Food and Agriculture Organization of the United Nations

FAO/China Intensive Training Course on Tilapia Lake Virus (TiLV)

Sun Yat Sen University, Guangzhou, China

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Session 2

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Fish necropsy & Sample collection for TiLV diagnosis

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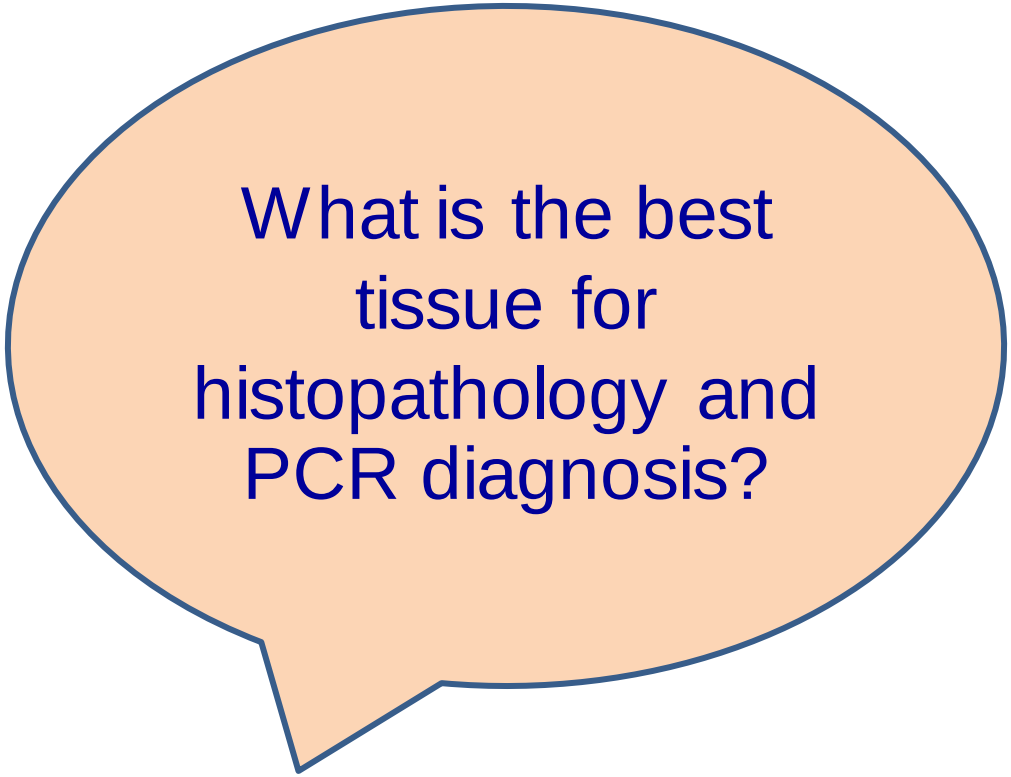
Objectives

- To be able to understand
  - ❖What are target tissues of TiLV?
  - ❖How to necropsy and collect samples?
  - ❖How to preserve tissues for histology?
  - ❖How to preserve tissues for PCR?

What are target tissues of TiLV?

- Liver
- Kidney
- Spleen
- Brain
- Gills
- Muscle
- Blood
- Mucus



How to necropsy and collect samples?

Record basic information

Collect fish samples

Collect samples for TiLV diagnosis
(non-destructive & destructive)

Preserve samples for histology & PCR

What need to be recorded?

- General information of fish farm
- Fish species, sizes, source
- Environmental parameters
- Disease history
- Clinical signs (both external and internal), abnormal behaviors (plus pictures, video)
- Mortality rate
- Others

Sample collection

Non-Destructive sampling

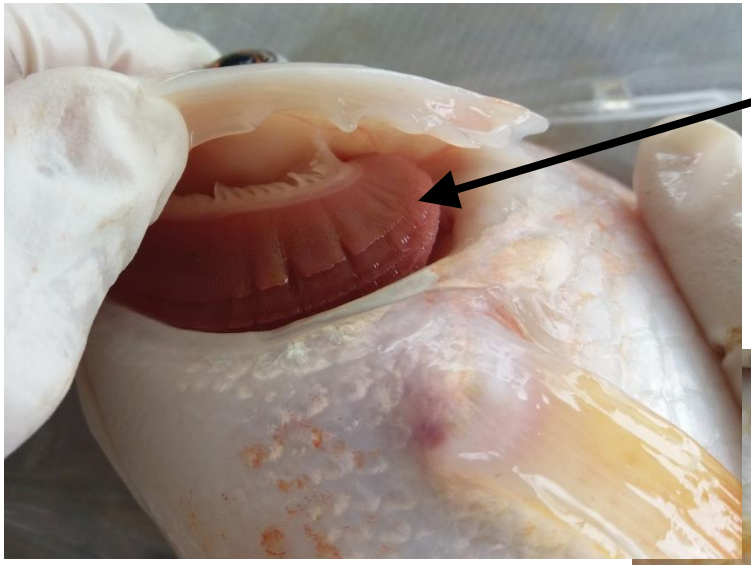
- Feces
- Gill filaments
- Blood
- Mucus
- Fins
- Liver biopsy

Destructive sampling

- Whole fish (for small fish such as fry, fingerlings)
- Some parts of the body (liver, spleen, kidney, gills, etc.)

