SECTION I.1

INTRODUCTORY CHAPTERS

CHAPTER I.1.1.

SAMPLING METHODS

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, which 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 I.1.6. Human safety in the veterinary microbiology laboratory). 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. Frequently, a combination of blood samples for serology and tissues from dead or culled animals for microbiological culture 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 (2, 4, 8, 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. In birds, a wing vein (brachial vein) is usually selected. In small laboratory animals, the vena auricularis or vena retroorbitalis maybe useful to obtain blood samples or it maybe obtained by heart puncture. 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 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 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.

b) Faeces

At least 10 g of freshly voided faeces should be selected. Faeces for parasitology should fill the container and be sent refrigerated to prevent hatching of parasite eggs and should arrive at the laboratory within 24 hours. 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 (2, 4, 8). 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 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. 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. 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 examinations required. 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 slaughter houses 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.

B. SAMPLE SIZE

When investigating a case of clinical disease, the samples collected should be representative of the condition being investigated and the lesions observed. When developing a programme of surveillance and monitoring for animal health, 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* (9). 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 assumed to be present in a certain percentage of the animals. The following formulae can give approximate numbers but a specific sampling program for the planned surveillance program should be based on complete formulas available in the references (1, 3, 11) or by the use of a program (FreeCalc) available off the internet: http://www.ausvet.com.au/content.php?page=res_software#freecalc.

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 sample 59 animals to be 95% confident of finding at least one positive, assuming that both the sensitivity and specificity of the test were 100%. As most diagnostic tests do not have specificity and sensitivity of 100%, the number of samples collected must be adjusted to the sensitivity and specificity of the test that will be used (see also Chapter I.1.3. 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 possible that a positive test result is a false positive.

