Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

VIRTUAL COURSE

26 March to 15 April 2021

Design of an Active Surveillance for Tilapia Lake Virus (TILV) Disease and Its Implementation

TCP/INT/3707: Strengthening biosecurity (policy and farm level) governance to deal with Tilapia lake virus

Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

CHECKLIST 6

02 April 2021

Diagnostic methods of aquatic animal diseases-Level III

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TCP/INT/3707: Strengthening biosecurity (policy and farm level) governance to deal with Tilapia lake virus

Checklist #6. Diagnostic testing

Components for accurately diagnosing disease status:

(i) procedures, and their sensitivity and specificity
(ii) interpretation of results
(iii) competent laboratories

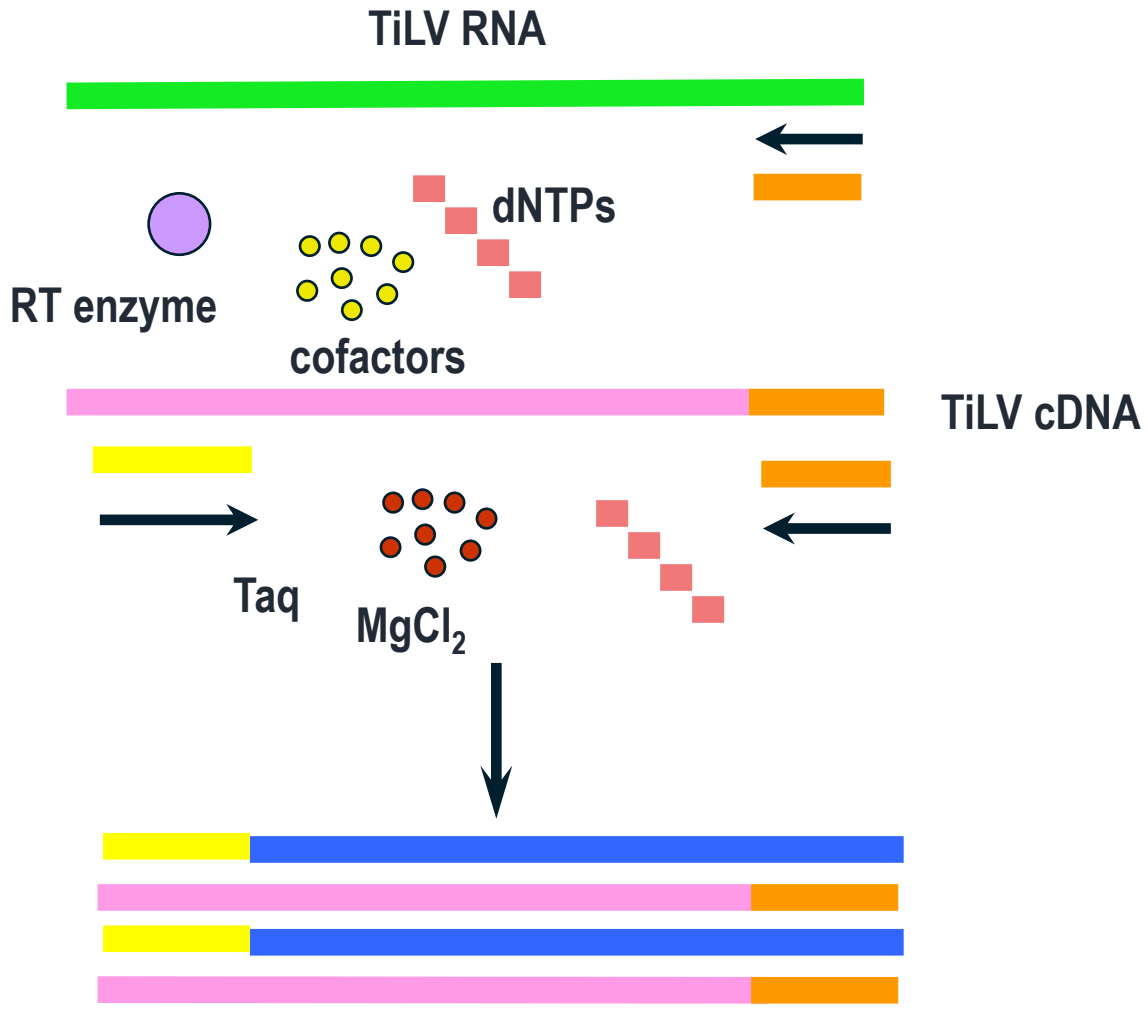
Diagnostic methods (Level III)

- Conventional RT-PCR, RT-qPCR and iiPCR
- Reverse transcription loop-mediated isothermal amplification (RT-LAMP)
- In situ hybridization (ISH)
- Immuno-detection
- Cell-culture system
- TEM examination

Conventional RT-PCR

Reverse transcription (RT)

PCR: Denature, anneal and extend for 25 - 40 cycles; generate amplified products of a specific size

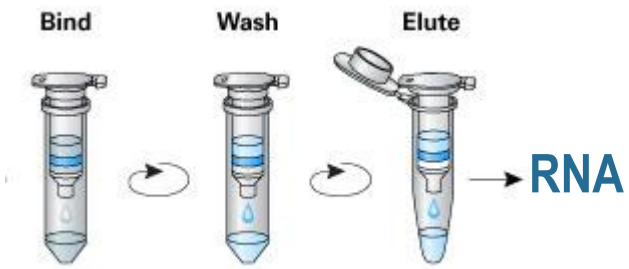


Conventional RT-PCR

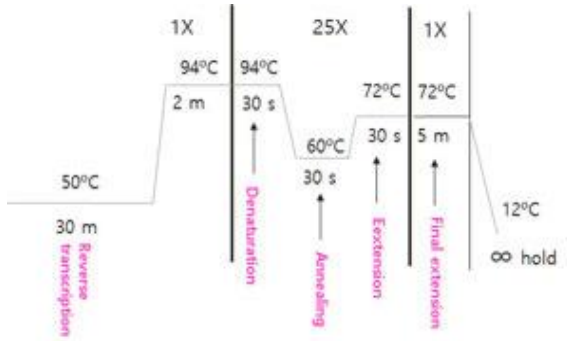
RNA extraction → RT-PCR → Endpoint Analysis

