



# **Better Training for Safer Food BTSF**

## **INSPECTION AND SAMPLING PROCEDURES**

**GIUSEPPE BOVO**

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# OVERVIEW

**AIM OF THE INSPECTION**  
**NATURE OF THE SUSPICION**  
**RESTRICTIONS ON SUSPICION**  
**INSPECTION PROCEDURES**  
**GENERAL SAMPLING PROCEDURES**  
**VHS/IHN SAMPLING**  
**KHV SAMPLING**  
**ISA SAMPLING**  
**EUS SAMPLING**

## **AIM OF THE INSPECTION**

**Inspection and sampling procedures of aquatic animal farms are different according to the purposes of the inspection :**

- **TO CONFIRM A SUSPICION**
- **TO DEMONSTRATE THE ABSENCE OF THE PATHOGEN IN ORDER TO ACHIEVE THE FREE STATUS**

## **NATURE OF THE SUSPICION**

**Suspicion could be based on one or more of the following factors:**

**UNUSUAL MORTALITY**

**EVIDENCE OF CLINICAL SIGNS**

**WATER SOURCE BELONGING TO THE SAME RIVER  
CATCHMENT WHERE INFECTION WAS FIRST  
DETECTED**

**RECENT INTRODUCTION OF LIVE FISH FROM AN  
INFECTED SITE**

**RECENT INTRODUCTION OF FISH PRODUCTS FOR  
PROCESSING OR DISPOSAL**

**COMMON TRANSPORT SUCH AS LIVE AND DEAD FISH  
AND FISH FOOD DELIVERY**

**SHARED EQUIPMENTS WITH AN INFECTED SITE  
SUCH AS GRADERS, FISH BOXES, NETS,**

# TEMPORARY RESTRICTIONS

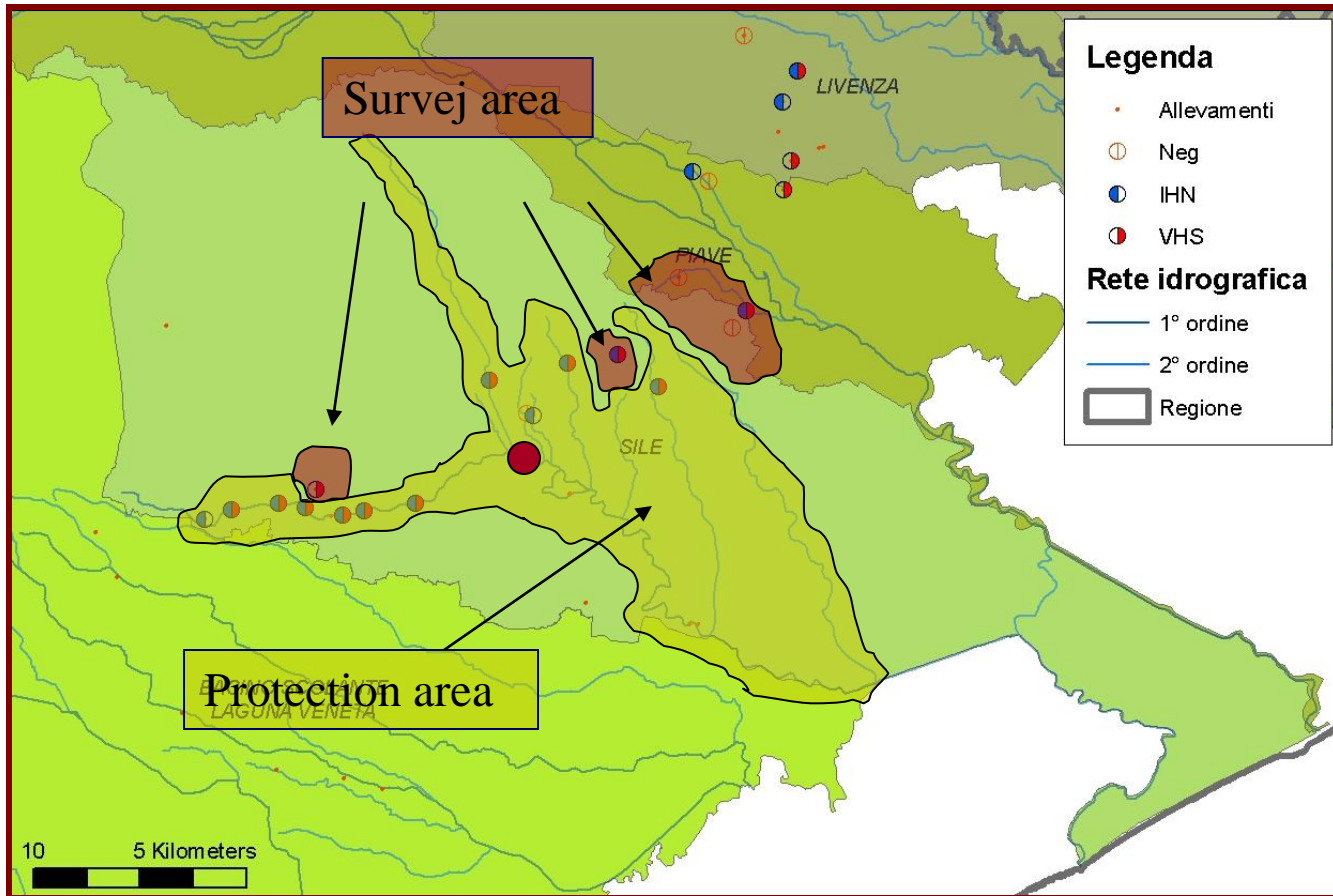
**Following a suspicion of a listed disease, waiting for the final lab results, the CA shall apply the following restrictions :**

