CHAPTER 1.1.2.

COLLECTION, SUBMISSION AND STORAGE OF DIAGNOSTIC SPECIMENS

INTRODUCTION

Laboratory investigation of animal disease and health indicators is critically dependent on the quality and appropriateness of the specimens collected and submitted for analysis. This chapter sets out the general standards involved in specimen collection, submission, and storage. The individual disease chapters in this Terrestrial Manual *provide specific information on appropriate specimens needed to test for particular pathogens, biomarkers, or toxins. Sampling may be from individual animals, from animal populations, or from the environment for a variety of purposes, such as disease diagnosis, disease surveillance, health certification, and monitoring of treatment or vaccination responses. To ~~provide~~ ensure scientifically and statistically valid results the specimens collected must be ~~appropriate~~ suitable for the intended purpose, and adequate in quality, volume, and number for the proposed testing. Additionally, the animals and tissues sampled must ~~be appropriately representative of the condition being investigated~~ accurately represent the condition under investigation.*

Specimens must be collected using appropriate biosafety and containment measures in order to prevent contamination of the environment, animal handlers, and individuals doing the sampling as well as to prevent cross-contamination of the specimens themselves. Regulations should be followed, including for the sampling of protected animals. Care should additionally be taken to avoid undue stress or injury to the animal and physical danger to those handling the animal. Biological materials should be packaged to rigorously control for leakage~~,~~ and then labelled with strict adherence to the applicable regulations guiding their transport as outlined in Chapter 1.1.3 Transport of biological materials.

A. COLLECTION OF ~~SAMPLES~~ SPECIMENS

1. General considerations

Careful consideration must be given to the collection, containment, and storage of the specimens, including biosafety measures that must be in place to prevent contamination of the environment or exposure of other animals and humans to potentially infectious materials (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*). For information on transport of specimens see Chapter 1.1.3 *Transport of biological materials.*

The reliability of the diagnostic testing is critically dependent on the specimen(s) being appropriate, of high quality, and representative of the disease ~~process~~ or condition being investigated. Prior to sampling, consideration must be given to the type of specimen(s) needed, including the purpose of the testing and the test technologies to be used. The volume or quantity of specimen must be sufficient to perform initial testing, to perform any subsequent confirmatory testing and to provide sufficient residual specimen for referral or archival purposes.

The purposes of testing will be aligned with the purposes for which tests are validated, as listed in Chapter 1.1.6 *Validation of diagnostic assays for infectious diseases of terrestrial animals*~~, namely:~~.

~~i) Demonstration of freedom from infection in a defined population.~~

~~ii) Certification of freedom from infection or presence of the agent in individual animals or their products for trade/movement purposes.~~

~~iii) Eradication of disease or elimination of infection from defined populations.~~

~~iv) Confirmatory diagnosis of suspect or clinical cases.~~

~~v) Estimation of prevalence of infection or exposure to facilitate risk analysis.~~

~~vi) Determination of immune status of individual animals or populations (post-vaccination).~~

Epidemiologically appropriate sampling plans should be developed prior to collection of specimens, as described in Section B and Appendix 1.1.2.1. These will specify the number of animals or other sampling units to be sampled.

Specimens ~~must~~ should be collected according to a sound knowledge of the epidemiology and pathogenesis of the disease under investigation, or the disease syndrome to be diagnosed, by a trained and competent person, taking into consideration animal welfare, relevant regulations and ethics. In the absence of sufficient knowledge (for instance, unusual host species), the specimen collection strategy has to be adapted to maximise the chance of detecting the causative agent(s). ~~This will lead to the sampling of tissues or fluids most likely to contain the infectious agent or evidence of the infection.~~ Considerations will include the tissue predilection(s) or target organ, the duration and site of infection in each tissue type and the duration and route of shedding, or the time frame in which evidence of past infection, such as an antibody response, can be detected reliably by the tests to be deployed. Host ectoparasites and environmental specimens can also be considered for pathogen detection. ~~These considerations will also indicate~~ The method(s) of collection should also be considered when selecting the specimen type to be used. In many herd or flock-based disease investigations, it is beneficial to collect specimens from a healthy cohort for comparative epidemiological or baseline testing (e.g. case-control and cohort approaches for diagnostic testing) and for validation purposes.

Where chemical euthanasia, sedation or anaesthesia is required for animal restraint, the impact on animal welfare and scope for the chemical to influence ~~on~~ the test result (e.g. toxicology testing) must be considered. Some laboratory tests are not compatible with specific blood anticoagulants and tissue preservatives, such as heparin, formalin, dry ice (exposure of the test sample to elevated levels of CO_2), or even freezing. While it is critical to collect specimens as aseptically as possible, equal care must be taken to avoid contamination with detergents and antiseptic treatments used to clean the collection site on the animal, as these agents may interfere with the laboratory test procedures. Procedures requiring tissue culture of pathogens, as well as many molecular-based tests, can be negatively affected by chemicals or detergents commonly used in the manufacture or preparation of collection tools (e.g. chemicals used in manufacture of some types of swabs and detergents used in cleaning glassware).