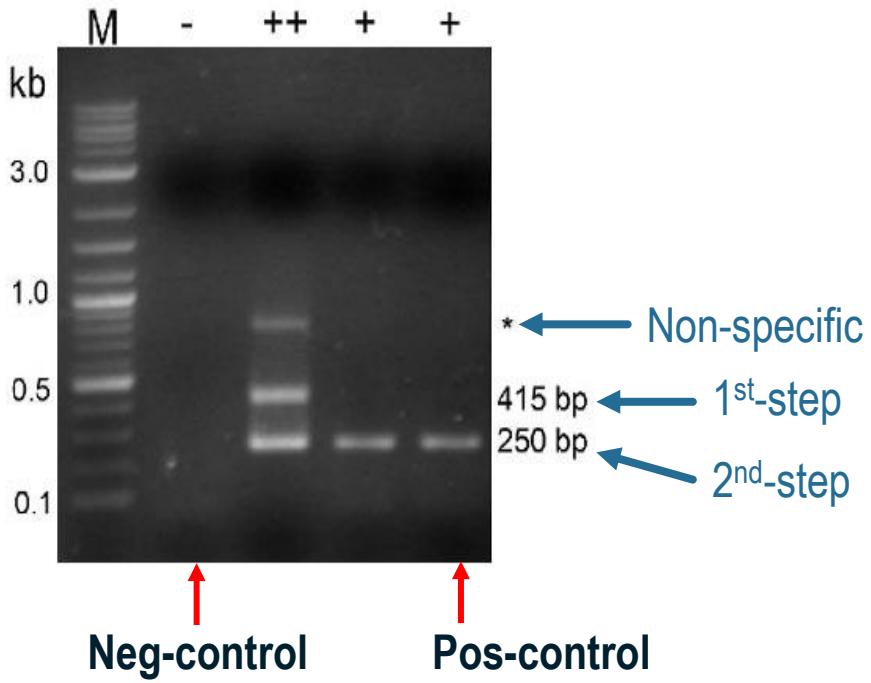
→ RNA

or



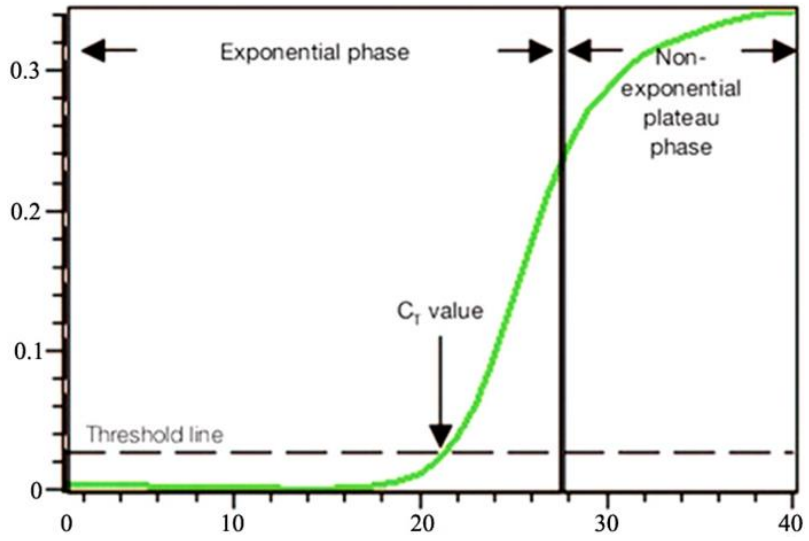
→ RNA





Same PCR profile for the 2-step

Real-time PCR (qPCR)



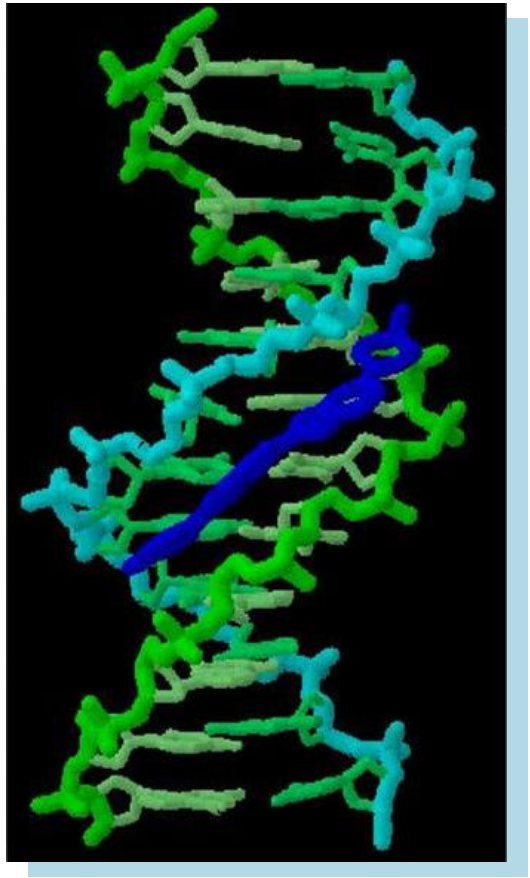
- Data taken at each amplification cycle
- Fluorescent detection of PCR products
- Rapid assay, high sensitivity (< 100 copies)
- Low risk of carry-over contamination (closed-tube)

Ct: The cycle number at which the fluorescence emission exceeds a fixed threshold