**NO AQUACULTURE ANIMALS MAY LEAVE OR ENTER THE SITE WITHOUT PERMISSION AND CONTINUED SURVEILLANCE OF THE SITE IS REQUIRED**

**FURTHER SAMPLING MAY NEED TO BE UNDERTAKEN ON SITES IN CONTACT WITH THE COMPARTMENT UNDER SUSPICION IF AN EXOTIC DISEASE IS SUSPECTED**

**WHERE IT IS SHOWN THAT DISEASE MAY HAVE BEEN SPREAD TO OR FROM DIFFERENT SITES THESE SHOULD ALSO HAVE MOVEMENT CONTROLS APPLIED**

# CONTAINMENT AREA



# EPIZOOTIC INVESTIGATION

**The compartment under suspicion should be inspected for evidence of clinical disease in all fish holding facilities. An epizootic investigation shall be aimed at :**

**DETERMINE THE ORIGIN AND MEANS OF CONTAMINATION**

**INVESTIGATING WHETHER DURING THE RELEVANT PERIOD ANIMALS FROM THE SUSPECTED SITE HAS LEFT THE FARM**

**INVESTIGATING IF OTHER FARMS BECAME INFECTED**

# **INSPECTION PROCEDURE -1**

**APPLY PERSONAL BIOSECURITY MEASURES TO AVOID CONTAMINATION**

**CONTROL THE EXISTENCE OF UPSTREAM/DOWNSTREAM COMPARTMENTS**

**CHECK RECORDS CONCERNING WATER QUALITY PARAMETERS ( DISSOLVED OXIGEN, TEMPERATURE... ) STOCKING DENSITY , RECENT STRESFULL EPISODES ( GRADING, VACCINATION, NEW ARRIVAL, TREATMENTS), EXTERNAL VISITORS**

**CHECK THE PRESENCE OF ICHTHYOPHAGUS BIRDS**

**CHECK THE FARM REGISTER FOR:**

**SPECIES AND AGE OF FARMED FISH  
LIVE AND DEAD FISH MOVEMENTS  
RECORD OF DAILY MORTALITY  
RESULTS OF RISK-BASED SURVEILLANCE  
MORTALITY DURING TRANSPORT  
WATER EXCHANGE DURING TRANSPORT**



