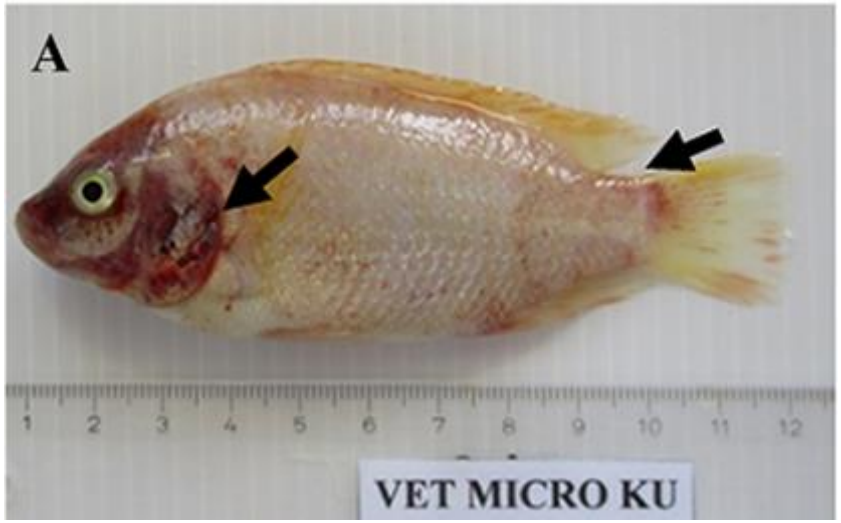
Cytopathic effects (CPE)

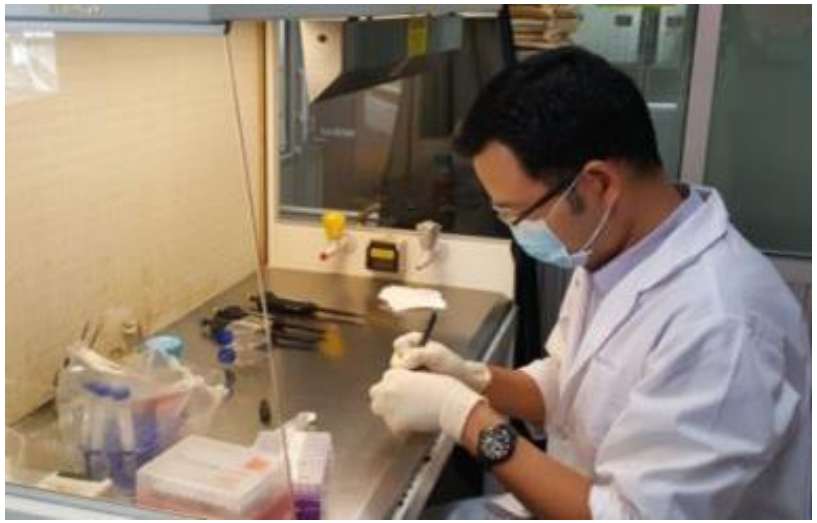
Morphological changes occurring in viral infection

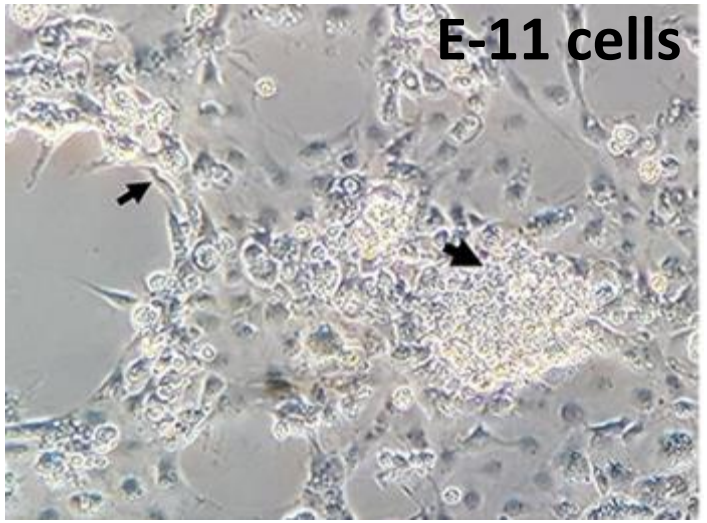
1. Rounding
2. Detachment
3. Syncytia or fusion
4. Shrinkage
5. Increased refractivity
6. Aggregation
7. Loss of adherence
8. Cell lysis or death



