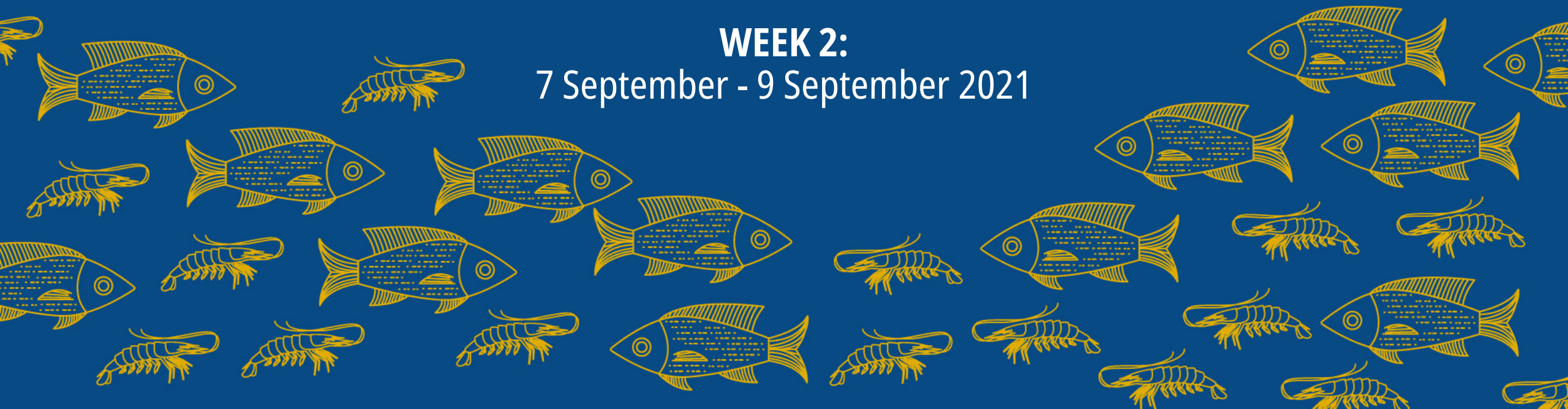
Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

TCP/EGY/3705: Enhancing biosecurity governance to support sustainable aquaculture production in Egypt

Virtual Course on an Active Surveillance Design Using a 12-point Checklist for Diseases of Aquatic Species



WEEK 2:
7 September - 9 September 2021

Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

8 September 2021

CHECKLIST 6

Field and laboratory preparation, checklists, and forms

Presented by

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TCP/EGY/3705: Enhancing biosecurity governance to support sustainable aquaculture production in Egypt

Outline

- To achieve optimal conditions for tilapia culture and the need for accurate record-keeping on water quality, fish health, and farm operation
- To identify gross signs of TiLVD-affected tilapia
- To conduct fish necropsy and examine for internal lesions of TiLVD-affected tilapia
- To sample TiLV-infected fish tissues (liver, spleen, brain, heart, kidney, gills and muscle) for diagnostic analyses
- To design a sample submission form for diagnosing TiLVD
- To develop checklists of supplies and equipment needed for TiLV diagnosis

Optimal temperature and water quality for Nile tilapia

Growth condition	Optimum	Range
Temperature (°C)	27-30 (warm water)	12-38
Dissolved oxygen (mg/L)	> 5	
Salinity (ppt)	5-10	<25
pH	6-9	5-10
Ammonia (NH_3) (mg/L)	< 0.1	
Nitrate (NO_3^-) (mg/L)	Keep < 27	n/a
Alkalinity (mg $CaCO_3$/L)	Keep <300	

Poor water quality can result suppression in immunity, increase risk for disease and mortality

Checklist for record-keeping of fish hatchery/farm

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, temperature, pH, NH_3, No_2^-			
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 (e.g. source and transfer of stock)			



A

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



B

(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

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

Supplies and data sheet for gross signs examination

Lab work	Equipment, supplies, data sheets	
External gross signs examination (Level I)	☐data sheet recording any abnormalities in behavior, body, skin, gills, eyes	☐disposable lab coats and gloves ☐camera ☐Compound microsope ☐microscope slides, coverslips ☐scalpel, forceps, scissors

External examination data sheet

1. behavior (normal, gasping, flashing, crowding in the water inlet/outlet, lethargic, swim erratically, etc).

_____

2. body appearance (normal, swelling, color change, etc)

_____

3. skin (normal, hemorrhagic, loss of scales, erosion, etc.)