Specific information on diagnostic test methodologies and the recommended specimens, preservatives, and specimen handling procedures can be found in the individual *Terrestrial Manual* disease chapters or through direct consultation with the laboratory that will be performing the required testing. Procedures for collection and submission of specimens are available from most diagnostic laboratories, including national and international authorities, where the information is frequently accessible via the specific laboratory's web page. The WOAH web page provides contact information for all WOAH reference laboratories[1].

It is critical not only to collect the most diagnostically appropriate specimens, but to also inform the laboratory of the associated disease epidemiology, aiding ~~in order for the~~ laboratory scientists ~~to~~ in assigning the most appropriate tests or panels of tests. Epidemiological information to be submitted with specimens is outlined in Section C of this chapter.

Where investigating diseases of unknown cause multiple different specimens that represent the different stages of the disease progression in an animal or the population of animals (e.g. the pre-clinical, early clinical, active clinical, chronically affected and convalescent phases) should be collected. Appropriate control specimens can also be collected whenever metagenomics and metaxonomics (microbiome exploration) are considered. Epidemiological considerations for sampling are particularly critical for diagnosing population-related diseases, as would occur with beehives, flocks, and herds. Epidemiological principles used for sampling are further introduced in Section B of this chapter. Investigation of pathogens transmitted at the livestock/wildlife/human interface may require a multi-pronged and interdisciplinary (One Health) approach.

1 https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

Specimens can be collected ante-mortem or post-mortem and specimen ownership aspects need to be clarified before sampling. Consideration related to transport of specimens and biosecurity are described in Chapter 1.1.3 *Transport of biological materials* and Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*, compliance with national and international regulations is needed for sampling and specimen transfer. Sampling of wild species is frequently subject to specific permits from wildlife authorities and should be aligned with existing wildlife management plans. Consideration of sampling of endangered wildlife protected by national or international regulations may require expert consultation, a risk analysis or a cost–benefit analysis and should consider practicalities of access to remote locations, specific expertise required, lack of basic data, carcasses degradation (scavengers or autolysis ~~autolyse~~). When possible, sampling invasiveness should be reduced and non-invasive alternatives should be sought, especially with wild animals (Schilling *et al.*, 2022). Consultation with an appropriate veterinary diagnostic laboratory ~~with expertise~~ or experts in wildlife ~~is critical for success~~ should be considered, and may include field training (WOAH, 2025).

Specific considerations regarding different specimen types are as outlined below.

2. Blood

Whole blood ~~samples~~ specimens may be collected for ~~haematology, clinical chemistry, toxicology,~~ direct examination for bacteria or parasites, PCR testing, untargeted metagenomic diagnostics or immunological testing, or for culture for bacteria or viruses. In addition, these specimens could be used for haematology, clinical chemistry or toxicology. Dependent on testing needs, whole blood, blood cells, and/or plasma samples can be obtained from whole blood collected into appropriate anticoagulants. In selecting the anticoagulant to be used the collector must be aware of the laboratory tests, including PCR-based diagnostics, clinical chemistry, and toxicology, which may be negatively affected by the presence of specific anticoagulants or preservatives. Specific disease chapters in this *Terrestrial Manual* provide guidance for individual tests and sample requirements. To be effective anticoagulants require that the collected blood be thoroughly mixed with the chosen anticoagulant during or immediately following its sampling from the animal.

To obtain serum, whole blood is collected without anticoagulants, and the clot is allowed to contract at ambient temperature protected from extremes of heat and cold for periods that may range from a few hours to ~~overnight~~ several hours, while extended periods, e.g. overnight, should be avoided. Clear serum can be decanted or collected by pipette following physical removal of the clot, ideally following gentle centrifugation to separate cell components from the serum. In the absence of a centrifuge, separation of the clot can be facilitated by tilting the freshly collected blood tube at an approximate 45-degree angle until the clot has retracted, “ringing” the clot with a sterile rod or pipette to separate the clot from the tube surface and then removing the clot with sterile forceps. The results of serological testing can be compromised by the quality of the ~~samples~~ specimens and bacterial contamination. ~~Bacterial contamination and red blood cell debris in serum samples can produce false positive reactions in agglutination type assays. Serological assays can be negatively impacted by haemolysis in the serum sample. Microbial contamination and haemolysis are significant concerns especially when obtaining blood and serum samples from post-mortem animals.~~ Frequent causes of haemolysed serum and plasma include exposure to excessive temperatures or time delays prior to separating sera from the red blood cells, blood collection using a needle of too small gauge, or failure to remove the needle when transferring the blood sample from the collection syringe.