TiLV viral isolation







Clinical specimens

- Organ and tissue
- Mucus

Virus cultivation in cell culture

Observe CPE in viral replicated cells

2. Laboratory animals

- 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

Veterinary Microbiology 207 (2017) 170–177

Contents lists available at ScienceDirect

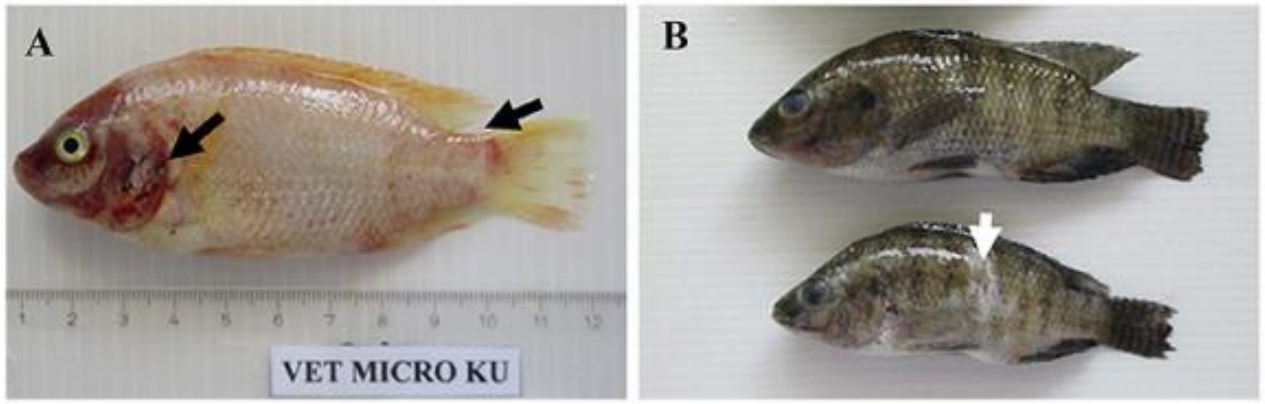
Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

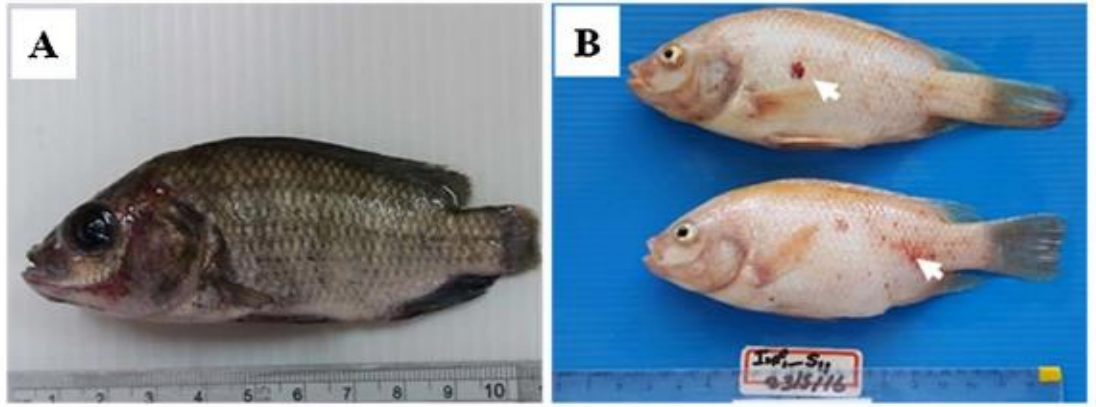
Experimental infection of Tilapia Lake Virus (TiLV) in Nile tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis* spp.)

