Food and Agriculture Organization
of the United Nations

Diagnostic requirements for TiLV

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Project Inception Workshop of GCP/RAF/510/MUL:
Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture
Southern Sun Myfair Hotel, 23-24 October 2018, Nairobi, Kenya

Diagnostic procedures for TiLV

1. **Histological examination**

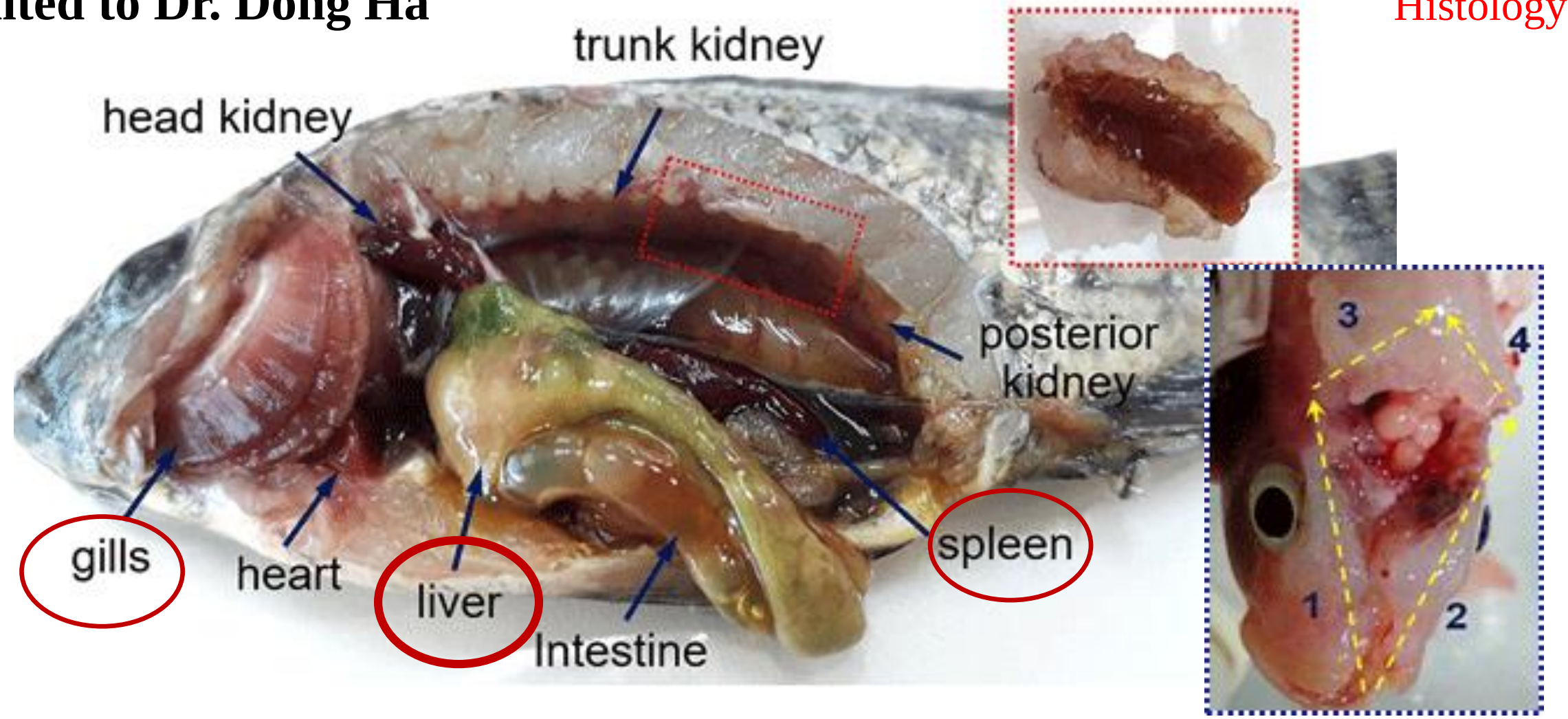
2. **RT-PCR**

3. **RT-qPCR**

4. **PCR laboratory design**

5. **Pondsite detection**

Credited to Dr. Dong Ha



Tilapia tissue sampling for histology:

Tissues from live or moribund tilapia preserve in 10% neutrally buffered formalin; do not sample dead or frozen fish**.**

What are target tissues of TiLV?

