BTSF ACADEMY

Organisation and implementation of training activities to strengthen understanding, implementation and enforcement of EU law in the area of Sanitary and Phytosanitary (SPS) standards in EU Member States and neighbouring non-EU countries

BTSF Programme on Sheep pox and Goat pox

Andy Haegeman

Valencia, Spain 30/01-01/02 2024

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Sheep and Goat Pox: Collection and transport of diagnostic sample material

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BTSF Programme on Sheep pox and Goat pox

Andy Haegeman, Nick De Regge

Valencia, Spain 30th January – 2nd February 2024

BTSF Sampling: Serology Vs Virology

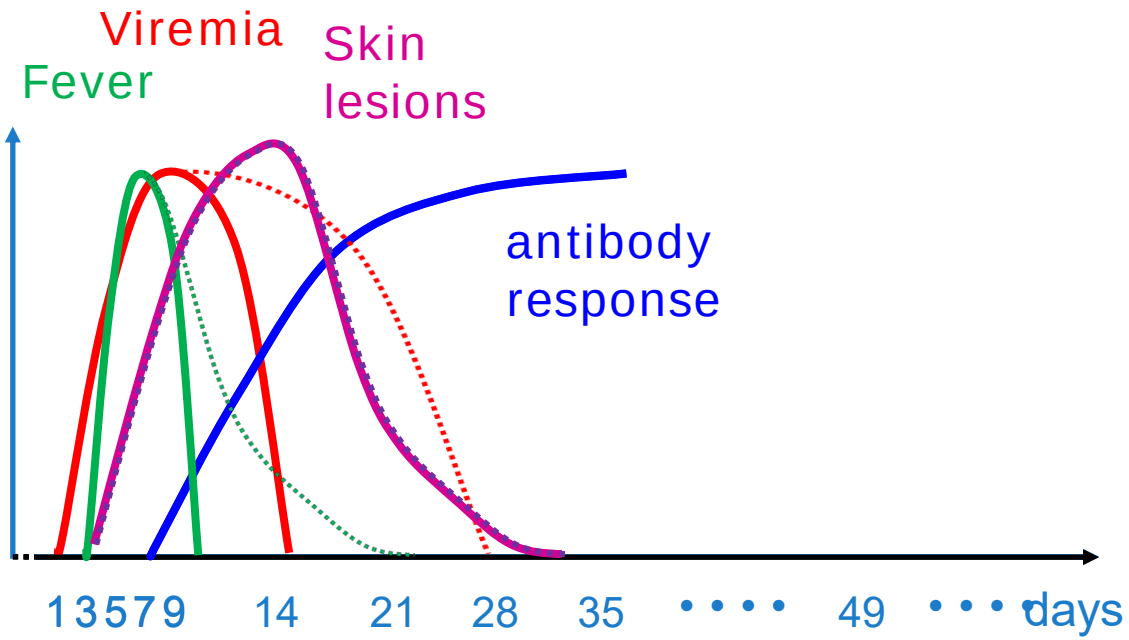
Serology (antibodies) → The host immune system needs time to react but is more long lasting → **Not suited** for early detection BUT **Suited** for exposure (vaccine or virus) in the past

Virology (Virus, genome) → Detecting the pathogen itself

- **Genome (PCR)** → Very sensitive: **Suited** for early detection !!! → as host clears the pathogen from its system → **Not suited** for detection in time
- **Virus isolation** → Less sensitive + labor intense: **Less suitable** for early detection

BTSF Sampling: link with virus and host dynamics

Sample type	Starts Days post-infection (dpi)	Disappears (dpi)
Blood (viraemia)	4-6	21-28
Viral DNA in saliva samples or in nasal and ocular discharge	6-8	56-64
Skin lesions	6	Several weeks
Urine and faeces	10 (urine) 4 (faeces)	15 (urine) 61 (faeces)
Serum antibodies	10	Several months



Babiuk et al. 2008
Bowden et al. 2009
Tuppurainen et al., 2017

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BTSF Sampling: General guideline

- Collect samples in sufficient number and volume
- Biosafety + Personal Safety +Animal Welfare
  - Personal Protection Equipment

    → Protect yourself from zoonotic agents (such as brucellosis, anthrax, tuberculosis, rabies, RVF)

    → Gloves: not only ware them but change them !