Puntanat Tattiyapong[a,b], Worawan Dachavichitlead[a,b], Win Surachetpong[a,b,*]

[a] Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand
[b] Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok, Thailand



Gross signs of fish from field outbreak



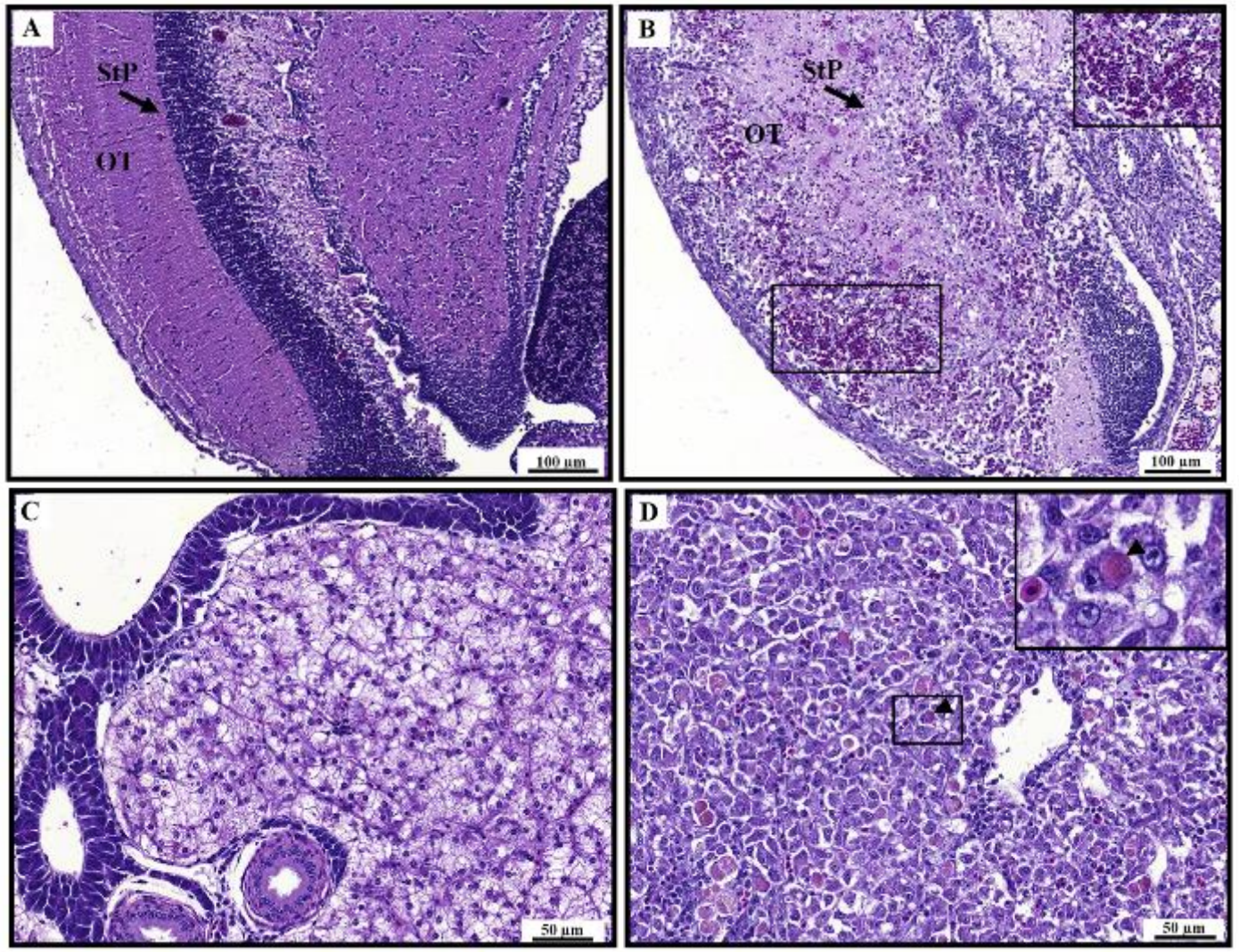
Gross signs of fish from lab challenge

Histopathology of TiLV-challenged fish

Normal **Infect**

Brain

Liver



Tattiyapong, Dachavichitlead, and Surachetpong 2017. *Veterinary Microbiology. 207: 170-177*