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

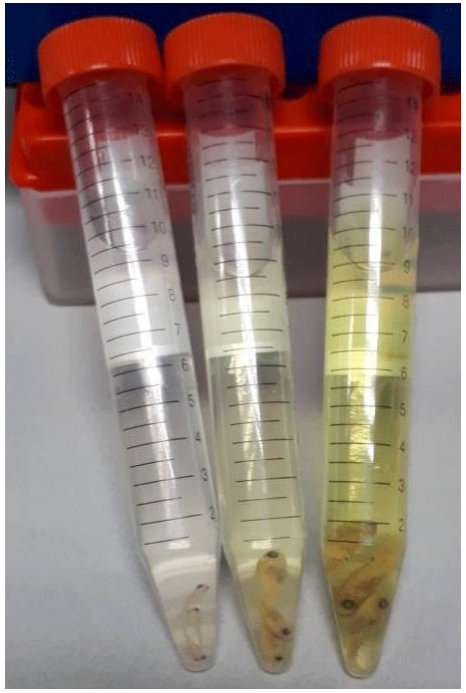
- **Mucus**

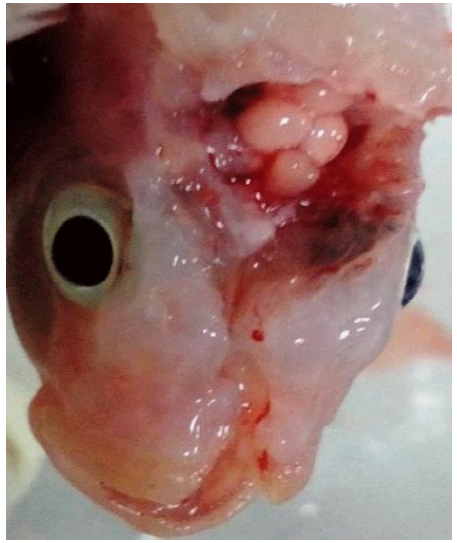
Credited to Dr. Dong Ha

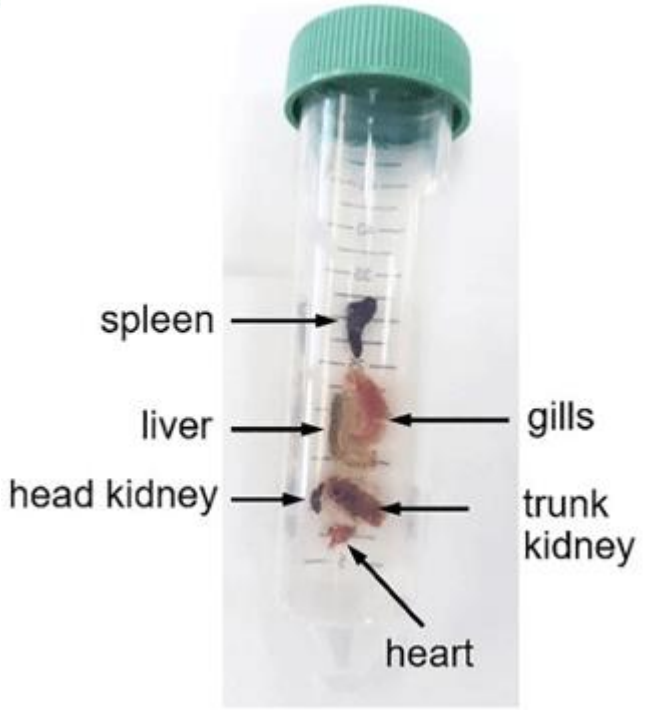
Histology

Large fish, perform necropsy

Smaller fish (< 2g), open the belly to expose the internal organs and preserve for histology and RT-PCR



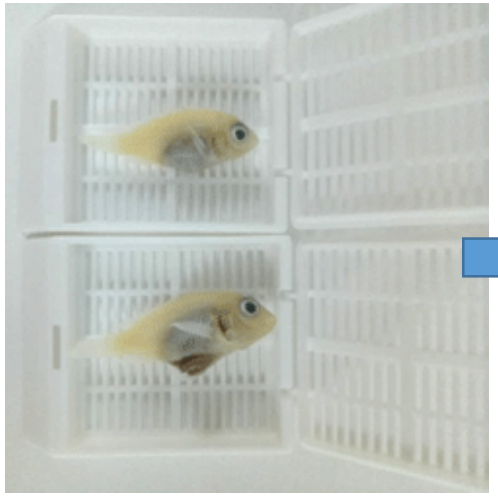




Laboratory work	Equipment, reagents, reference materials			
Necropsy	☐Laboratory gown ☐Mask ☐Groves ☐Dissecting kit -☐Scissor -☐razor blade -☐scalpel -☐Forceps ☐Dissecting try ☐Paper towels ☐Hand sanitizer ☐Trash bags	☐Projector & laptop computer	☐Live tilapia (prefer), ☐clove oil/or ice Or ☐10% buffered formalin ☐95% ethanol ☐70% ethanol ☐frozen tilapia (prefer 50-100g in size) (For RT-PCR/cell-culture analyses)	☐Tilapia dissection powerpoint ☐Dissection worksheet