# INSPECT THE DEAD FISH STOCKING ROOM



## **INSPECTION PROCEDURE 2**

**INSPECT ALL THE UNITS FOR CLINICAL SIGNS, IN PARTICULAR INLET AND OULET WATER PLAICES :**

**LETHARGY  
ABNORMAL SWIMMING BEHAVIOUR  
SKIN DARKENING  
SKIN HAEMORRHAGIES  
SKIN RED DOTS OR EROSIONS  
SWOLLEN ABDOMEN  
EXOPHTHALMUS  
SUNKEN EYES  
PERIORBITAL HAEMORRHAGIES  
SKELETAL DEFORMITIES  
RESPIRATORY PROBLEMS**

## **INSPECTION PROCEDURE - 3**

**COLLECT SAMPLES (10 FISH) IN CASE OF CLINICAL SIGNS**

**COLLECT A LARGER SAMPLE (150 FISH) IN CASE OF ABSENCE OF SIGNS**

**CHECK AVAILABILITY OF DEAD FROZEN FISH**

**INTERVIEW THE RESPONSIBLE PERSON OF THE RISK-BASED SURVEILLANCE AND ALL THE PERSONS ATTENDING THE ANIMALS**

# SELECT THE RIGHT FISH



# **GENERAL SAMPLING PROCEDURE -1**

**IN CASE OF SUSPICION SAMPLES SHOULD BE TAKEN AMONG MORIBUND FISH SHOWING EVIDENT CLINICAL SIGNS**

**FRESH DEAD FISH ARE ALSO SUITABLE FOR DIAGNOSIS**

**IT IS RECOMMENDED TO PREPARE COLLECTIVE SAMPLES DIRECTLY IN THE FARM**

**USE ONLY STERILE TOOLS FOR COLLECTING TISSUES**

**WHOLE FISH ARE ALSO ACCEPTABLE ( SENT ALIVE OR REFRIGERATED)**

**KEEP TISSUES, DEAD AND LIVING FISH AT  $<10^{\circ}$  C**



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## GENERAL SAMPLING PROCEDURES -2

### IDENTIFICATION OF SAMPLE :

**DATE**  
**FARM CODE**  
**SPECIES**  
**AGE**  
**TEMPERATURE**  
**TANK IDENTIFICATION N°**  
**CLINICAL DATA**  
**ADDITIONAL NOTES**





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Azienda Provinciale per i Servizi Sanitari  
Provincia Autonoma di Trento

485/E

Del. G. P. N. 1578/2007 - Programma di controllo della VHS e IHN dei pesci  
**VERBALE DI PRELEVAMENTO CAMPIONI**  
(in base alla Dec. 2001/183 CE)

All'Istituto Zooprofilattico Sperimentale delle Venezie  
Laboratorio di Ittiopatologia - Viale dell'Università 10  
35020 Legnaro (PD)  
tramite il Laboratorio di Trento

Codice azienda 020TN007 Riconoscimento: Dec. CE n. 427/08

Denominazione TROT.FIANA di VALENTI CLAUDIO  
Località FIANA Comune: BONDO  
Bacino idrografico: zona n. 8  
Provenienza acque: SORGIVA

Tipologia: X Azienda Incubatoio Laghetto Pesca Sportiva Acque Pubbliche

Stato sanitario:<sup>1</sup> ZR X ARZNR ASPC AI

Allevamento: X con riproduttori di soli riproduttori senza riproduttori

Specie allevata: X fario iridea X salmerino marmorata

|                           | N. vasca | T° acqua | N. esemplari interi | N. provette pool organi | N. provette liquidi ovarici |
|---------------------------|----------|----------|---------------------|-------------------------|-----------------------------|
| Avannotti<br>Specie Fario |          | 7        | 30                  |                         |                             |
| Trotelle<br>Specie cm     |          |          |                     |                         |                             |
| Trote<br>Specie cm        |          |          |                     |                         |                             |
| Riproduttori<br>Specie    |          |          |                     |                         |                             |