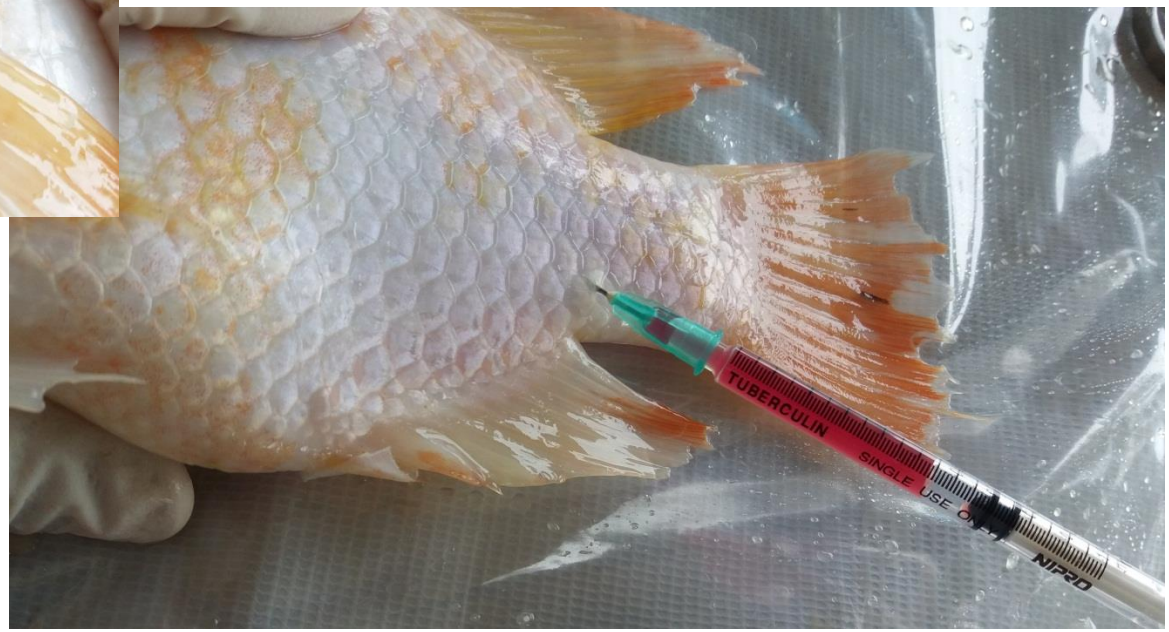
Non-destructive sampling

(Broodstock or ornamental fish)



Cut 2-3 filaments or collect mucus using a cotton swab

Blood collection



Destructive sampling for TiLV diagnosis

Small fish
(< 2 g)

Preserve whole fish (Slide A)

Large fish

Necropsy (Slide B)

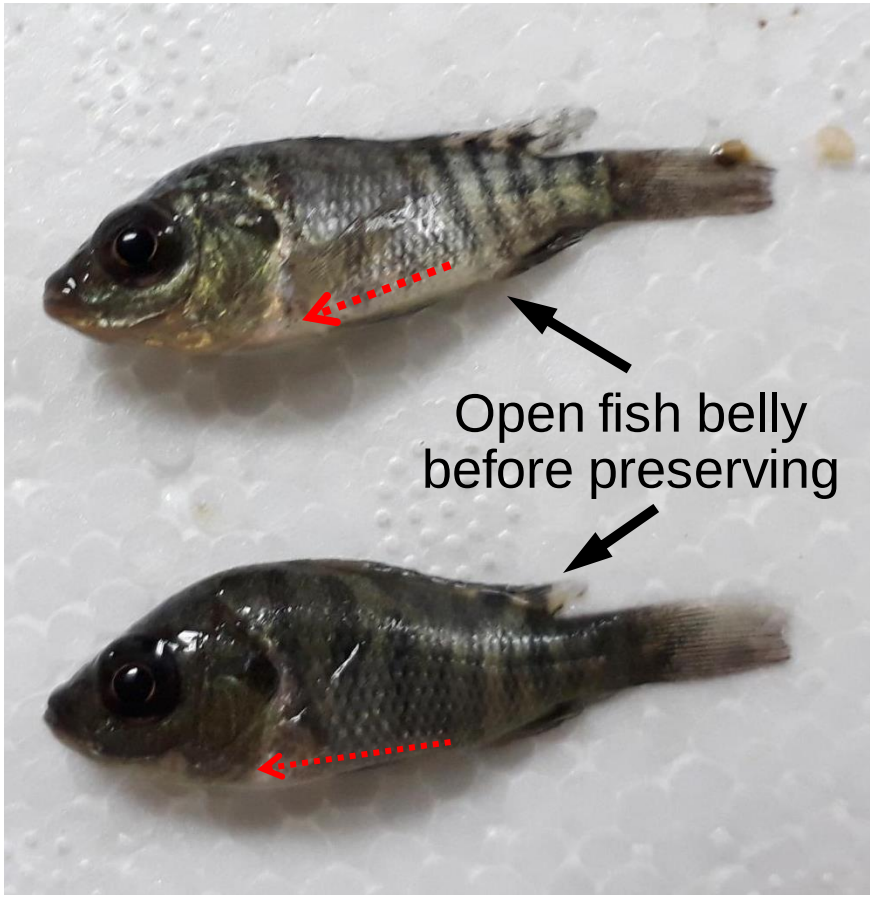
Liver, Kidney, Brain

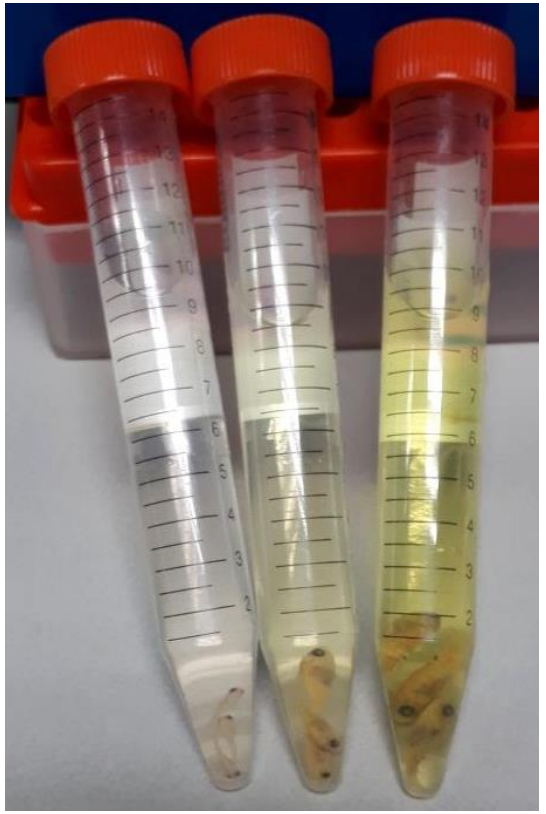
Preserve (Slide C)

Note: Liver, kidney and brain are recommended organs for TiLV diagnosis. However, other organs such as gills, spleen, muscle, mucus, etc. maybe possible for diagnosis as well.

