INVESTIGATING LOWER AIRWAY DISEASE

TRACHEAL WASH (TW) OR BRONCHOALVEOLAR LAVAGE (BAL)?

- **TW best for bacteriology**
- **BAL best for cytology**
- **TW cytology may represent the whole lung whereas BAL assumes lung disease to be diffuse**
- **BAL bacteriology generally very unreliable**

There are several advantages and disadvantages of each technique. Simply, TW samples are best for bacteriology, BAL samples are best for cytology. Tracheal washes are generally performed via an endoscope but a transtracheal aspirate may be performed to eliminate the possibility of contamination of the sample with oropharyngeal bacteria.

Cellular content of TW samples has less precise reference ranges than BAL samples although it has the advantage of representing all lung regions whereas BAL samples a small area and relies on the lung disease being diffuse and homogeneous in order to be diagnostically useful.

TW is useful as a quick and easy "screening" procedure when there is no particular suspicion of airway disease – e.g. pre-competition – as a TW with a non-inflammatory cytological picture (i.e. <10% neutrophils and no eosinophils) is reassuring and prior sedation is unnecessary. However, where there is prior suspicion of airway disease there is a strong argument for performing both TW and BAL – especially when either RAO or IAD are more likely. BAL samples are simple to collect and have a more consistent cytological pattern in healthy horses facilitating identification of the abnormal. However, it is impossible to take a BAL sample without nasopharyngeal contamination therefore bacteriology on BAL samples is rarely indicated.

As both techniques have their own strengths and weaknesses it is preferable for them both to be performed during a respiratory investigation. With few exceptions, we would recommend submission of TW samples for both cytology and bacteriology and BAL samples for cytology only.

TRACHEAL WASH

- Cytology is useful but the key unique benefit of tracheal wash is *bacteriology* – good technique is crucial to avoid nasopharyngeal contamination if culture results are to be believed
- sedation preferred to twitch to reduce movement and coughing (causes contamination of sample from endoscope)
- prior exercise preferable although not necessary if subsequent BAL is planned
- desensitise larynx with 5 mL local anaesthetic via endoscopic catheter (hold head low so doesn't run down the trachea, contaminating it)
- after 2-3 minutes pass the endoscope through the larynx and stop in the *upper* trachea
- hold head reasonably elevated and advance a new catheter out of the endoscope and inject 20-30 mL warm sterile saline onto the tracheal mucosa which will then flow away from the endoscope and collect in a pool at the level of the thoracic inlet
- advance the endoscope to within a few centimetres of the pool of fluid (not dipping into it as the endoscope will be contaminated with pharyngeal bacteria) and aspirate fluid via the catheter.
- divide the sample between plain and EDTA tubes

INVESTIGATING LOWER AIRWAY DISEASE

BRONCHOALVEOLAR LAVAGE

It is almost impossible to collect a BAL sample that is not contaminated by nasopharyngeal bacteria and therefore bacteriology is rarely interpretable. In contrast *cytologic* examination of BAL samples is significantly superior to tracheal wash samples.

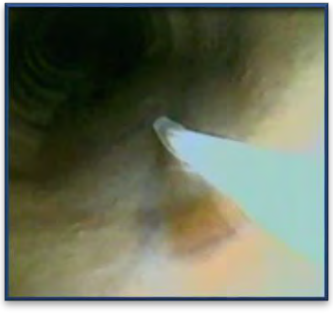
This author prefers a cuffed BAL catheter (e.g. Kruuse, Mila) without endoscopic guidance

BAL is a benign technique although coughing (sometimes quite violent) is to be unexpected during the procedure (pre-warn the owner). Horses with marked clinical signs of airway disease should probably not be subject to BAL until their clinical signs lessen in severity

It is customary to resume light exercise 24-48 hours after the procedure and more strenuous exercise may commence from 48-72 hours.

