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Review Article

Biological Safety in the Storage and Transport of Biological Specimens From Patients With Respiratory Diseases Used in Research Settings

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ABSTRACT

Major advances in genomics and proteomics have prompted the creation of biological specimen collections and biobanks for use in biomedical research. These specimen collections and the wealth of data they generate will allow longitudinal studies to be conducted and subproducts such as DNA or RNA to be obtained. They may even be used in future studies.

To ensure specimen integrity, from the outset it is necessary to define procedures for sampling, transport and storage, the subproducts to be obtained, and the end purpose, as well as to address biosafety issues and arrange for suitable equipment monitoring. Strict control of these conditions will confer added value on the specimens, as quality and traceability would be assured.

This article aims to provide a general overview of the recommendations concerning biological safety, transport, and storage of biological specimens for biomedical research into respiratory diseases in accordance with current legislation.

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Seguridad biológica en la preservación y el transporte de muestras biológicas obtenidas en el ámbito de las enfermedades respiratorias y destinadas a la investigación

Palabras clave:
Muestra biológica
Bioseguridad
Conservación
Biobanco
Enfermedades respiratorias

RESUMEN

Los grandes avances de la genómica y la proteómica han propiciado la creación de colecciones de muestras biológicas y de biobancos destinados a la investigación biomédica. Estas colecciones, y sus múltiples datos asociados, permitirán realizar estudios longitudinales, obtener subproductos como ADN o ARN e incluso prever estudios futuros.

Para mantener la integridad de las muestras se deberán diseñar desde el principio los procedimientos de obtención, transporte, subproductos que se obtendrán, condiciones de conservación, destino final y bioseguridad, así como disponer de una supervisión adecuada de los equipos. El control de todas estas condiciones proporcionará valor añadido a las muestras, ya que permitirá asegurar su calidad y trazabilidad.

Nos proponemos ofrecer una visión general de las recomendaciones relativas a la seguridad biológica, transporte y conservación de muestras biológicas destinadas a la investigación biomédica en el ámbito de las enfermedades respiratorias, de acuerdo con la legislación vigente.

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Introduction

Biological specimens—often with extensive associated information—are currently an essential and valued material for diagnostic studies, as well as a fundamental tool in the field of biomedical research.

For years, surplus material from biological specimens obtained during diagnostic procedures has been stored with a view to later diagnostic confirmation or possible use in future research. The design of research projects currently contemplates the possibility of taking advantage of these specimens for scientific ends. On obtaining the biological specimens for diagnosis or certain studies, it is therefore advisable to plan for storage in conditions to enable future use of the specimens in research projects.

From the ethical point of view, any study or research project that includes collection of biological specimens must obtain informed consent from the donor and receive the approval of the corresponding ethics committee. This body has the task of ensuring that the patients' best interests are observed and that any investigation does not interfere with any diagnostic procedures or clinical management (treatment, follow-up, course). From the point of view of biosafety, compliance with current legislation regarding the collection, transport, and use of human biological specimens is required.

Given the complexity both specimen and data collection and long-term storage, all processes need to be optimized. It will therefore be necessary to obtain, at the lowest possible cost, sufficient quantity of each specimen (whole blood, sputum, tissue biopsy, etc) to subsequently allow further sampling (of DNA, RNA, proteins, cells, etc) in the best possible conditions. It will also be essential that facilities be available for processing and storage in optimal safety conditions and at the optimum temperature. Good databases will also be required to manage all the associated information.

These ordered and controlled collections will ensure specimens are traceable and results reproducible. Such collections would also facilitate the formation of research networks or multicenter projects, and allow specimens to be shared or donated to other groups. Standard operating procedures that cover appropriate transport and storage of the samples, although costly from a logistical point of view, have proved decisive particularly in long-term and longitudinal studies in which the integrity of the stored samples is of overriding importance.

These are the factors that have driven the increasingly widespread creation of biobanks and biological specimen collections. These have been built up according to standard procedures, with transport and storage according to current recommendations, with the compilation of associated data, and in compliance with ethical and legal requirements.[1]

The present review focuses on the processes and recommendations concerning the biosafety, transport, and storage of biological specimens that are used in the specific field of respiratory diseases and that are intended for use in biomedical research. Specimens that, given their generic nature, are useful for any disease are also considered. Nevertheless, most recommendations will depend on the desired subproducts or subsequent analyses to which the specimens will be submitted. Therefore, many of them will also be common to other disease processes of nonrespiratory origin.

