Animal & Plant Health Agency

Livestock & Wildlife Disease Diagnosis at APHA

Guidance on sample and test selection

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Introduction

Background

This booklet is intended to assist veterinary practitioners with sample and test selection for diagnosis of common clinical presentations in livestock and wildlife. It has been compiled by Animal and Plant Health Agency (APHA) Species Expert Group members and other veterinary colleagues.

The main remit of Government-funded scanning surveillance at APHA is the detection of new and (re)emerging livestock disease threats. The types of threat include novel pathogens, novel diseases, novel presentations of known diseases, occurrence of diseases in novel species, strains or serotypes which are new or exotic to Great Britain; marked changes in endemic disease trends; rare, newly emerging or concerning antimicrobial resistances and threats to food safety or public health. Scanning surveillance findings are reported monthly in the Veterinary Record and quarterly on line: http://apha.defra.gov.uk/vet-gateway/surveillance/reports.htm

APHA funded provision of post mortem examination (PME) within the APHA scanning surveillance network in England and Wales includes the APHA Veterinary Investigation Centres (VIC), APHA Lasswade (poultry only) and non-APHA partner post mortem providers (SRUC VS, University of Bristol, Royal Veterinary College, University of Surrey and Wales Veterinary Science Centre, Aberystwyth). Further details are available at this link http://apha.defra.gov.uk/postcode/pme.asp

In order to maximise the surveillance value of this service, for which Defra provides a significant level of financial support, APHA requires every submission to be accompanied by a fully completed submission form available from this link: http://apha.defra.gov.uk/vet-gateway/surveillance/forms.htm. Species-specific forms are available.

Please fully complete the submission form and send with the submitted samples or animals. Scanning or emailing, the submission form when sending animals for post mortem examination is also acceptable, once there has been discussion with a Veterinary Investigation Officer (VIO), and the animals are accepted for post mortem examination. Similar information is needed by non-APHA partner post mortem providers.

Postal samples should be sent to APHA Penrith VIC or APHA Starcross VIC – addresses here: http://apha.defra.gov.uk/vet-gateway/surveillance/diagnostic/national-network.htm

For a list of available tests, their prices and other details, please refer to the current Disease Surveillance Price List:
https://science.vla.gov.uk/Tests/Default.aspx?SiteName=DST

Veterinary staff at the APHA VICs can give advice where the details in this guidance do not provide sufficient information, or where assistance is needed to investigate complex, unusual or problematic outbreaks of disease, even if you do not send samples to APHA. Veterinary staff at APHA VICs and Lasswade should also be contacted directly to discuss submissions for post mortem examination. VIC Contact Details: http://apha.defra.gov.uk/vet-gateway/surveillance/diagnostic/national-network.htm

Veterinary Investigation Diagnosis Analysis (VIDA)

The surveillance information you provide on the submission form is recorded in the VIDA database, together with any diagnosis reached. VIDA is a national database, providing analysis of all diagnostic submissions to APHA, SRUC Veterinary Services Disease Surveillance Centres and the network of non-APHA partner PME providers. Diagnoses follow strict criteria. VIDA allows monitoring of diagnoses, clinical syndromes and disease trends and epidemiological features associated with these. Submissions in which a diagnosis is not reached (DNR) after reasonable testing are also scrutinised to determine whether they provide any evidence of a new or re-emerging threat. The Species Expert Groups analyse and report these VIDA data.

Submission of animals for post mortem examination

Whilst submission of animals for post mortem examination (PME) remains a key part of surveillance, these are costly and, as Government funding has reduced in recent years, it is all the more important that the types and numbers of animals submitted are representative, of suitable quality, and that PME is the most appropriate means of investigating the disease problem. It is therefore essential that, if considering submission of animals for diagnostic PME to APHA, you speak to the designated PME provider for the premises from which the animals will be submitted. This can be found using the on-line postcode search tool: http://apha.defra.gov.uk/postcode/pme.asp. You will need to know the clinical history of the case with the date and estimated time of death of animal(s) (if there is mortality) to be submitted.

Selection criteria for post mortem examination

- Animals dead for more than 24 hours are likely to be of less diagnostic value and will usually only be accepted after careful consideration of factors likely to influence diagnostic value including storage temperature since death.
- Animals dead for more than 48 hours will not be accepted.
- Carcases which have been frozen may not be accepted since this limits their diagnostic value.
- In outbreaks, a maximum of three mammalian and 5-10 bird carcases may be submitted together from a single disease incident on each farm.
- Sometimes the clinical history indicates that diagnosis is best undertaken in the first instance by submission of samples rather than animals for PME.

Information in this guidance document provides general and species-specific guidelines on diagnosis of common disease presentations in livestock and selection of the most appropriate carcase or non-carcase material for different disease conditions.

Submission of live animals for post mortem examination

Occasionally it is advisable to submit live animals for clinical and post mortem examination, for example, investigations of enteric or nervous disease. Submission of a combination of live affected and freshly dead birds is often appropriate for flock health investigations in poultry and game birds. However, submission of a live animal or bird must be discussed with veterinary staff at the relevant PME provider within the APHA network at the same time that the PME is agreed, and is not possible where an APHA-funded carcase collection service is being used as they are not able to transport live animals. The decision must take account of the current welfare in transport legislation in England, Scotland and Wales. To ensure the wellbeing of any live animals, the private veterinary surgeon should agree to its transport and ensure supervision for the

journey in line with the current legislation which covers, inter alia, the transport of animals for non-commercial purposes - http://www.legislation.gov.uk/uksi/2006/3260/article/4/made

Notifiable diseases

It is important that all those involved with livestock health and production remain vigilant for signs of any notifiable disease. The Animal Health Act 1981 requires that anyone having in their charge an animal affected or suspected of having certain diseases must notify that fact to the veterinary authorities. The diseases covered by this legal requirement are known as notifiable diseases. If you suspect the presence of notifiable disease, you must immediately call APHA:

- In England via the Defra Rural Services helpline: 03000 200 301.
- In Wales on 0300 303 8268.
- In Scotland via the local APHA field office - see https://www.gov.uk/government/organisations/animal-and-plant-health-agency/about/access-and-opening for further contact details.

There is information on notifiable diseases of farmed livestock available on the following links, including information on their clinical signs and pathology:

- https://www.gov.uk/government/collections/notifiable-diseases-in-animals
- http://www.oie.int/?id=2493

Abbreviations

Ab Antibody
Ag Antigen
AGIDT Agar Gel Immunodiffusion Test
AT Agglutination Test
BAL Bronchoalveolar Lavage
BVD Bovine Viral Diarrhoea
CCN Cerebrocortical Necrosis
CEL Chicken Embryo Liver
CIT Citrate
CFT Complement Fixation Test
CIE Counter Immuno Electrophoresis
CNF Cytotoxic Necrotising Factor
CTM Charcoal Transport Medium
DAT Direct Agglutination Test
DEL Duck Embryo Liver
EDTA Ethylene Diamine Tetra-acetic Acid
ELISA Enzyme-Linked Immunosorbent Assay
EM Electron Microscopy
FAT Fluorescent Antibody Test
FAVN Fluorescent Antibody Virus Neutralisation
FPT Four Plate Test
HAT Haemagglutination Test
HAIT Haemagglutination Inhibition Test
Hb Haemoglobin
HEP Heparin
IBR Infectious Bovine Rhinotracheitis
ID Identification
IFAT Indirect Fluorescent Antibody Test
IHC Immunohistochemistry
IPMA Immunoperoxidase Monolayer Assay
IPX Immunoperoxidase Assay
OCD Osteochondritis Dissecans
PAGE Polyacrylamide Gel Electrophoresis
PBS Phosphate Buffered Saline
PCR Polymerase Chain Reaction
PCV Packed Cell Volume
PDNS Porcine Dermatitis and Nephropathy Syndrome
PED Porcine Epidemic Diarrhoea
PGE Parasitic Gastro-enteritis
PI$_3$ Parainfluenza 3
PME Post Mortem Examination
PMWS Postweaning Multisystemic Wasting Syndrome
PNP Porcine Necrotising Pneumonia
PoA Price on Application
PRCV Porcine Respiratory Coronavirus
PRRS Porcine Reproductive and Respiratory Syndrome
RBC Red Blood Cells
RBT Rose Bengal Test
RIA Radio Immuno Assay
RSA Rapid Slide Agglutination
RSV Respiratory Syncytial Virus
SAF Scrapie Associated Fibrils
SAT Serum Agglutination Test
SC Small Colony Variant
SNT Serum Neutralisation Test
SPF Specific Pathogen Free
TGE Transmissible Gastro-enteritis
VMAT Vaginal Mucus Agglutination Test
VTEC VeroToxic Escherichia coli
VTM Virus Transport Medium
VI Virus Isolation
VIC Veterinary Investigation Centre/s (formerly

LAT Latex Agglutination Test **LC** Large Colony Variant **MAT** Microscopic Agglutination Test **MIC** Minimum Inhibitory Concentration **MRT** Milk Ring Test **MZN** Modified Ziehl-Neelsen Stain **NEFA** Non-Esterified Fatty Acids **NPLA** Neutralising Peroxide Linked Assay	Regional Laboratories) **WBC** White Blood Cells

Colour codes for blood tubes

Stopper Colour	**Anticoagulant**
Red	None (for serum samples)
Green	HEP
Purple	EDTA
Grey	OXF
Blue	CIT

Sampling by discipline

Histology

Please note autolysis can severely compromise the ability to assess disease processes within tissues and therefore the submission of material which is obviously autolysed at the time of collection and fixation may not yield any useful diagnostic information.

Sampling

- Tissue samples should not be more than 1 cm thick
- Samples should be fully representative of the basic organ structure and include the junction between gross lesions and normal tissue
- Samples should be immersed in 10-20 times their volume of fixative as soon as possible
- Samples should be sent in an appropriately sized container with a wide opening
- Brain is best fixed whole allowing the pathologist to select appropriate sites
- Collect intestinal samples, as soon after death as possible (ideally within 30 minutes), from several sites of small and large intestine. Immersion fixation of gut tubes 1-2 cm in length is satisfactory, but avoid crushing with forceps. Gentle agitation of the sample in the fixative will help displace food material and allow fixative to enter the lumen

If the above guidelines are followed, primary fixation of most samples should take 24-48 hours – this time period will be extended if the fixative is cold (below 5˚C). However, whole brains will take longer - please discuss with your VIC.

Packing and sending

Material **must** be properly packaged. Packaging must conform to the postal regulations for packaging of pathological material (https://www.royalmail.com/sites/default/files/Guidance-Document-Infectious-Substances-171012.pdf) .

- Urgent cases can be sent immediately if the container is filled with fixative so that primary fixation occurs in transit. If non urgent, tissue can be initially fixed for 48 hours then sent in a reduced volume of fixative. This method is particularly appropriate for brain.

The **recommended** fixative for most cases is 10% neutral buffered formalin.