Member	Comment
USA	**Category**: Editorial **Proposed amended text:** …blood collection using a needle of too small **~~gauge~~ diameter/bore**, … **Rationale**: A needle’s diameter and gauge number are inversely related, which can cause confusion. Recommend using diameter/bore to be clearer. **Supporting Evidence**: not relevant

Whole blood should be collected aseptically, typically by venipuncture of the live animal. Depending on the animal and sampling situation jugular, caudal, brachial, cephalic, mammary veins or the vena cava may be used. Specific techniques for sampling small laboratory animals have been reviewed (Anon, 1993; Hem *et al.,* 1998). Care should be taken to collect and dispense blood samples as gently as possible to prevent damage to red blood cells, which causes haemolysis. Blood and sera are typically shipped and stored cool (or frozen in the case of sera) in non-breakable vials, tubes, or bottles; however, for some laboratory tests that require viable peripheral blood mononuclear cells, the blood must be packaged, transported and stored so as to prevent exposure to temperature extremes. For some tests, aliquots of specimens can be dried onto a piece of untreated or specifically treated commercial filter paper designed for stabilised sample transport and storage. However, in humid environments, the use of desiccants may additionally be required.

When collecting blood from animals of limited size or when cold storage is unavailable, specimens may be collected

using Nobuto filter paper strips. Blood collected with these strips can usually be used for virological and serological methods, however, verification of the method is advisable and should include assessment of storage time and temperature.

Member	Comment
USA	**Category**: Deletion/addition **Proposed amended text**: When collecting blood from animals of limited size or when cold storage is unavailable, specimens may be collected using ~~**Nobuto**~~ filter paper strips **or similar products**. **Rationale**: Recommend deleting "Nobuto" to account for other commercial brands and adding "or similar products" to include blood collection filter paper cards or disks. **Supporting evidence**: not relevant

3. Faeces

Faeces can be collected freshly voided or preferably directly from the rectum/cloaca for tests such as culture for microorganisms, parasite examination, or faecal occult blood determination; or can be collected for culture and molecular-based diagnostics from the rectum/cloaca using cotton, dacron, or gauze-tipped swabs, dependent on the volume of sample required by the specific test methodology. ~~Samples~~ Specimens collected on swabs should

be kept moist by placing them in the transport media recommended for use with the specific test to be performed, which may range from sterile saline to culture media containing antimicrobials or stabilisers. Faecal specimens should be kept chilled (e.g. refrigerated at 4°C or on ice) and tested ~~as soon after collection as possible~~ preferably within 24–48 hours to minimise the negative impacts on test results caused by death of the targeted microorganism, bacterial overgrowth or hatching of parasite eggs. Double-packaging of faecal ~~samples~~ specimens in screw cap or sealable containers that are subsequently contained within sealed plastic bags will help prevent cross-contamination of samples and associated packaging materials. Faeces contained only in rectal exam gloves, plastic bags, or rubber-stoppered tubes are unsuitable as they are very frequently comprised by bacterial growth with gas production that can rupture plastic bags, displace stoppers, and allow leakage of the specimen.

4. Urine

Urine sampling can be a non-invasive or minimally invasive method for detecting pathogens and physiological markers useful for surveillance of pathogens, renal function, systemic infections, and environmental toxins. It can provide crucial diagnostic information when blood or tissue sampling is logistically difficult or ethically undesirable. Urine samples can be collected during outbreaks, when non-invasive sampling is preferred for animal welfare or safety reasons, in longitudinal monitoring or from freshly deceased animals. Collection methods include spontaneous urination, manual expression, cystocentesis, catheterisation, absorbent material or surface swabbing. Samples can be kept refrigerated (for 24 hours), frozen, or conserved in RNA-stabiliser or other preservative.

~~45~~. Epithelium

Epithelial tissue in the form of biopsies or skin-scrapings; swabs of oral, nasal, pharyngeal, and gastrointestinal surfaces, as well as plucked hair or wool can be used variously for direct examinations or laboratory tests to identify surface parasites such as mites and lice, fungal, bacterial or viral infections, allergic reactions, and neoplasia. The specimens should be collected aseptically and preserved as specified for the intended test(s). Deep skin-scrapings obtained using the edge of a scalpel blade are useful for burrowing mites. Feather tips have been validated for use in the detection of viral antigen ~~for Marek's disease, and used as a sample for~~ or molecular detection of ~~additional~~ some avian diseases. Epithelial tissues, particularly those associated with vesicular lesions and collected into viral transport media, can be critical in the laboratory diagnosis of specific viral infections such as foot and mouth disease.

~~56~~. Ocular sampling

The surface of the eye can be sampled by swabbing or ocular scraping, ensuring that cells rather than mucopurulent discharge or lacrimal fluids are collected for testing. Specimens from the conjunctiva are typically collected by holding the palpebra apart and gently swabbing the surface of the eye with a cotton, dacron, or gauze swab that has been pre-moistened with sterile saline or equivalent media. Such swabs should be kept moist in saline or transport media specifically recommended for use with the testing to be performed. Biopsies from the third eyelid of sheep have been used as a lymphoid-rich tissue for prion detection. Aqueous humour serology is useful for the detection and measurement of antibodies and other substances related to eye infections.

~~67~~. Sampling the reproductive tract

Preputial and vaginal wash fluids and swabs of the cervix and urethra can be used as specimens for investigation of reproductive disease. The swabs should be kept moist following collection by placing in the recommended volume of transport media required by the laboratory test, typically sterile saline or specified culture media. Semen specimens are typically obtained using an artificial vagina or by extrusion of the penis and artificial stimulation. Avoid contamination of the specimen with antiseptic or detergent solutions used to prepare the animal/site for sampling.

