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2. Packing and shipping of materials for laboratory analysis with guide to shipping biological materials

A. Toffan¹ and S. Zrnčić²

¹ Istituto Zooprofilattico Sperimentale delle Venezie,
National Reference Centre for Fish, Molluscs and Crustacean Diseases, Legnaro, Padova, Italy

² Croatian Veterinary Institute, Laboratory for Fish, Molluscs and Crustacean Diseases,
Zagreb, Croatia

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2.1. Introduction

Accurate diagnosis demands properly collected and prepared samples for submission to the diagnostic laboratory. Fish must be alive when collected and fish showing signs of the disease in question are preferable and should be collected with minimal stress. It is a large advantage for the laboratory to start the examination with live samples. However, it is hardly possible to

keep seabass and seabream alive during transportation except in the situation when the laboratory is in close vicinity of the farm. If fish cannot be maintained alive, samples should be properly packed and shipped to the diagnostic laboratory as soon as possible after sampling. Dead fish are not suitable for disease diagnosis because they decompose rapidly after death and very often saprophytic bacteria overgrow the bacterial pathogen. Parasites also require a live host and even viruses survive in dead fish for a limited time.

2.2. Transport of fresh dead samples

Fish showing the signs of the disease should be collected alive from the population using a net or trap. The most suitable specimens for diagnostic purposes are fish with pronounced symptoms or moribund fish. They should be placed in a separate plastic bag and sealed. Each separate sample should be placed inside a larger, strong plastic bag and sealed (“double bagging”). The double bagged samples should be placed in a cooling box or insulated styrofoam box with sufficient freezer blocks (not ice cubes, which could melt and produce liquid inside the package, unless thermally sealed in strong plastic bags) to prevent the temperature inside the box from rising above 10°C during transport. Each sample should be labelled with a permanent ink marker. Any accompanying document should be placed in a separate, small plastic, leak-proof envelope placed inside the transport box. The document should contain all data about environmental and rearing conditions. A full record of environmental data, culture conditions (stocking, feeding), observations regarding fish behaviour, mortality patterns as well as the name and address and other contact details of the sender should be provided, either in writing or by e-mail sent to the laboratory in advance (Annex 3).

Sufficient inert material (e.g. used paper, paper threads, or plastic wrapping) should ensure that the samples do not move inside the box during transport.

Shipment should be delivered via fast overnight courier service.

2.3. Transport of material for histological examination

When it is not possible to send whole diseased fish to the laboratory for histology, organs already fixed in the appropriate fixative solution (most often buffered neutralized formalin BNF 10% 1:20 v/v) may be sent in tightly capped plastic bottles or tubes containing fixative. The container should be large enough to prevent squeezing of tissue pieces inside the tube or bottle. The diagnostician must be able to detect tissue changes indicative of the disease and not be confused by the post mortem decomposition process. This requires carefully-fixed tissue samples immediately after the death of the animal. To safeguard against spillage, the containers should be wrapped in plenty of absorbing material and placed in sealed plastic bags before placing them in an outer envelope or carton box. The outer package should be strong and made of stiff cardboard, polystyrene etc. Enough inert material (e.g. used paper, paper threads, or plastic wrapping) should ensure that the samples do not move about inside the box during transport.

2.4. Transport of samples for virological examination

For the diagnosis of viral diseases, sampling is sometimes performed on the farm and small portions of selected organs (i.e. anterior kidney, spleen, heart, gills and brain) are placed in plastic tubes containing transport medium. The tubes containing transport media (cell cultivation media with added foetal bovine serum, antibiotics, fungicides and buffer) may be provided by diagnostic laboratories a few days prior to sampling. The tubes containing tissue samples should be wrapped in plastic and sent with freezer blocks in a cool box to maintain the temperature below 10°C without freezing. Generally, organs from ten fish can be pooled in one

tube, bearing in mind that the volume of transport medium should be twice that of the organs. However, in some cases it is recommended to test individual fish samples. It is advisable to ask the laboratory staff for specific advice before pooling samples. Samples for virology should reach the diagnostic laboratory within 24-48 hours after sampling. A written record form should accompany samples, or be e-mailed to the laboratory.