Data prelievo: 04/11/08

ASPS-Provincia Trento  
Dr. Mario Lorenzini

Timbro e firma del prelevatore  
Dr. Mario Lorenzini

<sup>1</sup> ZR - zona riconosciuta; ARZNR - azienda riconosciuta in 2019 non riconosciuta; ASPC - azienda sottoposta a programma di controllo; AI - azienda infetta

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**recommended n°  
fish in each collective  
sample**

**VHS/IHN : 10**

**KHV: 2**

**ISA : 5**

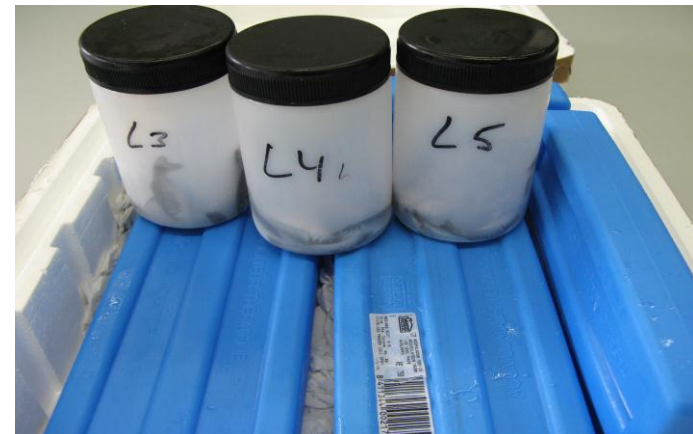
**EUS : 1**





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## SHIPMENT OF SAMPLES



## **IHN & VHS SAMPLING -1 (Decision 2003/183/EC)**

**Sampling to confirm VHS/IHN under suspicion must be performed taking in consideration the following :**

**CLINICAL INSPECTION BETTER PERFORMED OCTOBER-JUNE  
OR AT TEMPERATURE < 14° C**

**INSPECT ALL PRODUCTION UNITS IN PARTICULAR NEAR TO  
WATER OUTLET**

**IF RAINBOW TROUT IS PRESENT COLLECT ONLY THIS SPECIES  
ON THE CONTRARY COLLECT DIFFERENT SPECIES IN %**

**SELECT ONLY FISH SHOWING CLEAR CLINICAL SIGNS (   
LETHARGY, EXOPHTHALMUS, SKIN DARKENING, ERRATHIC  
SWIMMING )**

**IF FRESH DEAD FISH ARE AVAILABLE COLLECT THESE FISH  
PRIMARYLY.**

## IHN & VHS SAMPLING -2

**OVARIAN FLUID/TARGET TISSUES (0.5 GRAMS) FROM 10 FISH ARE COLLECTED AND SUSPENDED IN 4 ML OF TRANSPORT MEDIUM :**

**ANTERIOR KIDNEY  
SPLEEN  
HEART OR ENCEPHALON**

**WHOLE LIVING OR DEAD FISH ARE ALSO CONVENIENT PROVIDE TEMPERATURE IS MAINTAINED < 10° C**

**KEEP SAMPLES REFRIGERATED AT 4-10 ° C**

**THE LABORATORY MUST START INVESTIGATION < 48 HOURS POST SAMPLING .**

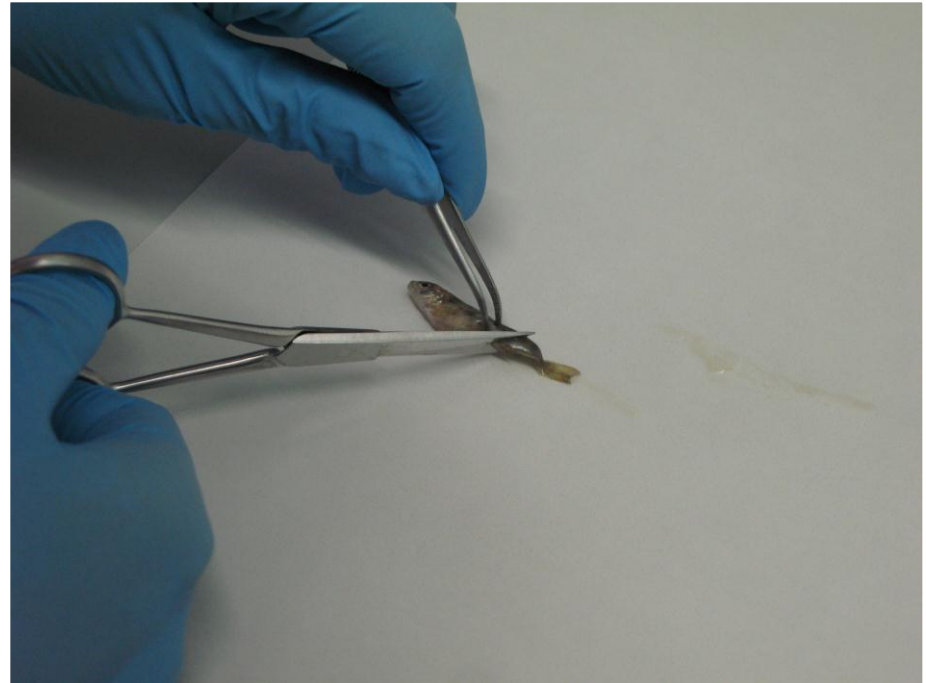