Origin and Collection of Biological Specimens

The Spanish legislation governing biomedical research defines a biological specimen as "any biological material of human origin that can be stored and that can hold information on the genetic make-up of a person."[2]

The most widely used specimens—and corresponding subproducts—in the field of respiratory diseases are described below. These include more generic specimens, common to a range of diseases (tissue biopsies, urine, peripheral blood), as well as specimens more specific to diseases of respiratory origin. It should be mentioned that not all specimen and procedure types defined here are used routinely in biomedical research, although all of them are of great diagnostic value.

- *Sputum* allows microbiological analysis of soluble factors and cell types.
- *Exhaled breath condensate* can be used to analyze the molecules in the lung parenchyma and airways.
- *Bronchial aspirate* specimens contain bronchial secretions that can be used for cytology and microbiological tests.
- *Bronchoalveolar lavage* is a diagnostic procedure performed during fiberoptic bronchoscopy to obtain material from the alveolar interstitium (cells, microorganisms, and soluble substances).
- *Protected catheter sampling* is a diagnostic procedure for studying bacterial infection.
- *Bronchial brushing* is an endoscopic technique for obtaining cells from the lower airways or from lesions of the lung parenchyma.
- *Transbronchial aspiration biopsy* is a diagnostic procedure for obtaining cells from different sites such as the mediastinum, pulmonary lesions, endobronchial lesions, and peribronchial and peritracheal hilar and mediastinal lesions.
- *Bronchial biopsy and transbronchial lung biopsy* are bronchoscopic techniques for obtaining bronchial and lung tissue, respectively.
- *Fine-needle transthoracic aspiration biopsy* is a technique for obtaining samples of cells (normally for diagnosis of peripheral pulmonary neoplasm) and bacteria (for diagnosis of pneumonia).
- *Surgical lung biopsy* is used for obtaining tissue.
- *Endoscopic ultrasound-guided fine-needle aspiration* is a technique for obtaining cell samples from lesions and lymph nodes of the lower mediastinum and aortopulmonary window.
- *Pleural fluid (thoracentesis)* involves drainage to collect samples for microbiology and cytology.
- *Pleural biopsy* is used for obtaining tissue.
- *Skeletal muscle biopsy* is used for obtaining muscle samples, particularly of leg muscles (quadriceps) for use in studies in patients with chronic respiratory diseases associated with metabolic disorders and exercise intolerance.
- *Samples from other parts of the body* may also be useful. These include biopsies of the skin and nasal mucosa, provided that there is a relation between those organs and the corresponding lung disease.

All these samples should be considered as potentially pathogenic and as such subject at all times to measures for preventing risks associated with exposure to biological agents,[3] and also subject to the biosafety recommendations of the World Health Organization.[4]

Table 1
Essential Standards for Cryopreservation

Temperature, °C	Biological Relevance
0 to +4	Temperature at which fresh specimens should be handled
–20	Limit of protein mobility, DNA stability
–80	RNA stability, tissue storage
–150 to –196	Recommended storage for cells

Storage of Biological Specimens

The collection of specimens for biomedical investigation should never interfere with the diagnosis or standard clinical management of the patient, a consideration of particular relevance when those specimens are obtained by invasive procedures. Specimens of potential diagnostic utility should first be analyzed by a pathologist, who will then decide which part of the specimen and how much material can subsequently be used for research.[5]

To ensure that specimens are of diagnostic value and, at the same time, can be used in research, a series of protocols should be followed. Some of these will be very general while others will be more specific to the type of specimen or the scope of the study. The quality of the specimens will be ensured by rapid collection and appropriate processing and transport to the laboratory. These aspects are as important as those of long-term storage.