Slide A: Preservation

Small fish < 2 g (open fish belly if possible)

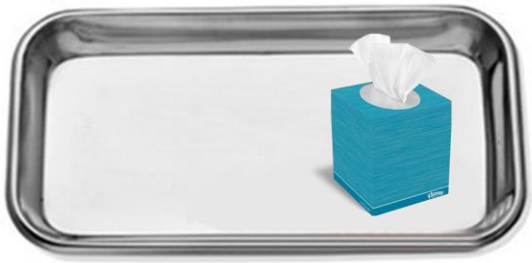




For PCR or histology

Slide B: Fish Necropsy

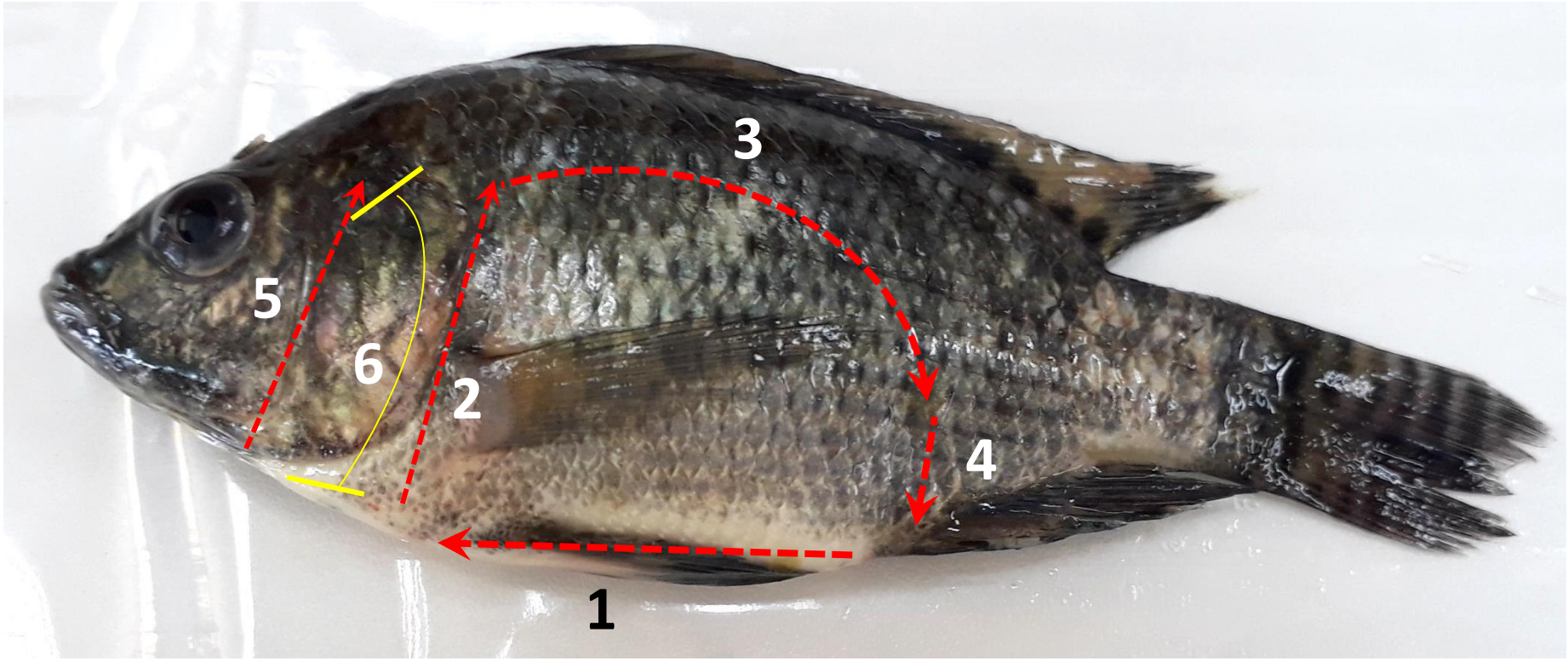
1. Preparation of scissor, forceps, tray, tissue paper for dissection
2. Terminate the fish by an overdose of clove oil (≥100 ppm) or ice
3. Disinfect the fish body surface with alcohol 70%
4. Dissect the fish (see picture below)
5. Collect target tissues for different purpose (histology, molecular analysis, TEM or virus isolation)