~~78~~. Nasal discharge, saliva, and vesicular fluids

Secretions can be collected directly or with swabs into a vial or tube~~, or can be collected using swabs~~. Vesicular fluids provide a highly concentrated source of pathogens for diagnostic testing, and can be collected from unruptured vesicles using a sterile needle and syringe, and immediately transferred to a securely sealed vial or tube. Specifically developed sampling tools, such as probang cups, can be used for collecting cellular material and mucus from the pharynx of livestock. Cotton ropes that animals are allowed to mouth and chew ~~have been validated for use in collecting saliva specimens from domestic swine~~ or food items discarded by the animals under investigation can be used to collect saliva specimens where applicable.

~~89~~. Milk

Milk can be collected from individual animals or from bulk milk in tanks pooled from multiple animals in a herd. The teat(s) used for sample collection should be cleaned, and any detergent thoroughly rinsed off before collection of the specimen. In collecting milk from individual teats, the initial stream must be discarded and only the subsequent

streams sampled. The method of preservation prior to testing varies with the requirements of the test; in some cases it will be critical to avoid freezing or addition of chemical preservatives. The individual disease chapters of this *Terrestrial Manual* or the advice of the testing laboratory should be consulted for appropriate specimen handling and preservation recommendations.

~~9~~10. Tissues collected at necropsy

The decision to conduct a post-mortem examination should be undertaken by qualified veterinarians or pathologists carefully considering human health and other risks. Necropsies should only be conducted by these trained individuals, although ~~paraveterinary staff~~ veterinary paraprofessionals may be trained to conduct post-mortem examinations for specific purposes. ~~Paraveterinary staff may be trained by veterinarians to conduct post-mortem examinations for specific purposes.~~ Importantly, the purpose of the necropsy is not only to collect specimens but to make informed observations regarding the pathology of the condition. Such observations are an important adjunct to epidemiological and clinical observations in the comprehensive veterinary investigation of the case or outbreak. It is useful for veterinary authorities to retain specialist veterinary pathologists to lead post-mortem investigations in important cases. Where this expertise is managed from a veterinary laboratory the methods employed should be formally described in the laboratory's Quality Assurance Manual and the capability should be recognised in the laboratory's scope of accreditation. Detailed procedures for conducting post-mortem examinations and tissue collection are available in most pathology textbooks and are additionally provided in many of the web-page accessible national laboratory testing guidelines. Specimens that are critical for the laboratory investigation of listed diseases are included in the chapters of the *Terrestrial Manual* relating to each disease.

Whether the necropsy is performed in a designated laboratory facility or in the field, appropriate biosafety and containment procedures should be followed to ensure operator safety and to provide non-contaminated and useful ~~tissues~~ ~~samples~~ specimens for testing as well as to protect the environment and other animals from potential exposure to pathogens. As a minimum requirement, the collector(s) must wear personal protective equipment that protects the skin and mucous membranes and that can be discarded or decontaminated. Specific information on biosecurity of wildlife necropsy is available (for instance, Wildlife Health Australia 2018). All remaining tissues or carcass parts and fluids should be contained and treated with an appropriate disinfectant or destruction method, and the immediate environment should be thoroughly disinfected.

~~Dependent~~ Depending on the suspected disease, condition of the carcass and facilities available for necropsies post-mortem specimens can be collected from one or multiple organs and tissues (including blood clots) and submitted to the laboratory as either fresh (no preservative) or preserved specimens for further laboratory testing. The process of carcass autolysis can destroy diagnostically relevant tissues and infectious agents and so should be considered prior to collecting and submitting post-mortem specimens.

For fresh specimens, particular attention must be paid to their handling and storage to avoid autolysis and overgrowth by bacterial and fungal contaminants. Ideally, freshly collected specimens are kept at a constant cool temperature from collection until processing for testing. Where such a cold chain cannot be provided fresh specimens for some test procedures can be collected into ~~fluids such as ethylene glycol that~~ transport medium that inhibits the growth of secondary organisms. This may, however, not be sufficient to maintain adequate quality of tissues susceptible to fast autolysis. Where such strategies are compatible with the subsequent test methods the option is mentioned in the relevant chapters of the *Terrestrial Manual* for each disease.

Preservation of post-mortem specimens is most frequently achieved by collection into formalin solution. This treatment, however, does not permit the testing of such specimens using culture or certain molecular methods. Where such chemical fixative is supplied to pathology staff by laboratories or competent authorities they must ensure adequate training in health and safety aspects of the use of such chemicals and training in compliance with regulations relating to the transport of such chemicals.

~~10~~11. Environment and feed

Environmental sampling may be of litter, bedding, water from troughs and drinkers, or feed which has been exposed to urine, faeces, and/or saliva of affected animals, or swabbed surfaces of facilities, ventilation ducts, drains or feed containers. If specialised equipment is available circulating air may be sampled. Environmental sampling of water, soil, and vegetation can provide valuable insights into pathogen presence and environmental contamination, particularly in ecosystems where direct animal sampling is difficult. Non-invasive techniques such as screening for environmental DNA (eDNA) are increasingly used to detect species ~~presence and track pathogen circulation~~ circulating in the environment, when direct sampling of the specimen is not feasible. However, eDNA limitations should be acknowledged, which include but are not limited to the following: ~~non-invasively.~~ proper collection requires sterile techniques, avoidance of cross-contamination, ~~and~~ the use of preservation methods appropriate to the target, and the use of a validated methodology in the laboratory.