Some of the basic recommendations—common to several societies such as the International Society for Biological and Environmental Repositories,[5] the Organization for Economic Cooperation and Development,[6] and the International Agency for Research on Cancer (IARC)[7]—are listed below:

1. *Standard operating procedures.* It is important that these procedures include reference to the temperatures of collection, transport, and storage. It is also essential to have registers and record incidents and deviations from protocol (for example, excessive transport time, longer collection time, or a temperature other than the one recommended).
2. *Fractionation and aliquoting.* Fractionation and aliquoting should be performed at the time of collection in order to avoid changes in temperature and subsequent handling that might affect the quality of the specimens or the expression of certain biomarkers.[8]
3. *Choice of ideal receptacles for specimens.* The choice of receptacle is important. and should be made before collection, taking into account how long the specimens are to be stored, the temperature at which they will be stored, and the processing and analyses which they will undergo. Another noteworthy point is the importance of labeling the specimen with a unique code that will remain fully legible in the storage and processing conditions. The labeling system should maintain the anonymity of the individual from who the specimen was taken. The informed consent document will accompany each specimen. All data will be anonymized or coded, or presented as completely anonymous, as stipulated in the Law for Biomedical Research.[2]
4. *Safeguards for storage equipment.* It is also necessary to have emergency or backup systems available in case failures in the storage systems occur.
5. *Optimum temperature ranges.* Certain types of specimen or process require temperature ranges that can be considered as standard. Table 1 shows the basic guidelines for cryopreservation. These temperatures are general recommendations that need to be reviewed according to the intended use of the specimens or the duration of storage.

Essential Characteristics for Conservation of Different Types of Sample

Biopsies of Solid Tissues

For solid tissue, members of the European Human Tumor Frozen Tissue Bank (TuBaFrost) have drawn up a series of widely accepted recommendations.[9] On collection, tissue should be stored in a closed receptacle and kept cool, without adding any liquid medium. The time between resection or collection of the sample and freezing should be recorded because, as discussed later, delays can negatively affect the quality of the samples.

Methods for freezing include submerging the specimens in liquid nitrogen or freezing in previously cooled isopentane. Freezing by immersion in liquid nitrogen, although quick and easy, is not the best option as it can lead to the appearance of artifacts that may affect certain cytological techniques. The procedure recommended by both the IARC and TuBaFrost is freezing in isopentane.[7,9] This procedure may be difficult to execute as it is not easy to have the necessary equipment available at the site where the biopsies and/or specimens will be obtained. For specimens that are to be used for histology, slow freezing of the tissue in a refrigerant at –80°C should be avoided as this makes it much harder to obtain frozen sections due to the formation of ice crystals. Definitive storage will depend on how the tissue is to be used in the future.

Prolonged ischemia time may lead to degradation of some of the additional samples that may be obtained (for example, RNA).[10] A study by Spruessel et al[11] showed how ischemia time affects the patterns of gene and protein expression by analyzing the changes that occur at ischemia times of between 5 minutes and 30 minutes. After 30 minutes of ischemia, gene and protein expression deviated by 20% from baseline. The study by Huang et al,[12] performed on specimens from patients with colon cancer, showed that gene expression patterns remained fairly stable at ischemia times of less than 20 minutes but were substantially affected at times greater than 40 minutes. In view of the results of these and other studies, TuBaFrost recommends that the time elapsed between resection and freezing should not exceed 30 minutes. In any case, provided fewer than 2 hours have elapsed, the specimen can be frozen, but the time elapsed between resection and freezing should be recorded. In accordance with these results and recommendations, it is appropriate to set a maximum time according to the end use of the tissue. If the time elapsed is greater than that established in the protocol, a decision is made either to accept the tissue, in which case the time elapsed will be recorded, or to discard the specimen.

For tissues to be used for DNA extraction, some studies show that stability at –80°C is maintained over time, whereas the integrity of the RNA declines after 5 years of storage.[13] Other authors have reported that DNA and RNA remain stable for about 10 years if the tissue has been stored under liquid nitrogen vapor.[14]

Peripheral Blood

Peripheral blood is one of the most routinely collected samples because it can be separated into several fractions and a wide range

of molecules can be studied. These samples can be used for studies of genomics, proteomics, metabolomics, and blood parameters. Blood is simple and cheap to obtain. Factors that may affect the stability of samples are mainly anticoagulants, stabilizers, temperature, time between extraction and processing, sterility, and endogenous degradation factors such as enzyme content.[15] It is very important to select an appropriate anticoagulant for blood samples as this choice will have a bearing on future analyses; the use of any anticoagulant induces cytokine production in vitro, for example.[16] When dextrose citrate is used, good quality DNA and RNA can be obtained and more lymphocytes are recovered for cell culture. The use of ethylenediaminetetraacetic acid (EDTA) gives good results when extracting DNA, but is problematic for cytogenetic studies. If immortalized cell lines are to be established, the blood should be collected into dextrose citrate. Heparin has a negative effect on T-cell proliferation and also affects DNA extraction and polymerase chain reaction amplification techniques. For metabolomic studies, the best anticoagulant is lithium heparin.

