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 5:

Review of field and laboratory checklist forms

ktangnelson@gmail.com

Melba.Reantaso@fao.org

Food and Agriculture Organization of the United Nations

1. Fish samples submission form

-provide a case identification number

Needed information:
(A) Farm location and reporting

-farm owner(s), site
-person(s) by whom the samples were collected
-date that samples were collected
-name(s) to whom the report will be sent
-name(s) to whom a service fee will be charged

(B) Fish

-species

- **Nile tilapia (*O. niloticus*)**
- **blue tilapia (*Oreochromis niloticus x O. aureus* hybrids)**
- **red tilapia (*Oreochromis* sp.)**
- ***Tilapia zillii***
- ***Sarotherodon galilaeus***
- ***Oreochromis aureus***

-strain (if known)

-life stage

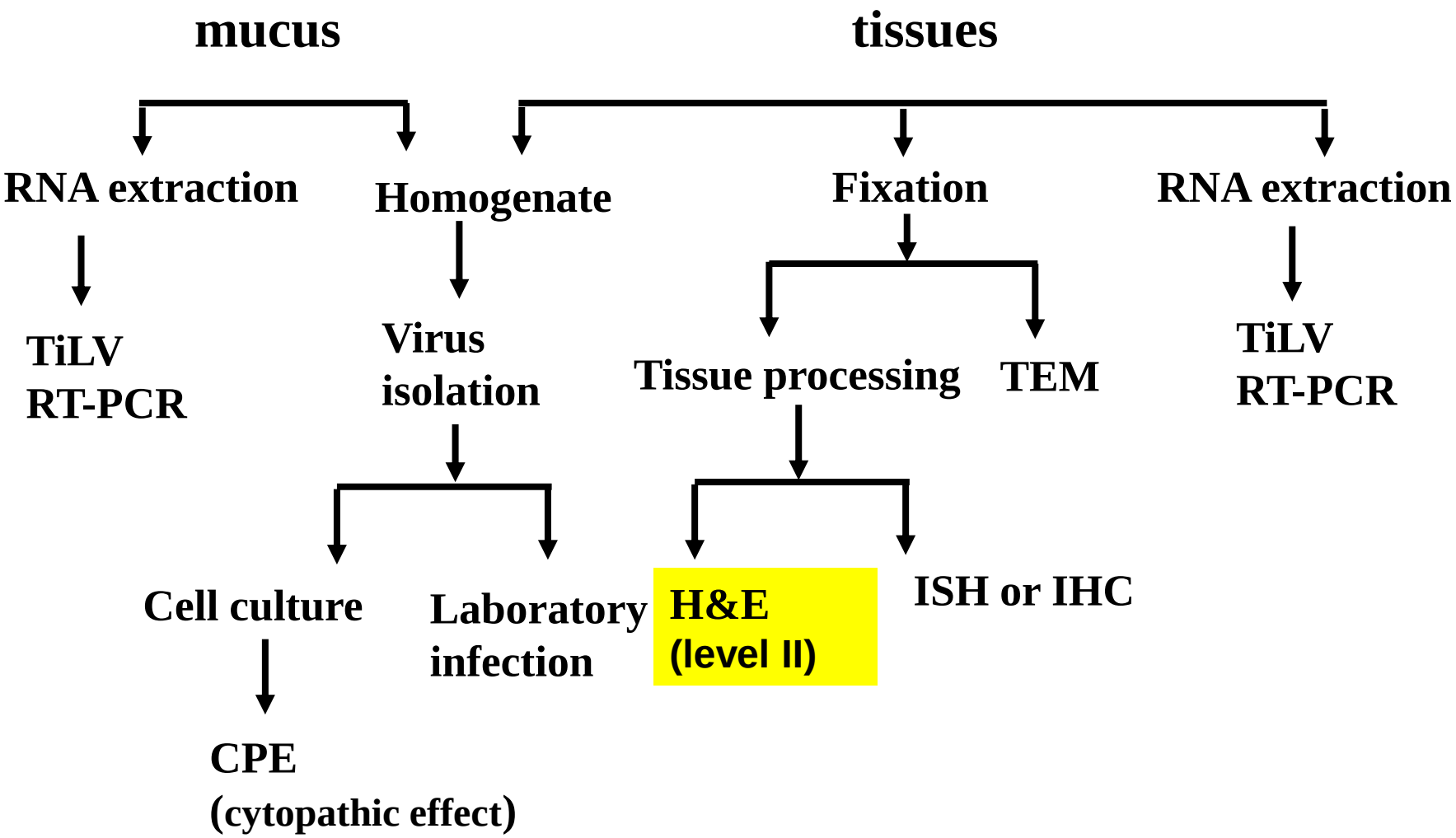
-sex

-Sample ID by the sender

(C) Type of tests to be run
-histology
-RT-PCR
-viral isolation
-other

(D) Type of the samples
-whole fish (larvae, fry)
-tissues: liver, brain, spleen, kidney, heart, gills
-mucus
-cell culture supernatant
-water

Fish samples for TiLV diagnosis



(E) Sample condition

-dead on the ice for < 12 hr, or >12 hr

-dead (kept on room temperature) for > 12 hr
-frozen
-live fish
-fixed in formalin, preserved in 70% ethanol
-fixed in glutaraldehyde (for TEM)
-preserved in 95% ethanol
-others (e.g. RNAlater)

(F) Purpose of testing

-increasing mortality
-moribund
-surveillance
-health certificate
-others

(G) History of treatment

-vaccination
-change water
-others

(H) Gross signs
-body color change
-loss of scales
-skin erosion
-popped eyes
-shrinkge of eyes
-other

(I) Mortality
-% cumulative mortality
-duration

Indonesia's definition for an outbeak: 10% mortality for 3 consecutive days

(A) diseased red tilapia showed hemorrhage (black arrows);

(B) diseased Nile tilapia showed skin erosion, hemorrhage on various parts of body, loss of scales, abdominal swelling, and swelling of the eyeball (exophthalmos);

(C) diseased wild tilapia (*Sarotherodon galilaeus*) showed shrinkage of the eye and loss of ocular functioning.

Gross signs include erosions and ulcerations in the skin and unilateral or bilateral ocular alterations (cataracts).

June, 6, 2017, Taiwan province
Mortality: 100's per day, for 2-weeks
clinical signs: abdominal swelling, hemorrhage, loss of scales