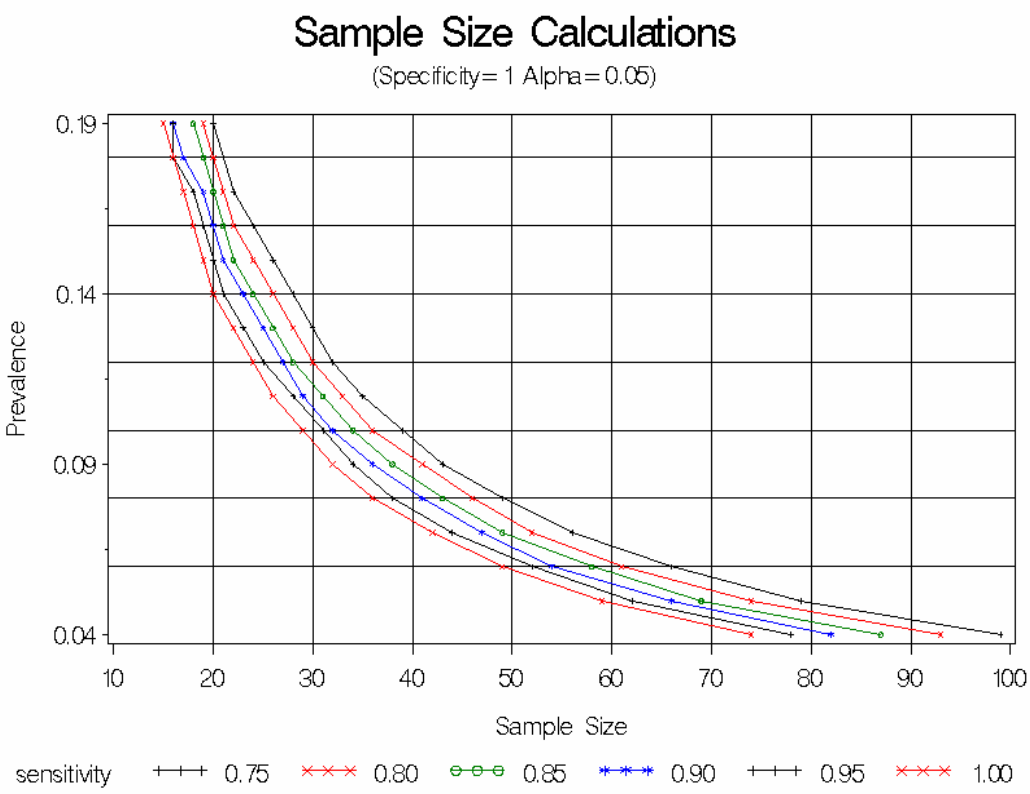


Fig. 1. *Minimum sample size required to be 95% confident of finding infection at various sensitivity and prevalence combinations*

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

i) Any vaccination given, and when given.

j) Other observations about the disease and husbandry

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 and must be obtained in advance for any biological material. This licence should be placed in an envelope on the outside of the parcel.

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 International Air Transport Association (IATA), Dangerous Goods Regulations (DGR) have explicit requirements for packaging and shipment of diagnostic specimens, by all commercial means of air transport (5, 6). In many countries, similar requirements are applicable to ground shipments and the postal service. These requirements for air transport are covered in detail in the IATA publications, 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 *Terrestrial Manual* went to publication and it should only be used as a guide for shipping. It is anticipated that there will be major changes in the DGR effective 1 January 2005. If there are significant changes in the DGR, a revised version of this chapter will be posted on the OIE web site: www.oie.int. Shippers must also always check the latest version of the IATA 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 (2, 4, 8).

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 which 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 (5, 6) lists the following exemption from the Dangerous Goods Regulations:

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.

There are also exceptions for some Biological Products and the shipper of these products is referred to the IATA Regulations for these requirements as some Biological Products are not exempted. The following is the DGR definition of Biological Products (5, 6):

Biological products are derived from living organisms. These are manufactured and distributed in accordance with the requirements of appropriate national governmental 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 and diagnostic products.

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

Samples sent for diagnostic purposes should be designated as UN 2814 or UN 2900 if they include material derived from humans or animals with a disease that can be readily transmitted and for which effective treatment and preventative measures are not usually available[1]. Infectious substances meeting this definition that affect humans, including zoonotic agents, are designated UN 2814; those affecting animals only are designated UN 2900.

Infectious substances shipped for diagnostic purposes that do not meet the criteria for assignment to UN 2814 or UN 2900 are assigned to UN 3373 and designated as 'Diagnostic Specimens'.

The IATA DGR contain 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 (5, 6).

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
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
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)
Hantaviruses causing hantavirus pulmonary syndrome	Variola virus
Hendra virus	Venezuelan equine encephalitis virus
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)	

1 The definition that has been proposed for the 2005 version of the DGR (5) is: "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 to humans or animals".

