PART 1

GENERAL INFORMATION

SECTION 1.1.

INTRODUCTORY CHAPTERS

CHAPTER 1.1.1.

COLLECTION AND SHIPMENT OF DIAGNOSTIC SPECIMENS

INTRODUCTION

The starting point for the laboratory investigation of an animal disease is the taking of samples. This first introductory chapter considers some of the general principles involved in sample collection, submission and storage. Each of the disease chapters of this Terrestrial Manual *provides specific information on sample collection for that particular disease. Samples may be taken from animals or the environment for a variety of purposes, such as disease diagnosis, disease surveillance, health certification or monitoring the response to treatment or vaccination. The samples collected should be appropriate for the intended purpose, and adequate in number and amount to provide statistically valid results. Diagnostic laboratories require the submission of appropriate samples that arrive at the laboratory in good condition. For disease diagnosis, the tissues sampled should be representative of the condition being investigated and the lesions observed. Samples should be taken with care, to avoid undue stress or injury to the animal or danger to the operator. Where appropriate, samples should be collected aseptically, and care should be taken to avoid cross-contamination between samples.*

The samples should be carefully packaged, labelled, and transmitted to the laboratory by the fastest practicable method, with the appropriate temperature control. There are specific requirements for the packaging and shipping of infectious substances, including diagnostic specimens that must be followed. If material is sent to a laboratory in another country, this laboratory should be consulted in advance to ensure that it is willing to receive the material and to obtain the appropriate import licence. All samples should be accompanied by a letter or submission form, which includes the name and address of the submitter, the origin of the material, the relevant history, animal identification and corresponding specimens, and the tests requested.

A. COLLECTION OF SAMPLES

Before taking samples, careful consideration should be given to the purpose for which they are required. This will determine the type and number of samples needed to provide valid results. When samples are taken from live animals, care should be taken to avoid injury or distress to the animal or danger to the operator and attendants. It may be necessary to use mechanical restraint, tranquillisation or anaesthesia. Whenever handling biological material, from either live or dead animals, the risk of zoonotic disease should be kept in mind and precautions taken to avoid human infection (see also Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities). Post-mortem examinations should be carried out under as aseptic conditions as is practicable. Care should be taken to avoid environmental contamination, or risk of spread of disease through insects or fomites. Arrangements should be made for appropriate safe disposal of animals and tissues.

Considerable skill and care are required to decide on the correct samples to be sent to the laboratory. The samples collected should be representative of the condition being investigated and the lesions observed. Also the stage of the disease and lesion development should be considered, as well as the type of test(s) that will be performed. Frequently, a combination of blood samples for serology and tissues from dead or culled animals for microbiological culture and pathological examination will be required. Recommendations for transport are described later in this chapter.

The disease chapters in this *Terrestrial Manual* provide guidance on samples that should be collected so that information will not be repeated here. In addition, procedures for sample collection and submission have been prepared by national and international authorities (3, 5, 9, 11, 12). These publications provide detailed recommendations of specific samples that should be collected from different species and for a wide variety of suspected diseases. They also provide information on post-mortem procedures, lists of appropriate media, and instructions on submission of samples. The laboratory that is going to perform the assay(s) should be contacted if there are specific questions concerning the type of sample that should be collected.

1. Sample collection from live animals

a) Blood

Blood samples may be taken for haematology or for culture and/or direct examination for bacteria, viruses, or protozoa, in which case it is usual to use anticoagulants, such as ethylene diamine tetra-acetic acid (EDTA) or heparin. They may also be taken for serology, which requires a clotted sample. Blood plasma is also used for some procedures. A blood sample is taken, as cleanly as possible, by venipuncture. In most large mammals, the jugular vein or a caudal vein is selected, but brachial veins and mammary veins are also used. Vena cava veins are also used in pigs. In birds, a wing vein (brachial vein) is usually selected. For techniques for sampling small laboratory animals, see refs 1 and 6. Blood may be taken by syringe and needle or by needle and vacuum tube (not easy in delicate veins but convenient in strong veins). Small quantities of blood are conveniently obtained by pricking with a triangular, solid-pointed needle. Ideally the skin at the site of venipuncture should first be shaved (plucked) and swabbed with 70% alcohol and allowed to dry.

For samples that are collected with anticoagulant, thorough mixing, using gentle agitation only, is necessary as soon as the sample has been taken. It may also be necessary to make a smear of fresh blood on a microscope slide; both thick and thin smears may be prepared. For polymerase chain reactions, EDTA is the preferred anticoagulant. For serum samples, the blood should be left to stand at ambient temperature (but protected from excessive heat or cold) for 1–2 hours until the clot begins to contract. The clot can then be ringed round with a sterile rod and the bottles placed in a refrigerator at 4°C. After several hours, or overnight, the sample can be centrifuged at about 1000 ***g*** for 10–15 minutes and the serum can be decanted or removed with a pipette. In order to establish the significance of antibody titres, paired serum samples will often need to be collected 7–14 days apart. An alternative method for collecting and transporting blood that is to be used for serology is to place a drop of blood on to filter paper, the blood is dried at room temperature and the sample can then be shipped unrefrigerated. Contact the laboratory to enquire if this method of collection is validated for the required tests.