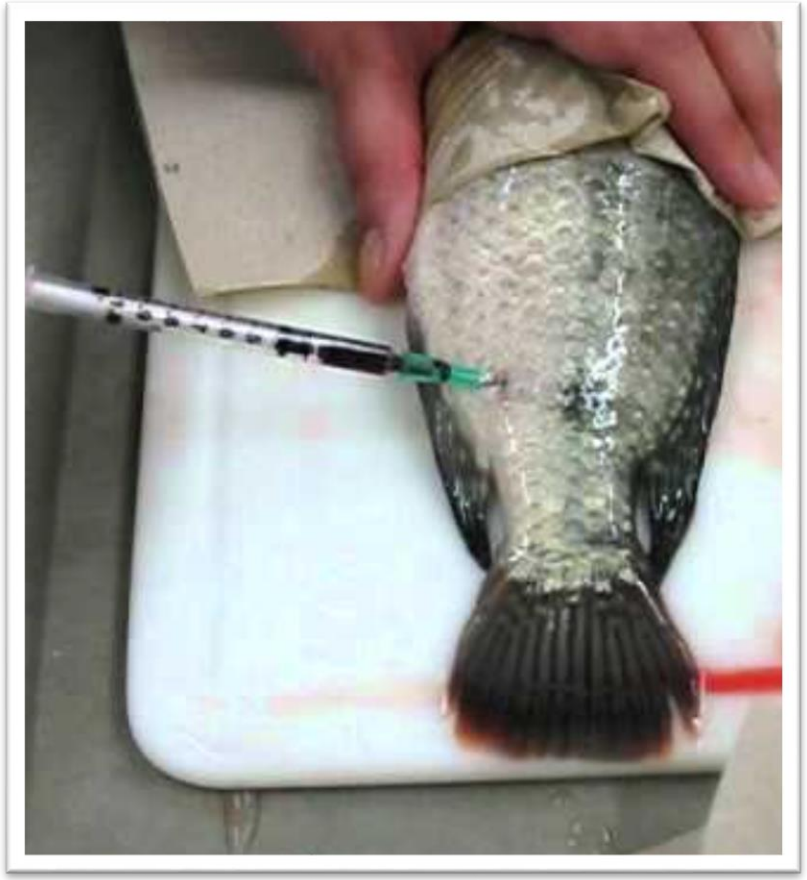
Serology

Classical techniques

- Complement fixation test
- Haemagglutination inhibition test
- Neutralization test

Advanced techniques

- Immunoassay (ELISA etc.)
- Western blot



Classical techniques

- **Neutralization test**
  - Detects the presence of viral neutralizing antibodies
  - Complete blockage of viral activity

    → No cell infection (no CPE)

JOURNAL OF
FISH DISEASES

Original Article

Validation of a serum neutralization test for detection of antibodies specific to cyprinid herpesvirus 3 in infected common and koi carp (*Cyprinus carpio*)

J Cabon, L Louboutin, J Castric, S Bergmann, G Bovo, M Matras, O Haenen, N J Olesen, T Morin

- ***Neutralizing antibodies were steadily detected in infected carp*** subjected to restrictive or permissive temperature variations over more than 25 months post-infection. The results suggest that this non-lethal diagnostic test could be used in the future to improve the epidemiological surveillance and control of CyHV-3 disease.

CVI
Journals.ASM.org

Development and Evaluation of a Blocking Enzyme-Linked Immunosorbent Assay and Virus Neutralization Assay To Detect Antibodies to Viral Hemorrhagic Septicemia Virus

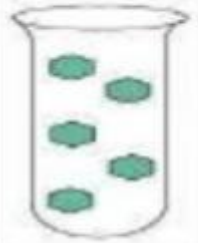
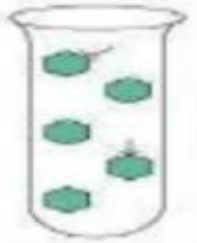
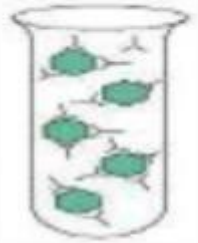
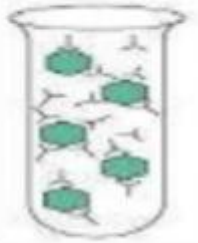
Anna Wilson,[a] Tony Goldberg,[a] Susan Marcquenski,[b] Wendy Olson,[c] Frederick Goetz,[d] Paul Hershberger,[e] Lucas Hart,[e] Kathy Toohey-Kurth[a,f]

TABLE 4 Results of VN assay[a]

VHS infection status	No. of known positives ($n = 28$)	No. of known negatives ($n = 34$)
Positive	12	0
Negative	16	34

[a] $n = 62$. The sensitivity is 42.9% and the specificity is 100%, both calculated from fish in the VHS-negative and VHS-positive groups.

