

Veterinary laboratory users guide

6th edition

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The policy on specimens accepted for testing and the tests available are correct at the time of printing but may be subject to change. Efforts will be made to notify all regular users of laboratory services of changes affecting them. If you are in doubt, please contact the laboratory for the latest information.

Contact details

Emergency animal disease watch hotline
1800 675 888
Biosecurity Queensland
13 25 23

Biosecurity Sciences Laboratory

Phone (07) 3708 8762
Fax (07) 3708 8860
Email bslcl@daf.qld.gov.au

Delivery address

Specimen Receipt (Loading Dock 12)
Biosecurity Sciences Laboratory
Health and Food Sciences Precinct
39 Kessels Road
COOPERS PLAINS QLD 4108

Postal address

Biosecurity Sciences Laboratory
Health and Food Sciences Precinct
PO Box 156
ARCHERFIELD BC QLD 4108

Operating hours

8 am to 5 pm, Monday to Friday

Receipt of samples outside these hours is by prior arrangement with the duty pathologist only.

Tick Fever Centre

Phone (07) 3898 9655
Fax (07) 3898 9685
Email tfc@daf.qld.gov.au

Street address

280 Grindle Road
WACOL QLD 4076

Postal address

PO Box 109
ARCHERFIELD BC QLD 4108

Operating hours

8 am to 4 pm, Monday to Friday

Send **all** diagnostic specimens to the Biosecurity Sciences Laboratory.

Foreword

The Biosecurity Sciences Laboratory (BSL) is dedicated to providing diagnostic testing services for infectious agents, toxins and other causes of disease in animal samples submitted by private veterinary practitioners, government veterinarians and inspectors. Its focus is on significant livestock and aquatic disease investigations, diseases of concern to public health and wildlife management.

The staff at BSL are discipline specialists, and include terrestrial and aquatic pathologists, microbiologists, virologists, parasitologists, molecular biologists, toxicologists, biochemists and serologists. The skills and experience of the staff offer an approach to diagnostic investigation with considerable depth. BSL is accredited by the National Association of Testing Authorities (NATA) for veterinary diagnostics and undergoes regular peer-reviewed assessments; this provides assurance of reliable testing by technically competent staff. The laboratory has the scientific expertise, capability and specialist equipment necessary to undertake veterinary testing for terrestrial and aquatic animal species.

BSL is funded by the Queensland Government and works in partnership with the national laboratory network, including the Australian Animal Health Laboratory in Geelong and other jurisdictional laboratories. In Queensland, the location of BSL within the Health and Food Sciences Precinct ensures close collaboration with our human health diagnostic colleagues. National links are maintained through involvement with the Animal Health Committee, the Australian Animal Pathology Standards Program and Animal Health Australia.

BSL provides a critical service in the diagnosis and management of emergency animal and aquatic diseases and has demonstrated its emergency readiness and capability in several significant responses, including those for equine influenza and white spot disease.

Underpinning a great veterinary diagnostic laboratory is the quality and appropriateness of the samples submitted. Submission of complete and suitable samples supports a thorough diagnostic work-up and improves the likelihood of reliable and meaningful results, which are needed for crucial decision-making. This users guide provides advice for sampling in a comprehensive and easy-to-navigate format and has been written to help submitters get the best possible service from BSL.

It gives me great pleasure to commend this guide to all those who access the services of BSL. I strongly encourage reference to the processes outlined in the guide, as this will optimise diagnostic outputs and support healthy and profitable livestock industries in Queensland.

Allison Crook

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Biosecurity Queensland
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Contents

Foreword	i
Acknowledgements	ii
Tables.....	vi
How to use this guide.....	vii
Abbreviations	viii
Part A Introduction.....	1
1 Laboratory guidelines.....	3
1.1 Policy on specimens accepted for testing and charges for tests	3
1.2 Principles of diagnostic laboratory use	6
1.3 Laboratory details.....	6
1.4 Collecting specimens for laboratory examination	8
1.5 Documentation and dispatch of specimens.....	11
1.6 Laboratory results.....	13
Part B Samples by discipline.....	15
2 Clinical biochemistry and nutritional biochemistry	17
2.1 Clinical biochemistry—collecting samples.....	17
2.2 Clinical biochemistry—tests available	19
2.3 Clinical biochemistry—interpreting results.....	21
2.4 Nutritional biochemistry—phosphorus analysis	23
3 Haematology and cytology	24
3.1 Haematology—collecting samples.....	24
3.2 Haematology—tests available	26
3.3 Haematology—interpreting results	27
3.4 Cytology.....	29
4 Histopathology.....	31
4.1 Collecting samples.....	31
4.2 Fixatives	33
4.3 Submitting samples.....	33
5 Microbiology	34
5.1 Collecting samples.....	34
5.2 Submitting samples.....	36
5.3 Interpreting results.....	37
5.4 Antibiotic sensitivity	37

6	Molecular diagnostics	38
6.1	Collecting samples.....	38
6.2	Submitting samples.....	38
7	Parasitology	39
7.1	Collecting samples.....	39
7.2	Submitting samples.....	41
7.3	Interpreting results.....	41
7.4	Total worm counts.....	43
7.5	Ticks—acaricide resistance testing.....	44
8	Serology.....	46
8.1	Collecting samples.....	46
8.2	Labelling samples	47
8.3	Submitting samples.....	47
8.4	Interpreting results.....	48
8.5	Bovine infertility investigations	51
9	Tick fevers of cattle	53
9.1	Collecting samples.....	53
9.2	Interpreting results.....	55
9.3	Tick fever vaccines	57
10	Toxicology	58
10.1	Collecting samples.....	58
10.2	Tests available	59
10.3	Interpreting results.....	60
10.4	Botanical identification of plants, fungi and cyanobacteria.....	62
11	Virology.....	65
11.1	General virus isolation	65
11.2	Collecting samples.....	65
11.3	Crustacean and mollusc virology	66
11.4	Submitting samples.....	67
	Part C Samples by disease or syndrome	69
12	Investigating disease in cattle, sheep, goats, pigs, camelids, deer and horses.....	71
12.1	General information.....	71
12.2	Samples by syndrome.....	72
12.3	Samples by disease	78

- 13 Investigating disease in poultry and birds 126**
 - 13.1 General information..... 126**
 - 13.2 Samples by syndrome..... 127**
- 14 Investigating disease in aquatic animals (finfish, crustaceans and molluscs)..... 128**
 - 14.1 Principles..... 128**
 - 14.2 Collecting specimens 128**
 - 14.3 Services offered 129**
 - 14.4 Necropsy 129**
 - 14.5 Histopathology 130**
 - 14.6 Bacteriology 132**
 - 14.7 Molecular diagnostics..... 133**
 - 14.8 Virology 133**
- Appendixes 135**
 - Appendix 1 Clinical biochemistry reference values 137**
 - Appendix 2 Haematology reference values 138**
 - Appendix 3 Suggested field necropsy kit 139**
 - Appendix 4 Equipment available from Biosecurity Sciences Laboratory 140**
- Index..... 141**

Tables

Table 7.1	Significant faecal egg counts in ruminants (epg of faeces).....	42
Table 7.2	Nematodes commonly associated with clinical helminthosis in ruminants in Queensland	44
Table 8.1	Interpretation of CFT and IHA test titres for diagnosis of melioidosis in goats.....	49
Table 10.1	Samples to collect from affected animals for toxicology	58
Table 10.2	Samples to collect from suspected sources of toxins.....	59
Table 10.3	Toxicology tests routinely available.....	59
Table 10.4	Maximum lead levels in animal tissues	61
Table 12.1	Samples by syndrome—cattle, sheep, goats, pigs, camelids, deer and horses	72
Table 13.1	Samples by syndrome—poultry and birds	127

How to use this guide

This guide has been written to help veterinarians and departmental field staff get the best possible service from the Biosecurity Sciences Laboratory (BSL). By following the procedures set out in this guide, you will contribute to more reliable and accurate results.

Part A (Section 1) details the policy and principles applied at BSL and provides general information about submitting samples to BSL. Please read it in conjunction with the specialist sections that follow.

Part B (Sections 2–11) provides information on tests and sampling by discipline. **Sections 2–8, 10 and 11** cover biochemistry, haematology, histopathology, microbiology, molecular diagnostics, parasitology, serology, toxicology and virology. Refer to them for information on sampling and submission of samples, available tests and interpretation of those tests. See **Section 9** for specific information about tick fevers of cattle.

Part C (Sections 12–14) covers the investigation of disease in various species.

When investigating disease in cattle, sheep, goats, pigs, camelids, deer and horses, refer to **Section 12**. See **Table 12.1** for a comprehensive list of samples for each syndrome identified in the affected group of animals. If you suspect exotic or reportable disease or need more detailed information on specific diseases, refer to **Section 12.3**. Diseases are listed alphabetically in this section, making it easy to navigate; however, specific information can also be found by using the index at the end of the book. The list of diseases is not exhaustive, so if you need more information about sampling, please contact the duty pathologist for advice.

When investigating disease in poultry and birds, refer to **Section 13**, in particular **Table 13.1**, for information on the samples required.

See **Section 14** for information on sampling of aquatic animals.

Appendixes 1 and 2 provide reference values for clinical biochemistry and haematology.

Appendix 3 has a list of items recommended for a field necropsy kit and **Appendix 4** has a list of laboratory forms, information sheets, media, swabs and testing kits available from BSL.

Always remember that this book is only a guide to sampling and interpretation of results; it cannot cover every possible circumstance. If you need more information or explanation of results, please contact the duty or case pathologist.

Abbreviations

A/G	albumin/globulin
AGID	agar gel immunodiffusion test
AIT	adult immersion test
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BC	bovine campylobacteriosis
BHV1	bovine herpesvirus 1
BSL	Biosecurity Sciences Laboratory
BVDV	bovine viral diarrhoea virus
CEM	contagious equine metritis
CFT	complement fixation test
CNS	central nervous system
CPK	creatine phosphokinase
CSF	cerebrospinal fluid
DAF	Department of Agriculture and Fisheries
DNA	deoxyribonucleic acid
EBL	enzootic bovine leucosis
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
epg	eggs per gram
FAT	fluorescent antibody test
FMD	foot-and-mouth disease
Hb	haemoglobin
HCT	haematocrit
HT-J	high throughput-Johne's
GGT	gamma glutamyltransferase
GLDH	glutamate dehydrogenase
GSH-Px	glutathione peroxidase
IATA	International Air Transport Association
IHA	indirect haemagglutination assay
LIMS	Laboratory Information Management System

LPT	larval packet test
LSD	Lumpy skin disease
MAT	microscopic agglutination test
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCV	mean cell volume
NATA	National Association of Testing Authorities
NBF	neutral buffered formalin
PBST	phosphate-buffered saline with Tween 20
PCR	polymerase chain reaction
PCV	packed cell volume
PCV2	porcine circovirus 2
PCVAD	PCV2-associated disease
PIC	property identification code
PPFR	plasma protein/fibrinogen ratio
qPCR	quantitative real-time PCR
RBC	red blood cells
RBT	rose bengal test
RCC	red cell count
RNA	ribonucleic acid
SST	serum separator tube
TCA	trichloroacetic acid
TEM	transmission electron microscopy
TFC	Tick Fever Centre
TSE	transmissible spongiform encephalopathy
VNT	virus neutralisation test
VTM	virus transport media
WCC	white cell count
WW	wet weight

The page features a white background with several large, overlapping geometric shapes. A green shape is in the top-left corner. A red shape is in the bottom-right corner. A pink shape is in the bottom-right corner, overlapping the red one. A dark purple shape is in the bottom-right corner, overlapping the pink one. The text 'Part A' is in green and 'Introduction' is in black, both in the top-right area.

Part A

Introduction

1 Laboratory guidelines

1.1 Policy on specimens accepted for testing and charges for tests

The Biosecurity Sciences Laboratory (BSL) is the veterinary laboratory of the Queensland Government Department of Agriculture and Fisheries (DAF). It provides the Queensland Government with surveillance data on disease occurrence in commercial livestock, aquatic animals and wildlife. The data is used to support Queensland's participation in national and international trade of animal products.

This policy has been developed to direct resources to areas that offer the most efficient means of gathering animal health surveillance data. Accordingly, preference will be given to investigating herd, flock or group disease problems affecting cattle, sheep, goats, horses, pigs, poultry, deer, camelids, finfish, crustaceans and molluscs in Queensland. Subject to the conditions outlined below, this will be undertaken free of charge to Queensland producers. Note that most users of the laboratory will not be charged for disease diagnostic services.

Specimens from animals outside Queensland will attract charges. Specimens from recreation or performance animals (e.g. greyhounds and thoroughbred horses), companion animals (e.g. dogs and cats) and non-commercial 'hobby farms' are regarded as low priority and, if accepted, will attract charges.

Specimens from any animal species in Queensland will be accepted regardless of background if a disease incident is considered to present an unacceptable risk to the health of either the community or commercial livestock. Subject to the conditions outlined below, specimens accepted under these circumstances will be processed free of charge.

Specimens accepted for processing

Acceptance of specimens and exemption from a service fee will be based on the following six criteria:

1. their public benefit component
2. the extent to which clinical history, owner information, epidemiological and other relevant information is provided
3. the appropriateness of specimens to the clinical history and disease(s) suspected
4. the duration of laboratory involvement in a long-running investigation
5. the depth of laboratory investigation required in solving complex, multifactorial diseases
6. the association of the specimens with a disease incident.

Specimens for health and export testing, for certain accreditation schemes or referred from other laboratories for testing will incur charges.

Public benefit component

A submission will be regarded as having substantial public benefit under the specific circumstances outlined below and will not attract charges.

Where there is clinical disease in one or more animals in Queensland under the following categories:

1. exotic or zoonotic disease
2. prohibited or restricted matter listed under the *Biosecurity Act 2014* (previously 'notifiable disease')
3. specified diseases and approved government programs
 - high morbidity and/or high mortality outbreaks of disease
 - diseases suspected of having public health significance, e.g. Hendra virus
 - progressive neurological disease in sheep and cattle, i.e. the National Transmissible Spongiform Encephalopathies Surveillance Program
4. new or emerging diseases, including investigations considered likely to provide new information or an improved understanding of animal health in Queensland
5. other targeted endemic disease surveillance programs under annual review.

Clients will be advised if a submission under these categories falls outside DAF requirements and is subject to charges.

Incomplete information

Submissions with incomplete information **will not** be processed or the results will be withheld until relevant information is provided. Clients will be advised to this effect in compliance with National Association of Testing Authorities (NATA) requirements.

A submission will be regarded as having incomplete information under the following circumstances:

- The information provided was insufficient to determine the public benefit component as described above.
- The information provided was insufficient to determine whether the specimens supplied were consistent with the clinical history and disease(s) suspected.
- Essential epidemiological data was not supplied. Essential information includes animal details, history and clinical signs of the animals and current outbreak data.

Inappropriate specimens

Submissions with inappropriate specimens **will not** be processed. Clients will be advised to this effect in compliance with NATA requirements.

A submission will be regarded as having inappropriate specimens under the following circumstances:

- Specimens are not consistent with the clinical history or disease(s) suspected.
- Specimens are sufficiently compromised, e.g. by autolysis (either directly by hours after death at collection or indirectly through poor preservation in transit), as to render them of no relevant diagnostic use.
- Sample presentation is hazardous by way of specimen containment, inclusion of sharps or external contamination as to render them unsuitable for processing.

The duration of laboratory involvement

When the laboratory is involved in the investigation of a major outbreak of endemic disease, charges may apply after the diagnosis has been established. The details of these charges and their application will be negotiated on a case-by-case basis.

The depth of laboratory involvement

The laboratory may become involved in the investigation of complex disease syndromes that are either multifactorial in nature or require special expertise or an experimental approach. Depending on the consequences for the industry, these investigations could be given 'special project' status. Alternative funding may be required under these circumstances to ensure proper design and costing for the project.

Specimens not associated with disease

Testing will incur charges where specimens are not associated with disease, e.g. health and export testing and accreditation scheme testing.

For an up-to-date schedule of test charges, contact the laboratory or visit business.qld.gov.au.

Testing for export or interstate movement of animals

It is the responsibility of the submitter to establish precisely what testing is required. Specific tests may be required and these may not always be available at this laboratory. Contacting the relevant authority in the state or country to which the animals are going is essential prior to submitting samples. Contact with the laboratory is recommended before sample dispatch and essential if there are a large number of samples. It is also important to allow sufficient time for testing to be completed prior to movement of the stock. Prices for specific tests are available at business.qld.gov.au or by contacting the laboratory.

1.2 Principles of diagnostic laboratory use

Some diseases in animals are diagnostically challenging for even the most skilled and experienced investigators. It is often impossible to distinguish between well-known disorders on clinical evidence alone. In such cases, the expertise and facilities of laboratories are available to help in obtaining a specific and accurate diagnosis.

To get the most from specialised laboratory services, follow the principles and techniques for the collection and submission of specimens outlined below. Remember that the service can only be as good as the specimens submitted to the laboratory. It is important to take the time to collect the right specimens in the correct manner and, if necessary, consult with the laboratory before submission.

- Choose freshly dead animals to send directly to the laboratory for necropsy. In some cases, live affected animals may be accepted. Consult with the laboratory before submitting either live or dead animals. Sample untreated animals rather than treated animals.
- Choose specimens for tests to cover the range of possible diagnoses in each case. Too many are better than too few. See **Sections 12–14** for detailed information on which specimens should be included.
- Fill out a **specimen advice sheet** (Form A) fully. Give as much detailed information on the history (including any treatment given) and clinical presentation as possible. Attach extra pages if necessary or email the laboratory with any additional details.
- Complete the **necropsy findings form** (Form B) for each necropsy.
- If in doubt, contact the laboratory by phone or email and ask to discuss the case with a veterinary pathologist.

1.3 Laboratory details

Location

The Biosecurity Sciences Laboratory (BSL) is located at Coopers Plains, Brisbane, and the Tick Fever Centre (TFC) is at Wacol, Brisbane.

Services offered

- BSL offers a broad range of diagnostic pathology services including necropsy and histopathology, clinical and nutritional biochemistry, haematology, microbiology, molecular diagnostics, parasitology, serology, toxicology and virology.
- TFC offers diagnosis of tick fevers and produces commercial tick fever vaccines. Please send orders for these vaccines directly to TFC.
- Please send **all** diagnostic specimens to BSL. Specialised testing from other laboratories will be requested by BSL if required.

Contact details

Biosecurity Sciences Laboratory

Phone (07) 3708 8762
Fax (07) 3708 8860
Email bslclo@daf.qld.gov.au

Delivery address

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Send **all** diagnostic specimens to BSL.

1.4 Collecting specimens for laboratory examination

General guidelines

- Ensure that you have an adequate supply of specimen advice sheets (Form A) and necropsy findings forms (Form B) at all times. These forms and sample numbering forms are available for download from business.qld.gov.au. Books of Forms A and B can also be supplied—contact BSL for details.
- Before collecting specimens, consider exotic disease, zoonotic disease and special arrangements that may be required for large numbers of specimens and specimens from experiments or research.
- Collect the clinical history and epidemiological data.
- Take ante-mortem specimens and post-mortem specimens.
- Select specimens to cover the range of possible diseases associated with the syndrome seen. See **Sections 12–14**.
- Use appropriate specimen collection containers. If using special media, check the expiry dates on the labels.
- Label the specimens with the owner's name, animal number and organ/sample.
- Ensure that all containers are correctly sealed.
- Complete all forms fully, making sure you include the date of collection.
- Package the specimens properly for transport. See **Section 1.5**.
- Do not include sharps or syringes in the package.

Things to consider before collection

Inadequate or dangerous specimens

Ensure that specimens meet the requirements of this guide. Specimens in syringes or containing sharps will be discarded without testing. Unsuitable specimens and inadequately labelled or unlabelled specimens may not be tested at the discretion of the pathologist.

Diseases due to prohibited or restricted matter

Early recognition of a serious or exotic animal disease is one of the most important factors in controlling the disease and reducing its economic and social impact on the whole community. The Queensland Government recognises this by prescribing some matter as 'prohibited' or 'restricted' under the *Biosecurity Act 2014*.

If a disease due to prohibited or restricted matter is suspected, whether you are an owner, a vet, laboratory staff or anyone else, report it to Biosecurity Queensland on 13 25 23 or call the emergency animal disease watch hotline on 1800 675 888.

Diseases dangerous to humans

Zoonoses are diseases that can spread from animals to humans, e.g. anthrax, salmonellosis, Hendra virus and Australian bat lyssavirus infection. Know the precautions to be taken and equipment required for sampling in these cases. To find out more about zoonotic disease, visit business.qld.gov.au.

Large numbers of specimens

If specimens for diagnostic tests exceed 20 (or 50 for health tests), make arrangements with the laboratory before collection. This will allow the laboratory to better plan its testing schedules and priorities. In some circumstances, limits may apply to the number of samples tested free of charge for certain diseases, e.g. mastitis and infertility. The laboratory will advise you of these limits.

Experiments or trials

Contact the laboratory before submitting samples for testing from experiments or trials.

Collecting data

- Take a full history of the case. Include observations on the circumstances of occurrence of the disease (epidemiology) in the group of animals involved and any treatments applied to them.
- Complete a careful clinical examination of the live affected animals available.
- Record these findings on the specimen advice sheet (Form A).

Collecting specimens

- If live affected animals are available, first decide which ante-mortem specimens you need to help you to reach a diagnosis. Check this guide or contact BSL for more information.
- If suitable subjects are to be necropsied, decide which post-mortem specimens are needed. Revise this decision if necessary, depending on the necropsy findings.
- Follow the principle of collecting sufficient specimens to cover the range of diseases that could account for the syndrome being investigated.
- For routine urinalysis for biochemical components, use a urine test strip at the time of collection.
- Once you have completed the various examinations, pause and review the case, checking that all organ systems have been examined.
- Before collecting a specimen, consult the appropriate section in this guide and consider:
 - use of suitable containers/tubes for each test
 - correct storage of each specimen before sending
 - specimen transport requirements
 - provision of relevant clinical information.
- Complete a specimen advice sheet and necropsy findings form fully and package the specimens correctly for transport before leaving the scene of the investigation.

Labelling specimens

Label all specimens clearly, preferably with a permanent marking pen (oil-based rather than water-based ink). Include the:

- owner's name
- animal number (identify individual animals with consecutive numbers, not names or IDs)
- tissue from which the sample was taken, e.g. SMITH (1) small intestine or SMITH (2) large intestine.

Where possible, use frosted-end slides for smears and write the animal number in pencil on the slide. Do not use marker pens.

Obscure cases

Occasionally, a necropsy will not give any clear indication of the possible cause of death or disease. Even under the best conditions, some animals will die and no satisfactory explanation will be found. Under Queensland field conditions, there is often a lack of clinical history and, in summer, rapid post-mortem decomposition. Both tend to reduce the chances of obtaining a diagnosis.

However, sending a range of specimens (chosen as appropriate from the material available) will provide some helpful information and some possible diagnoses can be ruled out. The diagnostic pathologist will consider the circumstances and the specimens available and decide on a reasonable number of tests.

For obscure cases involving aquatic animals (e.g. fish kills, complex diseases), consult the aquatic pathologist for advice on the range of samples that may help in the diagnostic investigation. The samples required could be both animal (e.g. live moribund or freshly dead fish) and environmental (e.g. water, sediment, feed).

Post-mortem decomposition

The time after death that specimens are taken is most important. Blood samples from dead animals are unsuitable for haematology and clinical biochemistry. Specimens for some purposes are not affected by decomposition, e.g. tissues for analysis for lead and arsenic. Fragile viruses and bacteria generally can only be isolated from specimens taken from the freshly dead animal. Decomposing tissues are not satisfactory for histopathology.

The rate of decomposition varies with seasonal and weather conditions and is faster with some diseases than with others. This makes precise rates impossible to provide to cover all cases. Even in winter in Queensland, most specimens must be obtained within 12 hours of death.

In aquatic animals (fish, crustaceans and molluscs), post-mortem decomposition is rapid. Therefore, specimens should be collected from humanely euthanased animals that are preserved in fixative, held in ice or kept refrigerated. Whole sick animals should be sent in live by aerated and cooled transportation. See **Section 14** for more information.

Handling specimens

- If specimens are held awaiting transport, any fresh material, sera and swabs in transport media should be refrigerated, not frozen. However, these specimens do deteriorate under refrigeration, so should be dispatched as soon as possible.
- Do not refrigerate blood smears, live ticks or fly larvae. Condensation of water on cold smears will rupture the blood cells and low temperatures may kill the ticks and larvae. Also, prolonged refrigeration of faecal samples can result in negative cultures for nematode larvae.
- Ensure that containers of fixed tissues are adequately sealed. Exposure to formalin fumes will result in inadequate staining of smears and may compromise microbial culture from fresh samples. In addition, any leakage of formalin is a health hazard to those who have to handle the package and samples.

1.5 Documentation and dispatch of specimens

- Complete forms fully. Use sealed plastic bags to protect forms from moisture.
- Do not send specimens in glass bottles, poor-quality plastic containers, zip-lock bags, syringes or gloves.
- Package specimens to prevent leakage in transit and include sufficient absorbent material to contain any leakage that might occur.
- Pack perishable specimens in insulated containers with cooler bricks or gel packs. Insulated containers with cooler bricks should reach the laboratory within 18 hours.
- Do not pack blood samples in direct contact with cooler bricks.
- Do not refrigerate or chill blood smears, live ticks, fly larvae or faeces for larval culture.
- Use a sealed container to protect blood smears from water and formalin fumes.
- Notify the laboratory of dispatch of urgent or important specimens.

Documentation

All relevant details must accompany specimens so that they can be efficiently handled. This is vital because the description given will help the pathologist determine what testing is appropriate.

Forms designed to obtain this information can be supplied by the laboratory as books and can also be downloaded from business.qld.gov.au. Instructions for the completion of these forms are on the cover of each book of forms and are also available at business.qld.gov.au.

Specimen advice sheet (Form A)

Use this form with all specimen submissions for diagnosis of disease, for testing for export, show, sale, movement or accreditation, for health testing and for experimental work. Send the form with the specimens and keep a copy for your records.

Necropsy findings form (Form B)

Attach this form to Form A to record necropsy findings. Send it with the specimens and keep a copy for your records.

- Use a separate form for each different species of animal in a submission and for each different reason for testing.
- Always give the ages of the animals and the number in the affected group. An estimate is better than no information and allows the laboratory to gauge the importance of the incident.
- State precisely and concisely what you see. Do not try to make your observations fit a textbook description of a disease. Email digital photos taken during the necropsy to enhance the description.
- State or estimate the time interval from death to post-mortem examination. Without this, it becomes very difficult to evaluate histopathological changes and the significance of isolation of some bacteria.

Dispatch

- The sender of specimens is responsible for the cost of transport.
- Specimen transport containers are not returned to the sender except at the sender's expense.
- Specimens must be properly packaged to prevent leakage and to maintain their usefulness for laboratory testing.
- There are packaging requirements for transporting biological specimens by road, rail or air. These requirements apply to all specimens sent by private veterinary practitioners to a laboratory. International Air Transport Association (IATA) packing instructions 650 and 620 apply to many of the specimens sent by veterinarians. All staff responsible for packaging and transport of biological specimens must be appropriately trained. (For more information on packaging requirements, search 'packaging and transport of pathology specimens' on the internet.)
- Place the accompanying documents (specimen advice sheet and necropsy findings form) in a sealed plastic bag and fix them securely to the outside of the package.
- Address the package to Specimen Receipt at BSL.
- If the samples are from a suspected case of a zoonotic disease (e.g. Hendra virus infection, melioidosis, brucellosis, anthrax), clearly write the name of the suspected disease on the specimen advice sheet and attach the paperwork to the exterior of the package in a sealed envelope.

1.6 Laboratory results

Availability of results

Detailed test results are confidential and will be provided only to the submitter of the specimens and to authorised government officers for disease control purposes. If specimens are sent by a private veterinarian, results will be provided to the veterinarian, not to the owner of the animals.

Laboratory reports

Submitters are sent laboratory reports by email, facsimile or post. Reports are generated through the Laboratory Information Management System (LIMS) and may be interim, final or supplementary. Interim reports show some test results with additional test results pending. Final reports have all tests completed. Supplementary reports have results of additional testing or additional information not provided on the final report.

If requesting laboratory reports to be sent by email, please include a generic email address for the veterinary practice rather than a personal email address.

Enquiries

Phone (07) 3708 8762

Email bslclo@daf.qld.gov.au

All enquiries to BSL are handled by interrogating LIMS directly. Please provide the following information to allow this to be done swiftly:

- job number
- submitter's surname and initial(s) and name of veterinary practice
- owner's surname or property identification code (PIC)
- date specimens were sent.

The pathologist responsible for the case may be consulted on request.



Part B

Samples by discipline

2 Clinical biochemistry and nutritional biochemistry

Clinical biochemistry is the analysis of the blood serum or plasma for a wide variety of substances (including enzymes, metabolites, electrolytes and trace elements) for use in the diagnosis and monitoring of disease. It also includes analysis of other body fluids (e.g. urine, effusions, cerebrospinal fluid, ocular fluid), tissue (e.g. liver, kidney, bone), rumen contents and calculi. Biochemistry tests should be accompanied by full haematology because evaluation of both together will optimise the recognition of characteristic disease patterns.

2.1 Clinical biochemistry—collecting samples

Blood

Serum is preferred for most clinical biochemistry assays, but heparinised plasma may also be used for most assays. Blood in EDTA is required for glutathione peroxidase (GSH-Px) analysis.

Collect blood from the jugular vein (cattle, sheep, pigs and horses), tail vein (cattle), ear vein (pigs) or wing vein or heart (poultry). Fill tubes containing anticoagulants first so that any clot formation is minimised.

Use plain tubes or serum separator tubes (SST) for the collection of serum. These can be a minimum of 5 mL but preferably 10 mL tubes. Fill the tubes to two-thirds capacity to provide a minimum of 2 mL of serum. Allow the blood in the plain tubes to clot at room temperature (30–60 minutes in summer and longer in winter) and ideally separate the serum by pouring off or centrifugation before transport to the laboratory. This is particularly important if the samples will not be received at the laboratory within 18 hours of collection. Invert SST tubes gently to mix the clot activators with the blood, then allow the blood to clot in a vertical position for 20–30 minutes and centrifuge **within 1 hour** of collection. Haemolysis and leakage of red cell components affects most analyses. Porcine blood is particularly prone to haemolysis if serum is not separated promptly.

Lithium heparin tubes contain anticoagulant, which allows the collection of blood plasma. Fill these tubes to the volume specified and thoroughly mix the blood with the anticoagulant by gentle swirling and inversion. Do not shake the tubes vigorously, as this may cause haemolysis.

Transport all samples to the laboratory at 4°C within 24 hours of collection. Attach a completed specimen advice sheet to each submission.

Factors leading to unsuitable samples or confusing results

- *Haemolysis*: Haemolysis interferes with most biochemical assays and is probably the single most important factor rendering samples unsuitable for analysis. It can be caused by:
 - prolonged contact of serum or plasma with red cells
 - water on needles, skin or blood containers
 - rough handling of samples, such as vigorous shaking or transport over rough roads
 - excessive negative or positive pressure on the syringe when drawing or transferring blood
 - inappropriate needle size (too small or too large)
 - exposure to excessive heat or cold.

- *Dead animals:* Marked changes occur in blood composition after death. Sample only live animals or animals immediately before euthanasia.
- *Treated animals:* Take samples needed to confirm the diagnosis before giving treatment. If this is not possible, record the treatment given on the specimen advice sheet.
- *Sample storage:* Exposure to light will cause the breakdown of bilirubin, reducing its concentration, so protect samples from natural and artificial light. Use aluminium foil to protect liver and blood samples for vitamin A and E assays.
- *Bacterial contamination:* The growth of bacteria will utilise urea and glucose in serum or plasma, reducing their concentrations. Collect samples using aseptic techniques and keep them cool in transit.
- *Serum/plasma separation:* Leakage of enzymes and electrolytes from red cells when serum or plasma is not separated promptly may lead to inaccuracies in many analyses.
- *Anticoagulants:* EDTA chelates calcium and magnesium, making plasma from EDTA blood samples unsuitable for calcium or magnesium analysis.

Ocular fluids

The eye is relatively isolated and protected after death, and ocular fluid samples—both aqueous humour and vitreous humour—are relatively easy to collect, so these can be of value in investigations up to 48 hours after death.

Note: Ocular fluid biochemistry should not be used as a diagnostic criterion but as an adjunct to other diagnostic information regarding the animal sampled. Results must be considered in relation to clinical history, gross pathology and the estimated time of death. Reference ranges are not available for many analytes, but extreme values are likely to be useful indicators of a particular disease or exposure to a toxin.