b) Faeces

At least 10 g of freshly voided faeces should be selected. Faeces for parasitology should fill the container and be sent to arrive at the laboratory within 24 hours. If transport times are likely to be longer than 24 hours, the sample should be sent on ice or refrigerated to prevent the hatching of parasite eggs. Screw top containers or sterile plastic bags should be used for shipment; avoid tubes with rubber stoppers as gas generated can result in blowing the stopper off the tube, ruining the integrity of the sample and contaminating other samples in the package. An alternative and sometimes preferable method is to take swabs from the rectum (or cloaca), taking care to swab the mucosal surface. The swabs should be visibly coated with faecal material; however, samples collected with a swab are inadequate for parasitology. Care should be taken when collecting swabs from small, delicate animals or birds to avoid injury to the animal; small swabs are commercially available that should be used. Swabs should be transported in appropriate transport medium. Faeces are best stored and transported at 4°C.

c) Skin

In diseases producing vesicular lesions, collect, if possible, 2 g of affected epithelial tissue as aseptically as possible and place it in 5 ml phosphate buffered glycerine or Tris-buffered tryptose broth virus transport medium at pH 7.6. Additionally, the vesicular fluid should be sampled where unruptured vesicles are present; if possible, vesicular fluid should be aspirated with a syringe and placed in a separate sterile tube. Plucked hair or wool samples are useful for surface-feeding mites, lice and fungal infections. Deep skin scrapings, using the edge of a scalpel blade, are useful for burrowing mites and, in birds, feather tips can be taken for detection of viral antigen where Marek's disease is suspected.

d) Genital tract and semen

Samples may be taken by vaginal or preputial washing, or by the use of suitable swabs. The cervix or urethra may be sampled by swabbing. Samples of semen are best obtained using an artificial vagina or by extrusion of the penis and artificial stimulation. The sperm-rich fraction should be present in the sample and contamination by antiseptic washing solutions should be avoided. Specific transport media and conditions are often required.

e) Eye

A sample from the conjunctiva can be taken by holding the palpebra apart and gently swabbing the surface. The swab is then put into transport medium. Scrapings may also be taken on to a microscope slide. The handles of metal-handled swabs are useful for this, to ensure that sufficient cells are removed for microscopic examination. Mucopurulent nasal and lacrimal discharges are rarely useful.

f) Nasal discharge (saliva, tears)

Samples may be taken with dacron, cotton or gauze swabs, preferably on wire handles as wood is inflexible and may snap. It may be helpful if the swab is first moistened with transport medium. The swab should be allowed to remain in contact with the secretions for up to 1 minute, then placed in transport medium and sent to the laboratory without delay at 4°C. Long protected nasopharyngeal swabs should be used to collect samples for some suspected viral infections.

g) Milk

Milk samples should be taken after cleansing and drying the tip of the teat, the use of antiseptics should be avoided. The initial stream of milk should be discarded and a tube filled with the next stream(s), a sample of bulk tank milk can be used for some tests. Milk for serological tests should not have been frozen, heated or subjected to violent shaking. If there is going to be a delay in submitting them to the laboratory, preservatives can be added to milk samples that are being collected for serological testing. If necessary, milk for bacterial examination can be frozen.

2. Sample collection at post-mortem

Samples of tissue from a variety of organs can be taken at post-mortem. Detailed procedures for conducting a post-mortem examination and collecting samples are described in most pathology text books; a guide to necropsy procedures has been published (10). Post-mortem techniques are also included in some of the national guidelines (3, 5, 9). A summary of these procedures will be provided here.

Animal health personnel should be trained in the correct procedures for post-mortem examination of the species of animals with which they work. The equipment required will depend on the size and species of animal, but a knife, saw and cleaver will be required, and also scalpel, forceps and scissors, including scissors with a rounded tip on one blade, for opening intestines. A plentiful supply of containers and tubes of transport media appropriate to the nature of the sample required should be available, along with labels and report forms. Containers should be fully labelled with the date, tissue and animal identification. Special media may be required for transport of samples from the field. The operator should wear protective clothing: overalls, washable apron, rubber gloves and rubber boots. Additionally, if potential zoonotic diseases are being investigated, the post-mortem examination should be conducted in a biological safety cabinet; if this is not possible, an efficient face mask and eye protection should be worn. If rabies or transmissible spongiform encephalopathies (TSEs) are suspected, it is usual to detach the animal's head.

Tissues may be collected for microbiological culture, parasitology, biochemistry, histopathology and/or immuno-histochemistry, and for detection of proteins or genome nucleic acids. In addition buccal, oropharyngeal or rectal (cloacal) swabs may be collected. The person conducting the post-mortem examination should have sufficient knowledge of anatomy and pathology to select the most promising organs and lesions for sampling. Each piece of tissue should be placed in a fully labelled separate plastic bag or sterile screw-capped jar. Swabs should always be submitted in appropriate transport media. Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents. Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation.