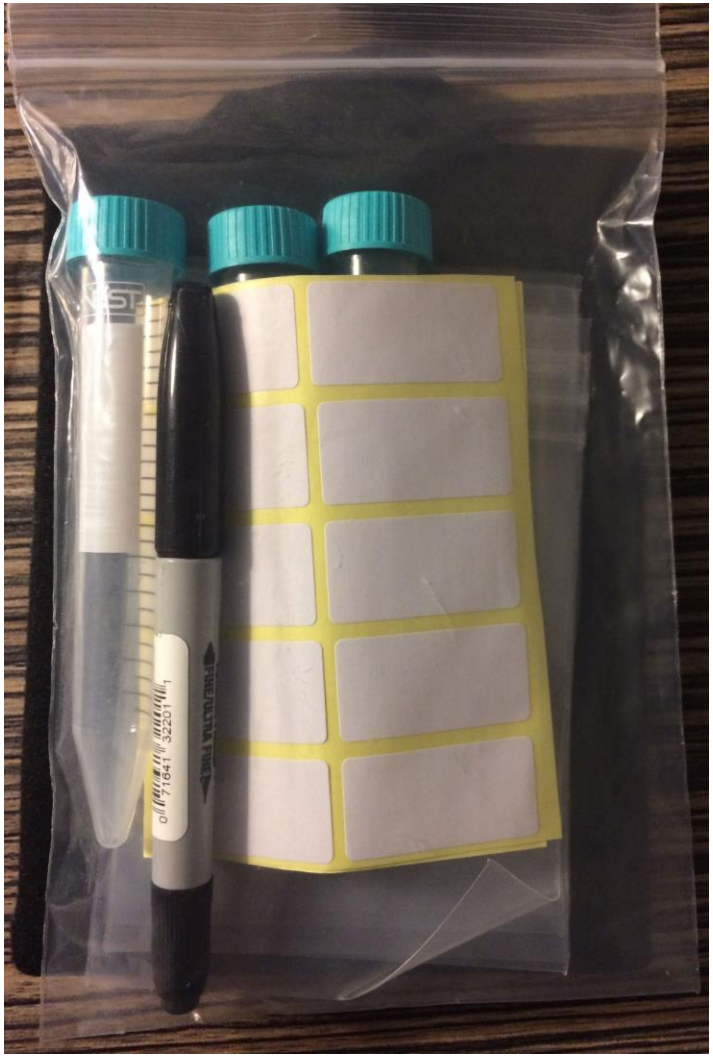
(j) Abnormal behavior

-gasping
-flashing
-scrapping
-crowding in the water inlet/outlet
-lethargic
-swim erratically
-swim in circles