add clove oil into water

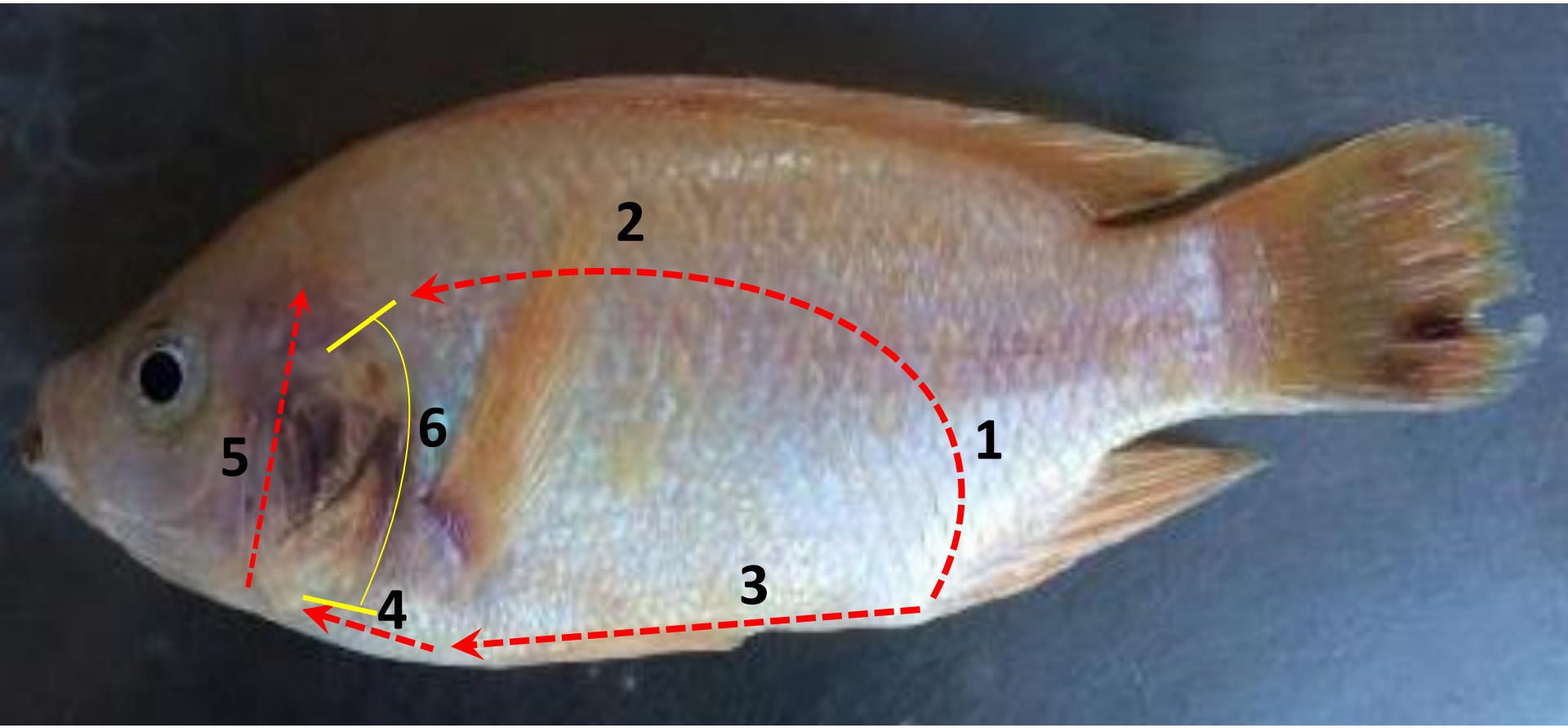
Slide B: Fish Necropsy

Slide B: Fish Necropsy



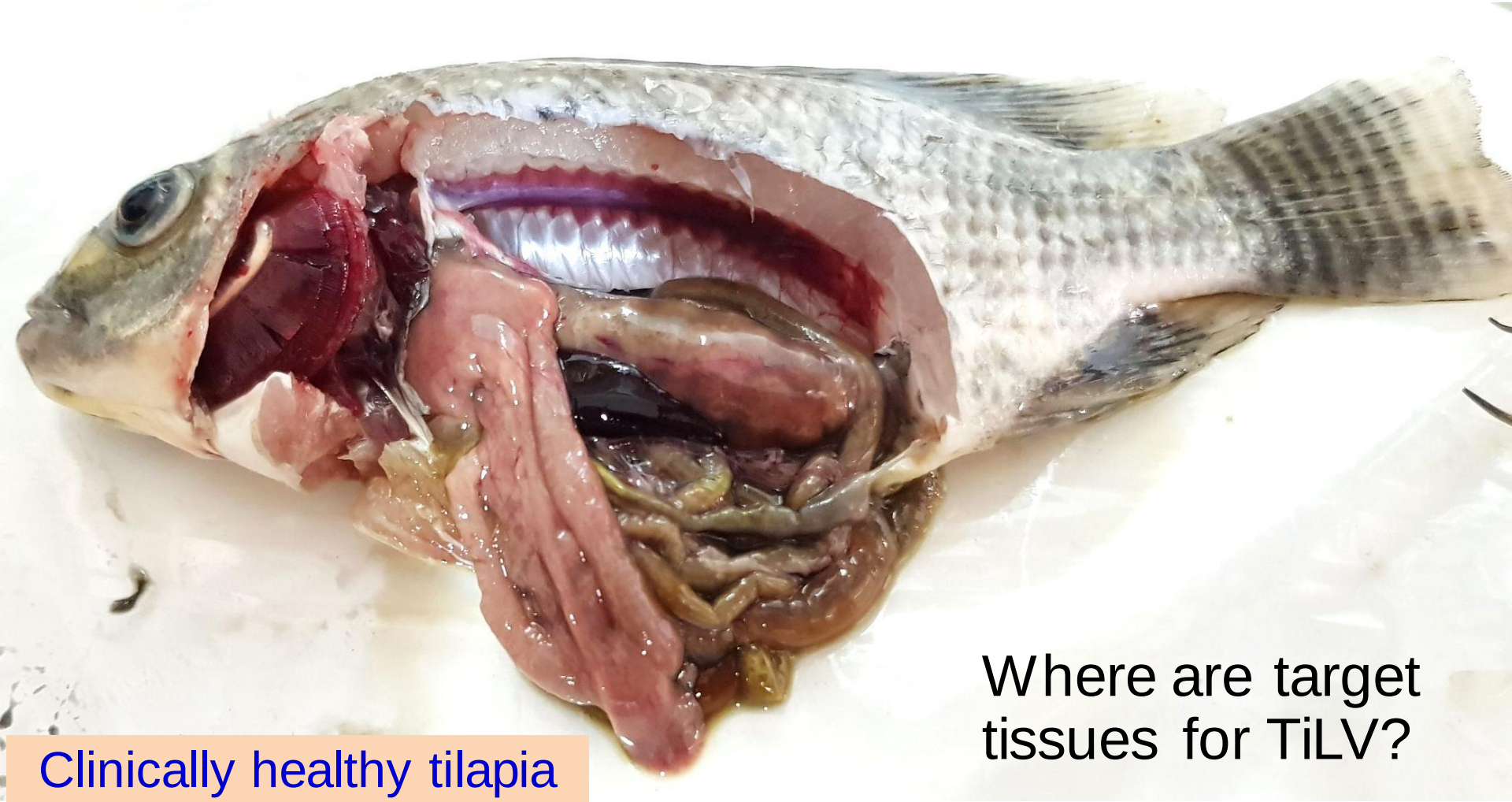
How to dissect a fish?