This sample has Ct =21, positive for TiLV

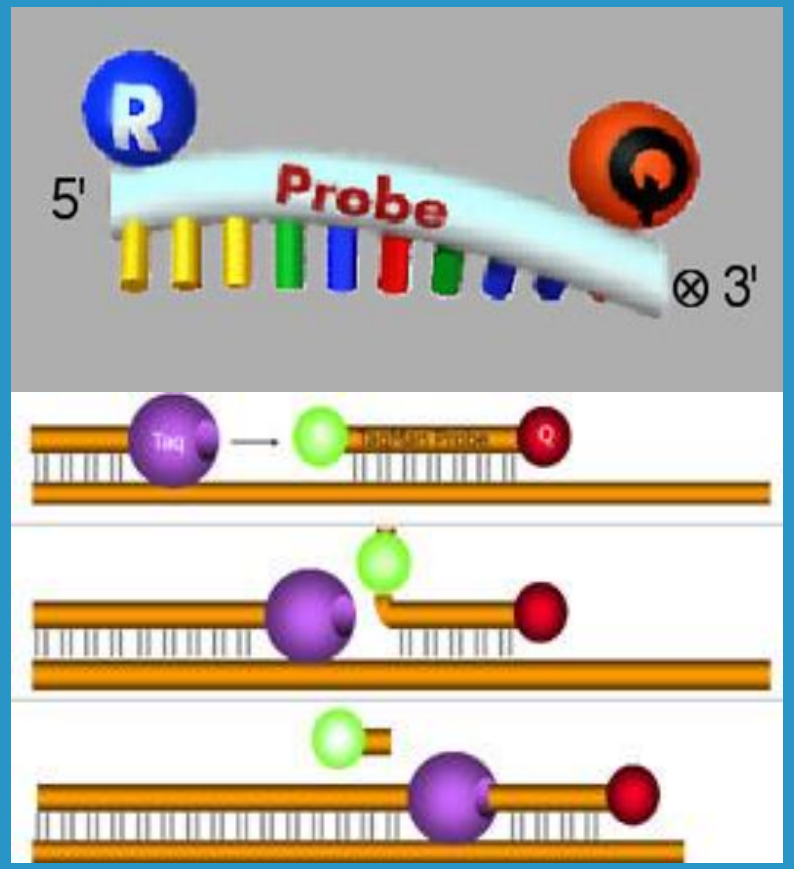
When Ct =40, not detected for TiLV

Fluorescence detection chemistry in qPCR

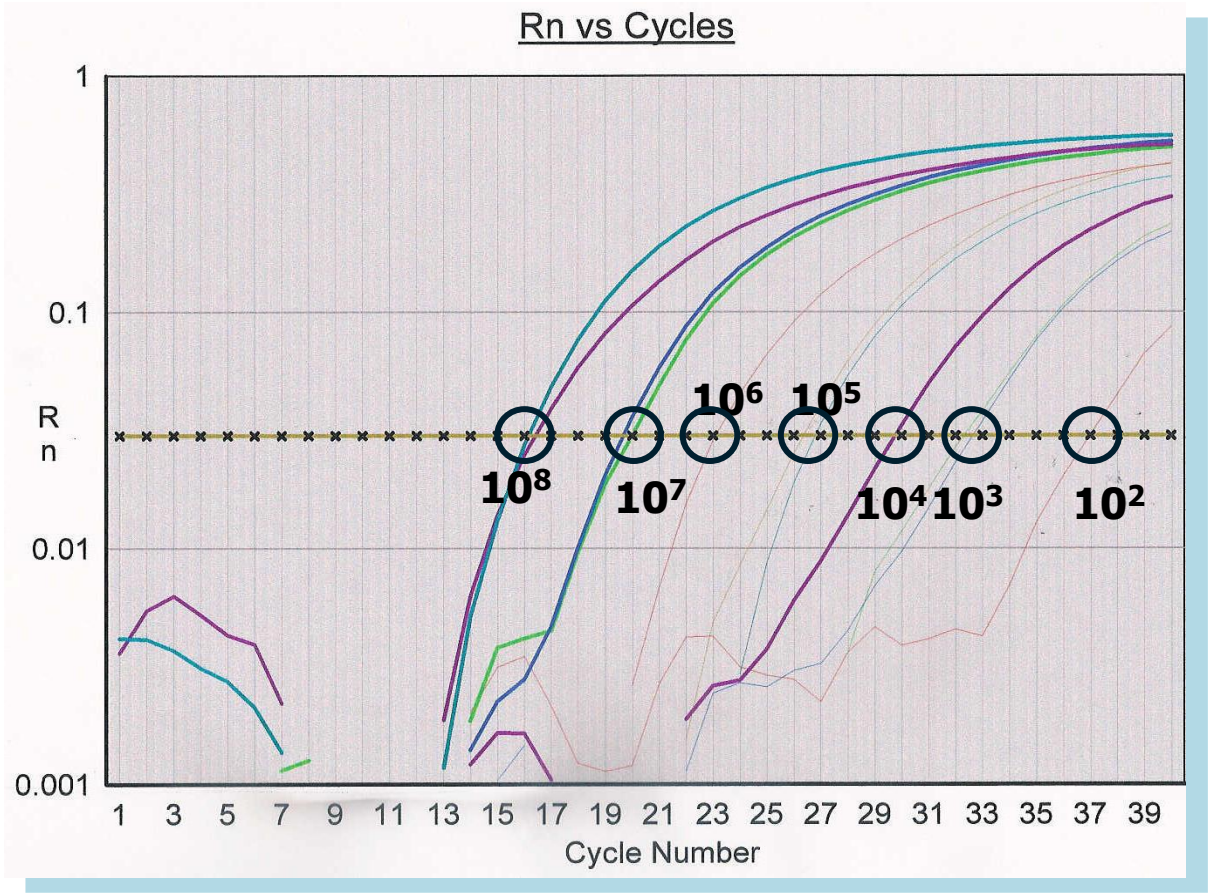


SYBR-Green, DNA-binding dye

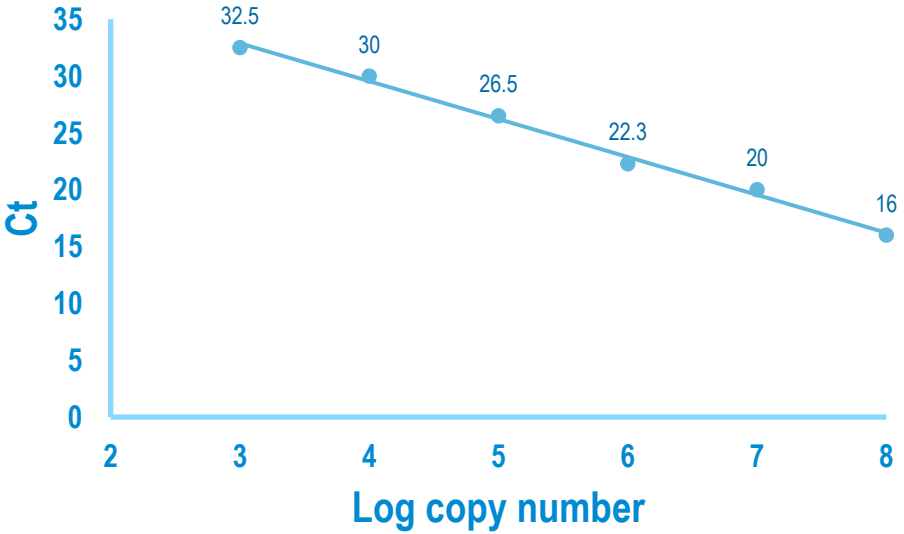