  - Mechanical restraint or sedation of the animal
- Sample Quality

  - Work aseptically to avoid cross-contamination between samples (disinfect the sample collection site, change needles, scalpels, gloves):

BTSF Sampling: Purpose: Diagnostics

Wound crusts → **Ideal to confirm clinical picture**

- Is suited for virus isolation and PCR due to high viral load + remains long time positive
- Easy to collect
- No need for the most sensitive test

- Extra care is needed in sampling, handling and analysis

→ High risk of contamination in the lab !! Extra cleaning is warranted !

Changing gloves also in the field !

Nodule and skin lesion

- Similar remarks but you need to take a biopsy !

→ Change biopsy needles after use !

→ Not always evident: location of nodule !!

(swabbing can be an alternative)

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BTSF Sampling: Purpose: Diagnostics

EDTA Blood

- Is suited for virus isolation and PCR
- Easy to collect

- Strength of viremia is more time dependent !

→ Sensitivity is more important

Swabs (buccal, nasal…)

Same remarks as for blood

→ Recent info: watchout for environmental or transfer contamination to determine individual status. Not an issue if you want to look at group (stable) status

BTSF Sampling: Some remarks on blood sampling

EDTA Blood

- Examine blood quality !!!! And make it traceable → linked to PCR results ?
  - ✓ Non-coagulated
  - ✓ Fresh blood
- Blood tubes: check expiration date
- Shaking after blood collection (EDTA mixed with blood)

Heparin blood can be used for virus isolation but NOT for PCR !

Whole blood on FTA cards can be used for PCR but NOT for virus isolation

→ Volume will also not be sufficient if you want (whole) genome sequencing

BTSF Sampling: Purpose: Diagnostics

Tissues, organs (cfr slaughterhouse…)

- Cut small pieces and put in Eppendorf tube
- Attention:

  → Large pieces are difficult to homogenize

  → Avoid Membranes and connective tissues: hard to homogenize and digest

  → Fat residues can cause problems during subsequent seps

BTSF Sampling: Purpose: Screening / surveillance

Serum

- Is suited for ELISA, virus neutralization, IPMA
- Easy to collect
- Can remain present for a prolonged period of time

- Developments during the course of the infection (+/- 2 weeks)
- → not suited for early detection

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Sampling: Purpose: Screening / surveillance

Serum

- ✓ 2 different types of tubes:

  - With clot activator
    - Advantage:
      - ✓ Better separation between red blood cells and serum
      - ✓ Less hemolysis (important for IPMA ~false negative results)

  - Without clot activator
    - Advantage:
      - ✓ Less expensive

BTSF Sample treatment

	Actions taken directly	Short time storage (48 hr)	Long time storage	Transport medium
Biopsy, wound crust tissue	/	2-8° C	-70°C (-20°C)	commercially available (20 (to 50%) glycerol in PBS)
EDTA blood	gently mixing	2-8° C	-70°C (-20°C)	
Swabs	External swabs: moisten before use with PBS	2-8° C	-70°C (-20°C)	Can be placed in transport medium
Serum	Simple tubes: 1/2 hr at RT + by low speed centrifugation 1000g for 15 minutes Activator tubes: low speed centrifugation Whole blood sample: in an upright position, overnight in the refrigerator and collect the serum in the morning	2-8° C	-20°C	

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BTSF Sample identification

- Samples should be individually labelled with a unique identifier (ID) and in a permanent way !