Bacteriology

Types of samples

- Portions of fresh tissue in clean containers are suitable if they are not autolysed or contaminated, are submitted same day or by overnight post and are kept cool during transport
- Purulent material is preferable to swabs
- Faeces samples (not just swabs) are essential if tests other than basic bacteriology are required.
- For anaerobic culture, fill container to brim or wrap tissues in cling film to exclude air
- Charcoal swabs are suitable for aerobic and anaerobic culture but where anaerobes are the target organism, commercial transport media are available that are aimed specifically at anaerobe preservation. Please discuss with the VIC
- Plain swabs required for fluorescent antibody test (FAT) e.g. for *Streptococcus suis* 2
- Plain swabs with wire or plastic stems for PCR tests (not wooden stems)

Sampling for aerobic bacteriology (request test code TC0101)

- Tissues to be sampled should be as fresh as possible
- Sear the surface of organs with a flame or heated scalpel blade prior to incision with a sterile scalpel and swab the incised surface
- In cases of serositis (pleurisy, pericarditis, arthritis etc), rub the swab on the lesioned serosal surface and avoid just dipping the swab in fluid exudate

Sampling for anaerobic bacteriology

- Follow similar guidelines to the above for aerobic bacteriology but, for anaerobic culture, request test code TC0528
- For diagnosis of clostridial enterotoxaemia, send a minimum of 1 ml of small or large intestinal contents. Do not add any preservative. Submit for *Clostridium perfringens* toxin ELISA (TC0035)
- For clostridial myositis or black disease in cattle or sheep, or *Clostridium novyi* infection (hepatitis) in pigs, take four impression smears from the cut surface of affected muscle or liver, air-dry and send in slide box for clostridial FAT (TC0032), or submit a portion of whole lesioned tissue in a sealed air tight container

Bacteriology

- Initial isolation of most bacterial pathogens in cultures occurs after 24 hours incubation following initiation of cultures at the laboratory
- Some exceptions are:

- *Haemophilus parasuis* - minimum of 2 days

- *Salmonella* spp. by enrichment - minimum 2 days

- *Campylobacter* spp. - up to 5 days

- *Brucella abortus* - minimum of 4 days

- *Mycobacterium avium paratuberculosis* (Johnes) – up to 16 weeks

- *Mycobacterium* species (TB) – 6 to 12 weeks

- *Avibacterium* spp and other avian *Pasteurellaceae* – minimum of 2 or 3 days

- Full identification of bacterial pathogens can take from one day to a week, and occasionally longer depending on the nature of the particular pathogen and the degree of contamination.
- Fastidious organisms exist such as *Mycoplasma, Brachyspira* and *Campylobacter* species and *Leptospira* serovars may require specialist techniques. Please contact your usual APHA VIC to discuss testing for these pathogens

Antimicrobial sensitivity

- Antimicrobial sensitivity will be initiated, if requested, once the pathogen has been obtained in pure growth which may require subculture
- Disc diffusion antimicrobial sensitivity testing (TC0401) takes 24 hours in most cases
- Minimum inhibitory concentrations (MICs) can be undertaken for some pathogens for selected antimicrobials. Please contact your usual APHA VIC for more information

Mastitis examinations (TC0544)

Misleading results are obtained if milk samples are contaminated

Follow this procedure to avoid contamination:-

1. Wash and dry your hands thoroughly
2. Wash teat to be sampled only if obviously dirty; dry immediately
3. Discard first two draws of milk
4. Clean end of teat:
    a) Use small piece of cotton wool, dampen with surgical spirit (80% spirit/20% water)
    b) Rub end of teat with “swab” until visibly clean
    c) Repeat using a second swab, make sure swab appears clean after use. If not, repeat using another clean swab; last swab should be spotless after wiping
5. To take sample:

Bacteriology

a) Open sterile sample bottle – **Keep lid clean, never place open-side down and preferably hold it facing downwards in crook of little finger, do not allow lid to touch teat**

b) Hold sample bottle at an angle to teat

c) Discard a further draw of milk

d) Collect 1-3 streams of milk to fill sample bottle at most half-full

e) Immediately replace lid carefully

6. Label sample bottle – **include cow's number, quarter, date, name of farm and farmer**

Mycology

- Request fungal culture (TC0101 Sabouraud's medium) on the submission form
- Fluids (e.g. foetal stomach contents) can also be examined for fungal hyphae by direct microscopy (TC0580)
- For ringworm (dermatophyte) culture (TC0080), submit hair plucks
- Dermatophyte cultures take up to four weeks

Serology

General

Serology is used to detect whether animals have been exposed to a particular pathogen. It is used in the diagnosis of disease and for monitoring the pathogen status of a group or herd/flock.

If animals are vaccinated against the pathogen in question, serology is of debatable value as antibody produced to vaccine cannot usually be distinguished from that produced to field infection. Only a few DIVA (Differentiation of Infected from Vaccinated Animals) vaccines are available for veterinary use (e.g. gE-deleted IBR vaccine) which allow a distinction to be made.

The possibility of maternally derived antibody being detected must be borne in mind in young animals; these may persist up to eight months of age (calves). Maternal antibodies interfere with interpretation and cannot be distinguished from antibody produced in response to active infection of the animal.

Diagnostic serology

- Sample several affected animals
- Single serology
    - presence of antibody only indicates exposure to the pathogen
    - does not indicate how recently the exposure occurred
    - if negative, rules out involvement of some pathogens
    - presence of antibody may be useful diagnostically if the animal(s) were supposed to be free from the pathogen
- Paired serology
    - sera tested from the same animals during acute and convalescent periods
    - detects seroconversion (seronegative to seropositive) or a significant rise in titre
    - establishes a temporal association of seroconversion and disease
    - acute samples must be collected within the three to four days of clinical signs occurring or animals will already have seroconverted
    - sampling interval can vary but should not be less than two weeks
    - is not usually useful in reproductive disease investigation as maternal seroconversion has usually already occurred by the time disease manifests
- Cohort serology
    - used where conventional paired serology problematic e.g. in pigs or poultry with no individual identification

- sera collected from groups of pigs or poultry at different ages within one management system
- assists in assessing the timing of exposure

Monitoring serology

Healthy animals may be tested for antibody to a pathogen to establish the status of a group or herd/flock with respect to that pathogen, so long as the animals are not vaccinated (unless a DIVA vaccine is used). The aim of the monitoring needs to be clear and is usually either:

a) to detect presence of pathogen – here the detection of a single seropositive animal is sufficient

b) to estimate prevalence of pathogen – here an estimate of the proportion of animals exposed to infection is needed and this usually involves testing a larger number of animals

In both situations, the numbers of animals tested from an epidemiological group depends on several factors including the suspected prevalence of infection, the degree of confidence needed in the results and the number of animals in the group. Epidemiological sample size calculation tables exist which assist in establishing the numbers of animals that should be sampled and tested for given group sizes, confidences and prevalences.

The number of animals which are tested is also influenced by the logistics of sampling, cost of the tests and the sensitivity and specificity of serological tests available.

Parasitology

Fresh faeces

- Submit in a wide mouthed, screw capped container sealed with insulation tape
- Submit 3g minimum for individual faecal egg count (TC0060), 40g for fluke egg examination (TC0061), and 50g for lungworm larvae examination (TC0062)
- Monitoring faecal egg counts in sheep (the composite faecal egg count, TC0668): submit 10 x 3g (minimum) as separate faecal samples from each group; these will be pooled at the laboratory
- Monitoring fluke egg counts in cattle and sheep (TC0689): submit 10 x 5g (minimum) as separate faecal samples from each group; these will be pooled at the laboratory

Blood

- For blood parasites (TC0256) submit 2ml whole blood in EDTA tube, or two thinly spread films air dried and fixed in methanol

Skin

- For skin parasites (TC0081) send multiple deep scrapings (i.e. firm enough to draw blood) and scabs, with hair/feathers (for mange/feather mites) or plucked underlying hair (for ringworm). Send fresh undamaged specimens of ticks, lice and fleas. All samples should be submitted in screw-capped containers; please do not submit the scalpel blade

Virology

Ruminant respiratory viruses

Polymerase Chain Reaction (PCR) tests permit rapid identification of IBR, PI3 and RSV in both affected live animals and carcases. If collecting tissue samples only, submit both fresh and formalin-fixed samples that can be subject to microbiology testing and histopathology respectively.

Animal selection

- Select recently affected animals
- Animals with mucopurulent nasal discharge are less likely to yield virus
- Broncho-alveolar washings (BAL) and guarded intranasal brush swabs are the preferred samples
- Nasal or ocular swabs are suitable for IBR, but are unlikely to detect PI3 or RSV
- Plain swabs must be used, but do not use swabs with a wooden stem
- Samples must be submitted as soon as possible after collection, certainly no longer than the day after collection

Carcases

- Submit intact fresh carcases, pluck or portions of lung tissue
- For the latter collect two or three blocks of lung tissue (2 cm cubes) from the junction between healthy and affected tissue
- Tracheal and/or bronchial swabs may also be collected
- Tissues or swabs should be forwarded APHA within 24 hours

Isolation of viruses from field cases is not routinely undertaken, is time-consuming and often difficult as some mammalian respiratory viruses survive poorly in transport. When virus isolation is required, it may be necessary for the samples to be submitted in virus transport medium (VTM). Consult APHA before submission.

Other mammalian viral diseases

- Sample as advised under the specific species sections
- For enteric viruses send intestinal contents or faeces, without VTM, rather than swabs
- For viral skin diseases send deep scrapings, fresh biopsies or aspirated fluid (if available) in screw-topped containers

Sampling by species and disease conditions

Avian

Poultry & game birds

Please refer to the Sampling by Discipline sections on pages 7 – 12 for general sampling information.

Please feel free to discuss avian investigations with a VIO or avian pathologist prior to submission and supply a fully completed avian submission form - http://apha.defra.gov.uk/vet-gateway/surveillance/forms.htm - that includes a good clinical history with affected bird age, morbidity and mortality patterns, information on medication and the vaccination programme and the type of husbandry system.

For post mortem examination, consider submitting a batch of birds that comprises some that have recently died and live, affected birds (if they are fit to travel and their welfare is not compromised). Individual birds may be all that is available from small flocks.

Recommended batch sizes for post mortem submissions of birds are:

- Up to 10 birds if they are less than 2 weeks old
- Up to 5 birds if they are more than 2 weeks old

Acutely affected and untreated birds are ideal candidates for post mortem examination. Fresh carcases should be submitted as post mortem autolysis occurs rapidly, particularly with chicks. Carcases should not be frozen as this leads to tissue damage and renders histopathology of very limited value.

For brain histology, fix one half of a sagittal section of the head with the brain *in situ* and retain the other half of the brain fresh (unfixed) for other tests.

For bacteriology, specialist avian pathogens may require a minimum of 2-3 days to achieve satisfactory growth, followed by identification. Definitive identification of some pathogens (such as *Avibacterium paragallinarum*) may require additional molecular testing such as 16S rRNA sequencing.

Parasitic infections caused by motile protozoa (*Spironucleus* – formerly *Hexamita*, and *Trichomonas/Tetratrichomonas*) are particularly prevalent in game birds. It is essential that live birds are submitted for an accurate diagnosis to be made of intestinal motile protozoan infections.

Serology can often be a useful diagnostic tool on a flock basis to help establish a diagnosis, particularly in some viral infections where virus isolation is difficult. This can include paired (cohort) serology. Information about available serology tests and packages, including test and sample types and costs is detailed under the 'Avian' section of the 'Disease Surveillance Tests' price list: http://science.vla.gov.uk/Tests/Default.aspx?SiteName=DST.

Note that for some serology tests (*Mycoplasma gallisepticum, M. meleagridis* and *M. synoviae* RSA) only fresh serum samples can be used; frozen or haemolysed sera are not suitable.

Provision of an accurate flock vaccination history is essential to aid interpretation of serological tests.

Avian

Avian virology

Specific information about available tests, including test and sample types and costs is detailed under the 'Avian' section of the price list on the APHA website:
https://science.vla.gov.uk/Tests/Default.aspx?SiteName=DST

Please also feel free to discuss investigations with a VIO or avian pathologist prior to submission.

Pools of tissue from each bird (brain and trachea in one pool; liver, lung, kidney and spleen in a second; intestinal tract in a separate pool) are useful for general virus isolation tests. Lymphoid tissue may also be required as a separate pool (thymus, spleen and/or bursa) depending on the investigation.

If swabbing (for both virus isolation and/or PCR testing) plastic or wire-stemmed swabs are required. Do not use wooden stemmed swabs as these contain substances that can interfere with PCR tests. PCR tests are available for IBV, aMPV and some other avian viruses.

Some viruses target specific organs that may be useful to sample, for example:

- Trachea, caecal tonsils, kidney, reproductive tract for Infectious Bronchitis virus (IBV) infections
- Spleen is required for Haemorrhagic Enteritis Virus detection in turkeys and Marble Spleen Disease in pheasants
- Bursae are useful for the diagnosis of Infectious Bursal Disease (Gumboro)
- Brain for viruses causing encephalitis
- Intestinal contents may be examined by electron microscopy (EM) when looking for enteric viruses or PAGE for rotavirus
- Skin/oropharyngeal samples for pox viruses

Avian

Birds (poultry, game birds and waterfowl)

In many cases, investigation requires the submission of birds for post mortem examination as described on page 15. The following is a brief guide to particular situations encountered in the field where the submission of samples and carcases is of diagnostic value. The list is not comprehensive; please contact a VIO or avian pathologist to discuss individual cases.