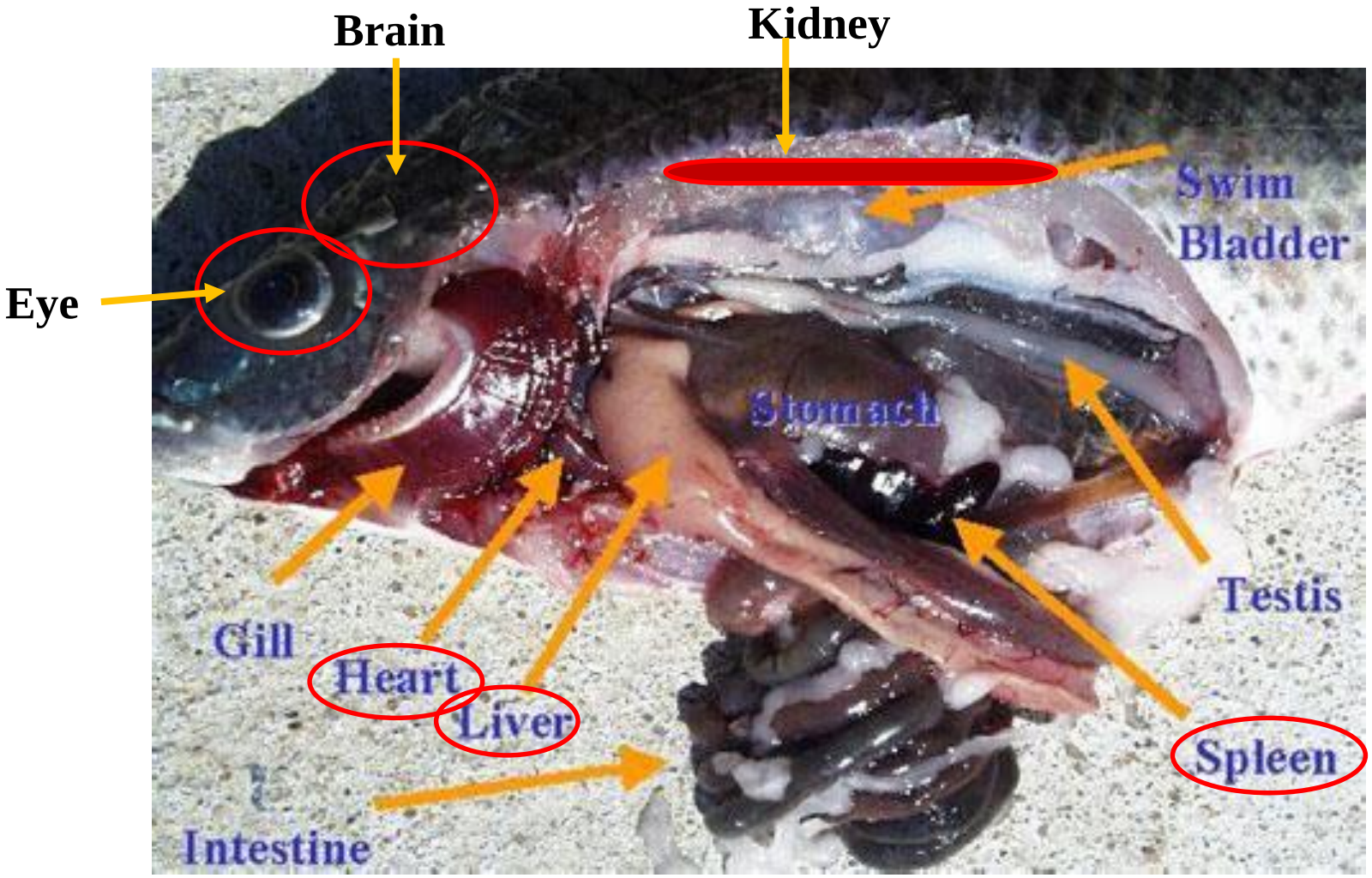
(K) Abnormal feeding pattern

-loss of appetite
-others

Sampling tools




CONVERSION
TABLE
VINTOP



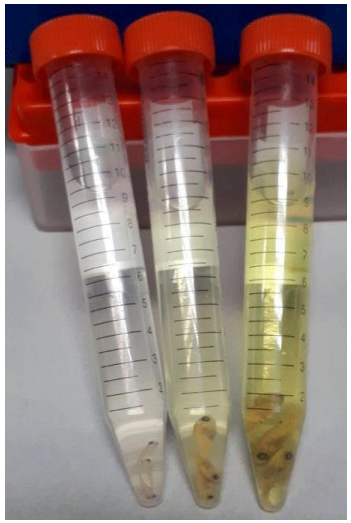
Tilapia tissue sampling for RNA extraction:

Fresh, frozen, or preserve in ethanol (70-95%)

Non-invasive: mucus

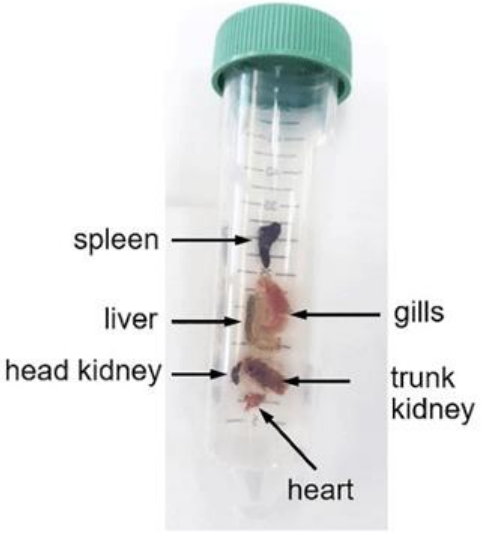
Credited to Dr. Dong Ha

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



Histology

Large fish, perform necropsy



- Where to send the samples?
- Positive results from the diagnostic lab
- Report to the competent authority?
  Notify to OIE?
- How to deal with the outbreaks?