TaqMan probe



Amplification of plot of standards 10^8– 100 copies

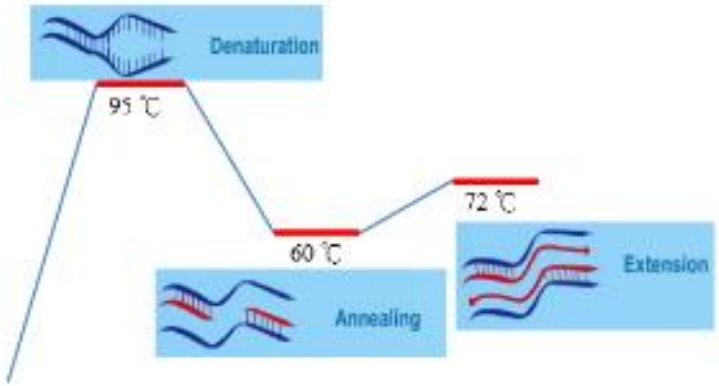


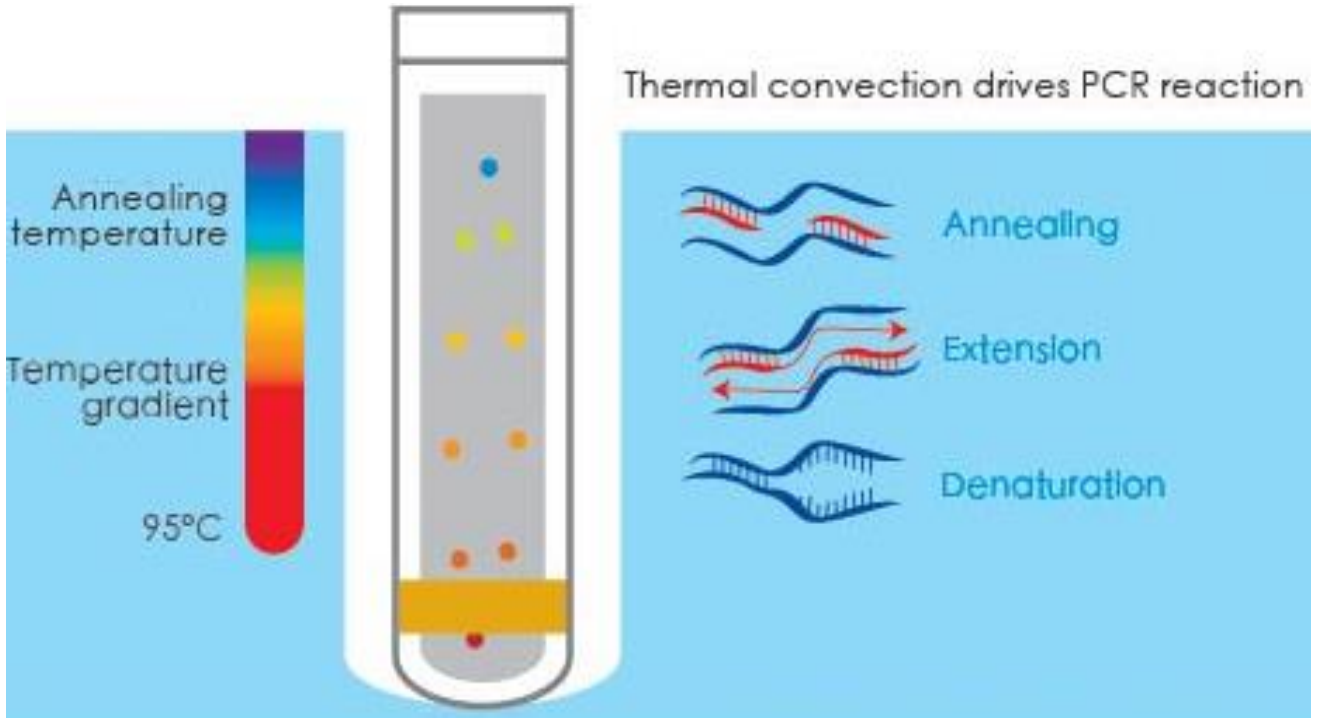
Standard curve showing an inverse relationship between: viral copy number and Ct value



Insulated isothermal PCR (iiPCR)