Ideally, do not submit the whole eyeball, as post-mortem degeneration and release of intracellular material into the aqueous humour and vitreous humour are likely to occur. Aqueous humour can be obtained by introducing a 21 gauge needle with syringe attached horizontally into the anterior chamber with the bevel of the needle facing the cornea. Take care to avoid the aspiration of iris tissue. Collect vitreous humour using a syringe with a 14 or 16 gauge 25 mm needle introduced vertically into the vitreous humour through the sclera caudal to the limbus, parallel to but avoiding the lens.

Transfer the samples to plain blood tubes without anticoagulant for transport. Most samples should be sent chilled (ruminal acidosis, ketosis, hypocalcaemia, hypomagnesaemia, salt poisoning, nitrate/nitrite). For cyanide and ammonia testing, place the samples into small sealed containers and freeze them immediately. Alternatively, for cyanide testing, send the whole eyeball chilled.

2.2 Clinical biochemistry—tests available

Standard serum biochemistry profile

- Calcium
- Magnesium
- Total protein
- Albumin
- Globulin
- Albumin/globulin (A/G) ratio
- Creatinine
- Urea
- Total, conjugated and unconjugated bilirubin
- GGT (gamma glutamyltransferase)
- GLDH (glutamate dehydrogenase)
- AST (aspartate aminotransferase)
- CPK (creatine phosphokinase)

Additional tests

The following tests are available on request. Tests marked with an asterisk (*) have special requirements, which are outlined below. Tests other than those listed may be available if prior arrangement is made and there is justification of the purchase of the necessary kits or reagents.

Serum/plasma

- Potassium, sodium, chloride
- ALT (alanine aminotransferase), ALP (alkaline phosphatase)
- Uric acid (birds only)
- *Glucose
- β -hydroxybutyrate (ketones)
- D-lactate
- *Inorganic phosphorus
- *Copper, zinc, iron, cobalt, manganese, molybdenum, selenium
- *Vitamins A and E

Whole blood (EDTA or lithium heparin)

- GSH-Px for assessment of selenium status
- *Ammonia (lithium heparin plasma)

Liver

- Copper, zinc, cobalt, manganese, molybdenum
- *Vitamins A and E

Kidney

- Copper, zinc, selenium

Urine

- Specific gravity
- pH
- Glucose, protein, β -hydroxybutyrate (ketones), urobilinogen, myoglobin, haemoglobin (semiquantitative)

Routine urinalysis for biochemical components is best done with a urine test strip at the time of collection.

Ocular fluid

- *Ammonia
- Calcium, magnesium
- D-lactate, β -hydroxybutyrate (ketones)
- Nitrate/nitrite, cyanide
- Sodium, chloride

Calculi (renal or biliary)

- Calcium, magnesium, phosphate, ammonia, carbonate, cystine, oxalate, urate

Body fluids

- Specific gravity
- Protein

Bone

- Ash
- Calcium, magnesium, phosphorus

Rumen contents

- *Ammonia

Tests with special requirements

Glucose

Collect blood samples for glucose into fluoride-oxalate (grey-top) tubes to prevent the utilisation of glucose by blood cells after collection. Fluoride-oxalate samples haemolyse very readily because the cells can no longer respire, so timely separation is especially important. Chilled plasma may be used for glucose estimation only if it is separated from blood within 1 hour of collection.

Inorganic phosphorus

Blood for inorganic phosphorus assay must be spun down and the serum/plasma separated from the clot within 2 hours of collection because leakage of phosphorus from the red cells causes phosphorus levels to be artificially elevated. Preferably, collect the blood into EDTA, then preserve it with trichloroacetic acid (TCA). See **Section 2.4** for more information.

Copper

The required volume of serum for copper testing is 2 mL, so if other testing is requested (e.g. serum profile or serology), duplicate samples of clotted blood/serum may be required.

Vitamin A and vitamin E

Vitamins A and E are destroyed rapidly after death, so submit liver samples from freshly dead animals. Freeze the liver samples immediately and deliver them frozen to the laboratory. Cover the outer container with aluminium foil to prevent light penetration. Similarly, wrap blood tubes for vitamin A and E assays in aluminium foil. Serum/plasma can be frozen, but whole blood should be chilled only.

Ammonia

Freeze rumen contents immediately. Keep the sample frozen until it arrives at the laboratory. Alternatively, acidify it by adding 1 part 0.2 N hydrochloric acid to 1 part rumen fluid, or by adding 3–5 drops of battery acid to 20 mL of rumen fluid. If the rumen fluid is not preserved immediately, the ammonia rapidly volatilises and is lost. Ocular fluid can be collected up to 24 hours after death. From the live animal, blood in lithium heparin is preferred. Separate the plasma as soon as possible, preferably within 5 hours of collection. Freeze ocular fluid and plasma before transport to the laboratory.

2.3 Clinical biochemistry—interpreting results

The minimum and maximum values provided on the laboratory report define the expected physiological ranges of biochemical parameters in normal adult animals for each species. Interpretation of these results should take into account other factors such as age, exercise, stress, diet, pregnancy and lactation, which can alter the values of blood and serum components. Clinical biochemistry reference values are provided in **Appendix 1**.

Calcium

Low serum calcium levels are often secondary to very low albumin levels because a significant fraction of serum calcium is bound to albumin.

CPK

Despite the stated normal ranges, CPK activities up to 2000 IU/L are not unusual in clinically normal animals. These usually result from mild muscle damage from restraint, intramuscular injections, recumbency, strenuous exercise or transport.

GGT

GGT can be used to monitor passive maternal antibody transfer via colostrum in neonatal calves and lambs. Pre-suckling calves have levels similar to that of adults (<32 IU/L). After suckling, levels may be >1000 IU/L. At 1–7 days of age, calves with levels of GGT <250 IU/L are likely to be colostrum deprived. Sampling of calves older than 1 week is unreliable, as GGT levels decline during weeks 2–4. Lambs at 7 days are likely to be colostrum deprived if GGT <100 IU/L.

D-lactate

D-lactate is not produced by mammalian tissues normally, but is produced by some bacteria. It accumulates in the rumen and then in the blood under certain conditions, including those of carbohydrate engorgement (grain overload, ruminal acidosis). Specimens needed for the test are fresh serum or fluoride-oxalate plasma. Levels between 0.4 and 1.0 mmol/L are equivocal, but levels >1.0 mmol/L are considered significant.

Phosphorus

The interpretation of blood inorganic phosphorus concentrations is difficult. Whole blood concentrations <1 mmol/L indicate some degree of phosphorus deficiency. However, values within the normal range of 1.13–2.25 mmol/L can be found in cattle with a low phosphorus intake but drawing heavily on body reserves. Consequently, faecal samples, which provide a better index of current phosphorus intake, should be taken in conjunction with whole blood samples. See **Section 2.4** for more information.

GSH-Px

GSH-Px is a selenium-containing enzyme. There is a good correlation between GSH-Px activity in red blood cells and tissue levels of selenium, so it provides a means of assessing selenium status. Herd sampling (10% of the herd or 20 animals, whichever is the lower number) provides a more reliable assessment than single animal analysis.

Urinalysis

Urinalysis should be performed on fresh urine. Because of the delay in samples reaching the laboratory, testing should be done in the field, e.g. with urine test strips. Submission of urine is still recommended where it may aid in the diagnosis of certain conditions.

Calculi analysis

Analysis of uroliths and biliary calculi is offered at BSL and the following components can be detected: calcium, magnesium, phosphate, ammonia, carbonate, cystine, oxalate, silicate and urate.

2.4 Nutritional biochemistry—phosphorus analysis

The three diagnostic measures—faecal nitrogen, faecal phosphorus and blood inorganic phosphorus—should be used together in diagnosing phosphorus deficiency.

Note: P-screen kits should be used for sample collection. These can be ordered from BSL.

Sampling guidelines

- Standardise sampling conditions.
- Avoid exciting the animals when taking the blood samples.
- Sample animals most at risk, e.g. lactating cows, first-calf heifers.
- Collect samples towards the end of the period of positive weight gain (usually March to May in Queensland).
- Do not sample animals that have access to a phosphorus supplement.

Blood samples

Blood for inorganic phosphorus assay must be spun down and the serum/plasma separated from the clot within 2 hours of collection, because leakage of phosphorus from the red cells causes phosphorus levels to be artificially elevated. Preferably, collect the blood into EDTA, then preserve it with TCA, which is included in the P-screen kit.

Interpreting results

Interpreting blood inorganic phosphorus concentration is difficult. Whole blood concentrations of less than 1 mmol/L indicate some degree of phosphorus deficiency. However, values within the normal range of 1.13–2.25 mmol/L can be found in cattle with a low phosphorus intake but drawing heavily on body reserves. Consequently, faecal samples, which provide a better index of current phosphorus intake, should be taken in conjunction with whole blood samples.

Faecal samples

Take samples from the rectum of at least 6 and preferably 10 animals in the herd or flock. Alternatively, take samples from freshly voided faeces in the paddock. Take about 250 g of faeces from each animal, mix in a plastic bag and send about 0.5–1.0 kg to the laboratory. Results of analysis of faecal samples from fewer animals or from supplemented animals cannot be interpreted and such samples will not be analysed.

Interpreting results

Faecal phosphorus is a reasonable indicator of the dietary phosphorus content of all types of diets for sheep and cattle, except where they are fed phosphorus supplements. As a guide, faecal phosphorus concentrations below 0.20% of the dry matter indicate dietary phosphorus concentrations that are at, or below, maintenance requirements. Diets high in nitrogen increase the animals' requirements for phosphorus.

3 Haematology and cytology

3.1 Haematology—collecting samples

Take blood as quickly and cleanly as possible from a major vessel into 5 mL or 10 mL EDTA tubes. EDTA is the anticoagulant of choice for haematological tests. Blood collected into lithium heparin anticoagulant is **not** suitable for haematology.

Commercially available blood collection tubes contain sufficient EDTA to prevent clotting of blood when the tube is filled to the mark on the label. Low sample volumes collected into standard 5 mL or 10 mL EDTA tubes may result in artefacts in haematology parameters and shrinkage of red blood cells. Gently mix the tube contents by repeated inversion immediately after sampling (do not shake). If lids or caps are removed to fill the container, ensure they are resecured to prevent leakage in transit.

Note: Differential leucocyte counts will not be done unless suitable smears accompany the blood samples.

Make at least two blood smears from the well-mixed EDTA sample, preferably within 5 minutes but up to 3 hours after collection. After this time, cells become distorted and the smears are not suitable for differential white cell counts.

Keep the EDTA samples cool during transport and ensure they reach the laboratory within 24 hours of sampling. Do not freeze them or allow contact with cooler bricks, as this will lyse the cells. Do not refrigerate smears, as this causes condensation, also resulting in cell lysis. Smears can be packed in the same container, provided they are put into slide holders inside a sealed container to separate them from other samples and are well wrapped to insulate them from chilling.

When sending samples from a number of animals, clearly label the EDTA sample and smears from each animal with that animal's number.

Unsuitable samples

- Blood collected into lithium heparin tubes
- Blood collected after death
- Clotted samples (inadequate mixing after collection)
- EDTA blood older than 4 days

Making blood smears

- Make sure the slides are dry and free from dirt and grease. Use a clean glass slide with a smooth edge as a spreader. Clean and dry spreaders carefully before reusing them.
- Thoroughly but gently mix the EDTA blood. Place a small drop of blood at the end of each of two slides.

- Place the spreader slide in front of the drop of blood and hold it at an angle of about 45° (see Figure 3.1). Draw the spreader back into the drop and allow the blood to run to the edges of the spreader slide. Maintaining the spreader at 45°, quickly and smoothly push the spreader right to the end of the slide to create a smear with a feathered edge. Repeat for the second slide.
- Where possible, use frosted-end slides and write the animal number on each slide in pencil. Do not use marker pens.
- Air-dry the slides away from flies and dust, put them into slide holders and then place these in an airtight container to avoid water condensation and exposure to formalin.

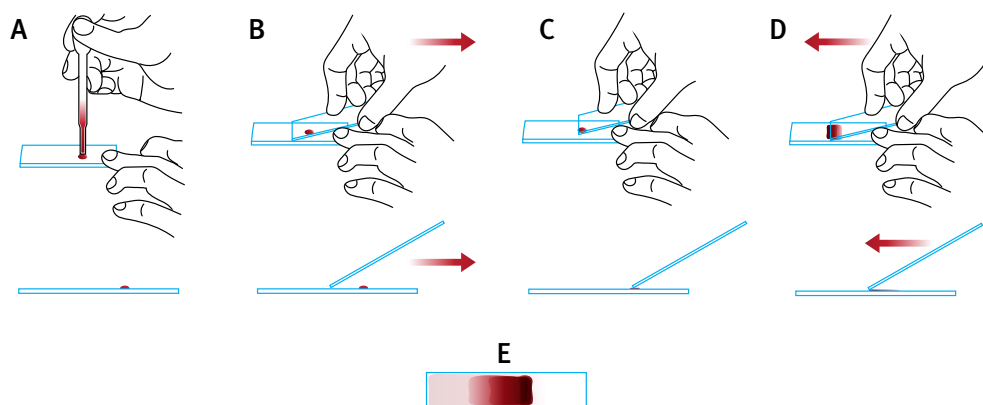


Figure 3.1 Making blood smears

Source: Cornell University College of Veterinary Medicine, Animal Health Diagnostic Centre, <https://ahdc.vet.cornell.edu/Sects/ClinPath/sample/test/hema.cfm#Bloodsmear>

3.2 Haematology—tests available

Standard profile

- Total red cell count (RCC)
- Haemoglobin (Hb)
- Haematocrit (HCT) or packed cell volume (PCV)*
- Calculation of red cell indices—mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC)
- Description of red cell morphology
- Total white cell count (WCC)
- Differential white cell count
- Platelet (thrombocyte) estimation
- Plasma fibrinogen
- Visual assessment of plasma appearance

* HCT and PCV are often used interchangeably to express the same value—the ratio of red blood cells (RBC) to plasma expressed as a percentage of the whole blood volume.

- HCT is a derived or calculated value obtained from automated haematology analysers:
$$\text{HCT} = (\text{RBC} \times \text{MCV}) \div 10$$
- PCV is measured directly from the proportion of cells in a volume of whole blood in a haematocrit tube after centrifugation.

Species variations

Goats

Caprine blood has unusually small erythrocytes compared to other animal species. The haematology analyser cannot accurately differentiate between erythrocytes and thrombocytes. Therefore, only a reduced haematology profile consisting of Hb, manual PCV, WCC, white cell differential (if a smear is provided) and fibrinogen will be reported.

Camelids

Camelids have ovoid red blood cells. The haematology analyser will therefore underestimate the MCV, so only a reduced haematology profile consisting of RCC, Hb, manual PCV, total WCC, white cell differential (if a smear is provided), platelets and fibrinogen and will be reported.

3.3 Haematology—interpreting results

When interpreting laboratory data, always consider the clinical signs of the animal, results of other diagnostic testing and suspected or known diagnoses. If the laboratory results do not fit with the clinical presentation, repeat testing on a fresh sample may be warranted. Remember that a haemogram is a snapshot in time. Bone marrow and blood cells respond rapidly (sometimes within minutes) to different situations, so repeat testing is worthwhile to monitor the progression or resolution of disease. Haematology reference values are listed in **Appendix 2**.

Stress leucograms

Stress leucograms are generally associated with release of corticosteroids in response to stress or pain. In cattle they can be associated with non-inflammatory conditions such as milk fever, ketosis, abomasal displacement and grain overload. Generally there is a leucocytosis ($<20 \times 10^9/L$) characterised by a neutrophilia without a left shift and a normal to low lymphocyte and eosinophil count.

Inflammatory leucograms

Bacterial infection

In general, there is leucocytosis due to neutrophilia. There is considerable species variation in the leucocyte response to bacterial infections. Pigs respond dramatically, with counts of $30\text{--}40 \times 10^9/L$ being common. Horses respond to a lesser degree, and counts of $25 \times 10^9/L$ are rarely exceeded. Cattle are less responsive. Total counts can be within the normal range, but there is a neutrophilia with a left shift (i.e. immature forms are present). Counts of greater than $20 \times 10^9/L$ are rarely obtained except in young cattle. In septicaemia, there is often a neutropoenia with predominance of band forms and toxic granulation.

Viral infection

Generally there is leucopoenia. The exception is bovine ephemeral fever, where there is a neutrophilia coinciding with the temperature rise.

Note: Blood samples older than 2 days will have reduced white cell counts because of rupture of leucocytes.

Neutrophilia

Neutrophilia is generally associated with suppurative bacterial infections, but can be seen in other conditions as mentioned above.

Note: Acute inflammation in cattle will often produce a neutropoenia with a left shift.

Eosinophilia

Eosinophilia is most commonly associated with antigen–antibody reactions, allergic responses and parasitic infections. However, the significance of eosinophilia must be interpreted with reservation, particularly in older cattle, which often have up to 20% eosinophils.

Monocytosis

Monocytosis is evidence of chronicity and is quite often associated with a moderate neutrophilia in persistent bacterial infections. It also occurs in the recovery phase of acute bacterial infections, babesiosis and anaplasmosis.

Lymphocytosis

Moderate lymphocytosis is often found in chronic infections and in stress conditions involving the reticuloendothelial system, e.g. tick fever. However, reactive lymphocytes found in these conditions are readily distinguishable morphologically from the immature lymphocytes of bovine leucosis. Normal cattle may have up to 7% atypical lymphocytes.

Bovine leucosis

Bovine leucosis in the terminal stages can usually be diagnosed haematologically. Although some cases have a normal blood picture, the majority show a marked lymphocytosis ($50\text{--}400 \times 10^9/\text{L}$) with numerous atypical lymphocytes, prolymphocytes and lymphoblasts.

Anaemia

Anaemia can be classified morphologically as normocytic normochromic, macrocytic or microcytic hypochromic.

Normocytic normochromic anaemia

Erythrocytes are normal in shape, size and haemoglobin content (MCV and MCHC within normal range). Normocytic normochromic anaemia is found in secondary anaemia of infection and malignancy, protein and vitamin B deficiencies and as the result of toxic suppression of the bone marrow by chemicals, drugs or plant poisons, particularly bracken and mulga ferns.

Macrocytic anaemia (usually regenerative)

Some of the red cells appear large and polychromatic; there is punctate basophilia and often some nucleated red cells (increased MCV and polychromasia).

- A mild to moderate response is found as the result of blood loss from haemorrhage, e.g. gastrointestinal lesions, haemonchosis or tick infestation.
- A more marked response is found when the anaemia is due to accelerated red cell destruction as in anaplasmosis, babesiosis (recovery phase), snakebite or neonatal haemolytic disease.

Microcytic hypochromic anaemia

Red cells are small, contain less haemoglobin and are often distorted (decreased MCV, decreased MCHC, hypochromasia and poikilocytosis). This is generally indicative of iron or copper deficiency.

Note: Young animals frequently have a physiological anaemia in the first few months of life (generally <4 months old). Calves and foals may remain microcytic but not anaemic for up to 1 year.

Plasma fibrinogen

As fibrinogen is an important component of the clotting cascade and one of the acute phase proteins of inflammation, high plasma fibrinogen concentrations are a good indicator of an inflammatory condition. This is particularly useful in pigs, which have a broad range for normal leucocyte values, and in cattle in the early stages of acute fibrinous inflammations such as pasteurellosis where the leucocyte response is minimal. Concentrations are also significantly increased in bovine ephemeral fever.

Plasma fibrinogen concentrations can be provided from unclotted blood in EDTA submitted for haematology. Samples submitted in lithium heparin are unsuitable for plasma fibrinogen.

Fibrinogen concentrations may be falsely elevated in cases of dehydration. The plasma protein/fibrinogen ratio (PPFR) is used to determine hyperfibrinogenaemia in dehydrated cases.

- PPFR $\geq 15:1$ = normal fibrinogen concentration.
- PPFR $>10:1$ and $<15:1$ = relative increase in fibrinogen.
- PPFR $\leq 10:1$ = true increase in fibrinogen.

3.4 Cytology

Suitable samples

- Aspirates/effusions
- Bone marrow smears
- Impression smears
- Fine-needle aspirates
- Urine

Collecting samples

Prepare all smears on thoroughly clean slides. Air-dry the slides and send them packaged in slide holders in a sealed container to avoid contact with dirt, water or formalin fumes.

Aspirates/effusions

Aspirates include material from abdominocentesis, thoracocentesis and arthrocentesis and samples such as tracheal washes. Samples of this nature are usually submitted for analysis, cytology and culture.

Ideally collect two samples, one into an anticoagulant such as EDTA for cytology and the remainder into a sterile tube for biochemical analysis and culture. This unpreserved sample can be used for cytology if the sample does not clot.

Where fluids are obviously of high cellularity, prepare smears from this sample immediately (within 30 minutes of collection). Use the same technique as for making a blood smear (see **Section 3.1**) or place a drop of the sample on one end of a slide and tilt it so the drop travels down the slide.

Centrifuge fluids with low cell counts to concentrate cells before making smears. Air-dry the smears.

Bone marrow smears

The standard sites for collection of bone marrow in large animals are the sternum or ribs (dorsal ends of 8th and 12th ribs in cattle). At necropsy, collection can be from any site, though the mid-shaft femur is recommended (except in aged animals, as this site may have insufficient cells for assessment).

Express aspirated material directly onto a glass slide. Spread it by placing a second slide at 90° and then drawing the two slides apart. In addition, place a larger sample of bone marrow in formalin for histopathology.

Impression smears

Gently dab the freshly cut surface of a lesion onto a glass slide. For ulcerated lesions, scrape off the superficial crust, scab and exudate before sampling. Air-dry the smear.

Fine-needle aspirates

Fine-needle aspirates are primarily performed on skin tumours, lumps or enlarged lymph nodes.

Take the sample using a 22–25 gauge needle (large-gauge needles cause blood contamination of the aspirate). Isolate the lesion between the thumb and forefinger and insert the needle into the lesion. Apply negative pressure to the syringe and move the needle about within the lesion in different directions. Before removing the needle from the lesion, slowly release the pressure on the syringe. There is usually sufficient material in the needle to prepare a smear using the same method as for a blood smear. To expel the sample from the syringe, remove the needle, draw air into the syringe, reattach the needle, then expel the aspirate onto the slide. Air-dry the smear.

Urine

Sediment examination of urine is generally not performed because there is usually a delay between collection and testing, and artefacts will occur in the urine, e.g. cell lysis, crystal formation in vitro.

4 Histopathology

The histology section at BSL prepares slides for microscopic examination so that terrestrial and aquatic veterinary pathologists can observe and interpret morphological changes in animal tissue. The tissue samples can be from specimens collected during field or laboratory necropsies or biopsies from live animals. Autolysis of tissues before collection or after collection (due to inadequate fixation) will compromise the ability of the pathologist to interpret the histological changes.

4.1 Collecting samples

Promptly fix samples in an adequate volume of 10% neutral buffered formalin to preserve the tissue. This is crucial, particularly for large whole organs such as brains.

Immerse samples in at least 10 times their volume of formalin. Place the formalin in the container to be used before adding the tissue samples. After completing the necropsy, gently shake the container to ensure that no tissues adhere to the container walls or other tissues, as adhering tissues will not be properly fixed. It is acceptable to use a single container for multiple samples from the same animal, provided there is sufficient volume of formalin. Where samples are taken from several sites in the same organ and the location of the sampling may be important to the diagnosis, use separate containers, clearly labelled, e.g. intestinal samples, spinal cord segments.

Formalin penetrates tissues slowly. To ensure complete fixation, cut blocks of tissue 5–10 mm thick. Thicker blocks of tissue will not be well penetrated by the formalin. Blocks cut thinner than 5 mm fix well, but buckle easily and are difficult to process. Generally, the size of the tissue blocks should be at least 2 cm² (and 5 mm thick). Wherever possible, the blocks should comprise tissue representative of the lesions seen, along with some normal tissue.

Cut 2 cm squares (including all layers) from the walls of large hollow organs such as the stomach and large intestine. Cut the small intestine into 15 mm lengths and gently flush the contents out with formalin before immersion in the fixative. Partly cut open any longer lengths of intestine to expose the mucosa before immersion.

Within several hours of death, degenerative changes due to autolysis, bacterial action, or both, take place. For best results, tissues must be preserved within a few hours of death. The gastrointestinal tract and gall bladder decompose most rapidly, so place samples from these sites in fixative as soon as possible after death (within 10 minutes for best results). Preserve tissue from aquatic animals as soon as possible after death.

Frozen samples are not suitable for histopathology as the formation of ice crystals disrupts the cells.

Collect a range of tissues at necropsy for histopathology so that the disease processes in the animal can be examined in detail and as complete a picture as possible can be obtained microscopically. Send samples from all tissues that appear abnormal. In addition, include samples from the major organs (heart, lung, liver, kidney, spleen), even if they appear normal. Include the brain (and also spinal cord) if the animal has neurological signs.

The gross appearance of tissues at necropsy can be misleading. For example, congestion is often misinterpreted as inflammation and autolysis may mimic necrosis. Histopathology can differentiate such changes. Histopathological findings can be essential supportive evidence for the results of other tests.

In general, the pathologist will only be able to offer comment on the significance of lesions when an appropriate range of adequately fixed samples has been submitted, together with a good history and a description of the clinical and necropsy findings.

Brain and spinal cord

If possible, submit the whole brain and spinal cord in cases of nervous dysfunction.

Obtain samples of cerebrospinal fluid for microbiology and cytology by needle puncture of the dura mater at the foramen magnum before removing the brain from the cranium. Take additional swabs and fresh samples of tissue for microbiology and molecular studies from the brain and spinal cord after exposure but before placing them into fixative.

Fix the brain intact in 10% buffered formalin. The container must be large enough to enable the brain to 'float'. It is very important to use adequate volumes of 10% buffered formalin—at least 2 L for cattle brains and at least 1 L for sheep brains. The brain should float with the cerebrum resting on the bottom so that the caudal brainstem is not bent or twisted.

Fix brains for **at least 3 days** at room temperature before sending them to the laboratory. Do not chill or freeze them. Take care to avoid damaging the fixed brain during handling and transport.

For brain removal techniques, visit business.qld.gov.au and search for 'National TSEs Surveillance Program (including information for veterinarians)'.

If complete spinal cord removal is not possible, obtain sections from the lumbar, thoracic and cervical parts of the spinal cord. Cut on either side of the selected vertebra with a saw or through the intervertebral discs with a knife. Stand the separated vertebra on one end. Grasp the dura mater with forceps and cut the spinal nerves around the cord on the outside of the dura using a scalpel or small pair of scissors and remove the length of cord. Place the three sections of cord into 10% formalin in separate containers labelled with the location of the spinal segment.

Gastrointestinal tract and other tubular organs

Ensure that the mucosal surface of tubular organs (gastrointestinal tract, uterus and bladder) is exposed by incising the wall before immersing in fixative. Handle only the edges of delicate tissues so that histological detail is not lost.

Other organs

Organs smaller than 1 cm in thickness can be fixed whole. Organs greater than 1 cm thickness must be sectioned. Slice tissue for fixation 0.5–1 cm thick and include part of the lesion along with adjacent apparently healthy tissue.

Eyes

Consult the laboratory before starting to investigate diseases that require the fixation of eyes.

4.2 Fixatives

Neutral buffered formalin

Use commercial premixed buffered formalin if possible, as this avoids any additional risks of handling when mixing neutral buffered formalin (NBF) from stock solutions.

Note: Stock solutions of formalin may vary in concentration. Follow the instructions carefully to achieve a correct concentration of 10% formalin.

Overstrength formalin results in poor fixation and causes excessive shrinkage and deposition of 'formalin pigments' in tissues. Understrength formalin results in inadequate fixation.

10% NBF is stable for 3 years when stored in a sealed container at room temperature away from heat and light.

Formaldehyde is regarded as carcinogenic in humans. Take extreme care with its use and storage. Only use 10% NBF in a well-ventilated area, and wear protective gloves, safety glasses and protective clothing. Minimise your exposure, and that of others, by careful handling and secure packaging of fixed samples.

Other fixatives

See **Section 14** for detailed information on fixatives suitable for samples from aquatic animals.

Alcohol (methylated spirits) is **not** a suitable fixative for histopathology.

4.3 Submitting samples

To prevent leakage, use screw-top containers with tightly fitting lids. The mouths of the containers should be wide enough for tissue to be easily withdrawn after fixation. **Do not squeeze large tissue samples into containers.**

Keep fresh tissue samples chilled from the time of collection until they are received at the laboratory, but **do not refrigerate fixed tissue samples.** Do not freeze samples being submitted for either gross or histopathological examination.

To avoid transporting heavy and dangerous volumes of formalin, keep fixed tissues moist during transport by placing them in securely sealed containers with a small amount (50 mL) of 10% formalin. Alternatively, wrap the tissues in formalin-soaked high-absorbency paper towels or swabs and place them in sealed containers. Do not transport formalin-fixed tissues in zip-lock bags.

5 Microbiology

A bacterial disease can only be diagnosed when a pathogenic organism can be demonstrated in association with pathological changes. This can be done using a smear, in culture, using molecular techniques or in tissue sections. Pathogenic organisms may be isolated from clinically normal animals, but recovery of the organism alone may not be significant without supportive pathology.

Wherever possible, submit formalin-fixed tissues concurrently for histopathology.

5.1 Collecting samples

Collect bacteriology samples from necropsies as soon as possible after death and immediately after opening the carcass. Collect samples aseptically and submit them promptly in individual sterile leak-proof screw-top plastic containers. Do not use non-sterile containers, gloves or fragile containers such as zip-lock bags when submitting material for bacteriological examination.

Sample fresh, active lesions. For a small lesion, submit the entire lesion or organ. For a large or widespread lesion, submit a portion of the affected tissue containing the lesion and surrounding tissue.

Alternatively, take an aspirate of the lesion and transfer it to a small sterile container or use swabs in a bacterial transport medium. If using swabs, sear the surface of the lesion or tissue with a hot spatula (heated using a small portable gas burner) and cut through the seared surface using a sterile scalpel blade, then swab the cut surface of the tissue.

To aid diagnosis, take and fix samples for histopathology at the same time.

Bacteria with special requirements

Anaerobes

Tissue samples and aspirate samples are best for anaerobic culture. Suitable specimens include blocks of affected tissue, pus from abscesses, discharges from wounds, pleural and peritoneal aspirates and joint fluid. Alternatively, swabs in bacterial transport medium can be used. All samples should be collected aseptically and placed in sterile, screw-top containers of suitable size to limit exposure of the sample to air. Avoid extremes of heat and cold and send the samples to the laboratory without delay.

Mycoplasma

Ideally, submit fresh tissue in mycoplasma media (Frey's transport media), which can be ordered from BSL. Chilled tissue, synovial fluid and milk samples received at the laboratory within 24 hours of collection are also suitable for culture.

Brachyspira

From live animals, collect faeces from the rectum. From recently dead animals, collect scrapings of the colonic mucosa or tied-off sections of the colon. Rectal swabs are not suitable. As *Brachyspira* is an anaerobic organism, place the samples in containers to reduce exposure to air and keep them chilled.

Dermatophilus

Select several scabs covering areas of fluid pus and send them in a sterile container. Smears made from the samples will be stained and examined microscopically, and aerobic culture will be performed.

Mastitis

Collect milk samples aseptically into sterile containers and submit them chilled. Take the samples immediately before milking, with as much time elapsed as possible after the last regular milking. Wash and dry the teat thoroughly and sterilise the teat end with 70% alcohol or undiluted teat dip solution. Discard the first few squirts, then collect the sample. If the samples cannot be delivered to the laboratory within 24 hours, freeze them at -20°C . Samples should be collected before treatment, but if antibiotics have been administered, record this on the specimen advice sheet.

- *Cattle*: If there is no aerobic growth after 24 hours, the samples will be cultured for *Mycoplasma*.
- *Goats*: Samples will be cultured for both aerobes and *Mycoplasma*.

Contagious equine metritis (*Taylorella equigenitalis*)

Use Amies charcoal swabs (CEM transport swabs, available from BSL). Do not use Stuart transport medium.

- *Stallions*: Collect swabs from the prepuce, urethral fossa and anterior urethra.
- *Non-pregnant mares*: Collect swabs from the cervix, clitoral sinus and clitoral fossa.
- *Pregnant mares*: Collect swabs from the clitoral sinus and clitoral fossa.
- *Mares with a vaginal discharge*: Collect vaginal swabs.

Refrigeration of the swabs is **not** recommended as *T. equigenitalis* survives longer at room temperature than at 4°C . However, avoid exposure of swabs to high temperatures.

Swabs should arrive at the laboratory and be plated out within 48 hours of collection.

Note: Samples should not be collected from horses that have been treated with antibiotics during the previous 7 days.

Faecal culture

Collect approximately 30 g of faeces directly from the rectum into a sterile, screw-top container. Alternatively, submit rectal swabs, heavily impregnated with faeces, in bacterial transport medium.

For Johne's disease faecal culture, see the special requirements under 'Johne's disease' in **Section 12.3**.

