 UTF/077/ZAM: Technical Assistance to the Zambia Aquaculture Enterprise Development (ZAED)

**Session 3:
Review of sample submission form**

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1. Fish samples submission form

-provide a case identification number (e.g. 19-xxx)

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

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(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

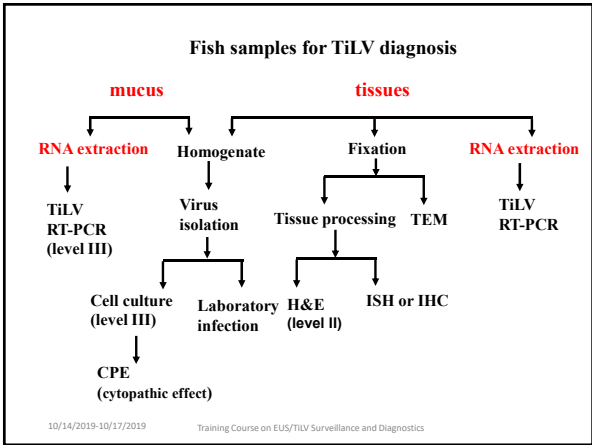
-Sample ID by the sender

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

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

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Diagnosis usefulness of fish condition

Specimen	Histology	RT-PCR	Immuno assay	Virus isolation
Live fish	++	++	++	++
Dead fish (>6 h at room temperature)	-	-	-	-
live fish in a plastic bag on ice for <6 h	+	+	+	+
Frozen fish (-20°C)	-	++	++	++
Formalin-fixed	++	-	++	-

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(F) Purpose of testing

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

(G) History of treatment

- vaccination
- change water
- others

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(H) Gross signs

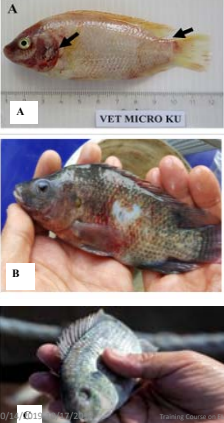
- body color change
- loss of scales
- skin erosion
- popped eyes
- shrinkage of eyes
- other

(I) Mortality

- % cumulative mortality
- duration

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

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(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.

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(j) Abnormal behavior

- gasp (gills problems)
- flashing (skin problems)
- scrapping
- crowding in the water inlet/outlet
- lethargic
- swim erratically
- swim in circles

(K) Abnormal feeding pattern

- loss of appetite
- others

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Sampling tools



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Inside the bags



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Credited to Dr. Dong Ha Histology

Tilapia tissue sampling for histology:
Tissues from live or moribund tilapia preserve in 10% neutrally buffered formalin; **do not sample dead or frozen fish.**

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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

spleen
liver
head kidney
gills
trunk kidney
heart

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Brain **Kidney** RT-PCR

Tilapia tissue sampling for RNA extraction:
Fresh, frozen, or preserve in ethanol (70-95%)

Non-invasive: mucus

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Laboratory and pondsite detection system



POCKIT™ Micro Plus Nucleic Acid Analyzer	
PCR Amplification Technology	Insulated isothermal polymerase chain reaction (iPCR)
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

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Thank you for your attention

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