Traditional PCR



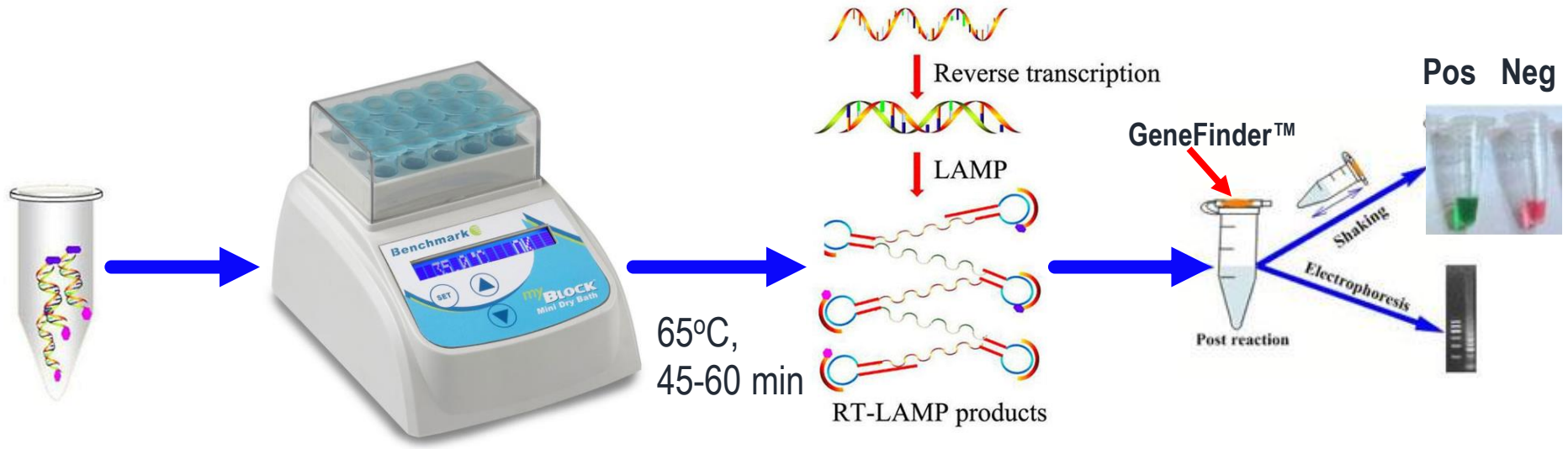


iiPCR technology provides isothermal heating at the bottom of special-designed capillary tubes to induce a natural thermal convection to drive the PCR reaction along with the temperature gradient in an insulated environment.

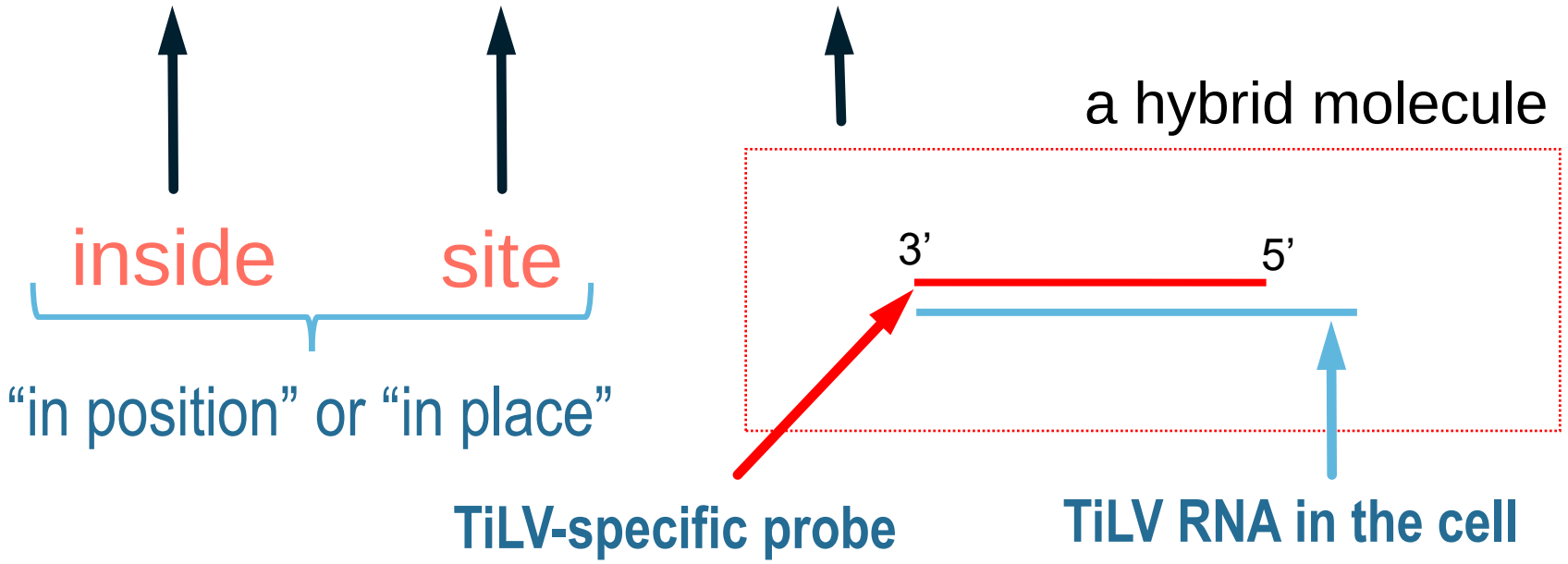
Pond-site diagnosing TiLV in a tilapia cage farm located in Lake Victoria

LAMP (loop-mediated isothermal amplification)

- Constant temperature (cost effective), field application
- Rapid (30 minutes - 1 hour)
- Have been applied in the detection of IPNV (infectious pancreatic necrosis virus), CyHV (cyprinid herpesvirus-3), TiLV, etc.



In - situ hybridization (ISH)



*“Basically, it involves **formation of a hybrid molecule** between a viral single-stranded RNA (or DNA) in the infected cell and a complementary single-stranded DNA (or RNA probe)”*

ISH

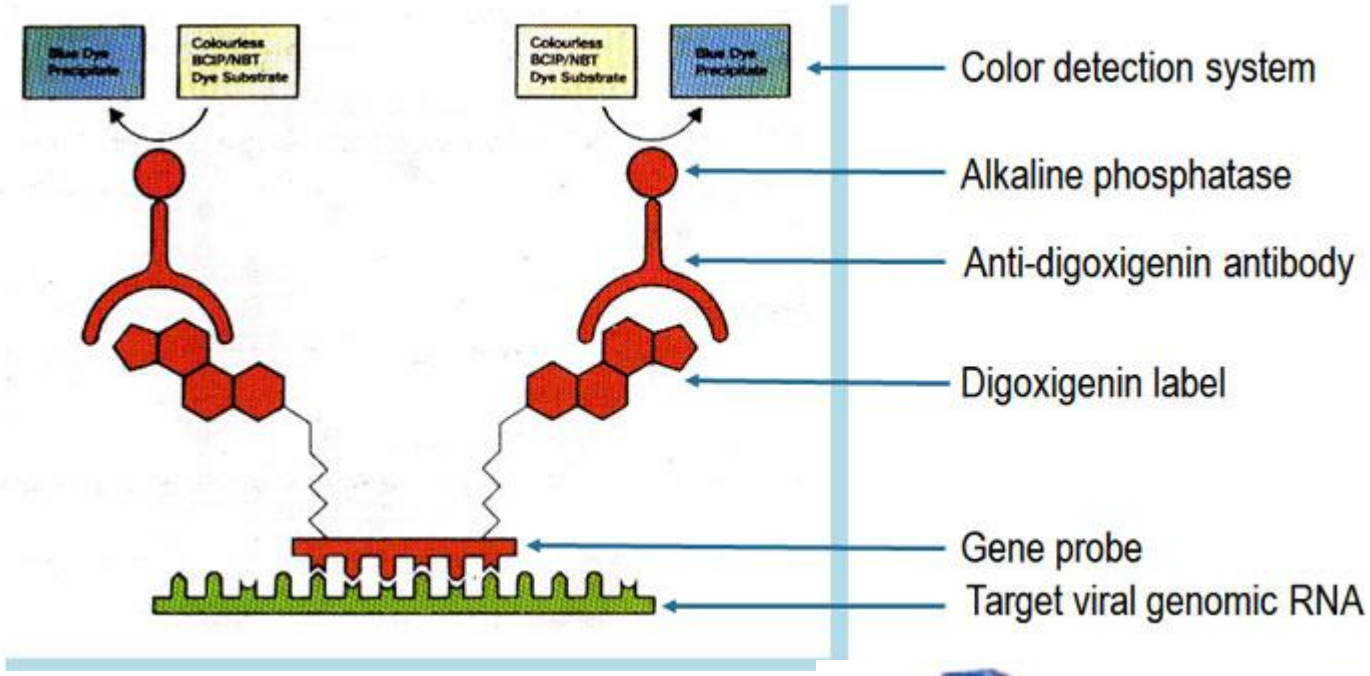
- To detect and confirm the presence of TiLV (through its RNA genome) in the tissues with histopathological lesions