Intestinal contents for clostridial toxin assays and culture

Express small intestinal contents into sterile screw-top containers or submit tied-off sections of the small intestine. Chill the samples. Swabs of intestinal contents in bacterial transport media are suitable for culture only, not toxin assays.

Smears

Use slides of standard size and ensure they are clean. Preferably use slides with frosted ends (for labelling). Ensure slides are packed in a slide holder and placed in a sealed container so that they do not break in transit and are protected from moisture and formalin vapour.

Exudates

Make and air-dry smears and submit them with a sample or a swab of the exudate. Aspirates submitted in syringes with needles pose a safety risk and will not be examined.

Intestinal mucosa and pathological lesions (e.g. suspected blackleg lesions)

Firmly press a slide onto the suspect area to make an impression smear. Air-dry the smear and place it in a slide holder for transport.

Preputial samples from bulls

See 'Bovine campylobacteriosis' and 'Trichomoniasis' in **Section 12.3** for specific requirements.

Fungal infections

Skin conditions

Take a skin biopsy from the margin of an active lesion. Submit half of this fresh for fungal culture and the other half fixed in formalin for histopathology. Alternatively, pluck hair and take deep skin scrapings from the peripheral areas of active lesions into sterile containers. Do not chill or freeze samples, as temperatures below 15 °C can be detrimental to fungal survival. Submit separate samples for parasitological examination.

Systemic conditions

Collect fresh samples from any affected organs into sterile containers and keep them chilled. Also submit fixed tissues for histopathology.

5.2 Submitting samples

In general, keep all samples for bacteriological examination, except smears, chilled but not frozen (2–8 °C) from the time of collection until they are received in the laboratory. This will ensure the minimum growth of contaminants.

Keep smears at room temperature and protect them from moisture and formalin vapour.

Allow for delays in transport over weekends and public holidays. Keep samples refrigerated until you are sure that transport to the laboratory will not be delayed.

Complete and attach a specimen advice sheet.

5.3 Interpreting results

The laboratory report will list the organisms cultured, often with a pathologist's comment about their significance. The history and clinical and pathological findings must be reviewed when determining the significance of a bacterial isolation. As several types of bacteria are common contaminants, differentiation of significant bacteria from contaminants needs to be carefully considered.

Negative results may not mean that infectious agents were not the cause of the disease, as some bacteria (e.g. *Mycoplasma*, *Campylobacter*) are difficult to isolate. Bacteria may not be recovered due to various factors that may reduce the viability of the organism.

Delays in transport to the laboratory and/or poor sampling technique (non-aseptic) can result in overgrowth of contaminants, which may mask the isolation of significant organisms.

5.4 Antibiotic sensitivity

Antibiotic sensitivities are provided with certain bacterial isolate results. A recommended panel of antimicrobials is tested based on the isolate and the animal species and anatomical site from which it was cultured.

The sensitivity method that is used at the laboratory is a standardised international method that provides precise guidelines for interpretation. This method has been standardised for testing rapidly growing pathogens and modified for testing of some fastidious bacteria.

Studies are not yet sufficient to develop reproducible, definitive standards to interpret sensitivity testing of some microorganisms. Such organisms should not be tested, as the results cannot be reliably interpreted. Examples of these types of organisms are anaerobes, haemophilic organisms (such as *Actinobacillus* spp., *Haemophilus* spp. and *Histophilus* spp.), slow-growing organisms (such as *Trueperella pyogenes*), *Mycoplasma* spp., exotic or zoonotic bacteria (such as *Brucella* spp. and *Burkholderia pseudomallei*) and yeasts or fungi. Other bacteria may not have sensitivities tested when antimicrobial treatment is not recommended for that animal or disease state, e.g. self-limiting diseases.

6 Molecular diagnostics

Molecular diagnostic techniques such as polymerase chain reaction (PCR), 16S sequencing and restriction enzyme analysis are applicable to all disciplines of veterinary diagnostics including virology, parasitology, bacteriology and mycology.

The advances in molecular diagnostics have allowed a quicker turnaround time in the diagnosis of a wide range of diseases of terrestrial and aquatic animals. In recent years, the use of real-time PCR, also known as quantitative real-time PCR (qPCR) or TaqMan® real-time PCR, has improved the diagnostic outcome of a number of animal diseases, especially those caused by non-cultivable pathogens. The advantages of real-time PCR are numerous, but the most acknowledged ones are rapidity, high sensitivity and specificity and limited cross-contamination due to the use of sealed tubes throughout the PCR process.

BSL routinely offers a range of molecular assays for the diagnosis of emerging, exotic and endemic animal diseases.

6.1 Collecting samples

Collect fresh samples, e.g. fresh tissues, whole blood (EDTA or lithium heparin), swabs in transport medium, body fluids, excreta and secretions. EDTA is preferred as an anticoagulant. Lithium heparin is known to be inhibitory to PCR, especially if the ratio of blood to lithium heparin is not correct. If using a lithium heparin tube, fill the tube with blood to the mark to ensure the correct ratio of blood to anticoagulant.

Faecal samples, contaminated samples and post-mortem blood samples are often inhibitory to PCR. Haemoglobin, charcoal, heparin, bile salts and proteases contained in products such as blood, faeces, milk etc. are known to be inhibitory. Some laboratory extraction methods will help remove the inhibitory effect of certain chemicals. When collecting samples, always take care to avoid contamination with faeces, soil, bile etc. If contamination occurs, record this on the specimen advice sheet.

Submit samples fresh, in either saline or transport medium, e.g. VTM. Avoid bacterial transport medium containing charcoal, e.g. Amies medium with charcoal, as charcoal is inhibitory to PCR. Formalin-fixed tissues or paraffin-embedded tissues are not recommended. Extended storage of tissues in 10% formalin damages DNA, which affects the integrity of the target sequence, and so also the efficacy of PCR.

Preserve samples from aquatic animals (i.e. finfish, crustaceans and molluscs) in at least 3 times the volume of 90% absolute ethanol, reagent grade. Alternatively, use commercially available nucleic acid carrier reagents.

6.2 Submitting samples

Keep samples chilled during transport to the laboratory. However, if the samples are not being sent to the laboratory within 24 hours, freeze them and keep them frozen during transport to the laboratory. Keep blood at 4 °C at all times.

7 Parasitology

7.1 Collecting samples

Faecal samples

Faecal samples may be sent for examination for:

- nematode eggs (eggs per gram) and culture for larval differentiation
- cestode (tapeworm) eggs
- coccidial oocysts
- cryptosporidial oocysts
- trematode eggs—liver fluke (*Fasciola hepatica*) and paramphistomes
- lungworm larvae.

For individual animals, collect faeces directly from the rectum and place them into a 25 mL screw-top container (or a 100 mL container for horses). For cattle, horses, pigs and deer, collect the faeces from the rectum with a gloved hand. For sheep and goats, insert a gloved finger into the rectum and hook out pellets. Samples taken from the ground, even if collected immediately, will be contaminated with free-living nematodes and their eggs, which will complicate the diagnosis.

Fill the container and close the lid tightly to minimise exposure of worm eggs to air, which would allow egg development.

Samples received in plastic gloves or other unsuitable packaging (e.g. zip-lock bags) will not be processed.

Where helminthosis is suspected, collect samples from several animals showing clinical signs. Bottle the samples separately, label them clearly and forward them to the laboratory with a full history including details of any recent anthelmintic treatment.

Pooled egg counts rather than individual counts can be used for flock or herd monitoring. Take samples from a range of animals, e.g. from 10 of the top animals and from 10 poorer animals. As this method is quicker than the conventional method, charges are reduced accordingly. Indicate on the specimen advice sheet whether individual or pooled testing is required.

Gastrointestinal tract

The total gastrointestinal tract (from sheep, goats and calves) may be submitted directly to the laboratory for total worm counts. See **Section 7.4** for more detailed information.

Examination of the whole gastrointestinal tract of avian species is particularly useful for the identification of both helminths and coccidia. Submit the entire gastrointestinal tract chilled in a sealed container.

Snails for identification

Dry shells of freshwater snails (intermediate hosts for flukes) are sometimes inadequate for identification. Send fresh live specimens between layers of moist paper towelling with a cooler brick or preserve snails in 70% alcohol in a small leak-proof container.

External parasites for identification

Ticks

Send large, fully engorged live ticks in ventilated, escape-proof containers. If possible, extract ticks with undamaged mouthparts, as these may be important for identification. Small ticks can be preserved in 70% alcohol.

Fleas, lice and other insects

Send specimens preserved in 70% alcohol or 10% formalin.

Fly larvae and similar soft-bodied larvae

Send specimens preserved in 70% alcohol, preferably after they have been dropped in boiling water to stretch them and preserve the colour.

Mange mites

Before sampling, clip the hair or wool as close to the skin as possible. Take skin scrapings from active areas at the edge of a lesion. Smear the skin with mineral oil and scrape with a sharp blade deeply enough to cause slight bleeding. If there are pustules, also submit material expressed from several of these. Dry scales, scurf and plucked hair are of little value. Sample several affected animals.

When taking scrapings for mycological (fungal) as well as parasitological examination, collect separate samples, as mineral oil renders samples unsuitable for mycological examination.

Send skin scrapings unpreserved in a sealed container.

Feather and hair mites

At necropsy, leave mites that are firmly attached in situ and snip out the skin where they are attached. After death of the host, many mites will start migrating and can be collected from the tips of wool, hair, fur and feathers. From live animals, collect individual mites using a small, wet brush or needle. Forward mites preserved in 70% alcohol or 10% formalin.

Internal parasites for identification

Unpreserved worms decompose very rapidly and are not suitable for identification.

Nematodes

In general, these can be placed directly in 10% formalin or 70% alcohol (70% alcohol containing 5% glycerine is better).

Lungworms found free in the bronchi (*Dictyocaulus* spp.) are very fragile and need to be properly prepared before being sent to the laboratory. Place lungworms in a large volume of normal saline for 16 hours at 4–8 °C, then transfer them to 10% formalin for transport to the laboratory. *Muellerius capillaris* in sheep occur in small nodules. Send a portion of lung containing these nodules in 10% formalin.

Trematodes (flukes)

Wash the flukes in saline and place them in 70% alcohol.

Cestodes (tapeworms)

Allow the worms to lie in tap water for 2 hours so that they die in an extended position. The scolex (head) is important for identification. If a worm is attached to the bowel wall, snip out the piece of wall and allow the worm to lie in tap water until it releases. If it remains attached, include the small piece of gut wall and attached scolex with the preserved specimen. Preserve the sample in 10% formalin. Do not preserve worms in methylated spirits, as the organs will not stain satisfactorily.

Coccidia

The species can usually be identified from morphology of the unsporulated oocysts in the faeces. Where identification requires measurement of the oocysts, the concentration/flotation technique will be used to concentrate the oocysts.

Suspected parasitic lesions

Excise the lesion with some surrounding tissue, being careful not to release its contents, especially if it is a fluid-filled cyst. Forward the unpreserved lesion to the laboratory in an insulated container with a cooler brick. If you expect a delay of several hours between sampling and arrival at the laboratory, preserve the sample in 10% formalin.

7.2 Submitting samples

Keep faecal samples cool but not frozen. Prolonged chilling at even 5 °C kills the eggs of most species and makes samples unsuitable for larval culture for strongyle egg identification. Refrigerate samples for lungworm larvae immediately.

Submit faecal samples as soon as possible after collection. Send them chilled in an insulated container, with cooler bricks wrapped in paper to prevent freezing of the samples.

Include a specimen advice sheet with a detailed history including information about recent anthelmintic treatments, drench resistance status, stocking rate, pasture availability, access to swampy areas and grazing rotation.

Samples remain suitable for egg counts for up to 3 days when stored at 4–8 °C. However, results of larval cultures are sometimes unsatisfactory from refrigerated samples.

For cattle faeces only: If the sample container is completely filled with faeces to exclude as much air as possible, the sample will remain suitable for egg counting and larval culture for several days without need for refrigeration or other preservation.

7.3 Interpreting results

Faecal egg counts

Faecal samples are routinely examined for nematode and cestode eggs and coccidial oocysts. If trematode or lungworm larvae examinations are required, please specifically request them. Quantitative estimations are made for nematode and trematode eggs and are expressed as eggs per gram (epg) of faeces. Larval differentiation by faecal culture takes 8–11 days and gives an indication of the genera of nematodes present. *Strongyloides* spp. eggs are noted if present but not expressed quantitatively. Results for coccidial oocysts are expressed semi-quantitatively ('negative', 'low', 'moderate' or 'high') and results for cestodes and lungworm larvae are expressed qualitatively only ('present' or 'not present').

Faecal culture and larval differentiation

The value of a faecal egg count is increased if the species of worms present can be identified. While some eggs are distinctive and can be identified to genus, most strongyle-type eggs are similar in shape and size and are not readily identifiable by microscopic inspection or measurement. However, infective larvae (L3) are morphologically distinct and readily differentiated. It is assumed that the various species of larvae recovered reflect the relative numbers of these species in the initial faecal egg count. Individual faecal samples can be cultured, but generally those samples with significant egg counts are bulk cultured.

Nematode eggs

Interpret egg counts with respect to the species of worms present, as different species have different pathogenic effects. Also, worm populations fluctuate rapidly and the results from one sampling may give a very narrow view of this dynamic situation.

Table 7.1 Significant faecal egg counts in ruminants (epg of faeces)*

Location	Parasite	Main clinical effect	Young sheep and goats		Young cattle	
			Significant†	Dangerous‡	Significant†	Dangerous‡
Abomasum	<i>Haemonchus</i>	Anaemia	500	2 000	200	1 000
	<i>Ostertagia</i> (cattle) <i>Teladorsagia</i> (sheep)	Diarrhoea	200	2 000	500	2 000
	<i>Trichostrongylus axei</i>	Diarrhoea	500	3 000	200	1 000
Small intestine	<i>Trichostrongylus</i>	Diarrhoea	500	3 000	500	3 000
	<i>Cooperia</i>	Diarrhoea	500	3 000	500	10 000
	<i>Nematodirus</i>	Diarrhoea	200	1 000		
	<i>Bunostomum</i>	Anaemia			200	500
	<i>Strongyloides</i> ‡	Diarrhoea (rare)				
Large intestine	<i>Oesophagostomum</i>	Mucoid diarrhoea	100	1 000	200	1 000
	<i>Trichuris</i> ‡	Diarrhoea (rare)				

* The counts are for animals with normal food intake and faeces of normal consistency. Correction factors are:

- × 0.5 for animals fasting >12 hours
- × 2 for soft, unformed faeces
- × 3 for diarrhoea.

† Significant = the lowest egg count that may indicate a significantly harmful infection. Dangerous = a high count that indicates significant production effects and may indicate pending fatalities.

‡ Eggs in faeces not quantified.

Cestode eggs

Tapeworms are not considered to be significantly pathogenic. However, very heavy burdens in lambs and kids may be harmful and specific therapy is recommended.

Coccidial oocysts

Coccidial oocysts are often found in faeces from normal, healthy animals and in these cases are of little significance. When large numbers are found in faeces from animals with a history of diarrhoea or dysentery (diarrhoea with blood), a diagnosis of coccidiosis should be considered.

Trematode eggs

Paramphistomes (stomach fluke)

Paramphistomes are a problem in some areas of Queensland. The immature stages in the small intestine may cause diarrhoea, but adult flukes cause little harm. When paramphistome eggs are found in faeces and there are clinical signs of diarrhoea and wasting, paramphistomosis should be considered in the differential diagnosis.

***Fasciola hepatica* (liver fluke)**

The migration of immature flukes through the liver and the presence of adult flukes in the bile ducts can cause a variety of clinical manifestations from mild ill thrift through to marked debility with anaemia, jaundice and bottle jaw, and sometimes sudden death. In addition to the results of the faecal examination, clinical signs, necropsy results and the distribution of *F. hepatica* should be considered before arriving at a diagnosis of fascioliasis.

Lungworm larvae

The presence of lungworm larvae in faeces indicates patent infection with adult parasites in the lungs. If this is associated with respiratory signs (e.g. coughing, dyspnoea), lungworm infection can be considered in the differential diagnosis.

7.4 Total worm counts

Total worm counts may be undertaken on freshly dead animals as part of a necropsy to determine the cause of death in individual animals. Elective slaughter and subsequent total worm counts on selected animals may also be used as an aid to diagnosis of helminthosis on a herd basis.

Gastrointestinal tracts from sheep, goats and calves can be submitted for total worm counts. However, tracts from adult cattle are difficult to handle. Contact the laboratory before sending such specimens for examination. Send the gastrointestinal tracts chilled if they will arrive at the laboratory within 48 hours; otherwise, preserve them with embalming fluid.

Collecting samples

The animal to be sampled should be freshly dead. Gastrointestinal nematodes deteriorate very rapidly after death and intestinal tracts from animals dead more than 8 hours are often unsatisfactory for total worm counts.

Locate the various organs of the gastrointestinal tract. Tie off the abomasum at both ends with string and sever the connection with the omasum. Tie off the rectum and remove the total tract from abomasum to rectum. At this stage, open the rumen and reticulum and check for stomach fluke in situ. If any are present, estimate the number and forward 20–30 to the laboratory for identification. If

embalming fluid is available, inject 20–50 mL into each of the abomasum, caecum and large colon. Mix the embalming fluid through the contents by kneading the outside of the organ.

Place the gastrointestinal tract into a thick plastic bag and add 300–500 mL of embalming fluid. Swill the embalming fluid around to ensure good contact with all the tract. Seal the bag, excluding as much air as possible. Place this bag inside another thick plastic bag for extra security and seal the second bag as well.

Send the preserved tract in an insulated container. Gastrointestinal tracts preserved with embalming fluid should be suitable for total worm counts for up to 3 weeks after removal from the animal.

Interpreting results

The number of worms required for significant production effects depends on many factors including the age, size and weight of the host, the duration of infestation, the age of the worms and the nutritional and immunological status of the host. Interpret results in the light of other knowledge such as clinical signs, production parameters and other laboratory findings.

Table 7.2 Nematodes commonly associated with clinical helminthosis in ruminants in Queensland

Animal	Nematode
Sheep	<i>Haemonchus contortus</i>
	<i>Trichostrongylus</i> spp. (of the small intestine)
	<i>Oesophagostomum</i> spp.
Goats	<i>Haemonchus contortus</i>
	<i>Trichostrongylus</i> spp. (of the small intestine)
Cattle	<i>Haemonchus placei</i>
	<i>Cooperia</i> spp.
	<i>Oesophagostomum</i> spp.

7.5 Ticks—acaricide resistance testing

An investigation can be initiated if field breakdown in acaricide control is suspected. If this is occurring with an acaricide group to which no resistance has been previously shown, contact the laboratory after collecting relevant history, but before visiting the property and sending samples. Two tests are currently available for resistance testing—the larval packet test (LPT) for organophosphate, synthetic pyrethroid, macrocyclic lactone and amidine resistance detection, and the adult immersion test (AIT) for fluzazuron resistance testing. Complete a resistant tick advice sheet for the resistance investigation. These sheets are available from BSL and at business.qld.gov.au. Usually, results will be available 7–8 weeks after receipt of suitable specimens.

Collecting samples

If possible, collect ticks before treatment or at least 49 days after treatment. Collect them in the late afternoon or early morning. Female ticks engorge in the last 12 hours of their cycle on the animal and most drop off in the early morning just after sunrise. At least 30–60 fully engorged ticks are required, but send a larger sample if you can collect them. Collect only the fat, fully engorged ticks, as half-engorged and small ticks lay few eggs. Avoid damaging or squeezing the ticks on removal.

Put live ticks in a ventilated, escape-proof container. Include 3–4 blades of green grass with the ticks. This helps maintain moist conditions for transport. Do not use cottonwool or damp paper towel. Keep the ticks away from all chemicals, sunlight and excessive heat. Ticks can die or their egg-laying can be compromised if they are not treated properly.

Complete a resistant tick advice sheet (available from BSL or at business.qld.gov.au). Supply as much information as possible, including current and previous acaricide use.

Dispatch the sample promptly using protective outer packaging such as cardboard. Keep the ticks cool but not refrigerated. At optimum temperatures, ticks will begin to lay eggs around 2–3 days after dropping from the animal.

Note: Ticks for AIT fluazuron testing must reach the laboratory before egg-laying commences.

8 Serology

Serology is available for a broad range of bacterial, viral and parasitic diseases. It is used to show the presence or absence of antibodies to a specific aetiological agent or group of agents. The presence of antibody indicates exposure to an organism, so may be related to the current clinical condition or may reflect an earlier unrelated infection. Antibodies may also indicate vaccination against a particular agent.

Ideally, collect two serum samples, the first in the acute stage of the disease and the second in the convalescent stage 3–4 weeks later (2 weeks for bovine ephemeral fever). The presence of antibody in the acute phase or absence of antibody in the convalescent phase can eliminate a diagnostic possibility. Many animals in endemic areas may have antibody to a given organism, so a single sample from an affected animal may not allow the serological test to be interpreted.

Because of the large number of serological tests run by the laboratory and limited resources of equipment and labour, some tests must be run as batches on particular days of the week. Samples for these tests are stored then tested together on the designated day. Contact the laboratory for information on turnaround times.

8.1 Collecting samples

Blood samples

Collect 5–8 mL of blood aseptically into a 10 mL blood collection tube with no additives, or a blood collection tube with clot activator or a serum separator tube (SST).

When requesting a range of serological tests, collect duplicate samples.

Avoid contamination of the tube and stopper. Remove any blood and foreign matter from the exterior of tubes before dispatch.

Allow the samples to clot at room temperature; this will take 30–60 minutes in summer and longer in winter. Separate the clot from the wall of the tube (by tapping the tube on the palm of your hand) before refrigerating the sample at 4 °C.

Ideally, pour or spin off the sera and place it into screw-top containers before sending the chilled samples to the laboratory. This is especially important for porcine blood, which haemolyses readily. Send the clots as well as the sera.

Invert SST tubes gently to mix the clot activators with the blood, then allow the blood to clot in a vertical position for 20–30 minutes. Centrifuge it **within 1 hour** of collection.

Avoid rough shaking of samples, high temperatures and contamination that could lead to bacterial growth.

Do not freeze the blood samples. Freeze separated sera if transport of the samples to the laboratory will be delayed.

Poor-quality samples result from haemolysis, insufficient quantity, bacterial contamination and failure of clot retraction, resulting in no serum yield. Causes of haemolysis include:

- prolonged contact of serum or plasma with red cells
- water on needles, skin or blood containers
- rough handling of samples (vigorous shaking or transport over rough roads)
- excessive negative or positive pressure on the syringe when drawing or transferring blood
- inappropriate needle size (too small or too large)
- exposure to excessive heat or cold.

Vaginal swabs

Sample collection kits for *Campylobacter* enzyme-linked immunosorbent assay (ELISA) can be ordered from BSL and include 15 × 4.5 mL phosphate-buffered saline containing 0.05% Tween™ 20 (PBST) and sterile swabs.

Collect samples from 10–20 infertile heifers or cows. Clean the perineum, then introduce the swab into the vagina as cranially as possible. Press the swab against the vaginal wall and turn it a few times to ensure full saturation. Cut off the cotton head and place it in PBST. Chill the samples and dispatch them to the laboratory as soon as possible.

Ear notches

Ear notches can be sent unpreserved and chilled, desiccated or frozen for bovine viral diarrhoea virus (BVDV) antigen-capture ELISA.

8.2 Labelling samples

Label samples sequentially (e.g. from '1' to '30') using a non-water-based pen. Keep a key list that correlates sample numbers with animal identification. Do not label samples with tag numbers, names etc., as this leads to confusion and errors in reading numbers in the laboratory.

Label paired sera consistently with the same sample number and the date of collection. If the paired samples are not sent at the same time, quote the previous job number.

Note: Sample numbering sheets are available at business.qld.gov.au.

8.3 Submitting samples

Transport samples to the laboratory at 4 °C, ideally within 24 hours of collection. Include a completed specimen advice sheet.

For bovine infertility investigations, also complete a cattle herd reproductive history form. These forms are available at business.qld.gov.au.

8.4 Interpreting results

Serological examinations may be of great value in making diagnoses if interpreted correctly. However, serological evidence of infection is generally not as significant as the actual isolation of the causative organism of the disease. If possible, try to detect the infective organism as well as antibody produced by the infected animal.

With some diseases, antibody may remain for long periods after recovery from infection and may be detected when investigating disease due to a different cause. Confusion in diagnosis may then arise if such results are not correctly interpreted. No serological test is 100% accurate. False positive and false negative reactions occasionally occur. Also, antibody may arise from vaccination and not infection. Final interpretation of results rests with the clinician, who can consider the circumstances of the case.

Serological results may be reported as:

- titres
- positive, suspect or negative
- strength of reaction (1+ to 3+).

Example: A titre of 16 means that this dilution of serum (1:16) is the highest dilution giving a positive result. More antibody is present in a serum that has a titre of 32 (1:32) than in a serum that has a titre of 16.

Agar gel immunodiffusion test

The agar gel immunodiffusion (AGID) test is used to detect the presence of antibody. The results are reported as positive or negative. When antibodies are present, the level of detection is rated from 1+ to 3+.

BVDV AGID test (for bovine pestivirus)

Antibodies to BVDV are detectable from 12–14 days after exposure to the virus. They reach a peak at about 2–3 months after infection, then slowly decline. Some animals will be very weak (1+) reactors after 12 months, while in others moderate to weak (1+ and 2+) antibody reactions may persist for years. Reactions of 3+ or greater generally do not persist for more than about 6 months in most animals. Similarly, reactions of >3+ are rarely found at times other than 1–3 months after infection.

This information is useful in the investigation of reproductive disease when groups of infected animals are sampled. If there are only seronegative animals, or almost all are weak positives (predominantly 1+ and some 2+ reactors), it is highly unlikely that BVDV is associated with the problem. However, the presence of a number of 3+ reactions (or stronger) will usually suggest that there has been transmission of BVDV within the group in the last 6 months. The finding of >3+ reactors usually indicates very recent infection.

Complement fixation tests

A complement fixation test (CFT) is conducted on doubling serum dilutions and titres are reported. Haemolysed or contaminated samples often give unreliable results in a CFT. The serum may be anti-complementary or give non-specific low titre positives, particularly with sheep and goat sera. In such cases, a further serum sample will need to be collected and sent for testing.

***Brucella ovis* CFT**

Titres of 8 are considered inconclusive in a flock with no history of brucellosis and no rams with lesions consistent with *Brucella ovis* infection. It is best to resample these animals in 2–4 weeks. However, a titre of 8 is considered a positive result in a flock with a history of brucellosis or rams with consistent lesions. A titre of 16 is considered positive regardless of the history or presence/absence of lesions.

Melioidosis serology (CFT and IHA test)

The *Burkholderia pseudomallei* CFT is quite specific and indicates active infection, while the indirect haemagglutination (IHA) test indicates past and current infection. Animals in melioidosis-endemic areas tend to have higher background IHA titres, so take care in the interpretation and diagnosis of serological tests. A more accurate diagnosis of melioidosis is likely if both the CFT and the IHA test are run and the results are compared as in Table 8.1. Although this table is specifically used for the diagnosis of caprine melioidosis, it can be used as a guide for diagnosis of melioidosis in other species. In camelids, however, positive serological results can be interpreted as evidence of exposure only, as no serological studies have been done in these species. Titres are variable and do not necessarily correlate with the clinical signs observed. If melioidosis is suspected, send samples suitable for culture of the organism.

Table 8.1 Interpretation of CFT and IHA test titres for diagnosis of melioidosis in goats

Test/titre		Interpretation
CFT	IHA	
Negative	Negative	Not infected (does not exclude infection in the 6 days prior to sampling)
Negative	40 to 160	Possibly natural agglutinins or cross-reaction with other infectious organisms May indicate past infection or exposure
Negative	320 or greater	Possibly melioidosis; symptoms may or may not be apparent Re-bleed in 14–21 days to check for a rising titre May indicate a chronic infection
8 or 16	40 to 160	Possibly melioidosis; symptoms may or may not be apparent Re-bleed in 14–21 days to check for a rising titre
32 or greater	320 or greater	Infected Likely to have clinical signs of acute melioidosis

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) is designed to detect the presence of antibody. The results are reported as positive, suspect or negative. A follow-up sample may be required to confirm the status of infection in an animal with a suspect result.

***Campylobacter* ELISA**

The *Campylobacter* ELISA is a herd test. Vaginal swabs from 10% of infertile heifers or cows (at least 10 individuals) are required. Bovine campylobacteriosis (BC) is unlikely to be present in the herd if all samples are negative. Finding 1 or 2 positives is consistent with BC. The disease in these cases may not be widespread in the herd. Finding 3 or more reactors indicates widespread infection in the herd. If several animals are reported as suspect, testing of 10 different animals should further clarify the BC status. Animals that have aborted as a result of BC and are sampled 1–12 weeks after the abortion will usually give positive results on the *Campylobacter* ELISA.

***Neospora* ELISA**

A negative result in an aborting cow is good evidence that *Neospora caninum* is not the cause of abortion. However, a positive result in a cow that has aborted indicates exposure but does not necessarily implicate neosporosis as the cause of abortion. Definitive diagnosis of *Neospora* abortion requires histological examination of the foetus. Serology may be useful as a herd test to compare the rates of serological positives in aborting versus non-aborting cows.

Leptospira microscopic agglutination test

The interpretation of serological results from the *Leptospira* microscopic agglutination test (MAT) is complicated by a number of factors including cross-reactivity of antibodies, antibody titres induced by vaccination and lack of consensus about the level of antibody titres that indicates infection.

Newer vaccines are more immunogenic and may produce titres as high as those produced by natural infection, with these titres persisting for 1–4 months after vaccination. In some animals, titres may decrease to be undetectable after 3 months, but in others high titres may persist for 6 months or more.

Titres to *L. hardjo* tend to be low even after natural challenge. Commonly, cattle can be actively infected with *L. hardjo* and shedding leptospire with antibody titres <1:100. Therefore, diagnosis of leptospirosis based on a single serum sample must be made with caution and with consideration of the clinical picture and vaccination history. Confirmation of acute infection is best achieved by detection of the organism in the urine or tissues by PCR.

High titres (1:3200 to 1:6400) to *L. pomona* are significant in unvaccinated cattle but are difficult to interpret in vaccinated animals.

When recent infection is suspected, the use of paired acute and convalescent titres is preferable and in cases of acute leptospirosis, a fourfold rise in antibody titre is diagnostic. Antibody titres can persist for months following infection and recovery, although there is usually a gradual decline in the antibody titre with time.

Leptospiral abortion

Detection of high titres to *L. pomona* (>1:1600) in **unvaccinated** cows that have recently aborted may be sufficient to establish a presumptive diagnosis of leptospiral abortion.

However, in *L. hardjo* infection in cattle, infected animals frequently have a poor antibody response to infection. Often, at the time of abortion (which may occur up to 12 weeks after infection), antibody titres may be quite low or negative. In these cases, the herd serological response to infection is often more helpful and submission of paired samples from up to 20 animals with a history of recent illness may be more useful in establishing the diagnosis.

Where reproductive losses are only detected as low calving percentages, *Leptospira* serology is not warranted, as abortions may have occurred many months previously. Titres in unvaccinated animals may indicate previous exposure but may not necessarily be linked to abortion. In vaccinated animals, interpretation of titres is very difficult and serological diagnosis of leptospiral abortion is not possible.

It is most useful to do serological testing on foetal serum or fluids (though titres may be very low) and demonstrate the presence of *Leptospira* in foetal tissues by PCR to confirm a diagnosis of leptospiral abortion.

Virus neutralisation tests

Virus neutralisation tests (VNT) can only be interpreted if paired samples are examined from affected animals during a disease outbreak. The first sample must be taken as early as possible in the disease and the second 3–4 weeks later (2 weeks later for bovine ephemeral fever).

When submitting paired samples, quote the job number of the first sample and use the same identification system. Note that tests are only done if paired samples are available.

Variations in antibody titre may aid in making a diagnosis. A fourfold rise in titre is significant, but under field conditions the rise may have occurred by the time the disease is investigated. If it appears that the disease is spreading at the time of investigation, sampling of in-contact animals may detect the occurrence of new infections.

8.5 Bovine infertility investigations

Submit serum samples from up to 20 animals from one property, preferably from those animals that have a history of recent illness, abortion or return to service. If possible, submit paired samples from the same individuals taken 3–4 weeks apart, as this will allow detection of rising and falling antibody titres. If you submit more than 20 samples from a single property, only the first 20 samples will be tested free of charge. Contact BSL for more information.

Complete and attach a cattle herd reproductive history form (available at business.qld.gov.au). This will provide important information for the interpretation of results by the submitter and the pathologist. At the discretion of the pathologist, in the absence of this form, the standard infertility panel will include the *Leptospira* MAT (for *L. hardjo* and *L. pomona* unvaccinated cattle only) and BVDV AGID (for bovine pestivirus). Any other testing (e.g. *Neospora* ELISA) will be done upon request but will incur a charge. If a completed form accompanies the submission, the pathologist may determine that further testing is warranted and this will also be performed free of charge on the first 20 samples.