Capability for TiLV diagnosis with RT-PCR

1) **Do you have a functional laboratory testing aquatic animal diseases: ☐Yes; ☐No**
   If yes,
2) **Do you have PCR diagnostic laboratory: ☐Yes; ☐No**
   If no:
   -Do you have access to a PCR diagnostic laboratory: ☐Yes; ☐No

3) Do you have trained staff that can add TiLV testing to your existing diagnosis work: ☐No

If yes, please mark the checklist below:

Country	Functional Lab	Access to a PCR lab	Add TiLV PCR	Reagents
Angola				
Egypt				
Ghana				
Kenya	No	Yes		
Nigeria				
Uganda	Yes	Yes		Nested RT-PCR: Verso 1-step RT-PCR Reddy mix kit 2X Reddy mix PCR master mix RT-qPCR: ~~SuperScript III first-strand synthesis system super mix~~ TaqMan fast virus 1-step master mix

Reagents for Fast PCR (40 min reaction)

RT-qPCR mix	Cat no. (ThermoFisher)
SuperScript III first-strand synthesis super mix	18080400
TaqMan™ Fast Virus 1-Step Master Mix (M-MLV reverse transcriptase & AmpliTaq Fast DNA polymerase)	4444432

Cycling profile at the fast mode for TiLV RT-qPCR)

Step	Stage	No of cycle	Temperature	Time
Reverse transcription (RT)	1	1	48°C	**5 min**
RT inactivation/initial denaturation	2	1	95°C	**20 sec**
Amplification	**3**	**40**	95°C	**1 sec**
			60°C	**20 sec**

Fish Farm Survey Form

1. Facility's name: _____

2. Address: District _____ Province _____

3. Facility area: ____; Culture area: ____; Number of tanks/ponds: _____

4. Pond/tank type: ☐ Outdoor; ☐ Indoor; ☐ Others: _____

5. Water management: ☐ Open; ☐ Closed; ☐ Semi-closed; ☐ Recirculating

6. Tilapia cultured: ☐ Nile tilapia; ☐ Hybrid tilapia; ☐ Other species_____

7. Source of broodstock: ☐ Domestic; ☐ Imported broodstock, countries: __ ;☐ Both

8. How many crops are produced per year? _____

9. Market: ☐ Domestic; ☐ International, countries: _____; ☐ Both

11. Is the facility fenced? ☐ Yes; ☐ No

12. Are visitors restricted from all or portions of the facility? ☐ Yes; ☐ No

13. Are there pets or other animals roaming freely in the facility:

☐ Yes, what kind of pet _____; ☐ No

14. Are vehicles disinfected prior to entering the facility?

☐ Vehicle body spray; ☐ Tire bath; ☐ None

15. Are hand- and foot-disinfection baths available for personnel entering the facility? ☐ Yes; ☐ No

16. Are separate supplies/equipment used for each tank/pond (or cluster of tanks/ponds)? ☐ Yes; ☐ No

17. what kind of feed is used? ☐ Commercial feed; ☐ Other feed _____

18. Is feed supplementation used?

☐ Probiotics (☐ licensed, ☐ non-licensed), type _____; how is it applied: __

☐ Special diets: _____

☐ Antibiotics/drugs: ☐ Yes, type: _____ ; ☐ No

19. Any unexplained mortality occurring recent years?

☐ No; ☐ Yes, percent mortality: _____; observed gross signs: __

20. Diseases/pathogens found in the facility:

☐ No; ☐ Yes, what disease/pathogen _____

21. Are samples sent for laboratory examination/tests?

☐ No; ☐ Yes, what are the tests: _____

22. Are samples analyzed at facility?

☐ No; ☐ Yes, what is the test kit: _____

23. Does the facility personnel know about tilapia lake virus:

☐ Yes; ☐ No

Information recorded	Yes	No	Comments
A. Feeding activities			
Date, time, tank/pond#			
Feed source			
B. Water quality management			
Date, time			
Salinity			
Algae blooms			
Dissolved O_2, pH, NH_3, No_2^-, temperature			
Water exchange			
Pipe flushing			
Filter back flushing			
C. Growth condition			
Weight, length, condition factor			
D. mortalities			
Date, time			
Gross signs			
Sampling for diagnosis			
E. Disinfection			
Date, chemical's name, concentration, treatment duration			
F. Human activities			
Date, name of visitor(s)			
G. Others			

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)

Food and Agriculture Organization of the United Nations