In some epidemiologic studies, whole blood is collected into EDTA only,[17,18] whereas in other cases, samples are mixed with different anticoagulants to keep future options open.[19]

Another factor that should be taken into account is the time between sampling and processing. How critical the time elapsed is will depend on the components of interest and their stability. Some studies have analyzed the importance of the preanalytical stage on the final results.[20] For certain parameters, a period of at least 24 hours before processing and analysis may cause substantial variations of up to 25% in the samples analyzed.[21] Cell viability decreases appreciably from 48 hours onwards, although decreased viability and lower cytokine secretion in samples processed after more than 24 hours have been observed in some studies, such as that of Bull et al,[22] in which 2 different processing times were compared (at 8 and 24 hours after sampling).

Blood Fractions

If cells are desired for DNA or RNA extraction, fractionation can be done by centrifugation to separate plasma, the leukocyte-platelet fraction, and the erythrocyte fraction. Aliquots are prepared of each of the fractions obtained and these are stored at –80°C.

Several methods are described in the literature for cases when cells are also required for subsequent immortalization. Lymphocytes transformed with the Epstein-Barr virus are a limitless source of DNA. There are several possibilities: extraction and immediate transformation or extraction, cell separation, and cryopreservation until future transformation. Studies have shown that long-term cryopreservation does not significantly affect B-cell viability or success rates for transformation.[23-25]

Cryopreserved cells are always stored at –150°C under liquid nitrogen vapor.[26] These samples are also transported under liquid nitrogen vapor in a dry receptacle to avoid any decline in cell viability.[27]

Serum, another fraction that can be obtained from whole blood, is the preferred fraction for clinical biochemistry or metabolic studies. It is just as important to preserve a portion of this sample for future studies. In large epidemiologic studies in which a large number of parameters are determined in serum, it is recommended to collect blood samples in siliconized plastic tubes.[18]

Urine

Urine is another sample commonly stored in collections,[13,15,18] as it is easy and inexpensive to obtain and can be used in a wide range of studies such as DNA, proteomics, and metabolomics. It is often stored for long periods at –80°C.[15,18]

Sputum

From sputum samples, and following the processing protocols of Djukanoviç et al,[28] it is possible to obtain both supernatant (used for determining soluble factors and inflammatory markers[29]) as well as cells.[30] Both types of product are stored at –80°C. DNA and/or RNA can be obtained from frozen sputum cell pellets.[27]

Exhaled Breath Condensate

Samples of exhaled breath condensate allow lung inflammation to be determined noninvasively.[31,32] The condensate contains a wide variety of mediators, such as adenosine, ammonium ions, hydrogen peroxide, isoprostanes, leukotrienes, nitrogen oxide, peptides, and cytokines. The American Thoracic Society and the European Respiratory Society have issued specific methodological recommendations that include storage temperature.[33] In some studies, such as the one reported by Lehtonen et al,[34] the samples were stored at –70°C until future use.

Bronchoalveolar Lavage Fluid

The usual technique for obtaining samples (cells, microorganisms, and soluble substances) from the alveolar interstitium is bronchoalveolar lavage.[35] The first 10 mL are processed separately to obtain the bronchial lavage fluid. The remaining fluid is deposited in siliconized tubes and transported on ice to the laboratory, where the samples are centrifuged at 400*g* for 5 minutes and the cells recovered. The supernatants are aliquoted and stored at –80°C.[36] The cell pellets are immediately frozen and stored at –80°C or –196°C, depending on the analyses or studies to be performed in the future.

Bronchial Aspirate

Bronchial aspirates can be used for diagnostic microbiology cultures and also for obtaining cells for subsequent molecular studies. In the latter case, the cells are stored at –80°C or in liquid nitrogen, depending on the studies that will be done later.

Other Techniques for Sample Collection

Specimens obtained by transbronchial aspiration biopsy and transthoracic fine-needle aspiration biopsy can all be considered as equivalent to tissue biopsy,[37,38] and so the recommendations described in the section Biopsies of Solid Tissue should be followed.