NB: Please rule out notifiable disease. If suspected, please use the contact details on page 5.

NB: Please discuss all cases of suspected or confirmed poisoning in food animals with a VIO, as voluntary measures to control contamination of the food chain may be requested. In rare circumstances statutory controls imposed under the Food & Environmental Protection Act (FEPA) may be required.

Diseases in poultry and game birds

<table>
<tr><th>Category/ Clinical signs/ Description</th><th>Condition/ cause</th><th>Sample type</th><th colspan="2">Recommended tests</th><th>Further information</th></tr>
<tr><td rowspan="6">Increased Mortality:

NB rule out notifiable disease</td><td>Consider bacterial, viral, fungal, metabolic / toxic causes</td><td>Carcases</td><td colspan="2">Post mortem examination</td><td></td></tr>
<tr><td rowspan="5">Systemic bacterial infections/ septicaemias</td><td rowspan="2">Carcases</td><td rowspan="2">Post mortem examination (>2 weeks: up to 5 birds
<2 weeks: up to 10 birds)</td><td>Post mortem examination farmed poultry and game birds over two weeks (TC0001)</td><td rowspan="5">For systemic infections, aseptic cultures from spleen, liver and heart valves (if abnormal) are often useful.</td></tr>
<tr><td>Post mortem examination farmed poultry and game birds up to two weeks (TC0021)</td></tr>
<tr><td>Fixed tissues</td><td>Histopathology</td><td>TC0008/ TC0010</td></tr>
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</table>

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
	Metabolic toxic causes	Blood	Example: glucose		Glucose requires OxF anticoagulant, Consult lab of choice for advice on individual lab test and sample requirements
		Tissues	Example: lead		Lead testing requires kidney (preferably). Consult lab of choice for advice on individual lab test and sample requirements
Increased mortality in pheasants	Consider coronavirus nephritis	Carcases, fixed kidney tissue	Post mortem examination Histopathology		
		Fresh caecal tonsil or swab	Infectious bronchitis virus/coronavirus	IBV RT PCR (single swab or sample, TC0787, pooled swabs, TC0887)	Kidney tissue can also be used
	Consider Marble Spleen Disease	Carcases, fixed spleen	Post mortem examination Histopathology		
		Fresh spleen	Virus detection		HEV/MSD AGIDT (TC0910)
Wet litter, abnormal faeces & caecal cores	Consider bacterial, parasitic, viral, toxic/nutritional causes; trauma	Carcases	Post mortem examination		
		Fixed intestine	Histopathology	TC0008/ TC0010	Please fix the material as soon as possible to reduce autolysis
		Faeces, caecal contents	Bacteriology: routine *Clostridium* spp *Brachyspira* (avian intestinal spirochaetosis)	Primary culture (TC0101) Anaerobic bacterial culture (TC0528) *Brachyspira* culture (TC0331 and TC0332)	Samples for *Brachyspira* culture should be sent if possible so that anaerobic conditions are maintained (for example in a full, screw cap sample pot. The pot should be thoroughly sealed with waterproof tape). Samples should preferably be received within 24 hours of being collected.

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
	Salmonellosis (statutory sampling)	Faeces, boot swabs or other samples as stipulated for the National Control Programme (NCP) guides	Statutory *Salmonella* culture	*Salmonella* isolation from routine CSPO submissions (TC0699S)	The current guidelines for sampling are given in the documents for the UK National Control Programme for Salmonella available on GOV.UK
	Salmonellosis (diagnostic sampling)	Faeces; internal organs (spleen liver, caecum) for pullorum disease and fowl typhoid	Culture	*Salmonella* culture (TC0025)	*S*. Pullorum and *S*. Gallinarum differ from other salmonellas in that they are best isolated from tissues. Serological tests are also available for *S*. Pullorum and Gallinarum
	Viral enteritis	Fresh intestine	Virus isolation	Virus isolation in tissue culture (TC0819)	
		Faeces, caecal contents	Rotavirus PAGE	Rotavirus antigen (TC0582)	
			EM for virus detection	Electron microscopy (TC0317)	
	Haemorrhagic Enteritis Virus of turkeys	Fixed spleen Fresh spleen	Histopathology Virus detection	TC0008/ TC0010 HEV/MSD AGIDT (TC0910)	
	Intestinal parasitism	Faeces or caecal contents	Egg/oocyst counts	Worm egg/coccidial oocyst count (TC0060)	
		Intestinal contents	Worm presence; worm identification	Microscopy (TC0580), parasite identification (TC0616)	
	Motile protozoan infection in game birds	Intestinal contents from freshly dead bird	Microscopy for protozoa	Microscopy (TC0580)	Material must be examined very fresh. Live or freshly dead birds

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
					should be submitted. Fixed intestine can also be of value for histopathology.
Lameness / recumbency	Consider viral, bacterial, deficiencies, toxic/nutritional, trauma See also under 'swollen head and upper respiratory disease' (for Mycoplasma testing) and 'nervous disease' below	Tissue (tendon, muscle, tibiotarsus, femoral head, skin); swabs of lesioned areas for bacteriology	Post mortem examination Histopathology	TC0008/ TC0010	
			Bacteriology:	Primary bacterial culture (TC0101) Antibiotic sensitivity – aerobe (TC0401)	Tissues and swabs must be taken aseptically
			Virology: Reovirus isolation	Virus isolation in tissue culture (TC0819)	
			Mycoplasma spp	DGGE/PCR (TC0672)	Samples for *Mycoplasma* detection should preferably be sent in *Mycoplasma* transport broth
	Rickets and other skeletal disorders	Affected bones	Histopathology	TC0008 / TC0010	For rickets, histopathology of growth plates required (such as proximal tibia)
		Clotted blood (serum)	*Mycoplasma* serology	*M. gallisepticum* and *M synoviae* Rapid Slide Agglutination (RSA, TC0306, TC0308); RSA flock screen (PC0932); or Western Immunoblotting (TC0749)	Minimum of 10 birds for flock screen. For RSA the serum must be freshly taken and not haemolysed or frozen The RSA is not recommended for use in game birds
Unevenness / poor condition in poultry	Consider enteric disorders (see also under wet litter), other systemic infections, nutrition	Carcases	Post mortem examination and other testing as indicated by findings and flock background		
Loss of	Consider	Serum	*Mycoplasma*	Egg drop	

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
production in Layers (i.e. egg drop & reduced egg quality) NB rule out notifiable disease	systemic infections and other causes		serology (as above), Infectious Bronchitis (see under respiratory disease below), Egg Drop Syndrome '76	syndrome '76 HAIT (TC0908)	
Swollen head and upper respiratory disease	Consider bacterial, viral, trauma	Carcases, fixed tissues, sinus swabs, bloods	Post mortem examination and tests as indicated below		
	Infectious coryza and other bacterial causes	Sinus swabs (or choanal cleft swabs in live birds)	Bacteriology	Primary culture (TC0101)	Take sinus swabs as aseptically as possible. Preferably use plain plastic or wire stemmed swabs pre-moistened in sterile distilled water, or alternatively charcoal transport swabs
	Mycoplasmosis	Conjunctival and tracheal swabs	*Mycoplasma* detection by DGE/PCR	TC0672	Swabs should preferably be sent in *Mycoplasma* transport broth. It is preferable to sample several birds. Costs can be reduced by inoculating pooled swabs into transport broth. The RSA is not recommended in game birds
		Clotted blood or serum	*Mycoplasma* serology (as above)		
	Avian meta pneumovirus (aMPV, ART, TRT)	Oropharyngeal swab	RT PCR	aMPV PCR for single swabs (TC0786), or for a pool of up to 5 swabs (TC0397)	Plastic or wire stemmed swabs must be used
		Clotted blood or serum	ELISA	ELISA (TC0940)	

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
	Infectious bronchitis and IBV-like gamma coronaviruses	Oropharyngeal or cloacal swabs	RT PCR	IBV PCR for single swabs (TC0787), or for a pool of up to 10 swabs (TC0887)	Plastic or wire stemmed swabs must be used. Positive results are followed up by sequencing of S1 gene to identify strain
		Clotted blood or serum	HAIT	HAIT for a specified single strain (TC0912), or for 3 specified strains (TC0640)	Interpretation of IBV serology requires knowledge of the IBV vaccination history. Paired serology is recommended
	Respiratory crypto-sporidiosis	Carcases or fresh or fixed heads	Histopathology	TC0008/ TC0010	*C. parvum* recognised in red grouse and occasionally other species
Respiratory disease	Consider bacterial, viral, fungal, parasitic and non-infectious causes. There is often a mixed aetiology. See under swollen head and upper respiratory infections	Carcases, fixed tissues, swabs, bloods	Post mortem examination, histopathology and tests as indicated below and under swollen head/upper respiratory disease as above		
	Tracheitis: Infectious laryngo-tracheitis (ILT)	Fixed trachea	Histopathology	TC0008/TC0010	In the chronic stages of the disease, confirmatory diagnosis of ILT may be difficult. May also cause upper respiratory tract disease.
		Fresh trachea	Virus isolation	Virus isolation in tissue culture (TC0819)	
		Blood from recovered birds	Serology by SNT	ILT SNT (TC0812)	
	Gapeworm (syngamosis)	Fresh or fixed tissue	Gross examination, histopathology	TC0008, TC0010	There may be pathology in lung tissue. Some *Syngamus*-like species in waterfowl and other species parasitise the bronchi and

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
					sometimes other sites.
	Bacterial and fungal pneumonia	Carcases, fixed and fresh tissues	Histopathology	TC0008, TC0010	A variety of infectious agents may be implicated including *E. coli*, *Ornithobacterium rhinotracheale* (ORT) and other *Pasteurella* –like organisms
			Primary bacterial culture, fungal culture	TC0101	
Non-specific findings; immune-suppression	Consider viral causes	Fixed lymphoid tissues	Histopathology	TC0008 / TC0010	Multiple bursas can be examined per slide.
Nervous disease NB rule out notifiable disease	Consider viral, bacterial, nutritional deficiencies / toxicities		Post mortem examination		*NB: rule out notifiable disease*
	Differentials include bacterial encephalitis in chicks (*Enterococcus* species), listeriosis, Marek's disease, Avian Encephalo-myelitis, crazy chick disease (Vitamin E deficiency)	Fixed brain or peripheral nerve	Histop athology	TC0008 / TC0010	*Nerve:* examination of nerves can be useful even if no gross lesions are observed. *Brain:* fix one half of a sagittal section of the head with the brain *in situ* and retain the other half of the brain fresh (unfixed) for other tests.
	Bacterial encephalitis	Swab of fresh brain	Bacterial culture	Primary culture (TC0101), *Listeria* culture (TC0663)	Aseptic sampling is essential
Neoplasia	Consider viral aetiology (Marek's disease; rarely Avian Leukosis Virus or other oncogenic viruses)	Fixed tumour, liver, spleen and nerve Fresh tumour tissue	Post mortem examination Histopathology TC0008 / TC0010		Retain fresh tissues frozen in case required for molecular testing, especially if unusual tumour distribution
Skin and feathers	Consider ectoparasites (mites, lice)	Collect parasites in water for identification	Microscopy	Ectoparasites-microscopic examination (TC0081)	Correct identification of mite species is important in

Avian

Category/ Clinical signs/ Description	Condition/ cause	Sample type	Recommended tests		Further information
					planning control measures
	Poxvirus (also sometimes see lesions in oral cavity)	Fixed lesion tissue	Histopathology	TC0008 / TC0010	'Dry' pox form refers to lesions on skin, 'wet' pox to lesions in oral cavity
		Fresh tissue	Electron microscopy or virus isolation	Electron microscopy-avian (TC0317); virus isolation for pox virus (TC0817)	
Unusual conditions	Any variations of the above – cause unknown	Carcases	Post mortem examination		Please contact one of our avian pathologists at APHA Lasswade for advice: Also keep fresh frozen material at -70°C for possible future analysis
		Histopathology on fixed affected tissue	Histopathology (TC0008, TC0010)		

Selected diseases of waterfowl (otherwise see above)

Category	Condition/ cause	Sample type	Recommended tests		Further information
Water fowl	Duck Viral Enteritis, Duck Viral Hepatitis, Goose Parvovirus	Carcases	Post mortem examination		Please contact the laboratory to discuss tissues of choice
		Fixed tissues	Histopathology	TC0008/ TC0010	
		Fresh tissues	Virus isolation	Virus isolation in duck/goose eggs (TC0820)	
		Clotted blood (serum)		DVE SNT (TC0906), DVH SNT (TC0907) GPV AGIDT (TC0302)	
	Duck septicaemia *(Riemerella anatipestifer)*	Carcases	Post mortem examination		
		Swabs	Bacteriology	Primary culture (TC0101)	Aseptic swabs of brain tissue are particularly useful

Cattle

Abortion and stillbirth

Whole fetus, placenta and maternal serum are the submission of choice. There is a statutory requirement to report all bovine abortion cases to the local APHA Field Service.