12. Invertebrates

12.1. ~~Arthropod insects~~ Ectoparasites

Ectoparasites are a valuable method for monitoring and diagnosing vector-borne pathogens, especially when direct sampling of animal hosts is not feasible. They can complement surveillance programmes and act as a proxy through xenosurveillance, although pathogen load detection may vary depending on the competence of the vectors. Ectoparasites are collected during outbreaks of vector-borne diseases or in areas where host animals are difficult to capture or sample, and for proactive vector surveillance in endemic or high-risk areas. Targeted vectors include ticks, mites, lice, fleas, mosquitoes, and flies, including tsetse flies. Collection methods include direct skin removal, trapping, baiting, stick traps and aspirators. Preservation and transport methods depend on the intended analysis, some ectoparasites such as ticks can be transported live to the laboratory whereas others require ethanol, DNA-stabilisers or cold chains.

~~11~~12.2. Honey bees

Adult moribund or recently dead bees can be collected in the vicinity of colonies. Live bees can be killed by freezing either shortly after collection or following receipt at the laboratory. Brood specimens are typically collected by removing a piece of brood comb showing abnormalities and including dead or discoloured brood followed by wrapping in a paper towel or newspaper rather than in foil or wax paper in order to help prevent microbial overgrowth. Alternately, diseased cells in a comb may be sampled using a toothpick or equivalent. A sticky board can be used to collect hive debris, including trapping of mobile parasites. More information on the specimens that need to be collected can be found in the disease-specific chapters of this *Terrestrial Manual* related to bees.

B. EPIDEMIOLOGICAL APPROACHES TO SAMPLING

To provide scientifically and statistically valid results specimens must be appropriate for the intended purpose for the proposed investigation, and adequate in quality, volume, and number. The range of purposes for which investigations supported by laboratory testing may be conducted ~~have been outlined in Section A above~~ can be found in chapter 1.1.6.

For the purpose of laboratory testing to establish a diagnosis, it is important to sample animals that are either clinically affected~~,~~ or suspected on good evidence to be infected or, for serology, to have been infected. Specimens that are most likely to give highest sensitivity and specificity to the investigation should be collected. In general, the stage of infection, as well as the route and duration of shedding, will determine the appropriate animal(s), stage of clinical disease, timeline for sampling, and tissue or anatomical site for sampling. In some cases, internal and/or external parasites may be useful in the confirmation of the condition. These criteria will be addressed through an understanding of the pathogenesis of the disease for known conditions and on a hypothesis of the pathogenesis of the diseases for conditions of unknown aetiology.

To detect evidence of infection in line with the other five purposes of testing as listed in ~~Section A above~~ chapter 1.1.6, the sampling should be done within the context of a surveillance programme. The criteria for the design and implementation of effective surveillance are described in Chapter 1.4 *Animal health surveillance* of the *Terrestrial Animal Health Code* (*Terrestrial Code*). Identification of animals for sampling in surveillance programmes may be targeted (risk-based) or random. Risk-based sampling based on epidemiological knowledge of the infection under study or on epidemiological observations of the population under study is intended to result in the most likely detection of infected individuals. For wild animal testing, the lack of validated tests for numerous wild species is frequently a limitation (Chapter 2.2.7 *Validation of diagnostic tests for infectious diseases applicable to wildlife*).

Inferences on the status of a population such as estimation of prevalence, immune status or disease freedom should be based on random sampling. Random sampling ensures that the animals sampled are representative of the population, within the practical constraints imposed by different environments and production systems. Additionally, random sampling allows extrapolation of the findings of the study to the overall population (with an appropriate confidence interval). The epidemiological principles for sample size estimation are addressed in Appendix 1.1.2.1.

The specific requirements for surveillance to demonstrate freedom from disease/infection, and the associated sampling requirements, are addressed in detail in Article 1.4.6 of Chapter 1.4 of the *Terrestrial Code*.

Sampling and laboratory testing may also be used in support of epidemiologically based diagnostics and studies such as case control, structured longitudinal, and cohort studies (Fosgate & Cohen, 2008; Mann, 2003; Pfeiffer, 2010). The number and selection of the animals to be sampled and the nature of the specimens will be part of the

study design. When wildlife population size is unknown, sampling strategies must rely on non-probabilistic or adaptive approaches. Convenience sampling, targeted sampling in high-risk areas or seasons, and opportunistic sampling (e.g. hunter-harvested or roadkill animals) are commonly used. Repeated sampling over time and space can help build a more representative picture. In such contexts, it is essential to document sampling effort, location, and selection criteria to support data interpretation and reduce bias. Where possible, combining multiple data sources (e.g. direct observation, camera traps, environmental DNA) can help infer population structure and pathogen dynamics.