If there is infertility, return to service and protracted calving in a herd, testing for bovine campylobacteriosis should also be considered. Vaginal swabs can be taken at the same time as blood samples. *Campylobacter* ELISA kits, which contain all the swabs and media needed to sample 15 animals, can be ordered from BSL. There is a charge for these kits, but no cost for the testing of the samples if they form part of an infertility investigation.

If there is abortion in a herd, collect and submit the aborted foetus and membranes to provide the best chance of reaching a definitive diagnosis. Alternatively, collect samples from the foetus and forward them to the laboratory. See 'Abortion/stillbirth' in **Table 12.1** for the recommended samples.

9 Tick fevers of cattle

Tick fevers of cattle are caused by three different organisms—*Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale*. *B. bovis* is the most common cause of tick fever outbreaks.

Always prepare samples from cases of suspected tick fever, even when confident the disease is involved. It is not possible to differentiate between the infections on the basis of clinical signs and gross pathological changes alone. These samples can also be a valuable resource for epidemiological and economic evaluations of the role of each tick fever parasite.

A diagnosis of clinical disease or cause of death is usually made by demonstrating the parasites microscopically in blood and/or organ smears and by ascribing a level of significance to the infection based on the number of organisms present, evidence of anaemia and history.

Sensitive PCR assays are available but are costly and not very useful in determining the significance of the infection.

Serological tests to detect specific antibodies are not of value in the clinical stage of the disease and are used only for research, epidemiological studies or detection of carriers, or to ascertain immune status after vaccination.

Diagnoses are sometimes not confirmed at the laboratory because samples are badly prepared or unsuitable. At other times, the samples are good, but confirmation is not possible because of factors associated with the disease itself. *Babesia* spp. may be difficult to detect during the recovery stage when parasitaemia falls rapidly, or when samples are collected after specific treatment has been administered. *A. marginale* is sometimes difficult to recognise in the recovering animal because of the presence of an intense anaemic response when punctate basophilic granules in red blood cells may resemble *A. marginale* and confuse the diagnosis.

9.1 Collecting samples

The samples required depend on whether the animals are alive or dead, the degree of decomposition of dead animals and the vaccination history.

Thin peripheral blood smears are ideal for detecting all three tick fever organisms and differentiating between species. Peripheral blood smears are most important for detection of *B. bovis*, because the organisms adhere to the capillary endothelium. The tail tip is a particularly useful peripheral site. Venous blood smears made immediately on collection of blood or from blood collected into EDTA tubes are satisfactory for detection of *B. bigemina* and *Anaplasma* spp., but are less suitable for *B. bovis*.

Organ smears are usually only useful for detection of *B. bovis* infections but may occasionally detect the other two organisms. Brain smears are extremely valuable in diagnosing *B. bovis* infections due to the tendency of this parasite to accumulate within brain capillaries.

Samples from treated cattle are frequently non-diagnostic.

Samples to collect

Acutely sick animals

- Thin peripheral blood smears from as many affected animals as possible

Dead animals

- Thin peripheral blood smears from an extremity
- Organ smears from (in descending order of preference) brain, kidney, heart muscle, spleen and liver

Previously vaccinated animals

- Peripheral blood smears and organ smears as above
- Serum and EDTA blood
- Sera from cohorts in the herd (for specific requirements, contact the TFC)

Complete a specimen advice sheet (including a detailed clinical and vaccination history) and send it with the samples. Clearly identify smears from different animals.

Preparing thin blood smears

- Hold the slide by the edges to avoid contamination of the areas where the blood smear will be, or has been made.
- Clip the hair from the tail tip or from a site on the ear and brush off any dirt.
- Prick the tail tip or ear with a sharp needle or scalpel blade to obtain capillary bleeding.
- Allow the blood to well up before touching a corner of the pusher slide onto the top of the drop of blood.
- Transfer the blood with the pusher slide onto one end of the specimen slide.
- Wipe the end of the pusher slide clean and dry.
- Place the pusher slide in front of the drop of blood and hold it at an angle of about 30–45°. Draw the pusher slide back into the drop and allow the blood to run to the edges of the pusher slide.
- Keeping the pusher slide at a 30–45° angle, move it smoothly forward in one quick movement, maintaining light pressure. This should spread an even film of blood onto the specimen slide.
- Wave the slide to air-dry the smear as quickly as possible, or gently heat it over a flame (e.g. a lighted match) if the weather is very cold or wet.
- Identify all slides and place them in a slide container. At all times keep prepared smears away from moisture, formalin vapour and insects. Do not refrigerate.

Preparing brain smears

- Using scissors or a scalpel blade, slice a piece of grey matter from the cerebral cortex. Alternatively, make a small hole in the skull and insert a 14–18 gauge needle attached to a 10 mL syringe into the outer grey matter of the brain. To avoid sampling white matter, which has far fewer capillaries, do not go too deep. Aspirate some brain tissue into the needle by drawing back on the syringe.

- Place or express a piece of brain tissue about the size of a match head onto a slide about 2 cm from one end.
- Take a second slide and lay it on top of the grey matter at right angles to the specimen slide.
- Press down gently and move the top slide sideways to the far end of the lower slide. This crushes the tissue and spreads it thinly.
- Wave the slide to air-dry the smear as quickly as possible, or gently heat it over a flame (e.g. a lighted match) if the weather is very cold or wet.
- Identify the slides and submit them in slide holders in conjunction with the thin blood smears.

Preparing visceral organ smears

- Make a fresh cut in the organ (kidney, heart muscle, liver or spleen), then squeeze out a little blood and make a thin blood smear as described above.
- Alternatively, make impression smears by lightly applying the freshly cut surface of a small piece of the organ to the surface of the slide in several places.

Unsuitable samples

- Smears exposed in transit to formalin or formalin vapour
- Smears with lysed red blood cells due to severe post-mortem decomposition, prolonged drying time or condensation on slides (as may occur if slides are refrigerated)
- Smears contaminated with bacteria during collection of blood and preparation of slides
- Smears stuck together or with a cover-slip applied when wet
- Organs too decomposed
- Dirty microscope slides

Packaging smears

When dry, place smears carefully into individual slide holders and then into a sealed container or zip-lock bag to avoid exposure to formalin or moisture during transit.

9.2 Interpreting results

When the laboratory indicates that no organisms were detected, the possibility of disease due to tick fever cannot be entirely excluded. Conversely, a positive finding does not invariably mean that tick fever is the primary cause of the presenting disease.

B. bovis is by far the most common cause of tick fever in Australia, but there is little correlation between clinical severity and the parasitaemia in circulating blood. Even in very sick animals, *B. bovis* is often present in small numbers only and, consequently, a positive finding in blood smears is usually reported as being significant, regardless of the level of parasitaemia.

With *B. bigemina*, interpretation of the results is often difficult because this parasite is sometimes found in clinically healthy animals. A report of a significant *B. bigemina* infection is generally based on 1% or more of red blood cells being infected. A parasitaemia of less than 1% may be regarded as significant if there is evidence of anaemia and the history is strongly suggestive of tick fever.

A. marginale infections are also difficult to interpret. A positive result is usually reported as being significant if more than 3% of red blood cells are infected, or where the infection is accompanied by marked anaemia and the history is suggestive of tick fever.

If only brain smears have been examined, the laboratory will indicate if parasites are present in small numbers (as in a normal carrier) or in significant numbers. Brain smears are very useful for differential diagnoses, as *B. bovis* commonly sequester in brain capillaries. *B. bigemina* is rarely seen in brain smears.

Differentiation of *B. bovis* and *B. bigemina* is difficult when gross morphological changes have occurred in the parasites. Also, badly degenerated parasites of both species can resemble *A. marginale*. These problems are likely when animals are sampled after treatment for tick fever or if sampling is delayed after death.

Finding *Babesia* spp. or *A. marginale* in animals less than 9–10 months of age would not often be considered significant, as young animals are considered to be quite resistant to the clinical effects of tick fever infection.

Clinical disease subsequent to *B. bovis* vaccination may be differentiated from *B. bovis* field infections by genotyping PCR tests if an EDTA sample is submitted.

Theileria orientalis

Theileria orientalis is a very common infection in Australia, and organisms are often present in readily detectable numbers in blood smears of clinically healthy cattle. It is rarely a cause of disease in Queensland.

A finding of *Theileria* may be considered significant if it is accompanied by a marked regenerative anaemia and other causes of regenerative anaemia have been ruled out.

The identification of the Ikeda variant by PCR testing on EDTA blood adds weight to the significance of the finding.

In Queensland, clinical *T. orientalis* infections are usually associated with the recent introduction of a susceptible animal to an area where the Ikeda variant is present. Some morphological forms of *T. orientalis* can be confused with *B. bovis* and degenerating *T. orientalis* can resemble *A. marginale*.

9.3 Tick fever vaccines

Tick fever vaccines are produced at the TFC and are available in both chilled and frozen forms.

For more information on the vaccines and how to order them, contact the TFC.

TFC contact details

Phone (07) 3898 9655
Fax (07) 3898 9685
Email tfc@daf.qld.gov.au

Street address

280 Grindle Road
WACOL QLD 4076

Postal address

PO Box 109
ARCHERFIELD QLD 4108

Operating hours

8 am to 4 pm, Monday to Friday

10 Toxicology

When poisoning is suspected, take a comprehensive range of specimens from affected animals. It is better to collect too many specimens than too few as it may not be easy to revisit the scene, and carcasses may be removed, buried or burned.

Also take specimens from suspect material following a thorough search of the area to identify possible poison sources, e.g. storage sheds, rubbish dumps, old batteries, dips, poisonous plants. BSL depends heavily on suggestions from the field investigator as to the likely poison or poisons involved, since exhaustive screening for all known poisons is impractical.

10.1 Collecting samples

Take animal specimens of approximately 200 g weight or include the whole organ if its weight is less than this. Submit fresh samples in clearly labelled, separate screw-top containers and keep them chilled.

Table 10.1 Samples to collect from affected animals for toxicology

	Species	Samples
Dead animals	Ruminants	Ocular fluid Ruminal and abomasal contents Fresh liver, kidney
	Pigs/horses	Ocular fluid Stomach contents Fresh liver, kidney
	Poultry/birds	Crop and gizzard contents or whole gastrointestinal tract Liver Brain
Live animals	All species	Faeces (200 g collected from the rectum) Urine (200 mL) Blood (clotted and EDTA) Vomit Hair (10 g shaved from the shoulder area)

Table 10.2 Samples to collect from suspected sources of toxins

Suspected toxin source	Samples
General	200 g of suspected material
Water	5 L sample together with 200 g of bottom mud from any apparent site of water pollution
Residues	Residue materials in tins, containers, paint scrapings etc. along with details on the labels of containers
Contaminated feed	500 g sample directly from areas visibly affected by dampness, staining, discolouration or mould
Feed additives	500 g sample
Plants	Plants for botanical identification—see Section 10.4 Cyanide poisoning: fresh leaves or preferably the whole plant wrapped in damp paper to prevent wilting and crushing; deliver the samples as quickly as possible to the laboratory Nitrate or oxalate poisoning: plant specimens, shade dried before submission; avoid using sealed plastic bags for transport as the plant rapidly 'sweats', becomes mouldy and decomposes, destroying both nitrate and oxalate
Cyanobacterial blooms	If possible, 1 L (minimum of 200 mL) of the most concentrated part of the algal bloom, chilled; preserved samples are preferred for identification of algal species but are unsuitable for toxin detection See Section 10.4

10.2 Tests available

Table 10.3 Toxicology tests routinely available

Toxin	
Arsenic	Liver, kidney, large volume of urine or faeces, ingesta, suspected sources, hair (in chronic cases)
Copper	Liver, kidney, serum
Cyanide	Plant (fresh and unwilted), rumen contents, liver, ocular fluid (send samples frozen)
Fluoride	Urine, bone (jaw bones with teeth), water, feed supplement
Lead	Liver, kidney, EDTA blood, faeces, suspected sources
Mercury	Liver, kidney, brain, environmental material
Nitrate/nitrite	Plant, rumen contents, ocular fluid, blood
Organophosphate pesticides	Serum, brain (avian), ingesta
Oxalate	Plant, rumen contents
Salt	Cerebrospinal fluid, serum, ocular fluid, feed
Selenium	Liver, kidney, hoof, hair, plant, serum
Urea	Feed, water (ammonia concentrations in frozen plasma, rumen fluid or ocular fluid or acidified rumen fluid provide a more reliable indicator)

Testing for other toxic agents is available by request at the Natural Toxins Laboratory and the Chemical Residue Laboratory (both located at the Health and Food Sciences Precinct, Coopers Plains). The Chemical Residue Laboratory accepts samples when no private laboratory is able to do the testing or if there is a public benefit involved. Charges are determined by the nature of the work required and prices are provided based on specific requests. Assays are available for anticoagulant rodenticides, carbamate pesticides, metaldehyde, mycotoxins, organochlorine pesticides, organophosphate pesticides, paraquat, phenolics, phosphine, pyrethrins, strychnine and 1080. Cyanobacterial toxin assay is not available at this laboratory. If potentially toxic cyanobacteria are identified, the samples can be forwarded to an external laboratory for toxin analysis.

10.3 Interpreting results

Acetylcholinesterase (organophosphate pesticides)

Organophosphates inhibit the enzyme acetylcholinesterase. The levels of acetylcholinesterase can be measured in serum or plasma and also in avian brain. Low levels (<25% of normal activity) are consistent with organophosphate poisoning. However, toxic effects are usually not observed until a level of cholinesterase inhibition of >50% is reached.

Normal serum levels in cattle are 150–400 IU/L. Levels <150 IU/L may indicate organophosphate poisoning.

Brain cholinesterase values in birds are difficult to interpret in the absence of species-specific reference ranges. Brain cholinesterase activity for 48 species of clinically normal wild birds from North America fell within the range of 7–38 $\mu\text{mol}/\text{min}/\text{g}$, but there was substantial variation within bird families and genera [Hill, EF 1988, *Journal of Wildlife Diseases*, vol. 24(1), pp. 51–61]. As there is so much interspecies variation, results that fall within this range do not exclude pesticide poisoning as a diagnosis.

Testing of ingesta and tissues for the presence of organophosphate pesticides is available at the Chemical Residue Laboratory.

Arsenic

Normal levels in bovine liver and kidney are <0.4 mg/kg wet weight (WW). Concentrations in bovine liver >10 mg/kg WW are considered diagnostic of acute poisoning. Concentrations <7.5 mg/kg WW are not considered diagnostic, but even low concentrations are indicative of exposure. All species are susceptible, but poultry are the most tolerant of commercial livestock. Urine and hair are the most useful specimens to assay if attempting to confirm low-level chronic inorganic arsenic exposure.

Copper

Large concentrations of copper can be stored in the liver and analysis supports a diagnosis of intoxication only when clinical signs and lesions are consistent. In chronic copper poisoning in sheep, liver levels >150 mg/kg WW (normal 25–100 mg/kg WW) and kidney levels >15 mg/kg WW (normal 4–6 mg/kg WW) are significant. Normal copper levels in blood are <16 $\mu\text{mol}/\text{L}$ in sheep. In acute copper toxicity, liver levels of 50–170 mg/kg WW and kidney levels of 6–12 mg/kg WW are diagnostic.

Cyanide of plant origin

Toxicity varies with plant type, rate of intake, animal species and physiological state of the plant. The lowest concentration of hydrogen cyanide in plant material suggestive of toxicity is 600 mg/100 g dry weight. Serum levels of cyanide $>3.0 \mu\text{g/mL}$ are potentially toxic (normal in all species $<0.5 \mu\text{g/mL}$).

Fluoride

Chronic fluorosis is the most common form of intoxication. Concentrations of fluorine of $<1500 \text{ mg/kg}$ in bones usually cause no adverse effects. Concentrations of 2000–13 000 mg/kg in cattle and 2000–8000 mg/kg in sheep have been reported in cases of chronic fluorosis. A urine concentration of 15–20 mg/L is considered to be significant (normal 1–5 mg/L).

Lead

Cattle are the species most susceptible to lead poisoning. Blood lead concentrations in cattle $\geq 1.7 \mu\text{mol/L}$ are indicative of clinical lead poisoning. However, diagnosis of lead poisoning should not be based on blood analysis alone. Kidney or liver lead levels $>5 \text{ mg/kg WW}$ are generally associated with clinical lead poisoning in ruminants. Concentrations $>10 \text{ mg/kg WW}$ are considered to be diagnostic, although fatal poisoning can occur with lower tissue levels. For calves, consider field evidence, clinical signs and the concentration of lead in the liver when interpreting concentrations in the kidney cortex $<20 \text{ mg/kg WW}$.

To assist in determining if lead residue levels could pose a food safety risk, the Australia New Zealand Food Standards Code sets maximum levels for lead (see Table 10.4).

Table 10.4 Maximum lead levels in animal tissues

Tissue	Maximum lead levels
Edible offal of cattle, sheep, pig and poultry	0.5 mg/kg
Meat of cattle, sheep, pig and poultry	0.1 mg/kg
Blood	$<0.24 \mu\text{mol/L}$

Cattle with elevated blood levels of lead and untested animals in exposed herds or mobs must be excluded from slaughter to ensure that there is no food safety or trade risk from food products derived from exposed animals.

Lead contamination is a reportable incident under Queensland legislation. Report any suspected exposure of food-producing animals to lead to Biosecurity Queensland on 13 25 23.

Nitrate of plant origin

Ingestion of plants containing high levels of nitrate only poses a problem to ruminant animals because the rumen converts nitrate to the more toxic nitrite. In hay, >1.5% potassium nitrate (KNO_3) in dry matter is considered dangerous. Higher concentrations are tolerated in green feeds. Cattle are more susceptible than sheep. Ocular fluid nitrate concentrations in poisoned cattle can be as high as 100–250 mg/L; however, 20 mg/L in ocular fluid (normal is 5 mg/L) and 25 mg/L in serum may be considered diagnostic of intoxication.

Oxalate of plant origin

In ruminants, the rumen flora can detoxify oxalates efficiently, but fatal poisoning can occur in animals when rumen flora is not adapted to oxalates in the diet. Concentrations of oxalic acid in plants less than 2% in dry matter are usually safe. More than 6% oxalic acid is considered dangerous. Intermediate concentrations must be judged after considering the field history. Supportive evidence of acute oxalate poisoning includes hypocalcaemia, increased concentrations of serum/plasma creatinine and urea and histological evidence of nephrosis with numerous oxalate crystals in renal tubules.

Salt

Chemical diagnosis of salt poisoning depends on detection of elevated concentrations of both sodium and chloride in cerebrospinal fluid, serum or ocular fluid. Water deprivation is necessary to induce acute salt poisoning. Supportive evidence for diagnosis includes histological evidence of cerebral oedema and tissue eosinophilia.

Urea

Ammonia concentrations in frozen plasma, rumen fluid or ocular fluid or in acidified rumen fluid provide a reliable indicator of urea poisoning. If these specimens are not available, the best course is to demonstrate excessive intake or incorrect concentration in feed by urea analysis of feed or supplement.

10.4 Botanical identification of plants, fungi and cyanobacteria

Poisonous plants, fungi and cyanobacteria are an important cause of disease and death in livestock. Although there may be extensive knowledge of poisoning syndromes with particular geographical or seasonal occurrences, laboratory evidence is often required to make a diagnosis. The mere presence of a known toxic species of plant, fungi or cyanobacteria does not necessarily mean that it is the cause of disease.

To help with diagnosis, provide a comprehensive range of specimens and a full history. Carefully inspect the area to tentatively identify the species present and to determine if these have been eaten in sufficient quantity to cause poisoning.

Plant poisoning incidents are influenced by:

- plant factors
 - palatability
 - stage of growth
 - physical condition and presence of disease
- animal factors
 - species, age and dominance
 - state of nutrition and degree of hunger
 - familiarity with surroundings
 - length and intensity of exposure
- environmental factors
 - availability of and access to water
 - season
 - rainfall history
 - mineral and nutrient content of the soil.

If plant poisoning is suspected, collect and submit the plant, fungi or cyanobacteria specimens and the relevant animal specimens. The laboratory may forward these for confirmation of identification, as this information can be critical to the diagnosis.

Collecting samples

Plants for botanical identification

- Select vigorous, typical plant specimens that include stems, leaves and fertile parts (flowers and fruits). Basal parts of grasses, sedges, ferns and bulbous plants are essential for identification.
- Ideally choose specimens 25–40 cm long and up to 26 cm wide (the approximate size of tabloid newspapers). Long and narrow specimens such as grasses can be folded one or more times.
- Label each specimen with a unique sequential number and press the specimens separately between sheets of absorbent paper and cardboard.
- For best results, press the specimens as soon as possible after being removed from the plant. Avoid using sealed plastic bags for transport, as the plant rapidly 'sweats', becomes mouldy and decomposes.
- Change the paper and cardboard regularly to ensure the plants dry quickly, preventing fungal attack.
- Place large, robust plant parts such as seeds or dried fruits into paper bags.
- Collect specimens in duplicate or take a photograph of each specimen before sending so that you match the identification provided by the herbarium to the plant.

- Record the following specimen details:
 - location of collection, preferably including GPS coordinates
 - habitat (site) information (e.g. open forest) and soil type
 - plant features including form, height, flower colour, bark type and leaf texture.

See Queensland Herbarium's *Collecting and preserving plants specimens, a manual* (second edition) for more detailed information.

Rapid technique for plant identification

When a rapid identification is required, email an image of the suspected poisonous plant for a tentative identification. This is not a substitute for a definitive identification, so still submit pressed specimens for confirmation.

Ruminal contents

Collect a representative sample into a 500 mL screw-top container. Also look for large, readily recognisable plant parts (e.g. intact leaves, fruits, seeds or flowers) and include these in a separate container.

Note: The examination of rumen contents is not a substitute for collection of suspect plants and needs to be done in conjunction with a field search for poisonous plants. Comparison of the rumen sample with known plant samples is much easier than identifying the plant fragments alone.

Fungi

For details on collection of fungi (e.g. mushrooms and toadstools) for herbarium identification, see Queensland Herbarium's *Collecting and preserving fungi specimens, a manual* (second edition).

Cyanobacteria (blue-green algae)

Collect 1 L of bloom material for possible toxin assay. Subsample 100 mL into a separate container and add 1 mL formalin (**not** alcohol) per 20 mL of subsample to prevent cell rupture and preserve the organism's structure for identification. Submit both samples chilled as soon as possible.

Note: Always wear protective clothing (including rubber gloves) and avoid contact between bloom material and skin or eyes. If contact occurs, wash the affected areas immediately with clean water.

See also 'Cyanobacterial poisoning' in **Section 12.3**.

11 Virology

Diagnosis of viral disease is based on a combination of tests including histopathology, transmission electron microscopy (TEM), serology, detection of virions by virus isolation, detection of viral DNA/RNA by PCR or detection of virus antigen by antigen-capture ELISA.

11.1 General virus isolation

Virus isolation is the cultivation and identification of virus following inoculation of a specimen into embryonated eggs or cell cultures susceptible to the relevant virus.

Note: It is not possible to screen for a wide range of viruses, so only submit specimens to be tested for specific viruses.

Failure to cultivate the virus does not rule out a viral aetiology and cultivation of a virus does not necessarily mean it caused the disease process. When interpreting the results, consider the history, clinical findings and other laboratory results.

11.2 Collecting samples

Collect samples for virus isolation aseptically using sterile instruments and sterile containers. Collect fresh tissues or swabs into virus transport media (VTM). If VTM is not available, chill the specimens and deliver them promptly to the laboratory, or freeze them at -80°C . Semen can be used for virus isolation of BVDV and bovine herpesvirus 1 (BHV1) and blood can be used for arboviruses.

Tissues and organs

Collect tissues and organs aseptically, using separate sterile instruments and containers for each tissue or organ. Avoid contamination between tissues. Take swabs from excretions, exudates, mucosal surfaces and orifices carefully, avoiding contamination from other sites.

Semen

Submit raw semen or extended semen. If submitting extended semen for exclusion of BHV1 as a requirement for international trade, send at least 2 straws for 1 cell type and 3 straws for 2 cell types.

Arbovirus isolation

Blood (EDTA or lithium heparin) and tissue samples are suitable specimens. EDTA blood is preferred for bovine ephemeral fever and lithium heparin for bluetongue virus and epizootic haemorrhagic disease virus. Chill but do not freeze blood samples.

Avian virus isolation

Provide fresh tissues and swabs in VTM or chilled. Alternatively, freeze the samples at -80°C .

Finfish virus isolation

The specific tissue depends on the disease to be diagnosed or the virus to be isolated. Unfixed spleen or kidney tissue from diseased fish is usually required for general virus isolation. Where fry are involved in a disease outbreak, whole fry submitted in VTM may be useful. Contact the aquatic pathologist at the laboratory for specific information.

Collect tissue samples from freshly euthanased, diseased fish. Place the samples in VTM and keep them refrigerated at 4 °C.

Provide information about the fish type as well as incubation conditions and outbreak details including the rate of mortalities so the laboratory can choose the specific cell line to target the virus involved in the outbreak. BSL maintains a range of fish cell lines that grow at lower (15–20 °C) or higher (25–28 °C) temperature ranges to target either subtropical or tropical fish viral pathogens.

11.3 Crustacean and mollusc virology

Specific known pathogens may be detected by PCR. However, for new or emerging viruses, histopathology and TEM are needed to detect and allow subsequent characterisation of the pathogens. Currently, no invertebrate cell line is available at BSL for virus isolation in crustaceans and molluscs.

Crustaceans

Submit gills, pleopods and/or hepatopancreas:

- unfixed
- fixed in 3% glutaraldehyde for TEM
- fixed in 10% Davidson's fixative or formalin for histopathology
- fixed in ≥70% ethanol for PCR.

Molluscs

Submit gills and visceral tissue containing mantle and hepatopancreas:

- unfixed
- fixed in 3% glutaraldehyde for TEM
- fixed in 10% Davidson's fixative or formalin for histopathology
- fixed in ≥70% ethanol for PCR.

11.4 Submitting samples

Chill all virological specimens before and during transport. If there will be more than 48 hours between collection and receipt at the laboratory, freeze all specimens except blood samples at $-80\text{ }^{\circ}\text{C}$. Freezing at $-20\text{ }^{\circ}\text{C}$ is not suitable, particularly for isolation of arboviruses (e.g. bluetongue virus) and herpesviruses (e.g. BHV1). These viruses have extremely poor survival at $-20\text{ }^{\circ}\text{C}$ with just a single freeze, and tissues for isolation must be kept chilled.

Clearly label all specimens. Pack chilled specimens in an insulated leak-proof container with sufficient cooler bricks to ensure that they are still cold when received at the laboratory. Check that screw caps are tight, especially after freezing. Prevent direct contact between cooler bricks and chilled specimens, so they do not freeze.

Part C

Samples by disease or syndrome



12 Investigating disease in cattle, sheep, goats, pigs, camelids, deer and horses

12.1 General information

- Gather and record history and other relevant information about the property and the environment before examining animals and taking samples.
- Examine and sample live clinically affected animals. Sampling of some unaffected herd mates may also be useful.
- Wherever possible, take ante-mortem **and** post-mortem specimens.
- Select specimens to cover the range of possible diseases associated with the syndrome seen. Refer to **Table 12.1** or contact the laboratory and ask to speak to the duty pathologist.
- When exotic disease or a particular disease is suspected, refer to **Section 12.3** for more detail about the samples needed for exclusion of that disease. The list of diseases is not exhaustive, so if more information is needed on sampling, contact the laboratory for advice.
- Fully complete the specimen advice sheet (Form A) and the necropsy findings form (Form B). Include as much information as possible to deliver an accurate picture of the disease to the pathologist. Also include detailed information about the property and the environment, particularly any changes that have occurred.
- Always quote job numbers of any previous related laboratory results.

12.2 Samples by syndrome

Table 12.1 Samples by syndrome—cattle, sheep, goats, pigs, camelids, deer and horses

Syndrome	Blood	Faeces	Urine	Ocular fluid*	Fresh tissues
Abortion/stillbirth Whole foetus and placenta preferred	Clotted, from dam Submit paired serum sample 3–4 weeks later				Full range of organst Include heart, lung, liver, spleen, kidney, placenta (including cotyledons in ruminants)
Acute febrile disease	Clotted, lithium heparin, EDTA, smear Submit paired serum sample 3–4 weeks later (2 weeks for bovine ephemeral fever)	Yes	Yes		Any lesion and full range of organst Include lymph nodes
Anaemia/haemorrhage	Clotted, lithium heparin, EDTA, smear	Yes	Yes		Full range of organst
Ataxia	Clotted, lithium heparin, EDTA, smear	Yes	Yes	Yes	Any lesion and full range of organst Include skeletal muscle, small portion of brain and spinal cord, liver, kidney
Congenital abnormalities Whole affected animal preferred	Clotted (dam)				As for abortion/stillbirth
Diarrhoea/gastrointestinal signs	Clotted, lithium heparin, EDTA, smear	Yes		Yes	Any lesion and full range of organst
Genital lesions/vaginal or preputial discharge	Clotted, lithium heparin, EDTA, smear Submit paired serum sample 3–4 weeks later				

* Ocular fluid includes either aqueous or vitreous humour, but aqueous is preferred. See 'Ocular fluids' in **Section 2.1**.

† Full range of organs includes heart, lung, liver, spleen and kidney.

Fixed tissues	Gastrointestinal tract	Other	Species/disease specific
Full range of organst Include heart, lung, liver, spleen, thymus, kidney, whole brain, skeletal muscle, placenta (including cotyledons in ruminants)	Foetal stomach contents, collected using aseptic technique	Foetal fluids or serum Vaginal swabs (guarded swab of uterine discharge preferred)	<i>Cattle</i> : vaginal mucus samples (swabs into PBST for <i>Campylobacter</i> ELISA; samples taken using infertility testing kit for <i>Tritrichomonas</i> and <i>Campylobacter</i> culture) <i>Sheep</i> : smears of placenta or vaginal discharge <i>Brucellosis</i> : fresh and fixed reproductive organs, milk
Any lesion and full range of organst	Small intestinal contents	Swab of lesion Bone marrow (collected aseptically)	<i>Cattle</i> : peripheral smears and/or organ smears for tick fever exclusion <i>Horses</i> : oral, nasal and rectal swabs for Hendra virus exclusion <i>Mastitis</i> : milk
Full range of organst Include bone marrow	Rumen contents	Bone marrow smears	<i>Cattle</i> : peripheral smears and/or organ smears for tick fever exclusion <i>Sheep</i> : peripheral smears for eperythrozoonosis
Full range of organst Include brain, spinal cord, peripheral nerve, skeletal muscle	Rumen and small intestinal contents	Cerebrospinal fluid Suspect plants for identification	<i>Cattle</i> : peripheral smears and/or organ smears for tick fever exclusion <i>Horses</i> : oral, nasal and rectal swabs for Hendra virus exclusion
As for abortion/stillbirth		Foetal fluids or pre-suckling serum	
Any lesion and full range of organst and gastrointestinal tract	Rumen, small and large intestinal contents		<i>Pigs</i> : scrapings of the colonic mucosa or tied-off sections of colon for <i>Brachyspira</i> culture <i>Coccidiosis</i> : impression smears or scrapings of intestinal mucosa
		Vaginal or preputial swabs in VTM Swabs of discharge (Amies)	<i>Cattle</i> : vaginal mucus samples (swabs in PBST for <i>Campylobacter</i> ELISA); vaginal/preputial samples taken using infertility testing kit for <i>Tritrichomonas</i> and <i>Campylobacter</i> culture

(continued)

Table 12.1 continued

Syndrome	Blood	Faeces	Urine	Ocular fluid*	Fresh tissues
Ill thrift/weight loss	Clotted, lithium heparin, EDTA, smear	Yes	Yes		Any lesion and full range of organs† Include liver, kidney, heart, lung
Infertility Sample up to 20 animals	Clotted Submit paired serum sample 3–4 weeks later				
Jaundice	Clotted, lithium heparin, EDTA, smear		Yes		Any lesion and full range of organs† Include liver, kidney
Lameness/ musculoskeletal signs	Clotted, lithium heparin, EDTA, smear Submit paired serum sample 3–4 weeks later (2 weeks for bovine ephemeral fever)				<i>Muscle lesions:</i> sample lesion and make impression smears <i>Foot lesions:</i> sample lesion and swab exudate
Lymphadenopathy	Clotted, lithium heparin, EDTA, smear				Any lesion and full range of organs† Include affected lymph node/s
Nervous signs	Clotted, lithium heparin, EDTA, smear	Yes	Yes	Yes	Any lesion and full range of organs† Include liver, kidney, heart, small portion of brain, spinal cord
Oedema	Clotted, lithium heparin, EDTA, smear	Yes	Yes		Any lesion and full range of organs† Include lymph nodes
Oral lesions/ salivation/nasal discharge	Clotted, EDTA, smear Submit paired serum sample 3–4 weeks later				Any lesion and full range of organs† Include spleen, liver, lung
Perinatal mortality Whole carcass may be submitted for necropsy	Clotted, EDTA, smear (if freshly dead)	Yes	Yes		Any lesion and full range of organs†

* Ocular fluid includes either aqueous or vitreous humour, but aqueous is preferred. See 'Ocular fluids' in **Section 2.1**.