Histology

Histology preparation



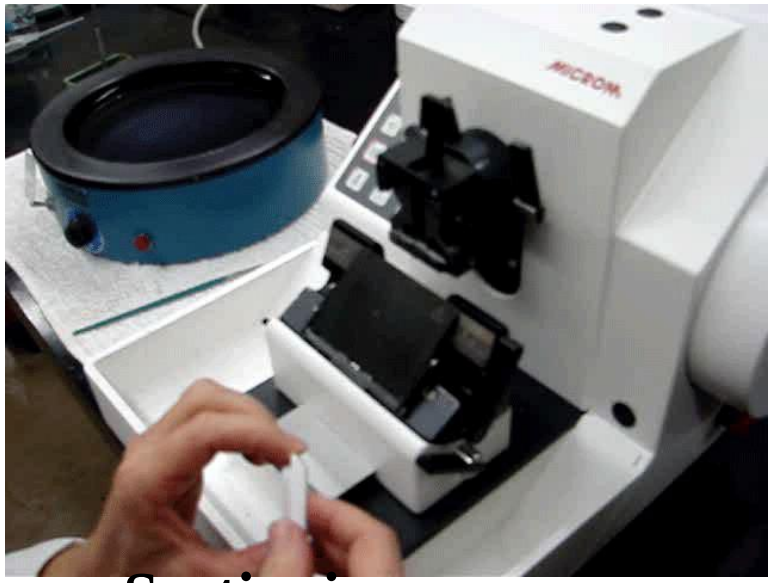
Processing

Embedding

Paraffin blocks

Credited to Dr. Dong Ha

Histology



Sectioning

Staining

H&E stained fish section

Laboratory work	Equipment, reagents, reference materials		
Histology	☐Paraffin wax ☐Histochemical stains (e.g. hematoxylin and eosin [H&E])	☐H&E slides from -☐TiLV-infected fish -☐Healthy fish ☐Microscope	☐Atlas of tilapia histology ☐Atlas of TiLV histopathology

Farm site sampling

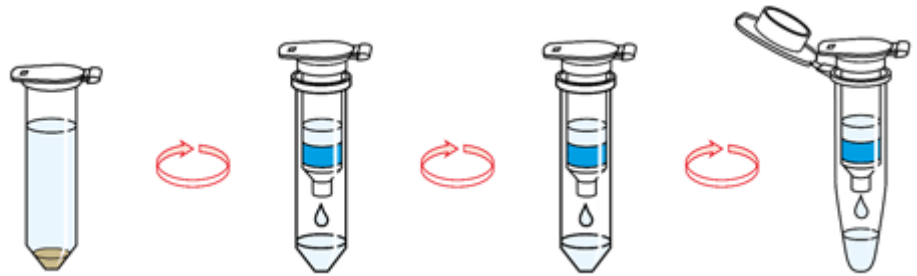
- **Fish need to be alive prior to fixation.**
- **Dead fish will be useless for histological analysis.**
- **Take formalin fixative and supplies to pondsite and do fixation on site; or**

  Hold fish in buckets or coolers with aeration prior to fixing in laboratory.
- **Never allow fish to sit in buckets without aeration.**
- **Never freeze or ice specimens before fixation.**