Category	Condition/ cause	Sample type	Recommended test	Further information
Adult	Most bacterial causes and mycotic abortion	Fetal stomach contents and placenta	Culture, stained smears and wet preparation (TC0101 for routine culture, TC0026 for *Campylobacter*, TC0580 for wet preparation for fungi)	Can be combined with PCRs for *Neospora caninum* and BVD virus (see below for sample requirements) under TC0015. If a full range of samples is submitted, testing will be carried out in a stepwise fashion. If there are no significant findings from step 1 (bacteriology/mycology), then the PCRs will be carried out as step 2. Tests additional to steps 1 and 2 (e.g. PCR for *Leptospira* sp.) can be commissioned, but will require additional samples and will attract an additional charge. Please discuss with a VIO.
	Neospora caninum	Fetal brain	PCR on fresh brain (TC0852)	A positive PCR result confirms *Neospora* infection of the fetus, but does not confirm that neosporosis was the cause of abortion. Confirmation of *Neospora* abortion can be achieved through histopathology on fixed tissue.
	Neospora caninum	Fixed fetal brain; Fixed fetal heart	Histopathology (PC0006)	
	BVD virus	Fetal spleen or thymus	PCR (TC0655)	
	IBR/BVD/ *L.hardjo/ N.caninum* (Maternal Serology)	Blood - clotted	Bovine abortion/stillbirth serology package A (PC0387) (*L.hardjo/ N.caninum*) Bovine abortion/stillbirth serology package B (PC0405) (IBR /BVD/ *L.hardjo/N.caninum*)	Paired samples are of limited diagnostic value. Single samples are useful in maintaining disease surveillance and can rule out neosporosis.
	Iodine deficiency	Fetal thyroid	Iodine assay	Consider in stillbirths and fetal death in last week of gestation.
		Fixed fetal thyroid	Histopathology (PC0006)	
	Investigation of cattle herd infertility requires a systematic approach. Laboratory investigations can be an important component of this approach. Please discuss individual herd problems and the potential for laboratory testing to inform an investigation with a VIO.			

Cattle

	When investigating suspected cases of bovine venereal campylobacteriosis, please note that *Campylobacter* culture and identification from sheath washings and vaginal mucus samples requires a specific sampling kit and submission form. Samples for this test (TC0098) should be sent to APHA Starcross VIC, ensuring arrival within 24 hours of sampling. Please obtain further details from a VIO before submitting these samples.

Enteric disorders

Category	Condition/cause	Sample type	Recommended test	Further information
Calves 1 – 5 days	*E. coli* (K99 +ve), *Salmonella*, cryptosporidia, rotavirus, coronavirus	Faeces (5g)	Enteric package for 1 – 5 day old calves (PC0069)	Individual components may be selected.
Calves 6 - 21 days	*Salmonella*, cryptosporidia, rotavirus, coronavirus	Faeces (5g)	Enteric package for 6 – 21 day old calves (PC0070)	Individual components may be selected.
Calves from 22 days	*Salmonella*, coccidiosis, PGE	Faeces (10g)	Enteric package for young ruminants (PC0071)	Individual components may be selected: *Salmonella* culture (TC0025) and worm egg/coccidial oocyst count (TC0060).
Adult	*Salmonella*, fasciolosis, Johne's disease	Faeces (40g) and Blood - clotted	Enteric package for adult cattle (PC0073)	Johne's disease testing is carried out by serology (ELISA). Additional tests such as PCR on faeces may be carried out for an additional charge but require additional faeces. Individual components may be selected: *Salmonella* culture (TC0025), fluke egg examination (TC0061) and Johne's disease antibody ELISA (TC0366).
All ages	Persistent BVD infection and Mucosal Disease	Blood – heparin or clotted	ELISA antigen (TC0772) and antibody tests (TC0390).	In calves ≤ 30 days old, consider use of BVD PCR (TC0655).
All ages	Acute BVD infection	Blood – clotted or heparin	Paired ELISA antibody test (TC0390) PCR (heparin blood) (TC0655) on acute sample	Diagnosis of acute infection by antibody ELISA requires paired acute and convalescent sera with an interval of 3 weeks
Adult	Winter dysentery (Coronavirus-associated diarrhoea)	Blood – paired clotted	Paired ELISA antibody test (TC0176)	Usually seen in housed adult dairy cattle. Usually characterised by high morbidity but low mortality, with spontaneous recovery in a few days.

Cattle

Ill thrift

Category	Condition/cause	Sample type	Recommended test	Further information
Adult, pre- and post-weaned	Endoparasitism	Faeces (50g)	Worm egg count and examination for fluke eggs (PC0064)	Individual components may be selected: Worm egg count (TC0060) and fluke egg examination (TC0061). A composite worm egg count (TC0688) is available, but is usually used for monitoring rather than diagnostic purposes. A composite fluke egg examination (TC0689) is available.
All ages	Trace element deficiency	Consult testing laboratory	Copper and GSH-Px (for selenium)	Sample at least 6 animals. Individual components may be selected. Liver copper assay may also provide useful information
All ages	Persistent BVD infection and Mucosal Disease	Blood - clotted	ELISA antigen (TC0772) and antibody tests (TC0390).	In calves ≤ 30 days old, consider use of BVD PCR (TC0655)
Adult	Adults: Johne's disease	Blood - clotted	ELISA antibody test (TC0366)	Additional tests such as PCR on faeces may be carried out for an additional charge.

Other syndromes

Category	Syndrome	Condition/cause	Sample type	Recommended test	Further information
All ages	Locomotor disorders	Nutritional myopathy	Consult testing laboratory		Consult lab of choice for advice on individual lab test and sample requirements
Adult	Downer cows	Downer cow	– Consult testing laboratory	Downer cow profile: Calcium, magnesium, phosphate, CPK, BHB and urea suggested	Always consider the other 'non-metabolic' causes of recumbency.
	Mastitis	Common mastitis pathogens	Milk	Culture (TC0544)	Aseptic technique in sample collection is essential. See page 10.
		Mycoplasma mastitis	Milk	DGGE/PCR (TC0672)	
	Herd level milk drop	There are many potential non-infectious and infectious causes of herd level milk drop in dairy herds. Investigation of problem herds requires a systematic approach. Laboratory investigations can be an important component of this approach. Please discuss individual herd problems and the potential for laboratory testing to inform an investigation with a VIO.			

Cattle

Respiratory disease

Category	Condition/cause	Sample type	Recommended test	Further information
Neonatal, pre- and post-weaned	Most bacterial causes of calf pneumonia	Guarded long nasotracheal swabs or BAL samples	Routine culture (TC0101)	
All ages	Mycoplasmosis	Guarded long naso tracheal swabs or BAL samples	DGGE/PCR (TC0672)	Do not use wooden stemmed swabs.
	IBR	Nasopharyngeal or ocular swabs	Multiplex Respiratory virus PCR (TC0019)	Swabs must be plain, not charcoal transport medium. Avoid sampling in chronic phase. Do not use wooden stemmed swabs
	RSV and PI3	BAL samples	Multiplex Respiratory virus PCR (TC0019)	Avoid sampling in chronic phase.
	IBR, RSV, P13, BVD, *Mycoplasma bovis* and *Histophilus somni* (Serology)	Blood - paired clotted	Bovine respiratory disease serology package A (PC0384) (IBR, RSV, P13, BVD) Bovine respiratory disease serology package B (PC0385) (IBR, RSV, P13, BVD, *Mycoplasma bovis* and *Histophilus somni*)	Paired acute and convalescent sera collected with an interval of 2 -3 weeks.
	Dictyocaulosis (lungworm)	Faeces (50g)	Baermann examination (TC0062)	Positive Baermann result indicates patent infestation. Baermann examination will be negative in the pre-patent phase. Positive antibody ELISA result indicates exposure in current/recent grazing season, but not necessarily current patent infestation. Haematology (EDTA blood) can provide useful supportive evidence of relative or absolute eosinophilia.
		Blood - clotted	ELISA antibody test (TC0507)	
	Malignant Catarrhal Fever	Blood - heparin	PCR for OvHV-2 (TC0747)	

Cattle

Sudden death

Always consider anthrax. Any suspicion of disease call APHA:

- In England via the Defra Rural Services helpline: 03000 200 301.
- In Wales on 0300 303 8268.
- In Scotland via the local APHA field office - see https://www.gov.uk/government/organisations/animal-and-plant-health-agency/about/access-and-opening for further contact details.

Category	Condition/cause	Sample type	Recommended test	Further information
Adult	Hypomagnesaemia (cows)	Eye fluid (preferably vitreous humour)	Consult lab of choice for advice on individual lab test and sample requirements	Blood samples from at least six cows in same cohort are useful to screen for blood magnesium concentrations.
Under two years	Blackleg (*Clostridium chauvoei*)	Four air-dried impression smears of cut surface of muscle lesion (or muscle lesion in a full sealed container to exclude air)	FAT (TC0032)	Submission of a carcase for post mortem examination is probably the preferred diagnostic approach.
		Fixed muscle lesion	Histopathology (PC0006)	
All ages	Lead poisoning	Kidney	Tissue lead (TC0246)	Please discuss all cases of suspected lead poisoning with a VIO. Please see footnote below.

Fixed = Tissue has been immersed in a suitable fixative such as 10% neutral buffered formalin.

Nervous disease

Investigation of fatal cases of nervous disease often requires examination of the whole brain. Submission of a carcase for post mortem examination is probably the preferred diagnostic approach in fatal cases.

Category	Condition/cause	Sample type	Recommended test	Further information
All ages	Hypomagnesaemia			Consult lab of choice for advice on individual lab test and sample requirements
	Nervous acetonaemia			Consult lab of choice for advice on individual lab test and sample requirements
	Lead poisoning	Kidney (from carcase)		Please discuss all cases of suspected lead poisoning with a VIO. Please see footnote below.
	Skin disease	Skin scrape/scab material	Microscopic examination (TC0081)	Histological examination of a fixed skin punch biopsy can be a useful diagnostic approach in more complex skin disease cases

NB: Please discuss all cases of suspected or confirmed poisoning in food animals with a VIO, as voluntary measures to control contamination of the food chain may be requested. In rare circumstances statutory controls imposed under the Food & Environmental Protection Act (FEPA) may be required.

Small ruminants (sheep, goats)

Abortion and stillbirth

We would like to encourage vets to take samples from aborted fetuses in the first instance of an abortion. However if abortions continue submission of a fetus plus placenta with **maternal** serum is recommended. VIOs at all investigation centres are happy to offer advice on cases and sample collection.

A summary of the samples most suitable for undertaking a complete abortion investigation is shown below. The tests are added sequentially, first looking for EAE, then undertaking bacteriology and toxoplasma testing.