_____

4. gills (normal, pale, tissue losses, etc)

_____

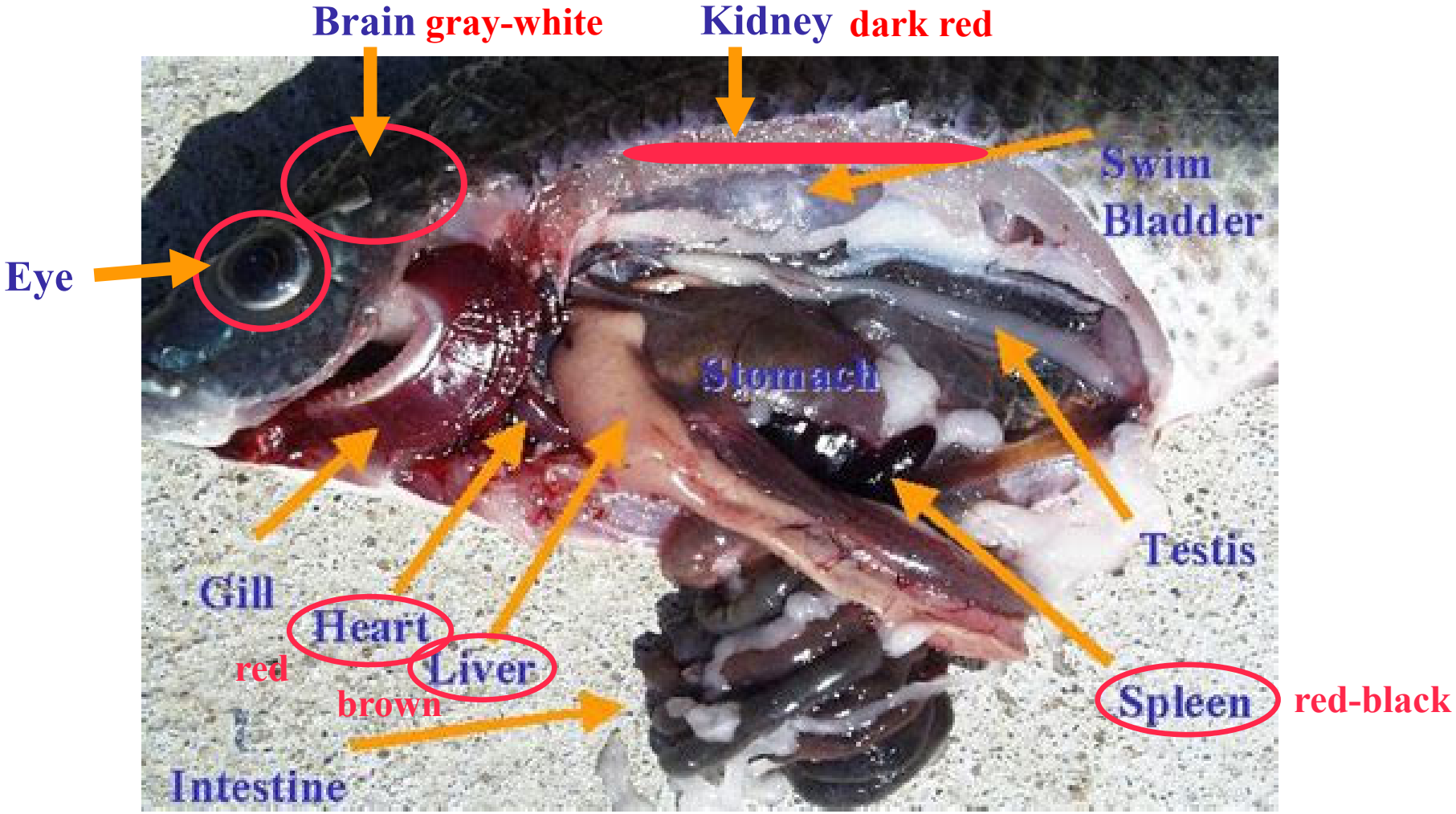
5. eyes (normal, exophthalmos, shrinking, cataracts, etc)

_____

6. photo/video: _____
7. skin scraping results: _____
8. gill scraping results: _____

Supplies for necropsy

Necropsy	☐fish nets ☐buckets ☐sedation chemicals (e.g. MS-222, 50-100 ppm) ☐measuring type/balance ☐sample jars, tubes or bags ☐sample ID labels ☐Permanent markers ☐95% ethanol	☐ laboratory gowns/latex groves/mask ☐dissecting kit (scissors, forceps, scalpels, etc) ☐dissecting tray ☐flame ☐sampling sticks, disposable razor blades ☐paper towel ☐trash bags