  → Use waterproof markers, labels which are suitable for -80°C storage…

- Samples should be well-packed (leak-proof)
- As much data as possible should be recorded

  ✓ Date of collection
  ✓ Species, gender
  ✓ Sample type
  ✓ Location/country of origin
  ✓ Clinical signs present

BTSF Sample shipment

- Samples should always be transported as quickly as possible to the laboratory
- Maintenance of cold chain is essential for sample quality, particularly for samples for virus isolation (see storage table above). Use refrigerants if necessary (gel packs, dry ice…)
- Transport according to international and national regulations

BTSF Sample shipment: international regulations

Recommendations on the Transport of Dangerous Goods (ST/SG/AC.10/1/Rev.21(Vol.I)

→ Basis for the development of regulations

- International Carriage of Dangerous Goods by Inland Waterways **ADN**
- International Carriage of Dangerous Goods by Road **ADR**

- International Carriage of Dangerous Goods by Rail **RID**
- International code of Maritime Dangerous Goods **IMDG**

- International Air Transportation Assocation **IATA**

IATA

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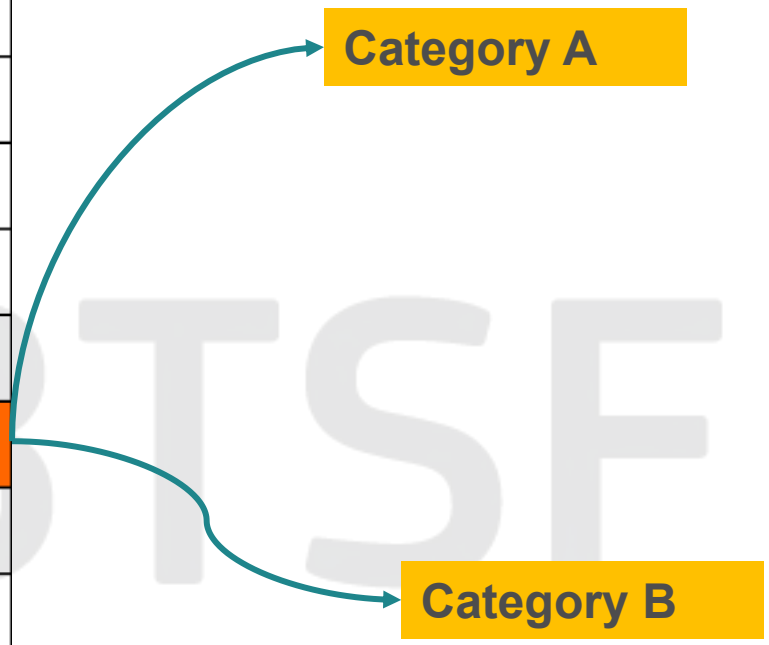
BTSF Sample shipment: international regulations

Class 1	Explosives
Class 2	Gases
Class 3	Flammable Liquids
Class 4	Flammable Solids
Class 5	Oxidising Substances & Organic Peroxide
Class 6	*Toxic and Infectious Substances*
Division 6.1	Toxic substances
Division 6.2	Infectious substances
Class 7	Radioactive Material
Class 8	Corrosives
Class 9	Miscellaneous Dangerous Goods (DRY ICE)



BTSF Sample shipment: international regulations

Category A → UN2900

An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening disease or fatal disease in otherwise animals

UN Number and Proper Shipping Name	Microorganism
UN 2900 Infectious substances affecting animals only	African swine fever virus (cultures only) Avian paramyxovirus Type 1 - Velogenic Newcastle disease virus (cultures only) Classical swine fever virus (cultures only) Foot and mouth disease virus (cultures only) Lumpy skin disease virus (cultures only) *Mycoplasma mycoides* - Contagious bovine pleuropneumonia (cultures only) Peste des petits ruminants virus (cultures only) Rinderpest virus (cultures only) Sheep-pox virus (cultures only) Goatpox virus (cultures only) Swine vesicular disease virus (cultures only) Vesicular stomatitis virus (cultures only)

Cultures ONLY !!!!