Slide B: Fish Necropsy



How to dissect a fish?

Slide B: Fish Necropsy

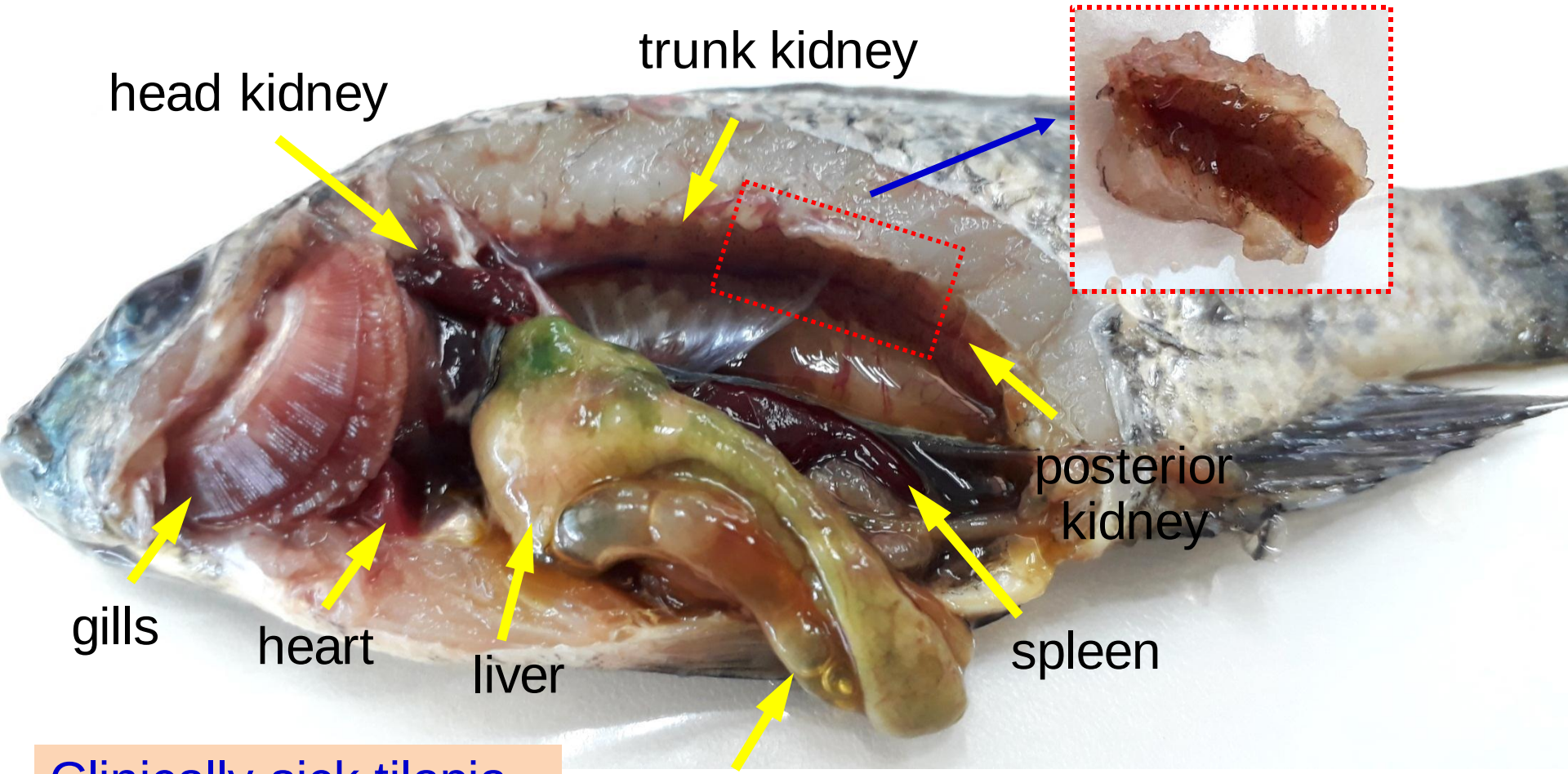


Clinically healthy tilapia

Where are target tissues for TiLV?

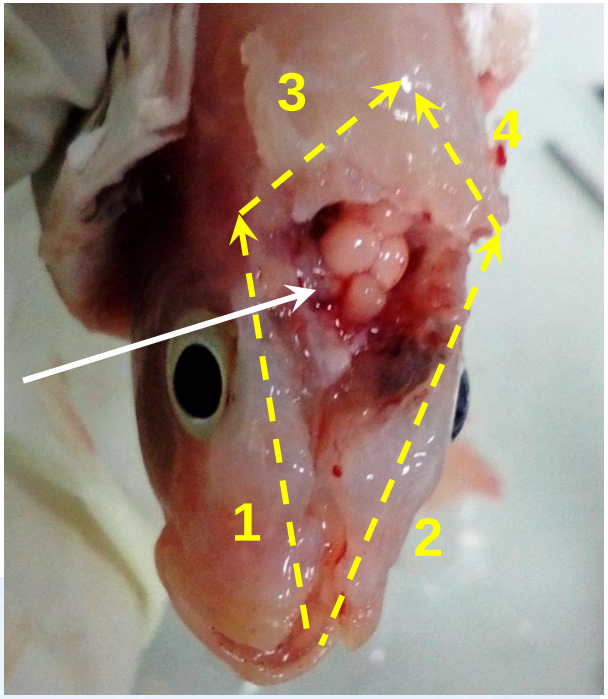
Slide B: Fish Necropsy

How to collect kidney?