In the case of cell cultures, the samples will be cryopreserved and stored in liquid nitrogen. In the case of blocks and cell pellets, the

Table 2
Recommended Temperatures for Long-term Storage, According to Sample Type

Biological Sample	Recommended Storage Temperature, °C
Urine	−80[a]/−150[a]
Whole blood with DMSO	−80[a,b]
Plasma	−80[a,b], −150[b]
Serum	−80[a,b], −150[b]
Leukocyte-platelet fraction	−150[a,b]
Lymphocytes/mononuclear cells	−150[a,b]
Cell lines	−150[a,b]
Homogenized tissues	−150[a]
Pleural lavage fluids	−80[a]/−150[a]

Abbreviation: DMSO, dimethyl sulfoxide.
The temperature of liquid nitrogen vapor (−150°C) is low enough to store any sample and storage in liquid nitrogen (−196°C) is unnecessary. Recommendation of the Australasian Biospecimen Network (ABN).
[a] Recommendations of the International Agency for Research into Cancer (IARC) of the World Health Organization.
[b] Recommendations of the ABN.

specimen is frozen rapidly and stored at −80°C or in liquid nitrogen according to the intended future use.

Table 2 summarizes the recommendations of the different international organizations regarding long-term storage temperatures.

Subproducts

Obtaining different products from the initial specimens (cells, tissues, blood, or urine) will be the ultimate objective of many proteomics and genomics studies. The scientific literature and the international organizations do not have a common set of recommendations for storage of DNA, RNA, and proteins, However, the scientific literature does, for the most part, describe storage of RNA at −70°C[39] or −80°C.[40]

In the case of DNA, there is less consensus and temperature recommendations vary according to anticipated duration of storage. Table 3 presents some of the recommendations of different studies concerning storage of DNA, with temperatures ranging from +4°C to −80°C. The table also shows the storage temperatures currently used in the main population genomics projects. Some of these projects store different aliquots of DNA at different temperatures. An example of this is the Spanish national DNA bank in Salamanca, which recommends storage at +4°C for periods of less than 2 months; −20°C for short-term storage (up to 1 year), and −80°C for periods in excess of 1 year.

There is also the possibility of storing drops of blood or other fluids on Flinders Technology Associates (FTA) treated filter paper at room temperature for subsequent DNA extraction. The study by Burger et al[41] shows that it is possible to obtain DNA from saliva samples stored on FTA paper kept at room temperature for 2 years, although Sigurdson et al,[42] in a similar study, were unable to obtain good quality DNA with the same type of sample stored for 7 years at room temperature. Rajendram et al,[43] who conducted a study with bacterial suspensions, concluded that good quality DNA can be obtained from these suspensions after 3 years of storage at room temperature. Finally, Galaal et al,[44] who stored ovarian tumor cells at room temperature on FTA paper, obtained good-quality DNA after 5 years and RNA after 6 months of storage.

Several studies have analyzed the stability of different proteins and metabolites after long periods of storage. In some cases, these studies have concluded that concentrations of different serum proteins do not vary when stored at −70°C.[45,46] In contrast, others such as the one by Basit et al,[47] concluded that storage at −70°C for more than 2 years can indeed affect serum concentrations of certain proteins. Shih et al[48] also reported an effect on serum lipid and lipoprotein levels as a function of storage time at a temperature of −70°C.

Table 3
Recommendations for Storage of DNA

Study	Temperature, °C				
	+4	−20	−40	−80	−196
EGP					X
FINRISK 2002	X	X			
GS-SFHS			X		
JFHS	X	X	X		
KORA-gen	X			X	
Life-Gene	X			X	
PMRP	X	X		X	
BNADN	X	X		X	
STAGE				X	
TwinGene				X	

Abbreviations: BNADN, Spanish National DNA Bank; EGP: Estonian Genome Project; FINRISK 2002, Finnish population-based study of risk factors for diseases of nonmandatory reporting; GS-SFHS, Generation Scotland, Scottish Family Health Study; JFHS, Joondalup Family Health Study; KORA-gen, Cooperative Health Research in the Region of Augsburg, Germany; Life-Gene, Swiss prospective cohort study of health and lifestyles; PMRP, Marshfield Clinic Personalized Medicine Research Project; STAGE, Swedish study of genes and environment in adult twins; TwinGene, Swedish study based on pairs of twins to determine environmental and genetic factors in cardiovascular diseases.