Development of VN for TiLV

Patient serum (dilution)	0	0	1/1000	1/100	1/10	1
Virus concentration	0	5000 pfu	5000 pfu	5000 pfu	5000 pfu	5000 pfu
Virus concentration						
CELL CULTURE serum/virus mixture	 No virus	 CPE	 CPE	 No CPE	 No CPE	 No CPE
		Infection		Neutralization		

Neutralization test (limitation)

- The test was not very specific
  - Fish has predominant IgM subtype in serum (low specificity)
  - Absence of affinity maturation in B-lymphocytes

ELISA

- **Immunoassay (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

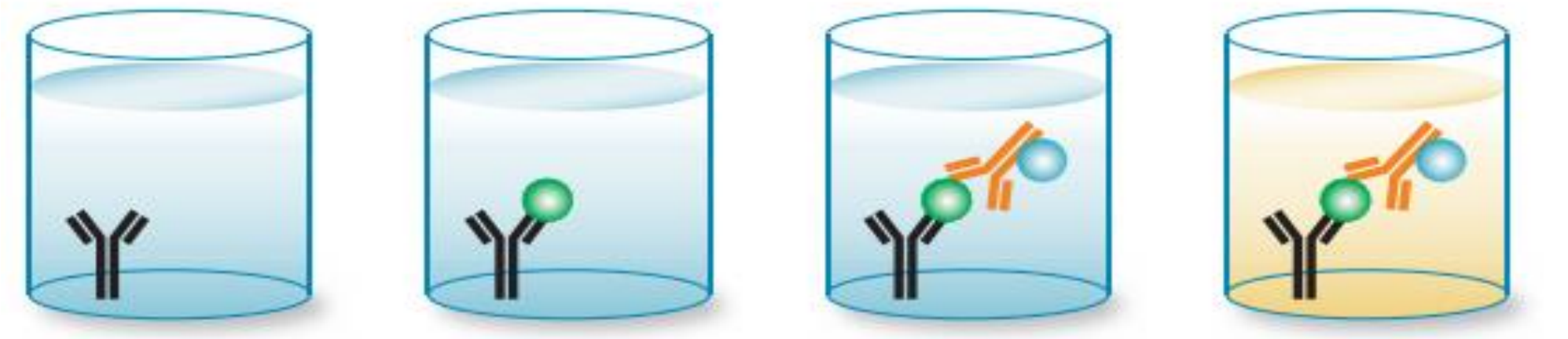


Plate is coated with a capture antibody

Sample is added, and any antigen present binds to capture antibody

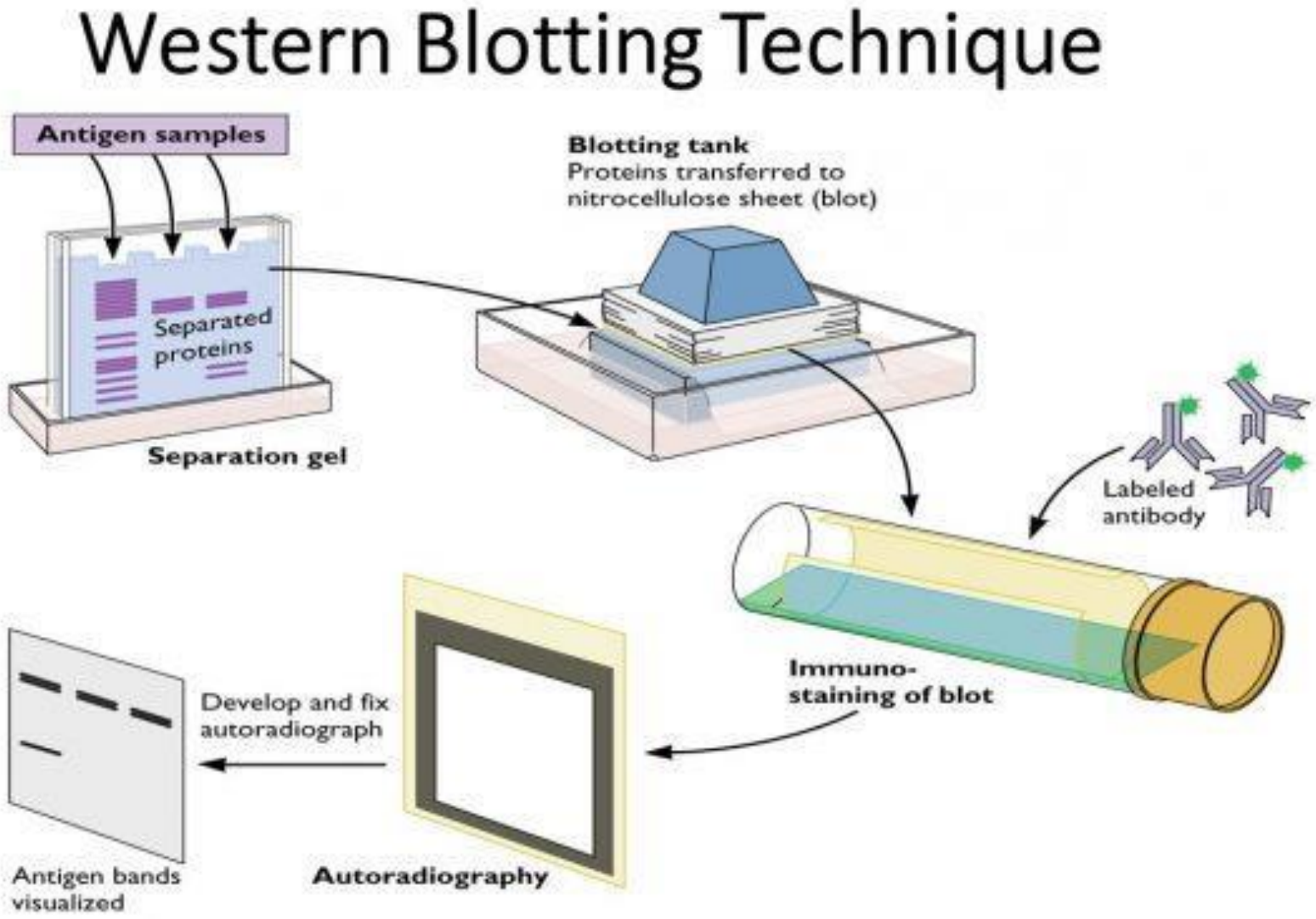
Enzyme-linked detecting antibody is added, and binds to antigen

Substrate is added, and is converted by enzyme to detectable form

Proteomic study

- **Western blot**
  - Identification of particular protein from a sample
  - Uses antibodies to detect target protein/antigen (Immunoblotting)