Where practical difficulties arise (e.g. bad weather conditions, holidays, laboratory problems) which make it impossible to process the samples within 48 hours post collection, it is acceptable to freeze the tissue specimens in transport medium at -20°C or below and carry out virological examination within 14 days. The tissue samples, however, must be frozen and thawed only once before the examination.

2.5. Transport of the samples for molecular analysis

Molecular techniques are widely used for the detection of many fish pathogens. Samples selected for nucleic acid-based diagnostic tests should be handled and packaged with care to minimize the cross-contamination among the sample degradation. Use of separate containers (plastic sample bags or bottles) and immersion of instruments in bleach when target tissues or organs are dissected should minimize sample cross-contamination. Target tissue should be cut to less than 0.5 cm in one dimension and submerged in the preservative solution: RNAlater™ for RNA viruses and RNAlater™ or ethanol 95% for DNA viruses. The ratio between samples and fixative should be 1:5 w/v. Small organs can be immersed whole in the solution while bigger organs are cut to fulfil required proportions. Manufacturers' instructions should be followed for the correct use of RNAlater™.

A water-resistant label, with the appropriate data filled out, should be placed in each package or container for each sample set. The major advantage of using RNAlater™ is that samples can be shipped at room temperature if the shipment lasts for less than 1 week. The samples preserved in this way may be stored up to 25°C for 1 week, at 4°C for one month, at or at -20°C indefinitely.

2.6. Guide to shipping biological materials

The transport of biological materials is subject to stringent requirements based on national and international legislation. It is governed by ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road). The packaging, labelling and dispatch of biological samples are regulated by the UN and described in the International Air Transport Authority (IATA) and the International Civil Aviation Organisation (ICAO) regulations. Under this regulation, specimens are classified into diagnostic specimens and infectious substances.

All biological materials must be properly packaged and checked in as luggage or transported via courier (carrying biological materials on board an aircraft is prohibited).

The appropriate steps to ship biological materials include:

- Classification
- Packaging
- Labelling
- Documentation

It is important to define the classification correctly as it determines how the goods should be packaged and labelled.

Biological materials fall into the following categories:

- Infectious substances
 - Category A infectious/potentially infectious substances
 - Category B infectious substances
- Diagnostic specimens
- Biological products

2.6.1. Infectious substances

Infectious substances are those known to contain, or are reasonably expected to contain, pathogens. Pathogens are defined as microorganisms or recombinant microorganisms that are known or are reasonably expected to cause infectious disease to humans or animals. However, they are not subject to the provisions of shipping if they are unlikely to cause human or animal disease. Infectious substances are subject to the regulations only if they are capable of spreading disease when exposure to them occurs.

2.6.1.1. Category A infectious substances

Category A infectious substances are capable of causing permanent disability, life-threatening or fatal disease to humans or animals when exposure to them occurs. Category A infectious substances have two shipping names: “Infectious substances, affecting humans” (UN 2814) or “Infectious substances, affecting animals” (UN 2900). The fish pathogens do not generally belong to this category.

2.6.1.2. Category B infectious substances

Category B infectious substances are infectious but do not meet the criteria for Category A. Category B infectious substances have the proper shipping name “Biological Substance, Category B” and the identification number UN 3373. Fish pathogens (virus and bacteria) fall into this category.

2.6.1.2.1. Packaging

Category B infectious substances must be triple packaged and compliant with IATA Packing Instruction 650. The maximum quantity for a primary receptacle is 500 ml or 500g and outer packaging must not contain more than 4L. or 4 kg.

2.6.1.2.2. Labelling

The outer container of all Category B infectious substance packages must display the following on two opposite sides:

- Sender’s name and address
- Recipient’s name and address
- The words “Biological Substance, Category B”
- UN 3373 label
- Class 9 label, including UN 1845, and net weight if packaged with dry ice

2.6.2. Diagnostic specimens

Any human or animal material including, but not limited to, excreta, secretions, blood and its components, tissue and tissue fluids, being transported for the diagnostic or investigational purpose but excluding live infected animals. Fish, organs, swabs belong to this category.

Diagnostic specimens must be assigned to UN3373 unless the source patient or animal has or may have a serious human or animal disease, which can be readily transmitted from one individual to another, directly or indirectly and for which effective treatment and preventable measures are not usually available, in which case they must be assigned to UN2814 or UN 2900.