Transport of Biological Specimens: Definitions and Classifications

First, we should define the most widely used terms in the international recommendations and legislation.

An infectious substance is defined as one that contains or may contain pathogenic agents (microorganisms or prions) that can cause disease in humans or animals. This definition is applied to all specimens except those explicitly excluded. Infectious substances are divided into 2 categories:

- Category A infectious substance: Exposure to this type of substance may cause permanent disability, be life-threatening, or cause a fatal illness in otherwise healthy humans or animals. Some of the numerous examples would include cultures of *Mycobacterium tuberculosis*, highly pathogenic avian influenza virus, *Chlamydia psittaci* (avian strains), and *Bacillus anthracis*. The substances of this group are assigned to UN 2814, which identifies dangerous goods. The official shipping name of UN 2814 is "infectious substance affecting humans."
- Category B infectious substance: These substances include all infectious substances that do not meet the criteria to be considered as category A. Category B substances are assigned to UN 3373, and the official shipping name is "biological substance, category B."

Exceptions are known as exempt specimens. These are specimens with a minimal risk of containing pathogenic agents. They should be transported in the same packaging as UN 3373 specimens (packaging with 3 elements, described below) and identified as "exempt human specimen." However, for a specimen to be considered exempt, a professional judgment based on the known medical history,

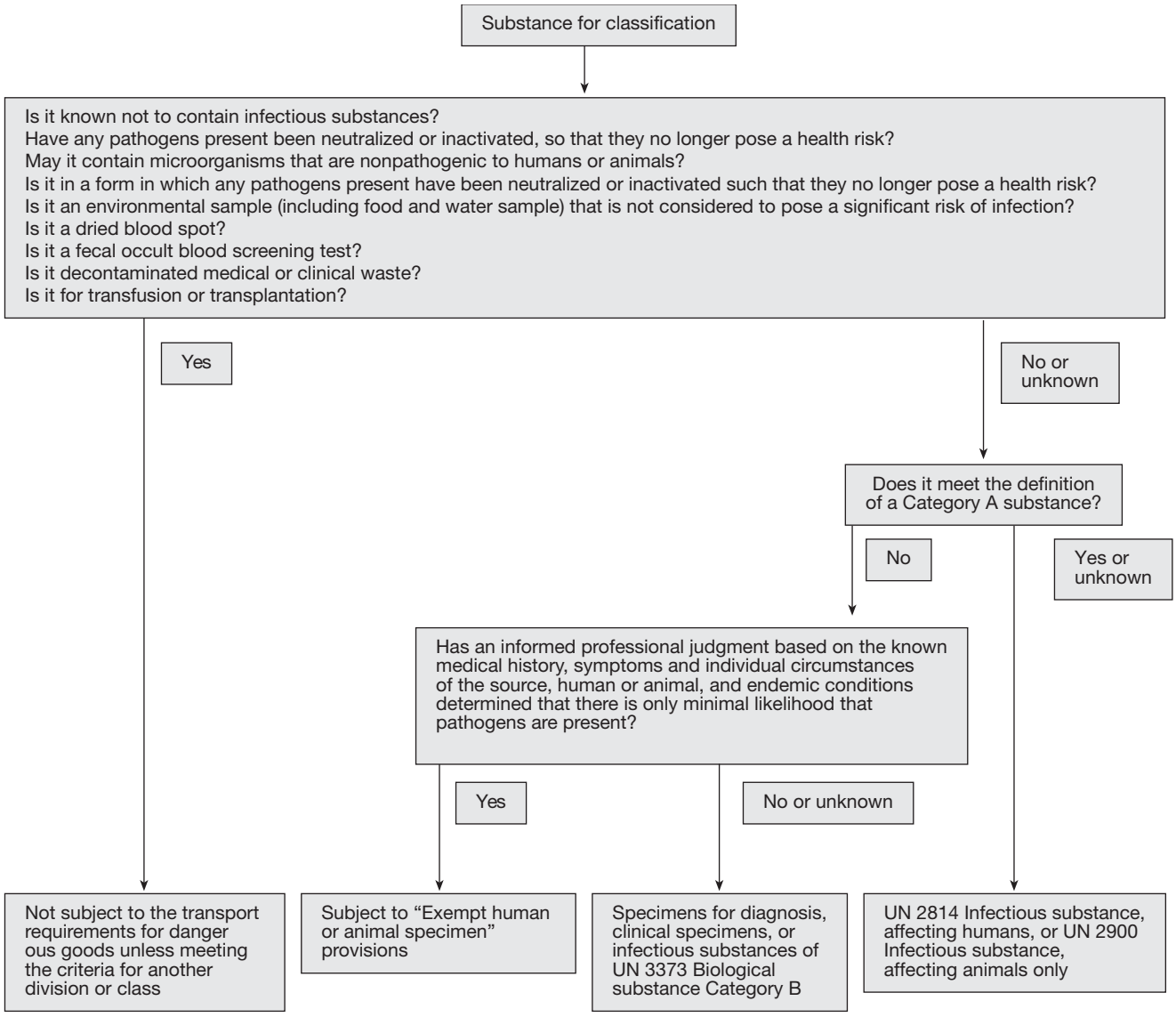

Figure 1. Flowchart for classifying biological specimens according to the UN classification. (Diagram taken from the World Health Organization *Guidelines on the transport of infectious substances, 2007-2008.*[49])