The tissues may be sent to the laboratory dry or in bacterial or virus transport medium, depending on the type of specimen and the examinations required; swabs should be sent in transport medium. After collection, the samples for microbiological examination should be refrigerated until shipped. If shipment cannot be made within 48 hours, the samples should be frozen; however, prolonged storage at –20°C may be detrimental to virus isolation. For histopathology, blocks of tissue not more than 0.5 cm thick and 1–2 cm long are cut and placed in neutral buffered 4–10% formalin, which should be at least ten times the volume of the tissue sample. For certain suspected diseases, larger portions of brain are required; the brain is sectioned using a sagittal cut, half is submitted fresh, on ice, and the other half is submitted in 10% buffered formalin. For scrapie, bovine spongiform encephalopathy and other TSEs, details of sample collection are provided in the individual disease chapters in this *Terrestrial Manual*. Store and pack formalin-fixed tissues separately from fresh tissues, blood and smears. Care should be taken to insure that formalin-fixed tissues are not frozen. Once fixed, tissues can be removed from formalin and, as long as they are kept moist and protected (e.g. by wrapping in formalin-soaked paper towels, then sealed in screw-capped jars), they can be forwarded to the laboratory without formalin.

3. Environmental and feed sampling

Samples may be taken to monitor hygiene or as part of a disease enquiry. Environmental samples are commonly taken from litter or bedding and voided faeces or urine. Swabs may be taken from the surface of ventilation ducts, feed troughs and drains. This kind of sampling is particularly important in hatcheries, artificial insemination centres and slaughterhouses in which specialised equipment is maintained. Samples may also be taken from animal feed, in troughs or bulk containers. Water may be sampled in troughs, drinkers, header tanks or from the natural or artificial supply.

4. Honey bees

Adult bees, either dead or moribund, may be collected in the vicinity of the colonies. Live bees should be killed by freezing. Brood samples are taken by removing a piece of brood comb that shows abnormalities. This should be wrapped in paper and placed in a box for transport to the laboratory. Hive debris may be collected for examination, preferably on a sticky board to trap mobile parasites.

B. SAMPLE SIZE

When investigating a case of clinical disease, the specimens collected should be representative of the condition being investigated and the lesions observed. When developing a programme of surveillance and monitoring for animal health in the absence of clinically evident disease, some general statistical sampling methods should be used. These sampling methods are needed to perform the scientifically based surveys specified in the OIE *Terrestrial Animal Health Code* (14). It is possible to calculate how many animals should be sampled from a herd/flock of a certain size, to achieve a 95% probability of detecting infection or previous exposure assumed to be present in a certain percentage of the animals. The following formulae can give approximate numbers, but a specific sampling programme for the planned surveillance programme should be based on complete formulas available in the references (2, 4) or by the use of a program (FreeCalc) available off the internet: http://www.ausvet.com.au/content.php?page=res_software#freecalc. All calculation examples provided in the following paragraphs can be calculated using FreeCalc. This software also includes “a “pooled prevalence calculator”, which describes the calculation of prevalence using pooled samples.

The following formula could be used to calculate the sample size n to detect at least one infection with a test that has a 100% sensitivity and specificity; where α is the significance level and 1–α is the level of confidence, p is the prevalence in the population. If disease were present in 5% of a herd of 500 animals, it would be necessary to collect specimens from 56 animals to be 95% confident of finding at least one positive, assuming that both the sensitivity and specificity of the test were 100%. In order to make a prediction of disease prevalence, it is critical that the sample be selected from the population by a formal random sampling procedure. As most diagnostic tests do not have specificity and sensitivity of 100%, the number of specimens collected must be adjusted to the sensitivity and specificity of the test that will be used (see also Chapter 1.1.4 Principles of validation of diagnostic assays for infectious diseases).

$$n = \frac{\ln(\alpha)}{\ln(1-p)}$$

In the above example α = 0.05, 1– α = 95%, p = 0.05 and n = 59

If the sensitivity (*Se*) is less than 100%, the above formula should be modified as follows:

$$n = \frac{\ln(\alpha)}{\ln(1-p.Se)}$$

In the above example with α = 0.05, p = 0.05, specificity (Sp) = 1 and *Se* = 0.95, a minimum of n = 62 animals instead of 59 would need to be sampled to have a probability of at least 0.95 of finding a positive animal. The increase in the sample size from 59 to 62 is due to the decrease in the sensitivity of the test from 1 to 0.95. The graph below gives the minimum sample size required for finding at least one positive for several sensitivity and prevalence combinations at α = 0.05 and Sp = 1.

If the test is known to have a specificity of less than 1, the positive results should be confirmed by a test with a higher specificity. If the prevalence is very low and the test used has a specificity of less than 1, it is very likely that a positive test result is a false positive.