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BTSF Sample shipment: international regulations

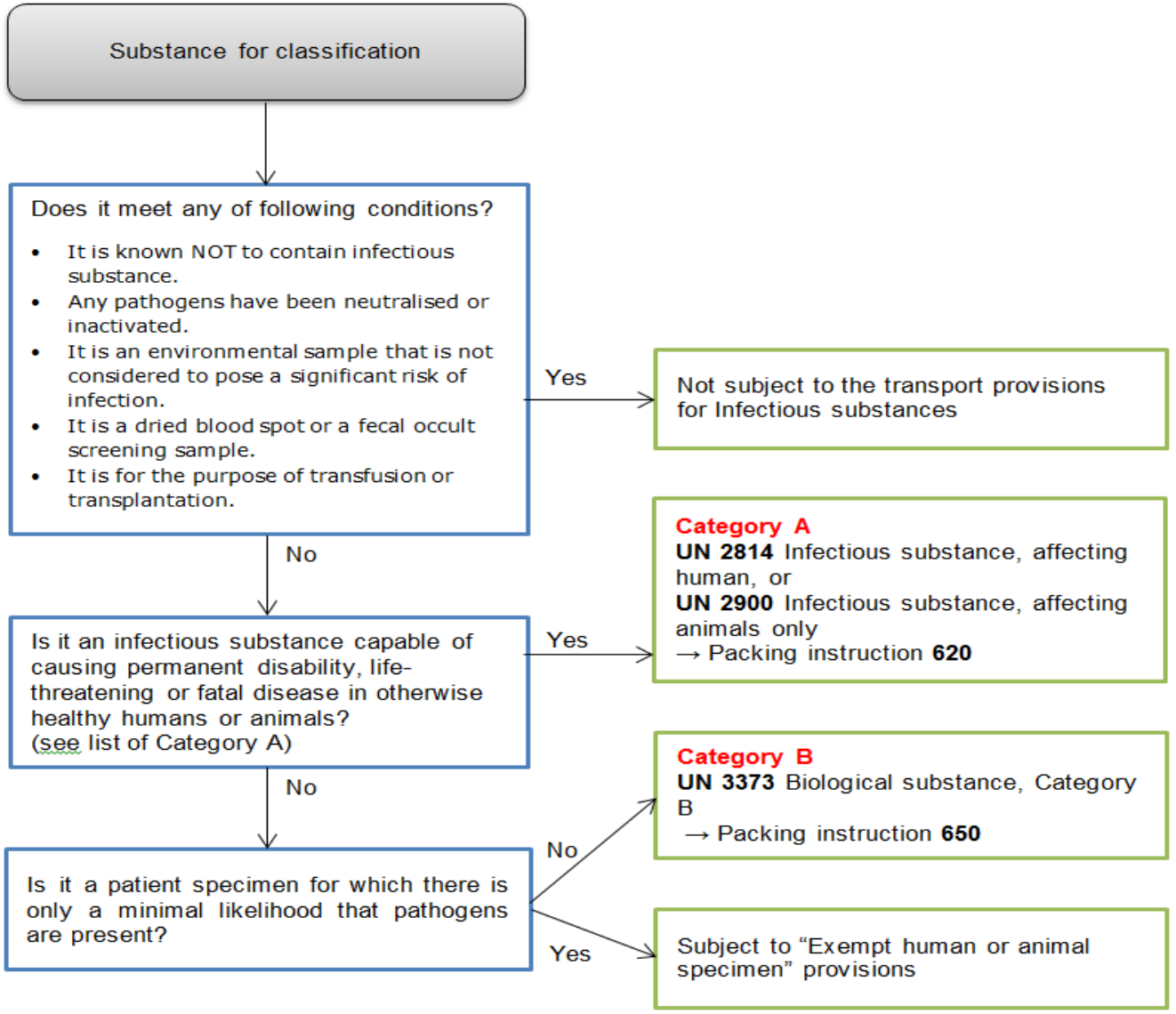
Category B → UN3373

An infectious substance which does not meet the criteria for inclusion in Category A