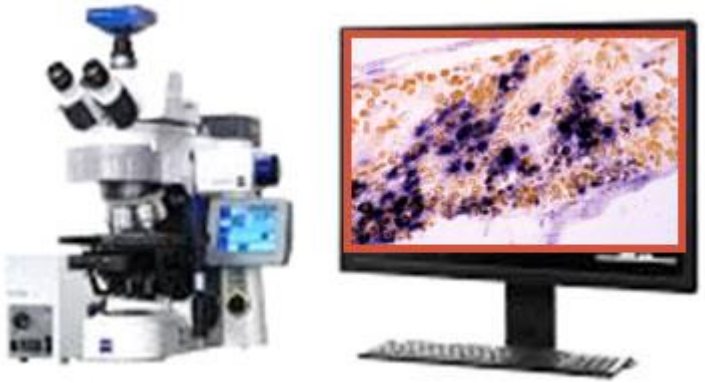
- To characterize tissue distribution of TiLV

ISH Incubator

- Temperature control
- Trough for water to maintain humidity
- Rack to hold slides
- Cover to maintain temperature & humidity

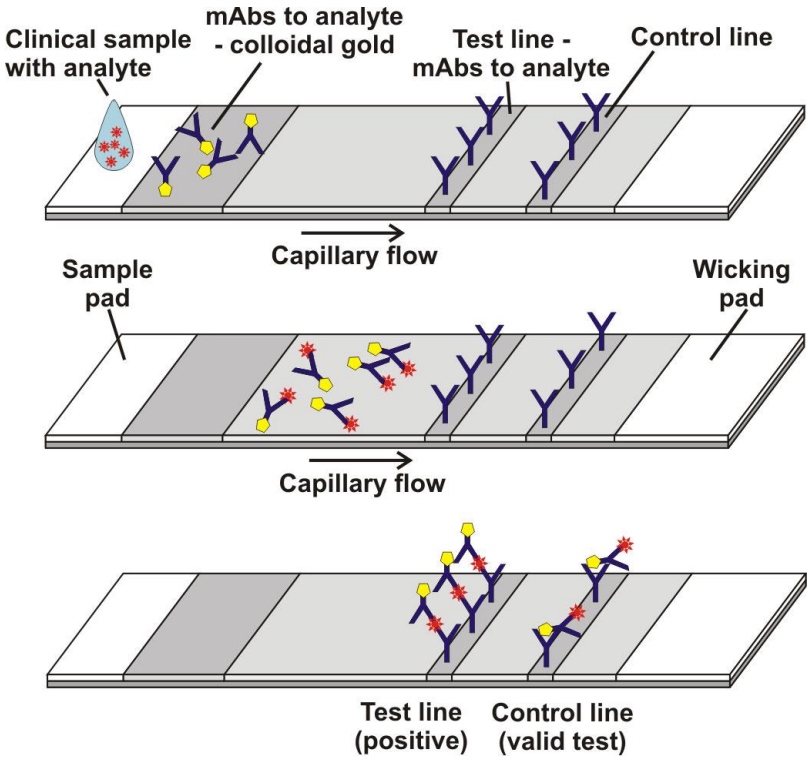
Detection of digoxigenin-labelled gene probe

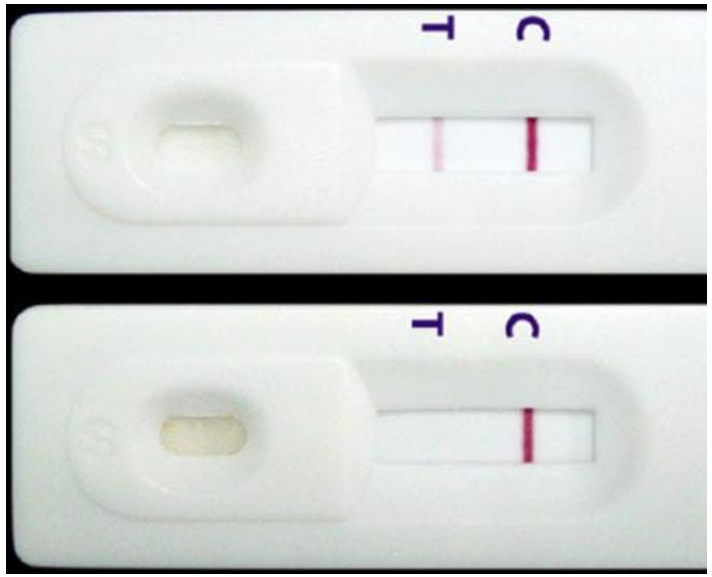




Immuno-detection

- lateral flow immunochromatographic assay (LFIA), or lateral flow assay (**LFA**), e.g. ISAV (infectious salmon anemia virus), results can be obtained in <10 min