RNA extraction

Automatic system

Manual protocol

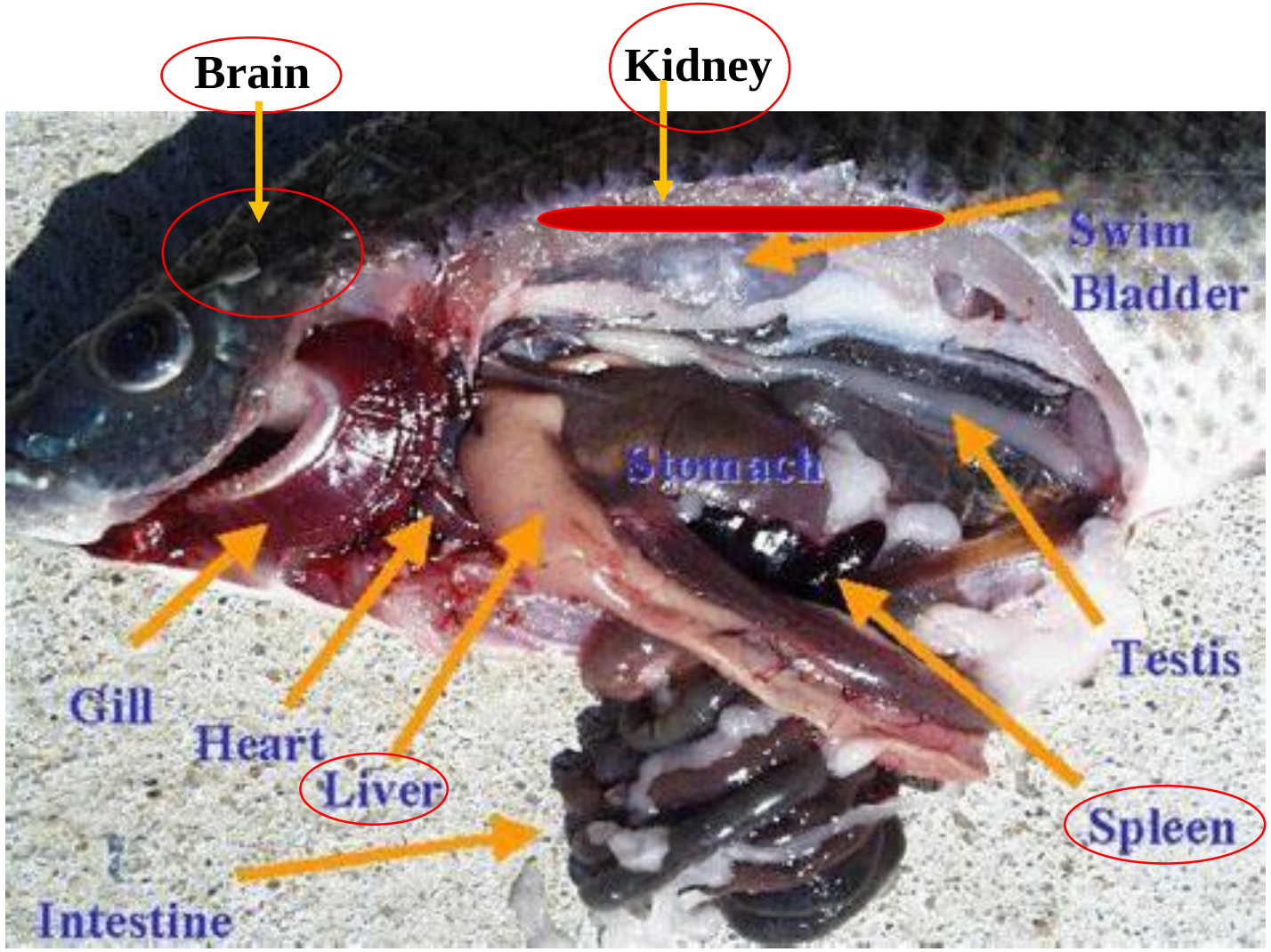


Add ethanol to tissue lysate

RNA binding to column

Wash RNA on column

Elute RNA from the column



Tilapia tissue sampling for RNA extraction:

Fresh, frozen, or preserve in ethanol (95%)