Sample	Test	Cause of abortion tested for
Placenta including multiple cotyledons and intercotyledonary membrane	Gross examination for placentitis and stained smear	Chlamydia (EAE), *Coxiella* (Q'fever) and *Brucella*
	PCR	*Toxoplasma gondii*
Foetal Stomach Contents collected aseptically via a syringe and needle or with a Vacutainer	Bacteriology	Bacterial causes including Campylobacter species and Salmonella
Foetal Fluid from thoracic or abdominal cavity	Antibody iFAT	*Toxoplasma gondii*
Spleen Fresh	PCR	Border's disease
Liver Fresh	Additional Bacteriology	Bacterial causes including Campylobacter species
Brain Fresh	PCR	SBV
Maternal blood Serum (red top)	Ab Elisa	*Chlamydia abortus*
	LAT	*Toxoplasma gondii*
	Ab Elisa PCR	Border disease

Small ruminants (sheep, goats)

Abortion and stillbirth contd

Category	Condition/cause	Sample type	Recommended tests	Further information
Adult	Enzootic abortion (*Chlamydophila abortus*)	Placenta	Gross examination, stained smears (TC0033)	A positive result on a stained smear confirms the diagnosis. Positive maternal serology indicates exposure
		Maternal Blood – single	Ab ELISA (TC0801)	
	Toxoplasmosis	Placenta	Gross examination PCR (TC0858)	PCR on placenta is the test of choice, if placenta is not available carry out IFAT on fetal fluid
		Thoracic fluid	IFAT on fetal fluid (TC0628)	
		Maternal blood – single	LAT on maternal blood (TC0383)	Positive maternal serology indicates exposure
	Campylobacter and other bacterial infections	Fetal stomach contents and liver	Culture and stained smears. (TC0101 for routine culture, TC0026 for campylobacter)	Send whole fetus and placenta if samples do not resolve a diagnosis

Downer cases

Category	Condition/cause	Sample type	Recommended tests	Further information
Adult	Downer / recumbent ewes Hypocalcaemia, pregnancy Toxaemia, hypomagnesaemia			NB – listeriosis should also be considered Consult lab of choice for advice on clinical chemistry lab test and sample requirements

Small ruminants (sheep, goats)

Enteric disorders

Category	Condition/cause	Sample type	Recommended tests	Further information
Small ruminants 1-5 days	Bacteria (*E.coli*, *Salmonella*), rotavirus, Cryptosporidia	Faeces	Enteric package 1-5 day old small ruminants including goats (PC0059): 5g faeces required	Individual components; Bacterial culture with identification of isolates (TC101) and sentest as appropriate, *Salmonella* culture (TC0025), rotavirus PAGE, Cryptosporidia smear (TC0033)
Small ruminants 6-21 days	*Salmonella*, rotavirus, Cryptosporidia	Faeces	Enteric package 6-21 day old small ruminants including goats (PC0066). 5g of faeces required	
Small ruminants over 3 weeks	Coccidia & PGE, *Salmonella*	Faeces	Enteric package young ruminants (PC0071) 10g of faeces required	Individual components may be selected: *Salmonella* culture (TC0025), Worm egg and coccidial oocyst counts (TC0060)
Adult	Johne's disease, *Salmonella*, fluke and PGE	Faeces	Enteric package adult sheep and goats (PC0075): (40g faeces).	Note that fasciolosis and Johne's disease do not always present with diarrhoea and individual components of the enteric package may be selected: Johne's disease smear (TC0776), Fluke eggs (TC0061), Worm egg count (TC0060), *Salmonella* culture (TC0025) Adult sheep usually acquire immunity to enteric parasites
		Blood	Blood (Johne's disease): ELISA (TC0366)	Diagnosis of Johne's disease in sheep may require post mortem examination as ELISA and faecal microscopy are less useful in this species compared to in cattle.

Small ruminants (sheep, goats)

Ill thrift

Category	Condition/cause	Sample type	Recommended tests	Further information
Young animals	Parasitism	Faeces	Worm egg count (TC0060)	Further information on investigation of anthelmintic resistance can be found at www.scops.org.uk
	Border disease	Blood – clotted	ELISA for antibodies (TC0292) PCR for virus (TC0755)	Often there is a history of abortions/hairy shaker lambs earlier in the year.
	Copper deficiency			Suggest sampling 5-6 animals **not** receiving concentrates. Consult lab of choice for advice on test and sample requirements
	Vitamin B_{12} (cobalt) deficiency			Suggest sampling 5-6 animals; animals should not be yarded for more than 6 hours prior to sampling as this may falsely elevate serum B_{12} levels. Consult lab of choice for advice on test and sample requirements.
Adult	Parasitism	Faeces	Worm egg count (TC0060), Fluke egg examination (TC0061) 40g faeces Parasite disease: Worm egg count and fluke egg examination (PC0064) Fluke egg examination (herd screen) (TC0689) Composite – 10 x 5g faeces	Further information on investigation of anthelmintic resistance can be found at www.scops.org.uk
		Serum	Ab ELISA (TC0678) Fluke Coproantigen test	Consult laboratory of choice for advice on sample requirements
	Johne's disease	Faeces	Johne's disease smear (TC0776)	Diagnosis of Johne's disease in sheep may require post mortem examination as ELISA and faecal microscopy are less useful in this species compared to in cattle.
		Blood - clotted	Ab ELISA (TC0366)	

Small ruminants (sheep, goats)

Category	Condition/cause	Sample type	Recommended tests	Further information
	Copper deficiency			Suggest sampling 5-6 animals **not** receiving concentrates. Consult lab of choice for advice on test and sample requirements.
	Chronic illnesses such as Maedi Visna and OPA	Blood- clotted Carcase	AGIDT (TC0373) Ab ELISA (TC1529) Post mortem examination	Serology works well for Maedi Visna, but need post mortem examination for Ovine Pulmonary Adenocarcinoma (OPA)

Locomotor disorders

Category	Condition/cause	Sample type	Recommended tests	Further information
Young animals	Nutritional myopathy		GSH-PX and Vit E CK and AST	Consult lab of choice for advice on test and sample requirements.
Young animals	Arthritis due to *Streptococcus dysgalactiae*	Joint aspirate	Culture (TC0101)	Joint ill in lambs most commonly affects the carpal and tarsal joints.
Young animals/adults	Arthritis due to *Erysipelothrix* spp.	Blood – Clotted	SAT (TC0361)	
		Joint fluid aspirate	Culture (TC0101)	
Adult	Maedi Visna	Blood – Clotted	AGIDT (TC0373) Ab ELISA (TC1529)	Sporadic cases in affected flocks. Nervous form of disease may present as in-coordinated gait
Suckled lambs	Chronic lead exposure	Carcase	Post mortem examination	Chronic low level exposure in lead mining areas can cause osteoporosis and brittle bones, manifesting as stiffness and pathological fractures

Skin disease

Category	Condition/cause	Sample type	Recommended tests	Further information
All ages	Orf	Scab from lesion	Electron microscopy (TC0082)	
	Sheep scab, lice	Skin scrape/wool pluck from margin of lesion	Microscopic examination (TC0081)	Please do not use liquid paraffin
	Dermatophilus	Wool pluck from abnormal wool	Microscopy (TC0033) culture (TC0101)	

Small ruminants (sheep, goats)

Respiratory disorders

Category	Condition/cause	Sample type	Recommended tests	Further information
Adult	*Maedi Visna*	Blood – clotted	AGID test (TC0373) Ab ELISA (TC1529)	Sheep affected with maedi-visna may have histological changes in other tissues including brain. Submission of a whole carcase may be required.
		Lung	Lung histopathology (PC0006)	
All ages	Pasteurellosis,	Lung	*Pasteurella* – culture (TC0101)	*Pasteurella* and *Manhemia* species most commonly
Adult	Ovine pulmonary adenocarcinoma	Lung	OPA – histopathology (PC0006)	

Nervous disorders

Scrapie is a notifiable disease and suspect cases should be reported call APHA:

- In England via the Defra Rural Services helpline: 03000 200 301.
- In Wales on 0300 303 8268.
- In Scotland via the local APHA field office - see https://www.gov.uk/government/organisations/animal-and-plant-health-agency/about/access-and-opening for further contact details.

Category	Condition/ cause	Sample type	Recommended tests	Further information
Adult	Hypocalcaemia, Pregnancy toxaemia, Hypomagnesaemia			NB – listeriosis should also be considered Consult laboratory of choice for advice on test and sample requirements.
All ages	CCN, listeriosis	Carcase	If deaths occur post mortem examination is indicated	NB – a blood test is not available to confirm disease in the live animal
	Cause uncertain	Carcase	If deaths occur post mortem examination will be more likely to yield a diagnosis - possibly at less cost	
	Louping ill	Serum	HAIT (TC0391)	
		Carcase	If deaths occur post mortem examination is indicated.	
	Lead	Blood or tissue biochemistry		Blindness, convulsions and head pressing can be seen. Consult laboratory of choice for advice on test and sample requirements.

Small ruminants (sheep, goats)

Category	Condition/ cause	Sample type	Recommended tests	Further information
Pre-/post-weaned	Drunken Lamb Syndrome / Nephrosis	Blood, but preferably a carcase	If deaths occur post mortem examination will be more likely to yield a diagnosis - possibly at less cost	Two distinct age groups affected: 10 days to 4 week old and 2-4 months old. Latest studies suggest the initial clinical signs are mainly due to metabolic acidosis in the younger age group not due to kidney failure/nephrosis.

Sudden death

Always consider Anthrax call APHA:

- In England via the Defra Rural Services helpline: 03000 200 301.
- In Wales on 0300 303 8268.
- In Scotland via the local APHA field office - see https://www.gov.uk/government/organisations/animal-and-plant-health-agency/about/access-and-opening for further contact details.

Category	Condition/cause	Sample type	Recommended tests	Further information
All ages	Clostridial enterotoxaemia / pulpy kidney	Small intestinal contents – fresh (do not add preservative)	*Clostridum* perfringens toxin test ELISA (TC0035)	ELISA on contents from affected portion of bowel - if not obviously affected send ileal contents bulked from at least 3 sites. Please remove contents from intestine prior to sending
All ages	Pasteurellosis	Lung and liver	Culture (TC0101)	Culture of multiple organs advised as systemic distribution
All ages	Other clostridial disease Fasciolosis / haemonchosis etc	Carcase	Post mortem examination of a fresh carcase at the VIC is more likely to yield a diagnosis and may be cheaper than submission of viscera	
All ages	Toxins and plant poisoning	Carcase and/or suspected substance/ plant	Post mortem examination of a fresh carcase at the VIC is more likely to yield a diagnosis and may be cheaper than submission of viscera	History may suggest the possibility of poisoning

NB: Please discuss all cases of suspected or confirmed poisoning in food animals with a VIO, as voluntary measures to control contamination of the food chain may be requested. In rare circumstances statutory controls imposed under the Food & Environmental Protection Act (FEPA) may be required.

Small ruminants (sheep, goats)

Mastitis

Category	Condition/cause	Sample type	Recommended tests	Further information
Adult	Staphylococci, pasteurellae, coliforms etc	Milk	Culture (TC0101)	Consider inadequate nutrition of the ewes as underlying cause

Pigs

General

Please feel free to discuss diagnostic investigations with a VIO prior to submission of samples and you must speak to a VIO or vet at non-APHA partner PME provider site before submitting pigs. Please provide a fully completed submission form that includes a full clinical history including information on medication and vaccination.

For post mortem examination, consider submitting a batch of up to three pigs, typical of the clinical problem being investigated. It is sometimes appropriate to submit pigs which have been euthanased or which are live (if they are fit to travel and their welfare is not compromised). This should be discussed with the VIO or vet at non-APHA partner PME provider site before submission. Individual pigs or samples may be all that it is possible to submit from small herds.

Disease and other information relating to pig surveillance can be found on the Vet Gateway: http://apha.defra.gov.uk/vet-gateway/surveillance/seg/pig.htm.

NB: Please discuss all cases of suspected or confirmed poisoning in food animals with a VIO, as voluntary measures to control contamination of the food chain may be requested. In rare circumstances statutory controls imposed under the Food & Environmental Protection Act (FEPA) may be required.

