Developed in association with **Tim Potter**, BVetMed MBA PhD MRCVS (Senior Clinical Director at Westpoint Farm Vets and Kingshay Farming and Conservation Limited).

With thanks to vets at Biobest, APHA Scientific, Axiom and SRUC for their input to this guide.

This sampling guide has been produced by Ceva Animal Health, manufacturers of a BRD treatment range, including **ZELERIS®**, **TULAVEN®**, **MELOXIDYL®** and owner of **RIDGEWAY BIOLOGICALS** – producers of autogenous vaccines.

FURTHER READING ON THIS TOPIC:

Caldow G, Bronchoalveolar lavage in the investigation of bovine respiratory disease, In Practice 2001;23:41-43.

AHDB Getting the most out of on-farm post-mortems.

Gibson D, Investigating respiratory disease, Vet times 2009

Lurier et al. Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach. Prev Vet Med. 2018 Jun 1;154:124-131.

Ben Strugnell BCVA Webinar PM of calves, October 2020

BRD SAMPLING GUIDELINES FOR BOVINE RESPIRATORY DISEASE INVESTIGATION

Ceva Animal Health Ltd,
Explorer House, Mercury Park, Wycombe Lane,
Wooburn Green, High Wycombe, Buckinghamshire, HP10 0HH
Tel: 01628 334056 **www.ceva.co.uk** ZEL020Aug24V1

RIDGEWAY BIOLOGICALS

BRD costs the UK cattle industry an estimated £80 million per year.

Prevention is always better than cure but when an outbreak does occur, finding the root of the problem and its association with husbandry, the environment and/or pathogens, is key to optimising treatment and preventing future outbreaks.

Collecting and sending the best possible range of samples to a laboratory improves the chances of getting the correct diagnosis for the BRD outbreak. This guide is intended to provide you with an outline as to what samples to collect and which tests to request.

PLEASE ALWAYS CHECK WITH YOUR DIAGNOSTIC LAB WHICH SAMPLES SHOULD BE TAKEN AND SUBMITTED, DISCUSS INTERPERTATION IF UNCLEAR.

EQUIPMENT NEEDED:

TRANSTRACHEAL WASH/ASPIRATE:
- Size 5 or suitable (stiff) sterile tubing
- Sterile saline

POST MORTEM:
- PM knife and sterile scalpel blades
- Universal pots
  - large & small
- Plain swabs
- Formol saline

MISCELLANEOUS:
- Virus transport media
- Purple top blood tubes
- Mycoplasma transport medium

DEEP NASOPHARYNGEAL SWAB (DNS):
- Antiseptic wipes
- Long plain cotton guarded swabs (2)

BRONCHOALVEOLAR LAVAGE (BAL):
- BAL catheter
- 50 ml phosphate-buffered saline
- 50 ml syringe for delivery and collection
- Plain sample collection pot

PAIRED SEROLOGY:
- Red top blood sample tubes

LABEL SAMPLES ACCURATELY

SUMMARY:

As a general rule swabs, transtracheal washes or bronchoalveolar lavage should be collected from at least four acutely infected animals to maximise the diagnostic potential of the samples. For paired serology blood samples, ideally at least six animals should be sampled and more may be useful in some situations, e.g. 12 samples if animals are under 4 months of age. Samples should be taken in the acute phase and then repeated 21 days later from the same calves.

