

Fish disease Laboratory at Keldur

Overview of diagnostic services

I. Dissection (fresh fish received)

- *Evaluation of macroscopic clinical signs*
 - Suggestive of:
 - Infectious diseases (viruses, bacteria, fungi, parasites)
 - Suboptimal environment, e.g. supersaturation of rearing water/gas bubble disease (GBD).
- Microscopic examination (if relevant).
- *Sampling for PCR, histopathology, bacterial/fungal (agar) and viral culture (on cell lines)*

II. Bacterial examination

- *Culture (non-specific)*
 - Blood agar (marine and freshwater – non-specific) - Most relevant bacteria grow on this medium.
 - FMM agar (marine and freshwater – semi-specific) - *Tenacibaculum* spp., *Flavobacterium* spp.
 - SKDM/KDM (semi-specific) - *R. salmoninarum*.
- *PCR methods*
 - Specific pathogens (see Table 1 below).
 - In case of unknown bacteria from culture, 16S is magnified with PCR then sequenced for identification.
- *ELISA (specific)*
 - *Renibacterium salmoninarum*.
- *Immunofluorescence (specific)*
 - *Renibacterium salmoninarum*.
- *Agglutination test (specific - most common fish bacteria)*
 - *Moritella viscosa*, *Aeromonas salmonicida*, *Vibrio anguillarum*, *Aliivibrio salmonicida*, *Yersinia ruckeri*.
- *Antibiotic resistance of bacteria - antibiotics tested:*
 - Enrofloxacin, Gentamicin, Sulfamethoxazole/ Trimethoprim, Tetracycline, Florfenicol, Trimethoprim, Compound Sulphonamide, Ampicillin, Chloramphenicol, Amoxicillin/clavulanic acid, Neomycin, Streptomycin, Oxylinic Acid, Oxytetracycline, Erythromycin

III. Viral examination

- *Cell culture (non-specific method)*
 - Cell lines: BF-2, EPC, CHSE, ASK etc.
 - Cultivable viruses.
 - E.g., IHNV, IPNV, VHSV, EHN, KHV, Rana, Noda, unknown viruses.
- *PCR methods (specific)*
 - Specific pathogens (see Table 1 below).

- Unknown viruses from culture.
- *Transmission Electron Microscope (non-specific method)*
 - Confirmation of the presence of viruses.
 - Presumptive identification of the viral type.
 - Basis for selection of primers for PCR.

IV. Parasites and fungi

- *PCR screening for specific parasites (see Table 1 below)*
- *Culture on agar plates (fungi – non-specific)*
- *Microscopic examination: Presumptive identification of fungi and parasites*
- *Confirmatory analysis: PCR and sequencing*

V. Histopathology

- *Evaluation of histopathological changes*
 - Presumptive identification (conventional staining).
 - Diseases/pathogens.
 - Environmental diseases/ suboptimal environment.
 - Nephrocalcinosis.
 - Quality assessment of fish.
 - *Specific staining e.g.*
 - Gram (*R. salmoninarum*).
 - PAS (fungal infections).
 - Ziehl Neelsen (*Mycobacterium* spp.).
 - Calcofluor white (Microsporidia, fungi).
- *Immunohistochemistry*
 - Specific antigen staining/identification of pathogens.
- *In situ hybridization*
 - Specific DNA/RNA staining/identification of pathogens.

VI. Transmission Electron Microscopy

- *Ultrastructure of pathogens.*

VII. E-DNA methods

- *Water/seawater samples*
 - Filtering, PCR, sequencing.