Tilapia tissue sampling for RNA extraction:

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

Non-invasive: mucus

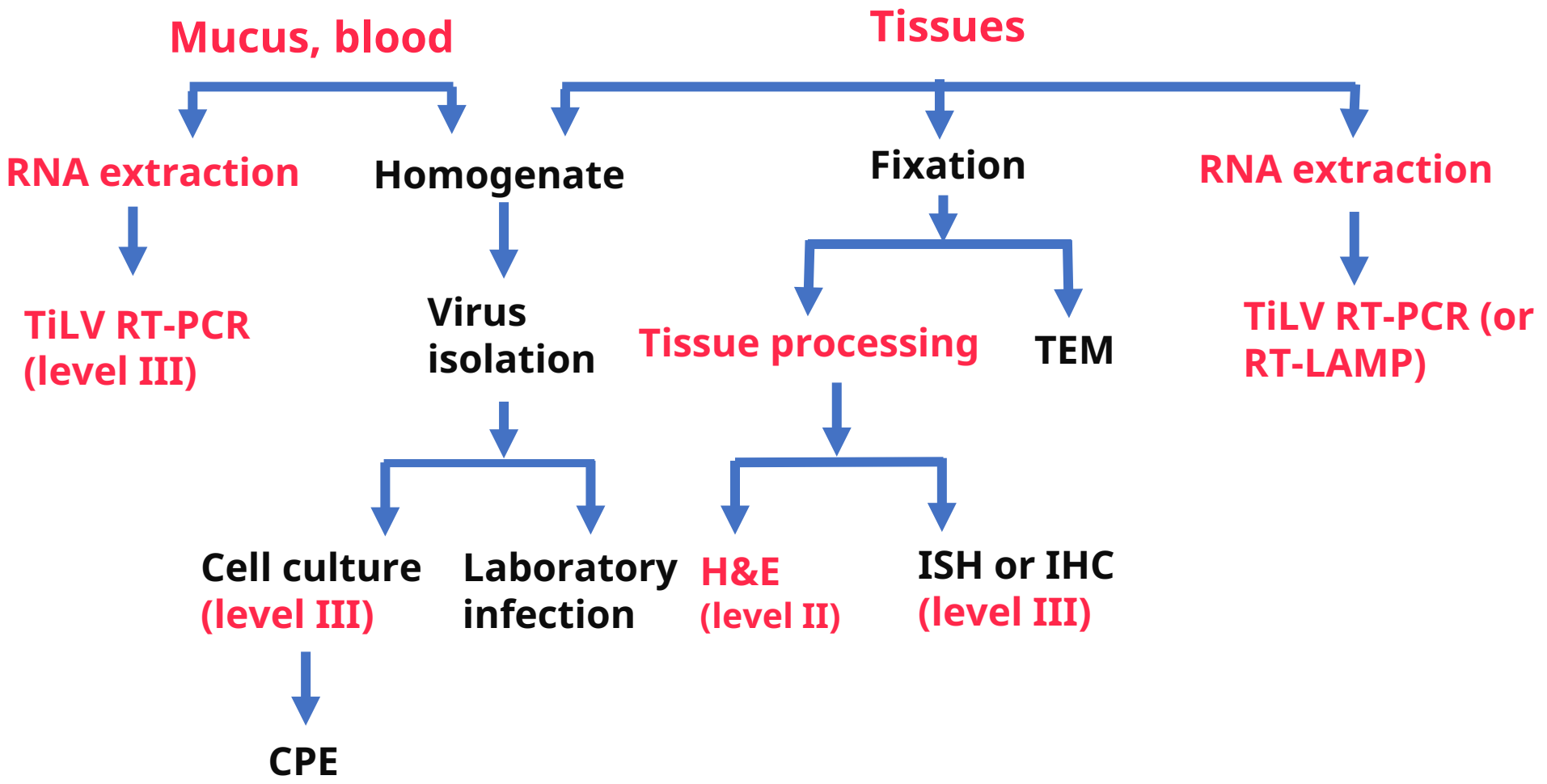
Data sheet for internal gross signs examination

Internal gross signs examination (Level I)	☐data sheet to record any abnormalities in the internal organs

Internal examination data sheet

1. visceral cavity (normal, acites, hemorrhage, etc)
2. Liver (normal, green, pale, watery, nodules, etc)
3. stomach (normal, empty, etc)
4. intestine (normal, empty, hemorrhage, fluid accumulation, etc)
5. spleen (normal, swollen, etc)
6. swimming bladder (normal, hemorrhage, etc)
7. kidney (normal, nodules, etc)
8. heart (normal, pale, etc)
9. brain (normal, reddness, etc)
9. gills (normal, hyperplasia, etc)
10. other tissues
11. photos/video