symptoms, and local endemic conditions should be sought. Some examples of specimens that can be considered exempt include derivatives of blood or urine samples used to measure cholesterol or hormone concentrations and blood-glucose levels, and samples taken for therapeutic drug monitoring.

To aid a simple classification of infectious substances for shipping, the World Health Organization, in its *Guidance on Regulations for the Transport of Infectious Substances 2007-2008*,[49] provides a flowchart that may be useful for assigning biological specimens to a given category for hazardous shipping (Figure 1).

Packaging According to Category

Infectious Substances Assigned to UN 3373

A triple packaging system will be used, even for local road transport. The packaging will comply with the 650 packaging instructions (P650) of the United Nations, summarized below. In general, for this group, packaging should be of good quality to avoid possible incidents, and will consist of three layers (a primary receptacle, secondary packaging, and an outer packaging or receptacle). In addition, the primary packaging will be wrapped in an absorbent material in case accidental spillage occurs. The outer packaging will be labeled with the corresponding symbol and with the official shipping name: "biological substance, category B." (There are certain specifications exclusive to air transport[50] which are not presented here.) Figure 2 shows UN 3373-compliant packaging.

Infectious Substances Assigned to UN 2814

Triple packaging will be used, even for local road transport. Category A infectious substances can only be transported in packaging/receptacles that meet the specifications corresponding to class 6.2 of the United Nations and packaging instruction 602 (P602), which are the same as for the UN 3373 category. In addition, these infectious specimens require consignment of the

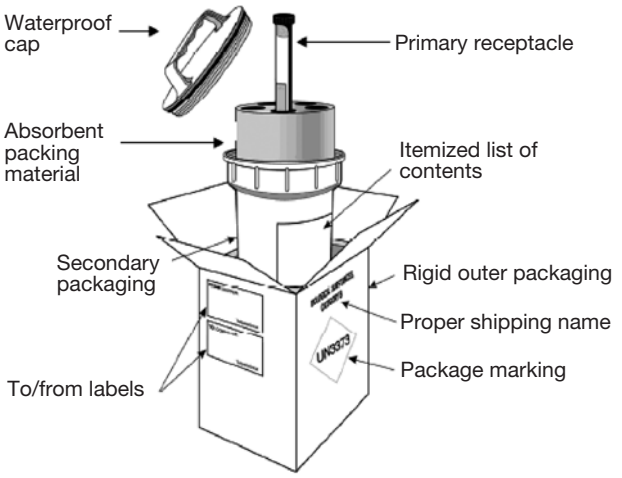

Figure 2. Illustration of UN 3373 packaging. (Diagram taken from the World Health Organization Guidelines on the transport of infectious substances, 2007-2008.[49])