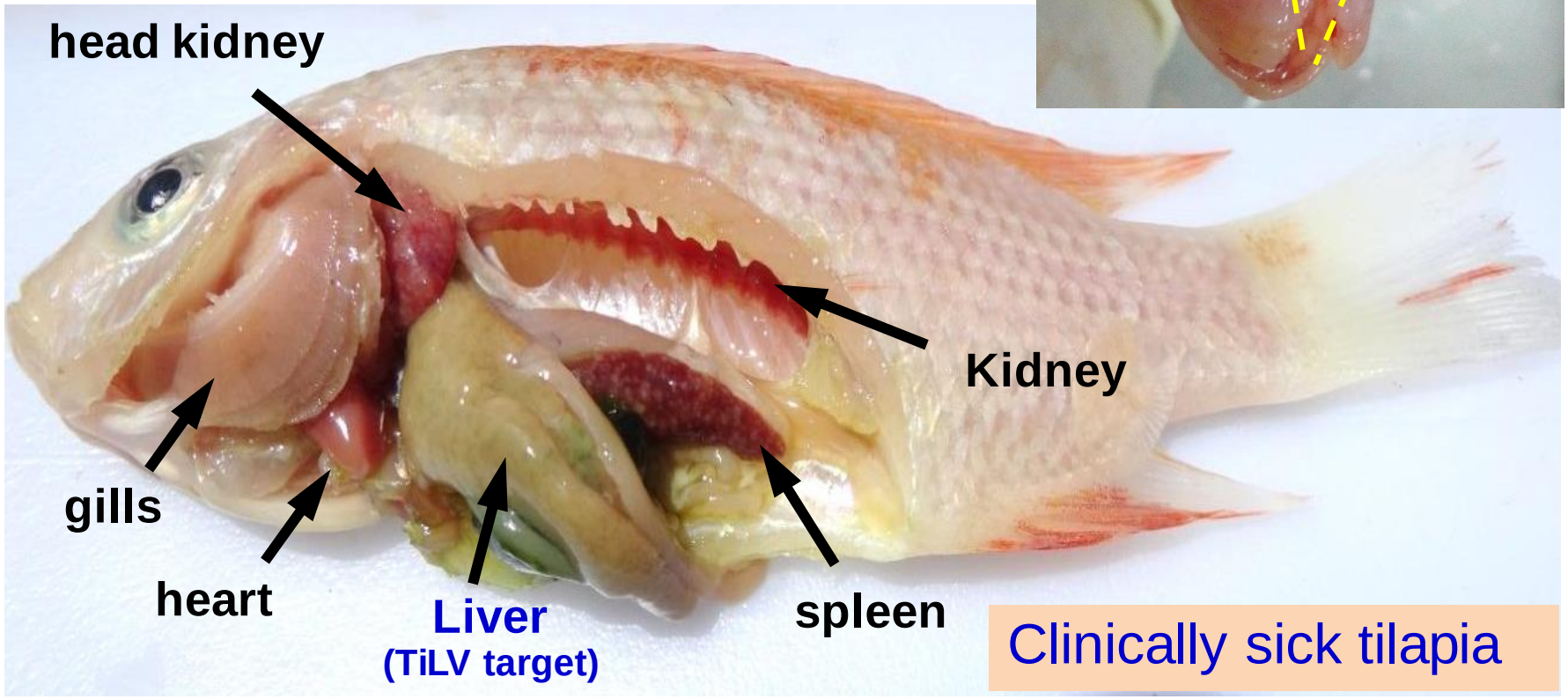


Clinically sick tilapia

Slide B: Fish Necropsy

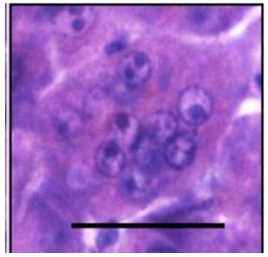
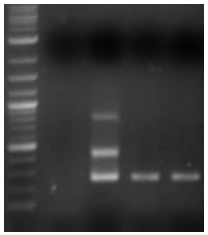
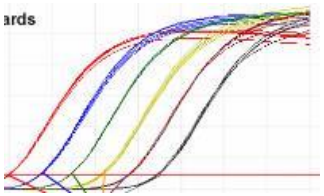


How to collect brain?



Clinically sick tilapia

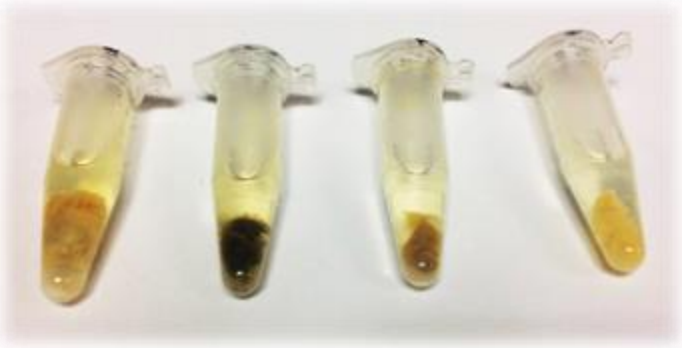
Slide C: Preservation

Purpose	Procedures
Histology 	• Fix in 10% neutral buffered formalin for 24h (sample: fixative, 1:10 (v/v)), then change to alcohol 70% for long-term preservation
Molecular analysis (PCR, RT-PCR, qPCR)  	• Alcohol 95% (sample: fixative, 1:10 (v/v)) • Trizol or RNA later (sample: fixative, 1:10 (v/v)) • Fresh sample, frozen sample