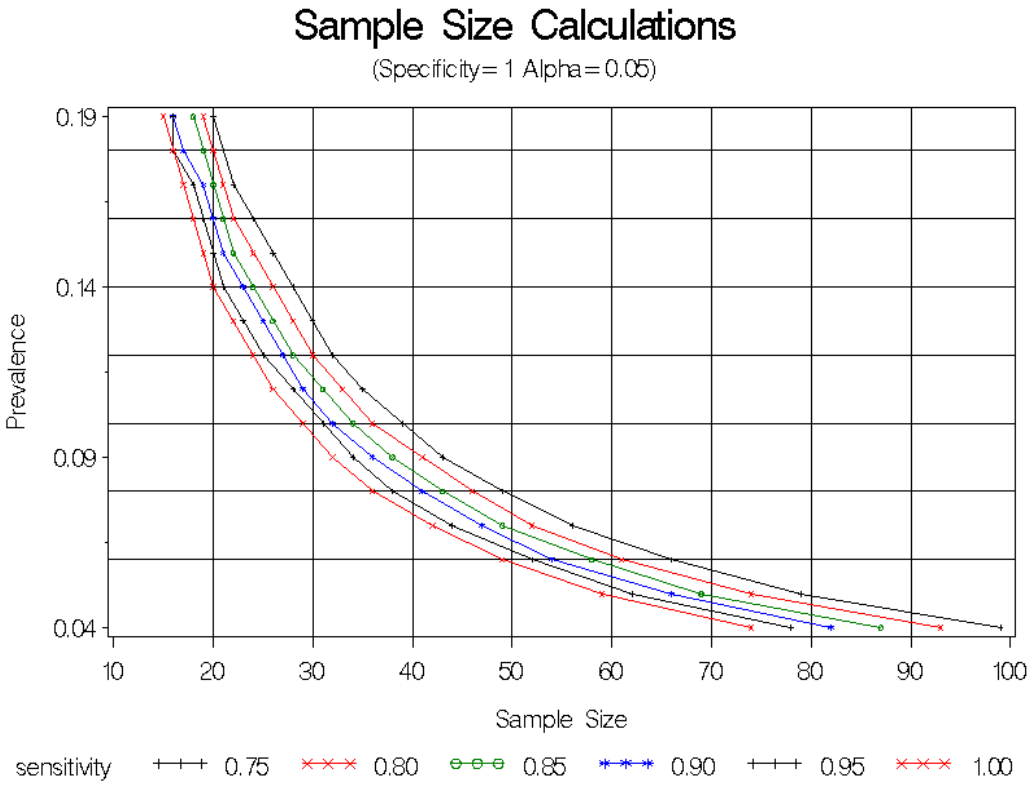


Fig. 1. *Minimum sample size required to be 95% confident of finding infection at various sensitivity and prevalence combinations.*

C. INFORMATION TO BE SENT WITH SAMPLES

It is essential that individual samples be clearly identified using appropriate methods. Marking instruments should be able to withstand the condition of use, i.e. being wet or frozen (use indelible marking pen). Pencil has a tendency to rub off containers and labels attached to plastic will fall off when stored at –70°C. Information and case history should always accompany the samples to the laboratory, and should be placed in a plastic envelope on the outside of the shipping container. As outlined in the following section on transport of samples, this information must also be inside the shipping container. The following are suggested items that should be addressed. It would be advisable to contact the receiving laboratory to determine if it has a submission form that it would like to have submitted with the samples or if it needs other information.

i) Name and address of owner/occupier and geolocation (latitude and longitude, if available) where disease occurred, with telephone and fax numbers.

ii) Name, postal and e-mail address, telephone and fax numbers of the sender.

iii) Diseases suspected and tests requested.

iv) The species, breed, sex, age and identity of the animals sampled.

v) Date samples were collected and submitted.

vi) List of samples submitted with transport media used.

vii) A complete history would be beneficial for the laboratory and should be included if possible. Some of the components of the history are:

   a) A list and description of the animals examined and the findings of the post-mortem examination.

   b) The length of time sick animals have been on the farm; if they are recent arrivals, from where did they originate.

   c) The date of the first cases and of subsequent cases or losses, with any appropriate previous submission reference numbers.

   d) A description of the spread of infection in the herd or flock.

e) The number of animals on the farm, the number of animals dead, the number showing clinical signs, and their age, sex and breed.

f) The clinical signs and their duration including the temperature of sick animals, condition of mouth, eyes and feet, and milk or egg production data.

g) The type and standard of husbandry, including the type of feed available, possible contact with poison or poisonous plants.

h) History of foreign travel by owner or of introduction of animals from other countries or regions.

i) Any medication given to the animals, and when given.

j) Any vaccines given, and when given.

k) Other observations about the disease, husbandry practices and other disease conditions present.

D. PACKAGING AND TRANSPORT OF SAMPLES

1. Approval to ship specimens

The laboratory that is going to receive the samples should be contacted to ensure that it has the capability to do the testing requested and to see if there are any special packaging or shipping requirements. It is essential to contact the receiving laboratory when material is sent to another country. A special import licence will usually be required for shipment of any biological material to other countries and must be obtained in advance. This licence should be placed in an envelope on the outside of the parcel.

Shipments must be made in accordance with the dangerous goods rules for the particular mode of transport. For air transport it is the International Civil Aviation Organization (ICAO) technical instructions for the safe transport of dangerous goods by air. These are reflected in the International Air Transport Association (IATA) Dangerous Goods Regulations which is the interpretation of ICAO instructions applied to shipments by air (7). These regulations have been described in a United Nations World Health Organization publication (13). The shipper is responsible for checking the variations guidelines to insure that restrictions are met.

2. Transportation of specimens

The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated. If dry ice is used, the additional packaging requirements must be met. Infectious substances, which can include diagnostic specimens, are not permitted to be shipped as checked luggage or as carry on luggage and must be shipped as cargo.