Exemptions

- 2.6.3.2.3.3. Substances in a form that any present pathogens have been **neutralised** or **inactivated** such that they no longer pose a health risk are not subject to this Code unless they meet the criteria for inclusion in another class.
- 2.6.3.2.3.5. Dried blood spots, collected by applying a drop of blood onto absorbent material, are not subject to these regulations.
- 2.6.3.2.3.8 Animal specimens for which there is a minimal likelihood that pathogens are present are not subject to these regulations if the specimen is transported in a packaging which will prevent any leakage and which is marked with the words "Exempt animal Specimen". The packaging should meet the following conditions/

BTSF Sample shipment: international regulations



Source: Belgian Biosafety Professionals

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BTSF Sample shipment: Packaging

Category A → P620

Triple packaging principle: Primary → secondary → Outer packaging

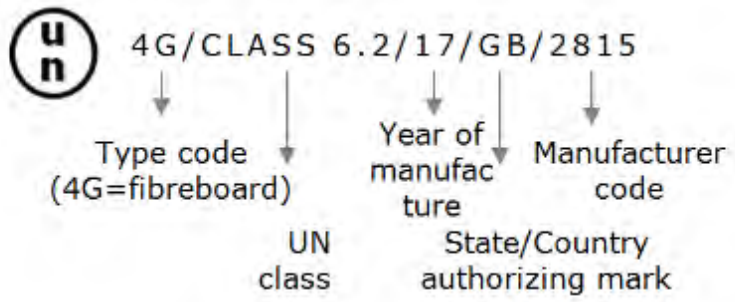
- Primary + secondary layer:
  - Leakproof
  - Withstand a pressure differential of not less than 95 kPa and temperatures in the range -40 °C to +55 °C.
- Absorbent material must be placed between the primary and secondary packaging. The absorbing material must be sufficient to absorb the entire contents of all primary receptacle.
- List of contents: between the secondary packaging and the outer packaging.
- No maximum quantity per package for surface transport. For air transport the quantity is limited to :
  - 50 ml or 50 g per package for passenger aircraft,
  - 4 litres or 4 kg per package for cargo aircraft.

BTSF Sample shipment: Packaging

Category A → P620

- The triple packaging system must be **UN approved**

→ UN packaging symbol and specification marking are required



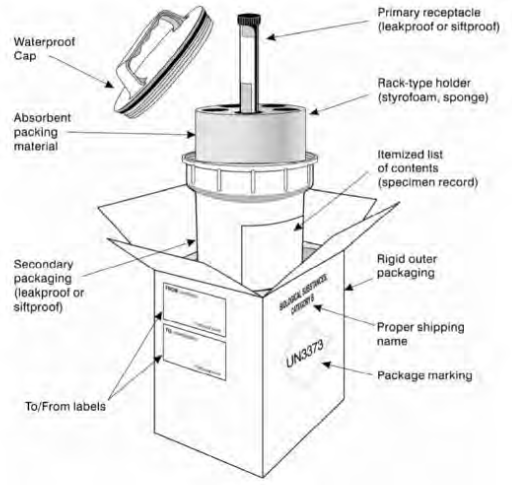
- It needs **to pass** drop test from a height of 9 m, puncture test with the fall of a steel rod on the package from a height of 1 m, stacking test (withstanding to a force applied to the top surface during 24 h) and internal pressure test.

BTSF Sample shipment: Packaging

Category B → P650

- The triple packaging system **does not need to be UN-certified**, so no UN packaging symbol and specification marking are required on the outer packaging.

- However, the completed package must be successfully pass the drop test from a height of 1,2 m.



(from: WHO Guidance on regulations for the transport of infectious substances 2015–2016)

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BTSF Sample shipment: Packaging

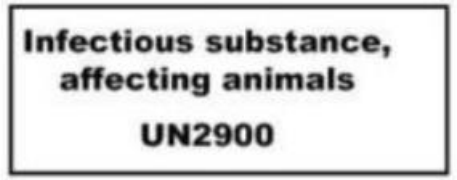
Category B → P650

Category A → P620

- Special provisions when sending refrigerated samples
  - Ice, dry ice or other refrigerant must be placed **outside the secondary packaging**(s) or in an overpack if any.
  - Interior support must be provided to secure the secondary packaging(s) in the **original position** after the ice or dry ice has dissipated.
  - If ice is used, the **outer packaging or overpack must be leakproof**.
  - If dry ice is used, the outer packaging or overpack must **permit the release of carbon-dioxide gas.**
  - The primary receptacle and the secondary packaging must **maintain their integrity** at the temperature of the refrigerant used.