Fish samples for TiLV diagnosis (level II and III)



Diagnosis usefulness of fish condition

SPECIMEN	HISTOLOGY	RT-PCR	IMMUNO ASSAY	VIRUS ISOLATION
Live fish	++	++	++	++
Dead fish (>6 h at room temperature)	-	-	-	-
Fresh dead fish in a plastic bag on ice for <6 h	+	+	+ (ELISA)	+
Frozen fish (-20ºC, or dry ice)	-	++	++ (ELISA)	++
Formalin-fixed	++	-/+	++ (IHC)	-

[Name of the laboratory] [contact information]

Sample submission form

Sample information	Completed by laboratory
Name of submitter: Address: Phone# Email: Collection site: Collection date: Click or tap to enter a date.	Case ID: Check in by: Receipt date:Click or tap to enter a date.
Name of owner: Farm address: Phone#: Email:	☐ same as the submitter

Reporting information	
Name of the recipient: ☐ same as the submitter Address: Phone#: Email:	**Send report through:** ☐Email: ☐Regular mail: ☐Courier: ☐Fax:

Animal information

Sample ID	Species	Strain	Age	Size(length, weight)	Sex	Choose an item.

*test selection is under dropdown-list: necropsy, bacteriology, parasitology, histopathology, molecular test, virology, others

Specimen type and quantity received

☐Blood Qty:	Tissue type: (fresh) Qty:	Tissue type: (frozen) Oty:
Tissue type: (fixed) Qty:	Histology slide Qty:	Other: Qty:

Number of animals per sample (pooled):

Reasons for submitting samples	☐ Disease outbreak; ☐ Surveillance; ☐ Import/translocation, country/state origin: ☐ Export/translocation, country/state destination: ☐ Others:

Clinical signs and mortality

History of treatment, vaccination, or drug administration

(A) Farm location and reporting

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

(B) Fish information: species

- Nile tilapia (*O. niloticus*)
- blue tilapia (*O. niloticus x O. aureus hybrids*)
- red tilapia (*Oreochromis sp.)*
- *Tilapia zillii* (wild cichlid in Africa and Middle East)
- *Sarotherodon galilaeus* (wild cichlid in Africa and the Levant)
- *Oreochromis aureus*
- strain (if known)
- life stage

(C) Type of diagnostic tests to be performed

- gross signs (level I)
- histology (level II)
- RT-PCR (level III)
- viral isolation and cell-culture (level III)
- other

(D) Type of the samples

- whole fish (larvae, fry)
- tissues: liver, brain, spleen, kidney, heart, gills, muscle
- mucus, blood
- cell culture supernatant
- pondwater
- feces

(F) Purpose of testing

- disease outbreak
- surveillance
- health certificate
- others

(G) Gross signs (level I)

- body color change
- loss of scales
- skin erosion
- popped eyes (exophthalmos) or shrinkage of eyes
- % cumulative mortality, duration
- others

(H) Abnormal behavior

- gasping (gills, environmental problems)
- flashing (skin irritation)
- scraping the body (parasite problems)
- crowding in the water inlet/outlet
- lethargic
- swim erratically, in circles

(I) Abnormal feeding pattern

- loss of appetite
- others

(J) History of treatment

- vaccination
- change water
- therapeutants
- others

Supplies and equipment for histology

Histology (Level II)	☐neutral buffered formalin (NBF) ☐fume hood ☐tissue processing machine ☐tissue embedding machine ☐microtome ☐staining solutions	☐compound microscope ☐microscope slides ☐coverslips

Supplies and equipment for PCR-based methods

PCR-based (level III)	RNA extraction ☐RNA extraction kit ☐Microfuge ☐pipettors (50-, 1000- µl) ☐filtered pipet tips ☐Eppendorf tubes ☐PCR tubes ☐test tubes racks	**Conventional RT-PCR** ☐PCR machine ☐Gel electrophoresis apparatus ☐Gel imaging system ☐Pipettors ☐RT-PCR enzymes kit ☐TiLV primers ☐ electrophoresis buffer, molecular marker, gel loading dye, ethidium bromide ☐positive control plasmid **RT-qPCR (real-time)** ☐Real-time PCR system ☐RT-qPCR enzymes ☐TiLV real-time primers ☐positive control plasmid

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Thank you for your attention!

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