3. Packaging

The shipper should ensure that the specimens are packaged so they arrive at the laboratory in good condition and there is no leakage during shipment. The Dangerous Goods Regulations (DGR) have explicit requirements for packaging and shipment of diagnostic specimens, by all commercial means of air transport (7, 13). In some countries, there are similar requirements for ground shipments and the postal service, but these requirements should be reviewed before shipping. These requirements for air transport are covered in detail in the IATA publication, which are updated every year. The shipper is expected to know and follow the procedures outlined in the current DGR. The following is a summary of the regulations at the time that this revision of the *Terrestrial Manual* was published and it should only be used as a guide for shipping. Shippers must also always check the latest version of the DGR prior to shipping diagnostic specimens. In addition, three of the national guidelines provide explicit directions for packaging and shipping diagnostic specimens and are based on IATA requirements (3, 5, 9).

The DGR outline the procedures for the shipment of infectious substances, which can include diagnostic specimens. Infectious substances are defined in the DGR as substances that are known or are reasonably expected to contain pathogens. Pathogens are defined as micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) or recombinant micro-organisms (hybrid or mutant) that are known or reasonably expected to cause disease in humans or animals.

The IATA (7, 13) lists the following exemption from the Dangerous Goods Regulations:

- *3.6.2.2.3.1 Substances which do not contain infectious substances or substances which are unlikely to cause disease in humans or animals are not subject to these Regulations unless they meet the criteria for inclusion in another class.*

- *3.6.2.2.3.2 Substances containing microorganisms which are non-pathogenic to humans or animals are not subject to these Regulations unless they meet the criteria for inclusion in another class.*

- *3.6.2.2.3.3 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 these Regulations unless they meet the criteria for inclusion in another class.*

- *3.6.2.2.3.4 Environmental samples* (*including food and water samples*), *which are not considered to pose a significant risk of infection, are not subject to these Regulations unless they meet the criteria for inclusion in another class.*

- 3.*6.2.2.3.5 Dried blood spots, collected by applying a drop of blood on to absorbent material, or faecal occult blood screening tests and blood or blood components that have been collected for the purposes of transfusion or for the preparation of blood products to be used for transfusion or transplantation and any tissues or organs intended for use in transplantation.*

- 3.*6.2.2.3.6 Patient specimens for which there is 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 human specimen" or "Exempt animal specimen", as appropriate. The packaging should meet the following conditions:*

  (*a*) *The packaging should consist of three components:*
  (*1*) *a leak-proof primary receptacle*(*s*)*;*
  (*2*) *a leak-proof secondary packaging; and*
  (*3*) *an outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm × 100 mm;*

  (*b*) *For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle*(*s*) *and the secondary packaging so that, during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the cushioning material;*

  (*c*) *When multiple fragile primary receptacles are placed in a single secondary packaging, they should be either individually wrapped or separated to prevent contact between them.*

*"**Note:** In determining whether a patient specimen has a minimal likelihood that pathogens are present, an element of professional judgment is required to determine if a substance is exempt under this paragraph. That judgment should be based on the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions. Examples of specimens which may be transported under this paragraph include the blood or urine tests to monitor cholesterol levels, blood glucose levels, hormone levels, or prostate specific antibodies* (*PSA*)*; tests required to monitor organ function such as heart, liver or kidney function for humans or animals with non-infectious diseases, or therapeutic drug monitoring; tests conducted for insurance or employment purposes and are intended to determine the presence of drugs or alcohol; pregnancy test; biopsies to detect cancer; and antibody detection in humans or animals. "*

There are also exceptions for some biological products and the shipper of these products is referred to the IATA Regulations for these requirements as not all biological products are exempt. The following is the DGR definition of Biological Products (7, 13):

> *"Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans 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.*"

The DGR state that infectious substances (including diagnostic specimens likely to contain animal or human pathogens) are designated as Category A and B and assigned to UN 2814, UN 2900 or UN 3373.

> Category A is defined as an: *"Infectious substance, which is transported in a form that when exposure to it occurs, is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals, indicative examples of substances that meet these criteria are given in Table 1 and 2".* Infectious substances meeting this definition that affect humans, including zoonotic agents, are designated UN 2814 and given the shipping name of "***Infectious substance, affecting humans***" those affecting animals only are designated UN 2900 and given the shipping name of "***Infectious substance, affecting animals***".

Infectious substances shipped for diagnostic purposes that do not meet the criteria for assignment to UN 2814 or UN 2900 are assigned to Category B and must be assigned to UN 3373 and designated as "DIAGNOSTIC SPECIMENS or CLINICAL SPECIMENS or *BIOLOGICAL SUBSTANCES CATEGORY B"*.

The IATA DGR contains an indicative list of pathogens that must be assigned to UN 2814 or UN 2900 (Tables 1 and 2). The pathogens on these lists cannot be assigned to UN 3373 (7, 13).