VIII. Miscellaneous pathogens

- *In case of previously unknown pathogens, we can identify those using various methodologies, e.g., microscopic examination of fresh preparations, histopathology, cell culture, bacterial/fungal culture, TEM → PCR, sequencing etc.*

Table 1. PCR-service at the Fish disease Laboratory – specific pathogens

Pathogen	Disease	Susceptible hosts	Type of PCR
VIRUSES			
ISAV-del	Infectious salmon anaemia (ISA)	AS	qPCR
ISAV-HPRO	No disease	AS	qPCR
SAV	Pancreas disease (PD)	AS, RT, BT	qPCR
PMCV	Cardiomyopathy syndrome (CMS)	AS	qPCR
IPNV	Infectious pancreatic necrosis	AS, VF	qPCR
IHNV	Infectious hematopoietic necrosis	AS, VF	qPCR
VHSV	Viral haemorrhagic septicaemia	AS, VS, LU, VF	qPCR
PRV	Heart and skeletal muscle inflammation (HSMI)	AS, AC, RT, BT, VF	qPCR
SGPV	Salmon gill poxvirus disease	AS	qPCR
KHV	Koi herpes virus disease	KC	PCR
Noda virus	Viral nervous necrosis	SO, LU, C, VF	qPCR
Eur. N-Atlantic Ranavirus	No disease name	LU	qPCR
Flavivirus (CluFV)	Lumpfish flavivirus disease	LU	qPCR
Totivirus (CluTV)	No disease name	LU	qPCR
Coronavirus (CluCV)	No disease name	LU	qPCR
Spring Viremia Carp virus	Spring viremia	KC, VF	PCR
BACTERIA			
<i>Yersinia ruckeri</i>	Red mouth disease	AS, AC, RT, VF	qPCR
<i>Aeromonas salmonicida</i>	Furunculosis (typical/atypical)	AS, AC, RT, LU, VF	PCR
<i>Vibrio anguillarum</i>	Vibriosis	AS, AC, RT, VF	qPCR/PCR
<i>Renibacterium salmoninarum</i>	Bacterial kidney disease	AS, AC, RT, BT	qPCR
<i>Tenacibaculum spp.</i>	Tenacibaculosis	AS, AC, RT, LU, VF	qPCR
<i>Moritella viscosa</i>	Winter ulcers	AS, AC, RT, LU, VF	qPCR
<i>Branchiomonas cysticola</i>	Epitheliocystis	AS, AC	qPCR
<i>Flavobacterium psychrophilum</i>	Flavobacteriosis	AS, AC, RT, VF	qPCR/PCR
<i>Pasteurella spp.</i>	Pasteurellosis	LU, VF	qPCR
<i>Piscirickettsia salmonis</i>	Piscirickettsiosis	AS, RT, VF	qPCR
<i>Vibrio aestuarianus</i>	Vibriosis	MO	qPCR
PARASITES			
<i>Gyrodactylus salaris</i>	Gyrodactylosis	AS	qPCR
<i>Parvicapsula pseudobranchicola</i>	Parvicapsulosis	AS, AC, BT, RT, VF	qPCR/PCR
<i>Neoparamoeba perurans</i>	Amoebic gill disease	AS, LU, VF	PCR
<i>Myxobolus cerebralis</i>	Whirling disease	RT, AS, AC, BT	qPCR/PCR
<i>Tetracapsuloides bryosalmonae</i>	Proliferative kidney disease	AS, AC, RT, BT	PCR
<i>Bonamia spp.</i>	Bonamiosis	MO	qPCR
<i>Marteilia spp.</i>	Marteiliosis	MO	qPCR
CONFIRMATION OF RESULTS – PHYLOGENY (FOR TRACKING SOURCE OF INFECTION)			
ISAV-del vs ISAV-HPRO	PCR on segment 6 (and 5 and 7 if needed) → Electrophoresis and sequencing		
Other viruses and bacteria	PCR covering certain areas of the genome → Electrophoresis and sequencing		
OTHER PATHOGENS			
Unknown pathogens	Selection of primers, semi-specific for certain groups of pathogens. Amplification in PCR and sequencing.		

Abbreviations: AS = Atlantic salmon; AC = Arctic charr; RT = Rainbow trout; BT = Brown trout; SO = Sole; C = Atlantic cod; KC = Koi carp; VF = Various fish species.