Technique:

- All horses should be well sedated with detomidine plus butorphanol
- Prefill 6 x 50 mL syringes with warm sterile saline
- Insert the BAL tube via the nostril and trachea until significant resistance to further passage is encountered
- Inflate the cuff with 5 mL of air
- Most horses will be coughing continuously at this point so wait for 20-30 seconds for coughing to reduce
- Sequentially attach the syringes and squirt the warm saline through the BAL tube
- Immediately following the last syringe of saline, re-aspirate the fluid. The first 10-20 mL can be discarded ('dead-space' in tube) then keep aspirating until no more fluid comes back (usually about half of the amount infused).
- A good sample is indicated by considerable white, stable foam as a result of surfactant. If there is an absence of stable foam then the technique should be repeated.
- pool all collected fluid rather than just randomly selecting one sample for laboratory analysis and place a sample in an EDTA tube *(plain tube needed for culture but culture rarely indicated in BAL)*



INTERPRETATION OF TRACHEAL WASH AND BRONCHOALVEOLAR LAVAGE SAMPLES

CYTOLOGY

The normal cellular constituents of TW and BAL fluid are shown below:

	TW	BAL
Neutrophils (%)	<20	< 5
Lymphocytes (%)	<10	20-50
Macrophages (%)	40-80	40-80
Eosinophils (%)	<2	<1
Mast cells (%)	<1	<2

Macrophages

- the most abundant cell in TW and BAL samples and are only of significance if they contain haemosiderin (an indication of recent haemorrhage).
- haemosiderophages indicate haemorrhage at any time up to a few weeks prior to collection.
- a few haemosiderophages is normal in exercising horses and EIPH is therefore a clinical diagnosis.
- in more sedentary horses the presence of haemorrhage may signify invasive pulmonary disease (i.e. neoplasia or abscessation).

Lymphocytes are the second commonest cell in TW and BAL and have little clear relevance to diagnosis.

Neutrophils

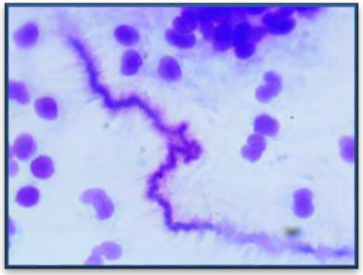
- Neutrophilia is the commonest abnormality in airway diseases.
- Neutrophil percentages are higher in TW than in BAL due to increased exposure to noxious stimuli that may result merely from housing or poor air hygiene.
- TW neutrophilia in affected by management and also by the wide range of reported results found in normal horses (3-83% neutrophils) and in horses with RAO (7-96% neutrophils).
- If BAL neutrophils account for > 10-15% of total cells then distal airway inflammation is present.
- Airway neutrophilia may reflect allergic lung disease (RAO, or summer pasture-associated RAO/SPARAO), inflammatory airway disease (IAD) or infectious lung disease (bacterial or viral). Generally the magnitude of the neutrophilia is highest in RAO/SPARAO.
- If BALF contains <5% neutrophils then one can be confident that there is no generalised distal airway disease.

Mast cells

- Not uncommon in inflammatory airway disease (will be missed unless special stains are employed)

Eosinophilia

- occasionally seen in IAD in young horses
- rare in RAO, or in rare cases of lungworm or eosinophilic interstitial pneumonitis.



Curschmann's spirals from an RAO case

INTERPRETATION OF TRACHEAL WASH AND BRONCHOALVEOLAR LAVAGE SAMPLES

BACTERIOLOGY

The potential for contamination from nasopharyngeal bacteria is a major confounding factor in the interpretation of TW bacteriology, necessitating careful technique

Squamous epithelial cells and/or the presence of plant material may indicate oro-naso-pharyngeal contamination and the bacteriology results are of little use.

It is important to interpret bacteriology in the light of concurrent cytology as even a profuse bacterial growth must be of dubious clinical relevance and merely indicate contamination when a non-inflammatory cytologic picture is found in the same sample.

Although the lower airways are virtually sterile in a normal horse, the passage of a BAL tube or endoscope inevitably results in oropharyngeal contamination and BAL samples are not recommended for bacteriological culture.

Submission for anaerobic culture is essential where pneumonia/pleuropneumonia is being investigated.

Culture of TW is more useful than culture of pleural fluid in cases with pleuropneumonia.

Pathogens commonly seen in IAD are listed below. Pneumonias may be associated with a far wider range of organisms.

Common pathogens in IAD cases	*Strep. equi subs zooepidemicus*
	Actinobacillus spp
	Pasteurella spp
Pathogens implicated in bronchopneumonias	*E. coli*
	Klebsiella pneumoniae
	Enterobacter spp
	Bordetella bronchiseptica
	Strep. pneumoniae
	Strep. dysgalactiae equisimilis
	Strep. equi subs equi
	Bacteroides spp
	Fusobacterium spp
	Peptostreptococcus spp
	Mycoplasma spp
Likely contaminants	*Pseudomonas aeruginosa*
	Staph. aureus
	Proteus spp
Definite contaminants	*coagulase negative Staphylococci*
	Bacillus spp
	Alternaria spp