Reproductive disease: with fetopathy e.g. abortion, stillbirth, weak piglets at birth

Category	Condition/cause	Sample type	Recommended tests	Further information
Adult	Suspected infectious cause	All aborted and stillborn piglets in litter (and euthanased weak where appropriate) including placentas	Abortion/stillbirth investigation (TC0011 one sow), (TC0012 two sows)	Diagnostic post mortem examination and stepwise diagnostic testing at discretion of VIO to include PRRS, leptospirosis and bacterial/fungal causes from the outset where material submitted is suitable
	Porcine Reproductive and Respiratory Syndrome (PRRS)	Fetal thymus, spleen or lung	PRRSv PCR (TC0718)	Fetal tissue preferable but sometimes virus only detected in serum from aborting sow. Detection of PRRS in abortions in vaccinated sows may need multiple samples and sows should be sampled at the time of abortion. PRRSv serology useful in sows if not vaccinated but only diagnostic if paired
		Maternal serum	PRRSv PCR (TC0718) (pooled PRRSv PCR not suitable for adult pigs)	

Pigs

	Porcine parvovirus PPV	Fetal heart	PPV PCR	Hearts of mummified fetuses can also be tested by PCR. Fetal serology by HAIT only useful if fetuses at ≥ 70 days gestation
		Fetal fluid (e.g. pleural)	PPV HAIT TC0375	
	Leptospirosis	Fetal kidney	Pathogenic *Leptospira* PCR (TC0856)	Autolysis interferes with the PCR test. Positive PCR results should be followed up with serology in recovered pigs or in contact pigs in the affected cohort to identify the infecting serovar. Histopathology (liver, kidney) can provide supporting evidence.
		Maternal sera as follow-up to positive PCR	*Leptospira* MAT 6 pools, 19 serovars (TC0399)	
	Swine influenza	Nasal swabs (plain) from sows	Swine influenza PCR (TC0771)	Consider if sows are showing transient pyrexia, malaise and/or respiratory signs. Pigs sampled for virus must be in the first few days of infection. Testing for virus is free of charge, for details see http://apha.defra.gov.uk/documents/surveillance/diseases/swine-influenza.pdf
		Maternal paired sera	Swine influenza HAIT serology (TC0160 four strains)	
	Erysipelas (and other bacterial causes including fungi)	Fetal stomach contents (liver is second choice)	Bacterial culture including fungal (TC0101)	Collect from stomach using a plain vacutainer to limit contamination. Do not pool from different fetuses. Histopathology on placenta also useful for confirming fungal placentitis
	PCV2-associated disease	Fetal heart – fresh and fixed	Histopathology (PC0006 and PCV-2 IHC if necessary)	If myocarditis detected by histopathology, PCV-2 IHC will be progressed

Pigs

Reproductive disease: infertility (no fetopathy)

Category	Condition/cause	Sample type	Recommended tests	Further information
Adult	Common infectious causes	Clotted sow bloods	Serology PRRSv ELISA (TC0412) PPV ELISA (TC0669) Erysipelas SAT (TC0361) Swine influenza HAIT TC0160 *Leptospira* Bratislava (TC0451) or All 19 Lepto serovars (TC0399)	Serology is unlikely to achieve a diagnosis but can rule out involvement of a pathogen. Serology is not advised for pathogens for which sampled sows are vaccinated. Paired serology is rarely possible as, by the time infertility manifests, the sow has seroconverted, it may be achievable by bleeding cohorts of sows or gilts at service and rebleeding. Many regular returns-to-service are physiological or managemental.
	PRRS	Clotted sow bloods	PRRSv PCR (TC0718)	Testing within 3-4 weeks of vaccination may detect live vaccine virus

Enteric disease

Category	Condition/cause	Sample type	Recommended tests	Further information
All ages	Unknown or suspected cause for differential diagnosis	Ideally affected untreated pigs early in the course of disease	Post mortem examination (TC0017 (neonatal) or TC0002, and tests at VIO discretion)	Batch of up to three pigs ideal. Live if welfare allows (important if pre-weaned), otherwise freshly dead. Where possible, submit untreated pigs. If treatment is failing, it may be appropriate to submit treated pigs also
Neonatal and pre-weaned	Clostridial enterotoxaemia	Intestinal contents (faeces second choice)	*Clostridium perfringens* toxin ELISA (TC0035)	Need 0.5ml intestinal contents per pig, do not pool. Types A (milder) and C are mainly diagnosed in pigs. Confirming a diagnosis of type A should include histopathology for supportive evidence.
	Colibacillosis (Enterotoxigenic *E.coli*)	Intestinal contents or faeces (swabs second choice)	Bacterial culture (TC0101) and (TC0829) *E.coli* fimbrex (K88) test typing	Further *E. coli* typing possible at extra charge (TC0040)
	Non-ETEC *E.coli* e.g. attaching and effacing *E.coli*	Live pigs	Post mortem examination (TC0017 (neonatal) or TC0002)	Culture results with histopathology essential for diagnosis and intestines must

Pigs

Category	Condition/cause	Sample type	Recommended tests	Further information
	(AEEC), Enterohaemorrhagic *E.coli* (EHEC)	Intestinal contents, faeces or swabs and fixed intestines	Bacterial culture (TC0101 and TC0829 *E.coli* fimbrex test typing and histopathology PC0006)	be fixed within minutes of death Further *E. coli* serotyping possible at extra charge (TC0040)
	Rotaviral enteritis	Intestinal contents or faeces	Rotavirus PAGE (TC0582)	
	Cryptosporidiosis	Intestinal contents or faeces	Cryptosporidium smear and ID (TC0492)	Rarely diagnosed in pigs, zoonotic
	Coccidiosis	Intestinal contents or faeces (min. 3g)	Coccidial oocyst count (TC0702). Speciation possible at extra cost (TC0648)	Absence of oocysts does not rule out a diagnosis of coccidiosis. Intestinal histopathology may be required to achieve a diagnosis and needs live affected pigs to be submitted
	Salmonellosis	Intestinal contents or faeces (swabs second choice)	*Salmonella* culture (TC0025)	Rare in neonates, uncommon preweaning. Zoonotic
	Transmissible gastro-enteritis	Intestinal contents or faeces	TGE/PED PCR (TC0198)	PCR is method of choice for diagnosis Last VIDA diagnosis in GB in 1999
		Paired sera	TGEv ELISA (TC0488)	
	Porcine epidemic diarrhoea	Intestinal contents or faeces	PEDV PCR (TC0398)	Note this is notifiable in England and Scotland: https://www.gov.uk/guidance/porcine-epidemic-diarrhoea-how-to-spot-and-report-the-disease PCR is method of choice for diagnosis Last VIDA diagnosis in GB in 2002, virulent strain has not been detected in UK
		Paired sera	TGE/PED ELISA (TC0377)	
	Hypogamma-globulinaemia (poor colostral antibody uptake)	Clotted bloods from piglets up to one-week-old	Gammaglobulin estimation (ZST) Consult lab of choice for advice on test and sample requirements.	Useful to determine if poor colostral antibody transfer is predisposing to enteric disease in neonatal piglets

Pigs

Weaners to about 6 weeks old (two weeks post-weaning)	*E.coli*, salmonellosis, rotaviral enteritis, TGE and PED	As above	As above	As above
	Bowel oedema (verocytotoxic *E.coli*)	Distal small intestinal contents or charcoal swab or faeces	Bacterial culture (TC0101), *E.coli* serotyping (TC0040)	Isolation of causative *E. coli* more likely from small intestine of untreated severe cases. Brain histopathology can provide supportive evidence
Growers to adults	Salmonellosis, bowel oedema, TGE and PED	As above	As above	As above
	Brachyspira hyodysenteriae (swine dysentery)	Intestine, intestinal contents or faeces	*Brachyspira* culture (TC00331) *Brachyspira* PCR (TC0495) *Brachyspira hyodysenteriae* FAT (TC0032)	Where *B. hyodysenteriae* isolated, tiamulin MIC testing should be considered – discuss with a VIO at your usual APHA VIC Fill container with freshly voided faeces or LI contents to below brim (anaerobic organism). Culture with PCR is recommended. FAT can provide early warning but positives require confirmation
	Brachyspira pilosicoli colitis	Intestine, intestinal contents or faeces	*Brachyspira* culture (TC0031) *Brachyspira* PCR (TC0495)	Fill container to below brim (anaerobic organism).
	Lawsonia intracellularis	Lesioned ileum and (on occasion) lesioned large intestine fresh and fixed	MZN smear (TC0033) Histopathology (PC0006) and silver stains	Several forms exist (necrotic ileitis, proliferative enteropathy, haemorrhagic enteropathy). MZN smears needs very fresh material and can be insensitive. PCR alone is not diagnostic
		Intestinal contents or faeces	Lawsonia PCR (TC0657)	
	Trichuris colitis or other nematodes (*Hyostrongylus, Ascaris* spp.)	Faeces	Worm egg count (TC0060)	Egg output can be low. *Hyostrongylus* species associated with anaemia and illthrift
	Gastric ulceration, intestinal torsion	Dead pigs	Post mortem examination (TC0017 (neonatal) or TC0002)	

Pigs

Respiratory disease

Category	Condition/cause	Sample type	Recommended tests	Further information
All ages	Unknown or suspected cause for differential diagnosis	Ideally affected untreated pigs or plucks	Post mortem examination (TC0017 (neonatal) or TC0002, and tests at VIO discretion)	Batch of up to three pigs/plucks ideal. Where possible, submit untreated pigs. If treatment is failing, it may be appropriate to submit treated pigs also. Severe cases early in the course of disease are ideal. Porcine respiratory disease commonly involves multiple pathogens.
All ages, especially post-weaning	PRRS	Fresh lung, (spleen, serum or lymph node)	PRRSV PCR (TC0718) Pooled PRRSv PCR on up to five sera (rearing pigs only) (TC0918)	Vaccinated pigs may become viraemic when undergoing challenge and positive PCR results require further investigation. PRRS vaccination history with timing of vaccination should be stated on submission. Sequencing differentiates vaccine-like virus strains from others and can assist investigations Serology useful if not vaccinated.
		Positive PCR tissue or serum	PRRSV ORF5 sequencing (TC0118)	
		Paired sera	Paired serology PRRSv ELISA (TC0412)	
	PCV2-associated respiratory disease	Lymph node and fixed lung	Histopathology (PC0006 and PCV-2 IHC if necessary)	If lymphoid lesions seen with viral inclusions, PCV-2 IHC is not progressed by VIO as not necessary
	Swine influenza	Pooled tonsil, trachea and lung (max 3 pigs) or plain nasal swabs (max 12 pigs) Paired sera	Swine influenza PCR (TC0771) Swine influenza HAIT (TC0160)	Do not pool tissues from different pigs. Pigs sampled for virus must be in the first few days of infection. Testing for virus is free of charge, see http://apha.defra.gov.uk/documents/surveillance/diseases/swine-influenza.pdf
	Enzootic pneumonia (*Mycoplasma hyopneumoniae*)	Fresh lung	*Mycoplasma* spp DGGE PCR (TC0672) Histopathology (PC0006) essential for interpretation of significance where *Mycoplasma* species detected by DGGE-PCR, especially in vaccinated pigs	Sample lung from cranioventral region at interface between consolidated and non-consolidated lung. Also detects *M. hyorhinis* which can cause EP-like lesions
		Paired sera	Paired serology TC0546	Serology useful if not vaccinated. *M. hyopneumoniae* vaccination history should be provided.

Pigs

Category	Condition/cause	Sample type	Recommended tests	Further information
	Bacterial respiratory pathogens - *Pasteurella multocida, Strep suis, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae , Haemophilus parasuis*	Fresh lesioned lung (large portion) or charcoal swab from cut surface of lesioned lung	Bacterial culture (TC0101)	Avoid contamination when sampling, sear surface of lung before incising and swabbing if possible, or use sterile scalpel to incise.
	Glässer's disease (*Haemophilus parasuis,* Hps)	Charcoal swabs from up to 4 sites	Bacterial culture for *Haemophilus parasuis* only (TC0103)	TC0103 allows up to four lesioned sites to be cultured for just Hps under one test code charge (e.g. lung, pleura, pericardium, joint). Where possible, sample untreated freshly dead pigs.
	Progressive atrophic rhinitis	Charcoal swab from lesioned nasal chamber	*Pasteurella multocida* – toxigenic strain ELISA (TC0623)	Uncommon nowadays in GB pigs

Systemic disease

Category	Condition/cause	Sample type	Recommended tests	Further information
All ages	Unknown or suspected cause for differential diagnosis	Affected pigs ideally untreated	Post mortem examination (TC0017 (neonatal) or TC0002, and tests at VIO discretion)	If pigs are euthanased for submission, it is useful to obtain clotted and EDTA bloods from them first
Mainly pre-weaned and early post-weaned	Iron deficiency anaemia	Typically affected pigs, batch of 3 ideal, euthanased or freshly dead	Post mortem examination (TC0017 (neonatal) or TC0002, and tests at VIO discretion)	Full haematology confirms anaemia supporting diagnosis Liver iron estimation if no serum available
		EDTA and clotted bloods	Full haematology and serum iron. Consult lab of choice for advice on test and sample requirements	
Mainly post-weaned pigs from 6 weeks old, occasionally younger preweaned pigs or young adults	Porcine circovirus 2- associated disease including PMWS/PDNS	Typically affected pigs, batch of 3 ideal, euthanased or freshly dead	Post mortem examination (TC0002 and tests at VIO discretion)	Post mortem investigation best to identify concurrent disease and, if not PCVAD, to establish diagnosis. PCV-2 vaccination history should be provided. If lymphoid lesions seen with viral inclusions, PCV-2 IHC is not progressed by VIO as not necessary.