† Full range of organs includes heart, lung, liver, spleen and kidney.

Fixed tissues	Gastrointestinal tract	Other	Species/disease specific
Any lesion and full range of organst			<i>Cattle</i> : vaginal swabs in PBST for <i>Campylobacter</i> ELISA
Any lesion and full range of organst Include liver, kidney	Rumen contents	Suspect plants for identification Water sample for cyanobacteria detection	<i>Cattle</i> : peripheral smears and/or organ smears for tick fever exclusion <i>Sheep</i> : peripheral smears for eperythrozoonosis
Any lesion and full range of organst Include brain, spinal cord, peripheral nerve, skeletal muscle	Rumen contents	Joint fluid (or swabs) from affected joints, fresh and into EDTA Suspect plants for identification	<i>FMD (ruminants and pigs)</i> : vesicular fluid, epithelium from vesicles, oral, nasal and tonsillar swabs in VTM or saline
Any lesion and full range of organst Include affected lymph node/s		Smears and swabs of lesions/affected lymph node/s	
Any lesion and full range of organst Include brain, spinal cord, skeletal muscle	Rumen and intestinal contents	Suspect plants or source of neurotoxin Cerebrospinal fluid	<i>Cattle</i> : peripheral smears and/or organ smears for tick fever exclusion <i>Horses</i> : oral, nasal and rectal swabs for Hendra virus exclusion <i>Chlamydiosis</i> : swabs or organ impression smears for <i>Chlamydia</i> FAT
Any lesion and full range of organst		Fluid from body cavities Samples and smears of oedema fluid Suspect plants for identification	<i>Pigs</i> : small intestinal contents; fixed brain, conjunctiva, stomach, spiral colon
Any lesion and full range of organst Include lymph nodes, thyroid, adrenal glands, brain		Nasal swabs Swabs of lesions	<i>FMD (ruminants and pigs)</i> : vesicular fluid, epithelium from vesicles, oral, nasal and tonsillar swabs in VTM or saline
Any lesion and full range of organst Include brain, skeletal muscle	Intestinal contents		<i>Cattle</i> : pre-suckling serum <i>Chlamydiosis</i> : swabs or organ impression smears for <i>Chlamydia</i> FAT

(continued)

Table 12.1 continued

Syndrome	Blood	Faeces	Urine	Ocular fluid*	Fresh tissues
Recumbency/ lingering death	Clotted, lithium heparin, EDTA, smear Submit paired serum sample 3–4 weeks later (2 weeks for bovine ephemeral fever)	Yes	Yes	Yes	Any lesion and full range of organs† Include liver, kidney
Respiratory signs	Clotted, EDTA, smear				Any lesion and full range of organs† Include lung, bronchial lymph nodes, pleural fluid
Skin lesions Provide detailed history and description of lesions	Clotted, EDTA, smear				Skin lesion (including multiple samples of crusts and skin scrapings) Liver, kidney
Sudden death/found dead	Clotted, EDTA, smear (if freshly dead)	Yes	Yes	Yes	Any lesion and full range of organs† Include liver, lung, kidney, brain

* Ocular fluid includes either aqueous or vitreous humour, but aqueous is preferred. See 'Ocular fluids' in **Section 2.1**.

† Full range of organs includes heart, lung, liver, spleen and kidney.

Fixed tissues	Gastrointestinal tract	Other	Species/disease specific
Any lesion and full range of organs† Include brain, spinal cord, skeletal muscle	Rumen and intestinal contents	Suspect plants/feed Water sample for cyanobacteria detection	
Any lesion and full range of organs† Include lung, bronchial lymph nodes		Swabs of lesions	<i>Cattle</i> : nasal swabs (in VTM or sterile saline); fresh and fixed trachea <i>Cattle and sheep</i> : faeces, if lungworm suspected <i>Horses</i> : oral, nasal and rectal swabs for Hendra virus exclusion
Skin lesion and full range of organs† Include some normal adjacent skin		Swabs of pustular lesions External parasites for identification (in 70% alcohol) Feed samples if deficiencies or toxicities are suspected	<i>FMD (ruminants and pigs)</i> : vesicular fluid, epithelium from vesicles, oral, nasal and tonsillar swabs in VTM or saline <i>LSD (cattle)</i> : skin lesion biopsy (fixed and fresh), scabs, vesicular fluid, skin scrapings
Any lesion and full range of organs† Include brain, skeletal muscle, lymph nodes	Rumen and intestinal contents	Swabs of lesions Fluid from body cavities Bone marrow (collected aseptically) Suspect plants/ feed Water sample for cyanobacteria detection	<i>Anthrax (do not open the carcass)</i> : thick air-dried blood smears from a peripheral blood vessel <i>Urea poisoning</i> : plasma, rumen contents, ocular fluid, frozen immediately and kept frozen in transit <i>Cyanide poisoning</i> : rumen contents, ocular fluid, liver, frozen immediately and kept frozen in transit <i>Cattle</i> : peripheral smears/ organ smears for tick fever exclusion

12.3 Samples by disease

Abortion in cattle

Infectious causes of abortion in cattle include many species of bacteria, viruses, protozoa and fungi. Diseases that cause a fever may also cause abortion. Non-infectious causes include chemical and plant poisonings, nutritional deficiencies, genetic factors and congenital abnormalities. The following is a general guide to the usual timing of abortions due to various causes:

- Early abortion (2–5 months)—trichomoniasis, bovine pestivirus infection
- Mid-term abortion (3–6 months)—neosporosis, bovine campylobacteriosis, bovine pestivirus infection, Akabane disease, Aino virus
- Late abortion (6–8 months)—leptospirosis, brucellosis, neosporosis, *Coxiella burnetii*, listeriosis
- Anytime abortion—mycoses, other bacteria (including *Salmonella*, *Yersinia*, *Trueperella*, *Pasteurella*, *Mycoplasma* spp., *Histophilus somni*), nitrate/nitrite poisoning

See individual disease entries in this section and ‘Abortion/stillbirth’ in **Table 12.1**.

Abortion in horses

Infectious agents responsible for equine abortion include *Streptococcus*, *Staphylococcus*, *Salmonella*, *Klebsiella* and *Leptospira* spp., *Escherichia coli*, *Chlamydia*, equine herpesvirus and fungi. Non-infectious causes include maternal pyrexia and malnutrition, cord compression abnormalities, equine amnionitis and foetal loss syndrome, endocrine dysfunction, endometrial incompetence and immunological factors.

For exclusion of equine herpesvirus and chlamydial abortion, perform a field necropsy and submit fixed and fresh foetal liver, lung and placenta to the laboratory for testing.

Equine herpesvirus associated with abortion or neurological signs is a reportable disease under Queensland legislation. Report all suspected and confirmed cases in horses to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Abortion in pigs

See ‘Reproductive failure in pigs’ in this section and ‘Abortion/stillbirth’ in **Table 12.1**.

Abortion in sheep and goats

Causes include brucellosis, campylobacteriosis, toxoplasmosis, listeriosis, *Coxiella burnetii*, pasteurellosis, chlamydiosis, melioidosis and conditions causing clinical illness in the ewe or doe, e.g. salmonellosis, hypocalcaemia, pregnancy toxæmia, septicaemia, enterotoxaemia in goats, toxic plants.

See individual disease entries in this section and ‘Abortion/stillbirth’ in **Table 12.1**.

Acetonaemia

See 'Ketosis' in this section.

Actinobacillosis and actinomycosis

Actinobacillus lignieresii causes granulomatous abscesses of the tongue, usually referred to as 'wooden tongue' in cattle. *Actinomyces bovis* causes granulomatous abscesses that most frequently involve the mandible, the maxillae or other bony tissues in the head and are known as 'lumpy jaw' in cattle. These organisms can also infect other sites and other species.

Samples required

- Smears of pus from deep parts of the lesion
- Unpreserved affected tissue and specimens of pus, chilled
- Formalin-fixed sections of recent lesions

Aflatoxicosis

Aflatoxins are produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus* on peanuts, soybeans, cottonseed, corn and other cereals either in the field or during storage when moisture content and temperatures are sufficiently high for mould growth. Aflatoxins can cause a range of clinical signs including nervous signs, diarrhoea, jaundice, haemorrhages and abortions.

Samples required

- Clotted blood
- Suspected foodstuff (100 g), urine, liver or milk for aflatoxin analysis
- Unpreserved and formalin-fixed tissues including liver and kidney

Note: As mycotoxin assays are difficult and expensive, they are only available when clinical and pathological evidence indicates that they are a likely cause of the disease problem.

Akabane disease

Infection of pregnant animals with Akabane virus may result in multiple congenital abnormalities including hydranencephaly and arthrogryposis. The distribution of Akabane virus varies within the limits of its presumed vector, *Culicoides brevitarsis*, but occurs endemically throughout much of Queensland. It only causes disease sporadically because most cows have been infected prior to their first pregnancy and are immune.

Samples required

- Foetal fluid (preferably pericardial or pleural) or pre-suckling serum from the newborn calf
- Serum from the dam
- Formalin-fixed tissues, full range (including brain, spinal cord and muscle)

Note: Specimens should also be submitted to exclude bovine pestivirus infection, which can also cause arthrogryposis and CNS lesions.

Anaplasmosis

Anaplasmosis is caused by *Anaplasma marginale*. Signs include rapid loss of condition, fever, marked anaemia and jaundice but no red urine. Because of the severe anaemia associated with *Anaplasma* infection, parasites may be difficult to detect in smears, so submit additional samples from herd cohorts.

See 'Tick fevers of cattle' in this section; also see **Section 9**.

Anthrax



Anthrax is a zoonotic disease causing serious illness and sometimes death in humans.

Animals are usually found dead. The carcass shows rapid putrefaction and there may be bloody discharges from the nose, mouth and anus. Live affected animals show weakness, staggering and rapid breathing. **Do not open the carcass.** If post-mortem examination of an animal with anthrax is made in error, the spleen will be swollen and soft and numerous haemorrhages will be present throughout the body.

In Australia, anthrax occurs mostly in parts of New South Wales and Victoria and has rarely been reported in Queensland. There was an isolated incident in Queensland on the Marlborough peninsula, north of Rockhampton, in 1993. In 2002 and 2017–2018, there were several confirmed cases of anthrax in south-western Queensland.

Samples required (in order of preference)

Cattle and sheep

- Thick air-dried blood smears from peripheral vessels (e.g. from the ear or tail)—make a small stab incision in the skin with a scalpel, collect the blood using a syringe, place a drop on a slide and air-dry; put any remaining blood into a plain blood tube
- Blood from a peripheral vein (e.g. jugular, brachial or mammary)—collect with a needle and syringe and transfer to an EDTA blood tube
- Blood that has pooled in the nasal cavity or other orifice (smears and swabs)
- Environmental samples (50 g soil) from the ground contaminated by nasal and anal discharges if none of the above are available

Other animals

- Smears from any affected organs (e.g. throat or lymph nodes in pigs)

Note: Send samples from more than one affected (or suspected) animal where possible. Treat any puncture site with a suitable disinfectant, e.g. a cottonwool swab soaked in formalin.

If the carcass is opened inadvertently, submit tissue smears and a range of fresh and fixed tissues to establish an alternative diagnosis after anthrax has been excluded.

Place all samples in sealed containers, identified, double-bagged and disinfected. **Do not**, under any circumstances, forward sharps (needles or blades) with the samples; dispose of them appropriately.

Contact the laboratory to warn them that you are submitting suspected anthrax specimens. Clearly label the samples with 'suspected anthrax' and attach the specimen advice sheet to the outside of the packaging.

Anthrax is a reportable disease under Queensland legislation. Report all suspected cases of anthrax in any species of animal to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

For more information on anthrax, visit business.qld.gov.au.

Arsenic poisoning

The most significant sources of arsenic are old arsenical dip solutions used in the past for control of cattle tick and sheep lice. Poisoning occurs when the animals ingest the chemical or absorb it through the skin. Severe gastroenteritis with haemorrhage, convulsions and death is seen in acute poisoning. Chronic poisoning may lead to ill thrift.

Samples required

- Faeces, urine (20 mL)
- Unpreserved liver and kidney, chilled
- Ingesta, suspected source of toxin
- Formalin-fixed liver, kidney and gastrointestinal tract

Arthritis/polyarthritis (joint ill)

Causes of arthritis include trauma, infection, nutritional imbalance and genetic factors.

Samples required

- Clotted blood, EDTA blood, smear
- Paired sera 3–4 weeks apart (2 weeks for bovine ephemeral fever)
- Joint fluid or swabs from affected joints, collected aseptically, chilled
- Joint fluid collected into EDTA for analysis
- Smears of joint fluid, for cytology

Note: Make smears for cytology immediately.

See also 'Bovine ephemeral fever', 'Caprine arthritis encephalitis', 'Glässer's disease' and 'Erysipelas' in this section.

Australian bat lyssavirus



Australian bat lyssavirus is a serious zoonotic disease. Only veterinarians and staff with current rabies vaccination, appropriate personal protective equipment and bat-handling experience should handle live bats.

Australian bat lyssavirus is a virus endemic in Australian bats that causes invariably fatal encephalitis in bats, humans and other animals. It is closely related to rabies virus and has been found in four common species of flying foxes as well as one species of microbat. Euthanase any bats that have a history of neurological signs or clinical illness, or have been in contact with a domestic animal. Carefully package the bats and submit them to the laboratory.

Samples required

- Whole carcass of the bat, including the head, chilled

If a necropsy has been performed, submit half of the brain fresh and the remainder fixed in formalin.

If you suspect that an animal is clinically ill with Australian bat lyssavirus infection, or if an animal has been bitten or scratched by a bat or other animal known to be infected with Australian bat lyssavirus, report this to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Babesiosis

Babesiosis is caused by *Babesia bovis* (most commonly) and *B. bigemina*. Signs include high fever, anaemia, jaundice, loss of condition and red urine. *B. bovis* infections often cause nervous signs, followed by coma and death.

See 'Tick fevers of cattle' in this section; also see **Section 9**.

Big knee in goats

See 'Caprine arthritis encephalitis' in this section.

Black disease (infectious necrotic hepatitis, *Clostridium novyi* type B)

Black disease is an acute toxæmia that usually occurs in cattle or sheep infected with liver fluke. Clinical signs are associated with severe liver disease, which is highly fatal in 1–2 days. Collect samples from freshly dead animals.

Samples required

- Large sample (5 cm block) of liver for anaerobic culture, chilled
- Impression smears from the cut surface of a lesion
- Formalin-fixed sample of liver containing lesions

Blackleg (*Clostridium chauvoei*)

Blackleg is a rapidly fatal, febrile disease of young, fast-growing cattle. At the site of infection, the affected muscles are dark red to black, dry and spongy, with a sweetish odour, and are infiltrated with gas bubbles. In some cases, the lesions are restricted to the diaphragm and heart. Collect samples from freshly dead animals.

Samples required

- Air-dried impression smears of the muscle lesion
- Swabs in transport medium of fluid exudate, muscle lesions and heart blood or liver
- Large sample (5 cm block) of the lesion for anaerobic culture, chilled
- Formalin-fixed sample of the muscle lesion

Bloat

Bloat is diagnosed on history and gross pathology, though it may be difficult to distinguish ante-mortem from post-mortem bloat. Collect samples to eliminate other causes of sudden death.

See 'Sudden death' in **Table 12.1**.

Blue-green algae poisoning

See 'Cyanobacterial poisoning' in this section.

Botulism (*Clostridium botulinum*)

Botulism is a rapidly fatal motor paralysis caused by ingestion of the toxin produced by *Clostridium botulinum*. It is commonly associated with phosphorus deficiency and bone-chewing in cattle but consumption of feedstuffs contaminated with toxin-containing carcasses may also lead to development of the disease.

Note: Diagnosis of botulism is usually made after elimination of other causes of flaccid paralysis as attempts at detecting the toxin are rarely successful and there are no characteristic gross or histologic lesions.

Collect samples from the first animals to show characteristic clinical signs, as these will have consumed the most toxin. Samples can be tested for the presence of toxin types C and D only.

Samples required

- Samples of suspected sources of toxin, chilled or frozen (e.g. feed, visibly contaminated water, animal and bird carcasses)
- Maggots from carcasses (may contain toxin at very high levels)

Cattle

- Small intestinal contents (50 mL), chilled
- Rumen contents (50 mL), chilled
- Unpreserved liver and spleen, chilled
- Faeces
- Clotted blood

Birds

- Alimentary tract contents (crop, gizzard, intestine), chilled
- Unpreserved liver and spleen, chilled
- Clotted blood

Note: Consider the possibility of post-mortem toxin formation in the carcass if samples are taken more than 24 hours after death.

Bovine campylobacteriosis (vibriosis)

Bovine campylobacteriosis is a venereally transmitted disease caused by *Campylobacter fetus* subsp. *venerealis* and is characterised by infertility and early embryonic death, a protracted calving season and occasionally mid-term to late-term abortion. Infected bulls show no clinical signs, but many become carriers and subsequently infect females at service. In a herd situation, demonstration of antibody in vaginal mucus is the preferred method of diagnosis, as it can be difficult to recover the organism from the prepuce. *C. fetus* subsp. *fetus* also causes abortion in cattle, but is not usually associated with infertility. *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* can also be isolated from aborted fetuses and placentas.

Individual bulls vary in their susceptibility to infection; some become permanent carriers, while others appear to be resistant to infection. In young bulls <3–4 years old, infection tends to be transient, but reinfection can readily occur. In bulls >3–4 years old, chronic infections are more likely to establish.

Samples required

Cows

- Vaginal mucus samples, for demonstration of *C. fetus* subsp. *venerealis* immunoglobulin A by ELISA, collected into PBST

Campylobacter ELISA kits containing PBST diluent and instructions for collection of vaginal mucus samples are available from BSL.

For abortion investigation, sample animals that have aborted 1 week to 3 months after the abortion.

For infertility investigation, to gain a representative sampling of the herd, collect at least 10 vaginal mucus samples from infertile heifers or cows. If possible, do this at the time of pregnancy testing when infertility is detected.

Bulls

- Preputial scrapings submitted in *Campylobacter* transport enrichment medium for bacteriology

Bull infertility testing kits (for both *Campylobacter* and *Tritrichomonas foetus*) are available from BSL.

Aborted fetuses

- Specimens as for diagnosis of abortion

Bovine enzootic haematuria

See 'Bracken fern poisoning' in this section.

Bovine ephemeral fever (three-day sickness)

Bovine ephemeral fever affects cattle and occasionally buffalo and is marked by fever, excessive salivation, shivering, shifting lameness and muscular stiffness. There is usually high morbidity and low mortality in susceptible populations. Bovine ephemeral fever is an arthropod-borne virus that is widespread in Queensland. The disease may cause serious production losses through deaths, decline in condition, decreased milk production, lowered fertility in bulls and occasional abortions.

Samples required

- Clotted blood, EDTA blood and smear
- Unpreserved spleen and lymph node, chilled
- Paired sera (acute and convalescing), 2 weeks apart

Bovine leucosis

See 'Enzootic bovine leucosis' in this section.

Bovine malignant catarrh

See 'Malignant catarrhal fever' in this section.

Bovine pestivirus infection (bovine virus diarrhoea, mucosal disease)

Bovine pestivirus infection in Australia is caused by BVDV type I. Infection with bovine pestivirus causes conception failure, embryonic mortality, abortion, congenital defects, stillbirths, perinatal mortality or the birth of persistently infected animals that are 'poor doers' and have a markedly reduced life expectancy. Persistently infected animals result from foetal infection between 30 and 90 days of gestation. These animals can present with ill thrift, scouring, lameness and a range of bacterial diseases that are secondary to the severe immunosuppression that the virus induces. Persistently infected animals provide a reservoir of the virus in the population. Bovine pestivirus infection is also implicated in bovine respiratory disease complex.

Samples required

Suspect persistently infected animals and aborted foetuses

- EDTA blood for PCR **or** clotted blood or lithium heparin blood for BVDV antigen-capture ELISA (ear notches also suitable)
- Unpreserved spleen or liver, chilled
- Serum from stillborn calves or pre-colostral serum from calves with congenital abnormalities
- Formalin-fixed tissues, full range, including gastrointestinal tract and any lesions

Note: The antigen-capture ELISA may produce false negative results in calves with maternal antibodies, so EDTA for PCR is preferred.

Herd serology

- Clotted blood from 20 cows for BVDV AGID

Bovine tuberculosis

Bovine tuberculosis is caused by the bacterium *Mycobacterium bovis*. It is a chronic disease with progressive emaciation, low-grade fluctuating fever, inappetence and weakness culminating in acute respiratory distress and enlarged lymph nodes. Australia was officially declared free of bovine tuberculosis in 1997.

Samples required

- Unpreserved sample of lesions, chilled
- Formalin-fixed portions of lesions
- Samples to exclude other possible differential diagnoses

If transport to the laboratory will be delayed for more than 24 hours, freeze the chilled samples and keep them frozen in transit.

Bovine tuberculosis is a significant zoonosis and is a reportable disease under Queensland legislation. Report suspected cases to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Bovine virus diarrhoea

See 'Bovine pestivirus infection' in this section.

Bracken fern poisoning and bovine enzootic haematuria

Bracken fern or mulga fern poisoning occurs mainly in cattle. Animals affected acutely may have fever and haemorrhagic disease due to bone marrow suppression caused by the toxin ptaquiloside. When there is prolonged intake of small amounts of the plant, cattle may develop urinary bladder neoplasia (bovine enzootic haematuria). Bracken fern poisoning is rare in horses, sheep and pigs and is associated with nervous signs due to thiamine deficiency induced by the thiaminase present in the plant.

Samples required

- Clotted blood, EDTA blood and smear
- Bone marrow smears
- Formalin-fixed tissues including liver, kidney, urinary bladder and bone marrow
- Urine
- Rumen contents, 200 g, chilled, for botanical identification

Brucellosis



Brucellosis is a serious zoonotic disease that causes illness in people. Exercise extreme care in handling infectious material. Pack the specimen advice sheet externally to the samples and clearly indicate on the sheet that brucellosis is suspected.

In general, the disease in animals causes reproductive failure, such as abortion or the birth of weak offspring in females and infection of the reproductive tract and sterility in males. There are several different species of *Brucella*, of which only two occur in Australia: *B. ovis* and *B. suis*. *B. abortus* no longer occurs in Australia as a result of a national eradication program between 1970 and 1989. *B. melitensis* and *B. canis* are exotic to Australia.

Brucellosis (with the exception of *B. ovis*) is a significant zoonosis and is a reportable disease under Queensland legislation. Report suspected cases to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Brucella suis

B. suis primarily affects domestic or feral pigs, causing infertility. It has not been reported in domestic pigs in Queensland since 1991, but is endemic in Queensland's feral pig population. Cattle and horses may be infected, especially if they share an environment with feral pigs. Infection has occurred in dogs that have had contact with feral pigs or have ingested feral pig meat. In pigs, the signs of infection with *B. suis* are variable, as the bacteria localises in other parts of the body as well as the reproductive organs. Sows show infertility, irregular oestrus and abortion. There can be heavy perinatal mortalities in piglets. In boars, orchitis of one or both testicles develops and lameness, incoordination and posterior paralysis may be seen. In dogs, clinical signs are similar to those for pigs, but subclinical infections also occur. Infected pigs and dogs are potentially a source of infection for humans. There are no specific clinical signs associated with *B. suis* infection in cattle and horses.

Samples required

- Clotted blood for serology (*B. suis* RBT and CFT)
- Sample or swab of lesion
- Unpreserved tissues, full range including reproductive organs, chilled
- Aborted foetus and foetal membranes
- Cervical swab (sow)

Brucella ovis

B. ovis infection causes epididymitis and infertility in rams, abortion in ewes and neonatal mortality in lambs.

Samples required

- Clotted blood for serology (*B. ovis* CFT; needed for diagnosis and accreditation)
- Semen
- Unpreserved and formalin-fixed epididymis, accessory glands including ampullae and seminal vesicles, and testis
- Aborted foetus and foetal membranes

Brucella abortus, *Brucella melitensis* and *Brucella canis* (exotic to Australia)

Samples required

- Clotted blood, EDTA blood
- Unpreserved and formalin-fixed lymph nodes (including mammary lymph nodes) and reproductive organs
- Aborted foetus and foetal membranes
- Milk
- Vaginal swabs

Caprine arthritis encephalitis (big knee)

Caprine arthritis encephalitis is a viral disease causing polyarthritis in adult goats and progressive paralysis in kids as well as pneumonia, mastitis and chronic wasting.

Samples required

- Clotted blood (needed for diagnosis and accreditation)
- Formalin-fixed tissues including brain, spinal cord, affected joints and lung

Caprine retrovirus infection

See 'Caprine arthritis encephalitis' in this section.

Caseous lymphadenitis (cheesy gland)



Caseous lymphadenitis is zoonotic. Take care when handling infected animals and samples.

Caseous lymphadenitis is a chronic, contagious disease caused by the bacterium *Corynebacterium pseudotuberculosis*. It is characterised by abscess formation in or near major peripheral lymph nodes or within internal organs and lymph nodes. Although caseous lymphadenitis is typically considered a disease of sheep and goats, it also occurs sporadically in other species.

Samples required

- Swabs or fresh tissue from active lesions, chilled

Cheesy gland

See 'Caseous lymphadenitis' in this section.

Chemical poisoning

See **Section 10**.

Chlamydial infections



Chlamydiosis is a zoonotic disease; it can cause serious illness and sometimes death in humans. Take care when handling infected animals and samples.

There are several species of *Chlamydia* that cause disease in a range of birds and domestic animals. Chlamydial infections affect multiple organs and can generate a variety of clinical manifestations, ranging from acute to chronic inflammation and from a severe to a mild or even subclinical course. Chlamydial abortion has been reported most commonly in sheep and goats but also in cattle, camelids and horses.

Samples required

- Smears of epithelial surfaces (nasal, ocular, rectal, vagina—use a dry swab, roll it onto microscope slides and air-dry)
- Tissue impression smears from liver, spleen and serosal surfaces
- Fresh tissues or swabs in saline

See also 'Sporadic bovine encephalomyelitis' in this section and 'Abortion/stillbirth' in **Table 12.1**.

Clostridial disease

Clostridial diseases are caused by anaerobic bacteria of the genus *Clostridium* that are widespread in the environment in soil and faeces. They form highly resistant spores that can survive in the environment for very long periods. They are also present in the gastrointestinal tract and as spores in tissues of healthy animals. Disease occurs when these bacteria enter the body (via cuts, abrasions or ingestion) and conditions in the body allow multiplication of the bacteria and/or toxin production.

Not all species of *Clostridium* cause disease, but those that do are usually fatal. They include:

- *C. tetani*—tetanus
- *C. septicum*—malignant oedema
- *C. chauvoei*—blackleg
- *C. perfringens* type D—enterotoxaemia (pulpy kidney)
- *C. novyi*—black disease
- *C. botulinum*—botulism.

Samples required

See the specific diseases in this section.

Clostridial organisms (e.g. *C. perfringens* types A–E and *C. difficile*) also produce enteritis and diarrhoea in many species of domestic animals and birds. See also ‘Diarrhoea/gastrointestinal signs’ in **Table 12.1** and ‘Diarrhoea/wet litter’ in **Table 13.1**.

Coccidiosis

Coccidiosis is usually an acute invasion of the intestinal mucosa by protozoa of the genera *Eimeria* or *Isospora*. It occurs mainly in young cattle, sheep, goats and pigs. Clinical signs include diarrhoea, fever, inappetence, weight loss, emaciation, nervous signs and in extreme cases, death. Subclinical infections occur frequently.

Samples required

- Faeces
- Impression smears or scrapings of intestinal mucosa from 5–10 different sites
- Formalin-fixed sections of intestine

Colibacillosis



Some strains of *Escherichia coli* (e.g. Shiga toxin-producing *E. coli* or STEC, verotoxigenic *E. coli* or VTEC) are zoonotic. Take care when handling infected animals and samples.

Diseases induced by *E. coli* are most common in young animals and may present as enteritis, enterotoxaemia or septicaemia.

Samples required

Live animals

- Faeces, chilled

Dead animals

- Unpreserved tissues or swabs (liver, kidney, small intestine, large intestine, mesenteric lymph node), chilled
- Small and large intestinal contents, chilled
- Formalin-fixed tissues, full range including brain

See also ‘Oedema disease’ in this section.

Contagious ecthyma

See ‘Scabby mouth’ in this section.

Contagious equine metritis

Contagious equine metritis is a highly contagious, venereally transmitted disease of horses caused by *Taylorella equigenitalis*. In infected mares it produces acute endometritis with a copious mucopurulent vulval discharge and early returns to service. In stallions there are no observable signs. The infection can be transferred at service or mechanically by veterinarians or handlers.

Samples required

Stallions

- Swabs from prepuce, urethral fossa and anterior urethra

Non-pregnant mares

- Swabs from cervix, clitoral sinus and clitoral fossa

Pregnant mares

- Swabs from clitoral sinus and clitoral fossa
- Vaginal swabs from mares that have vaginal discharge

Note: Use Amies charcoal swabs (CEM transport swabs, available from BSL). Do not use Stuart transport medium. Do not refrigerate the swabs, as *T. equigenitalis* survives longer at room temperature than at 4 °C. However, avoid exposure of swabs to high temperatures. Swabs should arrive at the laboratory within 48 hours of collection. Do not collect samples from horses that have been treated with antibiotics during the previous 7 days.

Contagious equine metritis was eradicated from Australia in 1985 and is a reportable disease in all states. Report any detection to Biosecurity Queensland on 13 25 23.

Contagious ophthalmia

See 'Infectious keratoconjunctivitis' in this section.

Contagious pustular dermatitis

See 'Scabby mouth' in this section.

Copper poisoning (toxaemic jaundice)

Chronic copper poisoning is the most common form, but acute and peracute copper toxicity may also occur. Sheep are the most susceptible, but toxicity is also seen in cattle and pigs. In primary chronic copper poisoning, accumulation of copper in the liver occurs due to excess copper ingestion usually in conjunction with low molybdenum and sulphur in the diet. Liver damage (e.g. pyrrolizidine alkaloid toxicity) may lead to further hepatic copper accumulation. A stressful event may trigger massive release of copper into the bloodstream, resulting in haemolytic anaemia, haemoglobinuria and jaundice.

Samples required

- Clotted blood, EDTA blood and smear
- Unpreserved liver and kidney (minimum 30 g)
- Formalin-fixed tissues including liver and kidney

Cryptosporidiosis



Cryptosporidiosis is a zoonosis. Take care when handling infected animals and samples.

Cryptosporidium spp. are protozoan parasites that are not host specific and cause diarrhoea primarily in neonatal calves but also in lambs, kids, foals and piglets usually between 1 and 4 weeks of age. Signs most commonly seen are mild to severe diarrhoea, anorexia and depression. The parasites commonly act in concert with other enteric pathogens. Asymptomatic infections are common.

Samples required

- Faeces (50 mL)
- Formalin-fixed abomasum/stomach and small intestine

Cyanide poisoning (prussic acid poisoning)

The most frequent cause of acute and chronic cyanide poisoning in livestock is ingestion of plants that either contain cyanogenic glycosides or are induced to produce cyanogenic glycosides by various environmental stressors. The cyanide risk is highest in young plants and regrowth, with stressed regrowth considered the most dangerous. Sorghum grasses are well known for their cyanide-producing potential. In the acute form of poisoning, there is rapid onset of excitement, dyspnoea, salivation and lacrimation, muscle fasciculations and spasms, convulsions, coma and death. Mucous membranes are initially bright red and then become cyanotic.

Chronic cyanide poisoning occurs in horses, cattle, sheep and goats as a toxic neuropathy syndrome and is typically associated with posterior ataxia or incoordination that may progress to irreversible flaccid paralysis and urinary incontinence. Foetal abnormalities and goitre have also been reported.

Samples required

Acute poisoning

- Fresh suspect plant material (fresh leaves or whole plant), wrapped in paper, chilled and delivered to the laboratory as soon as possible
- Rumen contents, frozen immediately and kept frozen in transit
- Fresh liver, frozen
- Ocular fluid (whole eyeball chilled or ocular fluid collected and put into a small sealed container and frozen)
- Clotted blood, EDTA blood and smears
- Full range of fresh and fixed tissues for alternative diagnoses

Chronic poisoning

- Formalin-fixed brain and spinal cord

Cyanobacterial poisoning (blue-green algae poisoning)



Cyanobacteria are toxic. Always wear protective clothing (including rubber gloves) and avoid contact between bloom material and skin or eyes. If contact occurs, wash the affected areas *immediately* with clean water.

