Handling of diseased fish for bacteriological examination

Use of small range media, incubators, an anaerobic jar, aseptic techniques, and the majority of the bacterial fish pathogens recovered as pure culture growth. Majority of clinical diseases .observes dense growth, except asymptomatic carrier states (BKD), only scant number of colonies may develop. Always include a kidney sample, it gives satisfactory results. Sample the materials by means of swabbing and inoculate media by swabs. Based on the pathogen, select the media for isolation.

Sample collection

- The Fish Pathology Section must be contacted to discuss whether samples are necessary, and if so, the appropriate type of sample and number of fish needed. Advance notice of sample submission by at least one week is preferred (serious disease). If advance notice is not given, samples may not be processed if other samples have priority.
- Samples that are not in an adequate condition (either substandard or improperly packaged) upon arrival may not be processed.
- In clinical cases of disease generally (0.5% mortality/day) 10 moribund fish or shellfish are a sufficient sample size to make a diagnosis.
- In case of no excessive mortality or clinical disease is apparent, a larger sample size of 60 animals may be necessary.
- Depending upon individual circumstances, sample sizes may vary between 10 and 60.

Sample size required to detect one or more infected specimens in populations (lots) with an assumed minimum prevalence of detectable infection of 5 and 10%. Calculations are based on a 95% level of confidence. For intermediate population sizes, use the sample size for the next larger population listed (Ossiander and Wedemeyer, 1973)

	Number of fish to be sampled when assumed Prevalence of detectable infection is	
Population size	5%	10%
50	29	20
100	43	23
250	49	25
500	54	26

1000	55	27
2500	56	27
5000	57	27
10000	57	27
100000	57	27
Over 100000	60	30

Transportation of samples

- Small fish must be received either alive or freshly dead (within 1-2 hours) on blue ice in a cooler.
- Fish must not be frozen. Bagged fish should not be in direct contact with blue ice or they will freeze.
- At least 10 moribund fish be placed in one or more large leak-proof plastic bags containing water. Seal the bags so space for air remains, without leakage.
- Label bags with fish status (moribund or healthy), incubator or raceway number, stock and species and enclose a Sample Submission Form.
- If the live fish do not survive transport, then the "dry" fish undergone less deterioration and contamination from the water and its bacterial flora will be processed instead.
- In a disease outbreak, 30 fish per lot of affected fish will be required for shipment (10 moribund, 10 healthy and 10 mart, but dry).
- Seminal and ovarian fluid samples collected in sterile test tubes and shipped in an insulated. Container on ice. Do not mix seminal and ovarian fluid samples.

Treatment of samples

A. Autopsy procedure:

External examination and sampling

- The bacteriological examination must be performed first. To maximize detection sensitivity, fish must be autopsied within 48 h of sampling.

- Note all gross abnormalities such as body discoloration, body distension, exophthalmia, ulcers, blebs, inflammation, hemorrhagic areas, etc.

- Inoculate the appropriate media and prepare stained smears with material from these lesions.

B. Disposal of samples

- All materials (fish carcasses or tissues, transport containers) should be autoclaved, incinerated, or otherwise sterilized before being discarded.

- Disinfect the outer surface of the fish by flooding with 70% ethanol. Equipments with by immersion in 100% ethanol and passing the instruments through a Bunsen flame allowing the alcohol to ignite and burn off.

- External lesions (ulcerations or abrasions) should be struck onto TSA. (For S W fish use TSA with 1% NaCI, a halophilic bacterial pathogen is suspected.)

- Stain the blood smears in Diff-Quike, observe on the microscope (1000X) for bacterial rods, erythrocytic inclusion bodies (EIB) and viral erythrocytic (VEIN cytoplasmic inclusions etc.

- Skin scrapes from lesion s mounted with a drop of PBS and cover-slip on a glass slide observe on compound microscope (40X and 200X) for bacteria, fungi, parasites.

- Wet mounts of gill filaments are made by (surgical scissors) to remove a portion of one gill arch, mounted in PBS, Look for gas bubbles in the capillaries, telangiectasia, hyperplasia, external parasites (bacterial, protozoal, fungal, metazoan).

- Mince the gill tissue, make a smear for Gram staining observe bacteria by oil immersion.

- If bacterial samples are to be taken they should be inoculated onto BMA or other growth medium (i.e., TYES from the kidney, spleen, visceral lesion, or other tissues if indicated.

- If bacteriologic samples, struck onto TSA from the kidney or visceral lesions before other samples are taken to avoid bacterial contamination.

- If *Phoma* (a fungus) is suspected, samples from the suspect lesion or air bladder should be struck onto PA.

- A spleen squash can be made by placing a cut section of the tissue with a drop of PBS on a glass slide and covering with a cover-slip.

- Clinical disease caused by the BKD agent can easily be detected using FAT or Gram stain when lesions are apparent.

- Bacterial problems due to Gram-negative bacteria such as furunculosis, ERM agentsr can be detected more efficiently by isolation on prepared media.

- Depending upon the nature of the lesion, bacteriological sampling, Gram staining or fixation for histology may be necessary.

- Histology samples should be taken as a backup measure, but only if moribund fish are available.

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