BTSF Sample shipment: labelling

Category A → P620

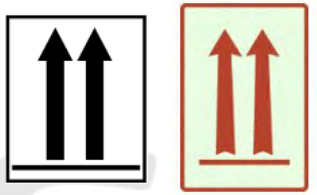


- **Proper shipping name and UN number(Category A label) + Net quantity**

→ Do NOT put technical name (organism name) on Proper Shipping Name label or anywhere on the outside of the package.

- **Hazard class 6 infectious substance lab**

- **UN package certification mark**
- **Orientation arrows (if volume >50 mL).**

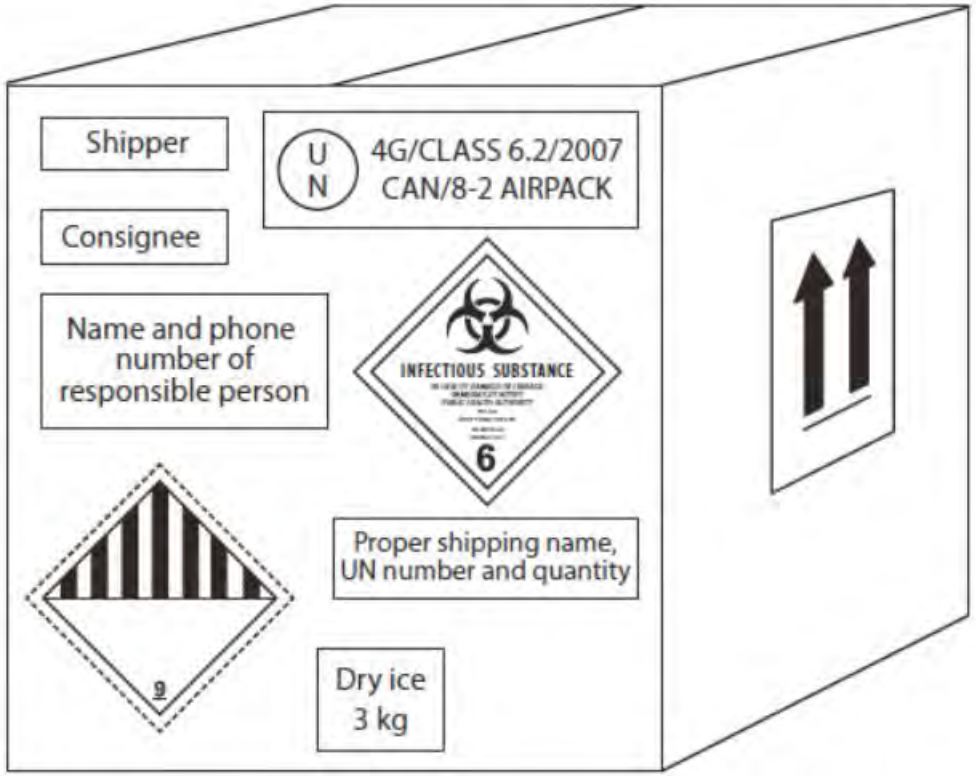


→ Either red or black orientation arrows may be used. Apply orientation arrows to two opposite sides of the outer packaging conform the orientation of the primary receptacle.

- **Name and address of shipper and consignee**
- **Name and phone of responsible person for shipment**
- **When dry ice is used: Net quantity of dry ice, "Dry Ice, UN 1845"**

BTSF Sample shipment: labelling

Category A → P620



BTSF Sample shipment: labelling

Category B → P650

- **Proper shipping name (at least 6 mm high)**

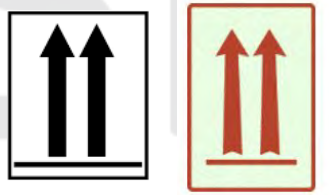
→ Do NOT put technical name (organism name) on Proper Shipping Name label or anywhere on the outside of the package.