C. INFORMATION TO BE SENT WITH SPECIMENS

~~Individual specimens must be clearly identified using appropriate methods. Marking instruments should or labels must be able to withstand the conditions of transport, storage and use, i.e. being wet or frozen. Use of an indelible marking pen is required. Pencil may rub off containers. Labels attached to plastic may fall off when stored at –70°C.~~ Proper labelling of specimens is essential to ensure accurate identification and reliable test results. The specimen markings and labels must be durable and able to withstand the expected transport and storage conditions, including changes in temperature, moisture, and handling. If labelling is not robust, critical information can be lost – for example, ink markings may rub off containers, or adhesive labels may loosen and fall off when specimens are frozen.

Information regarding the location and contact information of the submitter and the premises or area sampled, the case information and associated epidemiological information, as detailed below, should always accompany the specimens to the laboratory. Such documentation should be placed in a plastic envelope on the outside of the shipping container so as to be available for reference during transport and should also be duplicated inside the shipping container between the secondary and the outer packaging (see also Chapter 1.1.3 *Transport of biological materials*). It would be advisable to contact the receiving laboratory to obtain an appropriate submission form and other relevant shipping and handling information.

Necessary information includes:

1. Location and contact information

i) Name and address of owner/occupier of the animal owner and/or the sampled premises and the geolocation (latitude and longitude, if available~~)~~, where disease occurre~~d, with appropriate contact information (telephone number, e-mail address).~~ Note that this information can be sensitive in case of protected wild animal species.~~-~~

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

Note: This information can be sensitive in case of protected wild and domestic animal species and private individuals.

The level of urgency associated with the testing should be indicated, and if appropriate the laboratory should be contacted in advance of receipt of the specimen.

2. Case information

i) Disease agents suspected~~, and~~ tests requested and purpose of the sampling.

ii) Species, breed, sex, age and identity of the animals sampled, and trackability number when available.

iii) Date ~~samples~~ specimens were collected and submitted.

iv) List and type of ~~samples~~ specimens submitted with transport media used.

v) Case history:

a) The clinical signs and their duration, including the temperature of sick animals, condition of mouth, eyes and feet, and milk or egg production data as relevant. Potentially pictures of the animals can be attached.

b) A list and description of the animals examined and the findings of the ante- and post-mortem examinations.

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

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

vi) Ecological information

When sampling ectoparasites, trap locations and collection method, host association and environmental conditions of trapping are important associated metadata.

3. Epidemiological information

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

ii) The number of animals on the premise by species, the number of animals dead, the number showing clinical signs, and their age, sex and breed.

iii) The type and standard of husbandry, including biosecurity measures and other relevant factors potentially associated with the occurrence of cases.

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

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

vi) Vaccination history describing the type of vaccines used and dates of application.

vii) Other observations about the disease, husbandry practices and other disease conditions present as well as ecological observations.

Shipping from the field to the laboratory requires careful planning to preserve specimen integrity and ensure biosafety. Specimens should be placed in appropriate, containers following chapter 1.1.3 and international transport regulations (e.g. IATA). The cold chain should be maintained as appropriate for the specimen type and duration of transport. The choice of preservative, such as ethanol, proprietary nucleic acid preserving solution or formalin, should be compatible with the intended analysis. Accompanying documentation must be complete. Where appropriate sending samples should be compliant with international regulations, including CITES. Clear communication with the receiving laboratory prior to shipment is advisable to facilitate appropriate handling upon arrival and avoid delays that could compromise specimen quality.

D. RECEIPT, STORAGE AND ARCHIVES OF LABORATORY SUBMISSIONS

1. Reception of ~~samples~~ specimens

Receiving, unpacking and aliquoting specimens must be done in a way ~~to avoid cross-contamination in order~~ that avoids cross-contamination to guarantee reliable testing of samples and prevent exposure of personnel.

A risk assessment (RA) as outlined in chapter 1.1.4 should be performed before systems for handling biological agents and toxins are established ~~in order~~ to define the appropriate biosafety and laboratory biosecurity measures. The RA should lead to the development of stated policy and procedures for the operation of the whole process of receiving submissions to the laboratory.

Submissions delivered to the laboratory should be received in accordance with specified standard operating procedures by staff who are appropriately trained, and when possible are made aware of potential arrivals so that parcels are treated correctly upon reception. To enable appropriate specimen tracking the following information should be logged: a) the time of arrival, b) the sender, c) the person receiving the ~~samples~~ specimens, and d) the shipper with the tracking number. Where a specified chain of custody is required for the purposes of legal action or investigation the consignment should remain unopened and secured in a cool, dry place away from direct sunlight until authorised laboratory personnel are notified and available to receive the package and continue chain of custody. Laboratories should have a written operating procedure detailing the requirements to meet national legal requirements for such submissions.

1.1. Specimen reception area

Specimen reception areas should be equipped to facilitate the safe handling and processing of diagnostic submissions to avoid contamination of the work area, the personnel, cross-contamination among specimens and to allow easy disinfection in situations where specimen containers may have leaked. The specimen reception room should be clean with adequate bench space for organising submissions and paperwork. Depending on the number of submissions expected and depending on the RA the specimen reception area may be either a dedicated part of the diagnostic laboratory or a separate space outside the diagnostic laboratory.