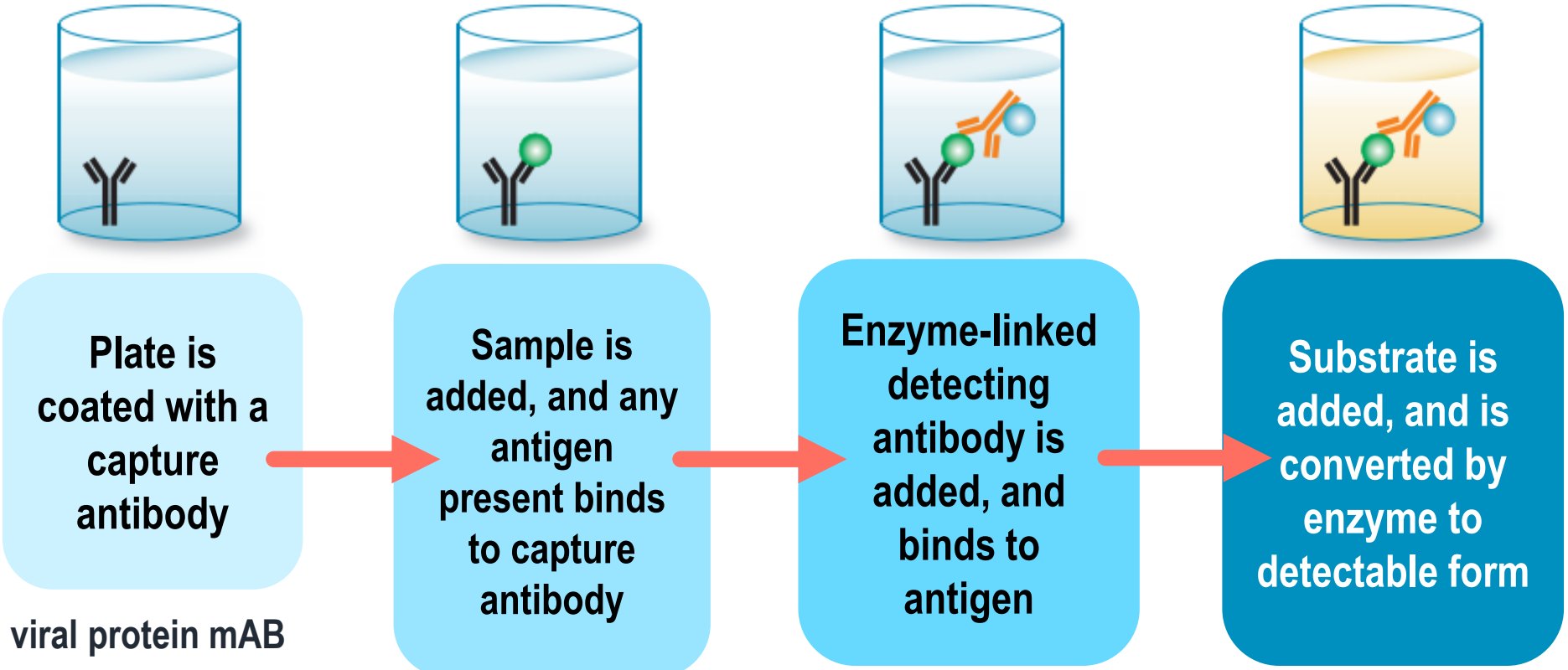


Positive

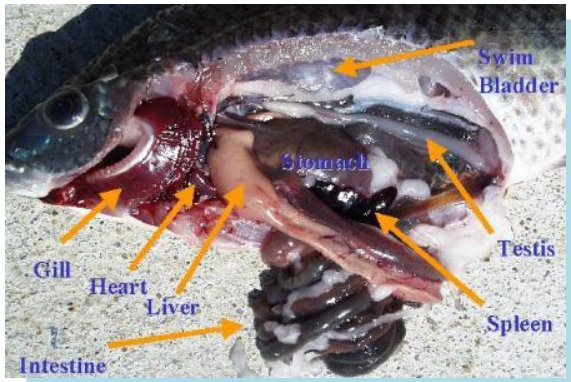
Negative

Enzyme-linked immunosorbent assay (ELISA)

- Uses antibodies and color change to identify an antigen (viral protein)
- Immobilize anti-viral protein mAB (capture antibody) on a solid phase
- Color development through an enzyme conjugate tagged on the reporter antibody



Virus isolation and inoculation to the cell-culture



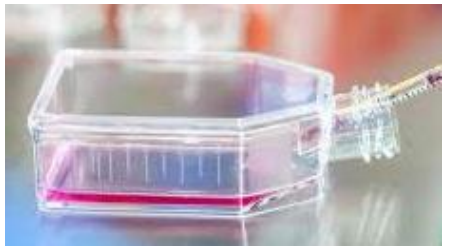
Sample the target tissues, e.g. Liver, brain, spleen