Pigs

Category	Condition/cause	Sample type	Recommended tests	Further information
		Formalin-fixed lymph nodes (inguinal, mesenteric) and other fixed tissues if lesioned	Histopathology (PC0006 and PCV-2 IHC if necessary to establish diagnosis)	PCVAD can have wider clinical presentation than illthrift, including mainly enteric or respiratory disease
Post-weaned pigs mainly from around 10-weeks-old	Porcine dermatitis and nephropathy syndrome (PDNS)	Typically affected pigs – euthanased or freshly dead	Post mortem examination (TC0002 and tests at VIO discretion)	Lesions of PDNS can resemble those of swine fever and, if in doubt, cases should be notified to APHA field services as suspect swine fever, especially if multiple cases are occurring with mortality
		Formalin-fixed lymph nodes (inguinal, mesenteric), skin and kidney	Histopathology (PC0006)	
Any age but especially pre post-weaned pigs	Bacterial septicaemia (e.g. erysipelas, streptococcal, pasteurellosis, *Klebsiella pneumoniae* and *Actinobacillus suis* and colisepticaemia in preweaned piglets)	Typically affected pigs – freshly dead	Post mortem examination (TC0017 or TC0002 and tests at VIO discretion)	Sudden or rapid death. Prior antimicrobial treatment may affect culture results.
		Fresh tissues or charcoal swabs from liver, lung, meninges	Bacterial culture (TC0101)	
	Haemophilus parasuis (Hps) septicaemia and/or polyserositis (Glässer's disease)	Charcoal swabs from up to 4 sites	Bacterial culture for *Haemophilus parasuis* only (TC0103)	TC0103 allows up to four sites to be cultured for just Hps under one test code charge (sites could include lung, meninges, liver, pleura)
	Polyserositis due to *Mycoplasma hyorhinis*	Plain swabs	*Mycoplasma* spp. DGGE_PCR (TC0672) – multiple swabs from same pig can be pooled	Polyserositis can include arthritis. Advisable to submit charcoal swabs for routine bacteriology and plain for *Mycoplasma* testing if no bacterial pathogen isolated
Any age	Toxicity	Affected pigs	Post mortem examination (TC0017 (neonatal) or TC0002, and tests at VIO discretion)	The two toxicities most frequently diagnosed in pigs at APHA are coal tar (liver necrosis) and bracken (cardiomyopathy and acute heart failure). Information on these are found on: http://apha.defra.gov.uk/vet-gateway/surveillance/seg/pig htm
	Neoplasia	Lesioned tissues	Histopathology (PC0006)	Individual pigs. Lymphoma most common neoplasia in pigs.

Pigs

Circulatory disease

Category	Condition/ cause	Sample type	Recommended tests	Further information
Any age	Unknown or suspected cause for differential diagnosis	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 or TC0002 and tests at VIO discretion)	Signs often sudden death or non-specific meriting submission of pigs.
Pre-weaned pigs	Thrombocytopaenic purpura (isoimmune disease – colostral antibodies to foetal thrombocyte antigens)	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 and tests at VIO discretion including haematology and bone marrow histopathology if submission allows)	Signs include weakness, anaemia, skin haemorrhages and death of non-pyrexic pigs up to about 30 days old. Gilt litters not affected. Lesions can resemble the swine fevers; a good clinical history and examination is usually sufficient to rule out concern, if in doubt, report cases to APHA field services
Pre and post-weaned pigs	*Mycoplasma suis* (eperythrozoonosis)	Blood (EDTA)	*Mycoplasma* DGGE/PCR (TC0672) Blood parasite detection (TC0826)	Full haematology detects anaemia and assists interpretation. Bone marrow and other histopathology assists interpretation
Any age	Endocarditis	Valve lesions or charcoal swab	Bacterial culture (TC0101)	Erysipelas and *Streptococcus suis* are the two main causes
Post-weaned pigs, occasionally pre-weaned	Mulberry heart disease (MHD) and Hepatosis dietetica (HD)	Typically affected pigs – freshly dead	Post mortem examination (TC0002) and histopathology	Usually presents as sudden death. If diagnosed, consider submitting bloods for vitamin E and selenium status from affected cohort. Consult lab of choice for advice on test and sample requirements. Main differentials for MHD: acute pulmonary oedema form of PCVAD, bracken poisoning, fumonisin toxicity. Main differentials for HD: coal tar toxicity, PCV2-associated hepatitis
		Fixed heart and lung (for MHD) Fixed liver (for HD)	Histopathology (PC0006)	

Pigs

Musculoskeletal disease

Category	Condition/cause	Sample type	Recommended tests	Further information
Any age but mainly post-weaned	Bacterial arthritis including erysipelas, *Streptococcus suis, Haemophilus parasuis*	Typically affected pigs – freshly dead	Post mortem examination (TC0017 or TC0002 and tests at VIO discretion)	PME allows diagnosis of osteochondrosis (OCD), an important differential in older pigs.
		Charcoal joint swabs or fluid aspirates	Bacterial culture (TC0101)	
		Paired sera	*Erysipelothrix* sp. SAT (TC0361)	It can be problematic isolating *Erysipelothrix* species from joints
		Charcoal joint swabs or fluid aspirates	Bacterial culture for *Haemophilus parasuis* only (TC0103)	Fibrinous polyarthritis may be part of Glässers disease. TC0103 allows up to four lesioned sites to be cultured for just Hps under one test code charge
	Infectious synovitis due to *Mycoplasma* spp e.g. *M. hyosynoviae or hyorhinis*	Plain joint swabs or fluid aspirates	*Mycoplasma* spp. DGGE PCR (TC0672) – multiple swabs from same pig can be pooled	*M. hyorhinis* usually younger postweaned pigs and may be part of polyserositis. *M. hyosynoviae* mainly older postweaned pigs or young breeding replacements. Histopathology on synovium provides supportive evidence
Post-weaned and young breeding pigs	Osteochondrosis dissecans, femoral epiphyseolysis, fractures	Typically affected pigs – freshly dead	Post mortem examination (TC0002 and tests at VIO discretion)	Gross pathology alone is often diagnostic. Bone analysis and histopathology would be considered if findings suggest an osteodystrophy

Nervous disease

Category	Condition/cause	Sample type	Recommended tests	Further information
Any age	Unknown or suspected cause for differential diagnosis	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 or TC0002 and tests at VIO discretion)	Where pigs are euthanased for submission, trauma to the brain due to the method of euthanasia limits full diagnostic investigation. Veterinary administration of barbiturate is ideal. Videos of typically affected pigs are a helpful diagnostic aid.
	Salt poisoning (water deprivation)	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 or TC0002 and brain histopathology)	Pigs are particularly sensitive to water deprivation-induced salt poisoning. Submission of heads of affected pigs would allow diagnosis of salt poisoning

Pigs

Category	Condition/cause	Sample type	Recommended tests	Further information
Neonatal	Congenital tremor (CT)	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 and histopathology on brain and spinal cord)	Clinical history and histopathology identify the type of CT. Classical swine fever is a cause of congenital tremor type A1 and, in CT outbreaks, the submitting vet must consider whether there are grounds to report as suspect swine fever to APHA field services. Atypical porcine pestivirus is a recently recognised cause of CT type A2 https://veterinaryrecord.bmj.com/content/180/2/42
Pre and post-weaned mainly	Bacterial meningitis	Typically affected pigs – freshly dead	Post mortem examination (TC0017 or TC0002)	*Streptococcus suis* and *Haemophilus parasuis* are the main bacterial causes. Cultures must include chocolate agar plates for *H. parasuis* isolation. Submission of heads of affected pigs would also allow diagnosis.
		Meningeal swabs (charcoal)	Bacterial culture (TC0101)	
		Meningeal swabs (plain)	*Streptococcus suis* 2 FAT (TC0032)	
Post-weaned pigs	Bowel oedema	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 or TC0002)	Verocytotoxigenic *E. coli* types involved. Histopathology on brain provide supportive evidence and may be indicated where clinical signs/gross lesions not typical. Isolation of causative *E. coli* more likely from untreated severe cases.
		Intestinal contents or faeces	Bacterial culture (TC0101)	
Any age	Viral polioencephalo-myelitis (e.g. porcine sapelovirus)	Typically affected pigs – live if welfare allows or freshly dead	Post mortem examination (TC0017 or TC0002)	Post mortem examination best option as uncommon and need to rule out differentials. Aujeszky's disease and Teschen disease are causes of viral encephalitis and are notifiable diseases. Submitting vet must consider whether there are grounds to notify severe outbreaks of nervous disease to APHA field services, especially if unresponsive to antimicrobial treatment. Sapelovirus PCR can be done at discretion of VIO if histopathology and other findings suggestive of involvement
		Fixed brain and spinal cord	Histopathology (PC0006 suspect lesions confirmed by IHC)	
		Fresh spinal cord and fresh brain (small portion of frontal cortex)	Sapelovirus PCR possible but not available as commercial test	

Pigs

Urogenital disease

Category	Condition/cause	Sample type	Recommended tests	Further information
Breeding females most likely	Cystitis and pyelonephritis	Typically affected pigs –freshly dead	Post mortem examination (TC0002)	Anaerobic culture essential to isolate *Actinobaculum suis* Urine samples need collection as aseptically as possible Histopathology allows diagnosis but not identification of causative organism
		Fresh kidney or urine	Bacterial culture aerobic (TC0101) and anaerobic (TC0528)	
		Fixed kidney	Histopathology (PC0006)	

Skin disease

Category	Condition/cause	Sample type	Recommended tests	Further information
Any age	Many conditions, e.g. exudative epidermitis, mange, ringworm, swine pox, parakeratosis, congenital conditions	Affected pigs	Discuss with APHA VIC	It is often helpful for images of the skin condition to be sent to the APHA VIC
		Charcoal swabs	Bacterial culture (TC0101) for *Staph. hyicus*	
		Hair plucks	Ringworm culture (TC0080)	
		Skin biopsies	Electron microscopy for pig pox virus (TC0082)	
		Skin/ear wax scrapings	Ectoparasite examination (TC0081)	

Miscellaneous and exotic farmed species

Please note the lists of differential diagnoses below concentrate on the more commonly seen conditions mainly in New World Camelids (NWC) and are not intended to be exhaustive. Other conditions may be involved.

Tests marked with an asterisk* are not validated for use in camelids.

Tests for other farmed species such as deer, bison, buffalo should use the tables for cattle or small ruminants as appropriate.