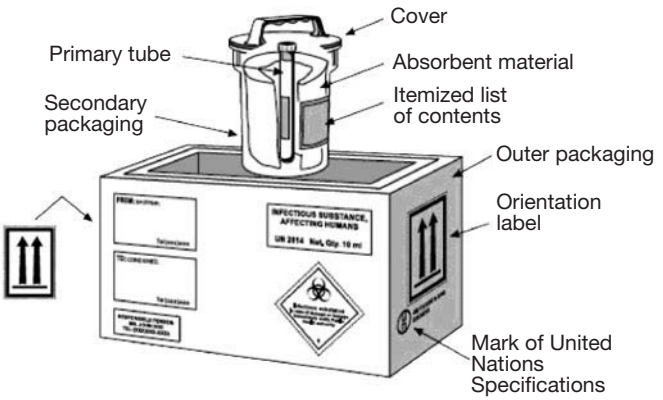

Figure 3. Schematic representation of UN 2814 packaging. (Diagram taken from the World Health Organization Guidelines on the transport of infectious substances, 2007-2008.[49])

packaging/receptacles in areas separated from other types of cargo.

Moreover, infectious substances labeled as UN 2814 will be packaged as follows:

1. Substances consigned at room temperature or above: the primary receptacles will be of glass, metal, or plastic. To ensure a leaktight seal, a heat seal, skirted stopper, or metal crimp seal will be used. If screw caps are used, they will be secured by adhesive tape or manufactured locking closure.
2. Substances shipped refrigerated or frozen: the ice, dry ice, or other refrigerants will be placed around the secondary receptacle or inside the outer packaging that contains one or several complete packages labeled according to regulations. Inner supports will be used to secure secondary packaging in its place after the ice or dry ice has melted or evaporated. If the refrigerant is ice, the outer packaging or overpack should be leakproof. If the refrigerant is dry ice, the outer packaging or overpack should permit the release of carbon dioxide. The primary receptacle and secondary packaging/receptacle should maintain their integrity at the temperature of the refrigerant used.
3. Substances shipped in liquid nitrogen: primary receptacles made of plastic and able to withstand very low temperatures will be used. The secondary packaging/receptacle should also be able to withstand very low temperatures and, in most cases, should be fitted over the primary receptacle individually. The same requirements will apply as those for the transport of liquid nitrogen. The primary receptacle and secondary packaging/container should maintain their integrity at the temperature of liquid nitrogen.
4. Lyophilized substances can also be transported in primary receptacles consisting of flame-sealed glass ampoules or rubber-stoppered glass flasks fitted with metal seals.

Whatever the intended temperature for the substance during transport, the primary receptacle or the secondary packaging/container should be able to withstand, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa and temperatures of –40°C to +55°C.

Figure 3 shows packaging corresponding to UN 2814.

Exportation and Importation of Biological Specimens

Now that the Spanish law on biomedical investigation has come into force, some changes have occurred in the importation and exportation of biological specimens. Any specimen to be transferred to third parties or any specimen entering Spain from abroad must meet the requirements of the royal decree concerning importation and exportation of biological specimens.[51] This royal decree specifies the conditions for importation and exportation of biological specimens and establishes a voluntary registry to allow continued importation and exportation, regardless of the end use (for diagnosis, analysis, or investigation). A biological specimen is considered as any material from humans or other organisms, as well as any substance, pathogenic or otherwise, that is to be used for diagnosis or investigation in humans, including infectious substances. Inscription in this registry obviates the requirement to present certain import and export permits. Registered importers and exporters should renew their documentation every 5 years or update it when there is a change in the type of sample for importation or exportation. Failure to renew the documentation or declare any variation in the type of samples will be grounds for removal from the registry of importers and exporters.

Conclusions

The main recommendations for the collection, processing, transport, and storage of biological specimens stipulate that all biobanks should, from the outset, have protocols for collection, handling, and storage so that the specimens can be used in the future. At the time when they are obtained, it is essential to ensure that they are correctly labeled and that sampling, transport, and processing is completed in as quickly as possible to ensure the stability of the molecules under study. It is also important to have appropriate equipment with sufficient safeguards to ensure proper storage and safe custody of the specimens. In the initial design of

protocols, it is important to bear in mind the factors that might influence the stability of the specimens.[52] The temperatures at which they are stored may compromise future study, so it is recommended to reserve some aliquots for storage in liquid nitrogen for future analyses of molecules unknown at present.[19] Finally, it should be remembered that it is also essential to be well aware of the ethical and legal framework regarding both sampling and handling, as well as the use/transfer of specimens.

Specimen collections that meet all these requirements will help maximize benefit from the major advances in the field of genomics.

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