Homogenize the tissues to release the viruses

Perform centrifuge to separate the viruses from tissue debris

Supernatant-containing TiLV

tissue debris

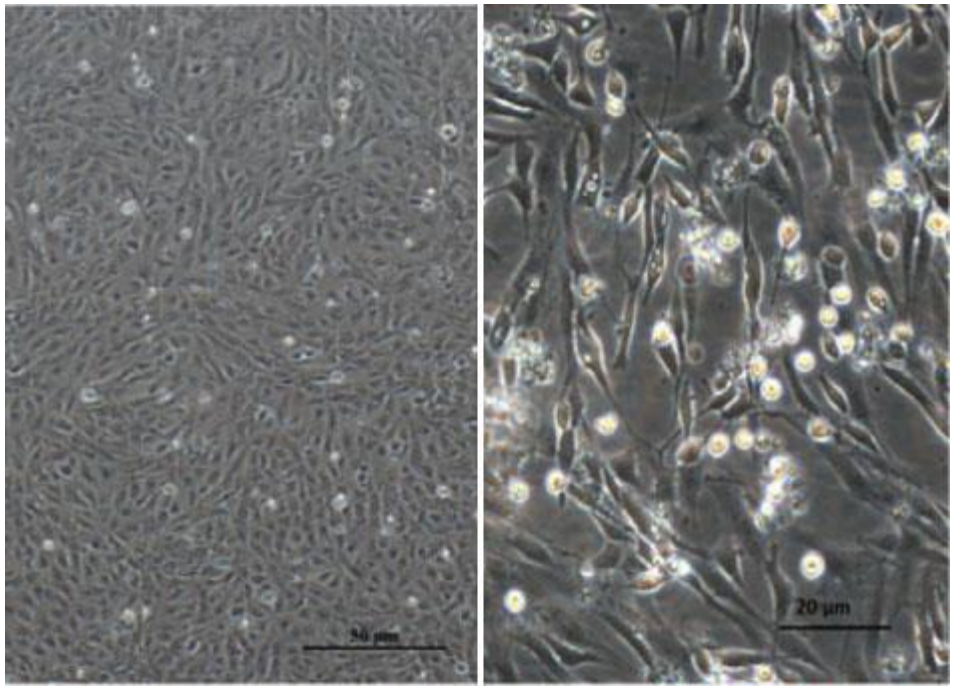


Inoculating the purified viruses to fish cell line

Virus-induced cytopathic effect (CPE)

Morphological changes

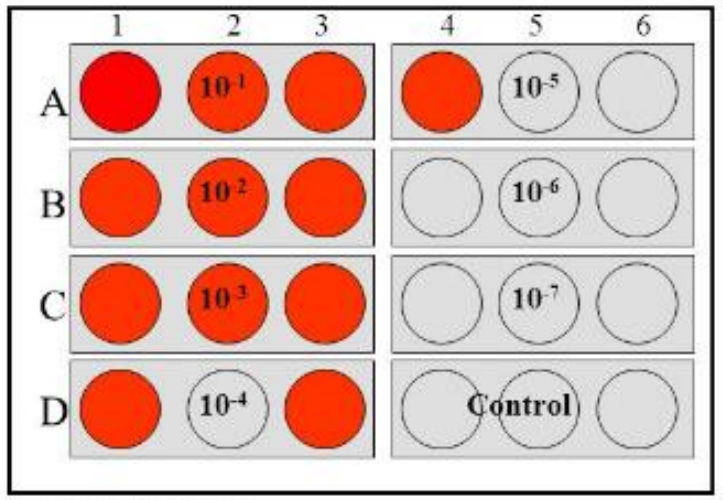
1. Rounding
2. Detachment
3. Shrinkage
4. Aggregation
5. Loss of adherence
6. Cell lysis or death



Control Virus-infected

Viral titer: 50% tissue culture infectious dose (TCID50)

TCID$_{50}$



Dilution	Infected	% Infected
10-1	3/3	100
10-2	3/3	100
10-3	3/3	100
10-4	2/3	66
10-5	1/3	33
10-6	0/3	0
10-7	0/3	0

Log PD = $\frac{66-50}{66-33}$ x (Log10)

Log PD = 0.48

Log Dilution above 50 % → **-4**

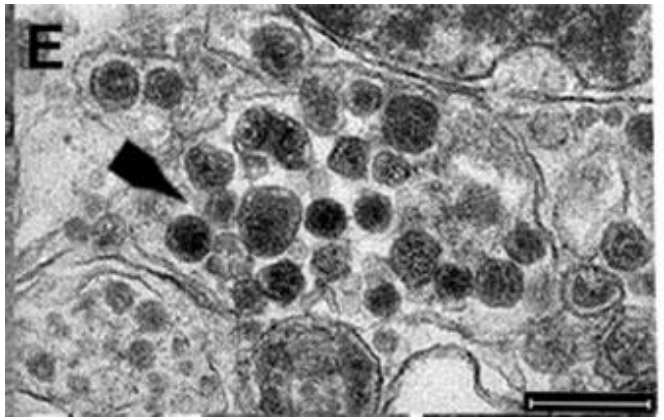
Infection $10^{-4.48}$

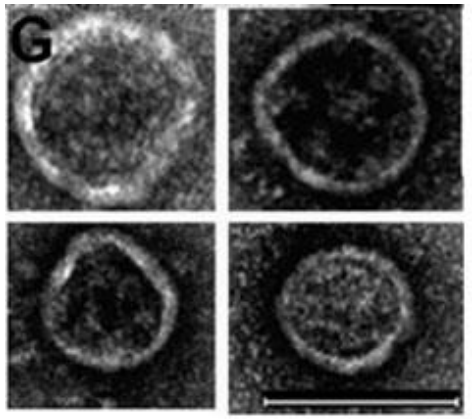
-4 + 0.48 (-1)

End point dilution assay (Reed-Muench method)

TEM examination

Disadvantage	• Not a sensitive diagnostic tool • EM is required, expensive • Laborious and complex procedures • need well-trained microscopists, usually run at core facility
Advantage	• Powerful magnifications and provide detailed information on the size and morphological features of virions • Confirms the presence of a virus(s)





Homework: Rate TiLV diagnostic methods according to the purpose of use

Method (Level category designated by FAO)	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	Fry/fingerings	Juveniles	Adults		
Gross signs (I)						
Histopathology (II)						
Isolation with cell-culture (III)						
PCR-based assays (III)						
In situ hybridization (III)						
Antibody-based assays (III)						
TEM (III)						

a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity;
b = the method is a standard method with good diagnostic sensitivity and specificity;
c = the method has application in some situations, but cost, accuracy, or other factors severely limit its application;
d = the method is presently not recommended for this purpose;
NA = not applicable

An example of test rating for infectious salmon anaemia virus (ISAV)

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance for infection with HPR-deleted ISAV				Presumptive diagnosis	Confirmatory diagnosis
	Fry	Parr	Smolt	**Adults**		
Gross signs	d	d	d	d	c	b
Histopathology	d	d	d	d	b	b
IFAT on kidney imprints	d	d	d	d	b	a
Immunohistochemistry	d	d	d	d	b	a
Isolation in cell culture with virus identification	b	b	b	b	b	a
RT-PCR	c	c	c	c	b	c
Real-time RT-PCR	a	a	a	a	a	b
Sequencing	d	d	d	d	d	a

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Thank you for your attention!

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TCP/INT/3707:
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This was also made possible with the support of the Norwegian Agency for Development Cooperation under the project GCP/GLO/979/NOR Improving Biosecurity Governance and Legal Framework for Efficient and Sustainable Aquaculture Production.

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