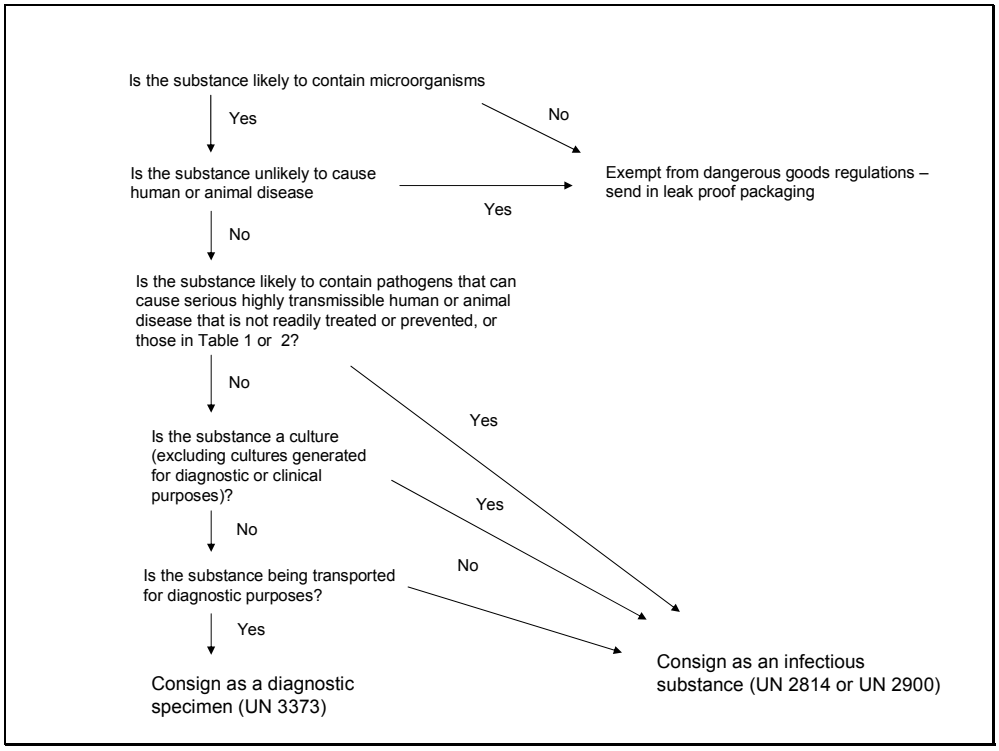
Table 2. Indicative examples of animal pathogens forbidden as diagnostic specimens that must be shipped as infectious substances affecting animals (UN 2900)

African horse sickness virus	*Mycoplasma mycoides* – Contagious bovine pleuropneumonia
African swine fever virus	Peste des petits ruminants virus
Avian paramyxovirus Type 1 – Newcastle disease virus	Rinderpest virus
Bluetongue virus	Sheep-pox virus
Classical swine fever virus	Goatpox virus
Foot and mouth disease virus	Swine vesicular disease virus
Lumpy skin disease virus	Vesicular stomatitis virus

Any infectious agent that can cause disease in humans or animals that has been amplified in culture and 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 (*laboratory stocks*) *are the result of a process by which pathogens are amplified or propagated in order to generate high concentrations, thereby increasing the risk of infection when exposure to them occurs. This definition refers to cultures prepared for the intentional generation of pathogens and does not include cultures intended for diagnostic and clinical purposes.*

The following flow chart summarises the classification of diagnostic samples.



The packaging of infectious substances and specimens from suspected serious animal diseases, UN 2814 or UN 2900, are outlined in packing instruction 602; 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 IATA regulations for further information on all UN 2814 or 2900 shipments.

The other group, UN 3373, covers 'Diagnostic Specimens'. This category has a lower risk and packages containing these specimens should be labelled as 'Diagnostic Specimens'; 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 compete procedure, as outlined in the most recent IATA Dangerous Good Regulations, must be followed (5, 6).

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 specimen should be put into the primary receptacle(s), which can be glass bottles, tubes or plastic containers.

iii) Adequate adsorbent material must be packed around the primary receptacle(s) to absorb all the fluid in the primary receptacle(s).

iv) If multiple primary receptacles are used they should be individually wrapped or separated so as to prevent contact.

v) The primary receptacle(s), and adsorbent material, are put into the secondary receptacle.

vi) The primary receptacle and the secondary packaging used for liquids must be leak-proof. The primary receptacle or secondary packaging must withstand, without leakage, an internal pressure differential of not less than 95 kPa in the range from –40°C to 55°C.

vii) The primary receptacle(s) used for solids must not leak (be siftproof).

viii) The primary receptacle must not contain more than 500 ml of liquids or 500 g of solid specimens. The outer packaging must not contain more than 4 litres for liquid specimens or 4 kg for solids.

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

x) 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 taped shut.

xi) 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.

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

xiii) 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.)

xiv) The package must be labelled Diagnostic Specimens. The 'Nature and Quantity of Goods' box of the airway bill must state 'DIAGNOSTIC SPECIMENS PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650'.

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.

Ê 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 (7).

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 (7).

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.

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