Abortion and stillbirth

Category	Condition/cause	Sample type	Recommended tests	Further information
Abortion and stillbirth (whole fetus/ stillborn, placenta and maternal clotted blood would be the submission of choice)	Bacterial causes (brucella, salmonella, campylobacter etc) and mycotic abortion	Fetal stomach contents or liver	Routine culture (TC0101) *Campylobacter* culture (TC0026) and wet preparation for funghi (TC0580)	Can be combined with PCRs for *Neospora caninum* and BVD virus (see below for sample requirements) under TC0015. If a full range of samples is submitted, testing will be carried out in a stepwise fashion. If there are no significant findings from step 1 (bacteriology/mycology), then the PCRs will be carried out as step 2. Tests additional to steps 1 and 2 (e.g. PCR for *Leptospira* sp.) can be commissioned, but will require additional samples and will attract an additional charge. Please discuss with a VIO.
	Chlamydia abortus, Coxiella burnetti	Fresh placenta	Stained smear exam (TC0033) PCR for *Chlamydia* (TC0417) PCR for Q fever* (TC0791)	Stained smear for *Chlamydia abortus* is routinely carried out as part of TC0015.
		Formalin fixed placenta	Histology (PC0006)	
	Neospora caninum	Formalin fixed heart and whole brain.	Histology (PC0006)	
		Fresh brain stem	Neospora PCR* (TC0852)	A positive PCR result confirms *Neospora* infection of the fetus, but does not confirm that neosporosis was the cause of abortion. Confirmation of *Neospora* abortion can be achieved through histopathology on fixed tissue (usually brain and heart).
	Leptospirosis	Fresh kidney	PCR for pathogenic leptospira* (TC0856)	

Miscellaneous and exotic farmed species

Category	Condition/cause	Sample type	Recommended tests	Further information
	BVD	Fresh foetal spleen or thymus.	PCR for BVD antigen* (TC0655)	
	Toxoplasma gondii	Placenta	PCR* (TC0858)	Rare cause of abortion. Cross reaction can occur between Toxoplasma and Neospora when using the IFAT test.
		Fixed tissues including brain	Histopathology (PC0006)	

Enteric disorders

Category	Condition/cause	Sample type	Recommended tests	Further information
< 7 days of age	Bacteria (*E.coli, salmonella*), rotavirus, cryptosporidia	Faeces (5g)	Enteric package (PC0059)	Individual components – routine culture and identification of isolates (TC0101) and sentest as appropriate, *salmonella* (TC0025), rotavirus PAGE* (TC0582), cryptosporidia (TC0492).
7-21 days of age	*Salmonella*, rotavirus, cryptosporidia	Faeces (5g)	Enteric package (PC0066)	As above but no routine culture or identification of isolates. Note cryptosporidiosis has been recorded as the cause of diarrhoea and death in animals up to 8 months of age.
> 7days of age	*Giardia*	Faeces (5g)	Flotation (TC0491)	
> 3 weeks of age	Coccidia, parasitic gastroenteritis	Faeces (10g)	Worm and coccidia count (TC0860)	Camelids only. Coccidiosis is more common between 3 weeks and 3 months of age. Nematodirosis can be a problem in young animals from 2 months of age. Haemonchonsis often presents as severe anaemia.
Adults	Liver fluke, coccidia, parasitic gastroenteritis	Faeces (40g + 10g)	Parasitology package (PC0864): Fluke egg count (TC0061) plus worm and coccidia count (TC0860)	Camelids only

Miscellaneous and exotic farmed species

Ill thrift/ weight loss

(Consider TB which is zoonotic in all age groups)

Category	Condition/ cause	Sample type	Recommended tests	Further information
Young animals	Coccidia, parasitic gastroenteritis	Faeces (10g)	Worm and coccidia count (TC0860)	Camelids only. Coccidiosis is more common between 3 weeks and 3 months of age. Nematodirosis can be a problem in young animals from 2 months of age. Haemonchonsis often presents as severe anaemia.
	BVD	Heparin blood or fresh lymphoid tissue (eg.spleen, thymus,lymph node).	PCR for BVD antigen* (TC0655)	
		Clotted blood	SNT for BVD antibodies* (TC1165)	
Any age	*Mycoplasma haemolamae*	EDTA blood	*Mycoplasma* DGGE/PCR (TC0672)	Can cause anaemia, vague signs or no overt disease. Note the causative organism can sometimes be detected on examination of a fresh blood smear (TC0256).
Adults	Liver fluke, coccidia, parasitic gastroenteritis	Faeces (40g + 10g)	Parasitology package (PC0864) Fluke egg count (TC0061) plus worm and coccidia count (TC0860)	Camelids only
Usually > 1 year of age	Johne's disease	Faeces (5g)	Detection of acid alcohol fast bacteria (TC0776)	Weight loss is the primary sign with diarrhoea only occurring terminally.
			Pool of up to 5 samples for PCR for *Map** (TC0693)	
			Liquid culture (TC0713)	Can take up to 12 weeks for result.
		Fixed ileo-caecal junction and associated lymph node.	Histology (PC0006)	Useful to differentiate from intestinal neoplasia and TB.
		Clotted blood	ELISA for Johnes disease antibodies* (TC0366)	Confirm positive ELISA results by PCR* (TC0693) or culture (TC0713).).

Miscellaneous and exotic farmed species

Category	Condition/ cause	Sample type	Recommended tests	Further information
Young animals/ adults	Trace element deficiency (eg B12/cobalt, GSH-Px/ selenium, copper)			Generally rare. Consult lab of choice for advice on individual lab test and sample requirements
Deer	Lungworm	Faeces (50g)	Baermann (TC0062)	Generally presents as weight loss rather than respiratory signs. Rarely recorded in NWC in the UK.

Other conditions

Category	Condition/cause	Sample type	Recommended tests	Further information
Nervous disorders	CCN/ encephalopathy	Carcase	Post mortem examination at a VIC	
	Meningitis/ encephalitis eg listeriosis	Carcase	Post mortem examination at a VIC	Often bacterial cause in young crias.
	Lead poisoning	Carcase	Post mortem examination at a VIC	Consult lab of choice for advice on individual lab test and sample requirements May present as a sudden death . Please discuss all cases of suspected lead poisoning in food producing animals with a VIO. Please see footnote below.
	Unknown cause	Carcase (plus clotted, EDTA and heparin bloods if available)	Post mortem examination at a VIC	Blood samples can be useful particularly when an assessment of hepatic or renal function is required.
Respiratory disease	Pneumonia	BAL	Culture (TC0101)	Viral infections of camelid respiratory system are rare. Consider TB which is zoonotic in all age groups
		Carcase	Post mortem examination at a VIC	
Skin disease	Parasites (*Chorioptes, Psoroptes, Sarcoptes, Demodex*, harvest mites,	Superficial and deep skin scrapes and hair plucks.	Microscopic exam (TC0081)	Suggest sampling of interdigital and margins of affected areas for mites. Sarcoptic mange represents a significant zoonotic risk. *Demodex* – rare.Prevalence of

Miscellaneous and exotic farmed species

Category	Condition/cause	Sample type	Recommended tests	Further information
	lice)			lice in NWC in the UK is unknown – likely to be low.
	Bacteria including CLA	Deep swab or tissue sample by biopsy.	Culture (TC0101)	CLA and *S.aureus* are rare in NWC – more common in Old World Camelids.
	Dermatophilus	Fresh moist scale and crust with hair pluck.	Stained smear exam (TC0033) Culture (TC0101)	Rare in NWC – more common in Old World Camelids.
	Dermatophytosis / ringworm	Hair pluck.	Wet preparation (TC0580)	Rare to uncommon and tends to be self limiting in NWC.
		Skin scale and scrape	Selective culture (TC0080)	
	Orf	Fresh skin tissue.	EM (TC0082)	Uncommon to rare.
		Formalin fixed tissue	Histology (PC0006)	
	Neoplasia	Formalin fixed skin tissue	Histology (PC0006)	Papillomas, fibropapillomas, squamous cell carcinoma, melanocytoma and mast cell tumours.
	Unknown skin disease	Formalin fixed tissue/ biopsy	Histology (PC0006)	Useful in assessing skin disease if other tests have failed to establish a diagnosis.
Sudden death Anthrax should be considered. TB can present as sudden death in camelids and should be included as a differential.	Clostridial enterotoxaemia	Small intestinal contents.	Clostridial toxin ELISA (TC0035)	
		Carcase	Post mortem examination at a VIC	
	Other clostridial diseases	Carcase	Post mortem examination at a VIC	
	Toxicities, poisoning	Carcase	Post mortem examination at a VIC	Copper and lead are the most common heavy metals involved in toxicities. See also lead poisoning in nervous disease section above. Please discuss all cases of suspected toxicities/poisoning in food producing animals with a VIO.
	(Nutritional) myopathy	Carcase	Post mortem examination at a VIC	Consult lab of choice for advice on individual lab test and sample requirements Can be associated with cardiac myonecrosis.
	Unknown cause	Carcase	Post mortem examination at a VIC	Hepatic lipidosis and abdominal disorders such as C3 ulceration and peritonitis can present as sudden death. A fresh carcase is more likely to yield a definitive diagnosis in

Miscellaneous and exotic farmed species

Category	Condition/cause	Sample type	Recommended tests	Further information
				these cases.
Musculo-skeletal disease (Note recumbency can result from nervous, parasitic, digestive, urinary, cardiovascular, metabolic or septic/toxic conditions)	(Nutritional) myopathy	Carcase	Post mortem examination at a VIC	Consult lab of choice for advice on individual lab test and sample requirements

NB: Please discuss all cases of suspected or confirmed poisoning in food animals with a VIO, as voluntary measures to control contamination of the food chain may be requested. In rare circumstances statutory controls imposed under the Food & Environmental Protection Act (FEPA) may be required.

Wildlife

Unusual and mass mortality of wildlife (all terrestrial vertebrate species) can be examined under the Diseases of Wildlife Scheme (APHA DoWS) and the AIV In Wild Birds Project. Carcases for post mortem can be submitted by veterinary surgeons, wildlife charities and members of the public. We will provide the person submitting the carcase with a written report.

We would ask for prior consultation before submission of carcases please. Cases accepted are generally examined free of charge with the submitter receiving copies of PM reports. New and emerging disease, zoonoses (in particular, mass mortalities of wild birds that may be caused by Avian Influenza Virus) and disease threats to livestock and biodiversity are the priorities in this Defra supported surveillance.

Any suspect malicious or accidental poisoning or misuse of pesticides or agrochemicals affecting wildlife should be reported to the Defra Wildlife Incident Investigation Scheme (WIIS) in the first instance (**Freephone 0800 321 600**).

Wild bird mortalities

APHA has a surveillance programme for wild bird mortalities, including testing for avian influenza viruses and the investigation of mass mortality incidents. Such incidents are defined as five or more wild birds of any species found dead at any location in England, Scotland and Wales. Members of the public are asked to remain vigilant for mass mortality incidents and report these to the Defra Helpline: **03459 33 55 77** or **08459 33 55 77**. Cases can also be discussed with vets at your usual APHA VIC in England or Wales, or your local SAC Disease Surveillance Centre in Scotland. Personal hygiene precautions should also be taken when handling dead garden and wild birds.

The table below provides basic guidance for frequently occurring incidents involving wildlife; it is not intended to be exhaustive.

Category	Condition/cause	Sample type	Recommended tests	Further information
Nervous disease in wild birds	Consider Avian Influenza Virus, West Nile Virus, Paramyxovirus, Avian botulism and poisoning	Carcases and live affected birds	PME (TC0004)	Clinical history essential. Virology may take several weeks
	Paramyxovirus, usually in wild pigeons and doves	Carcases and live affected birds	PME (TC0004)	Pigeons with PMV often show nervous signs e.g. torticollis
	Avian Botulism	Live affected birds are best. Carcases	PME (TC0004)	AB causes flaccid, floppy, paralysis. No sensitive laboratory tests – diagnosis based on clinical history
	Avian Influenza Virus	Carcases	PME (TC0004)	AIV may cause mass mortality In wild birds.
Mortality in garden birds	Garden bird salmonellosis	Carcases	Refer to the Garden Wildlife Health Scheme. Phone +44-20-7449-6685	Birds will be screened by APHA for West Nile Virus
	Garden bird trichomonosis	Carcases	Refer to the Garden Wildlife Health Scheme. Phone +44-20-7449-6685	Birds will be screened by APHA for West Nile Virus

Wildlife

Wild mammals

Category	Condition/cause	Sample type	Recommended tests	Further information
Nervous disease	Listeriosis, yersinosis, consider rabies	Carcases	PME (TC0004)	Laboratory testing and histopathology may take several days
Bats	Consider EBL (Bat rabies). Bacterial disease, parasites, trauma, cat predation, posioning	Carcases	PME (TC0004)	All bats submitted will be screened for rabies by APHA prior to further testing
Respiratory disease	Consider parasites, bacterial and viral infections	Carcases	PME (TC0004)	Lab testing including histopathology
Enteric disease	Consider parasites, bacteria (*salmonella*, yersiniasis and listeriosis) and viral disease	Carcases	PME (TC0004)	Lab testing