Not every cyanobacterial bloom is poisonous, but each bloom should be considered potentially poisonous until the algae have been identified and toxin assays completed. There are three known groups of cyanobacterial toxins, each produced by a number of different cyanobacterial species: those that produce acute liver damage (common), those that cause damage to the nervous system (rare) and those that cause more chronic effects.

Samples required

- Clotted blood, EDTA blood and smears
- Formalin-fixed tissues, full range including brain, kidney, liver and lung
- Unpreserved liver and kidney, chilled
- Unpreserved stomach/rumen contents, chilled

For identification of cyanobacteria

- 100 mL of the algal bloom, chilled and delivered to the laboratory in 24–48 hours or preserved with 1 mL 10% formalin to 20 mL of sample

For toxin detection (preserved samples are unsuitable)

- 1 L (minimum of 200 mL) of the most concentrated part of the algal bloom, chilled
- Rumen/stomach contents

Note: Toxin detection is not available at BSL, but samples can be forwarded to Queensland Health (Forensic and Scientific Services) for testing. The cost of this testing is the responsibility of the submitter.

Dermatophilosis (rain scald, lumpy wool)

Infection with *Dermatophilus congolensis* is characterised by exudative dermatitis with scab and crust formation. Cattle, sheep, goats and horses are affected most frequently. Predisposing factors include prolonged wetting by rain, high humidity, high temperature, and infection with ectoparasites. Most lesions associated with prolonged wetting of the skin are distributed over the head, dorsal surfaces of the neck and body, and upper lateral surfaces of the neck and chest.

Samples required

- Scabs from skin lesions
- Skin biopsy from affected area, chilled
- Skin biopsy from affected area, formalin fixed

Dermatophytosis (ringworm)



Some *Microsporium* and *Trichophyton* species are zoonotic. Take care when handling infected animals and samples.

Dermatophytosis is an infection of keratinised tissue (skin, hair and claws) by one of three genera of fungi collectively called dermatophytes. The most important animal pathogens worldwide are *Microsporium canis*, *M. gypseum*, *M. nanum*, *Trichophyton mentagrophytes*, *T. equinum* and *T. verrucosum*. These species are zoonotic, especially *M. canis* infections of domestic cats and *T. verrucosum* infections of cattle and lambs.

A common clinical sign of dermatophytosis is patchy hair loss. For most healthy adult hosts, dermatophyte infections are self-limiting, but in young or debilitated animals, infection may be persistent and widespread.

Samples required

- Skin scrapings, crusts and hair from active lesions; do not chill

Diamond skin disease

See 'Erysipelas' in this section.

Encephalomyocarditis virus infection

Encephalomyocarditis virus infection is an enteroviral disease of rodents that occasionally causes outbreaks of myocarditis in pigs and other animals. Spread through urine, faeces and saliva of infected rodents, it causes sudden death in young pigs and possibly reproductive losses (late abortion, mummification).

Samples required

- Formalin-fixed tissues, full range including heart and brain
- Fresh heart, liver, kidney, spleen and aborted fetuses

Enterotoxaemia (pulpy kidney, *Clostridium perfringens* type D)

Enterotoxaemia usually occurs when conditions in the gastrointestinal tract favour rapid growth of the bacteria *Clostridium perfringens* type D, i.e. high carbohydrate diets or lush green pastures. It is often associated with bloat. Clinical signs include diarrhoea, convulsions, paralysis, blindness or sudden death. At necropsy, animals may show minimal gross changes or sometimes small intestinal inflammation. Note that *C. perfringens* is a normal inhabitant of the intestine. It is also a common post-mortem invader and may be present in other tissues within 8 hours of death. Diagnostic confirmation of enterotoxaemia requires demonstration of the epsilon toxin in the intestinal contents.

Samples required

- Small intestinal contents in sterile containers; at least 10 mL from sheep and 40 mL from cattle from several different sites but particularly where the contents are creamy yellow or bloodstained

- Smears from the intestinal mucosa at multiple sites, especially where haemorrhages are visible (several sites can be smeared onto one slide)
- Formalin-fixed tissues, full range including liver, kidney and brain (whole)
- Urine

Enzootic bovine leucosis

Enzootic bovine leucosis (EBL) is a lymphoproliferative disease of cattle that is caused by a retrovirus, bovine leukaemia virus. A small proportion of infected cattle develop lymphoid tumours affecting all body systems.

EBL has been eradicated from the national dairy herd, but some beef herds in Australia still have a low level of EBL. Cattle are infected with bovine leukaemia virus through the transfer of blood and body fluids.

Samples required

- Serum (or milk) for EBL ELISA
- Formalin-fixed tissues including affected lymph nodes, spleen, thymus and intestine
- EDTA blood

Report any confirmed or suspected cases of EBL in animals to Biosecurity Queensland on 13 25 23.

Equine infectious anaemia

Equine infectious anaemia only infects horses and is characterised by recurrent fever, petechiae, anaemia, rapid loss of weight, weakness and oedema of the lower parts of the body. Infected horses that survive the acute disease remain viraemic carriers for life and can potentially transmit the infection to other horses. Transmission occurs by transfer of blood from an infected horse, e.g. by biting insects or contaminated needles.

Samples required

- Clotted blood, EDTA blood and smear
- Formalin-fixed tissues including liver, kidney, spleen and heart

Equine infectious anaemia is a reportable disease under Queensland legislation. Report all confirmed cases in horses to Biosecurity Queensland on 13 25 23.

Erysipelas (diamond skin disease)



Infection with *Erysipelothrix rhusiopathiae* is a zoonosis. Take care when handling infected animals and samples.

E. rhusiopathiae is a bacterium that causes fever, arthritis and lameness in sheep and fever, arthritis, skin lesions (diamond skin disease), valvular endocarditis and fatal septicaemia in pigs. Pregnant sows may abort. In sheep, the bacterium enters via wounds or skin abrasions. Infection in pigs is by ingestion of contaminated food or water or through minor skin abrasions.

Samples required

- Whole affected joint, unopened and chilled, or aseptically collected synovial membrane and synovial fluid/swabs from affected joints in separate sterile containers
- Joint fluid collected into EDTA for analysis
- Smears of joint fluid, for cytology (made immediately)

Additional samples from pigs

- Fresh samples of skin, valvular lesions, lung, liver, kidney and spleen in separate sterile containers
- Formalin-fixed tissues including skin lesions, heart, lung, liver and spleen

See also 'Arthritis/polyarthritis' in this section.

Fluorosis

Sources of fluorine are artesian bore water (fluorine becomes concentrated due to evaporation), feed supplements and pasture contamination by industrial emissions and superphosphate fertilisers. Chronic fluorosis causes dental and skeletal abnormalities including mottled, pitted teeth, uneven tooth wear, lameness, abnormal posture and emaciation. Subacute poisoning (usually associated with superphosphate fertiliser application to pasture) causes anorexia, thirst, diarrhoea, weakness, ataxia and death.

Samples required

- Bones (mandibles with teeth)
- Suspect feed and water
- Formalin-fixed tissues including bone lesions and kidney
- Urine

Foot-and-mouth disease

Foot-and-mouth disease (FMD) is a serious and highly contagious exotic viral disease that affects cloven-hoofed animals (particularly cattle, pigs, sheep and goats). The disease is clinically characterised by fever, salivation, lameness and the formation of vesicles and erosions in the mouth and nostrils, on the teats and on the skin between and above the hoofs. FMD can cause serious production losses and is a major constraint to international trade in livestock and livestock products.

FMD should be considered as a differential diagnosis whenever vesicles are seen in cloven-hoofed animals.

It is important to obtain a full range of samples to allow a definitive laboratory diagnosis. Do not omit some samples based on your lesion ageing assessment. Ideally, take samples from several affected animals and collect duplicate samples from each animal.

Samples required

- Vesicular fluid, carefully aspirated by syringe and needle from newly formed vesicles and placed in a sterile container without preservative
- Epithelium—up to 6 epithelial coverings of intact vesicular lesions (at least 2 cm²), or epithelial flaps or tags surrounding vesicles if unruptured vesicles are not available, in a sterile container without preservative
- Oral, nasal and tonsillar swabs in a sterile container without preservative
- Serum—approximately 20 mL of blood per animal collected into plain tubes (red tops)
- EDTA/lithium heparin blood (minimum 5 mL)
- Fresh and formalin-fixed tissues, full range of organs as well as lymph nodes, thyroid and adrenal gland

Note: Use separate sets of sterile instruments for each animal. Keep all unpreserved samples chilled and forward them to the laboratory as soon as possible.

FMD is a reportable disease under Queensland legislation. Report suspected cases of FMD in any susceptible animal species to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888 before collecting or sending any diagnostic samples.

Footrot

Footrot is a contagious bacterial disease of sheep and goats caused by the organism *Dichelobacter nodosus* in association with a number of other bacteria. Footrot will only occur where the organism is present and the right environmental conditions exist. For this reason, footrot is not considered to be a problem in Queensland. Diagnosis is usually made in the field based on history and clinical signs and requires the examination of a large number of animals in the flock. Laboratory testing is only used for confirmation if virulent footrot is suspected and may need to be referred interstate. Contact BSL for more information.

Ovine footrot is a reportable disease under Queensland legislation. Report suspected cases in sheep to Biosecurity Queensland on 13 25 23.

Fungal infections

See 'Mycoses' in this section.

Generalised glycogenosis

See 'Pompe's disease' in this section.

Glässer's disease

Glässer's disease is caused by *Haemophilus parasuis* and is characterised by fibrinous polyserositis, arthritis and meningitis. It can affect any age of pigs and is associated with a wide range of clinical signs. Outbreaks of disease are often linked to stress and may result in high morbidity and mortality.

As the organism is difficult to culture, samples should be collected from acutely ill, untreated pigs that have been recently euthanased and transported to the laboratory without delay.

Samples required

- Swabs from exudates and serosal surfaces in transport media (Amies)
- Fresh and formalin-fixed tissues, full range

Grain overload/poisoning

See 'Ruminal acidosis' in this section.

Grass tetany

See 'Hypomagnesaemia' in this section.

Haemonchosis

Haemonchosis is caused by infection of *Haemonchus placei* or *Haemonchus contortus* in cattle, sheep, goats and camelids. Goats and camelids are particularly susceptible and any age may be affected. Overcrowding and wet weather contribute to outbreaks of the disease. There may be anaemia, bottle jaw and rapid loss of condition, or animals may die suddenly in good condition.

Samples required

- Clotted blood, EDTA blood, smear
- Faeces (from several animals)

Hendra virus infection



Hendra virus is a zoonosis. Exercise extreme care in handling infectious material.

Hendra virus is a serious zoonotic disease that should be considered for any sick horse when the cause of illness is unknown and particularly where signs progress quickly and there is rapid deterioration. Affected horses may show a wide range of signs including rapid onset of illness with fever and increased heart rate, depression and rapid deterioration with respiratory and/or nervous signs.

Samples required

Live animals

- EDTA blood
- Swabs—nasal, oral, vaginal and rectal in VTM (or use a small amount of saline to prevent swabs drying out if VTM is not available)
- Urine (even a urine-soaked swab taken from the ground immediately after urination may be useful)
- Clotted blood

Dead animals

- Swabs—nasal, oral, vaginal and rectal in VTM (or use a small amount of saline to prevent swabs drying out if VTM is not available)
- Blood clot (obtained by cutting down onto the jugular vein)
- Submandibular lymph node, unpreserved

Note: Dead horses can be adequately sampled for Hendra virus exclusion without conducting a complete necropsy.

Sample from multiple sites to increase the diagnostic sensitivity of laboratory testing for Hendra virus exclusion. As a minimum, provide a nasal swab and whole blood (in EDTA).

Pack the specimen advice sheet externally to the samples and clearly indicate on it that Hendra virus is suspected.

For more detailed information, visit business.qld.gov.au.

Hendra virus infection is a reportable disease under Queensland legislation. Report suspected cases in any species of animal to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Hepatitis dietetica

See 'Mulberry heart disease' in this section.

Hypocalcaemia (parturient paresis, milk fever)

Parturient paresis is an acute to peracute, afebrile, flaccid paralysis of mature dairy cows that occurs due to low blood calcium levels (hypocalcaemia) usually within 72 hours of parturition. It is associated with changes in mental state and mild ataxia progressing to generalised paresis and circulatory collapse. It may be associated with imbalance of other minerals, namely magnesium, potassium and sodium. It can be a contributing factor to dystocia, uterine prolapse, retained foetal membranes, metritis, abomasal displacement and mastitis.

Samples required

- Clotted blood
- Ocular fluid

Hypomagnesaemia (grass tetany)

Hypomagnesaemic tetany is a complex metabolic disturbance characterised by hyperexcitability, muscular spasms, convulsions, respiratory distress, collapse and death. It occurs mainly when animals are grazed on lush grass pastures or green cereal crops. Adult lactating animals are most susceptible because of the loss of magnesium in milk.

Samples required

- Clotted blood
- Urine
- Ocular fluid

Hypophosphataemia

See 'Phosphorus deficiency' in this section.

Hypovitaminosis A (vitamin A deficiency)

Vitamin A deficiency occurs rarely but has been reported in extensive beef cattle following prolonged drought conditions or in feedlot animals that receive inadequate vitamin A in rations. Clinical signs of deficiency are wide ranging and include blindness, poor appetite and weight gain, defects of bone development, ataxia, neurological signs, increased susceptibility to infection, decreased fertility, abortions, stillbirths and birth of weak, blind or abnormal calves.

Samples required

- Serum, frozen and protected from light
- Unpreserved liver, frozen and protected from light

Neonates

- Formalin-fixed brain, eye and optic nerves

Note: Vitamin A is destroyed rapidly after death, so submit liver samples from freshly dead animals only. All samples should be frozen immediately and delivered frozen covered in aluminium foil to prevent light penetration.

Infectious bovine rhinotracheitis, infectious balanoposthitis and infectious pustular vulvovaginitis

Infectious bovine rhinotracheitis is caused by bovine herpesvirus 1 (BHV1); however, bovine herpesvirus subtype 1.2b is the only subtype identified in Australia. This subtype is associated with severe respiratory disease including fever, coughing and nasal discharge, but is not associated with abortion. Secondary bacterial infections may lead to pneumonia, especially in intensively managed livestock in feedlots. The venereal forms of the disease (**infectious balanoposthitis** and **infectious pustular vulvovaginitis**) result in pustular lesions in the prepuce and penile epithelium of the bull and vulva and vagina of the cow.

Samples required

Live animals

- Nasal swabs (preferably in VTM) from acute cases with serous nasal discharge
- Vaginal or preputial swabs (in VTM) if genital lesions are present
- Paired sera, 3–4 weeks apart

Dead animals

- Unpreserved trachea, lung, bronchial lymph nodes

Note: The detection of BHV1 by PCR in a diseased animal does not unequivocally mean that this virus is the cause of the illness. It may, for instance, be latent virus that has been reactivated due to stressful conditions. A confirmatory laboratory diagnosis must be made from a group of animals and must be accompanied by seroconversion from negative to positive, or a fourfold or higher increase in BHV1 specific antibody titres in paired serum samples collected 3–4 weeks apart.

Infectious keratoconjunctivitis (contagious ophthalmia, pinkeye)

Infectious keratoconjunctivitis of cattle, sheep, and goats is characterised by blepharospasm, conjunctivitis, lacrimation and varying degrees of corneal opacity and ulceration.

Samples required

- Conjunctival swabs in transport media (if possible remove surface contamination prior to swabbing)

See also 'Chlamydial infections', 'Mycoplasmosis', 'Infectious bovine rhinotracheitis' and 'Malignant catarrhal fever' in this section.

Infectious necrotic hepatitis

See 'Black disease' in this section.

Intestinal spirochaetosis in pigs

See 'Swine dysentery' in this section.

Johne's disease (paratuberculosis)

Johne's disease affects cattle, sheep, goats, alpaca, llama, camels and deer and is caused by *Mycobacterium avium* subspecies *paratuberculosis*. Although most infections occur in young animals, the disease has a long incubation period and clinical signs may not be seen until at least 2 years of age (possibly earlier in sheep and deer). The most common sign of Johne's disease is gradual weight loss leading to emaciation and eventual death. Chronic diarrhoea is often seen in cattle. The bacterium is shed in the faeces of infected animals, particularly when clinical signs are apparent, and young animals are infected either when suckling their dam or grazing contaminated pasture. Older animals, especially cattle, are less susceptible to infection.

Samples required

- Faeces (at least 20 g), chilled and delivered to the laboratory within 48 hours, or frozen at -80°C
- Unpreserved and formalin-fixed samples of ileum (proximal and distal), colon, ileo-caecal valve and ileo-caecal and mesenteric lymph nodes
- Clotted blood

Note: Confirmation of Johne's disease by culture takes 14–16 weeks.

Johne's disease is a reportable disease under Queensland legislation. Report suspected or confirmed infection or clinical disease to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Assurance programs

In Australia, a number of voluntary assurance programs facilitate control of Johne's disease and allow producers to endorse their negative Johne's disease status. Specific programs operate for different livestock species and industries and include J-BAS, Dairy Score, SheepMAP, GoatMAP and AlpacaMAP. For more information on these programs, visit the Animal Health Australia website.

To help clients meet the testing requirements of these programs, BSL offers the following tests.

Check test (cattle)

Faecal samples from 50 representative adult cattle over 2 years of age are pooled at the laboratory and tested using the high throughput–Johne's (HT–J) PCR.

Sample test (cattle)

Faecal samples from up to 300 adult cattle are pooled at the laboratory and tested using the HT–J PCR.

Note: Any positive HT–J PCR results will need to be confirmed by mycobacterial culture. Cultures can be conducted on both faeces and tissue samples from specific intestinal sites and have a turnaround time of 14–16 weeks.

Herd environmental culture (dairies only, for surveillance and as an alternative to the check test)

A faecal slurry sample representative of the whole herd is tested by mycobacterial culture. The slurry sample must be collected from the concrete yard/standing area in front of the milking shed following the protocol outlined on the Animal Health Australia website.

Sheep and goats

Serological and pooled faecal culture tests are available.

For more information on the management of Johne's disease in Queensland, visit business.qld.gov.au.

Joint ill

See 'Arthritis/polyarthritis' in this section.

Ketosis (acetonemia)

This is a metabolic disease of both beef and dairy cows characterised by ketonaemia, ketonuria and hypoglycaemia. High production and fatness predispose cows to the condition, but it may be precipitated by any reduction in feed quality or quantity and/or stress in late pregnancy and early lactation. It may also occur secondary to a wide variety of disease conditions. Affected cows typically show inappetence and nervous signs.

Samples required

- Clotted blood for β -hydroxybutyrate (ketone) analysis
- Blood in fluoride-oxalate for glucose estimation
- Ocular fluid or cerebrospinal fluid, chilled
- Urine or milk
- Formalin-fixed tissues, full range including liver, kidney, adrenal gland and brain

Note: If possible, check urine and milk for ketones in the field.

Lambing sickness

See 'Pregnancy toxaemia' in this section.

Lead poisoning

Common sources of lead include lead-acid batteries, sump oil, oil filters, lead paint on old buildings and lead shot. Cattle are most susceptible to poisoning. Signs vary according to the degree of intoxication, but animals may show a combination of nervous signs and gastrointestinal signs, which may include either constipation or diarrhoea.

Samples required

- Suspected source of lead
- Clotted blood, EDTA blood and smear
- Faeces (100 g)
- Urine
- Unpreserved liver and kidney (50 g)
- Formalin-fixed tissues, full range including brain

See also **Section 10** or visit business.qld.gov.au for more information.

Lead contamination is a reportable incident under Queensland legislation. Report any suspected exposure of food-producing animals to lead to Biosecurity Queensland on 13 25 23.

Leptospirosis



Humans may become infected with *Leptospira* through contact with water, food or soil contaminated with urine from infected animals. Take care when handling the urine of infected animals.

Leptospirosis is a zoonotic bacterial infection of livestock (cattle, pigs, sheep, horses, goats, camelids and deer) that causes abortions, stillbirths, neonatal weakness, infertility, loss of milk production and severe acute disease in young animals. Although 24 *Leptospira* serovars have been identified in Australia, only some are commonly associated with clinical disease in livestock:

- Cattle—*L. borgpetersenii* serovar hardjo (subtype hardjo bovis) and *L. interrogans* serovar pomona
- Pigs—*L. interrogans* serovar pomona and *L. borgpetersenii* serovar tarrassovi
- Horses—*L. interrogans* serovar pomona (sporadic)
- Sheep—*L. borgpetersenii* serovar hardjo (sporadic)

Samples required

- Clotted blood, EDTA blood and smear
- Midstream urine (5 mL)
- Unpreserved kidney and liver
- Formalin-fixed tissues, full range including liver and kidney
- Paired sera for leptospirosis serology (acute and convalescent samples 3–4 weeks apart)

Additional samples

Abortion

- Whole foetus, chilled, not frozen, **or** unpreserved foetal kidney, liver and lung
- Foetal fluids (pleural, peritoneal, pericardial)

Milk drop syndrome

- Milk samples for bacteriology

Where leptospirosis is suspected of causing abortion and/or mastitis, serological testing on a herd basis may be useful. Submit paired sera (collected 3–4 weeks apart) from up to 20 affected animals for leptospirosis serology. Choose those animals with a history of recent illness.

Listeriosis



Humans may become infected with *Listeria* through contact with infected animals or through contamination of food. Take care when handling infected animals and samples.

Infection with *Listeria monocytogenes* is often associated with feeding poor-quality silage. It produces characteristic syndromes in a wide range of animals including abortion and perinatal mortality, encephalitis or meningoencephalitis in adults and septicaemia in neonates. Neurological signs include dullness, torticollis, circling, unilateral facial paralysis and salivation followed by recumbency, paddling and death within a few days.

Samples required

Neurological disease

- Unpreserved brain stem and cerebellum, liver, spleen and kidney (or swabs), chilled
- Cerebrospinal fluid, chilled
- Formalin-fixed tissues, full range including brain

Note: It is important to preserve a portion of the brain stem for histopathology.

Abortion

- As for 'Abortion/stillbirth' in **Table 12.1**

Septicaemia

- Unpreserved liver, spleen and kidney (or swabs), chilled
- Formalin-fixed tissues, full range including lung, liver, spleen and kidney

Liver fluke (*Fasciola hepatica*)

The migration of immature liver fluke through the liver and the presence of adult fluke in the bile ducts can cause a variety of clinical manifestations in ruminants from mild ill thrift to marked debility with anaemia, jaundice and oedema and sometimes sudden death. Consider the distribution of *F. hepatica* in Queensland as well as findings of the faecal examination before arriving at a diagnosis of fascioliasis. Liver damage by migrating flukes may trigger black disease (*Clostridium novyi*).

Samples required

- Faeces (at least 30 g) from up to 10 animals

Note: Faecal egg counts are only of value in chronic fascioliasis where eggs are being excreted (patent infection); they are of no value in acute and subacute infections. Where animals are available for necropsy, the findings should be sufficient for diagnosis in the field (i.e. without sending further samples to the laboratory).

Lumpy skin disease

Lumpy skin disease (LSD) is an acute to chronic, highly infectious, generalised skin disease of cattle and water buffalo. The disease is caused by LSD virus, which is a capripoxvirus and is believed to be mechanically transmitted mostly by biting insects and ticks. Clinical signs include pyrexia, nasal and ocular discharges, hypersalivation and lymphadenopathy. Cutaneous nodules with necrotic centres (2–5 cm diameter) develop on the head, neck, limbs, udder, genitalia and perineum and fibrinous plaques eventually form over the top of the larger nodules (sit-fasts). Similar nodules may be found in the nasopharynx, trachea, bronchi, lungs, rumen, abomasum, renal cortex, testes and uterus. There is potential for significant economic loss due to high morbidity, drop in milk yield in cattle and hide damage, as well as restrictions on animal movements and international trade.

Samples required

Live animals

- Unpreserved and formalin-fixed skin lesion biopsies
- Scabs (from sit fast) or skin scrapings
- Vesicular fluid (if present) aspirated with a syringe
- EDTA blood (animals with fever)
- Clotted blood

Dead animals

- Unpreserved and formalin-fixed tissues of lesions in skin, respiratory tract, gastrointestinal tract, internal organs

LSD is a reportable disease under Queensland legislation. Report suspected cases of LSD in any susceptible animal species to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888 before collecting or sending any diagnostic samples.

Lumpy wool

See 'Dermatophilosis' in this section.

Lyssavirus

See 'Australian bat lyssavirus' in this section.

Malignant catarrhal fever (bovine malignant catarrh)

Malignant catarrhal fever is an infectious systemic disease affecting mainly ruminants and occasionally pigs. The disease in cattle is normally seen sporadically affecting single animals and is typically fatal. The principal carriers of the causative virus (ovine herpesvirus 2) are sheep, so there is usually a history of contact with sheep. The disease may range from peracute to chronic. Clinical signs are highly variable, but the main lesions are inflammation and necrosis of respiratory, alimentary or urinary mucosal epithelium. Fever, depression, oculonasal discharges, corneal opacity, salivation, reddening of the muzzle, dyspnoea, diarrhoea and nervous signs may be seen.

Samples required

- EDTA blood (for ovine herpesvirus 2 PCR at the Australian Animal Health Laboratory)
- Clotted blood
- Formalin-fixed tissues, full range including kidney, liver, urinary bladder, buccal epithelium, cornea, conjunctiva and brain
- Unpreserved spleen and liver
- Nasal swabs (for exclusion of infectious bovine rhinotracheitis)

Malignant oedema

Clostridium septicum, along with *C. chauvoei*, *C. perfringens*, *C. sordellii* and *C. novyi*, can produce malignant oedema. Malignant oedema results from wound contamination by soil. Deep puncture wounds, castration wounds and parturition injuries are high risk. Infection can also occur via the umbilicus in newborn animals and following injections if proper aseptic technique is not used. Production of toxin at the entry site causes extensive swelling, with accumulation of bloody or clear fluid, and tissue death followed by gangrene. The affected area is extremely painful. Toxins are absorbed into the bloodstream, causing fever, weakness, trembling and then death.

Samples required

- Air-dried impression smears of the lesion
- Swabs in transport medium of fluid exudate and lesion
- Large sample (5 cm block) of the lesion for anaerobic culture, chilled

Mastitis

Staphylococcus aureus, *Streptococcus uberis*, *Streptococcus dysgalactiae* and environmental *Streptococcus* spp. are the most common pathogens found in both clinical and subclinical mastitis in cattle.

Samples required

- Milk (15 mL collected into 25 mL sterile containers), chilled

Wherever possible, sample prior to treatment. If antibiotics have been used, record this on the specimen advice sheet. Take care to avoid contamination of the sample. Before taking the sample of foremilk, wash and dry the teat thoroughly and sterilise the teat end with 70% alcohol or undiluted teat-dip solution. Discard the first few squirts before collecting the sample. If the samples will not reach the laboratory within 24 hours of collection, store them frozen at -20°C .

Note: A maximum of 10 milk samples collected from clinically affected animals will be tested free of charge.

Melioidosis



Melioidosis is a zoonosis. Exercise care in handling infectious material.

Melioidosis is a zoonotic bacterial infection caused by the soil-dwelling bacterium *Burkholderia pseudomallei*. Melioidosis is regarded as endemic to parts of tropical Australia, but the disease is rare. All species can be affected, but goats, sheep, pigs and camelids seem to be more susceptible. Infection is acquired from contaminated soil or surface water rather than animal-to-animal contact, and outbreaks may occur following periods of heavy rain and flooding. Clinical signs are varied and include fever, weakness, anorexia, respiratory disease, nasal discharge, recumbency, mastitis and nervous signs, but subclinical infection is common. Abscesses frequently occur in internal organs and lymph nodes.

Samples required

- Swabs or unpreserved samples of lesions or discharges
- Serum for serology (CFT and IHA)
- Formalin-fixed lesions and full range of tissues

Pack the specimen advice sheet externally to the samples and clearly indicate on it that melioidosis is suspected.

See also 'Melioidosis serology' in **Section 8.4**.

Milk fever

See 'Hypocalcaemia' in this section.

Mineral deficiencies

Mineral deficiencies can be the cause of clinical disease. They can also contribute to, or predispose animals to, other diseases. Routinely offered assays include calcium, magnesium, phosphorus, copper, selenium, iron and zinc. Other assays are available on application. Evaluating 10–20 animals, rather than testing individual animals that are ill or have died from other diseases, will better reflect the mineral status within a group.

Samples required

- Clotted blood, full tubes (at least 2 mL of serum required for some assays), serum separated within 2 hours of collection as haemolysed samples may give inaccurate results in some assays
- EDTA for GSH-Px if selenium deficiency is suspected
- Fresh or frozen liver and kidney (minimum of 50 g of each)
- Ocular fluid (fresh or frozen)
- Bone for calcium/phosphorus/magnesium assay

Note: Charges will be applied when there is no disease investigation or a diagnosis has already been made and further health testing is requested.

See also 'Phosphorus deficiency', 'Hypocalcaemia' and 'Hypomagnesaemia' in this section.

Mucosal disease

See 'Bovine pestivirus infection' in this section.

Mulberry heart disease and hepatitis dietetica

Mulberry heart disease and hepatitis dietetica are associated with diets low in selenium or vitamin E and cause sporadic losses in rapidly growing pigs at 2–6 weeks of age. Death occurs suddenly, often precipitated by exercise. In mulberry heart disease, there is excess pericardial fluid and extensive haemorrhage in the epicardium and myocardium. In hepatitis dietetica, there is oedema and increased fluid in body cavities and the liver appears mottled.

Samples required

- Clotted blood, EDTA blood (for vitamin E analysis, wrapped in aluminium foil to protect from light)
- Formalin-fixed tissues, full range including heart, skeletal muscle and liver
- Unpreserved liver, frozen and kept frozen in transit, covered in aluminium foil to protect from light
- Unpreserved kidney, chilled

Mycoplasmosis

Mycoplasma infections cause a wide range of clinical conditions in pigs, cattle, sheep, goats and poultry.

Samples required

Pigs: enzootic pneumonia (*M. hyopneumoniae*)

- Unpreserved lung, chilled
- Formalin-fixed lung

Pigs: synovitis and arthritis (*M. hyosynoviae*)

- Unpreserved joint fluid, chilled

Pigs: serofibrinous polyserositis and fibrinous arthritis (*M. hyorhinis*)

- Unpreserved affected tissues or joints, chilled
- Unpreserved joint fluid, chilled
- Formalin-fixed tissues and lesions, full range

Cattle, sheep and goats: polyarthritis in calves, bovine mastitis and pneumonia, eye lesions and caprine and ovine arthritis and mastitis

- Unpreserved tissues, joint fluid, joint membrane, ocular fluid and milk, chilled
- Formalin-fixed tissues, full range

Note: Ideally, submit fresh tissue in mycoplasma media (available on request from BSL). Avoid submission of swabs for mycoplasmal culture. Chilled tissue, synovial fluid and milk samples are suitable for culture provided that they are received at the laboratory within 24 hours of collection.

See also 'Arthritis/polyarthritis', 'Mastitis' and 'Infectious keratoconjunctivitis' in this section.

Mycoses (fungal infections)

Systemic mycoses are infections with fungal organisms that exist in the environment, enter the host from a single portal of entry, and disseminate within the host, usually to multiple organ systems. Soil is the primary source of most infections, which can be acquired by inhalation, ingestion or traumatic introduction of fungal elements. Pathogenic fungi establish infection in apparently normal hosts but opportunistic fungi usually require a host that is debilitated or immunosuppressed to establish infection. Prolonged administration of antimicrobials or immunosuppressive agents appears to increase the likelihood of infection by the opportunistic fungi that cause diseases such as aspergillosis and candidiasis, which may be focal or systemic.

Samples required

- Unpreserved lesions for fungal culture
- If systemic infection is suspected, unpreserved liver, spleen and kidney for fungal culture
- Formalin-fixed lesions

Note: Samples for culture should not be chilled, as temperatures of less than 15 °C can be detrimental to fungal survival.

Mycotoxicoeses

Mycotoxins can produce a variety of syndromes—nervous, renal, hepatic or gastrointestinal disease, ill thrift, peripheral gangrene and infertility (oestrogenic effects).

Samples required

- Clotted blood, EDTA blood and smear
- Fresh and formalin-fixed tissues, including liver, kidney and any lesion
- Suspect source of mycotoxin

Note: As mycotoxin assays are difficult and expensive, they are only available when clinical and pathological evidence indicates that they are a likely cause of the disease problem. Other possible causes will need to be ruled out before testing for mycotoxins will be considered.

See also 'Aflatoxicosis' in this section.

Neonatal diarrhoea of calves

Cases of neonatal scours occur in both dairy and beef calves. The clinical presentation can range from mild diarrhoea without systemic disease to profuse, acute diarrhoea associated with rapid dehydration, severe metabolic disturbances and rapid death. The main aetiological agents are rotavirus, *Cryptosporidia*, *Salmonella* spp. and *E. coli*, which often occur in combination. Other factors such as inappropriate feeding management and inadequate colostrum intake may also contribute.