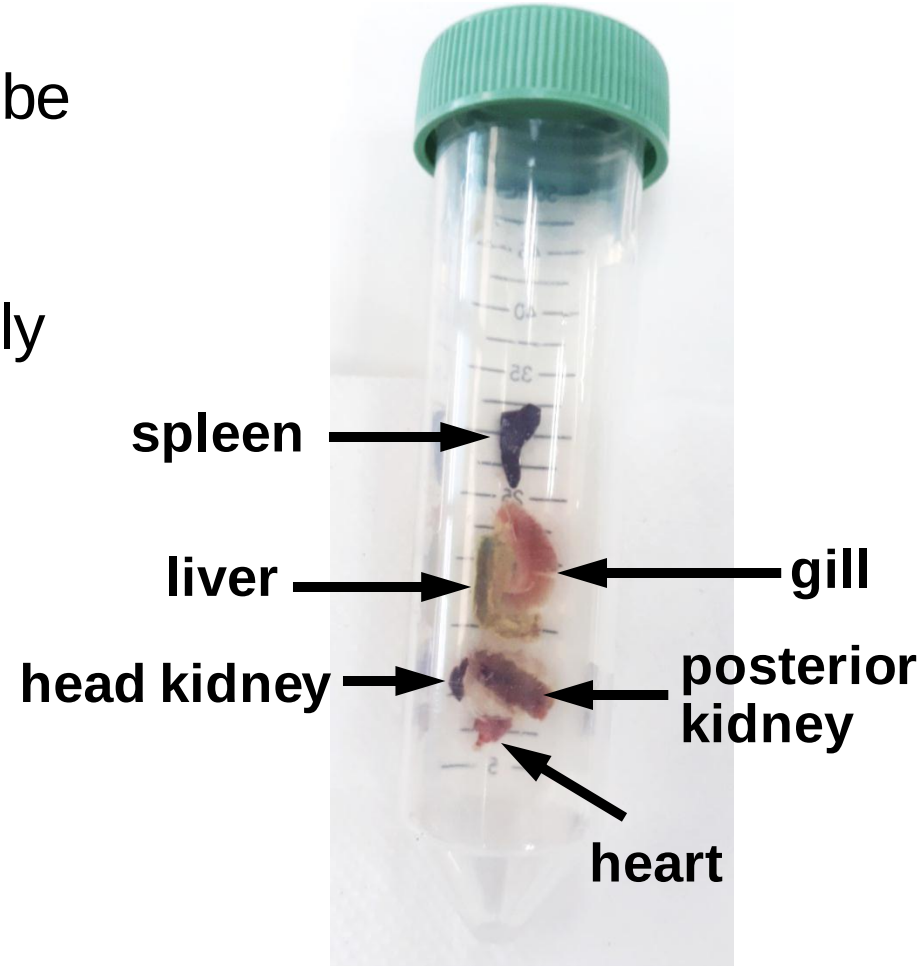
Slide C: Preservation

- For histology, all organs can be preserved in the same tube/ bottle
- For PCR, preserve individually or pool together

- Size of tissue: ~1 x 1 cm



Samples in alcohol 95% for PCR



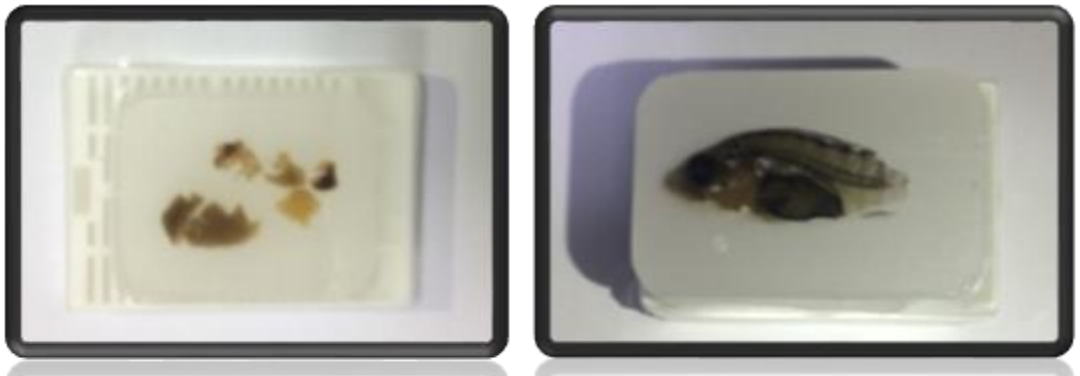
Samples in 10% NBF for histology

Slide C: Preservation

- Frozen samples can be used for PCR or viral isolation
- Deep frozen (-80 ^{o}C) is recommended if you aims for viral isolation
- -20 or -40 ^{o}C is sometimes acceptable if no -80 ^{o}C refrigerator available
- Deep frozen is useful for retrospective study

Frozen

Slide C: Preservation



Paraffin blocks

- Paraffin blocks can be preserved for many years for histology and ISH
- Useful for retrospective study

10% neutral buffered formalin (NBF)

Sodium phosphate, monobasic	4.0 gm
Sodium phosphate, dibasic	6.5 gm
Formaldehyde, 37-40%	100.0 ml
Distilled water	900.0 ml

Mix well

Label and date.

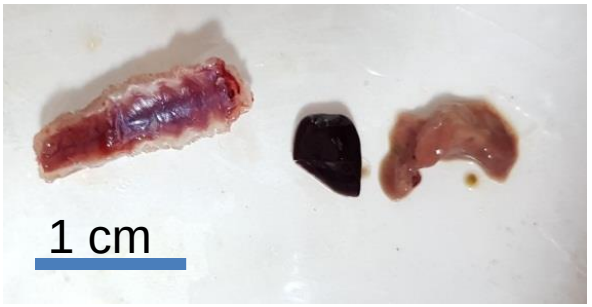
Sample preservation for histology

- Sample : NBF ratio 1:10 (v/v), keep at room temp.
- After 24 h, change to alcohol 70% ratio 1:10 (v/v) for long-term preservation, keep at room temp.