Non-invasive: mucus

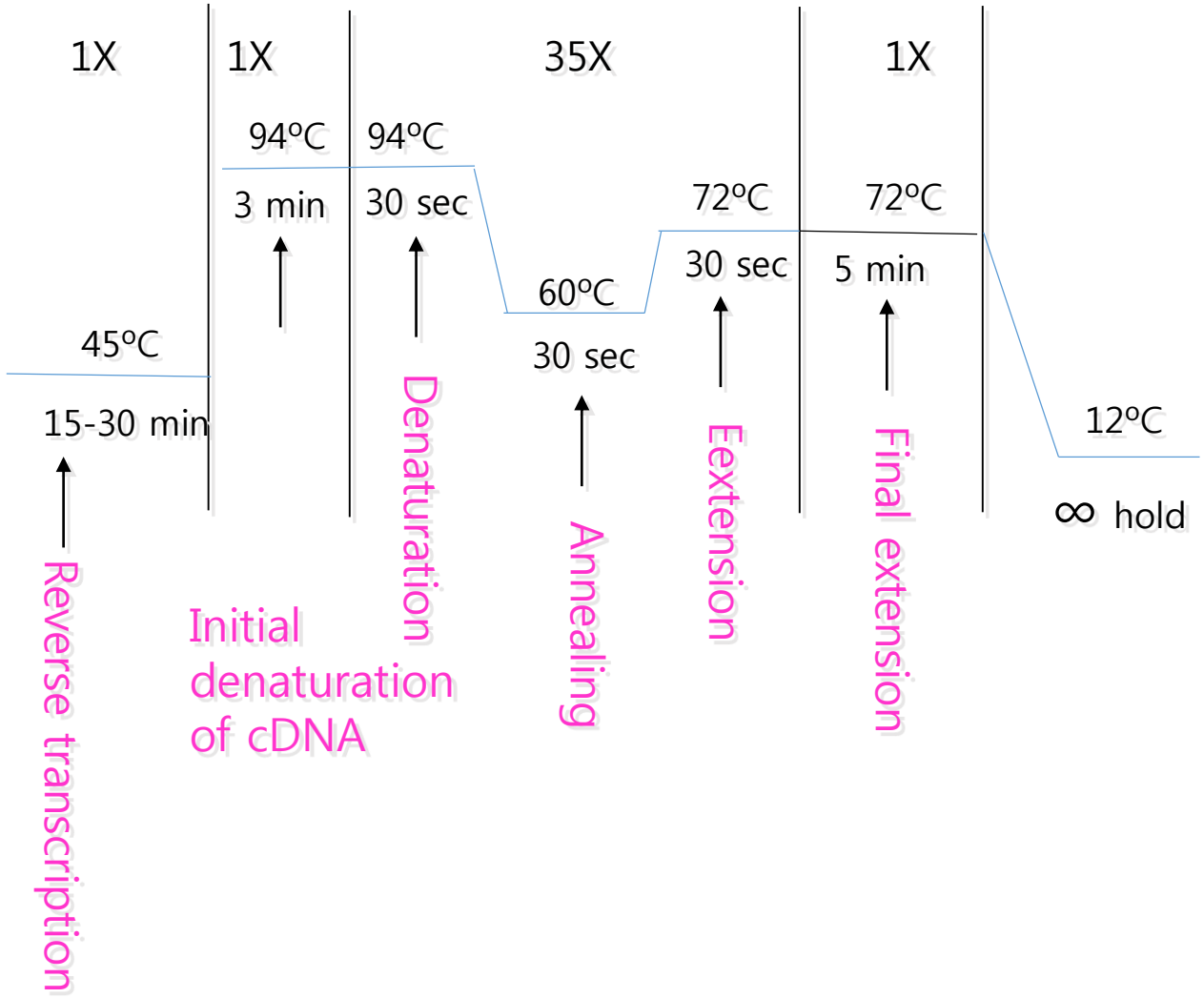
Laboratory work	Equipment, reagents, reference materials		RT-PCR
RNA extraction	☐Microfuges ☐Pipettors and aerosol barrier tips - ☐10-µl - ☐100-µl - ☐1000-µl) ☐RNA extraction kit -☐Absolute ethanol ☐Ice buckets ☐ice ☐Eppendorf tubes -☐1.5-mL, 15-mL ☐PCR tubes -☐0.2-mL, 0.5-mL ☐test tubes racks ☐PCR tubes racks ☐Vortex ☐Trash container	☐Spectrophotometer (e.g. nanodrop)	☐Tilapia tissues -☐TiLV-infected fish -☐Healthy fish (Win and/or Dong, or participants)

RT-PCR

Thermocycler:

Repeating cycles of three (general) steps (i.e. temperatures)

RT-PCR cycling profile



Gel electrophoresis

- **Separation through a matrix (agarose)**
- **Separates fragments based on the molecular weight difference**
- **Driven by the electric current, amplified products (DNA) are negatively charged**
- **loading buffer containing the track dyes (TD)-used to keep sample in the well and visualize the run**

Preparation of 10X gel loading buffer:

0.25% Bromophenol blue
0.25% Xylene Cyanol
25% Ficoll (type 400)