Samples required

Live animals

- Faeces, chilled (30 g)
- Faeces from 5–10 herd mates, chilled
- Clotted blood, EDTA blood and smear

Dead animals

- Formalin-fixed tissues, full range including sections of gastrointestinal tract from a freshly dead animal
- Faeces, chilled
- Small and large intestinal contents, chilled
- Unpreserved lung, liver, spleen and kidney, chilled

Neosporosis

Abortion in cattle due to *Neospora* usually occurs between 4 and 7 months of gestation, but may occur at any time from 3 months to term. Infected calves may be born showing incoordination and paralysis of the limbs, but this is uncommon. Adult cows have no clinical signs of illness following infection.

Samples required

- Whole foetus and placenta, chilled
- Formalin-fixed tissues including brain (even if autolysed), heart, skeletal muscle, tongue and diaphragm
- Foetal fluids (thoracic, abdominal)
- Clotted blood from dam

Serological testing

Seropositivity in an aborting cow does **not** confirm *Neospora* abortion. This can only be confirmed by histological examination of the foetus.

However, serological testing of multiple cows or heifers can be used to determine whether neosporosis is a major reproductive problem in a herd. Collect serum samples from up to 10 aborting cows and from a similar number of matched herd mates with normal gestation. If most cows in the aborting group are seropositive and few are seropositive in the normal group, neosporosis should be suspected as a cause of abortion in the herd. If most aborting cattle are seronegative, neosporosis is unlikely to be a major problem.

Note: This testing is done on a fee-for-service basis.

See also 'Abortion in cattle' in this section and 'Abortion/stillbirth' in **Table 12.1**.

Nitrate/nitrite poisoning

Nitrate/nitrite poisoning occurs most commonly from ingestion of plants that contain excess nitrate. The accumulation of nitrates in potentially toxic plants can be enhanced by certain environmental and soil conditions. Ruminants are particularly susceptible because the ruminal flora reduces nitrate to ammonia, with nitrite (~10 times more toxic than nitrate) as an intermediate product. Hungry stock are more likely to be affected. Acute intoxication is manifested primarily by methaemoglobin formation and resultant anoxia with rapid, weak heartbeat, muscular tremors, weakness and ataxia rapidly progressing to brown, cyanotic mucous membranes, dyspnoea and tachypnoea. Affected animals may die: suddenly without appearing ill, in terminal anoxic convulsions within 1 hour, or after a clinical course of 12–24 hours or longer. Blood may appear chocolate-brown in colour at necropsy.

Samples required

- Clotted blood, EDTA blood and smear
- Ocular fluid (withdrawn from the eye and transferred to a well-sealed container), chilled
- Rumens contents, chilled
- Suspect plants (shade-dried)

Avoid using sealed plastic bags for plant specimen transport, as the plant rapidly 'sweats', becomes mouldy and decomposes, destroying the nitrate.

Note: Delayed testing of samples may lead to inaccurate results. Samples need to be collected as soon as possible after death and tested immediately or chilled and sent promptly to the laboratory.

Nutritional myopathy

See 'White muscle disease' in this section.

Oedema disease

Oedema disease is a peracute enterotoxaemia caused by haemolytic *E. coli* affecting healthy, rapidly growing pigs at 4–10 weeks of age. There may be sudden death with no signs of illness, or neurological signs (ataxia, paralysis and recumbency) may be observed. Often there is subcutaneous oedema of the face and eyelids and oedema of the stomach wall and mesentery of the spiral colon.

Samples required

- Small intestinal contents or faeces, chilled
- Formalin-fixed tissues, full range including brain, stomach, small intestine, spiral colon and mesenteric lymph nodes

Orchitis and epididymitis in rams

Orchitis and epididymitis in rams may occur as a result of bacterial infections including *Brucella ovis*, *Actinobacillus seminis*, *Histophilus ovis* and *Trueperella pyogenes*. Epididymal enlargement may also follow trauma with formation of spermatic granulomas.

Samples required

- Semen, collected aseptically and submitted chilled for bacteriology
- Clotted blood
- Unpreserved epididymis, accessory glands including ampullae and seminal vesicles, and testis
- Formalin-fixed epididymis, accessory glands including ampullae and seminal vesicles, and testis

Note: The *A. seminis* CFT is not a reliable way of diagnosing *A. seminis* infection, as many normal rams will have antibodies.

See also 'Brucellosis' in this section.

Orf

See 'Scabby mouth' in this section.

Oxalate poisoning

Poisoning may occur where there is ingestion of oxalate-containing plants, most commonly by hungry animals when the rumen flora is unadapted to oxalate intake. Hypocalcaemia and nephrosis are the main effects, but there may also be pulmonary oedema and rumenitis. Plants containing more than 2.0% soluble oxalate have the potential to cause oxalate poisoning. Common plant sources include pigweed (*Portulaca oleracea*), setaria (*Setaria sphacelata*) and buffel grass (*Cenchrus ciliaris*).

Samples required

- Suspect plants
- Clotted blood, EDTA blood
- Rumen contents
- Formalin-fixed tissues including kidney and liver

Note: Shade-dry the plants and submit them in paper bags. Avoid using sealed plastic bags, as the plants 'sweat', leading to mould growth and decomposition, destroying any oxalate or nitrate that is present.

Parasites, external

See **Section 7**.

Parasites, internal

See **Section 7**.

Paratuberculosis

See 'Johne's disease' in this section.

Parturient paresis

See 'Hypocalcaemia' in this section.

Phosphorus deficiency (hypophosphataemia)

No single sign of phosphorus deficiency exists, but in most cases the presence of a number of visible signs will point towards a possible phosphorus deficiency. These include bone-chewing, broken bones, peg-leg disease, botulism and poor body condition of breeders.

Although depraved appetite (pica), generally seen as bone-chewing, is one of the most obvious signs of acute phosphorus deficiency in cattle, this behaviour is not specific to phosphorus deficiency, as cattle suffering from severe protein deficiency will also exhibit depraved appetite. There are no simple diagnostic tests to establish the phosphorus status of cattle. Whole blood inorganic phosphorus plus faecal nitrogen and phosphorus are the most convenient and least costly indicators that have diagnostic value.

Samples required

- Clotted blood samples from 20 animals (serum separated from the clot within 2 hours of collection)
- Bulk faecal sample from 6–10 animals

Note: P-screen test kits are recommended for diagnosis of phosphorus deficiency in cattle. These can be ordered from BSL.

See **Section 2.4** for detailed information on collecting samples and interpreting results.

Photosensitisation

Photosensitisation is the result of heightened sensitivity of the skin to sunlight. There are two types. Primary photosensitisation is caused by direct damage to the skin from chemicals in plants. Secondary photosensitisation is caused by damage to the liver that hinders the excretion of phyloerythrin, a product of the breakdown of chlorophyll, the green pigment in plants. Phyloerythrin can lodge in the skin, making it sensitive to sunlight. Most cases occurring in livestock are secondary photosensitisation. There is photophobia and itchiness, redness and oedema of lightly pigmented areas of skin exposed to sunlight (ears, eyelids, muzzle and udder). If severe, there may be necrosis and sloughing of the skin. Jaundice may also be observed in secondary photosensitisation. Plants commonly associated with photosensitisation include caltrop (*Tribulus terrestris*), lantana (*Lantana camara*), yellow-wood (*Terminalia oblongata*) and some *Panicum* spp., but many other plants may be involved.

Samples required

- Formalin-fixed skin biopsy
- Clotted blood, EDTA blood and smear
- Formalin-fixed tissues including liver and kidney
- Rumen contents
- Suspect plants for identification

Pinkeye

See 'Infectious keratoconjunctivitis' in this section.

Plant poisoning

The samples required will vary with the type of plant suspected of causing the toxicosis. History is very important for the diagnosis, particularly whether there has been access and noting if the plant has been eaten.

Samples required

Live animals

- Clotted blood, EDTA blood and smear
- Urine
- Suspect plant for identification and analysis (fresh plants for cyanide, dried plants for nitrate and oxalate)

Dead animals

- Formalin-fixed tissues, full range including brain and spinal cord if there are nervous signs
- Unpreserved liver and kidney, chilled
- Ocular fluid, chilled or frozen
- Rumen contents, at least 200 g, chilled

See also 'Bracken fern poisoning', 'Cyanide poisoning', 'Nitrate/nitrite poisoning' and 'Oxalate poisoning' in this section, and **Section 10**.

Polioencephalomalacia

Polioencephalomalacia is associated with production of thiaminases in the rumen, ingestion of thiaminases in plants or high dietary intake of sulfur resulting in cerebrocortical necrosis and neurological signs in sheep and cattle. Other toxic or metabolic diseases (acute lead poisoning, salt poisoning or water deprivation) can also result in polioencephalomalacia.

Samples required

- Formalin-fixed whole brain
- Samples for alternative diagnoses

Pompe's disease (generalised glycogenosis)

Pompe's disease is an inherited genetic disorder seen in Shorthorn and Brahman cattle. It causes progressive muscle weakness, ill thrift and neurological disease in young animals.

Samples required

- Formalin-fixed tissues, full range including skeletal muscle, heart, brain, liver and kidney

Note: Genetic testing for Pompe's disease is not available at BSL but is available interstate.

Porcine circovirus infection

Porcine circovirus 2 (PCV2) is considered to be a key agent in the development of a number of diseases in pigs collectively referred to as PCV2-associated disease (PCVAD). In outbreaks of systemic PCVAD, the predominant clinical signs are weight loss or emaciation, tachypnoea, dyspnoea, icterus and pallor in pigs of weaning and post-weaning age. Lymphadenomegaly may also be present. Due to the immunosuppressive nature of PCV2 infection, less severe cases of PCVAD may have clinical signs related to co-infection with secondary agents (bacterial or viral) rather than those directly associated with PCV2 infection.

PCV2 is widely distributed throughout the pig populations of Australia and New Zealand. Despite the fact that post-weaning multisystemic wasting syndrome has not been diagnosed in Australia, less severe manifestations of PCVAD have been identified. These include enteric and respiratory forms, and those with generalised lymphoid lesions. The occurrence of porcine dermatitis and nephropathy syndrome has also been reported in Australia. Pigs with low-grade PCVAD may not have grossly visible lesions, and the disease may be limited to the lymph nodes.

Samples required

- Formalin-fixed tissues, full range including lymph nodes
- Unpreserved lymph nodes
- Appropriate samples for the syndrome seen

Porcine parvovirus

Porcine parvovirus causes reproductive failure in pigs, mostly in gilts that have not been exposed to the virus before conception. They may return to oestrus, fail to farrow, farrow fewer pigs per litter or have a large proportion of mummified foetuses. Abortion is rare.

Samples required

- Whole mummified and stillborn foetuses, chilled (preferred), **or** formalin-fixed tissues from the foetus (including brain, lung, heart, liver, kidney, spleen and placenta)
- Unpreserved foetal brain, lung, liver, heart, kidney and placenta, chilled
- Swabs of lung and stomach contents

Note: Serology is of little value in diagnosing parvovirus infection in individual sows but can be used as a herd test in unvaccinated herds. Serology is not available at BSL.

See also 'Reproductive failure in pigs' in this section and 'Abortion/stillbirth' in **Table 12.1**.

Porcine proliferative enteropathy

The porcine proliferative enteropathy complex comprises porcine intestinal adenomatosis, proliferative haemorrhagic enteropathy, necrotic enteritis and regional ileitis. Porcine intestinal adenomatosis, necrotic enteritis and regional ileitis cause hyperplasia and inflammation in the lower small intestine, colon and caecum resulting in ill thrift and diarrhoea in weaner and grower pigs, while proliferative haemorrhagic enteropathy causes acute or subacute intestinal haemorrhage with anaemia, dysentery and death, mostly in young adult pigs. The disease is caused by *Lawsonia intracellularis*.

Samples required

- Formalin-fixed samples including multiple sections of affected small and large intestine
- Unpreserved sections of small and large intestine, chilled
- Intestinal contents or faeces, chilled (for exclusion of other causes of diarrhoea)

Pregnancy toxaemia of sheep, goats and cattle (twin lamb disease, lambing sickness)

This is a highly fatal disease of ewes and does in late pregnancy characterised by fatty infiltration of the liver, ketonaemia, ketonuria, hypoglycaemia and low liver glycogen. A rapid drop in the plane of nutrition of heavily pregnant ewes and does (especially those carrying twins) predisposes to this condition. Affected animals show inappetence and depression, often with nervous signs, progressing to recumbency and death. Pregnancy toxaemia in cattle is similar to the condition in small ruminants and typically it affects beef cows before calving and dairy cows after calving.

Samples required

- Clotted blood for β -hydroxybutyrate (ketone) estimation
- Blood in fluoride-oxalate for glucose estimation
- Ocular fluid or cerebrospinal fluid, chilled
- Urine or milk
- Formalin-fixed tissues, full range including liver, kidney, adrenal gland and brain

Note: If possible, examine urine and milk for ketones in the field.

See also 'Ketosis' in this section.

Prussic acid poisoning

See 'Cyanide poisoning' in this section.

Pulpy kidney

See 'Enterotoxaemia' in this section.

Rain scald

See 'Dermatophilosis' in this section.

Reproductive failure in pigs

Reproductive failure in pigs may present as abortion, weak neonates, stillbirth, mummification, embryonic death and infertility. Mummification is seen quite frequently because of the large litter size. If only a few foetuses die, abortion rarely occurs. Instead, mummies are delivered at term, along with live piglets or stillbirths. The cause may be non-infectious (environmental, toxic and nutritional) or infectious (viral and bacterial). Abortion should be differentiated from parturient and immediate post-parturient deaths due to hypoxia and managerial factors such as injury or stress, smothering and chilling.

See also 'Porcine parvovirus' in this section and 'Abortion/stillbirth' in **Table 12.1**.

Ringworm

See 'Dermatophytosis' in this section.

Rotavirus and coronavirus

Rotavirus is the most common viral cause of diarrhoea in calves and lambs. Rotavirus, often in association with other pathogens, also produces diarrhoea in unweaned and recently weaned pigs. Coronavirus may also be associated with diarrhoea in young calves.

Samples required

- Faeces or intestinal contents, chilled (swabs not suitable)
- Formalin-fixed samples of small and large intestine (collected immediately after death)

See also 'Neonatal diarrhoea of calves' in this section.

Ruminal acidosis (grain overload/poisoning)

Ruminal lactic acidosis develops in sheep and cattle that have ingested large amounts of unaccustomed feeds rich in carbohydrates that ferment readily in the rumen. The resulting production of large quantities of volatile fatty acids and lactic acid decreases rumen pH. Lactic acidosis can cause rumenitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death. The disease may present clinically or subclinically.

Samples required

- Fluoride-oxalate (grey-top) for D-lactate assay (clotted blood or ocular fluid can also be tested)
- EDTA blood and smear
- Clotted blood
- Ocular fluid
- Formalin-fixed tissues including samples from several sites in the forestomachs
- Small intestinal contents (for exclusion of enterotoxaemia)

Note: If possible, check rumen pH **immediately** after death and measure D-lactate in blood taken from live animals that are showing clinical signs. Blood samples from other animals in the affected group may be useful even if they are not showing obvious clinical signs.

Salmonellosis



Salmonellosis is a zoonosis. Take care when handling infected animals and samples.

Clinical disease due to *Salmonella* spp. usually presents as systemic septicaemia or enteritis. Young calves, piglets, lambs and foals may develop both the enteric and septicaemic forms. Adult cattle, sheep and horses commonly develop acute enteritis, and chronic enteritis may develop in growing pigs and occasionally in cattle. Pregnant animals may abort. Clinically normal carrier animals are common.

Samples required

Live animals

- Faeces
- Clotted blood, EDTA blood and smear

Dead animals

- Swabs or samples of fresh tissues including liver, kidney, lung, spleen and mesenteric lymph nodes
- Small and large intestinal contents
- Formalin-fixed tissues including liver, kidney, lung, spleen, small and large intestine and mesenteric lymph nodes

Some strains/serovars of salmonella (*S. abortus equi*, *S. abortus ovis*, and *S. enteritidis*, *S. pullorum* and *S. gallinarum* infection in poultry) must be reported under Queensland legislation. Report any detections of these diseases to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Salt poisoning (sodium toxicosis, water deprivation)

Salt poisoning is associated with excess salt intake in feed or water and/or inadequate access to water and is seen most commonly in pigs and cattle. Clinical signs include scouring, excessive thirst, nervous signs, prostration and rapid death. There may be evidence of gastroenteritis at necropsy.

Samples required

- Formalin-fixed tissues, full range including brain
- Ocular fluid, chilled

Scabby mouth (contagious pustular dermatitis, contagious ecthyma, orf)



Scabby mouth is a zoonosis. Take care when handling infected animals and samples.

Scabby mouth is an infectious skin disease of sheep and goats that affects primarily the lips of young animals. It is caused by a parapoxvirus and the disease is usually more severe in goats than in sheep. People are occasionally infected through direct contact.

Samples required

- Scabs and scrapings of underlying tissue, chilled

See also 'Foot-and-mouth disease' in this section.

Sodium monofluoroacetate (1080) poisoning

Sodium monofluoroacetate is the toxic constituent of compound 1080, the poison used for control of vertebrate pests such as dingoes, wild dogs, foxes, feral pigs and rabbits. The same toxin is present in a number of native Australian plants (various *Gastrolobium* spp. and *Acacia georginae*). Dogs are the most sensitive to this toxin, but cattle, sheep, pigs and horses may also be poisoned. Poisoned carnivores and pigs show nervous disturbance and gastrointestinal hyperactivity before death. Herbivores mostly show sudden collapse from heart failure. No abnormalities are seen at necropsy.

Samples required

- Stomach/rumen contents (200 g), frozen
- Unpreserved liver and kidney (200 g), frozen
- Suspected source (plants or bait)
- Rumen contents for botanical examination
- Formalin-fixed heart (ruminants)

Note: Samples for 1080 toxicity testing are forwarded to the Chemical Residue Laboratory and charges will apply. Testing is done in batches, so results may be delayed.

Sodium toxicosis

See 'Salt poisoning' in this section.

Sporadic bovine encephalomyelitis

Sporadic bovine encephalomyelitis is caused by *Chlamydia pecorum* and affects cattle and buffalo. It is most often seen in calves less than 6 months old and is characterised by neurological signs and polyserositis.

Samples required

- Tissue impression smears from surfaces of affected organs
- Unpreserved brain, liver, spleen and kidney, chilled
- Formalin-fixed tissues including brain, liver, spleen, kidney and other organs affected by serositis

Strangles in horses

Strangles is a highly contagious bacterial infection of horses and donkeys caused by *Streptococcus equi* subsp. *equi*. There is fever, depression, purulent nasal discharge, and swelling and abscessation of the lymph nodes of the throat. Infected horses should be isolated and strict hygiene procedures should be put in place to prevent spread of the disease to other susceptible horses.

Strangles is common in horses in Queensland and not reportable. Send samples for testing (nasopharyngeal swabs and swabs of discharge from abscesses) to private veterinary laboratories for diagnosis.

Strychnine poisoning

Strychnine is a vertebrate pesticide. Animals affected by strychnine show a rapid onset of neurological signs progressing to severe tetanic spasms with death ensuing in 1–2 hours.

Samples required

- Ingesta/vomit (200 g)
- Unpreserved liver and kidney (10 g)
- Urine (200 mL)
- Sample of suspected source

Note: Samples for strychnine toxicity testing are forwarded to the Chemical Residue Laboratory and charges will apply. Testing is done in batches, so results may be delayed. Because of the extreme toxicity of strychnine, traces detected in ingesta are sufficient to confirm toxicity.

Swine dysentery and intestinal spirochaetosis

Swine dysentery is caused by *Brachyspira hyodysenteriae*. It occurs most commonly in grower pigs, and typically produces mucohaemorrhagic diarrhoea. The severity of the diarrhoea may vary. The disease spreads gradually through the herd, resulting in decreased growth rates and ill thrift. Intestinal spirochaetosis is associated with infection with *B. pilosicoli* and produces a mild diarrhoea, inappetence and reduced growth rates in affected pigs.

Samples required

Live pigs (acutely ill and unmedicated)

- Faeces (at least 10–15 mL in a 20 mL container) taken from the rectum

Note: If less than this volume is submitted, the sample may become aerated and the organisms may be rendered non-viable.

Dead pigs

- Scrapings of the colonic mucosa or tied-off sections of the colon, chilled
- Faeces or large intestine contents
- Formalin-fixed tissues, including multiple sections of small and large intestine

Note: Tissues for histopathology need to be obtained from a freshly dead animal, as the gastrointestinal tract autolyses rapidly.

Faecal swabs are not suitable for *Brachyspira* culture, as they do not provide sufficient material and anaerobic conditions are compromised. Send samples chilled but not frozen within 24 hours of collection.

Tetanus (*Clostridium tetani*)

All mammals are susceptible to tetanus, though horses and lambs seem to be the most sensitive. Infection usually enters via a deep puncture wound or from dehorning and castration wounds. Clinical signs include muscle stiffness and tremor, whole-body rigidity, protrusion of the third eyelid, convulsions and death. There is no routine diagnostic test for tetanus. Diagnosis is based on clinical signs and history. A full range of specimens should be forwarded to eliminate other differential diagnoses.

Three-day sickness

See 'Bovine ephemeral fever' in this section.

Tick fevers of cattle (babesiosis, anaplasmosis)

Babesiosis is caused by *Babesia bovis* (most commonly) and *Babesia bigemina*. Signs include high fever, anaemia, jaundice, loss of condition and red urine. *B. bovis* infections often cause nervous signs, followed by coma and death.

Anaplasmosis is caused by *Anaplasma marginale*. Signs include rapid loss of condition, fever, marked anaemia and jaundice but no red urine.

Samples required

Live animals

- Thick and thin blood smears, preferably made from a peripheral site (e.g. tail tip)
- Clotted blood, EDTA blood
- Urine, chilled

Dead animals

- Peripheral blood smears
- Organ smears (brain, kidney, heart muscle, spleen, liver)
- Unpreserved kidney, heart, liver, brain and spleen, chilled
- Formalin-fixed tissues including kidney, heart, liver, spleen and brain

Under Queensland legislation, the presence of cattle ticks outside the cattle tick infested zone must be reported to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

See **Section 9** for more detailed information on the preparation of blood and tissue impression smears and advice on which animals to sample.

Toxaemic jaundice

See 'Copper poisoning' in this section.

Transit tetany

Transit tetany is characterised by staggering, recumbency, gut stasis, muscle spasms, coma and high mortality. It occurs where animals are transported long distances and are deprived of feed and water for long periods. It is most common in beef cows in late pregnancy, but recently calved cows, pregnant/lactating sheep and horses may also be affected. The specific cause is unknown; it may be an acute form of hypocalcaemia (and hypomagnesaemia). There is no typical post-mortem appearance, so samples should be submitted to rule out other causes of sudden death.

Samples required

- Clotted blood
- Ocular fluid, chilled

See also 'Hypocalcaemia' in this section.

Transmissible spongiform encephalopathy

Transmissible spongiform encephalopathies (TSEs) are not known to occur in Australia. The National Transmissible Spongiform Encephalopathies Surveillance Program is jointly funded by government and industry to demonstrate Australia's ongoing freedom from bovine spongiform encephalopathy and scrapie, and to provide early detection of those diseases should they occur. Live adult sheep and cattle with clinical signs consistent with TSEs (progressive neurological disease) may be eligible for testing.

Samples required

- Formalin-fixed brain (fixed whole in 10% buffered formalin in a container large enough to enable the brain to 'float')
- Unpreserved brain/spinal cord

For cattle brains, use at least 2 L of formalin. For sheep brains, use at least 1 L of formalin.

The brain should float with the cerebrum resting on the bottom so that the caudal brain stem (midbrain and medulla) is straight (not bent or twisted). Fix brains for at least 3 days at room temperature (do not chill or freeze) before sending them to the laboratory. Take care to avoid damaging the fixed brain during handling and transport.

For cattle, include a 2–3 cm length of unpreserved cervical spinal cord and/or medulla caudal to the obex. For sheep, include a 2–3 cm length of unpreserved cervical spinal cord and/or medulla caudal to the obex and the dorsal (top) third of the cerebellum.

Note: Remove only the dorsal third of the cerebellum. Removing more may damage the TSE standard site 2 (caudal cerebellar peduncles) and compromise histological evaluation. Refrigerate (4 °C) or freeze (–20 °C or –70 °C) the unpreserved samples as soon as possible. If submission to the laboratory could be delayed by more than 3 days, freeze unpreserved tissues.

For more detailed information, visit business.qld.gov.au.

TSEs are reportable diseases under Queensland legislation. Report any suspected cases to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

Trichomoniasis

Trichomoniasis is a venereal disease of cattle caused by the protozoan *Tritrichomonas foetus* and is characterised primarily by early foetal death and returns to service resulting in extended calving intervals. Abortions, usually in the first half of gestation, and pyometra may also occur. Bulls of all ages can remain infected indefinitely, but this is less likely in younger males. By contrast, most cows are free of infection within 3 months after breeding. However, immunity is not long-lasting and reinfection does occur. Transmission can also occur when the semen from infected bulls is used for artificial insemination. Diagnostic efforts are directed at bulls (preferably after at least 2 weeks sexual rest), because they are the most likely carriers. However, cows that have been pregnancy tested as empty, are known to have aborted, or have a uterine discharge, can also be sampled.

Samples required

Bulls

- Preputial samples, collected using Tricampers™ into sterile saline

Cows

- Vaginal mucus samples, collected using Tricampers into sterile saline

Infertility testing kits specifically designed for this purpose can be ordered from BSL. Detailed instructions for sample collection and handling are included in the kits.

Twin lamb disease

See 'Pregnancy toxaemia' in this section.

Tuberculosis

See 'Bovine tuberculosis' in this section.

Urea poisoning (ammonia toxicity)

Urea poisoning is most likely to occur when there is an abrupt change in the amount of urea consumed. Signs of poisoning include muscle twitching, grinding of the teeth, salivation, bloat, abdominal pain, frequent urination, forced rapid breathing, weakness, staggering, violent struggling and bellowing, and terminal spasms. In many cases, animals are found dead near the source of the urea supplement. Urea poisoning is difficult to confirm, since laboratory testing needs to demonstrate elevated levels of ammonia, which is volatile and easily lost from samples.

Samples required

- Plasma (not whole blood), spun off from blood collected into lithium heparin tubes, frozen immediately after collection
- Ocular fluid, frozen immediately after collection
- Rumen contents, frozen or preferably acidified (by adding 1 part of 0.2 N hydrochloric acid to 1 part of rumen fluid or adding 3–5 drops of battery acid to 20 mL of rumen fluid).

Note: For all these samples, use the smallest container in which they will fit (to minimise contact with air) with an airtight screw-top lid. Keep them frozen in transit.

Vibriosis

See 'Bovine campylobacteriosis' in this section.

Vitamin A deficiency

See 'Hypovitaminosis A' in this section.

Vitamin E deficiency

See 'White muscle disease' in this section.

Water deprivation

See 'Salt poisoning' in this section.

White muscle disease (nutritional myopathy)

White muscle disease is an acute, degenerative disease of cardiac and skeletal muscle caused by a dietary deficiency of selenium and/or vitamin E in young, rapidly growing calves, lambs and kids. It occurs when soils and diets are deficient in selenium. When cardiac muscle is primarily affected, animals may be found in respiratory distress, have cardiac arrhythmias, or be found dead. When skeletal muscle is primarily affected, there is stiffness of gait, weakness, and recumbency.

Samples required

- Formalin-fixed tissues including heart and skeletal muscle, affected and unaffected tissue

For selenium assay

- EDTA or lithium heparin blood (for GSH-Px)
- Unpreserved kidney, chilled

For vitamin E analysis

- Unpreserved liver (from freshly dead animal), frozen
- Serum/plasma, frozen (whole blood should not be frozen)

Note: Cover samples for vitamin E assay with aluminium foil to prevent exposure to light.

13 Investigating disease in poultry and birds

13.1 General information

- The first indication of disease may be a change in feed or water consumption or a drop in egg production. Observations of the sounds and activity of the flock may give some indication of the disease causing the problem.
- Obtain a complete history of the disease and all pertinent events leading up to the outbreak. Seek information on management factors that may have an effect on the diagnosis. A high percentage of 'disease' problems are non-infectious and related to management practices.
- When examining clinically affected birds, observe the general attitude of the birds as well as the clinical signs.
- Contact the laboratory before submitting whole birds for necropsy. Birds selected for necropsy should be representative of the presenting disease. Freshly killed affected birds are best. Send up to 10 birds <2 weeks of age and up to 5 birds >2 weeks of age.
- It is important to identify the most significant problems rather than becoming distracted by individual bird disorders. Look for patterns in pathology.
- Complete the specimen advice sheet and necropsy findings form fully. Include as much information as possible to deliver an accurate picture of the disease to the pathologist.
- Select specimens to cover the range of possible diseases associated with the syndrome seen. Refer to **Table 13.1** or contact the laboratory and ask to speak to the duty pathologist. It is better to take too many samples and discard those not required than to find that a sample important to the diagnosis is missing.

13.2 Samples by syndrome

Table 13.1 Samples by syndrome—poultry and birds

Syndrome	Birds for necropsy (live affected or freshly dead)	Fixed
Anaemia/ immunosuppression	Yes	Full range of organs* including thymus, spleen, bursa, bone marrow
Diarrhoea/wet litter	Yes	Full range of organs* including bursa, gastrointestinal tract (proventriculus, gizzard, duodenum, small intestine, colon, caeca)
Ill thrift/stunting	Yes	Full range of organs* including gastrointestinal tract, bursa, thymus
Lameness/ recumbency	Yes	Full range of organs* including brain, eye, peripheral nerve, bursa, any lesion, affected joints, tendons, muscles, bones
Neoplasia	Yes	Full range of organs* including brain, peripheral nerves, bursa, liver, spleen, kidney, ovary/testicle, proventriculus, gizzard, any lesion
Nervous signs	Yes	Full range of organs* including brain, spinal cord, peripheral nerves, skeletal muscle, proventriculus, gizzard
Production drop in layers (e.g. egg drop, reduced egg quality)	Yes	Full range of organs* including oviduct
Respiratory signs	Yes	Full range of organs* including head (transverse sections of nasal cavities and sinuses), larynx, trachea, lung, air sacs, lesion
Skin and feather conditions	Yes	Lesion
Sudden death/ increased mortality	Yes	Full range of organs*
Swollen head/ocular discharge/upper respiratory signs	Yes	Head (transverse sections of nasal cavities and sinuses), larynx, trachea, lung

* Full range of organs includes heart, lung, liver, spleen and kidney.

† *Mycoplasma* transport media is available on request from BSL.

14 Investigating disease in aquatic animals (finfish, crustaceans and molluscs)

14.1 Principles

As with terrestrial animals and wildlife, the diseases of aquatic animals are regarded as biosecurity events and can be biosecurity threats (disease emergencies). Certain aquatic animal diseases are listed as 'prohibited' and 'restricted' matter in the Biosecurity Act and must be reported to Biosecurity Queensland on 13 25 23 or to the emergency animal disease watch hotline on 1800 675 888.

The primary interest for the services of BSL is in farmed or commercial aquatic animals. Typically these are animals in the finfish, crustacean and molluscan groups, but more broadly any natural mass mortality of these aquatic animals would be investigated.

In this section, 'aquatic animals' refers to finfish, crustaceans and molluscs.

Two specific characteristics of aquatic animals influence the value of BSL services:

- **Aquatic animals are poikilotherms and autolyse rapidly after death, resulting in artefacts and loss of integrity of the specimen.**

For example, artefacts can begin to occur in fish gills as soon as 5 minutes after death. Live aquatic animals or specimens from freshly euthanased aquatic animals are required by the laboratory. In some circumstances, freshly euthanased fish or prawns may be transported packed with sufficient ice to maintain a suitable temperature while in transit to the laboratory. Always consult the duty aquatic pathologist before submitting such samples.

- **Many of the diseases of aquatic animals cannot be diagnosed from the clinical signs.**

For example, respiratory distress in finfish can be caused by environmental, parasitic, bacterial, fungal or viral causes, and laboratory analyses may be required to make a diagnosis.

Without a background in aquatic animal disease, it may be difficult to determine the best approach to take in an investigation. Contact the laboratory and consult the duty aquatic pathologist for advice on selecting specimens and sampling.

14.2 Collecting specimens

Key points

- Fish, prawns, crabs, oysters, scallops etc. decompose very rapidly after death. Send live animals or preserved specimens from freshly dead animals to the laboratory for the best results.
- Select aquatic animals from the population under investigation that represent the range of clinical signs seen. Provide at least 5 individuals from each pond, tank, cage, etc.
- Fully complete a specimen advice sheet.
- Consult the duty aquatic pathologist for advice on collection of samples.