The receiving room should contain an area dedicated to the unpacking of the specimens, with easily cleanable surfaces and trays and/or a biosafety cabinet, depending on the RA. There should be adequate and appropriate space to store specimens, either refrigerators or freezers, taking into consideration the

time the specimens are to be stored. Specimen registration equipment such as computers, printers or logbooks should be available. A bar code system can be used to identify and track the specimens.

1.2. Submission unpacking, registration and preparation for further processing

Consignments should remain unopened until transferred to the specimen reception area for further processing. The submission should be unpacked and opened according to defined standard operation procedures. Surface decontamination should be considered to avoid cross-contamination and be part of the designated procedures arising from the RA.

Information to be recorded at specimen log-in includes the delivery source, the date the submission was consigned, the condition of the outer package, and the condition of inner packages (noting the presence of leaks or breakage), the condition of the specimen material, the inner package temperature, and any specific requests from the sender.

Further activities may include labelling of specimen containers, transfer of specimens to the laboratory and storage of specimens.

Packaging material should be disposed of appropriately according to national regulations which may include autoclave destruction of all packaging materials, depending on the RA.

Personal protective equipment (PPE) should be provided to protect the personnel. Minimal PPE is a laboratory coat and gloves. Depending on the relevant RA respiratory protection or effective splash protection (e.g. protective glasses) may be required as well.

After it has been determined that the submission contains the appropriate paperwork matching the specimens and that the specimens are in good condition appropriately trained laboratory personnel become responsible for transfer to the appropriate laboratory area, including maintenance of the chain of custody of the specimens as required. Only properly contained registered (identified) specimens should be transferred into the diagnostic laboratory. It is good practice, and at times a requirement for biosafety and laboratory biosecurity dependent on the RA, to enclose the submitted specimen containers in a secondary container to transfer the specimens safely within the laboratory.

1.3. Emergencies

A comprehensive RA will identify credible and foreseeable emergency scenarios, and be the basis for preparing a response plan. In particular, leaky ~~samples~~ specimens represent a biohazard for the laboratory personnel and could contaminate the environment and other ~~samples~~ specimens. Written instructions on how to deal with broken or leaky tubes should be available in the ~~sample~~ specimen reception area. Personnel should be trained and regular emergency exercises and simulations should take place.

~~Samples~~ Specimens that are degraded or in a condition unacceptable for testing should be decontaminated and appropriately disposed of according to the laboratory's response plan as noted in the prior paragraph. Contaminated laboratory surfaces should be decontaminated with the appropriate disinfectant. ~~Sample~~ Specimen rejection and discrepancies between the ~~samples~~ specimens and accompanying paperwork should be resolved by contacting the sender immediately to resend a duplicate sample or to clarify paperwork.

2. ~~Sample~~ Specimen quality, storage and archives

Collections of well characterised specimens including infectious agents, infected tissues and fluids, as well as negative control tissues and fluids, are critical for future research and development efforts, for retrospective studies, epidemiological investigations, and for providing critical reference materials used in assay standardisation, validation, and proficiency testing programmes. In addition, specimens being investigated for legal purposes should be banked.

Materials routinely needed as reference standards and for assay validation are described in Chapter 1.1.5 *Quality management in veterinary testing laboratories*. The materials maintained in laboratory archives should be representative of the agents handled and the types of ~~samples~~ specimens used in the different testing methods performed, which would variously include fresh and fixed tissues and fluids, paraffin-embedded tissues, and stabilised or otherwise preserved cultures. The World Federation for Culture Collections (WFCC: *www.wfcc.info/collections*) is a useful source of information and reference documentation for developing, maintaining, and sharing culture collections, and has published a comprehensive guide for establishing and

operating microbial culture collections (WFCC, 2010). It is part of the remit of WOAH Reference Laboratories to supply reference materials.

The principal components of any laboratory archive include the appropriate means of stabilisation and storage, a complete system of documentation and inventory of the material stored, and implementation of biosafety and laboratory biosecurity measures needed to manage the collection.

2.1. Specimen quality

Volume, quantity and integrity of specimens as well as types of containers, visible contamination, storage history and proper labelling are the first steps of the quality check of any specimen type. Other quality criteria are sample-type dependent. Bacterial contamination and red blood cell debris in serum ~~samples~~ specimens can produce false-positive reactions in agglutination-type assays. Serological assays can be negatively impacted by haemolysis in the serum ~~sample~~ specimen. Microbial contamination and haemolysis are significant concerns, especially when obtaining blood and serum ~~samples~~ specimens from post-mortem animals.