Proteomic study



SDS-PAGE and Western blot analysis

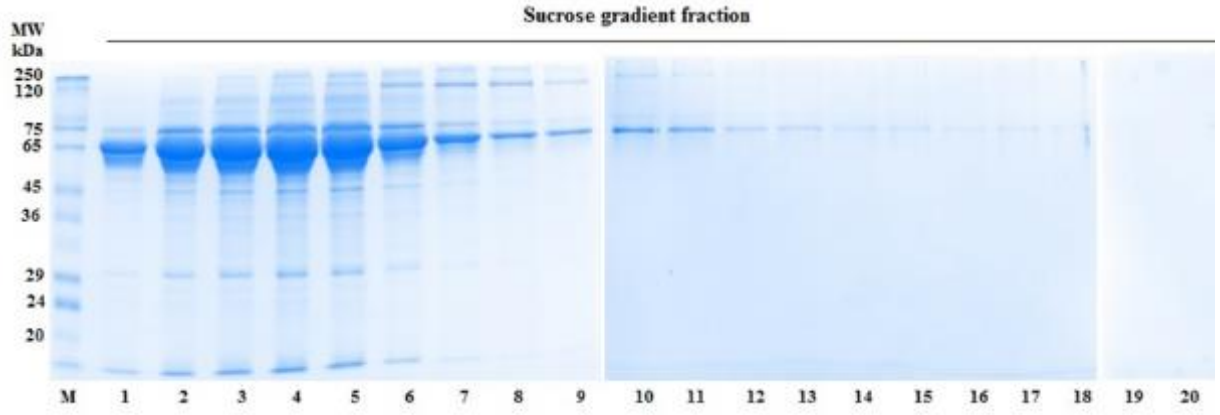
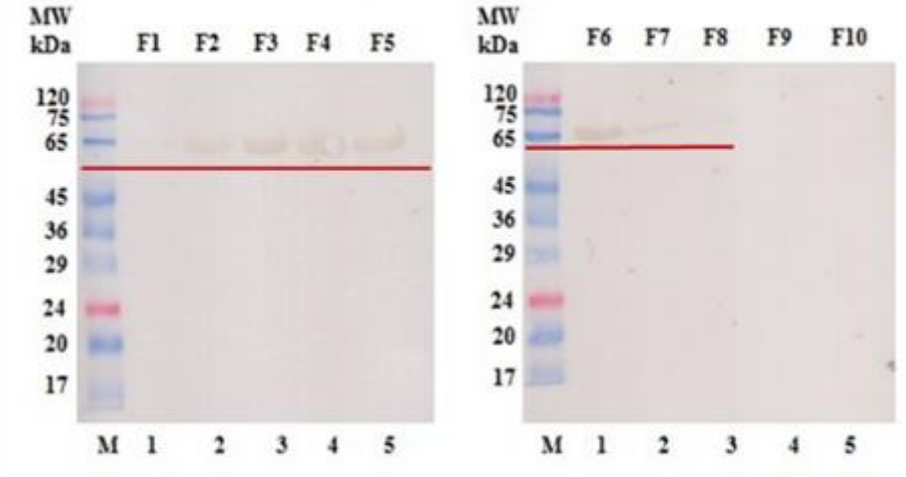
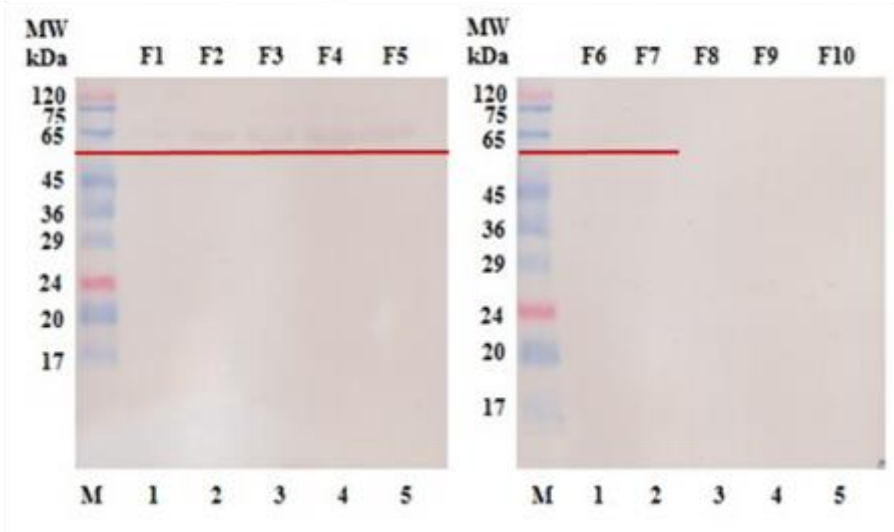


Fig 3. SDS-polyacrylamide gel analysis of viral particle in sucrose gradient containing PEG buffer. The viral particle were analyzed by SDS-PAGE and Coomassie brilliant blue R-250 staining.



Tilapia serum



Rabbit serum

Manuscript in preparation

General overview of diagnostic methods in virology

Direct methods

- Electron microscopy
- Light microscopy
- Immunofluorescence
- Molecular techniques

Indirect methods

- Cell culture
- Embryonated egg
- Laboratory animals

Serology

Classical techniques

- Complement fixation test
- Haemagglutination inhibition test
- Neutralization test

Advanced techniques

- Immunoassay (ELISA etc.)
- Western blot

Thank you