14.3 Services offered

Aquatic diagnostic pathology services for commercial finfish, crustaceans and molluscs include necropsy, histopathology, bacteriology (including antibiotic sensitivity testing), virology, molecular diagnostics and parasitology. Analytical services for other disciplines including biochemistry, haematology and toxicology may be discussed with the duty aquatic pathologist.

14.4 Necropsy

Send live animals directly to the laboratory for a detailed necropsy. This allows the pathologist to choose specimens for tests to cover the range of likely diagnoses.

Organise appropriate transport of the aquatic animals. Packing in water and oxygen in sealed plastic bags ensures that aquatic animals survive transport to the laboratory. Bubble aerators in water can be enough to provide oxygen to the animals over short distances.

Provide a detailed history, including information on water quality and epidemiological data, and complete a specimen advice sheet.

Field necropsy

This guide does not include detailed information on the anatomy and necropsy method for any of the different species of finfish, crustaceans or molluscs. Please follow these general guidelines and consult the resources listed below for specific details.

Open the branchial chamber of the fish and collect external samples from the gills to identify the presence of parasites or bacteria. Scrape skin and gill mucus from the surface, then smear or wet-mount it and observe it directly under a compound microscope. Record the presence of any parasites, bacteria or fungi and any other findings on the specimen advice sheet.

Similarly, wet-mount and directly observe crustacean gills under a microscopic. Record your findings.

Label the samples clearly with the date, owner, animal number or pond/tank number (i.e. site of sampling), specimen type and any other relevant information. Record all details on the specimen advice sheet and necropsy findings form and additional sheets as required.

Key points

- Record all necropsy information on the necropsy findings form.
- Use appropriate sample containers.
- Label all samples with the date, owner, animal number, origin and specimen type.
- Use a waterproof marker to label samples.
- Ensure containers are tightly sealed and placed in a second sealable bag or container.
- Complete all forms fully, and include the date of collection.
- Package specimens properly for transport.
- Contact the pathologist by phone and/or email to advise that chilled fresh specimens are being sent.

Resources

- European Association of Fish Pathologists, *The fish necropsy manual*
- Queensland Department of Agriculture and Fisheries, 'Management of aquaculture'
- Tasmania Department of Primary Industries, Parks, Water and Environment, *Guidelines and procedures manual for field sampling of fish for disease investigation and health monitoring*
- Australian Department of Agriculture and Water Resources, *Collection and submission of samples for investigation of diseases of fin fish*
- The Food and Agriculture Organization of the United Nations, *Asia diagnostic guide to aquatic animal diseases*

14.5 Histopathology

Histopathology remains the most common, and often most useful, laboratory analysis for diagnosis of aquatic animal disease.

Tissues must come from a freshly euthanased aquatic animal.

Finfish

Euthanase the moribund or live fish. Perform a complete external examination (including examination of skin and gill smears) and an internal examination before fixation.

Larvae

Preserve 25–50 individuals. Fine-mesh dip nets are useful when transferring and concentrating the larvae.

Juveniles and adults

Preserve 5–10 diseased individuals per tank, cage or pond. An additional 5–10 healthy individuals may be needed for comparison.

For finfish <4 cm total length, cut open the abdominal cavity, then displace the swim bladder and visceral mass away from the caudal kidney.

For finfish >4 cm total length, perform a full dissection. Remove individual organs and place them in fixative. Trim the organs to <1 cm to ensure complete fixation. Include gills, eye, heart, brain, head and caudal kidney, liver, spleen, stomach, pyloric caeca, anterior intestine, rectum, pancreas-omentum, gall bladder, swim bladder, gonad, skin, lateral line, skeletal muscle and any gross lesion.

Place the organs/tissues in fixative, ensuring that samples are immersed in at least 10 times their volume of fixative. Suitable fixatives include:

- 10% buffered neutral formalin
- 10% seawater formalin
- Bouin's fixative.

Crustaceans

Complete an external examination and examine the gill clips before fixation. Microscopic examination of wet-mounted larvae and post-larvae can be valuable to establish a diagnosis; do this in addition to histology.

Euthanase the moribund or live crustacean.

Larvae

Preserve 200–500 individuals. Use fine-mesh (50–200 µm) polyester screens to concentrate the larvae.

Post-larvae

Preserve 150–300 individuals. Use fine-mesh aquarium fish dip nets or sieves to concentrate the post-larvae.

Juveniles and adults

Preserve 5–10 diseased individuals per tank or pond. An additional 5–10 healthy individuals may be needed for comparison.

For juveniles (>3 cm length), inject fixative into the middle of the thorax (in particular the hepatopancreas) and the abdomen (muscle, hindgut, ventral nerve cord and gonad), then open the shell to facilitate entry of the fixative.

For adults (>15 cm length/width—adult prawns, crabs, crayfish etc.), perform a full dissection, then remove parts of the body or individual organs (<1 cm thick).

Place the organs/tissues in fixative, ensuring that the samples are immersed in at least 10 times their volume of fixative. Suitable fixatives include:

- Davidson's fixative
- 10% seawater formalin
- 10% buffered neutral formalin.

Molluscs (bivalve)

The typical commercial mollusc in Queensland is a bivalve and the following instructions are most relevant to that class of aquatic animals. If the investigation involves abalone or other gastropods or cephalopods (squids, octopus, nautilus etc.), please contact the aquatic pathologist to discuss sampling and the specimens to submit for diagnostic examinations.

Microscopic examination of wet-mounted larvae and early spat can be valuable to establish a diagnosis; do this in addition to histology.

Euthanase the moribund or live mollusc.

Larvae

Preserve 200–500 individuals. Use fine-mesh (50–200 µm) polyester screens to concentrate the larvae.

Spat

Preserve 150–300 individuals. Use fine (2–10 mm) sieves or fine-mesh aquarium fish dip nets to concentrate the spat.

Juveniles and adults

Preserve 5–10 diseased individuals per tank or line. An additional 5–10 healthy individuals may be needed for comparison.

For juveniles (1–2 cm length), cut a ‘V’ into the outer shell valves to allow rapid fixation of the internal visceral mass.

For larger juveniles (>2 cm length) and adults, remove the visceral mass from the shell valves. Make several scalpel blade cuts into the visceral mass or cut it into pieces no thicker than 1 cm.

Place the organs/tissues in fixative, ensuring that the samples are immersed in at least 10 times their volume of fixative. Suitable fixatives include:

- 10% seawater formalin
- 10% buffered neutral formalin
- Davidson’s fixative.

14.6 Bacteriology

Bacteriology is needed to confirm the diagnosis of bacterial diseases. Laboratory isolation of the putative pathogen also allows antibiotic sensitivity testing.

Use aseptic technique when sampling aquatic animals for bacteriology. Many bacterial pathogens are opportunistic and are normally present in the aquatic environment and on the surface of the aquatic animal or are part of the gut biota. To get meaningful bacteriological results, avoid contamination of samples from the animal surface or gut.

Live aquatic animal

Send the entire aquatic animal, freshly euthanased, in a sealed plastic bag and packed in ice to maintain a temperature of 4 °C or lower during transport. Avoid direct contact of the specimens with the ice. The specimen must reach the laboratory within 48 hours.

Necropsy

In finfish, generally sample the heart, kidney or spleen. Depending on the disease suspected, also sample skin ulcerations, gills, liver, brain or eye and any gross lesions.

Using aseptic technique, take swabs into microbiological transport media, or sample solid organs and fluids into separate sterile containers. Maintain these at 4 °C during transit.

Alternatively, aseptically sample an organ or tissue with a sterile loop or probe, then directly apply the loop or probe to an appropriate agar plate and ‘streak’ it across the plate.

For skin ulcers on fish, cauterise the edge of the ulcer and collect an inoculum from a scale bed or hypodermis at the ulcer’s leading edge using a swab, sterile loop or probe.

Suitable agar plate media include:

- for freshwater samples—blood agar, trypticase soy agar and freshwater Ordal's medium
- for marine samples—marine agar, blood agar, thiosulphate-citrate-bile salts-sucrose agar and seawater Shieh's medium.

For crustaceans or molluscs, haemolymph can be used to demonstrate bacteraemia. Disinfect the surface of the shell or mantle, then collect haemolymph using a sterile needle and syringe.

14.7 Molecular diagnostics

A number of molecular detection tests for viruses and bacteria of aquatic animals are available at BSL. Contact BSL or visit business.qld.gov.au to access the current list of tests.

Samples can be forwarded to external laboratories that offer tests for specific agents not available at BSL.

Molecular tests are sensitive, so avoid cross-contamination by using an appropriate aseptic technique. Use separate clean instruments or immerse instruments in a chlorine solution and rinse off excess chlorine in water between uses.

Live aquatic animal

Submit the entire aquatic animal, freshly euthanased, in a sealed plastic bag and packed in an insulated container with ice to maintain a temperature of 4 °C or lower during transit. Ensure the container is sealed to prevent leakage. The specimens must reach the laboratory within 48 hours.

Necropsy

The specific tissue required for molecular testing depends on the disease diagnosis or test required.

Place the tissues selected for testing in separate vials and freeze them as soon as possible for transport. Each sample should be no larger than a 2–3 mm cube. Alternatively, preserve tissues in 90% ethanol or an ammonia sulphate preservative medium (preservative to tissue ratio of 10:1).

Transport all unfixed samples chilled at or below 4 °C.

Crustacean or molluscan larvae can be pooled. The number of individuals per pool depends on animal size and total tissue mass. Contact the duty aquatic pathologist for guidance.

Keep samples frozen, in ethanol or in preservative medium prior to transport and testing.

14.8 Virology

Continuous cell lines for virus isolation are only available for finfish viruses. Contact the duty aquatic pathologist to discuss specific options to diagnose a viral disease or to isolate a specific finfish virus. Specific cell lines are used to screen for specific viral pathogens, e.g. SSN-1 for nodavirus. These cell lines need to be cultured up to a confluent state before inoculation with the sample. Therefore, virus isolation takes several weeks.

Live aquatic animal

Submit the entire aquatic animal, freshly euthanased.

Place the animal in a sealed plastic bag and pack it in an insulated container with ice to maintain a temperature of approximately 4 °C, but do not freeze it. Ensure the container is sealed to prevent leakage. The specimens must reach laboratory within 48 hours.

Necropsy

Depending on the disease to be diagnosed or the virus to be isolated, select tissues (approximately 1 g each) from the kidney, spleen, liver, pyloric caeca/pancreas, heart, brain and/or gills. Contact the duty aquatic pathologist for advice.

Place the tissues in VTM and transport them to the laboratory chilled. Do not freeze them.

Appendixes

Appendix 1 Clinical biochemistry reference values

Component	Units	Cattle		Sheep		Goat		Horse		Pig		Camelid	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Calcium	mmol/L	2.1	2.8	2.2	3.0	2.1	2.8	2.8	3.5	2.1	2.8	2.0	2.7
Magnesium	mmol/L	0.65	1.3	0.65	1.3	0.65	1.3	0.65	1.3	0.65	1.3	0.9	1.5
Potassium	mmol/L	3.5	4.5	3.9	5.4	3.5	6.7	2.4	4.7			3.7	6.2
Sodium	mmol/L	135	157	139	152	142	155	132	146			148	158
Total protein	g/L	60	85	60	80	60	80	60	90	60	95	47	73
Albumin	g/L	30	45	30	45	25	45	25	40	30	50	29	50
Globulin	g/L	30	45	30	40	30	40	30	45	30	40	11	30
A/G ratio		0.7	1.5	0.7	1.6	0.7	1.3	0.4	0.9				
Creatinine	μmol/L	40	220	40	220	20	220	40	170	40	250	97	283
Urea	mmol/L	2.0	8.5	2.0	8.0	3.5	10	4.0	9.5	3.0	8.5	3.2	11.8
Alkaline phosphatase	IU/L	25	200	30	200	30	200	40	250			31	779
Total bilirubin	μmol/L	1	10	1	7		<5	3	43	1	8		>5
Conjugated bilirubin	μmol/L	1	6								<4		
GGT	IU/L	10	35	30	70	25	70	0	15		<100	6	29
GLDH	IU/L		<40	0	20	0	20		0	20			<20
AST	IU/L	30	170	30	140	20	120	120	340	20	110	128	450
CPK	IU/L	10	200	10	190	30	190	30	190		<1000	8	89
Glucose	mmol/L	2.8	5.5	2.8	5.5	2.8	5.5	3.3	7.0			4.6	8.9
β-hydroxybutyrate	mmol/L	0	0.9	0	0.9	0	0.9						
D-lactate	mmol/L		<0.4		<0.4		<0.4						
Inorganic phosphorus	mmol/L	1.13	2.25	1.13	2.25	1.13	2.25	0.97	1.93	1.13	3.90	0.90	3.20
Serum copper	μmol/L	9.4	24.0	7.9	31.5	11.0		8.0	17.0	20.5	47.2		
GSH-Px	IU/g Hb	60	440	70	1000	60	750	50	220	40	200	70	300
Liver copper	mg/kg	25	100	25	100	25	150					25	100
Kidney copper	mg/kg	4.0	6.0	4.0	5.5	3.0	6.0					3.0	5.0

Appendix 2 Haematology reference values

Component	Units	Cattle		Sheep		Goat		Pig		Horse		Chicken	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Erythrocyte count	cell/pL	4.8	10.0	8.0	14.0			5.0	8.0	6.5	12.0	2.2	3.6
Haemoglobin	g/dL	9.50	14.50	10.00	15.00	9.00	14.00	10.00	15.00	11.00	19.00	7.50	11.00
Haematocrit	%vol	28	46	30	45	25	38	32	45	32	52	25	35
Mean cell volume	fL	39	65	30	45			50	60	40	60	100	120
Mean cell haemoglobin	pg	12.0	23.0	8.0	12.0			17.0	21.0	12.0	20.0	33.0	40.0
Mean cell haemoglobin concentration	g/dL			31.0	34.0			30.0	34.0				
Leucocyte count	cell/nL	4.0	20.0	4.0	12.0	4.0	13.0	11.0	22.0	5.5	12.0	10.0	25.0
Segmented neutrophil count	cell/nL	1.00	7.50	1.00	4.00	1.20	5.00	3.00	8.00	2.70	6.00		
Lymphocyte count	cell/nL	1.50	17.00	2.00	8.00	2.00	8.00	4.30	13.50	1.50	6.00		
Thrombocyte count	cell/nL	100	800	250	750			200	500	100	350		
Fibrinogen	g/dL	0.3	0.7	0.1	0.5	0.1	0.4	0.1	0.5	0.1	0.4	0.1	0.4

Component	Units	Llama		Camel		Alpaca	
		Min.	Max.	Min.	Max.	Min.	Max.
Erythrocyte count	cell/pL	9.90	17.70	6.50	12.00	9.10	13.80
Haemoglobin	g/dL	11.50	19.50	8.00	15.00	10.40	17.00
Packed cell volume	%vol	25	46	24	36	24	36
Mean cell haemoglobin	pg	9.8	12.7	26.0	40.0	22.0	29.0
Mean cell haemoglobin concentration	g/dL	37.7	49.0	12.0	22.0	10.6	12.7
Leucocyte count	cell/nL	8.9	22.0	8.0	20.0	5.7	32.9
Segmented neutrophil count	cell/nL	4.60	16.00	3.00	25.00	2.10	9.50
Lymphocyte count	cell/nL	1.20	7.80	5.70	32.90	0.90	4.40
Monocyte count	cell/nL			<2.10		<2.10	
Eosinophil count	cell/nL			<2.20		<2.20	

Appendix 3 Suggested field necropsy kit

The equipment listed here is sufficient to carry out a thorough necropsy of any species of livestock and to collect suitable specimens for submission to the Biosecurity Sciences Laboratory.

Basic equipment for necropsy

- Knives (skinning and boning)
- Steel
- Scissors—large and small
- Large rat-tooth forceps
- Scalpel handle and blades
- Bone-cutters (large tree-logging shears are good)
- Butchers saw or hacksaw
- Tomahawk or axe
- Gloves
- Overalls
- Boots

Equipment for specimen collection

- Book of specimen advice sheets (Form A)*
- Book of necropsy findings forms (Form B)*
- Insulated container(s) with cardboard outer for specimens
- Frozen cooler brick(s)
- Spatula for heating and searing organs
- Gas cylinder and burner
- Matches or gas-lighter
- Scalpel blades and handles for incising organs
- Sterile swabs
- Bacterial (Amies) and viral transport media (stored at 4 °C)
- Clean glass slides
- Slide holders and tissues for cleaning slides
- Oil pen and pencil for labelling
- Needles and syringes
- Assortment of large screw-top containers for fixed tissues, rumen contents etc.
- Sterile 5 mL and 25 mL screw-top containers for fresh tissues, body fluids and ocular fluids
- Plain (red-top) and EDTA (purple-top) tubes for blood collection
- Large plastic container or bucket with tightly sealing lid for fixing brains
- Quantity of 10% formalin
- Bottle of reagent strips for urinalysis
- Various sized zip-lock bags (to be used only for specimen containers, blood tubes and paperwork)

* Also available for download from business.qld.gov.au.

Appendix 4 Equipment available from Biosecurity Sciences Laboratory

Note: All forms are available from business.qld.gov.au.

Laboratory forms

- Specimen advice sheet (Form A), available as a book
- Necropsy findings form (Form B), available as a book
- Sample numbering sheet
- Equipment request form
- Cattle herd reproductive history form
- Resistant tick advice sheet

Information sheets

- Completion of specimen advice sheets, also included on cover of book of Form A
- Policy on specimens accepted for testing and service fee exemptions
- Transporting samples to BSL
- Submitting samples for serological investigation of infertility and abortion in cattle
- Tricamper sampling
- *Campylobacter* ELISA sampling
- Schedule of fees
- Advice on sampling and testing for Johne's disease in cattle
- Serological testing for Johne's disease in goats

Media/swabs

- Frey's transport media (*Mycoplasma* culture)
- CEM transport swab (*Taylorella equigenitalis* culture)

Kits (all with printed instructions)

- *Campylobacter* culture kit (bulls)
- *Tritrichomonas* PCR kit (diagnostic and health testing)
- *Tritrichomonas* culture kit (health testing)
- *Campylobacter* ELISA kit (cow herd test)
- P-screen kit (for phosphorus levels in cattle)



Index

1

1080 poisoning, 119

A

abortion/stillbirth, 72
 in cattle, 78
 in horses, 78
 in pigs, 78, 117
 in sheep and goats, 78
acaricide resistance testing, 44
 sample collection, 45
acetonaemia, 103
acetylcholinesterase
 brain assay, avian, 60
 serum/plasma assay, 60
actinobacillosis, in cattle, 79
Actinobacillus seminis, 112
actinomycosis, in cattle, 79
acute febrile disease, 72
aflatoxicosis, 79
agar gel immunodiffusion test, 48
AGID test, 48
Akabane disease, 79
ammonia
 ocular fluid assay, 21
 rumen contents assay, 21
 toxicity, 124
anaemia, 72
 avian, 127
 haematology, 28
anaerobic bacteria, culture, 34
Anaplasma marginale, see 'anaplasmosis'
anaplasmosis, 80, 121; see also 'tick fever'
 diagnosis, 53, 55
anthrax, 80
antibiotic sensitivity testing, 37
aquatic animals
 bacteriology, 132
 histopathology, 130
 molecular diagnostics, 133
 necropsy, 129
 specimen collection, 128
 virology, 66, 133
aqueous humour, sampling, 18
arsenic poisoning, 81
 interpretation, 60
 samples, 59
arthritis, 81
aspirates, cytology of, 29

ataxia, 72

Australian bat lyssavirus, 82

B

Babesia bigemina, see 'babesiosis'
Babesia bovis, see 'babesiosis'
babesiosis, 82, 121; see also 'tick fever'
 diagnosis, 53, 55
bacteriology, see 'microbiology'
big knee, 88
biochemistry profile, 19
birds
 disease investigation, 126
 syndromes, 127
black disease, 82
blackleg, 83
bloat, 83
blood sampling
 for clinical biochemistry, 17
 for haematology, 24
 for serology, 46
blood smears
 for haematology, 24
 for tick fever, 54
blue-green algae poisoning, 93
bone marrow smears, 30
botanical identification of plants, fungi and cyanobacteria, 62
botulism, 83
 avian, 127
bovine campylobacteriosis, 84
 serology, 50
bovine enzootic haematuria, 86
bovine ephemeral fever, 85
bovine leucosis, 95
bovine malignant catarrh, 106
bovine pestivirus infection, 85
 BVDV AGID, 48
bovine tuberculosis, 86
bovine virus diarrhoea, 85
Brachyspira, 120
 bacterial culture, 34
bracken fern poisoning, 86
brain, histopathology, 32
brain smears, for tick fever, 53, 54

- brucellosis, 87
 - Brucella abortus*, 88
 - Brucella melitensis*, 88
 - Brucella ovis*, 87, 111
 - serology, 49
 - Brucella suis*, 87
- Burkholderia pseudomallei*, 108
 - serology, 49
- C**
- calcium
 - hypocalcaemia, 99
 - serum/plasma assay, 21
- calculi, analysis, 22
- Campylobacter* ELISA, 50
- campylobacteriosis, in cattle, 84
- caprine arthritis encephalitis, 88
- caprine retrovirus infection, 88
- caseous lymphadenitis, 88
- cerebrospinal fluid, sampling, 32
- cestodes
 - faecal egg count interpretation, 42
 - identification, 41
- CFT, 49
- cheesy gland, 89
- chemical poisoning, see 'toxicology'
- chlamydial infections, 89
- clinical biochemistry, 17
 - blood sampling, 17
 - ocular fluid sampling, 18
 - reference values, 137
 - standard mammalian profile, 19
 - tests available, 19
- clostridial disease, 89
 - black disease (*Clostridium novyi* type B), 82
 - blackleg (*Clostridium chauvoei*), 83
 - botulism (*Clostridium botulinum*), 83
 - enterotoxaemia (*Clostridium perfringens*), 94
 - malignant oedema (*Clostridium septicum*), 107
 - tetanus (*Clostridium tetani*), 121
 - toxin assay, 35
- cobalt, biochemical assay, 19
- coccidia
 - identification, 41
 - oocyst interpretation, 43
- coccidiosis, 90
- colibacillosis, 90
- complement fixation test, 49
- congenital abnormalities, 72
- contagious ecthyma, 119
- contagious equine metritis, 91
 - bacterial culture, 35
- contagious ophthalmia, 101
- contagious pustular dermatitis, 119
- copper
 - deficiency, 108
 - poisoning, 60, 91
 - serum/plasma assay, 21
- coronavirus, 117
- CPK, serum/plasma assay, 21
- creatine phosphokinase, serum/plasma assay, 21
- cryptosporidiosis, 93
- CSF, sample collection, 32
- cyanide
 - interpretation, 61
 - poisoning, 92
 - samples, 59
- cyanobacteria
 - poisoning, 93
 - sample collection, 64
- cytology, 29
 - sample collection, 29
 - urine sedimentation examination, 30
- D**
- dermatophilosis, 93
 - bacterial culture, 35
- dermatophytosis, 94
 - fungus culture, 36
- diamond skin disease, 96
- diarrhoea, 72
 - avian, 127
 - neonatal, in calves, 110
- D-lactate, serum/plasma assay, 22
- downer cow, 76
- E**
- egg drop, in layers, 127
- ELISA, 50
- encephalomyocarditis virus infection, 94
- enterotoxaemia, 94
- enzootic bovine leucosis, 95
 - haematology, 28
- enzootic pneumonia, in pigs, 109
- enzyme-linked immunosorbent assay, 50
- eosinophilia, interpretation, 28
- epg, see 'faecal egg counts'

ephemeral fever, 85
epididymitis, in rams, 112
equine herpesvirus abortion, 78
equine infectious anaemia, 95
erysipelas, 96
external parasites, 40

F

faecal egg counts
 interpretation, 41
 larval differentiation, 42
 significance table, 42
faeces
 for bacterial culture, 35
 for parasitology, 39
 phosphorus assay, 23
Fasciola hepatica, 105
 epg interpretation, 43
 sample collection, 40
fascioliasis, 105
fever, 72
fibrinogen, interpretation, 29
fixatives, for histopathology, 33
fleas, 40
flukes, identification, 40
fluoroacetate poisoning, 119
fluorosis, 61, 96
foot-and-mouth disease, 96
footrot, 97
found dead, 76
fungal infections, 110
 dermatophytosis, 94
 samples for microbiology, 36
fungi, identification, 64

G

gamma glutamyltransferase, serum/plasma assay, 22
gastrointestinal signs, 72
generalised glycogenosis, 115
genital lesions, 72
GGT, serum/plasma assay, 22
Glasser's disease, 98
glucose, sample collection, 20
glutathione peroxidase, 22
grain overload/poisoning, 118
grass tetany, 100
GSH-Px, 22

H

haematology, 24
 anaemia, 28
 blood smears for, 24
 of camelids, 26
 differential white cell counts, 24
 fibrinogen, 29
 of goats, 26
 inflammatory leucogram, 27
 interpretation, 27
 reference values, 138
 sample collection, 24
 standard haematology profile, 26
 stress leucogram, 27
haemonchosis, 98
haemorrhages, 72
helminth
 faecal egg count interpretation, 42
 identification, 40
 significance table, 42
 total worm counts, 43
Hendra virus infection, 98
hepatosis dietetica, 109
histopathology, 31
 of aquatic animals, 130
 of brain and spinal cord, 32
 fixatives, 33
 of gastrointestinal tract, 32
 sample collection and preservation, 31
 sample submission, 33
hypocalcaemia, 99
hypomagnesaemia, 100
hypophosphataemia, 113
hypovitaminosis A, 100

I

ill thrift, 74
 avian, 126
immunosuppression, avian, 127
increased mortality, avian, 127
infectious balanoposthitis, 100
infectious bovine rhinotracheitis, 100
infectious keratoconjunctivitis, 101
infectious necrotic hepatitis, 82
infectious pustular vulvovaginitis, 100
infertility, 74
 in cattle, 84, 123
 in pigs, 116
 serological investigation, 51
internal parasites, 40
intestinal spirochaetosis, 121

J

jaundice, 74
 Johne's disease, 101
 joint ill, 81

K

ketosis, 103
 biochemical assay, 19

L

lambling sickness, 116
 lameness, 74
 avian, 127
Lawsonia intracellularis, 116
 lead
 maximum levels, 61
 poisoning, 59, 61, 103
 leptospirosis, 104
 serology, 50, 51
 lice, 40
 lingering death, 76
 listeriosis, 105
 liver fluke, 105
 epg interpretation, 43
 lumpy jaw, 79
 lumpy skin disease, 106
 lumpy wool, 93
 lungworm, 40
 larvae, interpretation, 43
 lymphadenopathy, 74
 lymphocytosis, interpretation, 28

M

maggots, 40
 malignant catarrhal fever, 106
 malignant oedema, 107
 manganese, biochemical assay, 19
 mastitis, 107
 microbial culture, 35
 melioidosis, 108
 serology, 49
 mercury, samples for toxicology, 59
 microbiology, 34
 antibiotic sensitivity, 37
 aquatic animals, 132
 interpretation of results, 37
 sample collection, 34

 sample submission, 36
 milk fever, 99
 mineral deficiencies, 108
 biochemical assays, 19
 mites, 40
 molecular diagnostics, 38
 aquatic animals, 133
 sample collection, 38
 sample submission, 38
 molybdenum, biochemical assay, 19
 monocytosis, interpretation, 28
 mucosal disease, 85
 mulberry heart disease, 109
 musculoskeletal signs, 74
Mycoplasma culture, 34
 mycoplasmosis, 109
 mycoses, 110
 fungal culture, 36
 mycotoxins, 109; see also 'aflatoxicosis'

N

nasal discharge, 74
 necrotic enteritis, in pigs, 116
 nematodes
 faecal egg count interpretation, 42
 identification, 40
 larval differentiation, 42
 neonatal diarrhoea of calves, 110
 neoplasia, avian, 127
 neosporosis, 111
 serology, 50
 nervous signs, 74
 avian, 127
 neutrophilia, interpretation, 27
 nitrate/nitrite poisoning, 59, 62, 111
 plant sampling, 59
 nutritional biochemistry, 23
 nutritional myopathy, 124

O

ocular discharge, avian, 127
 ocular fluid
 sampling for clinical biochemistry, 18
 sampling for toxicology, 18
 oedema, 74
 oedema disease, 112
 oral lesions, 74

orchitis and epididymitis, in rams, 112
orf, 119
organophosphate pesticide poisoning, 59, 60
oxalate poisoning, 59, 62, 113

P

paramphistomes, egg interpretation, 43
parasites, see 'parasitology'
parasitology, 39
 faecal samples, 39
 sample collection, 39
 sample submission, 41
paratuberculosis, 101
parturient paresis, 99
PCR
 sample collection, 38
 sample submission, 38
perinatal mortality, 74
phosphorus, serum/plasma assay, 21, 23
phosphorus deficiency, 113
 faecal assay, 23
 interpretation of results, 23
 serum/plasma assay, 21
photosensitisation, 114
pinkeye, 101
plant collection for botanical identification, 63
plant poisoning, 114
poisoning, see 'toxicology'
poisonous plants, sample collection and submission, 59, 63
polioencephalomalacia, 115
polyarthritis, 81
Pompe's disease, 115
porcine circovirus infection, 115
porcine intestinal adenomatosis, 116
porcine parvovirus, 116
porcine proliferative enteropathy, 116
poultry
 disease investigation, 126
 syndromes, 127
pregnancy toxemia, 116
preputial discharge, 72
production drop in layers, 127
proliferative haemorrhagic enteropathy, 116
prussic acid poisoning, 92
pulpy kidney, 94

R

rain scald, 93
recumbency, 76
 avian, 127
regional ileitis, 116
reproductive failure
 abortion/stillbirth, 72, 78
 infertility, 74
 in pigs, 117
respiratory signs, 76
 avian, 127
ringworm, 94
rotavirus, 117
ruminal acidosis, 118
 D-lactate, serum/plasma assay, 22

S

salivation, 74
salmonellosis, 118
salt poisoning, 59, 62, 119
scabby mouth, 119
selenium
 deficiency, 108, 109, 125
 samples for toxicology, 59
 whole blood assay, 22
serology, 46
 AGID test, 48
 bovine infertility investigations, 51
 CFT, 49
 ELISA, 50
 interpretation of results, 48
 MAT, 50
 melioidosis, 49
 sample collection
 blood, 46
 ear notches, 47
 vaginal swabs, 47
 sample labelling, 47
 sample submission, 47
 VNT, 51
skin and feather disorders, 127
skin lesions, 76
 avian, 127
snails, identification, 39
sodium monofluoroacetate poisoning, 119
sodium toxicosis, 119
sporadic bovine encephalomyelitis, 120
stomach fluke, egg interpretation, 43
strangles, 120

- strychnine poisoning, 120
 - stunting, avian, 127
 - sudden death, 76
 - avian, 127
 - swine dysentery, 121
 - swollen head, avian, 127
 - syndromes, avian, 127
 - syndromes of cattle, sheep, goats, pigs, camelids, deer and horses, 72
- T**
- tapeworm
 - faecal egg count interpretation, 42
 - identification, 41
 - tetanus, 121
 - Theileria orientalis*, 56
 - three-day sickness, 85
 - tick fever, 53, 121
 - blood smears, 54
 - brain smears, 54
 - interpretation of smears, 55
 - organ smears, 55
 - sample collection, 53
 - vaccines, 57
 - ticks, 40
 - acaricide resistance, 44
 - total worm counts, 43
 - interpretation, 44
 - sample collection, 43
 - toxaemic jaundice, 91
 - toxic plants, 114
 - toxicology, 58
 - interpretation of results, 60
 - sample collection, 58, 59
 - tests available, 59
 - transit tetany, 122
 - transmissible spongiform encephalopathy, 122
 - trematodes, identification, 40
 - trichomoniasis, 123
 - TSE, 122
 - tuberculosis, bovine, 86
 - twin lamb disease, 116
- U**
- upper respiratory signs, avian, 127
 - urea
 - ammonia assay, 21
 - poisoning, 59, 62, 124; see also 'ammonia'
 - urinalysis, 22
 - urolithiasis, 22
- V**
- vaginal discharge, 72
 - vaginal swabs, for serology, 47
 - vibriosis, 84
 - virology, 65
 - crustaceans and molluscs, 66, 133
 - sample collection, 65
 - sample submission, 67
 - virus isolation
 - arbovirus, 65
 - avian, 65
 - finfish, 66
 - sample collection, 65
 - virus neutralisation test, 51
 - vitamin A
 - deficiency, 100
 - liver assay, 21
 - vitamin E
 - deficiency, 124
 - avian, 127
 - liver assay, 21
 - vitreous humour, sampling, 18
 - VNT, 51
- W**
- water deprivation, 119
 - weight loss, 74
 - wet litter, avian, 126
 - white muscle disease, 124
 - wooden tongue, 79
- Z**
- zinc, biochemical assay, 19

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