- **Diamond shaped marking with the text "UN3373" at a 45° angle.**

→ Each side of the marking should measure at least 50 mm.

- **Orientation arrows (if volume >50 mL).**

→ Either red or black orientation arrows may be used. Apply orientation arrows to two opposite sides of the outer packaging conform the orientation of the primary receptacle.



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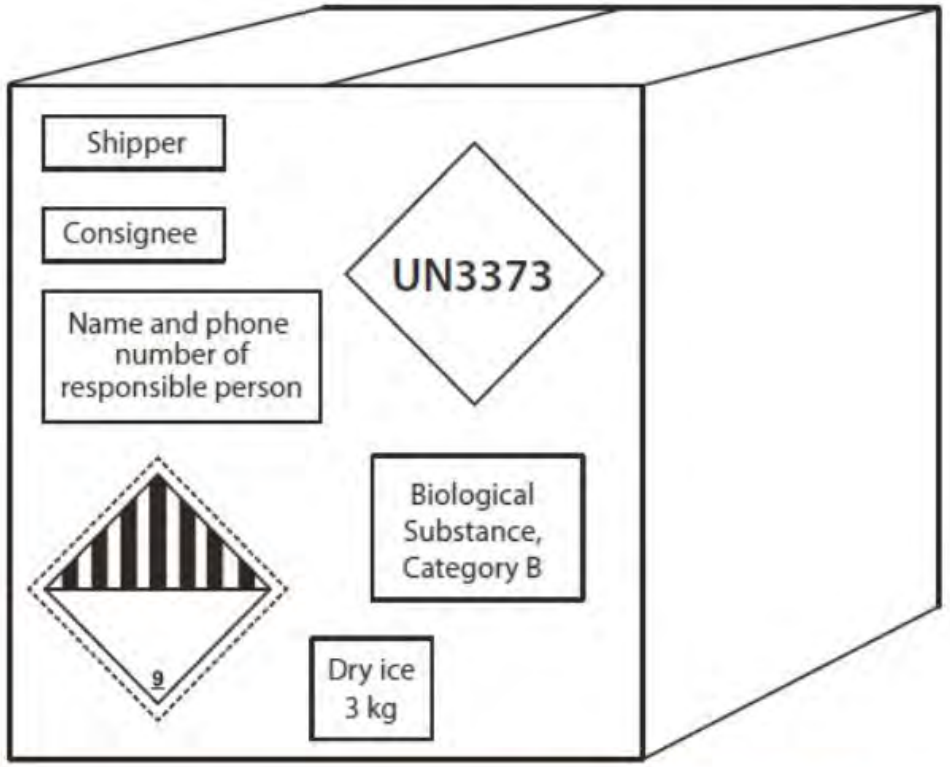
BTSF Sample shipment: labelling

Category B → P650

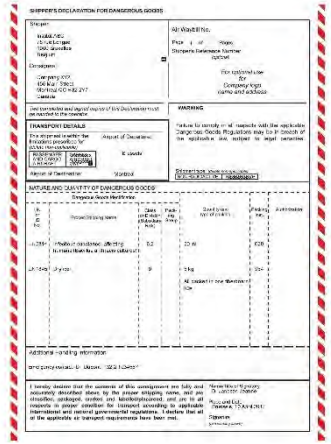


From: Solutions OP Ltd, https://solutionsop.co.uk/review-how-to-ship-lab-samples-in-clinical-trials/

BTSF Sample shipment: documents

- Road/Air/Railway bill – bill of lading (sea carrier)
- License, import/export permit (if required, depending on the type of material)
- Pro-forma invoice (customs)
- Packing list (customs)

Category B → P650



- Dangerous goods transport document (DGD)
- **Itemized content list: between the secondary packaging and the outer packaging.**
- Shippers' declaration of dangerous goods
- Air Waybill (form provided and filled out by carrier)

Category A → P620

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Thank you!

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