Table 1. Infectious substances affecting humans that must be designated UN 2814

Bacillus anthracis (cultures only)	Japanese encephalitis virus (cultures only)
Brucella abortus (cultures only)	Junin virus
Brucella melitensis (cultures only)	Kyasanur Forest disease virus
Brucella suis (cultures only)	Lassa virus
Burkholderia mallei – Pseudomonas mallei – Glanders (cultures only)	Machupo virus
Burkholderia pseudomallei – Pseudomonas pseudomallei (cultures only)	Marburg virus
Chlamydia psittaci – avian strains (cultures only)	*Mycobacterium tuberculosis* (cultures only)
Clostridium botulinum (cultures only)	Monkeypox virus
Coccidioides immitis (cultures only)	Nipah virus
Coxiella burnetii (cultures only)	Omsk hemorrhagic fever virus
Crimean-Congo hemorrhagic fever virus	Poliovirus (cultures only)
Dengue virus (cultures only)	Rabies virus (cultures only)
Eastern equine encephalitis virus (cultures only)	*Rickettsia prowazekii* (cultures only)
Escherichia coli, verotoxigenic (cultures only)	*Rickettsia rickettsii* (cultures only)
Ebola virus	Rift Valley fever virus (cultures only)
Flexal virus	Russian spring-summer encephalitis virus (cultures only)
Francisella tularensis (cultures only)	Sabia virus
Guanarito virus	*Shigella dysenteriae* type 1 (cultures only)
Hantaan virus	Tick-borne encephalitis virus (cultures only)
Hantavirus causing haemorrhagic fever with renal syndrome	Variola virus
Hendra virus	Venezuelan equine encephalitis virus (cultures only)
Hepatitis B virus (cultures only)	West Nile virus (cultures only)
Herpes B virus (cultures only)	Yellow fever virus (cultures only)
Human immunodeficiency virus (cultures only)	Yersinia pestis (cultures only)
Highly pathogenic avian influenza virus (cultures only)	

Table 2. Indicative examples of animal pathogens forbidden as diagnostic specimens that must be shipped as infectious substances affecting animals (UN 2900)

African swine fever virus (cultures only)	Peste des petits ruminants virus (cultures only)
Avian paramyxovirus Type 1 – Velogenic Newcastle disease virus (cultures only)	Rinderpest virus (cultures only)

Classical swine fever virus (cultures only)	Sheep-pox virus (cultures only)
Foot and mouth disease virus (cultures only)	Goatpox virus (cultures only)
Lumpy skin disease virus (cultures only)	Swine vesicular disease virus (cultures only)
Mycoplasma mycoides – Contagious bovine pleuropneumonia (cultures only)	Vesicular stomatitis virus (cultures only)

New or emerging pathogens must also be assigned to UN 2814 or UN 2900.

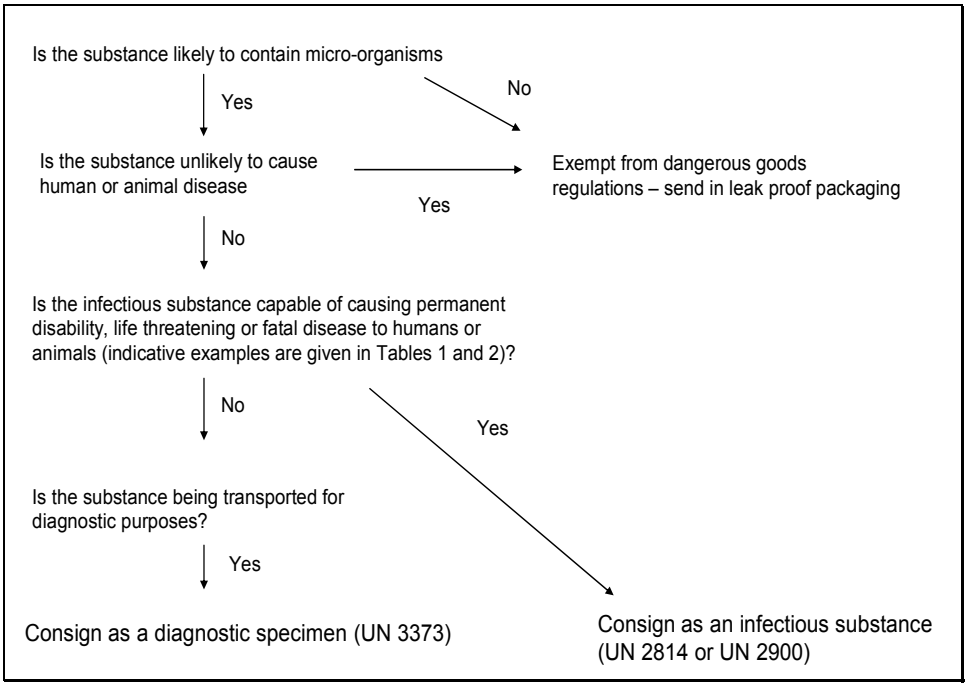
The following is the IATA definition of amplification in culture:

"Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include patient specimens."

"Patient specimens are those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention."

Note: Cultures of organisms that do not fit into the definition of Category A infectious substance can be transported as Biological Substances, Category B.

The following flow chart summarises the classification of *DIAGNOSTIC SPECIMENS* or *CLINICAL SPECIMENS* or *BIOLOGICAL SUBSTANCES CATEGORY B*.



Live animals must not be used to transport infectious substances.

Animal carcasses affected by pathogens of category A or which would be assigned to Category A in cultures only, must be assigned to UN 2814 or UN 2900 as appropriate. Other animal carcasses affected by pathogens included in Category B must be transported in accordance with provisions determined by the Competent Authority.