↓

Dissolve in 8 ml H_2O

↓

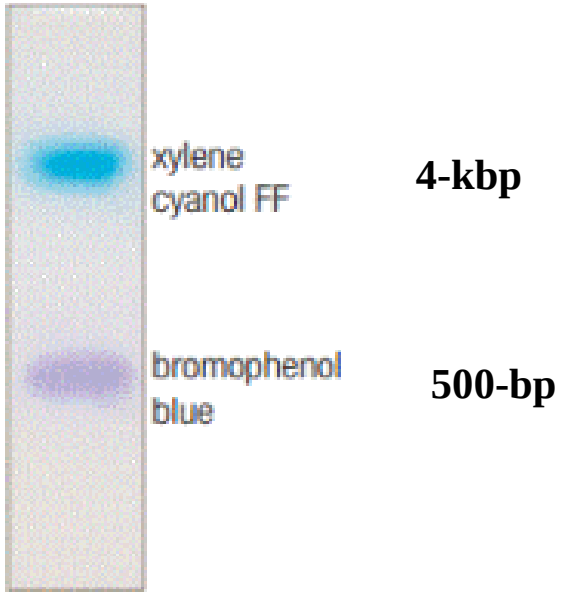
Adjust the volume to 10 ml with H_2O

↓

Dispense into 1 ml aliquots

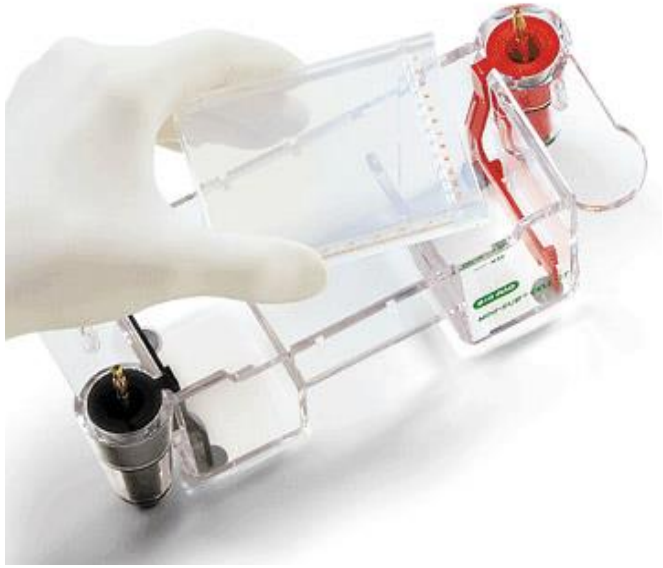
↓

Stored at 4°C for months



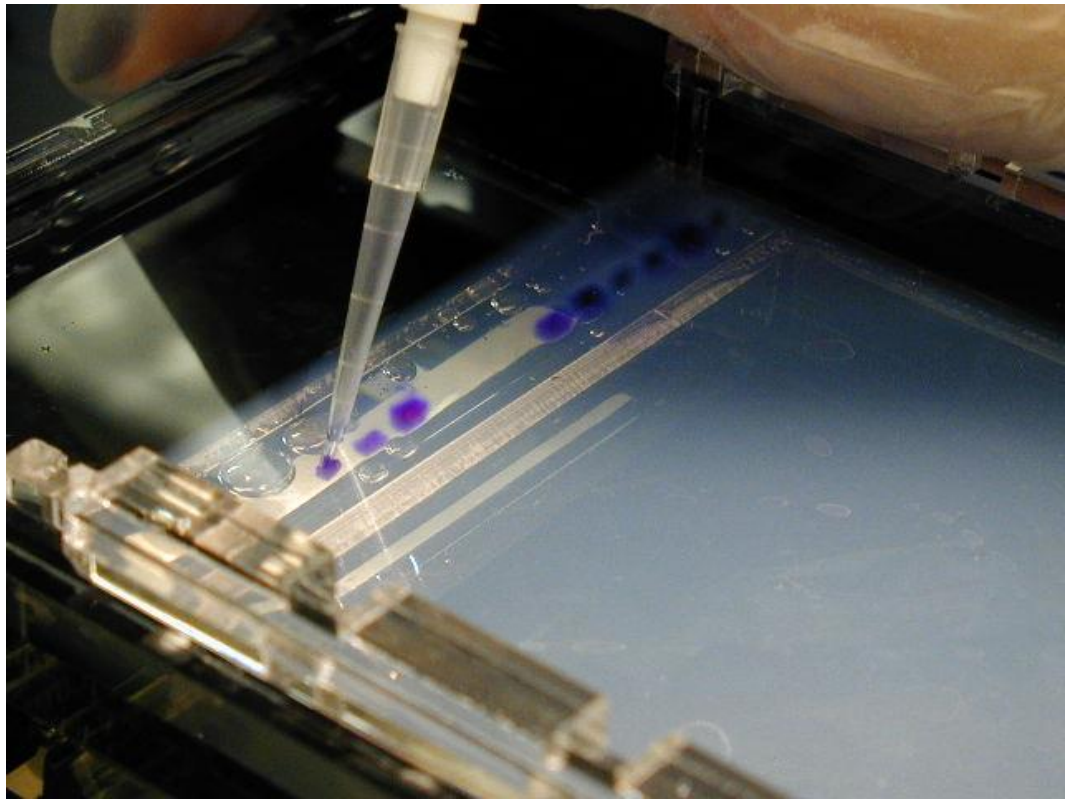
Agarose Electrophoresis

- **Place gel in gel box**
- **Pour buffer in box until gel wells are covered.**



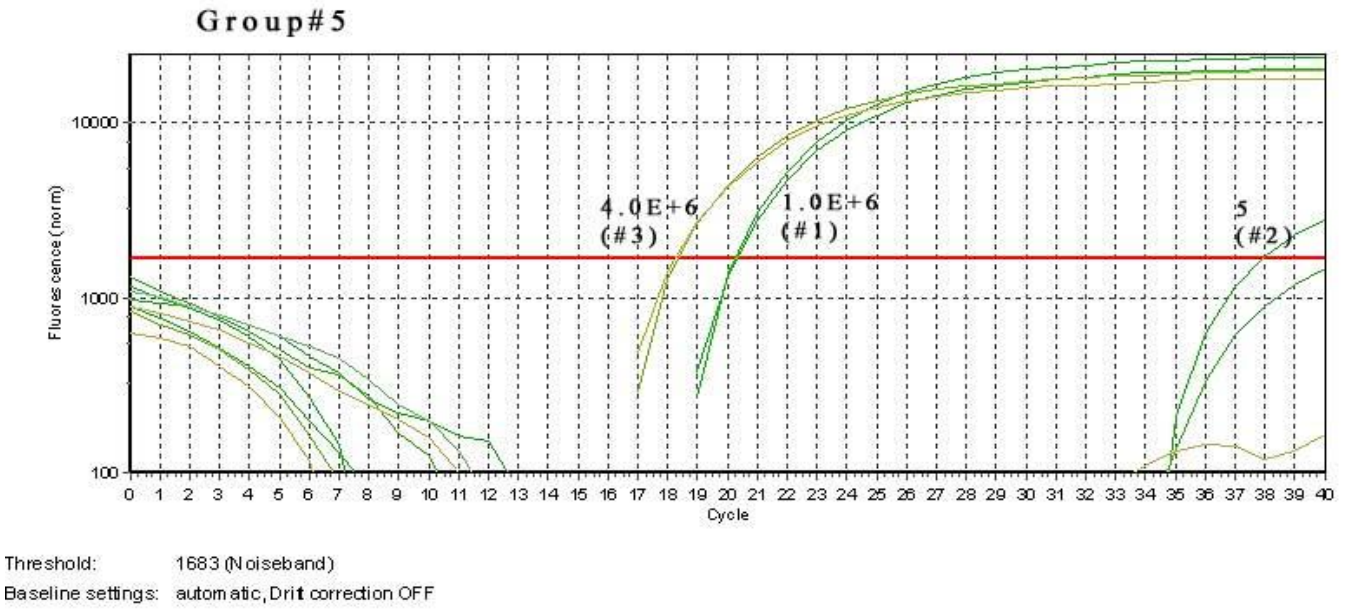
Load RT-PCR products

Place 5-10 ul of samples into appropriate wells



Laboratory work	Equipment, reagents, reference materials	
TiLV RT-PCR (conventional)	☐RT-PCR enzymes ☐TiLV-specific primers ☐ddH_2O ☐Pipettors and aerosol barrier tips -☐10-µl -☐100-µl ☐Agarose ☐Ethidium bromide (or equivalent dyes), ☐ Electrophoresis buffer ☐Molecular marker ☐Gel loading dye ☐positive control plasmid (or cDNA) (*Dong and/or Win)	☐PCR machine ☐Gel electrophoresis apparatus ☐Gel imaging system

Real-time RT-PCR (RT-qPCR)



Sample#1 and #3: virus-infected tissue

Sample #2 and #4: healthy animals

Laboratory work	Equipment, reagents, reference materials	
Real-time TiLV RT-PCR	☐RT-qPCR enzymes ☐TiLV real-time primers ☐ddH_2O ☐positive control plasmid (*Win) ☐Pipettors -☐10-µl -☐100-µl ☐Microfuge ☐0.2 qPCR tube Or ☐96 well plate ☐ plate covers	☐Real-time PCR system