Sample	Available Tests	Comments		
		A*	C*	
Deep nasopharyngeal swabs	Detection of both viruses and bacteria in the upper airways Culture and PCR possible Ideally sample at least 4 animals.	✔		**ADVANTAGES** Quick and easy and with minimal cost **DRAW-BACKS** Risk of contamination with commensal pathogens means a guarded swab should be used and results interpreted with caution Only represents pathogens in the upper airways
Bronchoalveolar lavage (BAL)	Culture, Cytology, PCR Ideally sample at least 4 animals. Lungworm diagnosis via parasite ID (adult or larval) or eosinophil count (Lurier)	✔	✔	**ADVANTAGES** Representative of lower airways Can be used for lungworm detection Can run PCR as well as culture **DRAW-BACKS** Need some training on completing the technique Moderate contamination risk from nasal passages
Transtracheal wash (aspirate)	Culture, Cytology, PCR Ideally sample at least 4 animals.	✔	✔	**ADVANTAGES** Considered representative of lower airways if muco-ciliary tract is functioning Can run PCR as well as culture **DRAW-BACKS** Training may be needed on the technique Some complications can occur (wound infection, local haemorrhage etc)
Blood samples	Paired serology Sample 12 animals if <4month old Sample 6 animals if >4 months old	✔	✔	**ADVANTAGES** Quick and easy to collect, send samples from acute cases at time of collection for lab to separate and store If an outbreak has already occurred, take a covalescent sample (day 21) and interpret results with caution **DRAW-BACKS** More useful for monitoring than treatment decisions Maternal antibodies can interfere until the animal is 6 months old
Post-mortem exam	PCR Bacteriology culture Cytology Histopathology	✔	✔	**ADVANTAGES** Can visualise the entire respiratory tract and take multiple samples alongside photographs Other relevant underlying pathology may become apparent **DRAW-BACKS** Ideally need to have a recently deceased animal or sacrifice an animal to sample

A* = Acute: Pyrexic with serous discharge
C* = Chronic: Disease is longer standing, potentially no longer pyrexic and have a muco-purulent discharge

BRD SAMPLING TECHNIQUES — AN OVERVIEW

DEEP NASOPHARYNGEAL SWAB

1. Ensure the calf is adequately restrained
2. Wipe the nares with an antiseptic wipe
3. Carefully advance the guarded nasopharyngeal swab through the ventral meatus to approximately 2/3 of the dorsal head length
4. Expose the swab by withdrawing the outer sleeve and firmly rotate through 360° against the pharyngeal wall for 20–30 seconds
5. Retract the swab back into the outer sheath, and gently remove the whole swab
6. For PCR send plain swabs. Otherwise, a swab in viral transport medium and a second swab for bacteriology (avoid commensal and environmental contamination) may be appropriate.

BRONCHOALVEOLAR LAVAGE (NON ENDOSCOPIC – CALDOW)

1. Ensure the calf is adequately restrained – sedation may be necessary
2. Introduce BAL catheter through the cleaned nares and advance through the ventral meatus until resistance is felt at the larynx
3. Upon inspiration, advance the catheter into the trachea – expect some coughing
4. Continue advancing until the catheter becomes wedged
5. Advance the lubricated inner tube through the catheter with gentle pressure until increased resistance is felt.
6. Inflate the balloon cuff (if using a catheter, it should be wedged) appropriately and inject 30-60ml of saline and aspirate
5. Immediately place aspirate in an appropriately sized universal container.

TRANSTRACHEAL WASH/ASPIRATE

1. Ensure the calf is adequately restrained – sedation may be necessary
2. Palpate ventral neck and select a location mid-way between the larynx and where the tracheal rings can no longer be palpated
3. Surgically prepare the site, infiltrate local anaesthetic and, when effective, make a small stab incision through the skin
4. Stabilise the trachea with one hand and with the other introduce a large bore (12) needle or catheter trochar, pass into the tracheal lumen between two rings and point distally. Thread the sterile tubing onto the needle and towards the lungs
5. Inject approx. 20ml sterile saline and immediately aspirate (you should get 3 – 5ml), repeat until 10 – 20ml of fluid has been collected. The fluid can be submitted for PCR, cytology and culture amongst other tests.

POST MORTEMS

Where possible whole carcass submission is preferred. If not feasible, then take multiple aseptic samples of the affected tissue(s):

IMPORTANT – samples should be taken at the margin of normal and abnormal so includes both tissues.