The packaging of infectious substances and specimens from suspected serious animal diseases, UN 2814 or UN 2900, are outlined in packing instruction 620; a Shippers Declaration of Dangerous Goods must be completed and submitted with these samples. There is also a requirement that the shipper receive training on the IATA-approved shipping procedures for UN 2814 and UN 2900 shipments. Due to the complexity of these guidelines the shipper is referred to the regulations for further information on all UN 2814 or 2900 shipments (7, 13).

The other group, UN 3373, covers 'Diagnostic Specimens or Clinical Specimens or Biological Substances Category B'. This category has a lower risk and packages containing these specimens should be labelled as 'Diagnostic Specimens or Clinical Specimens or Biological Substances Category B'; a Declaration of Dangerous Goods is not needed. IATA packing instruction 650 provides the guidelines for packaging infectious substances assigned to UN 3373 and the following is a summary of these packing instructions. However, the complete procedure, as outlined in the most recent IATA Dangerous Good Regulations, must be followed (7, 13).

i) Infectious substances assigned to UN 3373 'Diagnostic Specimens' must be packed in good quality packaging, which must be strong enough to withstand the shocks and loadings normally encountered during transport. Packaging must be constructed and closed so as to prevent any loss of contents, which might be caused under normal conditions of transport.

ii) The packaging must consist of three components:

- a primary receptacle;
- a secondary packaging; and
- a rigid outer packaging.

iii) For liquid substances:

- the primary receptacle(s) must be leak-proof and must not contain more than 1 litre; the secondary packaging must also be leak-proof;
- adequate adsorbent material must be packed around the primary receptacle(s) to absorb all the fluid in the primary receptacle(s);
- if multiple primary receptacles are used they should be individually wrapped or separated so as to prevent contact;
- the primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure of 95 kPa in the range of –40°C to 55°C (–40°F to 130°F);
- the outer packaging must not contain more than 4 litres. This quantity excludes ice, dry ice, or liquid nitrogen when used to keep specimens cold.

iv) For solid substances:

- the primary receptacle(s) must be sift-proof and must not exceed the outer packaging weight limit; the secondary packaging must be sift-proof;
- adequate adsorbent material must be packed around the primary receptacle(s) to absorb all the fluid in the primary receptacle(s);
- except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4 kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold;
- if there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then packaging suitable for liquids, including absorbent materials, must be used.

v) An itemised list of contents must be enclosed between the secondary packaging and the outer packaging.

vi) If shipped at ambient temperatures or higher, the primary receptacle must have a positive means of ensuring that it is leak proof, such as a leak proof seal, heat seal or skirted stopper. If screw caps are used they should be sealed with parafilm or tape.

vii) Prefrozen packs or dry ice can be packed around the secondary receptacle. If dry ice is used, there must be an internal support to secure the secondary receptacle in the original position after the dry ice has been dissipated. The outer packaging must permit the release of carbon dioxide. There are additional requirements if liquid nitrogen is used and these are described in the DGR.

viii) Packages containing diagnostic or clinical specimens are not required to have the net quantity marked on the outside of the package. However, where dry ice is used as a refrigerant, the net quantity of dry ice must be shown.

ix) The primary and secondary receptacles must be put into a shipping container with adequate cushioning material.

x) The packaging must be able to withstand a 1.2 metre drop test. (There are additional strength requirements for packaging used for UN 2900 and UN 2814 specimens.)

xi) At least one surface of the outer packaging must have a minimum dimension of 100 mm × 100 mm.

xii) For transport, the label **3373** must be displayed on the external surface of the outer packaging on a background of a contrasting colour and must be clearly visible and legible. The mark must be in the form of a square set an angle of 45° (diamond-shaped) with each side having a length of at least 50 mm, the width of the line must be at least 2 mm, and the letters and numbers must be at least 6 mm high. The proper

shipping name “Diagnostic specimen”, “Clinical specimen” or “Biological substance category B” in letters at least 6 mm high must be marked on the outer package adjacent to the diamond-shaped mark.

4. Shipping forms

All shipping forms, including the import licence and submission form must be put in an envelope attached to the outside of the shipping container. The forms and labels must be completed as outlined in the DGR and also put on the outside of the container.

E. PRESERVATION OF SAMPLES FOR PROLONGED STORAGE

Establishing a collection of samples for future studies can be very useful. This can include cultures for comparison with future isolates, tissue or serum samples that can be used for the validation of new tests and a collection of fixed tissues, or paraffin blocks, for future histological examination. Possibly the most useful collection is the storage of serum samples. These samples may be useful if a retrospective investigation is carried out to compare the present disease status with that of earlier times.

- **Serum banks**

Serum samples can provide information about the animals from which the sera were taken. The samples can be tested for a variety of constituents, such as immunoglobulins, trace elements, toxins, hormones and enzymes. If a sufficient number of serum samples have been collected at random from a population, comparisons can be made on the affect of sex, age, breed and geographical location. Results from this comparison can identify high risk groups, vaccination priorities can be established, and patterns and rates of disease determined (8).