# PREPARATION OF THE SAMPLES

**FISH < 4 cm:**

**REMOVE THE BODY  
BEHIND THE ANUS**

**MINCE WITH  
STERILE SCISSORS**

**SUSPEND 1:10 IN  
TRANSPORT  
MEDIUM**



## PREPARATION OF THE SAMPLES -2

**FISH 4 -6 cm**

**COLLECT VISCERA  
INCLUDING KIDNEY**

**MINCE WITH  
STERILE SCISSORS**

**SUSPEND 1:10  
IN TRANSPORT  
MEDIUM**



## PREPARATION OF THE SAMPLES -3

**FISH > 6 cm**

**COLLECT : SPLEEN  
ANTERIOR KIDNEY  
HEART/ENCEPHALON**

**SUSPEND 1:10 IN  
TRANSPORT MEDIUM**







## **FREEZING IN EXCEPTIONAL CASES**

**Where practical difficulties arise (eg. bad weather conditions, non-working days, laboratory problems) which make it impossible to inoculate cells within 48 hours after the collection of the tissue samples, it is acceptable :**

**TO FREEZE TISSUE SAMPLES IN TRANSPORT MEDIUM AT -20° OR BELOW**

**VIROLOGICAL EXAMINATION SHALL BE CARRY OUT < 14 DAYS**

**ONLY ONE THAWING/FREEZING CYCLE IS PERMITTED**

**RECORD MUST BE KEPT**

## **ISA SAMPLING -1**

**(Dec. 2003/466/EC)**

**INSPECT ALL UNITS OF THE FARM ( CAGES,  
TANKS, PONDS)**

**CHECK FOR FRESHLY DEAD, WEAK OR  
ABNORMALLY BEHAVING FISH**

**EXAMINE THOSE FISH FOR CLINICAL SIGNS AND  
POST MORTEM FINDINGS OF ISA**

**IF CLINICAL SIGNS ARE PRESENT COLLECT AT  
LEAST 10 FISH**

**IF CLINICAL SIGNS AND POSTMORTEM FINDINGS  
ARE NOT CONSISTENT WITH ISA SPECIFIC  
SAMPLING IS NOT NECESSARY BUT A  
DIFFERENTIAL DIAGNOSIS IS RECOMMENDED**