~~2.1~~ 2.2. Stabilisation and storage

The method of preserving tissues, fluids, and cultures will depend on their anticipated use(s). ~~Samples~~ Specimens and samples stored for periodic access such as assay reference materials should be aliquoted to avoid potential problems associated with repeated retrieval and return to storage. They may be stored separately from specimens or samples stored for historical, long-term preservation. Preservation of some aliquots for long-term nucleic acid preservation is desirable. Storage conditions should be managed to maintain viability, biochemical, and immunological properties ~~of the samples~~ to the maximum extent possible. Considerations for preserving the integrity of the specimens and samples must include protection from desiccation (e.g. as can happen in certain freezers), frequent or extreme temperature fluctuations, UV degradation, humidity, contamination, and the potential for loss of identification and associated archive documentation. Unique or valuable isolates and materials should be stabilised and stored using at least two different procedures and storage locations.

Storage at ultra-low temperatures (e.g. liquid nitrogen, cryopreservation in freezers at –140°C or lower) is considered the optimum method for long-term storage of biological materials. Storage at low-freezer temperatures of –80°C and –20°C is common for periods that may range from months to 5–10 years. Ultra-low freezing may not be a practical choice as it is expensive to maintain, but cost must be balanced with the fact that biological degradation of the ~~sample~~ specimens and samples over time is an increasing risk at warmer freezer temperatures. Reference materials that are to be accessed with any regularity should be stored in appropriately sized aliquots to allow access while minimising the number of times the "master stock" is handled. Repeated freezing and thawing ~~of samples~~ should be avoided as it can denature antigens, result in loss of viability of fastidious agents, and can precipitate the over-growth of contaminants or unwanted microorganisms in the sample.

Methods for stabilisation and storage of specimens and samples at room temperature range from commercially available technologies that largely target nucleic acid stabilisation, lyophilisation processes, to the relatively low-tech versions of drying fluids on filter paper disks or storage of biological ~~samples~~ specimens and samples in the presence of desiccating agents such as silica gel or grains of rice to absorb moisture.

Considerations such as speed at which a sample is frozen or chemically preserved, size and density of the material to be preserved, storage container and media, and ~~also~~ protocols for reconstitution, thawing, and reviving agents will vary with different tissues and agents. Whether the plan is to store samples frozen or at ambient temperature, for most tissues and groups of infectious agents there are specific preservatives, stabilisation protocols, and storage conditions that are considered optimal; the current published literature should be consulted.

~~2.2~~ 2.3. Documentation and Inventory

Agents and tissues maintained in an archive must be correctly identified and sufficient supporting data that characterises the ~~sample~~ specimen or agent must be recorded preferably in a machine-readable format (e.g. CSV [comma-separated values], JSON [java script object notation], XML, TXT [if structured properly], barcodes/QR codes, binary code). For reference materials, further documentation that authenticates the agent or tissue is required. The unique identity of the tissue, fluid, or agent and the storage location are best maintained in an electronic, machine readable format ~~or paper~~ inventory record which also documents the date the material was obtained, date and method of preservation, volume of material stored, source of the material including associated species, geographical location, and the

clinical history of the donor animal and the disease situation of the flock or herd. Additional information is extremely useful and generally includes the original method of isolation/recovery, characterisation (e.g. available data on biochemical properties, antibody or antigen titre, and genetic sequence) as well as additional history on handling of the material (e.g. number of passages for infectious agents and cell lines, dates the archived material was frozen-thawed or rehydrated and the dates it was transferred to different storage conditions or containers). Inventories are most often organised by assigning a unique identifying number or alphanumeric code to each sample (sample container) that is cross-referenced to a database or inventory log. Inventory records can be manual data logs, computerised spreadsheets, or specialised computer programs. However the records are managed, they must be kept current and the information entered must be version controlled, stored with a backup in case of local system failure and traceable to its source. The identification of the individual making an entry or modification to the sample inventory should be recorded.

~~2.3~~ 2.4. Biosafety and laboratory biosecurity

As a first step in establishing an archive, a laboratory biorisk assessment addressing biosafety and laboratory biosecurity issues, including any control or mitigation measures to be implemented, must be completed. As further defined in chapter 1.1.4, an appropriate risk assessment for archived ~~samples~~ specimens should address the technical competency needed of staff handling the tissues, fluids, and agents, with particular emphasis on those materials that are potentially infectious or toxic to workers, animals, and the environment in and around the laboratory. The laboratory should consider all biorisk management measures needed to protect the integrity of the ~~sample~~ specimen, as well as the health of the workers and environment, from the time the original ~~sample~~ specimen is received through the long-term storage and ultimate use or destruction of the material(s).

The appropriate level of laboratory biosecurity, including controlled access to the archived samples and inventory records, is an important consideration for laboratories maintaining biological inventories and archives. The laboratory should also have a back-up plan for the transfer or destruction of potentially dangerous archived materials in the event of power failures or other compromises to the storage environment. National and international regulations and legislation, including requirements for permits and licenses to receive, maintain, work with, and distribute specific agents and tissues must be followed for all laboratory archives. Current regulatory information ~~in regards to~~ on receiving and storage of biological materials can be found on the European Biological Resource Centre Network website (www.ebrcn.net/), in the WFCC guidelines (2010) and from relevant national government agencies.

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NB: FIRST ADOPTED IN 2008 AS COLLECTION AND SHIPMENT OF DIAGNOSTIC SPECIMENS.
MOST RECENT UPDATES ADOPTED IN 2013.