A serum bank is a catalogued collection of sera that are stored so as to preserve their immunological and other biochemical properties. Both the catalogue and the storage conditions are essential for a successful serum bank. Each individual sample should be fully documented and identified. The database should contain all relevant information about the origin of the sample and test results obtained. Additional data that may be of interest, such as weather conditions and the animal’s productivity may also be included. Accurate records are essential and must be obtained when the blood samples are collected. The first essential is the complete identification of the animal. The amount of detail recorded should be appropriate to the abilities of the operator, accuracy being more important than quantity of information. Although pooling of sera reduces documentation and storage space, it should be avoided as it greatly reduces the usefulness of the material. Care should be taken to collect the blood as aseptically as possible and sterility should be maintained during separation of the serum and all other manipulations. The serum bank catalogue should be well organised and maintained on a computer database with appropriate backup. A suggested methodology has been described in detail (8).

Sera may be stored for periodic use or kept in long-term storage for historical purposes and these two functions should be separated. Storage conditions should minimise loss of immunological and other biochemical properties of the sera. There are three methods: deep freezing, dry storage on paper disks at ambient temperature and lyophilisation (freeze-drying). For long-term storage of sera by deep freezing, a core temperature below –60°C should be maintained. The lower the temperature the better, but lower temperatures are more expensive to maintain. Liquid phase N_2 is at –196°C, vapour phase N_2 is at –100°C and an ultra-low deep freezer will maintain –90°C. Some serum banks have been maintained at –20°C, but the serum may deteriorate and not be suitable for detection of some properties, especially if stored for long periods at this temperature. Deep-freezers should have a system to provide a warning if the temperature rises due to mechanical break down or power failure. A stand-by generator is essential together with alternative cold storage space in case the contents of a freezer must be transferred. Paper disk storage is a simple and inexpensive method, but it allows only a small quantity of serum to be stored and the eluted serum is only suitable for a limited number of tests. The disks should be kept in a cool, dry atmosphere. They can probably provide satisfactory results for up to about 5 years. Lyophilisation is generally regarded as the best method for long-term storage of sera. If freeze-drying conditions are optimised the loss of serum characteristics are minimised. Lyophilisation requires expensive equipment and is a time-consuming process. Lyophilised vials should be stored at 4°C.

REFERENCES

1. ANON (1993). Removal of blood from laboratory mammals and birds. First Report of the BVA/FRAME/RSPCA/UFAW/Joint Working Group on Refinement. *Laboratory Animals*, **27**, 1–22.

2. CAMERON A.R. & BALDOCK F.C. (1998). A new probability formula for surveys to substantiate freedom from disease. *Prev. Vet. Med.,* **34**, 1–17.

3. CANADIAN FOOD INSPECTION AGENCY, LABORATORY DIRECTORATE: ANIMAL HEALTH (2002). Manual of Common Procedures. Section: Specimen Collection and Submission; Specimen Packaging; Specimen Transportation. Canadian Food Inspection Agency, Ottawa, Canada, 61 pp (http://www.inspection.gc.ca/english/animal/heasan/disemala/cpm-mpc/indexe.shtml).

4. CANNON R.M. & ROE R.T. (1982). Livestock Disease Surveys – A Field Manual for Veterinarians. Department of Primacy Industry, Water and Environment, Australia, 15 pp.

5. COOK R., BARTON M., GLEESON L. & MAIN C. (1996). AUSVETPLAN Management Manual: Laboratory Preparedness. Animal Health Australia, Canberra, 56 pp. http://www.aahc.com.au/ausvetplan/index.htm.

6. HEM A., SMITH A.J. & SOLBERG P. (1998). Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink. *Laboratory animals*, **32** (4), 364–368.

7. INTERNATIONAL AIR TRANSPORT ASSOCIATION (2006). Dangerous Goods Regulations, 44th Edition. International Air Transport Association, 800 Place Victoria, P.O. Box 113, Montreal, Quebec H4Z 1M1, Canada, 824 pp.

8. MOORHOUSE P.D. & HUGH-JONES M.E. (1981). Serum banks. *Vet. Bull*., **51**, 277–290.

9. NATIONAL VETERINARY SERVICES LABORATORIES (2006). Procedures for Collection and Submission of Specimens. National Veterinary Services Laboratories, Ames, Iowa, USA. http://www.aphis.usda.gov/vs/nvsl/html/shipping.html

10. STRAFUSS A.C. (1988). Necropsy: Procedures and Basic Diagnostic Methods for Practicing Veterinarians. Charles C. Thomas, Springfield, IL, USA, 244 pp.

11. VETERINARY LABORATORIES AGENCY (2003). Submission of Samples to the Veterinary Laboratories Agency. Veterinary Laboratories Agency, New Haw, Addleston, Surrey, United Kingdom, 27 pp. www.vla.gov.uk/servtovet/documents/Submissions.pdf

12. VETERINARY SERVICES (OF THE UNITED STATES DEPARTMENT OF AGRICULTURE) (2005). Regulations for Classifying Infectious Substances and Diagnostic Specimens, USDA Veterinary Services Notice NO. 06-02

13. WORLD HEALTH ORGANIZATION (2005). Guidance on regulations for the transport of infectious substances. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2005_22/en/

14. WORLD ORGANISATION FOR ANIMAL HEALTH (OIE: OFFICE INTERNATIONAL DES EPIZOOTIES) (2006). Terrestrial Animal Health Code. OIE, Paris, France, www.oie.int.

*
* *