2.6.2.1. Packaging

Diagnostic and clinical specimens must be triple packaged and compliant with IATA Packing Instruction 650 detailed in (Fig. 1). The maximum quantity for a primary receptacle is 500 ml or 500g and outer packaging must not contain more than 4 l or 4 kg.

2.6.2.2. Labelling

The outer container of all diagnostic/clinical specimen packages must display the following on two opposite sides:

- Sender's name and address
- Recipient's name and address
- The words "Biological Substance, Category B"
- UN 3373 label
- Class 9 label, including UN 1845, and net weight if packed with dry ice

2.6.3. Biological products

These are products derived from living organisms that are manufactured and distributed in accordance with the requirements of national governmental authorities which may have special licensing requirements, and are used either for prevention, treatment or diagnosis of disease in human or animals or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines and diagnostic products. Biological products transported for final packaging, distribution, or uses by medical professionals are not subject to shipping regulations. Biological products that do not meet these requirements must be assigned to UN 2814, UN 2900, or UN 3373, as appropriate.

2.6.3.1. Packaging biological products

Potentially hazardous biological materials must be packaged to withstand content leakage, shocks, temperature changes, pressure changes, and other conditions that can occur during transport. When ordering, specify what category of materials will be shipped: infectious substances, diagnostic specimens, dry ice, ice packs, etc. All biological materials must be triple packaged.

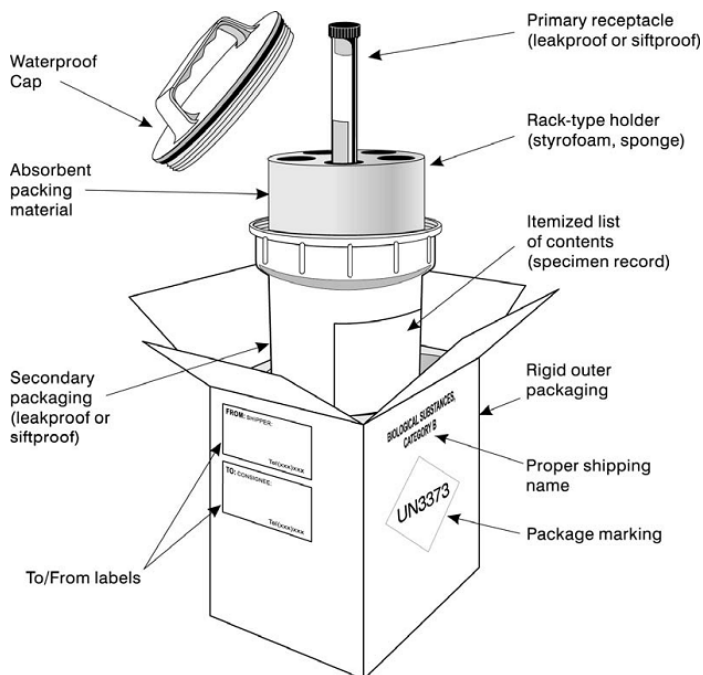


Fig. 2.1: Packaging of biological products

2.6.4. Shipping papers

Documentation required by transporter or operator must always be accessible without opening the package.

A Shipper's Declaration for Dangerous Goods must be completed when shipping a Category A infectious substance or a genetically modified organism or micro-organism assigned to UN 3245. A declaration is not required for shipments in which dry ice is the only hazardous material. A declaration is not required for Category B infectious substances or diagnostic/clinical specimens assigned to UN 3373. All shippers' declarations must be in English, typed, and printed in colour with red hatchings bordering the document. Three copies must be presented to the courier with a fourth copy retained by the shipper for at least 375 days. All shippers' declarations must conform to the standardized format provided by the courier company used for transport.

Considering that proper packaging and labeling are mandatory and have to comply with international regulations, it is always advisable to contact the chosen courier company beforehand to check the correctness of the procedure.

A specialized courier company should be used to transport Category substances (i.e. World Courier, PHSE or another company to which you are accustomed).

References

IATA, 2019. Infectious Substances Shipping Guidelines 15th Edition.

IATA, 2019. Dangerous Good Regulation 60th Edition.

WHO, 2015. A Guide for Shippers of Infectious Substances

https://www.who.int/ihr/infectious_substances/en/