To sear – place lung on a non-flammable metal surface (e.g. dehorner/palette knife) and heat the metal. Then place on a clean surface and cut through the sample before swabbing (pictorial guide in AHDB PM publication is useful):

- Sections (1 – 3 cm^2) of lung should be placed in a sealed container for bacterial culture and PCR
- Swabs from a seared and cut surface (sterile scalpel) for bacteriology (charcoal) and mycoplasma
- Several $1cm^3$ sections from the edge of lung lesions should be placed in 5 to 10 times the volume of formal saline for histopathology, also consider tracheal and cardiac samples
- Tracheal and spleen samples can be useful for PCR testing
- Examine the whole respiratory tract including the larynx and lymph nodes
- Liver samples can be taken for trace element testing and caecal/GIT samples for parasitology.

TOP TIPS:

- **Obtain a thorough clinical history, including the age of animal, any vaccines given with the date of vaccination and any other treatments.**
- **Make sure tests are requested for ALL likely pathogens.**
- **Avoid using wooden swabs they can interfere with PCR testing.**
- **Check with lab what pathogens are included in the PCR (bacteria and viruses) package and if you can pool samples, also be aware that PCR tests are very sensitive for IBR, interpret with caution.**
- **For post mortems, case selection is important, consider a calf that had acute disease and not a long-standing chronic case.**
- **Don't forget the non-typical BRD pathogens e.g. Salmonella dublin, Trueperella pyogenes, BVD etc.**
- **PCR testing can be run on a range of samples and these can be pooled in some circumstances (the lab should do this)**
- **Following PCR results, you may wish to culture (+/- sensitivity) or isolate a strain, so consider taking samples that can easily be stored.**

LUNG LESIONS RESTRICTED TO CRANIAL LOBES

YES
Bronchopneumonia

Presence of consolidation with fibrinous exudate in interlobular septa and pleura

YES

Fibrinous bronchopneumonia (e.g. *M. haemolytica* or *H. somni* *)

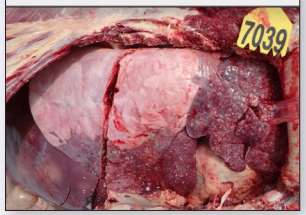
NO

Consolidation with purulent exudate in airways

Suppurative bronchopneumoni (*P. multocida*)

Consolidation with raised, white, sharply defined, crumbly areas of caseous necrosis

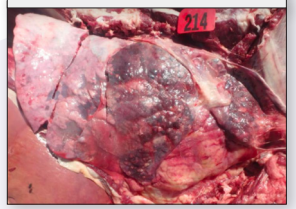
Caseonecrotic bronchopneumonia (e.g. *M. bovis*)(often associated with polysynovitis)



NO

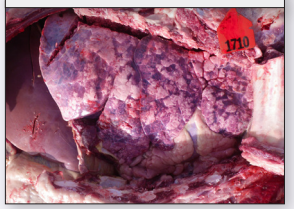
Multifocal lesions in all lobes, firm consolidation or abscesses

Embolic pneumonia (often associated with a thrombus in the vena cava)



Diffuse or lobular ("checkerboard") lesions, subtle firmness with alveolar and interlobular edema

Atypical interstitial pneumonia of feedlot cattle (endotoxemia, septicemia, respiratory viruses)



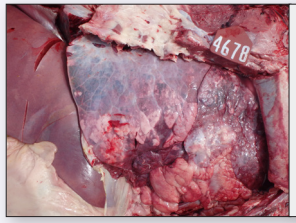
Cranio-ventral consolidation and interstitial lung lesions in the caudal lobes

Cranioventral lungs are slightly firm and colllapsed, dorsocaudal lungs are edematous, often with emphysema

Viral pneumonia (e.g.BRSV)

Cranioventral lungs are consolidated as a result of bacterial bronchopneumonia, dorsocaudal lungs are overinflated, often with alveolar and interlobular edema (and emphysema)

Viral pneumonia with secondary bacterial pneumonia or idiopatic (e.g. acute lung injury secondary to chronic bacterial pneumonia)



UNIVERSITY OF CALGARY

Developed by **Dr. Edouard Timsit,**
DVM, PhD, Dip. ECBHM

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**H. somni* pneumonia are not always fibrinous and are often associated with myocarditis and polysynovitis