PCR lab design

PCR lab design

Uni directional work flow

Rm#4 ← Rm#3 ← Rm#2 ← Rm#1

广东省水生动物疫病预防控制中心
Guangdong Aquatic Animals Disease Prevention Center

PCR lab design

Materials transporting chamber

广东省水生动物疫病预防控制中心
Guangdong Aquatic Animals Disease Prevention Center

PCR lab design

UV irradiation: 30min-1 hr after work

Air:, HEPA-filtration, rooms are positive pressure

Minimize Cross-Contamination & Gain Confidence in Your PCR Results

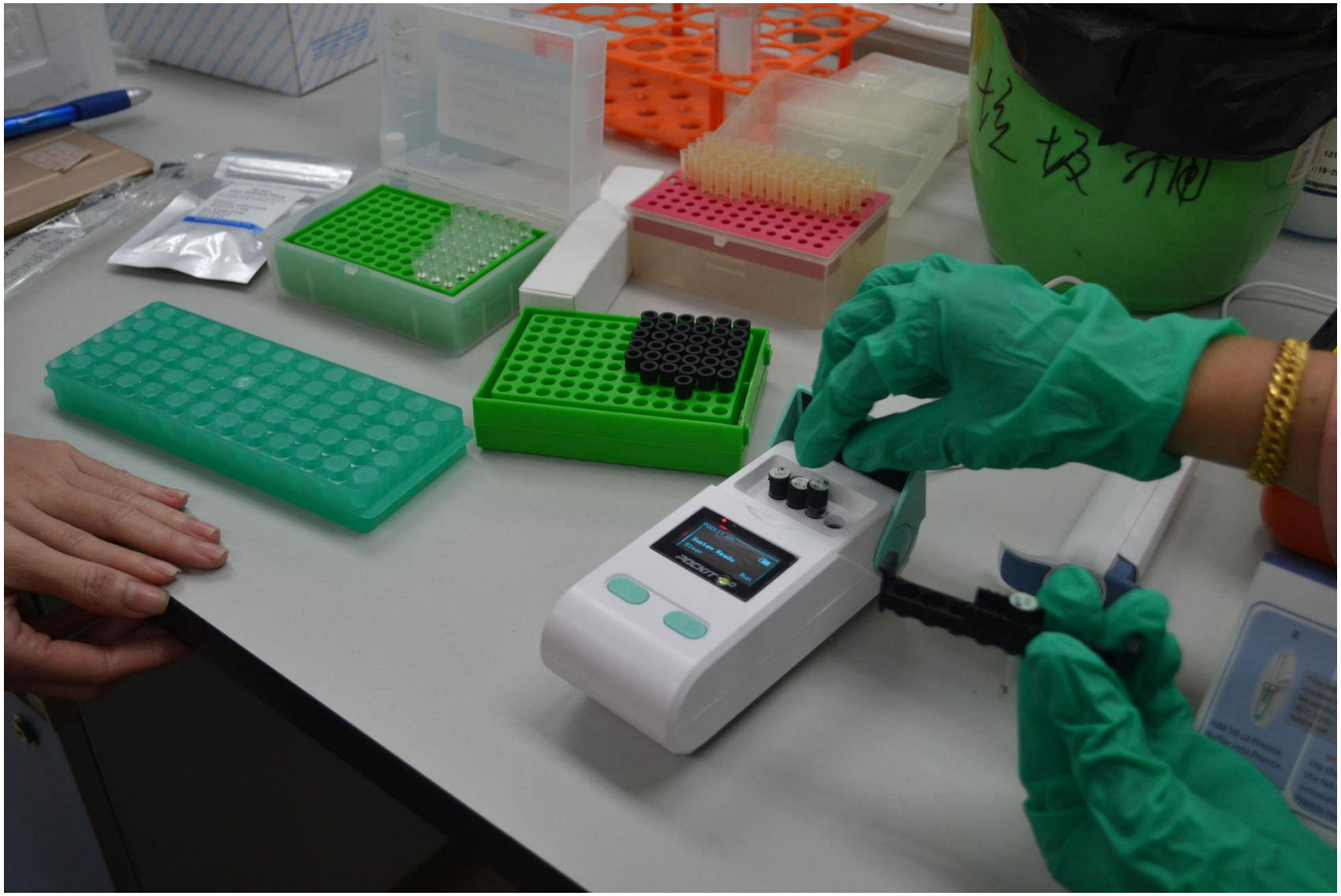
- **Separate rooms for each step**
- **Use laminar flow or biological hoods for each step**
- **Always separate post-PCR processing from all other steps**
- **Dedicated pipettors and lab coats for each work station**
- **Barrier tips for pipetting**
- **Specimens & reagents stored separately**
- **Gloves for operator safety as well as for contamination**
- **Experienced personnel**

- **Keep work areas and equipment clean**:
    1. decontaminate surfaces with chlorine or high pH detergent followed by alcohol wipe;
    2. decontaminate pipettors (wipe off exterior) & expose upside down to UV germicidal light for 15-30 min;
    3. decontaminate racks, centrifuge rotors and heat blocks

- **Run negative controls during extraction and RT-PCR**
- **Keep records of lot numbers & dates when reagents were dispensed and put into use**
- **Maintain a database to track history of farms & populations**

Pondsite detection system

POCKIT™ Micro Plus Nucleic Acid Analyzer	
PCR Amplification Technology	Insulated isothermal polymerase chain reaction (iiPCR)
Fluorescent Wavelength	520 nm (FAM)
Detection Target	DNA / RNA
Sensitivity	Detecting 10 copies per reaction
Throughput	1 - 4 samples per run
PCR Reaction Time	Approx. 45 minutes



Pondsite detection

Run

Run

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Thank you for your attention

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