What should be avoided

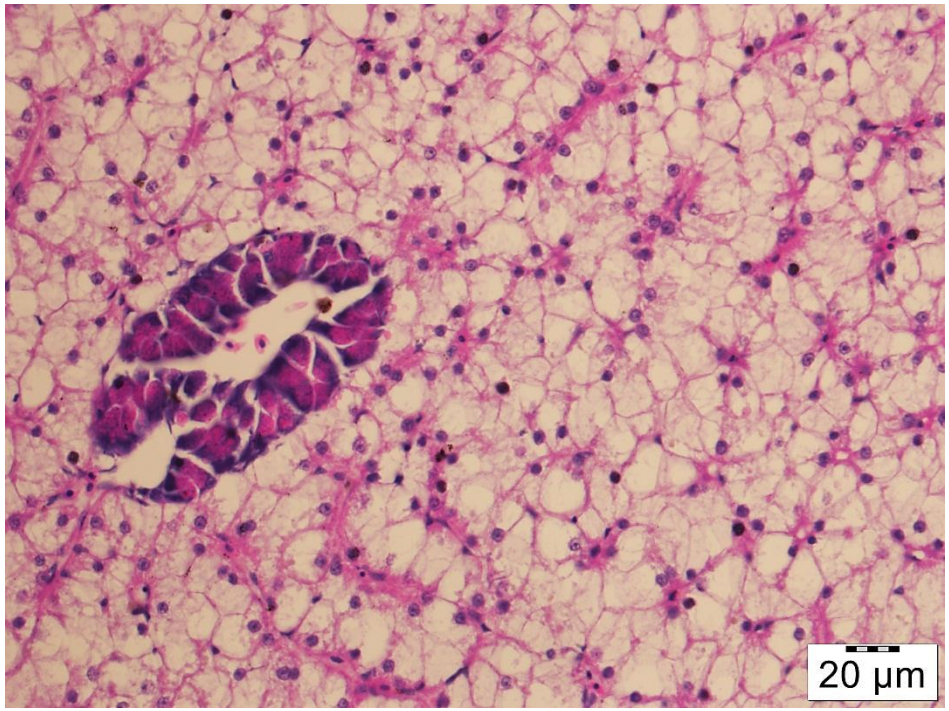
- Dead fish → post mortem change
- Dissection takes too long time → autolysis
- Contamination
- Physical destruction of tissue → not good for histology
- Tissue pieces are too big
- Fixative is not enough
- Sample in formalin 10% for too long → not good for ISH

Not enough fixative

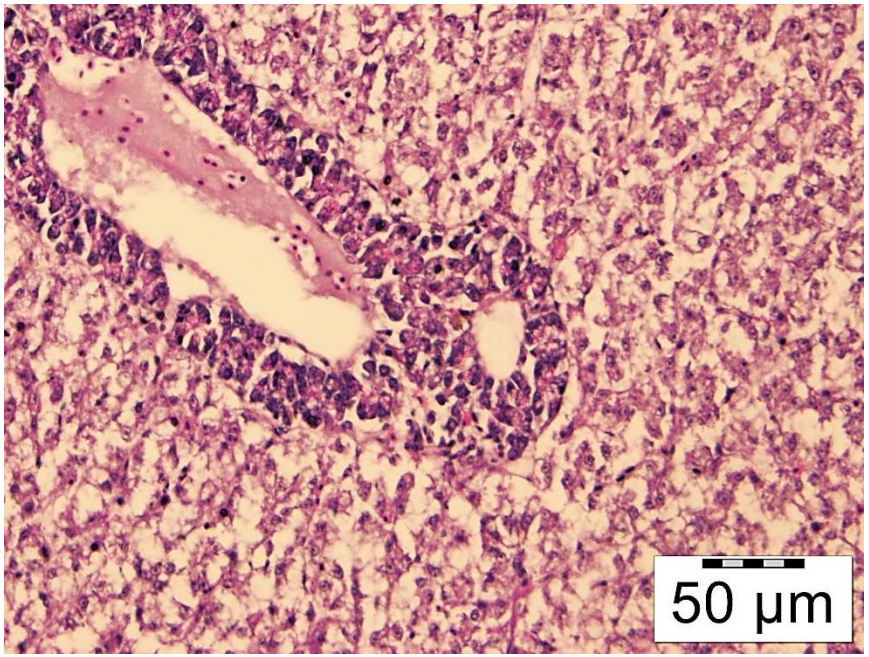


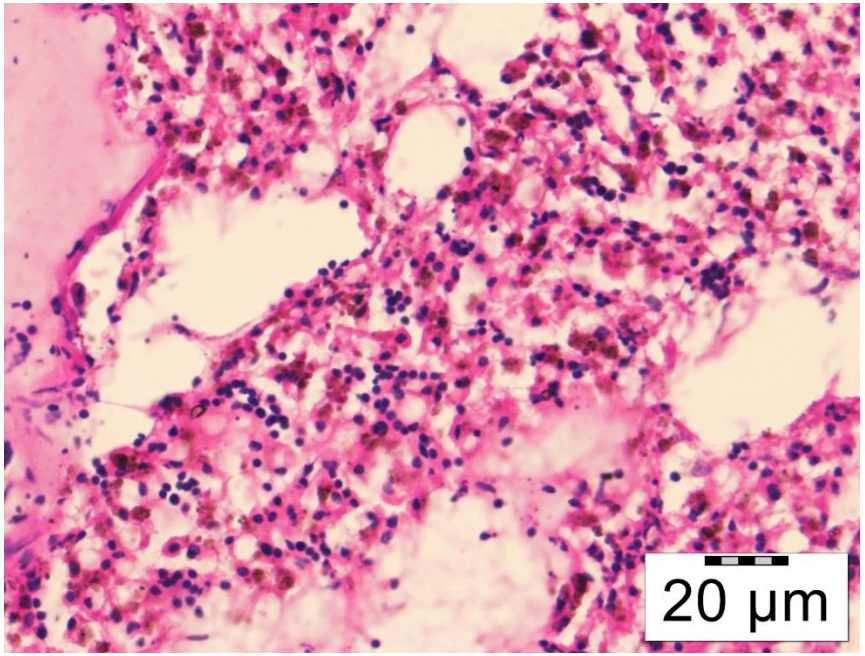
good size for preservation

Example



Normal liver
(standard preparation)





Poor fixing/post mortem change

Discussion

Question: If an unexplained mortality occurs in fish farm, how to preserve samples for later diagnosis/investigation?

Answer:

1……..

2……..

3……..

Thank you for your attention!