## **ISA SAMPLING -2** **(Dec. 2003/466/EC)**

### **SAMPLES FOR HISTOLOGY**

**REMOVE SMALL PIECES (0.5X 0.5) OF LIVER, MID-KIDNEY,  
HEART AND SPLEEN AND FIX IN BUFFERED 10%  
FORMALIN ( 1 TISSUE : 20 FIXATIVE )**

### **IMMUNOFLUORESCENCE**

**PREPARE IMPRINTS FROM KIDNEY TAKEN < 2 HOURS  
AFTER DEATH**

**ABSORBE BLOOD EXCESS ON ABSORBENT PAPER**

**MAKE LIGHT IMPRINTS ON POLY-L-LYSINE-COATED  
SLIDES**

**AIR DRY SLIDES AT ROOM TEMPERATURE**

**FIX < 72 HOURS OR STORE AT -20° C**

**SEND SLIDES TO THE LAB KEEPING DRY AND  
REFRIGERATED**

## **ISA SAMPLING -2**

**(Dec. 2003/466/EC)**

### **MOLECULAR TOOLS**

**REMOVE PIECES OF ANTERIOR KIDNEY OR MID-KIDNEY FROM EACH FISH AND TRANSFER INTO A MICROFUGE TUBE CONTAINING 1 ML OF RNAlater. KEEP REFRIGERATED/FROZEN**

### **VIRUS ISOLATION**

**REMOVE PIECES OF LIVER, ANTERIOR KIDNEY, HEART AND SPLEEN ( 1 GRAM ) AND TRANSFER INTO A TUBE WITH 9 ML TRANSPORT MEDIUM**

**PREPARE COLLECTIVE SAMPLES FROM < 5 FISH**

**KEEP SAMPLES REFRIGERATED UNTIL THEIR ARRIVAL AT THE LAB WHERE THEY SHALL BE PROCESSED < 72 HOURS**

**DUPLICATE SAMPLES SHALL BE TAKEN FOR CORROBORATIVE PURPOSES**

## KHV SAMPLING -1

**SELECT 10 MORIBUND OR FRESHLY DEAD CARPS  
DISPLAYING TYPICAL CLINICAL SIGNS.**

**COLLECT TISSUES FROM**

**CLINICALLY INFECTED FISH (PCR, VIRUS ISOLATION)**

**– GILL, KIDNEY SPLEEN**

**SUBCLINICALLY INFECTED FISH (PCR)**

**– GILL, KIDNEY, SPLEEN, GUT, ENCEPHALON**

**POOLING OF SAMPLES SHOULD BE AVOIDED OR LIMITED  
TO A MAXIMUM OF **TWO** FISH PER COLLECTIVE SAMPLE**

## **KHV SAMPLING -2**

**WHOLE FISH SHOULD BE SENT TO THE LABORATORY ALIVE OR KILLED AND PACKED SEPARATELY IN SEALED ASEPTIC CONTAINERS.**

**HOWEVER, IT IS HIGHLY PREFERABLE AND RECOMMENDED TO COLLECT ORGAN SAMPLES AT THE FISH PRODUCTION SITE.**

**WHOLE FISH OR SELECTED TISSUES SHOULD BE SENT TO THE LABORATORY IN REFRIGERATED CONTAINERS OR ON ICE**

**FREEZING OF FISH OR DISSECTED ORGANS SHOULD BE AVOIDED.**

## EUS SAMPLING

### FISH SELECTION AND POOLING

**SELECT 10 FISH WITH TYPICAL LESION IN EARLY STAGES OF INFECTION.**

**SEND SAMPLES TO THE LAB IN ICE-COOLED BOXES .**

**FISH COLLECTED FROM REMOTE AREAS SHOULD BE ANESTHETISED AND FIXED IN 10% BUFFERED FORMALIN FOR AT LEAST 1–2 DAYS.**

**DIAGNOSIS IS ACHIEVED USING THE HISTOLOGICAL TECHNIQUE AND OOMYCETE ISOLATION ON INDIVIDUAL FISH OR A GROUP OF A FEW FISH**

**THANK YOU FOR YOUR  
ATTENTION**

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